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The Effect of *Azotobacter* on Growth and Yield of Water Spinach in Growing Media Containing Gold Mine Tailings

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ABSTRACT

Gold mining provides economic benefits but results in the conversion of agricultural land and produces low-fertility tailings. Efforts to remediate tailings can be done through the utilization of the biofertilizer bacteria *Azotobacter* sp., which is capable of producing exopolysaccharides. This study aims to study the effects of biofertilizers containing a consortium of *Azotobacter* sp. strains S5, S6a, and S9 in enhancing the growth and yield of water spinach plants in growth media containing gold mine tailings. The experiment used a randomized complete block design with four treatments and six replications. The treatments consisted of; (A) control, (B) 1% biofertilizer, (C) 5% biofertilizer, and (D) 10% biofertilizer. The results of the experiment showed that the dosage of 5% biofertilizers increased plant height, leaf count, and fresh weight of plants, while the 10% biofertilizer increased the stem diameter. The findings of this experiment emphasize the importance of biofertilizers in enhancing the growth and yield of water spinach plants in media with low fertility.

Keywords: *Azotobacter* sp., biofertilizers, gold mining, tailings remediation, water spinach

Pengaruh *Azotobacter* terhadap Pertumbuhan dan Hasil Tanaman Kangkung pada Media Tanam Mengandung Tailing Tambang Emas

ABSTRAK

Penambangan emas memberikan manfaat ekonomi tetapi mengakibatkan konversi lahan pertanian dan menghasilkan tailing dengan kesuburan yang rendah. Upaya untuk meremediasi tailing dapat melalui pemanfaatan bakteri pupuk hayati *Azotobacter* sp., yang mampu memproduksi eksopolisakarida. Penelitian ini bertujuan untuk mengeksplorasi efek pupuk hayati yang mengandung konsorsium *Azotobacter* sp. strain s5, s6a, dan s9 dalam meningkatkan pertumbuhan dan hasil tanaman kangkung pada media tanam yang mengandung tailing tambang emas. Percobaan ini menggunakan rancangan acak kelompok dengan empat perlakuan dan enam ulangan. Perlakuan terdiri dari; (A) kontrol (B) 1% pupuk hayati (C) 5% pupuk hayati (D) 10% pupuk hayati. Hasil percobaan menunjukkan bahwa dosis 5% pupuk hayati mampu meningkatkan tinggi tanaman, jumlah daun, bobot segar tanaman, sedangkan dosis 10% pupuk hayati meningkatkan diameter batang. Temuan percobaan ini menekankan akan pentingnya pupuk hayati dalam meningkatkan pertumbuhan dan hasil tanaman kangkung pada media dengan kesuburan yang rendah.

Kata kunci: *Azotobacter* sp., penambangan emas, pupuk hayati, remediasi tailing, tanaman kangkung

INTRODUCTION

Indonesia is rich in natural resource includes gold mineral, a precious metal of high market value that is extensively exploited. Now, Indonesia is the 9th highest gold producer globally, with a total production of 70 tons in 2022 [1][2]. The positive impacts of gold mining include increased regional and national income, yet these activities also bring negative effects such as land use conversion[3]. The continuous exploration of gold mining areas leads to the conversion of agricultural land, plantations, and forests into mining sites, negatively impacting the environment.

Tailings are residual products after processing activities and gold mining has the potential to be an environmental pollutant if not managed properly. These tailings have poor physical, chemical, and biological properties, making them challenging to utilize. They are typically characterized by unstable structures, rapid permeability, high porosity, and a composition dominated by clay or sand (>50%). Additionally, tailings are nutrient-poor and have low organic matter content, leading to reduced microbial populations, particularly those crucial for nutrient cycling [4]. To address the challenges posed by tailings, organic materials can be added to improve their properties. Organic materials have been shown to improve the chemical and biological conditions of tailings, promoting plant growth [5]. Another promising approach is the use of biofertilizers, which contain microbial species capable of mobilizing nutrients and decomposing organic contaminants [6]. Among these biofertilizers, consortia containing multiple microbial strains are considered more effective in improving tailings conditions compared to single microbe applications [7].

There are three superior strains of *Azotobacter* sp. suitable for use as biofertilizers: *Azotobacter* sp. S5, *Azotobacter* sp. S6a, and *Azotobacter* sp. S9 [8]. These strains are resistance to Hg and can produce nitrogenase and organic acids in significant amounts. The genus of *Azotobacter* is a Plant Growth Promoting Rhizobacteria (PGPR), has shown potential for biofertilizer applications that can enhance plant growth and control certain plant pathogen [6]. These bacteria not only improve plant growth through nitrogen fixation, phosphate solubilization, and production of phytohormones (auxin, gibberellin, and cytokinin), but also play a role in bioremediation of heavy metals like Hg [9]. The ability of *Azotobacter* to produce exopolysaccharides (EPS) allows it to bind heavy metals, detoxify plants, and improve tailings aggregation[10]. Furthermore, *Azotobacter* activity can be enhanced by providing adequate carbon sources, often obtained from root exudates.

Water spinach emerges as a suitable candidate for testing in growing media containing tailings due to its tolerance to pollutants and high nutritional requirements [11]. This leafy vegetable's ability to thrive in media containing heavy metals makes it an ideal test subject for studying plant growth in challenging environments. Several studies have shown positive effects of adding organic matter on water spinach growth in tailings media but have not yet achieved optimal results, indicating the need for further research, especially in the addition of *Azotobacter* biofertilizers. This study aims to investigate the effects of *Azotobacter* biofertilizers on water spinach growth; the research will provide valuable insights into sustainable approaches for utilizing mining by-products in agriculture.

MATERIAL AND METHODS

The experiment was conducted at the greenhouse located in the Ciparanje experimental field, Universitas Padjadjaran, at an altitude of approximately 760 meters above sea level, from November 2023 to January 2024. The experiment was set up in Randomized Block Design with four treatments and six repetition (Figure 1). The treatments consisted of various doses of liquid biofertilizer, including: (A) Control, (B) 1% biofertilizer, (C) 5% biofertilizer, (D) 10% biofertilizer. The liquid biofertilizer composed of multi-strain of *Azotobacter*.

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Figure 1. The layout of pot experiment of water spinach at 28 days after planting

The growing medium was a mixture of gold mine tailings and cow manure (CM) fertilizer with the composition of 85% tailings and 15% CM fertilizer. This growing medium had a slightly acidic in pH (6.53), high organic matter content (3.82%), and low total-N (0.18%). The tailings were collected from Karanglayung Village, Tasikmalaya Regency of West Java. The water spinach seeds used in this experiment were of the Bangkok LP-1 variety produced by PT. East West Seed Indonesia.

The biofertilizer used in this experiment was a consortium of *Azotobacter* sp. S5, S6a, and S9 (1:1:1 ratio) were developed by the Soil Biology Laboratory. Biofertilizer application was carried out by mixing *Azotobacter* liquid inoculant (according to the treatment doses) with one kilogram of planting medium in each polybag. A week before sowing 6 seeds of water spinach in three planting holes with a depth of 5 cm. After 14 and 21 days after planting (DAP), chemical fertilizer of NPK (16-16-16) was added with the dose of 2.25 g plant^{-1}. The plants were maintained in the greenhouse for 28 days.

The parameters measured were plant height, number of leaves, stem diameter, and shoot fresh weight of water spinach. Plant growth parameters were measured at 7, 14, 21, 28 days after planting (DAP) while the biomass was analyzed at 28 DAP. All data sets were subjected to normality test prior to the analysis of variance at $p < 0.05$. If the treatments had a significant effect on parameter, then the Duncan's Multiple Range Test (DMRT) was conducted to determine differences between treatments. The statistical analysis performed by using IBM SPSS version 26.

RESULTS AND DISCUSSION

Plant Height

Table 1 shows that the biofertilizer treatment did not affect plant height at 7 DAP but 5% and 10% biofertilizer increased plant height at 14 to 28 DAP. Plants treated with 5% and 10% biofertilizer had similar plant height which is significantly higher compared to the control at 14, 21, and 28 DAP. At 28 DAP, application of 5% and 10% biofertilizer increased the plant heights up to 16.58% and 19.45% respectively compared to the control plants (Figure 2).

Table 1. The effect of various doses of biofertilizer on water spinach height

Treatment	Plant Height (cm) at			
	7 DAP	14 DAP	21 DAP	28 DAP
A : Control	4.80	5.36a	7.05a	8.02a
B : 1% biofertilizer	4.28	5.87ab	7.03a	7.89a
C : 5% biofertilizer	4.69	6.29b	8.36b	9.35b
D : 10% biofertilizer	5.13	6.39b	8.47b	9.58b

Note: Numbers in column followed by the same letter are not significantly different based on the DMRT at $p<0.05$.

Figure 2. Water spinach plants at 28 days after planting

Number of Leaves

The application of biofertilizers increased the number of leaves at 14, 21, and 28 DAP (Table 2). At 7 HST, plant with and without biofertilizers had two number of leaves. Application of 5% and 10% of biofertilizer resulted in a significantly different number of leaves at 28 DAP compared to the control. The increase of leaves number after those treatments were 19.95% and 30.86% respectively compared to untreated plants. The treatment with the lowest leaves number was the control, which had the same leaves number of plant with 1% of biofertilizer.

Table 2. The effect of various doses of biofertilizer on the number of water spinach leaves

Treatment	Number of Leaves at			
	7 DAP	14 DAP	21 DAP	28 DAP
A : Control	2.00	2.67a	3.70a	4.31a
B : 1% biofertilizer	2.00	3.25b	3.69a	4.28a
C : 5% biofertilizer	2.00	3.36bc	4.83b	5.17b
D : 10% biofertilizer	2.00	3.68c	4.96b	5.64b

Note: Numbers in column followed by the same letter are not significantly different based on the DMRT at $p<0.05$.

Stem Diameter

Increasing the dose of biofertilizer increased the stem diameter (Table 3). The stem diameter of plants without biofertilizer (control) significantly lower than that of plant treated with biofertilizer. Plants with 5% of biofertilizer had similar stem diameter with plants with 1% and 10% of biofertilizer. Plants treated with 10% of biofertilizer were able to achieve the highest stem diameter at 28 DAP, measuring 1.88 mm which is 15,34% higher than control plant.

Table 3. The effect of various doses of biofertilizer on the stem diameter of water spinach

Treatment	Steam Diameter (mm) at		
	14 DAP	21 DAP	28 DAP
A : Control	1.32a	1.57a	1.63a
B : 1% biofertilizer	1.42b	1.72b	1.75b
C : 5% biofertilizer	1.46bc	1.80bc	1.83bc
D : 10% biofertilizer	1.52c	1.85c	1.88c

Note: Numbers in column followed by the same letter are not significantly different based on the DMRT at $p<0.05$.

Shoot Fresh Weight of Water Spinach

The fresh weight of control plants was same with the plants treated with 1% biofertilizer (Table 4). The highest fresh weight was observed in plant treatment with 10% of biofertilizer (3.28 g), which is 29.67% higher than the control (2.53 g). However, the fresh weight of plant with 10% of biofertilizer did not different with dosage of 5% of biofertilizer.

Table 4. The effect of various doses of biofertilizer on the fresh weight of water spinach at 28 DAP

Treatment	Fresh Weight of Plants (g)
A : Control	2.53 ± 0.24a
B : 1% biofertilizer	2.58 ± 0.35a
C : 5% biofertilizer	3.22 ± 0.30b
D : 10% biofertilizer	3.52 ± 0.38b

Note: Numbers in column followed by the same letter are not significantly different based on the DMRT at $p<0.05$.

The results of this experiment indicated that the application of the *Azotobacter* sp. increased the growth parameters and yield (shoot fresh weight) of plants. Biofertilizer inoculation at the dosage of 5% was resulted in higher plant height (Table 1), leaf number (Table 2), and plant fresh weight (Table 4). Conversely, the best biofertilizer dosage for stem diameter increment was 10%. This is attributed to the beneficial mechanisms of *Azotobacter* as PGPR. The well-known mechanisms is nitrogen fixation by which the N_2 is enigmatically converted to NH_3 and then the gas will converted to NH_4^+ and NO_3^- that available for plants uptake [12] dequate nitrogen availability for plants can enhance all growth parameters in this experiment. Nitrogen stimulates cell development and division during the vegetative phase, thereby promoting stem, leaf, and root growth[13]. Additionally, the nitrogen sufficiency stimulates chlorophyll formation that related to photosynthesis and increase the leaves number and leaf surface area [14]. Therefore, the nitrogen levels in leaves must be sufficient (not excessive or deficient) to avoid delayed or retarded growth of water spinach plants [15].

The *Azotobacter* sp. also supports plant growth and root development through the production of phytohormones and exopolysaccharides [16]. The bacteria produce hormones such as cytokinin, gibberellin, or Indole-3-Acetic Acid (IAA) [17]. The IAA also known as natural auxin, can control cell expansion and division, thereby stimulating an increase in root weight, including hair roots, hair root branches, and lateral roots, which directly also increases plant fresh weight [18]. Phosphorus is the second most important nutrient for plants. In this experiment, the *Azotobacter* S5, S6a, and S9 consortium was supposed to solubilize the unavailable inorganic phosphate, thereby also played a role in stimulating water spinach growth. The result is consistent with the research, the *Azotobacter* consortium can produce higher levels of soluble phosphate compared to single isolates [19].

The application of NPK 16-16-16 fertilizer in this experiment supported plant growth. The NPK fertilizer provides nitrogen (N), phosphorus (P), and potassium (K), which are three essential macro-nutrients required by water spinach plants to optimize their growth, including plant height [20]. Moreover, organic matter in each pots have a significant role in providing organic carbon and energy for heterotrophic *Azotobacter*. The biofertilizer in this experiment has shown a positive impact on water spinach growth and yield. However, the plant yield were remarkably lower compared to the of water spinach cultivated in mineral soil, which produces a fresh weight of approximately 10 g $plant^{-1}$ until 30 g $plant^{-1}$.

CONCLUSION

This experiment verified that the application of the *Azotobacter* sp. contributed to the increase in plant height, leaf number, stem diameter, and fresh weight of water spinach in growth media containing gold mine tailings. The application of a 5% dose of the biofertilizer most enhanced the plant height, leaf number, and fresh weight of plants; while a 10% dose of the biofertilizer was needed to obtain higher stem diameter of water spinach plants. The research showed that application of 5% liquid inoculant of *Azotobacter* can be suggested to improve the growth water spinach planted in tailing enriched with 15% cow manure. However, it is likely that increased amount of cow manure is needed for better growth of water spinach.

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