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Integrated Management of Dry root rot of Greengram [Vigna Radiate(L.) Wilczek] Incited by *Macrophomina Phaseolina* (Tassi.) Goid

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Abstract: *Greengram (Vigna radiate L.) Wilczek is one of the important pulse crops grown in India and the crop is incited by a number of diseases. Dry root rot caused by Macrophomina phaseolina is an important disease causing maximum yield losses. The biocontrol agents along with neem formulatios were tested individually and in combinations for their effectiveness against root rot of greengram. In vitro studies on the effect of certain fungal (Trichoderma viride, T. harzianum, T. reesei, Gliocladium virens) bacterial antagonists(Pseudomonan fluorescens, Bacillus subtilis) showed that T. viride is more effective in inhibiting the pathogen (70%). Among the bacterial antagonists P.fluorescens was found significantly more effective in inhibiting the test pathogen(63.7%). Similarly, studies on in vitro effect of neem based commercial formulations viz., Starneem and Neem gold, Starneem was found to be more effective. In pot culture studies combined seed treatment with T. viride @ 4g/kg, P.fluorescens 10g/kg , Neem oil 3ml/kg and thiram 3g/kg seed was found to be highly effective in increasing seedling emergence(91.3%), plant dry weight (98.6%), shoot lemgth (73.9%) and root length (70.8%). Seed treatment with all four combinations was found to be superior in reducing dry root rot incidence (87.8%).*

Keywords: *Dry root rot, Greengram, Macrophomina phaseolina, Psedomonas fluorescens, Trichoderma viride.*

INTRODUCTION

Greengram *(Vigna radiata* L.) Wilczek is one of the important pulse crops grown in India. It is an excellent source of high quality proteins, rich in potassium and phosphorus. Being a pulse crop, it fixes atmospheric nitrogen and thereby enriches soil fertility and is also used occasionally as green manure cover crop and inter crop or mixed crop with many cereals. India is one of the leading countries in greengram production and diseases caused by various plant pathogens are major contributing factors for lowering crop yields. In India, it is estimated that more than 50 per cent of the crop losses are due to soil borne plant pathogens.

Dry root rot of greengram caused by *Rhizoctonia bataticola* (Taub) Butler (Pycnidial stage: *Macrophomina phaseolina* (Tassi.) Goid is one of the most destructive diseases in tropical and subtropical countries. The losses due to *M.phaseolina* are estimated to be around 10.8 to 24.1 per cent in India [1,2].

Management of soil borne fungal pathogens is difficult because of long-term survival and wide host range of the pathogens. These pathogens not only persist in the soil as saprophytes along with other soil organisms but also are transmitted from seed. Different fungicides are being used in the management of soil borne diseases including dry root rot of greengram. However, chemical management is uneconomical, hazardous; disturb the biological balance cause ground water pollution, leaves residues on food crops and results in development of resistance in pathogens to the chemicals and ultimately breakdown the varietal resistance.

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In view of the disadvantages of these fungicides and non-availability of resistant cultivars, there is a need to develop a method which is effective, economical and ecofriendly, to reduce the dry root rot of greengram. The use of bio-control agents and botanical products as potential bio-fungicides" may offer more environmental friendly and ecologically safe methods of protection of crop from soil borne plant pathogens. Therefore an attempt has been made to integrate management of dry root rot disease on greengram incited by *Macrophomina phaseolina* (Tassi.) Goid which have become a serious problem in hampering the production of the greengram in all growing areas of India. The present work was conducted to test the effect of various fungal and bacterial antagonists along with some neem based commercial formulations so as to identify an effective, economical and potential components of IDM for management of dry root rot under green house conditions.

MATERIALS AND METHODS

Isolation of Test Pathogen and Maintenance of Biocontrol Agents

The root rot pathogen *Macrophomina phaseolina* (Tassi) Goid) was isolated from greengram plant showing typical dry root rot symptoms collected during survey and pure culture of the pathogen were obtained by single hyphal tip method [3]. The effective bio control agents (*Trichoderma viride, T. harzianum, T. reesei, Gliocladium virens, Pseudomonan fluorescens, Bacillus subtilis)* required for study were obtained from Culture collection section, Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Tamil Nadu. India.

Mass Multiplication of the Pathogen

The inoculum of the test pathogen, *M. phaseolina* maintained on agar slants was further multiplied on sorghum grains. One hundred grams of sorghum seeds were washed thoroughly in tap water and soaked overnight in 250 ml conical flasks with addition of 20 ml of 4 per cent dextrose. The flasks were then autoclaved for 20 min at 15 lbs. After cooling the flasks at room temperature they were shaken well to separate the sterilized grains and were inoculated with disc of 4 day old culture of *M.phaseolina* and incubated at 28 ± 2°C for seven days in BOD incubator.

Testing the Antagonistic Activity of Fungal and Bacterial Bio-control Agents

The antagonistic activity of fungal and bacterial bio-control agents was tested against test pathogen, *M. phaseolina* following dual culture technique [4].

Dual Culture Technique for Fungal bio-control Agents

20 ml of molten PDA was poured in 90 mm Petri dishes and allowed to solidify. Seven days old fungal disc of the antagonist of size 9 mm was placed at one end of media on petriplate. A 9 mm disc of test pathogen of five days old was placed at the opposite end. Five replications along with suitable control were maintained. The plates were incubated at room temperature (28 + 1°C) till mycelial growth in the control plates covered the entire plate. The linear growth of the pathogen was measured and the percentage inhibition was calculated by adopting following formula.

$$\% \text{ inhibition of pathogen} = \frac{[\text{Growth of pathogen in control plate}] - [\text{Growth of pathogen in presence of tungal antagonist}]}{\text{Growth of pathogen in control plate}} \times 100$$

Dual Culture Technique for Bacterial Bio-control Agents

The antagonistic activity of *Pseudomonas fluorescens*, and *Bacillus subtilis* against the test pathogen *M. phaseolina* was tested following dual culture technique. A gentle superficial streak of one cm with bacterial bio-control agent was made at one end of the petriplate on PDA media by means of a sterilized inoculation loop. A nine mm PDA culture disc of the test pathogen was placed at the opposite end.

Five replications along with suitable control were maintained. The plates were incubated at room temperature (28 ± 1°C) till the mycelial growth in

the control plates covered the entire plate. The linear growth of the test pathogen was measured and the percentage inhibition was calculated.

Neem Based Formulations

Commercial neem based formulations of *Azadirachta indica* was used for present study. The details of commercial neem products are as follow (Table 1). Efficacy of neem oils were assessed by following Poisoned Food Technique.

Common name	*Trade name*	*Active ingradient*	*Concentrations ppm*
Neemgold (neem oil)	Neem gold	0.15%	100 200
Starneem (neem oil)	Starneem	0.15%	400 600 800 1000

POTCULTURE STUDIES

The pot culture experiment was conducted to know the individual as well as combined effect of bio-control agents, fungicide, and neem based formulations against test pathogen *M. phaseolina.* Earthern pots of size 12 inch diameter were used and filled with steam sterilized soil @ 2 kg/pot. The effective fungal and bacterial antagonists and neem based commercial formulations (Starneem) were selected to test against test pathogen. The standard fungicide, thiram was used as check for comparison. The experiment was conducted in a complete randomized block design with three replications.

Sowing and Treatments

Seed treatment with Thiram

The seeds of greengram cv ML-267 were treated with thiram (75 WP) @ 3g/kg seed using gum (5m1/kg) as sticker and the treated seeds were used for sowing.

Seed treatment with T. viride/ P. fluorescens

The talc based bio-control agents *T.viride* @ 4 g/kg seed and *P fluorescens* 10 g/kg seed were used for treating the seeds by using gum (5m1/kg) as sticker. The treated seeds were spread over a clean paper and dried in a cool shady place. The seeds were sown immediately after drying.

Combined seed treatment with Thiram, neem oil and bio-control agents

On the treatments involving combinations of thiram, neem oil, *T. viride* and *P fluorescens,* the seeds were first treated with thiram, followed by neem oil, and then mixed with *T.viride* and *P flurescens* at recommended doses using gum as sticker. The treated seeds were shade dried over a clean paper and used for sowing.

Termination of Experiment

The plants were grown for a period of 65 to 70 days *i.e.,* till the period of harvest and the data on seedling emergence, dry root rot incidence (%) and plant growth characters *viz.,* shoot length, root length, shoot and root dry weights were recorded

RESULTS AND DISCUSSION

In vitro Effect of Fungal and Bacterial Antagonists on Growth of *M.phaseolina*

In order to select a suitable fungal antagonist against the test pathogen *M. phaseolina,* fungal antagonists *Trichoderma viride*, *T.harzianum T.reesei* and *Gliocladium virens* were tested following dual culture technique.

The data presented in Table 2 revealed that all antagonists inhibited the growth of pathogen.

Table 2
In vitro* effect of bacterial and fungal antagonists on mycelia growth of *M. phaseolina

Sl. No.	Antagonist	*Radial growth (cm)	Inhibition over control
1.	*Pseudomonas fluorescens*	3.26	63.7
2.	*Bacillus subtilis*	5.5	38.8
3.	*Trichoderma viride*	2.7	70
4.	*Trichoderma harzianum*	2.9	67
5.	*Trichoderma reesei*	5.1	43.1
6.	*Gliocladium-virens*	4.9	45.5
7.	Control	9	–
	S.Em +	0.068	
	CD at 5%	0.196	

*Mean of five replications

However fungal antagonist *T.viride* inhibited highest growth of the pathogen (70%) followed by *T. harzianum* (67%), *G. virens* (45.5%) and *T. reesei* exhibited lowest inhibition (43.1%) and the bacterial antagonist *P.fluorescens* was significantly superior (63.7%) in inhibiting the growth of the pathogen over *B.subtilis* (38.8%).

Trichoderma sp. have been long known as effective antagonists against many soil borne pathogenic fungi and are the focus of recent research [5]. *In vitro* antagonism of *Trichoderma* spp. against *M phaseolina* has been well documented under laboratory conditions [6,7,8,9,]. In present study the antagonistic activity of *T. viride, T. harzianum, T. reesei* and *Gliocladium virens* tested against test pathogen *M phaseolina* employing dual culture technique indicated a maximum percent inhibition of *M phaseolina* in the presence of *T viride* followed by *T harzianum.* Thus indicating *T. viride* as highly effective, which was also reported earlier in cotton [10], in castor[11], in cowpea [12] and also in chickpea [13] on the same pathogen *M phaseolina.* The lower myceial pathogen growth may be due to antibiotics produced by the bio control agents, as has been reported by many workers [14,15]. There exists four forms of antagonism i.e., competition, antibiosis, parasitism and the growth inhibition of *M.phaseolina* could be attributed mainly due to antibiosis or hyper parasitism [16]. Most fungi have chitin â (1-3) glucanase as essential constituent in their cell wall. *Trichoderma* spp produce chitinase â (1-3) glucanase enzyme which degrades the cell wall leading to lysis of *Rhizoctonia* spp [17].

Antagonistic activity of six isolates of *Pseudomonas* spp. against *R.solani* in vitro was already reported [18]. The antagonistic activity of *P.fluorescens* was also reported on other pathogens, *Sclerotium rolfsii* [19] and *S.oryzae* [20].

In vitro Effect of Neem based Commercial Formulations against mycelial Growth of *M. phaseolina*

The data presented in table 3, revealed that both the neem based formulations inhibited mycelia growth of the pathogen completely at 800 and 1000 ppm concentrations. However, neem oil (Starneem) was found to be more effective in inhibiting the growth of the pathogen by 87.1 per cent at 600 ppm than neem gold with (75.1%) inhibition. Similar results were reported [21], on antifungal activity properties of commercial neem product, neem oil against *R.solani* which found to be effective in reducing the growth of the pathogen to the extent of 80-100 per cent *in vitro*. Leaf extract of *Azadirachta indica* was found effective in inhibiting spore germination of *Cerotelium fici* by 91.2 per cent [22]. Azadirachtin and other limonoids were quite effevtive in the control of plant diseases of diverse nature.

Table 3
Effect of neem based commercial formulations on the growth of *M. phaseolina*

Sl. No.	*Treatment*	*Concentration (ppm)*	**Colony diameter of M.phaseolina (cm)*	*Percentage inhibition of growth of M. phaseolina*
1.	Starneem	100	6.4	28.6
		200	4.2	53.3
		400	3.0	65.7
		600	1.1	87.1
		800	0.0	100.0
		1000	0.0	100.0
2.	Neem gold	100	6.4	28.6
		200	5.1	43.1
		400	3.7	58.0
		600	2.2	75.1
		800	0.0	100.0
		1000	0.0	100.0
3.	Control	–	9.0	0.0
	S.Em ±		0.027	
	CD at 5%		0.076	

*Mean of five replications

Pot Culture Studies

Effect of seed treatment with bio-control agents, neem oil (starneem) and thiram on seedling emergence, plant growth parameters and dry root rot incidence was studied in potculture with greengram cv. ML-267 in *M. phaseolina* infested soil.

Effect on Seedling Emergence

The data on germination and seedling emergence was recorded ten days after sowing. The data presented in Table 4 revealed that seedling

Table 4
Effect of seed treatment with biocontrol agents, neem oil (star neem) and thiram on seedling emergence of green gram cv. ML-267 in *M.phaseolina* infested soil in pots

S. No.	Treatment	*Seedling emergence (%)	Percentage increase over control
1.	*Pseudomonas fluorescens* (P.f) (10 g kg′ seed)	78.20 (62.17)	24.6
2.	*Trichoderma viride* (T.v) (4 g kg′ seed)	80.10 (63.51)	27.6
3.	Neem oil (Starneem) (3 ml kg′ seed)	76.20 (60.82)	21.4
4.	Thiram (3 g kg′ seed)	80.20 (63.58)	27.8
5.	*Pf* + *Tv*	84.0 (66.43)	33.9
6.	*Pf* + Thiram	81.20 (64.36)	29.4
7.	*P...f* + Neem oil (Starneem)	79.00 (62.73)	25.9
8.	*Tv* + Thiram	83.10 (65.73)	32.4
9.	*Tv* + Neem oil (Starneem)	80.47 (63.79)	28.2
10.	Neem oil (Starneem) + Thiram	80.32 (63.68)	28.0
11.	*P.f* + *Tv* + Thiram	84.37 (66.75)	34.4
12.	*Pf.* + *T. v.* + Neem oil (Starneem)	84.20 (66.59)	34.2
13.	*Pf* + Thiram + Neem oil (Starneem)	83.60 (66.12)	33.2
14.	Tv + Thiram + Neem oil (Starneem)	83.20 (65.36)	32.7
15.	*P.f* + *Tv* + Thiram + Neem oil (Starneem)	91.30 (72.88)	45.5
16.	Inoculated control	62.73 (52.39)	
	S.Em ±	0.690	
	CD at 5%	1.989	

Figures in parentheses are arc sign transformed values
*Mean of three replications

emergence was significantly improved in all the treatments compared to control (62.7%). Seed treatment with combined treatment of *P.fluorescens* + *T. viride* + neem oil + thiram gave maximum seedling emergence (91.3%) followed by seed treatment with *P. fluorescens* + *T.viride* + thiram (84.3%) and the minimum seedling emergence was recorded with neem oil (76.2%).

Effect on Growth Parameters Dry weight

As represented in Table 5 there was a significant increase in dry weight of plant by all the treatments when compared to control (1.49 g plant^{-1}) and the increase in dry weight ranged from (2.50 to 2.96 g plant-I). Combined seed treatment with *P. fluorescens, T.viride,* neem oil and thiram recorded maximum percentage increase in dry weight (98.6%) followed by combined seed treatment with *P. fluorescens, T. viride* and thiram (96.6%) and the minimum increase was observed in seed treatment with neem oil (67.7%) compared to control plants.

Effect on Root Length

Results in Table 5 revealed that all the treatments were found to be effective in increasing the root length of greengram plants ranging from 10.33 to 11.80 cm compared to control (6.93 cm). Combined seed treatment with *P. fluorescens,* T.*viride,* neem oil and thiram recorded maximum percentage increase in root length (70.8 %) followed by seed treatment with *P. fluorescens, T. viride* and thiram (68.4%) compared to control and the least per cent increase was observed in neem oil (Starneem) treated seeds (49%).

Two mechanisms have been advanced most frequently to explain the increased growth response induced by certain micro flora. The first hypothesis was that enhanced growth of plants induced by antagonists might be due to biological control of plant pathogens in the soil. The other hypothesis so far, not demonstrated clearly for any biological system was that a microbial agent produced growth regulatory metabolites [23]. Thus the rate of germination and dry weight of root and shoot were increased [24]. Similar reports of increase in vegetative growth using fungicide and *Trichoderma* spp. was also recorded in greengram [25], in chilly [26], in tomato [27] and also in cowpea [28].

Effect on Dry Root Rot Incidence

The effect of seed treatment with bio-control agents, neem oil and thiram on dry root rot incidence was recorded at 45 DAS and the data was presented in Table 6. The results revealed that individual as well as combined seed treatment with bio-control agents, neem oil and thiram significantly reduced dry root rot incidence ranging from 10 to 34.6 per cent compared to control (77.3%). Seed treatment with *P. fluorescens* + *T. viride* +neem oil + thiram recorded maximum percentage reduction of dry root rot

Table 5
Effect of seed treatment with certain biocnotrl agents, neem oil (star neem) and thiram on growth parameters of green gram cv.ML-267 in *M. phaseolina* infested soil in pots*

S. No.	Treatment	Dry weight (g/plant)	Increase over control (%)	Root length (cm)	Increase over control (%)	Shoot length (cm)	Increase over control (%)
1.	*Pseudomonas fluorescens (P f)* (10 g/ kg)′ seed)	2.57	72.4	10.47	51.0	24.67	43.5
2.	*Trichoderma viride (Tv)* (4 g/ kg seed)	2.73	83.2	10.67	53.9	25.10	46.0
3.	Neem oil (Stameem) (3 ml/kg seed)	2.50	67.7	10.33	49.0	24.40	41.9
4.	Thiram (3 g/kg seed)	2.77	85.9	10.67	53.9	25.00	45.4
5.	*Pf* + *T.v*	2.83	89.9	11.43	64.9	28.00	62.8
6.	*Pf* + Thiram	2.82	89.2	11.00	58.7	27.80	61.7
7.	*Pf* + Neem oil (Stameem)	2.79	87.2	10.50	51.5	27.50	59.9
8.	*T.v* +*Thiram*	2.85	91.2	11.20	61.6	29.00	68.7
9.	*T.v* +*Neem* oil (Stameem)	2.81	88.5	10.90	57.2	28.50	65.7
10.	Neem oil (Stameem) + Thiram	2.80	87.9	10.83	56.2	27.00	57.0
11.	*Pf* + *T.v* +*Thiram*	2.93	96.6	11.67	68.4	29.00	68.7
12.	*P f* + *T. v.* + Neem oil (Stameem)	2.92	95.9	11.53	66.3	29.60	72.1
13.	*Pf* + Thiram + Neem oil (Starne,em)	2.91	95.3	11.30	63.0	29.00	68.7
14.	T.v + Thiram + Neem oil (Stameem)	2.90	94.6	11.29	62.9	29.00	68.7
15	.. *Pf*+ *Tv* + Thiram + Neem oil (Stameem)	2.96	98.6	11.84	70.8	29.90	73.9
16.	Inoculated control	1.49		6.93		17.19	
S.Em ±		0.036		0.130		0.220	
CD at 5%		0.104		0.374		0.633	

* 45 days after sowing

Mean of three replications

incidence (88%) followed by *P. fluorescens* + *T viride* + thiram (83%) while neem oil was less effective (55.3%).

In the present investigation the percentage of dry root rot incidence was significantly reduced in the treatments where fungal and bacterial antagonist, neem oil (starneem) and fungicide were used in combination. Similar results were also obtained by earlier workers when *Trichoderma* spp. was used both as seed and soil treatments in combination with fungicide [26]. Chickpea wilt incited by *Fusarium oxysporum* can be effectively controlled by integration of *T. viride*, carbendazim and neem cakes [28], and combined application of carbendazim, *T. viride* and *P. fluorescens* were superior in management of Pigeon pea wilt disease incited by *Fusarium udum var cajani* [29]. Combination of PBP 4G (*T. viride*) for soil application and Pusa 5SD (*T.harzianum*) for seed treatment together with fungicide carboxin, provided the highest seed germination, shoot and root lengths and grain yield with the lowest incidence of wilt in chickpea under field conditions [30]. Application of biocontrol agents *viz., T. harzianum, B. subtilis* and *P. fluorescens* reduced the *Fusarium* wilt incidence of safflower both under greenhouse and field conditions [31]. Soil borne diseases of crops incited by species of *Fusarium* were cost-effective to be managed through integration of microbial antagonist, fungi toxi-cants or organic amendment [32].

Integration of biological, botanical and chemical control seems to be a very promising way of controlling pathogens with minimal interference with the biological equilibrium is one of the most attractive ways of reducing the amount of fungicide is the integration of sub lethal doses of chemicals with *Trichoderma* sp. which is resistant to high doses of chemicals [33,34,35,36,37,38].

Table 6
Effect of seed treatment with biocontrol agents, neem oil (star neem) and thiram on dry root rot incidence of green gram cv. ML-267 in *M.phaseolina* infested soil in pots

S. No.	Treatment	Dry root rot incidence (%)	Percentage decrease over control
1.	*Pseudomonas fluorescens* (P.f) (10 g/kg seed)	28.5 (32.8)	63.1
2.	*Trichoderma viride* (T.v) (4 g /kg seed)	27.4 (31.5)	64.5
3.	Neem oil (Starneem) (3 ml/kg seed)	34.5 (35.9)	55.3
4.	Thiram (3 g kg^{-1} seed)	29.2 (32.6)	62.2
5.	*P f* + *T.v*	21.0 (27.2)	72.8
6.	*Pf* + Thiram	23.2 (28.8)	69.9
7.	*Pf* + Neem oil (Stameem)	22.4 (2.82)	71
8.	*T v* + Thiram	20.1 (26.6)	73.9
9.	*T.v* + Neem oil (Starneem)	22.4 (28.2)	71
10.	Neem oil (Starneem) + Thiram	27.0 (31.3)	65
11.	*PI* + *T.v* + Thiram	13.3 (21.3)	82.7
12.	*Pf.* + *T v.* +*Neem* oil (Stameem)	14.2 (22.6)	81.6
13.	*P.f* + Thiram + Neem oil (Stameem)	14.4 (22.4)	81.3
14.	T v + Thiram + Neem oil (Stameem)	15.2 (23.0)	80.3
15.	*Pf* + T v + Thiram + Neem oil (Starneem)	9.4 (17.6)	87.8
16.	Inoculated control	77.3 (61.5)	
	S.Em +	0.290	
	CD at 5%	0.850	

Mean of three replications

Figures in parentheses arc sign transformed values

CONCLUSION

These present studies show that use of fungicide Thiram (3 g kg seed) and neem oil (Starneem) (3 ml kg' seed) provides initial protection to seed and seedlings from the attack of soil and seed borne pathogen, *M.phaseolina* in greengram and thereby helping in establishment and multiplication of antagonists *T. viride* (4g kg"' seed) and *P. fluorescens* (10g kg"' seed) which provide the protection throughout the crop growth period. This positive effect may be due to interaction effect of bio-control agents, fungicides and seed exudates.

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