



Effectiveness of encapsulated plant growth-promoting bacteria, *Acinetobacter* sp. and organic supplements on the growth and nutrient contents enhancement in chilli plant

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Received 9 November 2023; Received in revised form 30 April 2024; Accepted 16 June 2024

ABSTRACT

Aims: This study was aimed to determine the effectiveness of previously isolated plant growth-promoting bacteria (PGPR) (*Acinetobacter* sp. SR R-12) and organic supplements on the growth and nutrient content of chilli plant during its vegetative growth phase.

Methodology and results: The present study involved the encapsulation of a previously identified strain of *Acinetobacter* sp. (accession no: KB851199) using sodium alginate beads. A completely randomized design was employed, and four different treatments were administered, namely T0-encapsulated PGPR alone; T1-encapsulated PGPR + humic acid; T2-encapsulated PGPR + fish amino acid; and T3-encapsulated PGPR + humic acid + fish amino acid. After 5 days, these treatments were applied to chilli seeds that had already been sowed. The plants were harvested after 40 days after sowing, and various plant growth indicators and plant nutrient analyses were measured. The results showed that T2 treatment produced the highest plant height (55.75 cm), fresh weight (34.03 g) and dry weight (5.99 g) ($P < 0.05$). On the other hand, treatment T3 resulted in the highest fresh root weight (5.43 g), dry root weight (1.28 g) and leaf area (46.60 cm²). For plant nutrient analysis, T2 exhibited the highest levels of calcium (Ca) (9586.00 mg/kg), magnesium (Mg) (8747.75 mg/kg), iron (Fe) (188.90 mg/kg), zinc (Zn) (63.67 mg/kg), potassium (K) (50337.50 mg/kg), phosphorus (P) (8019.00 mg/kg), whereas T3 had the highest sodium (Na) (571.90 mg/kg) content. Overall, the findings suggest that T2 and T3 treatments have a significant positive effect on plant growth and development compared to the other treatments.

Conclusion, significance and impact of study: This study demonstrated the effectiveness of plant growth-promoting bacteria (PGPR) *Acinetobacter* sp. SR R-12 and organic supplements on enhancing various plant growth parameters and nutrient contents in chilli plants. These findings highlight the potential use of *Acinetobacter* sp. SR R-12 and organic supplements as a sustainable approach to promote chilli plants' growth and nutrient uptake.

Keywords: Chilli, encapsulation, fish amino acid, humic acid, plant growth-promoting bacteria

INTRODUCTION

Sustainable farming has led to new methods to increase agricultural productivity and reduce environmental impact. Chilli (*Capsicum annum* L.), a key crop in Malaysia, has been affected by chemical fertilizers and pesticides (Soares *et al.*, 2022). To address these issues, people are exploring how plant growth-promoting rhizobacteria

(PGPR) and organic additions like FAA and HA affect chilli plant development and nutritional composition. Plant growth-promoting rhizobacteria may increase crop yield, reduce synthetic pesticide use and promote sustainable agriculture. Syamsuddin *et al.* (2022) found that RB2NA1, RBNA14 and RB1NA1 rhizobacteria isolates help to develop chilli seedlings. These findings imply PGPR could bio-improve chilli farming. Plant growth-promoting

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rhizobacteria and organic additions may increase agricultural productivity, reduce pollutants and improve soil health, protecting the environment (Soares *et al.*, 2022). Despite agrochemical pollution and microbial decline, PGPR and organic supplements can help to mitigate the issue. Plant growth-promoting rhizobacteria and organic FAA and HA can make chilli cultivation sustainable. Organic plant nutrients can come from fish waste extract like FAA that consist of macro and micronutrients (Nanni *et al.*, 2021). Humic acid boosts chilli plant growth and production by improving soil structure, nutrient availability and water-holding capacity. Plant growth-promoting rhizobacteria and organic additives on chilli plants may increase crop production, reduce synthetic pesticide use and make agriculture more sustainable. Biobased technologies like PGPR and organic addition encapsulation can increase soil health and lessen conventional agriculture's environmental impact (Zamljen *et al.*, 2022). However, the evaluation of the effectiveness of PGPR, FAA and HA under the encapsulation technique is unknown. Based on the previous studies findings, the hypothesis of this study is, FAA and HA utilized in this study might exert a beneficial influence on the growth parameters and plant nutrient analysis of chilli plants along with PGPR. Thus, the aim of this study is to determine the effectiveness of plant growth-promoting bacteria (*Acinetobacter* sp.) and organic supplements (FAA and HA) on the growth and nutrient contents of chilli plant during its vegetative growth phase.

MATERIALS AND METHODS

Selection of beneficial bacteria for PGPR encapsulation

Bacteria were previously isolated from rice soil (Ramly *et al.*, 2023). The bacterial isolate was molecularly identified as *Acinetobacter* sp. SR R-12 with accession no: KB851199. It was chosen to be encapsulated as a potential bio-fertilizer due to its plant growth-promoting traits (Table 1).

Combination of HA, FAA and PGPR through encapsulation techniques

The encapsulation of PGPR was modified from (Bashan *et al.*, 2013). *Acinetobacter* sp. SR R-12, a pure culture of the bacteria, was inoculated into a liquid nutrient broth medium. It was cultured at 30 °C for 24 h. The encapsulation of bacteria within beads was conducted in a laminar flow environment with sterile conditions (Gullifa *et al.*, 2022). Fish amino acid and HA were combined in the solution based on their respective treatment. About 0.8 g of FAA and HA, respectively, was added into 200 mL of the alginate mixture of the solution while stirring using a magnetic stirrer. A sterile 20 mL syringe is employed to gradually transfer the solution into the 0.1 M calcium chloride (CaCl₂) solution. After being left in the CaCl₂ solution for five to ten minutes, beads formed

immediately (Coelho-Rocha *et al.*, 2018). After three sterile water rinses, the beads were dried for one to two hours. Then it was kept in an air-tight glass container.

Treatments application during chilli plant cultivation

The chilli plant cultivation was conducted at the greenhouse (No. 4) of UiTM Melaka, Campus Jasin. The chilli (*Capsicum annum* L.) variety from the Sakata SJ2-461F1 hybrid was chosen as the model plant organism in this experiment. The chilli seedling tray was collected at AliffRizqi Enterprise Agriculture before being transferred into 16" × 16" polybags. The soil was taken from the farm and combined well in a 4:1 ratio of topsoil to sand. The experiment was designed as a completely randomised design (CRD) with three treatments and four replications. The treatment's components consisted of T0: control with encapsulated PGPR only, T1: encapsulated PGPR and HA, T2: encapsulated PGPR and FAA, and T3: encapsulated PGPR with HA and FAA. A total of 12 polybags were used for the experiment. Each polybag with three chilli plant seedlings received 10 g for each treatment, individually. The crops were then watered in the early morning and late afternoon until 40 days after sowing (DAS).

Plant growth and plant nutrient analysis

The observations on growth parameters involved the vegetative stage of chilli including its plant height, the number of leaves, leaf area, fresh and dry weight of chilli plants, fresh and dry weight of roots, and plant nutrient analysis on chilli plants were measured after 40 days after sowing (DAS). The dry-ashing method was used to determine plant nutrient analysis (Akinyele and Shonkubi, 2015). Firstly, the plant materials were finely powdered, and 0.5 g of each sample was weighed and carefully placed into pre-weighed silica crucibles. The materials were ash-dried at 550 °C for 6 h in a muffle furnace and white ash was obtained (Allen, 1989). The ash was then dissolved in 10 mL of 10% HCl and filtered into 50 mL volumetric flasks. The resulting extracts were analysed using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Brand: Agilent) (Jorhem, 2000).

Statistical data analysis

The data for this project was analyzed through one-way ANOVA and followed with post hoc Tukey's B test using IBM SPSS Statistics version 26 software. The difference among treatments was considered significant at P<0.05.

RESULTS AND DISCUSSION

In this study, treatment T2, encapsulated PGPR + FAA produced the highest plant height (55.75 cm), fresh weight (34.03 g) and dry weight (5.99 g) (P<0.05) (Table 2). On the other hand, treatment T3, encapsulated PGPR, FAA and HA resulted in the highest fresh root weight (5.43 g), dry root weight (1.28 g) and leaf area (46.60

Table 1: Biochemical characteristics of rice soil plant growth-promoting bacteria isolates (Ramly *et al.*, 2023).

Isolates	Nitrogen fixation (+/-)	Phosphate solubilization efficiency (%)	Potassium solubilization efficiency (%)	Siderophore production (+/-)	Hydrolysing enzyme production (+/-)	Gram's stain (+/-)	Shape
SR N-2	+	52.33 ^{cd}	-	-	-	+	Bacilli
SR N-3	+	37.00 ^{de}	-	+	+	-	Cocci
SR R-2	+	61.00 ^c	52.00 ^{cd}	+	+	-	Bacilli
SR R-11	-	22.00 ^e	33.00 ^d	+	-	-	Bacilli
SR E-2	-	23.00 ^e	-	-	-	+	Bacilli
SR R-12	+	80.67 ^b	225.00 ^a	+	+	-	Cocci

Note: (+) Positive/presence and (-) Negative/absence. Values are means ± SE. Isolates with the same letters are not significantly different according to Tukey's HSD at P=0.05.

Table 2: The chilli plant growth parameters mean differences at P<0.05.

Treatments	Plant height (cm)	No. of leaves	Leaves area (cm ²)	Fresh weight chilli plant (g)	Dry weight chilli plant (g)	Fresh weight of roots (g)	Dry weight of roots (g)	Length of roots (cm)
T0	46.25 ^b	47.25 ^c	27.87 ^c	22.63 ^c	4.10 ^c	4.19 ^d	0.90 ^c	18.13 ^b
T1	49.88 ^b	72.75 ^a	41.48 ^b	30.63 ^b	5.19 ^b	4.60 ^c	1.07 ^{bc}	20.55 ^{ab}
T2	55.75 ^a	76.75 ^a	44.84 ^b	34.03 ^a	5.99 ^a	5.00 ^b	1.18 ^b	21.37 ^a
T3	49.75 ^b	64.75 ^b	46.60 ^a	30.64 ^b	5.38 ^b	5.43 ^a	1.28 ^a	20.15 ^{ab}

T0: Control treatment (encapsulated PGPR only); T1: Encapsulated PGPR + humic acid; T2: Encapsulated PGPR + fish amino acid; T3: Encapsulated PGPR + humic acid + fish amino acid.

cm²) (Table 2). *Acinetobacter* sp. was chosen for its ability to enhance plant growth. As a PGPR, *Acinetobacter* sp. is known to promote plant development through the mechanism of producing phytohormones and solubilizing phosphate (Keswani *et al.*, 2016; Zhang *et al.*, 2019). Previous studies have demonstrated that *Acinetobacter* sp. can increase crop yield in rice and tomatoes, with one study reporting a 14.66-15.68% increase in tomato plant height (Islam *et al.*, 2019; Moon, 2022). The strain of *Acinetobacter* sp. used in this research may have also contributed to the growth of the chilli plants. While Neelam and Tabassum (2023) found that *Acinetobacter* sp. can help chilli peppers grow in drought conditions, the current research demonstrates that the strain can also benefit chilli plants in normal conditions, as evidenced by the improved growth in T2 and T3. Overall, *Acinetobacter* sp. is a beneficial PGPR for promoting plant development.

Based on the current study, treatment T2, encapsulated PGPR and FAA showed the highest growth in chilli plant. Similarly, the research by Gauthankar *et al.* (2021) discusses the comparative assessment of amino acid composition in two types of marine fish silage, highlighting the role of amino acids in enhancing chilli plant growth. In addition, FAA also aids vegetative growth found occurred to *Lablab purpureus* (L.) sweet plant and *Asparagus officinalis* (Raihing and Vijayalakshmi, 2021; Xekarotakis *et al.*, 2021). Fish amino acids contain organic compounds like amino acids and peptides that act as a rich nitrogen source for plants (Ali, 2020). As a key nutrient, nitrogen plays an integral role in forming new cells and tissues needed for plant height and biomass accumulation. While, for the treatment T3, encapsulated PGPR with FAA and HA has been found to help increase

fresh weight, dry weight and leaf area in chilli plant in the current study. Similarly, Ichwan *et al.* (2022) found that red chilli, *Capsicum anuum* gave a positive response to the application of HA by increasing fruit weight by 44% and leaf area in dryland in Indonesia. Humic acid is a type of humic substance that is essential in improving soil properties, plant growth and agronomic parameters (Ampong *et al.*, 2022). It can enhance root hydraulic conductivity and plant growth by activating plant responses to abiotic stress (Olaetxea *et al.*, 2020). The application of HA has been found to increase the ability of soils to retain water and enhance chilli plant growth and soybean under different cultivation periods (Ichwan *et al.*, 2022; Meerza and Ali, 2023). Furthermore, encapsulation has been shown to have positive effects on the shelf life of encapsulated organic supplements, suggesting that encapsulation may help increase the shelf life of other encapsulated substances (Debbarma *et al.*, 2022). This provides further evidence in favour of the hypothesis that the encapsulated FAA and HA utilized in the present investigation might exert a beneficial influence on the growth parameters of chilli plants along with PGPR.

For plant nutrient analysis, the encapsulated PGPR and FAA from treatment T2 show the highest P content (8019.00 mg/kg), K content (50337.50 mg/kg), Ca content (9586.00 mg/kg), Mg content (8747.75 mg/kg), Na content (537.60 mg/kg), Al content (256.10 mg/kg), Fe content (188.90 mg/kg) and Zn content (63.37 mg/kg). While treatment T3, which is encapsulated with PGPR, HA and FAAs, shows the highest sodium (Na) (571.90 mg/kg) content in plants compared to the other treatments (P<0.05) (Table 3). Likewise, the *Acinetobacter pitii* has

Table 3: The chilli plant tissue analysis of macronutrients and micronutrients based on different treatments at P<0.05.

Treatments	Macronutrient (Mg/kg)					Micronutrient (mg/kg)			
	N	P	K	Ca	Mg	Na	Al	Fe	Zn
T0	5342.05 ^c	59.00 ^d	0.222 ^c	8086.25 ^d	0.001 ^d	569.48 ^b	233.40 ^b	113.75 ^d	50.69 ^c
T1	8672.22 ^c	8317.50 ^b	0.101 ^c	9571.75 ^c	8289.75 ^c	560.90 ^c	175.77 ^c	145.85 ^b	64.54 ^b
T2	9079.63 ^{ab}	8019.00 ^a	50377.50 ^a	9586.00 ^a	8747.75 ^a	537.60 ^d	256.10 ^a	188.90 ^a	63.37 ^a
T3	8776.53 ^{ab}	7078.50 ^c	48427.50 ^b	9667.25 ^b	8411.2 ^b	571.90 ^a	150.90 ^d	125.10 ^c	47.54 ^d

T0: Control treatment (encapsulated PGPR); T1: Encapsulated PGPR + humic acid; T2: Encapsulated PGPR + Fish amino acid; T3: Encapsulated PGPR + humic acid + fish amino acid.

been found to assist in mineral solubilization and mobilization, especially for nutrients like P, K, Zn, Fe solubilization and mobilization in rice (Othman *et al.*, 2022; Hossain *et al.*, 2023). Whilst FAA are rich in various macro and micronutrients that can enhance mineral uptake when supplied to plants. A study done by Ajayakumar *et al.* (2023), showed that liquid rhizobium and FAA have contributed macronutrient enzyme activity in blackgrams, a legume plant. In contrast, the use of FAA in conjunction with mycorrhizal fungi and liquid organic fertilizer, but it has also been shown to enhance the growth and yield of red chilli plants (Pradana *et al.*, 2021). Apart from FAA, a study showed the integrated use of HA and PGPR also led to higher potato productivity (Ekin, 2019). This suggests that the combination of FAA with PGPR and HA can have a synergistic effect on plant nutrient uptake and utilization. The bio-stimulatory effects of amino acids on specific enzyme activities in chilli plants indicate their potential role in enhancing nutrient utilization (Zamljen *et al.*, 2022). Overall, the results demonstrate the effectiveness of integrating natural agents to enhance chilli plant nutrition. Optimizing PGPR-organic supplement combinations holds promise to support sustainable crop production through balanced mineral nutrition.

CONCLUSION

The results showed that treatment T2, which included encapsulated PGPR and FAA, had the highest increase of P, K, Ca, Mg, Fe and Zn, along with other growth parameters such as plant height, fresh weight and dry weight of chilli plant. Treatment T3, which included HA, FAA and encapsulated PGPR, demonstrated the highest leaf area, Na content, fresh and dry root weight. These findings suggest that using organic supplements can improve chilli production and that optimizing PGPR-organic supplement

combinations can help to intensify chilli cultivation sustainably. However, further fieldwork is necessary to confirm the effectiveness of these treatments in varied climate conditions. This research provides valuable insights into the sustainable use of biofertilizers in chilli cultivation and the potential for natural growth boosters to enhance plant growth and nutritional status globally.

ACKNOWLEDGEMENTS

The author would like to thank the Malaysian Ministry of Higher Education (MOHE) for this research under the Fundamental Research Grant Scheme (FRGS) (Ref: FRGS/1/2023/STG02/UITM/02/4), Faculty of Plantation and Agrotechnology, UiTM, Jasin branch, Melaka, Malaysia.

CONFLICTS OF INTEREST

The author has no conflicts of interest to declare.

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