Scientia Horticulturae 323 (2024) 112552

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effect of rhizospheric bacteria on growth, physiological attributes and melatonin contents of *Trigonella foenum-graecum* L.

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ARTICLE INFO

Keywords:
Antioxidants
Fertilizers
Inoculants
Rhizobacteria
Varieties

ABSTRACT

Chemical fertilizers are economically important but environmentally these are unfit for the living organisms. Therefore, to solve this problem rhizobacteria were used as a substituent. The aim of this research was to investigate the effect of rhizospheric bacteria on growth, physiological attributes and endogenous melatonin contents of fenugreek (*Trigonella foenum-graecum* L.) varieties. Inorganic fertilizers (Urea and DAP) were used in experiment for comparison with bio-fertilizers (*Bradyrhizobium joponicum and Burkholderia cepacia*). It was found that plants under treatment of bacteria and fertilizers have more growth in comparison to control plants. Significant changes were observed in growth of plants. Rhizospheric bacterial amendments significantly improved endogenous melatonin level in all studied fenugreek varieties. Plants under bacterial treatments have represented positive changes in all the parameters which were studied as compare to control plants. From these results it is found that bacterial inoculants have beneficial effects on four varieties of fenugreek. These results suggested that beneficial bacteria can be essential and significant in reducing risks to ecosystems around the world.

1. Introduction

Fenugreek (*Trigonella* foenum-*graecum* L.) belongs to family Fabaceae (Leguminoseae). It is known as Greek hay. It is mostly used as spice in food and has been used as a folk remedy for hundreds of years. The presence of alkaloids in fenugreek seeds gives it the ability to treat illnesses (Zandi and Basu, 2016). Fenugreek leaves are also being used as food for humans and also as cow fodder (Badar et al., 2016). As it can better grow in nitrogen rich soil, it is preferred to grow together with grasses and silage to maintain soil nitrogen levels (Chaichi et al., 2015). It is grown in different countries throughout the world, including China, India, Pakistan, Egypt, Ethiopia, Morocco, Ukraine, Greece, Turkey, and Canada, where it is utilized as a medicinal plant (Dadrasan et al., 2015).

Globally, food security is the most pressing issue, and it is anticipated that climate change, fast population expansion, and arid land scarcity will exacerbate this problem over the next 50 years, putting pressure on agricultural land and the food business (Kumar et al., 2017). Sustainable agriculture is the only tool that can combat poverty and hunger in order to resolve this issue. Farmers are using synthetic fertilizers carelessly to get high productivity, that is ultimately resulting in loss of soil natural fertility and environmental issues (Ansari et al., 2017). Utilization of N and P in inorganic form is thus essential for crop growth. The use fertilizers which contain phosphorus and nitrogen promote the growth of fenugreek. The favorable effects of P fertilization on fenugreek seed output were reported (Kumar et al., 2011).

Plant growth-promoting rhizobacteria (PGPR), which make up the majority of soil microorganisms, aid plants by boosting their growth (Aqeel et al., 2023). As PGPRs have beneficial effects on plant growth and development, bio-fertilizers can replace agro-chemicals (Bhattacharyya and Jha, 2012). PGPRs can be utilized as bio-fertilizers rather than synthetic fertilizers in developing agricultural nations like Pakistan and India to boost economic production with less environmental harm (Singh et al., 2011). Investigating soil bacteria and its potential to promote plant growth is therefore crucial. To elaborate soil-borne microbial communities is a conceivable strategy (Zainab et al., 2021).

Despite being a necessary component for plants, phosphorus (P) is

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https://doi.org/10.1016/j.scienta.2023.112552
Received 15 August 2023; Received in revised form 19 September 2023; Accepted 2 October 2023
Available online 7 October 2023

typically not immediately accessible to plants because of its non-bioavailability state in soil. *Burkholderia cepacia* solubilize phosphate (P) from insoluble soil let plants use it for a variety of metabolic processes (Kumar et al., 2020). It is an organism which produces lot of secondary metabolites; these compounds include anti-bacterial, anti--fungal, insecticidal, or herbicidal characteristics (Jung et al., 2018). *Bradyrhizobium japonicum* make symbiotic association with root of plant and make available atmospheric nitrogen to plants, because nitrogen in the air is in molecular form which plant cannot use. As the rhizobial associations increase number of nodules also increase and fix maximum air nitrogen, mostly rhizobial associations have been seen in leguminous plants. Although these bacteria help in biological nitrogen fixation, also produce beneficial hormones and chemicals which are also increase the growth of crops (Shang et al., 2021).

Natural antioxidant melatonin (MT, N-acetyl-5-methoxytryptamine) are synthesized by plants and microorganisms. It has numerous functions in plant growth regulation and abiotic stress resistance (Guo et al., 2023). This substance controls photosynthesis, plant growth and development, flowering and seed germination, and increases plants' ability to withstand stress. Melatonin was easily able to cross morpho-physiological hurdles due to its hydrophilic and lipophilic properties, which led to its quickly transit into plant cells (Sadak et al., 2020). Due to its effective transport via cell pores, melatonin has a substantially higher antioxidant potential than other antioxidants. Melatonin influences the expression of several genes involved in physiological and biochemical processes in addition to its antioxidant function (Mohamadi Esboei et al., 2022).

Now a day's beneficial changes in soil are done by application of inorganic chemicals, these are continuously disturbing environment and humane health. It improves crop production and also results in sustainable agriculture. However, these methods are damaging environment and human health, because in these methods farmers use fertilizers and chemicals to get high harvest (Baez-Rogelio et al., 2017). The only way to get quality foods is to move towards organic agriculture. Rhizosphere is the part of soil where microbes make interaction with plants and affects the root system (Kumar et al., 2020). Plant growth promoting rhizobaceria are being applied to plants as bio-fertilizers to solve above problems related to chemical fertilizers (Youssef and Eissa, 2014).

The symbiotic associations made by *B. japonicum* with root of plant, in this way atmospheric nitrogen can be available to plants. Nitrogen in the air is in molecular form and not available to plants. As the Rhizobial associations increase number of nodules also increase these nodules help in to fix maximum air nitrogen. Mostly Rhizobial associations have been seen in leguminous plants. Although these bacteria help in biological nitrogen fixation, they also produce beneficial hormones, chemicals and are also, increase the growth of crops (Shang et al., 2021). *Burkholderia cepacia* is a strong pesticide against the soil born plant pathogens. It is much beneficial and important bio fertilizer for rice crop grown in low pH and low soil fertility. Different strains of *B. cepacia* have been identified; they can enhance the crop yield. It also has phosphorus solubilizing abilities and can change unavailable phosphorus to available form for plants (Jung et al., 2018). Therefore, the objective of the study was to determine the effect of rhizobacteria on growth, physiology and melatonin contents of fenugreek varieties.

2. Materials and methods

The pot experiment was conducted to investigate the effect of rhizospheric bacteria on growth and physiology of fenugreek (*Trigonella foenum-graecum* L.) varieties. Experiment was completely randomized with three replicates for each treatment. This experimental design was comprised of total 5 treatments which included BR (*Bradyrhizobium japonicum*), BC (*Burkholderia cepacia*), BR + BC (*Bradyrhizobium japonicum* + *Burkholderia cepacia*), synthetic fertilizer treatment (SF) and control (C) for each fenugreek variety, four varieties of fenugreek were used. Total 15 pots (width 21 cm, height 23 cm) were used for each variety. Pots were arranged in four rows each row contain single type of variety.

2.1. Collection of soil

Soil was collected from agricultural land located in Bhiyanwala Muridke, Sheikhupura. Soil collection method used by Chaichi et al. (2015) was followed. Soil was collected from top layer (up to 30 cm) before it was added in pots, sieved with 5 mm sieve mesh, sterilized with 70 % ethyl alcohol before filling in pots.

2.2. Collection of seeds

The seeds of fenugreek varieties (i.e., fenu-2001, fenu-2002, fenu-2003 and fenu-2004) were obtained from Vegetable Research Institute, Ayub Agricultural Research Institute, Faisalabad. The seeds were gently rinsed with distilled water after being cleaned with chlorine bleach. The seeds were cleaned with a solution of 70 % ethanol and 3 % hydrogen peroxide, followed by rinsing in distilled water. In each pot 14 seeds were sown in three rows with 3 cm spacing between rows and each plant (Chaudhary et al., 2021).

2.3. Collection of bacteria

The experiment was performed with two bacterial strains; *Bradyrhizobium japonicum* and *Burkholderia cepaci.* The bacterial strains were obtained from Fungal Culture Bank, Institute of Agriculture, University of Punjab, Lahore. Both strains were further sub-cultured on LB media.

2.4. Preparation of suspension

Bacterial colonies were picked by toothpick and added in 50 mL of distilled water, which was further diluted to inoculate the soil. The suspensions of both bacteria were prepared in separate flasks; a mixture of both bacteria was also prepared in separate. Mixture of two fertilizers were prepared by mixing DAP (Di ammonium phosphate) and Urea with 1:1 in distilled water (Nosheen and Bano, 2009).

2.5. Inoculation

Inoculation method used by Wang et al. (2019) was applied for the bacterial suspension inoculation. Already prepared bacterial suspensions were measured in measuring cylinder carefully. For each pot, 25 mL of inoculation material were employed. Firstly, the plants in each pot were thinned to 5 and then each plant was inoculated with 5 mL of bacterial suspension. Bacterial suspension was applied to root zone of each plant circularly. Same amount of distilled water was used for control plants, and for plants treated with fertilizer, similar amount of fertilizer mixture was used. Plants were inoculated two times in their life cycle. First inoculation was done after 35 days of sowing of seeds. Second inoculation was done after 30 days of first inoculation (Bairva et al., 2012).

2.6. Growth parameters

At maturity of plants, growth parameters were measured for the number of leaves, nodules, shoot length (cm), root length (cm), total length of the plant (cm), shoot fresh weight (g), root fresh weight (g), total fresh weight (g), shoot dry weight (g), root dry weight (g), total dry weight (g), leaf width (cm), leaf surface area (cm^2), number of branches, inter-nodal distance (cm), leaf petiole ratio (g), number of inflorescence per plant, number of pods per spike, number of seeds per spike, length of spike (cm), length of pod (cm), weight of 100 seeds.

Table 1
Significance of mean square values of different growth parameters of four varieties of fenugreek.

SOURCE	DF	Shoot Length (cm)	P	Root Length (cm)	P	No. of Leaves	P	No. of Nodules	P	Shoot Fresh Weight (g)	P	Root Fresh Weight	P	Shoot Dry Weight (g)	P
Variety	3	2272.40	.0000 ***	27.28	.0004 ***	18.78	.0000 ***	151.84	.0000 ***	28.43	.0000 ***	0.22	.0000 ***	0.24	.0000 ***
Treatments	4	108.13	.0001 ***	6.28	.16 ns	416.12	.0000 ***	112.30	.0000 ***	62.29	.0000 ***	0.05	.0000 ***	0.43	.0000 ***
Variety × Treatments	12	26.72	.056 ns	6.28	.097 ns	245.43	.0000 ***	59.71	.0000 ***	18.44	.0000 ***	0.0097	.0000 ***	0.11	.0000 ***
Error	40	13.69		3.64		34.46		4.83		1.77		0.0012		0.002	
	DF	Root Dry Weight (g)	P	Leaf Surface Area (cm^2)	P	Leaf Width (cm)	P	No. of Branches	P	Inter-nodal Distance (cm)	P	Leaf Petiole Ratio / Weight (g)	P	Leaf Petiole Ratio / Length (cm)	P
Variety	3	0.0010	.0000 ***	0.13	.0029 **	0.14	.033 *	51.39	.0000 ***	17.13	.0000 ***	0.39	.010 *	0.008	.011 *
Treatments	4	0.0055	.0000 ***	0.16	.0003 ***	0.06	.25 ns	3.54	.0042 **	4.83	.0004 ***	2.05	.0000 ***	0.01	.0003 ***
Variety × Treatments	12	0.0012	.0000 ***	0.22	.0000 ***	0.08	.072 ns	4.90	.0000 ***	2.38	.0029 **	0.27	.0044 **	0.007	.0006 ***
Error	40	0.0001		0.023<-		0.04<-		0.78<-		0.75<-		0.09<-		0.001<-	
	DF	Leaf Shoot Ratio / Weight (g)	P	No. of Inflorescence / Plant	P	No. of Pods / Spike	P	No. of Seeds / Spike	P	Spike Length (cm)	P	Seed Weight / 100 (g)	P	Pod Length (cm)	P
Variety	3	1.94	.0000 ***	370.77	.0000 ***	52.55	.0000 ***	416.06	.0001 ***	0.27	.1142 ns	5.93	.0000 ***	0.008	.0000 ***
Treatments	4	0.13	.0007 ***	54.4	.0000 ***	43.31	.0000 ***	138.14	.0271 *	1.33	.0000 ***	4.64	.0000 ***	0.004	.0000 ***
Variety × Treatments	12	0.08	.0007 ***	46.96	.0000 ***	6.21	.1046 ns	77.68	.0977 ns	0.10	.6264 ns	1.01	.0000 ***	0.01	.0000 ***
Error	40	0.02<-		5.88<-		3.66<-		45.05<-		0.12<-		0.02<-		0.0001<-	

ns = non-significant; *, **, *** significant at 0.05 level, DF = Degree of Freedom, *P* = Significance.

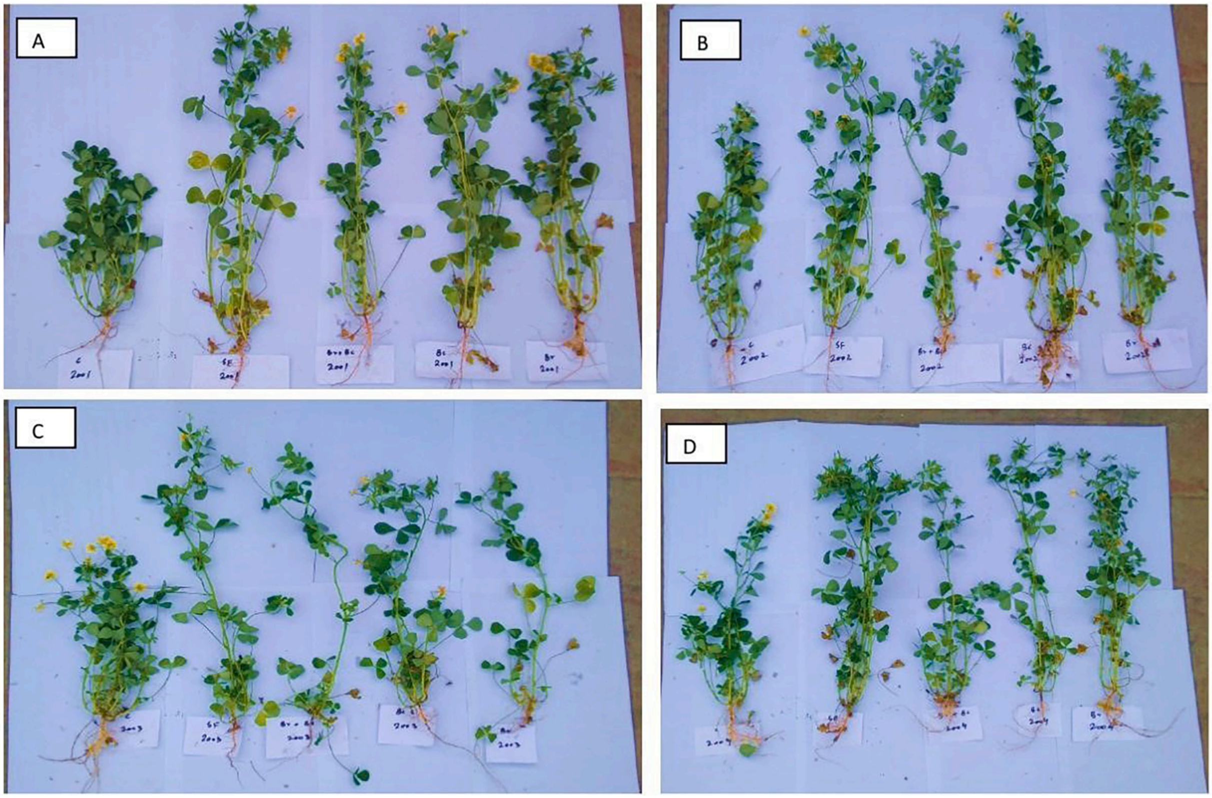

Fig. 1. Comparison of shoot length between four fenugreek varieties. A: Fenu-2001, B: Fenu-2002, C: Fenu-2003, D: Fenu-2004.

2.7. Physiological parameters

The physiological parameters including chlorophyll a, b, carotenoids and relative water content were also determined. Arnon (1949) method was used to determine chlorophyll a, b, and carotenoids. For this purpose, 0.1 g fresh leaves of each plant were grinded in pre chilled mortar. It was then given 5 mL of 80 % acetone, and filtered in test tubes, the filtrate left for 24 h in refrigerator, then 2 mL of this filtrate absorbance was read at 663, 645 and 480 nm using a spectrophotometer (Hitachi-220 Japan).

The Chlorophyll a and b and Carotenoids were calculated by the following formula:

$$Chl.\,a\,(mg\,g-1\,f.\,wt.) = (12.7\,(OD663) - 2.69\,(OD645))\,x\,V\,/\,1000\,x\,W$$

$$Chl.\,b\,(mg\,g-1\,f.\,wt.) = (22.9\,(OD645) - 4.68\,(OD663))\,x\,V\,/\,1000\,x\,W$$

$$Carotenoids\ (mg\ g-1\ f.\ wt.) = (OD\ 480 + 0.114\ (OD663) - -\ 0.638\ (OD645)$$

OD = Optical density

V = Volume of the extract (mL)

W = Weight of fresh leaf tissue (g).

The method of Jones and Turner (1978) was used for the determination of RWC (relative water content) by using following formula:

$$RWC\ (\%) = [(FW - -\ DW)\,/\ (TW - -\ DW)] \times 100$$

Here FW, DW, and TW are the fresh, dry and turgid weights, respectively.

2.8. Total phenolic contents (mg/g)

Phenolic contents were determined by following the method of Julkunen-Tiitto (1985). The fresh leaves (0.5 g) of plants were crushed in a pre-chilled mortar with 10 mL of phosphate buffer with a pH of 7.8 added. The filtrate was then filtered, centrifuged at 6000 g for 24 min at 4 °C, and the supernatant was removed and stored in a deep freezer for future use (for protein and enzymatic activity).

2.9. Total protein contents (mg/ml)

The Bradford (1976) technique was applied to determine the total soluble protein. Bradford solution was created by combining 100 mg of Coomassie Brilliant Blue with 50 mL of 95 percent ethanol, 100 mL of 85% phosphoric acid, and 1 L of distilled water to make the desired volume. After that, the extract that had already been made was added to 5 mL of Bradford solution, which was then vortexed. A UV/VIS spectrophotometer was used to measure the absorbance at 595 nm.

2.10. Antioxidants (mM/g f. wt.)

The method of Chance and Maehly (1955) was used to calculate the amount of catalase (CAT) and per-oxidase (POD). 1 mL of 50 mM phosphate buffer (pH 7.0), 1.9 mL of 5.9 Mm hydrogen peroxide (H_2O_2), and 0.1 mL of enzyme extract are all included in 3 mL of CAT reaction solution (discussed in Total Soluble Proteins). For a maximum of 120 s, multiple variations in the absorbance at 240 nm were monitored every 30 s. The 50 mM phosphate buffer (pH 5.0), 20 mM guaiacol, 40 mM hydrogen peroxide (H_2O_2), and 0.1 mL of enzyme extract were all included in the 2 mL POD reaction solution.

The APX (Ascorbate Peroxidase) activity was performed using method of Nakano and Asada (1981). 2.7 mL of 50 mM potassium phosphate buffer, 0.1 mL of enzyme extract, and 0.1 mL 7.5 mM of ascorbic acid and 0.1 mL of 300 Mm hydrogen peroxide (H_2O_2) were used to prepare a reaction mixture. A quartz cuvette containing 3 mL of the reaction solution was used to test it in a UV/VIS spectrophotometer against a blank that contained 0.1 mL of distilled water in place of the

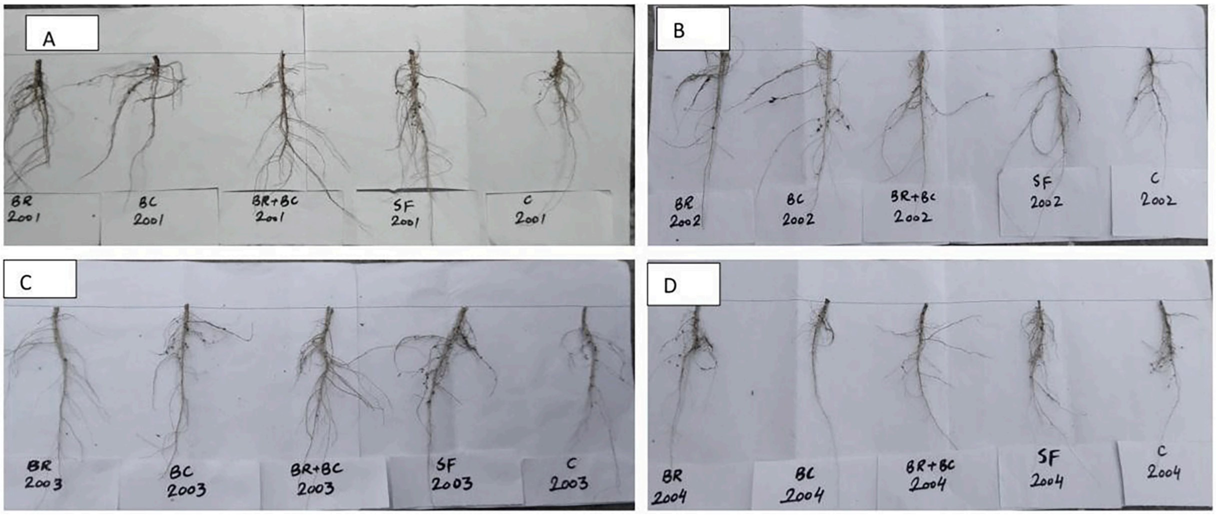

Fig. 2. Comparison of root length between four fenugreek varieties. A: Fenu-2001, B: Fenu-2002, C: Fenu-2003, D: Fenu-2004.

enzyme extract. Readings were recorded at intervals of 30 s between 0 and 60 s at a wavelength of 290 nm.

2.11. Inorganic ions

According to the method of Wolf (1982), 0.1 g of powdered, oven-dried plant material was added to test tubes from each replicate, mineral ions contents were calculated. These test tubes received 2 mL of sulfuric acid and were then placed in a digestion flask for 24 h. Hydrogen peroxide was dropped into the digestive flask while it was being heated on a hot plate, and the substance eventually became colorless. The substance was then filtered, and distilled water was used to create a filtrate volume of 50 mL. Flame Photometer (Sherwood Model 360) was used to take readings for Ca^{2+}, K^{+} and Na^{+}.

2.12. Determination of melatonin

The approach developed by Byeon and Back (2014) was used to estimate endogenous melatonin in plant leaves. Plant leaves (0.2 g) were chopped with liquid nitrogen before being mixed with 1 mL of methanol. This solution was transferred to centrifuge tubes and kept at 4 °C overnight. The supernatant was collected in fresh tubes after centrifugation at 10,000 g for 10 min. After evaporating the supernatant, the residual residues were dissolved in 0.5 mL of methanol. The solution was filtered using a 0.22 m needle filter to get a clean sample. Melatonin concentrations were determined using high-performance liquid chromatography with a fluorescence detector system (Rigol L3000). A Kromasil C18 column (250 mm 4.6 mm particle size, Eka Nobel, Bohus, Sweden) was used for the experiment. The mobile phase was a 40:60 blend of methanol and ultra-pure water. The injection volume was 10 L,

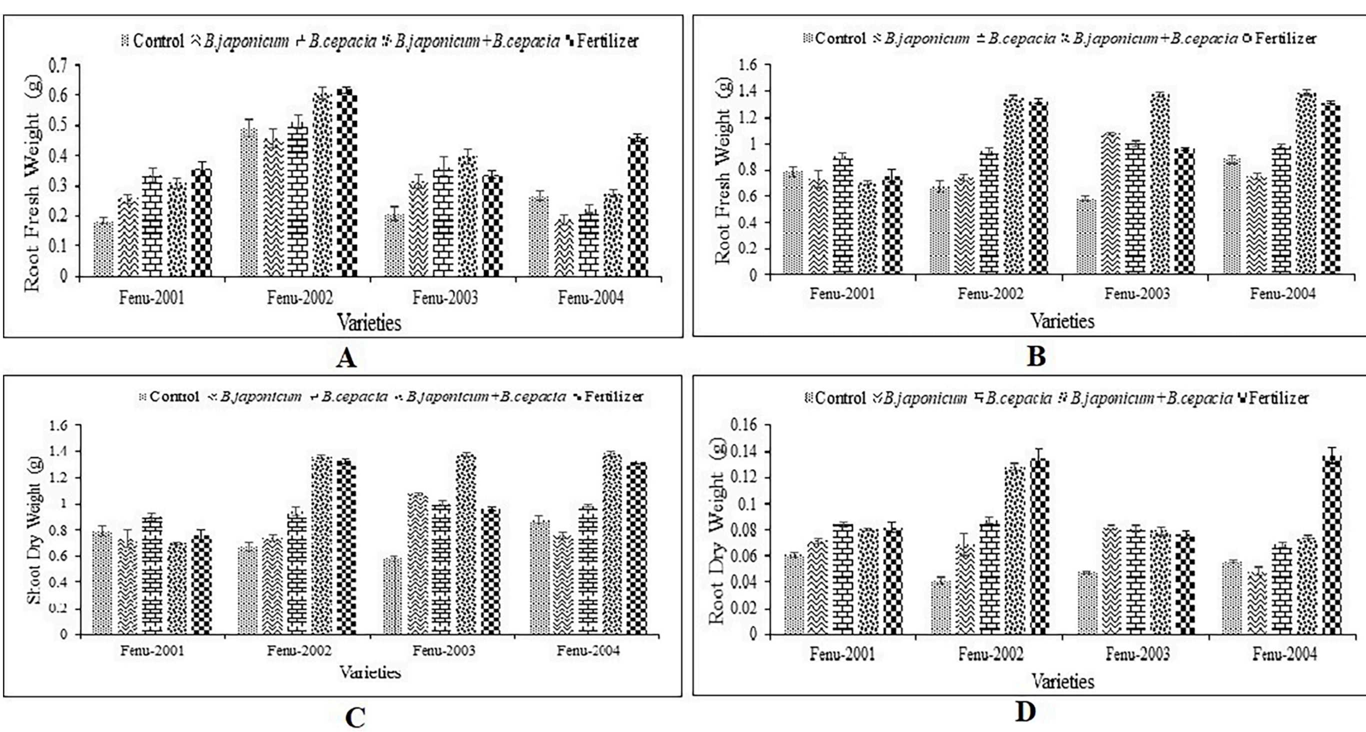

Fig. 3. Growth parameters of four fenugreek varieties. (A) Shoot fresh weight (B) Root fresh weight (C) Shoot dry weight (D) Root dry weight.

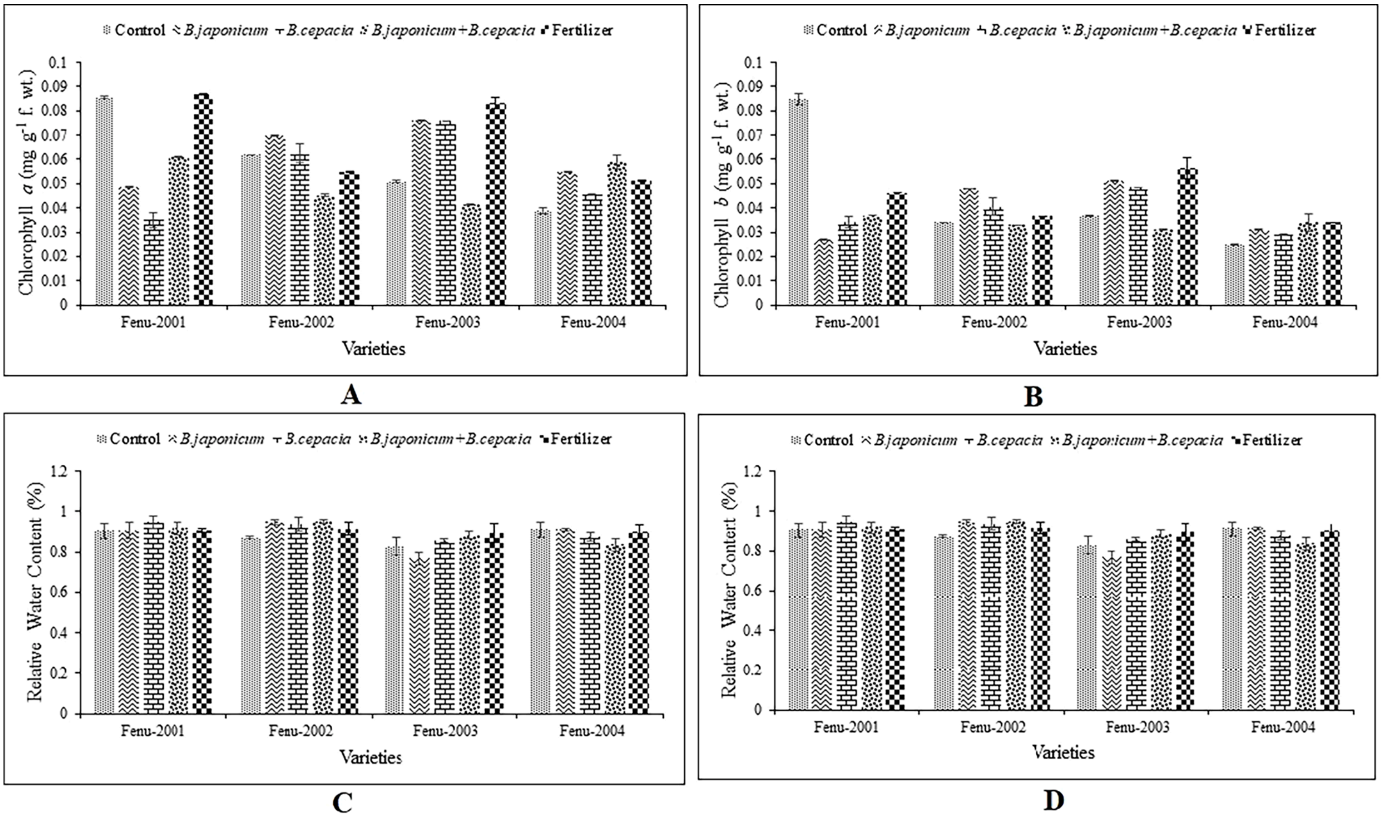

Fig. 4. Physiological parameters of four fenugreek varieties. (A) Chlorophyll a (B) Chlorophyll *b* (C) Carotenoids (D) Relative water content (%).

and the flow rate was 0.8 mL per minute1. The column temperature was held constant at 30 °C, and fluorescence detection was performed at excitation wavelengths of 280 nm and emission wavelengths of 340 nm. A standard curve was built using known melatonin values, and peak values acquired for samples were compared to the standard curve to estimate melatonin concentration in the samples.

2.13. Statistical analysis

Computerized software Co-Stat - version 6.303 (Cohort software, 1988–2004) was used for statistical analysis. ANOVA was performed to check the significance of results. The level of significance was kept at 5 %.

3. Results and discussion

3.1. Growth parameters

According to the study's findings, each type of fenugreek was affected differently by bacterial and fertilizer treatments (Table 1). In comparison to control plants, fenu-2001 had the shortest shoot lengths across all treatments, while fenu-2004 had the longest shoot lengths (Fig. 1). Root growth displayed a similar pattern (Fig. 2). It was discovered in fenu-2003 that combining the inoculation of both bacteria led to greater root growth when compared to other treatments. In tropics and temperate regions, *B. joponicum* inhabit the soil and enter plant roots. These bacteria are responsible for the formation of nodules, coexist symbiotically with plants, and fix atmospheric nitrogen. It has nitrogenase, an enzyme that fixes nitrogen. It has the ability to convert molecule nitrogen into ammonia (Purwani et al., 2021). The main source of phosphate is organic phosphate; since organic substances (phospholipids, nucleic acids, etc.) are complex, soil bacteria must break them down into simple, diffusible chemicals. According to Alori et al. (2017), some taxa of *Pseudomonas*, Cyanobacteria, and *Bacillus* have the ability to both dissolve and mineralize phosphate.

In the present study, it was found that combining bacteria treatments was significantly more effective than using control plants or other treatments. For shoot fresh weight, all four fenugreek varieties showed appreciable growth in plants treated with a combination of bacteria. For mixed bacterial treatment, fenu-2002 and fenu-2003 were found to have the highest root fresh weights. Fenu-2002, Fenu-2003, Fenu-2004, and Fenu-2002 for root dry weight all produce comparable results (Fig. 3). The growth parameters are improved by chemical fertilizers as well as nitrogen-fixing bacteria, which fix atmospheric nitrogen (Soumaya et al., 2016). After giving plants rhizobia treatment for 21 days, the experimental plants' roots grew more than those of the control plants' roots (Badar et al., 2016). Two bacteria were used to treat collard plants, and it was discovered that treated plants had more leaves than untreated plants (Helaly et al., 2020). The fresh weights, dry weights, and number of nodules of chickpea that had received bacterial inoculation all improved (Gopalakrishnan et al., 2016). It was found that plants treated with bacterial strains increased their shoot fresh weight, demonstrating the importance of bio-inoculants for crop growth (Zafar-ul-Hye et al., 2015). In comparison to control plants, broccoli treated with bio fertilizer and humic acid has larger leaves. Bio-fertilizers that fix nitrogen raise the soil's nutrient content, resulting in more plant biomass (Al-Taey et al., 2019).

Because microorganisms produce growth-promoting substances that affect the root mass, it was found that plants treated with vermicompost, nitroxin, and cow dung had more fresh weight than control plants. As a result, it should be noted that the dry weight of the strawberry aerial part produces significantly different results for the treatment of bacteria than nitrogen fertilizers (Wu et al., 2017). The highest values were found when the two bacteria *Azospirillum brasilense* and *Burkholderia cepacia* were combined (De Andrade et al., 2019). Six bacterial strains were inoculated into maize seedlings using the

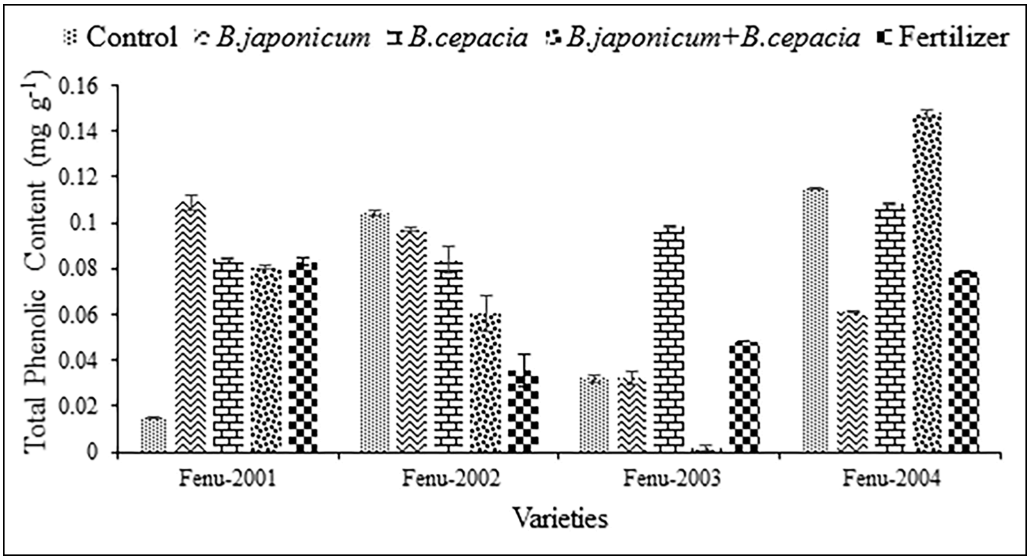

Fig. 5. Total phenolic content (mg g^{-1}) in four fenugreek varieties.

hydroponic culture technique. The root and shoot dry weight of maize is significantly affected by all strains, but two strains did not exhibit any beneficial effects on dry weights (De Sousa et al., 2021).

Results for leaf surface area and branch count were consistent across all treatments (Table 1). A careful record of the number of inflorescences per plant, the number of pods per spike, the number of seeds per spike, and the weight of 100 seeds (g) revealed that plants with single, combined and fertilizer inoculations produced higher yields than controls (Table 1). *Streptomyces sp*. inoculated buckwheat plants had a higher number of inflorescences than other plants that weren't treated with the bacteria (Kadyrova et al., 2020). Bean plants treated with PRM (PGPR, Rhizobacteria, and Mycorrhiza) had different plant yields. Bean plants received single, double, and triple inoculant treatments; it was discovered that the triple treatment resulted in the highest number of recoded pods (Raklami et al., 2019). As opposed to non-inoculated plants, it was observed that *Azospirillum* treatment of plants increased the number of grains and had a positive impact on the number of seeds per spike (Karimi et al., 2021). In contrast to other controls, plants treated with bacteria and exposed to light had higher seed weights of 100 (Soliman, 2019). Comparisons were also made between inoculated plants exposed to light and those that were not (Soliman, 2019).

3.2. Physiological parameters

For all of the fenugreek varieties, physiological parameters including chlorophyll a, b, carotenoid, and relative water contents were estimated under various conditions (Fig. 4). It was discovered in the current study that all bacterial inoculations were generally advantageous for chlorophyll a and chlorophyll *b*. The levels of carotenoids were essentially the little bit change across all treatments. It was found that bacterial inoculations had positive effects on all varieties of fenugreek regardless of relative water content. When compared to plants under control, the percentage of relative water content in bacterial treatments was higher.

Pseudomonas indica and *Azobacter* inoculated plants produced higher chlorophyll a content than control plants (Baishya et al., 2015). However, it was discovered that *Artemisia annua* plants treated with *Pseudomonas indica* and *Chroococcum* had higher levels of chlorophyll *b* than control plants (Arora et al., 2016). The bar graph in Fig. 4 shows that

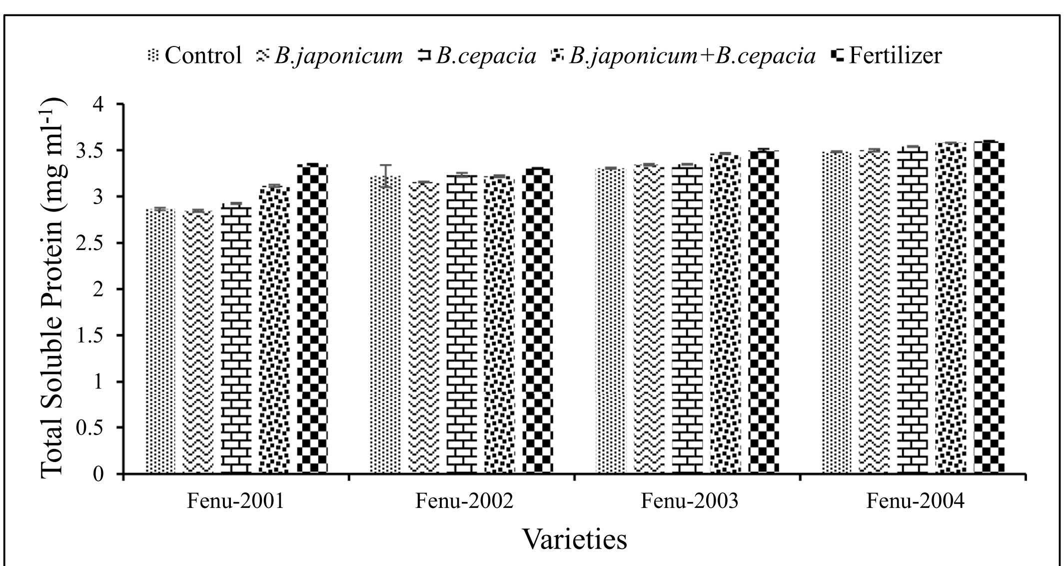

Fig. 6. Total soluble proteins (mg ml^{-1}) in four fenugreek varieties.

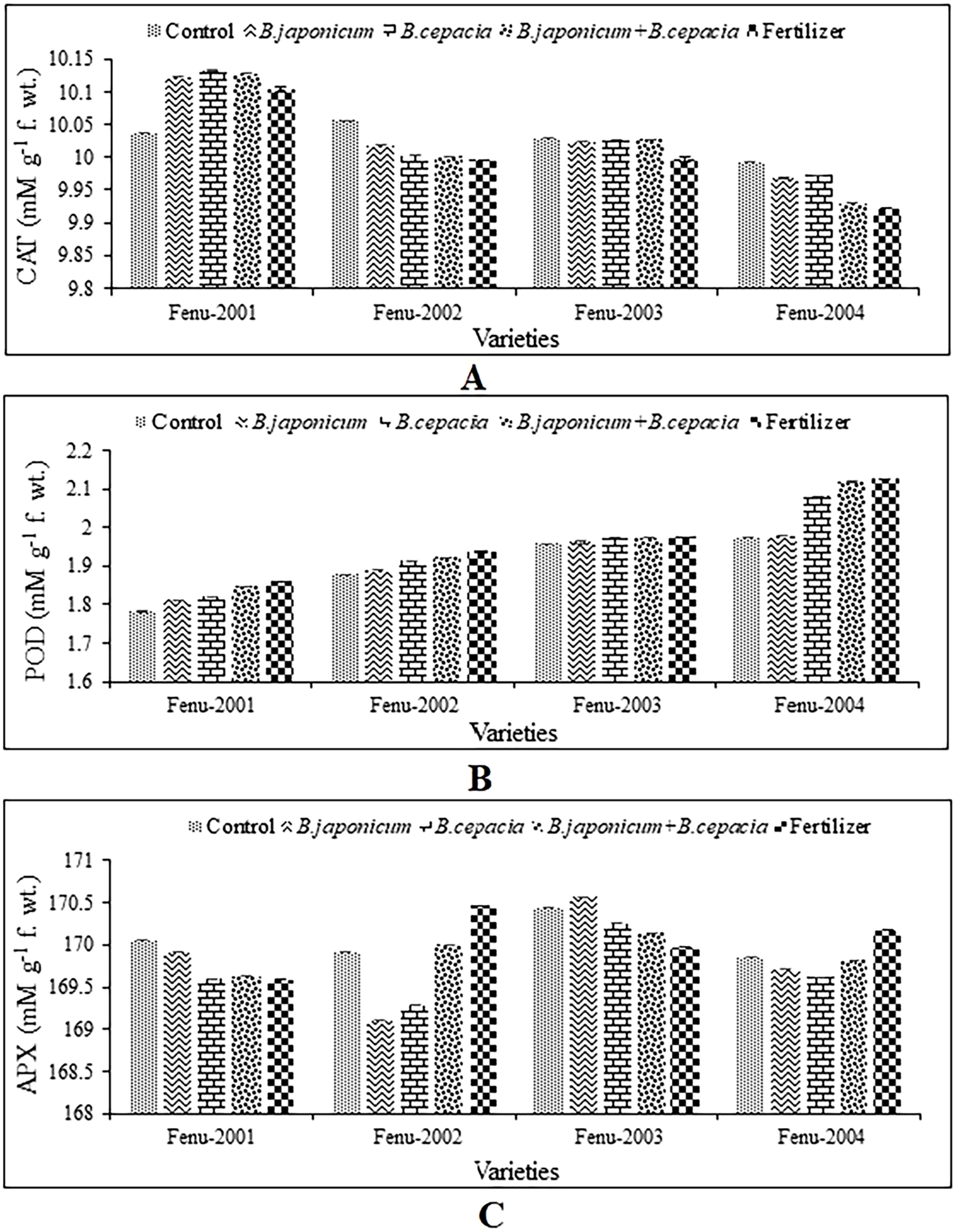

Fig. 7. Antioxidants in four fenugreek varieties. (A) Catalase (B) Peroxidase (C) Ascorbate peroxidase.

Fenu-2003 has significantly better results than other varieties. According to research by Arora et al. (2018), plants treated with the bacteria *Psuedomonas indica* and *Azobacter* showed an increase in carotenoids. Additionally, it was noted that wheat inoculated with PGPR displayed increased relative water content in arid and semi-arid regions of Pakistan (Rashid et al., 2021).

3.3. Total phenolic contents

A variety of plant functions depend heavily on bio-chemicals. These aid in both representing the contrast between variously treated plants and the beneficial effects of biological fertilizers. In comparison to control plants, it was found that fenu-2004 demonstrated a significant variation in phenolic contents under bacterial treatments, followed by fenu-2001 and fenu-2002 (Fig. 5). According to Singh and Patel (2016) Buckwheat plants inoculated with *Azospirillum* sp. *and Azotobacter* sp. have significant effect on synthesis of total phenolic content.

3.4. Total soluble proteins

All fenugreek varieties' total soluble protein content was calculated. When compared to the control, it was discovered that fenu-2004 had the most significant results while fenu-2001 had the lowest values (Fig. 6). Mung bean plants treated with *Trichoderma, Rhizobium*, Mycorrhiza, and their combination produced more total soluble protein than untreated plants (Kaur and Kumar, 2020).

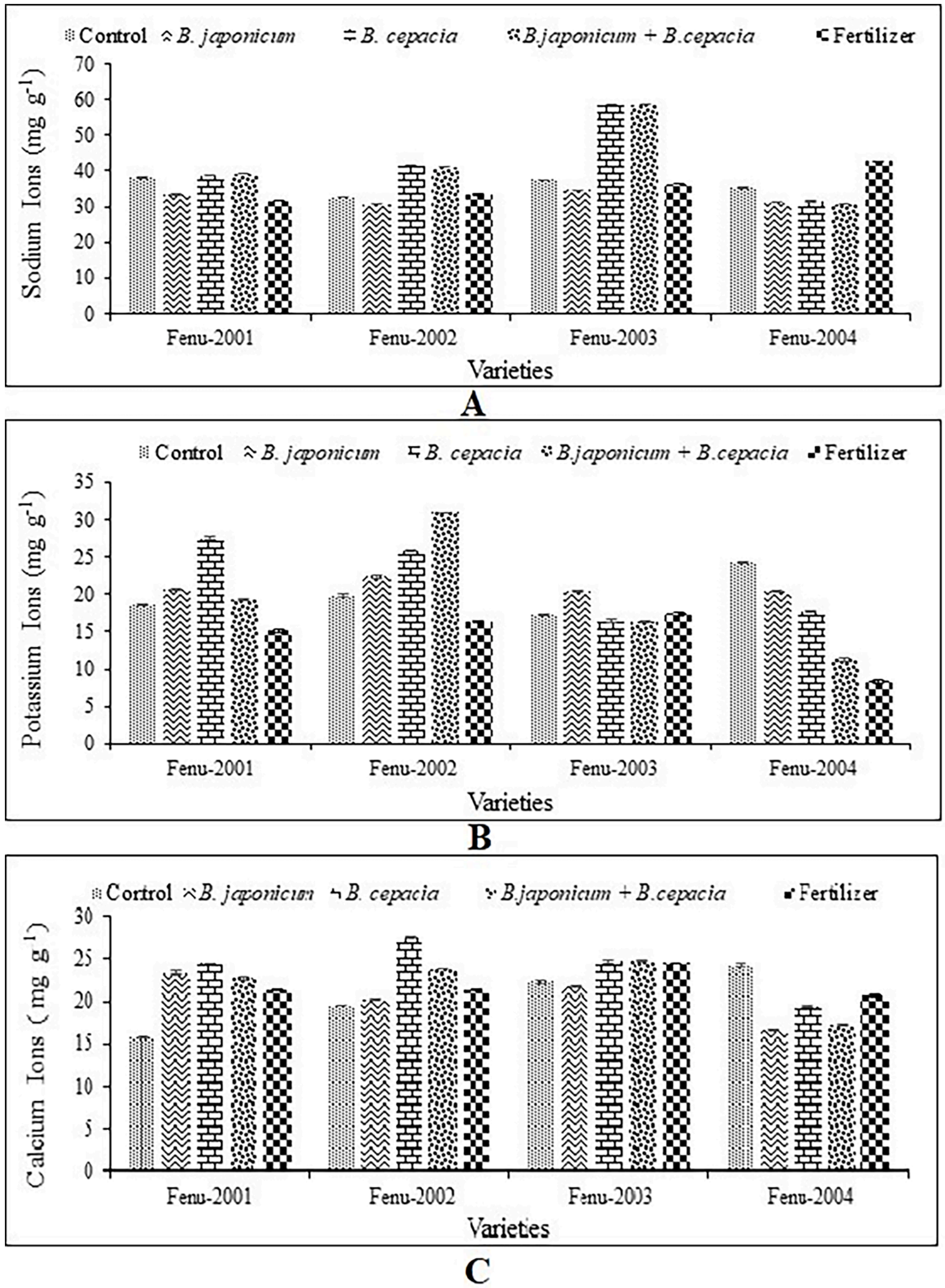

Fig. 8. Inorganic ions (mg/g) in four fenugreek varieties. (A) Sodium (B) Potassium (C) Calcium.

3.5. Antioxidants

To make an estimation of fenugreek's stress response, the antioxidant content was also measured. It was discovered that fenu-2001 had significantly more catalase than any of the other treatments, while fenu-2004 had the least amount. The amount of peroxidase was highest in fenu-2004 and lowest in fenu-2001, according to the peroxidase graph. All of the bacterial treatments had different ascorbate peroxidase concentrations (Fig. 7). Different treatments were used to calculate the CAT activity in the leaves of maize plants, maize plants exhibit an increase in CAT activity at the end of the crop. (Chaudary et al., 2021). It was determined that different bacterial inoculations increased the amount of per-oxidase that maize plant leaves produced, causing an increase in POD levels at the end of the crop (Chaudary et al., 2021). Plants inoculated with *Azospirillum lipoferum* and *Azotobacter chroococcum* have produced more ascorbate per-oxidase in saline soil conditions (Abdel Latef et al., 2020).

3.6. Inorganic ions

In plants, sodium, calcium, and potassium ions serve a variety of purposes. Healthy plants are better able to take up ions from the soil. According to mineral analysis, it was discovered that all varieties of fenugreek plants treated with bacteria and fertilizer had higher levels of sodium ions than control plants, similar outcomes were seen for potassium ions. The amount of calcium in plants that had been treated with fertilizer and bacteria was the same as that previously mentioned, but it was found that when fertilizer and bacteria were combined, the amount of calcium was higher than in controls and other treatments (Fig. 8). *Bacillus subtilis* inoculation to plants reduces the uptake of calcium and potassium ions in saline conditions as compared to sodium ions (Abd Allah et al., 2018). Bacterial treated potato plants have higher potassium levels than untreated plants (Tahir et al., 2019).

3.7. Melatonin content

Treatment with *B. japonicum* and *B. cepacian*, either alone or in combination with fenugreek varieties, dramatically enhanced endogenous melatonin concentrations. Although *B. japonicum* had a somewhat greater influence on the melatonin content of fenugreek varieties than *B. cepacian*. Two natural PGPR*, Enterobacter* 64S1 and *Pseudomonas* 42P4, were given to *Arabidopsis thaliana* plants grown in drought conditions to assess the impact of the inoculation. Variables such as plant growth, cellular membrane damage, leaf defensive chemicals, and endogenous MT levels were evaluated under both drought and watering conditions. The results demonstrated that the natural strains of *Pseudomonas* 42P4 and *Enterobacter* 64S1 enabled *A. thaliana* plants to produce more endogenous MT under drought conditions. These natural strains enhanced plant development, decreased oxidative and membrane damage, and improved drought resistance in the *Arabidopsis* plants (Jofre et al., 2023).

4. Conclusion

Artificial fertilizers are economically important but the long term use of these chemicals is damaging for different environments. From the study, it was observed that the variety Fenu 2002 and Fenu 2004 had best response in terms of growth and physiochemical factors respectively. The treatment in which both bacterial strains were applied showed significant positive response on growth and physiochemical attributes of fenugreek. It was concluded that *Bradyrhizobium japonicum* and *Burkholderia cepaci* can be used as biofertilizers, as it can provide nitrogen and phosphorus to plant in a safe way. The use of such beneficial microbes can reduce hazardous effects on ecosystems. However, a detailed study with field experiments is required prior to its proper application to obtain maximum benefits.

Credit author statment

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Declaration of Competing Interest

All the authors declare no conflict of interest

Data availability

Data will be made available on request.

Acknowledgment

The authors are grateful to the deanship of scientific research at King Khalid University for supporting this work under the grant number (R.G. P2/326/44).

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