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Efficacy of *Trichoderma hamatum*, *Rhizobium* and validamycin 3%L against Soil Borne Diseases of Green Gram

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ABSTRACT: Use of biocontrol agents against soil borne pathogens is gaining importance in the present situation for eco-friendly management of diseases. *Fusarium oxysporum* and *sclerotium rolfsii* are the major yield limiting factors of green gram. For eco-friendly management of diseases caused by these, bio-agents and plant protectant were evaluated against the soil borne diseases in pot culture experiment. The combination enhanced the plant growth parameters and decreased the percent disease incidence. The Treatment with seed treatment and soil application of *Trichoderma hamatum* enriched vermicompost, *Rhizobium* and Validamycin 3% L showed good result in case of *F. oxysporum* with higher no. of pods (11.0), no. of grains (11.0), 1000 grain weight (41.73g), leaf no. (9.66), shoot length (48.61cm), root length (23.65cm), dry matter (11.86g), chlorophyll content (3.20mg) and lowest disease incidence (5.30%). In case of *S. rolfsii* the same treatment was also found to be best with highest no. of pods (10.83), no. of grains (10.67), 1000 grain weight (41.79g), leaf no. (9.50), shoot length (48.37cm), root length (23.62cm), dry matter (11.85g), chlorophyll content (3.16 mg) and lowest disease incidence (5.65%). Soil application of bio agents enriched with vermicompost showed additive effect over the seed treatments.

Keywords: *Trichoderma hamatum, Rhizobium* sp., *Sclerotium rolfsii, Fusarium oxysporum,* Validamycin *3%* L.

INTRODUCTION

Pulses are always the first preference for Indians due to protein rich diet, low cost and long-time storage. In India, the production of pulses is far below the requirement to meet even the minimum level of per capita consumption which requires that agricultural scientists should evolve the strategy to improve its production to meet the protein requirement of increasing population of the country. The productivity of pulses in India is considerably low when compared with the average global mean productivity of 496.4 kg ha^{-1} (FAOSTAT, 2014). Mungbean or green gram, *Vigna radiata* (L.) Wilczek, an important pulse crop, is an excellent source of low cost and high-quality protein (Taylor *et al*., 2005) contributing about 14% of the total protein of average diet of an Indian. It is rich in vitamin A, B, C, niacin and minerals such as calcium, phosphorus and potassium which are necessary for human body (Rattanawongsa, 1993). Since mungbean roots fix atmospheric nitrogen through symbiosis with nitrogen-fixing rhizobia, this crop is valuable both economically as well as nutritionally and is widely used in different cropping systems (Yaqub *et al*., 2010). Use of several antagonistic species of *Trichoderma* (*T. viride, T. harzianum*) against a range of economically important soil borne plant pathogens have been well documented. In recent years, the search of biological control agents for the management of dreaded soil borne diseases has been advocated widely. Since the biocontrol agents are applied either to seed or soil or both, there is every possibility of interaction and interference that would arise with the commonly used fungicides. The full expression of potential biocontrol is considered in terms of rhizosphere competence, suppression of pathogens, tolerance to pesticides, competitive saprophytic ability, adaptability to environment etc. Combined application of biocontrol agents with commonly used fungicides may result either in synergism/antagonism between these two. Various fungicides are recommended for the management of major soil borne pathogens. Though few studies about the sensitivity of biocontrol agents with certain fungicides are available, studies with special reference to commercially available biocontrol agents, compatibility of *Trichoderma* with other living organisms with modern inputs in plant protection like fungicides is pre-requisite for disease management. Hence, there is need to test the compatibility of fungicides with *Trichoderma* sp., their influence on rhizosphere microflora of various crops (Shashikumar *et al*, 2019). Therefore, *Trichoderma* spp. have been used as a microbial antagonist for the management of root diseases of various field crops (Padamini, 2014). Another approach to suppress the soil-borne diseases is to use organic amendments (Bonanomi *et al.*, 2018).

Pandey *et al.* (2011) reported that when fungal antagonists were used in combination with organic amendments, their antagonistic efficacy was enhanced. These bio-intensive methods can be used to keep the economic threshold level of diseases below without harming the agroecosystem of soil and also promoting the growth and productivity of mungbean.

Fungal based BCAs have gained wide acceptance next to bacteria (mainly, *Bacillus thuringiensis*), primarily because of their broad-spectrum efficacy in terms of disease reduction and yield increase (Copping and Menn 2000). In this context, *Trichoderma* spp have been the cynosure of many researchers who have been contributing to biological control pursuit through use of fungi (Ahmad and Baker 1987; Aziz *et al*., 1997). Furthermore, *Trichoderma* spp share almost 50% of the fungal BCAs market, mostly as soil/growth enhancers and this makes them interesting candidates to investigate (Whipps and Lumsden 2001). According to Punja and Utkhede (2003), *Trichoderma* spp are the most widely studied mycoparasitic fungi. In addition to the well-recognized mycoparasitic nature of *Trichoderma* fungus, induction of resistance against pathogens in plants has also been reported by Benhamou *et al.* (1999).

Hence the present study was carried out to select some local strains of *Trichoderma* effective against soil borne pathogens of green gram. The aim of the study was to assess the effectiveness of microbial antagonists (*Trichoderma hamatum* and Rhizobium) on soil borne diseases in greengram in terms of disease severity, growth parameters under pot culture condition.

MATERIALS AND METHODS

The pot experiment was conducted in the Department of Plant Pathology, College of Agriculture, OUAT, Bhubaneswar during rabi seasons, 2021&2022. Field soil was collected and divided into two parts equally. Two parts were sterilized for further use. Soil, sand and FYM were mixed in 2:1:1 ratio. Forty-eight pots were filled up with sterilized soil which was inoculated with *Fusarium oxysporum* and *Sclerotium rolfsii*. Seed treatment with Validamycin @ 2ml/litre was done and dried for 30min which was followed by treatment with *Rhizobium sp@* 10ml / kg of seed and shade dried for 1hr. *Trichoderma hamatum* enriched vermicompost @ 20g / kg of soil was applied in the pot. Locally isolated and identified *Trichoderma hamatum* was used in the experiment.

Treatment details

T1-No. seed treatment

T2- Seed treatment with Validamycin @ 2ml / litre and *Rhizobium* sp@ 10ml / kg of seed

T3-T2 + soil application *Trichoderma hamatum* enriched vermicompost @ 20g / kg of soil at 7DAS

T4- T2 + soil application *Trichoderma hamatum* enriched vermicompost @ 20g / kg of soil at 7DAS +14DAS

T5- T2 + soil application *Trichoderma hamatum* enriched vermicompost @ 20g / kg of soil at 7DAS + 14DAS+ 21 DAS

T6- T2 + soil application *Trichoderma hamatum* enriched vermicompost @ 20g / kg of soil at 14 DAS + 21 DAS

T7- T2 + soil application *Trichoderma hamatum* enriched vermicompost @ 20g / kg of soil at 14 DAS + 21 DAS +28 DAS

T8- T2 + soil application *Trichoderma hamatum* enriched vermicompost @ 20g / kg of soil at 7DAS+ 14 DAS +21 DAS +28 DAS

Data on various yield attributing characters along with percent incidence of the disease were recorded in both the seasons and were subjected to pooled analysis.

RESULTS AND DISCUSSION

Pooled data (2021 & 2022) revealed that in case of *F.Oxysporum*, seed treatment with *Rhizobium sp@* 10 ml / kg of seed & Validamycin 3%L @ 2 ml / litre and soil application of *Trichoderma hamatum* enriched vermicompost @ 20 g /kg of soil at weekly interval of 7,14, 21 &28DAS was found to be the best with highest no. of pods (11.0), no. of grains (11.0), 1000 grain weight (41.73g), leaf no. (9.66), shoot length(48.61cm), root length(23.65cm), dry matter (11.86g), chlorophyll content (3.20mg) and with lowest disease incidence (5.30%) followed by the seed treatment with *Rhizobium sp@* 10 ml/kg of seed & Validamycin 3%L @ 2 ml / litre and soil application of *Trichoderma hamatum* enriched vermicompost @ 20 g/kg of soil at an interval of 7,14 &21 DAS with higher no of pods (10.33), no. of grains per pod(9.66),1000 grain weight (41.31g), no. of leaves(9.0), shoot length(41.16), root length (21.98 cm), dry matter (11.03g), chlorophyll content(2.88mg) and with less disease incidence (14.10%).

Similarly, the pooled data (2021 & 2022) revealed that in case of *S. rolfsii*, seed treatment with *Rhizobium sp@* 10 ml/kg of seed & Validamycin 3%L @ 2 ml/litre and soil application of *Trichoderma hamatum* enriched vermicompost @ 20 g / kg of soil at weekly interval of 7,14, 21 &28DAS was found to be the best with highest no of pods (10.83), no of grains (10.67), 1000 grain weight (41.79g), leaf no. (9.50), shoot length(48.37cm), root length(23.62cm), dry matter(11.85g), chlorophyll content (3.16 mg) and with the least disease incidence (5.65%) followed by the seed treatment with *Rhizobium sp@* 10 ml/kg of seed & Validamycin 3%L @ 2 ml / litre and soil application of *Trichoderma hamatum* enriched vermicompost @ 20 g/kg of soil at an interval of 7,14 & 21 DAS with higher no. of pods (10.61), no. of grains per pod(9.16), 1000 grain weight (41.36g), no. of leaves (8.83),shoot length (43.89cm), root length (21.92cm), dry matter (10.97g), chlorophyll content(2.98 mg) and with lower disease incidence (14.13%).

Kumar (2014) conducted experiment on the effect of different combinations of vermicompost, biofertilizers and chemical fertilizers on growth, productivity and economics in chickpea and reported that application of RDF @ 5.0 ton/ha VC + Rhizobium + PSB significantly increased growth and yield attributes of chickpea over control. The significantly maximum net profit (Rs. 40086) and benefit cost ratio (2.12) was recorded by application of RDF + @ 3.0 ton/ha VC + Rhizobium + PSB on control. Negi *et al*. (2021) experimented the

effect of seed biopriming with different bioagents including plant growth promoting rhizobacteria (PGPR-1), rhizobial biofertilizer (*Rhizobium* strain B1) and biological control agent (*Trichoderma viride*) on plant growth, seed yield and incidence of diseases in French bean cv. Contender conducting a field experiment. Under field conditions in both the years, field emergence (95.18%), plant height at 30 days after sowing (34.09 cm), plant height at final harvest (56.99 cm), days to pod harvest (52.33), harvest duration (18.67), pod length at final harvest (16.83 cm), number of pods per plant (20.17), dry pod weight (2.72 g), pod yield per plant (38.64 g), number of seeds per pod (7.17), seed yield per plant (20.76 g), seed yield per plot (875.33 g), seed yield per hectare (23.34 q), 100 seed weight (34.19 g), quality of harvested seeds were recorded significantly higher after seed biopriming with PGPR-1 + *Rhizobium* strain B1 (T4) as compared to carbendazim seed treatment and untreated control. This treatment combination also reduced the incidence of major diseases like, Rhizoctonia root rot and Angular leaf spot significantly as compared to carbendazim seed treatments and untreated control. They concluded that seed biopriming of French bean cv. Contender with PGPR-1+*Rhizobium* strain B1 @ 109cfu/ml for 8 hours was an effective treatment which significantly improved plant growth, pod yield, seed yield, seed quality and seed vigour and reduced disease incidence as compared to seed treatment with carbendazim @ 0.2% as well as untreated control under field conditions. The present findings also showed a similar trend and thus confirms the above earlier reported findings.

Similar observation was earlier reported by El-Wahab *et al.* (2018) who tested efficacies of *Trichoderma viride*, *Rhizobium leguminosarum* and the fungicide Topsin M 70% individually and/or their mixtures *in vitro* and greenhouse conditions to control damping-off and root rot diseases of pea plants (*Pisum sativum* L., cv. *Master P*) caused by *Rhizoctonia solani*. The ability of tested *Rhizobium leguminosarum* and *Trichoderma viride* to exhibit plant growth promoting Rhizobacteria (PGPR)-properties including ability to solubilise -P and production of IAA, as well as production of siderophores, hydrocyanic acid (HCN) and secretion of cell-wall degrading enzymes (chitinase and protease) were investigated. Also, under *in vitro* conditions the effect of Topsin M 70% on growth of *R. solani, T. viride, R. leguminosarum* and their mixtures was determined. The fungicide effective concentration was found to range from 10 to 50 ppm for *R. solani* and *T. viride* mycelial growth being 83.30, 100, 76.08 and 100% at 40 and 50 ppm of Topsin M 70%. The same trend was obtained with *R. leguminosarum* that showed maximum tolerance at 40 and 50 ppm of Topsin M 70% with the average of 52.19 and 59.85% inhibition over control, respectively. Additionally, greenhouse condition experiments were conducted on sandy clay soil at Etay El Baroud Agricultural Research Station, Beheria Government, Egypt to study singly the effect of seed soaking in *T. viride, R. leguminosarum* or their mixtures with the soil drench fungicide inpots after sowing of pea cv. Master P in concern. Significant decrease of damping-off and root rot of pea was obtained. Numbers of survived plants, shoot length as well as, fresh and dry weight were recorded. The combined treatments *R. leguminosarum* + Topsin M 70%, *T. viride* + Topsin M 70% and *R. leguminosarum* + *T. viride* + Topsin M 70% were the most effective ones resulting the least percentage of total damping off and the highest percentage of healthy plants being 96.67 and100.00%, respectively. Seed treatment with *T. viride, R. leguminosarum* and drenched fungicide individually or in mixture improved plant growth as indicated by the increased growth parameters and the physiological activities (phytosynthetic pigments, peroxidase and polyphenol oxidase), especially in combined treatments. Peroxidase and polyphenol oxidase activities were increased in the different treatments- even, the fungicide drench treatment.

The present investigation resulted in identifying efficacy of *T. Hamatum* in reducing the diseases of mung bean caused by *F. oxysporum* and *S. rolfsii*. This strain of *T.hamatum* was found to be compatible with Validamycin indicating its possibility to be included in the integrated disease management strategy. Commercial formulation of *T. hamatum* in the vermicompost base was found to be best with higher cfu value and better storability. Use of such *T. hamatum* can be recommended for management of wilt caused by *F. oxysporum* and root-stem rot caused by *S. rolfsii* in mung bean in order to avoid hazardous chemicals which are eco-friendly and economical.

Table 1: Effect of *Trichoderma hamatum* enriched vermicompost, *Rhizobium* sp and Validamycin 3% L on green gram in *Fusarium oxysporum* inoculated soil (2021 & 2022 pooled).

Treatments	No. of pods	No. of grains/pod	1000 grain weight (g)	Leaf no.	Shoot length (cm)	Root length (cm)	Dry matter (g)	Chlorophyll content (mg)	Disease incidence (%)
T1 No. Seed treatment	3.66	3.33	25.76	3..33	28.70	7.73	5.66	1.23	96.13
T2 ST	6.66	6.00	31.23	6.33	32.83	13.83	8.80	2.33	40.13
T3 ST + SA @7DAS	7.33	6.33	32.25	6.66	34.06	14.75	9.20	2.73	28.96
T4 ST + SA @7 & 14DAS	8.33	8	37.93	7.66	36.40	18.50	10.66	2.92	14.33
T5 ST + SA @7,14 &21DAS	10.33	9.66	41.36	9.00	41.16	21.98	11.03	2.88	14.10
T6 ST + SA @14 &21 DAS	7.66	7.33	37.26	7.33	35.04	17.06	9.62	2.82	20.13
T7 ST + SA @14,21&28DAS	9.00	8.66	38.72	8.66	37.90	20.68	10.58	2.98	14.26
T8 ST + SA @7,14,21&28DAS	11.00	11.00	41.73	9.66	48.61	23.65	11.86	3.20	5.30
SE(m) ±	0.40	0.44	0.15	0.31	0.29	0.24	0.17	0.09	0.13
CD (0.05)	1.23	1.33	0.47	0.94	0.90	0.73	0.52	0.27	0.41

ST- Seed treatment with Validamycin @2ml/ Litre followed by *Rhizobium sp*@ 10ml/kg of seed
SA-Soil application of *Trichoderma hamatum* enriched vermicompost@20g/kg of soil

Table 2: Effect of *Trichoderma hamatum* enriched vermicompost, *Rhizobium* sp and Validamycin3% L on green gram in *Sclerotium rolfsii* inoculated soil (2021 & 2022 pooled).

Treatments	No of pods	No. of grains per pod	1000 grain weight (g)	Leaf no.	Shoot length (cm)	Root length (cm)	Dry matter (g)	Chlorophyll content (mg)	Disease incidence (%)
T1 No. Seed treatment	3.50	3.50	25.60	3.16	28.60	7.70	5.83	1.27	96.35
T2 ST	6.66	5.83	31.31	6.16	32.88	13.88	8.92	2.30	40.19
T3 ST + SA @7DAS	7.16	6.33	32.62	6.50	34.08	14.74	9.20	2.72	28.98
T4 ST + SA @7 & 14DAS	8.33	7.33	37.90	7.50	36.31	18.46	10.54	2.84	14.41
T5 ST + SA @7,14 &21DAS	10.16	9.16	41.36	8.83	43.89	21.92	10.97	2.98	14.13
T6 ST + SA @14 &21 DAS	7.50	7.33	37.21	7.00	35.03	17.02	9.61	2.72	20.30
T7 ST + SA @14,21&28DAS	9.33	8.33	38.67	8.33	37.73	20.69	10.58	2.91	14.32
T8 ST + SA @7,14,21&28DAS	10.83	10.67	41.79	9.50	48.37	23.62	11.85	3.16	5.65
SE(m) ±	0.30	0.32	0.22	0.21	0.30	0.22	0.11	0.08	0.14
CD (0.05)	0.92	0.99	0.69	0.64	0.91	0.67	0.33	0.26	0.42

ST- Seed treatment with Validamycin @2ml/ Litre followed by *Rhizobium sp*@ 10ml/kg of seed
SA-Soil application of *Trichoderma hamatum* enriched vermicompost@20g/kg of soil

CONCLUSION AND FUTURE SCOPE

Integrated application of *Trichoderma hamatum* enriched vermicompost, Rhizobium and Validamycin 3%L has maximised the yield attributing characters@7, 14, 21 and 21 DAS. Synthetic fertilisers and pesticides have greatly increased crop yield, but their widespread usage has resulted in environmental issues such as soil salinity, pathogen resistance and other issues. Green technology, particularly microbial applications, may provide superior alternatives to chemicals. In comparison to chemical pesticides, the popularisation of biopesticides has been extremely sluggish. At the end it may be concluded that the application of Trichoderma can be considered as a safer way for managing diseases which modifies the morphological characters of the plant to adapt to the situation.

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Conflict of Interests. None.

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