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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

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Abstract

Sclerotium rolfsii is a soil-borne plant pathogen that causes root diseases in hundreds of plant species. It also causes collar rot disease in chickpea (*Cicer arietinum* L.). The present pot study was carried out to investigate the effect of soil amendment with dry biomass of a weed *Chenopodium album* L. and two antagonistic fungi, namely *Trichoderma harzianum* and *T. viride*, on growth and yield of chickpea variety Noor 2009 in soil infected with *S. rolfsii*. The pathogen-contaminated soil was amended either with 1, 2, or 3% *C. album* dry biomass, *T. harzianum*, and *T. viride* alone, or combinations of either of the two *Trichoderma* species and plant dry biomass. The lowest shoot and root dry biomass and grain yield of chickpea were recorded in *S. rolfsii* inoculation alone without any soil amendment (positive control). Plant growth and yield were significantly and gradually increased over positive control with an increase in *C. album* dry biomass application in the soil. Likewise, soil application of either of the two *Trichoderma* species significantly enhanced plant growth and yield over positive control under biotic stress of *S. rolfsii*. Combined application of either *T. harzianum* or *T. viride* with 3% dry biomass of *C. album* also proved highly effective in alleviating biotic stress of *S. rolfsii* on growth and yield of chickpea.

Keywords: Biological control, *Chenopodium album*, Chickpea var. Noor 2009, Collar rot, *Trichoderma* spp

Background

Sclerotium rolfsii is a soil-borne phytopathogen commonly found in tropical and sub-tropical regions of the world (Sumi et al. 2018). It causes collar rot, root rot, foot rot, and wilt diseases in more than 500 plant species (Sun et al. 2020). Of these, collar rot caused by *S. rolfsii* is the major limiting constrain in chickpea cultivation, which causes considerable yield losses up to 45% (Sarkar et al. 2014). The pathogen has the capability of producing excessive sclerotia that may persist in the soil for several years in the form of resistant resting structures under humid weather condition (Harlton et al. 1995). The typical

symptoms of the disease include rapid plant wilting with dark brown lesions at the stem base which later on girdles the main stem. Infected plant tissues also show a white mycelial growth that often radiates over the soil surface (Acabal et al. 2019). Because of resistant sclerotia formation, the control of collar rot pathogen is not easy, and it implies a combination of biological, cultural, and chemical approaches (Dwivedi and Prasad 2016).

The use of chemical fungicides is an effective management strategy, but keeping in view the complexities arising from their usage has led the scientists to adopt some alternative practices (Shrestha et al. 2020). In recent years, the application of natural plant products and bio-control agents have provided a promising alternate to

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the existing fungicides for more effective disease control with less hazardous impact on environment, wild life, human health, and beneficial microbes (Javaid et al. 2020). Products obtained from plants are a rich source of bioactive substances that are eco-friendly, curative, and protective against many fungal diseases (Khan and Javaid 2020). *Chenopodium album* belongs to family *Chenopodiaceae* is widely distributed in Asia, Europe, and North America (Krak et al. 2016). It contains flavonoids, isoflavonoids, aldehydes, saponins, polyphenols, apocarotenoid, and alkaloids (Mahour et al. 2008) with diverse antifungal activities against *Fusarium oxysporum*, *F. solani*, *Pythium aphanidermatum*, *Ascochyta rabiei*, *Rhizoctonia solani*, *Alternaria radicina*, and *A. dauci* (Semina et al. 2016 and Alkooranee et al. 2020).

Trichoderma genus has long been recognized as a bio-control agent to control fungal pathogens (Rabinal and Bhat 2020). The application of *Trichoderma* spp. against *S. rolfisii* has been received a great attention because of their excellent root colonization abilities, production of antifungal metabolites, and lysis of sclerotia (Hirpara et al. 2019). The mechanism through which they suppress the growth of fungal pathogens includes induction of plant defense responses, production of cell wall degrading enzymes, antibiosis, mycoparasitism, and competition for nutrients and niches (Wonglom et al. 2019). Despite the proven antagonistic potential of *Trichoderma*, there is still a need to further evaluate its efficacy by using available chickpea varieties grown in different geographical regions. Therefore, the present study was carried out to investigate the antifungal potential of *C. album* in combination with two *Trichoderma* species, namely *T. harzianum* or *T. viride* to control collar rot disease of chickpea.

Materials and methods

Pot trial

For possible management of collar rot disease and subsequent improvement in growth and yield of chickpea, pot soil was amended with dry biomass of *C. album* and two antagonistic fungi, namely *T. harzianum* and *T. viride*, the protocol given by Javaid et al. (2017) was generally followed with some modifications.

Inoculum of *S. rolfisii* was prepared on pearl millet seeds. The seeds were moderately boiled and water was strained then seeds were air dried to remove moisture from the surface. Seeds were packed in polythene bags and autoclaved for 30 min at 121 °C. Afterward, seeds were cooled and inoculated with discs of *S. rolfisii* and incubated for 7 days at 28 °C.

In the pot trial, there were following 13 treatments:

T₁ = Negative control

T₂ = Positive control [*Sclerotium rolfisii* (SR)]

T₃ = 1% *C. album* biomass + SR

T₄ = 2% *C. album* biomass + SR

T₅ = 3% *C. album* biomass + SR

T₆ = *T. harzianum* (TH) + SR

T₇ = *T. viride* (TV) + SR

T₈ = 1% *C. album* biomass + TH + SR

T₉ = 2% *C. album* biomass + TH + SR

T₁₀ = 3% *C. album* biomass + TH + SR

T₁₁ = 1% *C. album* biomass + TV + SR

T₁₂ = 2% *C. album* biomass + TV + SR

T₁₃ = 3% *C. album* biomass + TV + SR

Each treatment had five replicates and pots were arranged in a completely randomized design.

The soil used in the experiment was sandy loam in texture having pH 7.7, 95 mg kg⁻¹ potassium, 6.3 mg kg⁻¹ phosphorous, and 0.84% organic matter. Soil was fumigated with formylene-dipped cotton swabs and was covered with polythene sheet for 1 week to eliminate any kind of pathogens and insects. Thereafter, polythene sheet and cotton swabs were removed and soil was left for 1 week to evaporate any traces of the fumigant.

Earthen pots of 27 cm diameter were filled with 5 kg fumigated soil in each pot. Inoculum of *S. rolfisii*, prepared on pearl millet seeds, was thoroughly mixed in soil of each pot at 50 g per pot. After that, pots were irrigated and left for a week under natural environmental conditions for development of the pathogen in the soil. The pathogen was mixed in the soil of all the pots except those of negative control. In negative control, however, same amount of autoclaved pearl millet seeds was mixed in the pot soil. After 1 week, inocula of *T. harzianum* and *T. viride* were mixed in soil of the respective pots at 50 g per pot. In remaining pots, same amount of boiled pearl millet seeds was mixed. Pots were again left for 1 week after irrigation with a good quality tap water. After that, dry biomass of *C. album* was mixed at 1, 2, and 3% (w/w) in soil of respective pots, irrigated with tap water and left for 1 week for stabilizing the conditions.

Seeds of chickpea variety Noor 2009 were surface sterilized by 1% sodium hypochlorite solution for 3 min. After thorough washing with sterilized water, 20 seeds of each variety per pot were sown. Once seeds were germinated, thinning was done to sustain 10 uniform seedlings per pot. Pots were watered according to the requirement of the crop.

Plants were harvested at maturity. Data for total pod and seed weight, and root and shoot length were recorded. Plant materials were placed in an oven at 60 °C to constant weight and finally root and shoot biomasses were recorded in gram.

Physiological bioassays

Total chlorophyll and carotenoids extraction were carried out by mixing of 500 mg fresh leaf material (at

flowering sage) in 80% of chilled ethanol (10 ml). Mixture was homogenate at 800 rpm for 15 min using centrifuge machine. The supernatant was evaluated for chlorophyll content. The absorbance was checked for chlorophyll a at 645 nm, for chlorophyll b at 663 nm, and for carotenoids at 470 nm by UV-spectrophotometer. The observed values were analyzed quantitatively, and the amount of chlorophyll was estimated by using the formula of Lichtenthaler and Buschmann (2001). The quantities of chlorophyll and carotenoid content were expressed as mg g⁻¹ of fresh plant weight.

Leaves and root samples of each treatment were collected at flowering stage for enzyme assays. One

gram of sample from each treatment was grinded in pre chilled liquid nitrogen with 0.1 M sodium phosphate buffer (4 ml) using pestle and mortar. Centrifugation of the mixture was done for 15 min at 1000 rpm. The supernatant was considered as crude enzyme extract for evaluating phenyl alanine ammonia lyase (PAL) and catalase (CAT) activities (Ramanujam et al. 2012). Estimation of phenyl PAL and CAT activities were carried following procedures of Dikerson et al. (1984) and Beers and Sizer (1952), respectively. The enzyme activity was expressed as U min⁻¹ mg⁻¹ of protein. For estimation of phenolic content, methodology of Malik and Singh (1980) was adopted and

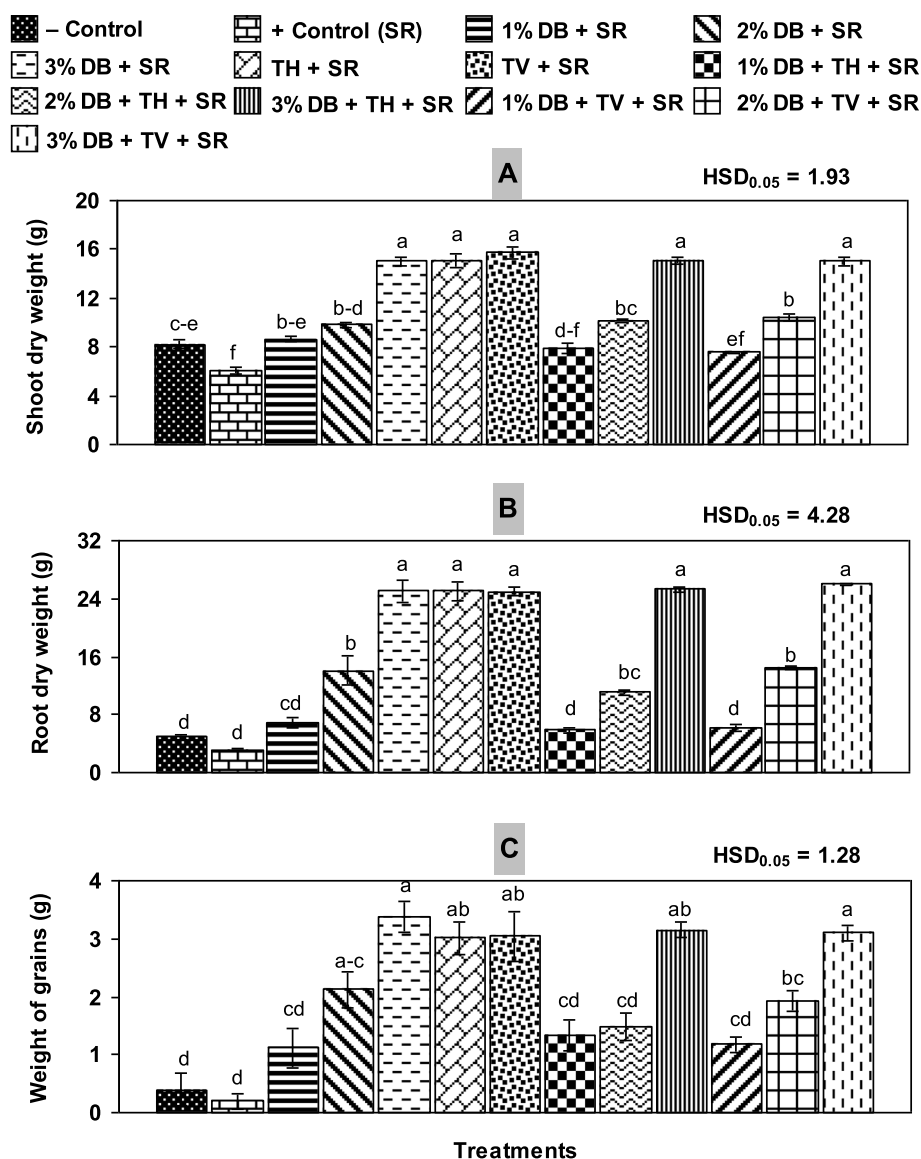


Fig. 1 Effect of *Sclerotium rolfsii* (SR), *Trichoderma harzianum* (TH), *T. viride* (TV), and dry biomass (DB) of *Chenopodium album* on growth and yield of chickpea var. Noor 2009. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference ($p \leq 0.05$) as determined by Tukey's HSD test

the quantity of total phenol was expressed in mg g^{-1} of fresh plant weight.

Statistical analysis

All data were subjected to analysis of variance (ANOVA), followed by Tukey’s HSD test to delineate the treatment means at $p \leq 0.05$ using computer software Statistix 8.1.

Results and discussion

Effect of soil amendments on plant growth and yield

Shoot dry weight in negative control was 8.2 g that was decreased to 6.02 g on inoculation of *S. rolf sii* resulting in 26% decrease in positive control as compared to negative control. Application of dry biomass of *C. album* (DBC) significantly ($p \leq 0.05$) increased shoot dry weight that gradually increased by increasing dose of DBC. As a result, there was 42–148% increase in chickpea shoot dry weight due to different doses of DBC over positive control. Separate applications of *T. harzianum* and *T. viride* also enhanced shoot dry weight tremendously by 148 and 159% over positive control, respectively. The

effect of combined application of *C. album* + *T. harzianum* and *C. album* + *T. viride* was similar to that of their separate applications, and there was no any synergistic effect of the combined application of DBC and the biological control agents (Fig. 1a). *S. rolf sii* inoculation reduced root dry biomass by 34% than the negative control. Application of 3% dry biomass of *C. album* alone or combined with application of either *T. harzianum* or *T. viride* exhibited tremendous increase in dry biomass of root that was 681–712% higher than positive control. The effect of separate application of either *T. harzianum* or *T. viride* was similar to effect of 3% *C. album* amendment (Fig. 1b). The lowest grain yield viz. 0.20 g was recorded in positive control. Different soil amendment treatments increased the yield variably and significantly. It was gradually and significantly increased with an increase in DBC as soil amendment. Grain yield was increased to 1.12, 2.12, and 3.37 g due to 1, 2, and 3% soil amendments with DBC that was 460, 960, and 1585% higher than the positive control, respectively. Applications of *T. harzianum* and *T. viride* either alone or combined with 3% *C. album* biomass had an effect on yield

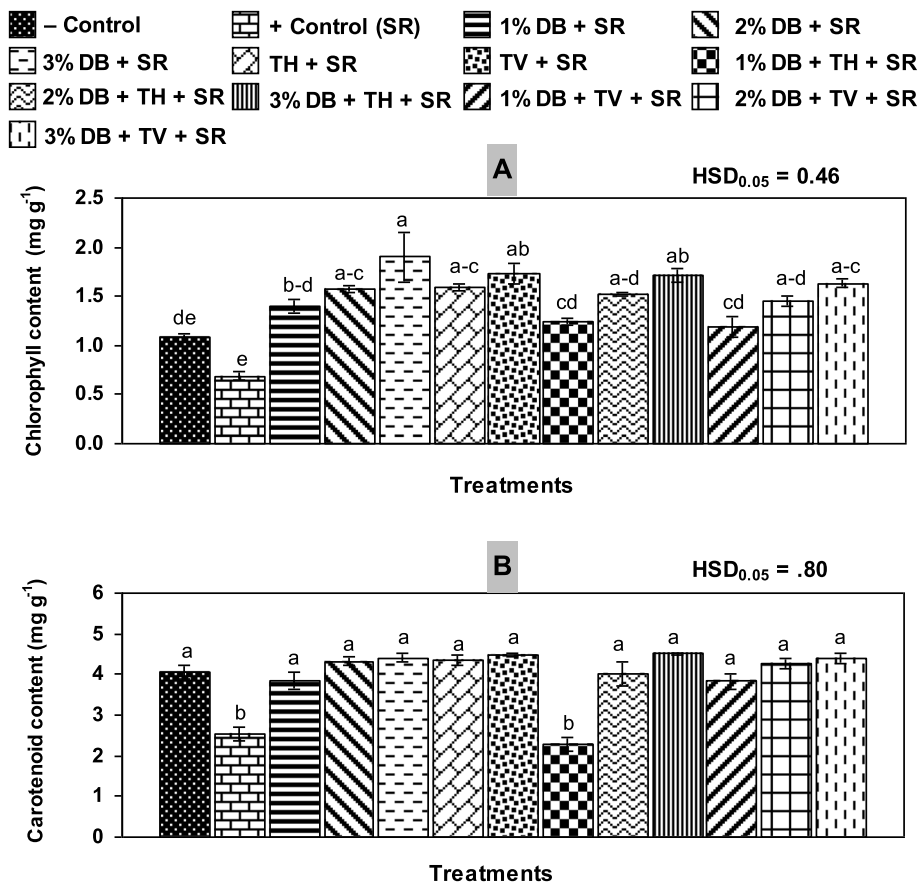
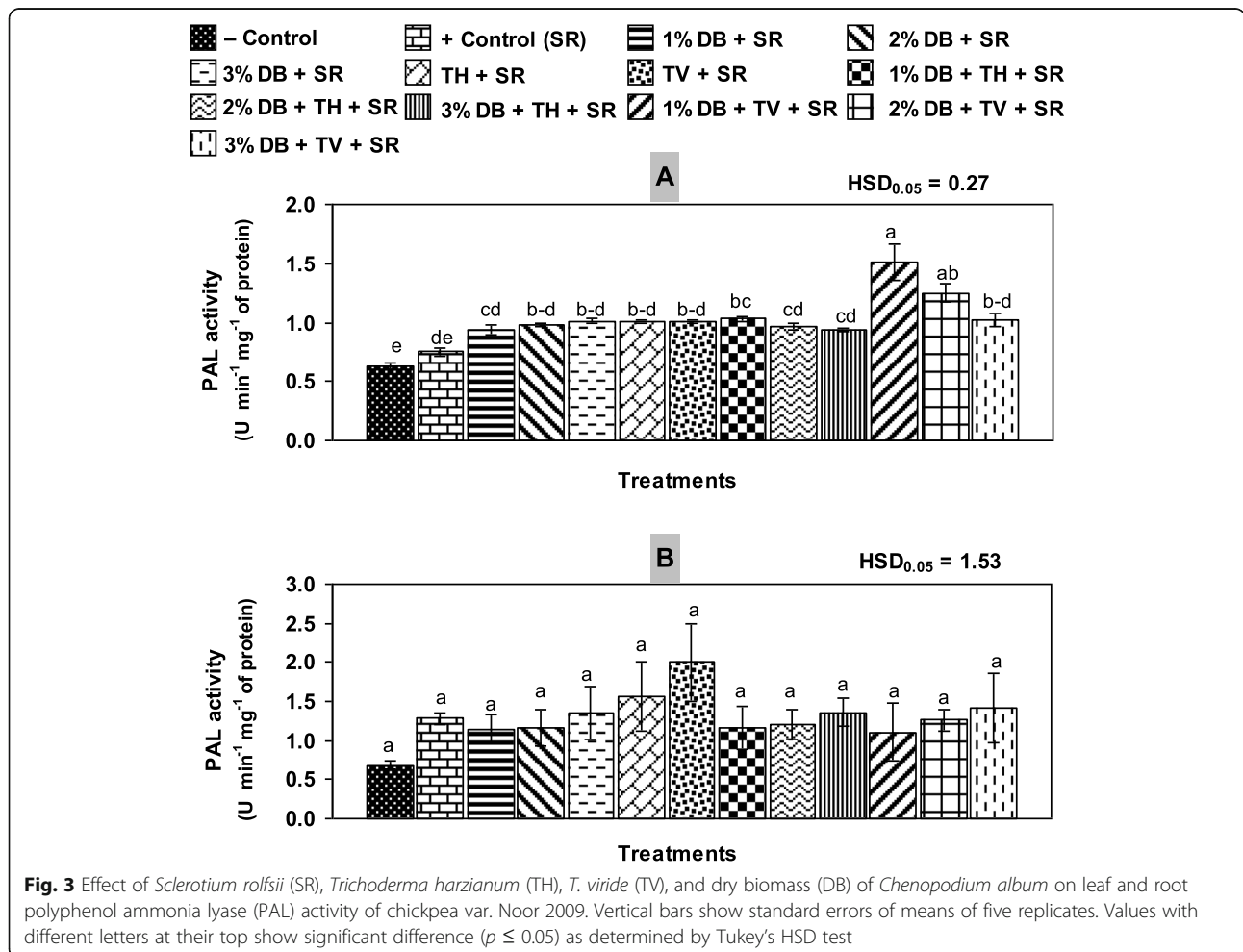


Fig. 2 Effect of *Sclerotium rolf sii* (SR), *Trichoderma harzianum* (TH), *T. viride* (TV), and dry biomass (DB) of *Chenopodium album* on leaf chlorophyll and carotenoid content of chickpea var. Noor 2009. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference ($p \leq 0.05$) as determined by Tukey’s HSD test

that was very much similar to the effect of 3% DBC alone amendment (Fig. 1c).

The use of organic amendments can enhance suppressive ability of the soil against soil-borne pathogens (van Elsas and Postma 2007). The effect, however, differed with respect to environmental conditions and the type of pathogen (Janvier et al. 2007). There were many successful stories where soil organic amendments such as dry biomass of *Datura metel*, *Coronopus didymus*, and *Withania somnifera* controlled charcoal rot of mung bean caused by *Macrophomina phaseolina*, collar rot of chilies caused by *S. rolfsii*, and basal rot of onion caused by *Fusarium oxysporum* f. sp. *cepae*, respectively (Javaid and Saddique 2011; Javaid and Iqbal 2014 and Akhtar and Javaid 2017). The phenomenon of disease suppression has been commonly related to change in physicochemical properties, microbial populations and related processes in the soil (Bonanomi et al. 2010). In the present study, application of 3% dry biomass of *C. album* alone or combined with *T. harzianum* and *T. viridi* markedly enhanced root and shoot growth, pod weight, and grain yield of chickpea variety Noor 2009 under biotic stress

of *S. rolfsii*. The decomposition of the dried biomass material of *C. album* possibly released some chemical constituents in the rhizospheric soil especially saponins, which in turn suppressed the growth and spread of the target pathogenic fungus. Javaid and Rauf (2015) reported that application of 3% leaf dry biomass of *C. album* significantly reduced incidence of basal rot of onion by 63% and plant mortality by 89% and improved crop growth of onion significantly. Similar work was done by Riaz et al. (2010) against *Fusarium oxysporum* f. sp. *gladioli* in which leaf residues of different plants significantly decreased disease incidence as well as disease severity. Results of the present study showed that when either *T. harzianum* or *T. viridi* were used in combination with 3% dry biomass of *C. album*, the effect on plant growth and yield was same as that of application of 3% dry biomass alone. By contrast, recently Javaid et al. (2017) reported that application of dry biomass of a brassicaceous weed *Sisymbrium irio* in combination with *T. harzianum* gave significantly higher crop growth and yield of mung bean when compared to application of either alone.



In the present study, individual application of either *T. harzianum* or *T. viridi* significantly enhanced crop growth and yield under stress of *S. rolfii*. Earlier studies showed that *Trichoderma* spp. were very beneficial fungi and had the ability to control many pathogens by various biological means (Vinodkumar et al. 2017). The modes of action of *Trichoderma* were competition, antagonism and production of some compounds in the soil against fungal pathogens (Jorge 2014). Recently, Liu et al. (2020) reported that *Trichoderma afroharzianum* reduced incidence of *Syringa* powdery mildew of *Syringa oblata*. They also induced production of abscisic acid that resulted in shedding of infected leaves and formation of new ones. Other recent studies also showed that different isolates of *Trichoderma* spp. had the ability to reduce the severity of foliar and root diseases (Muthukathan et al. 2020), presumably by inducing systemic resistance in plants (Maurya et al. 2008).

Effect of soil amendments on plant physiology

The lowest chlorophyll content (0.69 mg g⁻¹ FW) was recorded in positive control. The application of dry

biomass of *C. album*, *T. harzianum*, and *T. viride* significantly increased the chlorophyll content than in the positive control. The highest chlorophyll content (1.9 mg g⁻¹) was recorded in 3% dry biomass of *C. album*, followed by treatments inoculated with *T. harzianum* and *T. viride* separately or combined with 3% dry biomass of *C. album* (Fig. 2a).

The lowest carotenoid content (2.30 mg g⁻¹) was revealed in *T. harzianum* plus 1% dry biomass of *C. album* and positive control (2.53 mg g⁻¹) treatments. In general, in the rest of the treatments, application of dry biomass of *C. album*, *T. harzianum*, and *T. viride* significantly increased the carotenoids content as compared to positive control. Carotenoid content in these treatments was in the range of 3.83 to 4.48 mg g⁻¹ (Fig. 2b).

The lowest PAL activity in both leaf and root, i.e., 0.63 and 0.68 U min⁻¹ mg⁻¹ of protein, respectively, was recorded in negative control. All the *S. rolfii*-inoculated treatments increased PAL activity to variable extents. PAL activity in leaves and roots of positive control was 19% and 88% higher over negative control, respectively. In leaves, PAL activity was further increased by

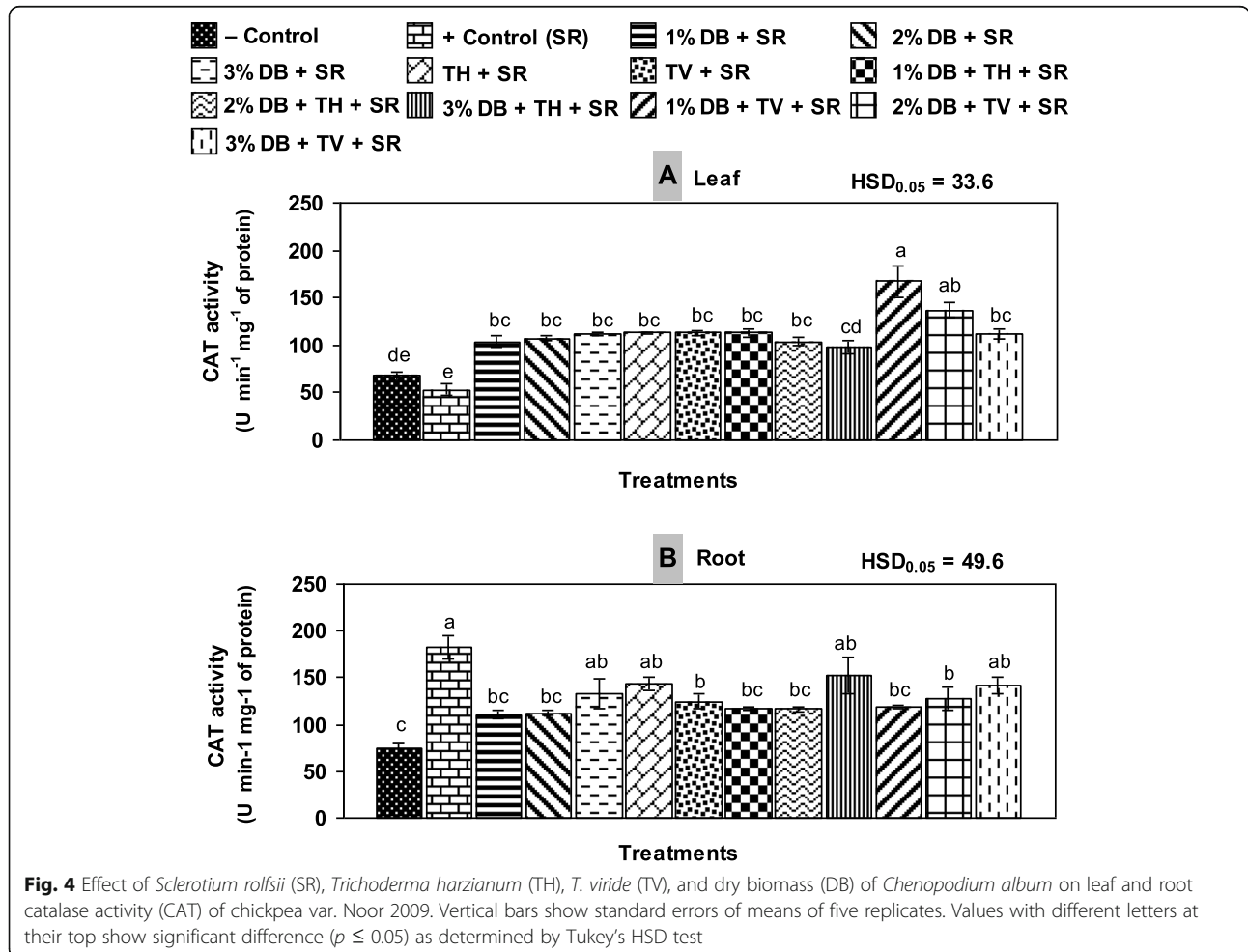


Fig. 4 Effect of *Sclerotium rolfii* (SR), *Trichoderma harzianum* (TH), *T. viride* (TV), and dry biomass (DB) of *Chenopodium album* on leaf and root catalase activity (CAT) of chickpea var. Noor 2009. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference ($p \leq 0.05$) as determined by Tukey's HSD test

application of soil amendments in *S. rolfii* inoculated plants that was 49–98 and 25–67% higher than negative and positive control treatments, respectively (Fig. 3a). However, in roots, PAL activity was marked higher than either negative or positive control treatments only in *T. viride* inoculated plant (Fig. 3b).

In leaves, the effect of *S. rolfii* inoculation on CAT activity was insignificant. By contrast, in root *S. rolfii* inoculation increased CAT activity to its highest value of 182 U min⁻¹ mg⁻¹ of protein as compared to 74 U min⁻¹ mg⁻¹ of protein in negative control. In general, CAT activity in leaves was significantly higher in *C. album*- and *Trichoderma*-amended treatments as compared to negative and positive control treatments. However, the highest CAT value (167 U min⁻¹ mg⁻¹ of protein) was recorded in 1% *C. album* + *T. viride* treatment (Fig. 4a). In roots, CAT activity in different *C. album* and *Trichoderma* treatments were 50–105% higher than negative control and 16–39% lower than positive control (Fig. 4b).

Data presented in Fig. 5 indicate that *S. rolfii* inoculation increased phenolic content in leaves and roots. The effect was more pronounced and significant in leaves

than in roots where 40% increase in phenolic content was evident in leaves over negative control. Application of different concentrations of *C. album* dry biomass as soil amendment significantly enhanced phenolic content in leaves as well as in roots over control. Similar effect was observed on phenolic content of leaf due to application of *T. harzianum* and *T. viride*, either alone or in combination with 3% dry biomass of *C. album*. In roots, the highest phenolic content was recorded in *T. harzianum* + 3% biomass of *C. album* that was 58% and 45% higher than phenolic content in negative and positive control treatments, respectively.

Application of dry biomass of *C. album*, *T. harzianum*, and *T. viride* not only enhanced the growth of tomato plants but also protected them from disease by stimulating resistance through inducing greater photosynthetic pigment, phenolics, and activities of enzymes probably to appropriate level to get rid of pathogen pressure (Awan et al. 2018). Similar results have been obtained previously due to effect of soil amendment or biocontrol agents in *Vigna mungo* and *V. radiata* against charcoal rot caused by *Macrophomina phaseolina* (Javaid et al.

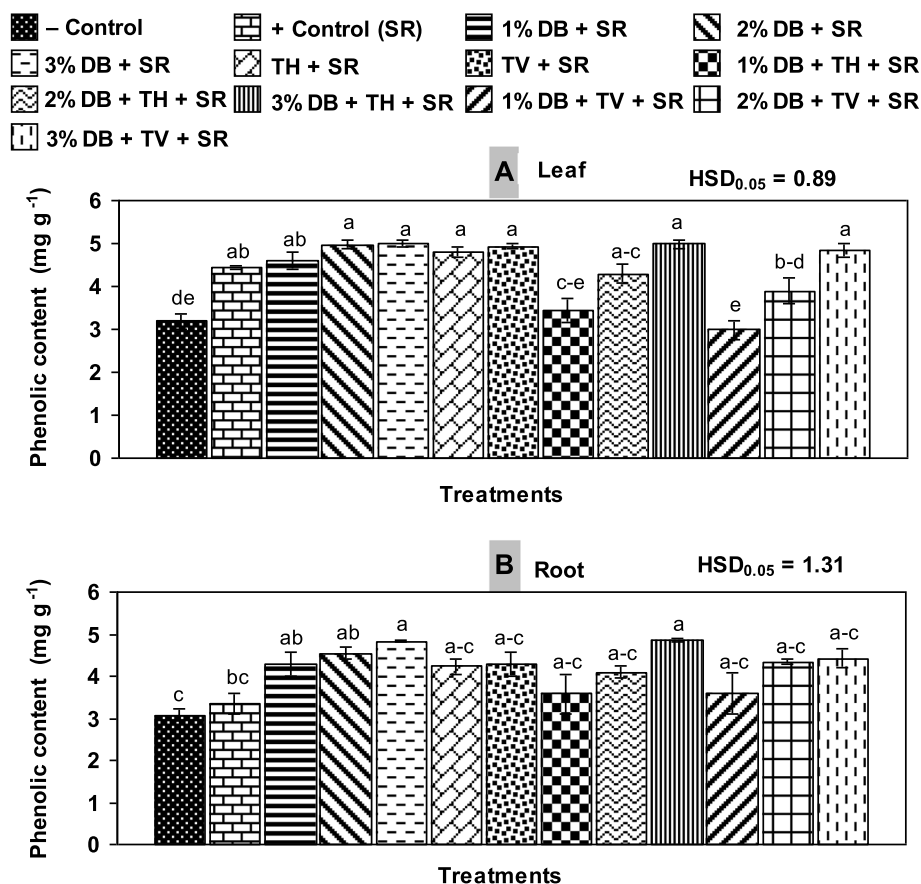


Fig. 5 Effect of *Sclerotium rolfii* (SR), *Trichoderma harzianum* (TH), *T. viride* (TV), and dry biomass (DB) of *Chenopodium album* on leaf and root phenolic content of chickpea var. Noor 2009. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference ($p \leq 0.05$) as determined by Tukey's HSD test

2018; Shoaib et al. 2018) and in chickpea against collar rot incited by *S. rolfsii* (Shoaib et al. 2019). Fungicidal action of dry biomass of *C. album* or *Trichoderma* spp. either alone as well as their synergism may act as immune booster to strengthen physiological attributes in favor of better plant health by conserving root system function (Shoaib et al. 2019).

Conclusion

This study concluded that application of 3% dry biomass of *C. album* either alone or combined with either of the two *Trichoderma* species viz. *T. harzianum* and *T. viridi*, as soil amendment, resulted in the highest crop growth and yield of chickpea under the stress of *S. rolfsii*.

Abbreviations

MEA: Malt extract agar; ANOVA: Analysis of variance; DB: Dry biomass; CAT: Catalase; POX: Peroxidase; PPO: Polyphenol peroxidase; SR: *Sclerotium rolfsii*; TH: *Trichoderma harzianum*; TV: *T. viride*

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Authors' contributions

AA conducted the study. AJ supervised the work, analyzed the data, and approved the final manuscript. AS supervised the part of study related to plant physiology. IHK contributed in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors are agreed to publish this paper.

Competing interests

The authors declare that they have no competing interests.

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