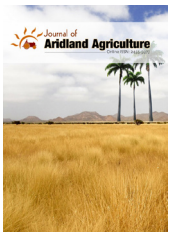
Journal of Aridland Agriculture 2024, 10: 20-27
doi: 10.25081/jaa.2024.v10.8614
https://updatepublishing.com/journal/index.php/jaa

Research Article



ISSN: 2455-9377

Potential of *Bacillus stercoris* B.PNR2 to stimulate growth of rice and waxy corn under atrazine-contaminated soil

Khanitta Somtrakoon[1]*, Preamkamon Prasertsom[1], Aphidech Sangdee[1], Rattana Pengproh[2], Waraporn Chouychai[3]

[1]Department of Biology, Faculty of Science, Mahasarakham University, Kantharawichai, Maha Sarakham, 44150, Thailand, [2]Department of Biology, Faculty of Science, Buriram Rajabhat University, Buriram Province, 31000, Thailand, [3]Department of Science, Faculty of Science and Technology, Nakhonsawan Rajabhat University, Nakhon Sawan, 60000, Thailand

ABSTRACT

The presence of atrazine residue in agricultural soil may affect crop growth and the activity of plant growth-promoting bacteria. Therefore, this study investigated the impact of atrazine contamination on indole-3-acetic acid (IAA) production by *Bacillus stercoris* B.PNR2. Subsequently, the ability of *B. stercoris* B.PNR2 to stimulate the seedling growth of rice cultivars RD6 and Leum Pua, as well as the waxy corn cultivar Muang Tam, under atrazine contamination, was determined. The results showed that *B. stercoris* B.PNR2 produced IAA under various atrazine concentrations, and atrazine was not toxic to *B. stercoris* B.PNR2 cells. Atrazine at 20 mg/kg of soil did not affect the shoot and root dry weight of rice cultivars RD6 and Leum Pua, as well as the waxy corn cultivar Muang Tam grown in atrazine-contaminated soil without receiving a bacterial inoculum. The application of *B. stercoris* B.PNR2 did not stimulate the germination and growth of any of the plants used in this study. The application of *B. stercoris* B.PNR2 decreased the shoot and root dry weight of waxy corn grown under atrazine-contaminated soil. Additionally, the chlorophyll b and total chlorophyll content in rice cultivar RD6, grown under atrazine-contaminated soil, decreased to only 162.6 ± 4.2 and 616.0 ± 55.8 µg/g fresh weight, which was related to the increase in proline content to 343.6 ± 41.6 µg/g fresh weight. In conclusion, it can be stated that soaking seeds with *B. stercoris* B.PNR2 was not an appropriate means of inoculation to stimulate the growth of plants in this study.

Received: September 25, 2023
Revised: February 29, 2024
Accepted: March 03, 2024
Published: March 13, 2024

***Corresponding author:**
Khanitta Somtrakoon
E-mail: khanitta.s@msu.ac.th

KEYWORDS: Atrazine, *Bacillus stercoris*, Corn, Plant growth-promoting bacteria, Phytotoxicity, Rice

INTRODUCTION

Atrazine (6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine) is a herbicide that is commonly applied to manage unwanted vegetation in various crop farming practices, including cornfields and sugarcane fields (Singh *et al.*, 2018). Atrazine has been heavily used for more than 50 years, with approximately 70,000-90,000 tons of atrazine being used globally per year (He *et al.*, 2019). Toxicity of atrazine has been reported in non-target living organisms, including mammals, aquatic animals, and non-target plants (Nwani *et al.*, 2010; Singh *et al.*, 2018; He *et al.*, 2019). The toxicity of atrazine in animals usually leads to the induction of oxidative stress (Nwani *et al.*, 2010) and endocrine disruption (Singh *et al.*, 2018). The extensive use of atrazine results in its contamination of soil and surface water (Singh *et al.*, 2018; He *et al.*, 2019). Atrazine has also been detected in paddy fields at levels ranging from 0.002 to 0.015 mg/kg (Arora *et al.*, 2014). The contamination of atrazine in paddy fields can occur through rice-corn intercropping (Riyanto *et al.*, 2021) and rice-corn rotation (Breidenbach *et al.*, 2017).

Contamination by several herbicides, such as atrazine, in agricultural soil affects the growth of non-target plant production (Zhang *et al.*, 2017). In general, atrazine exerts toxicity on plants by inhibiting photosynthesis and inducing oxidative stress. Symptoms such as chlorosis, a reduction in biomass, and impeded growth are typically observed in plants exposed to atrazine (Sánchez *et al.*, 2017; Singh *et al.*, 2018; Rostami *et al.*, 2021). Furthermore, the residues of atrazine and other herbicides in the soil continue to exert a toxic effect on crops planted after atrazine application in agriculture. For instance, the contamination of isoxaflutole along with atrazine led to a decrease in plant stand, biomass, and yield in soybeans by 7%, 49%, and 42%, respectively (Soltani *et al.*, 2011). The presence of atrazine residue in corn fields resulted in a decreased potato yield when planted in Serra do Salitre, Minas Gerais

State, Brazil (Reis *et al.*, 2018). Sunflower yield was significantly reduced by atrazine residues at concentrations of 3.0 and 6.0 kg/hectare 60 days after atrazine application (Brighenti *et al.*, 2002). Atrazine, at a concentration of 50 mg/kg, reduced the number of secondary metabolites and the plant dry weight of *Andrographis paniculata* (Tripathi *et al.*, 2021). Atrazine is also toxic to rice, an economically important crop in Thailand and other countries in Southeast Asia (Promkhambut *et al.*, 2023). The residues of atrazine in paddy soil resulting from rice-corn rotation (Breidenbach *et al.*, 2017) may lead to a decrease in rice yield due to the reported toxicity of atrazine to rice (Ma *et al.*, 2019). For instance, exposure to 0.2 mg/L of atrazine for 6 days resulted in a decrease in shoot length, root length, dry weight, and chlorophyll content in rice (Ma *et al.*, 2019).

Utilizing plant growth-promoting bacteria (PGPB) is one way to enhance plant growth for agricultural purposes because PGPB can increase crop yield, improve soil fertility, and help plants withstand biotic and abiotic stresses (Ramakrishna *et al.*, 2019). The application of PGPB has been successful in promoting the growth of plants under atrazine contamination. For example, the bacterial strain CIMAP-7, with the ability to solubilize phosphate, produce IAA, generate siderophores, produce 1-aminocyclopropane-1-carboxylate deaminase, and tolerate 1 mg/L of atrazine, can reduce atrazine levels in the soil. It also supports the growth of *A. paniculata* in contaminated soil, as indicated by the increase in total chlorophyll, carotenoid, and protein contents in the plant. Furthermore, bacterial strain CIMAP-7 mitigated stress in *A. paniculata* by reducing stress enzymes, proline content, and malondialdehyde accumulation in plants grown under atrazine contamination (Tripathi *et al.*, 2021). *Pseudomonas chlororaphis* PAS18, possessing the ability to produce IAA, could alleviate the toxic effects of atrazine at a concentration of 20 mg/kg on *Pennisetum americanum*. Additionally, *P. chlororaphis* PAS18 alleviated atrazine-induced stress by enhancing photosystem II repair and antioxidant defenses in the plants (Jiang *et al.*, 2020).

However, the ability of *B. stercoris* B.PNR2 (accession number OP592213) to stimulate the growth of rice and corn under atrazine contamination has not been tested previously (Pengproh *et al.*, 2023). Thus, the objective of this study was to investigate the stimulation of growth in rice RD6, rice Leum Pua, and the waxy corn cultivar Muang Tam under atrazine contamination. The ability of *B. stercoris* B.PNR2 to produce IAA under atrazine stress was also determined because the achievement of PGPB used as inoculants in agriculture is influenced by toxic contaminants in the soil (Lopes *et al.*, 2021). If plant growth-promoting bacteria can grow and exert plant growth-promoting activity under unfavorable environmental conditions, they will be useful as microbial inoculants in agriculture.

MATERIALS AND METHODS

Preparation of Bacterial Suspension

Bacillus stercoris B.PNR2 was cultured on nutrient agar and incubated at 37 °C for 24 hours. The bacterial suspension was prepared by pouring 2-3 mL of 0.85% (w/v) sodium chloride onto the bacterial colony, scraping the bacterial colony with an inoculating loop, and transferring the bacterial suspension into a sterile tube. The absorbance of the bacterial suspension was determined with a spectrophotometer at a wavelength of 600 nm and adjusted to 0.5 using 0.85% (w/v) sodium chloride. The viable bacterial count, determined by counting on nutrient agar, was approximately 10^7 cfu/mL. This bacterial suspension was prepared for use in the following experiment.

Toxicity of Atrazine on *Bacillus stercoris* B.PNR2

The nutrient broth was prepared and mixed with atrazine to achieve final concentrations of atrazine (containing 80% w/w of 6-chloro-N2-ethyl-N4-isopropyl-1,3,5-triazine-2,4-diamine, Weethong brand, imported by V.C.S. Agro Chem Company Limited, Thailand) at 0, 10, 20, and 30 mg/L. One milliliter of a 24 hour culture of bacterial inoculum (with an optical density of 0.5 at a wavelength of 600 nm) was transferred into 4 mL of the test medium and incubated at 37 °C for 48 hours. Bacterial growth was determined by measuring the optical density at a wavelength of 600 nm. The effect of atrazine concentration (0-80 mg/L) on IAA production was investigated by preparing a cell suspension of *B. stercoris* B.PNR2 as described above and inoculating it into nutrient broth containing 2 g/L of tryptophan. The culture was then incubated at 37 °C for 36 hours. IAA production was quantified using the method outlined by Ahmad *et al.* (2008).

Preparation of Seeds

Seeds of the rice cultivars RD6 and Leum-Pua, as well as the waxy corn cultivar Muang Tam, were received from a farmer in Nadi Sub-District, Suwannakhuha District, Nong Bua Lamphu Province. The seeds were surface-sterilized by soaking them in 0.3% sodium hypochlorite for 5 minutes, two times, and then rinsed by immersing them in sterilized distilled water for 1 minute, three times. Afterward, the rice seeds were soaked in a bacterial cell suspension of *B. stercoris* B.PNR2 (with an optical density of 0.5 at a wavelength of 600 nm) for 24 hours before use.

Preparation of Atrazine-contaminated Soil

The soil was sterilized by autoclaving it at 121 °C for 15 minutes, repeated three times before use. Commercial atrazine (Weethong brand, imported by V.C.S. Agro Chem Company Limited, Thailand) was used to prepare atrazine-contaminated soil. The commercial atrazine powder was thoroughly mixed with the dry soil to achieve a final atrazine concentration of 20 mg/kg, while soil without atrazine served as the control. This soil was then subdivided into trays, with each tray containing 100 g of soil for use in the next experiment.

Experimental Pot

Seeds of the rice cultivars RD6 and Leum-Pua, as well as the waxy corn cultivar Muang Tam, were planted in trays containing atrazine-contaminated soil and non-contaminated soil. The

experiment was conducted using a completely randomized design with one factor. The atrazine concentration in the soil was either 0 or 20 mg/kg, and the method used to stimulate plant growth was seeds receiving either distilled water or a bacterial cell suspension. There were four treatments per plant, with each treatment conducted in five replicates, and each replicate contained 15 plants. The seeds were left to germinate and grow in the experimental tray under the greenhouse for 12 days (for waxy corn) and 14 days (for rice). Seed germination was observed daily, and a seed was considered to have germinated when its coleoptile was observed to emerge. The percentage of seed germination and the mean time of germination were also calculated using the formulas provided by Chuanren *et al.* (2004). Seedling growth was assessed on the 14th day for rice and the 12th day for waxy corn in the experiment. This assessment included measurements of shoot growth (length, fresh weight, dry weight), root growth (length, fresh weight, dry weight), carotenoid content, chlorophyll a content, chlorophyll b content, total chlorophyll content, and proline content. Carotenoid, chlorophyll a, chlorophyll b, and total chlorophyll were determined using the methods described in Lichtenthaler (1987) and Sardoei and Rahbarian (2014). The equation described in Lichtenthaler (1987) was employed to calculate each photosynthetic pigment in the plant's shoot. Proline content in the plant's shoot was analyzed according to the methods outlined by Bates *et al.* (1973) and Ábrahám *et al.* (2010). Soil samples from the five replicates of each treatment were combined and sent for analysis of their physical and chemical characteristics at The Soil Fertilizer Environment Academic Development Project, Department of Soil Science, Faculty of Agriculture, Kasetsart University, Thailand.

Statistical Analysis

A one-way ANOVA and the Duncan's test were used for variance analysis and pairwise comparison, respectively.

RESULTS AND DISCUSSION

Atrazine affects the production of IAA by *B. stercoris* B.PNR2

There are several reports of isolating IAA-producing bacteria (James & Singh, 2018). However, the successful application of plant growth-promoting bacteria in agricultural sites may be limited by the toxic contaminants present in the soil (Lopes *et al.*, 2021). The results of this study revealed that atrazine was not toxic to *B. stercoris* B.PNR2 cells because the optical density of the bacterial cell suspension, when cultured in a nutrient broth mixed with 10, 20, and 30 mg/L of atrazine, did not differ significantly from that of the bacterial cell suspension grown in nutrient broth without atrazine addition (Table 1) ($P > 0.05$). Several atrazine-tolerant bacteria have been isolated from agricultural areas, and they are more tolerant to atrazine than the *B. stercoris* B.PNR2 used in this study. For example, *Pseudomonas* sp. strains AACB, *Pseudomonas* sp. strains TTLB, and *Arthrobacter* strains PPKB, which were isolated from the epiphytic roots of *Acorus calamus*, *Typha latifolia*, and *Phragmites karka*, respectively, tolerated 50 mg/L of atrazine (James & Singh, 2018). *B. licheniformis* ATLJ-5 and *B. megaterium* ATLJ-11, isolated from woodland soil, were found to tolerate 1,000 mg/L of atrazine (Zhu *et al.*, 2019). Moreover, *B. stercoris* B.PNR2 can produce IAA when exposed to 5-80 mg/L of atrazine, and the ability to produce IAA by this bacterial strain did not significantly differ between non-contaminated and atrazine-contaminated conditions (Table 2). This suggests the potential use of *B. stercoris* B.PNR2 as a microbial inoculant in agricultural areas with atrazine residues.

Mean Germination Time and Germination Percentage

Atrazine did not decrease the germination percentage in rice cultivars RD6, Leum Pua, and the waxy corn cultivar Muang Tam. The germination percentages of rice RD6, rice Leum Pua, and waxy corn cultivar Muang Tam grown in atrazine-contaminated soil without receiving *B. stercoris* B.PNR2 were 98.7±1.33%, 89.3±5.81%, and 80.0±7.60%, respectively. These values did not significantly differ from those grown in non-contaminated soil (Table 3). Soaking seeds with the bacterial suspension of *B. stercoris* B.PNR2 did not increase the germination percentage of these plants grown in both atrazine-contaminated and non-contaminated soil (Table 3). Additionally, atrazine did not prolong the mean germination time of rice cultivars RD6, Leum Pua, and the waxy corn cultivar Muang Tam. The application of the bacterial suspension of *B. stercoris* B.PNR2 did not accelerate the mean germination time or increase the germination percentage of rice cultivar Leum Pua and waxy corn cultivar Muang Tam under both non-contaminated and atrazine-contaminated conditions.

Even though plant growth-promoting activity, especially auxin production by bacteria, is usually related to seed germination (Fiodor *et al.*, 2023), this effect did not occur in our study. For example, plant growth-promoting bacteria, including *Serratia marcescens* AF8I1, *P. fluorescens* AF8I4, *P. putida*

Table 1: Growth of *Bacillus stercoris* B.PNR2 under 0-30 mg/L of atrazine for 48 hours (Mean±SE)

Atrazine concentration (mg/L)	Optical density (660 nm)
0	0.25±0.004[a]
10	0.26±0.012[a]
20	0.26±0.014[a]
30	0.28±0.008[a]

Different lowercase letters show significant differences ($P < 0.05$) between the levels of atrazine concentration

Table 2: Indole-3-acetic acid production by *Bacillus stercoris* B.PNR2 under various atrazine concentrations (Mean±SE)

Atrazine concentration (mg/L)	IAA (µg/mL)
0	3.9±0.69[a]
5	3.8±0.37[a]
10	3.3±0.16[a]
20	3.5±0.24[a]
40	3.7±0.40[a]
80	3.8±0.41[a]

Different lowercase letters indicate significant differences ($P < 0.05$) between the levels of atrazine concentration

Table 3: Mean germination time and germination percentage of rice cultivar RD6, rice cultivar Leum Pua, and waxy corn cultivar Muang Tam grown in non-contaminated and atrazine-contaminated soil (Mean±SE)

Treatment	Mean germination time (days)	Germination percentage
Rice cultivar RD6		
Non-contaminated + Water	6.3±0.12[a]	98.7±1.33[a]
Non-contaminated + B.PNR2	6.3±0.17[a]	92.0±3.89[a]
Atrazine + Water	5.8±0.13[b]	98.7±1.33[a]
Atrazine + B.PNR2	5.5±0.18[b]	94.7±1.33[a]
Rice cultivar Leum Pua		
Non-contaminated + Water	7.3±0.38[a]	89.3±4.52[a]
Non-contaminated + B.PNR2	7.8±0.60[a]	86.7±5.96[a]
Atrazine + Water	6.6±0.25[a]	89.3±5.81[a]
Atrazine + B.PNR2	6.8±0.28[a]	94.7±3.26[a]
Waxy corn cultivar Muang Tam		
Non-contaminated + Water	3.2±0.15[a]	76.0±6.18[a]
Non-contaminated + B.PNR2	3.4±0.25[a]	68.0±4.42[a]
Atrazine + Water	3.0±0.15[a]	84.0±4.52[a]
Atrazine + B.PNR2	3.1±0.03[a]	80.0±7.60[a]

Different lowercase letters indicate significant differences ($P < 0.05$) between treatments within the same plant species. B.PNR2 refers to *Bacillus stercoris* B.PNR2

AF1I1, *Klebsiella aerogenes* AF3II, and *B. cereus* AF8II1, could increase relative seed germination in carrots (Fiodor *et al.*, 2023). Plant growth-promoting bacteria isolate T22 also increased germination in maize seeds (Rudolph *et al.*, 2015). However, exposure to atrazine usually inhibits the germination percentage and seedling growth of crops (Burhan & Shaukat, 2000). For example, atrazine at concentrations of 10-100 mg/L inhibited seed germination in pearl millet, wheat, turnip, carrot, corn, and mustard (Burhan & Shaukat, 2000). The possibility of seed germination inhibition may be explained by the inhibition of enzyme amylase activity, thereby repressing the movement of reducing sugar (Burhan & Shaukat, 2000). However, seed germination inhibition was not observed in our study. Surprisingly, atrazine contamination in the soil accelerated the mean germination time in rice cultivar RD6 to only 5.5-5.8 days. However, the mean germination time of rice cultivar RD6 did not significantly differ between seeds soaked in water or a bacterial suspension of *B. stercoris* B.PNR2 (Table 3).

Root Growth and Shoot Growth

The response of rice cultivar RD6, rice cultivar Leum Pua, and waxy corn cultivar Muang Tam to atrazine and the bacterial suspension of *B. stercoris* B.PNR2 was different. Waxy corn tended to tolerate atrazine because the shoot and root dry weight of plants grown under atrazine-contaminated soil was higher than that of those grown in non-contaminated conditions. Based on plant dry weight, the immersion of waxy corn seeds in the bacterial suspension of *B. stercoris* B.PNR2 decreased the growth of waxy corn seedlings in atrazine-contaminated soil. The shoot dry weight and root dry weight of waxy corn were only 0.11±0.005 and 0.03±0.003 g, respectively, when planted in atrazine-contaminated soil (Table 4). Decreases in shoot dry weight and root dry weight were not observed in the waxy corn cultivar Muang Tam when planted in non-contaminated soil and treated with *B. stercoris* B.PNR2. Tolerance to atrazine in corn was not surprising because atrazine was widely used in corn fields, and it was metabolized into non-toxic metabolites in corn tissue (Cherif *et al.*, 2001). There were three possible reactions to metabolize atrazine into the non-toxic product, including a 2-hydroxylation reaction, N-dealkylation, and conjugation with glutathione (Cherif *et al.*, 2001). Meanwhile, rice cultivar Leum Pua seemed to tolerate atrazine, and treating the seeds of the plant with the bacterial suspension of *B. stercoris* B.PNR2 significantly increased the shoot fresh weight and root fresh weight of the plant. However, it did not increase the shoot dry weight, root dry weight, shoot length, and root length of rice cultivar Leum Pua. Atrazine was not directly used in rice fields, but the tolerance of rice RD6 to atrazine may come from the uptake of atrazine residue in paddy fields, which can activate the epigenetic mechanism of atrazine degradation in plants (Ma *et al.*, 2019). The application of the bacterial suspension of *B. stercoris* B.PNR2 increased the fresh weight of the shoot and root of rice cultivar RD6 when planted in non-contaminated soil, but this effect was not observed in atrazine-contaminated soil. Moreover, the shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, and root length of rice cultivar RD6 that received a bacterial suspension of *B. stercoris* B.PNR2 and were grown under atrazine-contaminated soil did not significantly differ from those grown without receiving a bacterial suspension. The application of the bacterial suspension of *B. stercoris* B.PNR2 only showed a trend toward increasing the shoot length of plants grown on atrazine-contaminated soil (Table 4).

Figure 1 shows the growth of rice cultivar RD6, rice cultivar Leum Pua, and waxy corn cultivar Muang Tam under non-contaminated and atrazine-contaminated soil. The outcome of using plant growth-promoting bacteria to stimulate plant growth depended on several factors, including the inoculation method, inoculum size, the ability to colonize roots, and the physiological state of the plant (Lopes *et al.*, 2021). The stimulation of rice and corn growth by *B. stercoris* B.PNR2 was not observed clearly in this study. This can be explained by the inappropriate amount of inoculum size or improper inoculation method. This phenomenon was observed when *B. subtilis* JN005 was inoculated into seeds of rice variety Xiangwanxian No 12. The growth of seedlings that received *B. subtilis* JN005 at a concentration of 1×10^7 cfu/mL was higher than that of seedlings that received other inoculum sizes (Zhu *et al.*, 2022). This study used seed inoculation methods to inoculate cells of *B. stercoris* B.PNR2 into the seeds of rice cultivar RD6, rice cultivar Leum Pua, and waxy corn cultivar Muang Tam. However, the root exudate compounds, which influenced microbial colonization on plant roots, changed with plant genotype and plant age (Lopes *et al.*, 2021). Moreover, abiotic factors in the soil impacted the composition of root exudates and thereby changed the interaction between plants and microbes (Lopes *et al.*, 2021). This can lead to the failure of using plant growth-promoting bacteria to stimulate plant growth.

Chlorophyll, Carotenoid and Proline Contents

In general, photosynthetic organelles in plants are targeted by atrazine phytotoxicity, often resulting in a decrease in chlorophyll content in plants exposed to atrazine (Yang & Zhang, 2020; Salem & El-Sobki, 2021). However, atrazine did not affect the chlorophyll, carotenoid, and proline contents of the rice cultivar Leum Pua and waxy corn cultivar Muang Tam because the amounts of pigments and proline in the shoots of both plants grown in non-contaminated and atrazine-contaminated soil did not differ significantly (Table 5). Immersion of the seeds of rice cultivar Leum Pua and waxy corn cultivar Muang Tam in a bacterial suspension before planting neither increased nor decreased the chlorophyll, carotenoid, and proline contents in the shoots of both plants (Table 5). Furthermore, atrazine did not exhibit toxicity towards the chlorophyll and carotenoid contents in the shoots of rice cultivar RD6. The amounts of chlorophyll and carotenoid contents in the shoots of rice cultivar RD6 grown in atrazine-contaminated soil, without seed soaking in a bacterial suspension, did not significantly differ from those grown in non-contaminated soil ($P>0.05$). However, the application of a bacterial suspension with the seeds of rice cultivar RD6 tended to decrease chlorophyll b and total chlorophyll content in the plant shoot when grown in atrazine-contaminated soil. This trend corresponds to the proline content in the shoot of rice cultivar RD6 because proline contents in the shoot increased when seeds were soaked with the bacterial suspension of *B. stercoris* B.PNR2 under both non-contaminated and atrazine-contaminated conditions. Lower proline content was detected in rice cultivar RD6 grown in non-contaminated soil (72.2±23.2 μg/g FW) and atrazine-contaminated soil (198.6±35.2 μg/g FW) when it did not receive a bacterial suspension. The proline content in the shoots of rice cultivar RD6 increased to 172.2±112.2 in non-contaminated soil and 343.6±41.6 in atrazine-contaminated soil when the seeds received a bacterial suspension of *B. stercoris* B.PNR2 before

Table 4: Shoot growth and root growth of rice cultivar RD6, rice cultivar Leum Pua, and waxy corn cultivar Muang Tam grown in non-contaminated and atrazine-contaminated soil (Mean±SE)

Treatment	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Rice cultivar RD6						
Non-contaminated + Water	10.0±0.20^{b}	0.06±0.001^{b}	0.02±0.001^{a}	12.5±0.48^{a}	0.05±0.002^{b}	0.01±0.001^{b}
Non-contaminated + B.PNR2	8.5±0.59^{c}	0.07±0.002^{a}	0.02±0.001^{a}	9.5±0.74^{b}	0.06±0.004^{a}	0.01±0.001^{b}
Atrazine + Water	10.3±0.23^{b}	0.07±0.001^{a}	0.02±0.001^{a}	10.2±0.41^{b}	0.06±0.003^{a}	0.02±0.002^{a}
Atrazine + B.PNR2	11.3±0.22^{a}	0.07±0.001^{a}	0.02±0.001^{a}	10.4±0.30^{b}	0.06±0.003^{a}	0.02±0.001^{a}
Rice cultivar Leum Pua						
Non-contaminated + Water	11.6±0.50^{a}	0.079±0.002^{b}	0.02±0.001^{a}	10.8±0.44^{a}	0.06±0.003^{b}	0.02±0.002^{a}
Non-contaminated + B.PNR2	12.5±0.47^{a}	0.085±0.002^{a}	0.02±0.001^{a}	11.0±0.42^{a}	0.08±0.005^{a}	0.02±0.001^{a}
Atrazine + Water	11.4±0.32^{a}	0.074±0.002^{b}	0.02±0.001^{a}	11.4±0.36^{a}	0.07±0.004^{b}	0.02±0.002^{a}
Atrazine + B.PNR2	11.6±0.38^{a}	0.087±0.002^{a}	0.02±0.001^{a}	10.9±0.34^{a}	0.08±0.004^{a}	0.02±0.002^{a}
Waxy corn cultivar Muang Tam						
Non-contaminated + Water	15.2±0.73^{a}	0.62±0.025^{a}	0.09±0.005^{b}	17.0±1.93^{a}	0.33±0.022^{a}	0.04±0.003^{b}
Non-contaminated + B.PNR2	14.0±0.61^{a}	0.67±0.023^{a}	0.10±0.004^{b}	15.9±0.85^{a}	0.40±0.026^{a}	0.04±0.004^{b}
Atrazine + Water	15.3±0.61^{a}	0.56±0.016^{a}	0.18±0.066^{a}	16.0±0.69^{a}	0.35±0.021^{a}	0.05±0.004^{a}
Atrazine + B.PNR2	14.6±0.70^{a}	0.62±0.023^{a}	0.11±0.005^{b}	14.6±0.62^{a}	0.32±0.024^{a}	0.03±0.003^{b}

Different lowercase letters show significant differences ($P < 0.05$) between treatments within the same plant species. B.PNR2 refers *Bacillus stercoris* B.PNR2

Table 5: Chlorophyll, carotenoid, and proline content in the shoot of rice cultivar RD6, rice cultivar Leum Pua, and waxy corn cultivar Muang Tam grown in non-contaminated and atrazine-contaminated soil (Mean±SE)

Treatment	Chlorophyll a (μg/g FW)	Chlorophyll b (μg/g FW)	Total chlorophyll (μg/g FW)	Carotenoid (μg/g FW)	Proline (μg/g FW)
Rice cultivar RD6					
Non-contaminated + Water	819.0±98.1^{a}	259.0±22.3^{a}	1078.0±114.2^{a}	150.1±22.1^{a}	72.2±23.2^{c}
Non-contaminated + B.PNR2	813.1±55.2^{a}	268.6±16.7^{a}	1081.7±39.0^{a}	186.2±7.3^{a}	172.2±112.2^{b}
Atrazine + Water	621.4±150.1^{a}	216.7±24.4^{ab}	838.1±131.9^{ab}	121.3±47.4^{a}	198.6±35.2^{b}
Atrazine + B.PNR2	453.4±51.6^{a}	162.6±4.2^{b}	616.0±55.8^{b}	107.5±8.7^{a}	343.6±41.6^{a}
Rice cultivar Leum Pua					
Non-contaminated + Water	737.7±93.2^{a}	389.4±11.1^{a}	1127.2±99.1^{a}	124.1±15.2^{a}	155.4±10.6^{a}
Non-contaminated + B.PNR2	576.1±45.9^{a}	298.1±69.1^{a}	874.2±115.6^{a}	105.6±6.9^{a}	141.3±14.9^{a}
Atrazine + Water	551.5±30.0^{a}	235.1±13.3^{a}	786.6±43.3^{a}	123.1±2.6^{a}	190.9±6.8^{a}
Atrazine + B.PNR2	608.8±65.8^{a}	257.5±84.6^{a}	866.4±128.2^{a}	94.3±34.3^{a}	151.3±42.6^{a}
Waxy corn cultivar Muang Tam					
Non-contaminated + Water	360.4±50.0^{a}	95.5±11.0^{a}	456.0±60.9^{a}	83.5±9.2^{a}	140.0±9.1^{a}
Non-contaminated + B.PNR2	494.9±66.4^{a}	135.6±22.7^{a}	630.5±89.0^{a}	118.3±10.4^{a}	219.5±61.7^{a}
Atrazine + Water	643.7±111.2^{a}	147.4±27.7^{a}	791.1±53.9^{a}	147.3±23.0^{a}	11.8±53.9^{a}
Atrazine + B.PNR2	526.5±66.7^{a}	148.4±19.6^{a}	674.9±86.4^{a}	124.0±13.8^{a}	85.9±14.3^{a}

Different lowercase letters show significant differences ($P < 0.05$) between treatments within the same plant species. B.PNR2 refers to *Bacillus stercoris* B.PNR2

Table 6: Physical and Chemical Characteristics of Atrazine-contaminated Soil

Treatment	pH	Organic matter (g/kg)	Available P (mg/kg)	Available K (mg/kg)	Available Mg (mg/kg)
Beginning soil	6.2	3.45	66.2	47.5	34.7
B.PNR2 + rice cultivar RD6	6.7	4.19	66.4	31.6	29.5
B.PNR2 + rice cultivar Leum Pua	6.6	4.28	66.4	36.3	29.9
B.PNR2 + waxy corn cultivar Muang Tam	6.7	4.36	65.0	36.4	27.3
DW + rice cultivar RD6	6.8	4.36	68.4	37.0	29.5
DW + rice cultivar Leum Pua	6.7	4.20	71.8	43.6	30.6
DW + waxy corn cultivar Muang Tam	6.9	3.95	65.0	44.9	26.0

Abbreviation: B.PNR2 and DW mean immersed seeds with *Bacillus stercoris* B.PNR2 and distilled water respectively

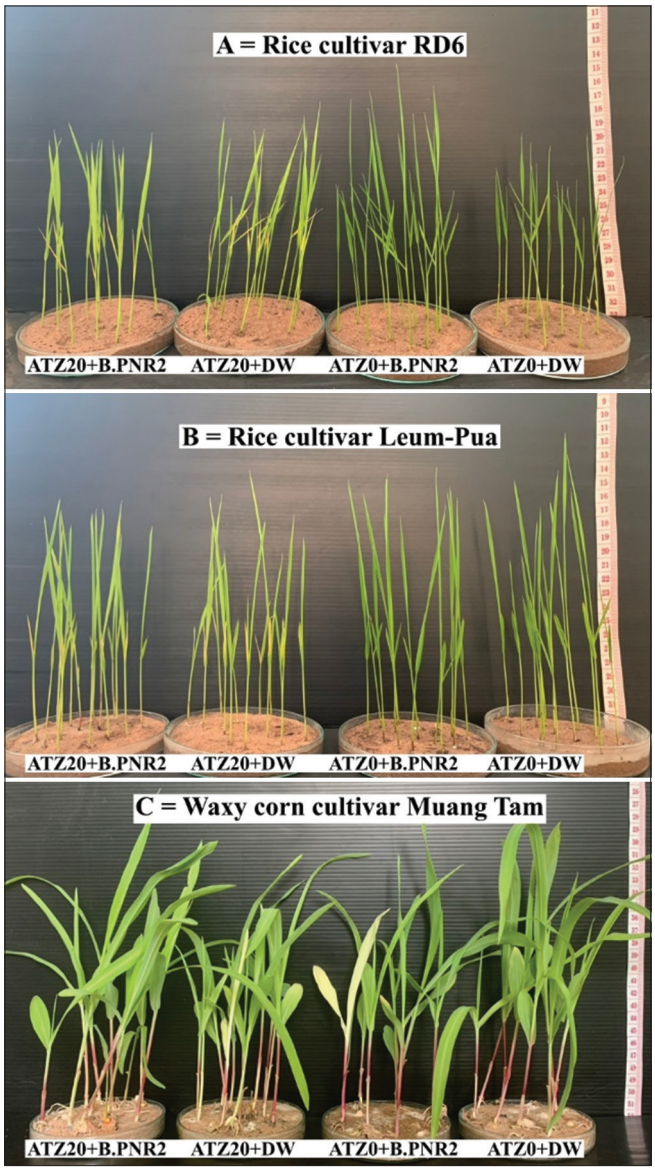


Figure 1: Seedlings of a) rice cultivar RD6, b) rice cultivar Leum-Pua and c) waxy corn cultivar Muang Tam were grown in atrazine-contaminated and non-contaminated soil with the addition of *Bacillus stercoris* B.PNR2 (Abbreviation: ATZ 20, ATZ 0, PNR2, and DW, representing atrazine 20 mg/kg, atrazine 0 mg/kg, *Bacillus stercoris* B.PNR2, and distilled water, respectively)

planting (Table 5). The unsuccessful use of *B. stercoris* B.PNR2 as a plant growth-promoting bacteria for rice cultivar RD6 was confirmed because oxidative stress was detected by determining proline molecules. Proline acts as an antioxidant molecule used to protect plants from oxidative stress (Din *et al.*, 2020). The failure to use plant growth-promoting bacteria may result from several reasons. For example, plant growth-promoting bacteria may fail to compete with other microorganisms in the rhizosphere, they may struggle to colonize the roots (Hossain *et al.*, 2023), and they may encounter unfavorable abiotic conditions in the soil (Lopes *et al.*, 2021).

The organic matter in the soil increased from the initial level of 3.45 g/kg to approximately 3.95-4.36 g/kg in soil planted with rice cultivar RD6, rice cultivar Leum Pua, and waxy corn cultivar Muang Tam (Table 6). Generally, plant litter incorporated into the soil is decomposed by soil microorganisms, and some of it can be transformed into organic matter in the soil (Xu *et al.*, 2022). Immersing plant seeds in a bacterial suspension of *B. stercoris* B.PNR2 did not increase organic matter in the soil. Furthermore, available potassium and magnesium decreased in soil planted with rice cultivar RD6, rice cultivar Leum Pua, and waxy corn cultivar Muang Tam. The soil pH and available phosphorus showed a consistent trend in soil planted with rice cultivar RD6, rice cultivar Leum Pua, and waxy corn cultivar Muang Tam compared to the pH and available phosphorus levels in the soil at the beginning of the experiment (Table 6).

CONCLUSIONS

B. stercoris B.PNR2 is an IAA-producing bacteria that can tolerate growth in 30 mg/L atrazine and continue to produce IAA even when exposed to 80 mg/L atrazine in nutrient broth. However, immersing seeds in a *B. stercoris* B.PNR2 suspension and planting them in atrazine-contaminated soil did not enhance the growth of seedlings for rice cultivar RD6, rice cultivar Leum-Pua, and waxy corn cultivar Muang Tam. To address this, changes in inoculum size, reduced seed soaking time, or the selection of new IAA-producing bacteria should be considered.

ACKNOWLEDGEMENT

This research project was financially supported by Thailand Science Research and Innovation (TSRI).

REFERENCES

Ábrahám, E., Hourton-Cabassa, C., Erdei, L., & Szabados, L. (2010). Methods for determination of proline in plants. In R. Sunkar (Eds.), *Plant Stress Tolerance. Methods in Molecular Biology* (Vol. 639, pp. 317-331) Totowa, NJ: Humana Press. https://doi.org/10.1007/978-1-60761-702-0_20

Ahmad, F., Ahmad, I., & Khan, M. S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research, 163*(2), 178-181. https://doi.org/10.1016/j.micres.2006.04.001

Arora, S., Mukherjee, I., Kumar, A., & Garg, D. K. (2014). Comparative assessment of pesticide residues in grain, soil, and water from IPM and non-IPM trials of basmati rice. *Environmental Monitoring and Assessment, 186*, 361-366. https://doi.org/10.1007/s10661-013-3380-3

Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil, 39*, 205-207. https://doi.org/10.1007/BF00018060

Breidenbach, B., Brenzinger, K., Brandt, F. B., Blaser, M. B., & Conrad, R. (2017). The effect of crop rotation between wetland rice and upland maize on the microbial communities associated with roots. *Plant and Soil, 419*, 435-445. https://doi.org/10.1007/s11104-017-3351-5

Brighenti, A. M., Moraes, V. J., Oliveira Jr., R. S., Gazziero, D. L. P., Voll, E., & Gomes, J. A. (2002). Persistence and phytotoxicity of the herbicide atrazine applied on corn crop on successive sunflower crop. *Planta Daninha, 20*(2), 291-297. https://doi.org/10.1590/S0100-83582002000200016

Burhan, N., & Shaukat, S. S. (2000). Effects of atrazine and phenolic compounds on germination and seedling growth of some crop plants. *Pakistan Journal of Biological Sciences, 3*(2), 269-274. https://doi.org/10.3923/pjbs.2000.269.274

Cherif, M., Raveton, M., Picciocchi, A., Ravanel, P., & Tissu, M. (2001). Atrazine metabolism in corn seedlings. *Plant Physiology and Biochemistry, 39*(7-8), 665-672. https://doi.org/10.1016/S0981-9428(01)01281-5

Chuanren, D., Bochu, W., Wanqian, L., Jing, C., Jie, L., & Huan, Z. (2004). Effect of chemical and physical factors to improve the germination rate of *Echinacea angustifolia* seeds. *Colloids and Surfaces B: Biointerfaces, 37*(3-4), 101-105. https://doi.org/10.1016/j.colsurfb.2004.07.003

Din, B. U., Amna, Rafiquee, M., Javed, M. T., Kamran, M. A., Mehmood, S., Khan, M., Sultan, T., Munis, M. F. H., & Chaudhary, H. J. (2020). Assisted phytoremediation of chromium spiked soils by *Sesbania sesban* in association with *Bacillus xiamenensis* PM14: A biochemical analysis. *Plant Physiology and Biochemistry, 146*, 249-258. https://doi.org/10.1016/j.plaphy.2019.11.010

Fiodor, A., Ajijah, N., Dziewit, L., & Pranaw, K. (2023). Biopriming of seed with plant growth-promoting bacteria for improved germination and seedling growth. *Frontiers in Microbiology, 14*, 1142966. https://doi.org/10.3389/fmicb.2023.1142966

He, H., Liu, Y., You, S., Liu, J., Xiao, H., & Tu, Z. (2019). A review on recent treatment technology for herbicide atrazine in contaminated environment. *International Journal of Environmental Research and Public Health, 16*(24), 5129. https://doi.org/10.3390/ijerph16245129

Hossain, M. A., Hossain, M. S., & Akter, M. (2023). Challenges faced by plant growth-promoting bacteria in field-level applications and suggestions to overcome the barriers. *Physiological and Molecular Plant Pathology, 126*, 102029. https://doi.org/10.1016/j.pmpp.2023.102029

James, K., & Singh, D. K. (2018). Assessment of atrazine decontamination by epiphytic rootbacteria isolated from emergent hydrophytes. *Annals of Microbiology, 68*, 953-962. https://doi.org/10.1007/s13213-018-1404-5

Jiang, Z., Jiang, D., Zhou, Q., Zheng, Z., Cao, B., Meng, Q., Qu, J., Wang, Y., & Zhang, Y. (2020). Enhancing the atrazine tolerance of *Pennisetum americanum* (L.) K. Schum by inoculating with indole-3-acetic acid-producing strain Pseudomonas chlororaphis PAS18. *Ecotoxicology and Environmental Safety, 202*, 110854. https://doi.org/10.1016/j.ecoenv.2020.110854

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: the pigments of photosynthetic biomembranes. In R. Douce & L. Packer (Eds.), *Methods in Enzymology* (Vol. 148, pp. 350-382) Cambridge, US: Academic Press Inc. https://doi.org/10.1016/0076-6879(87)48036-1

Lopes, M. J. S., Dias-Filho, M. B., & Gurgel, E. S. C. (2021). Successful plant growth promoting microbes: Inoculation methods and abiotic factors. *Frontiers in Sustainable Food Systems, 5*, 606454. https://doi.org/10.3389/fsufs.2021.606454

Ma, L. Y., Zhang, N., Liu, J. T., Zhai, X. Y., Lv, Y., Lu, F. F., & Yang, H. (2019). Uptake of atrazine in a paddy crop activates an epigenetic mechanism for degrading the pesticide in plants and environment. *Environment International, 131*, 105014. https://doi.org/10.1016/j.envint.2019.105014

Nwani, C. D., Lakra, W. S., Nagpure, N. S., Kumar, R., Kushwaha, B., & Srivastava, S. K. (2010). Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish *Channa Punctatus* (Bloch). *International Journal of Environmental Research and Public Health, 7*(8), 3298-3312. https://doi.org/10.3390/ijerph7083298

Pengproh, R., Thanyasiriwat, T., Sangdee, K., Saengprajak, J., Kawicha, P., & Sangdee, A. (2023). Evaluation and genome mining of *Bacillus stercoris* isolate B.PNR1 aspotential agent for Fusarium wilt control and growth promotion of tomato. *The Plant Pathology Journal, 39*(5), 430-448. https://doi.org/10.5423/PPJ.OA.01.2023.0018

Promkhambut, A., Yokying, P., Woods, K., Fisher, M., Yong, M. L., Manorom, K., Baird, I. G., & Fox, J. (2023). Rethinking agrarian transition in Southeast Asia through rice farming in Thailand. *World Development, 169*, 106309. https://doi.org/10.1016/j.worlddev.2023.106309

Ramakrishna, W., Yadava, R., & Li, K. (2019). Plant growth promoting bacteria in agriculture: Two sides of a coin. *Applied Soil Ecology, 138*, 10-18. https://doi.org/10.1016/j.apsoil.2019.02.019

Reis, M. R., Aquino, L. Â., Melo, C. A. D., Silva, D. V., & Dias, R. C. (2018). Carryover of tembotrione and atrazine affects yield and quality of potato tubers. *Acta Scientiarum. Agronomy, 40*(1), e35355. https://doi.org/10.4025/actasciagron.v40i1.35355

Riyanto, D., Yustisia, Anshori, A., & Srihartanto, E. (2021). Application of rice-corn intercropping as an optimization of the land use utilization and increasing of farmer income in Playen, Gunungkidul. *E3S Web of Conferences, 232*, 01033. https://doi.org/10.1051/e3sconf/202123201033

Rostami, S., Jafari, S., Moeini, Z., Jaskulak, M., Keshtgar, L., Badeenezhad, A., Azhdarpoor, A., Rostami, M., Zorena, K., & Dehghani, M. (2021). Current methods and technologies for degradation of atrazine in contaminated soil and water: A review. *Environmental Technology & Innovation, 24*, 102019. https://doi.org/10.1016/j.eti.2021.102019

Rudolph, N., Labuschagne, N., & Aveling, T. A. S. (2015). The effect of plant growth promoting rhizobacteria on seed germination and seedling growth of maize. *Seed Science and Technology, 43*(3), 507-518. https://doi.org/10.15258/sst.2015.43.3.04

Salem, R. E. M. E., & El-Sobki, A. E. A. (2021). Physiological and biochemical parameters as an index for herbicides damage in wheat plants. *Egyptian Academic Journal of Biological Sciences, 13*(2), 25-35. https://doi.org/10.21608/eajbsf.2021.182445

Sánchez, V., López-Bellido, F. J., Cañizares, P., & Rodríguez, L. (2017). Assessing the phytoremediation potential of crop and grass plants for atrazine-spiked soils. *Chemosphere, 185*, 119-126. https://doi.org/10.1016/j.chemosphere.2017.07.013

Sardoei, A. S., & Rahbarian, P. (2014). Effect of different media on chlorophyll and carotenoids of ornamental plants under system mist. *European Journal of Experimental Biology, 4*(2), 366-369.

Singh, S., Kumar, V., Chauhan, A., Datta, S., Wani, A. B., Singh, N., & Singh, J. (2018). Toxicity, degradation and analysis of the herbicide atrazine. *Environmental Chemistry Letters, 16*, 211-237. https://doi.org/10.1007/s10311-017-0665-8

Soltani, N., Mashhadi, H. R., Mesgaran, M. B., Cowbrough, M., Tardif, F. J., Chandler, K., Nurse, R. E., Swanton, C. J., & Sikkema, P. H. (2011). The effect of residual corn herbicides on injury and yield of soybean seeded in the same season. *Canadian Journal of Plant Science, 91*(3), 571-576. https://doi.org/10.4141/CJPS10110

Tripathi, P., Yadav, R., Das, P., Singh, A., Singh, R. P., Kandasamy, P., Kalra, A., & Khare, P. (2021). Endophytic bacterium CIMAP-A7 mediated amelioration of atrazine induced phytotoxicity in *Andrographis paniculate*. *Environmental Pollution, 287*, 117635. https://doi.org/10.1016/j.envpol.2021.117635

Xu, Y., Liu, K., Yao, S., Zhang, Y., Zhang, X., He, H., Feng, W., Ndzana, G. M., Chenu, C., Olk, D. C., Mao, J., & Zhang, B. (2022). Formation efficiency of soil organic matter from plant litter is governed by clay mineral type more than plant litter quality. *Geoderma, 412*, 115727. https://doi.org/10.1016/j.geoderma.2022.115727

Yang, L., & Zhang, Y. (2020). Effects of atrazine and its two major derivatives on the photosynthetic physiology and carbon sequestration potential of a marine diatom. *Ecotoxicology and Environmental Safety, 205*, 111359. https://doi.org/10.1016/j.ecoenv.2020.111359

Zhang, J. J., Gau, S., Xu, J. Y., Lu, Y. C., Lu, F. F., Ma, L. Y., Su, X. N., & Yang, H. (2017). Degrading and phytoextracting atrazine residues in rice (*Oryza sativa*) and growth media intensified by a phase II mechanism modulator. *Environmental Science & Technology, 51*(19), 11258-11268. https://doi.org/10.1021/acs.est.7b02346

Zhu, H., Zhou, H., Ren, Z., & Liu, E. (2022). Control of *Magnaporthe oryzae* and rice growth promotion by *Bacillus subtilis* JN005. *Journal of Plant Growth Regulation, 41*, 2319-2327. https://doi.org/10.1007/s00344-021-10444-w

Zhu, J., Fu, L., Jin, C., Meng, Z., & Yang, N. (2019). Study on the isolation of two atrazine-degrading bacteria and the development of a microbial agent. *Microorganisms, 7*(3), 80. https://doi.org/10.3390/microorganisms7030080