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# Efficacy of bioagents, biofertilizers and soil amendaments to manage root rot in greengram

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## ABSTRACT

The potential of soil amendaments, bioagents and biofertilizers was evaluated for management of root rot caused by *Macrophomina phaseolina (Rhizoctonia bataticola)*. Bioagents and biofertilizers i.e.*Trichoderma harzianum*, *Pseudomonas fluorescens,* Phosphate solubilizing bacteria and *Rhizobium phaseoli* exhibited significant inhibition of *Rhizoctonia bataticola*. Maximum inhibition was achieved by *Trichoderma harzianum* i.e. 80.5%. Compatibility amongst bioagents and biofertilizers were tested *in vitro.* Amongst them *Trichoderma harzianum* and *Rhizobium phaseoli* were found compatible to each other. In green house, seed treatment with the bioagents and biofertilizers and also application of soil amendments recorded good number of nodules. Application of FYM and seed treatment with *R. phaseoli* at 25 g/kg + *P. fluorescens* at 10 g/kg recorded maximum number of nodules (15.3). Different seed treatments with soil amendments were used for controlling root rot incidence. Among them ($T_6$) FYM with seed treatment of *T. harzianum* 4 g/kg seed + PSB at 25 g/kg seed recorded the lowest root rot incidence (26.0%) followed by ($T_3$) *R. phaseoli* at 25 g/kg seed + *P. fluorescens* at 10 g/kg seed which recorded 32.0% root rot incidence whereas, maximum incidence of the disease was recorded in the control (83.3%).

**Keyword:** Bioagents and Biofertilizers, Mungbean, Root rot, Soil amendments.

## INTRODUCTION

Green gram [*Vigna radiata* (L.) Wilczek] is one of the most important pulse crops in India. Dry seed yield of greengram is declining due to disease and insect pest. Root rot caused by *Macrophomina phaseolina* (Tassi) Goid [*Rhizoctonia bataticola* (Taub.) Butler] is one of the most important diseases causing numerous losses in mungbean [*Vigna radiata* (L.) Wilczek]. Chemical control achieved by treatment with fungicides gave greater variation in effectiveness of fungicides (Muthukrishnan, 1989).In addition application of chemicals may detrimental to beneficial biocontrol agents.

Biological control is one of the viable, ecofriendly propositions, which can substantially minimize the disease (Cook, 1985). Biological control of plant pathogens using fungi and bacteria can be successfully utilized especially within the frame work of integrated disease management system. The use of organic amendments is one of the successful control methods of soil borne diseases. The addition of organic matter in soil provide disease control through variety of mechanism such as providing antimicrobial compounds during decomposition (Mansoor,2007) reduced the inoculum density of the soil borne plant pathogens through changes in the general microbial balance by different mechanism (Klein, 2011).

Interaction between *Trichoderma* and *Pseudomonas* may operate independently or together and their activities can result in the suppression of the plant pathogens (Elad *et al*., 1986; Singh *et al*., 2013). An important factor of the improving biocontrol in the rhizosphere is to add mixtures or combination of biocontrol agents. Micro-organisms from rhizosphere have received considerable attention as potential protecting agents against soil borne pathogens. Different bioagents and biofertilizers like *P. fluorescens, Trichoderma viride, Rhizobium phaseoli* and Phosphate solubilizing bacteria (*Pseudomonas striata*) have good prospectus in future as it gives very high cost benefit ratio. These biofertilizers and bioagents in mungbean help in reduction of causing root rot pathogen. The capacity and potential of bioagents and biofertilizer to colonize the rhizosphere and rhizoplane can acts as a barrier for entrance of pathogen. Present investigation was carried out to see the potential of bioagents, soil amendments and biofertilizers for control of root rot in greengram.

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## MATERIALS AND METHODS

All isolates of *P. fluorescens, Trichoderma viride, Rhizobium phaseoli* and Phosphate solubilizing bacteria (*P. striata*) were screened for potential antagonistic activity against pathogenic fungi *M. phaseolina* using dual culture technique. In Petri plates containing 20 ml PDA media and loopful of fresh culture of biofertilizer and bio-agent was streaked 3 cm away from fungal mycelial disc at sides towards the edges of Petri plates and then 5 mm disc of the fungal culture was placed at the centre and Petri plates were incubated at $28^0$C. Plates inoculated with *M. Phaseolina* without bacteria were maintained as control. The per cent inhibition of test fungus with each bio-agent and biofertilizer was calculated. Three replicated plates were maintained for each biofertilizer/bioagents. The per cent growth inhibition was calculated.

Biofertilizer like as PSB, *Rhizobium phaseoli* and bioagent as, *P. fluorescens* and *T. harzianum* were tested *in vitro* for their interaction study between them through cross streak plate assay (Anandraj and Delopierre, 2010). Interaction was tested by streaking *Rhizobium phaseoli* at one end of the plate and *P. fluorescens* at other end of plate. After inoculation of these plates at $28 \pm 1^0$c inhibition zones were measured.

**Green house study:** To see the biocontrol ability of *P. fluorescens*, *R. phaseoli*, *T. harzianum* and PSB in green house, the study was conducted by using seeds of mungbean of variety Kopergaon. Mass multiplication of *M. phaseolina* was carried out in potato dextrose broth at room temperature for two weeks. Sterilized sandy soil mixed with *M. phaseolina* inoculated artificially (100 g inoculum/kg soil).The mixture was incubated for 5 days at room temperature for colonization of fungus in soil. Amended soil was transferred to 8 cm diameter plastic pots each containing 300 g soils. Pots were kept at 50 per cent 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. Pots were kept in greenhouse in factorial RBD. Seed treatment with bio-agent and biofertilizer alone and in combination was tested for its efficacy against test pathogen. Soil amendments like FYM, compost, neem seed cake and ground nut cake were added to increase efficiency of biofertilizer and bio-agent. Sterilized sandy soil mixture artificially inoculated with *M. phaseolina* (100 g /kg soil) was treated as control. Experimental design was factorial randomized block design having four main factor and seven sub factor and three replications. Soil amendments, M1 – FYM at 9 g /kg soil, $M_2$ – Compost at 9 g /kg soil, $M_3$ – neem seed cake at 9 g /kg soil, $M_4$ – groundnut cake at 9 g /kg soil. Biofertilizers and bioagents factor, $T_1$ – *Rhizobium phaseoli* at 25 g/kg seed, $T_2$ – *P. fluorescens* at 10 g/kg seed, $T_3$ – *R. phaseoli* at 25 g/kg seed + *P. fluorescens* @ 10 g /kg seed, $T_4$– *T. harzianum* @ 4 g/kg seed, $T_5$ – *P. striata* at 25 g/kg seed, $T_6$ – *T. harzianum* at 4 g/kg seed + *P. striata* at 25 g /kg seed, $T_7$ – Control.

## RESULTS AND DISCUSSION

***In vitro* growth inhibition:** The results indicated that the use of bioagents and biofertiilzers provided significant inhibition of *M. phaseolina.* The differences are statistically significant in inhibiting the growth of the test pathogen. Amongst these, *Trichoderma harzianum* (80.5%) was found most promising in inhibiting the growth of *M. phaseolina* (*Rhizoctonia bataticola)* at 7 days after inoculation followed by *P. fluorescens* (Table 1). Inhibition of pathogen may be because of biocontrol mechanism adopted by *T. harzianum* and antibiosis activity *of P. fluorescens.* The results corroborated with the findings of Natarajan and Rao (1996) who studied potential use of *Trichoderma* as an effective biocontrol agent against *R. bataticola* in most of the crop plants. Lokesha and Benagi (2007) also found that both *Trichoderma harzianum* and *P. fluorescens* significantly inhibited the mycelial growth of *Rhizoctonia bataticola* in dual culture technique. Observations in current study pertaining to inhibition of *M. phaseolina* by *T. harzianum* and *P. fluorescens* support this view.

Compatibility study was undertaken in which antagonism or synergism was tested among all isolates to see

**TABLE 1:** *In vitro* antagonistic study of *Rhizobium phaseoli, Pseudomonas fluorescens,* Phosphate solubilizing bacteria *and Trichoderma harzianum* against *Rhizoctonia bataticola*

| Treatments | Mycelial growth (mm)DAI | | | Growth inhibition %DAI | | |
|---|---|---|---|---|---|---|
| | 3 | 5 | 7 | 3 | 5 | 7 |
| *Rhizobium phaseoli* | 53.3 | 78.2 | 80.0 | 27.8 | 5.1 | 4.6 |
| *Pseudomonas fluorescens* | 45.0 | 50.1 | 70.1 | 39.1 | 39.2 | 26.4 |
| *P. striata* | 58.1 | 60.8 | 61.6 | 21.3 | 26.3 | 16.4 |
| *Trichoderma harzianum* | 11.3 | 14.6 | 16.5 | 84.7 | 82.4 | 80.5 |
| Control | 73.9 | 82.4 | 83.9 | 0 | 0 | 0 |
| S.E(m)± | 1.02 | 0.99 | 0.50 | | | |
| CD (P=0.01) | 4.58 | 4.45 | 2.25 | | | |

whether they are compatible to each other or play an antagonistic effect against each other. It was observed that *R. phaseoli* and *P. fluorescens* show minimum zone of inhibition (1.1 mm) followed by *R. phaseoli* and *T. harzianum* (1.3 mm) (Table 2). This proved that *R. phaseoli* and *P. fluorescens and R. phaseoli* and *T. harzianum* were compatible with each other. Similar results were recorded by Anandaraj and Delopierre (2010) who studied the compatibility of *R. phaseoli* and *P. fluorescens* through cross steak plate assay. The inoculants were found compatible with each other and were able to grow simultaneously without any inhibition in growth.

Soil amendments have an ability to influence secondary metabolites which are deleterious to pathogen. There were significant differences amongst soil amendments. FYM was found most effective to increase maximum No. of nodules per plant (9.7) as against groundnut cake (7.1) (Table 3) (Bonanomi *et. al*., 2007). Increase in nodulation may be because of increasing Rhizobial population in soil due to different mechanisms operated with the addition FYM (Lukade, 1992) and also the synergism observed between *Rhizobium* and *P. fluorescens* in earlier experiment. Among all the treatments, i.e. *rhizobium phaseoli* at 25 g / kg seed + *P. fluorescens* at 10 g/kg seed found effective to give maximum nodules (12.2). The interaction effect of soil amendments and treatments was also significant. Among them treatment combination of FYM + *Rhizobium* at 25 g/kg. + *P. fluorescens* at 10 g/seed showed maximum number of nodules (15.3). These results showed that these bioagents and biofertilizers individually or in combination could provide a broad spectrum of plant growth promoting activity which results in nodulation (Yadav *et.al*., 2013). Combined application of *Rhizobium* and FYM also reported an increase in yield by 729 kg / ha nodulation, N and P uptake at 60 days as well as *Rhizobium* population (Kundu, 1998).

Data presented in Table 4 showed that there was significant difference in root rot incidence in different treatments. Among all the treatments, *T. harzianum* at 4 g / kg + PSB at 25 g/kg gave significantly superior performance to give less root rot incidence (34.0%). The results of this study supports earlier observations that a combination of biofertilizers and bioagents with different mechanism of disease control will have an additive effect and results in enhance in disease control compared to individual application (Guetsky *et al*., 2002) These findings are also in support with Singh *et.al.,* (2008) who reported that *T. harzianum*

**TABLE 2:** Interaction between *Rhizobium phaseoli, Pseudomonas fluorescens,* Phosphate solubilizing bacteria and *Trichoderma harzianum* on different media.

| Medium | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | *R. phaseoli / T. harzianum* | *P. striata / T. harzianum* | *R. phaseoli / P. fluorescens* | *P. striata / P.fluorescens* |
| YEMA | 1.3 | 1.3 | 1.1 | - |
| Kings' B | - | - | 2.2 | 2.0 |
| PDA | 2.1 | - | - | - |
| Pikovaskayas | - | 1.4 | - | 1.3 |

**TABLE 3:** Effect of different treatments on number of nodules per plant in mungbean seedling in pot culture.

| Sr. No. | Treatment Details | Nodules / plant | | | | Mean |
|---|---|---|---|---|---|---|
| | | FYM | Compost | Neem cake | Gr. Nut cake | |
| $T_1$ | *Rhizobium phaseoli* at 25g / kg. | 12.7 | 9.7 | 9.7 | 10.0 | 10.5 |
| $T_2$ | *Pseudomonas fluorescens* at 10g / kg. | 12.7 | 9.0 | 9.0 | 7.7 | 9.6 |
| $T_3$ | *Rhizobium phaseoli at* 25 g/kg seed + *P. fluorescens* at 10g / kg | 15.3 | 11.0 | 10.7 | 11.7 | 12.2 |
| $T_4$ | *Trichoderma harzianum* at 4g / kg. | 5.7 | 4.0 | 6.0 | 6.7 | 5.2 |
| $T_5$ | Phosphate Solubilizing Bacteria at 25 g/kg seed | 6.3 | 7.3 | 7.7 | 6.7 | 7.0 |
| $T_6$ | *T. harzianum* +PSB *at* 25g /kg seed | 11.0 | 8.7 | 8.3 | 6.7 | 8.7 |
| $T_7$ | Control | 4.0 | 3.0 | 2.3 | 2.7 | 3.0 |
| | Mean | 9.7 | 7.5 | 7.7 | 7.1 | |
| | | Soil Amendment | Biofertilizer and Bioagent | Soil Amendment x Biofertilizer and Bioagent | | |
| | SEm ± | 0.25 | 0.18 | 0.5 | | |
| | CD ( P=0.05) | 0.69 | 0.52 | 1.38 | | |

**TABLE 4.** Effect of different treatment on percent root rot incidence in Mungbean seedling in pot culture (60 DAS).

| Sr. No. | Treatment Details | Root Rot incidence (%) | | | | Mean |
|---|---|---|---|---|---|---|
| | | **FYM** | **Compost** | **Neem cake** | **Gr. Nut cake** | |
| $T_1$ | *Rhizobium phaseoli* at 25 g / kg | 58.7 (50.0) | 57.7 (48.8) | 61.3 (51.5) | 62.7 (52.3) | 60.1 |
| $T_2$ | *P. fluorescens* at 10 g / kg seed | 49.0 (44.4)* | 53.3 (46.9) | 56.7 (48.8) | 59.7 (50.6) | 54.5 |
| $T_3$ | *Rhizobium phaseoli* at 25 g /kg seed + *P. fluorescens* at 10 g/kg seed. | 32.0 (34.4) | 43.3 (41.2) | 39.0 (38.6) | 45.7 (42.5) | 40.0 |
| $T_4$ | *Trichoderma harzianum* at 4 g / kg seed | 38.7 (38.4) | 48.3 (44.0) | 46.7 (43.1) | 52.0 (46.1) | 46.4 |
| $T_5$ | *PSB* at 25 g / kg seed | 66.7 (54.7) | 66.0 (54.3) | 65.7 (54.1) | 71.7 (57.8) | 67.5 |
| $T_6$ | *T. harzianum* at 4 g/kg seed + *PSB* at 10 g / kg seed | 26.0 (30.7) | 36.7 (37.3) | 31.3 (34.0) | 42.0 (40.4) | 34.0 |
| $T_7$ | Control | 83.3 (66.0) | 86.7 (68.6) | 83.0 (65.6) | 87.7 (69.4) | 85.2 |
| | Mean | 50.6 | 56.0 | 54.8 | 60.2 | |
| | | Soil Amendment | Biofertilizer and Bioagent | Soil Amendment x Biofertilizer and Bioagent | | |
| | SE m ± | 0.31 | 0.41 | 0.83 | | |
| | CD(p=0.05) | 0.89 | 1.18 | 2.36 | | |

* Value in parenthesis are in arc sin.

exhibited maximum (75.6 %) inhibition of *Rhizoctonia solani* and also gave maximum control of the disease (72.7%). Neha Dev and Dawande (2010) obtained effective control of *Rhizoctonia solani* by using *Trichoderma* spp and *Pseudomonas fluorescens*. Similarly, Shankar and Jeyarajan (1996) also reported that *T. harzianum* significantly reduced *M. phaseolina* root rot incidence in sesamum. Ebenezer and Yesuraja (2000) tested fifteen antagonists against root rot causing *Macrophomina* in green gram. *T. viride* and *T. harzianum* reduced the growth of pathogen (42-33 and 42-25 mm) in dual culture. The interaction effect of soil amendments and treatments was significant at 60 DAS. The treatment combination of FYM +*T. harzianum* at 4 g / kg + PSB at 25 g/ kg seed gave minimum root rot incidence in mungbean seedling (26.0 %) followed by FYM + *Rhizobium phaseoli* at 25 g/kg seed + *P. fluorescens* at 10 g/kg seed show minimum root rot incidence (32.0 %). Therefore this study shows that the approach of using certain compatible combinations of soil amendments, bioagents and biofertilizers for controlling test pathogen has potential benefit in managing plant health.

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