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### Authors' Contributions

ES: designed and conducted the experiments, analyzed the data, and wrote the manuscript; MH: participated in writing of the article; M: developed the study idea and design; FS: critically revised the manuscript; H: performed the experiments

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Competing Interests

No competing interests have been declared.

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© The Author(s) 2020. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits redistribution, commercial and noncommercial, provided that the article is properly cited. **ORIGINAL RESEARCH PAPER in ECOLOGY** 

# Increasing the Growth and Production of Irrigated Rice Through the Integrated Application of Rice–Duck–*Azolla*

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## Abstract

The application of integrated agriculture is an effort to reduce dependence up on agrochemicals and increase the absorption of nutrients, especially nitrogen, to increase plant growth and production. Azolla is an algae that can provide nitrogen for rice, while ducks can increase the availability of N, P, and K, as well as the efficiency of nitrogen use. This research aimed to evaluate the role of ducks and Azolla in increasing the growth and production of rice plants. This research was conducted from October 2018 to February 2019 using an experimental method with a nonfactorial randomized block design. The combinations of nitrogen fertilizers, *Azolla*, and ducks (K) were as follows:  $K1 = 115 \text{ kg N} \text{ ha}^{-1}$ , without ducks, without Azolla;  $K2 = 86 \text{ kg N ha}^{-1}$ , without ducks, 1,000 kg Azolla ha<sup>-1</sup>;  $K3 = 86 \text{ kg N ha}^{-1}$ , 3,000 ducks ha $^{-1}$ , 1,000 kg *Azolla* ha $^{-1}$ ; K4 = 58 kg N ha $^{-1}$ 3,000 ducks ha<sup>-1</sup>, 1,000 kg Azolla ha<sup>-1</sup>; K5 = 29 kg N ha<sup>-1</sup>, 3,000 ducks ha<sup>-1</sup>, 1,000 kg Azolla ha<sup>-1</sup>; and K6 = without N fertilizer, 3,000 ducks ha<sup>-1</sup>, 1,000 kg Azolla ha<sup>-1</sup>. The results showed that the application of 58 kg N ha<sup>-1</sup>, 3,000 ducks ha<sup>-1</sup>, and 1,000 kg Azolla ha<sup>-1</sup> (K4) was able to decrease the N fertilizer application by 50% and increase the rice growth and yield by 12.17% and 20.32%, respectively. Therefore, the integrated application of rice-duck- Azolla would support sustainable agriculture.

## Keywords

sustainable agriculture; ecology; nitrogen

## 1. Introduction

Modern agriculture requires high external inputs, such as inorganic fertilizers and pesticides (Reijntjes et al., 1992), and leads to water, soil, and air pollution, which does not support the concept of sustainable agriculture (Peng, 2011). In addition, modern agriculture can also increase production costs and reduce farmers' incomes (Reijntjes et al., 1992). To reduce the high external inputs on rice cultivation, integrated agriculture concepts have been developed (Mandavi et al., 2016).

Integrated agriculture is the mutually beneficial integration of crops and livestock (Manjunatha et al., 2014), an example of which is rice–*Azolla* application (Brouwer et al., 2014; Cheng et al., 2015; Feyisa et al., 2013; Kollah et al., 2016; Mahaligam et al., 2014; Teng et al., 2016; Xu et al., 2017; Yuan et al., 2012).

Applying *Azolla* with rice plants can increase the plant height, number of productive tillers, leaf area, dry weight of the rice plants (Castro et al., 2003), growth, and rice plant production (Mahaligam et al., 2014; Mujio et al., 2016). *Azolla* forms a symbiotic relationship with the cyanobacteria-producing *Anabaena azollae*, which can fix nitrogen in the air, reaching up to 30–60 kg ha<sup>-1</sup> (Kollah et al., 2016).

Furthermore, based on the results of the research, rice–duck application can increase rice growth and production (Xu et al., 2017), maintain rice production stability without applying fertilizers and pesticides (Su et al., 2012), increase the availability of N, P, and K (Li et al., 2008; Long et al., 2013), preserve the environment (Yuan et al., 2012), and increase soil biodiversity (Teng et al., 2016). The use of ducks can increase nitrogen uptake in grains by 17.8%, reduce nitrogen loss by 6.52% (Li et al., 2008), and reduce the use of nitrogen fertilizers by 50% (Baigi et al., 2013).

Rice–duck systems can decrease N leakage because the activity of the ducks changes the oxidized layer of the soil surface, reduces the lower layers, and appears to favor the nitrification and denitrification of N fertilizers under flooding conditions. This may inhibit nitrification, resulting in an increase in the accumulation of N in the soil and microbial activities around the roots (Gao et al., 2019). Rice–duck farming also increases the accumulation and translocation of nutrients in the rice due to duck activities that improve the physical soil properties (Liu et al., 2017).

While the research on integrated agriculture with rice is ongoing, the research on rice–duck–*Azolla* is still limited. This research aims to evaluate the application of rice–duck– *Azolla* in increasing the growth and production of rice plants.

## 2. Material and Methods

This research was conducted in the irrigated rice fields of Tanah Periuk Village, Muara Beliti District, Musi Rawas Regency from October 2018 to February 2019. The research used a nonfactorial randomized block design with combinations of nitrogen fertilizer, *Azolla*, and duck (K) treatments as follows: K1 = 115 kg N ha<sup>-1</sup>, without ducks, without *Azolla*; K2 = 86 kg N ha<sup>-1</sup>, without ducks, 1,000 kg *Azolla* ha<sup>-1</sup>; K3 = 86 kg N ha<sup>-1</sup>, 3,000 ducks ha<sup>-1</sup>, 1,000 kg *Azolla* ha<sup>-1</sup>; K4 = 58 kg N ha<sup>-1</sup>, 3,000 ducks ha<sup>-1</sup>, 1,000 kg *Azolla* ha<sup>-1</sup>; K5 = 29 kg N ha<sup>-1</sup>, 3,000 ducks ha<sup>-1</sup>, 1,000 kg *Azolla* ha<sup>-1</sup>; and K6 = no N fertilizer, 3,000 ducks ha<sup>-1</sup>, 1,000 kg *Azolla* ha<sup>-1</sup>.

Land preparation was performed at 20 days before planting. The land was plowed, harrowed, and leveled. Furthermore, the land was divided into three replications, with five  $5 \times 4$ -m plots in each. The distance between plots was 50 cm. The distance between replications was 100 cm, with a total area of 500 m<sup>2</sup>.

The distribution of 100 g Azolla m<sup>-2</sup> was conducted 20 days before rice planting. Azolla seedlings were not planted with other plants or attacked by pests. Azolla propagation was carried out in tarpaulin ponds at the research site. Seedlings of the Inpari 42 variety were sown in 12 × 4-m seed trays. Before sowing, rice seeds were soaked in water for 24 hours, then drained and ripened for 48 hours. Germinated seeds were stocked and spread in seed trays with a density of 25 g m<sup>-2</sup>.

Fifteen days after sowing, two seedlings were planted in each planting hole, with a distance of  $25 \times 25$  cm and depth of 5 cm. At 30 days old, six ducks per plot were released from 7 a.m. to 5 p.m. Western Standard Time (WIB). The ducks were released 20–65 days after planting the rice. Rice plant cultivation included replanting, fertilizing, and water regulating. Replanting was conducted 10 days after planting. Nitrogen fertilizers, KCl and SP-36, were provided for the treatments, at a dose of 100 kg ha<sup>-1</sup>. Until 80 days after planting, a 5-cm water level was maintained.

After the *Azolla* reached full maturity in the rice field, 80% was ploughed into the soil and the rest was spread over the land surface. The ducks were harvested from the rice field at 75 days after rice planting and rice was harvested 95 days after planting.

The variables include the *Azolla* biomass, plant height, number of tillers, leaf chlorophyll content (observed using a SPAD chlorophyll meter 42 days after planting), dry straw weight (g), number of productive tillers, panicle length (cm), number of grains panicle<sup>-1</sup>, number of full grains, number of empty grains, 1,000-grain weight, harvest index, and grain weight clump<sup>-1</sup>. The N contents of the leaves, grains, straw, *Azolla*, and duck manure were observed using the Kjeldahl method. The P and K contents of the *Azolla* and duck manure were observed using spectrophotometry and flamephotometry methods.

The data were analyzed using an analysis of variance (ANOVA) followed by the least significant difference (LSD) test at the 5% level (Gomez and Gomez, 1984).

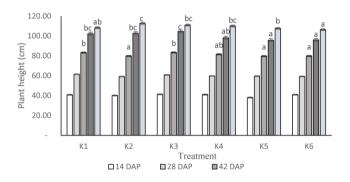
## 3. Results

The application of nitrogen fertilizers, ducks, and *Azolla* significantly affected the plant height at 42, 56, and 92 days after planting (at harvest), but had no significant effect on the plant height at 14 and 28 days after planting (Figure 1). The K3 treatment produced the highest average plant height at 14, 28, 42, and 56 days after planting. The K2 treatment produced the highest average plant height at 92 days after planting.

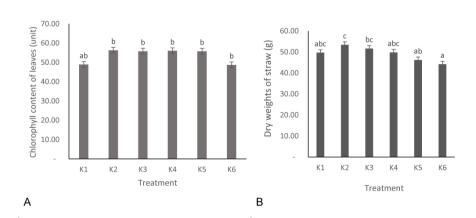
The application of nitrogen fertilizers, ducks, and *Azolla* had a significant effect on the leaf chlorophyll content and dry straw weight. The highest leaf chlorophyll content was in the K4 treatment (Figure 2A), while the highest dry straw weight was in the K2 treatment (Figure 2B).

The application of nitrogen fertilizers, ducks, and *Azolla* significantly affected the number of tillers (Figure 3A), number of productive tillers (Figure 3A), panicle length (Figure 3B), number of grains panicle<sup>-1</sup> (Figure 3C), number of full grains (Figure 3D), percentage of empty grains (Figure 3E), 1,000-grain weight (Figure 3F), harvest index (Figure 3G), and grain weight clump<sup>-1</sup> (Figure 3H). The highest number of tillers, panicle length, and grain weight clump<sup>-1</sup> were found in the K3 treatment. The highest number of productive tillers, panicle grain<sup>-1</sup>, full grain rice, 1,000-grain weight, and harvest index were found in the K4 treatment. The highest percentage of empty grains was found in the K6 treatment.

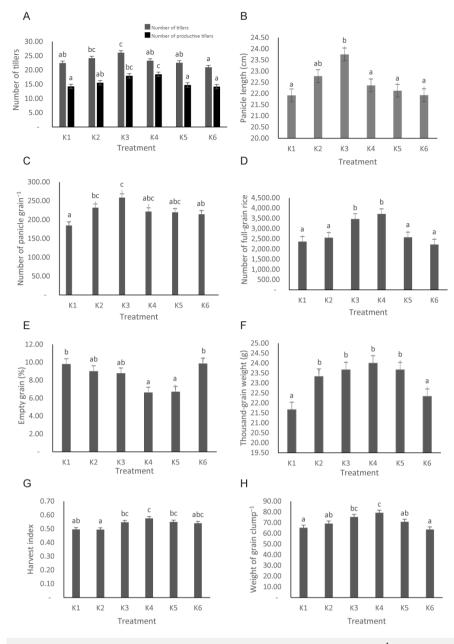
The application of nitrogen fertilizers, ducks, and *Azolla* significantly affected the N content of the straw, but had no significant effect on the N content of senescent leaves or grains (Figure 4). The highest N contents of the straw and grains were

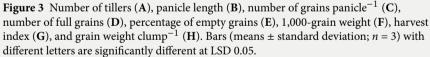


**Figure 1** Average heights of rice plants at 14, 28, 42, 56, and 95 days after planting. Bars (means  $\pm$  standard deviation; n = 3) with different letters are significantly different at LSD 0.05.



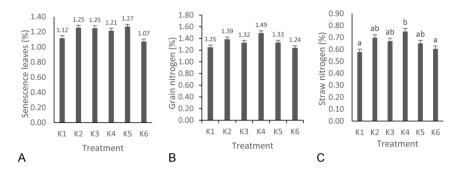
**Figure 2** Leaf chlorophyll contents (**A**) and dry straw weights (**B**). Bars (means  $\pm$  standard deviation; n = 3) with different letters are significantly different at LSD 0.05.

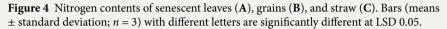


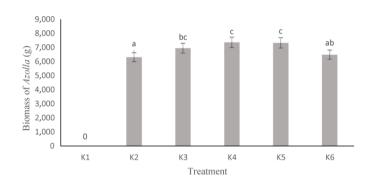


found in the K4 treatment (0.75% and 1.49%, respectively), while the highest N content of the senescent leaves was in the K5 treatment (1.27%). The lowest N contents of the senescent leaves and grains were found in the K6 treatment (1.07% and 1.24%, respectively), while the lowest N content of the straw was in the K1 treatment (0.58%).

The application of nitrogen fertilizers, ducks, and *Azolla* significantly affected the growth of *Azolla*. The K4 treatment produced the highest *Azolla* biomass, with an average biomass buried in the experimental plots of 7,360 g. The lowest *Azolla* biomass was found in the K2 treatment, with an average biomass buried in the experimental plots of 6,300 g (Figure 5). The results show that 100 g of dried *Azolla* contained 20.12% organic C, 3.07% total N, 1.20% total P, and 3.44% total K. The nutrient contents of *Azolla* was obtained after being applied with rice plants (Table 1).







**Figure 5** *Azolla* biomass at 30 days after planting. Bars (means  $\pm$  standard deviation; n = 3) with different letters are significantly different at LSD 0.05.

Treatment	Nutrient content (kg ha <sup>-1</sup> )			
	Total N	Total P	Total K	Organic C
K2	19.341	7.560	21.672	126.756
K3	51.927	35.664	38.839	1,101.520
K4	53.186	36.156	41.059	1,109.769
K5	53.063	36.108	40.922	1,108.964
K6	50.485	35.100	38.032	1,092.064
Average	45.600	30.118	36.105	907.815

Table 1 Nutrient contributions from Azolla and duck manure in each treatment.

The application of 3,000 ducks ha<sup>-1</sup> for 45 days produced 14,850 kg ha<sup>-1</sup> of manure. The results indicate that 100 g of duck manure contained 32.38% organic C, 1.03% total N, 0.92% total P, and 0.53% total K. The total nutrient content of the duck manure being applied in each treatment is shown in Table 1. The application of 3,000 ducks ha<sup>-1</sup> and 1,000 kg *Azolla* ha<sup>-1</sup> provided an average of 907.815 kg organic C ha<sup>-1</sup>, 45.600 kg total N ha<sup>-1</sup>, 30.118 kg total P ha<sup>-1</sup>, and 36.105 kg total K ha<sup>-1</sup> for rice plants.

## 4. Discussion

The availability of nutrients, especially nitrogen, affects plant growth and production. The application of nitrogen fertilizer, ducks, and *Azolla* significantly affected the growth and production of rice plants. The treatment including 58 kg N ha<sup>-1</sup>, 3,000 ducks ha<sup>-1</sup>, and 1,000 kg *Azolla* ha<sup>-1</sup> (K4) enhanced the rice plant growth by 12.17% and rice production by 20.32% compared to the treatment of 115 kg N ha<sup>-1</sup>, without ducks, and without *Azolla* (K1). The addition of 3,000 ducks ha<sup>-1</sup> and 1,000 kg *Azolla* ha<sup>-1</sup> elevated the plant growth and production due to the increase in the

availability of organic C by 907.815 kg ha<sup>-1</sup>, total N by 45.600 kg ha<sup>-1</sup>, total P by 30.118 kg ha<sup>-1</sup>, and total K by 36.105 kg ha<sup>-1</sup>. The application of ducks increased the availability and absorption of N and the P and K availability in the soil (Long et al., 2013; Teng et al., 2016). This indicates that the grazing of the ducks enriched the nutrients of the soil, probably through their excreta. Other mechanisms may also be involved. For example, the movement of the ducks in the rice field enhances the aeration of the soil and prevents the accumulation of harmful gases in the rhizosphere (Hossain et al., 2005).

The removal of chemical toxins, such as methane (CH<sub>4</sub>), from the soil in rice–duck integrated farming causes a significant improvement in the soil quality and provides favorable conditions for root growth (Baigi et al., 2013). The application of *Azolla* with rice plants could increase the nitrogen availability (Raja et al., 2012) and uptake (Baigi et al., 2013).

The availability of nitrogen, in accordance with the needs and stages of plant development, increased the ability of the plants to absorb nitrogen. This might be due to the role of nitrogen in improving rice growth, internode elongation, photosynthesis, metabolism, and assimilated production (Ghoneim et al., 2018). The increase in the ability of plants to directly absorb and use nitrogen affected the growth and production of plants, because those that were able to grow optimally in the vegetative phase could increase their growth in the generative phase. The appropriate and balanced availability of nitrogen could reduce the fertilizer dosage, increase plant growth, maintain production, and reduce dissolved nutrients (Alavan, 2015).

The lowest N contents of the leaves and grains were found in the treatment without nitrogen fertilizer, 3,000 ducks  $ha^{-1}$ , and 1,000 kg *Azolla*  $ha^{-1}$  (K6), while the lowest N content of straw was in the treatment with 115 kg N  $ha^{-1}$ , without ducks, and without *Azolla* (K1). The application of nitrogen fertilizer without ducks or *Azolla* reduced the ability of the plants to absorb nitrogen and increased nitrogen loss, thereby reducing the growth and production of the rice plants. The intensive use of inorganic nitrogen in high doses reduced the productivity of rice plants as there was a decrease in the ability of the plants to absorb and use nitrogen (Peng, 2011).

The application of ducks and *Azolla* without nitrogen fertilizers were not able to provide the nitrogen required by rice plants. Therefore, rice plant production was reduced (K6 treatment). The application of an inorganic fertilizer to the soil could rapidly increase the availability of nutrients and nitrogen, as a macro nutrient, needed by rice plants at the stages of plant growth and production (Fageria, 2014; Sainju, 2013). A low nitrogen availability is correlated with the ability of plants to absorb and use nitrogen.

The differences in the applications of nitrogen fertilizers significantly affected the growth of the *Azolla* (Figure 5), as an increase in the nitrogen content has been shown to inhibit *Azolla* growth (Sadeghi et al., 2013). *Azolla* harvesting was only done when the *Azolla* was 30 days old. At the beginning of the research, there was an increase in the intensity of sunlight. A high light intensity could inhibit the photosynthesis process, which would inhibit the growth of *Azolla* (Sadeghi et al., 2012).

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