EFFECTS OF MANURE TYPES AND INORGANIC FERTILIZERS ON AGRONOMIC CHARACTERS AND BIOMASS PRODUCTION OF *Azolla Microphylla*

Supartoto[1]*, A Nareswara[2]
[1] Lecture of Agrotechnology Department, Jenderal Soedirman University, Purwokerto, Central Java, Indonesia, 53123
[2] Alumny of Agrotechnology Department, Jenderal Soedirman University, Purwokerto, Central Java, Indonesia, 53123

Email : supartoto@unsoed.ac.id

Abstract. The objectives of this experiment were to: (1) determine the effect of manure types on population growth and biomass yield of *Azolla microphylla*, (2) determine the effect of dolomite and phosphate fertilizer on population growth and biomass yield of *Azolla microphylla*, and (3) determine the interaction between manure and fertilizer phosphate + dolomite on population growth and biomass yield of *Azolla mycrophylla*. The experiment was conducted in July to September in Dagan Village, Bobotsari District, Purbalingga Regency, at altitude. ±185 m above sea level. The study used a complete randomized block design (RCBD) consisting of 2 factors and 3 replications. The first factor were the type of manures Liquid Organic Fertilizer chicken manure (P1) and goat manure manure Liquid Organic Fertilizer (P2). The second factor were inorganic fertilizer that is without treatment (V0), dolomite fertilizer (V1), SP 36 fertilizer (V2) and dolomite fertilizer + SP 36 fertilizer (V3). The variables observed were *Azolla* growth rate, *Azolla* doubling time, chlorophyll content, fresh weight, and dry weight of the *Azolla* biomass. The results showed that the types of manures did not give different effect on the growth and yield of Azolla plants. The addition of dolomite + SP 36 fertilizer was able to increase the growth and yield of *Azolla*, affected the chlorophyll content (43,94% increased), Azolla fresh weight (20,49% increased), and dry weight of *Azolla* biomass (25,77% increased). There was no interaction between combination treatments of types of manure and inorganic fertilizers on all of growth and yield variables observed.

Keywords: *Azolla mycrophylla,* manure, inorganic fertilizer

1. Introduction

Azolla is a fast-growing floating fern. *Azolla* is a fix-free Nitrogen fern because it has a mutualism symbiotic with *Cyanobacteria* (*Anabaena Azollae*) [1,2]. One of Azolla species that often to be cultivated is *Azolla microphylla*. Nitrogen fixing potential of cyanobacterial symbiont varies between 30 and 60 kg N ha−1 which designates *A. microphylla* as an important biological N source for agriculture and animal industry [3]. This species has advantages compared to other Azolla plant species, including high protein content and fast growth with a doubling time of 4-7 days [4].

Azolla has several benefits, including in agriculture, *Azolla* can be used as green fertilizer and an environmentally friendly alternative nitrogen source suitable for rice cultivation. The ability to fix N from the air in *Azolla* exceeds its needs, so that some of the fix nitrogen is released in the media or growth environment [5].

Azolla also serves as an alternative feed or additional feed containing high protein. *Azolla* has a fairly high protein content of 28.12% dry weight, 10-15% minerals, and 7-10% amino acids [6]. *Azolla* in animal husbandry is also one of the breakthroughs in feed ingredients used as alternative feed ingredients for poultry [7,8].

Given the enormous potential of *Azolla*, good *Azolla* cultivation techniques are needed in order to obtain maximum yields. However, the development of *Azolla* in the community still faces obstacles. One of the obstacles that occur during *Azolla* cultivation experienced by the community is that *Azolla* cultivated in tarpaulin ponds will gradually shrink and turn yellow. This is thought to be due to lack of nutrients needed by *Azolla*. The lack of nutrients in *Azolla* planting media occurs because farmers in cultivating Azolla farmers have difficulty fertilizing *Azolla* properly. The difficulty of fertilizing *Azolla* is because people do not know the type of fertilizer that is suitable for *Azolla* plants.

To overcome this, it can be done by applying liquid organic fertilizers and / or inorganic fertilizers. Liquid organic fertilizer has advantages over solid fertilizer, namely the nutrients contained in it are more easily absorbed by plants [9]. Other additional fertilizers needed in the cultivation of *Azolla* are dolomite and SP 36. Dolomite in *Azolla* cultivation can maximize the growth of Azolla and dolomite is added because it is one of the fertilizers that contain calcium (Ca) and magnesium (Mg). Calcium for plants is beneficial to activate the formation of root hairs [10]. Magnesium plays a role in the formation of chlorophyll and the process of photosynthesis. The addition of dolomite to *Azolla* cultivation can cause *Azolla* to grow well and remain green. SP 36 is a source of phosphate, an inorganic P source, which is needed in the formation of ATP in photosynthesis [11].

The objectives of this study are: (1) knowing the effect of applying the type of manure on population growth and *Azolla* yield, (2) knowing the effect of adding dolomite fertilizer and phosphate fertilizer on population growth and *Azolla* yield, and (3) knowing the interaction between manure and phosphate + dolomite fertilizer on the growth and yield of *Azolla*.

2. Methods

This experiment used tarpaulin ponds, 2 m x 1 m in size, and 40 cm water depth. The experiment was done in Dagan Village, Purbalingga, at dry season (Juli-September) located at 300 m above sea level altitude. The experiment was designed in a Completely Randomized Block Design, two factors (types of manure and type of inorganic fertilizer) and three replicates. Types of manure, consisting of: (1) liquid organic fertilizer (LOF) chicken manure, and (2) LOF of goat manure, both made of 20 kg of chicken manure or goat manure solved in 50 litters of water. Types of organic fertilizer, consisting of: (1) without inorganic fertilizer, (2) phosphate, (2) dolomite, and (3) phosphate and dolomite. The observed variables were population growth rate, doubling time, chlorophyll content, fresh weight and dry weight of Azolla. LOF was applied at rate of 1 litter per m2 of pond surface per weeks. Dolomit was applied once at rate 1000g/m^2 at the beginning of experiment, and SP 36 as source of Phosphate was applied once, 1 week after application of Dolomit at rate 660g/m^2 of pond surface. Data was analysed with F test, followed by DMRT when it was significant.

3. Result and Discussion

The result of analysis was presented in Table 1. Table 1 showed that there were no effects of types of manure in all variable observed. Types of inorganic fertilizer on the other sides, significantly affected chlorophyll content, fresh weight and dry weight of Azolla biomass.

Table 1. The effect of treatments on growth and biomass weight of Azolla

Treatments	GR (m^2/d)	DT (mg/l)	Chl (IU)	FW (g)	DW (g)
		Manure types			
LOF Chicken	0,13a	11,17a	10889a	4687,1a	99,90a
LOF Goat	0,12a	12,08a	10753a	4367,5a	91,96a
		Inorganic fertilizer types			
Control	0,12a	13,59a	8188b	4102,4a	81,98a
Dolomit	0,13a	12,27a	10156ab	4495,0ab	97,90b
Phospat	0,13a	12,20a	10352ab	4569,0ab	101,65b
Dolomit + phosphat	0,13a	12,17a	11786a	4942,9b	103,11b
Interaaction	NS	NS	NS	NS	NS

Note:
1) GR growth rate; DT = Double time; Chl = Chlorophyll content, FW = Fresh weight; and DW = dry weight
2) In a collum, number followed by the same letter was not significantly difference

3.1. Effect of Manure Type on the Growth and Yield of Azolla microphylla

Table 1 shows that manure treatment had no significant effect on all observed variables. This happens because the nutrient content of chicken manure liquid organic fertilizer (LOF) and goat manure LOF is relatively low and the same level (Tabel 2), so it does not make different plant growth. This result is in line with the previous findings that the most suitable fertilizer for Azolla growth is cow dung fertilizer and the least suitable fertilizer is liquid fertilizer [11].

Tabel 2. The content of Chicken and goat LOF (20kg/50 litters water)

No	Parameters	Unit	Chicken LOF	Goat LOF	Standar
1.	N total	%	0.03	0.03	
2.	P_2O_5 total	%	0.004	0.003	3-6
3.	K_2o total	%	0.01	0.04	
4.	Ca total	mg/l	44.34	44.04	-
5.	Mg total	mg/l	43.27	3.53	-

Source: Primer data, Laboratorium Balai Pengkajian Teknologi Pertanian (BPTP) Yogyakarta.

3.2. Effect of Dolomite and Phospate on *Azolla microphylla* plants

3.2.1. Chlorophyll Content

Dolomite and SP 36 have a significant effect on the chlorophyll content of Azolla. The combination of dolomite and phospate differed markedly from the control treatment, but was no different from individual dolomite and SP 36 treatments (Figure 1).

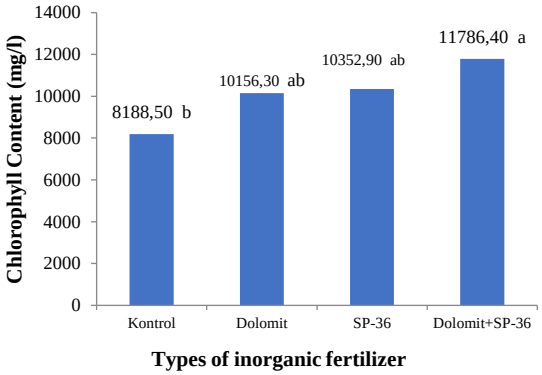

Figure 1. The influence of dolomite and phospat on the content of chlorophyll Azolla

The chlorophyll content in the combined treatment of dolomite and SP 36 fertilizers was 11786.40 mg/l, an increase of 43.94% compared to the control. This is thought to be because the application of dolomite fertilizer and SP 36 is able to increase nutrients in the growing media which affects the chlorophyll content. Dolomite addition can supply Mg elements which are essential elements that make up chlorophyll or green leaves [12]. Addition of SP 36 increased a source of inorganic phosphate in that is an essential element in the photosynthesis, stimulates the production of more photosynthate [13]. Increasing Photosynthate will increase carbohydrate supply for bacteria (*A. azzolae*) that is responsible for the nitrogen fixation. Nitrogen plays an important role in the formation of chlorophyll and makes leaves green [14].

3.2.2. *A. microphylla* Fresh weight

Table 1 shows that the application of dolomite fertilizer and phosphate fertilizer has a significant effect on the fresh weight of Azolla. The average value of fresh weights in each treatment is fully presented in Figure 2.

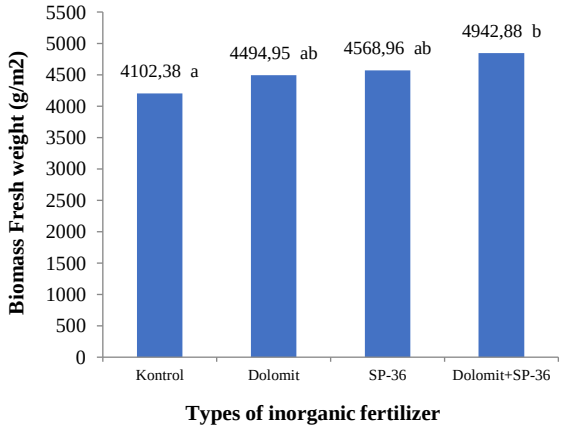

Figure 2. The influence of dolomite and phosphate on Fresh weight of Azolla

The combined treatment of dolomite and phosphate fertilizer has an average fresh weight of 4942.88 g / m^2, while the average fresh weight of the control treatment is 4102.38 g / m^2. The application of dolomite in Azolla planting media increased Ca and Mg elements, while the application of SP 36 fertilizer will increase P element, so Azolla can grow better than when

living in planting media without dolomite and phosphate fertilizer. The application of dolomite and SP 36 were thought to be able to increase the nutrient content in the planting media, which affects plant growth, and increase the fresh weight of Azolla.

The same result was reported, that dolomite and SP 36 have a significant effect on the fresh weight of Azolla [15]. The availability of calcium (Ca) in the growing media, causes Azolla to form cell walls well and root growing points will grow well, so that Azolla growth can be maximized [16]. The availability of Mg elements in growing media can increase leaf greenness, nitrogen absorption, CO_2 fixation which all lead to increased assimilate in its use as energy and cell formation as well as to be stored in the form of carbohydrates. Mg also plays a role in regulating the formation of sugar and starch from the remodeling of carbohydrate compounds, as well as increasing total dissolved solids which also causes an increase in fresh weight [17]. Giving SP 36 to planting media can increased P elements. Enriched of planting media with Phosphate element tend to increase the productivity of *A. microphylla* and *A. Azollae* activity [18]. Nitrogen fixation by *A. Azollae* greatly affects the growth of Azolla because the nitrogen fixation will be distributed to Azolla cells which will then be used for Azolla growth.

3.2.3. *A. microphylla* dry weight

Table 1 shows that the application of dolomite and SP 36 fertilizer has a significant effect on the dry weight content of Azolla. This is in accordance with the previous results that the application of dolomite fertilizer and SP 36 in Azolla planting media has a real effect on increasing plant growth compared to controls [15]. The magnitude of the average value of dry weight content in each treatment is fully presented in Figure 3.

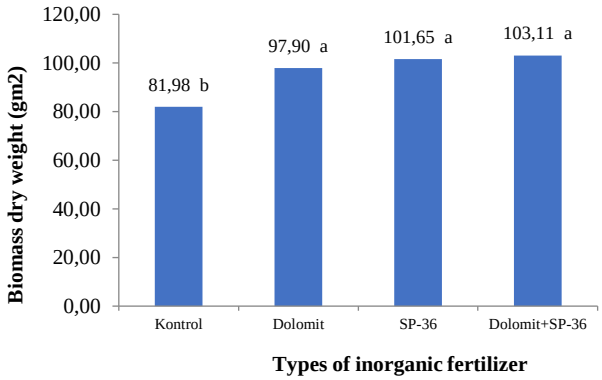

Figure 3. The influence of dolomite and phosphate on dry weight of Azolla

Inorganic fertilizer treatment had a significant effect on the dry weight of Azolla, but the addition of dolomite, SP 36, and the combination of the two did not differ markedly. The average dry weight that differs markedly from the control is directly proportional to the average result of the fresh weight. This result is in accordance with the previous results which state that the fresh weight and dry weight of plants are always directly proportional [19, 18].

3.3. Interaction of types of manure and inorganic fertilizer

There was no interaction between types of manures and types of inorganic fertilizer found in all observed variables.

4. Conclusion

1. The type of manure that applied as liquid organic fertilizer has no effect on the growth of *Azolla microphylla*
2. Application of dolomite and phospate increased leaf chlorophyll levels, fresh weight, and dry weight of biomass of *A. microphylla*, but had no effect on population growth rate and doubling time. The combined treatment of dolomite 1000 g/m2 and phosphate 660 g/m2, increased leaf chlorophyll content, fresh weight of azolla, and dry weight of azolla compared to controls, by 43.94%, 20.49%, and 25.77% respectively.
3. There was no interaction between the type of manure and the type of inorganic fertilizer at all observational variables.

Acknowledgement

The authors want to thanks to the Dean of Faculty Agriculture Unsoed and the parent of Andelira Nareswara, for providing all research facilities including moral and technical support during the work of this experiment.

References

[1] De Vries, Sophie and Jan De Vries. 2018. *Azolla: A Model System for Symbiotic Nitrogen Fixation and Evolutionary Developmental Biology*. In Current Advances in Fern Research. Springer International Publishing AG. Pp 21-46.

[2] Effendi, Irwan, Aditya Pranata, dan Feliatra. 2019. The Effect of Sun Light Intensity on the Growth of Azolla microphylla and its Symbiont Anabeana Azollae in Brackish. J. Phys.: Conf. Ser. 1351 012096. IOP Publishing Ltd. Pp 1-6.

[3] Kollah, B., Patra, A.K. & Mohanty, S.R. 2016. Aquatic microphylla *Azolla*: a perspective paradigm for sustainable agriculture, environment and global climate change *Environ Sci Pollut Res* **23**, 4358–4369 (2016). https://doi.org/10.1007/s11356-015-5857-9

[4] Paulus, J.M. 2010. Pemanfaatan Azolla Sebagai Pupuk Organik Pada Budidaya Padi Sawah. Warta-Wiptek. No 36: 0854-0667.

[5] Sudjana, B. 2014. Pemanfaatan Azolla untuk Pertanian Berkelanjutan. Jurnal Ilmiah Solusi. 1(2): 72-81.

[6] Wardani, R.E., Prayogo, & Agustono. 2017. Potensi Penambahan Azolla sp. dalam Formulasi Pakan Ikan Lele (Clarias sp.) terhadap Nilai Kecernaan Protein dan Kecernaan Energi Menggunakan Teknik Pembedahan. Journal of Aquaculture and Fish Health. 6(2): 94-100

[7] Raras, A., R. Muryani & W. Sarengat. 2017. Pengaruh Pemberian Tepung Azolla Fermentasi (Azolla microphylla) terhadap Performa Ayam Kampung Persilangan. Jurnal Peternakan Indonesia. 19(1): 30-36.

[8] Supartoto, Roesdiyanto dan Dalhar Shodiq, 2018. Kaji terap pemanfaatan Azolla microphylla sebagai hijauan pakan itik di kelompok ternak itik kelurahan Gandasuli Brebes. Jurnal Ilmiah Ultras Vol. 2 No. 1, Desember 2018. Pp 52-61.

[9] Rahmah, A., Munifatul I., & Sarjana P. 2014. Pengaruh Pupuk Organik Cair Berbahan Dasar Limbah Sawi Putih (Brassica chinensis L.) Terhadap Pertumbuhan Tanaman Jagung Manis (Zea mays L. Var. Saccharata). Buletin Anatomi dan Fisiologi. 22(1): 65-71.

[10] Jovita, D. 2018. Analisis Unsur Makro (K, Ca, Mg) Mikro (Fe, Zn, Cu) Pada Lahan Pertanian Dengan Metode Inductively Coupled Plasma Optical Emission Spectrofotometri (ICP-OES). Skripsi. Universitas Lampung, Bandar Lampung

[11] Zakarya, Irnis Azura, Nor Anis Nadhirah Md Nasir, Najiha Razali, Mahamad Hakimi Ibrahim, and Madalina Boboc. 2023. Assessment of Water Quality and Nutrient Uptake of Azolla in Different Fertilizers. IOP Conf. Series: Earth and Environmental Science 1216 (2023) 012039. IOP Publishing Ltd. Pp 1-9.

[12] Zarmiyeni, Farida A. & Majedi. 2013. Pengaruh Pemberian Emras terhadap Pertumbuhan dan Hasil Tanaman Buncis (Phaseolus vulgaris L.) pada Lahan Rawa Lebak. Rawa Sains. 3(2): 123-129

[13] Lambers, H, F. Stuart Chapin III, and Thijs L. Pons 2008. Plant Physiological Ecology 2nd edition. Springer, UK. 624ps.

[14] Setyani, Y.H., S. Anwar & W. Sla,et. 2013. Karakter Fotosintesik dan Serapan Fosfor Hijauan Alfafa (Medicago sativa) pada Tinggi Pemotongan dan Pemupukan Nitrogen yang Berbeda. Animal Agriculture Journal. 2(1): 86-96.

[15] Bernas, S.M., Dwi S., Agus H., Ucuk S., Indah. 2008. Dampak Pemberian Kapur dan Fosfat pada Air Lebak dari Berbagai Sumber terhadap Produksi Tanaman *Azolla pinnata* R. *Prosiding Pertemuan Ilmiah Tahunan Himpunan Ilmu Tanah Indonesia*. Universitas Sriwijaya, Palembang.

[16]1Pramita, I., Periadnadi & Nurmiati. 2015. Pengaruh Kapur dan Dolomit terhadap Pertumbuhan Miselium dan Produksi Jamur Kuping Hitam (*Auricularia polythrica* (Mont.) Sacc). *Journal of Natural Science*. 4(3): 329-337

[17] Wirawan, B.D.S., Eka T.S.P., Prapto Y. 2016. Pengaruh Pemberian Magnesium, Boron dan Silikon terhadap Aktivitas Fisiologis, Kekuatan Struktural Jaringan Buah dan Hasil Pisang (Musa acuminata) Raja Bulu. Vegetalika. 5(4): 1-14

[18] Utama, P., Dewi F. & Ganes N. 2015. Pertumbuhan Dan Serapan Nitrogen Azolla microphylla Akibat Pemberian Fosfat dan Ketinggian Air yang Berbeda. Agrologia. 4(1): 41-52.

[19] Royani, K. 2013. Pertumbuhan Vegetatif Anggrek Larat (Dendrobium phalaenopsis) yang Ditanam pada Media Limbah Sagu Segar dan Kompos Sagu Hitam. Skripsi. UNDIP, Semarang