ABSTRACT - A pot experiment was carried out to check the effect of Coronopus didymus (L.) Sm. dry biomass application (1%, 2% and 3% w/w) and two species of Trichoderma (T. viride and T. aureoviride) on growth and physiology of mungbean [Vigna radiata (L.) Wilczek] under biotic stress of Macrophomina phaseolina (Tassi) Goid. Inoculation of M. phaseolina (positive control) reduced plant survival, shoot and root length as well as plant dry biomass by 22%, 52%, 61% and 64%, respectively, over the negative control (without any amendment). There was 100% plant survival in treatments with T. aureoviride alone or in combination with 1% and 2% C. didymus biomass. Likewise, T. viride in combination with 2% biomass also showed 100% plant survival. Application of 3% C. didymus biomass had a pronounced effect on crop growth resulting in 101%, 233% and 342% increase in shoot length, root length and plant biomass, respectively, over the positive control. Sole inoculation of either of the two Trichoderma spp. significantly enhanced various plant growth parameters over the positive control. In general, in combination with 2% biomass of C. didymus, both Trichoderma spp. proved to be the best choice for improving mungbean biomass under stress of M. phaseolina. Activity of defense related enzymes viz. peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) was generally higher in treatments in which 1% C. didymus biomass was applied either alone or combined with Trichoderma spp. in M. phaseolina inoculated soil.

Keywords: biological control, soil amendment, swinecress, Vigna radiata.
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Pot experiment

Pot trial was carried out by amending the soil with *M. phaseolina*, dried leaves of *C. didymus* and two species of *Trichoderma*, namely *T. viride* and *T. aureoviride*. The protocol given by Javaid and Saddique (2011) was generally followed with some modifications. Soil was filled (2.0 kg pot⁻¹) in earthen pots (20 cm diameter and 15 cm deep) and inoculated with inoculum of *M. phaseolina* (15 g pot⁻¹) and watered. Likewise, *T. viride* and *T. aureoviride* inocula were also mixed in respective potted soil and watered. Later, after a week, soil was amended with *C. didymus* leaf biomass at 1%, 2% and 3% in the respective pots. Positive control consisted of the fungus (*M. phaseolina*) only whereas negative control was devoid of any inoculation or amendment. After 10 days, surface sterilized healthy mungbean seeds of uniform size were sown in each pot (10 seeds pot⁻¹). A total of 13 treatments were used in the pot study: T₁: Control; T₂: + Control [only *M. phaseolina* (MP)]; T₃: MP + 1% dry biomass of *C. didymus* (DBC); T₄: MP + 2% DBC; T₅: MP + 3% DBC; T₆: MP + *T. aureoviride* (TA); T₇: MP + 1% LDB + TA; T₈: MP + 2% DBC + TA; T₉: MP + 3% DBC + TA; T₁₀: MP + *T. viride* (TV); T₁₁: MP + 1% DBC + TV; T₁₂: MP + 2% DBC + TV; T₁₃: P + 3% DBC + TV. All the treatments were replicated thrice in a completely randomized design, kept under natural environmental conditions and watered whenever required.

Physiological tests

Various physiological tests were carried out after 35 days of growth just prior to flowering. For all the physiological parameters of the study, fresh leaves were taken from pot grown mungbean plants and, immediately after picking of leaves, physiological tests were performed on the fresh leaves. Total protein content was checked in leaf tissues (0.5 g), following the protocol of Baskaran et al. (2009) by measuring absorbance at 650 nm using bovine serum albumin (BSA) as standards. Peroxidase (PO) activity was determined spectrophotometrically by using pyrogallol as a substrate (Kumar and Khan, 1982). The increase in absorbance resulting from formation of oxidized product (purpuurogallin) was recorded at 420 nm. The reaction mixture [(2 mL of 0.1 M phosphate buffer (pH 6.8) + 1 mL of pyrogallol + 1 mL of 0.05 M H₂O₂)] was mixed with enzyme extract (0.5 mL). After incubation at 25 °C, 2.5 N H₂SO₄ (24.5 mL of H₂SO₄ + 100 mL of distilled water) was added in the reaction mixture. For estimation of polyphenol oxidase activity (PPO), the enzyme extract (100 μL) was mixed with 0.1 M of pH 7.0 sodium phosphate buffer (1.5 mL). The reaction started when 200 μL of 0.01 M catechol was added. The absorbance of the sample was measured at 30 sec interval for 3 min at 495 nm (Mayer et al., 1965). For determination of phenylalanine ammonia-lyase (PAL) activity, the reaction mixture [0.4 mL of enzyme extract + 0.1 M sodium borate buffer (pH 8.8) + 0.5 mL of 12 mM L⁻¹ phenylalanine] was incubated for 1 h in light at 25 °C and the reaction was stopped by incubation at 47 °C for 10 min. The amount of resulting trans-cinnamic acid was calculated after measuring absorbance of the samples at 290 nm (Dickerson et al., 1984).

Harvesting and data collection

After harvesting data on the number of surviving plants, shoot and root length, and plant dry weight were recorded.

Statistical data analysis

All the data were subjected to Analysis of variance (ANOVA) followed by the LSD test to separate treatment means at 5% level of significance.

RESULTS AND DISCUSSION

Effect of treatments on plant growth

ANOVA showed that there was a significant (P = 0.05 and 0.001) effect of treatments on plant survival, shoot length, root length and dry weight of mungbean plants (Table 1). There was 100%
survival of plants in the negative control but that rate was reduced to 78% in the positive control. Application of 1% *C. didymus* further reduced survival percentage to 37%, which was increased to 67% and 73% by increasing the dose of *C. didymus* to 2% and 3%, respectively (Figure 1). Shoot length in control was 19.3 cm. Inoculation of *M. phaseolina* significantly reduced this growth parameter to 9.2 cm. In general, the effect of all the *C. didymus* biomass amendment treatments was significant on shoot length as compared to the positive control treatment. Application of 1%, 2% and 3% *C. didymus* biomass gradually increased shoot length to 14.9, 17.6 and 18.6 cm, respectively (Figure 2A). Root length in the negative control was 12.33 cm but it significantly decreased to 4.77 cm in positive control. Application of 1% *C. didymus* biomass had an insignificant effect on root length as compared to the positive control. However, further increase in *C. didymus* biomass to 2% and 3% significantly increased root length to 11.70 cm and 15.93 cm, respectively (Figure 2B). Inoculation of *M. phaseolina* caused a 64% decline in dry biomass of mungbean. Application of 1% biomass of *C. didymus* failed to change the adverse effect of *M. phaseolina* on the biomass of mungbean. However, a further increase in the dose of *C. didymus* biomass as soil amendment significantly increased plant biomass up to 342% over the positive control (Figure 2C).

Earlier, Coelho de Souza (2004) studied the antimicrobial activity of crude methanolic extracts of *C. didymus* against seven microorganisms and a significant result was achieved. Likewise, Iqbal and Javaid (2012) reported that methanolic extracts with concentrations of 15 mg mL⁻¹ of leaf, stem, inflorescence and root of *C. didymus* reduced the biomass of *Sclerotium rolfsii* by 67%, 26%, 40% and 58%, respectively. Similarly, a 4% methanolic extract of *C. didymus* reduced biomass of *Fusarium moniliforme* by 48% (Javaid et al., 2018). Khan et al. (2010) studied the effect of *C. didymus* leaf incorporation on *Fusarium corm rot* of gladiolus. Different doses of this weed species significantly reduced disease incidence and mortality in gladiolus. Javaid and Iqbal (2014) reported that a 3% dose of dry leaves of *C. didymus* significantly

**Table 1** - Analysis of variance for the effect of different treatments of *C. didymus* biomass, *Macrophomina phaseolina*, *Trichoderma aureoviride* and *T. viride* on growth and physiological parameters of mungbean

<table>
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<th>Root length</th>
<th>Plant biomass</th>
<th>PO</th>
<th>PPO</th>
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<td>Mean squares</td>
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<td>31.42**</td>
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<td>1.073**</td>
<td>0.00032**</td>
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*, **, significant at $P\leq0.05$ and 0.001, respectively. PO: Peroxidase. PPO: Polyphenol oxidase. PAL: Phenylalanine ammonia lyase.

Vertical bars show standard errors of means of five replicates. Values with different letters at the top show a significant difference ($P\leq0.05$) as determined by the LSD Test.

**Figure 1** - Effect of soil amendment with dry biomass of *Coronopus didymus* (DBC), *Macrophomina phaseolina* (MP) and two *Trichoderma* spp. species [*T. aureoviride* (TA) and *T. viride* (TV)] on survival percentage of mungbean plants.
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C. didymus biomass abruptly declined shoot length to 12.1 cm. The effect of T. viride alone or combined with 1% and 2% C. didymus biomass on shoot length was less pronounced as compared to the effect of similar treatments with T. aureoviride (Figure 2A). Inoculation of T. aureoviride alone or combined with 1% and 2% C. didymus biomass significantly enhanced root length by 134-180% over the positive control. However, the effect of T. aureoviride + C. didymus biomass was less pronounced as there was 82% increase in root length over the positive control. Likewise, inoculation of T. viride alone or combined with different doses of C. didymus biomass significantly enhanced root length by 124-185% over the positive control (Figure 2B). Sole inoculation of either of the two Trichoderma species significantly increased mungbean biomass. However, the positive effect of T. aureoviride was more pronounced than the effect of T. viride. In combination with different doses of C. didymus, the two Trichoderma species showed different behaviours on plant biomass of mungbean. In combination with 2% C. didymus biomass, both species had a similar and highly pronounced effect on mungbean biomass. In combination with 3% C. didymus biomass, T. viride had a similar effect while T. aureoviride showed a markedly diminished effect on mungbean biomass (Figure 2C). Inhibition of pathogenic fungal growth by Trichoderma spp. occurs by physical as well as chemical interactions in which a variety of chemicals are released by Trichoderma spp., inducing localized or systemic resistance responses in plants (Harman et al., 2004). Faster metabolic rate of Trichoderma spp., competition for food and space, enzymatic antibiosis, release of secondary antimicrobial metabolites and physiological conformation are...
the key factors involved in the antagonistic interaction of Trichoderma spp. with pathogenic fungi (Verma et al., 2007).

**Effect of treatments on plant physiology**

ANOVA illustrates that different treatments had a significant effect (P=0.001) on the activities of peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) of mungbean leaves (Table 1). The difference in PO activity of the negative and positive control treatments was insignificant. Addition of 1% C. didymus biomass alone or combination with T. aureoviride significantly increased PO activity. An increase in the dose of C. didymus biomass alone or combined with either of the two Trichoderma species gradually decreased PO activity (Figure 3A).

Similar to that of PO activity, difference in polyphenol oxidase (PPO) activity between negative and positive control was insignificant and application of 1% C. didymus biomass alone or combined with T. aureoviride significantly enhanced this activity. Higher doses of C. didymus biomass significantly reduced PPO activity (Figure 3B). The highest PAL activity was recorded in 1% C. didymus + T. aureoviride treatment. Generally, the increase in C. didymus biomass adversely affected this parameter (Figure 3C).

Plants infected by M. phaseolina only showed an insignificant effect in enzyme activity (POX, PPO and PAL) in the positive control as compared to the negative control as the susceptible host does not have the ability to detect the threat posed by pathogen (Fortunato et al., 2015). A directly proportional relationship was found between enzyme production and disease suppression after application of different management agents attributed to a higher production of reactive oxygen species (ROS) and antioxidant enzymes in order to overcome stress. As the plant gets rid of the stress, enzyme production is also reduced with increase in biomass of C. didymus alone or along with either of the two Trichoderma species.

The present study concludes that in combination with 2% biomass of C. didymus, both Trichoderma spp. species have been proven to improve mungbean biomass under stress of M. phaseolina.

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REFERENCES


