EFFECT OF *Senna occidentalis* DRY BIOMASS AND *Penicillium oxalicum* ON GROWTH OF MASH BEAN UNDER *Macrophomina phaseolina* STRESS

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ABSTRACT

Charcoal rot disease caused by Macrophomina phaseolina (Tassi) Goid. is a highly destructive disease of mash bean [Vigna mungo (L.) Hepper] that causes significant yield losses annually. In this study, leaf dry biomass of Senna occidentalis [1, 2 and 3% (w/w of leaf biomass/soil)] and a biological control agent Penicillium oxalicum Currie and Thom were examined in a pot trial for their potential to improve growth of mash bean under biotic stress of M. phaseolina. M. phaseolina inoculation alone reduced shoot and root dry biomass by 19% and 67%, respectively. Soil amendments variably increased both shoot and root growth under biotic stress of M. phaseolina. There was 33–43% and 25–75%; 29% and 150%; and 38–43% and 34–125% increase in shoot and root biomass of mash bean due to different doses of S. occidentalis, P. oxalicum, and S. occidentalis + P. oxalicum, respectively, over positive control. Soil amendment with 3% leaf dry biomass of S. occidentalis combined with P. oxalicum significantly improved plant biomass as compared to rest of the treatments. This study concludes that soil amendment with 3% leaves of S. occidentalis either alone or in combination with P. oxalicum can be used for better crop growth of mash bean in M. phaseolina contaminated soil.

Keywords: Biological control, charcoal rot, *Penicillium oxalicum, Senna occidentalis,* soil amendment.

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INTRODUCTION

Mash bean is a commonly grown pulse crop in various regions around the world and is an extremely praised legume in Pakistan. In addition to being high in protein, it is also incredibly rich in all nutrients required for normal human diet (Dahiya *et al*., 2015). It is believed that mash bean antioxidant potential can reduce the risk of cardiovascular diseases and prevent certain types of cancers (Agouni *et al*., 2009). Mash bean is widely grown by farmers in dry land areas because it can thrive in drought-prone areas and can bear temperature up to 42 ºC. In Pakistan, it is a minor crop and cultivated on an area of 15.2 thousand hectares with a total production of 118.8 thousand tons (Pakistan Economic Survey, 2017-2018). Its cultivation is targeted chiefly in Punjab and Balochistan (Achackzai and Taran, 2011). Unfortunately, total production of this major summer pulse remains far below the national requirements because of many constrains including fungal diseases.

M. phaseolina causes charcoal rot disease in mash bean and the fungus can also infect about 500 other plants (Javaid *et al.,* 2018; Nafady *et al.,* 2019). The fungus produces hard and black microsclerotia (Crous *et al*., 2006), which germinate between 20-40 °C and infect root tissues. Synthetic fungicides being used to control fungal diseases also pollute environment and cause ill effects on human health (Westlund *et al.,* 2018). Therefore, some environmental friendly alternatives are required to combat the menace. Use of natural antifungal constituents of plants and biological control agents have been proved successful in controlling plant diseases in recent years (Javaid *et al.,* 2017a).

Among the preferred disease management alternatives, microbes have been recognized considerably for their antifungal potential, which have made significant contribution in every sphere of pesticide use (Singh *et al*., 2017). Substances or metabolites secreted by some fungal species are lethal to other life forms. Myco-biocontrol agents have attracted the attention since 1963 (Turhan and Gossmann, 1994). *Pencillium* spp. are generally present in soil and some species of the genus produce toxins that are harmful for growth of other microbes (Alam *et al*., 2011). *Pencillium* species could induce resistance mechanisms in plants against pathogens and can inhibit pathogens by degrading and penetrating their cell walls through antifungal compounds and extracellular metabolites (Sempere and Santamarina, 2010). *Penicillium oxalicum* is a soil-borne, xerotolerant and mesophillic fungal species that has wide pH tolerance (Pascual *et al*., 1997). It has been reported as promising fungal biocontrol agent against many diseases including diseases caused by *M. phaseolina* (Hamdi *et al*., 2018). *P. oxalicum* is involved in the reduction of various diseases of tomato caused by *Phytophthora parasitica, P. infestans* and *Botrytis cinerea* as well as wilt diseases caused by *Fusarium oxysporum* in melons and watermelons (Larena *et al*., 2003; Sabuquillo *et al*., 2006; De Cal *et al.,* 2008). Besides, the antifungal potential of *P. oxalicum* has also been documented against *Aspergillus niger*, responsible for root rot of onion (Khokhar *et al*., 2012), and *Fusarium solani*, causal agent of root rot of okra (Zia Ullah *et al*., 2015).

Senna occidentalis L. is a weed of family Fabaceae and is native to Central and South America while commonly found in tropics (Keay, 1989). It has long been used as a medicinal plant for its high value in treating whooping cough, measles, hepatitis, convulsions and poisonous snake bites (David *et al*., 1991; Nuhu and Aliyu, 2008). Antifungal activity of *S. occidentalis* has been observed in various studies. Davariya and Vala (2011) tested the antifungal activity of different parts of *S. occidentalis* against *Candida albicans, Aspergillus clavatus* and *A. niger* and reported significant reduction in growth of these fungi. Extracts of *S. occidentalis* also showed antifungal potential against *F. oxysporum, P. oxalicum* and *Aspergillus tamari* (Ettu *et al.*, 2011). Aqueous and ethanol extracts of *S. occidentalis* showed very high inhibition activity against coffee berry disease caused by *Colletotrichum kahawae* (Handiso and Alemu, 2014), and chloroform sub-fraction of leaf inhibited 92-97% biomass of *M. phaseolina* (Javaid *et al*., 2017b). Therefore, in the present study, the effect of leaf dry biomass of *S. occidentalis* and *P. oxalicum* was evaluated on growth of mash bean in *M. phaseolina* inoculated soil.

MATERIALS AND METHODS

Preparation of fungal inoculum

Millet (*Pennisetum glaucum* L.) seeds were purchased from the local market and after thoroughly washing under tap water, boiled for 30 min to make them soft. One kilogram of boiled millet seeds were autoclaved for 30 min in plastic bags, inoculated with actively growing culture of *M. phaseolina* and incubated at 28 ^{o}C for two weeks. Likewise, inoculum of *P. oxalicum* was prepared.

Preparation of pots

Sandy loam soil was fumigated with formalin for one week. Earthen pots of 25 cm deep were filled with fumigated soil @ 2 kg pot^{-1}. Pot soil was inoculated by thorough mixing of 10 g pot^{-1} of *M. phaseolina* inoculum prepared on millet. Pots were irrigated and left for one week for spread of the pathogen inoculum.

After one week of *M. phaseolina* inoculation, *P. oxalicum* inoculation was done at 10 g pot^{-1}. Pots were irrigated and left for one week. In order to maintain the same quantity of pear millet seeds in soil, boiled seeds were mixed in soil in treatments where fungal inocula were not mixed. One week after *P. oxalicum* inoculation, dried and crushed leaves of *S. occidentalis* was mixed in pot soil at 1, 2 and 3% (w/w). In positive control only pathogen inoculum was added while negative control treatment was without of all viz. *M. phaseolina*, *P. oxalicum* or *S. occidentalis* dry leaves. There were 9 treatments in total, each with three replicates. After irrigation, pots were irrigated left for 15 days under natural environmental conditions prior to sowing of seeds. Pots were regularly watered whenever required to keep the pot soil moist.

Ten surface-sterilized mash bean seeds were sown per pot. Pots were irrigated with tap water in such a way that some water stress conditions were there for the development of disease.

Harvesting and data collection

Harvest of mash bean plants was taken after two months of sowing. Plants were uprooted from the soil carefully and washed under tap water. Data regarding shoot and root growth in terms of length (cm) and fresh biomass (g) were recorded. For dry biomass, shoot and root were dried in an electric oven at 60 ^{o}C and weighed.

Statistical analysis

Standard errors of means of three replicates were calculated. All the data were analyzed by ANOVA followed by LSD test ($P \leq 0.05$) using software Statistix 8.1.

RESULTS AND DISCUSSION

Data regarding the effect of *M. phaseolina,* soil amendments with different doses of dry leaf material of *S. occidentalis* and *P. oxalicum* inoculation on shoot growth is shown in Fig. 1 & 2. In positive control, inoculation of *M. phaseolina* significantly reduced the length, fresh weight and dry weight of mash bean shoot by 12%, 15% and 19% over negative control, respectively. Application of *S. occidentalis* leaves and *P.*

oxalicum, separately as well as in combinations, significantly improved shoot growth as compared to both the control treatments. There was 8–9% and 23–24% increase in shoot length, and 12–28% and 31–50% increase in shoot fresh weight, and 8–15% and 33–43% increase in shoot dry weight over negative and positive control treatments, respectively, due to different doses of *S. occidentalis* biomass application in pot soil. Likewise, *P. oxalicum* inoculation increased shoot length, and fresh and dry biomass by 17%, 5% and 4% over negative control, and 32%, 24% and 29% increase in shoot dry biomass over positive control, respectively. Treatments with inoculation of *P. oxalicum* in combination with different doses of *S. occidentalis* enhanced shoot length by 8–17% and 22–32%, shoot fresh biomass by 22–38% and 44–61%, and shoot dry biomass by 12–15% and 38–43% over negative and positive control treatments, respectively.

Data concerning the effect of *M. phaseolina,* dry leaf biomass of *S. occidentalis* and *P. oxalicum* on root growth of mash bean is illustrated in Fig. 3. *M. phaseolina* inoculation significantly reduced the root fresh and dry biomass by 45% and 67% over negative control, respectively. All the soil amendment treatments showed higher root biomass over positive control. Different doses of leaf biomass of *S. occidentalis* enhanced fresh and dry biomass of root by 21–56% and 25–75% over positive control, respectively. The highest increase in fresh and dry biomass of root i.e. 68% and 150%, respectively, was exhibited by *P. oxalicum* alone. Application of 2% and 3% leaf biomass of *S. occidentalis* in combination with *P. oxalicum* showed better effects on root growth than application of these doses without the biological control agent.

In the present study, *M. phaseolina* inoculation markedly reduced root and shoot growth. The negative effect of the pathogenic fungus was more pronounced and significant on root growth than on shoot growth. This reduction in root growth of mash bean may be attributed to damaging of root by the pathogen. The damaged root system was possibly less efficient in absorption of water and nutrients and consequently resulted in reduced shoot growth. The reduced plant growth may also be attributed to negative effects of fungal toxins such as phaseolinone (Al-Ahmadi *et al.,* 2018). Reduced plant growth due to *M. phaseolina* has also been reported in other legumes namely mungbean (Javaid *et al.,* 2017a), chickpea (Manjunatha *et al.,* 2013), soybean (Perez-Brandán *et al.,* 2012) and cowpea (Oyewole *et al.,* 2017).

Soil application of *S. occodentalis* leaf material noticeably enhanced shoot and root growth over negative as well as positive control treatments. This increase in plant growth could be attributed to two factors. Firstly, the added plant material might release nutrients during the decomposition process which were absorbed by the plant and consequently plant growth in mash bean was increased. Secondly, various antifungal constituents were released by the decomposing plant material of *S. occidentalis* resulting in suppression of the pathogenic fungus, consequently plant growth was enhanced. Recently, Javaid *et al.* (2017b) reported *in vitro* antifungal activity of leaf extract of *S. occidentalis* against *M. phaseolina.* They also identified various compounds such as 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester and 9,10-dimethyltricyclo[4.2.1.1 (2,5)]decane-9,10-diol, which were likely to be responsible for antifungal activity against the pathogen. Inhibitory effects of leaf extract of this plant have also been reported against other fungal species namely *Aspergillus niger*, *A. flavus, Mucor* sp. *Candida albicans* and *Fusarium oxysporum* (Hussain and Deeni, 1991; Ali *et al.,* 1999; Abo *et al.,* 2000).

Inoculation of *P. oxalicum* exhibited pronounced effect on shoot and root growth of mash bean under biotic stress of *M. phaseolina.* The effect was more pronounced on root growth than on shoot growth. Earlier, there are reports of management of powdery mildew of strawberry (De Cal *et al.,* 2008), and tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* and *Verticillium dahliae* by application of *P. oxalicum* in the soil (Larena *et al.,* 2003; Sabuquillo *et al.,* 2006).

Combined application of *S. occidentalis* leaf dry biomass and *P. oxalicum* also found beneficial in improving crop growth of mash bean in *M. phaseolina* contaminated soil. However, in the present study, the effect of combined application was generally similar to the effect of separate application of the two soil amendments. In contrast, recently Javaid *et al.* (2017a) found that combined application of dry leaves of *Sisymbrium irio* and a biocontrol fungus *Trichoderma harzianum* had significantly higher effects in improving crop growth and yield of mungbean in *M. phaseolina* inoculated soil as compared to separate application of dry leaves and the biocontrol fungus.

CONCLUSION

The present study concludes that application of 3% dry leaves of *S. occidentalis* alone or in combination with *P. oxalicum* can improve crop growth of mash bean in *M. phaseolina* contaminated soil.

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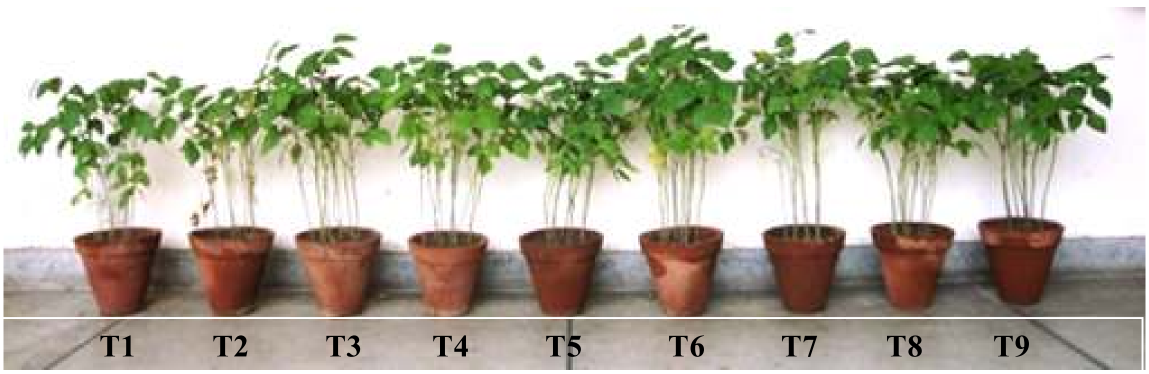

Fig. 1: Effect of *Macrophomina phaseolina* (MP), dry leaf biomass of *Senna occidentalis* (LBS) and a biological control agent *Penicillium oxalicum* (PO) on shoot growth of mash bean.

T_1 − control
T_2 + control (MP)
T_3 1% LBS + MP
T_4 2% LBS + MP
T_5 3% LBS + MP
T_6 PO + MP
T_7 1% LBS + MP + PO
T_8 2% LBS + MP + PO
T_9 3% LBS + MP + PO

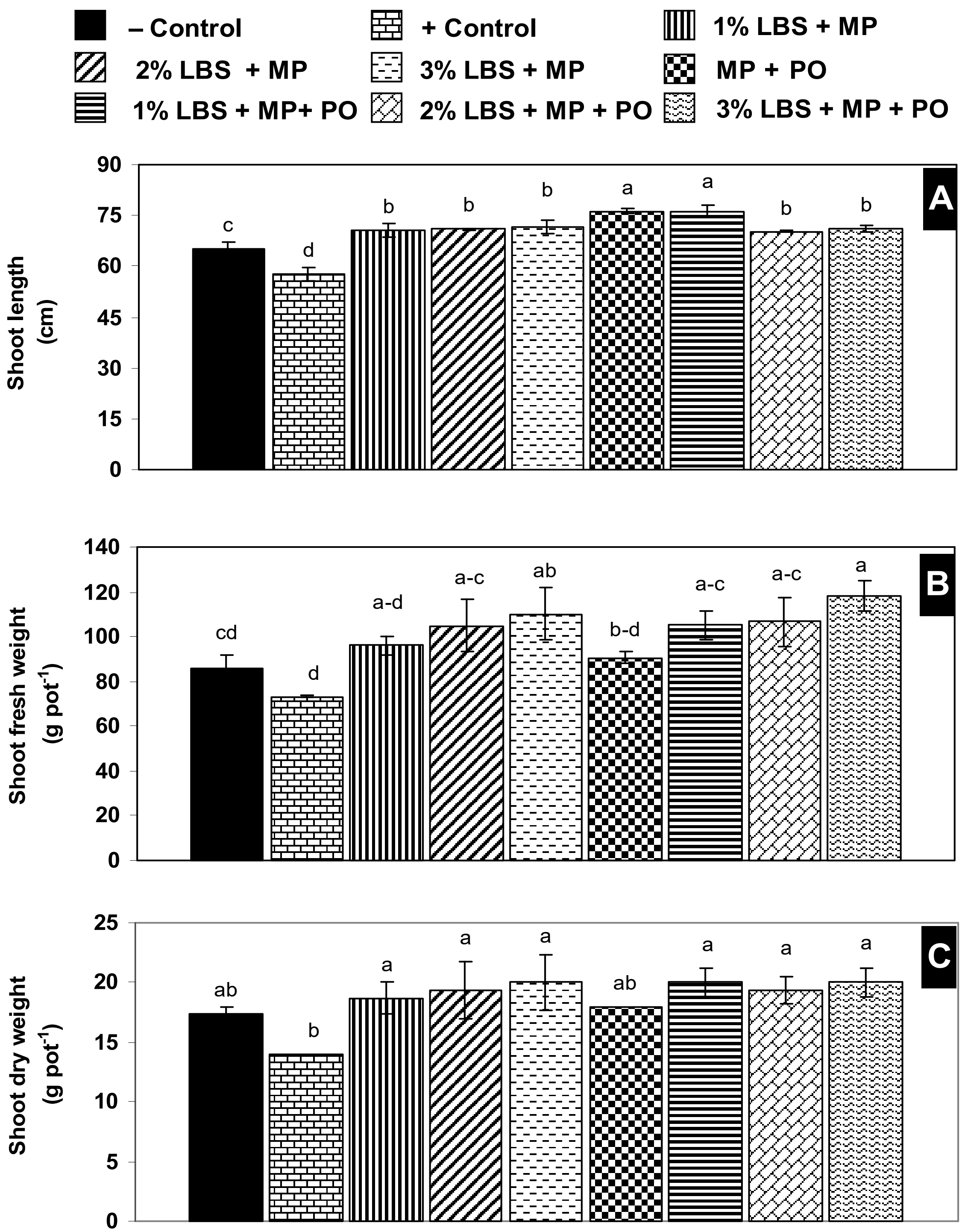

Fig. 2: Effect of dry leaf biomass of *Senna occidentalis* (LBS) and *Penicillium oxalicum* (PO) on shoot growth of mash bean under biotic stress of *Macrophomina phaseolina* (MP). Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

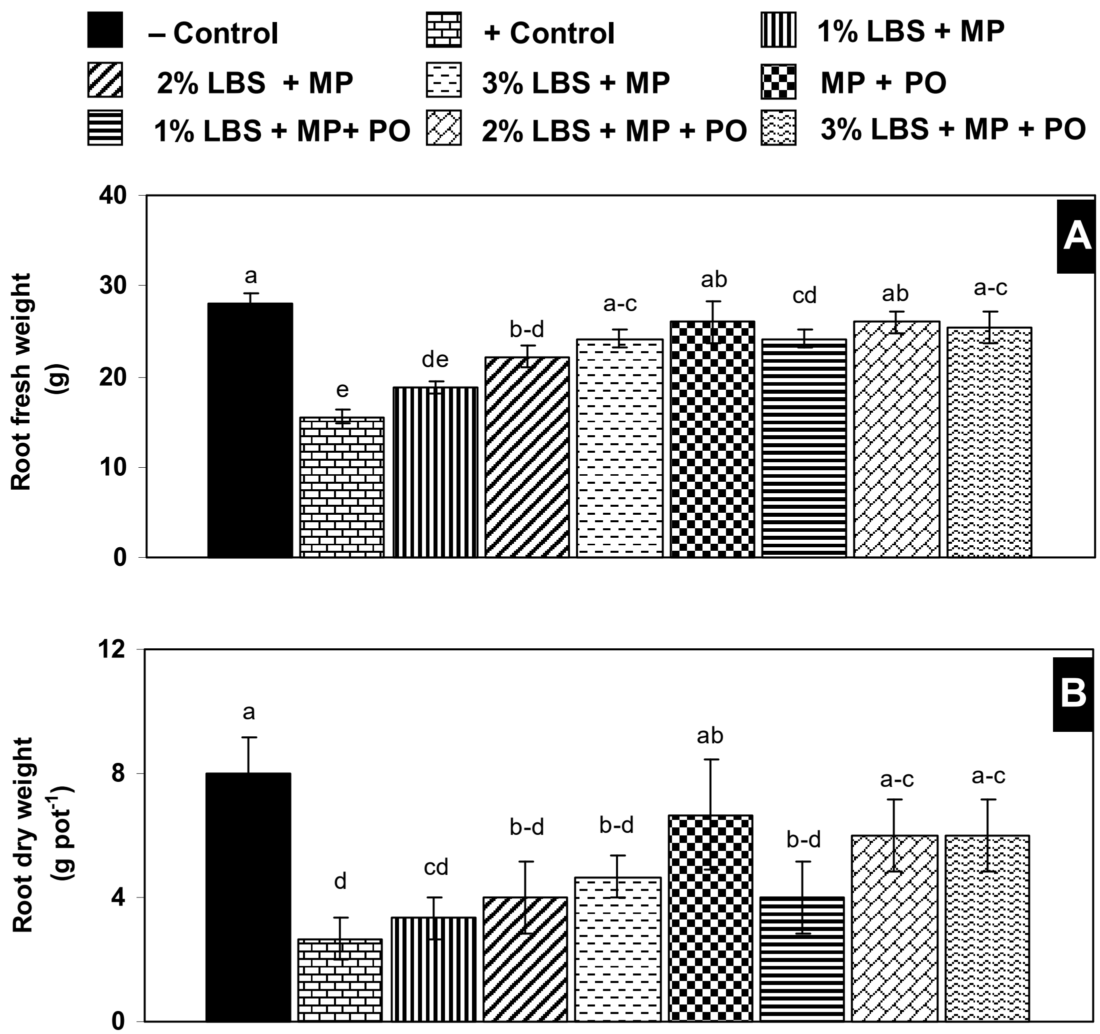

Fig. 3. Effect of dry leaf biomass of *Senna occidentalis* (LBS) and *Penicillium oxalicum* (PO) on root growth of mash bean under biotic stress of *Macrophomina phaseolina* (MP). Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.