

Original Research Article

Eco-friendly management of root rot disease in Cowpea

ABSTRACT

The research was conducted on evaluation of bio-agents and organic amendments against *Macrophomina phaseolina* of cowpea at the Department of Plant Pathology, S. D. Agricultural University Sardarkrushinagar, Gujarat during 2018-2019. Seven known antagonists were tested *in vitro* for their antagonism to *M. phaseolina* using by dual culture method. In this method, *T. harzianum* (Sardarkrushinagar) and *T. viride* (Sardarkrushinagar) were appeared as strong and 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 growth of mycelium was recorded in the extract of neem cake followed by mustard cake. Vermicompost and FYM were found less effective against *M. phaseolina*.

KEYWORDS: *M. phaseolina*, cowpea, antagonists, organic amendments, *in vitro*

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is locally known as chowli, lobiya, southern pea or black eye pea in India (Singh, 1998). Tropical Africa is considered as native of cowpea. It is grown extensively in West Africa, Brazil and India (Stele and Mehra, 1980). It has been referred to as “poor man’s meat” because of its high protein content (20 to 25%). It contains large high 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) (Chatterjee and Bhattacharya, 1986).

Cowpea is infected by many diseases caused by viruses, bacteria and fungi. Among the fungal diseases, 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 often makes cultivation of cowpea uneconomical (Singh *et al.*, 2012). Incidence of this disease ranging from 5- to 39 per cent (Ushamalani *et al.*, 1997). *M. phaseolina* (Tassi.) Goid. is a soil borne plant pathogen with a very wide host range. *M. phaseolina* (Tassi.)

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Goid. attacks a variety of oilseeds, legumes and vegetable crops. Its micro-sclerotia are formed in senescing shoot tissues, survive well in soil. The fungus is soil borne and poses great problem in managing the disease.

The hazardous effect of chemicals used in plant disease management has diverted plant pathologists to find out the alternative methods having little or no adverse effect on the environment. Notable success of disease control through the use of antagonistic microorganism in the laboratory, glass house and field have been achieved during past several years and based on this information, there is a possibility of developing biological control of plant disease under field conditions. Nowadays the commercial formulations of some of the bio-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, antagonists microorganism etc is the only alternative way of managing the plant pathogens without use of chemicals. Its good for sustainable disease management.

MATERIAL AND METHODS

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 (Rangaswami, 1972). 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 $5 \pm 2^\circ\text{C}$ 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. The cultural characters were recorded right from initiation of growth 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. The pathogen was identified as described in 'Illustrated Genera of Imperfect Fungi' by Barnett

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Efficacy of different bio-agents against pathogen *in vitro*

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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.

Table 1 : List of bio-agents tested against *Macrophomina phaseolina in vitro*

Sr. No.	Treatment	Name of the bio-agent
1	T ₁	<i>Trichoderma viride</i> (Navsari)
2	T ₂	<i>Trichoderma harzianum</i> (Sardarkrushinagar)
3	T ₃	<i>Trichoderma viride</i> (Junagadh)
4	T ₄	<i>Trichoderma harzianum</i> (Junagadh)
5	T ₅	<i>Trichoderma harzianum</i> (Anand)
6	T ₆	<i>Pseudomonas fluorescense</i> (Sardarkrushinagar)
7	T ₇	<i>Trichoderma viride</i> (Sardarkrushinagar)
8	T ₈	Control (Test pathogen only)

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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. Paired cultures were incubated at $28 \pm 2^\circ\text{C}$. The plates inoculated only with test pathogens served as control. The experiment was conducted with three repetitions of each treatment.

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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/cm² pressure for 20 minutes and considered as cent per cent 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 at 28 ± 2°C temperature. A control was also maintained by growing the pathogen on organic amendment free medium.

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

Sr. No.	Treatment	Organic amendment	Concentrations (%)		
			5	10	20
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	-	-	-

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 (1947).

$$PGI = \frac{C - T}{C} \times 100$$

Where,

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

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RESULTS AND DISCUSSION

Identification of pathogen

After purification of the pathogen as described under materials and methods, morphological and cultural characters of the fungus grown on PDA, were studied for identification and compared with those described in the literature. 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 (1981). The results obtained by Agarwal (1993) and Tandel (2004) also similar with our present finding. Thus, the causal organism of root rot of cowpea was confirmed as *Macrophomina phaseolina* (Tassi) Goid.

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 $28 \pm 2^\circ\text{C}$ temperature (Plate I). 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 IIA). The sclerotia formed in culture were black and hard (Plates II B and C). The pycnidia were not produced in culture. The morphology of *M. phaseolina* causing dry root rot of green gram was studied by Agrawal (1993).

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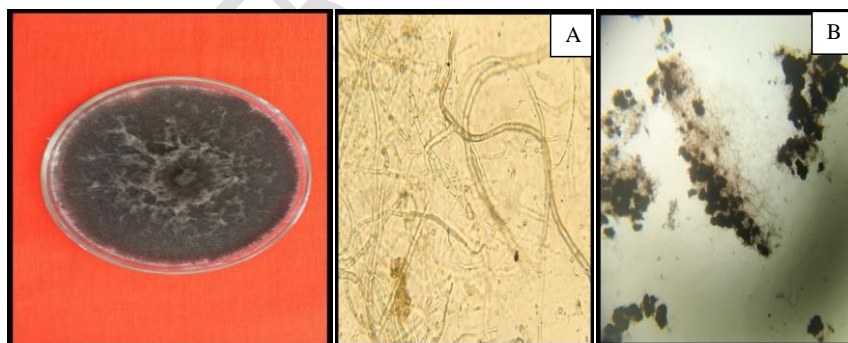


Plate I : Pure culture of *M. phaseolina* on PDA

Plate II : Microphotograph of mycelium and sclerotia of *M. phaseolina*

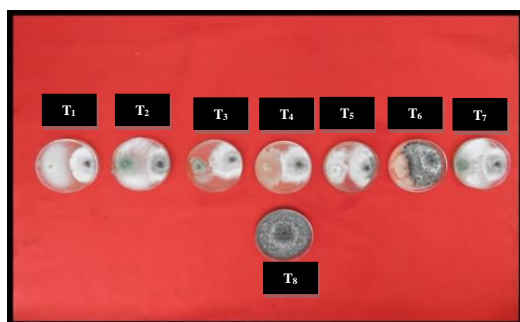
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Efficacy of different bio-agents against pathogen *in vitro*

The results are presented in Table 3 and (Plate III) revealed that, all the antagonists tested against *M. phaseolina* were effective in checking the growth of *M. phaseolina*. 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).

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Treatment	
T ₁	= <i>Trichoderma viride</i> (Navsari)
T ₂	= <i>Trichoderma harzianum</i> (Sardarkrushinagar)
T ₃	= <i>Trichoderma viride</i> (Junagadh)
T ₄	= <i>Trichoderma harzianum</i> (Junagadh)
T ₅	= <i>Trichoderma harzianum</i> (Anand)
T ₆	= <i>Pseudomonas fluorescence</i> (Sardarkrushinagar)
T ₇	= <i>Trichoderma viride</i> (Sardarkrushinagar)
T ₈	= Control (Test pathogen only)

Plate III : Efficacy of different bio-agents 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.

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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.

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

Tr. No.	Bio-agents	Colony diameter of pathogen (mm)	Growth inhibition over control (%)
T ₁	<i>Trichoderma viride</i> (Navsari)	34.18	52.15 ^{abc} (62.34)
T ₂	<i>Trichoderma harzianum</i> (Sardarkrushinagar)	27.93	56.11 ^a (68.90)
T ₃	<i>Trichoderma viride</i> (Junagadh)	40.87	47.60 ^{cd} (54.53)

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T ₄	<i>Trichoderma harzianum</i> (Junagadh)	38.54	48.89 ^{bcd} (56.76)
T ₅	<i>Trichoderma harzianum</i> (Anand)	42.31	46.52 ^{de} (52.65)
T ₆	<i>Pseudomonas fluorescense</i> (Sardarkrushinagar)	43.49	42.06 ^e (44.87)
T ₇	<i>Trichoderma viride</i> (Sardarkrushinagar)	31.81	53.49 ^{ab} (64.6)
T ₈	Control	90.00	4.05 ^f (0.49)
S.E.m.±			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.

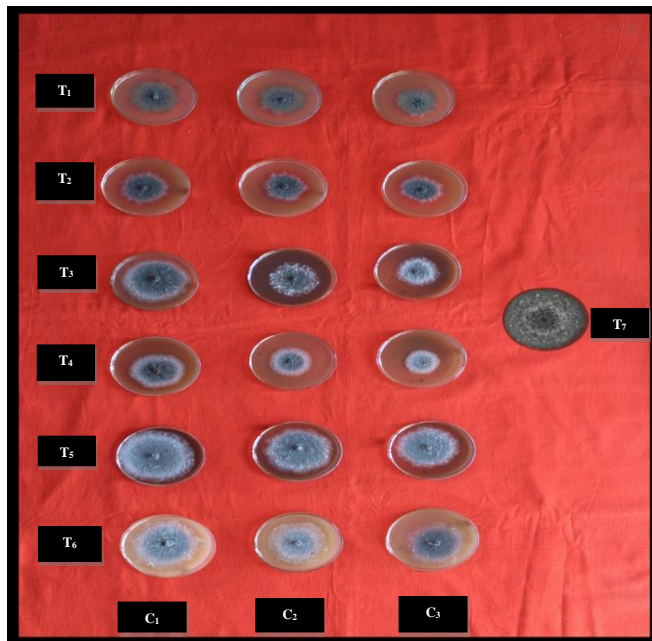
These results are in harmony with earlier workers Thombre and Kohire (2018) reported *T. harzianum* most effective and recorded significantly highest mycelial growth inhibition (77.59 %) of the *M. phaseolina* over untreated control. Kumar *et al.* (2020) also reported that maximum growth inhibition 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.

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 IV, V) indicated that organic extracts produced significant inhibitory effect on the fungal growth.

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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 VI : Efficacy of different organic amendments against *M. phaseolina* in vitro

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

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

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Tr. No.	Organic amendments	Growth inhibition (%)			Mean
		Concentration (%)			
		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.

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 more or less similar to the work done by earlier workers Makwana (2010) who concluded that neem cake @ 10 per cent with maximum (53.69 %) growth inhibition against *M. phaseolina*. Meena *et al.* (2014) also concluded that maximum mycelial growth inhibition (52.40 %) was recorded in neem cake (*Azadirachta indica*) 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.

Conclusion

Microscopic examination and tissue isolation from stem and root of infected plant yielded culture of *M. phaseolina*. Seven known antagonists were tested *in vitro* for their antagonism with *Macrophomina phaseolina* by dual culture method. Among them, *Trichoderma harzianum*

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(Sardarkrushinagar) and *Trichoderma viride* (Sardarkrushinagar) ~~were appeared as strong and~~ 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 **recorded by** the extract of neem cake (54.30%) followed **by and** mustard cake (51.63%).

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