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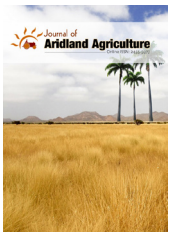

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Assessing the efficacy of exogenous auxin and indole-3-acetic acid-producing bacteria in promoting growth of *Vigna radiata* (L.) R. Wilczek. in used engine oil-contaminated soil

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ABSTRACT

This study aimed to compare the activity of synthetic phytohormones (naphthalene-acetic acid (NAA) and indole-butyric acid (IBA)) and indole-3-acetic acid-producing bacteria (*Bacillus stercoris* B.PNR1, *Bacillus stercoris* B.PNR2, and *Paenibacillus* sp. BSR1-1) to stimulate the growth of mung bean (*Vigna radiata* (L.) R. Wilczek) planted in used engine oil-contaminated soil. The results revealed that both synthetic phytohormones significantly stimulated mung bean growth in soil contaminated with used engine oil. Mung beans treated with IBA or NAA showed a 76.25±2.63 and 81.25±3.50% survival rate, compared to a survival rate of only 66.25% in the absence of seed treatment. Furthermore, the shoot length (20.54-20.84 cm), shoot fresh weight (0.276-0.279 g), shoot dry weight (0.025-0.025 g), and shoot dry weight per pot (0.190-0.204 g) of mung beans grown in used engine oil with seeds treated with IBA or NAA were higher than those of mung bean seedlings without seed treatment. In contrast with the shoot, NAA did not stimulate the growth of the mung bean root as much as the shoot. *Paenibacillus* sp. BSR1-1 tended to promote root growth to the best extent because the root length (7.61±0.25 cm) of mung bean grown in used engine oil was higher than that grown with receiving other bacterial isolates or NAA and IBA; however, root dry weight per pot of mung bean with seed treated with *Paenibacillus* sp. BSR1-1 was not significantly different from those treated with IBA and NAA.

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INTRODUCTION

Engine oil is used for multiple purposes, including lubrication, heat and power transfer, engine part protection, component cleaning, and various other applications (Raţiu *et al.*, 2022). When engine oils are contaminated in the soil, they have the potential to be hazardous to humans and the environment because the used engine oil contains various hazardous compounds (polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and different metallic elements) (Stout *et al.*, 2018; Raţiu *et al.*, 2022). Soil contamination with used engine oil significantly impedes agricultural activities in affected regions, primarily attributable to the physical properties and chemical composition of used engine oil (Ahamefule *et al.*, 2017; Raţiu *et al.*, 2022). Oil contamination in soil usually decreases nutrient availability and limits nutrient uptake (da Correa *et al.*, 2022). Also, used engine oil was toxic to the seed germination process, such as decreasing total germination, increasing time to germinate, and weakening seedling health (Ahamefule *et al.*, 2017; Osuagwu *et al.*, 2017; Oyedeji *et al.*, 2018). This toxic impact from used engine oil was observed in several crops, including *Vigna radiata* (L.) Wilczek, *Phaselous vulgaris* (Ngozi *et al.*, 2017), *Glycine max* L. Merril (Ahamefule *et al.*, 2017), *Arachis hypogea* (L.), *Zea mays* (L.) (Osuagwu *et al.*, 2017), and *Vigna unguiculata* L. (Walp.) (Oyedeji *et al.*, 2018).

Exogenous phytohormones and plant growth-promoting bacteria are employed to enhance plant growth and mitigate

chemical toxicity, particularly in plants cultivated under conditions of chemical stress, such as exposure to heavy metals and petroleum hydrocarbons (Šípošová *et al.*, 2021; Rafique *et al.*, 2023; Zheng *et al.*, 2023). The alleviated mechanism of exogenous phytohormones to adverse impact of abiotic stress encompasses to reduce of reactive oxygen species, increase antioxidant enzyme activity, and enhance plant biomass and photosynthetic efficiency (Šípošová *et al.*, 2021; Zhou *et al.*, 2024). The mechanisms of plant growth-promoting bacteria and exogenous phytohormones to stimulate plant growth were similar. For instance, *Rhodococcus erythropolis* CDEL254, which possesses the capacity to produce indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC)-deaminase, ammonia, and phosphate solubilization, has been shown to enhance the growth of *Lolium perenne* L. cv. Pearlgreen shoots. Furthermore, it aided in removing petroleum hydrocarbons from the soil, surpassing the growth and remediation potential observed in soil without bacterial inoculation (Ptaszek *et al.*, 2020). *Bacillus subtilis* strain PM32Y was a plant growth-promoting bacterium that could tolerate petroleum hydrocarbons, and this bacterium possesses the activity of ACC-deaminase enzyme. These bacteria demonstrated the ability to enhance the growth of *Medicago sativa* L. Additionally, when *Medicago sativa* L. was cultivated and inoculated with *Bacillus subtilis* strain PM32Y, it effectively promoted the removal of petroleum hydrocarbons from crude oil-contaminated soil (Rafique *et al.*, 2023).

The exogenous phytohormone used in this study belongs to the auxin group, which includes IBA and NAA. Both IBA and NAA have been used to promote plant growth and relieve the adverse effects from abiotic stress in plants. For instance, IBA has been observed to alleviate the toxicity of lead and cadmium in *Acutodesmus obliquus* and *Zea mays* (L.), respectively (Piotrowska-Niczyporuk *et al.*, 2020; Šípošová *et al.*, 2021). Meanwhile, NAA has demonstrated the ability to stimulate shoot growth in *Vigna sinensis* (L.) when cultivated in endosulfan-sulfate-contaminated sand (Somtrakoon & Kruatrachue, 2014). In this study, *Paenibacillus* sp. BSR1-1 (accession number OQ255601), *Bacillus stercoris* B.PNR1 (OP592212), and *Bacillus stercoris* B.PNR2 (OP592213) were used as representative plant-growth promoting bacteria. *Paenibacillus* sp. BSR1-1 has been demonstrated to have the ability to promote growth of *Arachis hypogaea* (L.) when cultivated under low water irrigation (Somtrakoon *et al.*, 2022), while *Bacillus stercoris* B. PNR1 and PNR2 have been shown to stimulate the growth of *Solanum lycopersicum* L. (Pengproh *et al.*, 2023). Research on using exogenous phytohormones and IAA-producing bacteria to stimulate plant growth in used engine oil-contaminated soil has been relatively limited. Thus, the exogenous phytohormones (IBA and NAA) and IAA-producing bacteria on growth enhancement of mung beans planted in used engine oil-contaminated soil were assessed.

MATERIALS AND METHODS

Bacterial Inoculum Preparation

The bacteria used were *Bacillus stercoris* B.PNR1 (accession number OP592212), *Bacillus stercoris* B.PNR2 (accession number OP592213), and *Paenibacillus* sp. BSR1-1 (accession number OQ255601). The 24-hour culture of each bacterial isolate was used to prepare the bacterial suspension for the IAA production test and the pot experiment. The details of bacterial preparation were described in Somtrakoon *et al.* (2024). Briefly, each bacterial isolate grown on the nutrient agar was suspended in 0.85% NaCl to achieve an optical density of 0.5 at 660 nm using a spectrophotometer.

IAA Production

The ability of *Bacillus stercoris* B.PNR1, *Bacillus stercoris* B.PNR2, and *Paenibacillus* sp. BSR1-1 to produce IAA was tested using a 24-hour culture of bacterial inoculum. One mL of bacterial inoculum was transferred to a tube containing 4 mL of nutrient broth + 2 g/L of tryptophan + 0%, 1%, 2%, 3%, and 4% NaCl. The mixture was incubated at 37 °C for 48 hours, and IAA production was determined as described by Ahmad *et al.* (2008). To test the effect of pH and temperature on IAA production, the medium and bacterial inoculum were prepared using a method similar to that described for the effect of NaCl. However, for the pH test, the medium consisted of nutrient broth + 2 g/L of tryptophan, with pH adjusted to 4, 5, 6, 7, 8, and 9. For the temperature test, the medium was nutrient broth + 2 g/L of tryptophan and incubated at 30 °C and 35 °C.

Soil Preparation

The soil was collected from Ban Don Wiang Chan, Kantharawichai District, Maha Sarakham Province, Thailand, and used engine oil was obtained from automobile repair shops near Mahasarakham University. The soil was air-dried in sunlight and sieved to remove stones and debris, and then it was sterilized in an autoclave. The soil contaminated with used engine oil was prepared by dissolving it in dichloromethane and pouring it into the soil to achieve a final concentration of used engine oil at 2% (w/w). Leave it at room temperature to allow the dichloromethane to evaporate. The control soil for the experiment was prepared similarly, except that only dichloromethane was added to the soil, and it was left to evaporate before use. The soil was divided into seedling trays, with each seedling tray containing 80 g of dry soil.

Seed Preparation

Mung bean seed preparation was performed by selecting complete seeds, which were surface-sterilized according to the methods described in Somtrakoon *et al.* (2024). Subsequently, the seeds were soaked in a solution of indole butyric acid (Sigma-Aldrich, China) or 1-naphthalene acetic acid (Sigma-Aldrich, India) at a concentration of 0.1 mg/L and soaked in the cell suspension of *Bacillus stercoris* B.PNR1, *Bacillus stercoris* B.PNR2, and *Paenibacillus* sp. BSR1-1 for 12 hours. Alternatively, they were soaked in distilled water for use as control experiments. After soaking, mung bean seeds were planted in soil contaminated with used engine oil, and the day of transplantation marked the beginning of the experiment (Day 0).

Pot Experiment

The experiment pot used a factorial completely randomized design with 2x6 factors. The first factor was the used engine oil concentration (0% and 2% w/w), and the second factor was the seed stimulants, including sterilized distilled water, 0.1 mg/L IBA, 0.1 mg/L NAA, and the cell suspensions of *Bacillus stercoris* B.PNR1, *Bacillus stercoris* B.PNR2, and *Paenibacillus* sp. BSR1-1. The seeds were transferred and planted in seedling trays for 10 days. Each seedling tray contained 10 seeds. Each seedling tray represented one replicate, and the experiment was conducted with eight replicates. At the end of the experiment, shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, and chlorophyll content in the shoots were determined. Chlorophyll content was determined and calculated using the methods and formulas described by Lichtenthaler (1987) and Sardoei and Rahbarian (2014).

Statistical Analysis

Two-way analysis of variance (ANOVA) was used to test the difference between treatments, and the least significant difference (LSD) test was used for pairwise comparison. IBM SPSS Statistics Version 29 for Windows was utilized.

RESULTS AND DISCUSSIONS

Sodium Chloride, pH, and Temperature on IAA Production

IAA production is one of the most important characteristics of plant growth-promoting bacteria. IAA production by microorganisms depends on several factors, including pH, temperature, sodium chloride, incubation time, tryptophan concentration, and bacterial strain (Dasri *et al.*, 2014; Chandra *et al.*, 2018; Myo *et al.*, 2019; Xa *et al.*, 2022). This study found that *Bacillus stercoris* B.PNR1 and *Bacillus stercoris* B.PNR2 could produce IAA over a more comprehensive pH range. The suitable pH for IAA production by these two isolates was pH 6-9 and pH 5-8, respectively, with IAA concentrations ranging from 27.43 to 32.30 µg/mL and 31.51 to 36.82 µg/mL, respectively. *Paenibacillus* sp. BSR1-1 appeared to produce IAA ranging from 29.86 to 60.88 µg/mL between pH 5-8 (Table 1). In addition, the optimum temperature for IAA production by *Bacillus stercoris* B.PNR1, *Bacillus stercoris* B.PNR2, and *Paenibacillus* sp. BSR1-1 was between 30-35 °C. According to another study, a bacterial isolate DPY-05 produced the highest IAA at a temperature range of 30-37 °C, and the production of IAA usually decreased at temperatures of 20 °C and 45 °C (Dasri *et al.*, 2014). Moreover, the three bacterial isolates (*Bacillus stercoris* B.PNR1, *Bacillus stercoris* B.PNR2, and *Paenibacillus* sp. BSR1-1) used in this study were able to produce a high amount of IAA in the presence of low concentrations of sodium chloride (0 and 1% sodium chloride). The concentration of IAA production by *Bacillus stercoris* B.PNR1, *Bacillus stercoris* B.PNR2, and *Paenibacillus* sp. BSR1-1 in the presence of 0-1% NaCl ranges from 25.02-33.72, 33.37-34.96, and 35.43-48.47 µg/mL, respectively (Table 1), and high concentrations of sodium chloride (3-4% NaCl) typically reduced IAA production. Producing IAA under a wide range of physical environments is a necessary characteristic of plant growth-promoting bacteria to be used as a bioinoculant under field conditions, in which bacteria probably face unfavorable conditions for the growth and expression of plant growth-promoting activity (Lopes *et al.*, 2021). The other plant growth-promoting bacteria that produced IAA under various environmental conditions include *Paenibacillus cineris* TP-1.4, which produced more than 5 µg/mL of IAA at a pH range from 2 to 10. *Bacillus megaterium* MQ-2.5 produced more than 20 µg/mL of IAA under pH 7-9, and *Klebsiella pneumoniae* OM-17.2 produced more than 40 µg/mL of IAA under pH 7-9. Additionally, *Klebsiella pneumoniae* OM-17.2 produced IAA within a wide range of sodium chloride concentrations between 0 and 5% (Xa *et al.*, 2022). In addition to these factors, another important aspect used to study IAA production by microorganisms is culture formulation, which is relevant for future work because it is the best criterion to consider for large-scale IAA production (Myo *et al.*, 2019; Bunsangiam *et al.*, 2021).

Table 1: The pH, temperature, and NaCl concentration on IAA production by *Bacillus stercoris* B.PNR1, *Bacillus stercoris* B.PNR2, and *Paenibacillus* sp. BSR1-1

Factors	IAA (µg/mL)		
	Bacillus stercoris B.PNR1	*Bacillus stercoris* B.PNR2	*Paenibacillus* sp. BSR1-1
pH			
4	1.51 ± 0.53^{Bc}	17.63 ± 6.76^{Ac}	13.16 ± 1.15^{Ae}
5	17.92 ± 0.84^{Cb}	34.15 ± 0.89^{Ba}	51.11 ± 1.31^{Ab}
6	27.43 ± 0.31^{Ca}	36.82 ± 0.35^{Ba}	60.88 ± 2.13^{Aa}
7	31.57 ± 0.36^{Ba}	36.50 ± 1.01^{Ba}	49.63 ± 1.74^{Ab}
8	32.30 ± 1.32^{Aa}	31.51 ± 0.53^{Aa}	29.86 ± 0.64^{Ac}
9	30.06 ± 0.69^{Aa}	24.76 ± 2.19^{ABb}	19.17 ± 0.39^{Bd}
Temperature			
30	47.28 ± 3.19^{Aa}	42.09 ± 3.62^{Aa}	26.90 ± 0.86^{Ba}
35	34.76 ± 1.94^{Ab}	30.03 ± 1.66^{Ab}	29.98 ± 3.50^{Aa}
NaCl			
0	33.72 ± 6.54^{Ba}	33.37 ± 5.57^{Bab}	48.47 ± 1.35^{Aa}
1	25.02 ± 0.32^{Aab}	34.96 ± 4.25^{Aa}	35.43 ± 9.80^{Ab}
2	19.46 ± 1.20^{Ab}	20.53 ± 8.91^{Ab}	23.14 ± 2.52^{Ab}
3	12.03 ± 2.87^{Ab}	17.77 ± 1.56^{Ab}	17.98 ± 0.90^{Ac}
4	10.21 ± 2.88^{Ab}	17.31 ± 0.33^{Ab}	16.90 ± 0.76^{Ac}

Denotes: Different lower-case letters in the same column indicate significant differences between each factor of the same isolate ($P<0.05$), and different capital letters in the same row indicate significant differences between isolates in the same factor ($P<0.05$)

Growth of Mung Beans Grown in Used Engine Oil-Contaminated Soil

The activity of the IAA-producing bacteria used in this study was compared to stimulating the growth of mung beans grown under used engine oil-contaminated soil with synthetic auxin, including NAA and IBA. Used engine oil has been reported to be toxic to mung beans (Ngozi *et al.*, 2017). This study reported that in the absence of seed treatment, 2% (w/w) contamination of used engine oil did not decrease the percentage of survival and growth of mung bean shoots and roots (Table 2). The survival percentage, shoot dry weight, and root length of mung beans without seed treatment when grown in non-contaminated soil

Table 2: Growth of shoot and root of mung bean seedlings grown in non-contaminated and used-engine oil-contaminated soil

Treatment	Survival (%)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot dry weight/ Pot (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Root dry weight/ Pot (g)
Non-Contaminated									
DW	63.75 ± 5.65^{Ab}	15.41 ± 0.64^{Bb}	0.221 ± 0.008^{Bab}	0.023 ± 0.001^{Aa}	0.151 ± 0.014^{Ab}	5.23 ± 0.25^{Aab}	0.067 ± 0.003^{Bb}	0.017 ± 0.001^{Ba}	0.128 ± 0.022^{Aa}
B.PNR1	48.75 ± 2.95^{Bc}	13.71 ± 0.94^{Bc}	0.176 ± 0.013^{Bb}	0.018 ± 0.001^{Ab}	0.091 ± 0.010^{Bc}	5.80 ± 0.30^{Bb}	0.066 ± 0.005^{Bb}	0.013 ± 0.001^{Bb}	0.069 ± 0.006^{Bb}
B.PNR2	32.50 ± 5.90^{Ad}	12.08 ± 0.94^{Ac}	0.185 ± 0.008^{Ab}	0.019 ± 0.007^{Ab}	0.063 ± 0.013^{Ac}	4.90 ± 0.33^{Bb}	0.054 ± 0.006^{Bb}	0.011 ± 0.001^{Bb}	0.042 ± 0.009^{Bb}
BSR1-1	46.25 ± 7.78^{Bcd}	14.51 ± 0.79^{Bbc}	0.191 ± 0.010^{Bb}	0.020 ± 0.001^{Aab}	0.096 ± 0.016^{Bc}	5.14 ± 0.25^{Bab}	0.058 ± 0.005^{Bb}	0.012 ± 0.001^{Bb}	0.096 ± 0.016^{Bb}
IBA	56.25 ± 8.00^{Bbc}	17.59 ± 0.61^{Ba}	0.233 ± 0.012^{Ba}	0.023 ± 0.001^{Aa}	0.131 ± 0.023^{Bbc}	4.66 ± 0.21^{Bb}	0.080 ± 0.006^{Bab}	0.016 ± 0.001^{Ba}	0.103 ± 0.019^{Ba}
NAA	82.50 ± 3.66^{Aa}	16.81 ± 0.50^{Bab}	0.234 ± 0.009^{Ba}	0.023 ± 0.001^{Aa}	0.190 ± 0.010^{Aa}	4.76 ± 0.17^{Bb}	0.087 ± 0.005^{Aa}	0.016 ± 0.001^{Ba}	0.131 ± 0.009^{Aa}
2% Used-Engine Oil									
DW	66.25 ± 5.65^{Ab}	18.06 ± 0.53^{Ab}	0.262 ± 0.011^{Aab}	0.024 ± 0.001^{Aab}	0.161 ± 0.017^{Ab}	5.80 ± 0.19^{Ac}	0.116 ± 0.006^{Aa}	0.023 ± 0.001^{Aa}	0.152 ± 0.018^{Aa}
B.PNR1	63.75 ± 5.96^{Ab}	18.74 ± 0.44^{Ab}	0.232 ± 0.011^{Ab}	0.020 ± 0.001^{Ab}	0.131 ± 0.014^{Ab}	6.76 ± 0.22^{Ab}	0.108 ± 0.006^{Ab}	0.020 ± 0.001^{Ab}	0.131 ± 0.014^{Aa}
B.PNR2	38.75 ± 5.49^{Ac}	13.79 ± 1.09^{Ac}	0.183 ± 0.014^{Ac}	0.016 ± 0.001^{Ac}	0.064 ± 0.009^{Ac}	7.08 ± 0.26^{Aab}	0.104 ± 0.008^{Ab}	0.022 ± 0.002^{Aab}	0.085 ± 0.016^{Ab}
BSR1-1	66.25 ± 1.83^{Ab}	20.76 ± 0.52^{Aa}	0.250 ± 0.011^{Ab}	0.022 ± 0.001^{Ab}	0.145 ± 0.007^{Ab}	7.61 ± 0.25^{Aa}	0.118 ± 0.006^{Aa}	0.020 ± 0.001^{Ab}	0.126 ± 0.008^{Aa}
IBA	76.25 ± 2.63^{Aab}	20.54 ± 0.32^{Aa}	0.276 ± 0.009^{Aab}	0.025 ± 0.001^{Aa}	0.190 ± 0.010^{Aab}	5.99 ± 0.20^{Ac}	0.129 ± 0.005^{Aa}	0.021 ± 0.001^{Ab}	0.160 ± 0.012^{Aa}
NAA	81.25 ± 3.50^{Aa}	20.84 ± 0.34^{Aa}	0.279 ± 0.009^{Aa}	0.025 ± 0.001^{Aa}	0.204 ± 0.011^{Aa}	6.19 ± 0.18^{Abc}	0.097 ± 0.005^{Ab}	0.020 ± 0.001^{Ab}	0.169 ± 0.007^{Aa}
Soil x Method	ns	**	ns	ns	ns	**	**	**	ns

Denotes: Different lower-case letters in the same column indicate significant differences between seed treatment methods under the same soil condition ($P<0.05$), and different capital letters in the same column indicate significant differences between the soil conditions of the same seed treatment methods ($P<0.05$). Abbreviations: ns denotes not significant differences ($P>0.05$), **denotes highly statistically significant differences ($P<0.01$), B.PNR1, B.PNR2, and BSR1-1 denote *Bacillus stercoris* B.PNR1, *Bacillus stercoris* B.PNR2, and *Paenibacillus* sp. BSR1-1, respectively

and used engine oil-contaminated soil were 66.25-63.75%, 0.023-0.024 g, and 5.23-5.80 cm, respectively (Table 2). Moreover, the shoot length (18.06±0.53 cm), shoot fresh weight (0.262±0.011 g), and root dry weight (0.023±0.001 g) of mung beans grown in used engine oil-contaminated soil without seed treatments were significantly higher than those grown in non-contaminated soil (Table 2). This contrasts with our previous study (Somtrakoon *et al.*, 2010), which revealed that used engine oil was toxic to the growth of shoots and roots of mung beans grown in soil contaminated with 1-3% of used engine oil. However, Chouychai *et al.* (2007) reported that 1-3 % of used engine oil-contaminated soil only decreased the root length of mung beans but did not affect the germination rate or shoot length. The variability in phytotoxicity of used engine oil may be attributed to several factors, including the environmental testing conditions (Boutin *et al.*, 2010), the source of the tested seed, the source of used engine oil collection, and the chemical components in used engine oils. These chemical components of used engine oil can vary depending on the lifetime of use and the objective of use (Hönig *et al.*, 2020; Grimmig *et al.*, 2021). Suppose the volatile fraction was still found to be low in the engine oil, and the toxicity test was done in an open environment. In that case, the phytotoxicity should be lower than in engine oil with a high volatile fraction, and the toxicity test was done in a closed environment (Henner *et al.*, 1999; Mackinnon & Duncan, 2013). However, this study did not test with used engine oil and seed from the same source as the previous study. The exact component and level of each component found in used engine oil should be determined in the future for consistent results.

However, the toxic effect of used engine oil in this study appeared on chlorophyll content because the content of chlorophyll a, chlorophyll b, and total chlorophyll in the shoot of mung bean grown without seed treatment was lower than that of mung bean grown in non-contaminated soil (Table 3). In general, the toxic effects of used engine oil have been reported to be toxic to the chlorophyll of plants. The toxic effect on chlorophyll can ultimately reduce root and shoot growth because it is a synthetic apparatus of plants and plays an essential role in plant productivity (Baruah *et al.*, 2014). For example, exposure to 0.2% (v/v) used motor oil decreased the chlorophyll a content of *Pseudokirchneriella subcapitata* after exposure for only 96 hours (Ramadass *et al.*, 2015). Used engine oil at 1-5% (w/w) also decreased the total chlorophyll content of *Amaranthus hybridus* L. (Odjegba *et al.*, 2002). Decreasing chlorophyll content in the leaves of plants may be caused by the inhibition of chlorophyll synthesis by the aliphatic, aromatic, and high molecular weight compounds (Baruah *et al.*, 2014). The presence of petroleum-derived products in the soil inhibits plant growth by reducing nutrient and water uptake (Rusin *et al.*, 2015). Decreasing leaf area and percentage of seed germination in *Phaseolus vulgaris*, *Zea mays* L., *Solanum lycopersium*, and *Sorghum saccharatum* have been reported when these plants were cultivated in 2% used engine oil (Ngozi *et al.*, 2017). Used engine oil is usually more toxic than unused motor oil because used oil usually contains high concentrations of toxic components, including polycyclic aromatic hydrocarbons, compared to unused oil (Wong & Wang, 2001).

Figure 1 expresses the characteristics of mung bean seedlings grown under non-contaminated soil and used engine oil-contaminated soil with various seed stimulants. The application of seed treatment found that the use of IBA and NAA was more effective in promoting mung bean growth in both used engine oil-contaminated and non-contaminated soil compared to using plant growth-promoting bacteria (*Bacillus stercoris* B. PNR1, *Bacillus stercoris* B. PNR2, and *Paenibacillus* sp. BSR1-1). The survival percentage of mung beans grown in soil contaminated with used engine oil was 76.25 % and 81.25 %, respectively, when the seeds were treated with IBA and NAA, compared to only 66.25 % for seeds without treatment. The growth of mung beans in used engine oil-contaminated soil was increased when seeds were treated with NAA and IBA, which increased the shoot dry weight and root dry weight. Among three bacterial isolates, *Paenibacillus* sp. BSR1-1 seems to stimulate the growth of mung beans grown under used engine oil-contaminated soil to a greater extent than the other two plant growth-promoting bacteria. Shoot and root length of mung beans with seed treated with *Paenibacillus* sp. BSR1-1 were 20.76±0.52 and 7.61±0.25 cm, respectively (Table 2). The ability to stimulate shoot length was similar to IBA and NAA, but seeds treated with *Paenibacillus* sp. BSR1-1 could promote root length to a greater extent than IBA and NAA. Seeds treated with IBA and NAA also gave shoot dry weight/pot (0.190-0.204 g) when mung beans grown in used engine oil contaminated soil more than all isolates of plant growth-promoting bacteria (0.064-0.145 g) or without seed treatment (0.161 g).

In this study, synthetic phytohormones seem to promote the growth of mung beans better than plant growth-promoting bacteria. This may be due to the activity of plant growth-promoting bacteria may retard by unsuitable environmental conditions in the soil because unfavorable environmental conditions in soil may border plant metabolisms and change the composition of root exudates, which affect the interaction between plant and microorganisms (Lopes *et al.*, 2021). Moreover, this study used seed inoculation to stimulate the growth of mung beans without re-inoculation; this may not contain the number of plant growth-promoting bacteria suitable for stimulating the growth of the plants. Re-inoculation is usually recommended to keep suitable cell densities to stimulate plant growth (Lopes *et al.*, 2021). When used at specific concentrations of synthetic auxins, IBA and NAA are expected to promote more remarkable growth in mung beans than in seeds treated with bacterial cell suspension. This is because

Figure 1: Characteristics of mung bean seedlings grown on a) non-contaminated soil and b) used engine oil-contaminated soil with seeds immersed in distilled water, *Bacillus stercoris* B.PNR1, *Bacillus stercoris* B.PNR2, and *Paenibacillus* sp. BSR1-1, IBA, and NAA solution

Table 3: Chlorophyll content in the shoot of mung bean seedlings grown in non-contaminated and used-engine oil-contaminated soil

	Chlorophyll a (μg/g FW)	Chlorophyll b (μg/g FW)	Total chlorophyll (μg/g FW)
Non-Contaminated			
Distilled water	103.51 ± 12.99^{Aab}	99.78 ± 10.14^{Aa}	203.30 ± 6.38^{Aa}
B.PNR1	117.18 ± 7.97^{Aa}	55.45 ± 5.84^{Ab}	172.63 ± 5.95^{Ab}
B.PNR2	97.52 ± 6.07^{Ab}	18.72 ± 2.26^{Bc}	116.24 ± 8.00^{Ac}
BSR1-1	56.7 ± 5.11^{Ac}	61.5 ± 7.51^{Ab}	118.19 ± 4.54^{Ac}
IBA	55.1 ± 1.64^{Ac}	27.73 ± 9.16^{Bc}	82.84 ± 9.23^{Ad}
NAA	44.72 ± 1.69^{Ac}	56.46 ± 6.98^{Ab}	101.19 ± 8.66^{Bcd}
2% Used-Engine Oil			
Distilled water	37.77 ± 1.49^{Bab}	52.75 ± 1.57^{Bb}	90.52 ± 2.87^{Bb}
B.PNR1	40.50 ± 4.98^{Bab}	57.71 ± 8.16^{Aab}	98.22 ± 12.91^{Bb}
B.PNR2	50.12 ± 3.54^{Ba}	70.65 ± 3.28^{Aab}	120.78 ± 6.53^{Aab}
BSR1-1	48.97 ± 2.06^{Aa}	59.44 ± 10.60^{Aab}	108.41 ± 12.64^{Aab}
IBA	32.14 ± 5.61^{Bb}	61.87 ± 5.95^{Aab}	94.01 ± 1.91^{Ab}
NAA	54.49 ± 1.99^{Aa}	74.94 ± 2.47^{Aa}	129.43 ± 4.33^{Aa}
Soil x Method	**	**	**

Denotes: Different lower-case letters in the same column indicate significant differences between seed treatment methods under the same soil condition ($P<0.05$), and different capital letters in the same column indicate significant differences between the soil conditions of the same seed treatment methods ($P<0.05$). Abbreviations: **denotes highly statistically significant ($P<0.01$), B.PNR1, B.PNR2, and BSR1-1 denote *Bacillus stercoris* B.PNR1, *Bacillus stercoris* B.PNR2, and *Paenibacillus* sp. BSR1-1 respectively

they stimulate plant growth immediately, without waiting for the roots to be colonized before becoming active.

CONCLUSIONS

The factor affecting IAA production by microorganisms is an important consideration when planning to use plant growth-promoting microorganisms in real-world situations. Although the IAA-producing bacteria used in this study could produce IAA optimally in the laboratory, their activity to promote mung bean growth was still limited compared to the synthetic auxin. More experiments and additional physical factors, as well as the reinoculation of inoculum, should be investigated in future work to support the potential use of IAA-producing inoculum in agricultural applications.

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