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ALLEVIATING STRESS OF *SCLERTIUM ROLFSII* ON GROWTH OF CHICKPEA VAR. BHAKKAR-2011 BY *TRICHODERMA HARZIANUM* AND *T. VIRIDE*

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is attacked by *Sclerotium rolfsii* at the seedling stage and the resulting collar rot disease significantly reduces the survival percentage of the seedlings and ultimately yield of the crop. In order to reduce environmental pollution caused by the use of synthetic fungicides, this study was carried out to use two biocontrol agents, namely *Trichoderma harzianum* and *T. viride,* against *S. rolfsii*, and to investigate their effect on plant growth, yield and physiology of chickpea var. Bhakkar-2011. *S. rolfsii* inoculation reduced dry weight of shoot, root and grains by 21.4%, 36.5% and 49%, respectively, over negative control. *T. harzianum* and *T. viride* increased shoot dry weight by 120% and 362%, root dry weight by 132% and 138%, and grain yield by 1109% and 572%, respectively, over positive control (*S. rolfsii* inoculated only). The effects of the pathogen and the two biocontrol agents were also studied on chlorophyll, carotenoid and phenolic contents as well as on activities of antioxidant enzymes *viz*. peroxidase (POX), phenylalanine ammonia lyase (PAL) and catalase (CAT). *S. rolfsii* inoculation suppressed chlorophyll and carotenoid contents while both the *Trichoderma* spp. increased these parameters many folds. Phenolic content and activities of POX, PAL and CAT were generally increased due to *S. rolfsii* but became normal due to application of *Trichoderma* spp. This study concludes that *T. harzianum* and *T. viride* are the potential biocontrol agents for control of collar rot of chickpea var. Bhakkar-2011.

Keywords: Biocontrol, chickpea, *Sclerotium rolfsii, Trichoderma harzianum*, *Trichoderma viride.*
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INTRODUCTION

Sclerotium rolfsii Sacc. is a serious pathogen in tropical and sub-tropical areas with a wide host range of about 500 species of plants (Jacob *et al.,* 2018). Being soil-borne in nature, it is responsible for collar rot, an emerging plant disease that may incite 65–90% mortality of chickpea seedlings. High soil moisture in combination with 28-30°C temperature are the favorable environmental conditions for its growth and infection (Pravi *et al.,* 2015; Rajani *et al.,* 2019). At the initial stages of infection, it may result in stem decay followed by wilting and plant death. At advanced infectious stages, the stem near the soil line bears white mycelial growth and tan to brown sclerotial bodies (Queiroz *et al.,* 2017; Zheng *et al.,* 2020). The pathogen can survive in plant tissues or in plant debris that upon conducive conditions attack the chickpea collar region (Tarafdar *et al.,* 2018).

Management of *S. rolfsii*is is somewhat challenging due to its prolific growth, wide host range, and having the capability to form abundant sclerotia that may remain in the soil up to 7 years under harsh conditions (Rodriguez-Kabana *et al.*, 1980; Bholanath and Papiya, 2017). Many efforts have been employed to control this devastating pathogen but met with limited success due to lack of sufficient information (Lal *et al.,* 2015). Different fungicides are in practice but they are not economical in the long run because they damage the environment, pollute the atmosphere and their repeated use also leaves detrimental effects on beneficial microorganisms (Kumar *et al.,* 2018). Of the various methods used to manage *S. rolfsii*, the use of biocontrol agents gave promising results with little or no hazardous effects on the environment (Rajani *et al.,* 2019; Javaid *et al.*, 2020; Sharf *et al.,* 2021). In current years, biocontrol of soil-borne diseases by microbial antagonists has been widely reported (Rabinal and Bhat, 2020; Shoaib *et al.,* 2020). *Trichoderma* spp. are widespread saprophytic, soil-inhabiting filamentous fungi that have the ability to antagonize various pathogenic fungi resulting in reduced disease incidence (Chen *et al.,* 2016; Khan *et al.,* 2021). Mycoparasitism, cell-wall degrading enzymes and antifungal compounds production, antibiosis and competition for space and nutrients are the possible mechanisms of actions of these fungi against the fungal pathogens (Divya and Sadasivan, 2016; El-Sobky *et al.,* 2019; Khan and Javaid, 2020). The present study was undertaken to assess the beneficial effects of the application of two *Trichoderma* species on growth and physiology of chickpea variety Bhakkar-2011 growing in *S. rolfsii* contaminated soil.

MATERIALS AND METHODS

Pot trial: The effectiveness of the two *Trichoderma* species against *S. rolfsii* on chickpea var. Bhakkar-2011 was scrutinized under soil treatment conditions following methods described by Javaid *et al.* (2020). Inoculations of the *Trichoderma* species and *S. rolfsii* were conducted according to the following combinations: T_1: negative control; T_2: positive control [*S. rolfsii* (SR)]; T_3: *T. harzianum* (TH) + SR and T_4= *T. Viride* (TV) + SR. There were five replications per treatment. *T. harzianum*, *T. viride* and *S. rolfsii* were separately propagated on pearl millet seeds in conical flasks, and the grain cultures were used as inocula for the respective treatments. Soil was fumigated with wet balls of cotton wool dipped in formalin, and was filled in the pots (5 kg/pot). For T_2–T_4, sterilized soil was inoculated with *S. rolfsii* (50 g/pot) propagated on the pearl millet grains, and kept for 7 days for the pathogen establishment. For T_1, the pots were mixed with the same quantity of boiled grains without any inoculum. However, in the case of T_3 and T_4, the pathogen inoculated pots were also inoculated (50 g/pot) with TH and TV, respectively and kept for another 7 days. Chickpea seeds were surface sterilized with 1% NaOCl solution and were sown at 10 seeds/pot). All the pots were watered regularly, arranged randomly and kept under the natural environmental conditions. The plants were harvested at maturity and, shoots and roots were separated. Roots were washed gently over a sieve using tap water. Data regarding root and shoot lengths, as well as dry weights of shoots, roots, pods and grains were recorded.

Estimation of chlorophyll and carotenoid contents: Leaf sample was grounded in 80% ethanol and the homogenized sample was centrifuged at 10,000 rpm for 5 min, while the supernatant was assessedfor chlorophyll a, chlorophyll b, and carotenoids by recording absorbance at 645 nm, 663 nm, and 270 nm, respectively. Total chlorophyll content and carotenoids were calculated using the formula specified by Lichtenthaler and Wellburn (1983).

Estimation of phenolic content: Total phenolic content of ethanolic leaf extract was estimated using Folin-Ciocalteatou reagent and taking absorbance of the mixture at 765 nm (Singleton and Rossi, 1965).

Estimation of antioxidant enzymes: CAT activity of the reaction mixture (leaf extract + 75 mM phosphate buffer + 112 mM H_2O_2) was examined as change in absorbance as a result of H_2O_2 consumption at 240 nm (Havir and McHale, 1987).

For POX assay, a reaction mixture was prepared by adding 25 mM phosphate buffer, 20 mM pyrogallol and 20 mM hydrogen peroxide in the leaf extract. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm (Kar and Mishra, 1976).

PAL activity was analyzed in the reaction mixture consisted of leaf extract, 0.1 M sodium borate buffer and 12 mM phenylalanine. A change in absorbance was recorded at 270 nm spectrophotometrically (Cochrane *et al.*, 2004).

Statistical analysis: One-way ANOVA was applied to analyze the data regarding root and shoot growth, grain yield, chlorophyll, carotenoid and phenolic contents, and activities of POX, PAL and CAT, followed by the application of LSD test to determine significant differences among the treatments mean at $P \leq 0.05$ using Statistix 8.1.

RESULTS AND DISCUSSION

Effect of *S. rolfsii* and *Trichoderma* spp. on plant growth and yield: Application of *S. rolfsii* (T_2) reduced all the growth and yield related parameters when compared with uninoculated negative control treatment (T_1). Addition of the pathogen reduced shoot length and biomass by 17.3% and 21.4%; root length and biomass by 26.10% and 36.5%, and dry weights of pods and grains by 39% and 49%, respectively, over T_1 (Fig. 1 and 2). *S. rolfsii* is a highly problematic soil-borne fungal pathogen and is also responsible for causing similar reductions in growth and yield of many other plant species including chickpea (Khan *et al.,* 2020), mungbean (Sun *et al.,* 2020) and tomato (Sahu *et al.,* 2019).

Both the *Trichoderma* species improved plant growth and yield under the stress of *S. rolfsii.* Boost in growth and yield due to the inoculation of *Trichoderma* species was generally significantly higher than T_1 and T_2. Earlier studies have shown that different species of *Trichoderma* have the potential to alleviate the stress of various pathogens and improve crop growth and yield (Akrami*et al.,* 2011; Liu *et al.,* 2020). Physical as well as chemical mechanisms are involved in the control of fungal pathogens and an increase in plant growth by application of *Trichoderma* spp. Different substances are released by *Trichoderma* spp. which provoke resistance in the host against the disease-causing agents (Gary *et al.,* 2004). Some species of *Trichoderma* also act as antagonists against pathogenic fungal species (Doley and Jite, 2012; Al-Ani and Mohammed, 2020). Recently, it has been reported that *Trichoderma* species suppress the growth of *M. phaseolina* through its DNA disintegration (Khan and Javaid, 2020; Khan *et al.,* 2021).

T. harzianum increased shoot length by 98% and 139%, shoot dry weight by 82% and 132%, root length by 29% and 74%, root dry weight by 40% and 120%, pod weight by 280% and 533% and grain yield by 504% and 1109% over negative and positive control treatments, respectively. *T. harzianum* is known for its biocontrol

activity against a number of phytopathogenic fungi. It is known for its biocontrol activity against *Sclerotinia sclerotiorum* in soybean as reported by Zhang *et al.* (2016), *Macrophomina phaseolina* in mungbean (Javaid *et al.,* 2017), *Fusarium oxysporum* f. sp. *cepae* in onion (Akhtar and Javaid, 2018) and *Fusarium solani* in olive trees (Amira *et al.,* 2017). Earlier, Rekha *et al.* (2012) reported that metabolites of *T. harzianum* limited the formation of zoospore and germ tube, and the growth of mycelium of *S. rolfsii*. Youssef *et al.* (2016) reported that the protection provided by *T. harzianum* against *Rhizoctonia solani* was related to increase in activities of guaiacol peroxidase, ascorbate peroxidase, catalase and superoxide dismutase. Recently, Bader *et al.* (2020) demonstrated that native Argentina strains of *T. harzianum* produce auxin indole 3-acetic acid, solubilize phosphate, enhance growth and control tomato wilt caused by *F. oxysporum*. The aspartic protease P6281 produced by *T. harzianum* plays a vital task in mycoparasitism on plant pathogenic fungal species (Deng *et al.,* 2018).

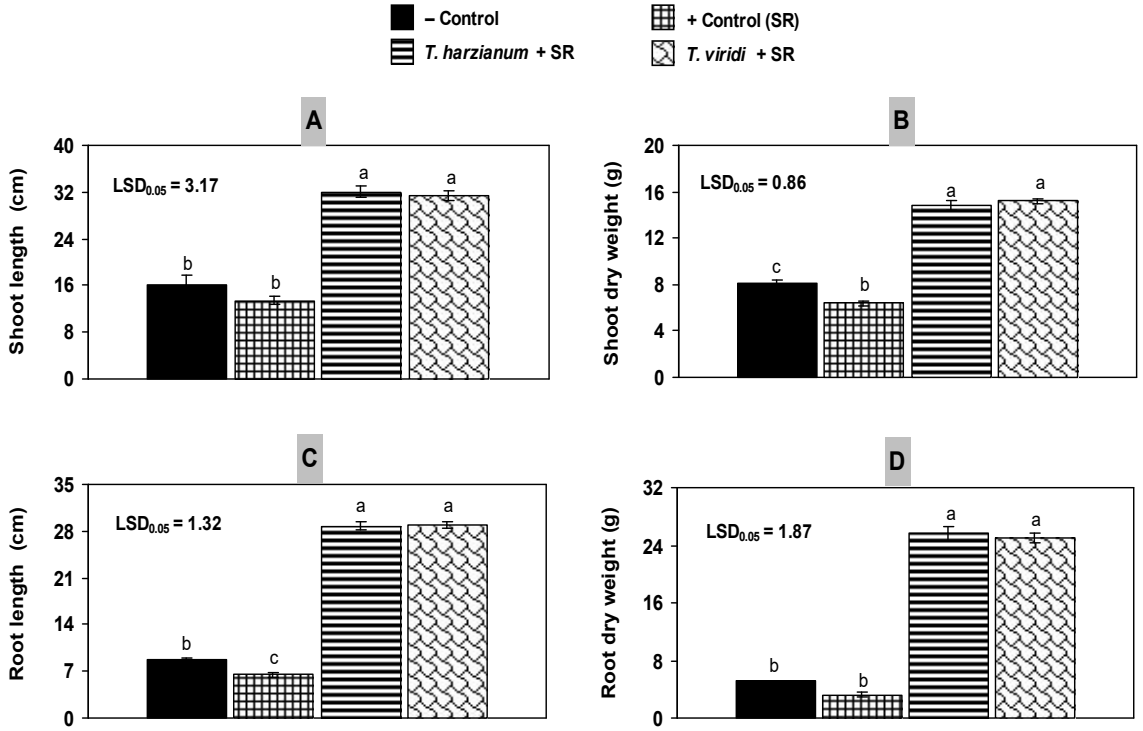

Fig. 1: Effect of *Sclerotium rolfsii* (SR), *Trichoderma harzianum* and *T. viride* on shoot and root growth of chickpea var. Bhakkar-2011. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

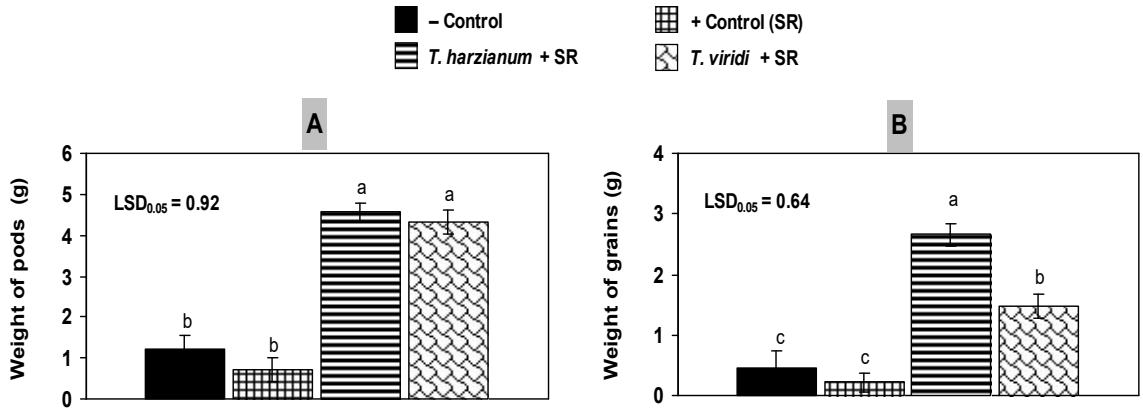

Fig. 2: Effect of *Sclerotium rolfsii* (SR), *Trichoderma harzianum* and *T. viride* on pod and grain yield of chickpea var. Bhakkar-2011. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

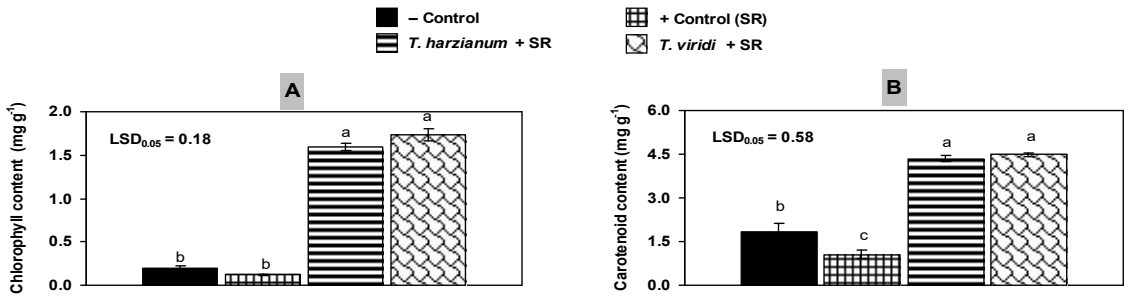

Fig. 3: Effect of *Sclerotium rolfsii* (SR), *Trichoderma harzianum* and *T. viride* on leaf chlorophyll and carotenoid contents of chickpea var. Bhakkar-2011. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

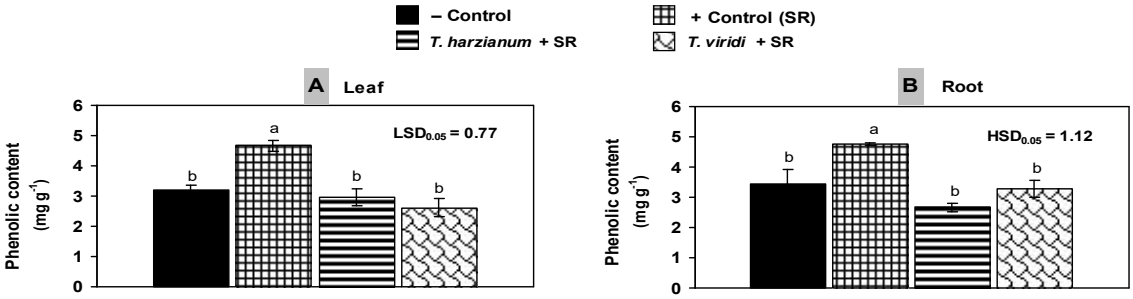

Fig. 4: Effect of *Sclerotium rolfsii* (SR), *Trichoderma harzianum* and *T. viride* on phenolic content of leaf and root of chickpea var. Bhakkar-2011. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

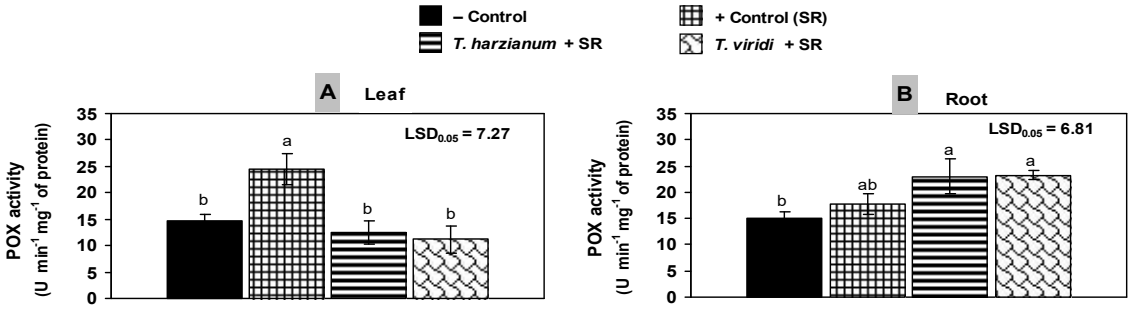

Fig. 5: Effect of *Sclerotium rolfsii* (SR), *Trichoderma harzianum* and *T. viride* on peroxidase activity (POX) of leaf and root of chickpea var. Bhakkar-2011. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

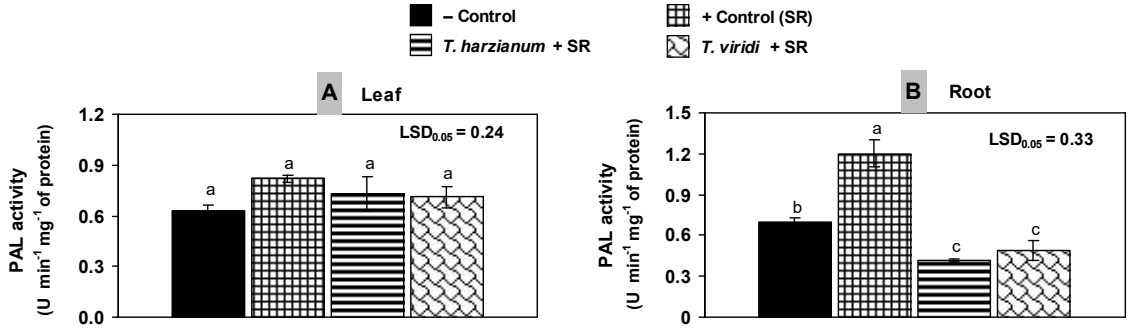

Fig. 6: Effect of *Sclerotium rolfsii* (SR), *Trichoderma harzianum* and *T. viride* on phenylalanine ammonia lyase activity (PAL) of leaf and root of chickpea var. Bhakkar-2011. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

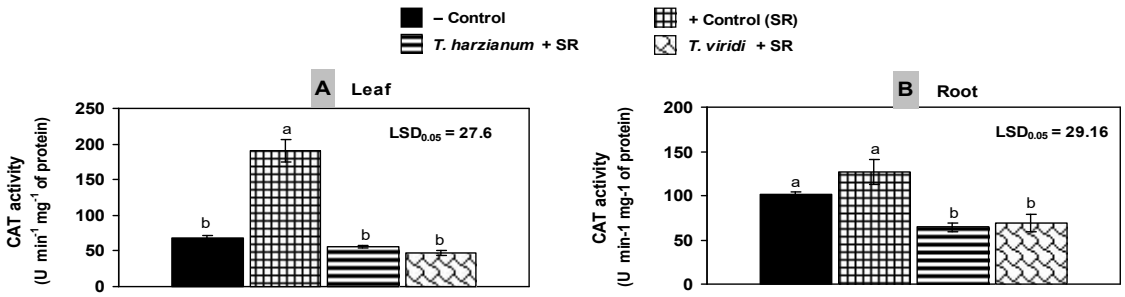

Fig. 7: Effect of *Sclerotium rolfsii* (SR), *Trichoderma harzianum* and *T. viride* on catalase activity (CAT) of leaf and root of chickpea var. Bhakkar-2011. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

T. viride application was also proved highly beneficial resulting in an increase of 94% and 135% in shoot length, 87% and 138% in shoot dry weight, 104% and 176% in root length, 194% and 362% in root dry weight, 260% and 500% in pod weight and 236% and 572% in grain yield over T_1 and T_2, respectively. Earlier reports indicated that *T. viride* has the potential to control *in vitro* development of other pathogenic fungi namely *M. phaseolina* and *F. oxysporum* (Javaid *et al.,* 2014, 2018). It has been found effective in controlling brown blotch disease of cowpea through the production of an antifungal compound viridian (Bankole and Adebanjo, 1996). It is also known to control seed-associated fungi, namely *Fusarium moniliforme* and *Aspergillus flavus* through production of lipolytic, pectinolytic proteolytic and cellulolytic enzymes (Calistru *et al*., 1997). It was also proved as a highly effective biocontrol agent against root pathogens of soybean namely *Pythium arrhenomanes* and *F. oxysporum* f. sp. *adzuki* resulting in improved crop growth (John *et al.,* 2010).

Effect of *S. rolfsii* and *Trichoderma* spp. on plant physiology: Photosynthetic pigments were significantly decreased under pathogenic stress (T_2) as compared to un-inoculated control (T_1).However, either of the *Trichoderma* species proved very effective in tremendously improving the said attributes up to 5-folds as compared to T_2. Reduction in the photosynthetic pigments in T_2 indicated that biotic stress might cause irregularities in photosynthesis and respiration rates, which generally causes an intensified level of reactive oxygen species (ROS). Hence reduced chlorophyll content is a typical symptom of stressed plants incurred by the pathogen (Nafisa *et al.,* 2020). Likewise, total phenolic content was increased and activities of PAL, CAT and POX were significantly higher in T_2 with respect to T_1, which is in agreement to the earlier studies (Hossain *et al.,* 2016; Shoaib *et al.,* 2018; Nafisa *et al.,* 2020). Higher enzyme activities may be correlated with higher ROS accumulation in the stressed cells as enzymes contribute to lowering the ROS levels. Furthermore, higher CAT and POX may show their higher consumption as a result of oxidative stress. However, it seems that the chickpea plant might not be able to handle stress posed by the pathogen, which resulted in disease in plants. Biocontrol agent's inoculations seem effective as either species of *Trichoderma* has been known to proliferate faster, colonizes root surface and secrete cell wall hydrolyzing enzymes along with bioactive compounds, which likely to limit pathogen growth as well enhanced plant resistance. Besides, *Sclerotium* spp. are known to secrete some lectins, which can stimulate *Trichoderma* to coil around pathogen hyphae (Srivastava *et al.,* 2015). Therefore, treatments provided with biocontrol organisms in the form of *T. viride* and *T. harzianum* normalized the effects on chickpea plants, which would have to face in case of pathogen attack.

Conclusion: This study clearly indicates that *T. harzianum* and *T. viride* have pronounced the potential to increase crop growth and yield of chickpea var. Bakhar-2011 many folds under the biotic stress of *S. rolfsii* by regulating plant physiology.

Author's contribution: Amna Ali carried out experimental work. Arshad Javaid supervised the whole work, wrote a part of the paper, and did the statistical analysis. Amna Shoaib did physiological studies. Iqra Haider Khan contributed to the writing of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

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