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Potential of Plant Growth Regulators to Enhance Arsenic Phytostabilization by *Pennisetum purpureum* cv. Mott

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ABSTRACT

The limited translocation of arsenic from contaminated soil to plant biomass is one way to decrease human exposure to arsenic (As). Plant growth regulators (PGR), including salicylic acid, indole butyric acid, and calcium, have been reported to alleviate toxicity and decrease the accumulation of heavy metals in many plants. Thus, this study has investigated the effect of plant growth regulators, including salicylic acid, salicylic acid + calcium chloride, indole butyric acid, and indole butyric acid + calcium chloride, to stimulate the growth and phytostabilization of *Pennisetum purpureum* cv. Mott grew in arsenic-spiked soil. The results showed shoot growth, root growth, and total chlorophyll content of *P. purpureum* cv. Mott grown in non-spiked soil were not significantly different from those grown in arsenic-spiked soil. Only the root-to-shoot ratio of plants grown under arsenic-spiked soil (0.28) was higher than that of non-spiked soil (0.19). Exogenous plant growth regulator application of each formula did not stimulate the growth of plants grown under both soil conditions. The most suitable plant growth regulator was indole butyric acid + calcium chloride, as the highest arsenic accumulation in plant roots was detected (47.38 mg/kg). It corresponds with the arsenic bioaccumulation factor, translocation factor, and efficiency, which were 4.52, 0.06, and 9.77% when using exogenously indole butyric acid + calcium chloride. Meanwhile, arsenic's translocation factor and efficiency were low when using the other formulae of plant growth regulators. Thus, 0.001 mM indole butyric acid + 20 mM calcium chloride may be used for the cultivation of *P.*

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purpureum cv. Mott as a forage crop in areas with low levels of arsenic contamination because it could limit the amount of arsenic entering the food chain.

Keywords: Arsenic, Napier grass, phytoremediation, plant growth regulator

INTRODUCTION

Arsenic is a hazardous metalloid naturally found in the Earth's crust with an average concentration of 1–2 mg/kg (Sanyal et al., 2020). Increases in the concentration of arsenic in the soil and groundwater usually come from geological reactions and anthropogenic activity including mining, smelting, or other industrial activities (Mateo et al., 2019; Mitra et al., 2017). Arsenic can be a contaminant in agricultural areas when using inorganic arsenic as a pesticide or defoliant (Wani et al., 2017). Millions of people worldwide are in danger from arsenic toxicity, and exposure to arsenic is a public health concern in several countries in Asia, including China, India, Thailand, and Vietnam (Mitra et al., 2017; Sanyal et al., 2020). In Thailand, the average concentration of arsenic in agricultural soil ranges from less than 0.005 to 64 mg/kg, and the mean value was 5.8 ± 6.5 mg/kg (Sukreeyapongse et al., 2009). The maximum concentration of arsenic in soil and agricultural soil specified by the Office of the National Environment Board of Thailand was 3.9 mg/kg (Weerasiri et al., 2014). The most prevalent inorganic forms of arsenic in the environment were arsenite ($H_2AsO_4^-$; As^{III+}) and arsenate (H_3AsO_3; As^{V+}); however, arsenic in the organic form was seldom detected in the environment (Sanyal et al., 2020). The toxicity of arsenic depends on the prevalent form in the environment. For example, arsenate is less toxic, while arsenite is more toxic and movable than arsenate because it has a higher ability to bind with functional biomolecules, such as sulfhydryl groups and cysteinyl residues (Berg & Borges, 2020; Mateo et al., 2019). Contamination of arsenic in soil is an environmental concern because arsenic is classified as a Group I human carcinogen, and it has several toxic effects on humans, including hypopigmentation, pigmentation, keratosis, and skin cancers (Sanyal et al., 2020). The route for human arsenic exposure is mainly from drinking contaminated water, eating contaminated food, direct skin contact with contaminated soil, and inhaling contaminated dust (Loukola-Ruskeeniemi et al., 2022). Biomagnification of arsenic along the food chain is a major concern of arsenic exposure in humans because arsenic has been reported in food crops in Poaceae plants, such as rice, maize, sorghum, and wheat (Upadhyay et al., 2019).

Based on the toxic health effects and biomagnification capacity along the food chain of arsenic, arsenic remediation from contaminated sites to decrease human contact with arsenic is a priority task. Among several arsenic decontamination methods, phytoremediation is feasible, environmentally friendly, and cost-effective in removing arsenic from contaminated sites (Mateo et al., 2019). Several phytoremediation mechanisms can be used

for arsenic decontamination, including phytoextraction and phytostabilization. Several terrestrial plant species have been used to remove arsenic from contaminated soil via phytoextraction, including *Bambusa bambos*, *Pennisetum purpureum*, *Vetiveria zizanioides* (Sampanpanish & Suwattiga, 2017), *Zea mays* (Mehmood et al., 2021), *Salix purpurea* 'Fish Creek', *Festuca arudinacea*, *Medicago sativa*, and *Brassica juncea* (Yanitch et al., 2020). The phytostabilization of arsenic has been reported less than phytoextraction, but it is still interesting. In addition, some plants have been reported to phytostabilize arsenic, including dwarf Napier grass (Boonmeerati & Sampanpanish, 2021; Kowitwiwat & Sampanpanish, 2020) and the halophyte *Acanthus ilicifolius* (Sarath et al., 2022). These plants were reported for use in mine tailing remediation. Even though the success of phytostabilization of arsenic differed from site to site, the accomplishment of the process depends on several factors in the environment, such as water, nutrients, oxygen soil structure, pH, and presence of other toxins (Hauptvogl et al., 2020). For example, the arsenic phytostabilization by *Phragmites australis* and *Arundo donax* were increased when compost was an amendment in planted soil (Castaldi et al., 2018).

Phytostabilization promises to be ecosystem friendly due to promoting the soil development process, microbial diversity, and self-reliance for environmental restoration as a long-term goal (Zine et al., 2020). The main hindrance to arsenic phytoremediation is plant growth inhibition due to the high concentration of arsenic, which also retards the phytoremediation efficiency (Mateo et al., 2019). Plant growth under arsenic contamination usually causes oxidative stress (A. P. Singh et al., 2017) and reduced photosynthetic pigments in plants (Kumari & Pandey-Rai, 2018). Decreasing metal toxicity to plants is important to improve the phytostabilization process, especially for mine waste remediation (Zine et al., 2020). In the case of arsenic contamination, the application of exogenous plant growth regulators, including salicylic acid, auxin, and calcium, is one way to improve plant growth by alleviating arsenic toxicity in plants grown under arsenic stress (He et al., 2021; Maghsoudi et al., 2020; R. Singh et al., 2018, 2020). The plant growth regulators in this study could protect plants from arsenic stress with several mechanisms. Salicylic acid could alter the plant's physiological and metabolic processes and alleviate the environmental stress on plants (Arfi et al., 2020). The salicylic acid application can enhance antioxidant enzymes and decrease arsenic accumulation in the plant (Maghsoudi et al., 2020). Exogenous auxin also mitigates arsenic toxicity in the plant by decreasing the arsenic translocation from the root to the shoot and promotes plant growth by increasing plant biomass, relative root length, relative root fresh weight, relative root length (He et al., 2021), and other negative impacts from arsenic toxicity (Piacentini et al., 2020). Meanwhile, calcium can act as a secondary

messenger and control signal transduction in plants grown under stress and non-stress conditions. Modulating key antioxidant enzymes and stabilized membranes by calcium plays an important role in protecting plants grown under environmental stress (R. Singh et al., 2018, 2020). Exogenous calcium application also reduced arsenic uptake and increased antioxidant enzymes in a plant under arsenic stress (Rahman et al., 2015). For the reasons described above, the objective of this study was to investigate the roles of salicylic acid and indolebutyric acid application alone or in combination with calcium to stimulate the growth and phytostabilization of arsenic by *Pennisetum purpureum* cv. Mott in a pot experiment. *Pennisetum purpureum* cv. Mott (dwarf Napier grass) was used as a model plant to stabilize arsenic from the soil in this study because this plant species has been reported to remediate arsenic via phytostabilization (Kowitwiwat & Sampanpanish, 2020). Moreover, this plant can grow in several soil types and weather conditions, it is resistant to many pests, and its biomass is used for animal feed and as a substrate for bioethanol production (Ishii et al., 2015). These plant growth regulators have been reported to decrease arsenic accumulation in plants in the Poaceae family, such as rice and wheat (He et al., 2021; Maghsoudi et al., 2020). Suppose these three plant growth regulators could increase arsenic phytostabilization of *P. purpureum* cv. Mott, the shoot biomass of the grass will be safe to be used for feed for livestock or bioenergy production in the future.

MATERIALS AND METHODS

Preparation of Arsenic-Spiked Soil

The soil used in this study was collected from an agricultural area in Muang Pluai Sub-District, Srisomdej District, Roi-Et Province, Thailand. The soil was air dry for two weeks, sieved with a 2 mm sieve, and sent for analysis of its physical and chemical characteristics at the Central Laboratory Thailand, Ltd. (Khon Kaen branch). The texture of the soil was loamy sand with 81.9% sand, 13.16% silt, 4.95% clay, pH 5.71, cation exchange capacity was 3.42 mg/100g, and the background level of arsenic in the soil was 0.809 mg/kg. Then, 1 kg of soil was weighed and used in each pot for the experiment. One kg of soil in each pot was spiked with 1,000 ± 0.005 g/l of standard arsenic solution (ChemSupply, Australia) and left to dry at room temperature for five days. The soil was mixed thoroughly several times to ensure it was homogenous before use. Soil samples were collected to determine the arsenic concentration in the soil after spiking, and the final concentration of the arsenic in the soil was 12.92 ± 0.54 mg/kg. Soil without arsenic spiking was used in the control pots. The soil pH after being spiked with the standard arsenic solution was approximately 3.9, and the pH of the control soil without arsenic addition was adjusted to 3.9 with a 2% (w/w) nitric acid solution (ANaPURE, New Zealand). Soil moisture content after soaking the soil with water was 11.67%.

Pot Experiment

A farmer kindly provided *P. purpureum* cv. Mott in Phon-Ngam Sub-District, Kamalasai District, Thailand. Cuttings of *P. purpureum* cv. Mott were cut into similar sized (approximately 8-10 cm per piece) and immersed in 1 mM salicylic acid (Sigma-Aldrich, USA), 0.001 mM indolebutyric acid (Fluka, China), 1 mM salicylic acid (Sigma-Aldrich, USA) + 20 mM calcium chloride (Ajax Finechem Pty Ltd., New Zealand), 0.001 mM indolebutyric acid (Fluka, China) + 20 mM calcium chloride (Ajax Finechem Pty Ltd., New Zealand) or water for three days. Then, similar-sized cuttings were planted into arsenic-spiked soil or non-spiked soil according to the treatments described below. One cutting was planted in each experimental pot. The experiment was performed with a completely randomized design (CRD) with 2 x 5 factors. The first factor was two levels of arsenic contamination (non-spiked soil and arsenic-spiked soils), and the second factor was five levels of plant growth regulator application (no plant growth regulator, salicylic acid, salicylic acid + calcium chloride, indole butyric acid, and indole butyric acid + calcium chloride). There were 10 treatments, and each treatment was performed in eight replicates, as described below:

1. Non-spiked soil + plant
2. Non-spiked soil + plant + salicylic acid
3. Non-spiked soil + plant + indole butyric acid
4. Non-spiked soil + plant + salicylic acid + calcium chloride
5. Non-spiked soil + plant + indole butyric acid + calcium chloride
6. Arsenic-spiked soil + plant
7. Arsenic-spiked soil + plant + salicylic acid
8. Arsenic-spiked soil + plant + indole butyric acid
9. Arsenic-spiked soil + plant + salicylic acid + calcium chloride
10. Arsenic-spiked soil + plant + indole butyric acid + calcium chloride
11. Arsenic-spiked soil

After transferring the cuttings into the experimental pots, 240 ml of 1/16 concentration Hoagland modified basal salt mixture solution (PhytoTech Labs, USA) was poured into each pot at the beginning of the experiment. Water was irrigated to the experimental pots every day for seven days after transplantation, and then irrigation was changed to every three days until the end of the experiment. Then, 10 ml of each formula of the plant growth regulators (1 mM salicylic acid, 0.001 mM indolebutyric acid, 1 mM salicylic acid + 20 mM calcium chloride, 0.001 mM indolebutyric acid + 20 mM calcium chloride) was poured into the soil planted with *P. purpureum* cv. Mott again on days 15 and 25 after transplanting. The experiment was terminated 41 days after transplanting.

Plant Growth Parameters, Arsenic Remaining in Soil, and Plant Biomass

At the end of the experiment, the plant growth parameters were determined, including leaf number, stem number, shoot length, root length, shoot fresh weight,

shoot dry weight, root fresh weight, root dry weight, and chlorophyll contents in the leaves. Total chlorophyll, chlorophyll *a*, and chlorophyll *b* contents were determined according to the methods described in Huang et al. (2004). Specific root length and root-to-shoot ratio were calculated from root length/root dry weight and root dry weight/ shoot dry weight according to the formulae described in Calvelo Pereira et al. (2010) and Xu et al. (2018), respectively. Soil and plant biomass were sent for analysis at the Central Laboratory Thailand, Ltd. (Khon Kaen branch) by the in-house method based on EPA3052 using inductively coupled plasma-mass spectrometry (ICP-MS) (Agilent Model 7500, Japan). Then, the bioconcentration factor (BCF) and translocation factor (TF) of arsenic were calculated by the formulae described in Hammami et al. (2016). The bioconcentration factor was calculated from the total arsenic concentration in the harvested plant/total arsenic concentration in planted soil, and the translocation factor was calculated from the total arsenic concentration in the shoot/ total concentration in the root (Hammami et al., 2016). Moreover, the translocation efficiency (TE%) was calculated by the equation described in Hammami et al. (2016):

$$TE\% = [\text{arsenic concentration in shoot} / \text{arsenic concentration in whole plant}] \times 100$$

Statistical Analysis

A two-way analysis of variance (ANOVA) was used for variance analysis among treatments for the arsenic toxicity to plant growth. One-way ANOVA was used for variance analysis among treatments of the phytoremediation experiment. The least significant difference (LSD) method was used for pairwise comparisons of means. The data are shown as mean ± standard error (SE), and the statistical differences are shown as $P \leq 0.05$.

RESULTS AND DISCUSSION

Growth of Plants Under Arsenic Contamination

In general, the toxic effects of arsenic exposure on terrestrial plants are similar, such as causing oxidative stress from reactive oxygen species production (A. P. Singh et al., 2017), reducing total chlorophyll content (Kumari & Pandey-Rai, 2018), inducing chlorosis, reducing relative water content in the leaf (Rahman et al., 2015), alteration of auxin biosynthesis, and distribution in plant roots (Piacentini et al., 2020). In this study, these two factors, arsenic and plant growth regulator, affected plant growth differently, and there was no interaction between the factors. Arsenic decreases the shoot growth, chlorophyll *b*, total chlorophyll content, and root fresh weight of *P. purpureum* cv. Mott. While plant growth regulator application did not alter almost all plant growth of *P. purpureum* cv. Mott. Only applying salicylic acid and salicylic acid + calcium decreases the number of leaves per plant and chlorophyll content in leaves (Table 1). The growth of the plants in both arsenic-spiked and non-spiked soil was similar, and chlorosis was not detected by the naked eye (Figure 1).

Table 1
The main effect of arsenic and plant growth regulator on Napier growth traits (data shown as mean ± SE)

	Leave number / plant	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Chlorophyll *a* (mg/ml)	Chlorophyll *b* (mg/ml)	Total chlorophyll (mg/ml)
Arsenic (factor 1)										
Without As	24.2 ± 1.23a	36.2 ± 1.43a	49.9 ± 1.66a	6.3 ± 0.26a	34.8 ± 1.46a	14.5 ± 0.87a	1.2 ± 0.12a	28.2 ± 2.56a	16.7 ± 1.24a	44.9 ± 3.47a
With As	19.6 ± 1.23b	31.9 ± 1.43b	38.5 ± 1.66b	5.0 ± 0.26b	35.2 ± 1.46a	7.9 ± 0.87b	1.1 ± 0.12a	22.8 ± 2.44a	11.0 ± 1.18b	33.8 ± 3.31b
F-test	*	*	**	**	ns	*	ns	ns	**	*
Plant growth regulator (factor 2)										
No plant growth regulator	24.8 ± 1.94a	34.6 ± 2.26a	50.7 ± 2.62a	6.1 ± 0.41a	36.6 ± 2.31a	11.5 ± 1.38a	1.4 ± 0.18a	27.6 ± 3.86a	19.00 ± 1.86a	46.6 ± 5.23a
Salicylic acid	17.7 ± 1.94b	37.1 ± 2.26a	44.3 ± 2.62a	5.9 ± 0.41a	37.9 ± 2.31a	12.7 ± 1.38a	1.2 ± 0.18a	21.5 ± 4.32a	9.99 ± 2.08b	31.5 ± 5.84a
Salicylic acid + calcium chloride	19.9 ± 1.94ab	34.9 ± 2.26a	40.8 ± 2.62a	4.9 ± 0.41a	33.7 ± 2.31a	9.9 ± 1.38a	0.9 ± 0.18a	24.8 ± 3.86a	12.4 ± 1.86b	37.2 ± 5.23a
Indole butyric acid	25.2 ± 1.94a	29.5 ± 2.26a	43.0 ± 2.62a	6.0 ± 0.41a	32.9 ± 2.31a	11.6 ± 1.38a	1.2 ± 0.18a	25.2 ± 3.86a	14.6 ± 1.86ab	39.8 ± 5.23a
Indole butyric acid + calcium chloride	21.9 ± 1.94ab	34.2 ± 2.26a	42.2 ± 2.62a	5.4 ± 0.41a	34.0 ± 2.31a	10.4 ± 1.38a	1.0 ± 0.18a	28.4 ± 3.86a	13.2 ± 1.86b	41.6 ± 5.23a
F-test	*	ns	ns	ns	ns	ns	ns	ns	*	ns
F-test As x Plant growth regulator	ns	*	ns	ns	ns	ns	ns	ns	ns	ns

Note. Different lowercase letters show significant differences within each factor. Abbreviations: ns, *, ** denote non-significance ($P > 0.05$), statistical significance ($P < 0.05$), and high statistical significance ($P < 0.01$) of each factor, respectively

(a)

(b)

Figure 1. *Pennisetum purpureum* cv. Mott grew under: (a) non-spiked soil; and (b) arsenic-spiked soil in the absence of any plant growth regulators

The concentration of arsenic in this study seemed to be low, but it was in the range of the arsenic background levels in agricultural soil in Thailand (Weerasiri et al., 2014). *Pennisetum purpureum* cv. Mott has been reported to tolerate arsenic toxicity as it can grow and accumulate in both aboveground and belowground parts when growing in mine tailings with 68 ± 2.65 mg/kg of arsenic (Sampanpanish & Suwattiga et al., 2020). However, when compared between the same plant growth regulator application, shoot growth and root growth of the grass in arsenic-spiked soil were not significantly different from those grown in soil without arsenic. However, using salicylic acid, indole butyric acid, and indole butyric acid + calcium chloride decreased the shoot fresh weight and root fresh weight of *P. purpureum* cv. Mott grown in soil contaminated with arsenic compared to those without arsenic contamination (Tables 2 and 3). The shoot fresh weight and root fresh weight of *P. purpureum* cv. Mott was around 35.0–37.2 g and 6.9–8.7 g, respectively, when salicylic acid, indole butyric acid, or indole butyric acid + calcium chloride were applied in arsenic-contaminated soil. The addition of salicylic acid tended to decrease the stem number and leaf number of *P. purpureum* cv. Mott grew in arsenic-spiked soil. This effect was not detected in the leaf number of plants grown in non-spiked soil (Table 2). Shoot length, shoot fresh weight, and shoot dry weight of *P. purpureum* cv. Mott grown in soil with and without arsenic contamination were around 31.4–38.0 cm, 46.2–55.1 g, and 5.4–6.8 g, respectively (Table 2). Root length, root fresh weight, and root dry weight of *P. purpureum* cv. Mott grown in soil with and without arsenic contamination

were around 34.0–39.2 cm, 8.8–14.3 g, and 1.3–1.5 g, respectively (Table 3). Applying plant growth regulators (salicylic acid, salicylic acid + calcium chloride, indole butyric acid, or indole butyric acid + calcium chloride) did not stimulate the growth of the shoots and roots of *P. purpureum* cv. Mott grown under both soil conditions, even though auxin, salicylic acid, and calcium have previously been reported to stimulate the growth of other plants grown under arsenic contamination.

For example, exogenous application of indole-3-acetic acid and salicylic increased the relative dry weight, relative shoot length, and relative root elongation in rice grown in soil contaminated with arsenic at 28.7 ± 1.52 mg/kg (He et al. 2021). Exogenous application of calcium chloride also increased rice growth by restoration of chlorophyll damage, enhancing the dry weight and antioxidant system in rice seedlings after exposure to 0.5 and 1 mM of disodium arsenate (Na_2HAsO_4) (Rahman et al., 2015). However, the shoot and root fresh weight of plants grown in arsenic-contaminated soil tended to be lower than those grown in non-contaminated soil. It corresponds to the lower root/shoot ratio of *P. purpureum* cv. Mott grew in arsenic-spiked soil, especially for plants with salicylic acid and without plant growth

Table 2
Shoot growth of Pennisetum purpureum *cv. Mott in the presence of plant growth regulators under non-spiked and arsenic-spiked soil (data shown as mean ± SE)*

Treatment	Stems number/plant	Leave number/ plant	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
Non-spiked soil					
No plant growth regulator	3.8 ± 0.30	26.1 ± 1.54aA	38.0 ± 2.08aA	55.1 ± 2.19aA	6.8 ± 0.46aA
Salicylic acid	2.9 ± 0.50	20.8 ± 3.12aA	39.8 ± 4.07aA	51.4 ± 4.05aA	6.6 ± 0.72aA
Salicylic acid + calcium chloride	2.8 ± 0.47	22.2 ± 2.79aA	32.1 ± 2.07aA	42.8 ± 3.68aA	5.1 ± 0.52aA
Indole butyric acid	4.0 ± 0.36	26.2 ± 1.63aA	32.8 ± 2.86aA	51.1 ± 4.10aA	7.0 ± 0.74aA
Indole butyric acid + calcium chloride	3.5 ± 0.68	25.4 ± 3.91aA	38.5 ± 4.29aA	49.0 ± 2.95aA	6.0 ± 0.52aA
As-spiked soil					
No plant growth regulator	3.2 ± 0.60	23.5 ± 3.52aA	31.4 ± 2.87aA	46.2 ± 4.77aA	5.4 ± 0.34aA
Salicylic acid	1.5 ± 0.18	14.6 ± 1.48bA	34.4 ± 2.35aA	37.2 ± 1.57aB	5.2 ± 0.42aA
Salicylic acid + calcium chloride	2.0 ± 0.36	17.5 ± 2.14abA	37.6 ± 2.98aA	38.8 ± 3.78aA	4.8 ± 0.64aA
Indole butyric acid	3.2 ± 0.35	24.1 ± 2.68aA	26.2 ± 3.37aA	35.0 ± 4.28aB	5.0 ± 0.56aB
Indole butyric acid + calcium chloride	2.6 ± 0.60	18.4 ± 2.50abA	29.8 ± 0.10aA	35.4 ± 3.16aB	4.8 ± 0.56aA

Note. Different lowercase letters show significant differences ($P < 0.05$) between plant growth regulators within the same As concentration; different capital letters show significant differences ($P < 0.05$) between with and without As at the same plant growth regulator

Table 3
Root growth of Pennisetum purpureum *cv. Mott in the presence of plant growth regulator under non-spiked and arsenic-spiked soil (data shown as mean ± SE)*

Treatment	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Root-to-shoot ratio	Specific root length (m/g)
Non-spiked soil					
No plant growth regulator	34.0 ± 2.43aA	14.3 ± 2.14aA	1.3 ± 0.19aA	0.19	0.27
Salicylic acid	39.7 ± 4.72aA	16.8 ± 2.23aA	1.3 ± 0.18aA	0.19	0.32
Salicylic acid + calcium chloride	31.5 ± 1.97aA	12.0 ± 2.28aA	0.9 ± 0.19aA	0.18	0.33
Indole butyric acid	34.0 ± 3.16aA	15.6 ± 2.33aA	1.5 ± 0.26aA	0.21	0.23
Indole butyric acid + calcium chloride	34.9 ± 2.16aA	14.0 ± 1.89aA	1.2 ± 0.20aA	0.20	0.28
As-spiked soil					
No plant growth regulator	39.2 ± 3.61aA	8.8 ± 1.48aA	1.5 ± 0.34aA	0.28	0.26
Salicylic acid	36.0 ± 3.22aA	8.7 ± 1.52aB	1.2 ± 0.33aA	0.23	0.30
Salicylic acid + calcium chloride	35.8 ± 3.61aA	7.9 ± 1.28aA	0.9 ± 0.16aA	0.18	0.42
Indole butyric acid	31.9 ± 3.50aA	7.5 ± 1.72aB	1.0 ± 0.26aA	0.20	0.32
Indole butyric acid + calcium chloride	33.1 ± 2.07aA	6.9 ± 1.56aB	0.8 ± 0.29aA	0.18	0.38
As	ns	**	ns	-	-
Plant growth regulator	ns	ns	ns	-	-
As x Plant growth regulator	ns	ns	ns	-	-

Note. Different lowercase letters show significant differences ($P < 0.05$) between plant growth regulators within the same As concentration; different capital letters show significant differences ($P < 0.05$) between with and without As at the same plant growth regulator

regulators. The low root-to-shoot ratio means the plant root was not healthy enough to produce more shoots (Bobeautong et al., 2017). A reduction in the root/shoot ratio has been observed in *Brassica juncea*. It may be due to higher arsenic accumulation in the root as it is in direct contact with the sand, damaging the plant roots (R. Singh et al., 2020). Even though a high concentration of arsenic was observed in the roots of *P. purpureum* cv. Mott receiving indole butyric acid + calcium chloride, the root/shoot ratio of the plant was similar to plants grown in non-spiked soil and receiving the same plant growth regulator formula.

The total chlorophyll contents in the leaves of *P. purpureum* cv. Mott were similar between plants grown under non-spiked soil and arsenic spiked soil. The plant growth regulators had no effects on the total chlorophyll content in the leaves of plants grown under both soil conditions (Table 4). It may be due to the low concentration of arsenic used in this study that did not

Table 4
Chlorophyll content in leaves of Pennisetum purpureum *cv. Mott in the presence of plant growth regulator under non-spiked and arsenic-spiked soil (data shown as mean ± SE)*

Treatment	Chlorophyll *a* content (mg/ml)	Chlorophyll *b* content (mg/ml)	Total chlorophyll content (mg/ml)
Non-spiked soil			
No plant growth regulator	25.2 ± 3.51aA	24.8 ± 5.02aA	50.0 ± 6.59aA
Salicylic acid	22.5 ± 0.27aA	8.8 ± 0.38bA	31.4 ± 0.10aA
Salicylic acid + calcium chloride	29.5 ± 4.35aA	15.2 ± 0.91bA	44.7 ± 5.26aA
Indole butyric acid	31.0 ± 5.58aA	19.6 ± 1.58abA	50.6 ± 4.09aA
Indole butyric acid + calcium chloride	33.0 ± 9.91aA	14.8 ± 3.64bA	47.9 ± 13.39aA
As-spiked soil			
No plant growth regulator	30.0 ± 2.88aA	13.1 ± 1.47aB	43.2 ± 4.31aA
Salicylic acid	20.6 ± 2.94aA	11.1 ± 0.93aA	31.7 ± 3.84aA
Salicylic acid + calcium chloride	20.2 ± 8.21aA	9.5 ± 3.14aB	29.8 ± 11.34aA
Indole butyric acid	19.4 ± 5.05aA	9.7 ± 2.60aB	29.0 ± 7.64aA
Indole butyric acid + calcium chloride	23.8 ± 3.63aA	11.5 ± 2.06aA	35.3 ± 5.65aA

Note. Different lowercase letters show significant differences ($P < 0.05$) between plant growth regulators within the same As concentration; different capital letters show significant differences ($P < 0.05$) between with and without As at the same plant growth regulator

damage the photosynthetic pigment in *P. purpureum* cv. Mott and arsenic was rarely translocated from the root to shoot in this study. A greater arsenic concentration was detected in the roots of *P. purpureum* cv. Mott than in the shoots (Table 5). Only the plant growth regulator and arsenic affected the chlorophyll *b* content in the leaves of the plant. In non-contaminated soil, the chlorophyll *b* content in the leaves of plants receiving all plant growth regulators, except indole butyric acid, was decreased when compared with plants that did not receive plant growth regulators. Arsenic contamination decreased the chlorophyll *b* content in the leaves of plants that received salicylic acid + calcium chloride, indole butyric acid, and did not receive plant growth regulators. However, a reduction in the total chlorophyll content has been observed in *Artemisia annua* after exposure to 100 µM of arsenic, and the application of 100 µM of salicylic acid could increase the total chlorophyll content and biomass of *A. annua* grown under arsenic contamination (Kumari & Pandey-Rai, 2018).

Arsenic Concentration in Soil and Plants

The arsenic concentration used in this study seems to be low to *P. purpureum* cv. Mott, as the plant grew normally in

Table 5
As remaining in soil and plants under various plant growth regulators

Treatment	As remaining in soil (mg/kg)	As in shoot biomass (mg/kg)	As in root biomass (mg/kg)	Bioconcentration factor	Translocation factor	Translocation efficiency (%)
No As	0.81	0.02	0.38	0.49	0.05	7.43
Un plant soil	12.92 ± 0.54a	-	-	-	-	-
No plant growth regulator	11.71 ± 0.48a	3.27 ± 0.27a	19.20	1.92	0.17	17.50
Salicylic acid	11.28 ± 0.30a	2.69 ± 0.12a	10.15	1.14	0.26	26.78
Salicylic acid + calcium chloride	12.70 ± 0.95a	2.50 ± 0.47a	15.03	1.67	0.13	20.02
Indole butyric acid	12.16 ± 0.64a	2.68 ± 0.15a	18.74	1.46	0.18	24.66
Indole butyric acid + calcium chloride	11.07 ± 1.34a	2.70 ± 0.35a	47.38	4.52	0.06	9.77

Note. Different lowercase letters show significant differences ($P < 0.05$) between plant growth regulators within the same As concentration

12.92 ± 0.54 mg/kg of arsenic in the soil. The removal of arsenic from the soil by translocation of arsenic from the soil into the aboveground tissue of the plant in this study was poor because the concentration of arsenic detected in the soil after 41 days of transplantation was not significantly different from the soil without planting (Table 5). Furthermore, there was no significant difference between the arsenic concentrations in the shoot biomass of *P. purpureum* cv. Mott (2.50–3.27 mg/kg) in the experimental pots with and without application of each plant growth regulator (Table 5).

In contrast, the highest arsenic concentration in the roots of *P. purpureum* cv. Mott was detected in plants receiving exogenous indole butyric acid + calcium chloride as a plant growth regulator. It corresponds with the bioconcentration factor of arsenic in plants also observed in Napier grass grown under arsenic-spiked soil and receiving indole butyric acid + calcium chloride. Using indole butyric acid + calcium chloride also limits the translocation of arsenic from the root to the shoot of *P. purpureum* cv. Mott compared to other plant growth regulators. The translocation factor and translocation efficiency of arsenic were only 0.06 and 9.77% when the plant received indole butyric acid + calcium chloride as plant growth regulators (Table 5). In general, the aim of using a plant growth regulator was to decrease the translocation of metal from the root to the shoot of the plant.

For example, exogenous auxin, such as indole-3-acetic acid, can reduce arsenic translocation in rice by lowering the arsenic concentration in the upper leaves and internodes, but higher concentrations of arsenic were detected in the lower leaves and internodes of rice (He et al., 2020). Exogenous application of salicylic acid decreased the arsenic accumulation in the shoot of rice by 27% (A. P. Singh et al., 2017). Exogenous application of calcium chloride also decreased the arsenic uptake by 47% and 21% in the shoot and root of rice seedlings exposed to 1 mM arsenic, respectively (Rahman et al., 2015). In this study, the application of salicylic acid alone or indole butyric acid alone did not stimulate the accumulation of arsenic in the root of *P. purpureum* cv. Mott. Increasing the arsenic accumulation in the plant roots in this study may be influenced by indole butyric acid in combination with calcium chloride. Normally, calcium is an essential mineral nutrient in plants and acts as a secondary messenger that mediates cell and plant development and alleviates arsenic toxicity by reducing its uptake in the plant (Rahman et al., 2015).

Moreover, an appropriate amount of exogenous calcium should be helpful to increase the integrity of the plant membrane (Boorboori et al., 2021). However, applying salicylic acid in combination with calcium chloride did not stimulate the accumulation of arsenic in the root of *P. purpureum* cv. Mott in this study. It may be due to the interaction of salicylic acid and calcium, which has been reported to be involved in

hormonal signal transduction (Medvedev, 2005). The different interactions between calcium with other plant growth regulators, salicylic acid, and indole-3-acetic acid, on arsenic accumulation, may depend on different mechanisms of each plant growth regulator on plant water uptake. Indole-3-acetic acid and calcium ion have been reported to increase water uptake, while salicylic acid has been reported to adjust osmotic pressure and decrease plant transpiration (Khushboo et al., 2018; Saruhan et al., 2012; Votrubová &Votruba, 1986).

The most likely mechanism for arsenic phytoremediation in this study was phytostabilization when considering the amount of arsenic detected in the root and shoot biomass of *P. purpureum* cv. Mott, translocation factor, and translocation efficiency. Successful arsenic phytostabilization was reported for *P. purpureum* cv. Mott when using other organic amendments, such as cow manure and acacia wood-derived biochar (Kowitwiwat & Sampanpanish, 2020). Moreover, ethylenediaminetetraacetic acid (EDTA) was shown to increase the translocation and accumulation of arsenic in the aboveground tissue of *P. purpureum* cv. Mott (Boonmeerati & Sampanpanish, 2021).

CONCLUSION

Successful application of plant growth regulators varied with concentration, type of plant growth regulator, and plant species (Piacentini et al., 2020; Rafiq et al., 2017). The combination of indole butyric acid and calcium chloride was the most suitable for arsenic phytostabilization of *P. purpureum* cv. Mott in this study. However, the application of these plant growth regulators to restrict the arsenic translocation to the aboveground parts of the plant tissue should be further studied under field experiments, especially regarding the effects of other biotic and abiotic factors in the environment.

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