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Plant growth-promoting effect of seed biopriming of local *Bacillus thuringiensis* isolates on *Cicer arietinum* L.

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Abstract
The present investigation aimed to evaluate the influence of seed biopriming with *Bacillus thuringiensis* (Bt) isolates on germination and early seedling growth of chickpea (*Cicer arietinum* L., variety Jaki-9218). Twenty-one indigenous Bt isolates, along with a standard Bt-HD-1 strain, were tested under laboratory conditions using the paper towel method. Results revealed that several Bt strains significantly enhanced seed performance compared to the untreated control. Notably, PDKV-Bt-SBn-2, PDKV-Bt-SAk-12, PDKV-Bt-SDA-1, and PDKV-Bt-SA-22 recorded faster emergence, higher germination rates, longer root and shoot lengths, and superior vigour indices. Improvements included a reduction of nearly one day in emergence, 13-14% higher germination, and up to 9-11 cm greater seedling length compared to control. These findings demonstrate that indigenous Bt strains not only promote early establishment but also enhance vigour and resilience. Hence, Bt-based biopriming represents a promising, cost-effective, and eco-friendly strategy for sustainable chickpea production under diverse agro-climatic conditions.

Keywords: *Bacillus thuringiensis*, PGPR, Seed biopriming, Chickpea

1. Introduction

Chickpea (*Cicer arietinum* L.) is the third most important food legume globally, valued for its high protein content (18-24%), carbohydrates, fiber, vitamins, and essential minerals, making it a vital component of human diets (Jukanti *et al*., 2012) [8]. It is cultivated in over 50 countries, with India contributing more than 70% of global production (FAO, 2020). Chickpea improves soil fertility through symbiotic nitrogen fixation with *Rhizobium* spp., reducing the need for synthetic fertilizers (Varshney *et al*., 2013) [21]. Chickpea is highly sensitive to moisture stress, particularly during the early and reproductive stages, when declining soil moisture significantly affects yield (Gaur *et al*., 2010) [4]. In addition, the crop is vulnerable to *Fusarium wilt*, insect pests, drought, and extreme temperature fluctuations, all of which contribute to substantial yield losses (Varshney *et al*., 2013) [21]. To overcome these challenges and ensure proper and uniform germination, seed vigor, and viability under adverse conditions, seed priming has emerged as one of the most effective strategies. Unlike pesticides, which provide only temporary solutions, seed priming enhances stress tolerance and promotes sustainable crop establishment. Additionally, it is cost-effective, eco-friendly, and improves resilience against biotic and abiotic stresses, making it a promising approach for chickpea cultivation (Farooq *et al*., 2019 [3]; Harris *et al*., 2001) [7].

Seed germination is a critical phase in the plant life cycle, influenced by hormones, gene regulation, and biochemical processes, with its success directly affecting plant growth and yield (Zhao *et al*., 2021) [22]. Seed biopriming, a pre-sowing technique, involves hydrating seeds with beneficial microorganisms such as plant growth-promoting rhizobacteria (PGPR) to enhance germination, vigor, and stress tolerance. Methods include seed coating, pelleting, and hardening, which improve hydration efficiency, shorten imbibition time, and accelerate germination (Prasad *et al*., 2016 [16]; Mahmood *et al*., 2016) [12]. Microbial inoculants, particularly PGPR, improve nutrient uptake, root architecture, and stress resilience, boosting crop yield by 12-20% under challenging conditions (Pérez-Jaramillo *et al*., 2016) [15]. Soil, a diverse microbial ecosystem, plays a central role in nutrient cycling and disease suppression. PGPR-based microbial fertilizers serve as eco-friendly alternatives to chemicals, enhancing soil fertility and crop productivity (Chen *et al*., 2021; Saleem *et al*., 2021) [19]. These microbes promote plant growth directly by producing phytohormones such as auxins and gibberellins and by increasing nutrient availability through solubilization and mineralization processes (Backer *et al*., 2018 [1]; Basu *et al*., 2021) [2]. Traits like zinc solubilization (Kamran *et al*., 2017) [9], improved water absorption, and stress adaptation further strengthen seedling establishment and resilience (Van Oosten *et al*., 2017) [20].

Bacillus thuringiensis (Bt) is a Gram-positive, spore-forming bacterium well known for its insecticidal crystal proteins, but is increasingly recognized as a Plant Growth-Promoting Rhizobacterium (PGPR). Several studies have demonstrated that Bt enhances plant growth through direct and indirect mechanisms (Raddadi *et al*., 2007 [17]; Qi *et al*., 2016; Mishra *et al*., 2022 [14]; Marisel *et al*., 2024 [13]; Longatto *et al*., 2025) [11]. Bt strains produce indole-3-acetic acid (IAA), which stimulates root elongation and lateral root development, leading to improved nutrient and water absorption. Ammonia production contributes to nitrogen nutrition and pathogen suppression, while phosphate solubilization through the secretion of organic acids enhances phosphorus availability in soils. Bt also secretes siderophores, which chelate iron, improving its uptake by plants and simultaneously limiting pathogen growth (Raddadi *et al*., 2007). Certain strains possess ACC deaminase activity, which reduces stress-induced ethylene levels, thereby promoting root development under abiotic stresses (Glick, 2012; Glick, 2014) [5, 6]. Moreover, Bt forms biofilms, ensuring strong root colonization and long-term persistence in the rhizosphere (Mishra *et al*., 2022) [14]. In crops like chickpea, Bt inoculation has improved nutrient uptake, nodulation, and grain yield, especially in association with Rhizobium (Ramesh *et al*., 2014) [18]. Their application by seed biopriming as biofertilizers or bio stimulants in integrated nutrient management systems can significantly reduce the dependency on chemical inputs, thereby supporting eco-friendly and cost-effective farming practices.

2. Material and methods

Seed biopriming was undertaken using twenty-one indigenous isolates of *Bacillus thuringiensis* (Bt) along with a standard Bt-HD-1 strain to evaluate their potential in enhancing germination and early growth of chickpea (*Cicer arietinum* L.). The chickpea variety Jaki-9218 was used as the seed material owing to its adaptability and agronomic importance. The experiment was conducted under laboratory conditions using the paper towel method, which allowed for controlled evaluation of seed performance. The laboratory-based approach provided a rapid assessment of the germination response.

Table 1: List of indigenous *Bacillus thuringiensis* strains used in the present study

SN	Isolate No.	Location of isolation	MAU Accession No.
1	PDKV-Bt-SA-6	Amravati Dist.	NAIMCC-B-03505
2	PDKV-Bt-SA-20	Amravati Dist.	NAIMCC-B-03509
3	PDKV-Bt-SGd-1	Gadchiroli Dist.	NAIMCC-B-03502
4	PDKV-Bt-SA-18	Amravati Dist.	NAIMCC-B-03508
5	PDKV-Bt-SAk-6	Akola Dist.	NAIMCC-B-03506
6	PDKV-Bt-SAk-9	Akola Dist.	NAIMCC-B-03507
7	PDKV-Bt-SGn-5	Gondia Dist.	NAIMCC-B-03504
8	PDKV-Bt-SBn-2	Bhandara Dist.	NAIMCC-B-03503
9	PDKV-Bt-SY-4	Yavatmal Dist.	NAIMCC-B-04032
10	PDKV-Bt-SGn-4	Gondia Dist.	NAIMCC-B-04037
11	PDKV-Bt-I-3	Insect cavaver	NAIMCC-B-04036
12	PDKV-Bt-SUd-2	Udgir, Dist. Latur	NAIMCC-B-04033
13	PDKV-Bt-SJa-2	Jalkot Dist. Sambhaji nagar	NAIMCC-B-04038
14	PDKV-Bt-SJa-1	Jalkot Dist. Sambhaji nagar	NAIMCC-B-04034
15	PDKV-Bt-SHi-1	Hingoli Dist.	NAIMCC-B-04035
16	PDKV-Bt-SDa-2	Darati, Umarkhed, Dist. Yavatmal	NAIMCC-B-03973
17	PDKV-Bt-SDa-1	Darati, Umarkhed, Dist. Yavatmal	NAIMCC-B-03972
18	PDKV-Bt-SA-22	Amravati Dist.	NAIMCC-B-03974
19	PDKV-Bt-SMa-1	Mahur Dist. Nanded	NAIMCC-B-03977
20	PDKV-Bt-SAk-12	Akola Dist.	NAIMCC-B-03976
21	PDKV-Bt-SUd-1	Udgir, Dist. Latur	NAIMCC-B-03975

2.1 Experimental Design

The experiment consists of 23 treatments, including 21 indigenous isolates of *Bacillus thuringiensis* (Bt) along with a standard Bt-HD-1 strain and a control, which was replicated three times and allocated in a Completely Randomized Block Design.

Table 2: Details of layout

Crop	Chickpea (*Cicer arietinum* L.)
Genotype	Jaki-9218
Season	Zaid 2025
Place	Molecular Biology and Nanotechnology laboratory, Biotechnology Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
Number of treatments	23 (21+1+1)
Number of replications	3
Number of seeds per replication	10
Method of experiment	Paper towel method
Days of observation taken	Up to 8 days

2.2 Preparation of media

1. The following components were weighed and dissolved in 1 litre of distilled water for the preparation of the Nutrient broth medium:
   - Manitol agar - 10.0 g
   - K_2HPO_4 - 0.5 g
   - $MgSO_4$ - 0.2 g
   - NaCl - 0.1 g
   - $CaCO_3$ - 4.0 g
   - Yeast extract - 0.1 g
2. The medium was autoclaved at 121°C for 15 minutes under 15 psi pressure.
3. The cooled broth was inoculated with fresh overnight cultures of Bt isolates.
4. The inoculated flasks were incubated at 28°C for 180 rpm for 3 days to achieve optimal bacterial growth.
5. The seeds of chickpea were surface disinfected by dipping them in 70% ethanol for a short duration.
6. The disinfected seeds were rinsed thoroughly with sterile distilled water to remove traces of ethanol.
7. The sterilized seeds were absorbed in bacterial suspension (1×10^7 cells/ml) prepared from the incubated cultures.
8. The soaking was maintained for 2 hours.
9. For the control, seeds were soaked in sterile distilled water for the same duration and under identical conditions.
10. The treated seeds were removed from the suspension and spread on sterile petri plates.
11. The seeds were air-dried under aseptic conditions for 48 hours to achieve a suitable moisture content for storage or sowing.
12. The bio primed and control seeds were placed between moist germination papers for the paper towel method.

3. Results and discussion

An experiment entitled "Effect of seed-biopriming of plant growth-promoting bacteria (PGPR) *Bacillus thuringiensis* on chickpea (*Cicer arietinum* L.)" focused on exploring the plant growth-promoting potential of indigenous *Bacillus thuringiensis* isolates. The key observations are summarized below.

3.1 Days to emergence

The shortest germination period was observed in strain PDKV-Bt-SBn-2 (2.50 days) and PDKV-Bt-SAk-12, closely followed by PDKV-Bt-SA-6 (2.5 days), PDKV-Bt-SGd-1 (2.5 days), PDKV-Bt-SY-4 (2.5 days), and PDKV-Bt-SUd-1 (2.5 days), which were remarkably faster than the control (3.50 days). PDKV-Bt-SAk-6 also demonstrated early germination with 3.00 days, indicating its potential to promote rapid seedling establishment. Rapid germination provides an advantage in field conditions by ensuring uniform crop stands and reducing vulnerability to environmental stress. Compared to the control, which showed the slowest germination, these strains improved emergence by almost one full day, a considerable gain for seedling vigor and early growth potential in chickpea.

3.2 Gemination rate (%)

The highest germination rate (100%) was recorded for PDKV-Bt-SGd-1, PDKV-Bt-SBn-2, PDKV-Bt-SY-4, PDKV-Bt-SHi-1, PDKV-Bt-SDa-1, PDKV-Bt-SDa-2, PDKV-Bt-SUd-1, and PDKV-Bt-SAk-12, all outperforming the control (86.67%). These strains ensured complete seed germination, which directly contributes to higher stand establishment. Among them, PDKV-Bt-SBn-2 combined high germination with the shortest germination period, indicating a synergistic effect on seed performance. In contrast, the control lagged significantly in both germination speed and rate, highlighting the clear benefits of Bt biopriming. The 13-14% improvement over control in germination percentage is agriculturally important, as it translates to denser, more uniform crop populations, potentially leading to better yields in chickpea cultivation. Similar result found by Marisel et.al. (2024) [13] when interaction of *B. thuringiensis* B3 with lettuce and tomato seeds significantly boosted germination and early plant growth.

3.3 Root length (cm)

PDKV-Bt-SAk-12 showed excellent root development (9.5 cm), making it the top performer overall, followed by PDKV-Bt-SDa-2 (8.6 cm) and PDKV-Bt-SDa-2 (8.4 cm), all significantly exceeding the control (6.2 cm). Enhanced root growth in these strains suggests improved nutrient and water uptake capabilities, which is vital under stress conditions. Compared to the control, these top strains increased root length by 2-3 cm, a substantial improvement in early seedling vigor. Longer roots can improve plant anchorage and facilitate better establishment, offering advantages in drought-prone or nutrient-poor soils where chickpea is often cultivated. Similar result found by Marisel et.al. (2024) [13] when inoculation with the Bt B3 strain notably increased germination by 20% to 25%.

Table 3: Effect of Seed-Biopriming of *Bacillus thuringiensis* strains on Chickpea by the paper towel method

SN	Isolate	Days required for gemination	Germination rate (%)	Root length (cm)	Shoot length (cm)	Plant height (cm)	Vigor index
1	PDKV-Bt-SA-6	2.67	93.34	6.3	10.2	16.5	1540.11
2	PDKV-Bt-SA-20	3.17	93.34	7.4	15.4	24.8	2128.15
3	PDKV-Bt-SGd-1	2.67	100	7.2	14.4	21.6	2160.00
4	PDKV-Bt-SA-18	3.17	93.34	6.1	10.8	16.9	1577.45
5	PDKV-Bt-SAk-6	3.00	93.34	9	13.5	22.5	2100.15
6	PDKV-Bt-SAk-9	2.83	93.34	7	12.3	19.3	1801.46
7	PDKV-Bt-SGn-5	3.50	93.34	6.8	11.6	18.4	1717.46
8	PDKV-Bt-SBn-2	2.50	100	7.4	18.5	25.9	2590.00
9	PDKV-Bt-SY-4	2.67	100	7	12.5	19.5	1950.00
10	PDKV-Bt-SGn-4	3.33	93.34	6.3	11.2	17.5	1633.45
11	PDKV-Bt-I-3	3.50	86.67	6	10.6	16.6	1438.72
12	PDKV-Bt-SUd-2	3.50	86.67	6.5	11.6	18.1	1568.73
13	PDKV-Bt-SJa_2	3.33	93.34	6.4	9.8	16.2	1512.11
14	PDKV-Bt-SJa-1	3.50	93.34	6.8	12.1	18.9	1764.13
15	PDKV-Bt-SHi-1	3.17	100	7.1	12.5	19.6	1960.00
16	PDKV-Bt-SDa-2	3.00	100	8.6	16.2	24.8	2480.00
17	PDKV-Bt-SDa-1	3.00	100	8.4	17.4	25.8	2580.00
18	PDKV-Bt-SA-22	3.33	93.34	7.6	20	27.6	2576.18
19	PDKV-Bt-SMa-1	3.50	86.67	5.9	9.4	15.3	1326.05
20	PDKV-Bt-SAk-12	2.50	100	9.5	16	25.5	2550.00
21	PDKV-Bt-SUd-1	2.67	100	8	15.5	23.5	2350.00
22	HD-1	3.50	86.67	6.1	11	17.1	1482.06
23	Control	3.50	86.67	6.2	10.6	16.8	1456.06
SE(m)±		0.10	0.26	0.11	0.24	0.26	-
CD 5%		0.31	0.76	0.31	0.69	0.76	-
Test of significance (p=0.05)		S	NS	S	S	S	-

3.4 Shoot length (cm)

The maximum shoot length was observed in PDKV-Bt-SA-22 (20.0 cm), followed closely by PDKV-Bt-SBn-2 (18.5 cm) and PDKV-Bt-SDA-1 (17.4 cm). These strains clearly outperformed the control, which recorded only 10.6 cm. PDKV-Bt-SAk-12 also exhibited a strong shoot elongation of 16.0 cm, indicating robust above-ground growth. The improvement over control ranged from 6.8 cm to 9.4 cm, nearly doubling shoot length in some cases. Enhanced shoot growth is essential for better light interception and early canopy development in chickpea. The superior shoot elongation in these top-performing strains demonstrates the growth-promoting potential of Bt biopriming, which can lead to faster establishment and greater biomass accumulation compared to untreated seeds. Similar result found by Longatto *et al*. (2025) [11] when inoculation with the Bt RZ2MS9 strain significantly boosted 15 cm shoot length.

3.5 Seedling length (cm)

PDKV-Bt-SA-22 recorded the tallest plants (27.6 cm), followed by PDKV-Bt-SDA-1 (25.8 cm) and PDKV-Bt-SBn-2 (25.9 cm). The control plants reached only 16.8 cm, indicating that Bt strains enhanced height by 9-11 cm. PDKV-Bt-SAk-12 also performed well, reaching 25.5 cm (Table 4.6) (Figure 4.6) (Plate 4.13). The increased plant height suggests that these isolates improve nutrient uptake efficiency and promote vigorous shoot elongation during early growth stages. This can be advantageous for competitive weed suppression and early pod development in chickpea. The strong correlation between plant height and other vigor traits in these strains underlines the overall growth-promoting effect of Bt-based seed biopriming compared to untreated control plants.

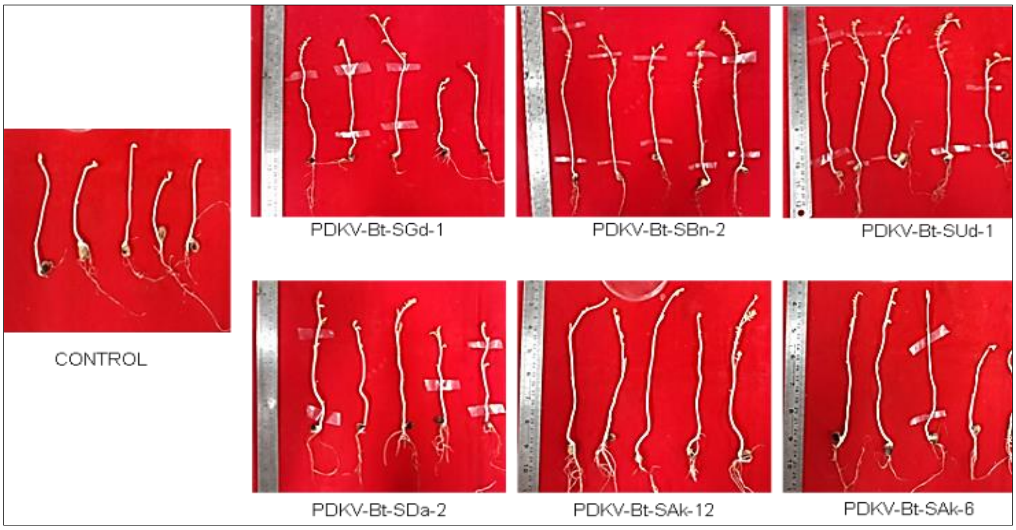

Fig 1: Effect of seed biopriming of local *Bacillus thuringiensis* strains on chickpea by paper towel method

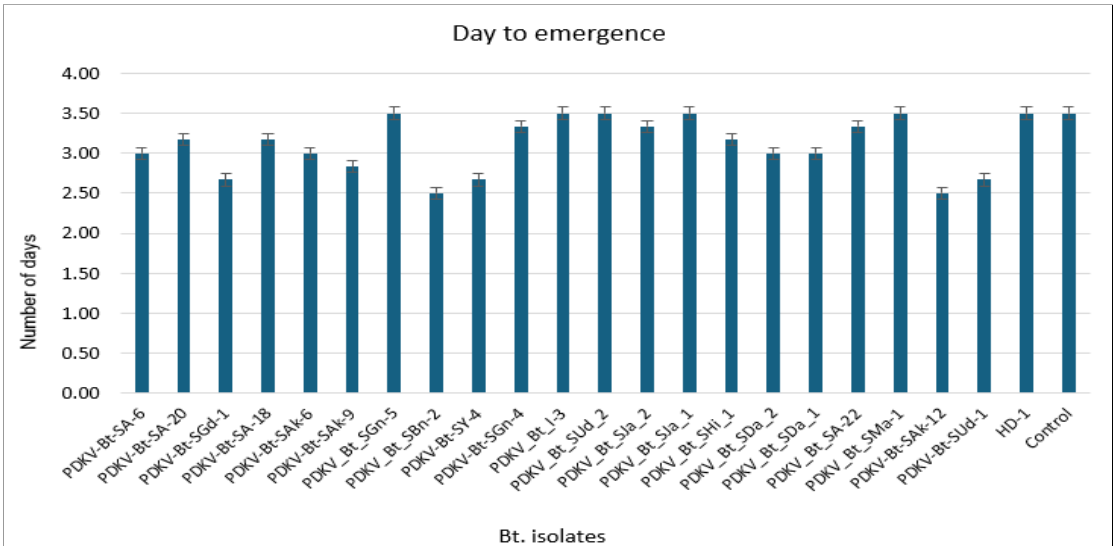

Fig 2: Effect of seed biopriming of local *Bacillus thuringiensis* isolates on emergence of chickpea by paper towel method

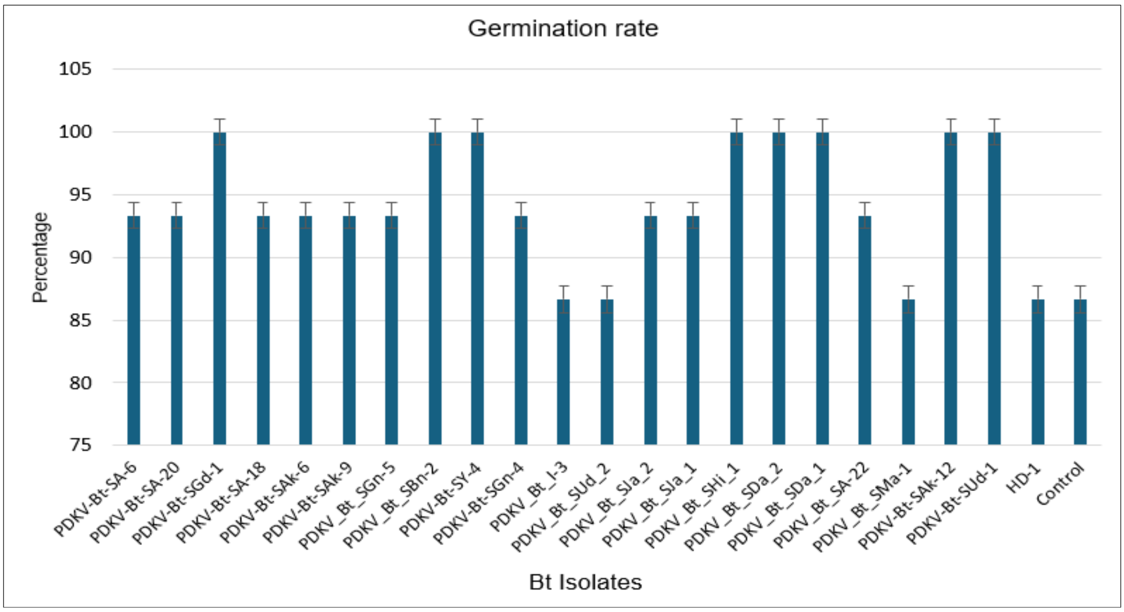

Fig 3: Effect of seed biopriming of local *Bacillus thuringiensis* isolates on germination rate (%) of chickpea by paper towel method

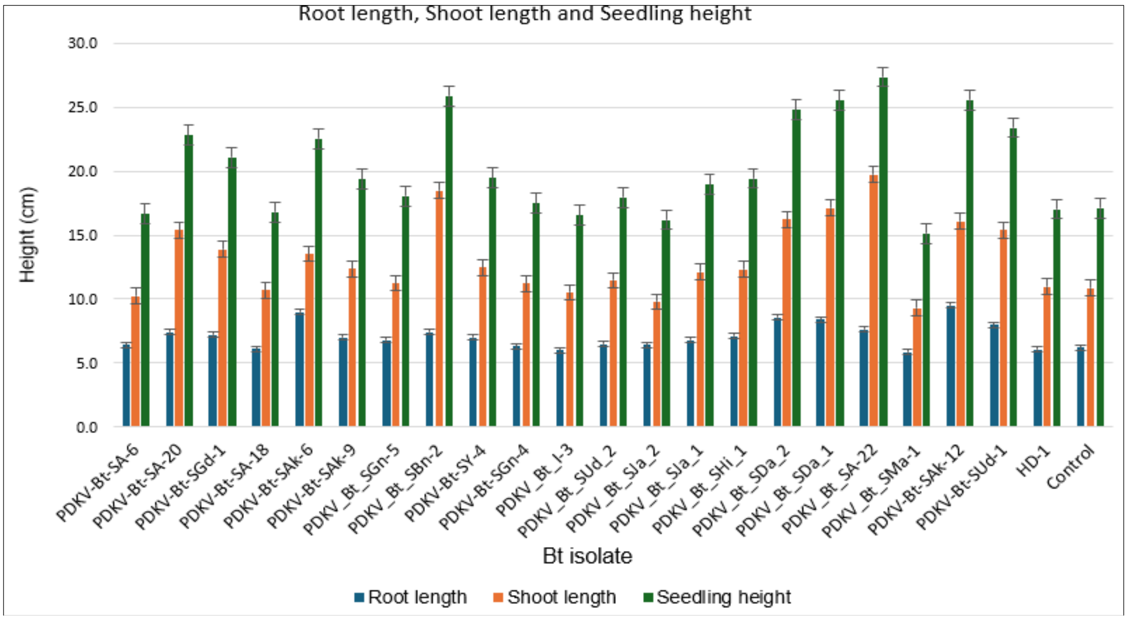

Fig 4: Effect of seed biopriming of local *Bacillus thuringiensis* strains on root length, shoot length, and seedling height of chickpea by paper towel method

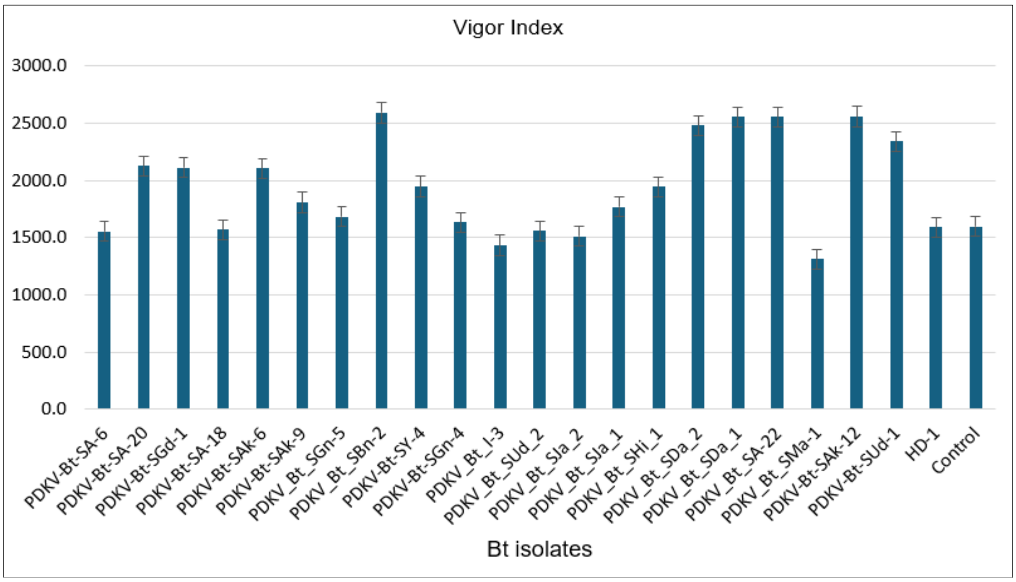

Fig 5: Effect of seed biopriming of local *Bacillus thuringiensis* isolates vigor index of chickpea by the paper towel method

3.6 Vigor index

The highest vigor index was achieved by PDKV-Bt-SBn-2 (2590.00), closely followed by PDKV-Bt-SDA-1 (2580.00) and PDKV-Bt-SA-22 (2576.18). These values were significantly higher than the control (1456.06), showing an increase of over 1100 units in seedling vigor. PDKV-Bt-SAk-12 also displayed a high vigor index of 2550.00. The vigor index integrates both germination percentage and seedling growth, making it a comprehensive measure of seedling performance. The exceptional performance of these strains indicates their strong potential in enhancing early plant establishment and field performance, which is crucial for achieving higher chickpea productivity, especially under challenging agro-climatic conditions.

4. Conclusion

Studying indigenous *Bacillus thuringiensis* isolates can improve plant health and plant growth, and Inoculating seed with beneficial indigenous bacteria can enhance nutrient availability and protect plants from harmful pathogens. Indigenous bacteria can offer valuable information about the local microbial community and its functions within different ecosystems. These bacteria possess distinctive enzymes or metabolic pathways that can be utilized for biotechnological purposes in agriculture.

Seed biopriming with *Bacillus thuringiensis* strains significantly enhanced chickpea germination, root and shoot growth, seedling length, and vigor index compared to the control. Strains such as PDKV-Bt-SBn-2, PDKV-Bt-SAk-12, and PDKV-Bt-SDA-1 consistently outperformed others, demonstrating rapid emergence, higher germination rates, and robust seedling vigor. These improvements highlight Bt's strong potential as an eco-friendly bioinoculant for promoting early establishment, resilience, and productivity in chickpea cultivation under diverse agro-climatic conditions.

5. Future prospects

The seed biopriming of indigenous *Bacillus thuringiensis* strains can be recommended for field planting as an environmentally friendly strategy to improve seed germination and initial seedling growth. Comprehensive field trials conducted across various environmental conditions, utilizing multiple seed lots of different cultivars, will be essential to determine the effectiveness of these strains as possible bio-priming seed treatments for enhancing crop productivity.

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