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Effect of lettuce seed pelleting with plant growth-promoting bacteria on seed quality and bacterial viability after storage

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Abstract Storage of seeds pelleted with plant growth-promoting microbes affects their quality, shelf life and viability of bacteria in controlled conditions. After 6–12 months of storage, it was found that the seeds pelleted with 1×10^8 CFU/ml of *Enterobacter* sp. had a higher germination percentage, which was different in comparison to the seeds in all other experimental groups. From the 4^{th} month onwards, after storing seeds in ambient conditions, their germination percentage began to decline more dramatically than the seeds stored under a controlled environment. The growth change of the seeds stored in both conditions was in the same direction. For seeds before storage (0 months) and after 12 months of storage, seeds pelleted with 1×10^8 CFU/ml of *Enterobacter* sp. in both conditions had a longer root length and the difference was statistically significant compared to seeds treated with all other methods. The viability of *Enterobacter* sp. pellets was higher than pellets with other isolates throughout the 4 months of storage. After 1 month of storage, the pellets of *Stenotrophomonas* sp. and *Burkholderia* sp. showed a significant reduction in bacterial viability. Therefore, using 1×10^8 CFU/mL of *Enterobacter* sp. is the recommended type and concentration level for pelleting 'Red Oak' lettuce seeds to enhance storage and seed utilization for the highest cultivation effectiveness.

Keywords: Seed enhancement, Seed treatments, Adhesive, Polymer, Seed quality

Introduction

Lettuce (*Lactuca sativa* L.) is a widely cultivated and popular vegetable consumed around the world. Lettuce is an economically important vegetable consumed throughout the year, especially during festivals. Therefore, it can be considered a vegetable that is highly demanded by the market, and the demand trend continuously increases. Lettuce is considerably rich in vitamin A, vitamin C, calcium, iron, protein, carbohydrates, etc. (Kim *et al*., 2016). However, in an

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industrial lettuce cultivation system, every process must be arranged so that the production system is driven in a precise direction. Furthermore, the seedling process is a very important step in preparing enough seedlings to meet consumer demand. Therefore, early-stage seedlings must be vigorous and free of disease. However, lettuce seeds are small and flat and have little food to accumulate in them. Consequently, the seedlings are prone to a low germination rate, low vigor and inconsistent growth, which lead to higher seedling nursery production costs for farmers (Siri, 2015).

From the abovementioned problems, seed pelleting is a solution to the problem of small seed sizes or uneven shapes. Pelleting makes the seeds larger, with a round and even shape. Additionally, seeds that have a crooked shape or unusual size become more suitable for planting by agricultural machinery, resulting in more convenient and faster cultivation (Hill, 1999; Taylor and Harman, 1990; Taylor, 2003; Pedrini *et al*., 2017; Kangsopa *et al*., 2018). Pelleting also protects seeds from unsuitable environmental conditions (Siri, 2015). Furthermore, active ingredients can be added to the pelleting materials, especially microorganisms that promote plant growth, to reduce the use of chemicals applied to the seeds. These microorganisms produce plant hormones that support plants in finding and absorbing more food from the soil. They can function as atmospheric nitrogen fixers (Lakshminarayana *et al*., 1992), stimulate the dissolution of insoluble phosphates (Kundu and Gaur, 1980), the synthesis of compounds iron (Glick *et al*., 2007) and organic amino acid production (IAA), which enhances root and shoot growth of the seedlings, etc. (Oteino *et al*., 2015). Kangsopa *et al.* (2015) reported that lettuce seeds pelleted with *Pseudomonas fluoresces* 31-12 had relatively longer shoot and root lengths. As a result, the 45-day-old lettuce seedlings grown from the pelleted seeds had a heavier shoot fresh weight and shoot dry weight. An experiment by Jomkhame and Atnaseo (2021) with 'jasmine105' rice grains soaked with bacteria discovered that *Pseudomonas* sp., *Enterobacter* sp. and *Burkholderia* sp. can induce crown root formation in rice seedlings, resulting in an increase in root fresh weight. At the same time, the viability of bacteria in pellets affects the germination and growth-promotion mechanisms of plants. Kangsopa (2018) reported that *P. fluorescens* 31-12 applied to pelleted lettuce seeds survived on the pellets for 3 months when stored at under 4 ℃. However, a report on using bacteria to pellet seeds for commercial purposes has not been found. Therefore, this approach needs to be researched further to enhance seed quality after storage and to monitor bacterial viability.

This research aimed to study the effect of lettuce seed quality and monitor the viability of bacteria after the pelleting process, and storage in different environmental conditions.

Materials and methods

This experiment was conducted at the Seed Technology Laboratory and the greenhouse of the Division of Agronomy, Faculty of Agricultural Production, Maejo University. 'Red Oak' leaf lettuce seeds, which were used in these experiments, were cultivated in 2021. The experiment was conducted between June 2021 and August 2022.

Microbe preparation

Two of the microbial isolates used in the experiment, *Burkholderia* sp. and *Enterobacter* sp. (Jomkhame and Atnaseo, 2021), were collected from forest soils in the Huai Hong Khrai Royal Development Study Center under the royal initiative in the Doi Saket District, Chiang Mai Province. *Stenotrophomonas* sp. was collected from soil surrounding kale roots in farmer plots in the San Sai District of Chiang Mai Province. These bacteria were identified based on a DNA sequence comparison of the 16s rRNA gene to the National Center for Biotechnology Information (NCBI) database. The study performed preliminary tests of the response of all three isolates of bacteria, which showed that the concentrations of *Stenotrophomonas* sp., *Burkholderia* sp. and *Enterobacter* sp. at concentrations of 1×10^7, 1×10^8 and 1×10^8 CFU/ml, respectively, were the appropriate concentrations for use in this experiment. Each bacterial isolate was cultured in 50% TSA (trypticase soy agar) (HiMedia®) for 24 h before being suspended in 0.85% NaCl solution and adjusted 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. Then, 1 mL of the bacterial culture was mixed with 99 mL of 0.1% CMC (carboxymethyl cellulose), which served as a binding agent.

Pelleting lettuce seeds with plant growth promoting bacteria

Red Oak leaf lettuce seeds were surface sterilized with 1% sodium hypochlorite (NaOCl) for 1 min and washed with sterilized distilled water three times before being dried with sterilized tissue paper. The bilayer matrix for lettuce seed pelleting was prepared using calcium sulfate ($CaSO_4$)-zeolite (40 g $CaSO_4$, 90 g zeolite) (Jeephet *et al*., 2022). Then, 0.4% w/w aqueous-carboxymethyl cellulose was prepared as a binder. Ten grams of lettuce seeds were used for each experimental method. Then, lettuce seeds were pelleted with each of the bacterial isolates previously prepared. This experiment consisted of 5 treatments: 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). Pelleted seeds were then placed at room temperature for 48 h to reduce the seed moisture to 7%.

Seed storage

All treatment methods were packed in aluminum foil bags (10 × 15 cm, W × L) and then stored either under ambient conditions or controlled conditions (with a temperature of 4 ℃ and 80% relative humidity). The seeds were randomly collected to inspect their quality every 2 months for 12 months consecutively.

Seed measurement

Seed quality tests under laboratory conditions

Fifty lettuce seeds pelleted with microbes and non-pelleted seeds in each experiment group were tested for their germination rate using the top of paper method with 4 replicates. Then, the box was placed in the germination incubator at 25 ℃ with 80% relative humidity, 180 μE light intensity, and 24-h lighting. The various methods of recording data were performed as follows:

For radicle emergence percentage, the root germination of seeds in all experimental groups was randomly assessed on day 3 after seeding. Counting started when seeds germinated with roots 2 mm in length (Mis *et al*., 2022). To study the radicle emergence speed, (root/day) germinated seeds with 2 mm root lengths were counted daily from day 1 to day 3 after planting and then the radicle emergence speed was calculated (Jeephet *et al*., 2022). For germination (%), the number of normal seedlings was identified after seeding for 4 days (first count) and 7 days after seeding (final count) (ISTA, 2018). To determine the speed of germination (seedling/day), the number of seeds that germinated into normal seedlings was checked daily from days 4–7 after seeding (AOSA, 1983). The shoot and root length of 10 seedlings (cm) in each experimental group were randomly assessed on day 7 after seeding by measuring the shoot length from the base of the seedling to the tip of the seedling leaf and measuring the root length from the base of the shoot down to the end of the roots of the seedling with 4 replicates.

Assessment of bacterial viability

Seeds treated in each experimental group under each storage condition were randomly examined for bacterial viability every month for 4 months

consecutively. Twenty seeds were used in the test with 4 replicates. In all experimental groups, the seeds were placed in a glass tube filled with 2 mL of distilled water. The tubes were sterilized using an autoclave. The tubes were then shaken to ensure that the seeds fell out of the pellets and werhn set aside for 5 min. Subsequently, the tubes were shaken continuously again for 1 min and set aside for 1 min. Then, 1 ml of the clear liquid of the suspended solids was transferred to the spread plate. The prepared seeds in each experimental group were incubated at 30℃ for 24 h in an incubator. At the end of the specified time, the number of bacteria colonies was counted (Thomma and Sirithorn, 2012).

Statistical analysis

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

Results

Radicle emergence and radicle emergence speed

In the controlled conditions, non-pelleted seeds showed high radicle emergence throughout the 12-month storage period. The difference between the radicle emergence of the seeds pelleted with 1×10^8 CFU/ml *Enterobacter* sp. and the non-pelleted seeds stored over 12 months was not statistically different. Over a 12-month randomized test (Table 1), the radicle emergence rate of seeds pelleted by only one pelleting material was considerably reduced when compared to seeds in all other groups.

In ambient conditions, before the storage of the non-pelleted seeds, the radicle emergence rate was high but not statistically different from seeds pelleted with 1×10^8 CFU/ml *Enterobacter* sp. After 2–6 months of storage, the radicle emergence rate was not statistically different. After 8–12 months of storage, the radicle emergence rate of the non-pelleted seeds was higher than that of the seeds pelleted in all other experimental groups. Nevertheless, after 8 months of storage under ambient conditions, the radicle emergence of seeds was reduced considerably compared to seeds stored under controlled conditions (Table 1). The radicle emergence speed showed high variability in both storage conditions. Mostly, non-pelleted seeds tended to have a higher root/day speed. From both storage conditions, when seeds were randomly inspected at the 10^{th}

and 12th month, the difference between the radicle emergence speed of non-pelleted seeds and pelleted seeds in all other experimental groups was not statistically different (Table 2).

Table 1. Radicle emergence of lettuce seeds after pelleting with different types of plant growth-promoting bacteria after storage for 12 months and tested under laboratory conditions

Treatment [1]	Radicle emergence (%)						
	0	2	4	6	8	10	12
Controlled condition							
T1	96 a [2, 3]	97 a	95 a	87 a	84	78 a	55 a
T2	93 b	92 b	90 b	79 b	75	59 c	38 c
T3	92 b	92 b	91 b	80 b	76	62 bc	48 b
T4	92 b	93 b	92 b	81 b	78	63 b	47 b
T5	94 ab	94 ab	93 ab	80 b	79	65 ab	50 ab
F-test	**	**	*	*	ns	*	*
CV.(%)	6.39	6.45	6.32	6.10	5.62	5.36	5.68
Ambient condition							
T1	96 a	93	92	82	72 a	50 a	42 a
T2	93 b	91	91	80	63 b	41 b	30 b
T3	92 b	94	93	80	69 b	46 b	34 b
T4	92 b	93	92	81	65 b	45 b	35 b
T5	94 ab	92	91	82	66 b	47 ab	34 b
F-test	**	ns	ns	ns	*	*	*
CV.(%)	6.39	6.54	6.84	6.12	5.32	5.16	5.41

ns, *, **: Not significantly different; significantly different at $P \leq 0.05$ and $P \leq 0.01$, respectively, [1] T1 = Unpelleted seeds, T2 = $CaSO_4$-zeolite, T3 = pelleted seed + *Stenotrophomonas* sp. $1x10^7$ CFU/ml, T4 = pelleted seed + *Burkholderia* sp. $1x10^8$ CFU/ml and T5 = pelleted seed + *Enterobacter* sp. $1x10^8$ CFU/ml., [2] Data are 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.

Germination and speed of germination

In the controlled condition, before storage, there was no statistical difference in the germination percentage of pelleted seeds in any experimental group. After 6–12 months of storage, seeds pelleted with 1×10^8 CFU/ml *Enterobacter* sp. had the highest germination percentage, which was statistically different when compared to the germination percentage of pelleted seeds from other experimental groups, followed by the germination percentage of seeds pelleted with 1×10^8 CFU/ml *Burkholderia* sp. From 0 to 10 months, the germination percentage of both non-pelleted seeds and pelleted seeds in all experimental groups was not statistically different. Twelve months after storage, seeds covered with 1×10^8 CFU/ml *Enterobacter* sp. showed the highest speed of germination, and this was statistically different compared to the speed of germination of seeds treated in all other groups (Table 3).

Table 2. Radicle emergence speed of lettuce seeds after pelleting with different types of plant growth-promoting bacteria after storage for 12 months and tested under laboratory conditions

Treatment [1]	Radicle emergence speed (root/day)						
	0	2	4	6	8	10	12
Controlled condition							
T1	22.31 [2]	21.37 a	20.21	19.14 a	17.12	14.55	12.33
T2	18.47	17.61 b	16.15	15.94 c	14.23	13.11	11.12
T3	18.75	17.34 b	16.44	15.74 c	14.33	13.74	11.52
T4	18.12	17.84 b	16.39	15.89 c	14.39	13.87	11.59
T5	18.22	18.04 b	16.87	16.32 b	14.47	13.98	11.84
F-test	ns	**	ns	*	ns	ns	ns
CV.(%)	6.33	7.20	8.21	6.23	7.11	7.45	7.51
Ambient condition							
T1	22.31	20.02 a	19.65	18.11 a	16.08 a	13.23	11.12
T2	18.47	16.18 b	15.64	14.30 c	14.10 b	11.44	10.01
T3	18.75	16.12 b	15.44	15.02 b	14.23 b	12.41	10.23
T4	18.12	16.13 b	15.67	15.18 b	14.38 b	12.55	10.26
T5	18.22	16.67 b	15.93	15.23 b	14.52 b	12.58	10.48
F-test	ns	*	ns	*	*	ns	ns
CV.(%)	6.33	6.12	6.41	6.13	6.20	6.27	6.32

ns, *, **: Not significantly different; significantly different at $P \leq 0.05$ and $P \leq 0.01$, respectively, [1] T1 = Unpelleted seeds, T2 = $CaSO_4$-zeolite, T3 = pelleted seed + *Stenotrophomonas* sp. $1x10^7$ CFU/ml, T4 = pelleted seed + *Burkholderia* sp. $1x10^8$ CFU/ml and T5 = pelleted seed + *Enterobacter* sp. $1x10^8$ CFU/ml, [2] Means within a column followed by the same letter are not significantly at $P \leq 0.05$ by DMRT.

Table 3. Germination percentage of lettuce seeds after pelleting with different types of plant growth-promoting bacteria after storage for 12 months and tested under laboratory conditions

Treatment [1]	**Germination (%)**						
	0	2	4	6	8	10	12
Controlled condition							
T1	98	96 b [2, 3]	96	80 b	71 b	67 b	52 b
T2	96	96 b	95	82 b	70 c	69 b	54 b
T3	98	97 ab	97	83 b	72 b	69 b	52 b
T4	97	97 ab	96	84 ab	78 ab	70 ab	56 ab
T5	99	98 a	97	88 a	79 a	72 a	59 a
F-test	ns	**	ns	*	*	*	*
CV.(%)	8.76	4.40	8.65	4.22	5.02	5.10	3.02
Ambient condition							
T1	98	95	83	71	55 c	45 c	28 c
T2	96	94	84	70	64 b	52 b	37 b
T3	98	97	85	71	65 b	53 b	38 b
T4	97	96	85	70	67 ab	58 ab	41 b
T5	99	96	84	71	69 a	59 a	47 a
F-test	ns	ns	ns	ns	*	*	*
CV.(%)	8.76	6.03	8.98	8.20	6.13	5.24	7.16

ns, *, **: Not significantly different; significantly different at $P \leq 0.05$ and $P \leq 0.01$, respectively, [1] T1 = Unpelleted seeds, T2 = $CaSO_4$-zeolite, T3 = pelleted seed + *Stenotrophomonas* sp. $1x10^7$ CFU/ml, T4 = pelleted seed + *Burkholderia* sp. $1x10^8$ CFU/ml and T5 = pelleted seed + *Enterobacter* sp. $1x10^8$ CFU/ml, [2] Data are 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.

In the ambient conditions, 0–6 months after pelleting and storage, the germination percentage of seeds in all treatment groups was not statistically different. After 8–12 months of storage, it remained true that the seeds pelleted with 1×10^8 CFU/ml of *Enterobacter* sp. had a higher germination percentage, which is different from seeds in all other treatment groups. From the 4th month onwards, after being stored in ambient conditions, the germination percentage of seeds began to decline earlier than seeds stored under the controlled conditions. The speed of germination of seeds after storage in months 0–10 showed no statistical difference. The random test of the seeds at the 12th month showed that those pelleted with 1×10^8 CFU/ml *Enterobacter* sp. still had a higher speed of germination than that of all other treatment groups (Table 4).

Table 4. Speed of germination of lettuce seeds after pelleting with different types of plant growth-promoting bacteria after storage for 12 months and tested under laboratory conditions

Treatment [1]	Speed of germination (seedling/day)						
	0	2	4	6	8	10	12
Controlled condition							
T1	22.32	21.41	21.56	20.19	19.18	17.52	15.33 b[2]
T2	21.20	20.65	20.64	20.01	19.13	16.23	15.34 b
T3	21.24	20.45	20.32	20.10	22.21	17.14	15.21 b
T4	21.11	20.44	20.36	20.12	21.56	17.22	15.28 b
T5	21.62	21.02	20.74	21.01	22.54	17.30	16.19 a
F-test	ns	ns	ns	ns	ns	ns	*
CV.(%)	3.21	3.44	3.30	3.02	2.13	5.41	7.23
Ambient condition							
T1	22.32	21.02	20.98	18.18	13.89	12.46	7.59 c
T2	21.20	21.11	20.74	17.09	16.33	14.14	10.20 b
T3	21.24	21.23	20.85	16.11	15.42	14.03	10.01 b
T4	21.11	21.17	20.87	17.12	16.18	13.19	11.23 ab
T5	21.62	21.25	20.89	17.16	16.24	14.05	11.34 a
F-test	ns	ns	ns	ns	ns	ns	*
CV.(%)	3.21	3.65	3.71	3.42	4.42	5.17	6.32

ns, *, **: Not significantly different and significantly different at $P \leq 0.05$, respectively.
[1] T1 = Unpelleted seeds, T2 = $CaSO_4$-zeolite, T3 = pelleted seed + *Stenotrophomonas* sp. $1x10^7$ CFU/ml, T4 = pelleted seed + *Burkholderia* sp. $1x10^8$ CFU/ml and T5 = pelleted seed + *Enterobacter* sp. $1x10^8$ CFU/ml, [2] Means within a column followed by the same letter are not significantly at $P \leq 0.05$ by DMRT.

Shoot and root length

The experimental results for the shoot and root lengths of seeds stored under both controlled and ambient conditions were similar. Before storage (0 months) and after storage for 12 months, seeds pelleted with 1×10^8 CFU/ml *Enterobacter* sp. had a significantly longer shoot length than seeds in all other experimental groups. Regarding the test of root length, before storage, the root lengths of non-pelleted seeds and pelleted seeds in all treatment groups were

not significantly different. From the 2nd to 12th month, the root length of seeds pelleted with 1×10^8 CFU/ml *Enterobacter* sp. was longer and statistically different compared to pelleted seeds in all other groups (Tables 5, 6).

Table 5. Shoot length of lettuce seeds after pelleting with different types of plant growth-promoting bacteria after storage for 12 months and tested under laboratory conditions

Treatment [1]	Shoot length (cm)						
	0	2	4	6	8	10	12
Controlled condition							
T1	1.08 b [2]	1.10 c	1.02 d	1.09 c	0.87 e	0.75 d	0.71 d
T2	1.14 b	1.23 b	1.16 c	1.15 bc	1.03 d	0.98 c	0.94 c
T3	1.21 b	1.22 b	1.19 c	1.18 bc	1.10 c	1.02 c	0.96 c
T4	1.25 a	1.26 b	1.24 b	1.23 b	1.12 b	1.04 b	1.01 b
T5	1.27 a	1.34 a	1.29 a	1.26 a	1.15 a	1.07 a	1.03 a
F-test	*	**	*	*	*	*	**
CV.(%)	3.45	3.20	3.48	3.22	3.08	3.12	3.05
Ambient condition							
T1	1.08 b [2]	0.94 b	0.98 d	0.94 c	0.77 c	0.62 c	0.54 d
T2	1.14 b	1.19 b	1.11 c	1.12 b	0.94 bc	0.86 bc	0.73 c
T3	1.21 b	1.18 b	1.16 bc	1.14 b	0.97 b	0.87 bc	0.79 bc
T4	1.25 a	1.21 b	1.19 b	1.14 b	0.99 b	0.94 b	0.80 b
T5	1.27 a	1.23 a	1.20 a	1.19 a	1.05 a	0.98 a	0.82 a
F-test	*	*	*	*	*	**	**
CV.(%)	3.45	4.03	3.65	3.84	3.24	3.07	3.12

*, **: Significantly different at $P \leq 0.05$ and $P \leq 0.01$, respectively.
[1] T1 = Unpelleted seeds, T2 = $CaSO_4$-zeolite, T3 = pelleted seed + *Stenotrophomonas* sp. $1x10^7$ CFU/ml, T4 = pelleted seed + *Burkholderia* sp. $1x10^8$ CFU/ml and T5 pelleted seed + *Enterobacter* sp. $1x10^8$ CFU/ml, [2] Means within a column followed by the same letter are not significantly at $P \leq 0.05$ by DMRT.

Bacterial viability of pelleted seeds when stored in different conditions for 4 months

In the controlled conditions, the bacterial viability of seeds pelleted with Enterobacter sp. was higher than seeds pelleted with other isolates during 4 months of storage. Seeds pelleted with *Stenotrophomonas* sp. and *Enterobacter* sp. exhibited similar bacterial viability in the pellets. After 1 month of storage, the bacterial viability of seeds pelleted with *Stenotrophomonas* sp. and

Enterobacter sp. decreased dramatically and continued to decrease throughout the randomized test after 4 months of storage (Figure 1A).

In ambient conditions, seeds pelleted with *Enterobacter* sp. still had higher viability than seeds pelleted with other isolates. The randomized test of the seeds pelleted with *Stenotrophomonas* sp. and *Enterobacter* sp. showed that after 1 month of storage, the bacterial viability decreased more than those of the controlled conditions. In particular, the bacterial viability of seeds pelleted with *Enterobacter* sp. showed a markedly rapid decrease. Seeds pelleted with any of the three bacteria isolates showed continuously decreased bacterial viability after 4 months of storage (Figure 1B).

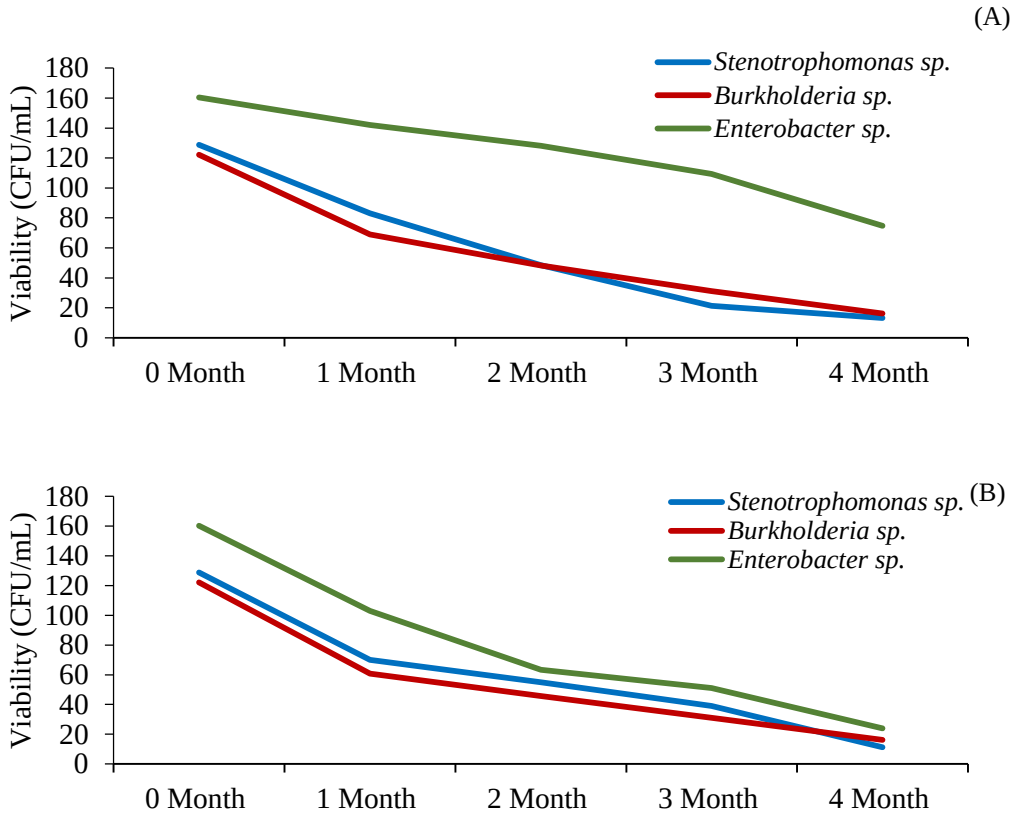

Figure 1. Viability of plant growth-promoting bacteria attached to pelleted lettuce seeds after 4 months of storage. (A) Storage under controlled conditions and (B) Storage under ambient conditions

Table 6. Root length of lettuce seeds after pelleting with different types of plant growth-promoting bacteria after storage for 12 months and tested under laboratory conditions

Treatment [1]	Root length (cm)						
	0	2	4	6	8	10	12
Controlled condition							
T1	5.54	5.34 b [2]	5.23 d	4.32 c	4.25 c	4.11 d	4.03 c
T2	6.79	6.68 b	6.45 c	5.49 bc	5.36 bc	5.13 c	5.01 b
T3	6.67	6.76 ab	6.54 bc	5.60 b	5.42 b	5.20 b	5.13 b
T4	6.71	6.76 ab	6.59 b	5.67 b	5.59 b	5.34 b	5.16 b
T5	6.84	6.86 a	6.66 a	5.71 a	5.68 a	5.46 a	5.21 a
F-test	ns	**	*	**	*	*	*
CV.(%)	4.77	4.31	4.12	4.06	4.14	4.22	4.09
Ambient condition							
T1	5.54	5.25 c	5.19 d	4.14 d	3.50 e	3.06 d	2.95 d
T2	6.79	6.61 b	6.24 c	5.37 c	4.13 d	3.89 c	3.25 cd
T3	6.67	6.73 b	6.48 b	5.42 b	4.56 c	3.94 bc	3.49 bc
T4	6.71	6.74 b	6.61 b	5.46 b	4.84 b	3.98 b	3.55 b
T5	6.84	6.81 a	6.74 a	5.64 a	4.87 a	4.02 a	3.61 a
F-test	ns	*	**	**	*	*	*
CV.(%)	4.77	4.32	4.35	4.24	4.11	4.46	4.15

ns, *, **: Not significantly different and significantly different at $P \leq 0.05$, respectively.
[1] T1 = Unpelleted seeds, T2 = $CaSO_4$-zeolite, T3 = pelleted seed + *Stenotrophomonas* sp. $1x10^7$ CFU/ml, T4 = pelleted seed + *Burkholderia* sp. $1x10^8$ CFU/ml and T5 = pelleted seed + *Enterobacter* sp. $1x10^8$ CFU/ml, [2] Means within a column followed by the same letter are not significantly at $P \leq 0.05$ by DMRT.

Discussion

The study of lettuce seeds pelleted with plant growth-promoting bacteria involves studies of the storage period and potential of bacterial viability on the pellets. Storage conditions can affect both seed quality and bacterial viability. Therefore, the inspection of seed quality is very important to ensure seed quality for commercial use.

Inspection of the non-pelleted seeds stored in both controlled and ambient conditions during the 3 days of seeding showed that, during the 12-month storage, radicle emergence and the radicle emergence speed were higher than those of the seeds in all other treatment groups. Siri (2015) argued that pelleted seeds had a higher ability to slow down the absorption of moisture, oxygen and other substances suitable for seed germination. For 3–4 days, the pelleted seeds had a chance to obtain the optimum factors for faster germination compared to the non-pelleted seeds. When checking seed quality after storage for more than 4 months, the seeds pelleted with 1×10^8 CFU/ml *Enterobacter* sp. and stored under controlled conditions showed a higher radicle rate than seeds in other experimental groups. Seed quality monitoring from days 4 to 7 showed an increase in the number of normal seedlings. As a result, although the pelleted seeds initially had slow radicle emergence, when the roots germinated, they absorbed suitable substances from the additional bacteria attached to the

seeds. The seeds that were stored under ambient conditions showed no difference in normal seedling appearance from 0 to 6 months. However, from 6 months of storage onwards, seeds pelleted with 1×10^8 CFU/ml *Enterobacter* sp. had a higher germination rate throughout a 12-month randomized test. *Enterobacter* sp. used to pellet seeds had a better tendency to promote seed germination than seeds in the other groups. *Enterobacter* sp. has been reported to have the potential to produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which controls ethylene by metabolizing ACC and then converting it to α-ketobutyric and ammonia, thereby promoting higher seed germination (Glick *et al*., 2007; Guo *et al*., 2011; Jha *et al*., 2011). This may provide a better mechanism for ACC production than non-pelleted seeds and seeds pelleted with other types of bacteria. Seed vigor was tested through assessment of speed of germination, and the randomized tests over the period of 0–10 months showed that the speed of germination of seeds under both storage conditions were not obviously different. However, after the test at 12 months of shelf life, seeds pelleted with 1×10^8 CFU/ml *Enterobacter* sp. showed better results than seeds in all other treatment groups. The results of growth in lettuce seeds pelleted with different strains and concentration levels of bacteria were as follows. For growth changes of lettuce seedlings, over 12-month storage under both conditions, seeds pelleted with 1×10^8 CFU/ml *Enterobacter* sp. had a longer shoot length and root length than the non-pelleted seeds and seeds pelleted with other strains of bacteria. Therefore, when seeds can germinate completely into a normal seedling, they have a complete root and shoot structure. *Enterobacter* sp. is also effective in producing IAA, which can stimulate radicle emergence, cell division and elongation in the root tip (Ashrafuzzaman *et al*., 2009; Guo *et al*., 2011; Zhang *et al*., 2021). This increases the contact surface of the roots, which enables lettuce seedlings to transport nutrients more efficiently. In addition, *Enterobacter* spp. have a property that can dissolve phosphates. Therefore, it is important to increase the elongation of roots and shoots (Khalid *et al*., 2004).

From the experiment, the storage environment of the seeds had a significant effect on the viability of the bacteria. The decrease in bacterial viability had a corresponding effect on seed germination and growth of lettuce seeds each month after storage. The monthly inspection showed that the seeds pelleted with *Enterobacter* sp. had the second highest number of colonies on the Petri dishes after being stored under controlled conditions. Seeds stored in ambient conditions showed a significantly fast reduction in bacterial viability for all isolates because seeds under ambient conditions were exposed to unsuitable environmental conditions, such as temperature and relative humidity (Yang and Wen, 2017). These factors affected the viability of the bacteria in the

pellets. However, although the viability of the bacteria was rapidly reduced, Enterobacter sp. survived in the pellets longer than *Stenotrophomonas* sp. or *Burkholderia* sp. Therefore, using *Enterobacter* sp. to pellet lettuce seeds helps to promote the growth of lettuce seedlings more effectively than other strains of bacteria. However, for seeds pelleted with 1×10^7 CFU/ml *Stenotrophomonas* sp. and 1×10^8 CFU/ml *Burkholderia* sp., the viability of bacteria decreased rapidly under both controlled and ambient conditions. As *Stenotrophomonas* sp. and *Burkholderia* sp. are bacteria extracted from the soil surrounding the plants, this might make them unsuitable for promoting bacterial viability when used to pellet lettuce seeds. Kangsopa (2018) found that the seed pelleting method, together with *P. fluorescens* 31-12, showed that *P. fluorescens* 31-12 could coexist with lettuce seeds for two months; then, the viability of *P. fluorescens* 31-12 decreased in the population. Moreover, microorganisms can survive and grow in carrot and parsnip seeds during drum priming (Wright *et al*., 2003).

In conclusion, seed pelleting with 1×10^8 CFU/ml *Enterobacter* sp. can be stored for 12 months, with better germination percentage, speed of germination, shoot length and root length than other treatment groups. Therefore, lettuce seed pelleting with 1×10^8 CFU/ml *Enterobacter* sp. was recommended for pelleting Red Oak lettuce seeds.

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