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Efficacy and plant growth promoting activity of novel strains of *Bacillus* spp. to control collar rot of chickpea (*Cicer arietinum)* in India

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ABSTRACT

The present study was carried out during 2022 and 2023 at Agriculture College and Research Station, Bilaspur, Chhattisgarh, focused on exploring native rhizospheric bacteria for managing soil-borne pathogen affecting chickpea (*Cicer arietinum* L.). Collar rot, caused by *Sclerotium rolfsii*, is a major threat to chickpea production. The study evaluated 104 native rhizospheric bacterial isolates collected from various agro climatic zones in Chhattisgarh for their efficacy against *S. rolfsii in vitro*. Among these isolates, 18 exhibited significant inhibition of *S. rolfsii* mycelial growth. Notably, certain isolates demonstrated remarkable effectiveness, with *Bacillus subtilis* (BI 28), *B. tequilensis* (BI 22), *Bacillus subtilis* (BI 68), *Bacillus velezensis* (BI 43), *Bacillus subtilis* (BI 49), and *B. tequilensis* (BI 31) showing the highest inhibition percentages ranging from 80.37–85.56%. Further analysis of chickpea growth parameters revealed that most isolates positively influenced root and shoot biomass, as well as root and shoot length, compared to the untreated control. Moreover, *in vivo* experiments demonstrated that all isolates enhanced seedling emergence and reduced post-emergence mortality rates compared to the control group. The findings suggested that these isolates hold potential for integrated management practices against collar rot in chickpea cultivation.

Keywords: *Bacillus*, Chickpea, Collar rot, Mortality, Plant growth

Chickpea (*Cicer arietinum* L.), commonly known as gram, is an important pulse crop within the Fabaceae family, commanding significance not only in India but also across Asia and the globe. India contributes around 75% to the world's chickpea production. However, despite its economic importance, chickpea faces substantial threats from various pathogens, with soil-borne diseases such as *Fusarium* wilt (*Fusarium oxysporum* f. sp. *ciceri*), dry root rot (*Rhizoctonia bataticola),* and collar rot (*Sclerotium rolfsii*) emerging as major constraints to production (Haware *et al.* 1986, Nene *et al.* 1996). Collar rot has been identified from approximately 14 countries worldwide, manifesting symptoms such as rotting at the collar region of the plant, ultimately leading to plant collapse. Early stages of infection are characterized by whitish mycelium, with rapeseed-like sclerotia observed adhered to the mycelium around the collar (Nene *et al.* 1978). Two of the most common biocontrol agents are members of *Bacillus* and *Pseudomonas* genera. Both bacterial genera have important trait such as plant growth-promoting (PGP) properties (Santoyo *et al.* 2012). Studies have shown that strains of *Bacillus* spp., including *B. subtilis* G-1, *B. amyloliquefaciens* B2, and *B. subtilis* EPCO 8, exhibit inhibitory effects on the mycelial growth of *S. rolfsii in vitro* (Shifa *et al.* 2015). Furthermore, *Bacillus subtilis* and *Bacillus tequilensis* employ various mechanisms, such as the production of indole acetic acid (IAA) and extracellular hydrolytic enzymes, to promote plant growth (Baard *et al.* 2023). In the recent past, several researchers have reported that plant growth promoting rhizobacteria (PGPR) mediates modulation of systemic resistance against wilt and root rot pathogens in many crops including chickpea (Rudresh *et al.* 2005, Zaim *et al.* 2016, Bekkar *et al.* 2018, Kumari and Khanna 2019). In light of these findings, the evaluation of native bacterial antagonists against plant pathogens becomes imperative in the development and implementation of cost-effective and user-friendly techniques for managing *S. rolfsii.*

MATERIALS AND METHODS

Preliminary screening of microbes against S. rolfsii: The present study was carried out during 2022 and 2023 at Agriculture College and Research Station, Bilaspur, Chhattisgarh. Total 104 native rhizospheric microbes were screened by using modified dual culture technique (confrontation test) against *S. rolfsii in vitro* (Kotasthane

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et al. 2017). The plates were incubated at 27 ± 2ºC till the full growth in the control plate was observed and inhibition zone was measured. Per cent inhibition of pathogen by bacterial isolates is calculated by the formulae given by Vincent (1947):

$$\text{Per cent inhibition (\%)} = \frac{\text{Growth of pathogen in control - Growth of pathogen with different isolates}}{\text{Growth of pathogen in control}} \times 100$$

Plant growth promoting (PGP) activities in chickpea: Effective isolates were evaluated on chickpea for growth promoting (PGP) activities by studying different parameters i.e. germination per cent, root length, shoot length, fresh weight and dry weight of root and shoot etc. under *in vitro* and *in vivo* conditions.

Efficacy of bacterial isolates as seed dresser for management of collar rot of chickpea caused by Sclerotium rolfsii: Sick soil was prepared by mixing the sclerotia of *Sclerotium rolfsii* grown on sorghum grains with soil followed by seed treatment with bacterial strains prior to sowing. Percentage seed germination and post emergence seedling mortality (PESM) were calculated by following formula:

$$\text{Germination(\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seed sown}} \times 100$$

$$\text{Post emergence seedling mortality \% (PESM)} = \frac{\text{Number of seedlings died}}{\text{Total number of seedlings}} \times 100$$

Deployment of antagonistic microbes: Chickpea seeds were surface sterilized with 2% sodium hypochlorite, rinsed thoroughly in sterilized distilled water thrice and dried aseptically. The seeds were coated with microbial antagonist, viz. antagonistic bacterial isolates by using 10% talc based formulation for 30 min. Total 14 treatments were made which includes 10 bacterial isolates and azoxystrobin alone and in combination with bacterial isolate. Fifty seeds/pot were sown in 25 cm diameter cement pots, each containing three kg of sick soil. Three replications of each treatment were maintained in the experiment. Pots without bacterial antagonists (untreated) served as control.

Percentage seed germination and post emergence seedling mortality (PESM) were calculated by using the following formula:

$$\text{Germination(\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seed sown}} \times 100$$

$$\text{Post emergence seedling mortality \% (PESM)} = \frac{\text{Number of seedlings died}}{\text{Total number of seedlings}} \times 100$$

RESULTS AND DISCUSSION

Antagonistic effect of bacterial isolates against chickpea collar rot pathogen Sclerotium rolfsii: Based on the size of the mycelia growth in confrontation test (dual culture technique), 18 strains were found to be highly efficient against the fungal pathogen while others had limited or no antimicrobial activity against *S. rolfsii*. Isolates i.e. *Bacillus subtilis* (BI 28) exhibited maximum suppression of mycelial growth (85.56%) followed by *Bacillus tequilensis* (BI 22) (84.81%) and *Bacillus subtilis* (BI 68) (83.33%) which were statistically at par among themselves (Table 1 and Fig. 1). Growth suppression of 82.22%, 81.11% and 80.37% was recorded by isolates i.e. *Bacillus velezensis* (BI 43), *Bacillus subtilis* (BI 49) and *Bacillus tequilensis* (BI 31), respectively. *Bacillus velezensis* NC318 produces several antifungal compounds, viz. Lipopeptide, polyketides and siderophore which inhibit the mycelial growth and sclerotia germination of *Sclerotium rolfsii* (Bidima *et al.* 2022). α-1,3-glucanase (EC 3.2.1.84) and β-1,3-glucanase (EC 3.2.1.39) are the enzymes produced by various *Bacillus* spp. responsible for the degradation of chitin in fungal cell (Planas 2000). In agreement to present findings, Shifa *et al.* (2015) and Suneeta *et al.* (2016) found different isolates of *Bacillus* i.e. *B. subtilis* G-, *B. amyloliquefaciens* B2 and *B. subtilis* EPCO 8 effective against *S. rolfsii* under *in vitro* condition. Antagonistic activity of *B. velezensis* is due to production of various secondary metabolite clusters that have antimicrobial properties (Alenezi *et al.* 2021).

Table 1 Efficacy of different bacterial isolates on mycelial growth of *S. rolfsii*

Bacterial isolates	Mycelial growth (mm)	Per cent inhibition over control
Bacillus tequilensis (BI 22)	13.67	84.81 (67.05)*
Bacillus spp. (BI 23)	51.33	42.96 (40.93)
Bacillus tequilensis (BI 24)	24.67	72.59 (58.41)
Bacillus subtilis (BI 28)	13.00	85.56 (67.64)
Bacillus tequilensis (BI 31)	17.67	80.37 (63.68)
Bacillus velezensis (BI 35)	57.78	35.80 (34.60)
Bacillus velezensis (BI 36)	49.00	45.55 (42.43)
Bacillus spp. (BI 41)	54.33	39.62 (38.99)
Bacillus velezensis (BI 43)	16.00	82.22 (65.04)
Bacillus tequilensis (BI 44)	43.67	51.11 (45.83)
Bacillus subtilis (BI 49)	17.00	81.11 (64.23)
Bacillus spp. (BI 65)	49.33	45.18 (42.22)
Bacillus subtilis (BI 68)	15.00	83.33 (65.88)
Bacillus velezensis (BI 69)	47.67	47.03 (43.28)
Bacillus subtilis (BI 72)	19.33	78.52 (62.51)
Bacillus tequilensis (BI 77)	44.00	51.11 (45.61)
Bacillus velezensis (BI 78)	17.33	80.74 (63.98)
Bacillus velezensis (BI 79)	26.00	67.22 (54.32)
Control	90.00	-
CD (*P*=0.05)		3.048
CV		3.54

*Mean of three replication values in parentheses are arcsine transformed.

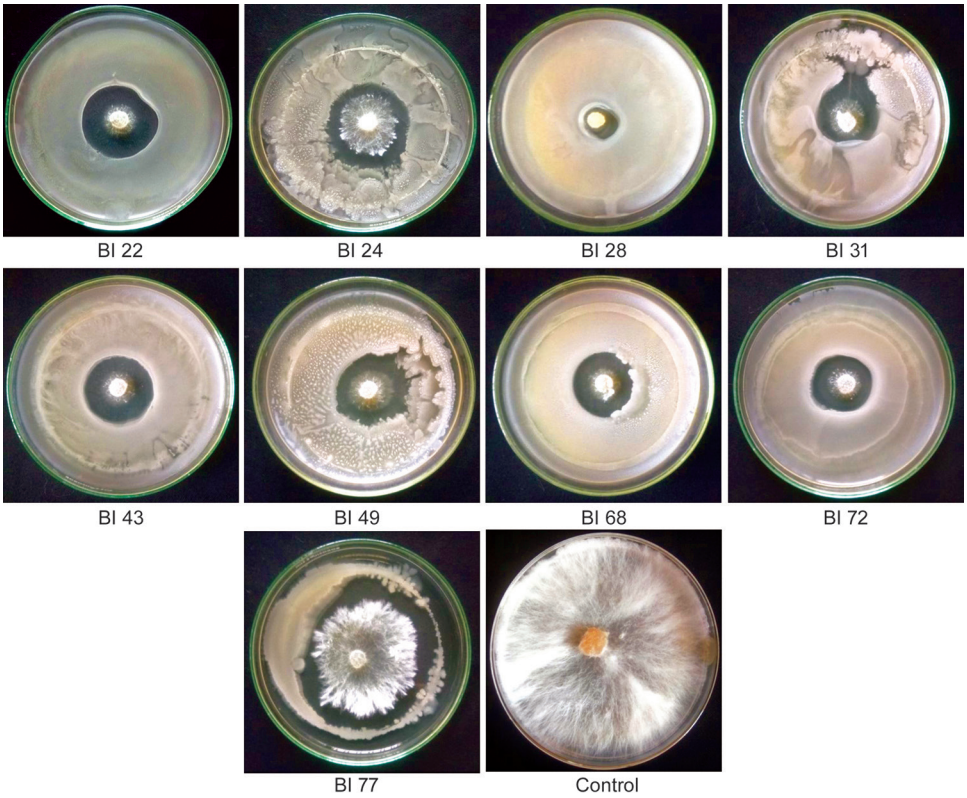

Fig. 1 Confrontation assay to evaluate the efficacy of different bacterial isolates to suppress the growth of *S. rolfsii.*

Plant growth promoting (PGP) activities in chickpea: Data on seed germination recorded from chickpea presented in Table 2, Fig. 2 indicate that all isolates had growth promoting effect on chickpea. Germination percentage in all the treatments including control exhibited 100% germination *in vitro*. After 96 h of incubation, maximum root length (cm) was recorded in isolate *B. velezensis* (BI 43) (3.43) followed by *B. tequilensis* (BI 31) (3.33), *B. velezensis* (BI 78) (3.27), *B. subtilis* (BI 68) (3.23) and *B. velezensis* (BI 36) (2.75) over control (2.13) and statistically at par with each other.

Data from pot experiment revealed that, seeds inoculated with different isolates, produced enhanced shoot and root over control after one month of growth. Maximum shoot and root length (cm) were recorded from isolate *B. velezensis* (BI 43) (23.27, 28.50) followed by *B. tequilensis* (BI 31) (22.13, 22.00), *B. velezensis* (BI 78) (22, 21.83) and *B. subtilis* (BI 68) (21.17, 21.67) which were significant superior over control (17.67, 17.50). Similarly, maximum fresh shoot weight and dry shoot weight (g) recorded in isolate *B. velezensis* (BI 43) (2.44 g, 0.577 g) followed by *B. tequilensis* (BI 31) (2.09 g, 0.389 g) and *B. velezensis* (BI 78) (2.03 g, 0.374 g) which were statistically superior over control (1.23, 0.221). Similarly, maximum fresh and dry weight (g) of root was recorded from isolate *B. velezensis* (BI 43) (1.687 g, 0.182 g) followed by *B. tequilensis* (BI 31) (1.443 g, 0.160 g) and *B. subtilis* (BI 68) (0.763 g, 0.086 g) and were significantly superior over control (0.182 g, 0.051 g). Least fresh and dry weight of root was recorded in treatment of *B. subtilis* (BI 72) (0.363 g, 0.066 g).

Root scanner data presented in the Table 2, Fig. 2 revealed that total root length (cm), surface area (cm^2), average root diameter (mm), root volume (cm^3), tips and forks were recorded higher in isolate *B. velezensis* (BI 43) (943.63 cm, 292.12 cm^2, 0.98 mm, 7.19 cm^3, 16467 and 23679) followed by isolate *B. tequilensis* (BI 31) (899.67 cm, 89.42 cm^2, 0.31 mm, 0.70 cm^3, 7274 and 7280) over control (308.71 cm, 28.86 cm^2, 0.29 mm, 0.21 cm^3, 3298 and 2635). The enhanced plant growth promoting properties of isolates of *Bacillus* spp. may be due to the secretion of plant growth hormones such as indole acetic acid (IAA), chelation of iron by producing compounds such

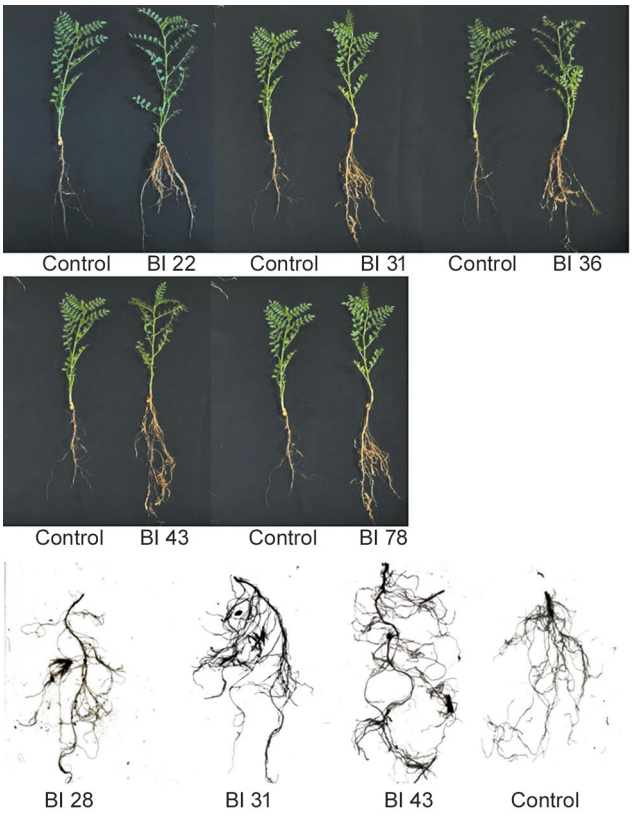

Fig. 2 *In vitro* evaluation of different isolates of *Bacillus* spp. on plant growth promoting activity in chickpea after 1month. (A) Simple image; (B) Root scanner image.

Table 2 Effect of different isolates of *Bacillus* spp. on plant growth promoting activities in chickpea using seed inoculation

Treatment	After 96 h (Plate experiment)			After 1 month (Pot experiment)						Root scanner data			
	Germination (%)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Length (cm)	Surface Area (cm^2)	Average Diameter (mm)	Root Volume (cm^3)	Tips
B. subtilis (BI 28)	100	00.00	2.47	19.85	19.83	1.43	0.256	1.433	0.122	630.22	58.93	0.29	0.43	9552
B. subtilis (BI 49)	100	00.00	2.43	19.67	19.17	1.38	0.247	0.586	0.085	510.11	56.74	0.35	0.50	2620
B. subtilis (BI 68)	100	00.00	3.23	21.17	21.67	1.64	0.298	0.763	0.086	549.45	51.61	0.29	0.38	6886
B. subtilis (BI 72)	100	00.00	2.40	18.50	18.90	1.33	0.236	0.363	0.066	461.27	38.37	0.26	0.25	7101
B. tequilensis (BI 22)	100	00.00	2.53	19.90	20.19	1.56	0.252	0.692	0.073	515.37	51.58	0.31	0.41	6600
B. tequilensis (BI 31)	100	00.00	3.33	22.13	22.00	2.09	0.389	1.443	0.160	899.67	89.42	0.31	0.70	6274
B. tequilensis (BI 77)	100	00.00	2.20	18.10	17.67	1.27	0.225	0.370	0.053	447.27	37.11	0.26	0.24	4786
B. velezensis (BI 36)	100	00.00	2.75	20.17	20.33	1.58	0.289	0.562	0.091	506.99	51.81	0.32	0.42	6276
B. velezensis (BI 43)	100	00.00	3.43	23.27	28.50	2.44	0.577	1.687	0.182	943.63	292.12	0.98	7.19	16467
B. velezensis (BI 78)	100	00.00	3.27	22.00	21.83	2.03	0.374	1.170	0.096	570.07	51.58	0.28	0.37	7648
Control	100	00.00	2.13	17.67	17.50	1.23	0.221	0.182	0.051	308.71	28.86	0.29	0.21	3298
CD (P=0.05)			0.63	3.50	3.55	0.24	0.086	0.183	0.019					
CV			13.51	10.09	10.09	8.619	16.524	12.817	11.210					

as siderophores, mobilization of nutrients and antagonistic potential against phytopathogens (Panhwar *et al.* 2012, Sreevidya *et al.* 2016). Similarly, Alenezi *et al.* (2021) reported plant growth promoting activity of the secondary metabolites produced by *B. velezensis. Bacillus tequilensis* (PBE1) promotes plant growth by producing indole acetic acid (IAA), hydroxymate-type siderophores and phosphate solubilizing. Treatment with isolates resulted in enhanced physical parameters such as root length, shoot length, number of branches, fresh weight and dry weight in tomato plants (Bhattacharya *et al.* 2019). Inoculation with *B. subtilis* increased uptake and accumulation of P and plant growth by cucumber plants (Garcia-Lopez and Delgado 2016).

Management of collar rot of chickpea caused by Sclerotium rolfsii: All the treatments significantly increased the emergence percentage (60.28–72.83) and decreased mortality (76.05 to 31.69) due to collar rot of chickpea over control (Table 3, Fig. 3). Seed treated with isolates i.e. *B. tequilensis* (BI 31) (72.83), *B. tequilensis* (BI 22) (72.83), *B. subtilis* (BI 49) (72.14) and *B. velezensis* (BI 43) (72.13) were found to be significantly effective in increasing the percent germination over control (58.93). There was an increase in germination percentage ranging from 6.62–19.08% over control (Table 3). However, seeds treated with azoxystrobin @0.3% (82.55) alone, azoxystrobin (@0.15%) (80.47) and combination of isolate *B. tequilensis* (BI 22) and half dose of azoxystrobin (@0.15%) (86.72) exhibited significantly higher percent emergence over other treatments including control (58.93) which were at par with each other. Other isolates i.e. *B. tequilensis* (BI 77) (60.28%), *B. subtilis* (BI 28) (61.03%), *B. subtilis* (BI 68) (63.11) and *B. tequilensis* (BI 24) (63.78%) were also able to increase the per cent emergence of chickpea significantly over control (58.93). Higher per cent emergence in all treatments over control might be due to less intensity of pre-emergence seed rotting and seedling mortality over control.

Fig. 3 Evaluation of isolates of *Bacillus* spp. against collar rot of chickpea under pot condition.

Table 3 Effect of seed treatment with isolates of *Bacillus* spp. on collar rot incidence in chickpea under pot condition

Treatment	Per cent seeding emergence*	Per cent increase over control	PESM %*	Per cent reduction over control
B. subtilis (BI 28)	61.03 (51.36)*	3.44	76.05 (60.72)*	11.17
B. subtilis (BI 49)	72.14 (58.16)	18.31	54.62 (47.69)	36.20
B. subtilis (BI 68)	63.11 (52.59)	6.62	75.47 (60.88)	11.85
B. subtilis (BI 72)	69.36 (56.54)	14.96	46.60 (43.87)	45.57
B. tequilensis (BI 22)	72.83 (58.60)	19.08	46.66 (43.06)	45.50
B. tequilensis (BI 24)	63.78(53.11)	7.60	68.51 (55.92)	19.98
B. tequilensis (BI 31)	72.83 (58.63)	19.08	31.69 (34.20)	62.98
B. tequilensis (BI 77)	60.28 (50.22)	2.29	61.82 (51.83)	27.79
B. velezensis (BI 43)	72.13(58.11)	18.31	36.88 (37.34)	56.92
B. velezensis (BI 78)	70.75 (57.32)	16.70	49.69 (44.79)	41.96
B. tequilensis (BI 22) + Azoxystrobin 23% SC (0.15%)	86.72 (68.61)	32.04	5.77 (13.78)	93.26
Azoxystrobin 23% SC (0.15%)	80.47 (63.85)	28.61	11.40 (19.59)	86.68
Azoxystrobin 23% SC (0.3%)	82.55 (65.34)	26.76	9.52 (17.91)	88.88
Control (Untreated)	58.93 (50.13)	00.00	85.62 (68.67)	00.00
CD (*P*=0.05)	6.24		7.364	
CV	6.475		10.232	

*Mean of three replication values in parentheses are arcsine transformed. PESM %, Post emergence seedling mortality.

Data presented in Table 3, on mortality indicated that all the isolates showed significantly reduced post emergence seedling mortality (31.69–76.50) over control (85.62). Maximum per cent reductions in mortality over control was observed in isolate *B. tequilensis* (BI 31) (62.98) followed by *B. velezensis* (BI 43) (56.92), *B. subtilis* (BI 72) (45.57) and *B. tequilensis* (BI 22) (45.50). Least post emergence seedling mortality (PESM) was observed in isolate *B. tequilensis* (BI 31) (31.69%) followed by *B. velezensis* (BI 43) (36.88) which were at par with each other and significantly superior over other isolates i.e. *B. subtilis* (BI 72) (46.60), *B. tequilensis* (BI 22) (46.66) and *B. velezensis* (BI 78) (49.69) including control (85.62).

Combination of isolate *B. tequilensis* (BI 22) and half dosage of azoxystrobin @0.15% had least post-emergence mortality (5.77) as compared to the half dose of fungicide i.e. azoxystrobin @0.15% (11.40) and azoxystrobin @0.3% alone (9.52) as positive control and were at par with each other. Isolate *B. tequilensis* (BI 22) combined with half dosage of azoxystrobin @0.15% was significantly superior to other isolates in terms of post emergence seedling mortality. Similarly, Bhattacharya *et al.* (2019) found *Bacillus tequilensis* (PBE1) most effective against *Fusarium oxysporum* for tomato wilt disease management. Inoculation with *Bacillus* spp. enhance the plant defence enzymes and induce expression of pathogenesis-related (PR) genes (Mageshwaran *et al.* 2022). In agreement to present findings, Zhou *et al.* (2022) recorded strong antagonistic property of *B. velezensis* BR-01 against a variety of fungal plant pathogens i.e. *Magnaporthe oryzae, Ustilaginoidea virens* and *Fusarium.*

The bacterial soil microbes isolated in the study were found to have potential bio control and plant growth activities. *In vitro* experiments indicated some promising isolates have antagonistic activity against *S. rolfsii*. The pot experiment revealed that *B. tequilensis* (BI 31) and *B. velezensis* (BI 43) treated seeds had lowest pre emergence and post emergence seedling mortality due to collar rot disease of chickpea plants. The current study provides insight into the possibilities of using potential rhizospheric microbes for collar rot disease in chickpea plants. This approach presents an environmentally friendly method for managing collar rot disease while contributing to the maintenance of plant and soil health.

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