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Management of root and collar rot (*Macrophomina phaseolina* (Tassi) Goid.) of OKRA (*Abelmoschus esculentus* (L.) Moench) through bioagents, oil cakes and fungicides

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Abstract
The *in vitro* evaluation of five different bioagents against *Macrophomina phaseolina*, incitant of root and collar rot of okra, revealed that *Trichoderma viride* was the most effective with highest growth inhibition (73.06%) followed by *T. harzianum* (68.89%). Among the oil cake extracts, neem cake extract had highest growth inhibition with 33.89 and 38.89 per cent at 10 and 20 per cent concentration, respectively. Among the different fungicides tested, tebuconazole had cent per cent growth inhibition at both concentration followed by carbendazim 12% + mancozeb 63% with 89.63 and 91.86 per cent growth inhibition at 500 and 1000 ppm, respectively. Out of the eight different treatments tested *in vivo* through pot culture studies, seed treatment with *T. viride* combined with soil application of *T. viride* enriched FYM recorded the least mortality percentage (26.55%) and highest vigour index (1643).

Keywords: okra, root and collar rot, bioagents, oil cakes, fungicides

1. Introduction
Okra [*Abelmoschus esculentus* (L.) Moench] is an important vegetable crop belonging to *Malvaceae* family and is widely cultivated in the tropical and subtropical parts of the world. It is valued for its tender green fruit and is rich in vitamins, calcium, potassium and crude fibre. Okra is affected by many pests and diseases at different stages of growth. Major diseases affecting the crop are powdery mildew, leaf spot, root rot and yellow vein mosaic (Rangaswamy and Mahadevan, 2014) [12]. *Macrophomina phaseolina* inciting root and collar rot is an emerging problem in okra in many parts of Gujarat and may account for 25-30 per cent plant mortality under field conditions. *Macrophomina phaseolina* is one of the most damaging primarily soil borne pathogens having heterogeneous host specificity and infects about 500 plant species in more than 100 families throughout the world. It causes stem canker, seedling blight, charcoal rot, dry root rot, wilt, leaf blight, stem blight and damping off in different crops (Singh *et al.*, 1990) [16]. It can assume epidemic form in areas with low rainfall and prolonged dry spell. Being soil borne in nature the disease is difficult to manage using conventional disease management strategies. Hence, an integrated strategy involving biocontrol agents, organic amendments and fungicides needs to be developed in order to tackle the pathogen. The present investigation has been carried out to find out the most effective biocontrol agent, organic amendment and fungicides along with their combinations to develop an effective integrated strategy to effectively manage the disease.

2. Materials and Methods
2.1 *In Vitro*
The *in vitro* studies were laid out in completely randomized design with four repetitions each. Observation on the radial growth (mm) was recorded from 24 hrs of incubation at 28±1 °C till the complete growth of test pathogen in control plates. Percent growth inhibition (PGI) over control was calculated by using following formula given by Vincent (1927) [20].

$$\text{Per cent growth inhibition (PGI)} = \frac{C - T}{C} \times 100$$

Where, C = Colony diameter in control plates (mm)
T = Colony diameter in treated plates (mm)

Biocontrol agents

Antagonistic effect of different species of *Trichoderma viz., T. viride, T. harzianum, T. virens, T. atroviride and T. asperellum* was tested by dual culture technique (Dennis and Webster, 1971) [2] for their antagonism against the root and collarrot pathogen *M. phaseolina.* The *Trichoderma* isolates were collected from Biocontrol Laboratory, Department of Plant Pathology, AAU, Anand. Seven days old culture of the bioagent and the pathogen were used. Mycelial disc of five mm diameter cut from the periphery of both antagonist and test pathogen were placed at 50 mm apart from each other in Petri plates. In control, only test pathogen was kept in the centre of Petri plate. The Petri plates was incubated at 28 ± 1 ^{0}C in BOD incubator.

Oil cakes and fungicides

The efficiency of FYM, vermicompost, four different oil cake extracts and seven different fungicides were tested against *M. phaseolina* by "Poisoned Food Technique" (Kumar *et al.*, 2011) [9]. Each of the extract/fungicides was tested at two different concentrations. The aqueous extract of the different oil cakes was prepared as per Yelmame *et al*. (2010) [20]. Thirty gram well ground powder of each cake was suspended in 150 ml sterile distilled water in flask and left for 25 days. The flasks were shaken for thorough mixing and dissolution of the content. After 25 days the flasks were thoroughly shaken and contents were filtered through double layered muslin cloth and autoclaved for 20 minutes. The autoclaved extracts were individually added in previously sterilized melted and cooled potato dextrose agar medium as per required concentration at the time of pouring in Petri plates and mixed thoroughly.

2.2 *In vivo*

The two most effective bioagents and fungicides were tested in pots with different treatment combinations by seed treatment, soil drenching and/or soil application. Experiment was laid out in completely randomized design with three repetitions each. Five seeds of okra cultivar Parbhani Kranti were sown per pot and three pots were maintained per repetition. Germination percentage, shoot and root length (cm), seedling vigour index and per cent seedling mortality due to *M. phaseolina* were recorded till 45 days after sowing. The percentage mortality and seedling vigour index were calculated by the formula given by Pandey *et al.* (1989) [11] and Baki and Anderson (1973) [1], respectively.

$$\text{Per cent mortality} = \frac{\text{No. of seeds / seedlings rotted} \times 100}{\text{Total no. of seeds sown}}$$

Vigour index = (Mean root length + Mean shoot length) x Per cent germination

2.3 Statistical analysis

Data obtained from various experiments were analyzed by appropriate statistical procedures as per Steel and Torrie (1980) [17].

3. Results and Discussion

3.1 *In vitro*

3.1.1 Bioagents

The effect of different *Trichoderma* sp. tested against *M. phaseolina* are presented in table 1. The results revealed that all the antagonists screened were significantly superior to control with percentage growth inhibition varying from 61.94 to 73.06 per cent. Out of the five *Trichoderma* spp., *T. viride* was significantly superior to the rest of the treatments and had the maximum growth inhibition (73.06%) while the lowest inhibition percentage was that of *T. virens* (61.94%). The other three species *T. harzianum, T. atroviride* and *T. asperellum* showed moderate inhibition of 68.89, 63.89 and 63.61 per cent, respectively. The treatments *T. atroviridae, T. asperellum and T. virens* were at par with each other with 32.50, 32.75 and 34.25 mm growth of the pathogen, respectively.

Results were in agreement with Kumar *et al.* (2015) [8] who reported that different isolates of *T. viride* and *T. harzianum* reduced the mycelial growth of *M. phaseolina* by 63.33 to 81.62 and 65.4 to 81.25 per cent, respectively. According to Reetha *et al.* (2014) [13] different isolates of *T. viride* inhibited the mycelial growth of *M. phaseolina* by 48.36 to 74.21 per cent. As per the findings of Tandel *et al.* (2014) [18], *T. viride* and *T. harzianum* have shown a growth inhibition of 77.38 and 78.57 per cent respectively against *M. phaseolina*.

Table 1: Effect of different *Trichoderma* spp. on mycelial growth of *M. phaseolina* under *in vitro* conditions

Sl. No.	Biocontrol agent	Average Colony Diameter (mm)*	Growth Inhibition (%)
1	*Trichoderma atroviride* (AAU isolate)	32.50	63.89
2	*Trichoderma harzianum* (AAU isolate)	28.00	68.89
3	*Trichoderma virens* (AAU isolate)	34.25	61.94
4	*Trichoderma viride* (TNAU isolate)	24.25	73.06
5	*Trichoderma asperellum* (AAU isolate)	32.75	63.61
6	Control	90.00	-
	S. Em±	0.952	
	CD (0.05)	2.829	
	CV (%)	4.72	

*Average of four replications.

3.1.2 Oil cakes

The results revealed that all the tested extracts had only low to moderate inhibitory effects with growth inhibition ranging from 25.28 to 38.89 and 9.17 to 33.89 per cent at 20 and 10 per cent concentration respectively (Table 2). Extracts of neem cake exhibited the highest inhibition of 33.89 and 38.89 per cent at both 10 and 20 per cent concentration, respectively. At ten per cent concentration, least mycelial growth was observed in neem cake extract (59.50 mm) which was at par with FYM (61.75 mm) and castor cake (63.75 mm). The next in order was groundnut cake (28.06% growth inhibition) which was at par with mustard cake (23.33%).

At 20 percentage concentration, neem cake had the highest growth inhibition percentage (38.89%) which was at par with mustard cake (36.94%) and groundnut cake (35.00%). The next best in order was castor cake (33.06%) and FYM

(31.94%). Vermicompost had the highest mean mycelia growth (67.25-81.75 mm) and least growth inhibition percentage (9.17-25.28%) at both the concentration. This reveals that the organic amendments have less direct toxicity against *M. phaseolina.* Hence the beneficial effects of soil application of organic amendments may be attributed to other factors like stimulating the growth of beneficial soil microflora, improving plant vigour and/or improving the host plant resistance by the metabolites released on decomposition of these amendments. Also some of the toxic metabolites could have been lost during autoclaving which added to the reduced growth inhibition of the extracts.

Table 2: Effect of organic amendment extracts on mycelial growth of *M. phaseolina* under *in vitro* conditions

Tr. No.	Organic Amendment	Average Colony Diameter (mm)*		Growth Inhibition (%)	
		Concentration (%)		Concentration (%)	
		10	20	10	20
1	Neem Cake	59.50	55.00	33.89	38.89
2	Castor Cake	63.75	60.25	29.17	33.06
3	Mustard Cake	69.00	56.75	23.33	36.94
4	Groundnut Cake	64.75	58.50	28.06	35.00
5	Vermicompost	81.75	67.25	9.17	25.28
6	FYM	61.75	61.25	31.39	31.94
7	Control	90.00	90.00	-	-
	S. Em±	1.57	1.43		
	CD (0.05)	4.61	4.21		
	CV (%)	4.47	4.46		

*Average of four replications.

Similar conclusion has been made by several other investigators. Dhingani *et al.* (2013) [3] has reported that out of several organic extracts tested neem cake had the maximum mycelial growth inhibition (59.40%) followed by farm yard manure, castor cake and mustard cake. Similarly, Dubey *et al.* (2009) [4] has reported that autoclaved neem extracts had growth inhibition of 19.7, 30.6 and 42.3 per cent at one, five and ten percentage, respectively.

3.1.3 Fungicides

The results revealed that all the tested fungicides significantly reduced the mycelial growth of fungus and had high growth inhibition percentage ranging from 80.37 to 100 per cent (Table 3). Among the tested fungicides tebuconazole had cent per cent growth inhibition at both 100 and 500 ppm. It was followed by carbendazim 12% + mancozeb 63% with 91.86 and 89.63 per cent inhibition at 1000 and 500 ppm. The next best in order was carbendazim 25% + mancozeb 50% at 1000 ppm which was at par with 1000 ppm each of fenamidon 10% + mancozeb 50% and trifloxystrobin (25%) + tebuconazole (50%) with 88.89 percentage inhibition. Carbendazim 25% + mancozeb 50% (500 ppm), trifloxystrobin 25% + tebuconazole 50% (500 ppm), fenamidon 10% + mancozeb 50% (500 ppm) and carboxin 37.5% + thiram 37.5% had moderate percentage growth inhibition of 87.86, 85.56, 84.81 and 84.08 percentage respectively. Carbendazim (500 ppm), carboxin 37.5% + thiram 37.5% (100 ppm) and carbendazim (100 ppm) recorded higher mycelia growth and low growth inhibition of 83.33, 80.74 and 80.37 per cent respectively.

Table 3: Effect of different fungicides on *M. phaseolina* under *in vitro* conditions

Sl. No.	Fungicides	Concentration (ppm)	Average Colony Diameter (mm)*	Growth Inhibition (%)
1	Carbendazim	100	17.67	80.37
		500	15.00	83.33
2	Carbendazim12% +Mancozeb 63%	500	9.33	89.63
		1000	7.33	91.86
3	Carbendazim 25% + Mancozeb 50 %	500	11.33	87.86
		1000	9.67	89.26
4	Fenamidon 10% + Mancozeb 50 %	500	13.67	84.81
		1000	10.00	88.89
5	Tebuconazole	100	0.00	100.00
		500	0.00	100.00
6	Trifloxystrobin (25%) + Tebuconazole (50%)	500	13.00	85.56
		1000	10.00	88.89
7	Carboxin 37.5 % + Thiram 37.5 %	100	17.33	80.74
		500	14.33	84.08
8	Control	-	90.00	-
	S. Em±		0.413	
	CD (0.05)		1.192	
	CV (%)		4.49	

*Average of four replications.

Veena *et al.* (2014) [19] has reported that tebuconazole at 250, 500, 750 and 1000 ppm completely inhibited the growth of *M. phaseolina* under *in vitro* conditions. Similarly Kanwal *et al.* (2012) [7] has reported cent per cent growth inhibition of *M. phaseolina.* According to Hussain *et al.* (2014) [5], carbendazim (100 ppm) had a growth inhibition of 54.6 per cent.

3.2 *In Vivo*

The two best fungicides and biocontrol agents were evaluated for their efficacy in managing the root and collar rot of okra. The organic amendments were not included for the *in vivo* study as they showed less than 50 percentage growth inhibition under *in vitro* conditions. The results are presented in table 4.

Table 4: Effect of different treatments on root and collar rot incidence in okra under pot conditions.

Tr. No.	Treatments	Mortality (%)
T_1	Seed treatment with *Trichoderma viride* TNAU isolate @ 10 g/ kg seeds	37.21# (36.57)
T_2	Seed treatment with *Trichoderma harzianum* AAU isolate @ 10 g/ kg seeds	46.90 (53.32)
T_3	T_1 + Soil application of *T. viride* enriched FYM ($2x10^8$ cfu/g) @ 100 g/ pot	26.55 (19.98)
T_4	T_2 + Soil application of *T. harzianum* enriched FYM ($2x10^8$ cfu/g) @ 100 g/ pot	41.14 (43.28)
T_5	Seed treatment with Carbendazim 12%+ Mancozeb 63% (75 WP) @ 3 g/kg seeds	26.55 (19.98)
T_6	T_5 + Soil drenching with tebuconazole @ 1.5 ml/ L.	41.14 (43.28)
T_7	Soil drenching with tebuconazole @ 1.5 ml/ L twice at 15 days interval.	52.75 (63.37)
T_8	Control (Untreated check)	89.964 (100.00)
	S. Em±	1.94
	CD (0.05)	5.83
	CV (%)	7.43

Figures in the parenthesis are retransformed values while those outside are arc sine transformed values

Out of the eight treatments, seed treatment with *T. viride* combined with soil application of *T. viride* enriched FYM and seed treatment with carbendazim12% + mancozeb 63% had the least mortality percentage (26.55%). It was followed by seed treatment with *T. viride* which had 37.21 per cent mortality. The next best in order was seed treatment with *T. harzainum* combined with the soil application of *T. harzianum* enriched FYM which was at par with seed treatment with carbendazim12% + mancozeb 63% along with soil drenching with tebuconazole (41.14% mortality). Seed treatment with *T. harzianum* and soil drenching with tebuconazole twice at 15 days interval had percentage morality of 46.90 and 52.75 per cent respectively.

Similar trend was observed in the plant vigour as well (Table 5). Highest plant vigour was observed in seed treatment with *T. viride* combined with application of *T. viride* enriched with FYM (1643). It was followed by combined application of *T. harzianum* through seed and soil (1346) and seed treatment with *T. viride* (1322). The next best in order was seed treatment *T. harzianum* (1305), seed treatment with carbendazim12% + mancozeb 63% (1195.83), seed treatment with carbendazim12% + mancozeb 63% combined with soil drenching with tebuconazole (1182) and soil drenching with tebuconazole twice (617). The control plants had the lowest plant vigour (504).

Table 5: Effect of different treatments on plant vigour

Tr. No.	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
1	83.82 (98.84)#	10.97	5.10	1322
2	83.82 (98.84)	11.13	4.43	1305
3	83.82 (98.84)	12.66	7.17	1643
4	77.68 (95.45)	11.43	5.67	1346
5	83.82 (98.84)	11.07	3.20	1196
6	83.82 (98.84)	11.00	3.10	1182
7	52.75 (63.36)	9.47	2.40	617
8	50.75 (59.97)	7.90	2.23	504
S. Em±	5.367	0.274	0.109	
CD (0.05)	16.09	0.820	0.326	
CV (%)	12.39	4.46	4.52	

Figures in the parenthesis are retransformed values while those outside are arc sine transformed values

According to Jambhulkar *et al.* (2015) [6], seed treatment with *T. harzianum* followed by the soil application of *T. harzianum* enriched FYM was effective in controlling the root rot of chickpea (PDI-5.8). It was followed by seed treatment with carbendazim + mancozeb combined with the soil application of neem cake (8.1%) which was at par with seed treatment with *T. viride* combined with the soil application of *T. viride* emriched FYM (PDI-8.2). Similar trend was observed in vigour index as well. Sayyad *et al.* (2015) [14] has reported that among bioagents, seed treatment with *T. viride* had the lowest dry root rot incidence in chickpea (31.1%) followed by seed treatment with *T. harzianum* (35.3%). According to Sharma *et al.* (2013) [15] seed treatment with carbendazim + mancozeb along with weekly irrigation had the lowest root rot incidence (16.36%) in sunflower. Nagamani *et al.* (2011) [10] has reported that seed treatment with carbendazim @ 2 g/kg seeds + seed treatment with *T. viride* @ 4 g/kg seed + soil application of FYM fortified with *T. viride* recorded lowest per cent disease incidence (4.33%) followed by seed treatment with *T. viride* @ 4 g/kg seed + soil application FYM fortified with *T. viride* (8.21%). Thus, it can be concluded that bioagents and fungicides are effective in managing the root and collar rot of okra as well as other diseases caused by *M. phaseolina* in different crops. There is a need to develop region specific integrated management strategies to effectively manage the soil borne pathogens which are difficult to manage by the conventional strategies.

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