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Effect of *Bacillus* spp. seed dressing on germination and growth of rice cultivar Khao Dawk Mali 105

Supatra Khamriang[1, 2,*], Wantana Sinsiri[1], Naris Sinsiri[1] and Suwita Saepaisan[3]

[1]Faculty of Technology, Mahasarakham University, Mahasarakham, Thailand
[2]Faculty of Agriculture, Ubon Ratchathani University, Ubon Ratchathani, Thailand
[3]Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand
[*]Corresponding author: supatra.kh@ubu.ac.th.

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Abstract

In Thailand, the Khao Dawk Mali 105 rice cultivar is particularly susceptible to dirty panicle disease, which severely impacts yield and quality. Farmers often resort to intensive fungicide use to manage this issue, leading to suboptimal grain quality and potential health risks from chemical residues. Consequently, there is a need for sustainable alternatives to improve seed quality and disease resistance. This research explores the potential of *Bacillus* spp. as a biocontrol agent and plant growth promoter to address these challenges. The study employs a Completely Randomized Design (CRD) with four replications and six treatments, including one control (water) and five *Bacillus* isolates (SR-SHP19, SK-KH3, BR-BD12, SK-KK4, UBU-1). These *Bacillus* isolates used to coat Khao Dawk Mali 105 rice seeds. Experimental conditions were maintained at 28 ± 2°C. The results indicate that *Bacillus* treatments significantly improved seedling vigor, particularly in 7-day-old seedlings. Isolates SK-KK4 and SK-KH3 were especially effective, enhancing biochemical composition with higher levels of sugars, free amino acids, chlorophyll, and carotenoids compared to the control. While overall germination rates were not significantly different, the improvements in seedling vigor and biochemical properties are promising for rice cultivation. *Bacillus* spp. seed dressing offers a viable alternative to chemical fungicides, potentially reducing environmental impacts and health risks. *Bacillus* treatments promote stronger and more resilient plants capable of withstanding disease and environmental stress, aligning with sustainable agricultural practices. Further research is necessary to optimize *Bacillus* spp. applications in field conditions which will ultimately contribute to improved rice production and food security.

Keywords: Rice seed, *Bacillus* spp., Seeding vigor index, Seed dressing

1. Introduction

As a primary grain crop, rice constitutes the principal daily source of food energy for over 50% of the global population. Rice production in Thailand is affected by a number of limitations. In particular quality organic rice seeds requires comprehensive research on seed technology and organic seed production, which currently lacks diversity. Much of the research efforts have concentrated on seedling development until the acquisition of high-quality seeds. Subsequent endeavors aim to enhance seed quality, encompassing improvements in germination rates and overall seed quality enhancement. However, there are limitations to augmenting the value of Thai organic seeds [1]. For instance, farmers encounter various problems while growing rice including low yields and high production costs. Moreover, the quality of rice seeds has deteriorated, and currently, rice seeds are at least twice as expensive as rice for consumption [2]. The lack of good quality seeds is a major issue for rice production in Thailand. The demand for rice seeds is approximately 1 million tons per year, but the government produces only about 100,000 tons per year to meet farmers' needs [3]. Most farmers prefer to collect seeds for personal use in locally procured containers, such as storage sacks and barns [4], or they purchase them from nearby stores. The quality of seeds used for planting varies based on storage conditions, leading to uneven or deteriorated seed quality in the next planting cycle, resulting in decreased rice production. However, if high-quality seeds are planted, they are likely to yield a high harvest [5].

Thailand faces issues with dirty panicle disease in rice (*Oryza sativa* L.) which is a significant challenge for rice production. This disease poses a substantial risk to rice cultivation, often resulting in significant yield losses, while severe infections can cause yield losses and reduced seed quality such as the germination percentage [6]. Fungal pathogens infecting the panicle contribute to a reduction in both the quality and quantity of rice yield. Organic seed quality for rice production in Thailand remains relatively constrained due to the intricate nature of cultivation processes, necessitating comprehensive research on seed technology and organic seed production, which currently lacks diversity. Much of the research efforts concentrate on seedling development until acquiring high-quality seeds. Subsequent endeavors aim to enhance seed quality, encompassing improvements in germination rates and overall seed quality enhancement [7]. Farmers frequently resort to intensively spraying fungicide in paddy fields, which results in harvested rice grains for inferior quality and prone to breakage. In light of these challenges, it is crucial to enhance seed quality to increase its value, resulting in improved germination and uniform seedling growth.

Due to the issues that have occurred in the last few years, there have been reports demonstrating that various bacterial species, including *Bacillus*, could act as biocontrol agents and plant growth-promoting agents [8, 9]. Plant growth-promoting bacteria are known to enhance plant growth by a variety of mechanisms; for example, by the production of phytohormones (indole-3-acetic acid (IAA), gibberellic acid, and ethylene) [10]. A diverse group of biocontrol agents (BCAs) exist in nature; among which, bacteria are considered ideal owing to their rapid growth, ease of handling, and aggressive colonizing characteristics. *Bacillus* spp. in particular are a useful biocontrol candidate since they are gram-positive, ensure endospore formation, and are tolerant to heat and desiccation, which are good features required for storage and field application [11].

Given the aforementioned significance, enhancing seed quality through biological methods using environmentally friendly bacteria reduces the reliance on chemicals and helps improve the resistance of rice seedlings to diseases and pests, resulting in stronger plants and better growth. Moreover, utilizing bacteria in agriculture promotes sustainable farming practices. It opens opportunities for the study and development of new bacterial strains with high efficacy to promote plant growth, which is highly beneficial for organic farming. Therefore, this research aims to study the effects of *Bacillus* isolates SR-SHP19, SK-KH3, BR-BD12, SK-KK4, and UBU-1 on the germination, growth, and biochemical composition of Khao Dawk Mali 105 rice seedlings under laboratory conditions.

2. Materials and methods

2.1 Location

The experiment was conducted from June 2021 to March 2023 at the National Biological Control Research Center Laboratory, Department of Agronomy, Faculty of Agriculture, Ubon Ratchathani University (UBU), Thailand.

2.2 Experimental material

2.2.1 Preparation of pure cultures of Bacillus spp.

Inoculation of pure cultures of *Bacillus* spp. were obtained from the Department of Plant Pathology and Microbiology, Faculty of Agriculture, Northeast Biotechnology Research Center, Ubon Ratchathani University, Thailand, A total of 531 isolates were selected from cultivation Khao Dawk Mali 105 rice growing area in the lower northeastern region in Thailand. Their efficacy in managing rice spotted disease was assessed using the Dual Culture Bioassay method. Antagonistic bacteria were acquired in both Cell Suspension (CS) and filtrate forms. Bacterial Cell-free Culture Filtrate (CFF) proved effective in inhibiting the fungal hyphae responsible for rice spotted disease [12]. which have been preliminarily tested for controlling dirty panicle on rice disease using five isolates namely *Bacillus* isolate SR-SHP19, *Bacillus* isolate SK-KH3, *Bacillus* isolate BR-BD12, *Bacillus* isolate SK-KK4, and *Bacillus* isolate UBU-1 which were cultured in Trypticase soy agar (TSA) medium and then incubated at 30℃ for 48 hours. Subsequently, the cultures were centrifuged, and the supernatant was discarded. The pellet were resuspended in sterile distilled water at a concentration of 5 milliliters per dish. Sterile glass rods were used to streak the colonies of *Bacillus* spp. onto the dishes. Each isolate was mixed with water to adjust the concentration to 10^8 colony-forming units per milliliter. This suspension was then mixed with 1% Methylcellulose, 25 g in volume, to coat the rice seeds for the next step.

2.2.2 Rice seed preparation for testing

The rice seeds of Khao Dawk Mali 105 variety, harvested from farmer's fields, were initially cleaned by soaking in distilled water to remove floating debris. They were then further cleaned by treating with 10% sodium hypochlorite solution for 3 minutes. Subsequently, they were rinsed with sterile distilled water and boiled twice for 3 minutes each time.

2.2.3 Sand preparation for testing

Sand was sifted through a 2-millimeter sieve and then rinsed with tap water. Subsequently, the sand was sterilized by baking at 180℃ for 3 hour. After sterilization, the sand was placed in a plastic container, with a weight of 300 g.

2.2.4 Effect of Bacillus spp. on seed germination and seedling growth of Khao Dawk Mali 105 rice at 7 and 14 days after sowing.

The experimental design employed a Completely Randomized Design (CRD) with four replications and six treatments, including T1 Control (water), T2 *Bacillus* isolate SR-SHP19, T3 *Bacillus* isolate SK-KH3, T4 *Bacillus* isolate BR-BD12, T5 *Bacillus* isolate SK-KK4 and T6 *Bacillus* isolate UBU-1. Take the prepared *Bacillus* spp. to coat the rice seeds at a ratio of 25 g to 50 g. Then lay out the seeds coated with *Bacillus* spp. to dry in a sterile cabinet for 24 hours. Subsequently, plant the treated seeds in plastic boxes, with 50 seeds per box, at a depth of 0.5 centimeters below the sand surface. Place the plastic boxes at a temperature of 28±2℃, with 13 hours of daylight per day. Water the seeds every 2 days and record the results after 7 and 14 days.

2.2.5 Testing the effect of Bacillus on the growth and development of rice seedlings at the laboratory level, 21 days after sowing.

Prepare *Bacillus* spp. as in experiment 2.2.4 Keep the plastic box was at a temperature of 28+2℃ with 13 hours of daylight per day, water every two days, record the results at the end of 21 days, and then analyze the biochemical results of the rice seedlings for the amount of total free amino acid content, sugar estimation and total chlorophyll and carotenoid content.

2.3 Data recording

The investigation of the germination percentage seeding vigor index, energy of germination, speed of germination, and the fresh weight of the shoot and root (g) involved cleaning and weighing the shoots and roots of the rice plants, weighing the dry weight of shoot and root (g) after collecting the samples thoroughly washing them with water, and then baking them at 70℃ for 72 hours. The samples were weighed to determine the dry weight at 7 and 14 days after sowing. Additionally, analysis includes measuring root and shoot length (cm), fresh and dry weight of root (g), fresh and dry weight of shoots (g). Analyses of sugar estimation (mg/g FW), free amino acid (mg/g FW), total chlorophyll (μg/g FW) and total carotenoids (μg/g FW) were conducted at 21 days after sowing.

Germination results are calculated using a formula adapted from ISTA [13], and the resulting ratio is then multiplied by 100 [9]:

$$\text{Percentage normal seedling} = \frac{\text{normal seedling germinated}}{\text{Total number of seeds per replicate}} \times 100$$

Seedling vigor index [14]

$$\text{Seedling vigor index} = \text{germination (\%)} \times (\text{Shoot length} + \text{Root length})$$

Energy of germination [15]

$$\text{germination Energy} = \text{Percentage of seeds germination at 72 h}$$

Speed of germination [16]

$$\text{Speed of germination (\%)} = \frac{\text{Number of seeds germination at 72 h}}{\text{Number of seeds germination at 168 h}} \times 100$$

All data was subjected to statistical analysis for significance using analysis of variance (ANOVA) to assess the variance in means among the experimental groups. Differences between means were further evaluated using Duncan's New Multiple Range Test (DMRT) at a 95% confidence level with the Statistix version 9.0 program.

3. Results

3.1 Effect of Bacillus spp. on seed germination and seedling growth of Khao Dawk Mali 105 rice at 7 and 14 days after sowing

The results of seed coating with *Bacillus* spp. at 7 and 14 days after planting, showed that the germination of Khao Dawk Mali 105 under laboratory conditions exhibited no statistical difference from the control. All control sets demonstrated germination percentages in the range of 85-91 and 89-93 percent, surpassing the standard for rice seed germination percentages. The Rice Department [16] stipulates that rice seeds must have germination rates greater than or equal to 80 percent. This finding is in line with Tidarat et al. [17] which investigated the germination rates of Khao Dawk Mali 105 rice seeds treated with *Bacillus subtilis* in laboratory conditions, indicating no statistically significant difference compared to untreated seeds. Regarding the germination index of rice seeds, all treatments had significantly different effects on the germination index of rice seedlings. Seed coating with *Bacillus* spp. did not affect the germination index under laboratory conditions of Khao Dawk Mali 105 (Table 1) just like the experiment of Jakkrapong et al. [18] which experimented with coating lettuce seeds with *Pseudomonas fluorescens* and *Bacillus subtilis*, and found that germination rates showed no statistically significant variance under controlled laboratory conditions.

Table 1 Effect of *Bacillus* spp. on seed germination and germination index of Khao Dawk Mali 105 rice at 7 and 14 days after sowing.

Treatments	Seed germination (%)		Germination Index	
	7 DAS*	14 DAS	7 DAS	14 DAS
Control (water)	91.50	92.50	6.53	3.30
Bacillus isolate SR-SHP19	88.50	92.50	6.32	3.30
Bacillus isolate SK-KH3	89.00	93.50	6.36	3.34
Bacillus isolate BR-BD12	86.50	89.50	6.18	3.19
Bacillus isolate SK-KK4	91.00	93.00	6.50	3.32
Bacillus isolate UBU-1	85.50	89.50	6.53	3.19
F-test	ns	ns	ns	ns
C.V. (%)	5.89	3.94	5.87	3.99

The same letters or without letters within the same column do not differ significantly, NS= Non Significant

Upon examining the energy of germination, diverse experimental outcomes were observed across all employed methods compared to the control set. Each isolate of *Bacillus* exerted varying effects on the germination energy of rice seeds. Correspondingly, in line with the seedling growth index, the Seeding Vigor Index indicated that the *Bacillus* isolate SK-KK4 notably influenced the index, registering at 1831.80, surpassing that of the control set. However, no statistically significant differences were observed for the speed of germination (Table 2).

Table 2 Speed of germination, germination percentage seedling vigor index and energy of germination of Khao Dawk Mali 105 rice after coating seed whit *Bacillus* spp. at 14 days after sowing.

Treatments	Energy of germination	Speed germination	Seeding vigor index
Control (water)	74.50^{a}	81.12^{a}	1495.10^{d}
Bacillus isolate SR-SHP19	73.00^{a}	82.62^{a}	1685.90^{bc}
Bacillus isolate SK-KH3	72.00^{a}	81.07^{a}	1801.40^{ab}
Bacillus isolate BR-BD12	70.00^{a}	81.11^{a}	1551.80^{cd}
Bacillus isolate SK-KK4	64.50^{ab}	70.95^{ab}	1831.80^{a}
Bacillus isolate UBU-1	51.00^{b}	65.46^{b}	1350.10^{e}
F-test	*	ns	**
C.V. (%)	15.04	14.21	5.64

The same letters or without letters within the same column do not differ significantly, *= Significant at 5% level of probability, **= Significant at 1% level of probability, NS= Non Significant

The evaluation of rice seedling growth over 14 days involved measuring shoot height, root height, fresh weight, and total dry weight of roots and shoots. The data, presented in Table 4 reveals statistically significant differences in mean root height and root fresh weight between those treated with *Bacillus* and those untreated. *Bacillus* isolates SR-SHP19, SK-KH*3*, BR-BD12, and SK-KK4 influenced root length, ranging from 9.25 to 11.26 centimeters, and had varying effects on root fresh weight, with the highest ranging from 2.00 to 2.28 g per plant, surpassing the control set without *Bacillus* treatment. Regarding root height and dry weight, as well as the fresh and dry weight of the plants, no differences were observed, although they exhibited statistical significance (Table 3)

Table 3 Effect of *Bacillus* spp. on seed germination and seedling growth of Khao Dawk Mali 105 rice at 14 days after sowing.

Treatments	Growth parameters (cm)		Root weight (g./plant)		Shoot weight (g./plant)	
	Root height	Shoot height	Fresh weight	Dry weight	Fresh weight	Dry weight
Control (water)	8.39^{c}	7.95	1.79^{c}	0.315	0.123	0.021
Bacillus isolate SR-SHP19	9.25^{bc}	9.80	2.15^{b}	0.285	0.147	0.019
Bacillus isolate SK-KH3	11.66^{a}	8.58	2.05^{b}	0.315	0.138	0.021
Bacillus isolate BR-BD12	9.27^{bc}	8.67	2.10^{b}	0.39	0.134	0.026
Bacillus isolate SK-KK4	10.73^{ab}	9.40	2.28^{a}	0.375	0.132	0.025
Bacillus isolate UBU-1	8.57^{c}	8.85	1.91^{c}	0.345	0.152	0.023
F-test	**	ns	**	ns	ns	ns
C.V. (%)	10.67	9.15	4.01	3.56	8.71	12.31

The same letters or without letters within the same column do not differ significantly, **= Significant at 1% level of probability, NS= Non Significant

3.2 Testing the effect of Bacillus spp. on the growth and development of rice seedlings at the laboratory level, 21 days after planting.

The effects of *Bacillus* spp. on the growth and development of rice seedlings over a 21-day period post-planting include stem length, root length, and the quantity of fresh and dry weights of both roots and stems. The data is reported in Table 5, revealing that *Bacillus* isolates SK-KH3 and SK-KK4 had the greatest impact on root length, measuring 16.37 and 16.08 centimeters, respectively. Moreover, compared to the control group, it was found that *Bacillus* isolate SR-SHP19 had the most pronounced effect on stem length, measuring 14.70 centimeters (Table 4). In the evaluation of the fresh weight of rice seedlings at the 21-day post-planting stage, *Bacillus* isolate SK-KK4 significantly influenced root weight at 3.32 g, surpassing the control group. However, no statistically significant differences were observed in the measurement of dry weight, mirroring the findings of the fresh weight of the stems. Nonetheless, when assessing the fresh weight of the rice stems, *Bacillus* isolate SR-SHP19 exerted the most substantial influence at 0.15 g, surpassing that of the control.

Table 4 Effect of *Bacillus* spp. on seed germination and seedling growth of Khao Dawk Mali 105 rice at 21 days after sowing.

Treatments	Growth parameters (cm)		Root weight (g./plant)		Shoot weight (g./plant)		Seed germination
	Root height	Shoot height	Fresh weight	Dry weight	Fresh weight	Dry weight	
Control (water)	13.26^{bc}	11.84^{e}	2.69^{e}	0.16	0.38	0.02^{b}	92.50
Bacillus isolate SR-SHP19	13.68^{b}	14.70^{a}	3.18^{b}	0.17	0.35	0.15^{a}	92.50
Bacillus isolate SK-KH3	16.37^{a}	12.93^{d}	3.06^{c}	0.18	0.42	0.05^{b}	93.50
Bacillus isolate BR-BD12	13.89^{b}	13.03^{d}	3.07^{c}	0.21	0.48	0.04^{b}	89.50
Bacillus isolate SK-KK4	16.08^{a}	14.23^{b}	3.32^{a}	0.19	0.39	0.04^{b}	93.00
Bacillus isolate UBU-1	12.84^{c}	13.29^{c}	2.91^{d}	0.18	0.42	0.04^{b}	89.50
F-test	**	**	**	ns	ns	*	ns
C.V. (%)	3.30	0.97	1.73	2.58	4.73	8.05	3.94

The same letters or without letters within the same column do not differ significantly, *= Significant at 5% level of probability, **= Significant at 1% level of probability, NS= Non Significant

Table 5 illustrates the effect of *Bacillus* spp. cells on the biochemical parameters of rice seedlings, which shows that *Bacillus* isolate BR-BD12 had a significant impact. Consequently, the highest sugar content was significantly different and higher than the control, with values of 6.62 and 3.88 mg/g FW, respectively. Additionally, *Bacillus* isolate SK-KK4 affected the total free amino acid content, which was significantly different from the control at 2.83 mg/g FW. This finding aligns with the report of Jayaraj et al. [19] which indicates that *Bacillus subtilis* AUBS1 induced the accumulation of thaumatin and β-1-3-glucanases [10]. Furthermore, *Bacillus* isolate SK-KH3 had an effect on chlorophyll content, with the highest recorded level being 42.66 µg/g FW, which is significantly higher than the control. Moreover, regarding carotenoid levels, *Bacillus* isolate SK-KH3 exhibited the greatest effect, with a recorded amount of 168 µg/g FW, statistically significantly different from the control.

Table 5 Effect of *Bacillus* spp. on the biochemical parameters of Khao Dawk Mali 105 rice at 21 days after sowing.

Treatments	Sugar Estimation (mg/g FW)	Free Amino acid (mg/g FW)	Total Chlorophyll (µg/g FW)	Carotenoids (µg/g FW)
Control (water)	3.88[d]	1.45[c]	30.69[d]	0.52[d]
Bacillus isolate SR-SHP19	4.76[c]	1.34[c]	39.05[bc]	0.75[cd]
Bacillus isolate SK-KH3	5.78[b]	2.51[ab]	42.66[a]	1.20[b]
Bacillus isolate BR-BD12	6.62[a]	1.79[bc]	35.79[c]	1.68[a]
Bacillus isolate SK-KK4	3.99[d]	2.83[a]	42.02[ab]	0.91[c]
Bacillus isolate UBU-1	4.32[cd]	1.22[c]	36.2[c]	1.23[b]
F-test	**	**	**	**
C.V. (%)	6.23	16.19	3.83	9.16

The same letters or without letters within the same column do not differ significantly, **= Significant at 1% level of probability

4. Discussion

This study finds that under laboratory conditions, Khao Dawk Mali 105 seeds coated with *Bacillus* spp. at 7 and 14 days after planting exhibited no statistical difference from the control. All control sets demonstrated germination percentages in the range of 85-93 percent, surpassing the standard for rice seed germination percentages. The Rice Department [16] stipulates that rice seeds must have germination rates greater than or equal to 80 percent. This is in line with Tidarat et al. [17] which investigated the germination rates of Khao Dawk Mali 105 rice seeds treated with *Bacillus subtilis* in laboratory conditions, indicating no statistically significant difference compared to untreated seeds. As for the germination index of rice seeds, it was found that all treatments had significantly different effects on the germination index of rice seedlings. Seed coating with *Bacillus* did not affect the germination index under laboratory conditions of Khao Dawk Mali 105, just like the experiment of Jakkrapong et al. [17]. which experimented with coating lettuce seeds with *Pseudomonas fluorescens* and *Bacillus subtilis* and found no statistically significant variance for germination rates under controlled laboratory conditions. This is a good trend for promoting the growth of rice seedlings, as Xie et al. [22] reported that *Bacillus subtilis* species are bacteria that interact with plant root zones, exhibiting plant growth-promoting properties (PGPR, plant growth-promoting rhizobacteria). *B. subtilis* cells are effective in promoting the growth of rice seedlings more than the control group that received Hoagland solution nutrients. To demonstrate that the enhanced growth originated from alterations in the chemical composition within the cells, the measurement of biochemical substances within the rice cells were measured. The experiment's findings revealed an increase in the levels of total sugar, free amino acids, total chlorophyll content, and the quantity of carotenoids in rice cells treated with *Bacillus subtilis*. During the stable growth phase (stationary phase), *Bacillus* sp. demonstrates the capability to produce Polyglutamic acid (PGA), a substance released by bacteria outside the cell, resulting in heightened viscosity [23]. This could be attributed to the presence of L-glutamate in PGA, serving as an excellent nitrogen source for plant growth. When combined with rice, which inherently contains a high nitrogen content, it fosters growth promotion. Furthermore, nitrogen is a vital constituent of numerous molecular substances in plants and plays a pivotal role in both the growth and quality of rice production, particularly as a component of chlorophyll. [24] which is a green substance found in various parts of plants. The results of the study show that when rice received PGA, the amount of chlorophyll increased dramatically. This is clearly different from rice seedlings that did not receive PGA because plants synthesize carbohydrates from photosynthesis. Chlorophyll is a molecule that absorbs light energy. A low photosynthesis rate will cause plants to grow slowly. Glycine and glutamic acid are basic substances in the process of tissue formation and chlorophyll synthesis. The creation of chlorophyll begins with the use of glutamate, which is a solid substance. The plant will convert glutamate into 5-aminolevulinic acid (ALA), which then changes to chlorophyll. However, it has been reported that wheat seedlings grown under normal conditions respond more to photosynthesis than to the use of amino acids. [25, 26] Amino acids play an important role in promoting and stimulating strong plant roots. Consequently, plants experience an increased rate of photosynthesis and develop a robust root system, enabling them to quickly absorb nutrients into the stems and leaves. As a result, the plant accumulates food for various parts of its structure, breaking it down into energy for growth. When the rate of photosynthesis increases, plants accumulate sugar and more amino acids within cell tissues. [24]

Bacillus spp. treatments demonstrate a significant impact, resulting in noteworthy increase in sugar content, total free amino acids, total chlorophyll, and carotenoids compared to the control. The growth-promoting abilities of *Bacillus subtilis* in crop plants are well established. In this study, the treated plots showed increased grain yield compared to untreated plots. Similarly, Kumar et al. [28] reported that the paddy rhizospheric isolates of *Bacillus* produced indole-3-acetic acid and were capable of solubilizing soil organic phosphates in addition to reducing the severity of sheath blight disease in paddy [29].

5. Conclusion

Bacillus spp. was not found to exert any noticeable influence on seedling height, plant length, fresh weight, and dry weight. However, a comparison with the control set revealed that *Bacillus* spp. positively impacted the germination percentage and led to an increase in root length compared to the control. Additionally, significant enhancements were observed in terms of sugar content, total amino acid content, chlorophyll content, and carotenoids when compared to the control set. Based on the outcomes of this experiment, it is concluded that *Bacillus* spp. holds potential for stimulating the growth and development of Rice Cultivar Khao Dawk Mali 105 seedlings. Nevertheless, further research is warranted to explore the effects of such substances on rice growth at greenhouse and plantation levels.

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