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Efficacy of fungicides, bioagents and organic amendment to manage root rot in green gram

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Abstract
Greengram (*Vigna radiata* L.) is one of the most important conventional pulse crop grown in the Vidarbha region of Maharashtra. It plays an important role not only in human diet but also in improving soil fertility by fixing atmospheric nitrogen. Root rot of greengram caused by *Rhizoctonia bataticola* is an economically important disease of greengram. In pot culture experiment, the seed treated with Thiophanate methyl @ 0.1% which was recorded least (29.62) percent disease incidence at 30, 60 and 90 DAS. *In vitro* evalution of fungicide showed cent Percent growth inhibition (100%) radial mycelial growth of *Rhizoctonia bataticola* in all fungicide treatments. In respect of bioagent, *T. viride* showed (86.02%) highest growth inhibition against the pathogen. Different seed treatments and soil amendments were evaluated for counting dynamic population and inoculums density of *Rhizoctonia bataticola.* The propagules of pathogen was observed per gram of soil. Minimum number of propagules were noticed in T_4 treatment Thiophanate methyl (5.66 and 4.33%). Effect of various seed treatments on germination, shoot length, root length and seedling vigour index was recorded. Highest germination (81.66%), shoot length (10.77 cm), root length (9.27 cm) and also highest seedling vigour index (1636.46) were recorded in Thiram + Carbendazim.

Keywords: Bioagents, mungbean, root rot and soil amendments

Introduction
Mungbean/green gram [*Vigna radiata* (L.) Wilczek] is one of the most important pulse crops. It is grown in almost all parts of the country and belongs to family *leguminosae*. Mung bean is an excellent source of high quality protein. It is consumed in different ways as dal, halwa, snack and so many other preparations. Ascorbic acid (Vitamin-C) is synthesized in sprouted seeds of mung bean. The leguminous crops have the capacity to fix-atmospheric nitrogen through symbiotic nitrogen fixation. It is also used as green manure crop. It is grown in summer and kharif season in northern India and in southern India. In India, it is the third important pulse crop after chickpea and pigeonpea. The major fungal diseases which infect the mungbean are root rot (*Macrophomina phaseolina* (Tassi) Goid), web blight, *Rhizoctonia solani* Khun (*Thanatephorus cucumeris*), powdery mildew (*Erysiphe polygoni* DC), *Cercospora* leaf spot (*Cercospora canescens* Ellis and Martin) and anthracnose [*Colletotrichum dematium* and *C. lindemuthianum*). *Macrophomina phaseolina* (Tassi) Goid is one of the most virulent and destructive pathogen which incite diseases in wide range of hosts, while the symptoms produced were seedling rot, collar rot, leaf blight and pod rot in mothbean. Root rot incited by *Macrophomina phaseolina* (Tassi) Goid has been rated as most devasting disease of mungbean. The pathogen attacks on all parts of plant i.e. root, stem, branches, petiols, leaves, pods and seeds. Moreover, seed infection of *Rhizoctonia bataticola* (*M. phaseolina*) ranges from 2.2-15.7% which causes 10.8% in grain yield and 12.3% in protein content of seed in mungbean. The infected seeds act as an important source of primary inoculum for new areas. Soil and seed borne nature of the disease possesses problems for an effective disease management. Therefore, an attempt has been made to integrate management of root rot disease on mung bean [*Vigna radiata* (L). Wilczek] incited by *Macrophomina phaseolina* (Tassi) Goid which have become a serious problem in hampering the production of the mungbean in all growing areas of India (Rekha Kumari, 2012).

Materials and Methods
Green house study: To check the biocontrol ability of *P. fluorescens* and *T. harzianum* in green house, the study was conducted by using seeds of mungbean of variety AKM 8802.

Mass multiplication of *R. bataticola* was carried out in potato dextrose broth at room temperature for two weeks. Sterilized sandy soil mixed with *R. bataticola* inoculated artificially (100 g inoculum/kg soil).The mixture was incubated for 5 days at room temperature for colonization of fungus in the soil. Pots were kept at 50 percent water holding capacity by watering daily. Six seeds of mungbean were sown in each pot. After germination, four seedlings were kept per pot and excess were removed and discarded. In view of stastistical base, the pots were arranged in greenhouse as a CRD. Seed treatment with combination of fungicides and bioagent tested for their efficacy against test pathogen. Soil amendments like castor cake, neem seed cake were added to increase efficiency of bio-agent. Sterilized sandy soil mixture artificially inoculated with *R. bataticola* (100 g /kg soil) was treated as control. Experimental design was CRD having nine treatment and three replications.

Treatments details

T_1	Vitavax + Thiram @ (0.3%)
T_2	Carbendazim + Mancozeb @ (0.3%)
T_3	Thiram + Mancozeb @ (0.3%).
T_4	Thiophanate methyl @ (0.1%)
T_5	*Pseudomonas fluorescens* @ 10 gm/kg
T_6	*Trichoderma viride* @ 4 gm/kg.
T_7	Caster cake @ 10%
T_8	Neem cake @ 10%
T_9	Control

Efficacy of chemicals by poisoned food technique: Poisoned food technique was used to evaluate the efficacy of fungicides against pathogen *in vitro* condition. Potato dextrose agar medium was prepared and distributed at the rate of 100 ml in 250 ml conical flask, autoclaved at 1.05 kg/cm^2 for 15 min then before solidification of media different fungicides of desired concentration were incorporated aseptically in different flasks. These flasks shaken thoroughly and poured in petriplates 20 ml/plate like wise three plates for each treatment were maintained. One set of three plates was poured without any fungicides to serve as a control. After solidification of medium, the plates inoculated with seven days old pathogen separately. The 5 mm diameter mycelial disc selected from peripheral growth of the plate by sterilized cork borer was used for inoculating the plates by keeping one disc per plate in the centre in inverted position, so as to make the mycelial growth touch the surface medium. The inoculated plates were incubated at room temperature for seven days. The colony diameter of the fungal pathogen on medium was recorded and percent inhibition in each treatment was calculated by using following formula (Vincent, 1947) [19].

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

Where,

I = Percent inhibition
C = Growth of fungus in control (mm)
T = Growth of fungus in treatment (mm)

Efficacy of bio agents by dual culture technique: Autoclaved medium was poured into the sterilized glass petriplates and allowed to solidify. The 5 mm diameter discs of the above bioagents were cut from peripheral growth of the plate by using sterilized cork borer under aseptic condition and placed at one end of the medium, just opposite to it 5 mm disc of the pathogen was plated at another end 0.5 to 1.0 cm away from edge of petriplates. For this a week old culture of pathogen and bioagent was taken. Three replication for each pathogen and control i.e. without *Trichoderma viride* were maintained. The efficacy of bacterial bioagents i.e. *Pseudomonas fluorescens* tested by streaking method. Pathogen *R. bataticola* was placed in the center of petriplates and *P. fluorescens* was streaked both sides of the pathogen. Further plates were incubated and radial diameter was measured. The mycelial growth of pathogens was measured in treated and control plates and percent inhibition was calculated by using following formula.

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

Where,

I = Percent inhibition
C = Growth of fungus in control (mm)
T = Growth of fungus in treatment (mm)

Soil microflora propagules: The composite soil sample from each pot was collected during both the before sowing and after sowing and it is used for estimation of *Rhizoctonia bataticola* population using serial dilution technique. The media was prepared and sterilized in autoclave. 1g Soil sample was taken in 9 ml sterilized distilled water in the test tube, stirred well and serial dilution were made up to 10^{-3}

Seedling Vigour Index: Seeds were inoculated with pathogen *Rhizoctonia bataticola.* The inoculated seed was treated with fungicide and bioagents. Treated seeds were placed between paper rolls in three replication. In each replication 50 seeds were maintained and allow it for germination. The rolls were kept at 23±2^{0}C in seed germinator. The first count of normal seedlings was taken on the 3rd day and the second count on the 7th day. The germination percent was calculated. Normal seedlings were evaluated for seedling vigour index (ISTA, 1985).
Seedling vigour index = [Mean root length (cm) + mean shoot length (cm)] × percentage germination

Results and Discussion

Effect of different treatments on percent root rot incidence in greengram at 30, 45 and 60 DAS

In table 1. Presented that the effect of different seed treatments and application of organic amendments on root rot incidence of greengram was assessed at 30, 45 and 60 days under pot condition. There was a significant differences found among all treatments at respective period. Least percent disease incidence was recorded in Thiophanate methyl @ 0.1% (16.66%) at 30 DAS followed by Thiram + carbendazim @ 0.3% and was at par with other seed treatments except control. Severity at 45 DAS, minimum root rot incidence was observed in seed treatment with Thiophanate methyl @ 0.1% i.e. 27.78%. The same treatment was found effective at 60 DAS. Among tested bioagents *Trichoderma viride* (38.89%) was effective as compare to *Psuedomonas fluorescens* (50.96%) and in organic amendments Neem cake (35.22%) recorded better reduction of disease as compare to castor cake (47.22%). Thiophanate methyl (T4) was most effective treatment among all performed systemic, preventive and curative action was enhanced phytotonic and antifungal effect due to sulphur atom. Therefore they can effective against

pathogen. *Trichoderma* is a saprophytic fungus that grows on dead organic matter and cell wall of pathogenic fungi. It secretes a range of extracellular compounds, which inhibit pathogens through antibiosis. *Trichoderma* was found effective to control the disease. Neem cake which contains azadirachtin, salannin, nimbin, azadiradione as the major component might be responsible to reduce the disease. It acts as a biofertilizer and helps in providing the required nutrients to plants. Neem seed cake performs the dual function, acts as a soil enricher, reduces the population of soil pest and bacteria, provides macro nutrients essential for all plant growth and these plant nutrients inhibits the growth of soil borne fungi. The present findings supports the result of John priya (2010) [6] who reported that the Thiophanate methyl was found most effective against *Rhizoctonia bataticola* followed by Carboxin and Captan. Shankar and Jeyaranjan (1996) [17] reported that *Trichoderma* significantly reduced *M. phaseolina* root rot incidence in sesamum. Khan and Sinha (2006) [8] also reported that the integration of organic amendments and fungal antagonist (*T. harzianum*) against sheath blight of rice caused by *R. solani.* These results similar with findings of Deshmukh *et al*. (2016) [2] who stated that that the approach of using bioagents, organic amendments for controlling pathogens has potential benefit in managing disease with good plant health.

Efficacy of fungicides and bioagents against *Rhizoctonia bataticola in vitro*

In present study the results were indicated that the use of fungicides and bioagents provided significant inhibition of *Rhizoctonia bataticola*. The differences are statistically significant in inhibiting the growth of the test pathogen. The observations were recorded on 7th day for mean colony diameter and percent growth inhibition was calculated. All the treatments were found to be significantly superior over control. The combination of different fungicides were significantly superior over all the treatment with percent inhibition of the fungus, which restricted complete mycelia growth managing disease with good plant health. The combi product of fungicide which performed dual action to control the plant disease i. e. systemic and contact. These dual action which was totally inhibit the fungal growth. The bioagent *Trichoderma viride* was found most effective (86.02%) followed by *Pseudomonas fluorescens* (37.58%) as an effective with maximum colony diameter (52.12 mm) and supported the result of Kumari *et al*. (2012) [10]. There findings are in support of Natarajan and Rao (1996) [14] who studied potential used of *Trichoderma* as an effective biocontrol agents against *R. bataticola* causing diseases in most of the crop plants. Similar results were recorded by Monga and Sheoraj (2014) [13] who reported that Thiophanate-M, Carbendazim and MEMC are highly toxic to root rot pathogen showing complete inhibition at 50 ppm. Lokesha and Benagi (2007) [12] stated that *Trichoderma* and *P. fluroscens* significantly inhibited the mycelial growth of *Rhizoctonia bataticola* by (78.22%) and (76.66%) respectively. Lambhate *et al*. (2002) [11] reported that, Carbendazim, Thiophanate methyl @ 0.1, 0.2, 0.3 were found effective in inhibiting the growth of *M. phaseolina.* Nagamma (2012) observed that, Thiram @ 0.3%, Carboxin + Thiram 0.2% and Carbendazim @ 0.1% showed cent percent inhibition of *Rhizoctonia bataticola.*

Effect of different treatments on inoculam propagule density per gram of *Rhizoctonia bataticola*

A dynamic population and inoculam density per gram of soil influenced by various treatments was recorded. Data on inoculam density in rhizosphere as influenced by different treatments at initial count and final count are noted in Table 3.The propagules of pathogen were observed per gm of soil and the minimum number of colonies (5.66, 4.33) were noticed in the T_4 Thiophanate methyl i.e. significantly superior to over control. Maximum number of colonies were exhibited in control (9.00, 10.66). All the treatments were found effective against pathogen for reducing the population of *Rhizoctonia bataticola.* Fawole *et al*. (2008) [4] reported that the populations of fungi, bacteria and actinomycetes were reduced significantly by the application of the fungicide. Elnasikh (2011) [3] revealed that Neem seed cake positively affected the population of actinomycetes and the population of fungal pathogen. These results corroborates the present investigation. The present investigation are in line with Prabhu (2009) [15] stated that, Carbendazim @ 0.3% seed treatment reduced the population of *M. phaseolina* followed by seed treatments with Thiram. In Carbendazim seed treated plots, the initial population of *M. phaseolina* was 36 cfu/g dry weight of soil and it get reduced to 25.20 cfu/g of soil. Veena and Reddy (2016) [18] estimated the population dynamics of *Rhizoctonia bataticola* at two different intervals, initially 7 DAS and 45 DAS in all the treatments. They stated that decrease the level of pathogen population may be due to increased levels of antagonists and fungitoxicity. Whereas, the pathogen population was very high in control, which may be due to lack of competition with any other microbes and fungi toxicants in soil. The present observations are on the similar line of results quoted by several workers.

Effect of fungicides and bioagents seed treatments on seed germination, shoot length, root length and seedling vigour index

To see the effect of different fungicides and bioagents on seed germination, shoot length, root length and seedling vigour index, the greengram seed variety AKM 8802 were treated with different fungicides and bioagents kept in wet towel paper and observation were recorded. Data presented in Table 4. showed that under rolled paper method seeds of greengram AKM-8802 treated with fungicides and bioagents exhibited large variation in respect to seedling vigour index. T_3 Thiram + Carbendazim @ (0.3%) was found most effective for increasing the seed germination (81.66%), shoot length (10.77 cm), root length (9.27 cm) and seedling vigour index (1636.46) followed by T4 Thiophanate methyl @ (0.1%) with seed germination (73.33%) shoot length (9.96 cm) root length (8.45 cm) and seedling vigour index (1350.00). Germination percent in control was 52.00% i.e. lowest compared to all other treatments. *Trichoderma viride* as seed dressing exhibited good germination percent (70.00), shoot length (9.33), root length (7.55) and seedling vigour index (1181.60) as compared to *Pseudoumonas fluorescens*. Similar results were recorded by Koche *et al*. (2009) [9] confirms the present observation. Seed treatment with Thiram + Carbendazim (2:1) @ 3 g/kg increases the seed germination (85.00 to 79.00%). Giri and Chilkuri (2014) also found that seed treatment with Thiram + Carbendazim @ 3 g/kg increases the seed germination percent (85%), shoot length (11.17), root length (9.27) and seedling vigour index (1765.60) in greengram. These findings correlates the present results.

Table 1: Effect of different treatments on percent root rot incidence in greengram at 30, 45 and 60 DAS (pot culture)

S. No	Treatment Details	% Root Rot incidence			Average disease incidence	Percent disease control
		30 DAS	45 DAS	60 DAS		
T1	Vitavax + Thiram @ 0.3%	27.78 (31.8)*	47.22 (43.4)*	61.11 (51.4)*	45.37	32.88
T2	Carbendazim + Mancozeb @ 0.3%	27.78 (31.8)*	41.66 (40.2)*	61.33 (51.5)*	43.59	35.51
T3	Thiram + Carbendazim @ 0.3%	19.4 (26.2)*	36.11 (36.9)*	50.11 (45.1)*	35.20	47.93
T4	Thiophanate methyl @ 0.1%	16.66 (24.1)*	27.78 (31.8)*	44.44 (41.8)*	29.62	56.17
T5	*Pseudoumonas fluorescens* 10 g/kg	36.11 (36.9)*	52.78 (46.6)*	63.99 (53.1)*	50.96	24.61
T6	*Trichoderma viride* 4 g/kg	27.78 (31.8)*	36.11 (36.9)*	52.78 (46.6)*	38.89	42.46
T7	Castor cake @ 10%	30.56 (33.6)*	47.22 (43.4)*	63.88 (53.1)*	47.22	30.13
T8	Neem cake @ 10%	25.0 (30.0)*	33.44 (35.5)*	47.22 (43.4)*	35.22	47.89
T9	Control	50.0 (45.0)*	72.22 (58.2)*	80.55 (63.8)*	67.59	-
	F test	Sig	Sig	Sig		
	SE(m)	0.63	0.82	0.76		
	CD (p=0.01)	2.84	3.46	3.24		

*Values in parenthesis are arc sin.

Table 2: Efficacy of fungicides and bioagents against *Rhizoctonia bataticola* in *vitro*

Treatments	Fungicides and bioagents	Concentration (%)	Radial mycelial growth (mm)	Percent growth inhibition
T_1	Vitavax + Thiram	0.3	0.00	100.00
T_2	Carbandezium+ Mancozeb	0.3	0.00	100.00
T_3	Thiram+ Cabandazium	0.3	0.00	100.00
T_4	Thiophanate methyl	0.1	0.00	100.00
T_5	*Pseudomonas fluorescens*	-	52.12	37.58
T_6	*Trichoderma viride*	-	11.67	86.02
T_7	Control	-	83.51	
	'F' test		Sig	
	SE(M) ±		0.68	
	CD (P=0.01%)		2.87	

Table 3: Effect of different treatments on inoculam propagule density per gram of *Rhizoctonia bataticola* (cfuX10^3/g soil)

S. No.	Treatments	Propagule density per gram of soil. (cfuX10^3/g soil)	
		Initial count	Final count
T1	Vitavax + Thiram 0.3%	7.00*	4.66*
T2	Carbendazim + Mancozeb 0.3%	6.33	4.33
T3	Thiram + Carbendazim 0.3%	6.33	5.00
T4	Thiophanate methyl 0.1%	5.66	4.33
T5	*Pseudomanas fluorescens* 10 g/kg	8.00	5.66
T6	*Trichoderma viride* 4 g/kg	6.66	5.00
T7	Castor cake 10%	7.66	6.00
T8	Neem cake 10%	7.00	5.33
T9	Control	9.00	10.66
	F test	Sig	Sig
	SE(M) ±	0.59	0.63
	CD (P=0.01%)	2.44	2.60

*Average of three replications

Table 4: Effect of fungicides and bioagents seed treatments on seed germination, shoot root length and seedling vigour index.

Seed treatments	Dose g/kg seed	Germination %	Shoot length (cm)	Root length (cm)	Seedling vigour index
Vitavax + Thiram	3.00	71.66 57.8*	8.78	7.68	1179.52
Carbendazim+ Mancozeb	3.00	72.00 58.1*	9.11	8.26	1250.64
Thiram+ Carbendazim	3.00	81.66 64.6*	10.77	9.27	1636.46
Thiophanate methyl	1.00	73.33 58.9*	9.96	8.45	1350.00
Pseudoumonas fluorescens	10.0	64.66 53.5*	8.11	7.55	1012.57
Trichoderma viride	4.00	70.00 56.8*	9.33	7.55	1181.60
Control		52.00 46.1*			
F test		Sig			
SE (m)		0.27			
CD (P=0.01)		1.05			

*Values in parenthesis are arc sin

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