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Effects of natural extracts combined with *Burkholderia* spp. on the growth, yield, and quality of lettuce (*Lactuca sativa* Muir) under greenhouse conditions

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ABSTRACT

This study aimed to evaluate the effects of natural extracts and Burkholderia spp. bacterial strains on the growth, yield, and quality of lettuce (Lactuca sativa cv. Muir) under greenhouse conditions. The experiment was conducted using a completely randomized block design with two factors: (A) two natural extracts (catfish waste extract FE and soybean oil cake extract SE), and (B) three bacterial strains (MP1, MP2, MP3), and no bacterial strain. A total of 8 treatments were set up, with 6 replications per treatment. Agronomic parameters were recorded at 15, 35, and 55 days after sowing (DAS) to evaluate the growth-promoting effects of the extracts and bacterial strains on Muir lettuce. The yield components and quality parameters were determined at 55 DAS. The results demonstrated that the FE extract was more effective than the SE extract ($p<0.01$), enhancing plant growth, producing marketable head weights of 300 $g.plant^{-1}$ and 287 $g.plant^{-1}$, respectively. Similarly, the MP1 strain significantly enhanced the growth and the marketable head weight. The application of natural extracts with bacterial strains reduced postharvest weight loss to below 5% after a 15-day storage period. Additionally, the treatment combining FE extract and the MP1 strain significantly improved the quality of Muir lettuce by increasing both total soluble solids and vitamin C levels compared with the control treatments. Therefore, these findings indicate the efficacy of the combination of the catfish waste extract FE and the MP1 strain as a potential biofertilizer to enhance lettuce productivity under greenhouse conditions.

1. INTRODUCTION

A green-colored lettuce cultivar, Muir, is a short-term crop (45-55 days) that can be grown year-round and offers considerable economic benefits. Apart from being rich in vitamins A and C, this lettuce cultivar has a high water content (approximately 95%) and a mild, non-bitter taste. As a result, Muir lettuce is considered one of the best available quality cultivars (USDA National Nutrient Database, 2022; Johnny's Selected Seeds, 2024). Following the United Nations Sustainable Development Goals in 2015, utilising natural organic resources derived from agricultural activities as growing substrates and organic fertiliser plays an essential role, particularly in greenhouse cultivation systems. This approach not only helps reduce plant diseases caused by bacteria and fungi (Joos et al., 2020) but also minimises greenhouse gas emissions from agricultural wastes and contributes to environmental protection (Mustapha & El Bakali, 2021).

For cultivation under greenhouse conditions, the interaction between plants and beneficial rhizosphere bacteria, such as nitrogen-fixing and phosphorus-solubilising bacteria, is critical for nutrient mobilisation and release (Hayat et al., 2010). Some studies have shown that microbial communities inhabiting soils or growing substrates used under greenhouse conditions are less diverse than those of field soils or natural soils (Ibekwe & Kennedy, 1998; Yao et al., 2016; Song et al., 2018). Therefore, applying beneficial microorganisms to growing substrates under greenhouse conditions could enhance plant productivity while reducing reliance on chemical fertilisers (Albureikan, 2024). Among them, *Burkholderia* is one of the plant growth-promoting rhizobacteria that have different beneficial effects, such as promoting plant growth, including phosphate solubilization, production of indole acetic acid, siderophores (Pandey et al., 2005; Elshafie & Camele, 2021), and yield of cultivars (Divjot et al., 2021). Several studies showed the effectiveness of organic nutrients on either the plant growth and the yield or quality of cucumbers (Santiago-López et al., 2016), strawberries (Ahmed et al., 2021b), lettuce (Ahmed et al., 2021a; Trinh et al., 2024), and tomatoes (Ma et al., 2021). Some studies have shown that the application of organic liquid fertilizers enhanced yield and improved the chemical and nutritional quality of lettuce without significantly increasing nitrate accumulation by improving the plant's water and nitrogen use efficiency (Xu & Mou, 2017; Ahmed et al., 2021a). Muir lettuce is a leafy vegetable with high nutritional value; however, due to its short growing cycle, it is essential to provide nutrient input management and appropriate cultivation practices to ensure high-quality products for consumers. Few studies have investigated the effects of *Burkholderia* spp. and natural extracts on vegetable productivity. Consequently, the present study was conducted to evaluate the efficacy of natural extracts and bacterial strains applied to the cultivation of Muir lettuce under greenhouse conditions.

2. MATERIALS AND METHODS

2.1. Materials

Growing substrate and nutrition regimes: The growing substrate resulted from composting a mixture of chicken manure, melon crop residues, and a growing substrate used for melon plants at a ratio of 1:1:8 (w:w:w). The resulting compost had a pH of 7.40, EC of 2.61 mS.cm^{-1}, total N of 1.96%, total P of 0.38%, SOM of 49.16%, and a moisture content of 14% (Anh, 2022). Four kilograms of the growing substrate were placed into each plastic bag (17 x 33 cm) and used for growing Muir lettuce.

Lettuce cultivar: Muir lettuce (*Lactuca sativa*) seeds were purchased from Johnny's Selected Seeds (2024), America. The seeds were sown in seedling trays for 7 days before being transplanted into growing bags.

Nutrient sources: YaraMila complex contains 12N-11P_2O_5-18K_2O+2,7MgO+8S+TE (Yara, Norway), and liquid organic fertilizers from Bio TT01 extracted from catfish waste FE and from BioTT02 extracted from soybean oil cake SE. These liquid organic fertilizers containing 20% organic matter (Hai Ha Hi-tech Agricultural Technology Co., Ltd., Vietnam). The lettuce was fertilized at 10, 20, and 30 days after sowing (DAS), following the manufacturer's recommendations.

Bacterial inocula: Three beneficial strains of *Burkholderia* spp., designated MP1, MP2 and MP3 have been characterized as phosphorus-solubilizing bacteria (Ngoc, 2023) capable of producing auxin (Dung, 2023). The bacterial inoculum was adjusted to a density of approximately 10^6 CFU.g^{-1} of dried growing substrate and was applied by watering 2-3 cm away from the base of each lettuce plant when the first true leaf had developed.

2.2. Methods

Experimental design: The experiment was conducted using a completely randomized block design with two factors: (A) two natural extracts (catfish waste FE and soybean oil cake extract SE), and (B) three bacterial strains (MP1, MP2, MP3), and no bacterial strain (NS) (Table 1). A total of eight treatments were established, each with six replications. The experiment lasted for 55 days.

Table 1. A summary of the experimental treatment design

Experiment set up		Factor B			
		No bacterial strain (NS)	MP1	MP2	MP3
Factor A	Soybean oil cake extract (SE)	+	+	+	+
	Catfish waste extract (FE)	+	+	+	+

Growth and yield indices of lettuce: Agronomic and yield-component parameters were evaluated throughout the experiment. Plant height, leaf length, leaf width, and number of leaves were measured at

15, 35, and 55 DAS. At 55 DAS, fresh weight, dry weight, marketable head weight, and the percentage of weight loss after 15 days of storage were determined as described by Trinh et al. (2024).

Evaluation of lettuce quality: The edible leaves of Muir lettuce from the treatments were rinsed, drained, homogenized, ground, and filtered. The filtrate extract was directly used to determine total soluble solids and nitrate content. The total soluble solids were measured using a hand-held refractometer (Model 2231 E08, ATAGO, Japan). One drop of the filtered extract was placed in the sample well, and the reading was expressed as %Brix. Nitrate content was determined in accordance with the Vietnamese standard TCVN 8742:2011. Vitamin C content was analyzed following Chon et al. (2005) with a modification. Fresh Muir lettuce leaves were homogenized. Five grams of the leaves were ground in a porcelain mortar with 20 mL of 1% HCl. The extract was transferred to a 100 mL volumetric flask. The volume was made up to 100 mL with 1% oxalic acid, shaken, allowed to stand for 15 minutes, and filtrated through filter paper (Newstar, Ø 11 cm, pore size 25 µm). A 10 mL aliquot of the filtrate was titrated with 0.001 N 2,6-dichlorophenol indophenol (DIP) solution using a microburet until a persistent pink endpoint was observed.

Vitamin C content (X, mg.100 g^{-1} fresh weight) was calculated as:

$$X = ((a-b)*0.088*100\%*V_1)/(V_2*m)$$

Where: a = mean titrant volume for the sample (mL); b = mean titrant volume for the blank (mL); V_1 = initial extract volume (100 mL); V_2 = titrated volume (10 mL); m = sample weight (g); and 0.088 = mg ascorbic acid equivalent per mL of 0.001 N DIP solution.

2.3. Statistical analysis

All data were carefully checked for normal distribution using the Anderson–Darling (AD) test. A two-way analysis of variance (ANOVA) was performed. Means among treatments were compared using Tukey's post hoc test at the 5% significance level. All data were analyzed using Minitab software (version 16.2). The results are reported as the mean of six replications.

3. RESULTS AND DISCUSSION

3.1. Effect of natural extracts and bacterial strain on the growth of the Muir lettuce

Plant height increased during the growth period. The treatment inoculated with the MP1 strain had significantly higher plant height than other treatments at 15 DAS and 55 DAS, with values of 7.42 cm and 24.3 cm, respectively. Regarding the natural extract application, the plant height of the treatment applied with the soybean oil cake extract (SE) was higher (6.59 cm) than that of the treatment applied with the catfish extract (FE) (6.36 cm) at 15 DAS ($p<0.01$).

Table 2. Changes in the plant height during plant growth and development under greenhouse conditions

	NS	MP1	MP2	MP3	Average (A)
			At 15 DAS (cm)		
SE	6.05^{c}	7.37^{a}	6.27^{bc}	6.67^{b}	6.59X
FE	5.89^{c}	7.47^{a}	6.07^{c}	6.03^{c}	6.36Y
Average (B)	5.97C	7.42A	6.17BC	6.35B	
F(A) = **	F(B) = **	F(AxB) = **	CV (%) = 9.82		
			At 35 DAS (cm)		
SE	18.3	17.6	17.1	17.6	17.7
FE	17.8	17.2	17.4	16.7	17.3
Average (B)	18.1	17.4	17.3	17.2	
F(A) = ns	F(B) = ns	F(AxB) = ns	CV (%) = 5.80		
			At 55 DAS (cm)		
SE	23.8^{cd}	23.9^{bc}	23.0^{e}	21.1^{g}	22.9Y
FE	23.2^{de}	25.4^{a}	24.5^{b}	21.9^{f}	23.8X
Average (B)	23.5B	24.3A	23.8B	21.5C	
F(A) = **	F(B) = **	F(AxB) = **	CV (%) = 5.79		

*In either column or row, numbers followed by different letters are significantly different from the others by Tukey's post-hoc test; **Difference at p<0.01; ns: no significant difference; DAS: days after sowing; SE: soybean oil cake extract; FE: catfish waste extract; NS: no-bacterial strain; MP: Burkholderia strains*

At 55 DAS, the plant height of the treatment applied with the SE extract was lower with 22.9 cm than that of the treatment applied with the FE extract (23.8 cm) ($p<0.01$). The combination of the MP1 strain and the FE extract significantly increased plant height at 55 DAS to 25.4 cm, compared with the other treatments (Table 2). However, there were no significant differences of the plant height among the treatments applied with either bacterial strains or the natural extracts as well as the combination of the two factors when the plant was at 35 DAS (Table 2).

Number of leaves

At 15 DAS, only the treatment without bacterial inoculation had the significantly greater number of leaves compared with the other treatments. At 35 DAS, the treatments applied with the FE extract produced a greater number of leaves than those of other treatments ($p<0.01$). There was no interaction between the two factors at 15 DAS and 35 DAS. However, at 55 DAS, the number of leaves in the treatment inoculated with the MP1 strain was 36.7 leaves plant^{-1} and was significantly greater than that of the other treatments. Regarding the natural extracts, the FE treatment resulted in a significantly higher number of leaves (35.0 leaves.plant^{-1}) compared with the SE treatment (34.2 leaves.plant^{-1}). There was an interaction between the two factors at harvest, where the combination of the MP1 strain and the FE extract resulted in the highest number of leaves (38.2 leaves.plant^{-1}) (Table 3).

Table 3. Changes in the number of leaves during plant growth and development under greenhouse conditions

	NS	MP1	MP2	MP3	Average (A)
		At 15 DAS (leaves. plant $^{-1}$)			
SE	6.50	6.17	6.00	5.83	6.13
FE	6.83	5.67	5.83	5.67	6.00
Average (B)	6.67A	5.92B	5.92B	5.75B	
F(A) = ns	F(B) = **	F(AxB) = ns	CV (%) = 17.9		
		At 35 DAS (leaves. plant $^{-1}$)			
SE	16.5	16.8	16.2	16.5	16.5Y
FE	17.0	17.3	17.3	17.5	17.3X
Average (B)	16.7	17.1	16.8	17.0	
F(A) = **	F(B) = ns	F(AxB) = ns	CV (%) = 3.80		
		At 55 DAS (leaves. plant $^{-1}$)			
SE	34.3^{bcd}	35.2^{bc}	34.0^{bcd}	33.3^{cd}	34.2Y
FE	33.6^{cd}	38.2^{a}	35.8^{b}	32.3^{d}	35.0X
Average (B)	33.9BC	36.7A	34.9B	32.8C	
F(A) = **	F(B) = **	F(AxB) = **	CV (%) = 5.74		

*In either column or row, numbers followed by different letters are significantly different from the others by Tukey's post-hoc test; **Difference at p<0.01; ns: no significant difference; DAS: days after sowing; SE: soybean oil cake extract; FE: catfish waste extract; NS: no-bacterial strain; MP: Burkholderia strains*

Leaf length and leaf width parameters

At 15 DAS, the microbial inoculum treatments did not increase lettuce leaf length compared to the control. Simultaneously, there were no significant differences among the treatments using the natural extracts, nor in the interaction between these factors. At 35 DAS and 55 DAS, the treatments with either the MP1 strain or the FE extract produced longer leaves than other treatments ($p<0.01$). A significant interaction was observed between the two factors. Among all treatments, the combination of the MP1 strain and the FE extract produced the longest leaves (25.0 cm) (Table 4).

At 15 and 35 DAS, the leaf width of the treatments was significantly affected by the application of the bacterial strains, the natural extracts, and their combination. However, no interaction between the two factors was observed at 55 DAS (Table 5).

At 15 DAS, for the bacterial application, the leaf width of the no-bacterial strain and the treatment inoculated with the MP1 strain was significantly greater, at 3.98 cm and 3.95 cm, respectively, compared to the other treatments. The leaf width of treatments applied with the FE extract was also significantly greater (3.98 cm) than that applied with the SE extract (3.79 cm) ($p<0.01$). An interaction between two factors was observed, in which the treatment without bacterial inoculation combined with the FE extract (4.07 cm) and the treatment with the MP1 strain combined with the SE extract (4.03 cm) produced the widest leaves at this stage (p<0.01).

Table 4. Changes in the leaf length during plant growth and development under greenhouse conditions

	NS	MP1	MP2	MP3	Average (A)
		Leaf length 15 DAS (cm)			
SE	6.18	5.57	5.28	4.82	5.46
FE	6.13	5.35	5.07	480	5.35
Average (B)	6.18A	5.46B	5.18BC	4.81C	
F(A) = ns	F(B) = **	F(AxB) = ns	CV (%) = 12.8		
		Leaf length 35 DAS (cm)			
SE	15.0^{c}	16.1^{ab}	16.5^{a}	15.2^{bc}	15.7Y
FE	16.3^{a}	16.7^{a}	16.6^{a}	15.1^{c}	16.2X
Average (B)	15.7B	16.4A	16.6A	15.2AB	
F(A) = **	F(B) = **	F(AxB) = **	CV (%) = 5.23		
		Leaf length 55 DAS (cm)			
SE	20.9^{d}	22.5^{bc}	21.9^{bcd}	20.9^{d}	21.5Y
FE	21.2^{cd}	25.0^{a}	23.2^{b}	21.7^{cd}	22.8X
Average (B)	21.1C	23.7A	22.5B	21.3C	
F(A) = **	F(B) = **	F(AxB) = **	CV (%) = 6.75		

*In either column or row, numbers followed by different letters are significantly different from the others by Tukey's post-hoc test; **Difference at p<0.01; ns: no significant difference; DAS: days after sowing; SE: soybean oil cake extract; FE: catfish waste extract; NS: no-bacterial strain; MP: Burkholderia strains*

At 35 DAS, the leaf width of treatments applied with the FE extract reached 14.5 cm and was significantly wider than that of the SE extract (14.1 cm). The treatments inoculated with the MP1 and MP2 strains produced significantly greater leaf width, 14.8 cm and 14.6 cm (p<0.01), respectively. The combination of the MP1 strain and the FE extract produced the widest leaves (15.5 cm) (Table 5).

At 55 DAS, treatments applied with the MP1 or MP2 strain produced significantly wider leaves (15.7 cm and 15.5 cm, respectively) compared to other treatments. Similarly, treatments supplemented with the FE extract had significantly wider leaves (15.4 cm) than those supplemented with the SE extract (15.0 cm) (p<0.01) (Table 5).

Table 5. Changes in the leaf width during plant growth and development under greenhouse conditions

	NS	MP1	MP2	MP3	Average (A)
		At 15 DAS (cm)			
SE	3.70^{cd}	4.03^{ab}	3.83^{bcd}	3.60^{d}	3.79Y
FE	4.27^{a}	3.87^{bcd}	3.83^{bcd}	3.95^{bc}	3.98X
Average (B)	3.98A	3.95A	3.83B	3.78B	
F(A) = **	F(B) = **	F(AxB) = **	CV (%) = 6.10		
		At 35 DAS (cm)			
SE	14.3^{bc}	14.1^{bcd}	14.4^{bc}	13.5^{d}	14.1Y
FE	14.1^{bcd}	15.5^{a}	14.8^{b}	13.8^{cd}	14.5X
Average (B)	14.2B	14.8A	14.6AB	13.6C	
F(A) = **	F(B) = **	F(AxB) = **	CV (%) = 4.83		
		At 55 DAS (cm)			
SE	14.9	15.4	15.5	14.3	15.0Y
FE	15.2	16.1	15.5	15.0	15.4X
Average (B)	15.1BC	15.7A	15.5AB	14.7BC	
F(A) = **	F(B) = **	F(AxB) = ns	CV (%) = 4.57		

*In either column or row, numbers followed by different letters are significantly different from the others by Tukey's post-hoc test; **Difference at p<0.01; ns: no significant difference; DAS: days after sowing; SE: soybean oil cake extract; FE: catfish waste extract; NS: no-bacterial strain; MP: Burkholderia strains*

3.2. Effects of natural extracts and bacterial strains on the yield components of Muir lettuce

The analysis results in Table 6 showed a significant interaction between natural extracts and bacterial strains of the plant yield components. The fresh weight of the treatments applied with either the MP1 strain or the FE extract was significantly greater, at 334 (g.plant^{-1}) and 327 (g.plant^{-1}), respectively, compared to other treatments ($p<0.05$). The combination of the MP1 strain and the FE extract produced the highest fresh weight (355 (g.plant^{-1}) compared with that of other treatments (Figure 1, Table 6).

Table 6. Yield components at the harvest under greenhouse conditions

	NS	MP1	MP2	MP3	Average (A)
		Fresh weight (g.plant^{-1})			
SE	296^{d}	313^{c}	309^{c}	300^{d}	304Y
FE	299^{d}	355^{a}	347^{b}	308^{c}	327X
Average (B)	297D	334A	328B	304C	
F(A) = **	F(B) = **	F(AxB) = **	CV (%) = 6.85		
		Marketable head weight (g.plant^{-1})			
SE	275^{c}	291^{b}	286^{bc}	280^{bc}	283Y
FE	280^{bc}	321^{a}	311^{a}	288^{c}	300X
Average (B)	278C	306A	299B	285C	
F(A) = **	F(B) = **	F(AxB) = **	CV (%) = 5.31		
		Dried weight (g.plant^{-1})			
SE	7.80^{f}	8.58^{c}	8.32^{d}	8.04^{e}	8,32
FE	7.65^{g}	8.88^{a}	8.74^{b}	8.59^{c}	8,33
Average (B)	7.73D	8.73A	8.53B	8.31C	
F(A) = ns	F(B) = **	F(AxB) = **	CV (%) = 5.16		

*In either column or row, numbers followed by different letters are significantly different from the others by Tukey's post-hoc test; **Difference at $p<0.01$; ns: no significant difference; DAS: days after sowing; SE: soybean oil cake extract; FE: catfish waste extract; NS: no-bacterial strain; MP: Burkholderia strains*

Similar to the fresh weight parameter, the marketable fresh weight of the treatments applied with the MP1 strain or the FE extract was significantly greater, at 306 g.plant^{-1} and 300 g.plant^{-1}, respectively, compared to other treatments ($p<0.01$) (Table 6). With the interaction between the two factors, the treatments combining either the MP1 or MP2 strain with the FE extract resulted in the highest marketable fresh weight, at 322 g.plant^{-1} and 311 g.plant^{-1}, respectively ($p<0.01$).

Figure 1. Morphological variations of the lettuce at 35 DAS applied by (a) different natural extracts and (b) different bacterial strains

The dry weight of the Muir lettuce was also significantly influenced by the bacterial strains and by their interactions with the natural extracts. There was no significant effect of the natural extracts. The dry weight of the treatment applied with the MP1 strain was significantly greater than that of other treatments ($p<0.01$). The combination of the MP1 strain and the FE extract resulted in the highest dry weight of 8.88 g $plant^{-1}$, which was significantly different from those of the other treatments ($p<0.01$) (Table 6). After 15 days of storage, percentage weight loss differed among the treatments ($p<0.01$). The combination of the natural extracts and bacterial strains helps to reduce weight loss below 5%, while no bacterial strain treatments applied with SE or FE showed markedly higher percentage of weight loss rates of 24.5% and 23.1%, respectively ($p<0.01$), as shown in Table 6.

3.3. Effects of natural extracts and bacterial strains on the quality of Muir lettuce

3.3.1. The total soluble solids (%Brix) and vitamin C content

The total soluble solids of leaves applied with either the MP1 or MP3 strain were not significantly different from those of the no bacterial strain treatment, but were significantly higher than those of the MP2 strain. Likewise, the total soluble solids of leaves applied with the SE extract were significantly higher than those of the FE extract (Table 7). In addition, the interaction between the natural extracts and the bacterial strains on Brix content was observed in this study. Similarly, the natural extracts did not affect the plant's vitamin C content, whereas the application of the bacterial strains significantly improved it. However, the interaction of the two factors did not clearly show effects on the vitamin C content (Table 7).

3.3.2. The nitrate content in the lettuce

The treatments without a bacterial strain and those with the FE extract had higher nitrate content in the leaves than those with the bacterial strains or the SE extract ($p<0.01$) (Table 7). Although there was an interaction between the natural extracts and bacterial strains, the nitrate content in the leaves observed in this study remained low and complied with the World Health Organization (WHO) standard of 500 mg NO_3^-/kg fresh weight (Afali & Elahi, 2014). This result is in line with other studies on the application of organic liquid fertilizers, which enhanced the yield and improved nutritional quality of lettuce without significantly increasing nitrate accumulation by improving the plant's water and nitrogen use efficiency (Xu & Mou, 2017; Ahmed et al., 2021a).

The experimental results clearly showed interactions between the bacterial strains and the natural extracts, resulting in increased growth, yield, and quality of Muir lettuce. Several studies showed that the application of either effective microorganisms or organic fertilizers increased the growth, yield, and quality of the lettuce (Ma et al. 2021; Demir et al., 2024; Trinh et al., 2024). The previous study by Ahmed et al. (2021a) also reported that lettuce grown with an organic nutrient solution had 11.2% lower shoot fresh weight (163.9 ± 2.7 g vs 182.3 ± 2.2 g) but significantly higher levels of chlorophyll, carotenoids, phenolics, flavonoids, and antioxidant activity. In soil systems, it was observed that the application of an organic on strawberries enhanced vegetative growth, plant height (Rosadi & Catharina, 2022), and significantly increased flower number and yield compared to mineral fertilization (Ahmed et al., 2021b). Our results showed that the combination of the effective bacterial strains and the natural extracts significantly increased the growth, yield, and quality of the lettuce. In addition, the application of *Burkholderia* spp. strains proved to better enhance the growth and development of the plant in this study.

Burkholderia is a diverse and dynamic genus, containing both pathogenic species and those that form complex interactions with plants (Angus et al., 2014). However, the application of the genus *Bukholderia* spp. inoculants for sustainable agriculture has expanded in many regions worldwide (Hassen et al., 2016). Certain *Burkholderia* species can live in the rhizosphere and within host plants as endophytes (Chaturvedi et al., 2016). They showed improvements in plant growth and yield (Hwang et al., 2021) through various mechanisms. Several previous studies reported that *Burkholderia* spp. had the capacity to produce auxin, siderophore synthesis, to solubilize phosphate, to fix free nitrogen, and to enhance resistance to diseases and stresses (Din et al., 2019; Hwang et al., 2021; Azarmi-Atajan & Sayyari-Zohan, 2022). In this study, these bacterial strains had functional activities on producing phosphatase activity and auxin (Dung, 2023; Ngoc, 2023) together with the results of the no bacterial train treatments, the application of bacterial strains proved their beneficial effects on the growth, development and yield parameters of the Muir lettuce. Therefore, the application of the *Burkholderia* MP1 strain either independently or in combination with the MP1 strain and the natural extracts stimulated plant growth, yield, and crop quality in this study.

Table 7. Effect of the natural extracts and bacterial strains on the quality components of Muir

	NS	MP1	MP2	MP3	Average (A)
Percentage of weight loss after 15days storage (%)					
SE	24.5^{a}	3.43^{c}	4.85^{c}	2.97^{c}	8.93X
FE	13.1^{b}	3.37^{c}	2.15^{c}	1.91^{c}	5.14Y
Average (B)	18.8A	3.40B	3.50B	2.44B	
F(A) = **	F(B) = **	F(AxB) = **	CV (%) = 15.9		
Total soluble solids (%Brix)					
SE	2.30^{a}	2.27^{ab}	2.37^{a}	2.30^{a}	2.31X
FE	2.17^{b}	2.30^{a}	2.03^{c}	2.27^{ab}	2.19Y
Average (B)	2.23AB	2.28A	2.20B	2.28A	
F(A) = **	F(B) = **	F(AxB) = *	CV (%) = 4,73		
Vitamin C (mg.100 g^{-1})					
SE	17.0^{d}	19.1^{c}	20.8^{ab}	22.3^{a}	19.8
FE	17.9^{cd}	20.5^{ab}	20.0^{ab}	19.7^{bc}	19.5
Average (B)	17.5C	19.8B	20.4AB	21.0A	
F(A) = ns	F(B) = **	F (AxB) = **	CV (%) = 8.56		
Nitrate content (mg.kg^{-1})					
SE	318^{abc}	200^{d}	252^{cd}	191^{d}	240Y
FE	385^{a}	375^{ab}	310^{bc}	378^{ab}	362X
Average (B)	351A	288B	281B	284B	
F(A) = **	F(B) = **	F (AxB) = **	CV (%) = 4.95		

*In either column or row, numbers followed by different letters are significantly different from the others by Tukey's post-hoc test; **Difference at p<0.01; *Difference at p<0.05; ns: no significant difference; DAS: days after sowing; SE: soybean oil cake extract; FE: catfish waste extract; NS: no-bacterial strain; MP: Burkholderia strains*

Natural nutritional extracts may contain biostimulants (Chrysargyris et al., 2018) and other substances, including proteins, amino acids, phytohormones, antioxidants, and carbohydrates (Du Jardin, 2015). Therefore, the application of nutritional extracts could enhance root systems, flowering (Ali et al., 2019), fruit quality, and taste (Kapur et al., 2018), as well as product quality (Li & Mattson, 2015), leading to improved crop productivity. In this study, these natural extracts, which had the same organic matter content, were derived from catfish and soybean waste after tofu processing, respectively. A previous study showed that the application of these two natural extracts helped to reduce the use of 90% of inorganic fertilizers applied for growing Muir lettuce. There was no trend indicating the role of these extracts when each was applied with or without bacterial strains (Abdel-Mawgoud et al., 2010; Trinh et al., 2024). However, the combination of the natural extracts and Burkholderia MP strains, including the MP1 strain, significantly increased plant growth, yield, and quality. Therefore, the combination of the MP1 strain and the FE extracts proved to gain higher yield and quality of the Muir lettuce in this study.

In addition, some plant growth parameters, e.g., plant height and number of leaves, did not differ statistically among treatments at 35 DAS. These results seemed to be affected by nutrient regimes because the last application of natural extracts was on a 30-day-old plant. Therefore, it may not be clearly shown that the effects of the two factors on these parameters at 35 DAS. Finally, the nitrate content of leaves from treatments with the SE extract combined with the bacterial strain tended to be lower than that of the treatment without the bacterial strain. However, the fresh weight, marketable head weight, and the dry weight of the plant in these treatments tended to be greater than those of the no bacterial strain treatment. This result was similar to that of Davis (2009), which investigated the causes of reduced nutrient content in fruits and vegetables. It was concluded that higher yields were often associated with lower mineral concentrations because plants grew rapidly without proportionally accumulating nutrients-a phenomenon known as the "dilution effect".

4. CONCLUSION

This study demonstrated that the application of either the bacterial MP1 strain or the fish waste FE extract significantly improved the growth, development, yield, and quality of Muir lettuce. The combination of the *Burkholderia* MP1 strain and the catfish waste extract FE enhanced growth and produced the highest yield and quality in Muir lettuce. These findings demonstrated that the combination of the Burkholderia MP1 strain and the catfish waste extract FE can serve as an effective biofertilizer to increase lettuce productivity under greenhouse conditions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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