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Research article

Optimizing Peanut Seed Storage and Quality through Biological Coating with IAA-Producing *Enterobacter kobei* and *Agrobacterium radiobacter*

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Abstract

Peanut seeds are prone to deterioration during storage due to their high oil content, which makes them susceptible to lipid oxidation, leading to the formation of free radicals that accelerate cellular degradation. This process can alter the seed structure and nutrient reserves, ultimately reducing seed viability and lowering germination rates over prolonged storage periods. Therefore, seed coating techniques are crucial in mitigating seed deterioration, particularly through the application of IAA-producing bacteria that enhance seed quality and slow down the degradation process. This study aimed to evaluate the effects of coating peanut seeds with *Enterobacter kobei* and *Agrobacterium radiobacter* at a concentration of 10^7 CFU/mL on seed quality during a 4-month storage period under controlled (4°C with 50% RH) and ambient conditions (27°C±2 with 70%±5 RH). The results demonstrated that seeds coated with *E. kobei* at 10^7 CFU/mL and *A. radiobacter* at 10^7 CFU/mL exhibited significantly higher germination percentages, increased germination speed, and reduced mean germination time compared to non-coated seeds. These effects were particularly pronounced under controlled conditions, where coated seeds maintained superior quality and promoted seedling growth throughout the storage period. Furthermore, *E. kobei* at 10^7 CFU/mL and *A. radiobacter* at 10^7 CFU/mL significantly improved the shoot and root length as well as the shoot and root dry weight of peanut seedlings compared to the seedlings of non-coated seeds. Considering the overall results, it can be concluded that seed coating with *A. radiobacter* at 10^7 CFU/mL exhibited the most substantial enhancement in seed quality, making it the recommended approach for improving peanut seed germination, vigor, and seedling growth during a 4-month storage period. Notably, the coated seeds maintained high germination percentage and vigor throughout the entire 4-month storage duration, indicating extended seed longevity under both controlled and ambient conditions.

Keywords: peanut seed coating; IAA-producing bacteria; storage longevity; seed germination

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1. Introduction

The long-term preservation of high-quality peanut (*Arachis hypogaea* L.) seeds is a critical factor influencing germination rates and seedling vigor. Seed deterioration during storage remains a major challenge, particularly in tropical and humid climates where elevated temperature and relative humidity accelerate seed degradation (Phyo et al., 2004). This degradation manifests as loss of seed viability, reduced germination capacity, and biochemical changes within the seed (Vasudevan et al., 2014). The primary biochemical factors contributing to peanut seed deterioration include unsaturated fatty acids, which undergo oxidation, leading to the production of free radicals and seed rancidity; proteins and enzymes, which may degrade due to hydrolysis and oxidation, resulting in the loss of essential enzymatic activity for germination; reserve carbohydrates, such as starch and oligosaccharides, which may be broken down or structurally altered, affecting energy availability for germination; and phenolic compounds, which can undergo transformation, impairing cell viability (Asibuo et al., 2008). Collectively, these biochemical changes contribute to the decline in seed quality and germination potential over extended storage periods.

A promising approach for extending seed longevity and maintaining seed quality is biological seed coating technology (Rocha et al., 2019; Paravar et al., 2023). Seed coating involves applying a protective layer of coating materials or polymers around the seed, creating a barrier against unfavorable environmental conditions. These coatings reduce moisture absorption and gas exchange, thereby slowing oxidative processes that contribute to lipid and protein degradation (Halmer, 2008; Pedrini et al., 2017). Furthermore, seed coatings help preserve cellular integrity and minimize leakage of vital intracellular components, ensuring that seeds retain sufficient energy reserves for germination (Pedrini et al., 2017; Paravar et al., 2023). Additionally, coatings can modulate the release of growth-promoting substances and antioxidants produced by beneficial microorganisms incorporated within the coating, thereby delaying seed deterioration and enhancing germination under optimal conditions. Another advantage is the ability to mitigate fluctuations in temperature and humidity during storage, leading to prolonged seed viability and improved seedling vigor when planted. Several studies have demonstrated the efficacy of seed coating in enhancing seed storage longevity. da Silva et al. (2018) reported that coated peanut seeds maintained high germination and vigor for up to 90 days post-storage. Similarly, Ramya et al. (2024) found that soybean seeds stored for six months retained superior germination and seedling growth performance compared to non-coated seeds. Moreover, seed coatings serve as a microbial habitat, allowing beneficial bacteria to colonize and further enhance seed quality (Deaker et al., 2012). Notably, *Enterobacter kobei* and *Agrobacterium radiobacter* are bacterial species capable of producing indole-3-acetic acid (IAA), a phytohormone that stimulates enzyme and protein synthesis essential for germination, as well as promoting cell division and root elongation, thereby improving root system development and nutrient uptake efficiency (Malisorn et al., 2020; Noor et al., 2023). Despite these advancements, limited studies have explored peanut seed coating with plant growth-promoting bacteria (PGPB) that enhance seed germination and seedling vigor after storage (Rocha et al., 2019). Investigating the application of IAA-producing bacteria in seed coatings could provide a cost-effective strategy for farmers and peanut producers by improving seed quality and reducing production costs.

This study aimed to evaluate the effects of peanut seed coating with *E. kobei* and *A. radiobacter* on seed quality during storage. Key parameters assessed included

germination rate, seed vigor, and seed viability under different storage conditions. The findings from this study should contribute to the development of biological seed coating technology as a sustainable approach to prolong seed longevity and enhance crop production efficiency.

2. Materials and Methods

2.1 Location of experiment, duration, and seed quality

The experiment was conducted at the Seed Technology Laboratory, Soil and Environmental Microbiology Laboratory, and Modern Seed Technology Research Center under the Agronomy Program, Faculty of Agricultural Production, Maejo University. The initial germination rate of the peanut seeds used in the study was 50%, with a seed moisture content of 10±1%. The experiment was carried out over a period spanning March to December 2024.

2.2 Information on bacteria

The *Enterobacter kobei* (CP017181) and *Agrobacterium radiobacter* (NP_116306) strains used in this study were isolated from Chiang Muan district, Phayao province, Thailand, and identified through 16S rRNA gene sequencing. The indole-3-acetic acid (IAA) production of these bacterial strains was subsequently quantified using the method described by Ehmann (1977), with the results indicating IAA concentrations of 76.93 µM/mL for *E. kobei* and 58 µM/mL for *A. radiobacter*.

2.3 Microbial inoculum preparation

The *E. kobei* and *A. radiobacter* strains were initially cultured by inoculating a single colony into 5 mL of nutrient broth (NB) and incubating at 30°C with continuous shaking at 170 rpm for 48 h. Following this, 100 µL of the actively growing culture was transferred into a 250-mL Erlenmeyer flask containing 50 mL of NB and incubated under the same conditions for an additional 48 h to achieve sufficient bacterial growth. After incubation, the bacterial culture was centrifuged at 8,000x *g* for 5 min to pellet the cells, followed by one wash with 0.85% NaCl solution to remove residual media components (Jomkhame et al., 2022). The resulting bacterial suspension was then incorporated into the seed coating formulation and adjusted to a final concentration of 10^7 CFU/mL. The prepared bacterial coating mixture was subsequently used for coating peanut seeds to evaluate its effects on seed quality and germination performance.

2.4 Coating peanut seeds

The peanut seeds were initially surface-sterilized using 0.50% sodium hypochlorite (NaOCl) for 1 min to eliminate potential contaminants. Following sterilization, the seeds were rinsed three times with sterile distilled water and dried using sterile tissue paper to remove residual disinfectant. A total of 50 g of peanut seeds were coated with 0.2% (w/v) methyl cellulose (MC) using a rotary pan coater (model KSC-02D, CERES International Ltd., Bangkok, Thailand), operated at a spinning rate of 32 rpm to ensure uniform coating. A volume of 20 mL of coating solution was applied per treatment. The seeds were divided into four different treatment groups: T1 = non-coated seeds (control), T2 = seeds coated

with MC only, T3 = seeds coated + *E. kobei* 10^7 CFU/mL, and T4 = seeds coated + *A. radiobacter* 10^7 CFU/mL. Following the coating process, the treated seeds were subjected to moisture reduction using a forced-air oven (model KKU40-2) at 33°C for 6 h until the seed moisture content was reduced to 10±1%.

2.5 Seed storage

All treated seed samples were sealed in aluminum foil bags (10 × 15 cm, W × L) and stored under two different conditions: ambient conditions 27±2°C with 70±5% relative humidity and controlled conditions (4°C with 50% relative humidity). Seed quality was assessed at monthly intervals over a four-month storage period, with samples randomly collected for evaluation.

2.6 Seed testing in laboratory and greenhouse conditions

2.6.1 Sand testing

Sand with a uniform particle size (<0.05 mm) was sterilized and autoclaved at 121°C for 15 min to eliminate contaminants before being used as a germination medium for seed quality assessment. Each treatment consisted of four replicates, with 50 seeds per replicate. The germination test was conducted using plastic boxes (180 mm × 140 mm × 90 mm, L × W × H) as seed containers. The sand moisture content was adjusted to approximately 60%, and the germination boxes were filled with a 3 cm layer of sand. Seeds were carefully arranged on the sand surface and then covered with an additional 2 cm layer of sand. The germination boxes were placed in a controlled germination chamber set to 25°C, 80% relative humidity, and a light intensity of 180 µE, with continuous light exposure for 24 h to optimize germination conditions. The seed germination percentage was determined by counting the number of seeds that developed into normal seedlings, with the first count recorded on day 5 and the final count on day 10 (ISTA, 2023). The germination speed was evaluated by counting the number of seeds that developed into normal seedlings between days 5 and 10 (AOSA, 1983). The mean germination time was calculated by daily assessing normal seedlings over a 10-day period, based on the approach described by Ellis and Roberts (1980). Additionally, seedling shoot length, root length, shoot dry weight, and root dry weight were measured 10 days after sowing using a randomly selected sample of 20 seedlings, following the methodology outlined by Jeephet et al. (2022).

2.6.2 Peat testing

The germination performance of both coated and non-coated peanut seeds was evaluated using seed trays filled with peat moss (Klasmann-Deilmann GmbH, Ltd., Germany) as the germination substrate. The germination percentage was assessed five days after sowing, with the final germination count recorded on day 10 (ISTA, 2023). Speed of germination was determined by daily monitoring of normal seedling emergence from day 5 to day 10, in accordance with the AOSA (1983) method. The mean germination time was calculated based on daily assessments of normal seedlings over a 10-day period, following the approach outlined by Ellis and Roberts (1980). Additionally, shoot length, root length, shoot dry weight, and root dry weight were measured using the same methodology as applied in the sand germination test.

2.7 Statistical analysis

The germination percentage of all peanut seed treatments was arcsine-transformed to normalize the data prior to statistical analysis. All data were analyzed using one-way analysis of variance (ANOVA) with a completely randomized design (CRD). Differences among treatments were assessed using the least significant difference (LSD) test to determine statistical significance.

3. Results and Discussion

3.1 Germination and vigor

The study of peanut seed germination after coating with two isolates of IAA-producing bacteria and subsequent storage under different conditions for four months revealed that seed storage under controlled conditions (Figures 1A and 1B) maintained germination percentage better than storage under ambient conditions (Figures 1C and 1D). This trend was particularly evident after 3 to 4 months of storage, where seeds stored under ambient conditions exhibited a significant decline in germination percentage. Sand test results indicated that under controlled conditions, seeds coated with *Enterobacter kobei* at 10^7 CFU/mL and *Agrobacterium radiobacter* at 10^7 CFU/mL exhibited higher germination percentages both immediately after coating and after 4 months of storage (Figure 1A). Under ambient conditions, seed coating with *E. kobei* and *A. radiobacter* (both at 10^7 CFU/mL) led to significantly higher germination percentages than the non-coated control after 3 months of storage. By the fourth month, *A. radiobacter*-coated seeds demonstrated the highest germination percentage with statistically significant differences compared to all other treatments, as determined under the sand test (Figure 1B). In the peat test, all coated treatments (T2-T4) exhibited consistently higher germination percentages throughout the 4-month storage period compared to non-coated seeds. Notably, seeds coated with *A. radiobacter* at 10^7 CFU/mL maintained their germination percentage even after 4 months of storage, showing no significant decline compared to the initial germination before storage (0 month) (Figure 1C). Under ambient conditions, seed coating with *E. kobei* and *A. radiobacter* (both at 10^7 CFU/mL) preserved high germination percentages for up to 3 months, followed by a decline observed at 4 months. Notably, seeds coated with *A. radiobacter* showed no significant reduction in germination percentage relative to other treatments when evaluated using the peat test (Figure 1D).

The evaluation of speed of germination after the sand test indicated that seeds coated with *E. kobei* at 10^7 CFU/mL and *A. radiobacter* at 10^7 CFU/mL exhibited a higher speed of germination than non-coated seeds after 4 months of storage under both controlled (Figure 2A) and ambient conditions (Figure 2B). The peat test results demonstrated that seeds coated with *A. radiobacter* at 10^7 CFU/mL maintained a consistently high speed of germination throughout the 4-month storage period under controlled conditions (Figure 2C). Regarding storage under ambient conditions, it was observed that seeds coated with *E. kobei* at 10^7 CFU/mL and *A. radiobacter* at 10^7 CFU/mL maintained a high speed of germination during the first to third months. However, in the 4-month, seeds coated with *E. kobei* at 10^7 CFU/mL exhibited a decline in speed of germination, whereas seeds coated with *A. radiobacter* at 10^7 CFU/mL continued to show the highest speed of germination compared to other treatments (Figure 2D).

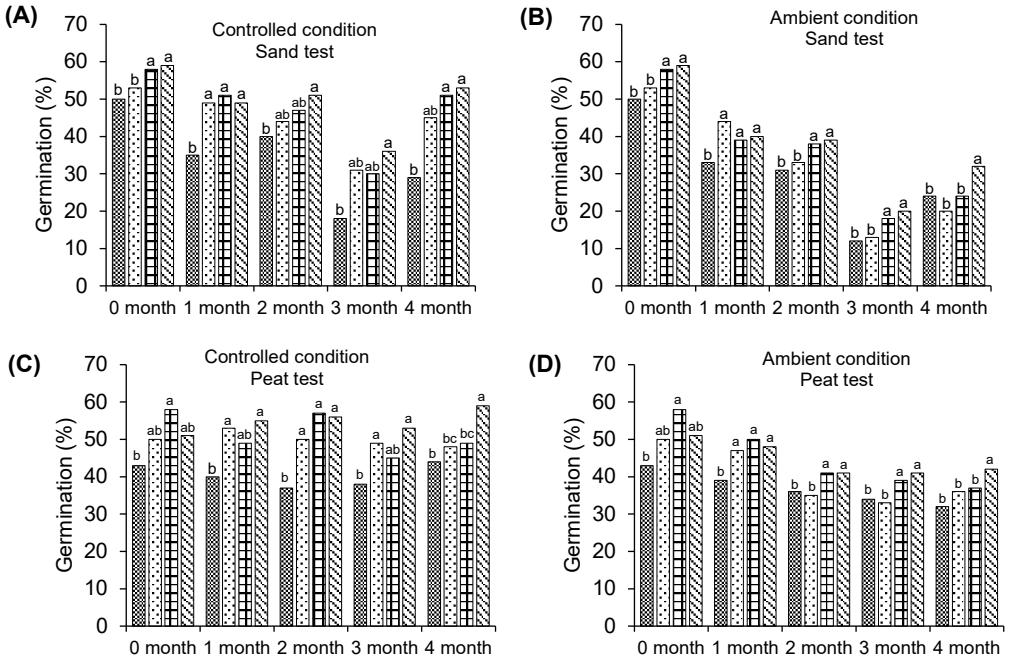

Figure 1. Germination percentage of peanut seeds after coating with *E. kobei* and *A. radiobacter* at concentration of 10^7 CFU/mL following storage under controlled (A, B; sand test) and ambient (C, D; peat test) conditions for 4 months. Means in each column within the same category followed by the same letter are not significantly difference at P≤0.05 by LSD.

The assessment of mean germination time after the sand test revealed that, throughout the 4- month storage period under both controlled (Figure 3A) and ambient conditions (Figure 3B) , seeds coated with *A. radiobacter* at 10^7 CFU/ mL exhibited a significantly faster mean germination time compared to non-coated seeds. For the peat test, seeds stored under controlled conditions from months 2 to 4 showed that seeds coated with *A. radiobacter* at 10^7 CFU/mL maintained a significantly faster mean germination time than non-coated seeds (Figure 3C). Regarding storage under ambient conditions, results indicated that from months 1 to 4, there were no statistically significant differences in mean germination time among the treatments when tested in the peat test (Figure 3D).

Seeds coated with *E. kobei* and *A. radiobacter*, which were capable of producing indole-3-acetic acid (IAA) at concentrations of 76.93 µM/mL and 58 µM/mL, respectively, demonstrated a significant improvement in germination percentage, speed of germination, and a reduction in mean germination time compared to non-coated seeds. This effect was particularly evident under controlled conditions, where seed quality was maintained throughout the 4-month storage period. In addition, the observed increase in germination percentage and speed of germination of peanut seeds during the 4-month storage period may be attributed to the natural breakdown of seed dormancy, a post-harvest physiological process. This dormancy release is driven by internal biochemical changes, such as a reduction in abscisic acid (ABA), a hormone that inhibits germination, and a rebalancing of

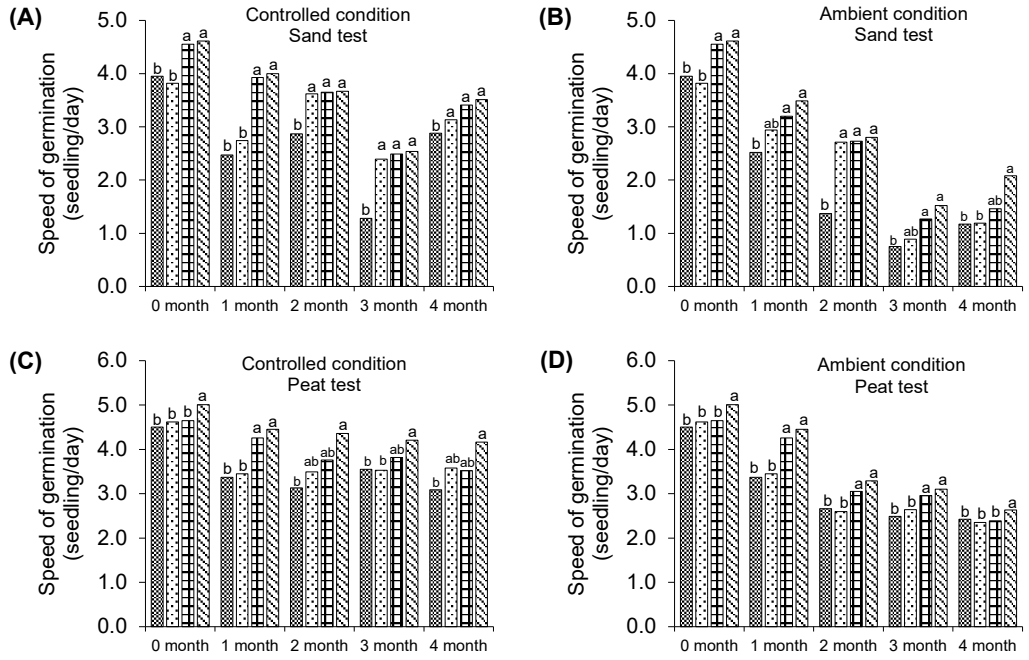

Figure 2. Speed of germination (seedlings/day) of peanut seeds after coating with *E. kobei* and *A. radiobacter* at concentration of 10^7 CFU/mL following storage under controlled (A, B; sand test) and ambient (C, D; peat test) conditions for 4 months. Means in each column within the same category followed by the same letter are not significantly difference at P≤0.05 by LSD.

growth regulators that enhance the seed's readiness to germinate (Bentsink & Koornneef, 2008). Moreover, seed coating with polymers and IAA-producing bacteria further facilitates water absorption and promotes the initiation of germination. This is particularly effective under controlled conditions, where stable humidity and temperature help maintain seed quality and enhance the performance of the coating treatment. In contrast, under ambient conditions, while germination percentage and speed of germination declined over time, they remained significantly higher than those of non-coated seeds. This suggests that the IAA produced by both bacterial isolates may play a crucial role in mitigating storage- induced stress and delaying seed deterioration. The primary mechanism by which bacterially-derived IAA enhances germination involves its ability to stimulate cell division during the germination process (Spaepen & Vanderleyden, 2011; Panneerselvam et al., 2021). Additionally, IAA induces the synthesis of hydrolytic enzymes such as amylase and protease, which accelerate the breakdown of seed storage reserves, enabling mitochondrial energy production to occur more rapidly, thereby promoting faster and more vigorous embryo development (Patten & Glick, 2002; Khan et al., 2016). Furthermore, IAA plays a key role in regulating gene expression associated with seed and seedling development, ensuring a more synchronized germination process and reducing the mean germination time (Solano et al., 2008). These findings highlight the potential of biological seed coating technology using IAA-producing bacteria as an effective strategy for extending seed storage longevity and preserving seed quality, particularly when compared to non-coated seeds (Bashan & de-Bashan, 2010; Bashan et al., 2014).

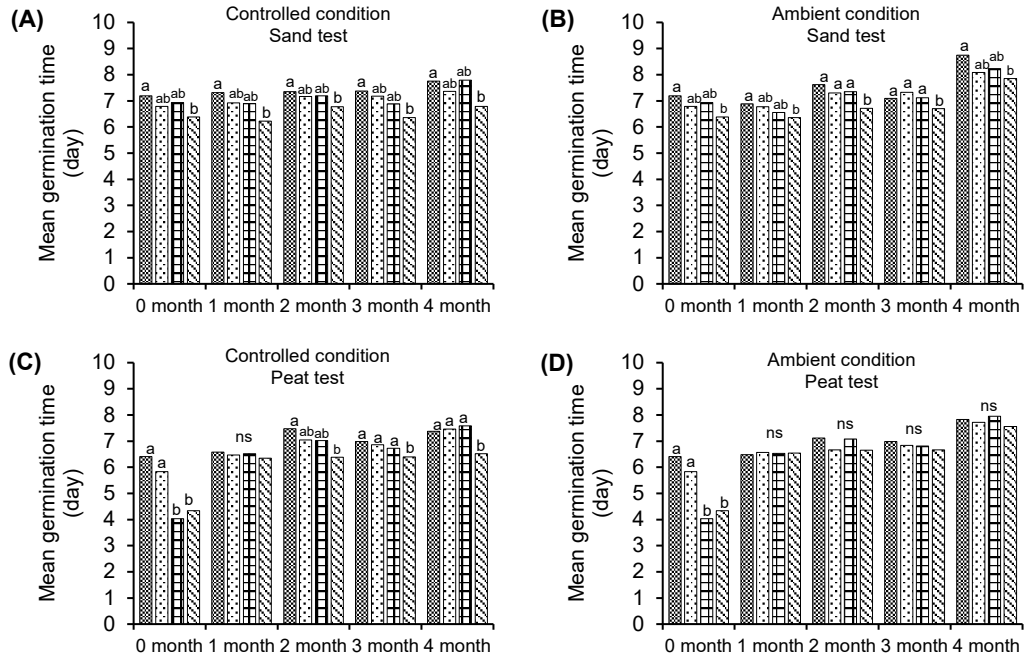

Figure 3. Mean germination time (day) of peanut seeds after coating with *E. kobei* and *A. radiobacter* at concentration of 10^7 CFU/mL following storage under control (A, B; sand test) and ambient (C, D; peat test) conditions for 4 months. Means in each column within the same category followed by the same letter are not significantly difference at P≤0.05 by LSD.

3.2 Seedling growth

All seed coating treatments exhibited a tendency to promote and maintain seedling growth quality after 4 months of storage under both conditions. The sand test results following storage under controlled and ambient conditions revealed that seed coating with *E. kobei* at 10^7 CFU/mL and *A. radiobacter at* 10^7 CFU/mL significantly enhanced shoot length compared to non-coated seeds (Table 1). For the peat test, seeds coated with *E. kobei* at 10^7 CFU/mL and stored under controlled conditions showed a significantly greater shoot length than non-coated seeds throughout the 1 to 4-month storage period. Under ambient conditions, seed coating with *E. kobei* at 10^7 CFU/mL and *A. radiobacter* at 10^7 CFU/mL consistently promoted superior seedling growth throughout the 4-month storage period, with statistically significant differences compared to non-coated seeds (Table 1). Figure 4 further illustrates the differences in seedling growth across the 4-month storage period under controlled conditions. Notably, seeds coated with *E. kobei* at 10^7 CFU/mL and *A. radiobacter* at 10^7 CFU/mL exhibited a clear trend of enhanced seedling development compared to non-coated seeds.

For root length assessment under sand conditions, seed coating with *A. radiobacter* at 10^7 CFU/mL throughout the 4-month storage period under controlled conditions significantly enhanced root length compared to non-coated seeds. Under

Table 1. Shoot length of peanut seedlings after coating seeds with IAA-producing bacteria for 4 months of storage

Treatment[1]	Shoot length (cm)									
	Sand test conditions					Peat test conditions				
	0	1	2	3	4	0	1	2	3	4
Controlled condition										
T1	14.35 b[2]	11.74 b	13.08 b	10.04 b	10.16 b	10.71 b	9.09 b	9.76 b	10.85 b	9.77 b
T2	15.13 a	12.78 b	14.44 ab	13.51 a	12.37 a	11.33 ab	9.30 ab	10.19 ab	11.74 a	10.14 a
T3	15.75 a	13.11 a	15.09 a	14.04 a	12.39 a	11.25 ab	9.64 a	10.47 a	11.66 a	10.23 a
T4	15.58 a	13.55 a	15.25 a	13.87 a	12.82 a	11.77 a	9.37 ab	10.31 ab	11.14 ab	9.90 b
F-test	**	**	**	**	**	**	**	*	**	**
CV.(%)	5.50	6.41	6.30	7.56	7.50	5.23	4.21	5.80	4.72	4.36
Ambient condition										
T1	14.35 b	14.85 b	12.97 b	9.89 b	8.07 b	10.71 b	9.16 b	9.02 b	10.12 b	9.21 b
T2	15.13 a	14.81 b	12.84 b	10.88 a	8.62 b	11.33 a	9.31 b	9.24 a	10.21 b	9.29 b
T3	15.75 a	15.26 a	13.90 a	10.94 a	9.88 a	11.25 a	9.61 a	9.62 a	11.42 a	9.25 b
T4	15.58 a	15.94 a	13.95 a	10.88 a	9.45 a	11.77 a	9.75 a	9.56 a	11.27 a	10.06 a
F-test	**	**	**	**	**	**	**	**	**	**
CV.(%)	5.50	7.54	7.22	9.78	10.25	5.23	6.78	4.12	5.47	4.22

*,**: significantly different at P≤0.05 and P≤0.01,respectively. [1]T1 = non-coated seed, T2 = coated seed with polymer only, T3 = coated seed + *Enterobacter kobei* 10^7 CFU/mL and T4 = coated seed + *Agrobacterium radiobacter* 10^7 CFU/mL. [2]Means within a column followed by the same letter are not significantly at P ≤ 0.05 by LSD.

ambient conditions, from month 1 to month 4, all coated seed treatments (T2-T4) exhibited significantly greater root length than non-coated seeds. In the peat condition, throughout the 4-month storage period under both controlled and ambient conditions, seed coating with *A. radiobacter* at 10^7 CFU/mL consistently resulted in significantly greater root length compared to non-coated seeds (Table 2).

Changes in shoot dry weight after the sand condition test indicated that seed coating with *E. kobei* at 10^7 CFU/mL and *A. radiobacter* at 10^7 CFU/mL significantly enhanced biomass accumulation in seedlings compared to non-coated seeds, following a 4-month storage period under both controlled and ambient conditions. For the peat condition test, seed coating with *A. radiobacter* at 10^7 CFU/mL continued to support biomass accumulation, resulting in a significantly higher shoot dry weight throughout the 4-month storage period compared to non-coated seeds. Similarly, under ambient conditions, all coated seed treatments (T2-T4) exhibited significantly greater shoot dry weight than non-coated seeds over the 4-month period, demonstrating the beneficial effects of seed coating on seedling growth (Table 3).

For root dry weight, the results indicated that under sand conditions, after 4 months of storage in controlled conditions, seed coating with *E. kobei* at 10^7 CFU/mL and *A. radiobacter* at 10^7 CFU/mL maintained significantly higher biomass accumulation in root dry weight compared to non-coated seeds. Under ambient conditions, seed coating with *A. radiobacter* at 10^7 CFU/mL continued to promote root dry weight accumulation, which remained significantly higher than that of non-coated seeds. For the peat condition test, seed storage under both controlled and ambient conditions demonstrated that seed coating with *E. kobei* and *A. radiobacter* at 10^7 CFU/mL resulted in significantly higher root dry weight accumulation compared to non-coated seeds (Table 4).

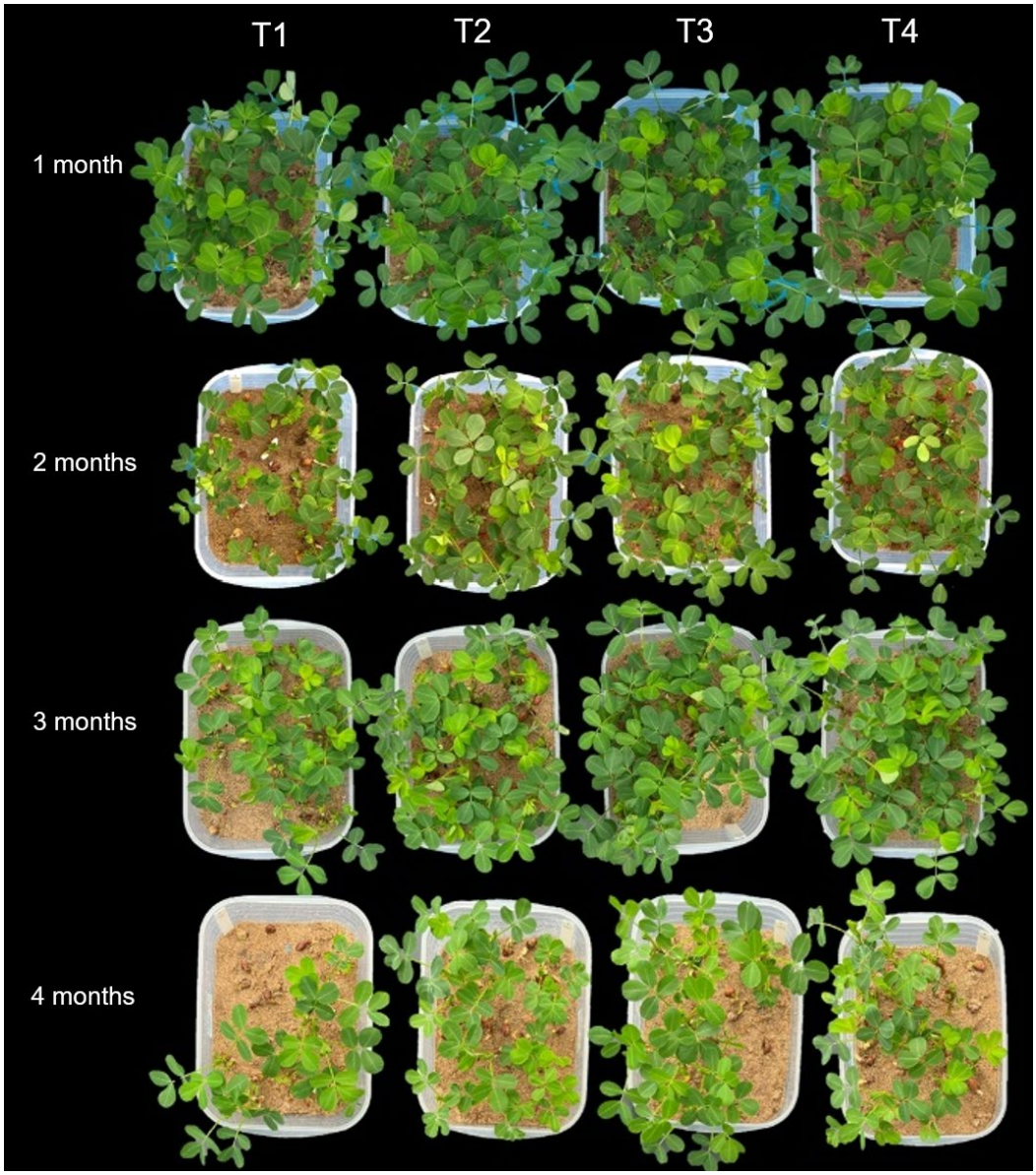

Figure 4. Seedling growth of peanut seeds after coating with *Enterobacter kobei* and *Agrobacterium radiobacter* at a concentration of 10^7 CFU/mL following storage under controlled conditions for 4 months. T1 = non-coated seed, T2 = coated seed with polymer only, T3 = coated seed + *Enterobacter kobei* 10^7 CFU/mL and T4 = coated seed + *Agrobacterium radiobacter* 10^7 CFU/mL.

Table 2. Root length of peanut seedlings after coating seeds with IAA-producing bacteria for 4 months of storage

Treatment[1]	Root length (cm)									
	Sand test conditions					Peat test conditions				
	0	1	2	3	4	0	1	2	3	4
Controlled condition										
T1	11.70 c[2]	12.96 b	10.24 b	8.98 b	10.56 b	13.90 b	11.38 b	14.76 b	10.23 c	9.98 c
T2	12.76 bc	14.29 a	10.63 b	9.58 ab	10.45 b	14.32 b	11.17 b	14.63 b	10.91 c	11.15 b
T3	13.55 a	14.48 a	10.98 a	9.51 ab	11.57 ab	14.12 b	11.03 b	15.65 ab	11.64 bc	13.60 a
T4	13.81 a	14.85 a	11.92 a	11.10 a	13.14 a	17.51 a	12.68 a	16.45 a	13.02 a	13.69 a
F-test	**	**	**	**	**	**	**	**	**	**
CV.(%)	4.54	7.40	11.12	15.25	9.02	10.22	12.44	9.04	9.60	8.56
Ambient condition										
T1	11.76 c	12.11 b	10.24 b	5.50 b	9.86 b	13.90 b	11.69 b	12.03 b	9.57 b	9.62 b
T2	12.76 bc	13.63 a	11.27 a	8.55 a	10.31 a	14.32 b	11.34 b	14.50 a	9.93 b	10.60 b
T3	13.55 a	13.84 a	11.12 a	8.82 a	10.14 a	14.12 b	14.95 a	14.21 a	10.02 ab	10.85 b
T4	13.81 a	13.29 a	11.17 a	8.98 a	10.28 a	17.51 a	14.15 a	14.53 a	12.31 a	12.50 a
F-test	**	**	**	**	**	**	**	**	**	**
CV.(%)	4.54	12.45	21.20	20.01	10.23	10.22	12.10	8.22	11.21	10.47

**: significantly different at P≤0.01. [1]T1 = non-coated seed, T2 = coated seed with polymer only, T3 = coated seed + *Enterobacter kobei* 10^7 CFU/mL and T4 = coated seed + *Agrobacterium radiobacter* 10^7 CFU/mL. [2]Means within a column followed by the same letter are not significantly at P ≤ 0.05 by LSD.

Table 3. Shoot dry weight of peanut seedlings after coating seeds with IAA-producing bacteria for 4 months of storage

Treatments[1]	Shoot dry weight (mg)									
	Sand test conditions					Peat test conditions				
	0	1	2	3	4	0	1	2	3	4
Controlled condition										
T1	272[2] b[3]	201 b	254 b	246 b	254 b	255 b	302 b	218 b	184 b	197 b
T2	315 a	315 a	317 a	284 ab	325 a	354 a	324 ab	315 a	307 ab	338 a
T3	335 a	282 a	318 a	315 a	341 a	344 a	310 ab	317 a	328 ab	324 a
T4	351 a	316 a	334 a	333 a	336 a	324 a	334 a	320 a	344 a	322 a
F-test	**	**	**	**	**	**	**	**	**	**
CV.(%)	10.20	11.32	7.20	9.24	7.32	12.32	8.10	10.57	5.50	12.21
Ambient condition										
T1	272[2] b[3]	234 b	225 b	244 b	278 b	255 b	265 b	217 b	239 b	208 b
T2	315 a	305 a	307 a	255 b	354 a	354 a	305 a	288 a	364 a	345 a
T3	335 a	315 a	315 a	357 a	374 a	344 a	317 a	294 a	347 a	358 a
T4	351 a	337 a	322 a	351 a	385 a	324 a	324 a	284 a	332 a	305 a
F-test	**	**	**	**	**	**	**	**	**	**
CV.(%)	10.20	13.11	9.23	9.56	9.54	12.32	12.24	10.27	7.51	9.32

**: significantly different at P≤0.01. [1]T1 = non-coated seed, T2 = coated seed with polymer only, T3 = coated seed + *Enterobacter kobei* 10^7 CFU/mL and T4 = coated seed + *Agrobacterium radiobacter* 10^7 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 ≤ 0.05 by LSD.

Table 4. Root dry weight of peanut seedlings after coating seeds with IAA-producing bacteria for 4 months of storage

Treatment[1]	Root dry weight (mg)									
	Sand test conditions					Peat test conditions				
	0	1	2	3	4	0	1	2	3	4
Controlled condition										
T1	80 b[2]	132 b	92 b	83 b	84 b	80 b	71 b	77 b	73 b	70 b
T2	100 a	141 ab	117 a	87 ab	82 b	81 b	74 b	97 a	75 b	72 b
T3	103 a	159 a	118 a	95 a	99 a	110 a	86 a	95 a	82 a	81 a
T4	112 a	162 a	113 a	92 a	104 a	113 a	87 a	98 a	83 a	82 a
F-test	**	**	**	**	**	**	**	**	**	**
CV.(%)	12.01	15.75	16.15	15.20	10.52	9.23	15.14	8.54	9.50	8.05
Ambient condition										
T1	80[2] b[3]	97 b	87 b	66 b	80 b	80 b	86 b	84 b	66 b	66 b
T2	100 a	129 a	95 ab	71 b	73 b	81 b	91 ab	85 b	68 b	64 b
T3	90 ab	124 ab	91 ab	85 ab	86 ab	110 a	103 a	95 a	84 a	63 b
T4	112 a	133 a	102 a	109 a	127 a	113 a	111 a	93 a	86 a	76 a
F-test	**	**	**	**	**	**	**	**	**	**
CV.(%)	12.01	14.54	15.12	18.54	16.87	9.23	13.20	11.14	6.21	10.54

**: significantly different at P≤0.01. [1]T1 = non-coated seed, T2 = coated seed with polymer only, T3 = coated seed + *Enterobacter kobei* 10^7 CFU/mL and T4 = coated seed + *Agrobacterium radiobacter* 10^7 CFU/mL. [2]Means within a column followed by the same letter are not significantly at P ≤ 0.05 by LSD.

The experimental results indicate that coating peanut seeds with IAA-producing bacteria played a crucial role in stimulating seedling growth by significantly increasing shoot and root length as well as shoot and root dry weight compared to non-coated seeds. This effect was particularly evident in controlled conditions, where the coated seeds maintained seed quality and promoted seedling development throughout the 4-month storage period. In contrast, under ambient conditions, although non-coated seeds showed a decline in growth parameters over time, seeds coated with *E. kobei* at 10^7 CFU/mL and *A. radiobacter* at 10^7 CFU/mL consistently outperformed non-coated seeds, demonstrating the vital role of IAA in enhancing seedling adaptability and maintaining growth quality even under suboptimal environmental conditions (Patten & Glick, 2002; Glick, 2012). IAA produced by *E. kobei* and *A. radiobacter* plays a key role in stimulating cell division and tissue expansion, particularly in the apical meristem, which is the primary site for plant growth (Solano et al., 2008; Spaepen & Vanderleyden, 2011). This corresponds with the findings in Tables 1 and 2, which show that shoot and root length of seedling in seeds coated with these bacterial isolates were significantly higher than in non-coated seeds. Under controlled conditions, the coated seeds consistently maintained high shoot and root lengths throughout the storage period, indicating that IAA plays a pivotal role in preserving seed quality and ensuring continuous seedling development without growth inhibition. Conversely, under ambient conditions, although shoot and root lengths declined over time, seeds coated with *E. kobei* at 10^7 CFU/mL and *A. radiobacter* at 10^7 CFU/mL still exhibited significantly greater shoot and root lengths than non-coated seeds. This suggests that IAA enhances water and nutrient uptake while mitigating seedling stress, allowing seedlings to continue developing even under less favorable environmental conditions (Solano et al., 2008; Kumla et al., 2020; Zhang et al., 2021).

Regarding shoot and root dry weight of seedling, seeds coated with *E. kobei* at 10^7 CFU/mL and *A. radiobacter* at 10^7 CFU/mL exhibited significantly higher dry weight than non-coated seeds, particularly in the fourth month of storage under controlled conditions. This indicates that IAA plays a key role in structural development and biomass accumulation in seedlings (Patten & Glick, 2002). Moreover, IAA stimulates the synthesis of hydrolytic enzymes such as amylase and protease, which promote the breakdown of seed storage reserves. This, in turn, enhances energy utilization via mitochondrial processes, accelerating seedling growth and strengthening structural development (Glick, 2012).

Under ambient conditions, although dry weight of peanut seedlings declined over time, the rate of reduction in seeds coated with *E. kobei* at 10^7 CFU/mL and *A. radiobacter* at 10^7 CFU/mL was lower than in non-coated seeds. This suggests that coating seeds with IAA-producing bacteria mitigates environmental stress effects and enhances root nutrient uptake, resulting in seedlings that retain higher dry weight than non-coated seeds (Bashan & de-Bashan, 2010). Furthermore, *E. kobei* at 10^7 CFU/mL and *A. radiobacter* at 10^7 CFU/mL can regulate the expression of genes associated with seed and seedling development, increasing resistance to adverse environmental conditions by reducing oxidative stress-induced damage during seed storage (Mugnier & Jung, 1985; Solano et al., 2008). These findings suggest that coating seeds with bacterial isolates, particularly *A. radiobacter* at 10^7 CFU/mL, effectively maintains peanut seed quality by enhancing seedling growth and vigor over extended storage periods.

4. Conclusions

The experimental results demonstrated that during the 4-month seed storage period under both controlled and ambient conditions, coating peanut seeds with *A. radiobacter* at 10^7 CFU/mL significantly increased germination percentage, germination speed, and reduced mean germination time compared to non-coated seeds. This effect was particularly evident in controlled conditions, where coated seeds demonstrated superior maintenance of seed quality and seedling growth throughout the storage duration. Additionally, *A. radiobacter* at 10^7 CFU/mL enhanced the shoot and root length as well as the shoot and root dry weight of peanut seeds, which significantly outperformed the non-coated seeds. The mechanism underlying this improvement is attributed to IAA's roles in stimulating cell division, promoting tissue expansion, mobilizing seed storage reserves, and enhancing nutrient uptake, which collectively contribute to faster and stronger seedling development. Based on these findings, the coated seeds with *A. radiobacter* at 10^7 CFU/mL exhibited extended longevity, maintaining high seed quality and vigor for up to 4 months under both storage conditions. Consequently, seed coating with *A. radiobacter* at 10^7 CFU/mL proves to be an effective approach for extending the storage longevity of peanut seeds, improving germination quality, and enhancing seedling vigor.

However, seed coating with *E. kobei* at 10^7 CFU/mL exhibited notable improvements in germination parameters, including germination percentage, speed of germination, and mean germination time, along with seedling growth performance in comparison to non-coated seeds under both controlled and ambient conditions. These findings suggest that *E. kobei* at 10^7 CFU/mL may also serve as a potential bacterial candidate for enhancing peanut seed quality through biological seed coating methods.

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6. Authors' Contributions

Saralee Prommayakul was primarily responsible for conducting the experiments, collecting and recording data, performing statistical analyses, and drafting the manuscript. Jiraporn Inthasan provided critical guidance on data recording and statistical interpretation, and contributed to the review and validation of the manuscript content. Vassana Viroonrat contributed to data management consultation, assisted in the interpretation of results, and participated in the final review of the manuscript. Jakkrapong Kangsopa conceptualized and designed the experimental framework, supervised the overall data collection and statistical interpretation, critically reviewed the manuscript, and managed the submission process to the journal. All authors have read and approved the final version of the manuscript.

7. Conflicts of Interest

The authors declare that there is no conflict of interest.

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