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Research article

Chitosan and probiotic bacteria promotion of yield, post-harvest qualities, antioxidant attributes and shelf life of broccoli heads

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Abstract

Importance of the work: Vegetables, such as broccoli, contain considerable amounts of health-promoting phytochemicals, nutrients and vitamins. Chitosan and probiotic bacteria are natural growth-enhancing agents for safely increasing production.

Objectives: To investigate the effect of chitosan and probiotics on the growth, yield and bioactive properties of broccoli.

Materials & Methods: Besides the control treatment, chitosan at 50 parts per million (ppm), 75 ppm, 100 ppm and 125 ppm and *Paraburkholderia* bacteria at 1.5×10^9 colony forming units/mL singly and in combination with each of the chitosan doses were applied twice (at 20 d and 40 d after transplanting) during the 2020–2021 season. The experiment was conducted in a randomized complete block design.

Results: Significantly maximum total phenols (391.71 mg gallic acid equivalents/100 g), flavonoids (0.66 mg quercetin/100 g), β-carotene (54.44 international units/100 g), ascorbic acid (52.77 mg/100 g) and total antioxidant activity (2,2-diphenyl-1-picrylhydrazyl; 74.36%) of broccoli were recorded in the chitosan at 100 ppm + *Paraburkholderia* treatment. The broccoli florets of that treatment had the highest dry matter (9.20%) and non-reducing sugar (1.43%) contents and the maximum shelf life of 7.33 d. However, the total sugar (5.30%) and reducing sugar (4.00%) contents were the greatest for the chitosan at 125 ppm + bacteria treatment. Furthermore, a yield increment of 31.00% over the control was observed in the chitosan at 100 ppm + *Paraburkholderia* treatment following superior shoot and root growth. A superior leaf chlorophyll content (0.44 mg/100 g) was measured in the same treatment.

Main finding: Shrimp shell chitosan and *Paraburkholderia* probiotic bacteria had a significant influence on plant growth, floret yield and the functional constituents of broccoli.

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Introduction

Broccoli (*Brassica oleracea* L. var. italica) is an edible green vegetable from the family Brassicaceae which originated from West Europe (Italy) and is a highly nutritive biennial and herbaceous cole crop which is enriched with various nutrients compounds, such as vitamins (A, B_1, B_2, C, K), minerals (calcium, potassium), antioxidants and health ameliorating phytochemicals (Bahorun et al., 2004). This protective effect of broccoli consumption has largely been attributed to its complement of phytochemicals, such as the vitamins C and E, the flavones quartering and kaempferol, the carotenoids, β-carotene, lutein and glucosinolates (Podsędek, 2007). However, to maintain these nutritional properties and to enhance the quality yield of broccoli, the balanced application of nutrients is one of the most important cultural practices. The use of chemicals has adverse outcome on non-target organisms and excess can spoil the surrounding environment. Furthermore, most of the chemicals are also too costly for marginal farmers (Popp et al., 2013) and non-chemical organic inputs are highly encouraged for small scale as well as commercial cultivation of vegetables (Sultana et al., 2022). However, naturalistic and beneficial microbe-based substitute approaches have long been used for improved crop production, stress alleviation and disease suppression even though their efficacy has been entirely alterable and attaining reproducible results over the years have been challenging (Chung et al., 2015; Flores-Félix et al., 2015). Chitosan and probiotics are examples of natural compounds and microbial communities. Chitosan (a polycationic polymer of β-1,4, linked D-glucosamine) is obtained from crustaceans and liquefiable in organic acids. Chitosan has antiviral, antibacterial and antifungal properties that are effective in reducing disease occurrence (Jitareerat et al., 2007; Al-Hetar et al., 2011). In addition, many observers indicated that the activity of key enzymes of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease) and nitrogen (N) transportation in the functional leaves is improved by chitosan application which ultimately promotes plant growth and development (Khan et al., 2002). Plant probiotics are mainly plant-associated microorganisms that enhance the growth of the host plants when applied in adequate amounts. A few plant growth promoting rhizoplane bacteria have reported phosphate-solubilizing capacity and exert an antagonistic effect against fungal propagules on the rhizoplane (Islam et al., 2012). Since environmental factors and the microbial community may vary widely from one locality to other, it is possible to have different outcomes from the use of corresponding microbial formulation at different sites. Very little information has been published relating to the influence of foliar applications of chitosan and plant probiotics on the growth, yield, biochemical and antioxidant activities of broccoli. Considering the above factors, the current study investigated the effect of chitosan and probiotics on the growth, yield and bioactive properties of broccoli and aimed to standardize chitosan-probiotic combinations for the successful cultivation of broccoli in tropical and sub-tropical environments, such as Bangladesh.

Materials and Methods

Ethical statement

Broccoli var. *BARI Broccoli-1* (registered broccoli variety of Bangladesh released by the Bangladesh Agricultural Research Institute) was used as plant material, and shrimp shell chitosan and *Paraburkholderia* bacteria were used as treatment materials. The laboratory and field experiments were carried out following the guidelines and recommendations of "Biosafety Guidelines of Bangladesh" published by the Ministry of Environment and Forest, Government of the People's Republic of Bangladesh (2005). The research works were strictly supervised by an advisory committee of the research work of Ms Samia Khanam with the approval of the Dean, Graduate Studies, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. The whole experimental activities were performed in a systematic and methodological order (Table S1) under their supervision and guidance.

Experimental site and design

The study was carried out in the research field and the laboratory of the Department of Horticulture, BSMRAU during October 2020 and April 2021. The soil in the experimental field was shallow red brown terrace soil in the Salna Series (Brammer, 1977) in Madhupur Tract (AEZ 28). Pretreated seeds of BARI *Broccoli-1* were sown in a plastic tray on 12 October 2020 and germinated within 11 d. The young, tender seedlings were transplanted into polybags (4 cm × 6 cm) to produce strong, healthy seedlings for planting in the main field. At 35 d, uniform seedlings were transplanted at 60 cm × 40 cm spacing in 2 m × 1.2 m plots on 16 November 2020. After seedling establishment, intercultural operations were

performed based on commercial guidelines (Azad et al., 2020). The experiment was laid out in a randomized complete block design with three replications.

Preparation and application of treatments

The required amount of chitosan powder (50 mg of chitosan for 50 parts per million, ppm, of 1.0 L solution) was dissolved in 0.1 N HCl (5 mL) and diluted in distilled water to prepare four different concentrations (50 ppm, 75 ppm, 100 ppm and 125 ppm) and the pH was adjusted to 6.5. For blank chitosan dose (control; 0 ppm), only 5 mL 0.1 N HCl was taken in a 1.0 L volumetric flask and distilled water was added to fill the volume up to 1.0 L. Freshly prepared chitosan solutions were sprayed onto the broccoli. For the probiotics, a single colony of bacteria (*Paraburkholderia fungorum* strain BRRh-4) from the regularly maintained bacterial culture plate was taken in a conical flask and cultured on 500 mL nutrient broth. Then, the bacterial suspension (0.5%) was diluted to a concentration of 1×10^9 colony forming units/ mL with sterile distilled water (Esitken et al., 2010). There were four concentrations of chitosan and a single dose of probiotic bacteria and combinations of the probiotic bacteria concentration with all four chitosan formulations, making a total of nine treatments that were applied. A blank dose of distilled water containing neither chitosan nor *Paraburkholderia* bacteria was used as the control. Therefore, the 10 treatments consisted of G_1: control, G_2: chitosan at 50 ppm, G_3: chitosan at 75 ppm, G_4: chitosan at100 ppm, G_5: chitosan at 125 ppm, G_6: *Paraburkholderia*, G_7: chitosan at50 ppm + *Paraburkholderia*, G_8: chitosan at 75 ppm + *Paraburkholderia*, G_9: chitosan at 100 ppm + *Paraburkholderia* and G_{10}: chitosan at 125 ppm + *Paraburkholderia*. The treatments were applied on the foliage of the standing crop starting on 20 d after transplanting, with a second spray at 40 d after transplanting.

Measurement of growth and yield characteristics

The broccoli plants in the experimental plots were carefully managed including all the necessary intercultural operations, such as weeding, irrigation, side dressing of fertilizers and manures, and pest and pathogen regulations according to commercial recommendations and guidelines (Azad et al., 2020). Flower head initiation started on 26 December 2020 and were ready to harvest during the following 15–20 d. The broccoli heads were harvested during 21–28 January 2021 by cutting the head along with inflorescence stalk at the stem joint. A measurement scale at harvest was used to determine plant height, leaf length and leaf breadth (all measured in centimeters) and canopy spread (measured in square centimeters). Number of leaves per plant was counted during harvest. Immediately after harvest, each plant was uprooted carefully with its rhizosphere soil, its root zone soil was washed in running water and the root length was measured (in centimeters). The chlorophyll content of the leaf at harvest was estimated according to Lichtenthaler (1987), based on a leaf sample of 0.5 g that was homogenized with acetone (90% volume per volume) filtered and then the volume was made up to 50 mL. The absorbance was measured spectrophotometrically at 645 and 663 nm against the blank to measure the chlorophyll content in milligrams per 100 g. Each harvested head was weighed (in grams) and its diameter (in centimeters) was estimated. The average individual head weight of each replication was converted to a hectare yield (in tonnes) and the yield increase compared to the control was calculated based on Equation 1:

$$\text{Percentage yield increase over control} = \frac{\text{Respective treatment yield - Control yield}}{\text{Control yield}} \times 100 \quad (1)$$

Determination of physiochemical and functional properties

The harvested broccoli heads were delivered to the laboratory to determine the dry matter, sugar (total, reducing and non-reducing), total flavonoids, total phenols, total antioxidant activity, β-carotene and ascorbic acid contents. Five broccoli heads from each replication were kept at room temperature (27±2°C) to determine their shelf life. Fresh broccoli samples were dried in an electric oven (SANYO Drying Oven, MOV202; Japan) at 72±3°C for 3 d to assess the dry matter content. The total sugar, reducing sugar and non-reducing sugar contents were estimated according to the procedure of Somogyi (1952), using Bertrand A, Bertrand B and Bertrand C solutions, respectively. The total phenol content was determined following Abdul-Hafeez et al. (2014) with some modification to their Folin-Cioucalteu method. A standard curve was made based on the absorbance of gallic acid to calculate the total phenol content expressed as milligrams (mg) of gallic acid equivalent (GAE)/ 100 g on a dry weight (DW) basis. The aluminum chloride colorimetric method (Pourmorad et al., 2006) with some modifications was used to determine the total flavonoid content and readings were taken at 420 nm using a spectrophotometer (APEL; ultraviolet-visible spectrophotometer PD– 303 UV; Japan) and from the standard curve the total flavonoid content was estimated in mg quercetin equivalent/100 g DW. Using 2, 2- dipheny

l-1- picrylhydrazyl (DPPH) assay (Gupta et al., 2018 with slight modification), the antioxidant activity was determined and a reading taken at 517 nm against a blank using the spectrophotometer. The inhibition concentration (IC_{50}) values were calculated using linear regression analysis and used to indicate antioxidant capacity. The antioxidant activity based on the DPPH free radical scavenging activity was expressed as a percentage. The ascorbic acid content was determined using the titration method (Shehata et al., 2019). Exactly 10 ml of prepared extract was placed in a conical flask. Then, 5 mL of 5% KI, 2 mL of 2% starch solution and 2 mL of glacial acetic acid were added to the extract, which was titrated with 0.001N KIO_3 solution. For, β-carotene content estimation, 1 g of sample was crushed and mixed thoroughly with 10 mL of acetone-to-hexane (4:6) solution. This sample was centrifuged, after which the optical density of the supernatant was measured using the spectrophotometer at 663 nm, 645 nm, 505 nm and 453 nm. The carotene content (in mg/100 g) was calculated using Equation 2:

$$\beta\text{-carotene} = 0.216\,(OD_{663}) + 0.452\,(OD_{453}) - 1.22\,(OD_{645}) - 0.4\,(OD_{505}) \quad (2)$$

where OD is the optical density at the various wavelength in nanometers.

Data analysis and interpretation

The collected data were tabulated and analyzed using the MSTAT-C statistical package program (Gomasta et al., 2016). One-way analysis of variance was performed. Data were presented as the average of three replicates ±SD, based on five observations per replication. The treatment means were compared based on Fisher's protected least significant difference (LSD) test, with significance tested at $p < 0.05$.

Results and Discussion

Plant height and leaf number

The applications of chitosan and plant probiotics significantly enhanced the plant height and number of leaves per plant of the broccoli (Figs. 1A and 1B). The tallest plant was measured in the G_9 treatment (68.07 cm) which was not significantly different from the G_3, G_4, G_5, G_7, G_8 and G_{10} treatments. The shortest plant was recorded for the control (53.17 cm) and was not significantly different to the G_2 and G_6 treatments. The maximum number of leaves was in the G_{10} treatment (14.97 leaves/plant) and was not significantly different from the other treatments, except for the G_1, G_2 and G_3 treatments, where both the G_1 and G_2 treatments had the same minimum number of leaves (10.70 leaves/plant). It has been reported that foliar application of chitosan and rhizobacteria alone significantly increased plant growth and development in different crops (Shrestha et al., 2014; Rahman et al., 2018a, b; Shaheen et al., 2019). Chitosan had a significant effect on plant growth which might have been due to an increase in the availability and uptake of water and essential nutrients through adjusting the cell osmotic pressure and reducing the accumulation of harmful free radicals by increasing antioxidants and enzyme activities (Geries et al., 2020).

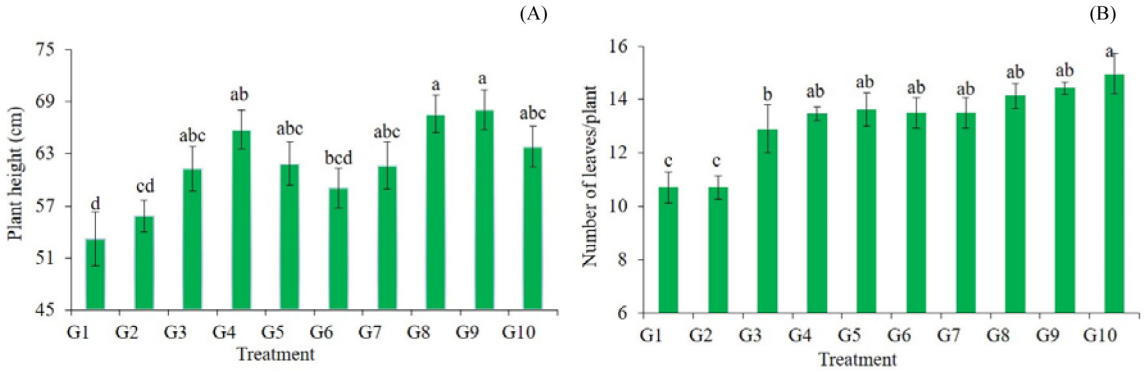

Fig. 1 Average plant height (A) and number of leaves per plant (B) of broccoli as influenced by chitosan and *Paraburkholderia* bacteria application, where error bars indicate ± SD of three replicates; different lowercase letters above bars indicate significant ($p < 0.05$) differences; G_1 = control, G_2 = chitosan at 50 ppm, G_3 = Chitosan at 75 ppm, G_4 = Chitosan at 100 ppm, G_5 = Chitosan at 125 ppm, G_6 = *Paraburkholderia*, G_7 = Chitosan at 50 ppm + *Paraburkholderia*, G_8 = Chitosan at 75 ppm + *Paraburkholderia*, G_9 = Chitosan at 100 ppm + *Paraburkholderia* and G_{10} = Chitosan at 125 ppm + *Paraburkholderia*

Plant growth-promoting bacteria improve plant growth parameters by increasing the levels of auxins and cytokinins and decreasing the levels of ethylene and abscisic acid in the plants, which result in enhanced cell division and elongation of plants (Ruzzi and Aroca, 2015).

Leaf growth and plant spreading

All the chitosan-probiotic combinations and single applications (except for the G_2 treatment) produced leaves that were not significantly different in length and breadth (Table 1). Among those treatments, the maximum leaf length and leaf breadth were in the G_9 treatment (52.37 cm) and G_5 treatment (23.40 cm), respectively. The minimum leaf length (42.47 cm) and leaf breadth (15.67 cm) were recorded in the control plants. Following these trends, the maximum and minimum canopy spread were measured in the G_8 treatment (114.27 cm^2) and G_1 treatment (85.94 cm^2), respectively. Mondal et al. (2012) considered that the application of chitosan increased the key enzyme activities of the nitrogen metabolism and improved the transportation of nitrogen (N) in the functional leaves as well as increasing photosynthesis, which enhanced plant growth and development. The rhizobacterial isolates of *Pseudomonas fluorescens* and *Bacillus subtilis* also produced significantly longer shoot lengths compared to the control plants with onion (Reetha et al., 2014). In addition, Mukta et al. (2017) reported that foliar application of chitosan and *Paraburkholderia* probiotic bacteria improved leaf growth and canopy spread in the treated plants compared to the non-treated plants in strawberry.

Root length

The root length of broccoli was significantly increased by the chitosan and *Paraburkholderia* BRRh-4 bacteria treatments (Fig. 2A). The longest root was derived from the G_9 treatment (26.03 cm), which was not significantly different to that of the G_4, G_5, G_8 and G_{10} treatments. The shortest root was observed in the control (19.33 cm), which was not significantly different to the G_2 treatment. These results were consistent with Sutariati et al. (2019) who reported that microbial bacterial inoculation increased the root length and the number of roots in onion plants. Chitosan application in certain doses induced root proliferation and root growth (Suwanchaikasem et al., 2023).

Leaf chlorophyll content

The leaf chlorophyll content of broccoli was significantly the greatest in the G_9 treatment (0.44 mg/100 g) and was not significantly different to the G_5, G_8 and G_{10} treatments (Fig. 2B). As per other variables, the control plants again produced the minimum leaf chlorophyll content (0.29 mg/100 g) on a fresh weight basis, which significantly different from all other treatments. An enhanced nitrogen metabolism and its translocation to functional leaves in the chitosan and *Paraburkholderia* bacterial-treated plants might have efficiently increased the leaf chlorophyll content compared to the control. The present findings were in agreement with Chibu et al. (2002) and Farouk et al. (2011), who reported that different concentrations of chitosan enhanced the chlorophyll content in vegetables and grain crops. Similarly, Hassnain et al. (2020) and Li et al. (2021) observed an increment in the leaf chlorophyll content of tomato and radish, respectively.

Table 1 Leaf growth and canopy spread of broccoli at harvest as influenced by foliar application of chitosan and *Paraburkholderia*

Treatment	Leaf length (cm)	Leaf diameter (cm)	Canopy spread (cm^2)
G_1 (control)	42.47±2.67^{b}	15.67±1.62^{c}	85.94±7.38^{d}
G_2 (chitosan at 50 ppm)	44.93±3.51^{b}	15.87±1.45^{c}	90.21±8.76^{cd}
G_3 (chitosan at 75 ppm)	48.93±2.97^{a}	18.70±3.40^{bc}	103.96±7.14^{ab}
G_4 (chitosan at 100 ppm)	51.30±2.38^{a}	21.20±0.61^{ab}	111.36±4.54^{ab}
G_5 (chitosan at 125 ppm)	50.83±2.93^{a}	23.40±0.89^{a}	110.78±6.13^{ab}
G_6 (*Paraburkholderia*)	48.80±2.88^{a}	20.53±2.42^{ab}	101.41±6.14^{bc}
G_7 (chitosan at 50 ppm + *Paraburkholderia*)	48.87±0.72^{a}	20.93±1.50^{ab}	104.89±9.15^{ab}
G_8 (chitosan at 75 ppm + *Paraburkholderia*)	51.53±2.61^{a}	22.40±1.47^{a}	114.27±9.31^{a}
G_9 (chitosan at 100 ppm + *Paraburkholderia*)	52.37±2.08^{a}	23.07±0.65^{a}	115.35±8.31^{a}
G_{10} (chitosan at 125 ppm + *Paraburkholderia*)	51.03±1.91^{a}	23.10±1.40^{a}	110.56±5.30^{ab}
Coefficient of variation (%)	4.58	8.90	6.66
LSD (0.05)	3.86	3.13	11.98
Level of significance	**	**	**

ppm = parts per million; LSD = least significant difference; ** = significant at $p < 0.01$.

Values are mean±SD of three replicates. Different lowercase superscripts above means in each column denote significantly ($p < 0.05$) different.

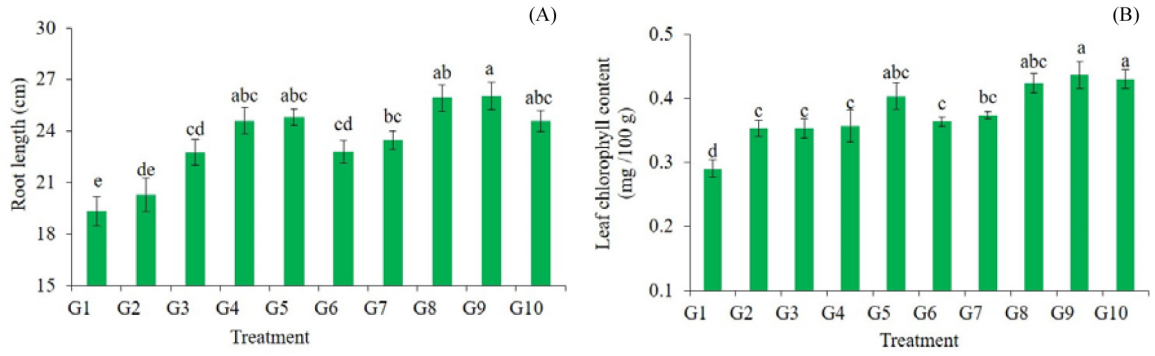

Fig. 2 Average root length (A) and leaf chlorophyll content (B) of broccoli as influenced by foliar application of chitosan and *Paraburkholderia*, where vertical error bars indicate ± SD of three replicates; Different lowercase letters above bars indicate significant ($p < 0.05$) differences; G_1 = control, G_2 = chitosan at 50 ppm, G_3 = Chitosan at 75 ppm, G_4 = Chitosan at 100 ppm, G_5 = Chitosan at 125 ppm, G_6 = *Paraburkholderia*, G_7 = Chitosan at 50 ppm + *Paraburkholderia*, G_8 = Chitosan at 75 ppm + *Paraburkholderia*, G_9 = Chitosan at 100 ppm + *Paraburkholderia* and G_{10} = Chitosan at 125 ppm + *Paraburkholderia*

Yield and yield attributes

Among the measured yield attributes single head weight and yield were enhanced significantly, whereas there was no significant improvement in the head diameter (Table 2 and Fig. 3A). The maximum single head weight (618.81 g) as well as the head yield (25.78 t/ha) of broccoli was in the G_9 treatment, with these values not being significantly different to those in the G_4, G_5, G_7, G_8 and G_{10} treatments. Control plants had the significantly lowest head yield (19.68 t/ha). The chitosan and probiotic treatments resulted in yield increments 5.49–31.00% over the control, with the maximum yield increment in the G_9 treatment. The, G_7 and G_8 treatments resulted in yield increases of 29.12% and 29.22%, respectively over the control (Fig. 3B). The head diameter of the broccoli was in the range 17.89–19.26 cm. The yield increases might have been due to significantly higher plant heights and numbers of leaves, with the combined application of chitosan and probiotic bacteria inoculation leading to a higher synthesis of photosynthates and their better translocation to the sink, as the rate of photosynthesis was significantly correlated with the growth of the broccoli head. El-Miniawy et al. (2013) mentioned that the nitrogen content in strawberry leaves significantly increased in their tested treatments with chitosan, compared to their control plants. Shaheen et al. (2019) reported that foliar spraying of potato plants with chitosan resulted in the heaviest total and marketable tuber yield. Similar trends in yield promotion through chitosan and probiotic applications were obtained by Yildirim et al. (2015) on broccoli and by Abdel-Mawgoud et al. (2010) on strawberry.

Table 2 Weight and diameter of broccoli heads at harvest as influenced by foliar application of chitosan and *Paraburkholderia*

Treatment	Head weight (g)	Head diameter (cm)
G_1 (control)	472.24±61.54^{c}	17.89±1.83
G_2 (chitosan at 50 ppm)	498.20±50.63^{c}	17.99±1.15
G_3 (chitosan at 75 ppm)	525.56±35.67^{bc}	18.86±1.59
G_4 (chitosan at 100 ppm)	582.13±39.40^{ab}	18.41±1.95
G_5 (chitosan at 125 ppm)	585.11±21.46^{ab}	18.21±1.00
G_6 (*Paraburkholderia*)	514.49±21.94^{c}	18.73±1.14
G_7 (chitosan at 50 ppm + *Paraburkholderia*)	609.94±20.34^{a}	18.93±1.29
G_8 (chitosan at 75 ppm + *Paraburkholderia*)	610.36±29.53^{a}	19.26±1.74
G_9 (chitosan at 100 ppm + *Paraburkholderia*)	618.81±25.21^{a}	19.12±1.71
G_{10} (chitosan at 125 ppm + *Paraburkholderia*)	586.28±34.80^{ab}	18.98±1.60
Coefficient of variation (%)	6.47	8.55
LSD (0.05)	62.20	2.73
Level of significance	**	ns

ppm = parts per million; LSD = least significant difference; ** = significant ($p < 0.01$); ns = not significant ($p > 0.05$)

Values are mean±SD of three replicates. Different lowercase superscripts above means in each column denote significantly ($p < 0.05$) different.

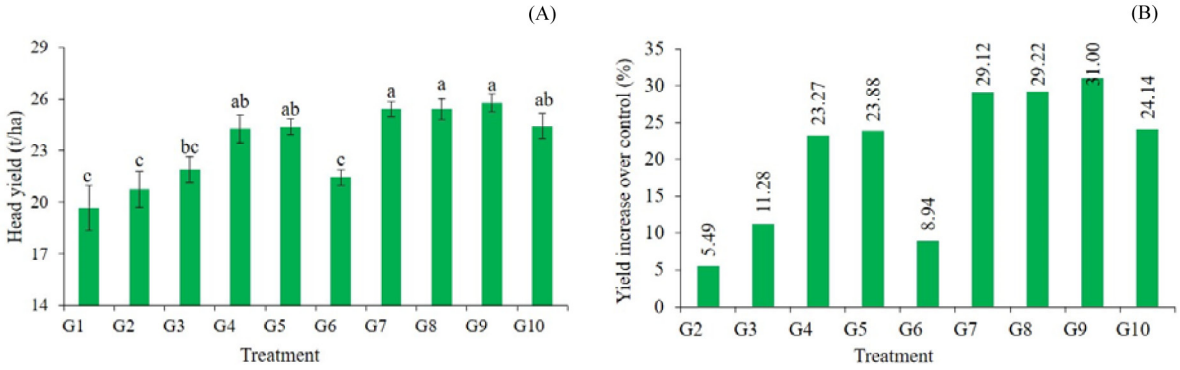

Fig. 3 Average head yield (A) and yield increase over control (B) of broccoli as influenced by foliar application of chitosan and *Paraburkholderia*., where error bars indicate ± SD of three replicates; different lowercase letters above bars indicate significant ($p < 0.05$) differences; G_1 = control, G_2 = chitosan at 50 ppm, G_3 = Chitosan at 75 ppm, G_4 = Chitosan at 100 ppm, G_5 = Chitosan at 125 ppm, G_6 = *Paraburkholderia*, G_7 = Chitosan at 50 ppm + *Paraburkholderia*, G_8 = Chitosan at 75 ppm + *Paraburkholderia*, G_9 = Chitosan at 100 ppm + *Paraburkholderia* and G_{10} = Chitosan at 125 ppm + *Paraburkholderia*

Dry matter and sugar content

The significantly maximum dry matter content was recorded in the G_9 treatment (9.20%), which was not significantly different to the G_3, G_5, G_8 and G_{10} treatments. The minimum head dry matter content was measured in the control (6.13%). Again, the total sugar and reducing sugar contents in the broccoli heads were the highest in the G_{10} treatment (5.30% and 4.00%, respectively), whereas the maximum non-reducing sugar content was recorded in the G_9 treatment (1.43%). The total, reducing and non-reducing sugar contents were significantly the lowest in the control (3.90%, 2.80% and 1.10%, respectively), as shown in Table 3. Increased vegetative growth with a higher number of leaves, greater canopy spread and higher leaf chlorophyll content in the chitosan and plant probiotic bacteria-treated broccoli plants efficiently extended photosynthetic CO_2 fixation in the leaves and its translocation to the inflorescence, resulting in an acceleration in dry matter accumulation in the broccoli. The chitosan and probiotics had a favorable effect on the nutrient uptake of elements through the rooting system in potato (Shaheen et al., 2019). Similarly, accumulation of higher photosynthates resulted in greater sugar contents in the broccoli heads. Trends in enhanced sugar (total, reducing and non-reducing) contents following the chitosan and probiotic treatments were also reported in tomato (Bona et al., 2017; Hernández et al., 2022) and strawberry (Rahman et al., 2018b; Nithin et al., 2020).

Table 3 Influence of chitosan and *Paraburkholderia* foliar application on dry matter content and sugar (total, reducing and non-reducing) contents of broccoli heads

Treatment	Dry matter content (%)	Total sugar content (%)	Reducing sugar content (%)	Non-reducing sugar (%)
G_1 (control)	6.13 ± 0.59^{d}	3.90 ± 0.35^{c}	2.80 ± 0.30^{d}	1.10 ± 0.17^{d}
G_2 (chitosan at 50 ppm)	7.87 ± 0.78^{c}	4.37 ± 0.06^{bc}	3.23 ± 0.15^{bcd}	1.13 ± 0.15^{cd}
G_3 (chitosan at 75 ppm)	8.43 ± 0.29^{abc}	4.47 ± 0.60^{bc}	$3.30\pm0.56^{a-d}$	1.17 ± 0.06^{cd}
G_4 (chitosan at 100 ppm)	7.53 ± 1.08^{c}	4.90 ± 0.30^{ab}	3.53 ± 0.42^{abc}	1.37 ± 0.15^{ab}
G_5 (chitosan at 125 ppm)	8.53 ± 0.86^{abc}	5.03 ± 0.15^{ab}	3.67 ± 0.21^{ab}	1.37 ± 0.06^{ab}
G_6 (*Paraburkholderia*)	7.77 ± 0.31^{c}	4.17 ± 0.40^{c}	2.93 ± 0.38^{cd}	1.23 ± 0.06^{bcd}
G_7 (chitosan at 50 ppm + *Paraburkholderia*)	7.93 ± 0.21^{bc}	5.27 ± 0.12^{a}	3.90 ± 0.17^{ab}	1.37 ± 0.06^{ab}
G_8 (chitosan at 75 ppm + *Paraburkholderia*)	9.00 ± 0.56^{ab}	5.23 ± 0.25^{a}	3.83 ± 0.32^{ab}	1.40 ± 0.10^{ab}
G_9 (chitosan at 100 ppm + *Paraburkholderia*)	9.20 ± 0.85^{a}	5.27 ± 0.71^{a}	3.83 ± 0.67^{ab}	1.43 ± 0.06^{a}
G_{10} (chitosan at 125 ppm + *Paraburkholderia*)	9.10 ± 0.60^{a}	5.30 ± 0.56^{a}	4.00 ± 0.46^{a}	1.30 ± 0.10^{abc}
Coefficient of variation (%)	8.01	8.89	11.72	7.90
LSD (0.05)	1.12	0.73	0.70	0.17
Level of significance	**	**	*	**

ppm = parts per million; LSD = least significant difference; * and ** = significant at 5% and 1% level of probability, respectively.
Values are mean±SD of three replicates. Different lowercase superscripts above means in each column denote significantly ($p < 0.05$) different.

Total flavonoid content

The total flavonoid content (TFC) was the highest and not significantly different in the G_9 and G_{10} treatments (0.66 mg QE/100 g DW) which was not significantly different to the G_5 and G_8 treatments. The lowest total flavonoid content was recorded in the control treatment (0.39 mg QE/100 g DW), as shown in Fig. 4A. As both chitosan and *Paraburkholderia* bacteria are key enzyme activators, they facilitate the enhanced accumulation of polyphenol oxidase and peroxidase enzymes in plants. Babu et al. (2015) reported that *Bacillus subtilis* and *B. Cereus* significantly activated the bioactive processes involved in the metabolism of increased phenols and flavonoids production in tomato fruit. Salimgandomi and Shabrangi (2016) reported that the functional properties of fruits are dependent on bioactive compounds that may be increased when plants are bio-fertilized with *Rhizobium* by impacting the plant's secondary metabolism.

Total phenol content

The total phenol content (TPC) of broccoli head significantly increased following treatment with chitosan and plant probiotic bacteria (Fig. 4B). The maximum TPC of broccoli on a dry weight basis was recorded in the G_9 treatment (391.71 mg GAE/100 g DW) which was not significantly different to the G_{10} treatment (383.55 mg GAE/100 g DW), followed by the G_8 treatment (353.66 mg GAE/100 g DW). The minimum TPC was in the control treatment (257.28 mg GAE/100 g DW) which was not significantly different to the G_6 treatment (270.88 mg GAE/100 g DW) followed by the G_2 and G_4 treatments. The treated plants showed a significant increase in the TPC in the broccoli head due to the application of chitosan that activated phenylalanine ammonia-lyase, a key enzyme in the phenol synthesis pathway (Romanazzi et al., 2017). Chamam et al. (2013) showed that *Azospirillum* sp. was able to modulate the phenolic compounds in rice. The effect of arbuscular mycorrhizal colonization on the concentration of anthocyanins was measured in strawberry fruits and other horticultural crops by Hernández et al. (2022), Mohammadi et al. (2021) and Jiménez-Gómez et al. (2017).

β-carotene and ascorbic acid content

Significant variations among the treatments were recorded for the β-carotene and ascorbic acid contents of broccoli, with increased doses of chitosan and *Paraburkholderia* BRRh-4 bacteria and their combinations stimulating the β-carotene and ascorbic acid contents of broccoli (Fig. 5A). The maximum amounts of β-carotene and ascorbic acid in the broccoli head were in the G_9 treatment (53.44 international units, IU/100 g and 52.77 mg/100 g, respectively) which were not significantly different to the G_8 and G_{10} treatments. The β-carotene and ascorbic acid contents were significantly the lowest in the control (33.37 IU/100 g and 29.50 mg/100 g). The minimum β-carotene and ascorbic acid contents were not significantly different among the G_6, G_2, G_3 and G_6 treatments and the control treatment. In addition, it was observed that the ascorbic acid content was lower than the β-carotene content in the broccoli. These results might have been due to the impact of the chitosan and probiotic bacteria on the photosynthetic process that is emphatically connected with the synthesis of sugars,

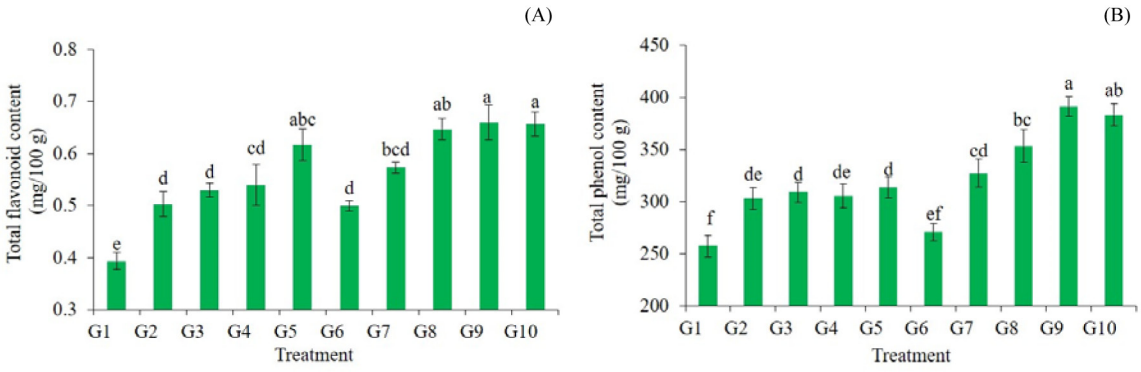

Fig. 4 Total flavonoid content (TFC) (A); and total phenol content (TPC) (B) of broccoli as influenced by chitosan and *Paraburkholderia* bacteria foliar application, where vertical error bars indicate ± SD of three replicates; different lowercase letters above bars indicate significant ($p < 0.05$) differences; G_1 = control, G_2 = chitosan at 50 ppm, G_3 = Chitosan at 75 ppm, G_4 = Chitosan at 100 ppm, G_5 = Chitosan at 125 ppm, G_6 = *Paraburkholderia*, G_7 = Chitosan at 50 ppm + *Paraburkholderia*, G_8 = Chitosan at 75 ppm + *Paraburkholderia*, G_9 = Chitosan at 100 ppm + *Paraburkholderia* and G_{10} = Chitosan at 125 ppm + *Paraburkholderia*

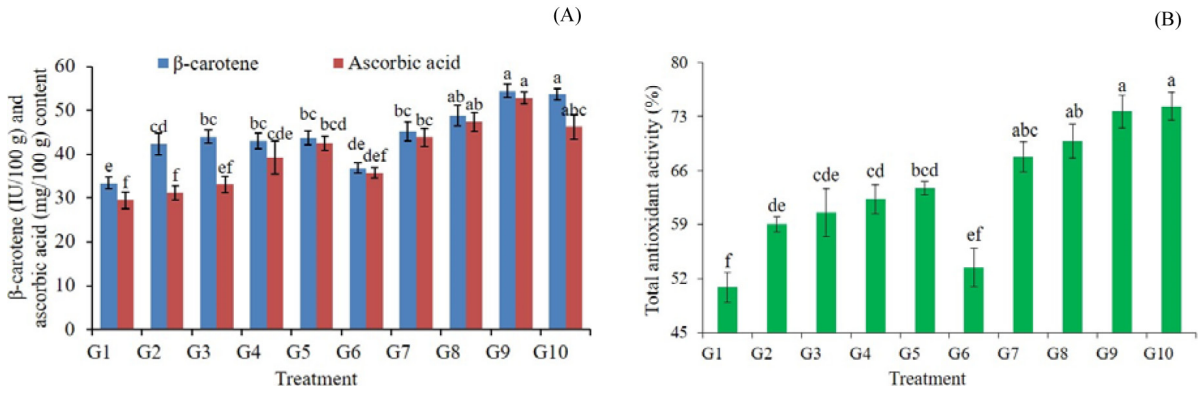

Fig. 5 β-carotene and ascorbic acid contents (A) and total antioxidant activity (B) of broccoli as influenced by foliar application of chitosan and *Paraburkholderia*, where error bars indicate ± SD of three replicates; different lowercase letters above bars indicate significant ($p < 0.05$) differences; IU = international units, G_1 = control, G_2 = chitosan at 50 ppm, G_3 = Chitosan at 75 ppm, G_4 = Chitosan at 100 ppm, G_5 = Chitosan at 125 ppm, G_6 = *Paraburkholderia*, G_7 = Chitosan at 50 ppm + *Paraburkholderia*, G_8 = Chitosan at 75 ppm + *Paraburkholderia*, G_9 = Chitosan at 100 ppm + *Paraburkholderia* and G_{10} = Chitosan at 125 ppm + *Paraburkholderia*

polysaccharides and vitamins. The current results were consistent with the findings of Parvin et al. (2019), who reported that the foliar application of chitosan significantly increased the vitamin C and lycopene (carotenoid) contents in tomato. Chitosan application in strawberry resulted in a high level of ascorbic acid accumulation (Wang and Gao, 2013). Bona et al. (2017) showed that tomato fruits had enhanced yield and increased concentration of vitamin C when they were inoculated with the strain *Pseudomonas* sp.

Total antioxidant activity

The total antioxidant activity (TAA) in the broccoli heads in terms of DPPH free radical scavenging assay varied significantly among the treatments due to the field application of chitosan and *Paraburkholderia* BRRh-4 bacteria (Fig. 5B). The maximum TAA based on the DPPH free radical scavenging assay in the broccoli head was in the G_{10} treatment (74.36%), which was not significantly different to the G_7, G_8 and G_9 treatments. The broccoli harvested from control plants had the significantly lowest TAA (50.93%), which was not significantly different to the G_6 treatment (53.49%). Increased levels of the total phenolic, flavonoid and ascorbic acid contents significantly accelerated the antioxidant activity of broccoli after foliar application of shrimp shell chitosan and *Paraburkholderia* BRRh-4 bacteria. Chitosan serves as a plant growth promoter perhaps due to an increase in the availability and uptake of water and essential nutrients through adjusting the cell osmotic pressure and reducing the accumulation of harmful free radicals by increasing antioxidant and enzyme activities (Al-Hetar et al., 2011). Furthermore, Rahman et al. (2018b) reported that the application of plant probiotic bacteria significantly increased the total antioxidant, carotenoid, flavonoid, phenolic and total anthocyanin contents in fresh strawberry fruits compared to their non-treated control.

Shelf life

Field application of chitosan and *Paraburkholderia* BRRh-4 bacteria significantly improved the shelf life of the broccoli heads compared to the control (Fig. 6). The maximum head shelf life of 7.33 d resulted from the G_9 treatment, which was not significantly different to the G_3, G_5, G_8 and G_{10} treatments. The minimum shelf life (4.90 d) was recorded for the control treatment. It is well recognized that chitosan has antiviral, antibacterial and antifungal properties when applied to plants (Al-Hetar et al., 2011) and it has been utilized to control diseases or reduce their spread by preventing the pathogens from penetrating the plant or to enhance plant native defense mechanisms (El Hadrami et al., 2010). Therefore, the shelf life of the broccoli increased. In addition, there was enhanced dry matter content in the broccoli head with improved amounts of phenolic and flavonoid contents in the chitosan and *Paraburkholderia* treatments that ultimately increased the shelf-life in vegetables, such as broccoli. Romanazzi et al. (2017) reported that chitosan can play a role as an antimicrobial agent, by inducing immunity against many plant pathogenic bacteria and fungi and is likely to be linked with prolonging the shelf life of fruits and vegetables in storage.

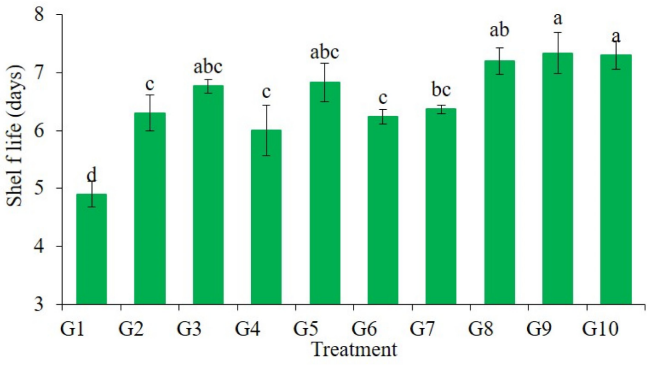

Fig. 6 Shelf life increment of broccoli floret as influenced by foliar application of chitosan and *Paraburkholderia* at vegetative stage in field, where vertical error bars indicate ± SD of three replicates, different lowercase letters above bars indicate significant ($p < 0.05$) differences; G_1 = control, G_2 = chitosan at 50 ppm, G_3 = Chitosan at 75 ppm, G_4 = Chitosan at 100 ppm, G_5 = Chitosan at 125 ppm, G_6 = *Paraburkholderia*, G_7 = Chitosan at 50 ppm + *Paraburkholderia*, G_8 = Chitosan at 75 ppm + *Paraburkholderia*, G_9 = Chitosan at 100 ppm + *Paraburkholderia* and G_{10} = Chitosan at 125 ppm + *Paraburkholderia*

Conflict of Interest

The authors declare that there are no conflicts of interest.

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