

# **Biofertilizer: *Azolla pinnata* in-combination with Inorganic Fertilizer on Growth and Yield of Rice**

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## **Abstract**

A study was conducted at MARDI Seberang Perai, Penang. This study aims to examine *Azolla pinnata* on the growth and performance of the MR 297 variety. The experiment consisted of five treatments; PK + *Azolla* (T1); NP + *Azolla* (T2); NK + *Azolla* (T3); NPK-Control (T4), and *Azolla* only (T5). Each treatment has four replications. The experimental design used was a complete randomized block design (RCBD), and all data collected were analyzed using one-way ANOVA with a statistically significant 0.05% test. For the average soil analysis between the beginning and end, all soil analyses showed decreased soil properties except Total N (%) and organic carbon (%). Treatment has a significant influence on the tiller number, the number of panicles, and the yield per pot. There is a significant influence on treatment on plant height and SPAD value in crop growth performance. There was no significant effect on N and P in plant nutrients. In contrast, there was a significant treatment effect on K. This study showed that *Azolla* could be used as an alternative fertilizer on rice fields because the soil treated with NK+*Azolla* shows a comparable result with soil treated with inorganic fertilizer without *Azolla* on the total yield.

## **Keywords**

Biofertilizer, sustainable farming, food security, soil fertility, plant nutrients

## **1. Introduction**

Rice (*Oryza sativa*) is an essential food for more than 3 billion people worldwide, especially Asia (Muthayya *et al.*, 2014). Besides the rice as a source of nutrient intake, it could also help in income and employment sources (Herman *et al.*, 2015). However, N fertilizers' continuous usage affects soil organic matter reserves, creating further N deficiency (Smithson & Giller, 2002). Long-term use of inorganic fertilizers in conventional agricultural systems would lead to soil acidification, nutrient imbalance, and organic matter loss (Miao *et al.*, 2011). Hence, this caused disturbances in organic nutrient's chemical and biological balance in soil (Amanullah & Hidayatullah, 2016). The balance of organic nutrients in the soil could provide nutrients required for plants to grow and give a good soil structure for roots and good aeration (Ball *et al.*, 2005).

*Azolla pinnata* has a historical role in agriculture. It has been recognized as a useful plant in Southern China and Northern Vietnam (Hove & Lejeune, 1996). According to Hove & Lejeune (1996), it has been used as bio-fertilizer and green manure for the rice crop due to its N-fixing abilities. Besides that, *Azolla* also has been recognized as poultry feed (Gouri *et al.*, 2012). *Azolla* is a substitute for Urea (Agustina, 2011). Based on chemical composition, *Azolla* is very effective for organic fertilizer to maintain soil fertility; each hectare requires 20 tons of *Azolla* in dry conditions. If *Azolla* were given every planting season, the level of use of artificial fertilizers would decrease. This is because the first

quarter of the *Azolla* elements are directly utilized by the soil (Maftuchah *et al.*, 1998). This quarter, equivalent to 65 kg of urea fertilizer. In the second and third growing seasons, *Azolla* substitutes a quarter to one-third of the fertilizer dose. The use of *Azolla* as a fertilizer and the fresh form, both also in the dry form and compost.

The addition of nitrogen by providing organic material can be done through the provision of manure and *Azolla*. The ability of *Azolla* to anchor N reaches 1.4 kg N/ha/day (Utami *et al.*, 2013). 50% (100 kg/ha) of nitrogen fertilizer dose and 1.13 tons/ha of *Azolla* gave good results on plant height parameters 2-6 DAT, the number of tillers 2-7 DAT (Nurmayulis *et al.*, 2011). According to Haryanto *et al.* (2008), fertilization with artificial fertilizers combined with *Azolla* can increase production by about 10-30% compared to fertilizing with urea fertilizer at the recommended dosage. Sisworo *et al.* (2011) added that the use of *Azolla* could save inorganic nitrogen fertilizer by 25-50%. For leaf area components, the total dry weight of plants is also higher than the treatment of *Azolla* without urea and urea without *Azolla* (Rahmatika, 2009). The highest R/C ratio value calculation is in the treatment of 75% N *Azolla* + 25% N urea, which is 4.96, meaning that every rupiah of investment in this farming produces income Rp. 4.96.

Provision of organic material can increase leaf area and plants' dry weight (Ying *et al.*, 1998). Along with the increase in leaf area, the biomass products produced are also high (Singh *et al.*, 1984). The *Azolla* layer on the surface of paddy fields can save urea use of 50 kg/ha, and if the development of *Azolla* is very high, it can save the use of urea fertilizer to 100 kg/ha (Gunawan *et al.*, 2014). Furthermore, giving *Azolla* compost at a dose of 6 tons/ha gives the best yield of 12.05 tons/ha of paddy or increases grain production weight by 21.03% (Gunawan *et al.*, 2014). The highest number of tillers at six weeks of age was also achieved in the administration of *Azolla* by 400 g/pot, which was 24 puppies, while the highest dry weight of stover was also in the administration of *Azolla* by 400 g/pot, which was 62.93 grams (Gunawan *et al.*, 2014).

Interestingly, *Azolla* has several unquestionable agronomic qualities that can fix atmospheric nitrogen, have very high productivity in the right environment, a high protein content, a herbicide effect, and decrease N-fertilizer volatilization (Kamalasanana *et al.*, 2002). For those reasons, *Azolla* started to attract attention again in the late 1990s. It is a component of integrated farming such as rice-fish-*Azolla*, rice-duck-*Azolla*, rice-duck-fish-*Azolla* or pig-fish-*Azolla* systems (Cagauan *et al.*, 2000).

There is still a lack of knowledge on rice growth and yield performances under *Azolla* incorporating local cultivation. Basic knowledge is important before new technology or recommendation be delivered for farmer practices. Therefore, the objective was to study the effect of *Azolla* on the growth and yield performances of the MR 297 rice variety.

## **2. Methodology**

### **2.1. Location and planting materials**

This research was conducted under the plant house at Malaysia Agriculture Research and Development Institute (MARDI) Seberang Perai, Pulau Pinang (5°32'29.0"N 100°28'00.3"E). This site was selected to provide the rice plant with a protected environment from heavy raindrops, prevent rice crops from strong winds and intense weather, affecting plant growth.

This research took about four months to be completed. It started from sowing rice for 20 days before transplanting, taking soil samples before transplant, and collecting data for analysis. Insecticides and fungicides were sprayed, and manual weeding for unwanted weeds was done. Besides, a total of 10 times data was collected, including plant height, Number of tiller and Soil Plant Analysis Development (SPAD meter) data until 110 days of transplant.

### **2.2. Treatment and Experimental Design**

The plastic pot was filled with 11 kg of homogenized soil. After that, the soil was submerged with water. The water level was maintained at approximately 2 cm above the soil surface starts from planting until 14 days before harvest. This experiment consisted of 5 treatments with four replications arranged in Randomized Completely Block Design (RCBD). The treatments use for this study are shown in Table 1. The recommendations for fertilizer cultivation in Malaysia are based on the subsidiary fertilizer package and sustainable fertilizer package (Azmi *et al.*, 2008) where the recommended fertilizer rate is 104 kg N/ha: 42 P<sub>2</sub>O<sub>5</sub>/ha: 62 kg K<sub>2</sub>O/ha and 120 kg N/ha: 70 kg P<sub>2</sub>O<sub>5</sub> / ha: 80 kg K<sub>2</sub>O / ha. The fertilizer was applied in the experimental pots based on the sustainable fertilizer package of 121.63 kg N ha<sup>-1</sup>, 69.01 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, 122.20 kg K<sub>2</sub>O ha<sup>-1</sup> Urea, Triple Super Phosphate (TSP) and Muriate of Potash (MOP), respectively. The fertilizers were applied at 7, 25, 45 and 65 days after transplanting (DAT). 50 g of Azolla was placed in the experimental pots five days after transplanting.

**Table 1: Treatments of Azolla in combination with and/or without inorganic fertilizers**

Symbol	Treatments
T1	PK + <i>Azolla</i>
T2	NP + <i>Azolla</i>
T3	NK + <i>Azolla</i>
T4	Control (Standard fertilizer rate)
T5	<i>Azolla</i>

### 2.3. Rice Cultivation and Agronomic Practice

The rice seedlings have been raised in a germination tray for the nursery stage during the soil preparation. The rice seedlings of MR 297 were transplanted into the pot after 18 days of seed germination. The size of the plastic pot is 0.08 m<sup>2</sup>. The rice growth was observed from the seedling stage until the harvesting stage.

The treatment used straight fertilizer such as Urea (N), Triple Super Phosphate (P) and Muriate of Potash (K). Fertilization was applied four times at 7 DAT, 25 DAT, 45 DAT and 65 DAT.

**Table 2: Fertilizer rate table according to subsidized fertilization package**

Treatments	7 DAT			25 DAT			45 DAT			65 DAT		
							g/pot					
	Urea	TSP	MOP	Urea	TSP	MOP	Urea	TSP	MOP	Urea	TSP	MOP
T1 PK+Azolla	0	0.83	0.98	0	0	0	0	0.34	0.48	0	0.03	0.17
T2 NP+Azolla	0.43	0.83	0	0.64	0	0	0.78	0.34	0	0.27	0.03	0
T3 NK+Azolla	0.43	0	0.98	0.64	0	0	0.78	0	0.48	0.27	0	0.17
T4 NPK	0.43	0.83	0.98	0.64	0	0	0.78	0.34	0.48	0.27	0.03	0.17
T5 Azolla	0	0	0	0	0	0	0	0	0	0	0	0

**Table 3: Fertilization schedule according to plant age. Source: MARDI Brochure (MARDI Sebernas 307) Variety Padi Baharu**

Age (Day After Transplant)	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
7	24.50	47.92	74.00
25	36.80		
45	44.93	19.59	35.70
65	15.40	1.50	12.50
Total	121.63	69.01	122.20

## 2.4. Plant Growth

Plant height was monitored and recorded at 10 days intervals from planting until harvesting. The tiller's height with the longest leaf would be the height of the plant (Constantino *et al.*, 2015). The number of tillers was monitored and recorded at ten days intervals from planting until harvesting. SPAD value on rice leaf was measured by using Soil Plant Analysis Development (SPAD) meter. Soil Plant Analysis Development (SPAD) meter has been introduced as a popular, fast, and cheap technique to estimate N levels from leaf transmittance measurement (Wayayok *et al.*, 2017).

## 2.5. Rice Yield Component Measurement

The panicle number was determined by counting all the panicles from each plot sampling unit. The percentage of filled grain was determined by the number of filled spikelets over the total number of spikelets. The weight of filled grains was determined by weighed using electronic balance after dried. The moisture content of grains was determined by using a moisture meter. According to Klomklao *et al.* (2017), the measurement results have a standard uncertainty of 1.23% moisture content in the range of 14% to 20%.

## 2.6. Soil analysis

The soil samples were dried in a 60°C oven, ground to a fine size 2 mm before soil analysis. Soil samples were taken two times before planting and after harvesting.

### 2.6.1. Soil pH

Firstly, a ratio of 1:2.5 (soil/distilled water) was prepared in a 100 mL beaker. The mixture was then placed on a mechanical shaker to be shaken for 30 minutes at 150 rpm. After that, each beaker was left to stand for 1 hour before measuring soil pH using an electrode pH meter. Before reading, the pH meter was calibrated with buffer solutions (pH 4 and 7). Ensure that the electrode is immersed only in the suspension and not allow it to touch the soil surface to prevent it from breaking.

### 2.6.2. Available P

Firstly, a plastic vial weighed 5 g of soil samples and a 20 mL double acid mixture (0.05 M HCl + 0.025 M H<sub>2</sub>SO<sub>4</sub>). The plastic vial was then shaken for 10 minutes at 180 rpm. The solution was filtered using filter paper Whatman no. 2 and percolate collected in another plastic vial. Then, reagent A containing 6 g ammonium molybdate, 74 mL sulphuric acid and 0.1454 g potassium antimonyl was prepared in a 1-litre volumetric flask. Reagent B that contained 1.32 g ascorbic acid was mixed with 250 mL reagent A in a 250 mL volumetric flask. Next, 10 mL of sample percolate and 8 mL of reagent B was pipetted into a 50 mL volumetric flask. The solution was marked up with distilled water and shake for a few seconds. The sample was then analyzed using a spectrometer at 882 nm wavelength.

### 2.6.3. Total N

Total nitrogen was determined by using a CHN analyzer. 0.15 g of soil samples were weighed and packed with tin foil. The soil samples in the tin foil were then analyzed using the CHN analyzer, determining the soil samples' nitrogen and carbon content (%).

#### **2.6.4. Exchangeable K**

Firstly, place a 1.5 g scoop of soil sample into a 50-ml Erlenmeyer flask. Then, add 15 ml of extracting solution (1 N  $\text{NH}_4\text{OAc}$ , pH 7.0) by constant suction pipette. After that, shake the suspension on an oscillating shaker for 15 minutes. Filter through Whatman No. 2 filter paper into 15-ml funnel tubes. Acid washed filter papers should be used for Na extraction. Finally, determine K in the filtered extract via AA spectrophotometry, using a bulk standard containing 15 ppm of K respectively, diluting by the AA to make as many standards as the user specifies (Thomas, 1982). For calculations, the computer performs any necessary weight to volume dilutions during analysis (ppm in soil  $\times$  10).

#### **2.6.5. Cations Exchangeable Capacity (CEC)**

Firstly, 100 mL of 1N ammonium acetate was poured in a leaching tube containing 10 g of the soil sample, and the percolate was discarded. Then, 100 mL 95% ethanol was used to wash the remaining residues of ammonium acetate, and the percolate was also discarded. Next, 100 mL of 0.05M  $\text{K}_2\text{SO}_4$  was poured into the leaching tube, and the percolate was collected into a 100 mL volumetric flask. The leachate was marked up with 0.05M  $\text{K}_2\text{SO}_4$ . Next, 10 mL of the solution was pipette and mixed with 10 mL of 40% NaOH into a distillation apparatus. The distillate was collected in a 50 mL conical flask containing 10 mL of 2% boric acid. Then, the solution was titrated with 0.01M HCl until the solution in the conical flask has changed from green to orange (Houba *et al.*, 1988)

#### **2.6.6. Organic carbon**

Firstly, determine the moisture content of the air-dry soil ground to pass a 0.42 mm sieve. Weigh enough soil accurately to contain between 10 mg and 20 mg of carbon into a dry tared 250 mL conical flask (between 0.5 g and 1 g for topsoil and 2 g and 4 g for subsoil). Then, accurately add 10 mL 1 N  $\text{K}_2\text{Cr}_2\text{O}_7$  and swirl the flask gently to disperse the solution's soil. Add 20 mL concentrated  $\text{H}_2\text{SO}_4$ , directing the stream into the suspension. Immediately swirl the flask until the soil and the reagent are mixed. A 200 °C thermometer was inserted and heat while swirling the flask until the temperature reaches 135 °C (approximately ½ minute). After that, set aside to cool slowly on an asbestos sheet in a fume cupboard. Two blanks (without soil) must be run in the same way to standardize the  $\text{FeSO}_4$  solution. When cool (20–30 minutes), dilute to 200 mL with deionized water and proceed with the  $\text{FeSO}_4$  titration using either the "ferroin" indicator or potentiometrically with an expanding scale pH/mV meter or auto titrator.

#### **2.7 Statistical Analysis**

For the research of *Azolla* in paddy growth and yield component, all the collected data were tabulated and statistically analyzed by the Analysis of Variance (ANOVA) and Duncan's Multiple Range (DMRT) in SAS version 9.2.

### **3. Results**

#### **3.1. Effect of treatments on the soil chemical characteristics**

Table 4 shows the initial selected chemical properties of the soil used for the experiment. The soil pH was 5.2. Total N, available P and exchangeable K were, 0.16%, 66.6 mg/kg and 0.75 cmol(+)/kg, respectively. Table 5 shows the effect of treatments on the soil pH, CEC, total nitrogen, organic carbon, available P and exchangeable K. No significant effect was observed on those soil chemical characteristics between the treatments.

**Table 4: Initial chemical soil characteristics**

Characteristics	Value
	Initial
Soil pH (pH)	5.2
Total N (%)	0.16
CEC (cmol(+)/kg)	19.7
Organic carbon (%)	1.8
Available P (mg/kg)	66.6
Exchangeable K (cmol(+)/kg)	0.75

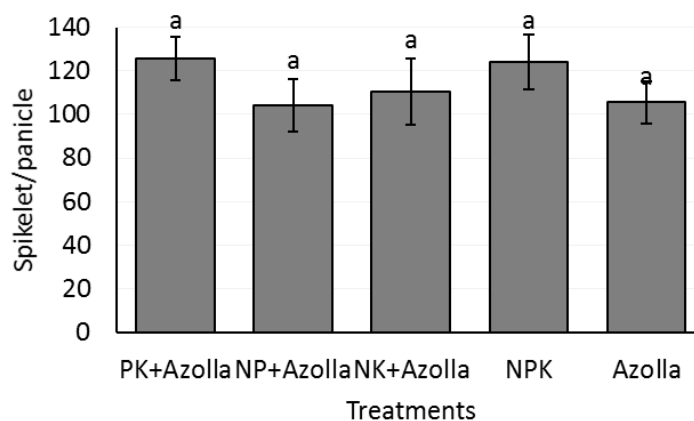
**Table 5: Mean soil analysis at harvest between treatments.**

Characteristic	Treatments				
	PK+ Azolla	NP+ Azolla	NK+ Azolla	NPK	Azolla
Soil pH	5.2a	5.0a	5.2a	5.2a	5.2a
CEC (cmol(+)/kg)	16.6a	18.3a	17.8a	17.6a	17.7a
Total Nitrogen (%)	0.16a	0.17a	0.19a	0.19a	0.17a
Organic carbon (%)	1.52a	1.70a	1.81a	2.02a	2.03a
Avail. P (mg/kg)	67.8a	55.9a	59.6a	55.8a	54.7a
Exc. K (cmol(+)/kg)	0.24a	0.23a	0.24a	0.25a	0.26a

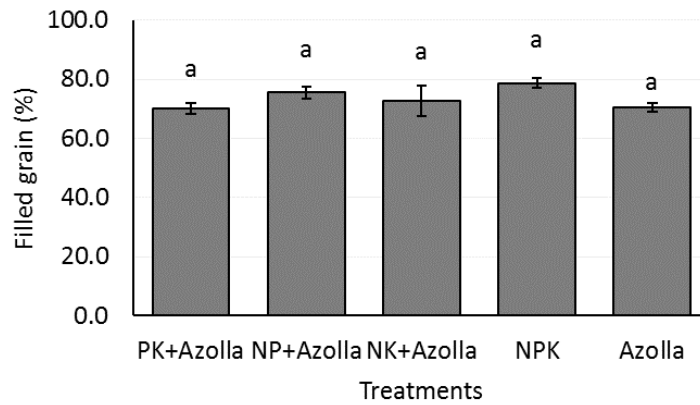
Means followed by the same letter in the same row are not significantly different (LSD's tests  $P > 0.05$ )

### 3.2. Effect of treatments on rice yield and rice yield components

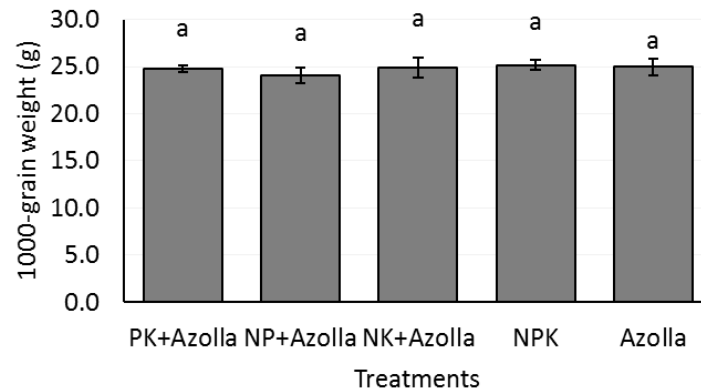
There was a significant effect of treatments on tiller number, panicle number and yield per pot. There was no significant effect of treatment on spikelet per panicle (Figure 1), percentage of filled grain (Figure 2), and 1000-grain weight (Figure 3). The highest means value for spikelet per panicle, filled grain (%), and 1000-grain weight (g) was 126 (PK+Azolla), 78.62 % (NPK Control) and 25.16 g (NPK Control), respectively.



**Figure 1: Response of different treatments on spikelet/panicle.** Means followed by the same letter are not significantly different (LSD's tests  $P > 0.05$ )



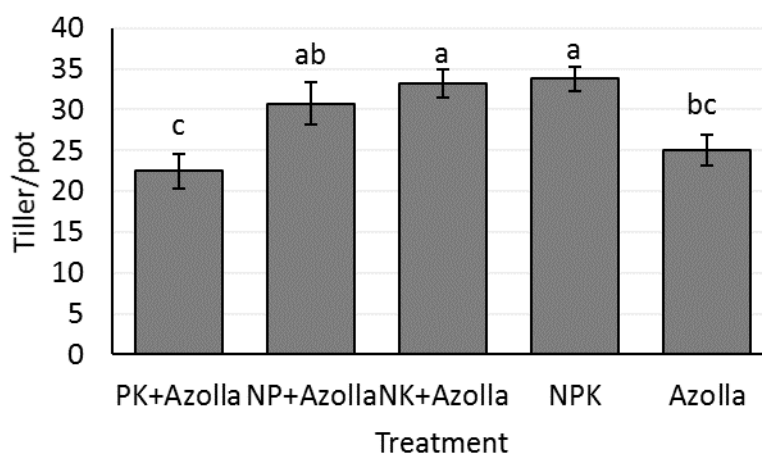
**Figure 2 : Response of different treatments on filled grain(%).** Means followed by the same letter are not significantly different (LSD's tests  $P > 0.05$ )



**Figure 3: Response of different treatments on 1000-grain weight (g)** Means followed by the same letter are not significantly different (LSD's tests  $P > 0.05$ )

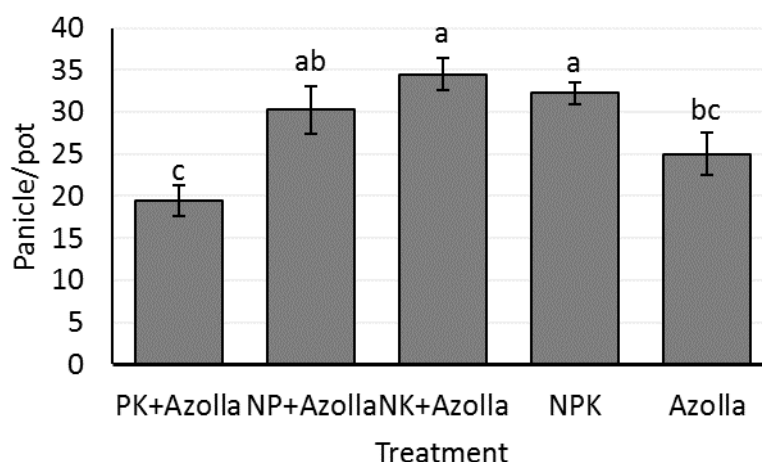
In this study, the application of NPK showed the highest tiller number (33.8), and no significant difference from NK+ *Azolla* and NP+ *Azolla* were 33.3 and 30.8, respectively. Soil treated with *Azolla* alone and PK+ *Azolla* produced the lowest tiller number (Figure 4).





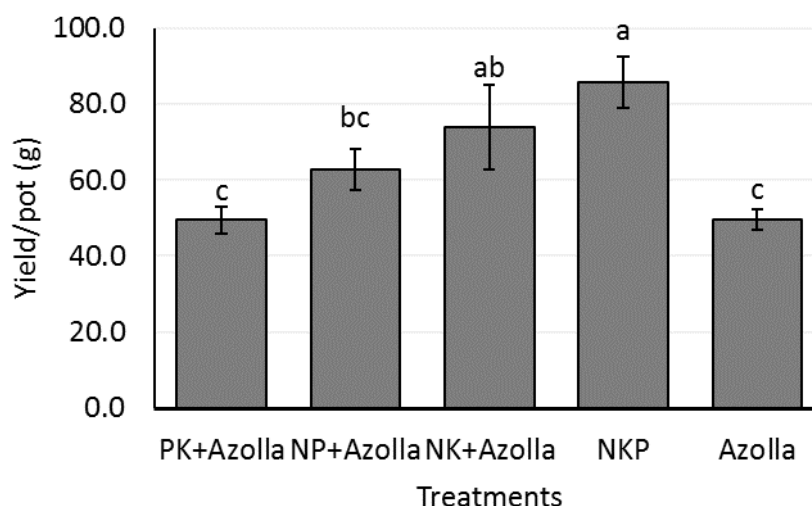
**Figure 4 : Response of different treatments on number of tiller.** Means followed by the same letter are not significantly different (LSD's tests  $P > 0.05$ )

NK+ *Azolla* treated to rice plants showed the highest number of panicles (35). However, it had no difference from NP+ *Azolla* (30) and NPK (32). Other than that, PK+ *Azolla* and *Azolla* produced the lowest number of panicles (Figure 5).



**Figure 5 : Response of different treatments on number of panicle.** Means followed by the same letter are significantly different (LSD's tests  $P > 0.05$ )

In this study, the application of NPK showed the highest grain yield (85.7 g/pot). However, no significantly different from NK + *Azolla* (73.8 g/pot). The yield difference was 16.1 percent. Results showed that PK+ *Azolla* (49.5 g/pot) produced the lowest grain yield (Figure 6).

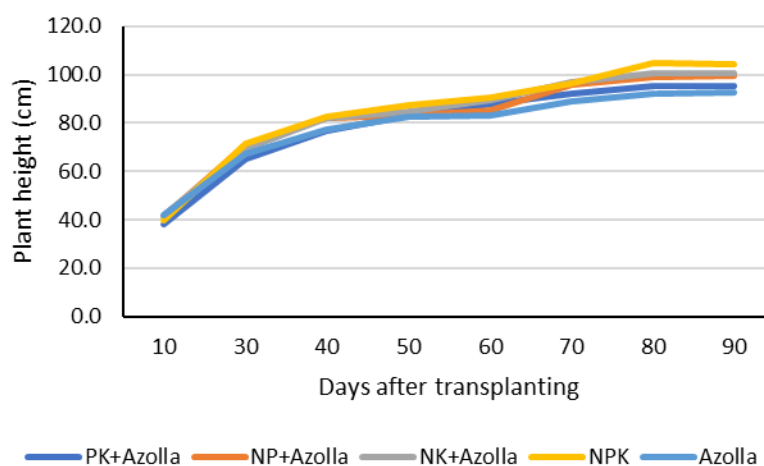


**Figure 6 : Response of different treatments on grain yield (yield/pot)** Means followed by the same letter are significantly different (LSD's tests  $P > 0.05$ )

### 3.3. Plant Growth Performances

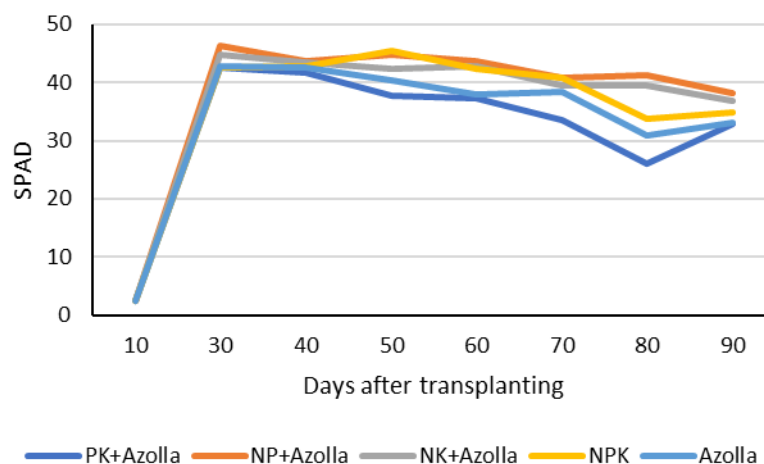
There was a significant effect of treatment and days on plant height and SPAD value.

Over time, the increase in plant height showed that Azolla alone and PK+ Azolla had lower plant height between 30 to 50 days after planting compared to other treatments (Figure 7). Similar results also showed between 60 to 90 days after planting. Overall, the application of NPK showed the highest plant height from the vegetative stage (10 DAT) to the maturity stage (90 DAT)



**Figure 7: Response of different treatments on plant height over time (days after planting)**

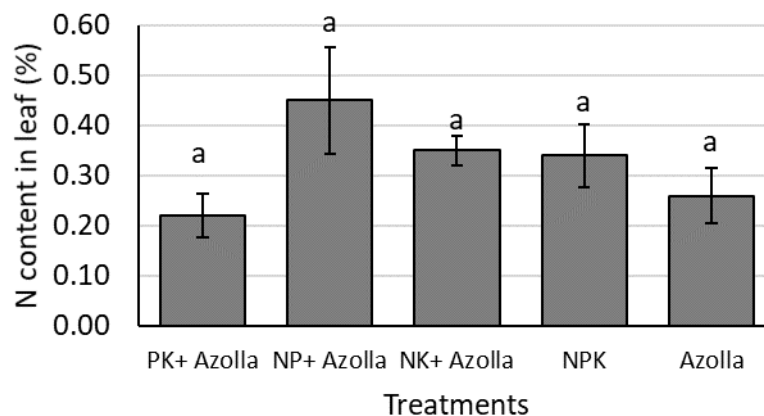
SPAD values increased rapidly in all treatments, from 10 to 30 DAT (Figure 8). Then they decreased slightly to 40 DAT (maximum tillering stage). The value decreased gradually until maturity for all treatments. Generally, rice plants treated with PK+ Azolla and Azolla showed the lowest SPAD values compared to other treatments.



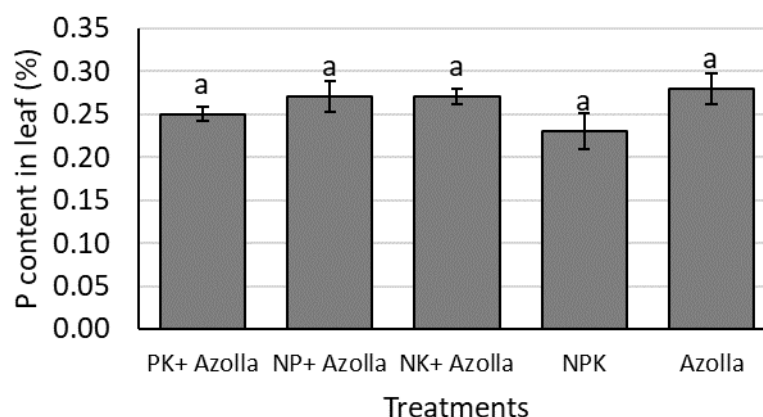
**Figure 8: Response of different treatments on SPAD value over time (days after planting)**

### 3.4. Plant Nutrient Composition

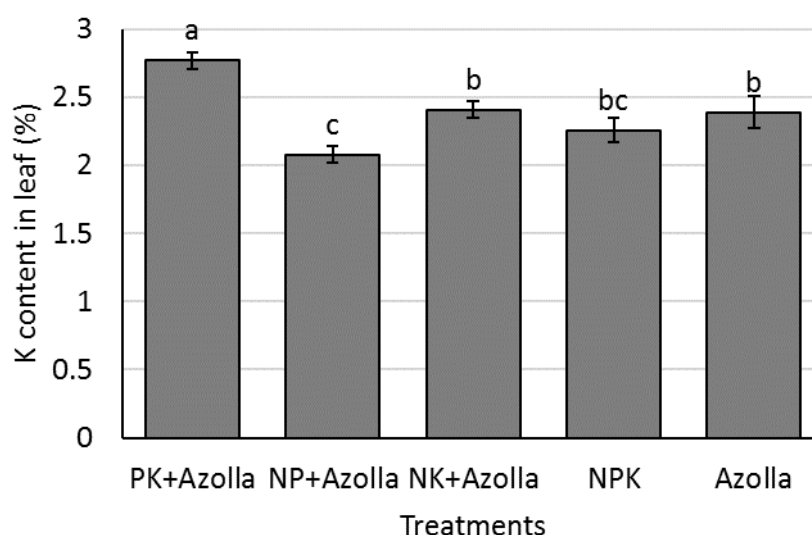
There was no significant effect of treatment on N (Figure 9) and P (Figure 10). On the other hand, there was a significant effect of treatment on K (Figure 11). The highest value for N and P were 0.44 % unit (NP+Azolla) and 0.28 % unit (Azolla only), respectively (Figure 9 and 10). The result showed that PK+ Azolla produced the highest K content in the leaf (Figure 11). On the other hand, NP + Azolla (T2) produced the lowest K content in the leaf (Figure 11).



**Figure 9: Response of different treatment on N content in leaf.** Means followed by the same letter are not significantly different (LSD's tests  $P > 0.05$ )



**Figure 10: Response of different treatment on P content in leaf.** Means followed by the same letter are not significantly different (LSD's tests  $P > 0.05$ )



**Figure 11 : Response of different treatment on K content in leaf.** Means followed by the same letter are significantly different (LSD's tests  $P > 0.05$ )

#### 4. Discussion

According to (Manickam *et al.*, 2020) pH value,  $\geq 5.0$  is suitable for rice cultivation which is not required for liming. In addition, Total N (%) between 0.1 – 0.25% is considered moderately low. In intensive rice cultivation, the indigenous nitrogen (N) in soils is insufficient, and N fertilization is required. The optimum total N for rice cultivation is 0.2 – 0.3 % in soils (Dobermann & Fairhurst, 2000). Addition of Azolla increase in total N from the initial value of 0.16%. Total N in the sufficient level as required by rice plant. However, Azolla may be a management practice to maintain N sources for crop growth for long-term management. Cation exchange capacity (CEC) is a soil chemical property that measures soil ability to hold nutrients. Soils with high CEC ( $\geq 20$  meq/100g) can hold more nutrients and benefits to rice plants (Manickam *et al.*, 2020). In this study, CEC was decreased at

harvest compared to the initial. CEC is soil pH-dependent, which CEC increased with increasing soil pH (Edmeades, 1982). Thus, a low pH will result in lower CEC. The present study showed that available P and exchangeable K were also reduced. According to Rawanake *et al.* (2013), the beneficial effect of the *Azolla* is after its decomposition, and humus is formed, increasing the soil's water-holding capacity and promoting aeration drainage, thus improving the physical and chemical properties of the soil and supply fixed nitrogen. This is also mentioned by Taha & El-Shahat (2017) that incorporation of *Azolla* increased the soil organic matter significantly upon its decomposition by the soil microorganisms that later released nutrients into the soil. These results could suggest that incorporating *Azolla* most probably not fully decomposed, and the soil chemical properties had no improvement.

In this study, a high number of tillers could be achieved by incorporating *Azolla* either with NK or NP as both showed comparable to NPK. This could suggest that *Azolla* could be an alternative to P and K fertilizer. However, without N, the number of tillers is significantly lower. This is in line with Razavipour *et al.* (2018), who reported that *Azolla*'s application resulted in a more significant tiller number. According to the authors, high tillering capacity is a desirable rice production trait, given that tiller number per plant is closely related to rice plants' panicles number production. A similar result was found in some panicles. A high number of panicles significantly contributed by NPK application followed by *Azolla* either with NK or NP. Incorporating *Azolla* alone into rice cultivation had no improvement on the number of the panicle. Besides, Shen & Tung (1985) also reported that *Azolla* alone did not influence the panicle's number. This is because the decomposition of *Azolla* in moist and flooded soil showed that the maximum amount of ammonium-N released stabilized after about 45 days after incorporating into the rice cultivation, indicating the N is not available for rice plants at the early growth stage (Shen & Tung, 1985). It is common for modern rice varieties to develop rice tillering as early as 40 – 45 days after planting.

In this study, the number of spikelets, filled grain (%), and *Azolla* treatments did not influence 1000-grain weight. According to Yoshida (1973), spikelet number is influenced by temperatures rather than nutrients application where spikelet number increase in the conditions of the temperature drops from 31°C to 25°. Jagadish *et al.* (2007) reported that spikelet numbers declined as the temperature increase from 29.6°C to 36.2°C. Besides, Yoshida (1981) mentioned that the 1000-grain weight is a constant characteristic because the hull's size controls grain size. Thus, the grain cannot grow more significant than that hull regardless of the rice plant's nutrient supply. This is supported by Oyange *et al.* (2020) that reported the spikelet number, filled grain (%) and 1000-grain weight depending on temperature. They observed a significant effect of *Azolla* at a temperature between 22.1 °C – 23.5 °C at the reproductive stage.

In the present study, incorporating NK+*Azolla* showed a slightly lower yield but comparable to NPK fertilizer. The improved nutrient uptake efficiency under *Azolla* was attributed to the enhanced N uptake in rice plants and improved rice grain yield (Yao *et al.*, 2018). However, according to Hou *et al.* (2019), N and K are two critical elements in improving rice grain yield than P. The effects of N on grain yield were interactively influenced by K application. Ye *et al.* (2019) reported that N's effects were stronger than P and K fertilizer in rice growth and development where rice was sensitive to N supply. This could suggest that comparable grain yield between NK+*Azolla* and NPK is not necessary contributed by *Azolla*, but sufficient N and K could contribute to sustainable rice grain yield.

The rice plant height increased with the advancement of growth stages. In this study, plant height was different at the vegetative stage (10 – 30 DAT) for all treatments. However, rice plants treated with NPK outperformed other vegetative to ripening stages (90 DAT). According to Kavitha & Subramaniam (2007), the increase in plant height might be due to enhanced nutrient levels in fertilizer, which leads to the continuous availability of nutrients in the available form to the rice plants. This could suggest that complete NPK fertilizer provides all essential nutrients for plant growth, while incorporating *Azolla* may not provide sufficient nutrients to promote greater plant height.

SPAD values decreased overgrowth stages (over time), especially at the ripening stage as the leaves turned yellow as the nitrogen in the leaves was utilized for grain growth (Putri *et al.*, 2016). This is in line with the present study; the SPAD value decreased for all treatments towards maturity.

According to Hou *et al.* (2020), N concentrations declined sharply with increased K rates due to the antagonistic relationships between  $K^+$  and  $NH_4$ . Further, they explained that it was also probable that the increase in K promoted the growth of leaves and diluted N concentrations in rice leaves. This could explain the high K concentration in rice leaves with PK+Azolla due to insufficient N. This could be true as NP+Azolla showed the lowest K concentration.

## 5. Conclusion

The experiment shows that Azolla can be used as a biofertilizer for rice planting as treatment NK+Azolla shows a comparable total yield result with inorganic fertilizer without Azolla. It can reduce the inorganic fertilizer, thus able to reduce the cost of the inorganic fertilizer.

## Declarations

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions.

All the authors contribute equally in this research developed, designed the study, financial support, summarized the data, data interpretation and critically revised the manuscript for important intellectual content. The corresponding author had full access to all the data in the study and was responsible for submission for publication.

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