Sains Malaysiana 53(9)(2024): 3071-3083
http://doi.org/10.17576/jsm-2024-5309-13

Synergistic Effect of Plant Growth Promoting Rhizobacteria and *Cirsium arvense* against Black Scurf Disease of Potato

(Kesan Sinergi Pertumbuhan Tumbuhan Menggalakkan Rizobakteria dan *Cirsium arvense* terhadap Penyakit *Scurf* Hitam Kentang)

KARAMAT ALI ZOHAIB, UZMA BASHIR*, IQRA HAIDER KHAN, ARSHAD JAVAID & WAHEED ANWAR

Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan

Received: 31 January 2024/Accepted: 6 July 2024

ABSTRACT

Potato (*Solanum tuberosum* L.) is an important cash crop of Pakistan. Black scurf of potato is a very important disease that is caused by *Rhizoctonia solani* Kühn. In this study, management of black scurf of potato was done through biocontrol method by using plant growth promoting rhizobacteria (PGPR) and dry biomass of a weed, *Cirsium arvense* (L.) Scop. *In vitro* antagonistic interactions were carried out to assess the potential of two strains of PGPR namely *Bacillus megaterium* ZMR6 and *Pseudomonas fluorescence* PF180 against the fungal growth. *P. flourescens* showed marked antagonistic activity causing 65% reduction in growth of the fungus as compared to 43% reduction due to *B. megaterium.* Likewise, in laboratory bioassays, methanolic extract of 2, 4 and 6% concentrations of leaf, stem, and root of *C. arvense* reduced biomass of *R. solani* by 64-71%, 42-53% and 26-47%, respectively. In pot experiment, the two PGPR species and different doses of *C. arvense* dry biomass (CDB) viz. 1, 2 and 3% (w/w) were used as soil amendment separately as well as in combination to control the disease. There were 13 treatments, which included a negative control; a positive control (*R. solani* only); 1, 2 and 3% CDB (separately) together with *R. solani,* two PGPR species (separately) plus *R. solani*; different combinations of two PGPR species and CDB together with *R. solani.* Potato variety Sante was used as test plant. The highest disease incidence (91%) and disease severity (rating scale 4) were observed in positive control (with *R. solani* only). *R. solani* significantly reduced biomass of tubers by 52% over negative control. All the treatments significantly enhanced tuber biomass by 18-166% over positive control. The best combination was 3% CDB + *P. florescence* where minimum disease incidence (3%) and severity (mean disease rating 0.2) were recorded. This treatment also showed the highest tubers yield that was 29% and 166% higher as compared to negative and positive control treatments, respectively. It concluded that *P. florescence* in combination with 3% dry biomass of *C. arvense* can control black scurf disease and enhance potato yield.

Keywords: *Bacillus megaterium;* black scurf; *Cirsium arvense;* potato; PGPR; *Pseudomonas fluorescence*

ABSTRAK

Kentang (*Solanum tuberosum* L.) ialah tanaman kontan yang penting di Pakistan. *Scurf* hitam kentang adalah penyakit yang sangat penting yang disebabkan oleh *Rhizoctonia solani* Kühn. Dalam kajian ini, pengurusan *scurf* hitam kentang dilakukan melalui kaedah biokawalan dengan menggunakan rizobakteria penggalak pertumbuhan tumbuhan (PGPR) dan biojisim kering rumpai, *Cirsium arvense* (L.) Scop. Interaksi antagonis *in vitro* telah dijalankan untuk menilai potensi dua strain PGPR iaitu *Bacillus megaterium* ZMR6 dan *Pseudomonas fluorescence* PF180 terhadap pertumbuhan kulat. *P. flourescens* menunjukkan aktiviti antagonis yang ketara menyebabkan 65% pengurangan dalam pertumbuhan kulat berbanding pengurangan 43% disebabkan oleh *B. megaterium*. Begitu juga, dalam bioasai makmal, ekstrak metanol 2, 4 dan 6% kepekatan daun, batang dan akar *C. arvense* mengurangkan biojisim *R. solani* masing-masing sebanyak 64-71%, 42-53% dan 26-47%. Dalam uji kaji pasu, dua spesies PGPR dan dos berbeza *C. arvense* biojisim kering (CDB) iaitu 1, 2 dan 3% (b/b) digunakan sebagai pindaan tanah secara berasingan serta dalam gabungan untuk mengawal penyakit. Terdapat 13 rawatan, termasuk kawalan negatif; kawalan positif (*R. solani* sahaja); 1, 2 dan 3% CDB (berasingan) bersama *R. solani*, dua spesies PGPR (berasingan) ditambah *R. solani*; gabungan berbeza dua spesies PGPR dan CDB bersama *R. solani*. Varieti kentang Sante digunakan sebagai tumbuhan uji. Insiden penyakit tertinggi (91%) dan keterukan penyakit (skala penarafan 4) diperhatikan dalam kawalan positif (dengan *R. solani* sahaja). *R. solani* dengan ketara mengurangkan biojisim

ubi sebanyak 52% berbanding kawalan negatif. Semua rawatan meningkatkan biojisim ubi dengan ketara sebanyak 18-166% berbanding kawalan positif. Gabungan terbaik ialah 3% CDB + *P. florescence* dengan kejadian penyakit minimum (3%) dan keterukan (min rating penyakit 0.2) direkodkan. Rawatan ini juga menunjukkan hasil ubi yang paling tinggi masing-masing iaitu 29% dan 166% lebih tinggi berbanding rawatan kawalan negatif dan positif. Ia membuat kesimpulan bahawa *P. florescence* dalam gabungan dengan 3% biojisim kering *C. arvense* boleh mengawal penyakit scurf hitam dan meningkatkan hasil kentang.

Kata kunci: *Bacillus megaterium; Cirsium arvense;* kentang; PGPR; *Pseudomonas fluorescence;* scurf hitam

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most widely consumed and produced tuberous crops in the world (Dourado et al. 2019). It is popular in Pakistan due to its diverse potential uses, nutrient capacity and easy availability to low-income consumers. It ranked third after rice and wheat and grown in different agro-ecological conditions of Pakistan ranging from plains to hilly areas (Khan, Nakano & Kurosaki 2019). It is a rich source of vitamins, carbohydrates, proteins, minerals and fats (Neela & Fanta 2019). Several biological constraints such as fungal pathogens negatively affect the crop production and tuber quality in the country (Majeed & Muhammad 2020). Black scurf of potato is caused by *Rhizoctonia solani* has become a destructive disease in recent years in the entire growing areas (Rafiq et al. 2024; Yang et al. 2017). Being soil- and seed-borne in nature, the pathogen has a high level of survival and a wide host range, which makes its management very complex (Swain, Naik & Mukherjee 2019). Once establishes in a field, it can remain there in the form of mycelium or sclerotia for extended time period leaving drastic effects on crop development from emergence to harvest (Salamone & Okubara 2020).

Presently, it is not possible to control the pathogen completely but its severity can be limited by following a combination of integrated approaches such as physical, cultural and chemical measures (Akhter et al. 2015). Nevertheless, each practice has its limitations and no single tactic is totally effective (Abbas et al. 2019). To date, biological control is considered as the best management strategy because of its low cost, immediate availability and low risk implementation (Zohora, Ano & Rahman 2016). The use of plant growth-promoting rhizobacteria (PGPR) belonging to the species of *Bacillus* and *Pseudomonas* genera have shown promising results for the effective control of various fungal plant pathogens (Hussain & Khan 2020; Sharf et al. 2021; Yu et al. 2017). These biocontrol agents could form spores which remain metabolically active and survive under harsh environmental conditions making them appropriate for the formulations of viable and stable products (Selim, Gomaa & Essa 2017). They also have the capability to reduce deleterious effects of plant pathogens by colonizing plant roots with an improved crop yield (Khedher et al. 2015). The mechanism by which PGPR exert beneficial effects on crop plants is through induce systemic resistance, improved nutrient uptake, enhanced synthesis of stimulatory phytohormones and the production of inhibitory substances to overcome the deleterious effects of the phytopathogens (Etesami & Adl 2020).

Natural plant products are well-known for their use in the management of plant diseases caused by soil-borne plant pathogens such as *Macrophomina phaseolina, Sclerotium rolfsii,* and *Fusarium oxysporum* (Akhtar & Javaid 2018; Jabeen et al. 2021; Javed et al. 2021). However, similar studies to control black scurf of potato are not common. *Cirsium arvense* L., commonly known as creeping thistle, belongs to family Asteraceae (Koc et al. 2015). It is a medicinal plant and often found as noxious weed grown throughout the tropical and subtropical world zones (Anser et al. 2018). It is used in folk medicines for the treatment of leukemia, ulcer, metrorrhagia, epitasis, eye infections, bleeding piles, tuberculosis, syphilis, skin sores gonorrhea and also found to be effective against diabetes (Sadat-Hosseini et al. 2017). It contains coumarins, flavonoids, tannins, phenolic acids, triterpenes, and sterols that might be responsible for antifungal, antioxidant and anti-inflammatory properties (Amiri, Yadegari & Hamedi 2018; Popova et al. 2018). However, its affect against black scurf disease of potato caused by *R. solani* is lacking. There are reports that crude extract and/or soil application of dry plant materials of allelopathic weeds of family Asteraceae can significantly reduce growth of soil-borne fungal plant pathogens such as *R. solani* (Rafiq, Javaid & Shoaib 2021) and *Macrophomina phaseolina* (Banaras, Javaid & Khan 2020; Banaras, Javaid & Shoaib 2020). Therefore, the present investigation was carried out to manage the black scurf of potato through soil application of dry plant biomass of *C. arvense* and two species of PGPR.

MATERIALS AND METHODS

PROCUREMENT OF FUNGAL PATHOGEN AND PGPR

Pure cultures of *R. solani* and two PGPR strains (*B. megaterium* ZMR6 and *P. fluorescence* PF180) were procured from Fungal Culture Bank of Pakistan. The fungal culture and bacterial strains were multiplied and stored at 4 ºC for further experimentation.

IN VITRO ANTAGONISTIC INTERACTION

Interaction of PGPR with *R. solani* was carried out on PDA (potato dextrose agar) in 9-cm Petri plates. For this, mycelial discs (5 mm diameter) of 7-day-old culture of *R. solani* were prepared and placed in the center of the

Petri plates. At the same time, bacterial colonies of *B. megaterium* ZMR6 and *P. fluorescence* PF180 were picked up with the help of sterilized loop and spread inside the Petri plates 2 cm away from the fungal plugs. The control plates were inoculated with only *R. solani.* Three replicates of each treatment were made and arranged in a completely randomized design. The whole experiment was placed at 28 ºC in an incubator for 6 days. Thereafter, radial growth of the pathogenic fungus was noted in the control and in the dually inoculated plated. The percentage inhibition in the growth of pathogenic fungus was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Radial growth in control - Radial growth in dual culture}}{\text{Radial growth in control}} \times 100$$

COLLECTION OF WEED PLANT

C. arvense leaves, stems and roots were collected from Lahore, Pakistan. The plant parts were washed thoroughly under running tap water, surface sterilized with 1% sodium hypochlorite and again washed with distilled water. The material was shade dried and kept in an electric oven at 40 ºC for 24 h and grinded into a fine powder form.

PREPARATION OF *C. ARVENSE* METHANOLIC EXTRACTS

One hundred grams of each dried plant part were soaked in airtight glass jars containing 250 mL methanol. After 2 weeks, the materials were passed through muslin cloth followed by double layered filter papers. In order to remove methanol, the filtrate was kept on a rotary evaporator at 45 °C and the remaining material was poured into pre-weighed containers. Next, the beakers were placed in an electric oven at 45 °C for the complete solvent evaporation. As a result, brown gummy masses of 4.7 g, 5.1 g and 4.5 g of leaves, stems and roots were obtained, respectively.

ANTIFUNGAL BIOASSAYS

For this, procedure given by Khan and Javaid (2020) was adopted with little modifications. Malt extract broth (MEB) was prepared in conical flasks of 250 mL and chloromycetin (50 mg 100 mL^{-1}) was added to avoid bacterial growth. Each flask contained 80 mL of autoclaved MEB. Methanolic extracts (2, 4 and 6 g) of different parts of *C. arvences* extracts were dissolved in 0.5 dimethyl sulfoxide (DMSO) and appropriate quantity of distilled water was added to raise the volume to 20 mL. These solutions were added to 80 mL MEB to prepare 2%, 4% and 6% (w/v) growth media. For control, 19.5 mL of distilled autoclaved water was mixed with 0.5 mL DMSO and added to 80 mL of MEB without having plant extract. After that, mycelial plugs (5 mm) of *R. solani,* prepared from 7-day-old culture, were inoculated in each flask and kept on an incubator shaker at 27 ºC for 10 days. Each treatment was replicated thrice. The fungal biomass from each flask was collected on pre-weighed filter papers and dried at 60 ºC in an electric oven for dry weight measurement.

POT TRAIL

A pot experiment was conducted in clay pots (27 cm diameter, 35 cm height) each having 6 kg of fumigated soil. Soil having pH 7.7 and sandy loam texture, 0.85% organic matter, 95 mg kg^{-1} exchangeable potassium and 5.6 mg kg^{-1} available phosphorus was used in the pot study. Soil was fumigated by placing cotton swabs dipped in formalin and then covered by polythene sheet for 7 days. Afterwards, cotton swabs were removed and the soil was left open for 7 days in order to vaporize any traces of formalin.

Pearl millet seeds were selected for the preparation of *R. solani* inoculum. First seeds were lightly boiled, air dried, filled in plastic bags and autoclaved at 121 °C for 30 min. After that the bags were cooled at room temperature and inoculated with *R. solani* discs and kept at 27 °C for 7 days. Thereafter, *R. solani* inoculated seeds (5 g kg^{-1} soil) were mixed thoroughly in the pots and left for 7 days after irrigation. A negative control was also prepared by adding the same amount of boiled pearl millet seeds but without pathogen inoculum. Likewise, powdered dry biomass of *C. arvense* was mixed in the respective pot soil in the ratio of 1%, 2% and 3% (w/w) and irrigated with tap water and left for one week.

The tubers of potato variety Sante were procured from Punjab Seed Corporation, Sahiwal, Pakistan and surface sterilized with 0.5% NaOCl solution. After 30 s, the tubers were washed thoroughly with sterilized water. Later, four tubers were sown in each pot. Following 13 treatments were used in completely randomized design with 5 replicates of each: T_1 (–) Negative control; T_2 (+) Positive control [*Rhizoctonia solani* (RS)]; T_3 1% CDB (*Cirsium* dry biomass) + RS; T_4 2% CDB + RS; T_5 3% CDB + RS; T_6 *B. megaterium* ZMR6 + RS; T_7 *P. fluorescence* PF180 + RS; T_8 1% CDB + ZMR6 + RS; T_9 2% CDB + ZMR6 + RS; T_{10} 3% CDB + ZMR6 + RS; T_{11}1% CDB + PF180 + RS; T_{12} 2% CDB + PF180 + RS; T_{13} 3% CDB + PF180 + RS.

After 90 days of sowing, the crop was harvested by emptying the pots carefully. The plants were washed and tubers were separated from roots and stems. Data regarding disease incidence, disease severity, tubers yield pot^{-1}, tuber size, and shoot, root and dry biomass were collected. Disease incidence was calculated by applying the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected tubers}}{\text{Total number of tubers observed}} \times 100$$

Disease severity was calculated with the help of 0–5 scale of disease severity which is based on percentage of disease symptoms shown on surface of tubers as shown in Table 1 (Ahmed et al. 1995).

STATISTICAL ANALYSIS

By using MS Excel office program, standard errors (SE) of means were calculated. Whole data obtained from pot trials and lab assays were subjected to analysis of variance, followed by separation of treatment means with the help of LSD test at $P \leq 0.05$ by using Statistix 8.1 computer software.

RESULTS

In vitro INTERACTIONS STUDY

Both the PGPR species markedly suppressed the growth of *R. solani* under *in vitro* conditions. However, *P. fluorescence* showed better antagonistic activity than *B. megaterium* causing 65% and 43% reduction in growth of *R. solani* over control, respectively (Figures 1 & 2).

ANTIFUNGAL BIOASSAYS

Methanolic extract of all the three parts of *C. arvense* showed inhibitory potential against growth of *R. solani.* However, inhibitory activity of the extracts variable with respect to the plant part used for extraction. Among the three plant parts, leaves extract showed the highest antifungal activity by suppressing 64-71% growth of the pathogen. On the other hand, different concentrations of stem and root extract reduced the biomass of *R. solani* by 42-53% and 26-47%, respectively (Figures 3 & 4).

POT TRIAL

No disease was observed in the negative control while the positive control showed the highest disease incidence (91%) and severity (mean rating 4.6). Application of weed dry biomass showed significantly lowered incidence (36 to 68%) and severity (mean scale 1.2 to 2.4) of disease over positive control. The disease gradually deceased with the increase in concentration of the dry biomass. In general, both the PGPR species significantly lowered the disease attack. However, *P. fluorescence* application proved more efficient than the *B. megaterium* with 14% and 32% disease incidence, and 1 and 2 rating scale of disease severity, respectively. *B. megaterium* together with different doses of weed biomass further reduced disease attack. *P. fluorescence* also showed a similar effect together with 2% and 3% doses of weed biomass. The lowest disease incidence (3%) and severity (mean rating 0.2) were recorded in treatments where *P. fluorescence* was applied with 3% weed dry biomass (Figure 5).

R. solani inoculation significantly reduced root length and biomass by 37% and 40% over negative control. The lowermost dose of weed biomass did not affect root growth under *R. solani* stress while the higher doses (2% and 3%) significantly enhanced root growth over positive control. Root growth in 3% weed biomass application was at part with that of negative control. Both the PGPR strains either alone or together with weed biomass improved root growth over positive control. However, the effect of *B. megaterium* was not much pronounced. On the other hand, application of *P. fluorescence* either alone or in combination with 3% weed biomass completely masked the effect of *R. solani* and showed root growth at par with negative control (Figure 6).

R. solani inoculation significantly reduced number and biomass of tubers by 37% and 52% over negative control. All the treatments significantly enhanced number of tubers by 47-84% and tuber biomass by 18-166% over positive control. The highest number and biomass of potato tubers were recorded in combined application of *P. fluorescence* and 3% weed biomass (Figure 7).

DISCUSSION

In the present study, two PGPR strains namely *P. fluorescence* and *B. megaterium,* and dry biomass of an asteraceous weed *C. arvense* were evaluated for their *in vitro* and *in vivo* potentials to control black scurf disease of potato. In general, all the applied organisms and the plant material markedly suppressed the growth of pathogen and enhanced quality and quantity of the product. In laboratory bioassays, both the PGPR strains caused a pronounced antagonistic activity against the pathogen *R. solani. P. fluorescence* showed a better antagonistic behavior than *B. megaterium* against the pathogen. In a recent report, these PGPR strains showed similar antagonistic behavior against *Sclerotium rolfsii* under laboratory conditions (Sharf et al. 2021). In pot trial, both the PGPR species significantly lowered incidence as well as severity of the black scurf disease of potato resulting in improved quality and quantity of potato. There are reports that PGPR not only suppress the growth of plant pathogens but also improve crop growth by enhancing nutrient uptake (Xiang et al. 2017). Generally, *Bacillus* spp. control the growth of plant pathogens by producing antifungal compounds (terpenes and polypeptide), cell wall degrading enzymes (chitinases), antibiotics such as iturin A (Romero et al. 2007; Shoda 2000). Certain volatile compounds such as 5-methyl-2-phenyl-1H-indole produced by *B. megaterium* suppressed sporulation, conidial germination, and germ tube growth of various *Aspergillus* spp. (Mannaa & Kim 2018). Earlier, *P. fluorescence* exhibited its antifungal activity in controlling growth of *Macrophomina phaseolina* and *R. solani* (Ayyanar et al. 2004; Shanmugam, Ramanathan & Samiyappan 2002). A number of mechanisms have been proposed regarding the antifungal activity of *P. fluorescence*. These include hydrolytic enzyme namely proteases, chitinase and ß-(1,3)-glucanase, which degrade cell wall of the pathogens (Goswami, Thakker & Dhandhukia 2016), production of antibiotic, secondary metabolites such as cyanides, siderophores and phytochromes, and formation of biofilms (Sahni, Prasad & Ranjan 2019; Saraf, Pandya & Thakkar 2014).

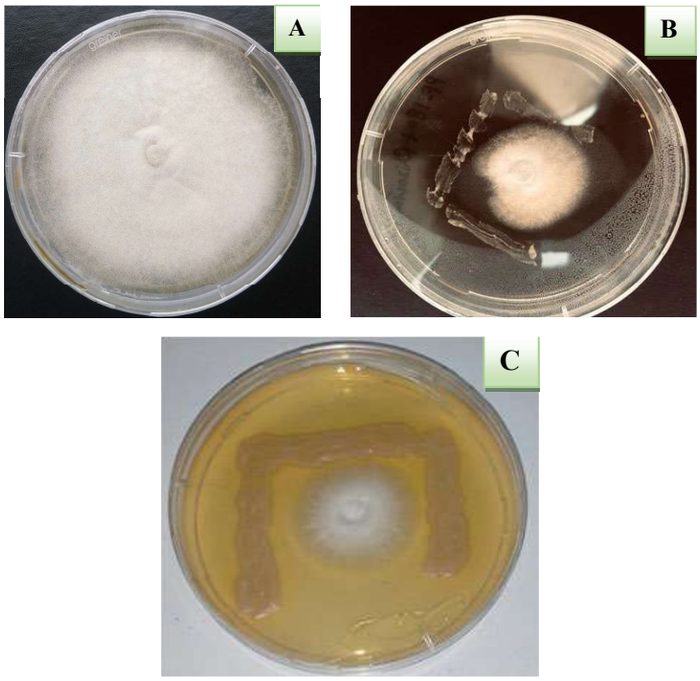

FIGURE 1. Inhibitory effects of antagonistic PGPR strains against growth of *Rhizoctonia solani* in dual culture bioassays. A): Pure culture of *R. solani*, B): *R. solani* co-cultured with *Bacillus megaterium* ZMR6, C): RS co-cultured with *Pseudomonas fluorescence* PF180

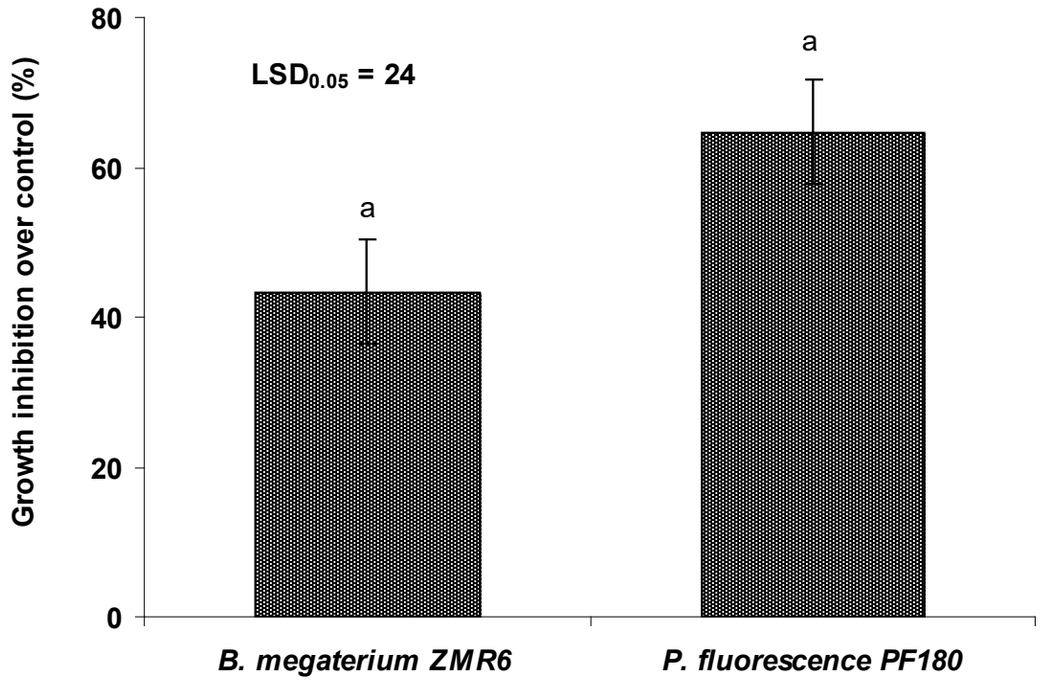

FIGURE 2. Percentage inhibition in radial growth of *Rhizoctonia solani* due to *Bacillus megaterium* ZMR6 and *Pseudomonas fluorescence* PF180. Same letters on bars show insignificant difference between the two treatments

TABLE 1. Disease severity rating scale for the evaluation of black scurf of potato under field conditions

Disease severity grades	Percentage of disease
0	No disease symptoms
1	< 1% tuber surface affected
2	1 to 10% tuber surface affected
3	11 to 20% tuber surface affected
4	21 to 50% tuber surface affected
5	> 50% tuber surface affected

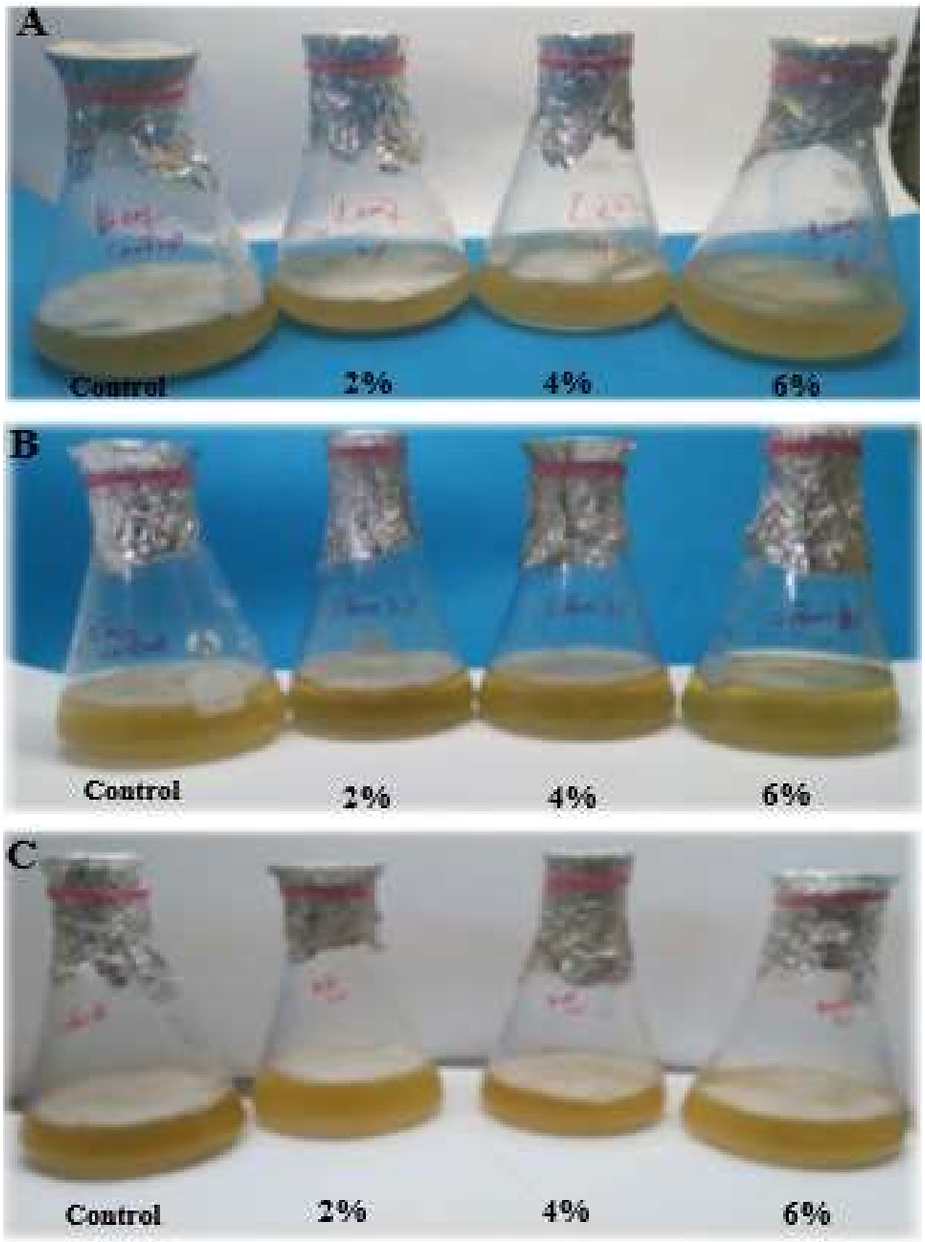

FIGURE 3. Effect of different concentrations of methanolic extracts of different parts A): leaf, B): stem, and C): root of *Cirsium arvense* on biomass of *Rhizoctonia solani*

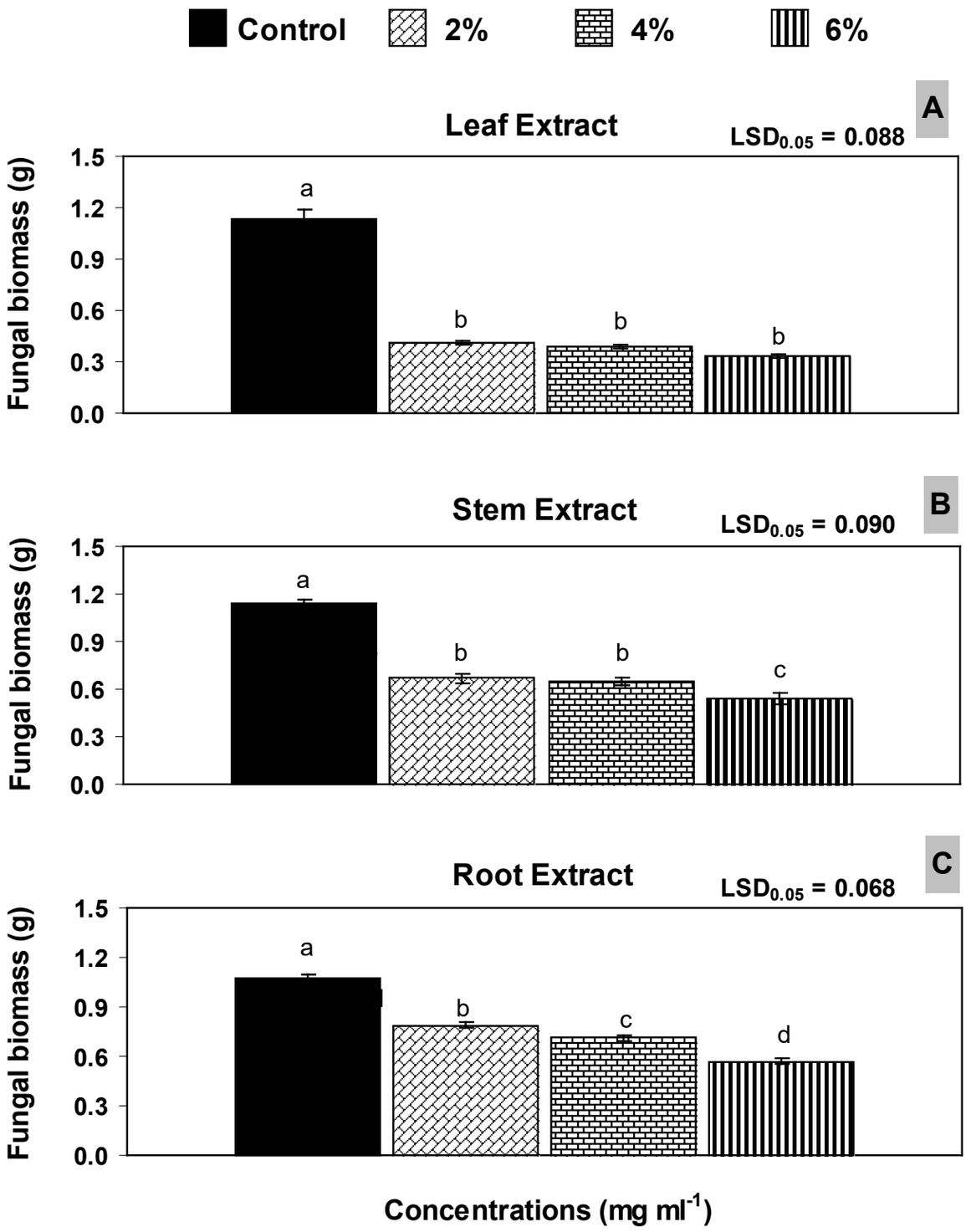

FIGURE 4. Effect of methanolic extracts of different parts of *Cirsium arvense* on biomass of *Rhizoctonia solani.* Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD test

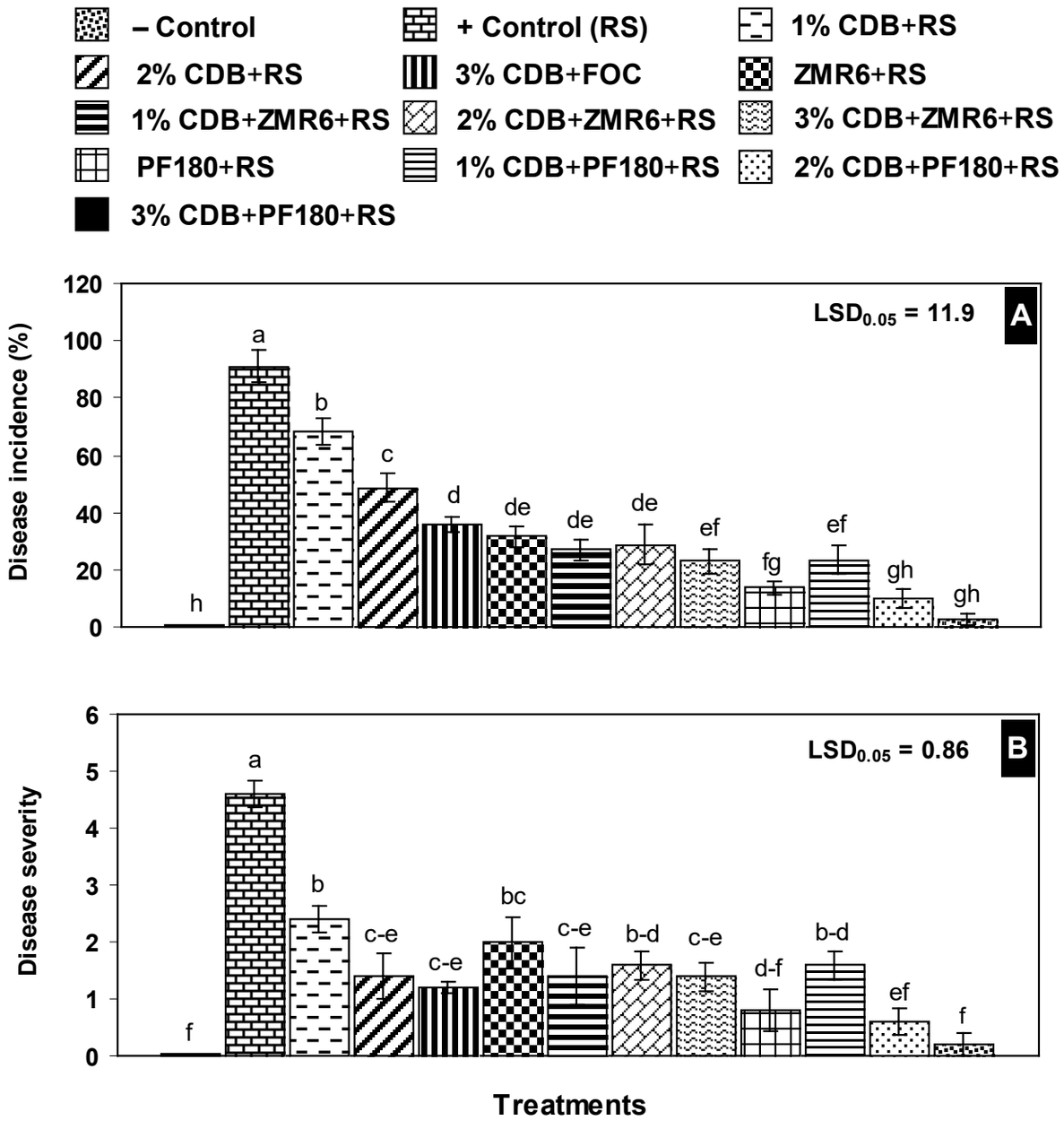

FIGURE 5. Effect of different doses of *Cirsium arvense* dry biomass(CDB) and inoculation of two species of PGPR (*Bacillus megaterium* ZMR6 and *Pseudomonas fluorescence* PF-180) on incidence and severity of black scurf disease of potato caused by *Rhizoctonia solani* (RS). Vertical bars show standard errors of means of four replicates. Values with differentletters at their top show significant difference (P≤0.05) as determined by LSD test

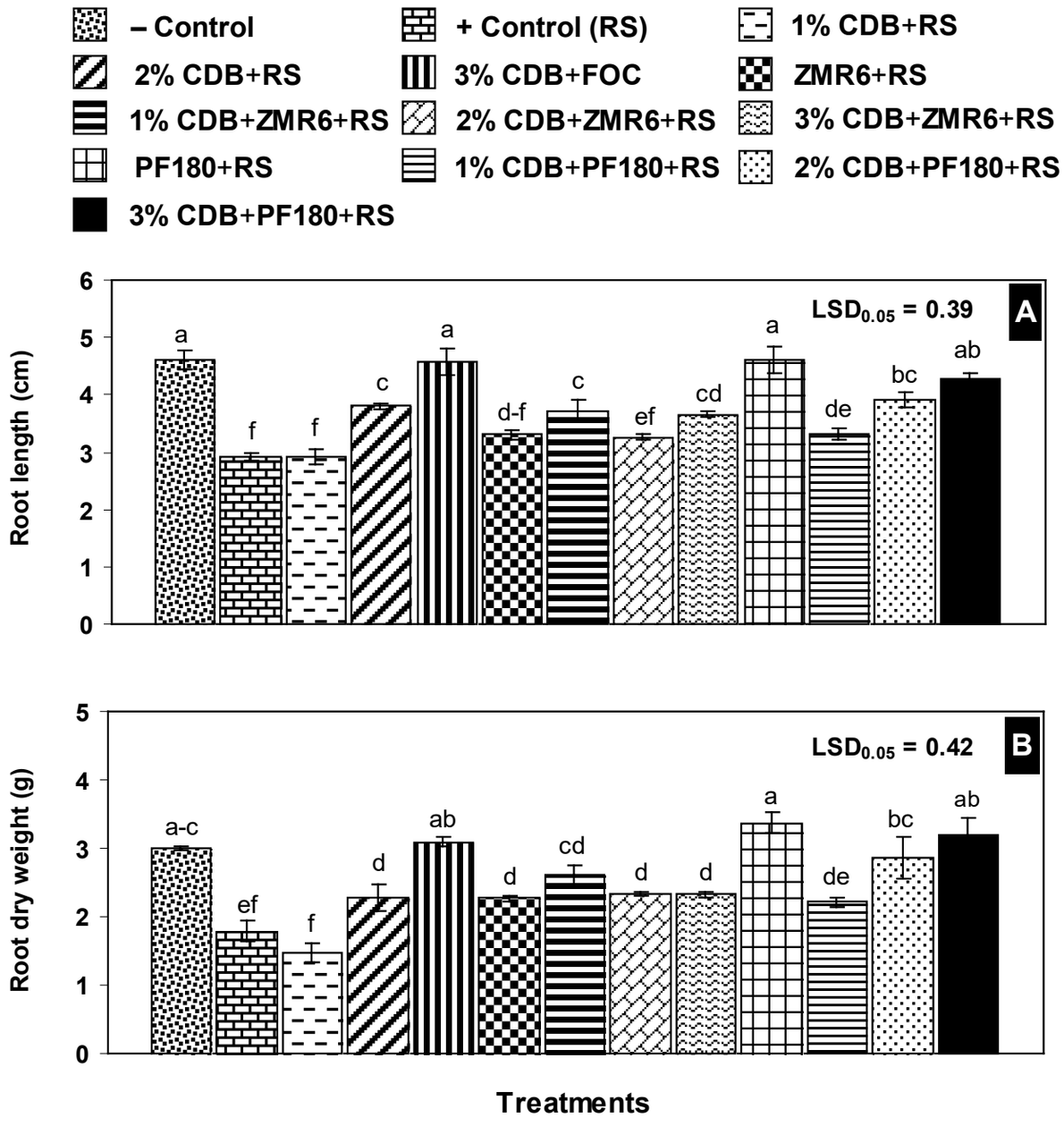

FIGURE 6. Effect of different doses of *Cirsium arvense* dry biomass (CDB) and inoculation of two species of PGPR (*Bacillus megaterium* ZMR6 and *Pseudomonas fluorescence* PF180) on root growth of potato in *Rhizoctonia solani* (RS) inoculated soil. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD test

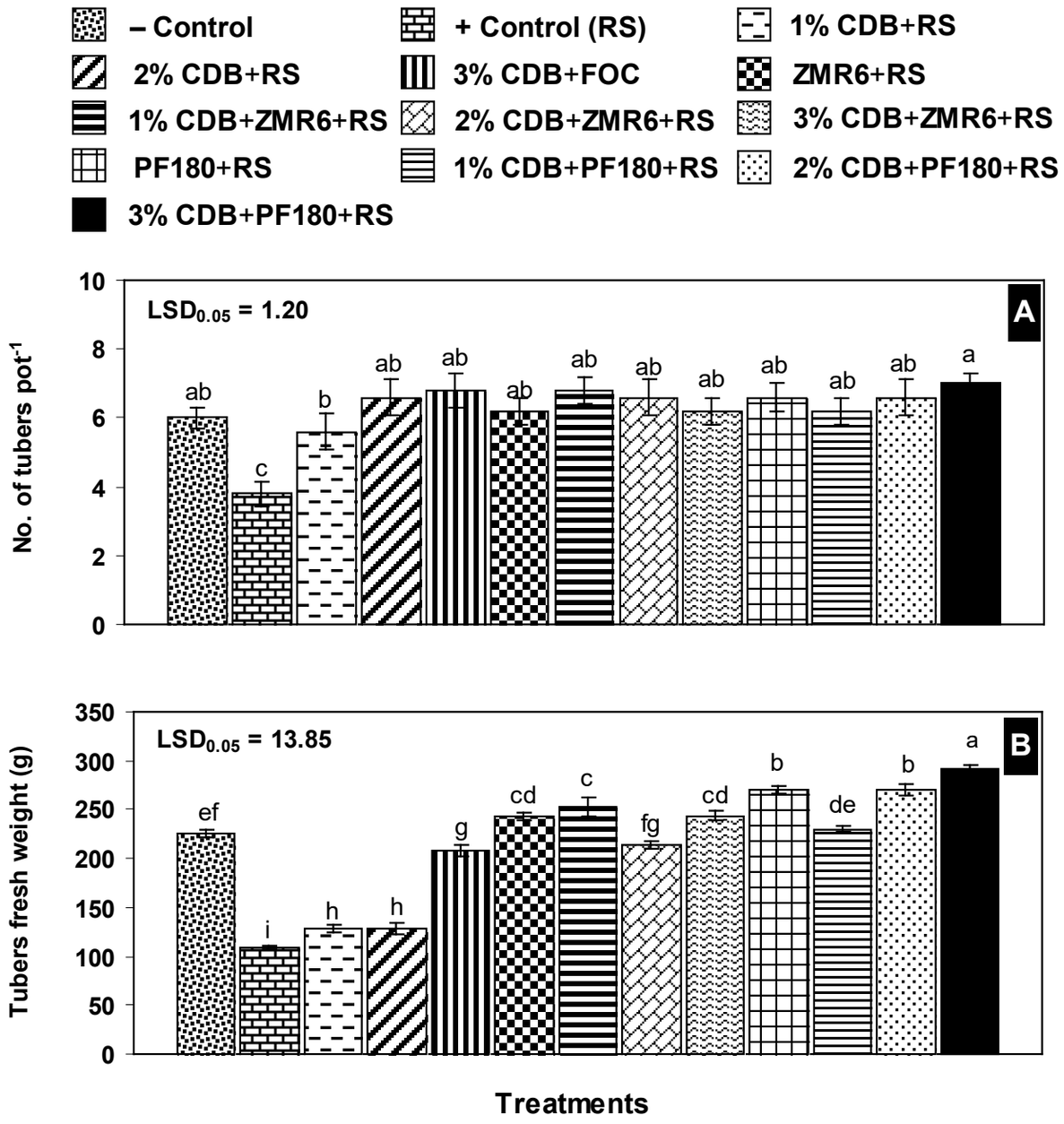

FIGURE 7. Effect of different doses of Cirsium arvense dry biomass (CDB) and inoculation of two species of PGPR (Bacillus megaterium ZMR6 and Pseudomonas fluorescence PF180) on number and fresh weight of potato in Rhizoctonia solani (RS) inoculated soil. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD test

Under laboratory conditions, extracts of all the three parts of *C. arvense* significantly declined growth of the pathogen. Khan et al. (2011a) also reported antifungal activity of this weed against *Aspergillus niger*. Polyacetylenic compounds along with aplotaxene gallic acid, tannin and taraxasterol present in this weed could be responsible for antifungal properties of *C. arvense* (Donalds 1994; Norton 2005). Compounds namely pectolinarigenin-7-O-glucopyranoside, ciryneol C acacetin and scopoletin identified in *C. arvense* whole plant exhibited antifungal behavior against many fungal species including *Candida albicans, C. glaberata Fusarium solani,* and *Aspergillus flavus* (Khan et al. 2011b). In the present study, leaf extract of *C. arvense* showed marked better antifungal activity than the extracts of other two parts of the test plant species. Earlier, Banaras et al. (2017) reported that a 5% leaf extract of this weed reduced biomass of *Macrophomina* by 74% as compared to 57% and 39% reduction due to stem and root extract, respectively. Leaves of *C. arvense* contain much higher concentrations of flavonoid and polyphenol than other parts of the plant that might be responsible for its better antifungal activity than stem and root (Mihaela 2014). Flavonoids have been recognized as widespread group of natural compounds with pronounced antimicrobial properties (Karasakal et al. 2015). Fatty acid methyl esters present in different parts of *C. arvense* could also be responsible for antifungal activity of *C. arvense* (Banaras et al. 2017). Similarly, other asteraceous weeds namely *Ageratum conyzoides, Sonchus oleraceous,* and *Carthamus oxycantha* contain a number of potent antifungal compounds to control growth of *Macrophomina phaseolina* and *Rhizoctonia solani* under *in vitro* conditions (Banaras, Javaid & Khan 2021, 2020; Rafiq, Javaid & Shoaib 2021). In pot trial, the highest disease incidence and severity were recorded in the positive control where *R. solani* was inoculated without any soil amendment. Different doses of *C. arvense* suppressed disease and significantly increased root length and biomass as well as number and biomass of tubers. The positive effect of weed biomass was gradually increased by increasing concentration of the weed biomass. In an earlier study, it has been found that soil amendment with dry biomass of an asteraceous weed *Sonchus oleraceous* significantly reduced charcoal rot disease in mash bean resulting in plant growth and yield (Banaras, Javaid & Shoaib 2020). It is possibly due to release of antifungal compounds from decomposing materials that reduced growth of pathogenic fungi in the soil.

CONCLUSIONS

Both the PGPR species significantly controlled *in vitro* growth of *R. solani*. Likewise, methanolic extracts of aerial and underground parts of *C. arvense* reduced biomass of the fungus by 26-71%. In pot trial, *P. fluorescence* together with 3% dry biomass of *C. arvense* exhibited the best biocontrol potential against black scurf disease of potato and markedly increased the plant growth and yield.

REFERENCES

Abbas, A., Khan, S.U., Khan, W.U., Saleh, T.A., Khan, M.H.U., Ullah, S. & Ikram, M. 2019. Antagonist effects of strains of *Bacillus* spp. against *Rhizoctonia solani* for their protection against several plant diseases: Alternatives to chemical pesticides. *Comptes Rendus Biologies* 342: 124-135.

Ahmed, I., Soomro, M.H., Khalid, S., Iftikhar, S., Munir, A. & Burney, K. 1995. Recent distributional trends of potato diseases in Pakistan. *National Seminar on Research and Development of Potato Production in Pakistan*, April 23-25, NARC, PSPDP, PARC, Islamabad, Pakistan.

Akhtar, R. & Javaid, A. 2018. Biological management of basal rot of onion by *Trichoderma harzianum* and *Withania somnifera. Planta Daninha* 36: e017170507.

Akhter, W., Bhuiyan, M.K.A., Sultana, F. & Hossain, M.M. 2015. Integrated effect of microbial antagonist, organic amendment and fungicide in controlling seedling mortality (*Rhizoctonia solani*) and improving yield in pea (*Pisum sativum* L.). *Comptes Rendus Biologies* 338: 21-28.

Amiri, N., Yadegari, M. & Hamedi, B. 2018. Essential oil composition of *Cirsium arvense* L. produced in different climate and soil properties. *Records of Natural Products* 12: 251-262.

Anser, M.R., Ahmad, I., Shah, S.H., Abuzar, M.K., Raza, M.S. & Malik, M.A. 2018. Weed control measures for controlling the density of Canada thistle (*Cirsium arvense* (L.) Scop. in wheat (*Triticum aestivum* L.). *Pakistan Journal of Botany* 50: 355-363.

Ayyanar, K., Mohan, L., Harish, S., Radjacommare, R., Amutha, G., Chitra, K., Karuppiah, R., Mareeswari, P., Rajinimala, N. & Angayarkanni, T. 2004. Biocontrol agents induce disease resistance in *Phyllanthus niruri* Linn. against damping-off disease caused by *Rhizoctonia solani. Phytopathologia Mediterranea* 43: 187-194.

Banaras, S., Javaid, A. & Khan, I.H. 2021. Bioassays guided fractionation of *Ageratum conyzoides* extract for the identification of natural antifungal compounds against *Macrophomina phaseolina. International Journal of Agriculture and Biology* 25(4): 761-767.

Banaras, S., Javaid, A. & Khan, I.H. 2020. Potential antifungal constituents of *Sonchus oleraceous* against *Macrophomina phaseolina. International Journal of Agriculture and Biology* 24(5): 1376-1382.

Banaras, S., Javaid, A. & Shoaib, A. 2020. Non-chemical control of charcoal rot of urdbean by *Sonchus oleraceous* application. *Planta Daninha* 38: e020216088.

Banaras, S., Javaid, A., Shoaib, A. & Ahmed, E. 2017. Antifungal activity of *Cirsium arvense* extracts against a phytopathogenic fungus *Macrophomina phaseolina. Planta Daninha* 35: e017162738.

Donald, W.W. 1994. The biology of Canada thistle (*Cirsium arvense*). *Rev. Weed Sciences* 6: 77-101.

Dourado, C., Pinto, C., Barba, F.J., Lorenzo, J.M., Delgadillo, I. & Saraiva, J.A. 2019. Innovative non-thermal technologies affecting potato tuber and fried potato quality. *Trends in Food Science & Technology* 88: 274-289.

Etesami, H. & Adl, S.M. 2020. Plant growth-promoting rhizobacteria (PGPR) and their action mechanisms in availability of nutrients to plants. In *Phyto-Microbiome in Stress Regulation. Environmental and Microbial Biotechnology,* edited by Kumar, M., Kumar, V. & Prasad, R. Singapore: Springer. pp. 147-203.

Goswami, D., Thakker, J.N. & Dhandhukia, P.C. 2016. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. *Cogent Food Agriculture* 2: 1127500.

Hussain, T. & Khan, A.A. 2020. *Bacillus subtilis* Hussain T-AMU and its antifungal activity against potato black scurf caused by *Rhizoctonia solani* on seed tubers. *Biocatalysis Agricultural Biotechnology* 23: 101443.

Jabeen, N., Javaid, A., Shoaib, A. & Khan, I.H. 2021. Management of southern blight of bell pepper by soil amendment with dry biomass of *Datura metel. Journal of Plant Pathology* 103(3): 901-913.

Javed, S., Mahmood, Z., Khan, K.M., Sarker, S.D., Javaid, A., Khan, I.H. & Shoaib, A. 2021. Lupeol acetate as a potent antifungal compound against opportunistic human and phytopathogenic mold *Macrophomina phaseolina*. *Scientific Reports* 11: 8417.

Karasakal, A., Demirci, A.Ş., Demirok, N.T. & Cabi, E. 2015. Antioxidant, antimicrobial activities and total flavonoid contents of *Cirsium bulgaricum* DC. leaf extracts. *Marmara Pharmaceutical Journal* 19: 43-51.

Khan, A., Amin, A., Khan, M.A. & Ali, I. 2011a. *In vitro* screening of *Cirsium arvense* for potential antibacterial and antifungal activities. *Pakistan Journal of Pharmaceutical Sciences* 24: 519-522.

Khan, Z.U.H., Ali, F., Khan, S.U. & Ali, I. 2011b. Phytochemical study on the constituents from *Cirsium arvense. Mediterranean Journal of Chemistry* 2: 64-69.

Khan, I.H. & Javaid, A. 2020. Comparative antifungal potential of stem extracts of four quinoa varieties against *Macrophomina phaseolina*. *International Journal of Agriculture & Biology* 24: 441-446.

Khan, M.F., Nakano, Y. & Kurosaki, T. 2019. Impact of contract farming on land productivity and income of maize and potato growers in Pakistan. *Food Policy* 85: 28-39.

Khedher, S.B., Kilani-Feki, O., Dammak, M., Jabnoun-Khiareddine, H., Daami-Remadi, M. & Tounsi, S. 2015. Efficacy of *Bacillus subtilis* V26 as a biological control agent against *Rhizoctonia solani* on potato. *Comptes Rendus Biologies Journal* 338: 784-792.

Koc, S., Isgor, B.S., Isgor, Y.G., Shomali, M.N. & Yildirim, O. 2015. The potential medicinal value of plants from Asteraceae family with antioxidant defense enzymes as biological targets. *Pharmaceutical Biology* 53: 746-751.

Mannaa, M. & Kim, K.D. 2018. Biocontrol activity of volatile-producing *Bacillus megaterium* and *Pseudomonas protegens* against *Aspergillus* and *Penicillium* spp. predominant in stored rice grains: Study II. *Mycobiology* 46(1): 52-63.

Mihaela, R.F. 2014. Chemical studies on plants of *Cirsium arvense* species. PhD Thesis. Craiova: University of Medicine and Pharmacy of Craiova.

Majeed, A. & Muhammad, Z. 2020. An overview of the common bacterial diseases of potato in Pakistan, associated crop losses and control stratagems. *Journal of Plant Pathology* 102: 3-10.

Neela, S. & Fanta, S.W. 2019. Review on nutritional composition of orange-fleshed sweet potato and its role in management of vitamin A deficiency. *Food Science & Nutrition Research* 7: 1920-1945.

Norton, N.A. 2000. Botanical heritage of dermatology. In *Dermatologic Botany,* edited by Avalos, J. & Maibach, H.I. Boca Raton: CRC Press.

Popova, Y.V., Mazulin, O.V., Mazulin, G.V. & Oproshanska, T.V. 2018. The phytochemical investigation of polyphenolic composition of herbs *Cirsium arvense* (L.) Scop. of Ukraine flora. *Farmatsevtychnyi Zhurnal* 2: 83-87.

Rafiq, M., Javaid, A. & Shoaib, A. 2021. Antifungal activity of methanolic leaf extract of *Carthamus oxycantha* against *Rhizoctonia solani. Pakistan Journal of Botany* 53(3): 1133-1139.

Rafiq, M., Shoaib, A., Javaid, A., Perveen, S., Umer, M., Arif, M. & Cheng, C. 2024. Exploration of resistance level against black scurf caused by *Rhizoctonia solani* in different cultivars of potato. *Plant Stress* 12: 100476.

Romero, D., Perez-Garcia, A., Veening, J.W., de Vicente, A. & Kuipers, O.P. 2007. Transformation of undomesticated strains of *Bacillus subtilis* by protoplast electroporation. *Journal of Microbiological Method* 66: 556-559.

Sadat-Hosseini, M., Farajpour, M., Boroomand, N. & Solaimani-Sardou, F. 2017 Ethnopharmacological studies of indigenous medicinal plants in the south of Kerman, Iran. *Journal of Ethnopharmacology* 199: 194-204.

Sahni, S., Prasad, B.D. & Ranjan, T. 2019. Biocontrol of *Sclerotium rolfsii* using antagonistic activities of pseudomonads. *Current Journal of Applied Science & Technology* 35(5): 1-9.

Salamone, A.L. & Okubara, P.A. 2020. Real-time PCR quantification of *Rhizoctonia solani* AG-3 from soil samples. *Journal of Microbiological Methods* 172: 105914.

Saraf, M., Pandya, U. & Thakkar, A. 2014. Role of allelochemicals in plant growth promoting rhizobacteria for biocontrol of phytopathogens. *Microbiological Research* 169: 18-29.

Selim, H.M., Gomaa, N.M. & Essa, A.M. 2017. Application of endophytic bacteria for the biocontrol of *Rhizoctonia solani* (Cantharellales: ceratobasidiaceae) damping-off disease in cotton seedlings. *Biological Sciences & Technology* 27: 81-95.

Shanmugam, V., Ramanathan, A. & Samiyappan, R. 2002. Interaction of *Pseudomonas fluorescens* with Rhizobium for their effect on the management of peanut root rot. *Phytoparasitica* 30: 169-176.

Sharf, W., Javaid, A., Shoaib, A. & Khan, I.H. 2021. Induction of resistance in chili against *Sclerotium rolfsii* by plant growth promoting rhizobacteria and *Anagallis arvensis. Egyptian Journal of Biological Pest Control* 31: 16.

Shoda, M. 2000. Bacterial control of plant diseases. *Journal of Biosciences & Bioengineering* 89: 515-521.

Swain, H., Naik, S.K. & Mukherjee, A.K. 2019. Comparative analysis of different biotic and abiotic agents for growth promotion in rice (*Oryza sativa* L.) and their effect on induction of resistance against *Rhizoctonia solani*: A soil borne pathogen. *Journal of Biological Control* 133: 123-133.

Xiang, N., Lawrence, K.S., Kloepper, J.W., Donald, P.A., McInroy, J.A. & Lawrence G.W. 2017. Biological control of *Meloidogyne incognita* by spore-forming plant growth promoting rhizobacteria on cotton. *Plant Disease* 101: 774-784.

Yang, S., Min, F., Wang, W., Wei, Q., Guo, M., Gao, Y. & Lu, D. 2017. Anastomosis group and pathogenicity of *Rhizoctonia solani* associated with stem canker and black scurf of potato in Heilongjiang Province of China. *American Journal of Potato Research* 94: 95-104.

Yu, Y.Y., Jiang, C.H., Wang, C., Chen, L.J., Li, H.Y., Xu, Q. & Guo, J.H. 2017. An improved strategy for stable biocontrol agents selecting to control rice sheath blight caused by *Rhizoctonia solani*. *Microbiological Research* 203: 1-9.

Zohora, U.S., Ano, T. & Rahman, M.S. 2016. Biocontrol of *Rhizoctonia solani* K1 by iturin A producer *Bacillus subtilis* RB14 seed treatment in tomato plants. *Advances of Microbiology* 6: 424.

*Corresponding author; email: uzma.iags@pu.edu.pk