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Research Note

Evaluation of bioagents for their compatibility in the development of consortium for enhanced efficacy

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ABSTRACT: The concept of development of microbial consortia for bio-control relies on the fact that bioagents under natural habitats live in communities with some benefits for plants. Till now no guideline has been published for the evaluation of bioagents to test their compatibility before developing bioagent consortium. In the present studies compatibility among biocontrol potential *Trichoderma-Pseudomonas* and *Trichoderma-Trichoderma* isolates was studied by dual culture, mixed formulations and using cell free cultures. In dual cultures all the combinations (14 nos.) were found compatible with each other as no isolate inhibited the growth of one-another i.e. absence of inhibition zone. All the mixed formulations of potential *Trichoderma-Pseudomonas* isolates (8 nos.) were found compatible with each other as they were growing simultaneously on the PDA without antagonizing the growth of other or formation of inhibition zone in their combinations. The cell free cultures of each *Trichoderma* and *Pseudomonas* isolates tested with each other using Food Poison Technique showed synergistic effects on their fresh mycelial weight among some combinations while majority showed no significant differences with their checks. Further, all the combinations (14 nos.) were tested for their effects on seed germination and vigour index of chickpea in glass-house. All the combinations showed significantly better seed germination while some combinations, *viz*., Th14+Psf173, TCMS36+Psf173, Th17+Th19, Th17+Psf2, Th17+TCMS36 and Th14+Psf2 showed a better plant vigour index (43.5 to 44.9%) as compared to their checks (28.8 to 41.5%). These guidelines could be used before developing bioagent consortium and evaluation in field for crop health management.

KEY WORDS: Chickpea, microbial consortia, *Trichoderma harzianum*, *Pseudomonas fluorescens*

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Plants rhizosphere microbiome is heterogenous and playing major role in defense responses against pathogens. Use of biological control agents (BCAs) is a potentially important component of sustainable agriculture. The principal biocontrol mechanisms involved include mycoparasitism, antibiosis, competition, and induced resistance (Arras and Arru, 1997; Elad and Freeman, 2002). *Trichoderma* and *Pseudomonas* are free living, nonpathogenic microorganisms which successfully colonize the soil, and the root region of host plant and compete with pathogens, suppress their growth, acting as growth promoting and/or antagonistic to pathogens (Sharma *et al*, 2012). Over the years, numerous studies have described the application of microbial consortia for plant disease management throughout the world. Studies revealed that plants treated with anagonistic microbial consortia showed a significant disease reduction when compared to using individual isolates. Application of bioagents in a consortium may improve efficacy, reliability and consistency of the bioagents even under diverse soil and environmental conditions. Diversity in biocontrol mechanisms offered by each bioagentin consortium may help in enhancing disease suppressiveness and may also strengthen the capabilities of the partners in an additive or synergistic manner. Biocontrol attributes also are more in consortia in comparison to using single isolates (Thakkar and Saraf *et al*., 2015). Studies on employing indigenous bacterial and fungal antagonistic consortia are very limited. The development of new biocontrol consortium requires various screenings of two or three microbial antagonists to prevent inhibitory action on each other. Antagonists for commercial use have to fulfill many different requirements. In relation to this, the present study had focused on the approach to develop guidelines for the evaluation of antagonistic microbial consortium could help in effective plant disease management.

Testing of compatibility of Biocontrol Agents (BCAs)

Selected isolates of *Trichoderma harzianum* - Th14, Th17, Th19, and TCMS36 and *P. fluorescens* Psf-2 and Psf-

173 were obtained from Biocontrol Laboratoty, Department of Plant Pathology, GBPUA&T, Pantnagar.

These strains were evaluated for their compatibility by three different methods to ensure their compatibility under *in vitro* conditions. In the first method, compatibility testing between *T. harzianum* and *P. fluorescens* isolates was conducted using dual culture method (Sivakumar *et al*., 2000). A 5 mm disc from 4 days old young culture of *T. harzianum* isolates grown on PDA medium was placed on onside of 9cm petri dish containing PDA on opposite side *P. fluorescens* isolates were streaked. The plates were incubated at 28±2°C under incubator and examinedevery day for inhibition zone; if the growth of both antagonists is overlapping it was interpreted as a compatible interaction whereas incompatible interaction was evident by a zone of inhibition between the two species.

In second method, five days old *T. harzianum* and two days old cultures of *P. fluorescens* were taken for preparing culture suspension. A 5mm of *T. harzianum* isolates and a loopful of *P. fluorescens* isolates was taken using sterile needle and suspended in 10 ml sterile distilled water. Ten-fold serial dilution was prepared; from the 10^{-6} dilution one ml of each isolates plated in a petri dish containing PDA media. The plates were incubated @ 28±2 °C and growth inhibition of *Trichoderma* growth monitoredevery day. Plates with no inhibition zone interpreted as incompatible interaction and overlapping with one another considered as compatible interaction.

In the third method, non-volatile compounds produced by the *P. fluorescence* and *T. harzianum* were studied for its compatibility. Initially, *P. fluorescens* and *T. harzianum* isolates were inoculated in 150ml of sterilized PDB in 250 ml conical flasks and incubated at 28±2°C for 10 days. The culture filtrates were filtered through Whatman filter paper (No.44) to remove mycelial mats and then filtered using syringe filter at 0.22µ. The filtrates were added to PDB contained in a conical flask at the concentration of 20% (v/v). Then they were inoculated with mycelial plugs (0.5 cm diameter) of *Trichoderma* and incubated at 25±2°C for 7-days. Broth without filtrate served as control and the experiment replicated into two.

The experiment was a completely randomized design (CRD) with a factorial arrangement and four replications. The experiment was repeated once.

Greenhouse experiment

Soil and FYM mixture sterilized in an autoclave at 15 lbs pressure for 30min for three consecutive days, 1.5 kg of the mixture was filled in each pot (15×10cm). The following treatments were examined: (i) *T. harzianum* (Th14, Th17, Th19, TCMS 36), (iii) *P. fluorescens* (Psf2, Psf 173), chick pea (*Cicer arietinum* L.) seeds were surface sterilized with 1% sodium hypochlorite, rinsed twice with sterile distilled water. The seeds were coated with *T. harzianum* and P. *fluorescens* isolates singly, in combinations using 1% CMC (Carboxy Methyl Cellulose) as adhesive. Then, the treated seeds were dried overnight in sterile Petri dish. Consortia used as treatment and individual isolateand untreated used as control. The pots were kept under the greenhouse condition. The experimental set up was completely randomized.

Fusarium wilt pathogen of chickpea *Fusarium oxysporum* f. sp. *Ciceris* was multiplied on bajra (*Pennisetum typhoides* Pers.) seedsat 25±2 °C for 15 days. Fully colonized culture was blended well before use as inoculum. Pathogen inoculated at the rate of 20g per pot. Percent seed germination was recorded 10 days after sowing and final count after 30 days of sowing. Height (cm) was recorded to calculate vigour index percentage and seedling mortality up to 30 days by using following formulae given by (Kharb *et al*, 1994).

$$\text{Germination (\%)} = \frac{\text{Total number of germination seeds}}{\text{Total number of seeds sown}}$$

Vigour index (%) = Germination percentage × Seedling length on the day of final count

Vigour index mass = Germination percentage × seedling dry weight

Compatibility among BCAs

All four *T. harzianum* isolates (Th 14, Th17, Th19 and TCMS 36) and *P. fluorescence* combinations were found compatible with each other and used for further experimentation. Absence of inhibition zone around the *Pseudomonas* indicated typically that these bacterial biocontrol agents were compatible with *T. harzianum*.

Glass house experiment

Efficacy of consortia differed from individual isolates with respect to its effect on Vigor Index (Table 1) and seed germination of chickpea, consortium T_{10} (Th 19+ Th 14) exhibited maximum seed germination (98.33%) followed by (TCMS 36+ Psf 173) and (Th 17+ Psf 2) showed (96.67%). Developed consortium was superior over individual formulation. Individual isolates also resulted in significant reduction in mortality of seedlings over control (Fig. 1).

When *Trichoderma* used as seed treatment or soil application, it is reported to be more effective in acidic soil while *Pseudomonas* performs better in neutral and alkaline

Table 1. Efficacy of consortium under glass house condition

Treatments	Seed germination (%)	Plant height (cm) 60th DAS	Vigor Index
T_1 (TCMS 36+ Psf 173)	96.67	45.47	4395.58
T_2 (Th 19+ Psf 173)	91.67	45.50	4170.99
T_3 (Th 14+ Psf 173)	93.33	47.80	4461.17
T_4 (Th 17 + Psf 173)	88.33	44.30	3913.02
T_5 (Th 17+ Psf 2)	96.67	45.03	4353.05
T_6 (Th 17+ TCMS 36)	96.67	46.93	4536.72
T_7 (Th 19+ TCMS 36)	91.67	44.07	4039.90
T_8 (Th 14+ TCMS 36)	95.00	45.47	4319.65
T_9 (Th 17+ Th 19)	93.33	48.20	4498.51
T_{10} (Th 19+ Th 14)	98.33	42.23	4152.48
T_{11} (Th 14+ Th 17)	83.33	45.63	3802.35
T_{12} (TCMS 36) (Control)	91.67	41.27	3783.22
T_{13} (Th 19) (Control)	90.00	46.47	4182.30
T_{14} (Th 17) (Control)	88.33	42.43	3747.84
T_{15} (Th 14) (Control)	93.33	41.80	3901.19
T_{16} (Psf 2) (Control)	86.67	36.67	3178.19
T_{17} (Psf 173) (Control)	75.00	39.77	2982.75
T_{18} (Th 14+Psf 173) (Std. check)	93.67	41.17	3774.05
T_{19} (Control no treatment)	70.33	40.37	2839.22
CD (0.05)	5.39	9.156	

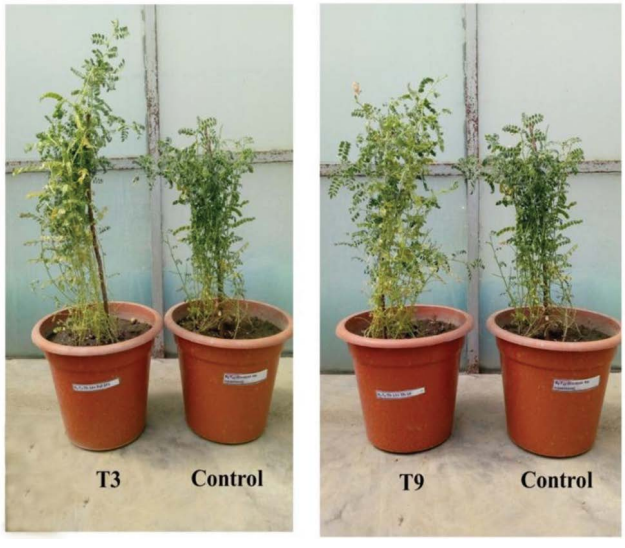

Fig. 1. Efficacy of consortia under glasshouse condition

soils. To test and develop microbial consortium there no proper guidelines has been described so an attempt was made to develop testing methods to develop consortia of these two fungal and bacterial antagonists, which could be equally effective in all types of soil. The results of present investigation indicated that most of the combinations were found to be compatible with one another. These results are similar to those of Manjula *et al*. (2004), who observed that fluorescent pseudomonads did not reduce the biocontrol ability of *T. harzianum* under *in-vitro* conditions.

Dandurand and Knudsen (1993) studied compatibility of *T. harzianum* and *P. fluorescens* 2-79 RN10 and they found that mycelial growth of *T. harzianum* was stimulated in presence of *P. fluorescens* 2-79 RN10, but this enhancement was not observed our study. Rini and Sulochana (2007) isolated 26 *Trichoderma* isolates and eight *P. fluorescens* and they tested for their compatibility and found that *T. viride/T. harzianum* and *P. fluorescens* were reported to be compatible and also they improved plant growth, as well as suppressed seedling disease of chilli and tomato.

Results on the biocontrol efficacy under glass house study demonstrated that all the individual as well as consortia induced significant disease against *Fusarium* wilt. The results are found similar with the studies conducted by earlier workers, who reported that increased biocontrol activity might be achieved by combining different isolates of biocontrol agents (Raupach and Kloepper, 1998; Manjula *et al*., 2004). The usefulness of combining *Trichoderma* spp.

with *Pseudomonas* was initially questioned by Hubbard *et al*. (1983). They reported that native populations of *P. fluorescence* reduced the biocontrol activity of *T. hamatum* when applied to control *Pythium* seed rot of pea. Such inhibitory action is due to iron competition between one another. In contrast, Dandurand and Knudsen (1993) found that the combination of *P. fluorescens* 2-79 and *T. harzianum* ThzID1 were no effect on each other in both enhancement as well as inhibition. Jhumishree *et al*. (2018) observed that significant enhancement in shoot, root length and vigour index of plants was observed on seed treatment with *Trichoderma* isolates and *P. fluorescens* as compared untreated plant. They found maximum plant vigor index was recorded on treatment with Tr-7, *i.e*., 3383.3 with 26.5cm shoot length and 7.3cm root length followed by 3296.7, 3066.7 and 2791.2 with Tr- 6, Tr-2 and Tr-1, respectively, in variety JG14 as compared to 1589 and 2149.3 in pathogen treated control and untreated control, respectively

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