International Journal of Food Science and Nutrition
www.foodsciencejournal.com
E-ISSN: 2455-4898
Received: 22-09-2026, Accepted: 22-10-2026, Published: 06-11-2026
Volume 11, Issue 4, 2026, Page No. 122-124

Antagonastic efficiency of *Bacillus* spp. isolates against *S. Rolfsii* incitant of collar rot of chickpea

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DOI: https://doi.org/10.66856/ijfsn.2026.11.4.11201

Abstract
Collar rot, caused by *Sclerotium rolfsii*, causes devastating seedling mortality in chickpea cultivation. This study evaluated the biocontrol efficacy of nine native *Bacillus* spp. isolates (B1–B9) from Chhattisgarh using a spore-selective heat-treatment isolation method and a talc-powder bioformulation (9×10^8 cfu/ml). In *in vitro* dual-culture screening, isolate B6 demonstrated the highest antagonistic efficiency, inhibiting *S. rolfsii* mycelial growth by 72.22%, which was statistically at par with isolate B3 (71.48%). In glass-house pot trials using infested soil, seed treatment with isolate B6 drastically suppressed the disease, reducing plant mortality to 17.03% (a 79.10% decrease over control) compared to the untreated control (81.54%). Furthermore, B6 significantly promoted plant growth, achieving the highest germination rate (96.66%) and plant vigour index (4233.70). These findings indicate that native *Bacillus* isolates B6 and B3 serve as powerful bio-control agents for sustainable chickpea collar rot management.

Keywords: *Bacillus* spp, *Sclerotium rolfsii*, collar rot, chickpea, antagonistic efficiency, biocontrol

Introduction
Chickpea (*Cicer arietinum* L.) is one of the world's most critical pulse crops, with India accounting for over 70 per cent of its global production. However, during early crop growth, seedling establishment is severely threatened by collar rot, a destructive and rapidly spreading soil-borne disease incited by *Sclerotium rolfsii* Sacc. (Nene *et al*., 1984) [9]. In India, collar rot mortality ranges from 54.7% to 95%(Kotasthane *et al*., 1976), causing devastating reductions in plant population. Due to its wide host range and ability to infect the root and collar zones, managing this pathogen is exceptionally challenging (Aycock, 1966; Punja, 1988) [2, 8]. Although fungicides and crop rotation can lower disease incidence (Kumar *et al*., 1997; Azhar *et al*., 2006) [3, 7], rising environmental concerns demand eco-friendly, sustainable alternatives. Seed-applied bacterial antagonists offer a practical solution to protect establishing seedlings. Because successful biological control depends entirely on identifying highly effective microbial strains, this study evaluates the antagonistic efficiency of native *Bacillus spp.* isolates against *S. rolfsii*.

Materials and Methods
- **Isolation:** Native *Bacillus* spp. were isolated from chickpea rhizospheric soil in Chhattisgarh using the selective heat-treatment method (80°C for 10 min) to target endospore-forming bacteria (Walker *et al*., 1998) [12]. Screened colonies were grown on King's B (KB) medium and stored in 50% glycerol at -80°C.

- **Bioformulation:** Isolates were grown in KB broth (9×10^8 cfu/mL) for 72 h at 150 rpm. A talc-powder carrier was prepared by adding 1% Carboxy Methyl Cellulose (CMC) and 1% $CaCO_3$ (pH 7.0), then autoclaved. The bacterial suspension (400 mL) was mixed per kilogram of talc carrier and air-dried.

- **Dual Culture Assay:** Antagonistic efficiency was evaluated *in vitro* using a composite PDA and KB medium. A sterilized glass funnel was used to apply a circular ring of *Bacillus* broth along the plate periphery, while a 5-mm *S. rolfsii* disc was placed at the center. Percent mycelial inhibition was calculated after 3 days relative to the untreated control.

- **Glasshouse Trial:** A pot experiment was laid out in a Completely Randomized Design (CRD) with three replications. Sterilized sandy loam soil (2 kg/pot) was inoculated with a 2-week-old *S. rolfsii* culture (25 g/kg soil) and incubated for 5 days. Chickpea seeds were coated with the talc formulation using 2% CMC as a sticker. Ten seeds were sown per pot. Seed germination was recorded at 10 days, plant mortality was evaluated every 15 days up to 45 days, and plant vigour index was calculated at uprooting based on root and shoot lengths.

Results
The evaluation of nine *Bacillus* spp. isolates recovered from diverse soil locations across Chhattisgarh revealed significant variation in their antagonistic efficacy against *Sclerotium rolfsii* under both *in vitro* and *in vivo* conditions. In the *in vitro* dual culture assay, isolate B6 demonstrated the highest biocontrol potential by reducing the mycelial growth of *S. rolfsii* by 72.22 per cent over the control, which was statistically at par with isolate B3 (71.48 per cent), while the minimum inhibition was recorded in isolate B1 (18.52 per cent). These findings are in strong agreement with Hassen Shifa *et al*. (2015) [10], who observed that *B. subtilis* G-1, *B. amyloliquefaciens* B2, and *B. subtilis* EPCO 8 were highly effective biocontrol agents in inhibiting the mycelial growth of *S. rolfsii* in dual culture assays, as well as Gholami *et al*. (2014) [4], who reported that *B. subtilis* subsp.
subtilis and *B. subtilis* subsp. *spizizenii* demonstrated the largest zones of growth inhibition against the pathogen. This strain-dependent variation in suppression is typically attributed to the well-documented potential of *Bacillus* strains, particularly *Bacillus subtilis*, to produce specialized

bioactive compounds and secondary metabolites antagonistic to plant pathogenic fungi (Gomashe *et al*., 2014) [5], a screening dynamic also observed by Suneeta *et al*. (2016) [11] when identifying five highly superior antagonistic strains out of 26 *Bacillus* isolates screened against the collar rot pathogen *in vitro*.

When transitioned to the greenhouse pot experiments via seed treatments, this biocontrol efficacy translated to a significant reduction in chickpea collar rot disease severity and an enhancement of plant growth metrics. Isolate B6 exhibited the lowest seedling mortality rate at 17.03 per cent, followed closely by B3 (22.68 per cent) and B5 (26.11 per cent), which were statistically at par with each other, while the untreated control suffered a maximum mortality of 81.54 per cent.

Furthermore, seed treatment with isolate B6 yielded the highest germination rate (96.66 per cent) and the maximum seedling vigour index (4233.70), followed by isolates B7 and B5, all performing significantly superior to the untreated control group (81.54 per cent germination and 2155.90 vigour index). These *in vivo* results align closely with the glasshouse experiments of Gholami *et al*. (2014) [4], who observed a 50 to 58.5 per cent reduction in root-rot severity in bean seeds pre-treated with various *Bacillus* subspecies. They also closely mirror the findings of Hassen Shifa *et al*. (2015) [10], who reported that groundnut seeds treated with *B. subtilis* G-1 effectively reduced the incidence of *S. rolfsii* stem rot while simultaneously driving significant increases in per cent germination, root/shoot length, and overall seedling vigour.

Table 1: Antagonastic efficiency of *Bacillus* spp. isolates against *S. rolfsii* by dual culture technique.

S.No.	*Bacillus* spp. isolates	Percent inhibition*
1	B1	18.52 (25.47)
2	B2	57.41 (49.24)
3	B3	71.48 (57.701)
4	B4	57.11 (49.11)
5	B5	64.81 (53.59)
6	B6	72.22 (58.17)
7	B7	62.59 (52.28)
8	B8	66.29 (54.50)
9	B9	59.63 (50.53)
	SEm ±	1.179
	CD (5%)	3.531

*Average of three replication
Figures in parentheses are angular transformation

Table 2: Efficacy of *Bacillus* spp. isolates against collar rot of chickpea under glass house condition.

S.No.	*Bacillus* spp. isolates	Per cent mortality*	Per cent decrease over Control
1	B1	54.76 (47.71)	32.85
2	B2	40.73 (39.61)	50.04
3	B3	22.68 (28.01)	72.18
4	B4	38.42 (38.26)	52.88
5	B5	26.11 (30.59)	67.98
6	B6	17.03 (24.19)	79.10
7	B7	35.55 (36.57)	56.39
8	B8	30.35 (33.34)	62.77
9	B9	32.40 (34.53)	60.26
10	Control (untreated)	81.54 (64.89)	0.00
	SEm ±	2.65	
	CD (5%)	7.88	

*Average of three replication
Figures in parentheses are angular transformation

Table 3: Effect of *Bacillus* spp. isolates on germination and vigour index of chickpea under sick pot condition.

S. No.	*Bacillus* spp. isolates	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index
1	B1	73.33 (8.6)	19.60	21.60	3021.19
2	B2	90.00 (9.5)	21.60	20.80	3816.00
3	B3	86.66 (9.3)	22.40	20.80	3743.71
4	B4	86.66 (9.3)	24.40	20.60	3899.70
5	B5	90.00 (9.5)	23.60	22.20	4122.00
6	B6	96.66 (9.8)	23.00	20.80	4233.70
7	B7	93.33 (9.7)	21.80	21.40	4031.85
8	B8	76.66 (8.8)	21.40	20.40	3204.38
9	B9	83.33 (9.1)	22.40	19.20	3466.52
10	Control (untreated)	73.33 (8.6)	14.20	15.20	2155.90
	SEm ±	0.189	1.118	0.518	
	CD (5%)	0.561	3.207	1.485	

* Average of three replication
** Average of five replication
Figures in parentheses are square root transformation

Conclusion

In the present investigation, nine *Bacillus* spp. isolates were isolated from soil samples collected from different locations of Chhattisgarh and evaluated for their antagonistic potential against *Sclerotium rolfsii* under *in vitro* and *in vivo* conditions. Under *in vitro* conditions, isolate B6 exhibited the highest inhibition of mycelial growth (72.22%) over the control, followed by B3 (71.48%), with both isolates being statistically at par. In contrast, the minimum inhibition of mycelial growth was recorded in isolate B1 (18.52%) using the dual culture technique.

Similarly, under *in vivo* pot conditions, seed treatment with *Bacillus* spp. isolates resulted in varying degrees of reduction in chickpea collar rot mortality. Among the nine isolates evaluated, B6 recorded the lowest plant mortality (17.03%), followed by B3 (22.68%) and B5 (26.11%), which were statistically at par with each other. The highest mortality among the bacterial treatments was observed in B1 (54.76%), whereas the untreated control recorded 81.54% mortality.

Overall, isolate B6 emerged as the most promising biocontrol agent, exhibiting superior antagonistic activity against *S. rolfsii* under *in vitro* conditions and the lowest plant mortality under *in vivo* pot conditions. Isolate B3 also demonstrated considerable biocontrol potential. These findings indicate that indigenous *Bacillus* spp., particularly isolates B6 and B3, could be exploited as potential biological control agents for the sustainable management of chickpea collar rot.

Future Scope

Further studies are required for molecular identification and characterization of the promising *Bacillus* isolates, particularly B6 and B3, to understand their mechanisms of antagonism against *Sclerotium rolfsii*. Their efficacy should be validated under field conditions across different agro-climatic regions. Additionally, the development of suitable formulations with improved shelf life and compatibility with other integrated disease management practices may help in their effective utilization as eco-friendly biocontrol agents for sustainable management of chickpea collar rot.

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