Utilization *Azollapinnata* as substitution of manure to improve organic rice yield and paddy soil health

M R Setiawati[1]*, P Suryatmana[1], Budiasih[2], N Sondari[2],L Nurlina[3], B A Kurnani[3]and E Harlia[3]

[1]Faculty of Agriculture, Universitas Padjadjaran, Jatinangor. Indonesia
[2]Faculty of Agriculture,Winaya Mukti University, Sumedang, Indonesia
[3]Faculty of Animal Husbandry, Universitas Padjadjaran, Jatinangor. Indonesia
*Email: m.setiawati@unpad.ac.id

Abstract. Organic rice has become the champion program of the Department of Agriculture in several districts in Indonesia, it is related to increasing awareness of consuming healthy foods which free from inorganic contaminants. Organic farming requires organic fertilizer supply in large quantities as a source of nutrition plants. *Azollapinnata* water fern is alternative organic matter in organic rice fields because of higher nutritional value compared to manure and can be cultivated in situ that reduces transportation costs. This research was conducted in the fields which first planted organic rice. The randomized block design was used, repeated three times with the treatment: cow manure 100% and the combination of Azolla + manure 25%, 50%, and 75% up to 100%. The results showed that Azolla 50% + cow manure 50% could increase plant height and tillers number, but the organic C content, total N, and C/N of soil was not different than the application of 100% cow manure. Although the yield of all treatments showed similarly,it could increase the dry grain rice weight as much as 19.17% compared with the 100% manure treatment. TheAzolla 50% + cow manure 50% treatment could increase the soil organic C content ranging from 1.3% to 1.7% which indicates the restoration of sick soil leads to healthy soil.

1. Introduction

The impact of the use of inorganic fertilizers results in a fairly high increase in crop productivity, but the use of inorganic fertilizers over a relatively long period generally has adverse effects on soil conditions. The soil becomes rapidly hardened, less able to store water and quickly becomes acid which will eventually decrease the productivity of the plant [1].Soil processing and N fertilization will directly increase the rate of decomposition process of soil organic matter and affect the soil ecosystem. Reduced soil organic matter will directly affect the soil quality (physical fertility, chemical and biological soil). Decreased levels of soil organic matter will affect the viability of organotrophic organisms that will ultimately affect organism life at subsequent tropical levels [2].

Efforts to restoring health and soil fertility can be pursued by increasing the soil organic matter content up to> 2%. According to [3] from the study of the Indonesian Center for Agricultural Land Resources Research and Development (BBSDLP) of the Ministry of Agriculture revealed that at present, about 73% of paddy fields (about 5 million ha) have very low C-organic (C-organic <2%), 22% had a moderate C-organic content (2–3% C-org) and 4% had a high C-organic content (> 3% C-org). Efforts to restore health and soil fertility can be done by utilizing local potentials such as animal manure or straw compost or green manures such as nitrogen-rich *Azolla pinnata* which may grow in wetland habitats.

Organic farming, especially organic rice requires proper and sufficient supplying of organic fertilizer in large quantities as a source of nutrition of rice plants. Organic fertilizers are often used manure of cows, goats, and chickens surrounding farmer fields, but it needed extra transportation costs. *Azolla pinnata* water fern is alternative organic materials that can be applied to organic rice fields, because of *Azolla pinnata* has higher nutritional value than manure. *Azolla pinnata* has a rich source of protein and essential amino acids (nitrogen 4–5 %, phosphorous 0.5–0.9%) and contain several vitamins such as vitamin-A, vitamin B-12 and beta carotene. It is also rich in minerals such as Calcium, Phosphorous, Potassium, Magnesium, Copper and Zinc etc. The protein composition of *Azolla*is 25–35% on dry weight basis [4]. Nitrogen fixation potential of the *Azolla anabaena*system has been estimated to be 1.1 kg N ha^{-1} day^{-1} and one crop of *Azolla*provided 20–40 kg N ha^{-1} to the rice crop in about 20–25 days [5].

In low land rice cultivation mineralization of organic nitrogen to ammonia is an important process [6]. *Azolla*helps to sustain soil nitrogen supply by returning N to the soil in quantities roughly equal to those extracted from soil by the rice plants [7]. After the decomposition of organic nitrogen content is made available to the rice plants. Field trials demonstrated that use of *Azolla*enhanced crop yield and crop N uptake significantly as compared to treatments without *Azolla*[8]. The aim of this research was to utilize *Azollapinnata* as substitution of manure to improve organic rice yield and paddy soil health.

2. Materials and Methods

This research was conducted at the altitudeof712 m above sea level during rainy season at Cilayung Village Jatinangor District, West Java in farmer rice Field. The soil texture was silty clay; low fertility soil which was slight acid (pH 6.36). Chemical characteristics of soil was low inorganic carbon (1.30%), low in total Nitrogen (0.22%), high in available P_2O_5 (49.51mg/kg) and total P_2O_5 (24.71mg/100g), and low in total K_2O (15.21mg/100g).

The field experiment comprising of cow manure as control and combination cow manure with Azolla; manure 100%, Azolla 25% + manure 75%, Azolla 50% + manure 50%, Azolla 75% + manure 25%, Azolla 25% + manure 100%, no fertilizer added. This experiment as was conducted completed randomized block design with four replications. The seed of paddy Ciherang variety was germinated with soaked water for 12 hours and then incubated in moisture cloth for two days. Germinated seed were planted in mixed of soil and compost on tray for seven days. The seeds were sown with a row to row spacing of 35 cm.

During the growth period liquid biofertilizer produce from Soil Biology Laboratory of Unpad registered as Bion-UP were added 5 L ha^{-1} dosage each week; these consist of nitrogen fixing bacteria *Azotobacterchroococcum*, *A. vinelandii*and*Azospirillum* sp., *Acinetobacter* sp. and PSM *Pseudomonas cepacia*and*Pencillium*sp. Liquid organic fertilizer were also applied from extract earthworms manure with dosage 10 L ha^{-1} two tofive weeks after planting (WAP). Diseases and pest were controlled by use of neem, garlic, and lemon grass based organic pesticides.

Observations on plant height and number of tiller were recorded from five selected randomly from each pot. The sampling of N-total and organic-C of soil, N content of plant were analysis on 6 WAP, the yield was collected after panicle were yellow ripe *from each plot on 16 WAP*. All data were subjected to analysis of variance (F test) at P <0.05% followed by Duncan's Multiple Range Test if the effect of treatments on experimental parameters was significant.

3. Results and Discussion

3.1. Effect of Azolla and manure application on organic-C, total-N and C/N

According to [9], the carbon to nitrogen (C/N) ratio of the organic wastes will largely determine the balance between mineralization and immobilization. The C/N ratio was the best predicting parameter for the potential amount of N that can mineralize from a crop residue. The initial soil C/N increased after receiving treatment of organic manure on the first rice planting season. Before planting rice in the first growing season C/N soil of 2.91 increased to 6.0 at the beginning of the second growing season.

Increased C/N occurs due to the supply of organic matter added to the soil. Organic material in the form of manure which is incorporated into the soil increases the organic soil C content from 0.66% to 1.3% thus increasing the C/N of the soil.

The organic C, N total, and C/N soil of the whole treatment were not different after being analyzed in 45 days after sowing. Increased organic C content and C/N soil ratio occurred in all plots compared to the soil at baseline, although in all treatments there was no difference. This means that Azolla as organic fertilizer can replace a manure or can be used as a mixture or substitution of manure. Increase in soil organic C in the soil is very beneficial. According to [10], C organic soil serves as a source of energy for the activity of microorganisms in the soil and also increase the ability of soil to hold nutrient elements or the capacity of soil cations exchange becomes high.

Table 1. Effectof azolla and manure application on organic-C, total-N and C/N of low land soil at 45 days after sowing

Treatments	Organic-C (%)	Total-N (%)	C/N
A = Azolla 0 % + Manure 100 %	1.8875 a	0.2525 a	7.5550 a
B = Azolla 25 % + Manure 75 %	1.7750 a	0.2275 a	7.8850 a
C = Azolla 50 % + Manure 50 %	1.7000 a	0.2025 a	8.7700 a
D = Azolla 75 % + Manure 25 %	1.7300 a	0.2300 a	7.5700 a
E = Azolla 25 % + Manure 100%	1.7875 a	0.2400 a	7.4325 a

Note: Numbers in a column followed by same letters were not significantly differ based on 5% Duncan's Multiple Range Test

Based on this study, according to[11]found a higher mineralization of clover and Lucerne green manures after the application of soil (C/N ratio 12 and 10, respectively) vetch and oat mixture (C/N ratio 31) and straw wheat (C/N ratio 55). For this reason, the increase in the soil microbial biomass-C as well as in the soil enzymatic activities was very different depending on the type of green manure applied to the soil. This difference in the C/N ratio of the different green manures is manifested in the different evolution of the soil C/N ratio. As a result, the mineralization of the organic matter applied to the soil will be carried out under good conditions of the mineralization versus the immobilization processes; aspect that is manifested in the highest values in soil microbial biomass and the soil enzymatic activities.

Efforts to restore health and soil fertility can be pursued by increasing the soil organic matter content up to> 2%. According to [3] from the study of the Indonesian Center for Agricultural Land Resources Research and Development (BBSDLP) of the Ministry of Agriculture revealed that at present, about 73% of paddy fields (about 5 million ha) have very low C-organic (C-organic <2%), 22% had a moderate C-organic content (2–3% C-org) and 4% had a high C-organic content (> 3% C-org). Efforts to restore health and soil fertility can be done by utilizing local potentials such as animal manure or straw compost or green manures such as nitrogen-rich Azolla which can grow in wetland habitats.

3.2. Plant height of paddy plant

The growth of rice plants at phase of two to eight Week After Planting (WAP) have not shown differences in plant height between treatments. This is because of the plant is still in adapting stage to the conditions of the end treatment. Differences of the height of new plants came out in rice plants at phase of 9 WAP to 10 WAP.

Table 2. Effect of azolla and manure application on height of paddy plant at 2 to 10 WAP

Treatments	Height of paddy plant (cm)						
	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP	9 WAP	10 WAP
A = Azolla 0 % + Manure 100 %	19.43a	23.14a	26.38a	33.3a	39.86 a	65.85a	69.30a
B = Azolla 25 % + Manure 75 %	20.76a	24.32a	28.55a	34.3 a	44.93a	69.43b	73.92 b
C = Azolla 50 % + Manure 50 %	19.20a	24.39a	27.83a	36.34a	42.71a	68.64 ab	72.51 b
D = Azolla 75 % + Manure 25 %	19.86a	24.13a	27.25a	34.59a	42.16a	66.41 ab	69.34 a
E = Azolla 25 % + Manure 100%	20.35a	24.74a	29.14a	35.99a	44.90a	69.60b	73.54 b

Note: Numbers in a column followed by same letters were not significantly differ based on 5% Duncan's Multiple Range Test

At the phase of 9WAP rice plants has entered the phase of stem growth, leaves, as well as the maximum fast saplings and root development. The rate of plant growth in a rapid phase growth requires more nutrients than the other growth phase of plant, and organic matter can supply nutrients needed on rapid phase growth of plant, because of the organic matter will be readily nutrient available through the easily decomposition process of organic matter. The decomposition of the organic material will be perfectly mineralized when the N content in organic material piles is sufficient its availability for the decomposer organismnutrient needed as long as mineralize process of the organic material. Azolla is organic material which rich in N elements (containing 3–5% N) and it can accelerate the process of mineralization of organic materials. Therefore, in rice plants on 9 and 10 WAP phase which the treatment by applying combine between manure and azolla showed the result of plant height was higher than the manure treatment. Acoording to [12] *A. pinnata*is organic material which has a rich source of protein and essential amino acids and provide the nitrogen 4–5 % source for plant.

3.3. Tillers number of paddy plant

Plants begin to form saplings together with the development of new shoots. The seedlings arise from axillary shoots on the stem node and replace the leaf place and grow and develop. This seed indicates the position of the first two branches flanking the main stem and the leaves. After growing (emerging), the first puppies bring up the secondary seedlings, and so on until the tillers are maximal. In this phase, there are two important stages namely the formation of active tillers followed by stem elongation. Both of these stages can overlap, the plants that have not formed the seedlings will experience stem extension, the fifth book of the stem under the panicle, extending only 2–4 cm before the formation of panicles. While young plants sometimes still form new saplings, so that will be came out very quickly the development of the canopy. In general, the seedling phase lasts for approximately 30 days. The number of tillers that given the manure and the organic fertilizer of Azolla and its combinations did not show any difference from the age of 5 WAP to 10 WAP (table 3).

Table 3. Effect of azolla and manure application on number of tiller paddy plant

Treatments	Number of tiller					
	5 WAP	6 WAP	7 WAP	8 WAP	9 WAP	10 WAP
A = Azolla 0 % + Manure 100 %	5.30 a	8.45 a	11.35 a	13.25 a	15.80 a	16.55 a
B = Azolla 25 % + Manure 75 %	5.95 a	8.80 a	11.75 a	14.70 a	17.05 a	17.75 a
C = Azolla 50 % + Manure 50 %	5.80 a	9.90 a	13.05 a	16.45a	17.50 a	18.15 a
D = Azolla 75 % + Manure 25 %	5.65 a	8.80 a	11.15 a	14.70 a	15.10 a	17.00 a
E = Azolla 25 % + Manure 100%	6.45 a	10.20 a	14.65 a	17.80 a	19.20 a	19.90 a

Note: Numbers in a column followed by same letters were not significantly differ based on 5% Duncan's Multiple Range Test

Number of tillers of rice plants one of which is influenced by nutrients available in the soil. As the plants are applicated by organic fertilizers in the form of manure equivalent to the growth of plants with other organic fertilizers such as Azolla and its combinations, it is suspected that the nutrients produced from both kinds of organic fertilizers and its combination have almost the similar on their

nutrient content.Environmental conditions and the availability of nutrients properly could be supporting the existing of microbes indigenous that obtain its energy sources from organic carbon, will be accelerating the process of decomposition of organic materials. This is confirmed by [13] that the number of tillers is closely related to the nitrogen content in rice plants.

3.4. Yield of paddy plants

Nutrient element is one of the factors that can influence rice yield. N and P elements are essential nutrients needed during the vegetative phase to productive (grain producing). The amount of nutrients can affect the growth, development, and yield of plants, especially N nutrient that plays a role in the formation of protein and increase the weight of rice grain. As the dosage and nutrient composition applicated are not appropriate condition, it will affect to the reduction of the rice crops yield.

The yield of paddy crop in Dry Harvested Grain and Dry Milled Grain weight per plot have no differ weight for all treatments (table 4). The weight of rice crop yielded affected by application of manure or Azollaand also its combination showed that there was not significant difference effectto the yield obtained.

Table 4. Effect of azolla and manure application on paddy yield

Treatments	Dry Harvested Grain per Plot (kg)	Dry Milled Grain per Plot (kg)
A = Azolla 0 % + Manure 100 %	3.13 a	2.05 a
B = Azolla 25 % + Manure 75 %	3.30 a	2.18 a
C = Azolla 50 % + Manure 50 %	3.73 a	2.42 a
D = Azolla 75 % + Manure 25 %	2.90 a	2.03 a
E = Azolla 25 % + Manure 100%	3.14 a	2.17 a

Note: Numbers in a column followed by same letters were not significantly differ based on 5% Duncan's Multiple Range Test

In general, all treatments of manure or Azolla and its combinations were capable ~~of~~ to produce 2.03–2.42 kg Dry Milled Grain Yield per plot or equivalent to 3.38 tha^{-1}–4.03 t ha^{-1}. Based on the description of Ciherang rice varieties which has an average yield about 6 t ha^{-1} and if it according to this experiment, the Dry Milled Grain yield in decreased about 32.8–43.6%. The declining of yield in organic farming is correlated to land conversion phenomenon that is changing from conventional farming to organic farming system. In the conversion of land to agriculture/organic rice will be reducing system of the dependence of plants on chemical fertilizers which called the adjustment phase. During this phase, there will be a decrease in yields due to conversion/change from chemical systems to biological systems, because of the nutrient source from derived from chemical fertilizer is more easily available than organic fertilizer. Plants are forced to experience "nutritional hunger" due to nutrients derived from chemical fertilizers are no longer available. The length of the initial phase of this organic soil adaptation depends on the historical conditions of the soil and the initial fertilization. For seasonal crops such as rice crops, a minimum of two years of conversion is required, while annual crops (excluding grasslands) require a minimum of three years of conversion [14].

4. Conclusion

The results of this study indicate that the use of Azolla 50% + manure 50% could increase plant heightbut not for increasing the number of tillers. The application of Azolla 50% + manure 50% was equivalent to 100% cow manure application on organic C, total N and C/N of soil. Utilization of Azolla as substitution of cow manure on organic rice farming has advantages for utilizing local potential to cultivated directly in the field and can be applied to the fields without composting. Using Azolla 50% + manure 50% can increase organic C content of soil 1.3% to 1.7%, which indicates that the restoration of sick soil leads to occured healthy soil.

Acknowledgements
AuthorsthankstoUniversitas Padjadjaran, Bappeda Sumedang district, Universitas Winaya Mukti and Kemenristekdikti through the Program Kemitraan Wilayah (PKW)which has supported usto carryout this experiment.

References

[1] Onunka N A, Chukwu L I, Mbanasor E O and Ebiniro C N 2012*Journal of Agriculture and Social Research (JASR)***12**(1): 183–194
[2] InghamER.2001.The foodweb andsoilhealth.SoilBiologyPrimer.www.statlab.iastate.edu [Online]
[3] Mulyani A, Setyorini D, Rochyati S and Las I 2012*Proc oncharacteristics and distribution of paddy fields are degraded in eight provinces of rice production centers*(Bogor: Fertilization Technology and Degraded Land Restoration) pp 99–110
[4] Parashuramulu S, Swain P S andNagalakshmi D 2013 *J Ani Feed Res* **3**(3): 129–132
[5] Watanabe I, Espianas C R,Berja N S and Alimagno B V 1977 *IRRI Res. Paper Ser.***11**: 1–15
[6] Sahrawat KL 1983 *Plant Soil* **42**: 305–308
[7] Cisse M and Vlek P L G 2003 *Plant Soil* **250**: 105–112
[8] Manna A B and Singh P K 1989 *Fert. Res.***18**: 189–199
[9] TejadaM and Gonzalez J L 2006*Eur.J.Agron.***25**:22–29
[10] Mohammadi K, Heidari G, Khalesro S and Sohrabi Y2011*African Journal of Biotechnology***10**(84): 19840–19849
[11] Maiksteniene S and Arlauskiene A 2004 *Agron.Res.***2**:87–97
[12] Singh PK and Subudhi B P R 1978 *India Farming* **27**: 37–39
[13] Grist DH1986. *Rice 6th edition*(New York: Longman) p 599
[14] Sharma AK 2002 *A Handbook of Organic Farming* (India: Agrobios) p 656