International Journal of Life Science Research Archive

ISSN: 0799-6640 (Online)

Journal homepage: https://sciresjournals.com/ijlsra/

(RESEARCH ARTICLE)

Azolla pinnata ammendment and P-solubilizing bacteria inoculation for improving Inceptisols fertility and rice (*Oryza sativa* L.) yield production

Pujawati Suryatmana [1, *], Wildan Maulana Ridho [2], Betty Natalie Fitriatin [1] and Mieke Rochimi Setiawati [1]

[1] *Department of Soil Science and Land Resources Management Faculty of Agriculture, Padjadjaran University, Jalan Ir. Soekarno, Km 21. Jatinangor. West Java, Indonesia.*

[2] *Agrotechnology Programe Study, Agriculture Faculty, Universitas Padjadjaran, Jalan Ir. Soekarno, Km 21. Jatinangor. West Java, Indonesia.*

International Journal of Life Science Research Archive, 2023, 05(02), 079–088

Publication history: Received on 30 October 2023; revised on 09 December 2023; accepted on 12 December 2023

Article DOI: https://doi.org/10.53771/ijlsra.2023.5.2.0104

Abstract

Continuous and long-term use of chemical fertilizers in lowland rice fields can have negative impacts on the environment, causing harm to aquatic life and a decrease in soil health. Applying *Azolla pinnata* and inoculating phosphate solubilizing bacteria (PSB) in organic farming is an appropriate strategy to increase soil nutrients and lowland rice production. *Azolla pinnata* plays a role in increasing N nutrients, while phosphate solubilizing bacteria (PSB) increase available phosphate in the soil. This study aims to assess the effectiveness of varying doses of *Azolla pinnata* and PSB inoculant in increasing soil available P, plant-P concentration, plant hegh, productive tiller, and rice yield. The experimental design used a Factorial Randomized Block Design of two factors in three replications. The first factor was *Azolla pinnata* application (0 ton ha^{-1}; 10 ton ha^{-1}; 20 ton ha^{-1}; 30 ton ha^{-1}), and the second factor was PSB inoculation (0 kg ha^{-1}; 12.5 kg ha^{-1}; 25 kg ha^{-1}). The experimental results showed that the *Azolla pinnata* application at a dose of 10 t ha^{-1} with inoculation of 12.5 kg ha^{-1} PSB significantly increased the highest soil available P by 48.3 ppm. The application of *Azolla pinnata* 30 t ha^{-1} significantly increased the P content of plants—0.26 mg g^{-1}. *Azolla pinnata* 30 t ha^{-1} application and PSB inoculation produced significantly the highest productive tillers of rice plants, 38 plants pot^{-1}. *Azolla pinnata* 10 tons ha^{-1} application with BPF inoculant 12.5 kg ha^{-1} significantly increased the highest grain yield, namely 81.47 g $pot.^{-1}$

Keywords: *Azolla pinnata*; Inceptisols; *Oryza sativa*; P-solubilizing bacteria

1 Introduction

Rice, the main carbohydrate-producing crop in Asia, is consumed and produced in large quantities in seven countries: China, India, Indonesia, Bangladesh, Vietnam, Thailand, and Myanmar. These countries produce an average of more than 30 million tons of rice. However, the increasing demand for rice means that more high-yield rice production is necessary. To achieve this, proper and environmentally friendly cultivation techniques are crucial.

Kavvadias (2010) states that cultivation practices, such as intensive planting seasons and continuous application of artificial chemical fertilizers without balancing organic material, can lead to soil fertility loss and degradation. This ultimately affects the ability of soil to provide nutrients to plants. The Incetisol order is a potential soil for development in Indonesia. The formation of Inceptisol soil order is mainly influenced by time, parent material, climate, vegetation, and topography. The process of transformation of minerals and organic materials and the size of eluviation and illuviation play a crucial role in the formation of most inceptisols (Fanning & Fanning, 1989).

* Corresponding author: Pujawati Suryatmana

Upland acid soils found in Inceptisols order areas in Indonesia have the potential for food crop production of about 102.8 million hectares, as reported by Mulyani et al. (2004). However, one of the major challenges in cultivating these soils is the high cost associated with maintaining and increasing their productivity, as they tend to decline after several years of cultivation. Inceptsols have problems with their characteristics, including low N and P content, acidic pH ranging from 4.5 to 5.5, and low to medium organic matter content (Nurjaya & Nursyamsi, 2013), their ability to store/hold water (Fanning & Fanning, 1989). Thus, efforts are needed to increase the productivity of the land to be used as soybean cropland. Yusuf et al.(2017) state that plants need nitrogen (N). Nitrogen functions in growing roots, stems, and leaves (Yusuf et al., 2017). Generally, to overcome the Inceptisol problem, chemical fertilization is applied, but on the other hand, the continuous use of chemical fertilizer without being balanced with organic fertilizer has a negative impact on reducing soil quality and crop yields (Garfansa et al., 2017). One alternative The solution to improve soil health and productivity of rice plants in Inceptisols is by applying two important components as suppliers of N and P nutrients, namely organic material that supplies N and bacterial inoculation, which can facilitate increased availability of soil P. These two components are *Azolla pinnata* and consortium isolate Phosphorus solubilizing bacteria.

Azolla is a free-floating water fern native to the tropics and temperate paddies (Subedi & Shrestha, 2015; Watanabe et al., 1980). Worldwide, it is represented by seven (7) recognized species, namely *A. nilotica, A. pinnata, A. filiculoides, A. mexicana, A. rubra, A. microphylla, and A. caroliniana* (Pereira et al., 2011). Azolla, a free-floating, N2-fixing aquatic fern, is an established N biofertilizer for flooded rice. (Majumdar et al 1993). *Azola pinnata* can have a symbiotic relationship with Cyanobacteria (*Anabaenna azollae*), which can fix N2 from the air so that the biomass from Azolla contains relatively higher N than conventional compost organic material. This makes it essential to use in paddies as a bio-fertilizer. Elmizan et al. (2014) showed that Azolla-based fertilizer given in green and compost form significantly increased the soil's C-organic N, P, K-dd, and CEC. Setiawati (2014) stated that applying 3 tons/ha of *Azolla pinnata* increased the N and P content of the soil. *Azolla pinnata*, as a green fertilizer, can meet half of the nitrogen needs of lowland rice, estimated at 90 to 120 kg N/ha, depending on the type of Azolla, type of rice, as well as soil fertility, and climate conditions (Elmizan et al., 2014). The chemical analysis of *Azolla mycrophylla* compost is as follows: pH 7.17, N 2.57%, P 0.34%, K 0.03%. (Lestari & Muryanto, 2018). Using 20 t/ha of Azolla can increase rice production yields equivalent to 30 kg/ha of N fertilizer and increase the organic content of the soil. An increase in organic matter in the soil can stimulate an increase in the population of heterotrophic, cellulolytic bacteria and urea hydrolysis activity (Yadaf et al., 2014). *Azolla pinnata* contains macronutrients such as Nitrogen 3.91%, Phosphorus 0.3%, Potassium 0.65%, C/N = 6, and B.O 39.905 %. Meanwhile Seleiman et al. (2022) reported that Azolla compost has pH 7.12; Organic C 35.83; N, P, and K: 3.17; 1.10 and 1.80 (%) respectively. Kurniawati et al. (2021), using *Azolla microphylla* liquid compost with different dosage levels, can increase the growth and yield of sweet corn plants. Azolla can also increase the absorption of several nutritional elements, such as Ca, Mg, and K (Bhuvaneshwari & Kumar, 2013). Several Azolla species have different abilities in returning P elements. *A. filiculoides* and *A. caroliniana* have the highest ability in returning P of 45% of what they have absorbed, *A. pinnata* (28%), and *A. mexicana* the lowest (19%). Based on the species, planting Azolla can supply P equivalent to 8-11 kg/hectare to rice plantings (Watanabe, 1987). Utilization of *Azolla pinnata* in rice planting can increase organic matter. An increase in organic matter in the soil can stimulate an increase in the population of heterotrophic, cellulolytic bacteria and urea hydrolysis activity (Yadaf et al., 2014).

Administering phosphate-solubilizing bacteria can help increase available phosphate, P uptake, and crop yields in rice plants (Saraswati et al., 2009). These microbes also can fix N in the air, produce phytohormones, and dissolve the bound P, making it available for lowland rice plants. Different genus such as *Pseudomonas* sp., *Bacillus* sp., *Mycobacterium*, *Flavobacterium* sp., and *Thiobacillus* sp. fall under the category of phosphate-solubilizing bacteria, each having unique characteristics and optimal environmental conditions that influence its effectiveness in dissolving phosphate (Simanungkalit et al., 2010). Phosphate solubilizing microbes have various benefits, including the release of organic acids that can form organic chelates or stable complexes with Al, Fe, or Ca cations, which bind P and make the $H_2PO_4^{2-}$ ion free from its bonds, thereby making it available to plants (Rao, 1977). PSB are capable of converting insoluble P into soluble forms that are bioavailable for plant uptake (Sharma et al., 2013; Paulucci et al., 2015; Khan et al., 2021). These microbes can extract P from its bonds with Al, Fe, Ca, and Mg, which was initially unavailable to plants, by secreting organic acids that can form stable complexes with P-binding cations in the soil. Phosphate solubilizing microbes can transform insoluble P to soluble orthophosphate forms then liberated $PO4^{3-}$, $HPO4^{2-}$, and $H2PO^4$. The most potential group of soil microbes that play a role in the phosphorus dissolution process such as *Pseudomonas putida*, *P. plecoglossicida*, *P. moteilii*, *P. asiatica*, and *Bacillus* spp. (Yu et al., 2022). The carboxyl groups of organic acids can bind P by replacing cations or competing for P adsorption sites, enhancing the soil absorption of $PO4^{3-}$ and increasing Pi solubilization (Zaidi et al., 2010)

This research aims to evaluate the effectiveness of *Azolla pinnata* and phosphate-solubilizing bacteria in increasing soil available P and plant P concentration, soil organic carbon, growth, and rice production.

2 Materials and Methods

The experiment was conducted at the Ciparanje Experimental Garden Greenhouse, located in the Faculty of Agriculture at Padjadjaran University in Jatinangor, Sumedang Regency. The altitude of the location is approximately 765 meters above sea level (masl), and the rainfall type is C3 according to Oldeman's classification. For the experiment, we used Ciherang rice seeds, a mixture of phosphate solubilizing bacterial cultures *Bacillus* sp. and *Pseudomonas* sp. The Colonies morphology of *Bacillus* sp. and *Pseudomonas* sp. are shown in fig 2 and 3, respectively; fresh *Azolla pinnata* (fig 4); seeding media (comprised of soil and compost); Inceptisol paddy soil, and essential fertilizers such as urea, SP-36, and KCl.

The research design used was a Randomized Factorial Block Design (RFBD) which consisted of two factors, with a total of 12 treatments and three replications (The experiment lay out is shown in fig 1.), first factor is the dose of fresh *Azolla pinnata* used (A) which consists of four levels, and the second factor is the dose of Phosphate solubilizing Bacteria (P) which consists of three levels, with each level as follows:

The first factor is the dose of *Azolla pinnata* (A):

a0 = No Azolla ;
a1 = Azolla 10 tons ha^{-1}(112.5 g/pot)
a2 = Azolla 20 tons ha^{-1}(225 g/pot)
a3 = Azolla 30t on ha^{-1}(337.5 g/pot) .

The second factor is the dose of solid Phosphate solubilizing bacteria - PSB (P):

h0 = No Phosphate solubilizing Bacteria (without PSB)
h1 = PSB inoculation 12.5kg ha^{-1} (0.14 g/pot
h2 = PSB inoculation 25kg ha^{-1}(0.28 g/pot)

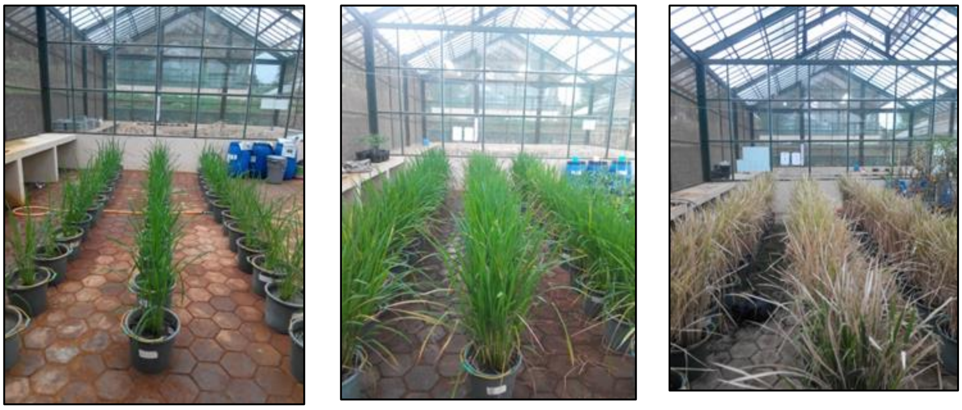

Figure 1 The lay out of the experimental

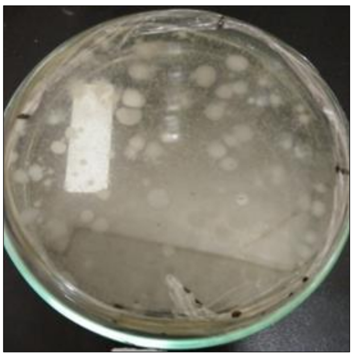

Figure 2 *Bacillus sp.*

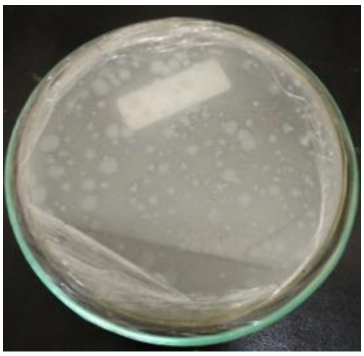

Figure 3 *Pseudomonas* sp.

Figure 4 Propagation of *Azolla pinnata* in production ponds (left) and *Azolla pinnata* morphology (right) (Suryatmana et al. 2021)

During the research, the following observations were made: the availability of P in the soil, plant P concentration, plant height, the number of productive tiller, and Rice yield.

2.1 Preparation of Rice Seeds and Planting Media

The rice seeds used in this process are of the Ciherang variety, which is certified and selected based on their excellent germination capacity. The first step is to sow the rice seeds, which are then grown until they reach 7 DAP (day after planting). The planting medium used is Inceptisol, which is obtained from rice fields around Ciparanje, the Experimental Garden of the Agricultural Faculty of Padjadjaran University. The topsoil is taken from a depth of 20 cm and air-dried for two weeks. Then, the soil is crushed and sifted to a size of 2 mm and put into a pot with a capacity of 10 kg. The next step is to flood the soil by adding water in a ratio of 2:1. After the soil is flooded, it is stirred repeatedly until it turns into mud.

2.2 Planting, fertilizing and maintaining plants

After the seeds have been sown for seven days, planting begins using the twin seedling method. For each seedling, a hole is dug and the roots are positioned in an L shape about 6-10 cm deep, allowing for proper root growth and nutrient absorption. Three types of fertilizer were used in the research: *Azolla pinnata* as a green fertilizer, a consortium of phosphate-solubilizing bacteria as treatment, and NPK inorganic fertilizer as essential fertilizer. The *Azolla pinnata* fresh was applied a week before planting by soaking fresh *Azolla pinnata* biomass. The consortium of phosphate-solubilizing bacteria was applied during the rice planting in experimental pots. Inorganic fertilizer was applied as the primary treatment by giving half of the recommended dose by the Department of Agriculture. The recommended dose for rice fertilizer in Jatinangor District, Sumedang Regency, is 300 kg/ha Urea, 50 kg/ha SP-36, and 50 kg/ha K

2.3 Observation and Sampling

The growth observations were conducted every week to monitor the progress of the plants. Two types of components were monitored - growth and yield components. Plant height and number of tillers were the key growth components observed, while the number of productive tillers and the weight of the harvested dry grain were the yield components observed. The plant samples were taken from the top fourth leaves of the plants that had entered the final vegetative phase or before the flowers appeared on the plants. The harvesting process was carried out when the rice grains were physiologically mature, which is characterized by a fully mature grain (every grain is mature, fully developed, hard, and yellow), the upper leaves drying out, and several dead leaves accumulating at the base of the plant.

2.4 Statistical analysis

Statistical Product and Service Solutions (SPSS) version 15.0 was used to analyze experimental data. An analysis of variance (ANOVA) was performed, and significant differences were assessed at a 5% significance level ($p < 0.05$).

3 Results and discussion

3.1 Initial physical and chemical properties of experimental soil

The soil used as a planting medium is rice field with the Inceptisol soil order from Ciparanje-Jatinangor. The experiment soil characteristics are shown in table 1.

Table 1 Characteristics of Experimental Soil (Incetisol order from Jatinangor)

No	Parameters	Unit	Result	Criteria
1	pH: H_2O	-	6.38	slightly acid
2	pH: KCl	-	5.83	-
3	C-organic	%	6.36	high
4	N-total	%	0.25	low
5	C/N	-	26	high
6	P_2O_5HCl 25%	mg/100g	80.95	high
7	K_2O HCl	mg/100g	20.34	low
8	P_2O_5 Bray 1	Ppm P	13.61	high
9	Al-dd	cmol kg^{-1}	0	-
10	H-dd	cmol kg^{-1}	0	-
11	cation exchange capacity (CEC)	cmol kg^{-1}	29.40	high
12	Base saturation	%	61.39	high
13	Al saturation	%	0	low
14	Cation arrangement:			
	K-dd	cmol kg^{-1}	0.35	low
	Na-dd	cmol kg^{-1}	0.33	low
	Ca-dd	cmol kg^{-1}	11.87	high
	Mg-dd	cmol kg^{-1}	5.50	high
15	Texture			
	Sand	%	13	Klay
	Dust	%	38	
	Klay	%	49	

Description: Chemical analysis at the Soil Fertility and Plant Nutrition Laboratory, Faculty of Agriculture, UNPAD (2021).

The paddy soil used in this study has a slightly acidic actual pH of 6.38, and a potential pH of 5.83. According to Grist (1986), acidic and flooded soil contains high amounts of aluminum (Al) and iron (Fe). These two elements can bind phosphorus (P) compounds, making them unavailable to plants. The soil has a high total organic carbon (C) content of 6.36%, and a medium nitrogen (N) content of 0.25%. The C/N ratio content is classified as high (26). The available P concentration is high, with 13.61 parts per million (ppm), and the potential P is high, with 80.95 milligrams per 100g of soil.

3.2 Plant height of Rice Plant

Plant height observations were conducted weekly from 1 WAP (week after planting) to 8 WAP (weeks after planting) during the plant's final vegetative stage is shown in fig 5. The result reveal that no significant differences in height were observed among treatments.

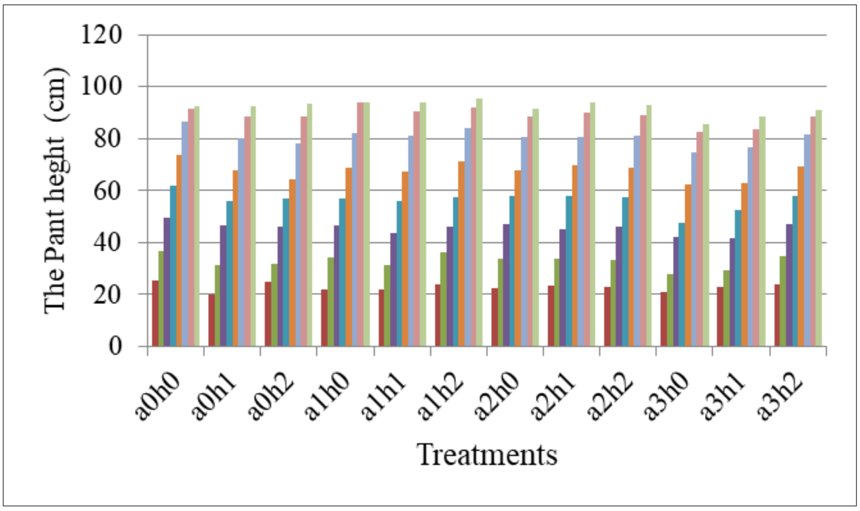

Figure 5 Plant growth as long as 8 WAP

It has been observed that the use of *Azolla pinnata* has no significant effect on the growth of plant height. Similarly, the application of phosphate-solubilizing bacteria does not contribute significantly to an increase in plant height. This is because the soil already has a relatively high content of nitrogen and phosphorus, which limits the effectiveness of the bacteria in fixing nitrogen or dissolving phosphate.

3.3 Soil availability-P

Table 2 displays the effects of *Azolla pinnata* and PSB treatments to soil availability-P. In the cultivation system, farmers often apply phosphate fertilizers to the soil in order to increase plant productivity. However, plants have a limited ability to absorb P from the soil because more than 80% of the P in the soil is immobile and unavailable to be absorbed. This is due to factors such as absorption, leaching, or conversion into organic forms. Based on an analysis of variance, it has been found that administering *Azolla pinnata* combined by phosphate-solubilizing bacteria can increase the available P in the soil. Further tests have shown that administering 10 tons ha-1 of *Azolla pinnata* with PSB consortium of 12.5 kg ha^{-1} Phosphate-solubilizing Bacteria can significantly increase available P compared to other treatments, with an average value of 48.3 ppm (table 2). However, increasing the application dose beyond 10 tons ha^{-1} of *Azolla pinnata* and 12.5 kg ha^{-1} of Phosphate

Table 2 The Effect of *Azolla pinnata* application and PSB to availability-P in soil (ppm)

Azolla pinnata	**PSB Inoculation (kg/ha)**		
(ton/ha)	h_0	h_1	h_2
0 (a_0)	28.87 a	33 a	40.2 a
	A	A	A
10 (a_1)	36.77 a	48.3 b	30.4 a
	B	C	A
20 (a_2)	32.5 a	31.53 a	29.3 a
	A	A	A
30 (a_3)	28.77 a	29.8 a	31.2 a
	A	A	A

Note: Numbers with the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% level. Uppercase letters are read horizontally and lowercase letters are read vertically.

Azolla pinnata is a potential source of organic soil P due to its high content of N and P elements compared to other composts. When *Azolla pinnata* decomposed, it can provide N and P nutrients. *Azolla pinnata* absorbs P elements where it is cultured and releases them upon decomposition. *Azolla pinnata* immersion supplies P and N to rice plants and increases P mobilization in the soil. Simultaneously embedded nutrients become more available to rice plants. *Azolla pinnata* supports microbial growth in the soil, especially microbes added by phosphate-solubilizing bacteria. Decomposed organic materials provide an energy source as polysaccharides and proteins, increasing microbial biomass and facilitating the availability of N and P for plants. Phosphate-solubilizing bacteria require nitrogen as ammonium to dissolve P bound by Al, Fe, and Ca. The availability and nature of the nitrogen used in the media greatly influence the ability of Phosphate-solubilizing Bacteria (BPF) to dissolve phosphate (Khan et al., 2021).

3.4 P-content of Rice Plant

The effects of *Azolla pinnata* and PSB inoculation to P-content of Rice plant are displayed in Table 3. The analysis of variance revealed that there was no interaction between the application of *Azolla pinnata* and the inoculation of the phosphate-solubilizing bacteria consortium. However, each treatment had an independent effect. The analysis showed significant differences in various applications of *Azolla pinnata* and phosphorus solubilizing bacteria when plant P concentration was increased. Applying 20 tons ha^{-1} of *Azolla pinnata* can significantly increase plant P concentration compared to *Azolla pinnata* treatment of 0 tons ha^{-1} (control), 10 tons ha^{-1}, and 30 tons ha^{-1}, with an average of 0.26%. The plant phosphate content was not significantly different between the 12.5 kg ha^{-1} and 25 kg ha^{-1} Phosphate-solubilizing bacteria inoculation treatments. However, it significantly differed from the control treatment, and the phosphate content increases (table 3). The presence of organic matter of *Azolla pinnata* in soil can increase the availability of phosphorus (P) by facilitating the conversion of organic P to inorganic P. Plants absorb phosphate in the form of orthophosphate, which includes HPO_4^{2-} and H_2PO^{4-}. Dead organisms, both plant and animal, can contribute organic material to the soil. Microbes are responsible for breaking down this organic material, resulting in the release of organic P that can then be mineralized.

Table 3 Effect of *Azolla pinnata* application and inoculant to P-content of Plant Rice (%)

Treatment	P-content (%)
Azolla application	
Without azolla (kontrol)	0.22 a
Azolla 10 ton/ha	0.22 a
Azolla 20 ton/ha	0.26 b
Azolla 30 ton/ha	0.24 a
PSB inoculation	
Without PSB (control)	0.22 a
PSB 12.5 kg/ha	0.25 b
PSB 25 kg/ha	0.24 b

Note: Numbers with the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% level

The application of *Azolla pinnata* or phosphate-solubilizing bacteria affected to supply available P, which plants can absorb directly. Studies state that Azolla is a nutrient-rich plant, especially in N, P, and K, and can be decomposed relatively quickly, thus making the nutrients it contains available to plants (Lumpkin, 1980). *Azolla pinnata* embedded in paddy soil undergoes a phosphate cycle, so organic P that was initially unavailable to plants will be converted into a more available form (HPO_4^{2-} or $H_2PO_4^-$). In the Phosphate cycle, dead plants and animals are a source of organic P for the soil.

Organic Phosphate in the soil is found in the form of inositol, phosphate phosphomonoesters, phosphodiesters (phospholipids and nucleic acids, H_2PO^{4-}), which plants can absorb, but most of it undergoes immobilization. Immobilization occurs because Al, Fe, and Ca ions bind to the P element. PSB can release these bonds by releasing organic acids. In the P cycle, microorganisms play a crucial role in transforming Phosphate until it becomes available to plants (Rao, 1977).

3.5 Productive Tiller

Table 4 presents the impact of *Azolla pinnata* and Phosphate-solubilizing bacteria to productive tillers.

Table 4 Effect of application of *Azolla pinnata* and Phosphate-solvent bacteria to productive tiller

Treatment	Produktive Tiller
Azolla pinnata	
Without Azolla (control)	31 a
Azolla 10 ton/ha	32 a
Azolla 20 ton/ha	34 b
Azolla 30 ton/ha	38 c
PSB inoculation	
Without PSB (control)	31a
PSB 12.5 kg/ha	34 b
PSB 25 kg/ha	34 b

Note: Numbers with the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% level.

Based on the analysis of variance, it showed that the application of *Azolla pinnata* independently was significantly different in increasing the number of productive tillers. However, the application of Phosphate-solubilizing Bacteria was similar, and there was no interaction between the two in increasing the number of productive tillers. In further tests, the application of *Azolla pinnata* 30 tons ha^{-1} showed the highest number of tillers with an average of 38. Table 4 shows that the application of *Azolla pinnata* can significantly increase productive tiller or paddy plant. This happens because the *Azolla pinnata* that has been buried experiences decomposition and releases N, P, S, and K, as well as micronutrients, which stimulate the growth of productive tillers in rice. According to Lupmkin (1989), when Azolla decomposes, it will provide N and P elements that can be absorbed by rice plants so that the N and P levels in the plants are sufficient to stimulate the growth of tillers. According to Murata & Matshushima (1978), apart from N and P, they significantly influence seedlings' growth. It requires an N level of 3.5% in plants to stimulate growth. If the N level in plants is 2.5%, seedling growth will stop, and if it is less than 1.5%, seedlings will die. If the P level in the main stem is less than 0.25%, the growth of the saplings will stop. According this research, *Azolla pinnata* and the consortium of phosphate-solubilizing bacteria were able to supply N and P at the required levels.

The number of productive tillers in rice plants is a crucial factor that significantly affects the yield, either positively or negatively. The ability to produce tillers in rice is influenced by genetics, cultivation process, planting distance, N fertilizer application, weed management, and water supply. The number of tillers in rice varies depending on the variety being grown. According to Grist (1986), different varieties have different tiller production capacities.

3.6 Grain yield of Paddy

Fig 6 depicts the impact of *Azolla pinnata* and PSB inoculation on the weight of harvested dry grains.

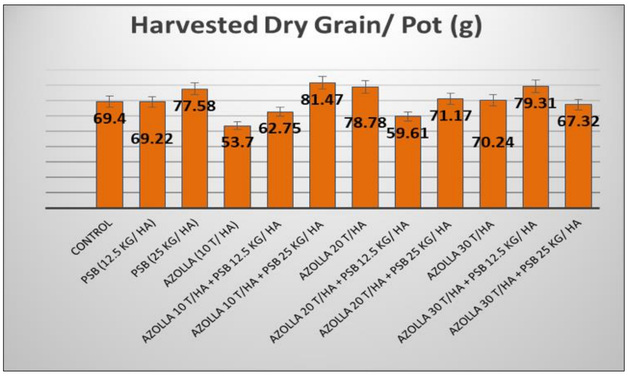

Figure 6 Effect of *Azolla pinnata* application and PSB inoculation to harvest dry grain of paddy.

Applications of *Azolla pinnata* and Phosphate-solubilizing Bacteria (PSB) has been found that there is no significant difference in increasing the weight of harvested dry grains (HDG).

The combination of *Azolla pinnata* at a rate of 10 tons/ha with PSB inoculant consortium at a rate of 25 kg/ha resulted in a higher dry grain yield of 81.47 g/pot compared to other treatments. However, the crop yields were not significantly different due to the lack of photosynthate rate from leaves to panicles during the generative period, which caused the grain-filling process to be suboptimal. As Salisbury and Ross (1995) suggest, plant photosynthesis is influenced by various environmental factors, such as water, CO_2, light, temperature, and nutrients. Therefore, plants that grow in an optimal environment have a higher photosynthetic capacity than those with limited water, nutrients, and light supplies. It's important to note that administering *Azolla pinnata* and the Phosphate-solubilizing Bacteria inoculant Consortium cannot overcome non-optimal environmental conditions.

4 Conclusion

The application of *Azolla pinnata* and a consortium of Phosphate-solubilizing bacteria (PSB) did not have a significant effect on plant height. However, when *Azolla pinnata* was applied independently at a rate of 20 t/ha, it resulted in significantly higher soil available phosphorus than other treatments. Applying *Azolla pinnata* at a rate of 30 tons/ha increased the number of highly productive tillers. On the other hand, inoculating with a consortium of Phosphate-solubilizing bacteria independently increased the number of productive tiller significantly compared to the control. When 10 tons/ha of *Azolla pinnata* was combined with 25 kg of PSB inoculant resulted in an 81.47 g/pot increase in dry grain rice yield. The application of *Azolla pinnata* and a consortium of Phosphate-solubilizing bacteria has the potential to increase lowland rice production and available P elements.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Alexander M. Introduction to Soil Microbiology. Jhon Wiley & Son, Inc, 1977. New York.

[2] Fanning DS, Fanning MCB. Soil Morphology, Genesis, and Clasification. John Wiley & Sons, 1989, New York.

[3] Elmizan, Muyassir, Fikrinda. Soil chemical properties, growth and yield of lowland rice (*Oryza sativa* L.) due to the application of Azolla (*Azolla pinnata* L.) in the form of green manure and compost. Journal of Land resources Management , 2014; 3 (1) : 441-446.

[4] Garfansa MP, Iswahyudi, Rohmah M, Awidiyantini R. Growth abd Yiels of Brown Rice Inpari Arumba variety on Wetland and Dryland. Jurnal Pertanian, 2022; 13(1): 25-3

[5] Grist, D H. Rice. Longman Inc. 6th Ed. 1986, New York. pp. 33-36

[6] Kavvadias, V. A. Soil Degradation. Athena. Soil Science Institute of Athens-National Agricultural Research Foundation, 2010, http://www.prosodol.gr/sites/prosodol.gr/files/Soil%20degradation%20by%20Victor.pdf.

[7] Khan N, Ali S, Shahid MA, Mustafa A, Sayyed RZ, Curá J. Insights into the interactions among roots, rhizosphere, and rhizobacteria for improving plant growth and tolerance to abiotic stresses: a review. Cells, 2021; 10:1551. doi: 10.3390/cells10061551

[8] Kurniawati H, Yulianingsih R, Wahda L. Efforts to Improve the Growth and Yield of Sweet Corn Plants by Providing *Azolla Microphylla* liquid organic fertilizer. PIPER, 2021; 17 (1): 1-7. http://jurnal.unka.ac.id/index.php/piper.

[9] Lestari SU, Muryanto. Analysis of some chemical element of *Azolla mycrophyla* compost. Journal of Agricultural scientific, 2018; 14(2): 60-65

[10] Lumpkin, T A. Collection, Cultivation of Azolla in Syimbiotic Nitrogen Fixation Technology. Marcel Dekker, 1987. New York Brusel.

[11] Majumdar J, Rajagopal V, Shantaram MV. Rock phosphate is an effective P carrier for Azolla. Int. Rice Res. Notes, 1993. 18(1): 40 -47

[12] Mulyani A, Hikmatullah, and Subagyo H. Characteristics and potential of acid dry land in Indonesia. Proceedings of the National Symposium on The Utilization of Soil Soil, 2004; 1: 1-32.

[13] Nurjaya , Nursyamsi D. Effectiveness of Direct Application of Phosphate Rock in Upland Acid Inceptisols Soils on Available-P and Maize Yield. J. Trop Soils, 2013, 18(1): 1-9

[14] Paulucci NS, Gallarato L, Reguera YB, Vicario JC, Cesari AB, de Lema MBG. Arachis hypogaea PGPR isolated from Argentine soil modifies its lipids components in response to temperature and salinity. Microbiol. Res. 2015; 173: 1–9. doi: 10.1016/j.micres.2014.12.012

[15] Pereira AL, Martins M. Oliveira MM, Carrapico F. Morphological and genetic diversity of the family Azollaceae inferred from vegetative characters and RAPD markers. Plant systematic and Evolution, 2011; 297: 213-226. DOI: 10.1007/s.00606-011-0509-0

[16] Rao, N S S. Soil Microorganisms and Plant Growth. Low Cost University Editions (LCUE),1977. India.

[17] Salisbury, F. B., and C. W Ross. Plant Physiology: Vol 3., 1995. ITB Publisher. Bandung.

[18] Saraswati, R., T. Prihatini, and hastuti R. D. Microbial Fertilizer Technology to Increase Fertilization Efficiency and Sustainability of Lowland Rice Production Systems. Center for Land and Agroclimate Development, 2004. Bogor - Indonesia.

[19] Setiwati MR. Increased soil N and P content as well as lowland rice yields due to the application of Azolla pinnata and phosphate solubilizing bacteria *Azotobacter chroococcum* and *Pseudomonas cepaceae*. Agrologia, 2014; 3 (1): 28-36.

[20] Seleiman MF, Elshayb OM, Nada AM, El-leithy SA, Baz L, Alhammad BA, Mahdi AHA. Azolla Compost as an Approach for Enhancing Growth, Productivity and Nutrient Uptake of Oryza sativa L. Agronomy, 2022; 12: 416. https://doi.org/10.3390/agronomy12020416

[21] Simanungkalit, RDM, Suriadikarta, DA, R, Setyorini, D and Hartatik, W. Organic Fertilizers and Phosphate Solubilizing Bacteria. Jakarta. PT. Library Hall, 2010. http://balittanah.litbang.pertanian.go.id/eng/dokumentasi/buku/pupuk/pupuk7.pdf

[22] Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer Plus. 2013; 2: 587. doi: 10.1186/2193- 1801- 2-587

[23] Subedi P, Shrestha J. Improving soil fertility through Azolla application in low land rice: A review. Azarian Journal of Agriculture, 2015; 2(2): 35-39.

[24] Watanabe, I. Summary Report of The Azolla Program of The International Network on Soil Fertility and Fertilizer Evaluation for Rice in Azolla Utilization. IRRI, 1987. Manila

[25] Yadaf, R K., G. Abraham, Y V. Singh, and P K. Singh. Advancements in the Utilization of Azolla-Anabaena System in Relation to Sustainable Agricultural Practices. Proc Indian Natn Sci Acad, 2014. India.

[26] Yu H, Wu X, Zhang G, Zhou F, Harvey PR, Wang L, Fan S, Xie X, Li F, Zhou H, Zhao X, Zhang X . Identification of the Phosphorus-Solubilizing Bacteria Strain JP233 and Its Effects on Soil Phosphorus Leaching Loss and Crop Growth. Front. Microbiol., 2022; 13 (Article 892533)

[27] Zaidi A, Ahemad M, Oves M, Ahmad E, Khan MS. Role of Phosphate-Solubilizing Bacteria in Legume Improvement. In Microbes for Legume Improvement; Springer: Cham, Switzerland, 2010; pp. 273–292. [CrossRef]