Sri Lanka Journal of Food and Agriculture (2016)
Kodithuwakku *et al*. 2(1): 69-72
ISSN 2424-6913

Short Communication

Efficacy of single and combined application of *Trichoderma* spp. and *Pseudomonas fluorescens* along with bio-fertilizer (Arbuscular Mycorrhizae - AM) on growth of nursery plants of black pepper (*Piper nigrum* L.)

R.D. Kodithuwakku*, W.M.R.W.B. Wijekoon, I.S. Kumari and D.P.P. De Silva

Abstract: An experiment was conducted to evaluate different combinations of bio-fertilizer and biocontrol agents on the vegetative growth of nursery plants of black pepper (*Piper nigrum* L.). Treated plants showed a significantly higher ($p<0.05$) leaf area, shoot height, root volume, shoot dry weight and root dry weight compared to the untreated control. The highest leaf area of 244.47 cm^2 was recorded in black pepper plants treated with *Trichoderma* sp. and *Pseudomonas fluorescens* while the lowest (118.82 cm^2) was recorded in the untreated control. The highest root volume was recorded in plants treated with *Trichoderma* spp. The highest shoot height, shoot dry weight and root dry weight were found in plants treated with *P. fluorescens*. Results showed that the combination of above three organisms has not shown a significant impact on growth ($p>0.05$) than that of the respective individuals. As certain biological control agents may mechanistically be incompatible, one strain may interfere with the mechanism of the other. Hence, only the mixtures composed of mechanistically compatible strains can give better results in biocontrol and bio-fertilizer activities. The results of the experiment confirmed that black pepper plants treated with different biocontrol agents and bio-fertilizer showed a higher vegetative growth in the plant nursery.

Keywords: Antagonism, growth promoters

Introduction

Among different strategies used for plant growth promotion, biological approaches are useful and an ominous choice. Fungi in the genus *Trichoderma* have been known since early 1920s for their ability to act as biocontrol agents against plant pathogens. These fungi colonize the root epidermis and outer cortical layers and release bioactive molecules that cause walling off of the *T.* thallus. At the same time, the transcriptome and the proteome of plants are substantially altered (Harman, 2006). As a consequence, in addition to induction of pathways for resistance in plants, increased plant growth and nutrient uptake would occur. However, at least in maize, the increased growth response is genotype specific, and some maize in-breds have responded negatively to some strains (Harman, 2006).

Research Station, Department of Export Agriculture, Matale, Sri Lanka
***** Corresponding Author: ruchdk@yahoo.com

Pseudomonas fluorescens is a potential candidate to be used as a plant growth promoting agent (Yogesh *et al.,* 2008). Besides being highly effective against sheath blight, *P. fluorescens* can also be used successfully as a seed or soil treatment for augmenting the growth of rice seedlings as an added benefit (Singh and Sinha, 2008). Hence, the aim of this study was to identify the efficacy of combined and single application of plant growth promoting agents such as *Trichoderma* sp. and *P. fluorescens* along with bio-fertilizer (Arbuscular Mycorrhizae-AM) on the nursery pants of black pepper (*Piper nigrum* L.).

Materials and Methods

Nursery plants of black pepper (*Piper nigrum* L.) variety GK 49, was used for the experiment. Soil was inoculated separately with mass-cultured *Trichoderma* spp., *Pseudomonas fluorescens* (substrate - rice seeds) and Arbuscular mycorrhizae (AM; *Glomus mosseae*), and incorporated at the rate of 10 g/kg, 20 g/kg and 75 g/kg of potting mixture, respectively, to form 10 treatments *i.e.* T1 - potting mixture + *Trichoderma* spp, T2 - potting mixture + AM, T3 - potting mixture + *P. fluorescens*, T4 - potting mixture + *Trichoderma* spp. + AM, T5 - potting mixture + *Trichoderma* sp. + *P. fluorescens*, T6 - *P. fluorescens* + AM, T7 - potting mixture + *Trichoderma* sp. + *P. fluorescens* + AM, T8 - potting mixture with no treatment, T9 - potting mixture + un-inoculated tea refuse, and T10 - potting mixture + un-inoculated rice. Three replicates were maintained in a Complete Randomize Design (CRD) with 15 plants per replicate. At the end of nursery period, leaf area, root volume, shoot and root dry weight and shoot height were recorded. Data were analyzed using ANOVA followed by a mean separation with Duncan's Multiple Range Test at $p=0.05$.

Results and Discussion

Treated plants showed significantly higher ($p<0.05$) vegetative growth when compared to the un-treated controls (Table 1). Application of bio control agent is enhanced plant growth of black pepper significantly at the nursery stage. The highest leaf area of 244.47cm^2 was recorded black pepper plants treated with *Trichoderma* sp. and *P. fluorescens* while the lowest (118.82 cm^2) was recorded in the un-treated control. The highest root volume was recorded in plants treated with *Trichoderma* spp. The highest shoot height, shoot dry weight and root dry weight were found those treated with *P. fluorescens*.

A number of mechanisms have been proposed for the plant growth promotion by *Trichoderma* spp. The importance of auxins for plant development has been long recognized, and redundancy for IAA biosynthesis is widespread in plants and among plant-associated microorganisms. Accumulation of auxins or increased responses to auxins may lead to diverse outcomes in plant, varying from pathogenesis to growth promotion (Harman *et al*., 2004).

Table 1. Mean growth data of five months old black pepper nursery plants when treated with bio control agents and bio fertilizer

Treatment	Leaf area (cm^2)	Shoot height (cm)	Root volume (ml)	Shoot dry weight (g)	Root dry weight (g)
Trichodrma sp. (T1)	219.74^{abc}	26.85^{ab}	1.89^{a}	2.09^{ab}	0.25^{a}
AM (*Glomus mosseae*) (T2)	195.46^{abcd}	25.47^{ab}	1.74^{ab}	1.73^{ab}	0.22^{a}
Pseudomonas fluorescens (T3)	239.23^{ab}	32.00^{a}	1.43^{abc}	2.27^{a}	0.22^{a}
Trichodrma sp.+AM (T4)	161.31^{cde}	23.93^{ab}	1.29^{bc}	1.78^{ab}	0.18^{ab}
Trichoderma sp. + *P. fluorescens* (T5)	244.47^{a}	28.2^{ab}	1.77^{ab}	2.01^{ab}	0.23^{a}
AM + *P. fluorescens* (T6)	236.97^{abc}	26.63^{ab}	1.61^{abc}	2.05^{ab}	0.19^{ab}
Trichodrma sp.+AM + *P. fluorescens* (T7)	166.57^{bcde}	22.70^{b}	1.10^{c}	1.42^{b}	0.15^{ab}
Control - no substrate applied (T8)	141.19^{de}	11.66^{c}	0.18^{d}	0.44^{c}	0.12^{b}
Control - pure tea refuse (T9)	118.82^{e}	10.22^{c}	0.02^{d}	0.21^{c}	0.10^{b}
Control - pure sorghum seeds (T10)	121.45^{de}	9.44^{c}	0.16^{d}	0.14^{c}	0.09^{b}

Within a column, means followed by the same letter are not significantly different by the DMRT at p=0.05

Plant growth is affected by a plethora of environmental factors, including light, temperature, nutrients, and microorganisms. The region around the root, the rhizosphere, is relatively rich in nutrients, because as much as 40% of plant photosynthesis products can be lost from the roots. Consequently, the rhizosphere supports large microbial populations capable of exerting beneficial, neutral, or detrimental effects on plant growth. In maize (*Zea mays* L.) plants, *Trichoderma* inoculation has affected the root system architecture, which was related to increased yield of plants by enhancing root biomass production and increased root hair development (Bjorkman *et al*., 1998; Harman *et al*., 2004).

The impact of application of biocontrol agents and bio-fertilizer in different combinations need to be studied further in detail. Certain biological control agents may mechanistically be incompatible, where one strain may interfere with the mechanism of the other. Thus, the mixtures composed of mechanistically compatible strains could give better results in biocontrol and bio-fertilizer activities. Results of the present study showed that the combination of above three organisms has not given significantly higher growth than that of the individual treatments. Vazquez *et al.* (2000) reported that soil micro-organisms influence AM fungal development and symbiosis establishment with no clear pattern of response. Bin *et al.* (1991) have also found that under certain restrictive conditions, high population levels of antagonistic bacteria in bulk soil suppressed a fungal biocontrol agent, but this

suppressive effect was reduced or eliminated when a high bacterial population was not present. Stockwell *et al.* (2011) reported that the efficacy of combinations of *P. fluorescens* A506, a commercial biological control agent for fire blight of pear, and *Pantoae vagans* strain C9-1 or *P. agglomerans* strain Eh252 rarely exceeds that of the individual strains.

Conclusion

The results of the experiment confirmed that *Trichoderma* spp., *P. fluorescens* and AM enhanced plant growth and showed the potential to serve as growth promoters of black pepper plants at the nursery. Application and efficacy of biocontrol agents and bio-fertilizer in different combinations need to be studied in detail to identify any antagonists that are commercially made available.

References

Bin. L., Kudsen, G.R. and Eschen, D.J. (1991): Influence of an Antagonistic strain *Pseudomonas fluorescens* on growth and ability of *Trichoderma harzianum* to colonize Sclerotia of *Sclerotinia sclerotiorum* in soil. *Phytopathol.*, 81(9): 994-1000.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. and Lorito, M. (2004): Trichoderma species opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol*., 2(1): 43-56.

Harman, G.E. (2006): Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathol.,* 96 (2): 190–194.

Bjorkman, T., Blanchard, LM., Harman, G.E. (1998): Growth enhancement of shrunken-2 sweet corn when colonized with *Trichoderma harzianum* 1295-22: Effect of environmental stress. *J. Amer. Soc. Hort. Sci.,* 123: 35–40.

Singh, R. and Sinha, A.P. (2008): Growth promoting activity of *Pseudomonas fluorescens* of rice seedlings. *Indian Phytophol.,* 61(2): 264-267.

Stockwell, V.O., Johnson, K.B., Sugar, D. and Loper, J.E. (2011): Mechanistically compatible mixtures of bacterial antagonistics improve biological control of fire blight of pear. *The Amer. Phytopathol. Soc.*, 101(1): 113-123.

Vazquez, M.M., Cesar, S., Azcon, R. and Barea, J.M. (2000): Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillium, Pseudomonas, Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. *Appl. Soil Ecol.*, 15(3): 261-272.

Yogesh, K.N., Garg, S.K. and Kumar, J. (2008): Plant growth promoting and biological activities of cold-tolerent *Pseudomonas fluorescens* isolates against root rot in pea. *Indian Phytopathol.,* 61(4): 461-470.