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EFFECT OF PREVIOUS SEASON APPLICATION OF *Sisymbrium irio* AND *Trichoderma harzianum* ON GROWTH OF BLACK GRAM IN *Macrophomina phaseolina* INOCULATED SOIL

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ABSTRACT

Macrophomina phaseolina is the cause of charcoal rot in green gram [Vigna radiata (L.) Wilczek] and black gram [Vigna mungo (L.) Hepper]. A pot experiment was carried out in March 2013 to study the effect of soil amendment with a biological control agent Trichoderma harzianum and dry biomass of a brassicaceous weed Sisymbrium irio on charcoal rot disease of green gram. The present experiment was a continuation of that study and carried out in the same pots in September 2013 to study the effect of the soil amendments on crop growth of black gram. In general, number of leaves, and various root and shoot growth parameters were significantly decreased due to M. phaseolina inoculation. Application of 1, 2 and 3% dry biomass of S. irio or T. harzianum separately failed to alleviate biotic stress of M. phaseolina. However, combined application of 1% dry biomass of S. irio and T. harzianum significantly increased number of leaves by 23% over positive control inoculated only with M. phaseolina. Various parameters of root and shoot growth were also higher in this treatment as compared to other soil amendment treatments.

Keywords: Biological control, *Macrophomina phaseolina,* mashbean, *Sisymbrium irio, Trichoderma harzianum, Vigna mungo.*

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INTRODUCTION

Charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid is an important disease that significantly reduces growth and yield of economical important crops especially in arid regions of the world (Khan and Shuaib, 2007; Chamorro *et al.,* 2015; Marroni, 2015). Two aspects have been documented to play major role in the fungal aggressiveness during the disease development. One is the fungus

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ability to adapt itself according to prevailing environmental condition in soil through changes in morphology, biochemistry and pathology. The other one is its aptitude to produce microsclerotia in saprophytic and pycnidia in pathogenic phase. In pathogenic phase, the fungus act as non-specific pathogen and can attack on a number of plants, therefore, causes massive yield losses (Beas-Fernández *et al.,* 2006). Up till now more than 500 plant species have been reported to be infected by this fungus (Rayatpanah and Dalili, 2012). In addition, the fungus exhibits potential to attack plants at almost all growth stages. However, death of young seedling occurs due to formation of dark irregular lesions on the epicotyls and hypocotyls that extends to the cotyledons. The adult plant dies due to blight and stem rot followed by blockage of xylem vessels (Beas-Fernández *et al.,* 2006). The presence of the pathogen in seed poses a serious risk to some overseas sprouting seed markets because disease may cause up to 100% yield losses (Iqbal *et al.*, 2010).

Different species of *Trichoderma* are well known as potential antagonists against plant pathogens and these fungi are found extensively in almost all agricultural soils as well as in other environments (Javaid *et al*., 2014). More than 50% of the *Trichoderma* species have been investigated for their potential to combat against fungal pathogens (Hjeljord and Tronsmo, 1998). It has been found that *Trichoderma* species induce systematic resistance in plant by activating plant defense system through a number of morphological and biochemical changes in it (Lelavthi *et al*., 2014). Among many species of *Trichoderma*, *T. harzianum* has always gained attraction of scientists because of diverse physiological traits and important chitinolytic system (Saba *et al*., 2012). Besides, *T. harzianum* is an active hyperparasite that has been successfully utilized in many bio formulations and extensively applied in field studies (Li *et al*., 2010; Ceia-Torres and Zuniga-Mendoza, 2014).

Members of family Brassicaceae are known for their antifungal properties (Riaz *et al.,* 2010; Sun *et al.,* 2011; Javaid and Iqbal, 2014). However, generally crop plants especially species of genus *Brassica* have been used in antifungal trials (Subbarao *et al.,* 1994; Troncoso *et al.,* 2005). In the present study, dry biomass of a brassicaceous weed *S. irio* and *T. harzianum* were used against *M. phaseolina* using mashbean as a test crop. This study was a continuation of a previous experiment where these treatments were used using mungbean as a test crop. This study was carried out in the pot soil of previous experiment to investigate the effect of treatments in next season.

MATERIALS AND METHODS

In March 2013, an experiment was conducted in plastic pots (15-cm diameter) each containing 1.0 kg sandy loam soil. Before pot filling, soil was sterilized by fumigating with formaline for one week. Inocula of *M. phaseolina* and *T. harzianum* were prepared on autoclaved pearl millet [*Pennisetum glaucum* (L.) R. Br.] seeds. Both the inocula were mixed in the soil at 15 g per pot. Control pots received the same quantity of boiled pearl millet seeds in each pot without any fungal inoculation. All the pots were irrigated and left for seven days for establishment of inocula. Thereafter, crushed dried leaves of *S. irio* were mixed in soil at 1%, 2% and 3% in the respective pots. Pots of positive control were prepared by inoculating them with *M. phaseolina* only whereas pots of negative control were without any fungal inoculum as well as dry powdered leaves of *S. irio*. In total there were nine treatments, i)- **-** Control, ii)- **+** Control [*M. phaseolina* (MP)], iii)- 1% *Sisymbrium* leaves (SL) + MP, iv)- 2% SL+ MP, v)- 3% SL+ MP, vi)- MP + *T. harzianum* (TH), vii)- 1% SL+ MP + TH, viii)- 2% SL+ MP + TH, ix)- 3% SL+ MP + TH. All the pots were irrigated and left for one week. Thereafter, green gram seeds were sown in these pots. This crop was harvested after 75 days of sowing.

After harvesting green grams in May 2013, pots were left fallow till September 2013. However, during this time, pots were regularly irrigated with tap water to maintain soil moisture at field capacity. The present study was started in September 2013 and black gram seeds were sown in the pots to investigate the effect of previous season soil amendments on crop growth. Black gram crop was harvested after 30 days of sowing. Data regarding number of leaves, plant height, and biomass of root and shoot were recorded.

All the data were analyzed by analysis of variance (ANOVA) followed by Duncan's Multiple Range Test using computer software COSTAT.

RESULTS AND DISCUSSION

Effect of soil amendments on shoot growth

Positive control treatment (inoculated with *M. phaseolina* only) significantly reduced number of leaves by 21% over negative control. Soil amendment with 1% dry biomass of *S. irio* enhanced number of leaves by 13% over positive control. However, further increase in leaf dry biomass amendment failed to enhance number of leaves over positive control. Inoculation of *T. harzianum* significantly reduced number of leaves by 41% and 25% over negative and positive control treatments, respectively. Combined application of *T. harzianum* and 1% dry biomass of *S. irio* significantly enhanced number of leaves by 23% over positive control. By contrast, combined application of *T.*

harzianum either with 2% or 3% dry biomass of *S. irio* significantly and adversely affected the number of leaves over positive control (Fig. 1A).

The highest length (37.68 cm), fresh weight (53.56 g) and dry weight (9.66 g) of shoot were recorded in negative control. *M. phaseolina* inoculation (positive control) significantly reduced length, fresh weight and dry weight of shoot by 10%, 11% and 15% as compared to negative control. Application of 1% dry biomass of *S. irio* as soil amendment failed to exhibit any significant effect on various shoot growth parameters. However, further increase in *S. irio* biomass as soil amendment adversely and significantly reduced all the studied shoot growth parameters. Likewise, *T. harzianum* alone inoculation significantly reduced all the shoot growth parameters over negative as well as positive control. In contrast, *T. harzianum* inoculation in combination with 1% dry biomass of *S. irio* significantly increased shoot length as well as markedly enhanced shoot biomass over positive control. Further increase in quantity of *S. irio* in combination with *T. harzianum* failed to induce any positive effect on shoot growth of mash bean (Fig. 1B-D).

Effect of soil amendments on root growth

The effect of *M. phaseolina* and *T. harzianum* inoculation, and soil amendment with dry biomass of *S. irio* exhibited an effect on various root growth parameters similar to that the effect on shoot growth. *M. phaseolina* inoculation had insignificant effect on root length but significantly reduced fresh and dry biomass of root as compared to negative control. Soil amendment with 1% dry biomass of *S. irio* had similar effect on these root growth parameters. Further increase in quantity of soil amendment gradually decreased various root growth parameters. Sole inoculation of *T. harzianum* did not alleviate biotic stress of *M. phaseolina* but further reduced various root growth parameters which were significantly lower than negative as well as positive control treatments. Combined application of *T. harzianum* and 1% dry biomass of *S. irio* markedly increased all the studied root growth parameters. Conversely, further increase in dry biomass of *S. irio* in combination with *T. harzianum* adversely affected root growth.

In the present study, *M. phaseolina* inoculation reduced shoot and root growth of black gram. Similar growth inhibition due to *M. phaseolina* has also been reported in other crops such as green grams, chickpea and strawberry (Javaid and Saddique, 2012; Manjunatha *et al.,* 2014; Chamorro *et al.,* 2015). Incorporation of 1% dry biomass of *S. irio* markedly enhanced number of leaves over positive control while have no pronounced effect on other root and shoot growth parameters under biotic stress of *M. phaseolina*. Further increase in biomass of *S.*

irio as soil amendment adversely affected plant growth possibly due to allelopathic effects (Hoagland *et al.,* 2008). Generally, *T. harzianum* alleviates biotic stress of pathogen and consequently enhances plant growth (Martínez-Medina *et al.,* 2011; Afzal, 2013). In contrast to many earlier studies, inoculation of *T. harzianum* significantly reduced plant growth over positive control. However, combined application of *T. harzianum* and 1% dry biomass of *S. irio* markedly enhanced crop growth.

CONCLUSION

The present study concludes that combined application of *T. harzianum* and 1% dry biomass of *S. irio* is the most suitable treatment for better crop growth of black gram under biotic stress of *M. phaseolina.*

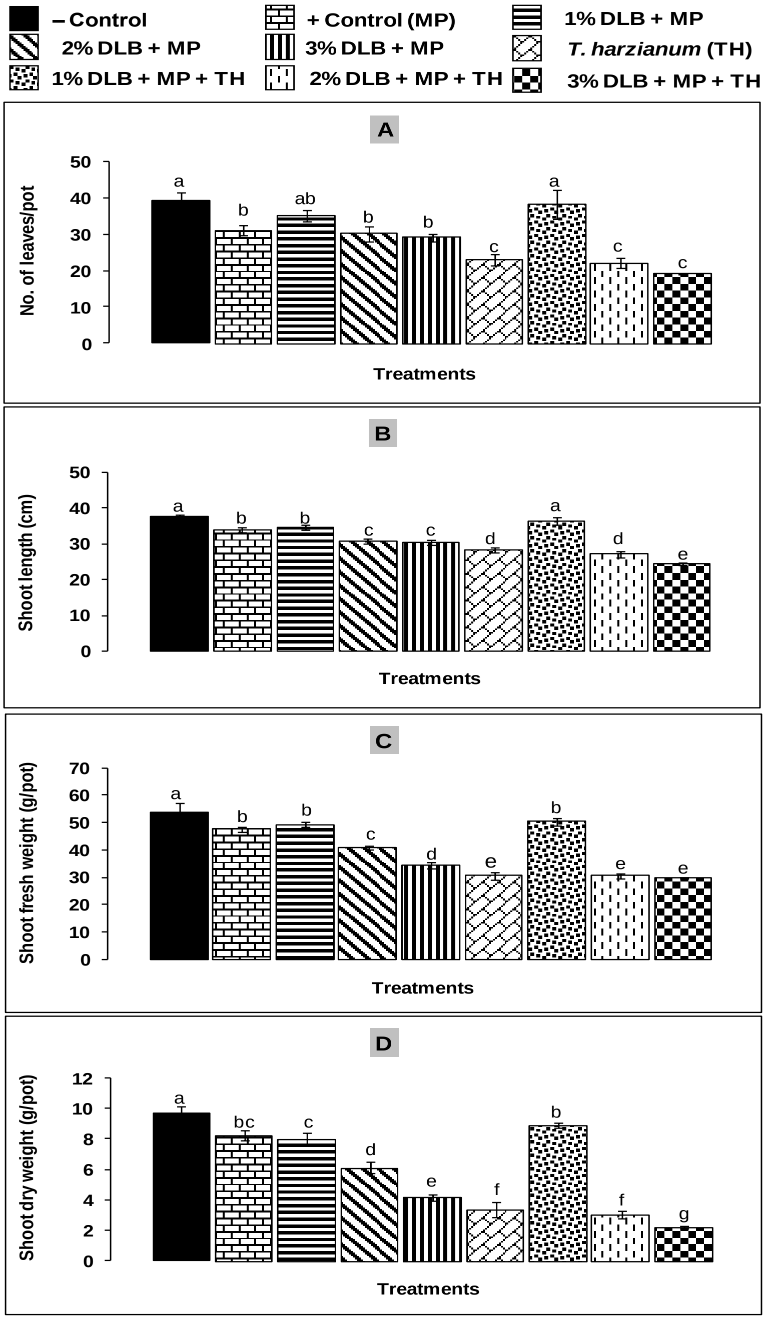

Fig on
shoot growth of black gram under biotic stress of *M. phaseolina.*

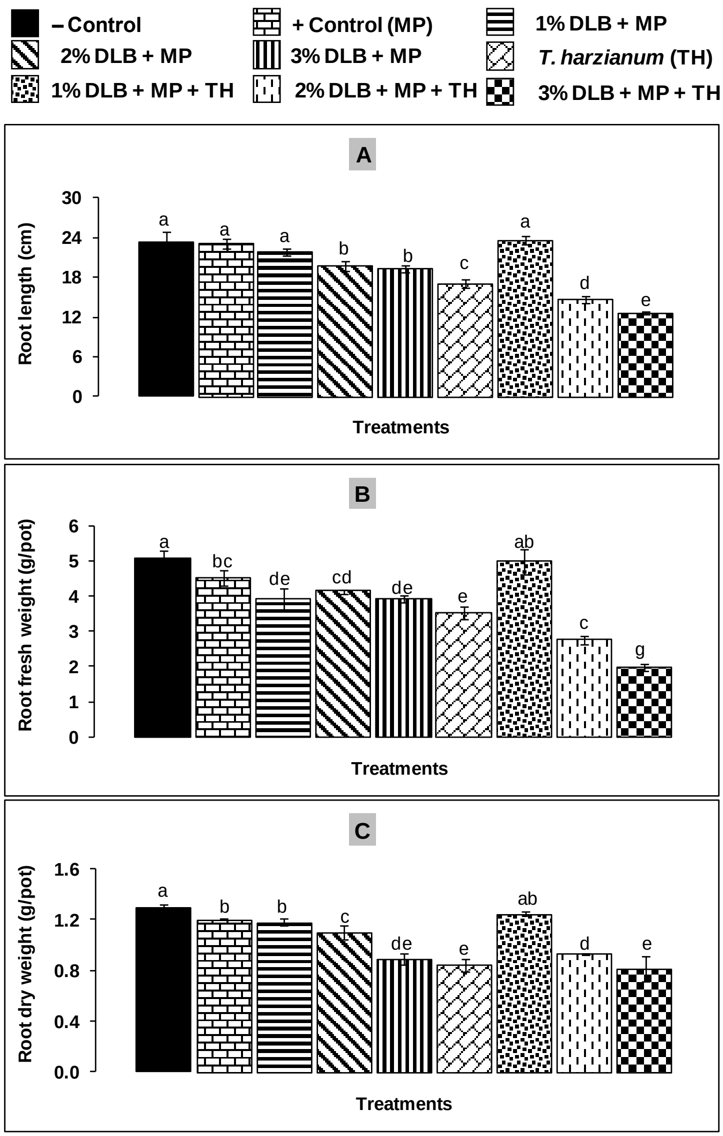

Figure 2. Effect of leaf dry biomass of *S. irio* and *T. harzianum* on root growth of black gram under biotic stress of *M. phaseolina.*

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