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EFFECTS OF SEED COATING WITH *TRICHODERMA* SPECIES ON COLONIZATION OF *MACROPHOMINA PHASEOLINA* AND THE GROWTH OF SUNFLOWER UNDER FIELD CONDITIONS

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ABSTRACT

The main emphasis of this research was to evaluate ability of *Trichoderma* species in field conditions to improve sunflower growth and reduce the disease severity cause by *Macrophomina phaseolina* (Tassi) Goid. Conidia of four different *Trichoderma* species namely, *T. pseudokoningii*, *T. longibrachiatum*, *T. harzianum* and *T. viridi* were coated on the seeds of sunflower before sowing in soil; soil was naturally infested with *M. phaseolina* (6-9 sclerotia per g of soil). The data of plant growth and *M. phaseolina* infection was recorded at 30 and 60 days of sowing. It was recorded that *T. viridi* treatment on sunflower elicited better growth of plant in terms of germination, plant length, plant weight, vigor index, petiole of the smallest and the largest leaf, area of small and the large leaf and stem diameter after 60 days of treatment. It was followed by *T. longibrachiatum*. The colonization of *Macrophomina phaseolina* was greatly reduced after 30 days of seed treatment with *T. harzianum.*

Keywords: Sunflower; *Trichoderma* species; Seed treatment; Plant growth.

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid, is an anamorphic soil born fungus which infects more than 500 species of 7 families worldwide including legumes, fruits, vegetables and fiber crops (Kunwar *et al.,* 1986; Sinclair & Backman, 1986; Salik, 2007). *M. phaseolina* infection on sunflower was first time reported in 1972 from Sri Lanka then reported from Uruguay, Australia, Yugoslavia, Argentina and Senegal, Hungary, USA, India, France, Egypt and Pakistan (Bhutta, 1997). The Fungus has a wide host range including both cultivated and wild plant species. In Pakistan, 67 economically important plant species have been reported to be infected by this fungus, which include cotton, rice, maize, okra, cucurbits, wheat etc. (Mirza & Qureshi, 1978; Shehzad *et al.,* 1988). *M. phaseolina* generally affects the fibrovascular system of the roots, blocking the transport of nutrients and water to the upper parts of plant, resulting in progressive wilting, premature dying, loss of vigor and reduction in yield. The appearance of minute, black microsclerotia was recorded when the epidermis of plant tissue was removed (Kolte, 1985; Hoes, 1985). Diseases of sunflower have been estimated to result in 12 % annual loss in yield in nearly 12 million hectares of the world (Kolte, 1985).

Trichoderma are commonly found in soil, root ecosystem and foliar environments, are considered as potent biological agents in the control of plant diseases and improvement of growth of crop plants (Malik *et al.,* 2005; Anis *et al.,* 2010). Recent studies showed that *Trichoderma* species induces localized and systemic resistance in plants against attack of variety of plant pathogens or damage caused by insects or treatment with different chemical inducers (Kuc, 2001; Oostendorp *et al.,* 2001). Strains of *Trichoderma* are well known in their ability to colonize roots but conidia of *Trichoderma* when applied to fruit, flowers and foliage can control plant diseases (Harman, 2000; Dubos, 1987; Elad, 1994). Many researchers reported that root colonization of *Trichoderma* strains caused increased levels of defence-related plant enzymes including chitinases, peroxidases, β-1,3-glucanases and lipoxygenase (Howell *et al.,* 2000; Yedidia *et al.,* 1999). The present study was attempted to use of *Trichoderma* species as a seed coating for the control of charcoal rot fungus on sunflower.

MATERIALS AND METHODS

Seed treatment with *Trichoderma* species

Trichoderma species were obtained from Karachi University culture collection (KUCC). Sunflower seeds (*Helianthus annuus* cv. Hysun-39) were surface sterilized with 0.5 % $HgCl_2$ (2 minutes), rinsed several times with sterile distilled water and then dried on sterile filter paper under laminar flow hood. Seeds were treated with *T. pseudokoningii* (1.9×10^8 conidia/ml), *T. longibrachiatum* (1.8×10^8 conidia/ml), *T. harzianum* (2.0×10^8 conidia/ml) or *T. viridi* (2.0×10^8 conidia/ml) by using 2 % gum arabic for 10 minutes. Treated seeds were air dried in laminar air flow hood.

Preparation of field

Micro plots were prepared in the Department of Botany, University of Karachi (properties of soil described in Table 1). The experiment consisted of seeds treated with four *Trichoderma* species and seeds treated with sterile distilled water were served as control. In each micro plot (4x4 sq.ft) ten seed for each treatment were sown randomly at the distance of 4-6 inches and area of sowing was tagged. Experiment was planed according to complete randomized designed and there were three plots (replicates). From each plot, two plants for each treatment were randomly harvested at 30 days of germination while from the remaining; two plants were harvested at 60 days. Plant height, plant fresh weight, area of small and large leaf, stalk of large and small leaf, vigor index and stem diameter were recorded at each observation. Mean of two plants was used for statistical analysis for each parameter except germination of seeds.

Table 1. Characteristics of the field soil.

Organic matter (%)	1.4
Sand (%)	73
Silt (%)	18
Clay (%)	9
Textural class	Sandy loam
pH	8.0
Electrical conductivity (dS m^{-1})	0.50
Available N (??ug/g)	0.06-0.09
Number of *Macrophomina phaseolina* sclerotia (per g soil)	6-9

Determination of *M. phaseolina* Infection

Root system was rinsed with tap water to remove dirt particles and these root samples were cut into small fragments of 1 cm long, disinfected in 1 % sodium hypochlorite (2 minute) and transferred to potato dextrose agar plates (5 fragments/plate) containing antibiotics and incubated for 7 days at 27±1°C. Infection severity was determined by colonization percentage.

Statistical analysis

Experiment was conducted according to randomized complete block design. Data of the experiment was subjected to mono-factorial or bi-factorial analysis of variance (ANOVA) depending upon the experimental design. While treatment means were compared using by post hoc tests including least significant difference and Duncan's multiple range test at $P < 0.05$.

RESULTS

Effect on growth of sunflower

Seeds of sunflower treated with different *Trichoderma* species produced various effects on growth parameters when observed at different time intervals. Seeds treated with *T. viridi* showed maximum germination (98 %) followed by *T. harzianum* (97 %), *T. longibrachiatum* (93 %) and *T. pseudokoningii* (88 %) significantly higher than the control ($P<0.01$). Plant length and weight were significantly ($P<0.001$) enhanced due to seed treatment with *Trichoderma* species in comparison to the control. In both observations plants were larger when seeds were treated with *T. viridi* (Fig.1). *T. harzianum* after 30 and 60 days interval ranked second highest (243 cm) in their response towards increment of plant length. Significant ($P<0.001$) plant weight was observed after 30 days interval when sunflower seeds treated with *T. longibrachiatum.* However, *T. viridi* gave maximum weight (170.3 g) after 60 days interval followed by *T. pseudokoningii* (162 g).

Petiole small and large leaf showed progressive result when sunflower seeds were treated with *T. harzianum* after 30 and 60 days interval whereas seeds treated with *T. viridi* gave significant increment ($P<0.001$) in vigour index after 60 days interval (Figure 1). However, treatment of sunflower seeds with *T. pseudokoningii* revealed least effective result in increment of vigor index and stalk small leaf (57, 1.4 cm respectively). Compared to control, area of large leaf (369 cm) was increased due to treatment of seeds with *T. viridi* after 30 and 60 days interval followed by seed treatment with *T. harzianum* after 30 and 60 days interval where treatment, time separately and their interaction showed significant response ($P<0.001$).

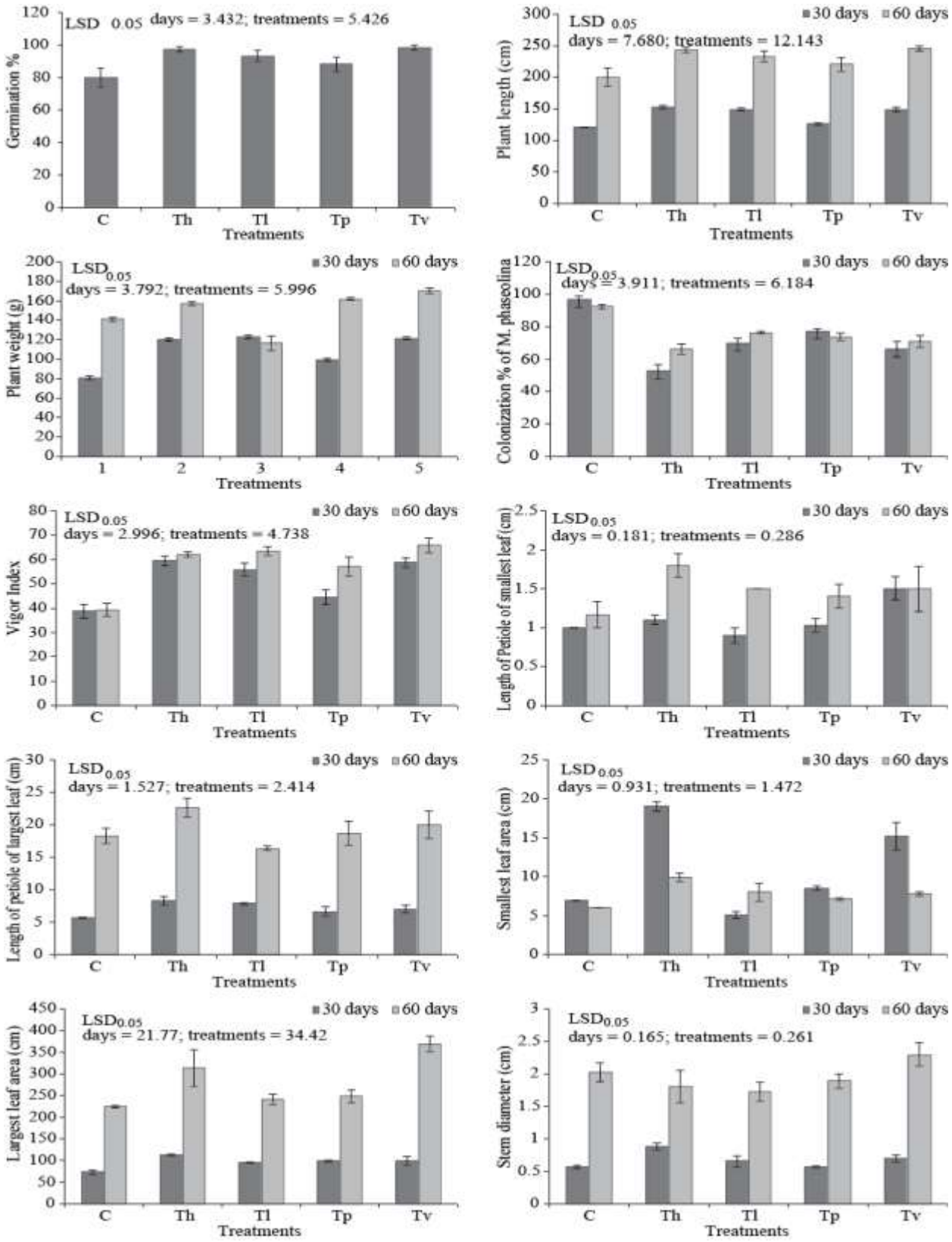

Fig. 1. Effect of seed treatment with *Trichoderma* species on germination %, plant length, plant weight, vigor index, stalk small leaf, stalk large leaf, smallest leaf area, largest leaf area and stem diameter.
C = control; Th = *T. harzianum,* Tl = *T. longibrachiatum*; Tp = *T. pseudokoningii;* Tv = *T. viridi.*

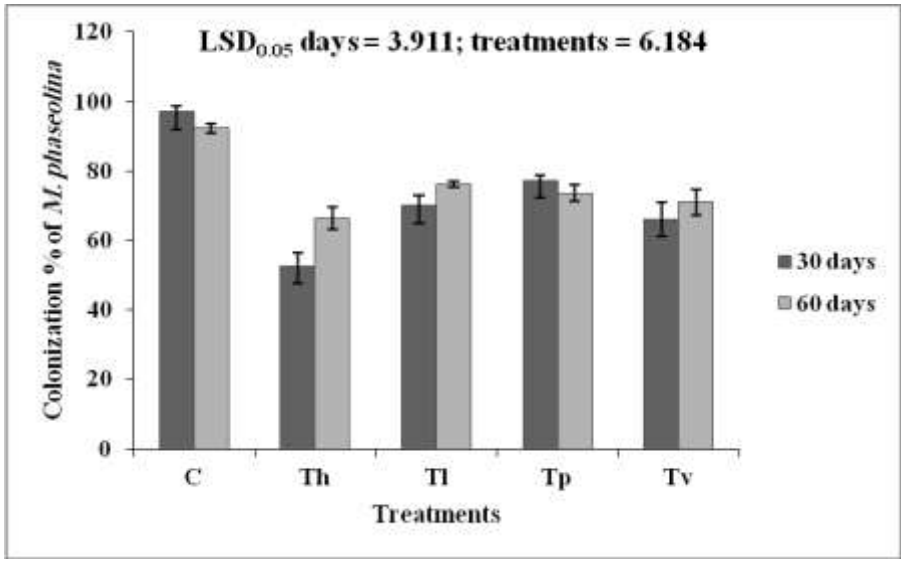

Fig. 2. Effect of seed treatment with *Trichoderma* species on colonization of roots by *M. phaseolina.* C = control; Th = *T. harzianum,* Tl = *T. longibrachiatum*; Tp = *T. pseudokoningii;* Tv = *T. viridi.*

Maximum stem diameter was recorded in plants treated with *T. harzianum* and *T. viridi* after 30 and 60 days interval. Least stem diameter was observed when *T. pseudokoningii* after 30 days and *T. longibrachiatum* after 60 days interval where interaction between time and treatment was non significant.

Effect on colonization of roots

All *Trichoderma* species significantly ($P<0.05$) reduced *M. phaseolina* colonization after 30 and 60 days interval, except for *T. pseudokoningii* in which maximum reduction was observed only after 60 days interval. However, greater reduction was observed at 30 days interval in case of seeds treated with *T. harzianum* (Fig. 2).

DISCUSSION

Trichoderma species are ubiquitous in soil. They successfully to combat phytopathogenic fungi (Fravel, 2005; Desai *et al.*, 2002) by producing antibiotic compounds, formation of specialized structures, and degradation of the cell wall of host called as mycoparasitism (Chet & Chernin, 2002; Steyaert *et al*., 2003; Benitez *et al*., 2004). The present experiment indicated towards the use of *T. harzianum* as it promoted growth of sunflower in large proportion *T. viridi* and *T. longibrachiatum.* Promotion in plant growth was frequently seen in field as well as in field crops (Harman, 2000). Many deleterious root microflora present in soil reduces plant growth by producing cyanide but *Trichoderma* spp., are resistant to cyanide and producing two different enzymes that are capable to degrading cyanide. This action of *Trichoderma* spp., leads to control root microflora, removing toxic metabolites produced by deleterious microflora and resulting in enhancement of root growth which improves the plant growth and provides resistance against stress (Ezzi & Lynch, 2002). Another importance of *Trichoderma* spp., is related towards increase uptake of variety of nutrients including copper, phosphorus, iron, cobalt, arsenic, cadmium, zinc, boron, aluminium, manganese and sodium from roots (Yedidia *et al.,* 2001). *Trichoderma* spp., also solubilize several plant nutrients like Fe^{3+}, Cu^{2+}, Mn^{4+}, Zn^{0}, which are unavailable to plants in certain soils (Altomare *et al.,* 1999). This mechanism results in enhancement in plant active uptake mechanisms. Harman (2000; 2001) reported that seed treatment with *Trichoderma* spp (T-22) gave maximum increment in plant growth as compared to plants without treated with *Trichoderma* spp.

Seed treatment applied as liquid or ground solids using adhesives known as stickers, which form covering around seed coat (Scott, 1989). This method affects the seed interface when some biocontrol agent stick to it which help in preventing the colonization of root infecting fungi and helps in germination and establishment of seed (Chang & Kommedahl, 1968). The present results elicited that colonization of *M. phaseolina* was greatly reduced due to seed treatment with *T. harzianum* after 30 days interval. During mycoparasitism, *Trichoderma* grows tropically towards fungi and produced cell-wall degrading enzymes and as a result, fungi produced low levels of an extracellular exochitinase. Diffusion of this enzyme catalyses the release of cell wall oligomers from the pathogenic

fungi which in turn induces the expression of fungitoxic endochitinases. As the pathogenic fungi come in contact, *T. harzianum* attach and coil around the pathogenic fungi producing appressoria. At this stage, *T. harzianum* releases several fungitoxic cell-wall degrading enzymes and peptaibol antibiotics which results in the dissolution of cell wall of pathogenic fungi. Now *T. harzianum* hyphae directly enter into the lumen of pathogenic fungi (Brunner *et al.*, 2003; Schirmbock *et al.*, 1994).

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