Effect of Seed Pelleting Application of Plant Growth Promoting Bacteria on Germination and Growth of Lettuce (*Lactuca sativa*)

Phetcharat Jeephet[1], Nararat Thawong[1], Chuthamat Atnaseo[1,2], Sutheera Hermhuk[1,2] and Jakkrapong Kangsopa[1,2*]

[1]Division of Agronomy, Faculty of Agricultural Production, Maejo University, Chiang Mai 50290, Thailand
[2]Modern Seed Technology Research Center, Faculty of Agricultural Production, Maejo University, Chiang Mai 50290, Thailand

ARTICLE INFO

Received: 7 Jul 2023
Received in revised: 16 Nov 2023
Accepted: 22 Nov 2023
Published online: 4 Jan 2024
DOI: 10.32526/ennrj/22/20230176

Keywords:
Seed enhancement/ Seed germination/ Carboxymethyl cellulose/ Plant growth promoting bacteria

*** Corresponding author:**
E-mail: jakkrapong_ks@mju.ac.th

ABSTRACT

Plant growth-promoting bacteria (PGPB) are commonly used to pellet seeds. Different bacterial strains affect germination and plant growth in varying ways. The objective of this experiment was to study the effects of seed pelleting with three strains of bacteria on changes in germination, vigor, seedling growth, and the plant growth of lettuce. The experiment followed a completely randomized design with four repetitions and five treatments: without pelleting (T1), pelleting with $CaSO_4$-zeolite only (T2), pelleting with 1×10^7 CFU/mL *Stenotrophomonas* sp. strain sk3 (T3), pelleting with 1×10^8 CFU/mL *Burkholderia* sp. strain 3-DB05 (T4), and pelleting with 1×10^8 CFU/mL *Enterobacter* sp. strain 4-RB05 (T5). *Burkholderia* sp. and *Enterobacter* sp. were more effective in producing indole-3-acetic acid (IAA), and pelleting seeds with these strains resulted in higher germination rates and seedling growth compared to unpelleted seeds when tested in both laboratory and greenhouse conditions. Seed pelleting with 1×10^8 CFU/mL *Enterobacter* sp. promoted plant growth and resulted in significantly higher leaf and root weight. Therefore, seed pelleting with 1×10^8 CFU/mL *Enterobacter* sp. strain 4-RB05 is recommended to improve the germination and plant growth of Red Oak Leaf lettuce seeds.

1. INTRODUCTION

Red oak leaf lettuce (*Lactuca sativa* L.) is a globally popular salad plant, experiencing increasing demand due to the growing emphasis on promoting health. This has elevated lettuce to a key vegetable in constant demand throughout the year (Kim et al., 2016). However, meeting market demands poses challenges, particularly in large-scale production under soilless systems using seeding machines. Lettuce seeds, with their small size, flat shape, and light weight, present difficulties for seeding machines, leading to issues, such as inconsistent germination rates, uneven growth, and low seedling vigor (Damrosch, 2016).

To address these challenges, seed pelleting technology emerges as a promising solution. Seed pelleting involves adding materials to seeds to increase weight and size, and to alter their shape, facilitating their uses with seeding machines (Taylor and Harman, 1990; Pedrini et al., 2017). Beyond enhancing practicality for seeding machines, seed pelleting provides protection against adverse environmental conditions (Siri, 2015) and offers an avenue to improve seed quality. This improvement is achieved by incorporating substances that promote plant growth into the seed, including plant nutrients, hormones, fungicides, biopesticides, and plant growth-promoting bacteria (Taylor and Harman, 1990; Wurr and Fellows, 1985; Contreras et al., 2008).

The focus of this study was to assess the impact of seed pelleting on the quality of red oak leaf lettuce seeds concerning plant growth-promoting bacteria (PGPB). Specifically, *Stenotrophomonas* sp., *Burkholderia* sp., and *Enterobacter* sp. were chosen for their roles in enhancing plant growth through nitrogen fixation, phosphate solubilization, and the production of growth-promoting substances (Kumar et al., 2023). *Stenotrophomonas* sp., recognized for its versatility, contributes to plant growth by aiding nutrient uptake and boosting stress tolerance (Kumar

Citation: Jeephet P, Thawong N, Atnaseo C, Hermhuk S, Kangsopa J. Effect of seed pelleting application of plant growth promoting bacteria on germination and growth of lettuce (*Lactuca sativa*). Environ. Nat. Resour. J. 2024;22(1):26-33. (https://doi.org/10.32526/ennrj/22/20230176)

et al., 2023). *Burkholderia* sp. and *Enterobacter* sp. share similar mechanisms, providing protection against pathogens and improving stress tolerance (dos Santos et al., 2022; Roslan et al., 2020). The incorporation of PGPB through seed pelleting aligns with well-established strategies, as seen in previous studies (Rocha et al., 2019).

Therefore, the objective of this research was to determine the effect of lettuce seed quality after seed pelleting on plant growth-promoting bacteria. Germination and vigor tests were conducted in both laboratory and greenhouse conditions. Ultimately, the implementation of this technology aims to promote self-sufficiency by enabling the development and in-country production of seed pelleting methods. This shift would significantly reduce dependence on imports, fostering a more sustainable and resilient agricultural system.

2. METHODOLOGY

2.1 Information of bacterial isolates

Three bacterial isolates used in this work were previously isolated, tested for IAA production and phosphate solubilization, and identified based on 16S rRNA gene as explained in Jomkhame and Atnaseo (2021). Two of these isolates were from forest soils in the Huai Hong Khrai Royal Development Study Center (Jomkhame and Atnaseo, 2021) and the 16s RNA of strains 3-DB05 and 4-RB05 were best matched to *Burkholderia* sp. (GenBank: KY048279.1) and *Enterobacter* sp. (GenBank: MN400348.1), respectively. Another strain sk3 was from Chinese kale (*Brassica oleracea* L.) rhizosphere in a farmer plots in the San Sai District of Chiang Mai Province, Thailand (Jeephet, 2022), which matched best to *Stenotrophomonas* sp. (GenBank: NR_118008.1). Essays for plant growth promoting activity of these bacteria were repeated prior to the start of this work whereby IAA production were quantified by colorimetric method using Salkowski's reaction, while phosphate solubilization index (PSI) was used to indicate level of phosphate solubility, which was calculated by dividing the diameter of the clear zone by that of the colony (Paul and Sinha, 2017) after 7 days of growth on Pikovskaya's agar (Jomkhame and Atnaseo, 2021). Plant growth promoting activity of the three isolate is summarized on Table 1.

Table 1. Phosphate solubilization index and indole-3-acetic acid (IAA) production (µg/mL) of *Stenotrophomonas* sp., *Burkholderia* sp., and *Enterobacter* sp.

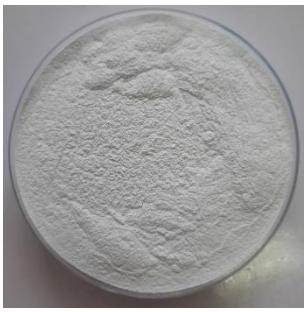
Bacterium	Phosphate solubilization index	IAA production (µg/mL)
Stenotrophomonas sp.	-	1.46
Burkholderia sp.	2.80	6.98
Enterobacter sp.	3.94	9.28

2.2 Microbes preparation

Bacterial isolates were cultured in 50% TSB (Trypticase Soy Broth) (Himedia®, India) for 24 h, suspended in 0.85% NaCl solution, and adjusted concentrations to 1×10^7 CFU/mL for *Stenotrophomonas* sp., 1×10^8 CFU/mL for *Burkholderia* sp., and 1×10^8 CFU/mL for *Enterobacter* sp. These bacterial concentrations were selected based on data from our previous work (Jeephet, 2022). Each bacterial suspension was mixed with a binding material (0.4% CMC carboxymethyl cellulose (CMC)) at the ratio of 1 mL bacterial culture: 99 mL of 0.4% CMC.

2.3 Pelleting lettuce seeds

The surface of the Red Oak leaf lettuce seeds was sterilized with 1% sodium hypochlorite (NaOCl) for 1 min, washed with sterilized distilled water three times, and dried with sterilized tissue paper. For each treatment, 10 g of lettuce seeds was pelleted with the bilayer matrix contained in the inner layer was 40 g calcium sulfate ($CaSO_4$) and in the outer layer was 90 g zeolite determined previously in Jeephet et al. (2022) using a rotary drum (SKK12, CERES International Ltd., Bangkok, Thailand) at spinning rate of 40 rpm. Each layer of the matrix was cemented by the binding material-bacteria mixture prepared as mentioned in 2.1 (Figure 1). Five treatments, which were without pelleting (T1), pelleting with $CaSO_4$-zeolite only (T2), pelleting with 1×10^7 CFU/mL *Stenotrophomonas* sp. (T3), pelleting with 1×10^8 CFU/ml *Burkholderia* sp. (T4), and pelleting with 1×10^8 CFU/mL *Enterobacter* sp. (T5) were applied to Red Oak leaf lettuce seeds. After receiving treatment, seeds were left at room temperature for 48 h to reduce the seed moisture to 7%.



(a) Calcium sulfate powder ($CaSO_4$)

(b) Zeolite powder

(c) Carboxylmethyl cellulose binding agent

(d) Pelleting machine

(e) Lettuce seeds

(f) Lettuce pelleted seeds

Figure 1. Characteristic of filler types (a-b), binder type (c), pelleting machine (d), Lettuce seeds (e), and Lettuce pelleted seeds (f)

2.4 Evaluation of seed quality

2.4.1 Seed quality examination under laboratory conditions

Treated seeds were germinated using the top of paper method with 50 seeds per replication and a total of 4 replications. A germination boxes were placed in a germinator under 25°C, 80% relative humidity, 180 µE light intensity, and 24-h lighting condition. Different data on seed quality was recorded using various methods, as described below.

For the first three days, the speed of radicle emergence was evaluated by counting the number of seeds for which there were appearances of radicle length of at least 2 mm (Mis et al., 2022). Sum of the ratio of numbers of seed with radicle to the total number of tested seeds from each of the first three days were recorded as the speed of radicle emergence. On the third day of seeding, radicle emergence percentage was recorded as a percentage of seeds with apparent radicle.

Similarly, the speed of germination was recorded as a sum of the ratio of numbers of germinated seeds to the total number of tested seeds of each day from the forth to the seventh day of seeding (Czabator, 1962). Germinated seeds were those seeds which developed into normal seedlings. Germination percentage was determined by counting the number of normal seedlings four days (first count) and seven days (final count) after seeding (ISTA, 2013).

On the seventh day of seeding, ten seedlings were randomly selected for evaluating shoot and root lengths. Shoot length was measured from the base of the shoot to the tip of the longest leaf, while the root length was measured from the base of the shoot to the end of the longest root (Baki and Anderson, 1973). The seedling length was measured from the root tip to the tip of the longest leaf. All measurements were aided by a ruler and expressed in millimeter.

2.4.2 Seed quality examination under greenhouse conditions

Treated seeds were sawn in a nursery tray using peat moss as media. For this test 50 seeds were used per treatment per replication with a total of four replications.

For the first three days, the speed of cotyledon emergence was evaluated by observing numbers of visibly emerged seedlings (Jeephet et al., 2022). Sum of the ratio of numbers of emerging seed to the total number of tested seeds from each of the first three days were recorded as the speed of cotyledon emergence. On the third day of seeding, cotyledon emergence percentage was recorded as a percentage of emerging seeds.

Assessments of the germination percentage and the speed of germination were identical to those in laboratory conditions. Ten randomly selected seedlings were used for measuring shoot length seven days after planting. Shoot length was assessed from media surface to the tip of the leaf.

2.5 Evaluating the effect of seed pelleting on plant growth under non-circulating hydroponic system

One seeds for each treatment was sown in 10 cm pots supported by perlite and vermiculite (70:30). Six pots containing seed undergone the same treatment were placed in a 35 × 50 × 8 cm tray containing 1 L of 10% plant nutrient solution prepared according to the method described by Colmer et al. (2006). The total of 5 trays were used, one for each treatment. The solution in each tray was changed every 3 days, and the experiment was conducted for 45 days. The plant height was evaluated on day 7, 14, 21, 28, 35, 42, and 45 after sowing. At 45 days after sowing, leaf and root fresh weights were assessed. Then, leaf and root were oven dried at 60°C for 72 h before dry weights measurements were taken.

2.6 Data analysis

The percentage of germination was arcsine-transformed to normalize the data before statistical analysis. All data were analyzed by one-way analysis of variance (ANOVA, complete randomized design), and the difference between the treatments was tested using Duncan's multiple range test (DMRT).

3. RESULTS AND DISCUSSION

3.1 Seed quality

After a three-day evaluation in the laboratory, there was no difference in the radicle emergence rate. None of the three bacteria isolates increased the radicle emergence rate of lettuce seeds. Seeds that were not pelleted had a higher radicle emergence rate compared to other methods, but there was no difference between pelleting seeds with 1×10^8 CFU/mL *Burkholderia* sp. and 1×10^8 CFU/mL *Enterobacter* sp.. Pelleting seeds with 1×10^8 CFU/mL *Burkholderia* sp. and 1×10^8 CFU/mL *Enterobacter* sp. resulted in a significantly higher germination rate compared to other methods (Table 2).

In greenhouse conditions, a 3-day evaluation after testing revealed that unpelleted seeds had a higher cotyledon emergence rate and speed of cotyledon emergence compared to other methods but did not differ from seeds pelleted with 1×10^8 CFU/mL *Burkholderia* sp. and 1×10^8 CFU/mL *Enterobacter* sp. There was no significant effect on emergence and vigor in the 3-day period. Seeds pelleted with 1×10^8 CFU/mL *Burkholderia* sp. and 1×10^8 CFU/mL *Enterobacter* sp. showed significantly higher germination rates of 10% and 14%, respectively, compared to unpelleted seeds. The speed of germination showed results similar to those of the germination test (Table 2).

This result showed that pelleting seeds with plant growth promoting bacteria could enhance germination of lettuce seeds by increasing germination rate both in laboratory and greenhouse conditions and by increasing speed of germination under greenhouse condition. Increasing germination rate and speed of germination was more pronounced when using isolates with higher level of IAA production (*Burkholderia* sp. and *Enterobacter* sp.) suggesting that IAA produced by these isolates contributed to the enhancement of germination. This may be attributed to the influence of IAA on accelerating seed germination through the regulation of endogenous hormones and sucrose metabolism (Zhao et al., 2020). Sucrose metabolism is crucial for the germination process, providing energy for emerging seedlings. Enzymes break down sucrose into glucose and fructose, fueling glycolysis to generate ATP. Additionally, sucrose metabolism contributes to the synthesis of essential molecules—nucleic acids, proteins, and lipids—necessary for building cell structures and supporting overall plant growth during germination (Weber et al., 1997; Xu et al., 2010; Hao et al., 2022).

3.2 Seedling growth

In laboratory conditions, the shoot length, root length, and seedling length were greater when seeds were pelleted with 1×10^8 CFU/mL *Burkholderia* sp. and 1×10^8 CFU/mL *Enterobacter* sp. compared to the other treatments but were not significantly different from 1×10^7 CFU/mL *Stenotrophomonas* sp. (Table 3). In greenhouse conditions, seed pelleting with 1×10^8 CFU/mL *Enterobacter* sp. resulted in significantly

higher seedling length compared to unpelleted seeds and seeds pelleted with pelleting materials only (Table 3). The experimental results clearly showed that seed pelleted with the 3 bacterial isolates promoted seedling growth in both test conditions, particularly pelleting with *Enterobacter* sp. Enhancement of seedling growth was observed in treatments with plant growth-promoting bacteria, suggesting that these bacteria play a role in this process. The IAA produced by these bacteria stimulates cell expansion, enhancing cell wall flexibility by breaking bonds between cellulose molecules, thereby influencing the permanent stretching of the cell wall. Additionally, IAA reduces cell turgor pressure, promoting increased water influx into the cells and facilitating cell expansion (Cosgrove, 2000). Therefore, it is anticipated that the IAA produced by both types of bacteria contributes significantly to the growth and development of vegetable seedlings compared to unpelleted seeds. Moreover, *Burkholderia* sp. and *Enterobacter* sp. have been reported to promote plant growth by influencing shoot and root length, producing phytohormones, and releasing siderophores. *Burkholderia* sp. produces auxins, cytokinins, and siderophores (Pal et al., 2022), while *Enterobacter* sp. produces auxins, cytokinins, and gibberellins, and facilitates nitrogen fixation (Roslan et al., 2020).

Table 2. Radicle emergence (RE), speed of radicle emergence (SRE), germination percentage (GE), speed of germination (SGE), cotyledon emergence percentage (COT), and speed of cotyledon emergence (SCOT) of lettuce after pelleting seeds with *Stenotrophomonas* sp., *Burkholderia* sp., and *Enterobacter* sp., tested under laboratory and greenhouse conditions.

Treatment[1]	Laboratory condition				Greenhouse condition			
	RE (%)	SRE (root/day)	GE (%)	SGE (seedling/day)	COT (%)	SCOT (root/day)	GE (%)	SGE (seedling/day)
T1	85[2]	43.80[a3]	89[bc]	21.40[a]	54[a 2/3]	9.08[a]	83[b]	10.27[c]
T2	82	32.02[b]	85[c]	19.82[bc]	39[b]	6.50[b]	90[ab]	11.07[b]
T3	83	31.76[b]	92[ab]	19.03[c]	41[b]	6.83[b]	91[ab]	11.09[b]
T4	85	34.37[ab]	94[a]	20.17[ab]	43[ab]	7.17[ab]	92[a]	11.62[ab]
T5	86	34.44[ab]	94[a]	20.01[ab]	48[ab]	8.07[ab]	95[a]	12.11[a]
F-test	ns	**	*	**	*	**	*	**
CV (%)	14.01	13.82	5.89	6.73	10.02	13.62	11.79	8.94

ns, *, **: Non-significantly different and significantly different at $p \leq 0.05$ and $p \leq 0.01$, respectively.
[1]T1=Unpelleted seeds, T2=$CaSO_4$-zeolite, T3=$CaSO_4$-zeolite + 1×10^7 CFU/mL *Stenotrophomonas* sp., T4=$CaSO_4$-zeolite + 1×10^8 CFU/mL *Burkholderia* sp., and T5=$CaSO_4$-zeolite + 1×10^8 CFU/mL *Enterobacter* sp.
[2]Data were transformed by the arcsine before statistical analysis, and back-transformed data are presented.
[3]Means within a column followed by the same letter are not significantly at $p \leq 0.05$ by DMRT.

Table 3. Shoot length, root length, and seedling length of lettuce seeds after pelleting seeds with *Stenotrophomonas* sp., *Burkholderia* sp., and *Enterobacter* sp., tested under laboratory and greenhouse conditions.

Treatment	Laboratory condition			Greenhouse condition
	Shoot length (mm)	Root length (mm)	Seedling length (mm)	Shoot length (mm)
Unpelleted seeds	10.5[c1]	54.5[bc]	65.0[bc]	10.2[bc]
$CaSO_4$-zeolite (P)	11.3[b]	47.7[c]	59.0[c]	11.0[c]
(P) + 1×10^7 CFU/mL *Stenotrophomonas* sp.	13.5[ab]	59.6[ab]	73.1[ab]	15.0[ab]
(P) + 1×10^8 CFU/mL *Burkholderia* sp.	17.1[a]	60.0[a]	77.1[a]	15.4[ab]
(P) + 1×10^8 CFU/mL *Enterobacter* sp.	17.7[a]	60.2[a]	77.9[a]	16.1[a]
F-test	**	**	**	**
CV (%)	8.44	14.21	10.59	8.41

**: Significantly different at $p \leq 0.01$.
[1]Means within a column followed by the same letter are not significantly at $p \leq 0.05$ by DMRT.

The good performance of both types of bacteria has led to studies that align with the findings of this experiment, as follows: Studies indicate that *Burkholderia* sp. enhances the growth of maize, while *Enterobacter* sp. promotes the growth of wheat (Glick, 2012; Kundan et al., 2015). This enhancement in plant

growth is attributed to increased shoot and root length, as well as improved nutrient uptake and utilization efficiency. Several investigations have explored the impact of *Burkholderia* sp. and *Enterobacter* sp. on plant growth through phosphate solubilization. Liu et al. (2018) discovered that *Enterobacter* sp. significantly increased shoot and root length, as well as phosphate uptake in wheat plants. These bacteria also produce other growth-promoting substances, such as phytohormones and siderophores, further contributing to plant growth and development (Glick, 2012). However, research on the phosphate-solubilizing ability of *Stenotrophomonas* sp. has yielded mixed results. Gholami et al. (2009) observed that *Stenotrophomonas* sp. can solubilize phosphate and enhance plant growth. Further investigations are needed to determine the potential of *Stenotrophomonas* sp. as a phosphate-solubilizing bacterium, as its efficacy seems to be strain-dependent and influenced by environmental factors.

3.3 Plant height and yield components under non-circulating hydroponic system

During the 7-day assessment period, the plant height of seeds pelleted with bacteria (T3-T5) was higher than that of unpelleted seeds. At 21-45 days after planting, seed pelleting with bacteria led to a significant increase in plant height compared to unpelleted seeds. During the 28-45-day period, seed pelleting with 1×10^8 CFU/mL *Enterobacter* sp. resulted in a significantly higher plant height than all other treatments. When comparing the height differences of plants at 7 and 45 days using different methods, seed pelleting with all methods (T2-T5) resulted in significantly higher plants than unpelleted seeds. In particular, seed pelleting with 1×10^8 CFU/mL *Enterobacter* sp. showed the most significant increase in plant height compared to the other treatments. However, seed pelleting with all bacterial methods increased plant height compared to the unpelleted seeds (Table 4).

Table 4. Plant height of lettuce seeds after pelleting with *Stenotrophomonas* sp., *Burkholderia* sp., and *Enterobacter* sp. tested under non-circulating hydroponic conditions.

Treatment	Plant height (cm)						
	7 days	14 days	21 days	28 days	35 days	42 days	45 days
Unpelleted seeds	1.10^{b1}	6.50^{b}	12.14	14.56^{b}	16.84^{c}	18.12^{d}	18.21^{d}
$CaSO_4$-zeolite (P)	1.15^{ab}	6.48^{b}	11.21	15.17^{ab}	17.77^{bc}	19.18^{c}	19.22^{c}
(P) + 1×10^7 CFU/mL *Stenotrophomonas* sp.	1.23^{a}	7.11^{ab}	12.08	15.89^{ab}	18.24^{b}	20.09^{bc}	20.15^{b}
(P) + 1×10^8 CFU/mL *Burkholderia* sp.	1.26^{a}	7.32^{a}	12.41	15.98^{ab}	18.59^{ab}	20.11^{b}	20.18^{b}
(P) + 1×10^8 CFU/mL *Enterobacter* sp.	1.25^{a}	7.01^{ab}	12.33	16.41^{a}	19.87^{a}	22.21^{a}	22.26^{a}
F-test	**	*	ns	**	*	*	*
CV (%)	8.32	13.82	15.89	16.23	17.21	17.98	19.21

ns, *, **: Non-significantly different and significantly different at $p\leq0.05$ and $p\leq0.01$ respectively.
[1]Means within a column followed by the same letter are not significantly at $p\leq0.05$ by DMRT.

This experiment tested lettuce growth under a non-circulating hydroponic system with a 10% nutrient solution concentration, restricting its growth parameters. The outcomes revealed a height and growth boost in lettuce when seeds were pelleted with three types of bacteria. The seed pelleting method proved effective in enhancing seed conditioning by preventing bacteria adherence to the seeds. This technique exhibited advantages over conventional seed planting. Shahab et al. (2009) and Weyens et al. (2009) reported that plant growth-promoting bacteria play a crucial role in producing IAA, which stimulates cell elongation, division, and differentiation. *Burkholderia* sp. and *Enterobacter* sp. in this experiment were found to produce IAA, leading to noticeable differences in plant height after 28 days.

After 45 days, seeds pelleted with *Burkholderia* sp. and *Enterobacter* sp. at concentrations of 1×10^8 CFU/mL showed significantly higher fresh shoot weight, dry shoot weight, fresh root weight, and dry root weight compared to unpelleted and pelleting material only. *Stenotrophomonas* sp. at 1×10^7 CFU/mL also resulted in higher fresh root weight and dry root weight (Table 5). Studies indicated that *Burkholderia* sp. and *Enterobacter* sp. have the potential to promote lettuce growth. The success of *Burkholderia* sp. and *Enterobacter* sp. in producing IAA contributed to consistent changes in plant height throughout the 45-day test period. *Burkholderia* sp. was particularly effective due to its reported role in fixing atmospheric nitrogen, providing essential components for plant development, and increasing nitrogen

availability, leading to enhanced photosynthesis and increased yield of lettuce plants (Coenye and Vandamme, 2003; Ghosh et al., 2016; Zhang et al., 2021). Similarities between *Enterobacter* sp. and *Burkholderia* sp. in producing growth-promoting substances, including IAA and cytokinins, were noted. These substances play a crucial role in stimulating root growth and overall plant development, affecting both root and leaf biomass. Inoculating *Enterobacter cloacae* MSR1 in *Pisum sativum* seeds, as reported by Khalifa et al. (2016), resulted in increased shoot and root length. *Enterobacter* sp. also shares similarities with *Burkholderia* sp. in promoting plant growth. Consistent with this, the inoculation with *Enterobacter* sp. J49 was observed to enhance the yield of maize crops (Anzuay et al., 2023).

Table 5. Fresh leaf weight, dry leaf weight, fresh root weight, and dry root weight of lettuce seeds after pelleting seeds with *Stenotrophomonas* sp., *Burkholderia* sp., and *Enterobacter* sp. tested under non-circulating hydroponic conditions.

Treatment	Fresh leaf weight (g)	Dry leaf weight (g)	Fresh root weight (g)	Dry root weight (g)
Unpelleted seeds	6.26^{c1}	0.41^{b}	3.87^{bc}	0.19^{c}
$CaSO_4$-zeolite (P)	6.21^{c}	0.44^{b}	3.73^{c}	0.35^{bc}
(P) + 1×10^7 CFU/mL *Stenotrophomonas* sp.	7.94^{b}	0.57^{b}	4.44^{b}	0.43^{b}
(P) + 1×10^8 CFU/mL *Burkholderia* sp.	8.54^{a}	0.62^{a}	4.49^{a}	0.49^{a}
(P) + 1×10^8 CFU/mL *Enterobacter* sp.	8.48^{a}	0.60^{a}	4.51^{a}	0.50^{a}
F-test	**	**	**	**
CV (%)	4.41	10.59	5.24	6.21

**: Significantly different at $p\leq0.01$

[1] Means within a column followed by the same letter are not significantly at $p\leq0.05$ by DMRT.

4. CONCLUSION

This study showed that *Enterobacter* sp. strain 4-RB05, which had the highest IAA production and PSI levels among the three tested strains, perform best in improving the germination rate in both laboratory and greenhouse conditions. When tested in a non-circulating hydroponic system for 45 days, seeds pelleting with 1×10^8 CFU/mL *Enterobacter* sp. resulted in higher plant height, fresh leaf weight, dry leaf weight, fresh root weight, and dry root weight compared to unpelleted seeds. It can be concluded that *Enterobacter* sp. at 1×10^8 CFU/mL has the potential to enhance the germination, vigor, and growth of red oak leaf plants. This resulted in higher plant height, fresh leaf weight, dry leaf weight, fresh root weight, and dry root weight compared to unpelleted seeds. Additional recommendations from this experiment include the possibility of seed pelleting with *Stenotrophomonas* sp. at 1×10^7 CFU/mL and *Burkholderia* sp. at 1×10^8 CFU/mL with lettuce seeds as alternative options. This is because the quality of germination and the yield of lettuce significantly increased compared to unpelleted seeds.

ACKNOWLEDGEMENTS

We would like to thank The National Research Council of Thailand (NRCT), for the financial support for this research. This project was conducting under the Research and Researcher for industries (RRI) project, 2021, [grant number: N41A640243]. The author would like to offer particular thanks to the Division of Agronomy, Faculty of Agricultural Production, Maejo University for materials and the use of laboratories and research sites.

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