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Effect of the Biofertilizer (*Azolla pinnata*) in Combination with Inorganic Fertilizers on Growth and Yield of Rice

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Abstract

This study was conducted at Malaysia Agriculture Research and Development Institute (MARDI), Seberang Perai, Penang (Malaysia), that aims to examine the impacts of the application of *Azolla pinnata* on the growth and performance of rice 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 had four replicates. 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. There is a significant effect on the tiller, panicles, yields, plant height, and SPAD value in crop growth performance. There was no significant effect ~~observed~~ on N and P among plant nutrients. In contrast, there was a significant treatment effect on K. This study concluded that the soil treated with NK + *Azolla* showed a comparable result with soil treated with inorganic fertilizer only for the total yield.

Keywords

Biofertilizer; Sustainable farming; Food security; Soil fertility; Plant nutrients

Introduction

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

Azolla pinatta has a historical role in agriculture. It has been recognized as a useful plant in Southern China and Northern Vietnam (Hove and Lejeune, 1996). According to Hove and Lejeune (1996), it has been used as biofertilizer 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 as organic fertilizer to maintain soil fertility; each hectare requires 20 tons of *Azolla* in dry conditions (Haryanto and Sisworo, 2008). If *Azolla* is applied every planting season, the use of artificial fertilizers decreases considerably. This is because the first quarter of the *Azolla* elements are directly utilized by the soil (Maftuchah *et al.*, 1998). This quarter is 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. *Azolla* is used as a fertilizer in fresh and dry form.

The nitrogen can be added to soil or plants by applying organic matter in the form of manure and or by application of *Azolla*. The ability of *Azolla* is to fix N by 1.4 kg N/ha/day (Utami, Purwanti and Putra, 2013). In an experiment, 50% (100 kg/ha) of nitrogen fertilizer and 1.13 tons/ha of *Azolla* gave good results in terms of plant height (2-6 Day After Transplanting (DAT)) and the number of tillers (2-7 DAT) (Nurmayulis *et al.*, 2011). According to Haryanto and Sisworo (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 *Azolla* could save inorganic nitrogen fertilizer by 25-50%. According to Rahmatika (2009), application of 25% N Azolla + 75% N urea, 50% N Azolla + 50% N urea and 75% N Azolla + 25% N urea gives results rice plant is better than 100% N Azolla and 100% N urea treatment.

Application of organic matter can increase leaf area and plants' dry weight (Ying *et al.*, 1998). Along with the increase in leaf area, the biomass produced is also high (Singh, Misra and Singh, 1984). The *Azolla* layer on the surface of paddy fields can save 50 kg ha^{-1} urea, and if the development of *Azolla* is very high, it can save the use of urea fertilizer to 100 kg ha^{-1} (Gunawan *et al.*, 2014). Furthermore, applying *Azolla* compost at a dose of 6 tons/ha gives the best yield of 12.05 tons ha^{-1} of paddy or it increases grain production weight by 21.03% (Gunawan *et al.*, 2014). The highest number of tillers at 6 weeks of age was also achieved when *Azolla* was applied by 400 g pot^{-1}. While the highest dry weight of stover was also observed when *Azolla* was applied by 400 g pot^{-1} (62.93 grams) (Gunawan *et al.*, 2014).

Interestingly, *Azolla* has several unquestionable agronomic qualities that can help fixing atmospheric nitrogen. It has very high productivity in the right environment, a high protein content, a herbicidal effect, and it decreases N-fertilizer volatilization (Kamalasanana, Premalatha and Rajamony, 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, Branckaert and van Hove,, 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 is suggested 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.

Methodology

Location and Planting Materials

This 4-month research was conducted in 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 plants with a protected environment from heavy rains, to protect rice crops from strong winds and intense weather, affecting the plant growth.

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 from planting time until 14 days before harvest. Only one rice plant for each pot. This experiment consisted of 5 treatments with 4 replications arranged in Randomized Completely Block Design (RCBD). The treatments used for this study are shown in Table 1. The recommendations for fertilizer application in Malaysia are based on the sustainable fertilizer package[1] (Azmi *et al.*, 2008). Table 2 shows the treatments, and it is based on the sustainable fertilizer package of 121.63 kg N ha^{-1}, 69.01 kg P_2O_5 ha^{-1} and 122.20 kg K_20 ha^{-1} using Urea, Triple Super Phosphate (TSP) and Muriate of Potash (MOP), respectively. The fertilizers were applied on 7, 25, 45 and 65 days after transplanting (DAT) (Table 3). 50 g of *Azolla* was placed in the experimental pots 5 days after transplanting.

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

Symbol	*Treatments*
T1	PK + *Azolla*
T2	NP + *Azolla*
T3	NK + *Azolla*
T4	Control (Standard fertilizer rate)
T5	*Azolla*

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 rice growth was observed from the seedling stage until the harvesting stage.

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

[1] Sustainable Fertilizer Package is the recommended rate use by the farmers locally.

was monitored and recorded at 10 days intervals from planting until harvesting. Soil Plant Analysis Development (SPAD) value on rice leaf was measured by using meter. Soil Plant Analysis Development (SPAD) SPAD meter has been introduced as a popular, fast, and cheap technique to estimate N levels from leaf transmittance measurement (Wayayok *et al.*, 2017).

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 dry weight of filled grains was determined by using electronic balance. The moisture content of grains was determined by using a moisture meter. According to Klomklao, Kuntinugunetanon and Wongkokua. (2017), the measurement results have a standard uncertainty of 1.23% moisture content in the range of 14% to 20%.

Table 2: Fertilizer rate table according to subsidized fertilization package

Treatments	*7 DAT*			*25 DAT*			*45 DAT*			*65 DAT*		
	g pot^{-1}											
	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_2O_5*	*K_2O*
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

Soil Analysis

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

Soil pH

~~Firstly, a~~ 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). It was ensured that the electrode was immersed only in the suspension and not allowed it to touch the soil surface to prevent breaking.

Available Phosphorus

5 g of soil samples and a 20 mL double acid mixture (0.05 M HCl + 0.025 M H_2SO_4) were added into a plastic vial. The plastic vial was then shaken for 10 minutes at 180 rpm. The solution was filtered using filter paper Whatman no. 2 and percolate was 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 shaked for a few seconds. The sample was then analyzed using a spectrometer at 882 nm wavelength.

Total Nitrogen

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 (%).

Exchangeable Potash

A 1.5 g of soil sample was placed into a 50-ml Erlenmeyer flask and 15 ml of extracting solution (1 N NH_4OAc, pH 7.0) was added by constant suction pipette. After that, the suspension was shaked on an oscillating shaker for 15 minutes and filtered through Whatman No. 2 filter paper into 15-ml funnel tubes. Acid washed filter papers were used for Na extraction. K in the filtered extract was determined using AA spectrophotometry (Thomas, 1982).

Cations Exchangeable Capacity (CEC)

Firstly, 100 mL of 1-N ammonium acetate was poured into 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 0.05 M K_2SO_4 was poured into the leaching tube, and the percolate was collected into a 100 mL volumetric flask. The leachate was marked up with 0.05 M K_2SO_4. Next, 10 mL of the solution was pipetted 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 0.01 M HCl until the solution in the conical flask had changed from green to orange (Houba *et al.*, 1988).

Organic Carbon

The moisture content of the air-dry soil ground was determined to pass a 0.42 mm sieve. Enough soil was weighed 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, 10 mL 1-N $K_2Cr_2O_7$ was accurately added, and the flask was gently swirled to disperse the solution's soil. 20 mL concentrated H_2SO_4 added, directing the stream into the suspension. The flask was immediately swirled until the soil and the reagent were mixed. A 200°C thermometer was inserted, and it was heat while swirling the flask until the temperature reached 135°C (for approximately ½ minute). After that, it was set aside to cool slowly on an asbestos sheet in a fume cupboard. Two blanks (without soil) were run in the same way to standardize the $FeSO_4$ solution. When cooled (for 20–30 minutes), it was diluted to 200 mL with deionized water and proceeded with the $FeSO_4$ titration using either the "ferroin" indicator or potentiometrically with an expanding scale pH/mV meter or auto titrator.

Statistical Analysis

For the research of *Azolla* in context of paddy growth and yield, 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.

Results

Effect of Treatments on the Soil's Chemical Characteristics

Table 4 shows the 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^{-1}, respectively. Table 5 shows the effect of treatments on the soil pH, CEC, total nitrogen, organic carbon, available P and exchangeable K at the time of harvest. No significant effect was observed on the 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^{-1})	19.7
Organic carbon (%)	1.8
Available P (mg kg^{-1})	66.6
Exchangeable K (cmol(+) kg^{-1})	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^{-1})	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^{-1})	67.8a	55.9a	59.6a	55.8a	54.7a
Exc. K (cmol(+) kg^{-1})	0.24a	0.23a	0.24a	0.25a	0.26a

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

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.

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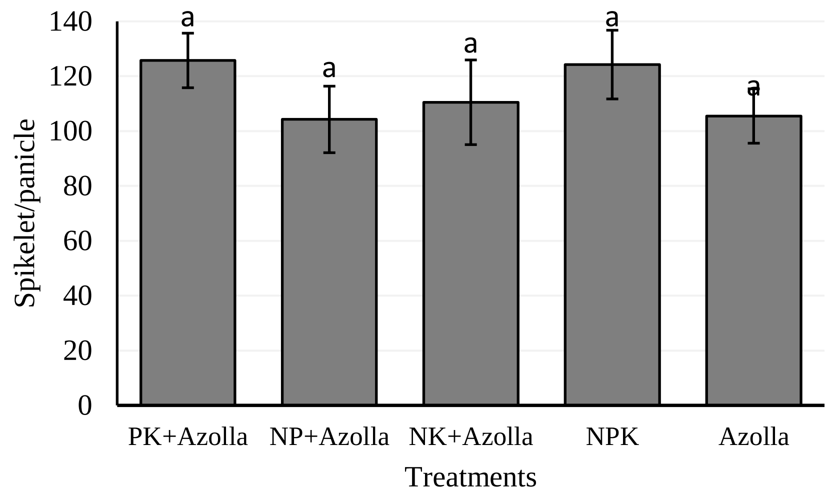

Figure 1: Response of different treatments on spikelet per panicle. Means followed by the same letter are not significantly different (LSD's tests P> 0.05).

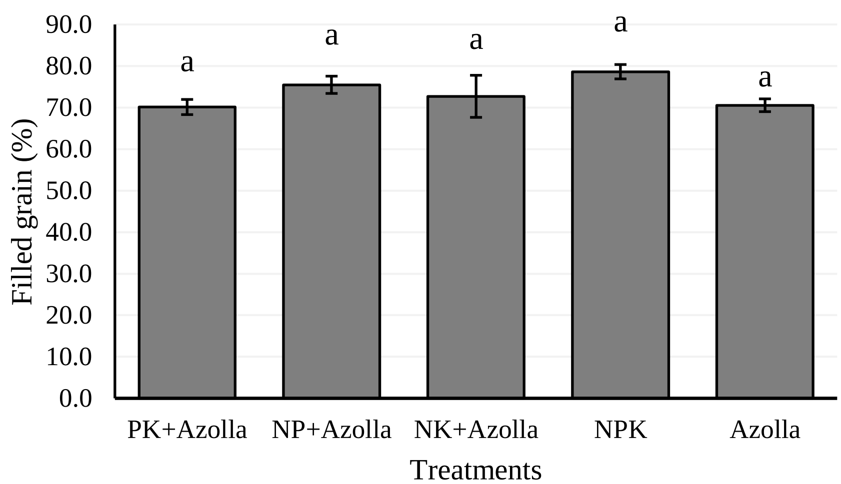

Figure 2 : Response of different treatments on filled grain (%). Means followed by the same letter are not significantly different (LSD's tests P> 0.05)

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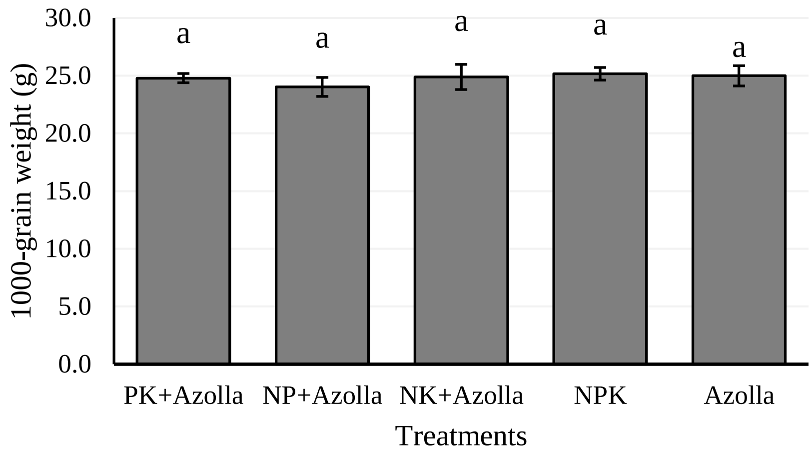

Figure 3: Response of different treatments on 1000-grain weight (g). Means followed by the same letter are not significantly different (LSD's tests P> 0.05)

In this study, the application of NPK showed the highest tiller number (33.8), and no significant difference from applying NK+ *Azolla* and NP+ *Azolla* that were yielded 33.3 tillers and 30.8 tillers, respectively. Soil treated with *Azolla* alone and PK+ *Azolla* produced the lowest tiller number (Figure 4). NK+ Rice plants treated to *Azolla* showed the highest number of panicles (35). However, it had no difference from plants treated with NP+ *Azolla* (30) and NPK (32). Other than that, PK+ *Azolla* and *Azolla* produced the lowest number of panicles in rice plants (Figure 5). In this study, the application of NPK showed the highest grain yield (85.7 g pot^{-1}). However, not significantly different from NK + *Azolla* (73.8 g pot^{-1}) treatment. The yield difference was 16.1%. Results showed that PK+ *Azolla* (49.5 g pot^{-1}) produced the lowest grain yield (Figure 6).

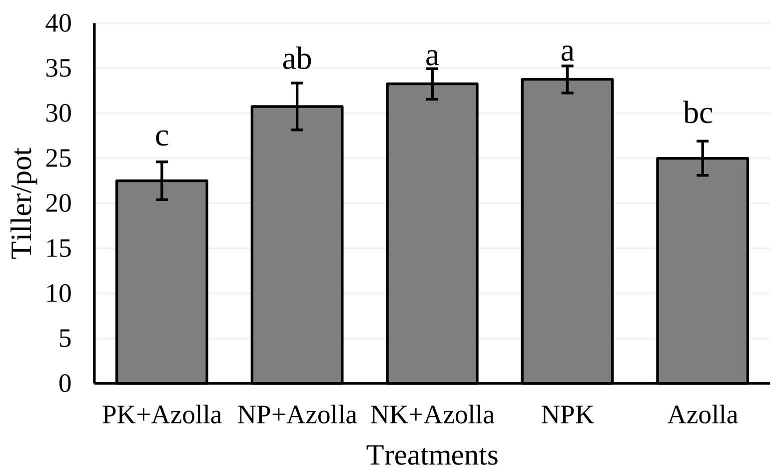

Figure 4: Response of different treatments on number of tillers. Means followed by the same letter are not significantly different (LSD's tests P> 0.05)

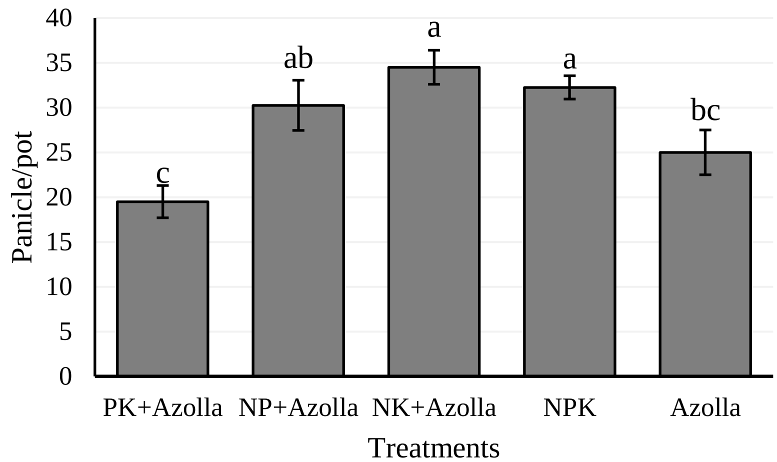

Figure 5: Response of different treatments on number of panicles. Means followed by the same letter are significantly different (LSD's tests P> 0.05)

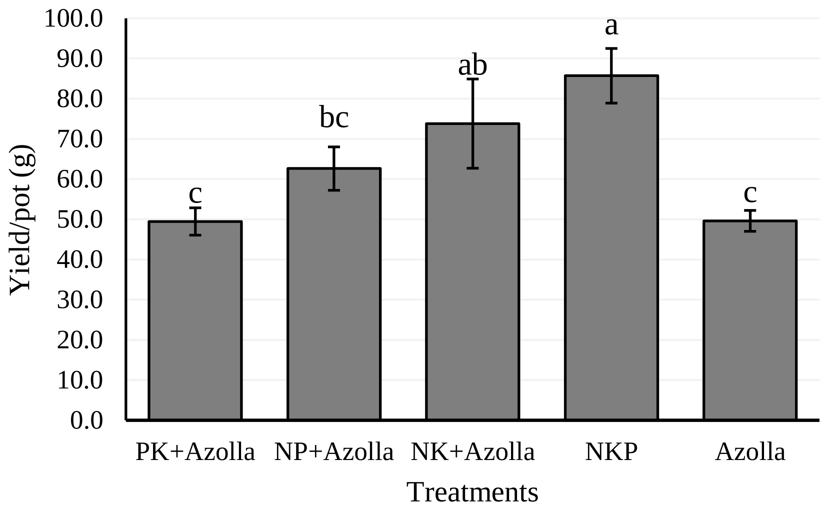

Figure 6: Response of different treatments on grain yield (yield/pot). Means followed by the same letter are significantly different (LSD's tests P> 0.05)

Plant Growth Performances

There was a significant effect of treatment and number of days on plant height and SPAD value. Over time, the increase in plant height showed that rice with *Azolla* alone and PK+ *Azolla* treatments had lower plant height at 30 to 50 days after planting than other treatments (Figure 7). Similar results were also obtained when kept for between 60 to 90 days after planting. Overall, the application of NPK yielded the highest plant height from the vegetative stage (10 DAT) to the maturity stage (90 DAT). SPAD values increased rapidly in all treatments from 10 to 30 DAT (Figure 8) and decreased slightly from 31 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.

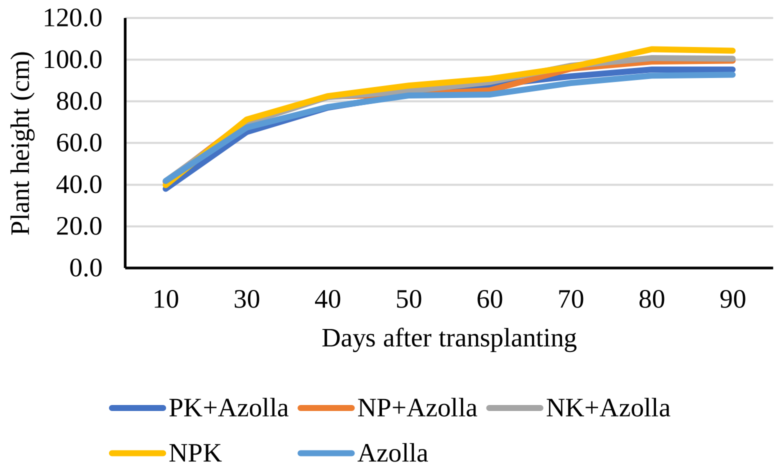

Figure 7: Response of different treatments on plant height over time (days after planting)

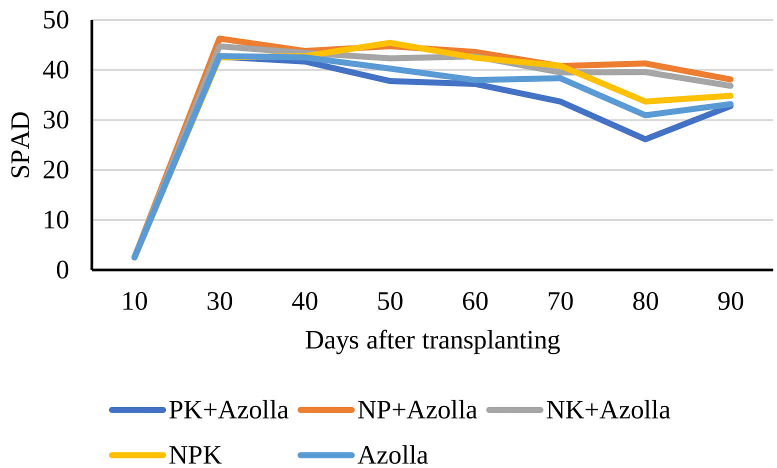

Figure 8: Response of different treatments on SPAD value over time (days after planting)

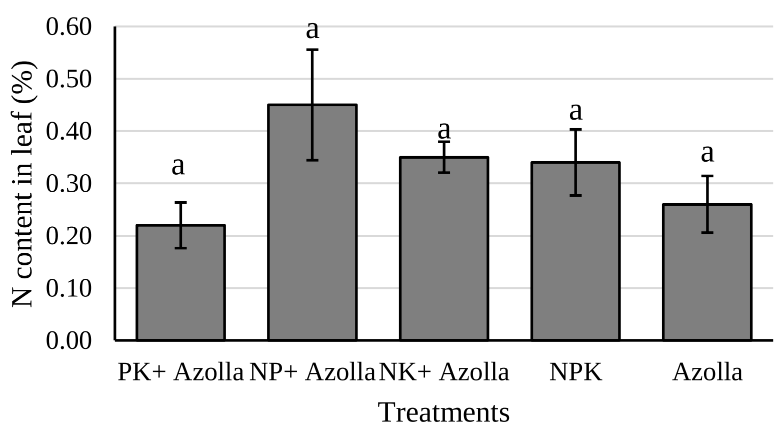

Figure 9: Response of different treatment on N content in leaf. Means followed by the same letter are not significantly different (LSD's tests P> 0.05).

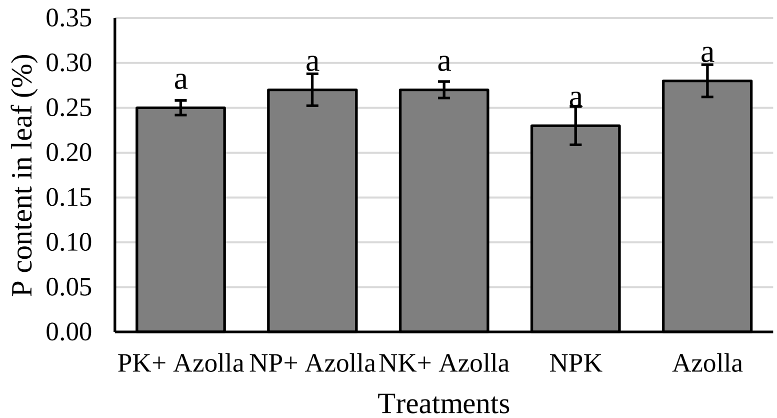

Figure 10: Response of different treatment on P content in leaf. Means followed by the same letter are not significantly different (LSD's tests P> 0.05).

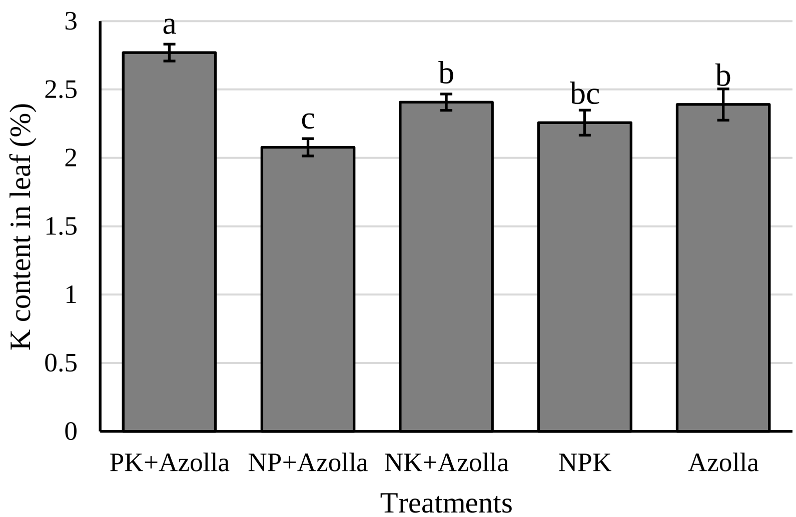

Figure 11: Response of different treatment on K content in leaf. Means followed by the same letter are significantly different (LSD's tests P> 0.05).

Plant Nutrient Composition

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

Discussion

According to Manickam *et al.* (2020), pH value ≥ 5.0 is suitable for rice cultivation, which is not required for liming. In addition, total N (%), which is 0.1 – 0.25%, is considered moderately low. In intensive rice cultivation, the 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 and Fairhurst, 2000). Addition of *Azolla* increases total N from the initial value of 0.16%. Total N is at 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 (≥20 meq/100 g) can hold more nutrients and benefits to rice plants (Manickam *et al.*, 2020). In this study, CEC decreased at harvest stage compared to the initial stage. CEC is soil pH-depend, which means the CEC increases 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 had also been reduced. According to Rawanake *et al.* (2013), the beneficial effect of the *Azolla* is after its decomposition, when humus is formed, increasing the soil's water-holding capacity and promoting aeration drainage, thus improving the physical and chemical properties of the soil fixing nitrogen. This is also mentioned by Taha and 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 suggest that incorporating *Azolla* most probably is not fully decomposed, hence the soil chemical properties had shown 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 result to NPK. This could suggest that *Azolla* could be an alternative to P and K fertilizers. However, without N, the number of tillers is significantly lower. This is in line with the observations of 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 the production of rice plants' panicles. A similar result was found in some panicles. A high number of panicles significantly is contributed by NPK application followed by *Azolla* either in combination of NK or NP. Incorporating *Azolla* alone into rice cultivation had no improvement on the number of the panicles. Besides, Shen and 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 had 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 and 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/panicles, percentage of filled grain, and 1000-grain weight were not affected by the treatments. According to Yoshida (1973), spikelet number is influenced by temperatures rather than nutrients' application. Spikelet number increases in the conditions of the temperature drop from 31°C to 25°C. Jagadish, Craufurd, and Wheeler (2007) reported that spikelet numbers declined as the temperature increased 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 significantly than hull regardless of the rice plant's nutrient supply. Oyange *et al*. (2020) supported this, who reported the spikelet number, filled grain (%) and 1000-grain weight depend 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 more important elements in improving rice grain yield compared to 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 fertilizers for rice growth and development where rice was sensitive to N supply. This could suggest that comparable grain yield between treatment NK + *Azolla* and NPK is not necessary contributed by *Azolla.*

The rice plant height increased with the advancement of growth stages. In this study, plant height differed 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 and 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 decrease overgrowth stages (over time), especially at the ripening stage as the leaves turned yellow and the leaves' nitrogen 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 decline sharply with increased K rates due to the antagonistic relationship 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.

Conclusion

Comparable grain yields on applications of NK + *Azolla* and NPK are not necessarily contributed by *Azolla*, but sufficient N and K could contribute to sustainable rice grain yield. Therefore, it can be concluded that *Azolla* can be used as a biofertilizer for rice planting. It can reduce the inorganic fertilizer inputs, thus ~~is able to~~ reducing the cost of the inorganic fertilizers.

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Authors' Declarations and Essential Ethical Compliances

Authors' Contributions (in accordance with ICMJE criteria for authorship)

Contribution	Author 1	Author 2	Author 3	Author 4
Conceived and designed the research or analysis	Yes	Yes	Yes	Yes
Collected the data	Yes	No	No	No
Contributed to data analysis & interpretation	Yes	Yes	Yes	Yes
Wrote the article/paper	Yes	Yes	Yes	Yes
Critical revision of the article/paper	Yes	Yes	Yes	Yes
Editing of the article/paper	Yes	Yes	Yes	Yes
Supervision	No	Yes	Yes	Yes
Project Administration	Yes	Yes	Yes	Yes
Funding Acquisition	Yes	Yes	Yes	Yes
Overall Contribution Proportion (%)	50	25	15	10

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Has this research used human subjects for experimentation? No

Research involving animals (ARRIVE Checklist)
Has this research involved animal subjects for experimentation? No

Research involving Plants
During the research, the authors followed the principles of the Convention on Biological Diversity and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. Yes

Research on Indigenous Peoples and/or Traditional Knowledge
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(Optional) PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)
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