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UTILIZATION OF MICROBIAL ANTAGONISTS WITH *THUJA ORIENTALIS* L. FOR THE CONTROL OF PATHOGNIC FUNGI

HIRA SARFARAZ[1], MARIUM TARIQ[2] AND SHAHNAZ DAWAR[1*]

[1]*Department of Botany, University of Karachi, Pakistan*
[2]*M.A.H.Qadri Biological Research Centre, University of Karachi, Pakistan*
[*]*Corresponding author's email: shahnaz_dawar@yahoo.com*

ABSTRACT

Pulses, vegetables and fruit crops are grown worldwide, are great source of nutrition and fiber which are necessary in human diet. Growers face various challenges of managing fungal pathogens and use different bacteria, fungi and yeast to resolve matter of disease. Applying antagonistic organisms gave much successful results in controlling various plant diseases under green house conditions. *Thuja orientalis* L. was collected from Department of Botany, University of Karachi, and used as soil amendment (1% w/w) in combination with bacterial antagonists such as *Bacillus subtilis, Rhizobium meliloti, Trichoderma harzianum* and *Paecilomyces variotii*. Results showed that *T. harzianum* and *P. variotii* in addition with *T. orientalis* @ 1% (w/w) plant powder gave significant results in growth parameters and in the control of root infecting fungi in mash bean and cowpea.

KEYWORDS: Fungal and bacterial antagonists, Crop plants, Growth improvement, Soil amendment.

INTRODUCTION

Management of diseases caused by soil borne root rot fungi is mainly dependent on fungicidal applications. However, these fungicides are not only produce hazards to the human health but also to the environment. Recently, microbial antagonists has a great influence in the control of many plant pathogens instead of chemical fungicides (Callan *et al*., 1990; Conway *et al*., 2001). Addition of fungal and bacterial antagonists in soil for controlling population of soil borne root infecting pathogens and plant parasitic nematodes has been reported by many researchers (Park, 1989; Saleem *et al.*, 2000). Antagonists like *Trichoderma* spp., *Paecilomyces lilacinus, Verticillium chlamydosporium, Bacillus* spp., *Stachybotrys atra, Pseudomonas aeruginosa, Gliocladium virens,* not only found to reduce the colonization of root rot pathogens and also enhance the growth of plant (Rodriguez- Kabana *et al*., 1984; Lumsden and Locke, 1989; Izhar *et al*., 1995; Lewis *et al.*, 1996; Bajwa *et al.*, 2003; Abdel-Monaim, 2014). According to Haram *et al.* (1996), microbial antagonists has an ability to control diseases caused by microorganisms due to the secretion of chitinolytic enzymes and production of inhibitory and toxic compounds for the control of soil borne pathogens (Bari, 2001). Production of chitinase enzymes produced breakage of β-glucan, chitin and polysaccharides of fungal cells, ultimately destroying pathogen (Howell, 2003). Apart from many fungi, there are many beneficial bacteria too, containing antagonistic properties which are helpful in reduction of soil borne pathogenic fungi (Nancy *et al.,* 1997). Now a days there are political pressure to remove hazardous chemicals from market which cause disturbance in natural ecosystems. Applying antagonistic organisms gave much successful results in controlling various plant diseases under green house conditions (Sivan, 1987; Rafi *et al.*, 2016; Rafi and Dawar, 2015).

Thuja orientalis L. belongs to the family cupressaceae, containing essential oil like carnphor, fenchone, isothujone, thujone, mono and sesquiterpenes. *T. orientalis* is used as antipyretic, antitussive, astringent, diuretic, refrigerant, gastrointestinal, asthma, skin infections, mumps, bacterial dysentery, arthritic pains, coughs, tumors, premature blandness and stomachic problems (Sokovic *et al.*, 2010; Yeung, 1985; Asili *et al*., 2007; Shimada, 1956; Biswas, *et al.*, 2011; Brown, 1995). Many soil borne diseases cause hazardous effect on crop. Most common root infecting fungi like *Fusarium* species, *Macrophomina phaseolinsa, Rhizoctonia solani* causes various complex root rot and wilt diseases on many leguminous and non-leguminous crops, resulting in the death of plants (Nancy *et al.*, 1997; Armstrong *et al*., 1976). Due to antifungal and antibacterial compounds present in *T. orientalis* plant, present study was carried out to evaluate its efficacy in combination with antagonistic microbes for the improvement of crop production and control of root infecting fungi.

MATERIALS AND METHODS

Thuja orientalis L. plant was obtained from Botany Department, University of Karachi in the sterilized polythene bag. Plants were ground into powder after drying in shade for 2 weeks. Ten treatments with 3 replicates each was placed on screen house bench of Botany Department in a randomized manner. Soil was analyzed for pH (7-7.6), moisture holding capacity (23.12%), total nitrogen (1.5%), *M. phaseolina* (4-6 sclerotia/g), *R. solani* (6-9%) and *Fusarium* spp., (3700 cfu g^{-1}) (Keen and Raczkowski, 1922; Mackenzie and Wallace, 1954; Sheikh and Ghaffar, 1975; Wilhelm ,1955; Nash and Snyder, 1962). Soil was amended with 1 % *T. orientalis* powder and seeds of mash bean and cowpea were treated with *R. meliloti* (158 x 10^7cells/mL), *Bacillus* sp. (65 x 10^7 cells/mL), *P. variotii* (19 x 10^3 conidia/mL) and *T. harzianum* (186 x 10^4 conidia/mL) cultures for 10-20 minutes and then sown in 300g pot. Un-treated seeds were also kept under screen house for comparison. Pots were regularly watered for upto thirty days which after uprooted for the observation of growth and root infecting fungi following the procedure of Kanwal *et al.* (2017). Data obtained were analyzed using COSTAT and means were compared by least significance difference at 5% probability level (Gomez and Gomez, 1984).

RESULTS

Use of microbial antagonists in combination with soil amended with *T. orientalis* at 1% (w/w) gave maximum enhancement in shoot weight, root length, root weight, number of nodules, leaves and reduction in root rot fungi when seeds of cowpea were treated with microbial antagonists such as *Bacillus subtilis, Rhizobium meliloti, Trichoderma harzianum* and *Paecilomyces variotii*. Shoot length, shoot weight and number of leaves were increased significantly ($p<0.01$) when soil was amended with 1 % (w/w) *T. orientalis* plant powder in combination with treated seeds with *P. variotii.* However, enhancement in root weight and length was recorded when seeds were treated with *T. harzianum* in addition with soil amendment by *T. orientalis* at 1% (w/w). Seeds treated with *R. meliloti* in combination with soil amended with 1 % *T. orientalis* powder showed maximum improvement in number of nodules in cowpea. Significant reduction of *Fusarium* spp. ($p<0.01$) was observed when seeds treated with *P. variotii* and soil amended with 1% *T. orientalis*. Similarly, *M. phaseolina* was significantly ($p<0.05$) suppressed when seeds were treated with *B. subtilis* in addition with soil amendment with *T. orientalis* at 1% (w/w) while *R. solani* was significantly ($p<0.001$) inhibited when seeds treated with *R. meliloti* in combination with soil amendment with *T. orientalis* at 1% (w/w) as compared to their alone effect (Fig. 1).

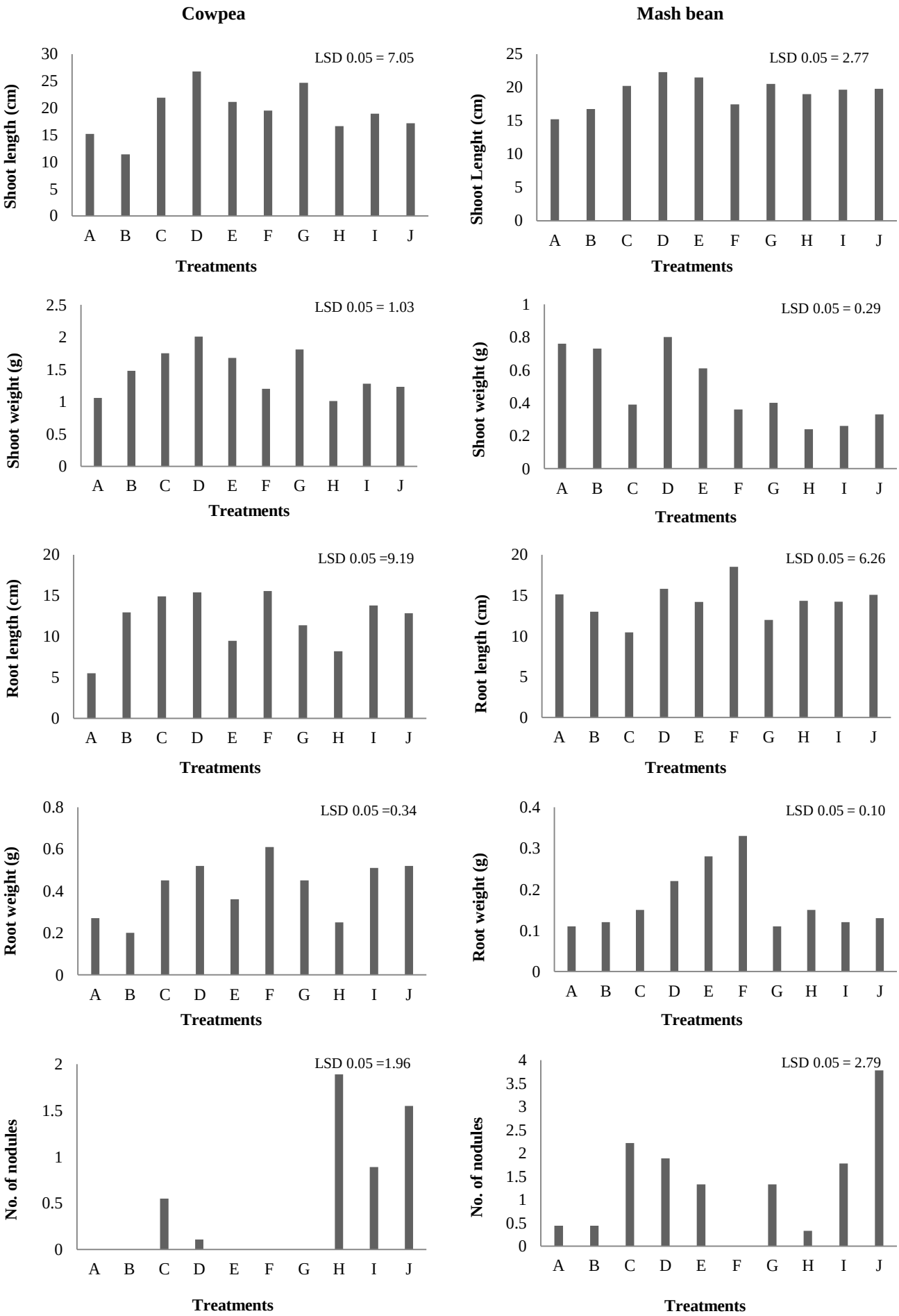
In case of mash bean, combined effect of microbial antagonists with *T. orientalis* gave improved growth and excellent control of infection caused by root rot fungi such as *Fusarium* spp., *R. solani* and *M. phaseolina* as compared to use alone. Shoot length and weight were significantly ($p<0.001$; $p<0.01$) enhanced when seeds were treated with *P. variotii* and soil was amended with *T. orientalis* at 1% (w/w). However, maximum root length and weight were observed when seeds were treated with *T. harzianum* and soil was amended with 1% (w/w) *T. orientalis* plant powder ($p<0.005$; $p<0.01$). Number of nodules were increased when *B. subtilis* used in combination with soil amendment with 1% *T. orientalis* while number of leaves were significantly ($p<0.001$) increased when seeds treated with *P. variotii* and soil was amended with *T. orientalis* at 1% (w/w). Microbial antagonists significantly reduced the infection of root rot fungi like *Fusarium* spp., ($p<0.05$), *R. solani* ($p<0.001$) and *M. phaseolina* ($p<0.05$) when seeds were treated with *P. variotii* and soil was amended with *T. orientalis* at 1% (w/w) (Fig. 1).

Combined effect of microbial antagonists *P. variotii* and *T. harzianum* and soil amended with *T. orientalis* at 1% (w/w) was found best in the control of root rot fungi and promotion of growth of mash bean and cowpea plants.

DISCUSSION

Various medicinal plants have been used for years in daily life to treat plant diseases all over the world due to enormous diversity in the phytochemicals. Biocontrol agents has been found effective and is becoming important day by day as one of the cheapest control measures. Soil amendment with 1 % *T. orientalis* in combination with microbial antagonists not only improved growth of cowpea and mash bean but also showed remarkable inhibition against root rot fungi like *Fusarium* spp., *M. phaseolina* and *R. solani*. In cowpea, significant increase in growth parameters like root length, shoot length and shoot weight was observed when seeds were treated with *B. subtilis, T. harzianum, P. variotii* and *R. meliloti* in combination with *T. orientalis* at 1% (w/w). Similar results were found by Rafi and Dawar (2014) when leguminous and non-leguminous seeds were primed with local tree seeds at different time intervals in combination with antagonists like *P. variotii, T. harzianum, R. meliloti* and *B. subtilis* showed improved growth and reduced the incidence of root rot fungi like *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp. Many researchers used *T. harzianum* for reducing disease development of *Botrytis cinerea* on cucumber and tomato (Dik and Elad, 1999; O'Neil *et al*., 1996; Uthkede *et al.,* 2000). Harman (2000) found that strain of *Trichoderma* (T-22) improves plant growth and decrease the infection of root rot fungi which showed the ability of antagonistic fungi (Altomare *et al.,* 1999). Seeds treatment with different microbial antagonists like *T. harzianum, G. virens* and *T. viride* in combination with soil amendment showed remarkable results against *R. bataticola*, which produced disease on chickpea plants (Dubey *et al.,* 2011). *Bacillus subtilis* was helpful in controlling the disease severity of tomato seeds from *R. solani* and *F. oxysporum* (Hernández-Rodríguez *et al.,* 2008). A strain (T-22) of *T. harzianum* was reported to trigger host defense mechanisms in cucumber plants through induction of defense enzymes (Yedidia *et al.,* 1999). Bacterial antagonists used in this study were found more effective and showed maximum inhibition in the colonization of *M. phaseolina*. According to Laura *et al.* (1998), different kinds of antibiotics and toxins were produced by bacteria showed inhibition against the growth of pathogenic organisms. Present results showed maximum increase in mash bean growth parameters like shoot weight, root length and root weight when seeds treated with *P. variotii, T. harzianum* and *B. subtilis* in combination with *T. orientalis* at 1% (w/w) and impressively reduction in the incidence of root rot fungi *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* spp., were also observed when seeds were treated with *P. variotii, T. harzianum* in addition with soil amendment with *T. orientalis* at 1% (w/w). Severity of *Fusarium* wilt caused by *Fusarium oxysporum* was reduced when cotton seeds were treated with *Gliocladium virens* and *Bacillus subtilis* (Zhang *et al.,* 1996).

Using plant sources containing antifungal activity is a good effort in developing viable mode of agriculture in organic farming system. Hence combination method gave more pronounced effect on yield of crops by improving plant weight and yield.


Cowpea
Mash bean
Shoot length (cm)
Shoot Lenght (cm)
LSD 0.05 = 7.05
LSD 0.05 = 2.77
Shoot weight (g)
LSD 0.05 = 1.03
LSD 0.05 = 0.29
Root length (cm)
LSD 0.05 =9.19
LSD 0.05 = 6.26
Root weight (g)
LSD 0.05 =0.34
LSD 0.05 = 0.10
No. of nodules
LSD 0.05 =1.96
LSD 0.05 = 2.79
Treatments
A B C D E F G H I J

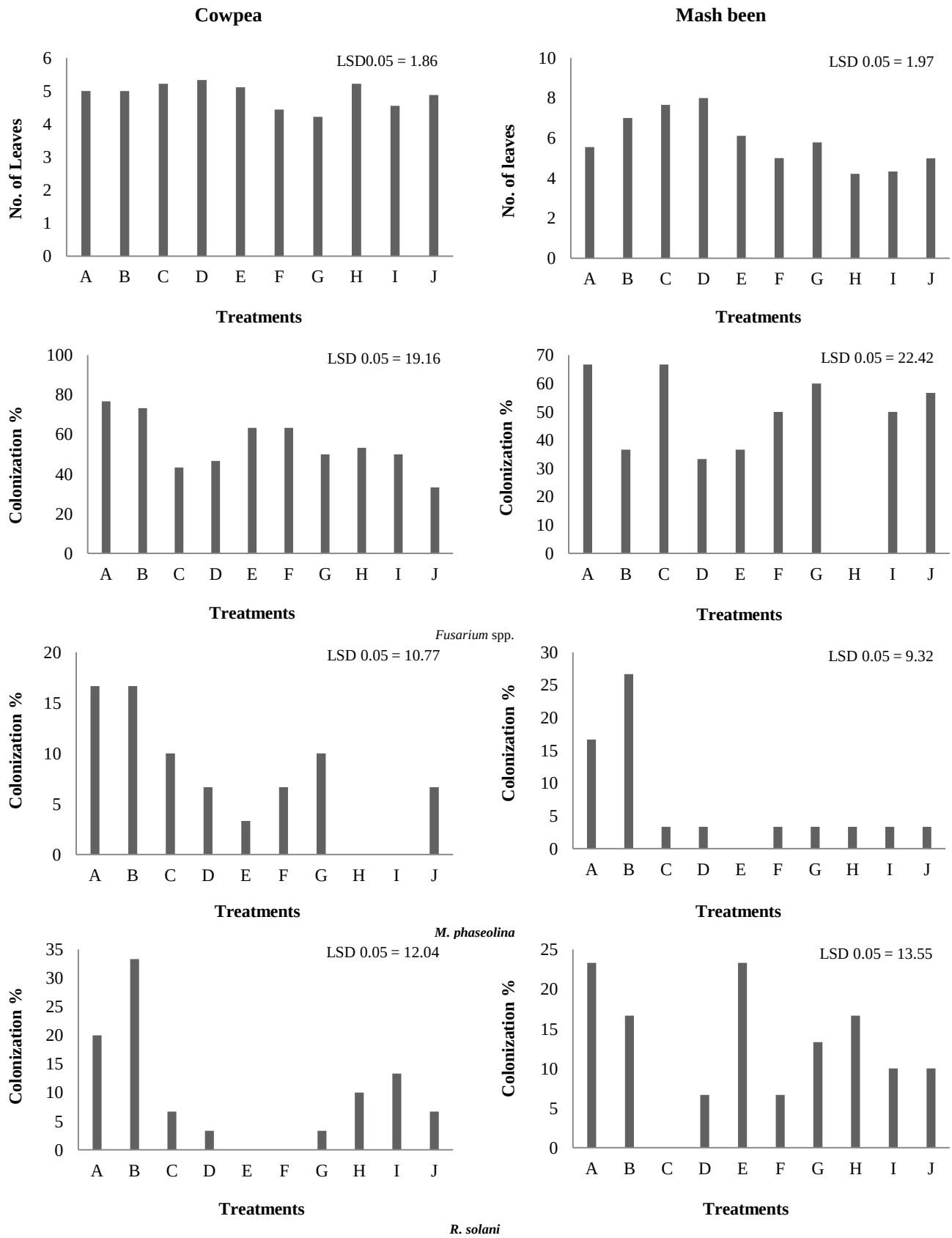


Fig. 1. Combined effect of microbial antagonists in combination with *T. orientalis* on growth parameters and in the control of root rot fungi on crop plants.

A = Control, B= Soil amendment with *Thuja orientalis* at 1% (w/w),C= Seed treated with *P. variotii,* D = Soil amendment with *Thuja orientals* at 1% (w/w) and seed treatment with *P. variotii*, E = Seed treated with *T. harzianum,* F = Soil amended with *Thuja orientalis* at 1% (w/w) and seed treated with *T. harzianum*, G= Seed treatment with *R. meliloti*, H = Soil amended with *Thuja orientalis* at 1% (w/w) and seed treated with *R. meliloti*, I = Soil amended with *Thuja oriental* at 1% (w/w) and Seed treated with *B. subtilis,* J= Soil amended with *Thuja orientalis* at 1% (w/w) and seed treated with *B. subtilis.*

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