*INT. J. BIOL. BIOTECH.*, 15 (3): 535-540, 2018.

# EFFECT OF *TRICHODERMA HARZIANUM* AND DRY LEAVES OF *ACACIA NILOTICA* SUBSP. *INDICA* ON GROWTH OF MASH BEAN IN *MACROPHOMINA PHASEOLINA* CONTAMINATED SOIL

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## ABSTRACT

*Macrophomina phaseolina* (Tassi) Goid. causes charcoal rot in mash bean (*Vigna mungo* L.) that produces significant yield losses especially in arid regions of the world. This study was carried out to assess the effect of soil amendment with a biological control agent *Trichoderma harzianum* and dry leaf biomass of *Acacia nilotica* subsp. *indica* on growth of mash bean in *M. phaseolina* inoculated soil. In a pot experiment, 1, 2 and 3% dry leaf biomass of *A. nilotica* subsp. *indica* (LBA) was mixed in soil alone as well as in combination with *T. harzianum*. There was 15–45% and 38–74% increase in shoot and root dry biomass over positive control (only inoculated with *M. phaseolina*), respectively, due to different doses of LBA. Likewise, *T. harzianum* inoculation alone increased 50% and 100% shoot and root dry biomass, respectively, over positive control. The highest shoot and root biomass i.e. 163–172% and 120–167% higher than positive control, respectively, was recorded in treatments with combined application of LBA and *T. harzianum*. The present study concludes that better crop growth in mash bean in *M. phaseolina* contaminated soil can be obtained by applying 2% dry leaf biomass of *A. nilotica* subsp. *indica* in combination with *T. harzianum.*

**Keywords:** *Acacia nilotica,* biological control, charcoal rot, *Macrophomina phaseolina*, *Trichoderma harzianum*.

## INTRODUCTION

Mash bean (*Vigna mungo* L.) Hepper is one of the most commonly grown pulses in tropical and sub-tropical regions of world that was originated from India and central Asia (Pratap and Kumar, 2011). It is a staple food crop in India, Bangladesh, Burma and Thailand (Fery, 2002). Mash bean is highly nutritional, palatable and easily digestible food legume. Its seeds contain about 24% protein, 1.3% fats and 60% carbohydrates (Ali *et al*., 2002). It can also be used as animal fodder in the form of hay and straw (Maqsood *et al*., 2001). This crop itself is a mini-fertilizer factory as it has the ability to fix atmospheric nitrogen through rhizobia living in its roots by forming nodules. It results in increase in soil fertility hence minimizes the use of expensive nitrogenous fertilizers (Nilanthi *et al.*, 2014).

The average yield of mash bean in Pakistan is very low as compared to its yield obtained in other countries. Various biotic and abiotic factors restrict mash bean productivity in Pakistan. Among these, charcoal rot caused by *Macrophomina phaseolina,* has significant importance in reducing the mash bean yield particularly in arid regions (Iqbal *et al.*, 2010). The pathogen has a broad geographic distribution and wide host range, while high temperatures and drought conditions provoke the disease. When infected plants wilted, abundant minute black sclerotia cause the rotted tissue to become blackened, thus the disease is named as charcoal rot (Mayek-Perez *et al*., 2001, Sarr *et al*., 2014). Sclerotia are hard enough to survive in soil over 10-15 years, and germinate at wide temperature range (20-40 °C) and infect subsequent crops (Luna *et al*., 2018). Up till now, chemical fungicide failed to mitigate this pathogen effectively (Gaige *et al*., 2010). Moreover, threat poses due to occurrence of pathogen strains resistant to fungicide has further increased public concerns on negative drawbacks of fungicides on health and environment. Under such circumstances, management of intricate plant pathogens through antagonistic microorganisms and allelopathic plants is a potential nonchemical, cheapest and effective method that can be successfully exploited in the framework of integrated disease management (Shoaib *et al*., 2018).

Recently, several successful investigations have been undertaken to confirm antimitotic potential of *Trichoderma* spp. against *M. phaseolina* both *in vivo* and *in vitro* (Aly *et al*., 2007; Gajera *et al*., 2012; Khaledi and Taheri, 2016, Shoaib *et al.*, 2018). Accordingly, *Trichoderma* spp. mainly promote seedling establishment, increase plant growth and elicit plant defense reaction against *M. phaseolina* through several mechanisms *viz.* production of bioactive compounds, formation of specialized structures, mycoparasitism, antibiosis and competition (Nederhoff, 2001).

Being store house of biochemicals such as nitrogen-containing compounds and phenolics, plant extracts and biomass may serve as environment friendly ways of managing the plant diseases (Banarus *et al*., 2017). Earlier, Javaid and Saddique (2011) found considerable reduction in root rot in mungbean and improvement in plant growth by amending the soil with dry leaf manure of *Datura metel*. In another finding, significant reduction in growth of *M. phaseolina* with extracts of *Imperata cylindrical* was recorded (Javaid *et al*., 2015). Likewise, leaf and stem extracts of *Launea nudicaulis* (asteraceous weed) was found as a potential antifungal agent against *M. phaseolina* (Banaras and Javaid, 2015). Similarly, all parts of *Cirsium arvense* exhibited antifungal activity against *M. phaseolina* (Banarus *et al*., 2017).

Many leguminous trees and shrubs such as *Albizia lebbeck*, *Prosopis juliflora* and *Acacia nilotica*) contain such compounds as tannin, alkaloids, glycosides, fatty acids and flavonoid and posses antimicrobial activity (Kokila *et al*., 2013; Amjad ur Rahman *et al*., 2014, Sana *et al*., 2016). Different parts of *P. juliflora*, *A. lebbeck* and *A. nillotica* have been utilized against many soil-borne phytopathogen (Shahid and Firdous, 2012; Ikkram and Dawar, 2013). *A. nilotica* is an ornamental and medicinal plant of tropical and sub-tropical regions of the world. The plant can grow over wider range of soil types, temperature (-1 to 50 °C) and pH (5-9) and is cultivated to reclaim soil from salanity stress, as a pioneer species in land rehabilitation and as a barrier to desertification. Moreover, wide range of biological activity due to presence of many active secondary metabolites has enlisted *A. nilotica* as a safe, biodegradable and renewable source of drugs with high therapeutic index (Rather *et al*., 2016). The current study was conducted to check impact of soil amendment with dry leaf biomass of *A. nilotica* subsp. *indica* in combination with *Trichoderma harzianum* on plant growth in mash bean in *M. phaseolina* contaminated soil.

## MATERIALS AND METHODS

Fungal inoculum was prepared on boiled and autoclaved seeds of pearl millet. After sterilization, fresh culture of *Macrophomina phaseolina* was inoculated in pearl millet seeds and incubated at 27 $^{o}$C for one week in an incubator. Likewise, inoculum of *Trichoderma harzianum* was prepared.

For soil sterilization, cotton balls soaked in formaldehyde were placed inside the heap of soil at different locations and covered with plastic sheet for one week. Afterward plastic sheet and cotton balls were removed and mixed the soil thoroughly. The soil was left for 2 days to evaporate the fumes of formaldehyde.

Fumigated soil (2 kg per pot) was added to earthen pots. Twenty gram of pearl millet based pathogen inoculum was mixed in soil of each pot except pots of negative control treatment and all pots were watered. For establishment of inoculum, pots were left for 7 days. After one week, 1%, 2% and 3% dried powdered leaves of *Acacia nilotica* subsp. *indica* were mixed in soil and left for 7 days. Thereafter, inoculum of biological control agent *T. harzianum* (10 g pot$^{-1}$) was mixed in soil of respective pots. For positive control, only pathogen was mixed in soil. Each treatment was replicated four times. Pots were irrigated and left for 7 days for establishment of fungal inocula.

Seeds of mash bean were soaked in water for 4 h. Twenty seeds were sown in every pot in uniform pattern. Pots were watered on regular basis to fulfill plant's water requirement. Thinning was done to maintain eight uniform plants per pot. Experiment was conducted using completely randomized design. After 60 days, plants were carefully harvested. Shoot length of each plant was recorded. Fresh weight of shoots and roots were measured on electric balance. After drying at 70 $^{o}$C, dry weights were recorded. Data was analyzed by ANOVA followed by LSD test using computer software Statistix 8.1.

## RESULTS AND DISCUSSION

The effect of *M. phaseolina*, soil amendment with dry leaf biomass of *Acacia nilotica* subsp. *indica* (LBA) and biological control agent *T. harzianum* on growth of mash bean is illustrated in Fig. 1–3. *M. phaseolina* inoculation markedly suppressed shoot fresh weight by 29%. Application of different doses of LBA markedly enhanced various growth parameters of shoot and root. In general, shoot and root growth was enhanced by increasing LBA dose. A 3% dose of LBA exhibited the highest stimulatory effect on plant growth in mash bean. There was 22–35%, 51–96% 15–45%, 36–92% and 38–74% increase in shoot length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight over positive control, respectively, due to different doses of LBA. Suppression of root rot pathogens (*Fusarium* spp., *Ralstonia solani* and *M. phaseolina*) along with growth improvement in okra, sunflower chickpea and peanut has also been documented due to seed, stem and leaves extract of *A. nilotica* (Rafi *et al*., 2015). Likewise, antifungal activity of methanolic leaf extract of *A. nilotica* subsp. *indica* has been reported against *Sclerotium rolfsii* due to presence of linolenic acid ester in leaves extract (Sana *et al*., 2016). The leguminous nature of *A. nilotica* also facilitates soil with organic carbon, total and available forms of N and P and improved plant growth (Bargali and Bargali, 2009). Furthermore, allelopathic effect of *Acacia* spp. can induce formation of stress proteins, which are responsible for activation of broad array of normal cellular processes that through signaling network of molecular and physiological responses can improve plant growth (El- Khawas, 2004).

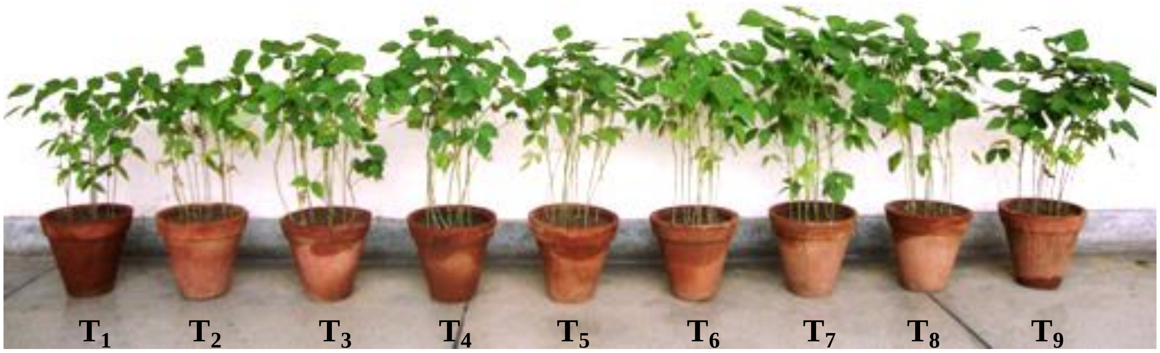


Fig. 1. Effect of *Macrophomina phaseolina* (MP), dry leaf biomass of *Acacia nilotica* subsp. *indica* (LBA) and a biological control agent *Trichoderma harzianum* (TH) on growth of mash bean.
**$T_1$** = control; **$T_2$** = + control (MP); **$T_3$** =1% LBA + MP; **$T_4$** = 2% LBA + MP; **$T_5$** = 3% LBA + MP; **$T_6$** = TH + MP; **$T_7$** = 1% LBA + MP + TH; **$T_8$** = 2% LBA + MP + TH; **$T_9$** = 3% LBA + MP + TH

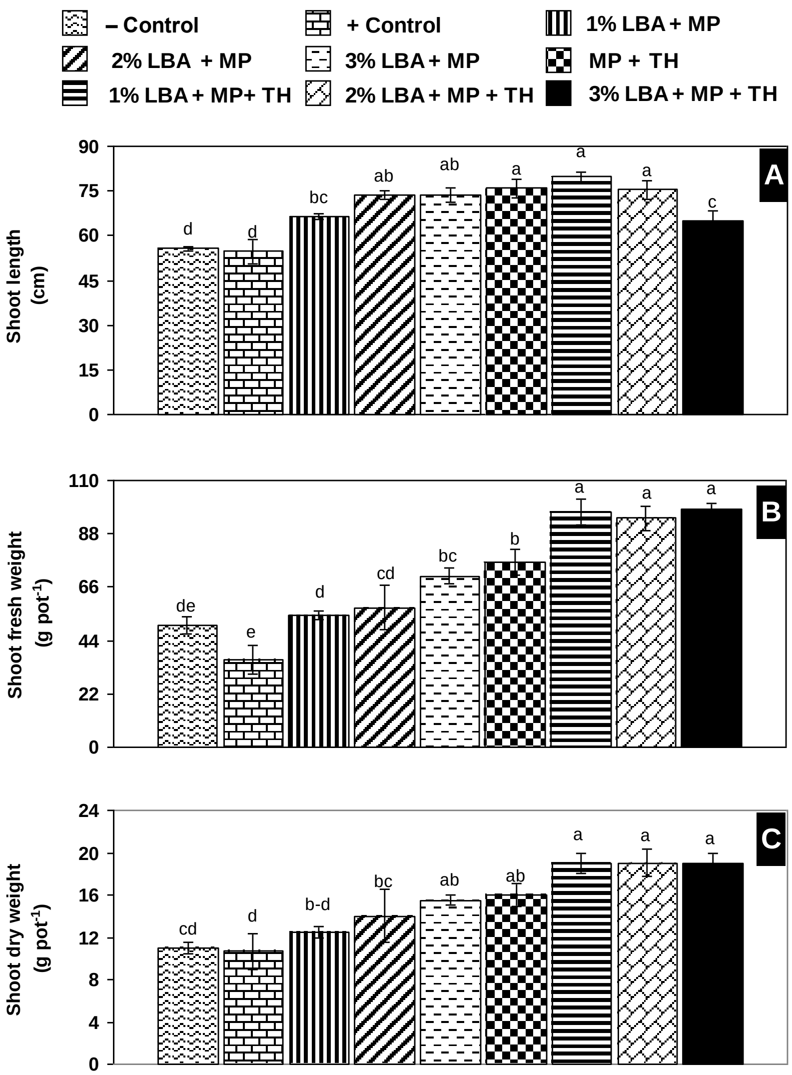


Fig. 2. Effect of *Macrophomina phaseolina* (MP), dry leaf biomass of *Acacia nilotica* subsp. *indica* (LBA) and a biological control agent *Trichoderma harzianum* (TH) on shoot growth of mashbean. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

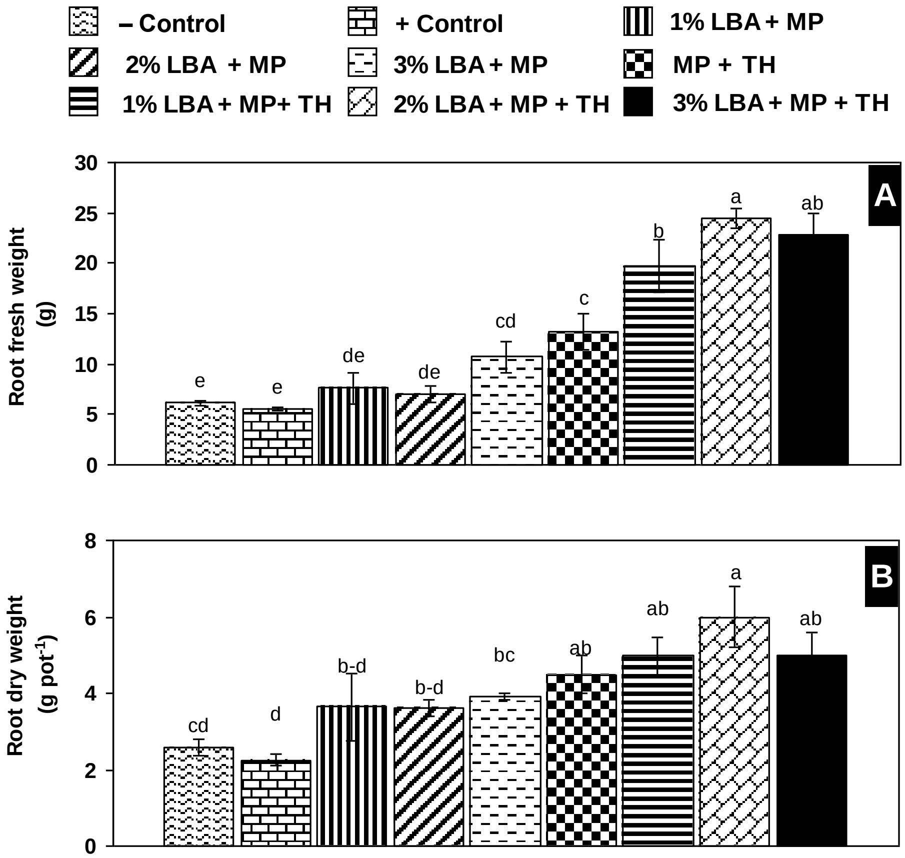


Fig. 3. Effect of *Macrophomina phaseolina* (MP), dry leaf biomass of *Acacia nilotica* subsp. *indica* (LBA) and a biological control agent *Trichoderma harzianum* (TH) on root growth of mashbean. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

Soil inoculation with *T. harzianum* showed highly pronounced and significant (P = 0.05) effect on shoot and root growth in *M. phaseolina* inoculated soil. There was 39%, 113%, 50%, 138% and 100% increase in shoot length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight over positive control, respectively, due to *T. harzianum* inoculation (Fig. 1–3). Significant reduction in charcoal rot disease and improvement in cow pea has been observed previously due to application of *T. harzianum* (Shoaib *et al*., 2018). Induction of resistance in host plant against pathogen along with activation of innate antagonistic mechanisms (nutrient competition, antibiotic production and mycoparasitism) in *Trichoderma* spp. might be the reasons in improving crop growth under pathogenic stress (Vinale *et al*., 2008).

Combined application of *T. harzianum* and LBA showed a better effect on growth of mash bean than their separate applications. In case of shoot length, the highest stimulatory effect was noted owing to combined application of 1% LBA and *T. harzianum* that was 46%, 20% and 5% higher than positive control, 1% LBA, and *T. harzianum* alone, respectively. Further increase in LBA dose along with *T. harzianum* reduced shoot length (Fig. 2A). In contrast, the effect of mutual use of *T. harzianum* and LBA on fresh and dry biomass of shoot was not affected by increasing the dose of LBA. Different doses of LBA in combination with *T. harzianum* increased dry biomass of mash bean shoot by 77–78%, 19–36% and 22–23% over positive control, different doses of LBA, and *T. harzianum* alone, respectively (Fig. 2 B&C). Likewise, combined application of different doses of LBA and *T. harzianum* enhanced dry biomass of mash bean by 120–167%, 28–66% and 11–34% as compared to positive control, different doses of LBA, and *T. harzianum* alone, respectively (Fig. 3 A&B). Similar to that of present study, recently Javaid *et al.* (2017) reported that 1% leaf biomass of *Sisymbrium irio* in combination with *T. harzianum* exhibited significantly greater shoot and grain biomass in mungbean as compared to their separated applications. Likewise, Javaid *et al.* (2018) reported that application of 2% dry biomass of *Coropus didymus* in combination with either *Trichoderma viridi* or *Trichoderma aureoviridi* proved better than their separate applications in improving mungbean growth under stress of *M. phaseolina.*

The present study concludes that soil amendment with *T. harzianum* and dry leaf biomass of *A. nilotica* subsp. *indica* has the potential to alleviate biotic stress of *M. phaseolina* and increase plant growth of mash bean.

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(*Accepted for publication June 2018*)