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Efficacy of *Trichoderma viride* Pers. Gray and neem cake amendment in different soils against blackgram root rot caused by *Macrophomina phaseolina* (Tassi.) Goid

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ABSTRACT: **Studies were conducted in a greenhouse to assess the efficacy of *Trichoderma viride* Pers. Gray and neem cake in different problems soils *viz.*, sandy, black hard pan, saline, sodic, alkaline, red crusted soil, acid soil and normal soil in suppressing the growth of blackgram root rot pathogen *Macrophomina phaseolina* (Tassi.) Goid. The biocontrol efficacy of *T. viride* I_3 plus neem cake application in uninfested soil was the maximum 60 days after inoculation in acid soil which showed the minimum (5.63%) root rot incidence as compared to *T. viride* I_3 alone (6.44%) and neem cake application alone (7.15%). In infested soil, the *T. viride* I_3 and neem cake plus pathogen inoculation recorded the minimum (6.47%) disease incidence in acid soil as compared to *T. viride* I_3 and pathogen inoculation (7.52%), and neem cake application plus pathogen inoculation (8.72%). In uninoculated control and in inoculated control of acid soil, the root rot incidence was 7.84 and 9.70 per cent, respectively.**

KEY WORDS: Biocontrol, *Macrophomina phaseolina*, neem cake, problem soils, *Trichoderma viride*

Macrophomina phaseolina (Tassi.) Goid infects a number of crops and causes root-rot disease in blackgram. The pathogen being soil-borne in nature, is very difficult to control. Fungicides provide certain degree of control against seed, air and soil-borne pathogens, but at the same time pollute the environment affecting the beneficial microorganisms. Considering the above facts, biocontrol agents are being used for disease control in the present day crop husbandry in an increasing scale. The use of *Trichoderma* spp. (Xu *et al.*, 1993; Boer *et al.*, 1998) and organic amendments (Suriachandraselvan, 1997) in plant disease management has been well documented. The perusal of the literature reveals that work on the efficacy of this biocontrol agent and organic amendments in different problem soils is meager. Therefore, in the present study, the efficacy of *T. viride* and organic amendment (Neem cake) in problem soils against this disease was assessed.

MATERIALS AND METHODS

Soil samples were collected from eight representative sites of problem soil areas of which two were from Madurai, and the rest from Ramanathapuram, Aruppukottai, Trichy, Paramakudi, Vandiyur and Thadiyankudisai in Tamil Nadu. The physio-chemical properties *viz.*, pH, EC

Table 1. Problem soils collected from different places of Tamil Nadu

S.No.	Places of collection	Types of problem soil	pH	EC (milli mhos/cm)	Organic carbon (%)
1.	Madurai	Sandy soil	7.5	0.2	1.004
2.	Aruppukottai	Black hard pan	8.8	0.8	1.175
3.	Ramanathapuram	Saline	8.5	4.5	0.881
4.	Trichy	Sodic	8.0	4.5	0.857
5.	Paramakudi	Alkaline	8.8	3.6	0.757
6.	Vandiyur	Red crusted	6.2	0.3	1.224
7.	Thadiyankudisai	Acid	5.2	0.1	1.934
8.	Madurai	Normal	7.8	0.07	0.857
	CD (P=0.05)				0.095

and organic carbon concerned with various soil types were also estimated (Table 1). The samples were collected from upper 15cm following the standard sampling procedure.

Isolation of pathogen

Blackgram plants showing the typical root rot disease symptoms were collected from the field at Agricultural College and Research Institute, Tamil Nadu Agricultural University (TNAU), Madurai. The pathogen was isolated by tissue segment method onto potato dextrose agar (PDA) medium and purified in plain agar medium by single hyphal tip method (Rangaswami, 1958).

Preparation of Sand-Maize inoculum of *M. phaseolina*

Sieved sand and maize powder were mixed @ 19:1 and filled into empty saline bottle upto 60 per cent of the volume. The moisture content was adjusted to 50 per cent vol./wt. basis and sterilized for 20 minutes at 1.04 kg/cm^2 pressure for two consecutive days and then inoculated with 10mm PDA culture disc of *M. phaseolina* and incubated for 15 days.

Effect of *T. viride* I_3 and Neem cake in problem soils on disease development

A pot culture experiment was conducted in the greenhouse, Department of Plant Pathology, Agricultural College and Research Institute, Madurai. Earthen pots (15cm) were filled with uniform quantity of problem soil and blackgram seeds surface sterilized with $HgCl_2$ (0.1%) and washed with repeated changes of sterile distilled water were sown in the pots @ 25 seeds. The *T.viride* I_3 (TNAU) formulation was obtained from Department of Plant Pathology, Agricultural College and Research Institute, Madurai was used as seed treatment for further studies. Following were the eight treatments:

Efficacy of *T. viride* and neem cake against root rot of blackgram

T_1 *Trichoderma viride* I_3
T_2 Neem cake application
T_3 *Trichoderma viride* I_3 + Pathogen inoculation
T_4 Neem cake + Pathogen inoculation
T_5 Pathogen inoculation
T_6 No treatment control
T_7 *Trichoderma viride* I_3 + Neem cake application
T_8 *Trichoderma viride* I_3 + Neem cake + Pathogen inoculation

These treatments were replicated three times.

Fully mature, surface sterilized blackgram seeds of uniform size were treated with *T.viride* I_3 (TNAU) commercial formulation @4g/kg. Untreated seeds served as control. In case of neem cake application, the pot culture soil was thoroughly mixed with the neem cake (5% w/w) and filled in 15cm earthen pots. The pots were maintained under glasshouse conditions.

Ten days after sowing, the pot soil was inoculated with the sand-maize inoculum of *M. phaseolina* (5% w/w). *M. phaseolina* inoculated soil alone was kept as control. Observations were recorded on mortality of the seedlings from 15-60 days after inoculation (DAI) at 15day interval.

Isolation of *Trichoderma* from problem soil

Trichoderma spp. was isolated from the soil by using *Trichoderma* selective medium (TSM) developed by Elad and Chet (1983) following dilution plate technique (Waksman, 1952). Transferred one ml of the respective soil suspension (10^4dilution) into each sterile Petri-plate by means of a sterilized pipette and poured 15ml of sterilized, melted and cooled TSM, rotated the plate gently for uniform suspension and allowed to solidify. The plates were incubated at room temperature (28±2°C) for five to seven days and observed for the development of the fungal colonies. *Trichoderma* colonies were counted and recorded. The individual colonies were identified based on the colony and morphological characteristics. The population was calculated as per gram of oven dry soil.

RESULTS AND DISCUSSION

The results presented in Table 2 indicated that in all the sets of treatments in uninfested soils the mortality of blackgram seedlings steadily increased from 15 to 60 days after inoculation (DAI). The mortality was comparatively less (5.63%) in blackgram seedlings raised from seeds treated with *T.viride* I_3 plus neem cake application in acid soil 60 DAI followed by sandy soil (7.92%), sodic soil(10.12%) and normal soil (12.94%) as compared to *T.viride* I_3 and neem cake application alone which recorded mortality of 6.44 and 7.15 percent in acid soil followed by sandy soil (8.63 and 9.36%), sodic soil (10.83% and 11.54%) and normal soil (13.65% and 14.38%), respectively.

However, the performance of soils which have been treated with combined application of *T.viride* I_3 and neem cake was not encouraging and the mortality was very severe in alkaline soil (15.87%), red crusted soil (18.67%), saline soil (21.54%), and black hard pan soil (24.31%) as compared to the*T.viride* I_3 and neem cake application alone in the problem soils. The mortality values with respect of the *T.viride* I_3 and neem cake application alone in the alkaline soil (16.56% and 17.25%), red crusted soil (19.47% and 20.16%), saline soil (22.25% and 22.94%) and black hard pan soil (25.00% and 25.71%) was relatively higher. In all the soils, the mortality was lesser in *T. viride* I_3 application, neem cake application and combined application of *T. viride* I_3 and neem cake in comparison to control.

The mortality of blackgram seedlings in the infested soils was severe (Table 3). It is concluded that the combined application of *T.viride* I_3 and neem cake application in acid soil, sandy soil, sodic soil and normal soil recorded very low mortality of blackgram seedlings namely 6.47, 9.75, 12.58 and 15.34 percent, respectively. Seedling mortality in *T.viride* I_3 and neem cake alone was 7.52 percent and 8.72 percent in acid soil followed by 10.67 percent and 11.61 percent in sandy soil, 13.49 percent and 14.40 percent in sodic soil and 16.25 per cent and 17.20 percent in normal soil, respectively.

However, where combined application of *T.viride* I_3 and neem cake was made, the seedling mortality was found comparatively higher in alkaline soil (18.15%) followed by red crusted soil (20.95%), saline soil (23.78%) and black hard pan soil (26.58%), respectively as compared to single application of *T.viride* I_3 and neem cake. These treatments recorded mortality to the extent of 19.08 percent and 19.99 percent in alkaline soil followed by 21.90 percent and 22.85 percent in red crusted soil, 24.73 percent and 25.64 percent in saline soil and 27.49 percent and 28.41percent in black hard pan, respectively.

Table 2. Effect of *T. viride* I_3 and neem cake in uninfested problem soils on root rot incidence in blackgram caused by *M. phaseolina*

Problem soil	Treatment	Mortality of seedlings (%) 60 days after inoculation (DAI)
Sandy soil	T_1	8.63 (17.08)
	T_2	9.36 (17.81)
	T_7	7.92 (16.34)
	T_6	10.05 (18.48)
Black hard pan soil	T_1	25.00 (30.00)
	T_2	25.71 (30.46)
	T_7	24.31 (29.54)
	T_6	26.40 (30.91)
Saline soil	T_1	22.25 (28.14)
	T_2	22.94 (28.61)
	T_7	21.54 (27.65)
	T_6	23.62 (29.07)
Sodic soil	T_1	10.83 (19.21)
	T_2	11.54 (19.85)
	T_7	10.12 (18.54)
	T_6	12.23 (20.47)
Alkaline soil	T_1	16.56 (24.01)
	T_2	17.25 (24.54)
	T_7	15.87 (23.47)
	T_6	17.96 (25.07)
Red crusted soil	T_1	19.47 (26.18)
	T_2	20.16 (26.68)
	T_7	18.67 (25.60)
	T_6	20.85 (27.16)
Acid soil	T_1	6.44 (14.70)
	T_2	7.15 (15.50)
	T_7	5.63 (13.72)
	T_6	7.84 (16.26)
Normal Soil	T_1	13.65 (21.68)
	T_2	14.38 (22.28)
	T_7	12.94 (21.08)
	T_6	15.07 (22.84)
	CD (P= 0.05)	0.988

Figures in parentheses are angular transformed values.

Table 3. Effect of *T. viride* I_3 and neem cake in infested problem soils on root rot incidence in blackgram caused by *M. phaseolina*

Problem soil	Treatment	Mortality of seedlings (%) 60 days after inoculation (DAI)
Sandy soil	T_3	10.67 (19.60)
	T_4	11.61 (19.92)
	T_8	9.75 (18.19)
	T_5	12.52 (20.72)
Black hard pan soil	T_3	27.49 (31.62)
	T_4	28.41 (32.21)
	T_8	26.58 (31.03)
	T_5	29.33 (32.79)
Saline soil	T_3	24.73 (29.82)
	T_4	25.64 (30.42)
	T_8	23.78 (29.18)
	T_5	26.55 (31.01)
Sodic soil	T_3	13.49 (21.54)
	T_4	14.40 (22.30)
	T_8	12.58 (20.77)
	T_5	15.32 (23.04)
Alkaline soil	T_3	19.08 (25.90)
	T_4	19.99 (26.55)
	T_8	18.15 (25.21)
	T_5	20.92 (27.21)
Red crusted soil	T_3	21.90 (27.90)
	T_4	22.85 (28.55)
	T_8	20.95 (27.24)
	T_5	23.76 (29.17)
Acid soil	T_3	7.52 (15.91)
	T_4	8.72 (17.17)
	T_8	6.47 (14.73)
	T_5	9.70 (18.14)
Normal Soil	T_3	16.25 (23.77)
	T_4	17.20 (24.50)
	T_8	15.34 (23.05)
	T_5	18.11 (25.18)
	CD (P=0.05)	0.688

Figures in parentheses are angular transformed values.

Table 4. Effect of *Trichoderma* population in different soils with blackgram plants

Problem soil	*Trichoderma* population (10^4) cfu g^{-1} oven dry soil					
	Days after sowing (DAS)					
	0	15	30	45	60	75
Sandy soil	22.22	45.39	51.90	54.11	58.37	78.15
Black hard pan soil	10.16	12.03	15.11	17.21	23.15	25.80
Saline soil	14.71	18.16	21.03	23.20	29.13	38.16
Sodic soil	19.35	41.09	45.35	47.16	48.79	70.19
Alkaline soil	13.61	25.75	32.43	37.11	44.15	55.86
Red Crusted soil	12.03	21.82	26.75	29.70	36.55	46.15
Acid soil	32.18	53.01	59.17	63.15	69.70	86.30
Normal Soil	20.43	29.48	34.03	44.11	51.85	63.12
CD (P=0.05)	1.009	3.654	5.632	5.788	6.318	7.032

The results on population density of *T.viride* I_3 in problem soils is presented in Table 4. In all the soils, the population density of *T.viride* I_3 steadily increased from 0 to 75 DAS. In acid soil, the *T. viride* I_3 population was maximum (86.30 X 10^4 cfu/g of oven dry soil) 75 DAS as compared to other soil types. It was followed by sandy soil (78.15 X 10^4 cfu), sodic soil (70.19 X 10^4 cfu) and normal soil (63.12X10^4cfu). The least (25.80X10^4cfu) population density of *T.viride* I_3 was observed in black hard pan soil, saline soil (38.16 X 10^4 cfu), red crusted soil (46.15X10^4 cfu) and alkaline soil (55.86X10^4 cfu).

The study revealed that seed treatment of *T.viride* I_3 plus neem cake application significantly reduced the blackgram root rot disease incidence. The efficacy of neem cake was increased by the introduction of *T.viride* I_3. Application of neem cake pre-colonized with *T. viride* I_3 reduced the root rot incidence in pigeonpea (Nakkeeran *et al.*, 1995). Samiyappan *et al.* (1987) reported that seed treatment of *T. viride* controlled root rot caused by *M. phaseolina* in greengram. Charcoal rot incidence in soybean was reduced by seed application of *T.viride* when applied alone or in combination with organic amendments (Muthusamy, 1989). Kousalya and Jeyarajan (1988) found that seed application of *T. viride* commercial product recorded the low root rot incidence in blackgram.

Mondal *et al.* (1996) reported that acidic pH (4.0 to 5.5) supported the maximum population of *T. viride* I_3 registering 50.1X10^4 and 51.5X10^4 cfug^{-1} oven dry soil. Rai and Upadhyay (1978) found that *T. viride* I_3 was the most frequent species in soil, which showed affinity with acidic soils containing high levels of organic matter. Boer *et al.* (1998) reported that growth of *Trichoderma* was strongly inhibited in alkaline soils but not in the acid soils.

It is concluded that seed treatment with *T.viride* plus neem cake amendment to soils, reduces root rot incidence in acid soils followed by sandy, sodic and normal soils. It is therefore possible that under such ecological conditions these biocontrol agents may colonize significantly to suppress the root rot incidence. However, black hard pan, saline, red crusted, and alkaline soils failed to support the *T.viride* population.

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