Application of Azolla and intermittent irrigation to improve the productivity and nutrient contents of local black rice variety

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Abstract. Black rice is a local rice variety that contains a high level of anthocyanin pigment. Anthocyanin has been reported to be very effective in reducing cholesterol levels as well as cancer cell invasion. One of the main problems in rice cultivation is lack of water. System of Rice Intensification (SRI) has shown to be able to increase rice productivity by increasing the number of tillers. This system is known as a water-efficient cultivation. Other rice cultivation barrier is related to the use of nitrogen fertilizer. One of replacement of nitrogen fertilizer is by adding azolla. The objective of this research was identifying growth and yield of organic black rice with intermittent irrigation and application of azolla. The plant material used was black rice Cempo variety from Sleman, Yogyakarta. This experiment utilized 4 dosages of azolla as the first treatment: 100 gm⁻², 200 gm⁻² and 400 gm⁻². The second treatment was water supply consisted of continuous flooded 2 cm; flooded 2 cm every 3 days; flooded 2 cm every 6 days. The results depicted that the application of azolla was able to increase the growth of black rice. Azolla of 200 gm⁻² with intermittent irrigation 3 days could be a good combination to improve plant growth, yield and properties of local black rice.

1. Introduction
Nitrogen is essential for improving plant growth and yield. On the other hand, excessive use of inorganic fertilizers will cause adverse effects to soil fertility. Therefore, currently the growers are interested to find alternative of inorganic fertilizer. Providing biofertilizer and organic fertilizer is a priority to solve this problem. The use of azolla as a biofertilizer has been successful in some countries as well as in Indonesia [1]. Azolla could be a substitution for urea and beneficial for maintaining plant growth [2]. Azolla is widely applied because of its ability to supply nitrogen with fixation capability of nitrogen reaching 1.2 kg ha⁻¹ daily [3].

Rice is the major cereal that consumed as the staple food by more than half the world's population. Various colors of rice depend on the pigment in the epidermis or aleurone. At this time, the black rice is widely consumed as a functional food. A functional food is food that naturally or through a specific process comprising one or more compounds which may have physiological functions that are beneficial to health. Black rice in Indonesia is widely available in a variety of areas with different local names. Black rice in Java known as: Cempo rice in Sleman; Gadog rice in West Java, Melik rice in...
Bantul, and Wulung in Solo. Black rice has a number of advantageous nutrients compared to white rice and red rice. It contained of high protein, vitamins and minerals which were varies among cultivars and planting sites [4]. Anthocyanin pigment has been reported to be very effective in reducing cholesterol levels in the human body [5], treatment of allergic reactions, and also inhibits cancer cell invasion [6].

Problems in rice cultivation are lack of water and nitrogen excessive fertilizer. SRI (System of Rice Intensification) is known as water-efficient cultivation system. This system applied young seedlings that are transplanted in wider planting distance and intermittent irrigation in vegetative phase [7]. This system is able to increase rice productivity through increasing number of tillers.

In order to support black rice as a functional food crop, it is recommended to cultivate this crop by organic method using organic fertilizers. One replacement for nitrogen fertilizer is azolla. Soil organic matter, soil pH and soil nutrients (N, P, K, Ca, Mg and Na) improved with the rate of azolla [8]. Azolla could boost seedling growth. The objective of this research was to increase the productivity of organic black rice [9]. Specific objectives were to identify the growth and yield, anthocyanin, antioxidant and protein contents as the response of different water supply system and azolla application.

2. Material and methods
The experiment was conducted in the net house located in Ngemplak village, Wedomartani, Yogyakarta. The plant material used was black rice Cempo variety from Sleman, Yogyakarta. Sources of plant nutrient are cow manure, Bio Natural™ phosphates (6.4 g/plant) and Z-ka (6.4 g/plant). Azolla was utilized to replace urea fertilizer. Rice were planted in plastic pot with 40 cm in diameter. This experiment employed 2 treatments. The first treatment was dose of azolla comprised three levels (100 g m⁻², 200 g m⁻² and 400 g m⁻²). The second treatment was water supply consisted of flooded 2 cm tall every day (continuous flooded); flooded 2 cm every 3 days; flooded 2 cm every 6 days. On the flowering and grain filling period, plants were supplied by 2 cm high of water for all treatments. Irrigation was stopped in 2 weeks before harvesting. The experiment employed Randomize Complete Block Design with three replications.

In order to investigate the soil fertility, soil pH, texture, soil chemical properties were analysed in this experiment. Soil chemical properties comprised Nitrogen (N) total, available P, exchangeable K, organic material, and cation exchange capacity. N was analysed by Kjehdahl method. To determine the differences in vegetative growth, plant height was measured. Generative and plant quality parameters covered dry grain yield, anthocyanin, protein and antioxidant.

3. Result and discussion
Azolla and intermittent performed significant effects on plant height, dry grain yield, anthocyanin, protein and antioxidant. Interaction occurred between azolla and intermittent to plant height, protein and antioxidant.

Interaction between azolla 200 g m⁻² and 400 g m⁻² with 3 days intermittent irrigation produced higher plant (Figure 1.). This was due to higher nitrogen availability. Besides, the low fertility of the soil (Table 1.) contributed to the plant response. It meant that in unfertile soil, addition of azolla will be beneficial to rice growth. Flooded soil will effect to mineral conditions in the soil. In flooded soil, there will be some changes in soil chemistry. Some of those changes were the decline in the value of oxidation reduction potential (redox) of some nutrients. A decrease in these value would be toxic to plants [10].
Figure 1. Interaction between azolla doses and intermittent water supply to plant height

Table 1. Soil analysis result

| No | Parameters                              | Regosol |
|----|-----------------------------------------|---------|
| 1  | Type                                    |         |
| 2  | Chemical properties                      |         |
|    | N total (%)                             | 0.36    |
|    | Available P (ppm)                       | 11.70   |
|    | Exchangeable K (me/100 g)               | 0.24    |
|    | Ca (me/100 g)                           | 11.20   |
|    | Organic Material (%)                    | 3.30    |
|    | Cation Exchange Capacity (%)            | 28.15   |
| 3  | Texture                                 |         |
|    | Silt (%)                                | 29.46   |
|    | Clay (%)                                | 22.55   |
|    | Sand (%)                                | 47.99   |
|    | pH H₂O                                  | 5.64    |

Table 2. Grain yield and anthocyanin content due to azolla doses

| Azolla dose | Dry grain yield (g) | Anthocyanin (ppm) |
|-------------|---------------------|-------------------|
| 100         | 73.77 b             | 6033 b            |
| 200         | 86.32 a             | 7187 a            |
| 400         | 82.34 ab            | 6625 a            |

Note: The same letter in the same column showed no significant difference

Table 3. Grain yield and anthocyanin content due to intermittent irrigation treatment

| Intermittent       | Dry grain yield (g) | Anthocyanin (ppm) |
|--------------------|---------------------|-------------------|
| Continuous flooded | 76.60 b             | 6459 b            |
| 3 day              | 84.60 a             | 6539 b            |
| 6 day              | 81.23 ab            | 6846 a            |

Note: The same letter in the same column showed no significant difference
The higher dry yield and anthocyanin compound were generated by application azolla 200 gm$^2$ and 400 gm$^2$. Intermittent 3 days significantly boosted the rice yield but it declined the production of anthocyanin. On the contrary, intermittent every 6 days yielded higher anthocyanin. Intermittent every 6 days meant longer dry period experienced by plants so rice faced water stress period and produced higher anthocyanin as the response. This result was in line with Daneshmand [11] who conveyed that anthocyanin increased under drought. Intermittent irrigation is a concept of water saving through the regulation of water conditions in the field. At intermittent irrigation, the land is arranged in flooded and dried conditions alternately according to land conditions and growth phases.

Paddy fields flooded after the cultivation process will have consequences for the physicochemical changes in the soil. In flooded soil, the oxygen levels declined drastically so that anaerobic microorganisms became active. As a result, organic matter would decompose slower and less perfect. In addition, Flooding had a real impact on soil microbial characteristics [12]. Conversely, intermittent irrigation produced a positive response to aerobic bacteria in the soil [13]. Intermittent irrigation created higher root oxidizing activity rate, higher root length density, greater plant biomass, as well as it improved plant yield [14]. Moreover, intermittent drainage contributes to the alleviation of CH$_4$ emission and thus may decline net global warming potential in paddy cultivation [15].

![Figure 2](image.png)

**Figure 2.** Protein content as the effect of azolla dan water supply

![Figure 3](image.png)

**Figure 3.** Antioxidant as the effect of azolla and water supply
Intermittent 3 to 6 days and azolla 100 to 200 g m$^{-2}$ can increase the percentage of protein and antioxidant. The greatest protein content exhibited by utilization of azolla 200 combined with 3 days intermittent water supply of 8.027%. This value was higher than those stated by Juliano [16] that protein content of rice was in the range of 7%. This record correlates with the vegetative growth. The protein content of milled rice was strongly influenced by degree of post-harvest processing and the soil conditions. Rice grown on soil rich in N elements will tend to have high levels of protein.

Treatment of 100 azolla combined with 6 days intermittent irrigation created the highest antioxidant. Lower nutrient and longer drying period lead to stress condition resulting more antioxidant. This revealed that antioxidant activity was consistent with levels of anthocyanin. The dark purple colour of black rice is due to the high anthocyanin content located in the pericarp layers. Anthocyanin contributed to the antioxidant activity [17]. Anthocyanin is a polyphenol compound that acts as an antioxidant [18] because it is very reactive towards reactive oxygen species (ROS) or reactive nitrogen species (RNS) [19,20]. The phenolic components on the anthocyanin pigment contributed to the antioxidant capacity of black rice [21].

4. Conclusion
Azolla and intermittent escalated the yield grain, anthocyanin, protein and antioxidant. Azolla 200 g m$^{-2}$ combined with intermittent irrigation 3 days could be a good combination to improve plant growth, yield and properties of local black rice.

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