Chenopodium album MITIGATES ADVERSE EFFECTS OF *Sclerotium rolfsii* ON CHICKPEA VAR. BAKHAR-2011

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ABSTRACT

Sclerotium rolfsii, a soil-borne pathogen of over 500 plant species, causes collar rot disease in chickpea plant and reduces its survival rate, growth and yield. This study was undertaken to assess the benefits of soil amendment with a weed Chenopodium album L*., on growth, yield and physiology of chickpea var. Bakhar-2011, against S. rolfsii. For this purpose, soil was sterilized by fumigation with formaline and then inoculum of S. rolfsii was mixed. After one week of pathogen inoculation, soil was amended with dry biomass of C. album aerial parts at 1, 2 and 3% (w/w), irrigated and left for one week for leaching. Thereafter, chickpea seeds were sown in the pots. Experiment was conducted in a completely randomized design with six replications. S. rolfsii significantly reduced dry biomass of shoot, root and grains of chickpea by 21, 36 and 50%, respectively, as compared to negative control (without fungus or C. album). Likewise, chlorophyll and carotenoid contents were also reduced by application of S. rolfsii. Application of different concentrations of dry biomass of C. album (DBC) significantly improved shoot and root dry biomass, grain yield, and chlorophyll and carotenoid contents of chickpea over S. rolfsii inoculated treatment (positive control). The positive effects of DBC on plant growth and yield were increased by increasing its concentration. In general, S. rolfsii increased phenolic content, and activities of phenylalanine ammonia lyase (PAL), catalase (CAT) and peroxidase (POX), which were gradually reduced with increasing concentration of DBC. This study concluded that application of 3% DBC improve resistance of chickpea var. Bakhar-2011 to S. rolfsii and increase crop growth and yield.*

Keywords: Chickpea var. Bakhar-2011; Environment friendly; Lamb's quarters; Natural fungicides; *Sclerotium rolfsii*

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INTRODUCTION

Sclerotium rolfsii is a widespread phytopathogenic fungus that commonly found in tropical, sub-tropical and warmer regions of the world causing collar rot, damping-off, stem canker and pod rot disease in over 500 host plant species (Rafi *et al.*, 2017). It produces a large number of sclerotia, the principle structures that can remain in the soil up to 7 years even under adverse environmental conditions (Rodriguez-Kabana *et al.*, 1980). The abundant growth rate of *S. rolfsii* makes it a well-suited facultative parasite of economically importance crops throughout the world (Tarafdar *et al.*, 2018). It produces a considerable mass of white fluffy mycelia which later on forms a uniform size of sclerotia. Upon maturity, the sclerotia turn from white to tan in color and become dark brown, and attack plants near the soil line (Paul *et al*., 2017; Kushwaha *et al*., 2019). The initial visible disease symptoms are the production of dark brown lesions on roots with progressive yellowing of leaves which ultimately wilt and result in plant death (Mahadevakumar *et al*., 2018). *Sclerotium rolfsii* causes southern blight in chili and caused 37% plant mortality and 44% yield losses in chili as reported by Javaid *et al.* (2020). Likewise, Khan *et al.* (2020) recorded 56% plant mortality and 58% reduction in dry biomass of chickpea var. CMS-2118-2508 seedlings due to *S. rolfsii* infection. Moreover, Ali *et al.* (2020) demonstrated that *S. rolfsii* caused 50% decline in yield of chickpea var. Noor 2009.

The control of *S. rolfsii* has become one of the major concerns in agriculture. Since many strategies are in practice, one of them is the use of natural plant products (Saeed *et al*., 2016). Recently, it has gained more importance because it reduces the dependency on synthetic chemical fungicides with beneficial effects on the environment (Ali *et al*., 2017). Various studies have shown great potential of using botanicals for the control of diseases caused by *S. rolfsii* (Iqbal and Javaid, 2012; Jabeen *et al*., 2014; De and De, 2019; Khan *et al.,* 2020). *Chenopodium album* belongs to family Chenopodicaeae, is an annual weed native to Asia, Europe and North America (Bajwa *et al*., 2019). It is commonly found in cultivated fields of Pakistan especially in wheat growing areas (Rehman *et al.,* 2020; Ijaz *et al*., 2020). It is a rich source of bioactive phytoconstituents such as aldehydes, alkaloids, apocarotenoids, linolenic acid and flavonoids that play crucial roles in suppressing the growth and reproduction of pathogenic fungi (Khomarlou *et al*., 2018). Previously, it has been demonstrated against a variety of fungal pathogens including *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani*, *Ascochyta rabiei*, *Macrophomina phaseolina* and *Sclerotinia sclerotium* (Sherazi *et al*., 2016; Ali *et al*., 2017; Alkooranee *et al*., 2020). Therefore, the present study was aimed to evaluate the potential benefits of *C. album* amendment on chickpea plants growth, yield and physiology in soil infected with *S. rolfsii*.

MATERIALS AND METHODS

Pot trial

A pot experiment was carried out to control collar rot disease in chickpea through soil amendment with dry biomass of *C. album* using chickpea varieties Bakhar-2011 as the test crop. The experimental design comprised of five different treatments in 6 replications. The pot experiment was performed following the method of Javaid *et al.* (2020). Inoculum of the pathogen (*S. roflsii*) was prepared on pearl millet seeds. Sterilization of the sandy-loam soil (pH 7.5, containing 95 mg kg^{-1} potassium, 6.3 mg kg^{-1} phosphorous and 0.84% organic matter) was conducted by treating heap of soil with cotton swabs impregnated with the formalin solution. After 7 days of soil fumigation, the soil was filled in the pots (5 kg pot^{-1}), artificially inoculated by *S. rolfsii* inoculum (10 g kg^{-1}) and incubated for 7 days for colonization of the fungus in the soil. After one week, the *C. album* dry biomass was thoroughly mixed at 1, 2 and 3% (w/w) in soil of respective pots, irrigated and left for one week for

stabilizing the conditions. In each pot, 20 seeds of chickpea were sown. The soil inoculated with the pathogen only was treated as a positive control, while soil mixed with boiled pearl millet seeds only served as negative control. The observations for physiological traits like total chlorophyll, carotenoids and total phenolic contents, and activities of POX, PAL and CAT were evaluated following protocols of Shoaib *et al.* (2020) from the triplicate samples of leaves and roots of chickpea collected from each replicate at 40 days after sowing. Data for total number of pods and seed weight, and root and shoot growth were recorded after 100 days sowing at maturity.

Statistical analysis

All the data were analyzed by ANOVA and the means were separated at P≤0.05 by applying LSD test using Statistix 8.1.

RESULTS AND DISCUSSION

Effect of *S. rolfsii* and *C. album* on plant growth and yield

Sclerotium rolfsii (positive control) markedly reduced shoot and root growth of chickpea as compared to negative control. There was 17% and 21% reduction in length and dry biomass of shoot due to *S. rolfsii* application. Likewise, 26% and 36% reduction in length and biomass of root was recorded due to the pathogen. Similar effect of *S. rolfsii* inoculation was also noted on weight of pods and grains where a reduction of 40% and 50% was noted with respect to negative control, respectively (Fig. 1–3). *S. rolfsii* attacks chickpea at seedling stage in tropical and sub-tropical regions of the world (Tarafdar *et al.,* 2018), and causes seedling mortality that ranges from 55–95% (Kokub *et al.,* 2007), which ultimately results in reduced plant biomass and yield (Khan *et al.,* 2020).

A significant boost in all the growth and yield parameters was observed due to application of DBC. The stimulatory effect was gradually increased with an increase in quantity of the DBC in the soil. There were significant increases of 29–142%, 41–134%, 74–329%, 120–650%, 200–692% and 300–1309% in shoot length and dry weight, root length and dry weight, pod weight and grain yield, respectively, was obtained due to 1–3% soil application of DBC as compared to positive control (Fig. 1–3). The enhanced crop growth and yield due to application of DBC could be due to the presence of various antifungal compounds in *C. album* which might lowered the fungal inoculum in the soil resulting in reduced rate of plant mortality which resulted in increased plant biomass production. Javaid and Amin (2009) found *C. album* extracts very effective in suppressing growth of *Macrophomina phaseolina.* Likewise, extracts and dry biomass of *C. album* also controlled *in vitro* and *in vivo* growth of *Fusarium oxysporum* f. sp. *cepae* causing basal rot disease in onion (Rauf and Javaid, 2013; Javaid and Rauf, 2015). An antifungal compound trans-2, transe-4 hexadienedial has been identified in leaves of *C. album* (Tahara *et al.,* 1994). In addition, it also contains various saponins and phenolics (Stuardo and Martin, 2008; Lavaud *et al.,* 2000), which could be responsible for its antifungal activity against *S. rolfii.*

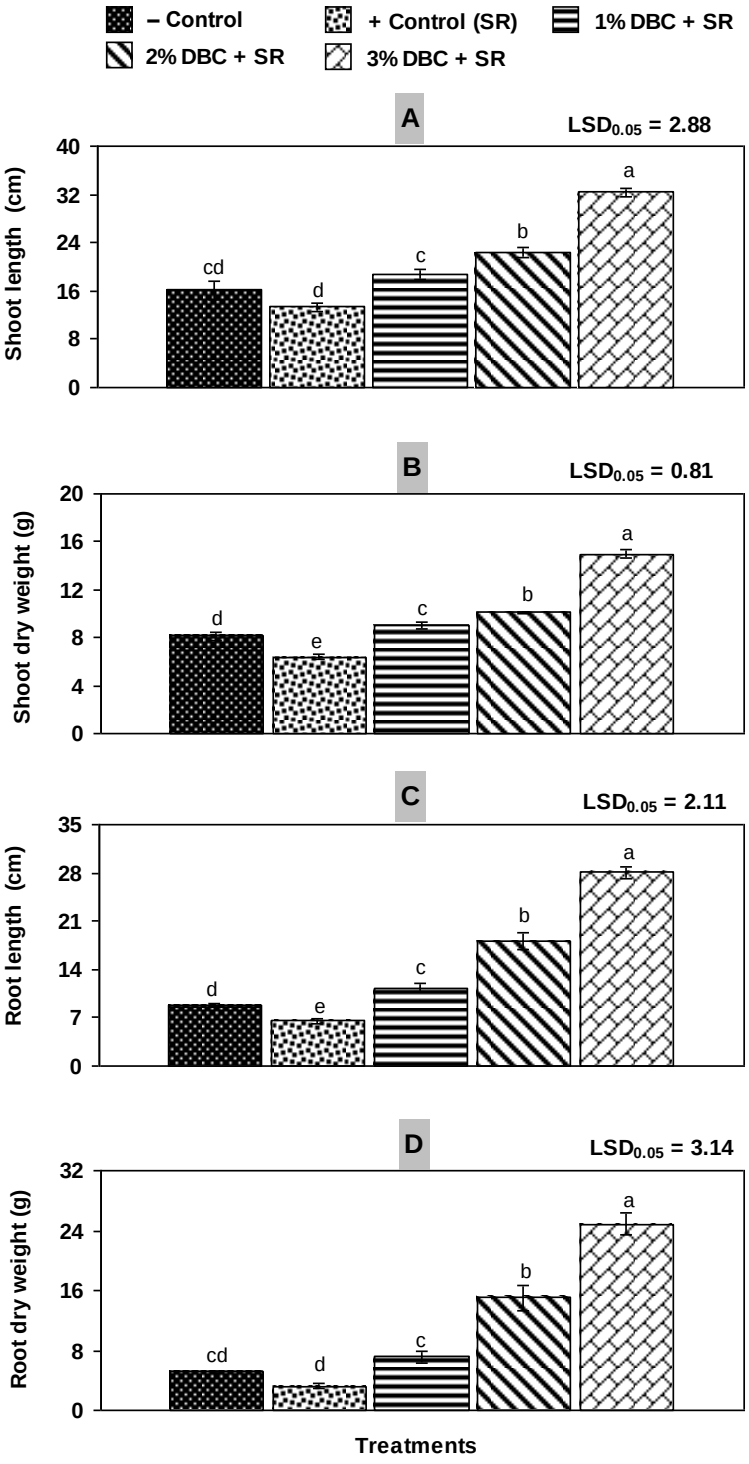

Fig. 1: Effect of *Sclerotium rolfsii* (SR) and dry biomass of *Chenopodium album* (DBC) on shoot and root growth of chickpea var. Bakhar-2011. Vertical bars show standard errors of means. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

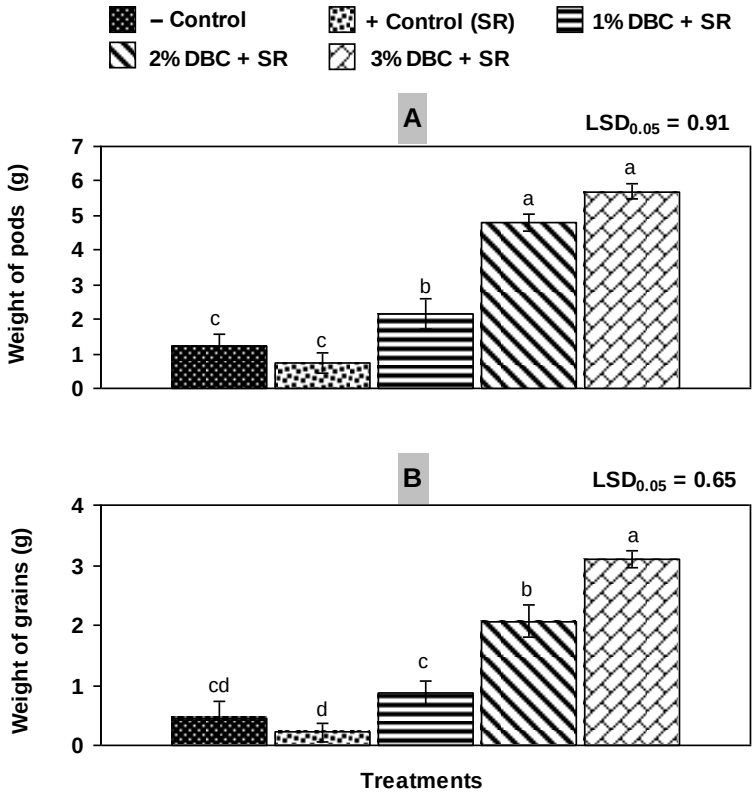

Fig. 2: Effect of *Sclerotium rolfsii* (SR) and dry biomass of *Chenopodium album* (DBC) on pod and grain yield of chickpea var. Bakhar-2011. Vertical bars show standard errors of means. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

Effect of *S. rolfsii* and *C. album* on plant physiology

The total chlorophyll and carotenoid contents in chickpea were 0.34 and 1.64 mg g^{-1} fresh weight, respectively, in negative control while these attributes were insignificantly decreased in the positive control. However, soil amendment with dry biomass of *C. album* significantly improved chlorophyll and carotenoid content as compared to positive control (Fig 1 A and B). The increase in crop growth and yield due to application of *C. album* dry biomass could be attributed to this increase in contents of photosynthetic pigments. Total phenolics and the activities of POX, PAL and CAT (stress markers) were significantly higher in the leaf of the positive control as compared to negative control. However, the DBC significantly decreased these stress markers over positive control, while these traits were insignificantly differ with respect to negative control. Similar results have been recorded earlier due to the effect of early blight pathogen (*Alternaria solani*) on susceptible varieties of the tomato (Awan *et al.,* 2019; Nafisa *et al.,* 2020). Accumulation of oxidative stress caused by pathogen might have caused oxidations of biomolecules (lipids, protein and DNA), that may stimulate innate immunity of the host resulting in the improvement of stress makers (Nafisa *et al.,* 2020). However, *S. rolfsii* causes

acidification of the host tissues due to production of oxalic acid, which through sequestration of calcium ions from host cell wall could cause disruption in regulatory functions of reactive oxygen species (ROS) and other associated signaling molecules. Therefore, antifungal programmed defense under pathogen attack appears not to be fully hard-wired, and plant suffered collar rot disease (Awan *et al.,* 2018; 2019). Soil amendment with DBC might have caused modulation in physiological pathways in host through reducing accumulation of ROS in the leaves directly by arresting the pathogen colonization in the soil and by conserving root system (Shoaib *et al.,* 2018).

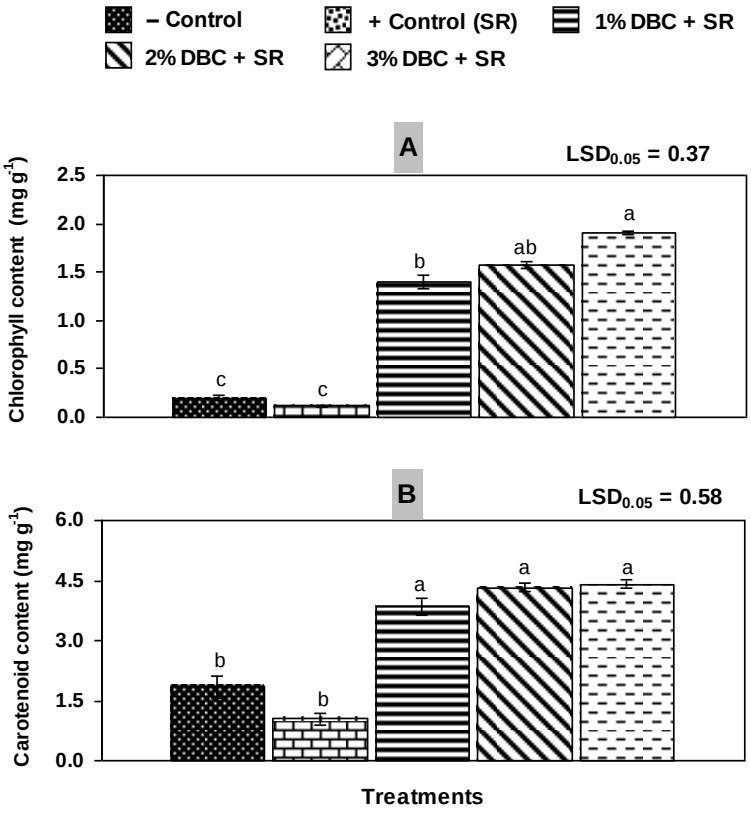

Fig. 3: Effect of *Sclerotium rolfsii* (SR) and dry biomass of *Chenopodium album* (DBC) on leaf chlorophyll and carotenoid contents of chickpea var. Bakhar-2011. Vertical bars show standard errors of means. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

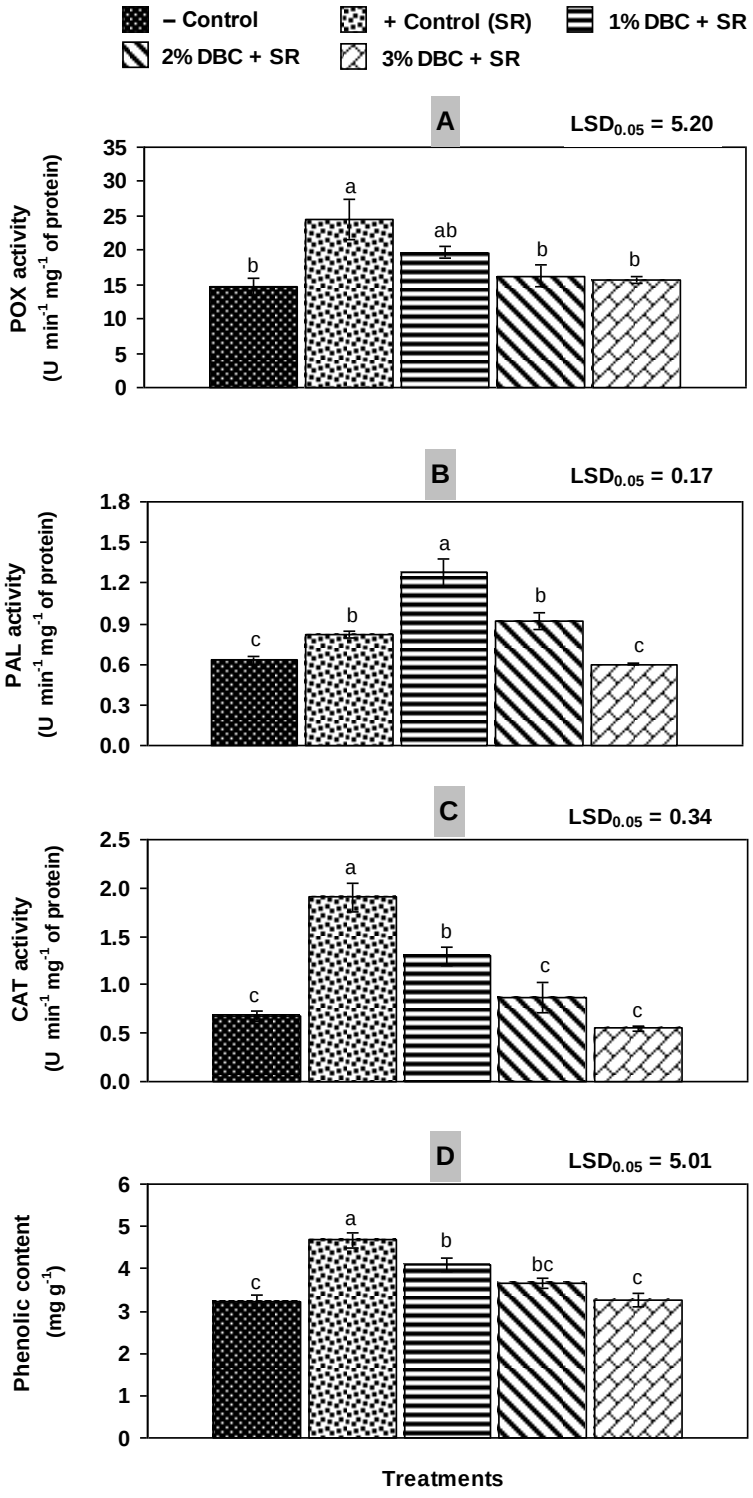

Fig. 4: Effect of *Sclerotium rolfsii* (SR) and dry biomass of *Chenopodium album* (DBC) on phenolic content, and activities of peroxidase (POX), polyphenol ammonia lyase (PAL) and catalase (CAT) in leaves of chickpea var. Bakhar-2011. Vertical bars show standard errors of means. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

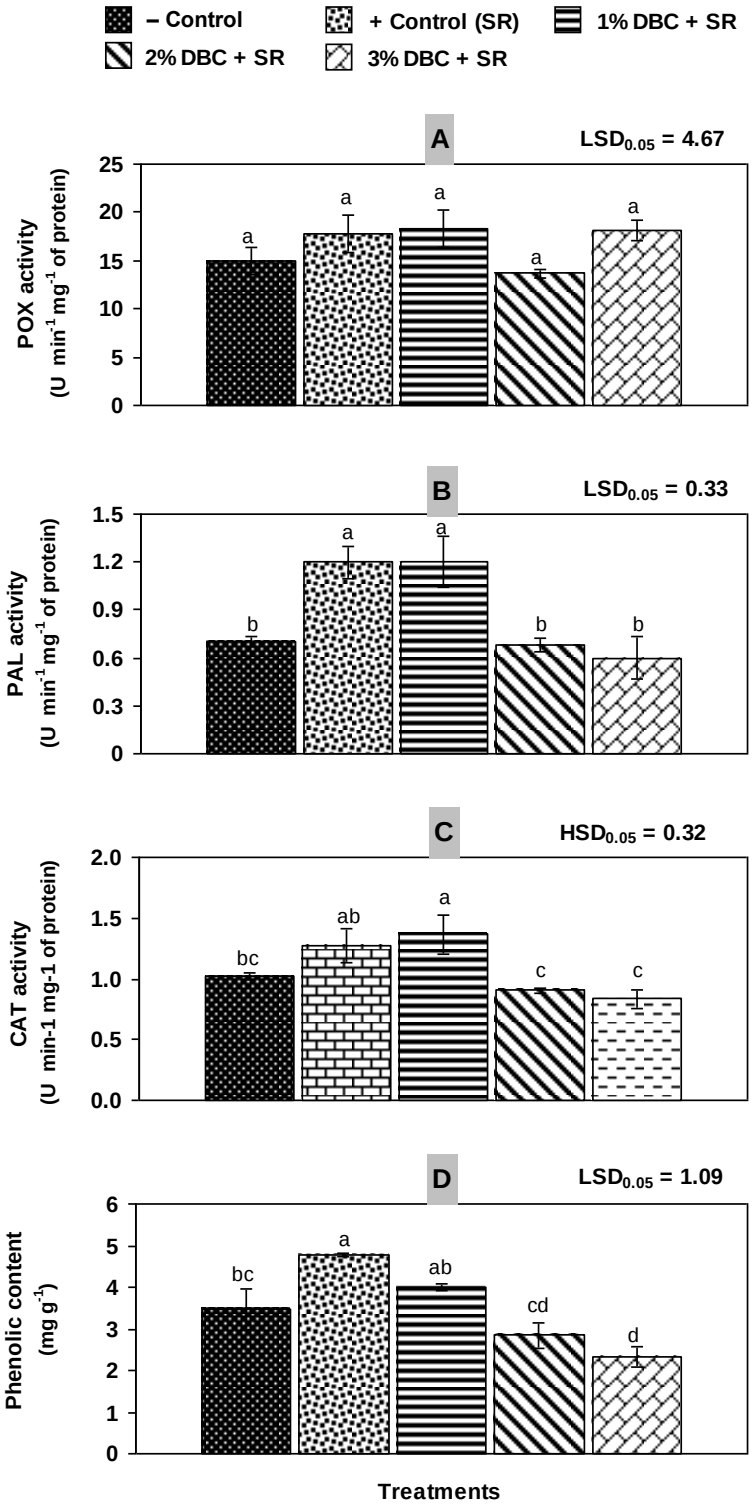

Fig. 5: Effect of *Sclerotium rolfsii* (SR) and dry biomass of *Chenopodium album* (DBC) on phenolic content, and activities of peroxidase (POX), polyphenol ammonia lyase (PAL) and catalase (CAT) in roots of chickpea var. Bakhar-2011. Vertical bars show standard errors of means. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

Conclusion

This study concluded that biotic stress of *S. rolfii* on growth, yield and physiology of chickpea var. Bakhar-2011 can significantly be overcome through addition of dry biomass of *C. album* at 3% (w/w) concentration.

Author's contribution

Amna Ali carried out experimental work. Arshad Javaid supervised the whole work, wrote a part of paper and also did statistical analysis. Iqra Haider Khan contributed in writing of the manuscript. Amna Shoaib did physiological studies.

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