Trichoderma as a recommended growth promoter for soybean cultivation in Pakistan

Sabra Reesham, *Arusa Aftab, Zubaida Yousaf, Nayyab Naeem, Zainab Maqbool and Zainab Shahzadi

Department of Botany, Lahore College for Women University Lahore, Pakistan
**Corresponding author's email: arusa.aftab@lcwu.edu.pk, zubaida.yousaf@lcwu.edu.pk*

Abstract

The aim of the study was to examine the impact of several strains of *Trichoderma* on soybean [*Glycine max* (L.) Merr.] growth promotion. The effects of three *Trichoderma* spp. *viz. T. hamatum*, *T. harzianum* and *T. viride* were examined both singly and in combination on growth and physiology of soybean. The growth-promoting effects of the *Trichoderma* spp. was estimated through morphological features, antioxidant assay, chlorophyll content, and nutritional contents (N, P, and K) estimation. The best morphological characteristics were displayed by the *T. hamatum*-inoculated plant, either singly or in combination. Combined application of *T. harzianum* + *T. hamatum* resulted in maximum total chlorophyll content. *T. hamatum* inoculation also resulted in maximum antioxidants potential through estimation of total phenolic content. Likewise, the highest antioxidant potential was found in the *T. hamatum* + *T. harzianum* inoculation as shown by total antioxidant and DPPH assays. It concluded that inoculation with selected plant growth promoting fungi could serve as a useful tool for soybean crop in Pakistan.

Keywords: Antioxidant, Morphology, Nutrients, *Trichoderma*.

Introduction

Soybean, an Asian origin legume plant, is a rich source of protein, as well as many nutrients. In addition, it also contains nine necessary amino acids in it. Vegetarians frequently replace soy for meat in their diets (Nair *et al.*, 2014). The Fabaceae family, also known as the Leguminosae family, is one of the most significant plant families because it produces products that humans utilize. Legumes, or legume plants, are fruits that are grown for food, green manures, and feed. It is the 3rd largest group of plants with 19,400 species (Ahmad *et al*., 2016). Legumes forms a symbiotic association with nitrogen fixing bacteria called rhizobia (Javaid, 2009).

Erratic and poor production of soybean crop in the field is partially due to poor seedling formation and higher temperatures in Pakistan (Voora *et al*., 2020). Delayed and unpredictable germination creates difficulties with use of fertilizers and weed control. The fungus can increase germination rate as well as seedling growth. It is a chief source of protein for humans and feedstock feeding as a high-quality animal feedstuff (Pagano and Miransari, 2016). Bio-inoculants enhance plant growth, yield, and soil nutrient enrichment by nitrogen-fixing, phytochrome production, control of plant disease caused by pathogens, and improved vegetative growth and root development even under a combination of stresses (Hossain and Sultana, 2020). Plant growth-promoting fungi modulate plant growth by producing phytohormones and volatile compounds (Hossain *et al.*, 2017). *Trichoderma* species occur worldwide in varied habitats, are a low-cost versatile biocontrol agent and also a good fertility promoter. *Trichoderma* spp. are efficient competitors for nutrition and living space (Bakhshandeh *et al*., 2020).

Inoculation of *Trichoderma* species result in the marked development of root and shoot organization and yield. They produce several antibiotics and secondary compounds that play a vital role in biocontrol (Khan and Javaid, 2020; Khan *et al.*, 2021). They are well-known for manufacturing of numerous lytic enzymes and antibiotics. These are extensively used in the biocontrol of soil-borne pathogens such as *Sclerotium rolfsii* and *Macrophomina phaseolina* (Ali *et al.*, 2020; Javaid *et al.*, 2021). These are among the most common fungi commercially available as bioinoculatants (Keswani *et al.*, 2014). They produce many antifungal enzymes, which are useful for biological control and other processes (Florencio *et al.*, 2015). The objectives of present studies were to check the effect of inoculation of *Trichoderma* spp. on growth, yield and various biochemical processes of soybean.

Materials and Methods

Procurement of materials

One-year-old, uniform size and healthy seeds of the soybean (ACC 24558-1) were procured from NARC, Islamabad. Healthy seeds were water soaked overnight. Three *Trichoderma* species namely *T. harzianum* FCBP-SF-1277, *T. viride* FCBP-SF-671, and *T. hamatum* FCBP-SF-907 were obtained from 1st Fungal Culture Bank of Pakistan (FCBP), University of the Punjab, Lahore and used in the present study.

Culture and inoculum preparation

Cultures of the three *Trichoderma* species were prepared following methodology of Florencio *et al*. (2015). Culture plates were prepared using PDA (potato dextrose agar) at 30 °C. Streptomycin sulphate was used to prevent the bacterial contamination. After culturing, the mycelia were transferred to PDA plates. The fungal inocula were prepared by adding one loop of each fungal species in glass jars, each containing100 mL distilled water. Jars were incubated at 37 °C. The prepared inocula were added to the respective pots twice, after 15 and 45 days of germination (Rakibuzzaman *et al*., 2021).

Planting method

Soybean seeds were sown during March and harvested in July. The experimental design consisted of 7 treatments with three replications following protocol of Marra *et al*. (2019). The experiment was done in 9 cm plastic pots filled with 2 kg of soil. The seven treatments were T1 (*T. harzianum*), T2 (*T. viride*), T3 (*T. hamatum*), T4 (*T. harzianum* + *T. viride*), T5 (*T. viride* + *T. hamatum*), T6 (*T. harzianum* + *T. hamatum*) and T7 (control).

Solvent extraction by maceration method

Ten grams of harvested soybean plant material was thoroughly ground. The extraction was carried out through maceration technique under a period of 8 days using petroleum ether, chloroform, hexane, ethyl acetate and methanol (Chung *et al*., 2010).

Morphological traits measurement

Different parameters like germination percantage, number of leaves, length of leaves, width of the leaves, the height of plant, color of leaves, number of flowers, number of pods and length and width of pods, were observed from germination to harvest (Vafadar *et al*., 2014).

Chlorophyll content

Chlorophyll content was measured using method of Singh *et al*. (2014). Fresh leaves and stem (0.1 g) were cut and grounded in 2 mL of 80% acetone. The homogenized mixture was centrifuged at 3000 rpm for 5 min and supernatant was collected. The procedure was repeated till the residue turned colorless. The collected supernatants were made up to 10 mL with 80% acetone. Absorbance of the solution was read by UV visible spectrophotometer at 645 nm and 663 nm wavelength. The 80% acetone was used as blank.

Chlorophyll a (mg g^{-1} fresh wt.) = $(12.7 \times A663 - 2.69 \times A645) / 1000 \times W \times V$

Chlorophyll b (mg g^{-1} fresh wt.) = $(22.9 \times A645 - 4.68 \times A663) / 1000 \times W \times V$

Total Chlorophyll (mg g^{-1} fresh wt.) = $(20.2 \times A645 - 8.02 \times A663) / 1000 \times W \times V$

Where:

V = Volume of extract (mL)

A = Absorbance at respective wavelengths

W = Fresh weight of the sample (g)

Antioxidant analysis

Antioxidant analysis was carried out by DPPH radical scavenging, total antioxidant and total phenolic content assays (Aftab *et al.*, 2019). Total antioxidant capacity of all the extracts was determined by following methodology of Vafadar *et al*. (2014). DPPH assay was performed following methodology of Shahidi *et al*. (2015). Total phenolic contents were determined following protocol of Shahidi *et al.* (2015).

Plant N, P, K analysis

Plants were got analyzed for N, P and K contents from Soil Survey of Pakistan Multan Road, Lahore Punjab, Pakistan.

Data analysis

Data was analyzed using SPSS 25 (IBM crop released in 2017) and Origin Pro.

Results

Morphological traits

The effect of individual or combined inocula of different *Trichoderma* spp. on soybean plant growth is presented in Fig. 1. The highest number of seed germination was measured on day 7 while the lowest number of seed germination was noted at day 10. The maximum height (cm) was observed in treatment T3 (50.1 ± 0.6), maximum number of leaves were recorded in treatment T6 (13.33 ± 3.05) while the maximum length of leaves and width of leaves (cm) were observed in treatment T3 (4.46 ± 3.2, 3.2 ± 1.21), however total number of flowers was noted in treatment T3 (10 ± 0) (Fig. 2).

The effect of treatments on different growth parameters of 60-days-old soybean is presented in Fig. 2. Maximum plant height (99.46 ± 5.35 cm) was observed in T3 (*T. hamatum*) followed by T2 (98.66 ± 15.02 cm). and the minimum plant height (64.23 ± 29.24 cm) was recorded in T6 (*T. harzianum* + *T. hamatum*). The maximum number of leaves (35.66 ± 3.05) was noted in T3 (*T. hamatum*). Maximum length (5.16 ± 0.83 cm) andwidth of leaves (3.13 ± 0.75 cm) were observed in T3. Total number of flowers were recorded at maximum in treatment T3 (*T. hamatum*) *i.e.* 10.66 ± 14.15. The highest number of pods (3 ± 0) were noted in T1, maximum length of pods (7.5 ± 0 cm) was measured in T3 and maximum width of pods was observed (0.9 ± 0 cm) in treatment T3.

The effects of individual or combined species of *Trichoderma* spp. on the growth parameters of 90 days old plants is shown in Fig. 2. Plant morphological traits among treatments were compared and observed. Maximum height (cm) of plant (113.16 ± 22.87) was found in treatment T3 while the minimum height (85.23 ± 30.11) were observed in T6. The maximum number of leaves was noted in T3 (42 ± 4.0). Maximum length of leaves was observed (7.56 ± 0.61) in T5, while maximum width of leaves was noted in T5 (4.4 ± 0.43). Maximum number of flowers was recorded in T4 (17.66 ± 15.04), maximum number of pods was noted (7.0 ± 3.6) in T3, while maximum length of pods (4.96 ± 2.23) was calculated in treatment T3 and width 0.86 ± 0.05 was noted in treatment T3. The maximum increase in root fresh and dry weight were observed in treatment T3 (1.50 ± 0.596, 1.17 ± 0.085), while maximum pods of dry weight and fresh weight also were noted in treatment T3 (1.41 ± 0.1, 0.67 ± 0.442). Two different color of flower was observed during experiment (purple and white), purple color of flower was observed in three treatments (T1, T2, T5) and white color of flower were noted in four treatments *i.e.* T3, T4, T6 and T7 (Fig. 3).

Chlorophyll content estimation

The effect of treatments on chlorophyll content is shown in Fig. 4. Wavelengths were recorded at 663 nm, 645 nm and 470 nm presented variations with the increase in chlorophyll concentration during the growth cycle of soybean. The chlorophyll content (chl a, chl b and total chl) showed significant results among the treatments. The highest chlorophyll content (6.39 mg g^{-1}) was recorded T6 (*T. harzianum* + *T. hamatum*). On the other hand, T4 exhibited the lowest chlorophyll content (0.835). Likewise, the highest (2.734 mg g^{-1}) and the lowest (0.442 mg g^{-1}) chlorophyll b contents were also recorded in T6 and T4, respectively. Similarly, maximum (10.11 mg g^{-1}) and minimum (1.943 mg g^{-1}) total chlorophyll contents were also found in T6 and T4, respectively, as shown in Fig. 4.

Total antioxidant assay (TAA)

ANOVA showed the significant effect of treatments on total antioxidant activity. Methanol extract exhibited the highest values ranging from 0 .737 in T3 to 0.607T2. In *n*-hexane extract, the highest antioxidant potential was found in T3 with value of 0.621 that can be considered as standard. The other values of *n*-hexane extract for this parameter were in the range of 0.31 in T2 to 0.03 T1. The peak antioxidant values of 0.68 and 0.63 in chloroform and ethyl acetate extracts were recorded in T6 (Fig. 5).

Radical scavenging activity by DPPH assay

Chloroform extract in T5 exhibited 99.93% followed by methanol extract (99.5%) in T1 and chloroform extract (89.5%) in T2, which can be considered as standard. By contrast, chloroform extract in T4 exhibited the lowest value (49.48%) as shown in Fig. 6.

Total phenolic content (TPC)

The highest (360.79 mg g^{-1}) and the lowest (1.11 µg g^{-1}) phenolic content were recorded in methanol extract of T3 and chloroform extract of T2, respectively. In general, TPC in methanol extracts of different treatments was in the range of 360.79 to 261.16. Similarly, TPC values of hexane extract ranged from 41.64 to 154.77, chloroform extract was from 1.11 0 to 227.38 and that of ethyl acetate extract was between 2.80 to 80.47, respectively (Fig. 7).

NPK analysis

Table 1 shows that NPK uptake was higher in all the *Trichoderma* treatments as compared to control. Highly significant difference was observed among the treatments for N uptake that was the highest in T6 followed by T3. The maximum and minimum P uptakes were found in T6 (10.16) T2 (4.71), respectively. The treatment T6 showed the highest (355.05) uptake of K, followed by T3 (319.6) and T7 (100.7).

Discussion

The current investigation was carried out to check the growth promoting effects of three species of *Trichoderma* on morphological traits and nutrients of soybean. The findings indicated the positive effects of *Trichoderma* spp. as growth promoting fungi. Application of *T. hamatum* gave the highest stimulatory effects on various studied parameters. *Trichoderma* species, either singly or in combinations, especially *T. hamatum* + *T. harzianum* and *T. viride* significantly enhanced germination over control. Earlier studies have also reported positive influence of *Trichoderma* inoculation on morphological traits of soybean (Alosio *et al*., 2019). Findings of this study are in line with a previous study where *T. hamatum* inoculation improved root growth and shoot growth of chili. The fungistatic potentiality of strain *T. hamatum* was noted against pepper early blight disease at a proficiency level of 60.61%. In pot experiments, the inoculation significantly increased different growth parameters. *Trichoderma* spp. produce extracellular gluconates, chitinases, lipases, and proteases. Moreover, some lytic enzymes are also produced *Trichoderma* spp. interact with pathogenic fungi. These fungi have no known detrimental effects on humans or other beneficial organisms (Saba *et al*., 2012).

Trichoderma species are known to promote plant growth in fruit, vegetable and forestry crops (Mao *et al*. 2020). Morphological parameters of plants are useful in the visual identification of plants. Aloisio *et al*. (2019) reported that *Trichoderma asperellum* enahnce growth of soybean plants. The strain of *T. asperellum* can be recommended as a plant growth promoter in soybean. The combination inoculum of *T. harzianum* + *T. hamatum* boosted the total chlorophyll content of the current study, probably leading to higher photosynthetic rates and therefore improved plant biomass. *Trichoderma* has also been exposed in the study of Akladious and Abbas (2021), that the application of *T. harzianum* T2 as a biofertilizer potential in maize development and exposed that application of the highest concentration of airdried mycelia.

The antioxidant is important to maintain human health against unfarmable reactive oxygen species (Rizzo, 2020). In this study, the antioxidant assay was performed to analyze the nutritional quality of soybean and observed the total antioxidant content was higher in T6 of methanol extract in comparison with other treatments. Scavenging activity in DPPH radicals' treatments treated with *T. hamatum* of chloroform extract was higher as compared to rest of the treatments. The increase in antioxidant content and DPPH could be due to the presence of higher secondary metabolites such as phenolic acids, isoflavones and anthocyanins. Singh *et al*. (2013) conducted an experiment on the extract of tomato fruit taken from *Trichoderma* treatments and found higher scavenging activity on DPPH radicals, than control. The total phenolic content (TPC) in methanol extracts of treatment T3 (*T. hamatum*) was found highly increased among treatments in the comparison of control. It could be due to their hydroxyl group polyphenolic substances have significant radical scavenging ability. In the present study, nutrients uptake (NPK) was significantly increased in inoculated plant especially T6 (*T. harzianum* + *T. hamatum*). The cooperative benefits of *T. hamatum* individual and combination inoculation apparently improved the relative growth, biomass, and nutrition assimilation of the host plant as compared to control. The combined application of *Trichoderma* species improved growth characteristics and also enhanced nutrients content (Tandon *et al*., 2018).

Conclusion

T. hamatum exhibited the most effective results in T3 and T6. The application of *T. harzianum* enhanced the morphological parameters when applied either individually or together with other *Trichoderma* species. Inoculation of *T. harzianum* + *T. hamatum* resulted the highest total chlorophyll content. Likewise, *T. hamatum* application resulted in the maximum potential of antioxidants in soybean. *T. hamatum* is recommended as the best biofertilizer either alone or together with other *Trichoderma* species such *as T. harzianum* for good growth of soybean crop in Pakistan.

Author's contributions

SR did research work, AA supervised the work and critically reviewed the manuscript, ZY did chemical analysis and reviewed the manuscript, ZM analyzed the data and contributed in paper writing, ZS helped in data curation.

Conflict of interests

Authors declare no conflict of interest.

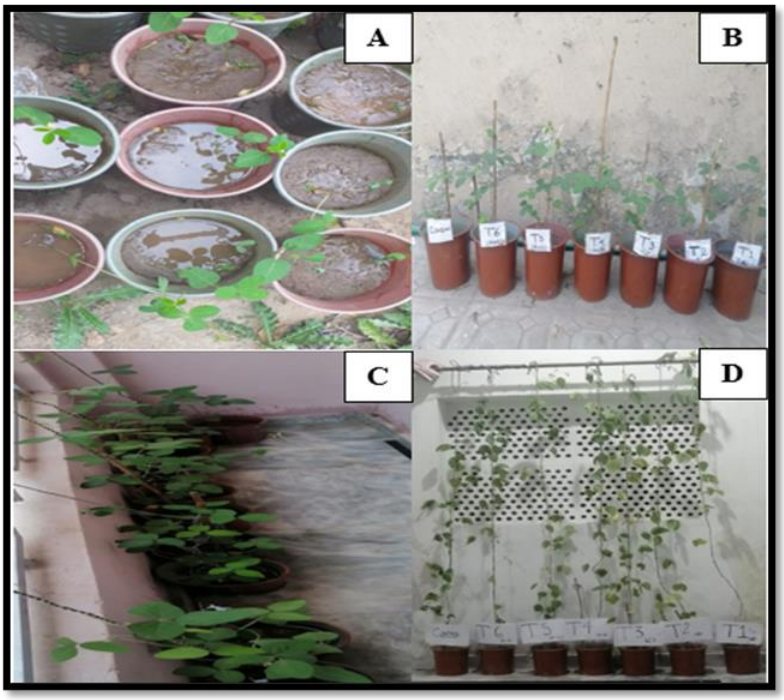

Fig. 1: Soybean plants at inoculation time (**A**), after 30 days (**B**), 60 days (**C**), and 90 days of sowing (**D**).

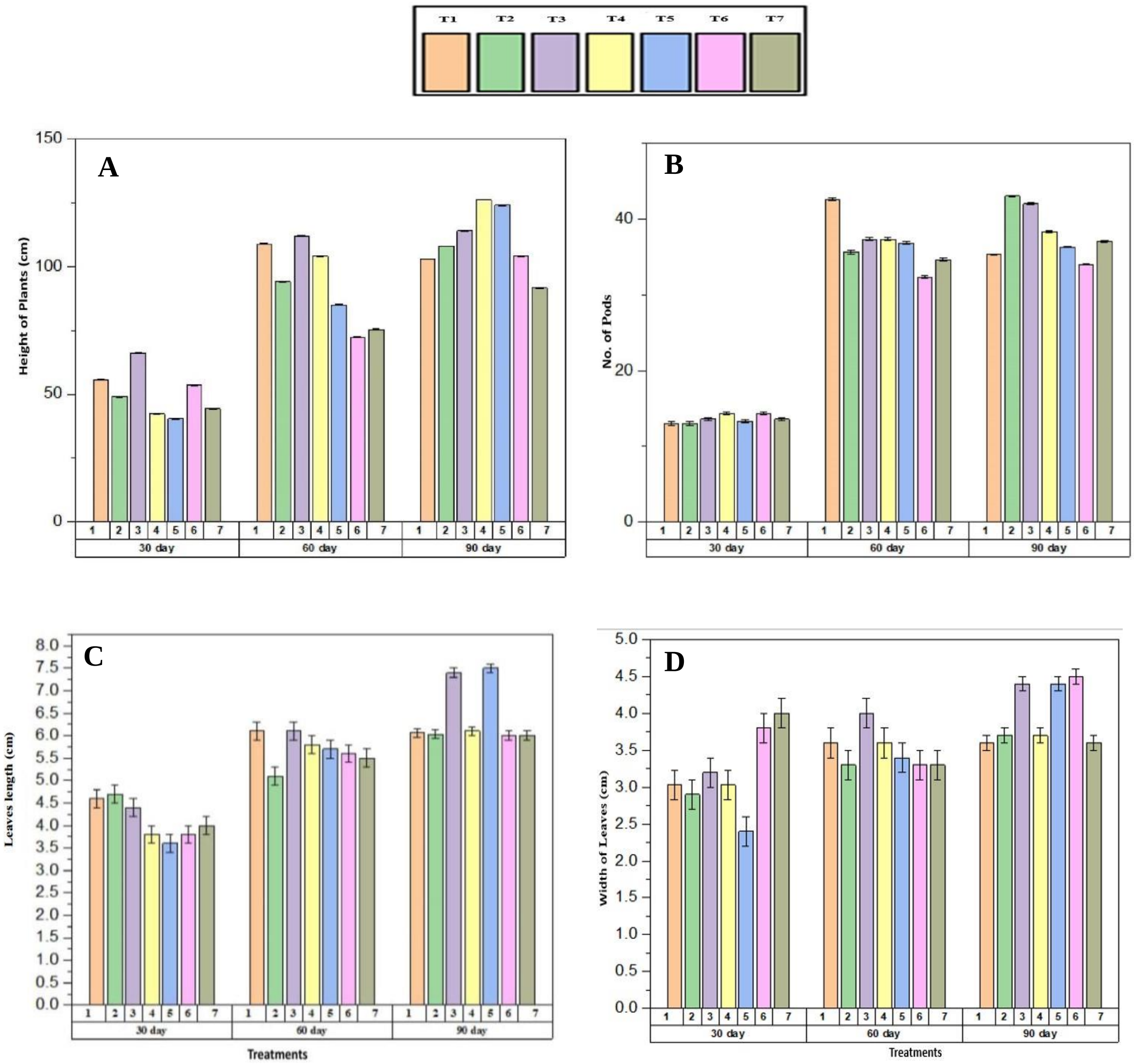

Fig. 2: Effect of different combinations of *Trichoderma* spp. on morphological traits of soybean. (**A**)- Height of plant (**B**)- No. of leaves, (**C**)- Length of leaf, (**D**)- Width of leaf. **T1** (*Trichoderma harzianum*), **T2** (*Trichoderma viride*), **T3** (*Trichoderma hamatum*), **T4** (*Trichoderma harzianum* + viride), **T5** (*Trichoderma viride* + *hamatum*), **T6** (*Trichoderma harzianum* + *hamatum*) and **T7** (control).

Table 1: The effect different *Trichoderma* spp. on NPK contents of soybean.

	Treatments						
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Nitrogen	3.5±0.09	3.28±0.14	3.7±0.09	3.61±0.03	3.5±0.07	3.8±0.03	3.11±0.02
Phosphorus	5.2±0.07	4.71±0.03	8.56±0.07	6.32±0.04	7.28±0.03	10.16±0.04	3.79±0.07
Potassium	287±5.9	249.5±4.07	319.6±6.62	259.1±7.33	254.9±8.06	355.1±15.4	100.7±0.44

T1 (*T. harzianum*), **T2** (*T. viride*), **T3** (*T. hamatum*), **T4** (*T. harzianum* + viride), **T5** (*T. viride* + *T. hamatum*), **T6** (*T. harzianum* + *T. hamatum*) and **T7** (control).

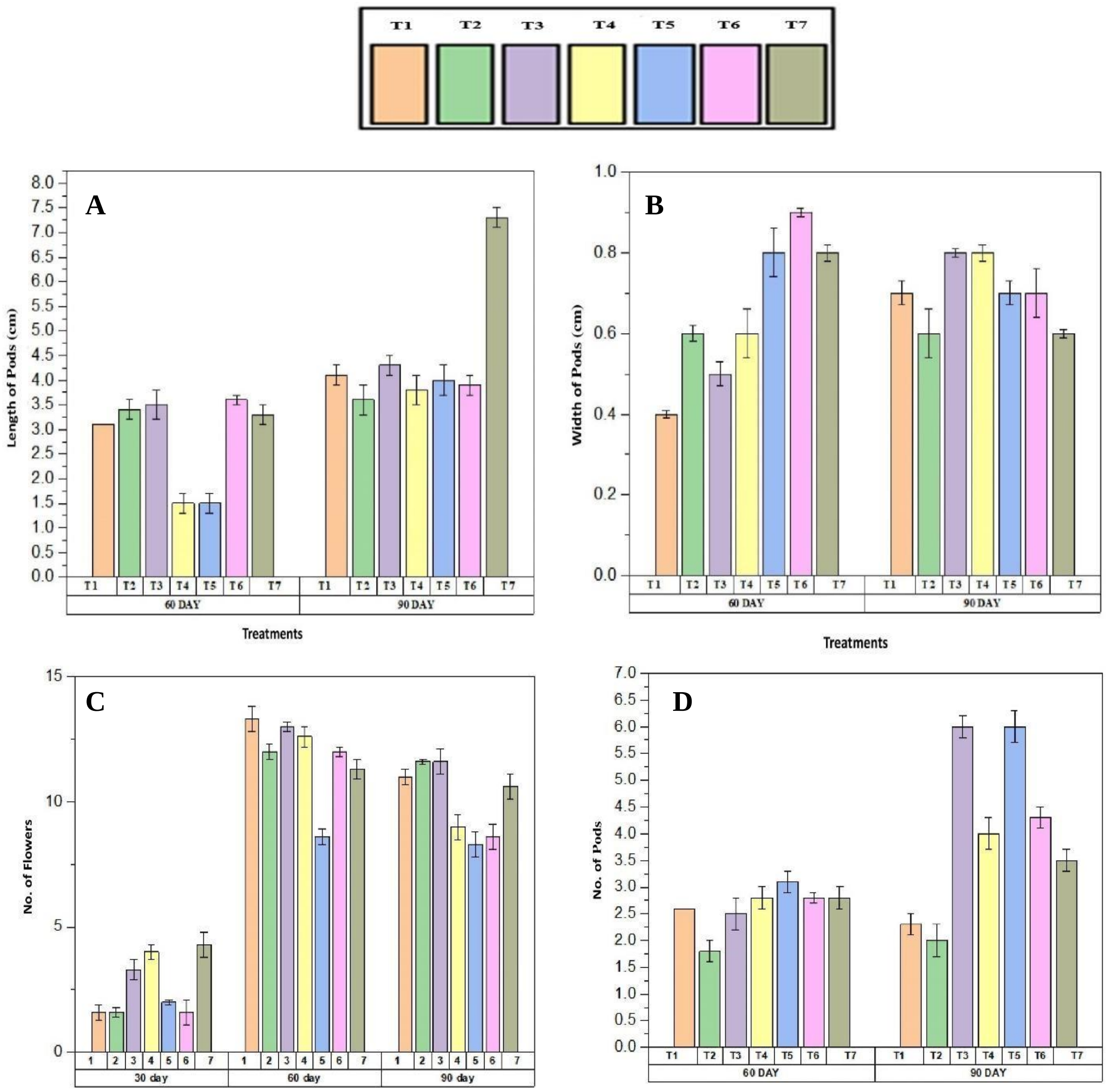

Fig. 3: Morphological traits of soybean. (**A**)- No. of flowers (**B**)- No. of pods, (**C**)- Length of Pod, and (**D**)- Width of pods). For details of treatments, see Fig. 2.

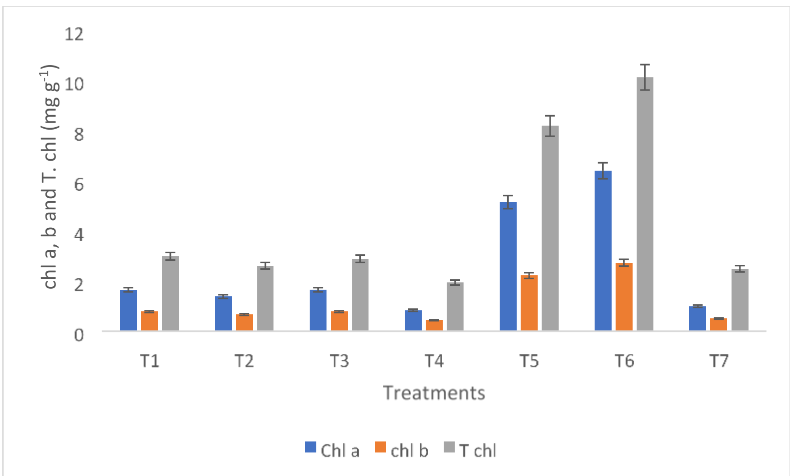

Fig. 4: Effect of inoculation of *Trichoderma* spp. on chlorophyll a, b and total chlorophyll contents of soybean.

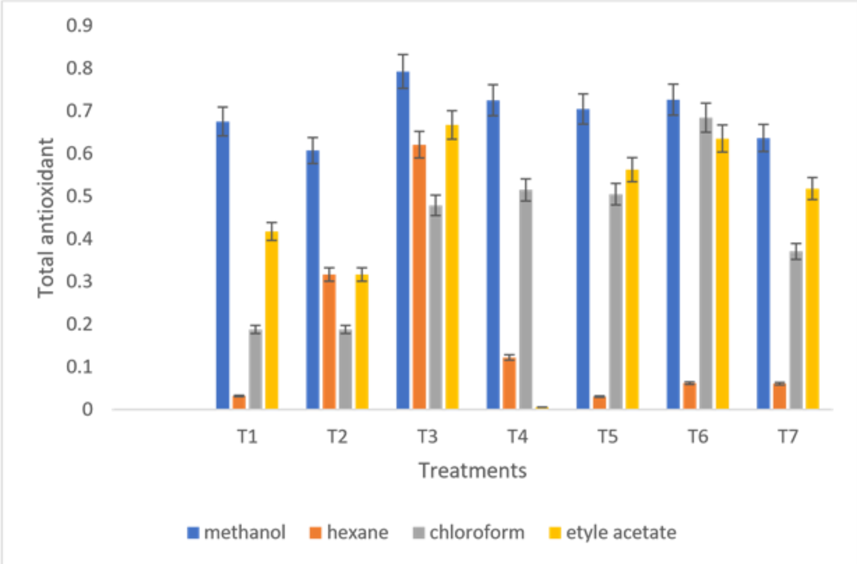

Fig. 5: Total antioxidant evaluation of various extracts of different treatments of soybean under different treatments. For details of treatments, see Fig. 2.

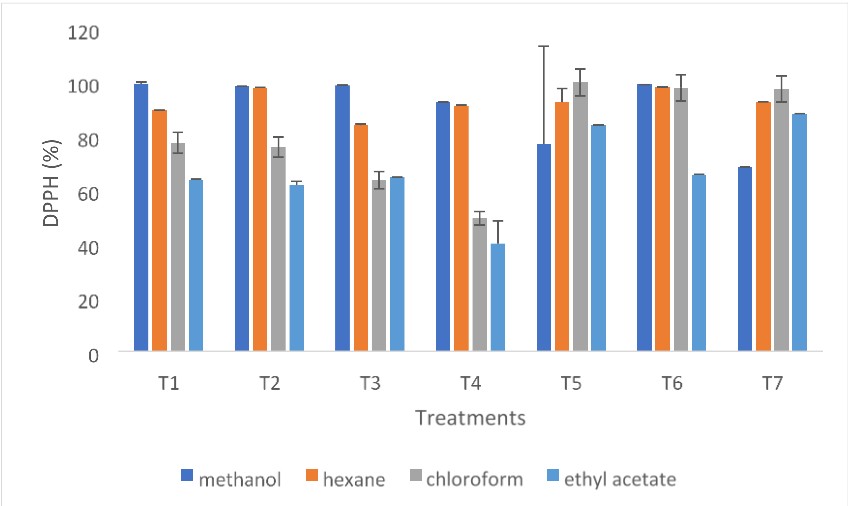

Fig. 6: Effect of application of different *Trichoderma* spp. on DPPH activity of various extracts of soybean. For details of treatments, see Fig. 2.

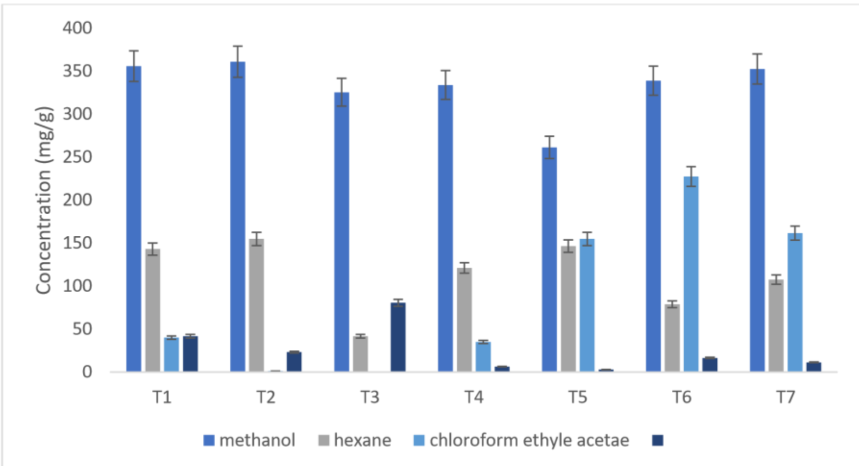

Fig. 7: Total phenolic contents estimation of selected extracts of soybean as affected by different *Trichoderma* spp. treatments. For details of treatments, see Fig. 2.

References

Aftab Z, Afzal M, Bushra M, Khan H, Khan S, 2019. Fistuloates A–C: New antioxidative aromatic compounds isolated from *Cassia fistula*. *J. Chem. Res.,* **43**: 516-521.

Ahmad F, Anwar F, Hira S, 2016. Review on medicinal importance of Fabaceae family. *Pharmacologyonline,* **3**: 151-157.

Akladious SA, Abbas SM, 2014. Application of *Trichoderma harzianum* T22 as a biofertilizer potential in maize growth. *J. Plant Nutr*. **37**(1): 30-49.

Ali A, Javaid A, Shoaib A, Khan IH, 2020. Effect of soil amendment with *Chenopodium album* dry biomass and two *Trichoderma* species on growth of chickpea var. Noor 2009 in *Sclerotium rolfsii* contaminated soil. *Egypt J Biol Pest Control,* **30**: 102.

Bakhshandeh E, Gholamhosseini M, Yaghoubian Y, Pirdashti H, 2020. Plant growth promoting microorganisms can improve germination, seedling growth and potassium uptake of soybean under drought and salt stress. *Plant Growth Regul*., **90**: 123-136.

Chung H, Canning C, Sun S, Zhou K, 2010. Comparison of different strategies for soybean antioxidant extraction. *J. Agric. Food Chem.,* **58**: 4508-4512.

Florencio C, Cunha FM, Farinas CS, 2015. Validation of a novel sequential cultivation method for the production of enzymatic cocktails from *Trichoderma* strains. *Appl. Biochem. Biotechnol*., **175**: 1389-1402.

Hossain MM, Sultana F, 2020. Application and mechanisms of plant growth promoting fungi (PGPF) for phytostimulation. *Organic Agric.*, **1**: 31.

Hossain MM, Sultana F, Islam S, 2017. Plant growth-promoting fungi (PGPF): Phytostimulation and induced systemic resistance. In: Singh D, Singh H, Prabha R (eds), *Plant-Microbe Interactions in Agro-Ecological Perspectives.* pp. 135-191.

Javaid A, 2009. Growth, nodulation and yield of black gram [*Vigna mungo* (L.) Hepper] as influenced by biofertilizers and soil amendments. *Afr. J. Biotechnol.,* **8**: 5711-5717.

Junior AFC, Chagas LFB, Colonia BSO, Miller LDO, de Oliveira JC, 2019. Trichoderma asperellum (UFT201) functions as a growth promoter for soybean plant. *Afr. J. Agric. Res.*, **14**: 1772-1777.

Keswani C, Mishra S, Sarma BK, Singh SP, Singh HB, 2014. Unraveling the efficient applications of secondary metabolites of various *Trichoderma* spp. *Appl. Microbiol. Biotechnol.*, **98**: 533-544.

Khan IH, Javaid A, 2020. *In vitro* biocontrol potential of *Trichoderma pseudokoningii* against *Macrophomina phaseolina*. *Int. J. Agric. Biol.,* **24**: 730-736.

Khan IH, Javaid A, Ahmed D, 2021. *Trichoderma viride* controls *Macrophomina phaseolina* through its DNA disintegration and production of antifungal compounds. *Int. J. Agric. Biol.,* **25**: 888-894.

Marra R, Lombardi N, d'Errico G, Troisi J, Scala G, Vinale F, Lorito M, 2019. Application of *Trichoderma* strains and metabolites enhances soybean productivity and nutrient content. *J. Agric. Food Chem.*, **67**: 1814-1822.

Nair R, Schafleitner R, Easdown W, Ebert A, Hanson P, D'arros HJ, Donough HKJ, 2014. Legume improvement program at AVRDC-The World Vegetable Center: Impact and future prospects. *Ratarstvo Povrtarstvo,* **51**: 55-61.

Pagano MC, Miransari M, 2016. The importance of soybean production worldwide. In Abiotic and biotic stresses in soybean production, *Pak. J. Agric. Sci.,* **6**: 1-26.

Rakibuzzaman M, Akand MH, Siddika M, Uddin AJ, 2021. Impact of *Trichoderma* application as bio-stimulator on disease suppression, growth and yield of potato. *J. Biosci. Agric. Res.*, **27**: 2252-2257.

Rizzo G, 2020. The antioxidant role of soy and soy foods in human health. *Antioxidants,* **9**: 631-635.

Saba H, Vibhash D, Manisha M, Prashant KS, Farhan H, Tauseef A, 2012. *Trichoderma*–a promising plant growth stimulator and biocontrol agent. *Mycosphere*, **3**: 524-531.

Shahidi F, Ambigaipalan P, 2015. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *J. Funct. Foods*, **18**: 820-897.

Singh AS, Lawrence K, Pandit S, 2014. Response of leaves, stems and roots of *Withania somnifera* to copper stress. *Pak. J. Bot.,* **4**: 63-69.

Singh SP, Singh HB, Singh DK, 2013. Effect of *Trichoderma harzianum* on mineral component and antioxidant activity of tomato fruits. *Vegetos*, **26**: 237-244.

Tandon A, Fatima T, Gautam A, Yadav U, Srivastava S, Singh PC, 2018. Effect of *Trichoderma koningiopsis* on chickpea rhizosphere activities under different fertilization regimes. *Open J. Soil Sci.,* **8**: 261.

Vafadar F, Amooaghaie R, Otroshy M, 2014. Effects of plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungus on plant growth, stevioside, NPK, and chlorophyll content of *Stevia rebaudiana*. *J. Plant Interact.*, **9**: 128-136.

Voora V, Larrea C, Bermudez S, 2020. Global market report: Soybeans. Winnipeg, MB, Canada: *Int. Inst. Sustain. Dev. J.,* **4**: 36-42.