Sharf et al. Egyptian Journal of Biological Pest Control (2021) 31:16
https://doi.org/10.1186/s41938-021-00364-y

Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Induction of resistance in chili against *Sclerotium rolfsii* by plant-growth-promoting rhizobacteria and *Anagallis arvensis*

Warda Sharf, Arshad Javaid*, Amna Shoaib and Iqra Haider Khan

Abstract

Background: *Sclerotium rolfsii* is a soil-borne fungal pathogen causing diseases in more than 500 plant species. It causes southern blight disease in chili. Chemical fungicides are used to control this disease, which also pollute the environment. The present study was designed to assess the potential of two species of plant-growth-promoting rhizobacteria (PGPR) viz. *Bacillus megaterium* and *Pseudomonas fluorescence*, and an allelopathic weed, *Anagallis arvensis* L., for the control of southern blight disease of chili.

Results: Initially, three PGPR strains, viz. *B. megaterium* OSR3, *B. megaterium* ZMR6, and *P. fluorescence* PF-097, were selected for their in vitro antagonistic assessment against *S. rolfsii* by dual culture technique on potato dextrose agar medium. OSR3 showed the highest antagonistic potential (68%), followed by PF-097 (54%) and ZMR6 (33%). In a pot experiment, the two best strains of PGPR, namely OSR3 and PF-097, and dried biomass of *A. arvensis* (DBA) in different concentrations (1, 2 and 3%) were used to manage southern blight disease of chili. In positive control treatment (*S. rolfsii* only), plant survival was low (73%) than the negative control (100%). OSR3, PF-097, OSR3 + 2% DBA, and PF-097 + 2% DBA significantly enhanced plant survival over positive control. The highest increase in chili growth over positive control was recorded due to OSR3, followed by PF-097 inoculations. Contents of carotenoid and chlorophyll were significantly decreased due to the fungal pathogen and improved due to PGPR strains. Application of the two PGPR strains and different concentrations of *A. arvensis* distinctly increased the catalase (CAT), peroxidase (POX), and polyphenol peroxidase (PPO) activities over positive control.

Conclusions: The present study concluded that PGPR strains *B. megaterium* OSR3 and *P. fluorescence* PF-097 can control southern blight disease effectively and increase growth and yield of chili.

Keywords: *Bacillus megaterium*, *Sclerotium rolfsii*, Biocontrol, Chili, Collar rot, *Pseudomonas fluorescence*

Background

Capsicum annum L. commonly known as chili belongs to family Solanaceae. Initially, its cultivation was limited to Southern and Central America which later on spread all over the world (Majid et al. 2016). In Pakistan, it is cultivated on 58.2 thousand hectares with an annual production of 74.6 thousand tons (Bashir et al. 2018). The crop is affected by many fungal, bacterial and viral diseases resulting in huge economic losses. Among the fungal diseases, *Sclerotium rolfsii*, responsible for southern blight in chilies, is of major concern (Javaid et al. 2020). The observed disease symptoms are yellowing of plant leaves and the formation of dark brown lesions at collar region near the soil line which further lead to wilting of the whole plant (Mahadevakumar et al. 2018). It produces enormous sclerotia, which persist in soil for many years in the form of infected plant debris. It has a

* Correspondence: arshad.iags@pu.edu.pk; arshadjpk@yahoo.com
Institute of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan

wide geographical host range, which makes its management challenging (Murthy et al. 2018).

The pathogen control may be achieved by applying tremendous volume of fungicides but their excessive usage has hazardous impact on environment as well as on human health (Keinath and DuBose 2017). Hence, non-chemical means of disease management include biocontrol agents and plant-based products that can serve as cheap and environmentally safe alternates to synthetic products (Wankhade et al. 2019). Recently, PGPR have attracted much attention in modern agriculture system because of their ability to promote plant growth and soil health (Mohamed et al. 2019). They have a distinct mechanism of action such as antibiosis, hyper parasitism, competition, and induced systemic resistance (Verma et al. 2019). The role of *Bacillus megaterium* and *Pseudomonas fluorescence* have been demonstrated against *Fusarium*, *Rhizoctonia*, *Pythium*, and *Verticillium* species (Omara et al. 2018; Guenoun et al. 2019; Zain et al. 2019). In addition to their ability to suppress the plant pathogens, these bacteria have the capacity to decompose organic matter in soil which plays an important role in plant production (Mohamed et al. 2019).

Anagallis arvensis, family Primulaceae, is an annual herb plant native to Europe, Africa and Asia (Soberon et al. 2017). Its chemical profile indicates the presence of sterols, terpenes, triterpenes, alkaloids, flavonoids, saponins, tannins, glycosides, and quinones with potent antifungal properties (Soberon et al. 2017). It is effective against several fungal plant pathogens including *Rhizoctonia solani*, *Rhizopus stolonifera*, *Helminthosporium sativum*, *Phytopthora drechsleri*, and *Pythium aphanidermatum* (Bahraminejad et al. 2011, 2013).

However, its role in controlling *S. rolfsii*, especially in combination with PGPR, has never been investigated. Therefore, the present study was carried out to determine the potential role of PGPR strains *B. megaterium* and *P. fluorescence*, and *A. arvensis* to control the southern blight disease of chili.

Methods

Procurement of microorganisms

The culture of pathogenic fungus *S. rolfsii* was obtained from Bio-fertilizers and Biopesticides Research Laboratory, Institute of Agricultural Sciences, University of the Punjab Lahore, Pakistan. Three PGPR strains, namely *B. megaterium* OSR3, *B. megaterium* ZMR4, and *P. fluorescence* PF-097, were obtained from the First Culture Bank of Pakistan. The fungus was sub-cultured on malt extract agar (MEA) plates and then incubated at 30 °C for 7 days. Sub-culturing of the bacterial strains was done on LBA (Luria-Bertani Agar) plates and then incubated at 37 °C for 24 h. Culture plates of fungal and bacterial strains were stored in a refrigerator at 4 °C.

In vitro antagonistic interaction

In vitro, the antagonistic isolates were screened using the dual culture technique on PDA (potato dextrose agar) medium, following the procedure of Mishra et al. (2017). Mycelia plug (6 mm) from the leading edge of a 7-day-old culture of *S. rolfsii* was placed in the center of a new PDA plate (9 cm). The bacterial strains PF-097, OSR3, and ZMR4 were inoculated by streaking 2 cm away from the fungal plug. In the control plate, only fungal disc was placed. Petri plates were placed at 30 °C for incubation for 7 days. Six replicates of each treatment were made. The diameter of the fungal colony was recorded using a scale. Percentage inhibition of the radial growth of *S. rolfsii* by bacterial strains was determined after 7 days of incubation by using the following formula:

$$\text{Growth inhibition}\ (\%) = (C - T) \times 100/C$$

where T and C represent radial growth of pathogen in treatment and control plates, respectively.

Pot experiment

A pot experiment was conducted by augmenting the soil with whole plant dried biomass of *A. arvensis* (DBA). Two bacterial strains namely PF-097 and OSR3 were selected from in vitro screening experiments and used alone as well as along with DBA. *S. rolfsii* (SR) inoculum was multiplied on pearl millet seeds, following the protocol of Javaid et al. (2020). For preparation of bacterial inocula, the two strains, namely OSR3 and PF-097, were cultured in LB broth media for 24 h at 30 °C. Culture flasks were then placed on the centrifuged machine at 4000 rpm for 20 min and the pellet was placed in autoclaved d.H_2O. The concentration of the bacterial inoculum was calibrated to 1×10^7 cfu ml^{-1} by quantifying optical density of 0.9 at 600 nm (Park et al. 2013).

Soil was disinfected by formalin solution (Javaid et al. 2020). Earthen pots of (30 cm diameter) were filled by disinfected soil at 8 kg per pot. The pearl millet-based inoculum of the pathogen was mixed in each pot at 40 g per pot (@ 5 g kg^{-1} soil), except in negative control. All the pots were irrigated and then left for 7 days for the development of inoculum of the pathogen. The whole plant dried material of *A. arvensis* was grinded thoroughly and mixed in the soil at 1, 2, and 3% (w/w), irrigated and left for 1 week. Thereafter, the bacterial inoculum suspension (100 ml pot^{-1}) of each of PF-097 and OSR3 was poured into the soil of respective pots and mixed thoroughly (Zhang et al. 2011). The pots were irrigated and then left for 7 days. After that, 15-day-old chili seedlings were sown in each pot (12 seedlings pot^{-1}). The pots were placed under natural environmental conditions in open sunlight.

The experiment was carried out in completely randomized design with triplicate set of each treatment and there were 13 treatments altogether:

T_1 Negative control
T_2 Positive control [only *S. rolfsii* (SR)]
T_3 1% DBA + SR
T_4 2% DBA + SR
T_5 3% DBA + SR
T_6 OSR3 + SR
T_7 1% DBA + OSR3 + SR
T_8 2% DBA + OSR3 + SR
T_9 3% DBA + OSR3 + SR
T_{10} PF-097 + SR
T_{11} 1% DBA + PF-097 + SR
T_{12} 2% DBA + PF-097 + SR
T_{13} 3% DBA + PF-097 + SR

All the chili plants were harvested after 90 days of transplantation. In order to check the effect of disease, number of plants survived in different treatments was recorded. After removing chili plants from the soil, the root and shoots were separated for measuring growth and biomass. Dry weight of chili plants were measured after drying at 70 °C. Data regarding yield was determined by taking number, and fresh and dry weight of fruits. Physiological changes in chili plants were determined at flowering stage, 40 days after transplanting of seedlings in the pots. Total chlorophyll content was determined following the procedure given by Arnon (1949). For this, the leaf extract prepared in 80% ethanol was centrifuged at 10,000 rpm and the extract was checked for measurement of chlorophyll a (645 nm), chlorophyll b (663 nm) and carotenoids (470 nm) on a spectrophotometer. Likewise, the, total protein content was estimated using the protocol of Lowry et al. (1951). For estimation of total protein content, leaf sample (0.5 g) was crushed in 10 ml of phosphate buffer (0.1 M, pH 7.5) in pre-chilled pestle and mortar in liquid nitrogen, followed by centrifugation at 3000 rpm for 10 min and addition of 1 ml of reagent C. The mixture was shaken for 10 min, added with 0.1 ml folinphenol reagent and finally incubated at room temperature for 30 min. The total protein content was estimated at 650 nm by using standard curve of Bovine serum albumin. The activities of antioxidant enzymes, namely catalase (CAT0), peroxidase (POX0), and polyphenol oxidase (PPO), in leaves of chili estimation were estimated, following the procedure given by Shoaib et al. (2019a).

Statistical analysis

In vitro experiment was conducted, using 6 replicates, while pot study was carried out, using 3 replicates of each treatment. In both the cases, standard errors of means were calculated, using MS Excel software. Data of both the experiments were analyzed by one-way ANOVA and the treatment means were delineated by applying LSD test ($P \leq 0.05$), using software Statistix 8.1.

Results

Interaction of PGPR with *S. rolfsii*

Among the three strains used, OSR3 showed the highest antagonistic activity and reduced growth of *S. rolfsii* by (68%). Likewise, PF-097 also proved a very promising antagonistic PGPR strain as it inhibited the growth of the fungal pathogen by (54%). In contrast, ZMR6 showed the least activity against *S. rolfsii* and retarded its in vitro growth by only 33% (Fig. 1). On the basis of their highly pronounced in vitro inhibitory activity against the fungal pathogen, PGPR strains OSR3 and PF-097 were selected for pot trial.

Effect of soil amendments on plant survival

In negative control, number of plants survived was 11, significantly reduced to 8 in positive control. Application of 1% plant biomass further reduced number of plants to 7. Increase in dose of dry biomass of *A. arvensis* (DBA) did not improve the situation. However, application of OSR3 significantly increased the plant survival up to the level of negative control. Application of PF-097 alone or combined with 2% DBA also markedly improved plant survival. In the rest of the treatments, number of plants survived was insignificantly different from the positive control (Fig. 2A).

Effect of soil amendments on shoot growth

Shoot length in negative control was 33.5 cm that was insignificantly reduced to 31.4 cm due to application of *S. rolfsii.* Application of DBA markedly reduced shoot length over negative as well as the positive control. By contrast, application of either of the two PGPR strains markedly enhanced this parameter. The effect on shoot length due to application of PF-097 was significant where (21%) increase in shoot length was observed over positive control. The effect of combined application of DBA and the PGPR strains was insignificant (Fig. 2B). Shoot dry weight in negative control was 22.6 g, significantly declined to 9.36 g in positive control (59% decrease). The effect of DBA was insignificant when used alone or combined with OSR3. However, sole application of OSR3 significantly enhanced shoot dry weight by 156% over positive control. Likewise, application of PF-097 also increased shoot biomass by 114% over positive control. Combined application of PF-097 and 2% DBA also significantly increased shoo dry weigh of chili b 69% over positive control (Fig. 2C).

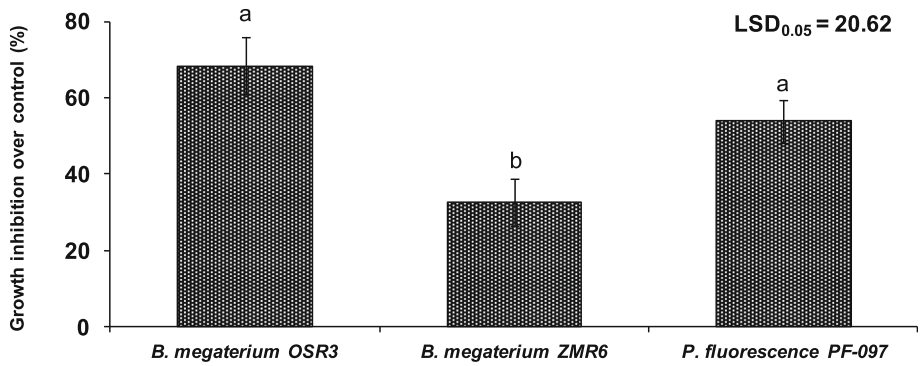

Fig. 1 Inhibition in radial growth of *Sclerotium rolfsii* due to interaction with different strains of *Bacillus megaterium* and *Pseudomonas fluorescence*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD test

Effect of soil amendments on root growth

The maximum root length (15.33 cm) was noted in negative control. Application of *S. rolfsii* suppressed the root length to 12.41 cm, 19% lower than that in negative control. Application of DBA either alone or in combination with PGPR strains further suppressed the root length (Fig. 3A). Root dry weight was the highest in negative control (11.1 g), diminished to 3.1 g in the positive control. Application of DBA did not improve the root dry biomass. Conversely, application of OSR3 improved the root biomass significantly and raised it (10.7 g) at par with the negative control. Application of PF-097 also improved the situation (7.45 g); however, the effect was much lower than that of OSR3. Application of PGPR along with DBA gave root biomass lower than sole application of the two PGPR strains (Fig. 3B).

Effect of soil amendments on yield

The highest number of fruits was noted in negative control. *S. rolfsii* application significantly reduced this number by 80%. Likewise, the pathogen reduced fresh and dry weight of chili by 89 and 87%, respectively, over negative control. Some soil amendment treatments increased various yield-related parameters over control; however, the effect was insignificant (Fig. 4).

Effect of soil amendments on plant physiology

The highest chlorophyll content (1.06 mg g^{-1} FW) was recorded in negative control. In general, all the *S. rolfsii* inoculated treatments, either alone or combined with different soil amendments, showed markedly lower chlorophyll content than the negative control. In positive control, chlorophyll content was 0.62 mg g^{-1} that was significantly lower than the negative control by 42%. Different doses of DBA insignificantly increased chlorophyll contents by 2–19%. The highest increase in this parameter over positive control was due to application of OSR3 (60%), followed by application of PF-097 (50%). Application of DBA along with either of the two PGPR strains reduced chlorophyll content than in PGPR strains alone (Fig. 5A).

Application of *S. rolfsii* in positive control resulted in an insignificant decrease in total protein content by 21% over negative control. All the soil amendments alleviated the biotic stress of *S. rolfsii* on this parameter and variably increased protein content by 15–69% over positive control. The highest increase was noted in OSR3 treatment (69%), followed by OSR3 + 1% DBA (60) as shown in (Fig. 5B).

Catalase (CAT) activity in negative control was (0.66 U min^{-1} mg^{-1}) protein, reduced to half (0.33 U min^{-1} mg^{-1}) on inoculation of *S. rolfsii.* All the soil amendment treatments, except PF-097 + 3% DBA, significantly increased this parameter over positive control. As compared to positive control, there was (129–544%) increase due to different treatments. Similar to that of chlorophyll content, the highest increase (544%) was recorded in OSR3, followed by PF-097 (428%) over positive control. Application of DBA negatively affected the activity of both the PGPR strains (Fig. 6A).

The effect of *S. rolfsii* application and different soil amendments on peroxidase (POX) activity was generally similar to that on CAT activity. In negative control, POX activity (1.5 U min^{-1} mg^{-1}) was decreased by 33% (1.01 U min^{-1} mg^{-1}) in positive control. Application of various soil amendments enhanced this parameter by (24–109%) over positive control. The highest increase was due to OSR3 application (Fig. 6B).

The effect of the pathogen inoculation as well as other soil amendments on polyphenol ammonia lyase (PPO) activity was not much different than of these amendments on CAT and POX activities. PPO was (0.52 U min^{-1} mg^{-1}) in negative control and reduced to (0.33 U min^{-1} mg^{-1}) due to *S. rolfsii* application, i.e., lowered by 42%. Soil amendments enhanced PPO by 27–184% over positive control. Application of OSR3 was the most effective treatment for improving PPO (Fig. 6C).

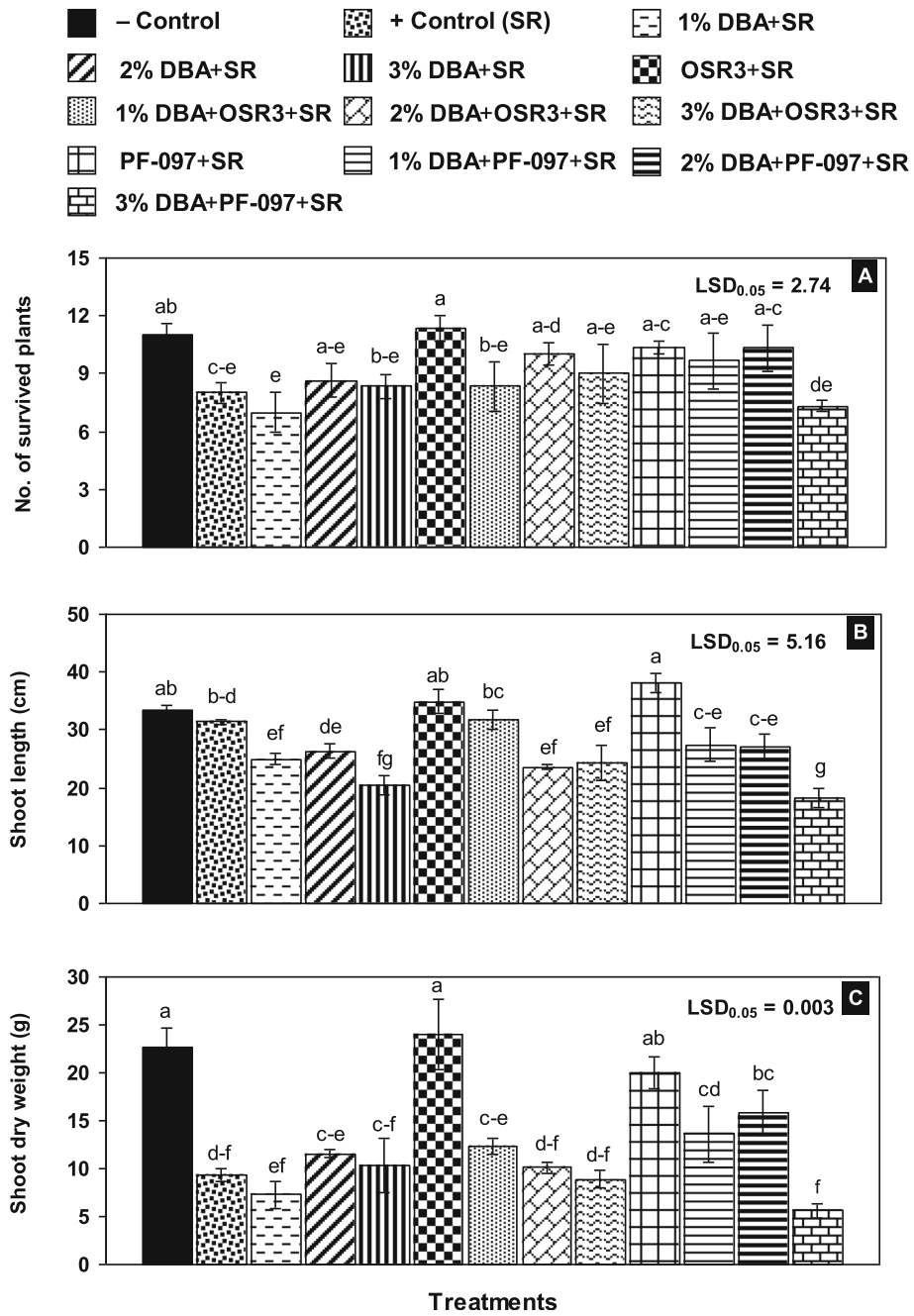

Fig. 2 Effect of different doses of dry biomass of *Anagalis arvensis* (DBA) and inoculation of two PGPR spp. *viz. Bacillus megaterium* OSR3 and *Pseudomonas fluorescence* PF-097 on survival of chili plants and shoot growth of chili in *Sclerotium rolfsi* (SR) inoculated soil. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD test

Discussion

In laboratory screening test, OSR3 exhibited the highest reduction (68%) in the growth of *S. rolfsii*, followed by PF-097 (54%). However, ZMR6 showed the least inhibition (32%) against the pathogen. PGPR have attained a lot of attention as bioagents against numerous plant pathogens because they not only have the ability to prevent plant diseases but also provide nutrients to the plant and enhance their growth (Xiang et al. 2017). *Bacillus* spp. inhibited the growth of many pathogens by producing several metabolites (terpenes and polypeptide) and cell wall degrading enzymes such as chitinases (Shoda 2000). Romero et al. (2007) reported that *Bacillus* spp. produce several antibiotics such as iturin A that

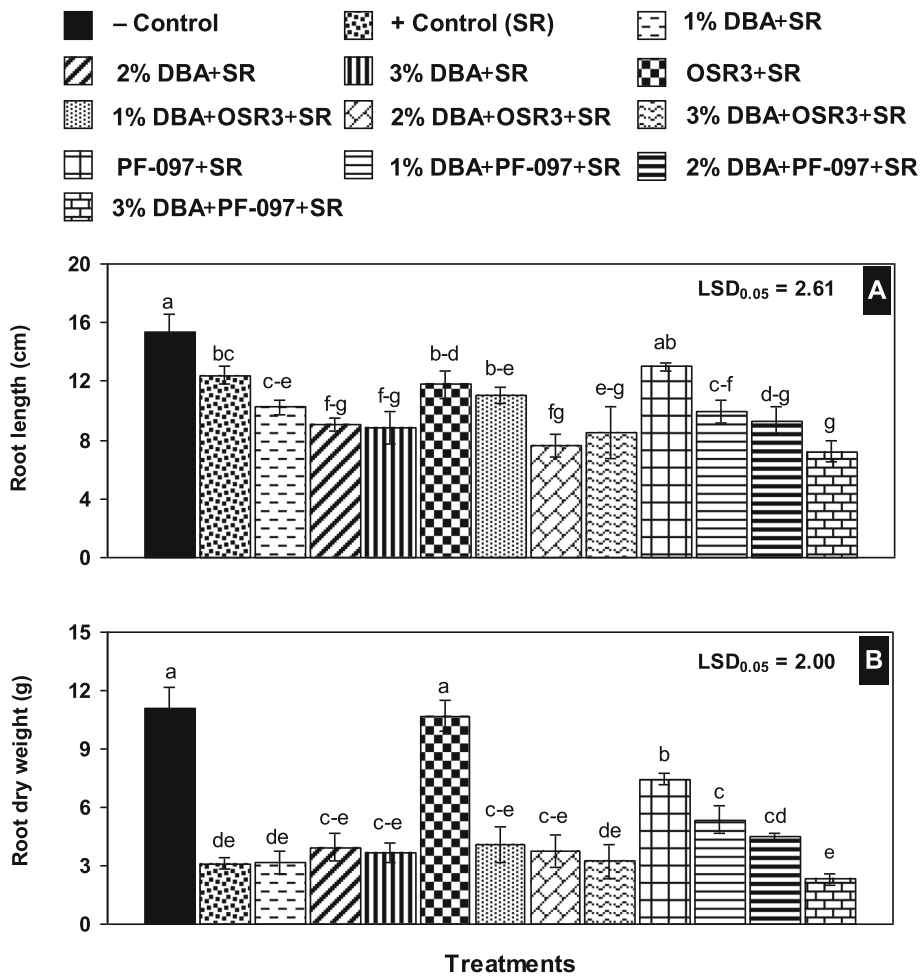

Fig. 3 Effect of different doses of dry biomass of *Anagalis arvensis* (DBA) and inoculation of two PGPR spp. *viz. Bacillus megaterium* OSR3 and *Pseudomonas fluorescence* PF-097 on root growth of chili in *Sclerotium rolfsi* (SR) inoculated soil. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD test

has the ability to inhibit the growth of *F. oxysporum*. Antagonistic potential of *B. megaterium* KU143 might be attributed to its volatile compounds (5-methyl-2-phenyl-1H-indole), which proved effective in suppressing germination of conidia, germ tube elongation, and sporulation of *Aspergillus* spp. in stored rice grains (Mannaa and Kim 2018). The antifungal potential of *P. fluorescnec* was ascribed to its ability to produce hydrogen cyanide, hydrolytic enzyme I, phosphate solubilization, and siderophore to suppress the pathogen's mycelial growth (Sahni et al. 2019). In a previous study, *P. fluorescence* showed potential antifungal activity against *Macrophomina phaseolina* (Shanmugam et al. 2002) and *R. solani* (Ayyanar et al. 2004). Hence, the antifungal activity in PGPR might be attributed to its potential to directly inhibit pathogen growth through siderophores and phytochrome production, which also play an essential role in enhancing plant growth (Bashan et al. 2005), and indirectly by degrading pathogen cell wall through hydrolytic enzyme, e.g., chitinase, proteases and ß-(1,3)-glucanase (Goswami et al. 2016), antibiotic, secondary metabolites (cyanide), and formation of biofilms (Saraf et al. 2014).

In the present study, when the bacterial isolates OSR3 and PF-097 were added in the soil, the southern blight disease of chili was reduced significantly, and growth attributes of the plants improved significantly. In the previous study, PGPR strains have been recommended to use as soil amendment to suppress soil-borne pathogens (Zheng et al. 2011). Soil application of *B. megaterium* was used to improve rice yield through suppressing brown spot caused by *Drechslera oryzae*, while the antifungal potential ascribed to production of lipopeptide and polyoxine antibiotics by biocontrol agent (Islam and Nandi 2007). Bhat et al. (2015) revealed that *P. fluorescence* exhibited ability to control southern blight disease of bell paper and to enhance the yield, probably through phosphate solubilization, phytochromes production, and nitrogen fixation. Sahni et al. (2008) proved that

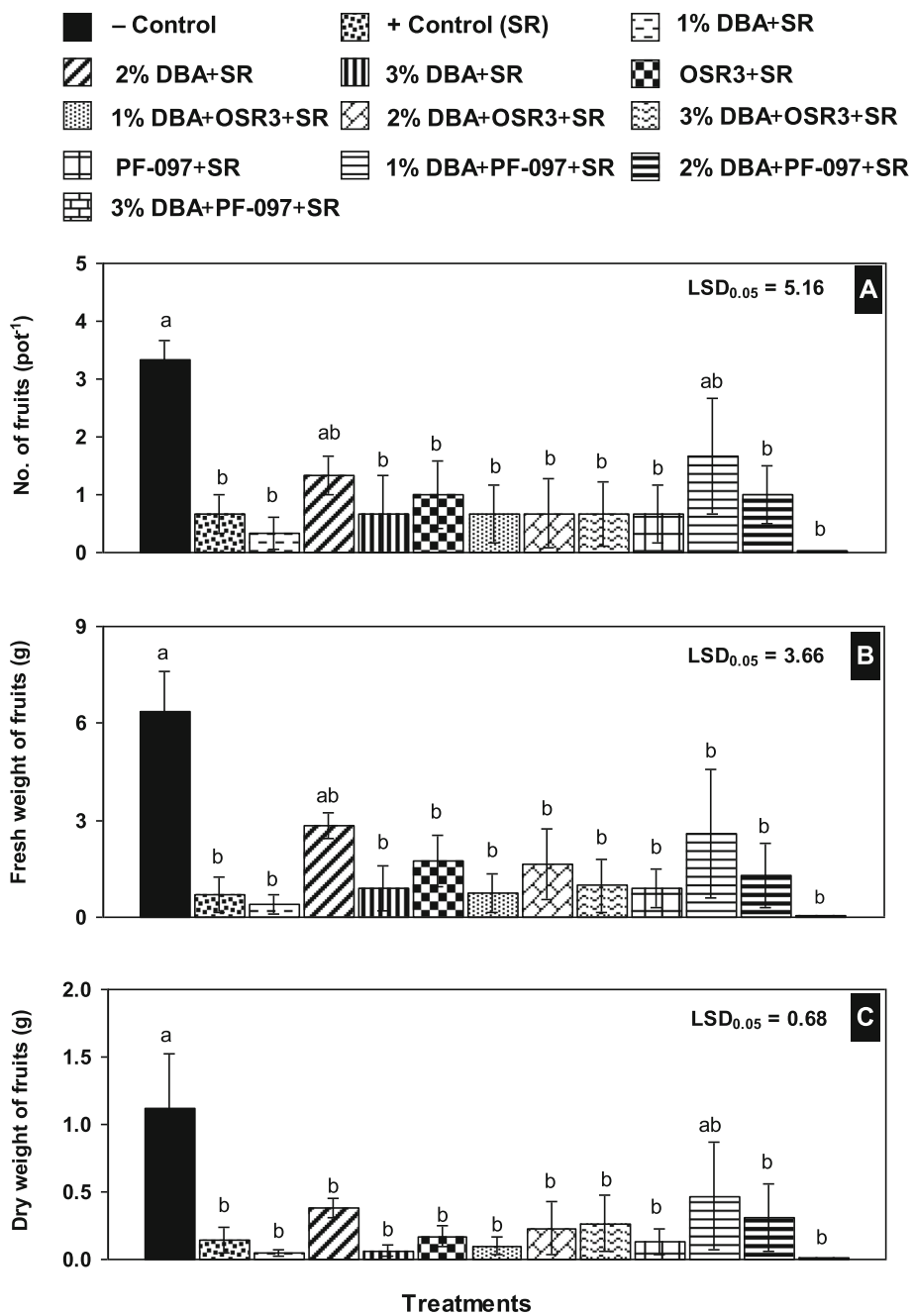

Fig. 4 Effect of different doses of dry biomass of *Anagalis arvensis* (DBA) and inoculation of two PGPR spp. *viz. Bacillus megaterium* OSR3 and *Pseudomonas fluorescence* PF-097 on yield of chili in *Sclerotium rolfsi* (SR) inoculated soil. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD test

Pseudomonas spp. was highly effective to control the collar rot disease caused by *S. rolfsii* and to improve nutrient uptake of the plant when used with vermicompost. Induction of systemic resistance, synthesis of antimicrobial compounds, production of growth hormones, and competition for niches important for pathogen are some of the mechanisms that the PGPR could employ to alleviate disease stress in chili (Awan et al. 2019).

The disease was managed and the growth/yield attributes were improved when either of PGPR strains (OSR3 or PF-097) was applied along with dry biomass of *A. arvensis*. Similar results have been obtained previously due to the combined effect of soil amendment or biocontrol agents in *Vigna mungo* and *V. radiata* against charcoal rot caused by *Macrophomina phaseolina* (Shoaib et al. 2018) and in *Cicer arietinum* against collar

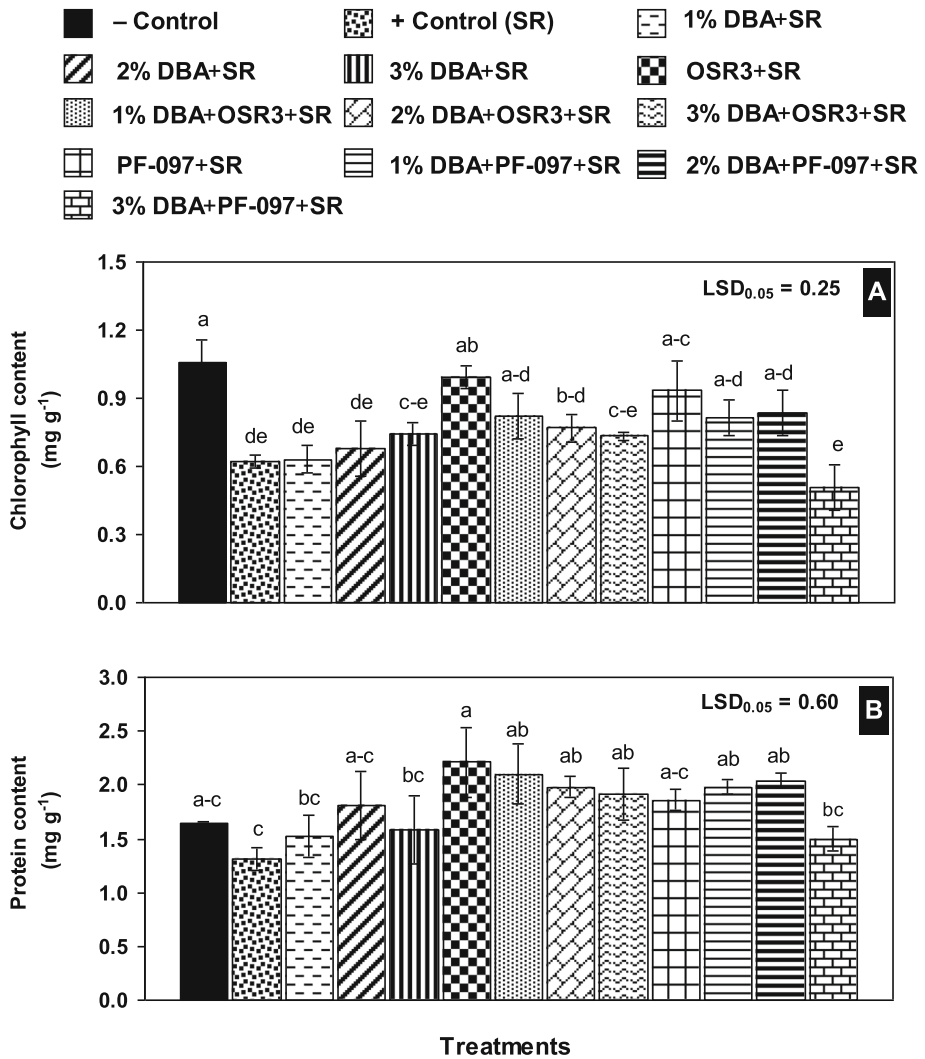

Fig. 5 Effect of different doses of dry biomass of *Anagalis arvensis* (DBA) and inoculation of two PGPR spp. *viz. Bacillus megaterium* OSR3 and *Pseudomonas fluorescence* PF-097 on chlorophyll and protein contents of chili in *Sclerotium rolfsi* (SR) inoculated soil. Vertical bars show standard errors of means of five replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD test

rot incited by *S. rolfsii* (Shoaib et al. 2019b). Likewise, combine application of PGPR strains such as *Pseudomonas* spp. and *Bacillus* spp. has been found highly antagonistic against Fusarium wilt caused by *F. oxysporum*. The biocontrol agents also increased plant height, and fruit and dry weight of tomato (Sundaramoorthy and Balabaskar 2013). Fungicidal action of dry biomass of *A. arvensis* and biocontrol bacteria may act as immune booster to increase resistance in chili plant against southern blight disease by conserving root system function, increasing nutrient availability and strengthening plant defense system in favor of better plant health (Shoaib et al. 2019b).

Results reveled that physio-chemical attributes in chili plants were drastically affected due to stress of *S. rolfsii* in positive control treatment than in the healthy plants in negative control, while application of plant biomass as organic amendment or bacteria as biofungicides exhibited variable influence on the investigated attributes. The pathogen stress in positive control treatments significantly decreased total chlorophyll content, which also resulted in reduction in the total protein content and activities of defenses related enzymes (CAT, POX and PPO). The obvious trend of reduction in physic-chemical attributes in plant under pathogen stress was reported earlier in many studied (Shoaib et al. 2018, 2019b; Awan et al. 2019). It could be attributed to chlorophyll degradation associated damaged to photosynthetic machinery that would cause disruption in the food manufacturing system. Plant immune system was likely to respond through over accumulation of reactive oxygen species (ROS) in response to *S. rolfsii*-induced

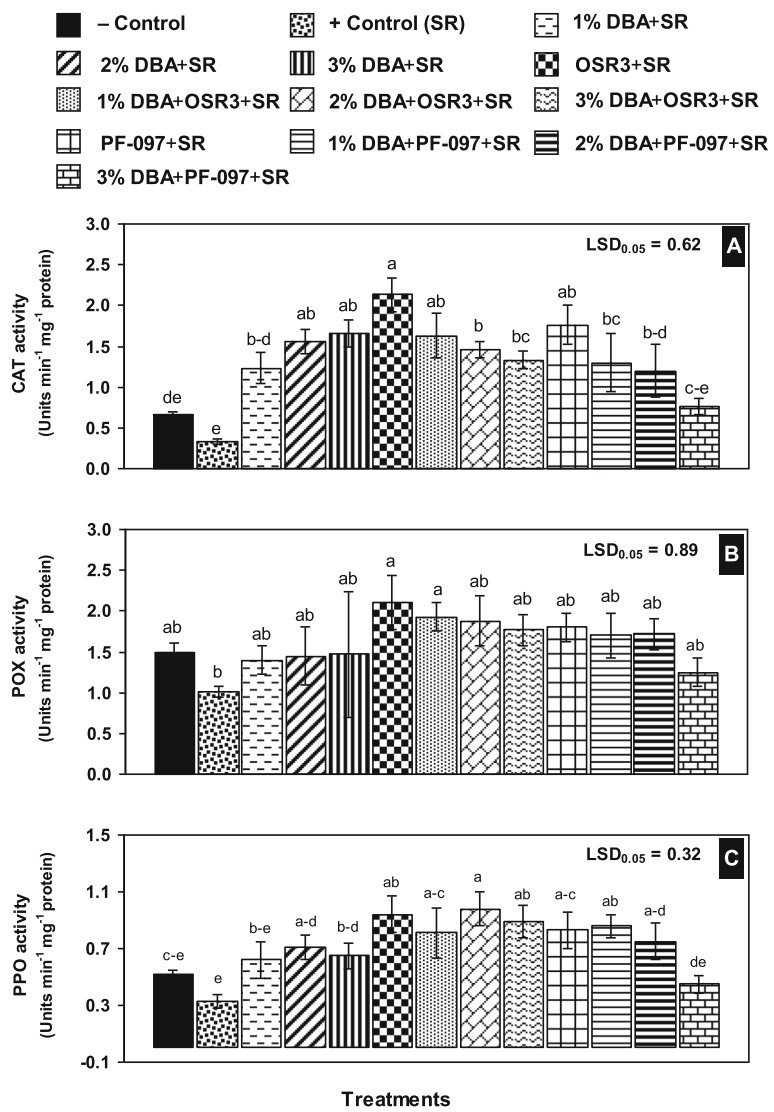

Fig. 6 Effect of different doses of dry biomass of *Anagalis arvensis* (DBA) and inoculation of two PGPR spp. *viz. Bacillus megaterium* OSR3 and *Pseudomonas fluorescence* PF-097 on activities of catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) of chili in *Sclerotium rolfsi* (SR) inoculated soil. 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 LSD test

stress. However, reduction in activities of defense related enzymes may indicate that the host defense system had weakened, hence unable to facilitate with required level of protection against ROS, which may lead to the general oxidation of cell contents, nucleotide damage, and ultimately cell death (Shoaib et al. 2019a; Awan et al. 2019).

Soil inoculation with either *B. megaterium* or *P. fluorescence* as well as in combination with 1 and 2% dry plant biomass of *A. arvenses* significantly improved total chlorophyll content, protein content, and activities of antioxidant enzymes. However, separate effect of either biocontrol agent displayed more pronounced improvement than the bilateral interaction with dry biomass of *A. arvenses*, while biocontrol agent along with higher dose (3%) of dry plant biomass exhibited adverse effect on different physio-chemical attributes. Moreover, *B. megaterium* was found to be more effective in improving the investigated physic-chemical attributes of the plants as compared to *P. fluorescence*. Therefore, total chlorophyll content, protein content, and activities of CAT, POX, and PPO were significantly improved by 60, 70, 600, 109, and 191%, respectively, due to single application of *B. megaterium* and by 30, 55, 400, 90, and 170%, respectively, due to interactive effect of *B. megaterium* with 1 or 2% dry plant biomass of *A. arvenses*. Likewise, *P. fluorescence* either alone or in combination with 1or 2% dry plant biomass of *A. arvenses* significantly improved total chlorophyll content, protein content, and

activities of CAT, POX, and PPO by 30–51, 42–56, 300–487, 70–78, and 132–159%, respectively. Improvement in total chlorophyll content could be ascribed to stimulatory effect of biocontrol agent on rubisco activity as reported formerly by (Awan et al. 2019; Shoaib et al. 2019b). Increase in protein content could be ascribed to more availability of free amino acid for synthesis of nitrogenous compound (Rosa and Maiti 1995). So, change in total protein content due to different treatments under pathogenic stress conditions revealed different levels of resistance acquired by host plant against *S. rolsii*. CAT and POX may contribute in resistance by limiting the damage caused by free radicals and also act like antibiotic against invading pathogen (Awan et al. 2019). Babu et al. (2015) also documented that tomato plants treated with antagonistic bacteria showed improved resistance against early blight, which was associated with increased synthesis of antioxidant enzymes (CAT, POX, and PPO). POX activity also increased rigidity of plant cell wall by synthesizing cell-wall polymers (lignin and suberin), and elevation in POX further indicated its role as physical barriers against pathogen stress (Khurshid et al. 2017). Increase in activity of PPO after incorporation of bio fungicides may signify its importance in plant defense by oxidizing phenolic compounds into quinones that could create toxic environment for pathogen and could react with it. Besides, PPO could activate plant defense mechanism by inactivating of pathogen pectolytic enzyme (Shoaib et al. 2018).

Conclusions

It is concluded that OSR3 was the best PGPR strain to control *S. rolfsii* both in vitro and in vivo and to increase crop growth of chili. Disease management by bacterial antagonists might be associated with induction of resistance in plant through enhanced activity of antioxidant enzymes. *A. arvensis* was not suitable to be used for control of collar rot disease of chili as it exerted negative impact on crop growth through allelopathy.

Abbreviations

PGPR: Plan-growth-promoting rhizobacteria; MEA: Malt extract agar; ANOVA: Analysis of variance; DBA: Dry biomass of *A. arvensis*; CAT: Catalase; POX: Peroxidase; PPO: Polyphenol peroxidase

Acknowledgements

Not applicable.

Authors' contributions

WS 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 paper writing. All authors have read and approved the manuscript.

Funding

Not applicable.

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

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 20 May 2020 Accepted: 8 January 2021
Published online: 15 January 2021

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