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Effect of Composted Animal Manure as Fertilizer on Productivity of *Azolla Pinnata* Grown in Earthen Ponds

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Abstract: *Azolla pinnata* contains a high nutritional value with a high protein content of 25%, so it can be used as an alternative to animal feed. The low production of Azolla is caused by the suboptimal use of fertilizers. This study aims were to determine the type of manure (composted manure from poultry, goats, and rabbit) which was the most optimal in producing Azolla. *Azolla pinnata* was planted at 50×50×10 cm (W×L×H) soil pools coated with plastic sheets. To maintain the acidity of the media, 10% (2 kg) of sludge fields was added to each pond. Water and animal waste compost (5 g/L) were added to each pond as a treatment. The treatments were: paddy mud (K0), paddy mud + poultry composts (K1), paddy mud + compost rabbit manure (K2) and paddy mud + compost goat manure (K3). Each treatment was repeated 5 times. Measurements made were pH of the media, area of cover, yield (g weight of dry matter) and crude protein content of Azolla. Harvesting was done every 14 days. The results showed that the Azolla dry matter production of K1, K2 and K3 treatments were greater ($p<0.01$) than K0 (20.80, 18.20, and 19.00 vs. 10.40 g/2,500 cm^2; respectively). Among the compost treatments, the crude protein content of K1 and K2 were higher than K0 and K3 (25.34 and 26.25 vs. 13.11 and 22.96%, respectively; $p<0.01$). Fertilizing with rabbit or poultry compost was the best in improving Azolla production and crude protein content.

Keywords: Organic Fertilizer, Animal Manures, *Azolla* Production, Crude Protein

Introduction

Azolla pinnata is a water fern plant that is potentially an alternative source of protein and minerals for livestock. *Azolla pinnata* has many benefits such as bio fertilizer, human food (Pabby *et al*., 2003), animal feed, fish feed and as medicinal supplements (Jain *et al*., 1992). Its protein content ranges from 21.4 – 37% (Alalade and Iyayi, 2006), with a protein digestibility of 84%, highly of total crude protein so it is potentially used as a protein supplement for livestock (Parashumaluru *et al*., 2013). *Azolla pinnata* has a short production cycle, with harvesting time of 7 – 20 days and fresh biomass production can reach up to 390 tons/ha/year (Ferentinos *et al*., 2002). *Azolla pinnata* has many benefits such as bio fertilizer, human food, animal and fish feed, as well as medicinal supplements (Mithraja *et al*., 2011). The use of animal manure as fertilizer for *Azolla pinnata* will reduce the costs of production but the type and rate of application of manure is still not known. However, there were few studies related to the right fertilizer for optimizing the growth of *Azolla pinnata*. In addition, most studies on Azolla's chemical composition and/or feeding values have not used Azolla biomass obtained from productive cultures, or not report biomass productivity data of analyzed plants.

The use of organic fertilizers in this study was performed due to organic fertilizers have advantages over inorganic fertilizers, including improving soil structure, adding nutrients, increasing nutrients and organic materials, improving the life of microorganisms. In addition, the nutrient content in organic fertilizer is released slowly so that it can benefit the plant (Samadi and Cahyono, 2005). The objectives of this study were to establish the type of compost used and the time of harvest for optimal growth and production of *Azolla pinnata* grown under earthen ponds. The composts used in this study were manure from poultry, rabbits, and goats.

Materials and Methods

Preparation of Ponds and Treatments

Twenty ponds were randomly divided into four treatment groups based on type of organic fertilizer used (compost made from poultry, rabbit and goat manure) to fertilize the water in the ponds. The treatments were: Control (K0) without compost addition, K1 (chicken excreta compost), K2 (rabbit feces compost) and K3 (goat feces compost). Each treatment consisted of five replicates. The basal media used were water with the addition of 10% mud (2 kg) obtained from paddy fields situated in the vicinity of the university. The media (with the respective fertilizer compost) were prepared and allowed to stand in the ponds for 7 days (to dissolve the fertilizer) before the inoculation of Azolla. Fresh *Azolla pinnata* (about 10 g fresh weight), were disinfected using potassium permanganate at 20 ppm for 60 min (Arizal, 2010) before inoculation onto the media. The experiment lasted for 42 days and the Azolla was harvested every 14 days (Ferentinos *et al*., 2002). Weekly measurements and samples taken were pH of water, area of water covered by Azolla (cover area), yield (dry matter), and crude protein of *Azolla pinnata*.

Measurements of pH, Crude Protein and Cover Area

Determination of pH was carried out by using pH-meter (Hannah). Determination of protein content was performed using the Kjeldahl method (AOAC, 2005). Cover area of *Azolla pinnata* was measured weekly using 60 cm ruler by measuring the length and width of the water surface covered by *Azolla pinnata*. Kinetics of dry matter production were determined by calculating the production of *Azolla pinnata* at every harvest The kinetics of production was carried out to find out the exact time for fertilization to be seen from the production of *Azolla pinnata* which has dropped dramatically. Production in forecast was done to determine the estimated production of *Azolla pinnata* for 5 weeks (4 weeks of production +1 week of rest).

Statistical Analysis

All data were analyzed using Statistical Analysis System (SAS) software using the model described below:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where, Y_{ij} is the Azolla response variable measured, μ is the overall mean. τ is the fixed effect of fertilizer and ε is the error. The differences between the means were compared using Duncan’s new multiple range test.

Result

Water pH

The pH of water in the ponds was measured for each pond on the different treatments and the means are shown in Table 1. The results showed that the average pH of water in ponds on treatment K0 day 7 and at day 14, were similar to the pH at day 0, suggesting that with any fertilization the alkalinity of the water tended to stabilized over time. This finding suggested that treatment with chicken manure as well as rabbit and goat feces compost stabilize the alkalinity of the water.

Cover Areas

The cover areas of the *Azolla pinnata* in each pond were obtained by measuring the area of *Azolla pinnata* per 2,500 cm^2 on the surface of the ponds using a ruler. The results of the measurement of the cover area are shown in Table 2. The cover area of Azolla in ponds for all fertilizer treatments was similar until Day 7, but on Day 14 the cover area of all fertilizer treatments were higher than those of the control, suggesting that fertilizer treatment increased the growth of Azolla after 14 days.

Production and Protein Content of Azolla pinnata

Production and crude protein content of *Azolla pinnata* with several kinds of fertilizer use can be seen in Table 3. The growth of *Azolla pinnata* was influenced by the amount of nutrient element dissolved in water especially element phosphorus (P). Chicken excreta and goat feces contain P which is sufficiently high for Azolla (3 and 2.46%, respectively) with production of *Azolla pinnata* 12.40 and 11.20 g/2,500 cm^2, respectively.

Kinetics Production

Azolla pinnata was harvested after the plant was two weeks old (fourteen days). The harvesting kinetic every fourteen days is listed in Table 4. There were a decrease in production of dry matter during the second and the third 14 days periods when the ponds were not fertilized after the initial fertilization. However the decline in production were more dramatic during the 3rd m 14-day period, suggesting that after the second 14 days harvest the ponds need to be fertilized to maintain the high production. The reduction dry matter production could be due to the lowering of available nutrients, especially phosphorus, for the Azolla and possibly the decline in pH.

Table 1: The pH of water in the treatment ponds, measured at 0, 7, and 14 days of growth period

Type of fertilizer	First day	Seventh day	Fourteenth day
Mud only (control / K0)	8.98±0.29	8.89±0.33	8.81±0.27
K1 (chicken excreta compost)	8.14±0.39	8.59±0.43	9.54±0.98
K2 (rabbit feces compost)	8.08±0.24	8.46±0.21	8.82±0.35
K3 (goat feces compost)	8.54±0.20	8.77±0.26	8.68±0.27

Table 2: Cover area (cm^2) of *Azolla pinnata* measured at 7 and 14 days of growth period

	Cover area (per 2,500 cm^2)		
Type of fertilizer	Day 0	Day 7	Day 14
Mud only (control/K0)	338.6±19.99	750±36.74	1,320^a±270.65
K1 (chicken excreta compost)	320.4±19.72	1,696±862.69	2,500^b±0
K2 (compost of rabbit feces)	338.6±19.99	1,465±348.93	2,500^b±0
K3 (goat feces compost)	338.6±19.99	1,560±210.36	2,500^b±0

Table 3: Production and crude protein content of *Azolla pinnata* at 7 days of growth period

Type of fertilizer	Dry weight of *Azolla pinnata* per 2.500 cm^2 (g)	The average crude protein content of *Azolla pinnata* (%)
Control (no fertilizer) (K0)	9.60^a±1.52	13.11^a±0.52
K1 (chicken excreta compost)	11.20^{bc}±0.84	25.34^c±0.89
K2 (compost of rabbit fecal)	9.40^a±0.55	26.25^c±1.23
K3 (goat fecal compost)	12.40^c±1.67	22.96^b±1.46

^{abc} Means with different superscript within the same column are significantly different (p<0.01).

Table 4: Production of *Azolla pinnata* harvested every 14 days (g DM) over a period of 42 days

Type of fertilizer	P1 (The first 14 days)	P2 (The second 14 days)	P3 (The third 14 days)	Mean
Mud (control / K0)	9.60±1.52	0.80±0.45	0.38±0.23	3.59^a±0.73
K1 (K0 + chicken excreta compost)	11.20±0.84	9.60±1.82	2.86±1.17	7.89^c±1.28
K2 (K0 + compost of rabbit feces)	9.40±0.55	9.60±1.14	2.46±0.44	7.15^{bc}±0.71
K3 (K0 + goat feces compost)	12.40±1.67	5.80±1.64	1.06±0.53	6.42^b±1.28
Mean	10.65^c±1.15	6.45^b±1.26	1.69^a±0.59	

^{abc} Means with different superscript within same column are significantly different (p<0.01)

Table 5: The total dry matter production of *Azolla pinnata* (g DM), over a period of 4 weeks (5 weeks production cycle)

Type of fertilizer	P1 (The first 14 days)	P2 (The second 14 days)	Total production per 28 days	Mean
Mud (control/K0)	9.60±1.52	0.80±0.45	10.40±1.05	5.20^a±1.01
K1 (chicken excreta compost)	11.20±0.84	9.60±1.82	20.80±0.99	10.40^c±1.22
K2 (compost of rabbit fecal)	9.40±0.55	9.60±1.14	20.00±1.04	9.50^{bc}±0.91
K3 (goat fecal compost)	12.40±1.67	5.80±1.64	18.20±1.59	9.10^b±1.63
Mean	10.45±1.15	6.45±1.27	16.90±1.17	

^{abc} Means with different superscript within same column are significantly different (p<0.01)

Production in Forecast

It was proposed that *Azolla* must be harvested after 14 days (2 weeks) inoculation and then it need to be fertilized to maintain its high production. It is suggested that each production period consists of 5 weeks ((2×2 weeks of harvest) +1 week of fertilization). The calculated amount of dry matter production per 5 weeks (including 1 week standing after fertilization) cycle is shown in Table 5.

Discussion

The increase in pH that has been observed shows that in all various sources of fertilizer produces a different pH after 1 week, an increase in pH occurs on day 7th and 14th. The pH value of media is an indicator of chemical fertility as a result of the nitrification and denitrification process by ponds water bacteria derived from manure that can produce OH^- subsequently increasing the pH of pond l water (Akhmar, 2007). *Azolla pinnata* can live at a pH of 3.5 to 10, but in water which is too acid there will be an adverse effect on growth. The pH of the water where *Azolla* was recorded ranged from 7.1 to 9.0 (mean = 7.8) (Serag *et al*., 2000).

Based on the two weekly observations made, it appeared that the pH of ponds water given compost (chicken excreta, rabbit feces, and goat feces) were higher compared to that of ponds that were not given compost. The decomposition process of organic material found in compost by microorganisms will also produce OH^- ions thus increasing the pH value in the pods water (Sawyer and Mc Carty, 1998). The higher pH of ponds water was probably due to the increased photosynthetic process by *Azolla pinnata* so that CO_2 was converted into $C_6H_{12}O_6$ which requires energy and hydrogen input. Energy is obtained from sunlight while hydrogen (H^+ ion) is obtained from ponds water, removing H^+ ions from ponds water can increase water pH (Hasan and Chakrabarti, 2009). All of the ponds used in this study had pH which is ideal for the growth of *Azolla pinnata*. In their study, Hasan and Chakrabarti (2009) showed that *Azolla pinnata* can live on pH 3.5 to 10.

The increase in the area cover, as a result of the fertilization process carried out is greater than the control. The availability of nutrients in the form of phosphate in planting medium can affect the growth of *Azolla pinnata*, because this plant efficiently absorbs phosphorus (P) early in the beginning of their growth (Lumpkin and Plucknett, 1982).

Insufficient concentration of phosphate can inhibit growth rate, nitrogen fixation and chlorophyll content in *Azolla pinnata*. The content of nutrients in chicken manure is known to be three times larger than other livestock manure because chicken manure is mixed with feces and urine (excreta) so that chicken manure has a nitrogen content ranging from 1.00 to 3.13%, in addition to phosphorus (P_2O_5) which range between 2.80 to 6.00%(Marschner, 1986). The high phosphorus content can increase *Azolla* growth.

Production of *Azolla pinnata* influenced by the P content contained in fertilizer. According to Lumpkin and Plucknett (1982), *Azolla pinnata* plants can grow rapidly because these plants can efficiently absorb P elements from the beginning of its growth.

The highest dry matter production of *Azolla pinnata* was obtained from the ponds on K3 treatment, which was 12.40±1.67 g and the lowest was from K2 treatment, which was not significantly different from that of the control (K0). Further test results showed that dry weight yield between treatments K1 and K3 showed no significant differences. According to Marschner (1986), the phosphorus element is influential in increasing the dry weight of plants to form the required pyrophosphate compounds as the main source of energy for plant growth and development. Element P is also used as a constituent of phospholipids for cell membrane structures.

The production of Azolla per ha per year can be projected as follows: After two harvests (2×14 days), the dry matter production will start to decline, therefore the ponds need to be fertilized again, followed by rest for 7 days, hence, it takes 5 weeks per cycle. In one year there are 52 weeks of which means that it is possible to run 10 cycles of harvest, with an annual production of 83.2 tones/ha/year (20.80g dry matter/2,500 cm^2, production = (100,000,000: 2,500) ×20.80 g ×10 = 83,200 kg/ha/yr).

Conclusion

It can be concluded that organic fertilizer both chicken feces compost and rabbit feces compost when used as fertilizer in ponds of Azolla, with an estimated production of more than 20 g BK/2,500 cm^2 per 5 weeks or projected as 83,200 kg/ha/year) and higher protein content compared to fertilization with goat feces compost. It was proposed that each production cycle consisted of 5 weeks period, with 1 week rest after fertilizer application followed by two 2 weeks growth period.

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Author’s Contributions

Ristianto Utomo: Coordinated the implementation of research work, conducted research, compiled the literary review, analyzed and interpreted the study findings, drew conclusions, contributed manuscript preparation and revisions main supervisor for Mr. Permadi.

Cuk Tri Noviandi: Contribution Second researcher, designed the research plan, organized the study, conducted research and contributed to result analysis and the writing manuscript, co-supervisor for Mr. Permadi.

Nafiatul Umami: Conducted research, compiled the literary review, analyzed and interpreted the results, contributed manuscript revisions, Third researcher, co-supervisor for Mr. Permadi.

Adhitya Permadi: Conducted research, contributed manuscript revisions.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and there are no ethical issues involved

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