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Field efficacy of *Trichoderma hamatum* and *Rhizobium* against wilt complex of green gram

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Abstract
Use of biocontrol agents against soil borne pathogen is gaining importance in the present situation for eco-friendly management of soil borne diseases. The seed treated with *Rhizobium* along with soil application of *T. hamatum* enriched Vermicompost was found to be most effective in reducing the incidence of wilt complex (*F. oxysporum and S. rolfsii*) of green gram. The same treatment combination was superior in case of growth parameters i.e., no of grain, no of pods per plant,1000 grain weight, leaf no, shoot length, root length, dry matter and chlorophyll content of green gram. In case of *F. oxysporum* inoculated soil this treatment was found to be best with 10.67 no of pods, 10.00 no of grains / pod, 40.40 g thousand grain weight, 8.5 no of leaves, 42.85 cm shoot length, 30.53 cm root length, 10.61 g dry matter, 3.62 mg chlorophyll content and with the least percent disease incidence of 7.05 followed by seed treatment with *Rhizobium* sp @ 10 ml / kg of seed and soil application of *Trichoderma hamatum* enriched vermicompost @ 20 g / kg of soil at an interval of 7, 14 & 21 DAS with 10.00 no of pods, 8.66 no of grains per pod, 38.11 g thousand grain weight, 7.50 no of leaves, 41.53 cm shoot length, 25.66 cm root length, 9.88 g dry matter, 3.17 mg chlorophyll content and 12.66`% of disease incidence. In case of *S. rolfsii* inoculated soil seed treatment with *Rhizobium* sp @ 10 ml / kg of seed and soil application of *T. hamatum* enriched vermicompost @ 20 g / kg of soil at weekly interval of 7, 14, 21 & 28 DAS was found to be the best with 10.50 no of pods, 10.50 no of grains / pod, 40.75 g thousand grain weight, 9.33 no of leaves, 45.00 cm shoot length, 31.46 cm root length, 10.35 g dry matter, 3.69 mg chlorophyll content and with the least percent disease incidence of 6.71 followed by seed treatment with *Rhizobium* sp @ 10 ml / kg of seed and soil application of *T. hamatum* enriched vermicompost @ 20 g / kg of soil at an interval of 7, 14 & 21 DAS with 9.33 no of pods, 8.66 no of grains per pod, 37.03 g thousand grain weight, 9.00 no of leaves, 39.26 cm shoot length, 30.30 cm root length, 9.00 g dry matter, 3.29 mg chlorophyll content and 14.99`% of disease incidence. Integrated Disease Management (IDM) approach was carried out to combat green gram wilt with a combination of bio agents and organic amendments. Soil application of bio agents enriched with Vermicompost showed additive effect over the seed treatments.

Keywords: *Trichoderma hamatum, Rhizobium* sp., *Sclerotium rolfsii, Fusarium oxysporum*

Introduction

Foods which provide high quality protein and are cheap in price are a demand for the poor living in developing countries like India where a large population is vegetarian (Saravanakumar *et al*., 2007) [15]. For this purpose, pulses are always the first preference for them 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} (FAO STAT., 2014) [9]. Mung bean 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) [17] 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) [14]. Since mung bean 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) [19]. The low productivity and poor quality of mung bean in India can be attributed to several biotic and abiotic constraints of which diseases caused by fungi are of great importance (Khan and Khan, 2001) [10]. Bioagents served as an ecologically safe and acceptable substitute for the fungicidal management of soil-borne diseases in recent years (Abada and Ahmad, 2014) [1].

Therefore, *Trichoderma* spp. have been used as a microbial antagonist for the management of root diseases of various field crops (Padamini, 2014) [11]. Another approach to suppress the soil-borne diseases is to use organic amendments (Bonanomi *et al.*, 2018) [5]. Pandey *et al*. (2011) [12] 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 below without harming the agroecosystem of soil and also promoting the growth and productivity of mung bean.

The major fungal diseases which infect pulses are Wilt (*Fusarium oxysporum*), Dry root rot (*Rhizoctonia bataticola*), Collar rot (*Sclerotium rolfsii*), Wet root rot (*Rhizoctonia solani*), Ascochyta blight (*Ascochyta rabiei*), Botrytis grey mould (*Botrytis cinerea*), Black root rot (*Fusarium solani*), Seed rot (*Aspergillus flavus*), Stem rot (*Sclerotinia sclerotiorum*), Crown rot (*Sclerotium rolfsii*), Foot rot (*Phacidiopycnis padwickii*) and Sclerotinia wilt (*Sclerotinia sclerotiorum*). 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 *et al*., 2000) [7]. 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 *et al*., 1987 and Aziz *et al*., 1997) [2, 3]. 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 *et al*., 2001) [18]. According to Punja and Utkhede (2003) [13], *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 (1999) [6]. 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 present study was to assess the effectiveness of microbial antagonists (*Trichoderma hamatum* and *Rhizobium*) on soil borne diseases in green gram 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 at 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 *Rhizobium* sp @ 10 ml / kg of seed was done and shade dried for 1hr and seeds were treated with *Trichoderma hamatum* enriched vermicompost as well as *Trichoderma hamatum* enriched vermicompost @ 20 g / kg of soil was applied in the pot.

Treatment details

T_1-No seed treatment

T_2- Seed treatment with *Rhizobium* sp @ 10 ml / kg of seed

T_3- T_2 + soil application *Trichoderma hamatum* enriched vermicompost @ 20 g / kg of soil at 7 DAS

T_4- T_2 + soil application *Trichoderma hamatum* enriched vermicompost @ 20 g / kg of soil at 7 DAS +14 DAS

T_5- T_2 + soil application *Trichoderma hamatum* enriched vermicompost @ 20 g / kg of soil at 7 DAS + 14 DAS+ 21 DAS

T_6- T_2 + soil application *Trichoderma hamatum* enriched vermicompost @ 20 g / kg of soil at 14 DAS + 21 DAS

T_7- T_2 + soil application *Trichoderma hamatum* enriched vermicompost @ 20 g / kg of soil at 14 DAS + 21 DAS +28 DAS

T_8- T_2 + soil application *Trichoderma hamatum* enriched vermicompost @ 20 g / kg of soil at 7 DAS+ 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 on disease incidence along with yield attributing characters (2021 & 2022) showed that in case of *F. oxysporum* inoculated soil this treatment was found to be best with 10.67 no of pods, 10.00 no of grains / pod, 40.40 g thousand grain weight, 8.5 no of leaves, 42.85 cm shoot length, 30.53 cm root length, 10.61 g dry matter, 3.62 mg chlorophyll content and with the least percent disease incidence of 7.05 followed by seed treatment with *Rhizobium* sp @ 10 ml / kg of seed and soil application of *Trichoderma hamatum* enriched vermicompost @ 20 g / kg of soil at an interval of 7, 14 & 21 DAS with 10.00 no of pods, 8.66 no of grains per pod, 38.11 g thousand grain weight, 7.50 no of leaves, 41.53 cm shoot length, 25.66 cm root length, 9.88 g dry matter, 3.17 mg chlorophyll content and 12.66`% of disease incidence. Similar observation was earlier reported by Gehad *et al.* (2018) [8] 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 solubilize-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. Ajit Fakira Mandale *et al*. (2021) [4] investigated to check the effect of liquid formulations of *Rhizobium* inoculation on Soil microbial population dynamics at periodic intervals in soil, nitrogen and phosphorus uptake by mung bean, growth and yield of mung bean as influenced by application of liquid *Rhizobium*. After considering all the parameters, inference could be drawn that *Rhizobium* application enhanced the growth leading to increase in yield of mung bean. It was observed that T_3: S.T.L. liquid *Rhizobium* @ 25 ml / kg of seed each had higher arithmetic value for growth parameters including germination, plant height, number of branches, number of leaves, LAI, root nodules and yield parameters like pods / plant, thousand seed weight and ultimately yield / ha. Other parameters including chemical and microbial parameters showed significant increase over the absolute control. Above investigation concluded that inoculation of liquid formulation of *Rhizobium* enhanced growth as well as yield of mung bean. Population of *Rhizobium* as influenced by inoculation of liquid formulations was enhanced significantly. Total N and P uptake by plant and grain sample was found to be significantly higher due to microbial inoculations. Similar observations on improvement in the yield attributing characters as well as yield were also seen in the present investigation. Pooled data (2021 & 2022) also indicated that in case of *S. rolfsii* inoculated soil seed treatment with *Rhizobium* sp @ 10 ml / kg of seed and soil application of *T. hamatum* enriched vermicompost @ 20 g / kg of soil at weekly interval of 7, 14, 21 & 28 DAS was found to be the best with 10.50 no of pods, 10.50 no of grains / pod, 40.75 g thousand grain weight, 9.33 no of leaves, 45.00 cm shoot length, 31.46 cm root length, 10.35 g dry matter, 3.69 mg chlorophyll content and with the least percent disease incidence of 6.71 followed by seed treatment with *Rhizobium* sp @ 10 ml / kg of seed and soil application of *T. hamatum* enriched vermicompost @ 20 g / kg of soil at an interval of 7, 14 & 21 DAS with 9.33 no of pods, 8.66 no of grains per pod, 37.03 g thousand grain weight, 9.00 no of leaves, 39.26 cm shoot length, 30.30 cm root length, 9.00 g dry matter, 3.29 mg chlorophyll content and 14.99`% of disease incidence. Sharma and Borah (2020) [16] studied the effect of seed inoculation treatments with biological agents on seed germination, field emergence and its effect on seed during summer season. Seeds of green gram variety Pratap (SG-1) were treated with microbial formulations of seven treatments which consisted of-*Rhizobium* (T_1), *Trichoderma harzianum* (T_2), *Trichoderma viride* (T_3), *Bacillus megaterium* (T_4), *Trichoderma harzianum* + *Trichoderma viride* + *Bacillus megaterium* (T_5), *Rhizobium* + *Trichoderma harzianum* + *Trichoderma viride* + *Bacillus megaterium* (T_6) and Control (T_7). Shoot length, root length and seedling dry weight were recorded after 20 days of sowing, observations on nodulation, yield and yield contributing characters were recorded at maturity and germination characteristics of the seeds were studied in the laboratory. They had observed that seeds treated with combined inoculation of *Rhizobium* @ 4 g + *Bacillus megaterium* @ 5 ml / 1000 ml of water + *Trichoderma harzianum* @ 5 ml / 1000 ml of water + *Trichoderma viride* @ 5 ml / 1000 ml of water (T_6) recorded significantly higher field emergence (91.25%), speed of emergence (42.14), seedling dry weight (1.67 mg), shoot length (25.48 cm), seed yield (992 kg ha^{-1}), Stover yield (1870 kg ha^{-1}), number of pods $plant^{-1}$ (37), number of seeds pod^{-1} (13.25), 100 seed weight (3.63 g), root length (9.22 cm) and nodulation (15). The present findings on improvement in yield and yield attributing characters in mung bean confirm the above mentioned earlier findings. This Integrated Disease Management (IDM) approach will be helpful for the farmers of the State to combat green gram wilt complex with a combination of bio agents and organic amendments. Soil application of bio agents enriched with Vermicompost showed additive effect over the seed treatments. Hence this will pave way towards an eco-friendly management approach for green gram diseases.

Table 1: Effect of *Trichoderma hamatum* enriched vermicompost and *Rhizobium* sp 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 (%)
T_1 No seed treatment	3.50	3.50	25.61	3.83	27.86	17.70	5.70	1.53	96.06
T_2 ST	5.66	5.16	33.46	5.00	30.03	19.57	7.35	2.29	41.71
T_3 ST+ SD @ 7 DAS	6.50	5.50	34.58	6.33	30.83	21.41	7.42	2.86	28.86
T_4 ST+ SD @ 7 & 14 DAS	9.00	7.33	36.01	7.66	32.90	24.40	8.71	2.94	14.78
T_5 ST+ SD @ 7,14 & 21 DAS	10.00	8.66	38.11	7.50	41.53	25.66	9.88	3.17	12.66
T_6 ST+ SD @ 14 & 21 DAS	7.33	6.16	36.14	7.00	32.03	24.25	8.31	2.74	22.27
T_7 ST+ SD @ 14, 21& 28 DAS	9.50	7.83	37.42	7.50	33.33	24.55	9.04	3.11	13.15
T_8 ST+ SD @ 7, 14, 21 & 28 DAS	10.67	10.00	40.40	8.50	42.85	30.53	10.61	3.62	7.05
SE(m) $\pm$	0.17	0.30	0.38	0.65	0.20	0.24	0.19	0.08	0.28
CD (0.05)	0.53	0.90	1.14	1.97	0.60	0.72	0.58	0.26	0.84

ST- Seed treatment with *Rhizobium* sp @ 10 ml/1kg of seed
SD- Soil drenching @ 20 g vermi bioagent /litre of water i.e., 40 ml/pot

Table 2: Effect of *Trichoderma hamatum* enriched vermicompost and *Rhizobium* on green gram in *Sclerotium rolfsii* 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 (%)
T_1 No seed treatment	3.50	3.83	26.55	4.00	29.08	14.03	6.31	1.54	96.02
T_2 ST	5.83	5.83	29.98	5.33	30.86	21.03	6.66	2.46	41.97
T_3 ST+ SD @ 7 DAS	6.33	6.50	32.33	6.50	31.95	22.18	7.56	2.99	29.23
T_4 ST+ SD @ 7 & 14 DAS	7.83	8.00	34.90	8.33	33.90	24.80	8.77	3.14	17.77
T_5 ST+ SD @ 7, 14 & 21 DAS	9.33	8.66	37.03	9.00	39.26	30.30	9.00	3.29	14.99
T_6 ST+ SD @ 14 & 21 DAS	7.00	7.16	33.83	7.50	32.41	26.89	8.38	2.83	22.07
T_7 ST+ SD @ 14, 21& 28 DAS	9.16	8.16	35.61	8.33	35.93	28.98	8.91	3.24	16.00
T_8 ST+ SD @ 7, 14, 21 & 28 DAS	10.50	10.50	40.75	9.33	45.00	31.46	10.35	3.69	6.71
SE(m) $\pm$	0.24	0.30	0.35	0.64	0.44	0.31	0.23	0.11	0.78
CD (0.05)	0.73	0.90	1.07	1.96	1.34	0.95	0.70	0.33	2.37

ST- Seed treatment with *Rhizobium* sp @ 10 ml/1kg of seed
SD- Soil drenching @ 20 g vermi bioagent /Litre of water i.e., 40 ml/pot

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