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Application of Azolla sp. Liquid Organic Fertilizer in Swamp Water for the Cultivation of Pangasius Catfish in an Aquaponic Floating Raft System

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Abstract: Fertilization has an important role in overcoming nutrient deficiencies for plankton growth as natural fish food and plant growth in aquaponic systems. This study aims to determine the optimal dose of Azolla sp. liquid organic fertilizer in the limed swamp water for the culture of pangasius catfish and lettuce plants using an aquaponic floating raft system. This study used a completely randomized design with four treatments and three replications. The treatments were the different doses of Azolla sp. liquid organic fertilizer, namely 5 mL L^{-1} (P_1), 10 mL L^{-1} (P_2), 15 mL L^{-1} (P_3), and 20 mL L^{-1} (P_4). Pangasius catfish (initial size 5 ± 0.5 cm) with a stocking density of 100 fish m^{-3} and lettuce plants that were sown for 23 days (3-4 lettuce leaves) and reared for 42 days. The results showed that of *Azolla* sp. liquid organic fertilizer at a dose of 5 mL L^{-1} (P_1) was the best dose with ammonia 0.027-0.104 mg L^{-1}, nitrate 2.823-7.968 mg L^{-1}, phosphorus 0.01-0.84 mg L^{-1}, potassium 1.28-5.94 mg L^{-1}, total dissolved solid 42.67-183.67 mg L^{-1}, dissolved oxygen 3.23-4.47 mg L^{-1}, pH 7.17-7.95, temperature 27.53-28.27°C, absolute growth of weight 14.40 g, absolute growth of length 7.44 cm, survival rate 89%, catfish production 1,394.58 g m^{-3} and total weight of lettuce plants 88 g.

Keywords: *Azolla sp.; Liquid Organic Fertilizer; Pangasius.*

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I. INTRODUCTION

A technological innovation applicable to catfish cultivation using swamp water as a culture medium is the aquaponics system. Aquaponics is the integration of recirculating aquaculture and hydroponics in one production system [1, 2]. Aquaponic systems have been developing since the 1980s, one of which is the deep-water aquaponics system, also called the floating raft system [3]. This deep-water culture involves suspending plants in polystyrene sheets, with their roots hanging down into the water. This method is the most common for large commercial aquaponics growing one specific crop (typically lettuce, salad leaves, or basil) and is more suitable for mechanization [1]. Aquaponics using floating raft culture is an eco-friendly system for food production using aquaculture and hydroponics to cultivate fish and crops without soil [2]. Aquaponics floating raft succeeded in producing a higher yield of basil plants [4].

Principally, aquaponics uses the fish waste in aquaculture (nutrients such as nitrogen-containing ammonia) as the food for plants grown in water (hydroponics) [3]. Swamp fish pond has a problem related to submerged flat soil and water pH value, resulting in low productivity of the pond [1]. Low pH is associated with low productivity and biodiversity (Boyd, 2020). The low productivity of swamp water results in insufficient nutrient availability, which can be overcome by fertilization. Fertilization of aquaculture ponds increases the productivity of phytoplankton, which is the food base of zooplankton and benthic animals, as a food source for fish and crustaceans. Organic fertilizer will refer to livestock excrement, agricultural waste, and by-products [5]. The liquid organic fertilizer was used in aquaculture ponds. Azolla is one of the aquatic plants that can potentially be used as a material for liquid organic fertilizer. Azolla pinnata contains almost all essential amino acids, minerals such as iron, calcium, magnesium, potassium, phosphorus, manganese, etc., apart from appreciable quantities of vitamin A precursor beta-carotene and vitamin B12 [6]. Liquid organic fertilizer from 25% Azolla and 25% beef bone meal contains C-organic 18.7%, N-total 0.51%, P-total 1.23%, and K-total 0.26% [7]. This study aims to determine the best dose of Azolla sp. liquid organic fertilizer on the limed swamp water for the culture of pangasius catfish and lettuce plants using an aquaponic floating raft system.

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II. MATERIALS AND METHODS

➢ *Experimental Design*

The experimental design used was a completely randomized design with four treatments and three replications. The treatments consist of different concentrations of Azolla sp. liquid organic fertilizer (LOF): 5 mL L^{-1} (P_1), 10 mL L^{-1} (P^2), 15 mL L^{-1} (P_3), and 5 mL L^{-1} (P_4).

➢ *Research Procedures*

Culture of fish using 12 concrete ponds measuring 1mx1mx1m. The ponds were cleaned and disinfected using potassium permanganate at a dose of 2.5 mg L^{-1} for 24 hours. At the top surface of the pond, 80cmx80cmx2cm styrofoam was placed, drilled with a 6 cm diameter hole, and plastic cups were installed as plant containers. Each styrofoam was filled with 16 plastic cups. The installed plastic cups were inserted into the fish culture water with the bottom of the cup submerged in water to a height of 5 cm. The center of the styrofoam was provided with a hole for the installation of an aerator hose. The catfish culture medium was taken from the reservoir ponds in the Aquaculture and Experimental Pond Laboratory, Aquaculture Study Program, Department of Fisheries, Faculty of Agriculture, Sriwijaya University. Before use, the water was first incubated for 1 day and limed with dolomite at a dose of 37.5 mg L^{-1}. The water in a culture pond with a water level of 70 cm was then incubated for 10 days

Liquid organic fertilizer is made from 15 kg of fresh *Azolla* sp., which was ground, then mixed with 6 kg of brown sugar, 15 L of water, and 600 mL of commercial probiotics. Fermentation was carried out for 14 days. Lettuce seeds were sown in a rockwool growing medium. The sowing process lasted for 23 days, and the lettuce seeds were transferred into a rearing pond containing fish. Catfish were stocked with a stocking density of 100 fish per m^{-3} [8].

The fish and lettuce were reared for 42 days. During the rearing period, the fish were fed commercial feed with a protein content of 39-41%. Feeding was done three times a day at satiation. Probiotics commercials were given every 14 days at a dose of 0.015 mL L^{-1}. The fish and plants were harvested at the end of the rearing period.

Observed parameters, including water quality parameters, consist of ammonia, nitrate, phosphorus, potassium, total dissolved solids, dissolved oxygen, pH, and temperature; growth performance, survival, and fish production; and total harvest of lettuce plants.

The data on water quality (ammonia, total dissolved solids, dissolved oxygen, and pH), growth performance, survival rate, fish production, and total harvest of lettuce were statistically analyzed using analysis of variance to detect if there was any significant effect due to experimental treatments. Post-hoc comparisons were performed using the Least Significant Difference test at a significance level of 0.05. Data on nitrate, phosphorus, and potassium at the initial and final stages of culture were analyzed descriptively. Data temperatures were analyzed using a T-test.

III. RESULTS AND DISCUSSIONS

➢ *Water Quality*

Analysis of variance results indicate that the varying dosages of *Azolla* sp. LOF had no significant effect on water ammonia levels during the 42-day rearing period. Ammonia levels increased by day 42 (Table 1). While these levels exceeded the limit established by the National Standardization Agency [9], which is <0.01 mg L^{-1}, they remained below the threshold set by the Government of the Republic of Indonesia [10]. For freshwater fish farming, which is 0.5 mg L^{-1}.

Table 1 Ammonia

Treatments	Ammonia (mgL^{-1})	
	Day-0	Day-42
P_1	0.027±0.001	0.104±0.008
P_2	0.026±0.001	0.099±0.004
P_3	0.027±0.001	0.098±0.014
P_4	0.026±0.002	0.102±0.004

The water ammonia and nitrate values presented in Tables 1 and 2 indicate that levels for both parameters increased across all treatments by the 42^{nd} day of the rearing period. This rise in ammonia and nitrate content is attributed to the *Azolla* sp. LOF applied during the rearing process contains 0.03% nitrogen (N). In addition to the fertilizer application, the increase in ammonia levels stemmed from fish metabolic byproducts and uneaten feed remaining in the rearing pond. Reference [11] noted that elevated ammonia concentrations result from fish metabolic waste (feces) and uneaten feed that settles on the pond bottom.

Treatments involving the application of *Azolla* sp. LOF at dosages of 10 mL L^{-1} (P_2), 15 mL L^{-1} (P_3), and 20 mL L^{-1} (P_4) showed a trend where nitrate levels increased as the dosage increased. However, a very high nitrate value was observed with the dosage 5 mL L^{-1} (P_1). The nitrification process, which converts total ammonia nitrogen into nitrate and nitrite, proceeds more efficiently when oxygen availability is relatively higher [12]. Nevertheless, the nitrate levels recorded during the rearing period remained within the limits for fish culture established by the Government of the Republic of Indonesia [10], which set a maximum threshold of 20 mg L^{-1}. Reference [1] recommends maintaining nitrate levels in aquaponic systems between 5 and 150 mg L^{-1} and performing water exchanges when levels exceed this range.

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Table 2 Nitrate, Phosphorus, Pottasium

Treatments	Nitrate (mgL^{-1})		Phosphorus (mgL^{-1})		Pottassium (mgL^{-1})	
	Day-0	Day-42	Day-0	Day-42	Day-0	Day-42
P_1	2.823	7.968	0.01	0.84	1.28	5.94
P_2	2.917	3.504	0.10	0.90	1.27	6.84
P_3	2.730	6.160	0.08	0.86	1.49	9.60
P_4	2.411	6.350	0.11	0.64	1.39	8.64

The water phosphorus (P) values shown in Table 2 indicate an increase across all treatments by the 42^{nd} day of the rearing period. This rise in water phosphorus content is attributed to the *Azolla* sp. LOF applied during the study, which contains 0.015% phosphorus. Phosphorus levels in natural waters are typically lower than nitrogen levels because phosphorus sources in aquatic environments are less abundant than nitrogen sources [13]. Furthermore, low water phosphorus levels are also due to the rapid absorption of phosphorus from the water by plankton [14]. The phosphorus levels recorded during the rearing period remained within the water quality standards established for fish culture. According to the water quality standards set by Government of the Republic of Indonesia [10]. Phosphorus content in water should be less than 1.0 mg L^{-1}. Phosphorus is essential for stimulating root development, particularly in seedlings and young plants, and can also accelerate flowering as well as seed and fruit maturation [15].

The water potassium (K) values presented in Table 2 indicate an increase across all treatments by the 42^{nd} day of the rearing period. This rise in water K levels is attributed to the *Azolla* sp. LOF was added during the study, which contains 0.28% potassium. Water K levels tended to rise further as the dosage of LOF increased. Potassium is an essential element for the growth of both plants and animals [13]. It is vital for aquatic organisms, as it aids in maintaining body fluids and electrolytes, preserving cellular balance, and regulating blood pressure [16]. Plants require potassium to strengthen their structure, thereby preventing lodging (toppling over) and the premature shedding of flowers and fruit [15]. Potassium nutrient concentrations in an aquaponic system featuring a tilapia density of 25 kg m^{-3} and 200 g fresh-weight lettuce plants reared for 30 days ranged from 3 to 14 mg L^{-1} [17].

Analysis of variance results indicate that the varying dosages of LOF significantly affected the Total Dissolved Solids (TDS) of the culture medium water on days 0 and 42 of the culture periods (Table 3). The LSD test results in Table 3 indicate that on day 0 of the rearing period, the water TDS value for the treatment involving the application of *Azolla* sp. LOF at a dose of 15 mL L^{-1} (P_3) was significantly higher than that of treatments P_1 and P_2, though not significantly different from P_4. On day 42, the water TDS value for the treatment with a POC dose of 20 mL L^{-1} (P_4) was significantly higher than those of the other treatments. These results show that TDS values increased in line with the dosage of LOF applied. The rise in TDS values during the rearing period is attributed to the application of LOF. Additionally, uneaten feed and fish metabolic waste (feces) can also increase the TDS of the rearing water. The TDS values increase after fertilizer is dissolved in water [18]. Reference [19] suggests that high TDS values can result from increased fish activity associated with growth and development, as well as the influence of uneaten feed and fish metabolic waste. The TDS values recorded during the rearing period remained within the freshwater quality standard set by the Government of the Republic of Indonesia [10], which specifies a maximum limit of 1,000 mg L^{-1}.

Table 3 Total Dissolved Solid

Treatments	Total Dissolved Solid (mgL^{-1})	
	Day-0	Day-42
P_1	$42.67^{ab} \pm 2.52$	$183.67^{a} \pm 4.16$
P_2	$40.67^{a} \pm 4.51$	$214.67^{b} \pm 4.16$
P_3	$50.67^{c} \pm 3.51$	$238.00^{c} \pm 2.00$
P_4	$49.00^{bc} \pm 3.00$	$252.00^{d} \pm 4.00$
$LSD_{\alpha 0.05}$	6.52	6.96

Numbers followed by different superscript letters in the same columns indicate significant differences at $LSD_{\alpha\ 0.05}$.

Analysis of variance results indicate that the treatments involving different dosages of *Azolla* sp. liquid organic fertilizer did not significantly affect dissolved oxygen levels on day 0 of the rearing period, but did have a significant effect on day 42. Dissolved oxygen values and the results of the LSD test are presented in Table 4.

Table 4 Dissolved Oxygen

Treatments	Dissolved Oxygen (mgL^{-1})	
	Day-0	Day-42
P_1	4.47 ± 0.06	$3.23^{b} \pm 0.06$
P_2	4.47 ± 0.21	$3.10^{ab} \pm 0.17$
P_3	4.40 ± 0.10	$2.93^{a} \pm 0.06$

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P_4	4.50 ± 0.10	2.90^{a} ± 0.10
LSD□0.05		0.20

Numbers followed by different superscript letters in the same columns indicate significant differences at $LSD_{\alpha\ 0.05}$.

The LSD test results in Table 4 indicate that on day 42, the dissolved oxygen level in the P_1 treatment (5 mL L^{-1} *Azolla* sp. LOF) was significantly higher than in treatments P_3 and P_4, but not significantly different from P_2. Dissolved oxygen levels tended to decrease as the dosage of LOF increased and also tended to decline by day 42 of the rearing period. LOF contains organic matter that undergoes nutrient decomposition [20]. A drop in dissolved oxygen concentration can be attributed to factors such as aquatic organism respiration and the decomposition of organic matter [21]. Overpopulation of aquatic organisms and excessive fertilization of aquatic plants can both contribute to reduced dissolved oxygen levels in the water [22]. The dissolved oxygen levels recorded on day 42 of the rearing period were lower than the standard established by the National Standardization Agency [9] for Siamese catfish *Pangasianodon hypophthalmus* culture specifically >4 mg L^{-1}; nevertheless, the fish tolerated these levels well, resulting in satisfactory survival rates (Table 7).

The initial pH of the floodplain swamp water was 4.6; however, after a 10-day incubation period, the water pH across all treatments reached levels optimal for Pangasius fish, ranging from 7.95 to 8.10 (Table 5). According to the National Standardization Agency [9], the optimal pH range for rearing Siamese pangasius catfish is 6.5–8.5. Analysis of variance results indicate that the treatments involving different dosages of *Azolla* sp. LOF did not significantly affect the rearing water pH on day 0, but had a significant effect on the water pH on day 42 of the rearing period. The pH values and the results of the LSD test at the 0.05 level are presented in Table 5.

Table 5 pH

Treatments	pH (Incubated Periods)	pH (Culture Period)	
		Day-0	Day-42
P_1	7.55-7.95	7.95 ± 0.10	7.17^{d} ± 0.03
P_2	7.65-8.10	8.02 ± 0.19	7.00^{c} ± 0.05
P_3	7.65-8.10	8.08 ± 0.13	6.82^{b} ± 0.06
P_4	7.55-7.95	7.90 ± 0.09	6.65^{a} ± 0.05
LSD□0.05			0.10

Numbers followed by different superscript letters in the same columns indicate significant differences at $LSD_{\alpha\ 0.05}$.

On day 42, the pH of the culture water in the treatment receiving Azolla sp. LOF at a dosage of 5 mL L^{-1} (P_1) was significantly higher than that of the other treatments. The pH value tended to decrease as the dosage of LOF increased and also tended to decline by day 42 of the culture period. This is likely due to the acidic nature of the LOF; consequently, continuous application at increasing dosages and higher quantities can lower the culture water pH, although the levels remained within the optimal range. Reference [18] noted that fertilizer addition can drastically alter water pH, with higher amounts of fertilizer resulting in lower pH levels. Nevertheless, the pH values throughout the culture period remained within the tolerance limits for rearing Pangasius catfish.

The t-test results in Table 6 indicate that the water temperature during the morning was significantly lower than during the day. Water temperatures throughout the rearing period ranged from 27.53°C to 28.63°C. These values remain within the tolerable range for pangasius catfish; according to the National Standardization Agency [9], the suitable range is 25–30°C. This is because the study was conducted outdoors, meaning water temperature was influenced by variations in sunlight intensity between the morning and the day. Sunlight entering the water undergoes absorption and is converted into heat energy) [13].

Tabel 6 Temperature

Treatments	Temperaturev (°C)	
	Morning	Mid day
P_1	27.53 ± 0.12^{a}	28.27 ± 0.15^{b}
P_2	27.77 ± 0.38^{a}	28.63 ± 0.49^{b}
P_3	27.53 ± 0.06^{a}	28.33 ± 0.12^{b}
P_4	27.53 ± 0.23^{a}	28.37 ± 0.29^{b}

Numbers followed by different superscript letters in the same row indicate significant differences based on a t-test at the 5% level.

- *Fish Growth, Survival and Production*

Analysis of variance results indicate that varying dosages of *Azolla* sp. LOF significantly affected absolute weight and length growth, fish survival, and pangasius catfish production during the rearing period.

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Table 7 Fish Growth, Survival and Production

Treatments	Absolute Growth		Survival (%)	Fish Production ($g\ m^{-3}$)
	Weight (g)	Length (cm)		
P_1	$14.40^b \pm 0.82$	$7.44^b \pm 0.09$	$89.00^b \pm 4.58$	$1,394.58^{bc} \pm 9.13$
P_2	$14.31^b \pm 0.36$	$7.41^b \pm 0.10$	$96.67^c \pm 3.51$	$1,509.82^c \pm 7.45$
P_3	$13.58^b \pm 0.89$	$7.24^b \pm 0.17$	$90.00^{bc} \pm 3.00$	$1,336.93^b \pm 4.90$
P_4	$10.64^a \pm 0.34$	$6.04^a \pm 0.13$	$78.67^a \pm 4.51$	$931.87^a \pm 6.25$
$BNT_{0,05}$	1.23	0.23	7.45	133.78

Numbers followed by different superscript letters in the same columns indicate significant differences at $LSD_{\alpha\ 0.05}$

The LSD test results in Table 7 indicate that the absolute weight and length growth of Pangasius catfish treated with *Azolla* sp. LOF at a dosage of 5 mL L^{-1} (P_1) was significantly higher than that observed at a dosage of 20 mL L^{-1} (P_4), though they did not differ significantly from treatments P_2 and P_3. Survival rates for Pangasius catfish treated with *Azolla* sp. LOF at 10 mL L^{-1} (P_2) was significantly higher than those in treatments P_1 and P_4, but did not differ significantly from treatment P_3. The observed survival rates were high, ranging from 78.67% to 96.67%. LOF serves not only as a food source for plankton but also plays a crucial role in fish health, given its capacity to regulate water quality parameters [23]. Fish survival is influenced by the quality of the rearing water, with the application of *Azolla* sp. LOF at a dosage of 10 mL L^{-1} (P_2) showed relatively better water quality, specifically regarding ammonia and dissolved oxygen levels, compared to the other treatments. Survival rates are influenced by abiotic and biotic factors, including the organism's ability to adapt to its environment [24].

The results of the LSD test presented in Table 7 indicate that pangasius catfish production with the application of Azolla sp. LOF at a dosage of 10 mL L^{-1} (P_2) was significantly higher than that observed in treatments P_3 and P_4, though not significantly different from P_1. This suggests that increasing the Azolla sp. LOF dosage up to 10 mL L^{-1} remains effective, whereas further increases in dosage result in reduced fish production.

- *Total Harvest Weight of Lettuce Plants*

Analysis of variance results indicate that the varying dosage treatments of Azolla sp. LOF had a significant effect on the total harvest weight of lettuce plants. The LSD test results in Table 9 indicate that the total harvest weight of lettuce plants treated with *Azolla* sp. LOF at a dosage of 5 mL L^{-1} (P_1) was significantly higher than that of the other treatments. This high total harvest weight is attributed to the application of Azolla sp. LOF provides the nutrients necessary to support lettuce growth. Supplying fertilizers containing N, P, and K along with utilizing fish waste as a supplementary fertilizer can effectively support plant growth by meeting nutrient requirements [25].

Table 9 Total Harvest Weight of Lettuce Plants

Treatments	Total Harvest Weight (g)
P_1	$88,00^c \pm 2,00$
P_2	$25,00^a \pm 3,00$
P_3	$34,00^b \pm 2,00$
P_4	$34,67^b \pm 2,08$
$BNT_{0,05}$	4,35

Numbers followed by different superscript letters in the same columns indicate significant differences iat $LSD_{\alpha\ 0.05}$

Applying *Azolla* sp. LOF at dosages of 10, 15, and 20 mL L^{-1} (P_2, P_3, and P_4) did not result in a higher total harvest weight compared to the 5 mL L^{-1} dosage (P_1). It is hypothesized that the 5 mL L^{-1} dosage (P_1) had already reached the plant's optimal requirement level; consequently, increasing the dosage further did not affect crop yield. This aligns with the findings that noted that while the dosage of fertilizer applied influences nutrient availability, a higher dosage does not guarantee increased plant growth, as plants have a limit to the amount of nutrients they can absorb for their survival and development) [26].

IV. CONCLUSIONS

The application of Azolla sp. liquid organic fertilizer at a dosage of 5 mL L^{-1} (P_1) proved to be the optimal rate, providing sufficient nutrients and maintaining water quality conducive to the growth, survival, and production of Pangasius catfish, as well as the yield of lettuce, in an aquaponic system.

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