Effects of Liquid Cow Manure Compost Content in Azolla Cultivation on Water Quality, Yield, Chemical Properties and Nutritional Contents

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Abstract

Azolla, a fast-growing aquatic fern, is an inexpensive source of biomass for biofertilizer, livestock feed, phytoremediation and biofuel production applications. The use of compost-based fertilizer to improve the cultivation of azolla can reduce production costs and enhance sustainability. While previous studies investigated the use of organic fertilizers for azolla growth, little work has been done to compare such use with chemical fertilizer use. In this work, the effects of liquid cow manure compost fertilizer, compared to urea fertilizer, on azolla growth, yield, chemical and nutritional compositions and water quality were studied.

The results revealed that the optimal application of compost fertilizer enhanced azolla growth and yield greater than that of urea fertilizer. The compost also improved nitrogen, phosphate, potash and protein content in azolla. Azolla treated with the liquid compost fertilizer could effectively remove phosphate from water, but the addition of the compost increased both phosphate and ammonia content and reduced dissolved oxygen. Thus, the liquid cow manure compost fertilizer was suitable for promoting azolla growth for livestock feed and biofuel applications rather than water treatment.

Keywords: Liquid cow manure compost, Water quality, Nutritional contents, Azolla.

Introduction

Azolla is an aquatic fern comprising seven living species in the *Salviniaceae* family and is commonly found in freshwater sources in Asia, Africa and the America.[19] Only *Azolla pinnata* has been found and cultivated in Thailand.[17]

Due to its capability to rapidly reproduce and grow and its rich amino acid and mineral profiles, azolla is considered a valuable and inexpensive source of biomass for various applications.[27] *Anabaena azollae*, a cyanobacterium, forms a symbiotic relationship with azolla and enables efficient nitrogen fixation.[22] Consequently, azolla has been widely used as a biofertilizer, especially in rice fields in southeast Asia.[25,26] Because of its high nutritional values, azolla has been proposed as a sustainable livestock feed.[7,11] Azolla can be cultivated in wastewater and its phytoremediation capabilities to remove various contaminants such as phosphate, zinc and petroleum hydrocarbons have been demonstrated.[10,12,20]

Azolla biomass also possesses a high quantity of energy-dense materials including cellulose, hemicellulose and lipids and therefore the use of azolla as biofuel has received significance.[13,15] Furthermore, an extensive carbon dioxide sequestration scheme by azolla has also been proposed.[8]

The cultivation of azolla is a paramount factor in determining the viability of various azolla applications. Azolla yield provided by appropriate growth methods should meet the increasing demands. Due to the superior intrinsic growth capability of azolla, studies on optimal growth methods and their relation to azolla quality have been conducted.[6,23] Studies that employ close containers can reveal optimal conditions. The use of organic matter such as manure and compost, in azolla cultivation can further improve sustainability and reduce production costs. Adzman et al[1] investigated the growth and nutrient content of azolla cultivated in open tanks exposed to direct sunlight in Malaysia.

Water depth, compost weight, water pH and light exposure time were varied. The results revealed that the azolla growth rate increased as the water depth and sunlight exposure increased. The growth was most favorable at a pH of 7 and a nutrient concentration of approximately 800 ppm. Cultivated in these optimal conditions, azolla possessed a high content of fat, fiber and proteins, which are suitable for biofuel and livestock feed applications. Similarly, Golzary et al[6] found that the optimal conditions for azolla growth were 22 °C, light intensity of 20 lux, 75% relative humidity and a pH of 6.4.

Zakarya et al[27] studied azolla production in plastic containers filled with nutrient-rich water and different types and amounts of fertilizers: commercial liquid fertilizer, cow dung, food waste compost and mushroom block fertilizer. After measuring the yield and chemical compositions of azolla, the authors concluded that cow dung was the most suitable for promoting azolla growth, while commercial liquid fertilizer was the least effective.

https://doi.org/10.25303/209rjbt2000205

The use of liquid cow manure compost in azolla cultivation and its performance relative to chemical fertilizers have not been thoroughly investigated. Cow manure compost is one of the most accessible and widely used organic fertilizers in Thailand.[24] Effective incorporation of cow manure compost would promote sustainable azolla cultivation among Thai farmers. Therefore, in this work, liquid cow manure compost was prepared and used in azolla production in various quantities. Water quality and azolla yields, chemical compositions and nutritional contents were monitored. The compost's performance was then compared to that of chemical fertilizer. Finally, the recommended liquid cow manure compost usage level was thus provided.

Material and Methods

To study the effects of liquid cow manure compost (LC), LC was first prepared by mixing 30 kg of dry cow manure, 3 kg of molasses, 1 kg of rice bran powder and 66 kg of water in a 100-liter opaque plastic container. The container was sealed and the composting process proceeded for 30 days. LC was sent to the Science Center at Valaya Alongkorn Rajabhat University for analysis. The properties of LC and the analytical method are displayed in table 1.

A completely randomized design was used in the experiment to investigate the effects of the use of LC in the production of azolla. The experiment proceeded from January to March 2024. Azollas were cultivated in 18 ponds, each with a width, length and height of 50, 100 and 30 cm respectively. Groundwater was used as a growth medium and its depth was set to 20 cm. Before introducing azolla plants, the groundwater was treated with six methods, each with three replications. The treatments were no addition (#1, control), an addition of 2.1 g of 46% urea fertilizer per 1 liter of water (#2) and an addition of 10 (#3), 15 (#4), 20 (#5) and 25 (#6) g of LC per 1 liter of water.

One hour after the treatment, 15 g of azolla plants were transferred to each pond. After 10 days of growth, the azollas were collected. The experiment was then repeated three more times. Water, prior to azolla transfer and after 10 days of azolla growth, was collected and sent to the Science Center at Valaya Alongkorn Rajabhat University so that its pH, dissolved oxygen (DO), electrical conductivity (EC), phosphate content and ammonia content were quantified. Air and water temperatures were also monitored during the experiment.

The fresh weight of the collected azollas was measured. Subsequently, the azollas were dried in an air oven at 70 ºC for 48 hours and then weighed. Dried azollas were suspended in water at a 1:10 dilution ratio and the suspension's pH and electrical conductivity were measured according to the methods described in table 1. The organic matter, total nitrogen content, total phosphate content, total potash content and carbon-to-nitrogen ratio of dried azollas were also quantified via methods according to table 1.

The nutritional contents of the dried azollas were analyzed as follows: the humidity, protein, fat, fiber and ash contents of the dried azollas were quantified via standard proximate analysis. The amount of calcium, magnesium, manganese and iron in dried azolla was determined by inductively coupled plasma-emission spectrometry (ICP-ES). Statistical analysis of the collected data was done via One-way ANOVA with the least significant difference (LSD) method. Pearson correlation coefficients between selected dependent variables that are significantly influenced by the treatments, were also calculated.

Results and Discussion

Azolla yields and chemical properties after 10 days of cultivation under different treatments are shown in table 2. The fresh and dry weights of azolla under treatments with urea and liquid compost were significantly higher than those of the control group. The treatments with 10 g/L of LC and urea fertilizer led to similar fresh and dry weights. As the concentration of LC increased, fresh and dry weights rose as well. Azolla's electrical conductivity and nitrogen content followed a similar trend. Regarding phosphate content, the urea treatment showed similar results to the control and all LC treatments led to higher phosphate content than the urea treatment.

Potash content in azolla under the urea treatment was similar to that under the control and the 10 g/L LC treatment and the 25 g/L LC treatment led to the highest potash content. Azolla's carbon-to-nitrogen ratio was the lowest under the treatment with urea and the highest under the treatment with 25 g/L LC. No treatment has effects on the pH or organic matter of azolla. These results revealed that the addition of LC could improve both the fresh and dry weight of azolla more than the use of urea fertilizer if enough LC was used.

Table 1
Analysis of liquid cow manure compost.

Compost properties	Values	Methods
pH	6.1	pH meter
Electrical conductivity (mS/cm)	4.7	Conductivity meter
Organic matter (%)	13.11	Walkley and Black
Total nitrogen content (%)	9.56	Kjeldahl method
Total phosphate content (%)	8.34	Colorimetric method
Total potash content (%)	10.93	Flame photometric method
Carbon-to-nitrogen ratio	12.05	Walkley and Black and Kjeldahl method
Microorganism count (cfu)	104	Standard plate count

Referring to the chemical properties of LC shown in table 1, the urea treatment and the 10 g/L LC treatment had a similar content of nitrogen. This is in agreement with the equal values of fresh and dry weight and nitrogen content of azolla under the urea and 10 g/L LC treatments. Additionally, as more nitrogen was added via increasing LC concentrations, the weight increased. A similar trend was also observed by Zakarya et al[27] where higher application rates of organic fertilizer led to a higher azolla growth rate. These results suggest that the careful application of nitrogen-providing fertilizers remains important to azolla cultivation despite the outstanding nitrogen fixation capabilities of azolla.

To further elucidate the effects and mechanisms of azolla growth promotion via the applications of urea fertilizer and LC, water quality before and after azolla cultivation was investigated as shown in table 3. Prior to the cultivation of azolla, the treatment had no significant effects on dissolved oxygen, which was due to every treatment using the same source of water. The higher amount of LC decreased the pH due to the acidity of LC. The pH values were in the range suitable for azolla growth as reported in other works[1,6,12] and thus might not be the influencing factors. On the other hand, phosphate and ammonium contents were significantly impacted by the treatments. There was no difference in the phosphate concentration in the water subjected to the control and urea treatments.

The addition of LC increased the phosphate level higher than the addition of the urea fertilizer and a higher LC amount led to greater phosphate concentration. This was due to the high phosphate content of the LC as prepared. The urea treatment and the 10 g/L and 15 g/L LC treatments led to equal ammonia concentrations in water prior to azolla cultivation, which was also greater than the control, while greater applications of LC led to higher ammonia concentrations. The increase in the electrical conductivity of water before cultivation was due to the increase in phosphate and ammonia concentrations. Correlation analysis, shown in figure 1, revealed a significant positive correlation between phosphate and ammonia content and fresh and dry azolla weight. Additionally, these factors correlated well with azolla N and P content, implying that azolla has used and internalized nutrients provided by the treatments. Therefore, these data suggested that phosphate and ammonia provided by the treatments were beneficial to azolla growth and the appropriate use of LC can improve azolla yield when compared to the use of urea fertilizer.

Azolla has been used as a phytoremediator for wastewater. The effects of azolla growth and different conditions on water quality are shown in table 3 and the correlation between azolla yield and water quality after cultivation is displayed in figure 2. For all treatments, a decrease in dissolved oxygen after 10 days of azolla growth was observed. Higher application rates of LC and greater azolla growth also led to further reductions in DO. While azolla can be used to improve water DO,[5] excessive growth, such as in the case of azolla bloom, can reduce water DO levels.[14,18] Dense layers of azolla can hinder the photosynthesis of other submerged plants and algae and impede oxygen diffusion, leading to a reduction in water DO.[2,9] In our work, azolla cultivation effectively removed water phosphate content after 10 days for all treatments.

This result was in concurrence with other reports[12,20] which revealed that azolla could utilize phosphate contents in water as a nutrient. However, in our experiments, the weight of azolla positively correlated with water phosphate concentration after cultivation, but this was simply because azolla weight also positively correlated with the amount of applied LC, which contained significant levels of phosphate. Additionally, this positive correlation was also true for ammonia content, but an increase in ammonia content after 10 days was also observed. This was likely because azolla can fix nitrogen and was less reliable on nitrogen content in water provided by the urea and LC treatments.[23] For these reasons, the promotion of azolla growth for water treatment applications via both urea and LC treatments should be carefully utilized.

Table 2
Azolla yields and chemical properties after 10 days of cultivation.

Treatment	Fresh weight (g/m^2)	Dry weight (g/m^2)	pH	EC (mS/cm)	N (%)	P (%)	K (%)	C/N	OM (%)
control	615.19e	52.14c	7.26	2.17d	2.08e	0.12e	3.90c	10.24d	75.01
urea	834.11d	53.82c	7.51	2.37c	2.58d	0.17e	4.11bc	6.78e	74.95
10g/L LC	801.34d	53.78c	7.30	2.44c	2.61d	0.30d	4.28b	10.56d	75.02
15g/L LC	1,045.35c	55.33bc	7.48	2.91b	3.05c	0.61c	4.33b	12.55c	75.45
20g/L LC	1,530,44b	59.06b	7.46	3.33ab	3.90b	0.74b	4.92ab	14.69b	75.89
25g/L LC	1,798.76a	72.91a	7.59	3.50a	4.56a	0.86a	5.27a	15.91a	75.90
p-value	**	**	ns	**	**	**	**	**	ns
SEM	188.98	3.16	0.05	0.22	0.38	0.13	0.21	1.37	0.18
%C.V.	41.92	13.39	1.71	19.61	29.70	66.80	11.64	28.43	0.59

Note: LC: liquid compost fertilizer, EC: electrical conductivity, N: nitrogen content, P: phosphorous content, K: potassium content, C/N: carbon-to-nitrogen ratio, OM: organic matter, ns: not significant ($p > 0.05$), *: significant at $p < 0.05$, **: extremely significant at $p < 0.01$, a-e: significant differences, SEM: standard error of mean and %C.V.: coefficients of variance (%).

Table 3
Water quality subjected to different treatments before and after 10 days of the cultivation of azolla.

Treatment	Air T (ºC)		Water T (ºC)		pH		DO (mg/L)		EC (mS/cm)		P_2O_5 (mg/L)		NH_3(mg/L)	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A
control	32	33	29	31.24	7.98bc	7.33a	4.21	3.55a	1.04d	0.23c	1.20c	0.77c	0.12ab	1.23a
urea	32	33	29	31.31	8.02c	7.98b	4.34	3.14ab	1.62c	0.43c	1.22c	0.69c	1.13b	1.62b
10g/L LC	32	33	29	31.66	7.86b	7.54ab	4.33	3.34a	2.11bc	2.98b	1.56b	0.75c	1.03b	1.76bc
15g/L LC	32	33	29	31.01	7.55ab	7.41ab	4.57	3.02b	2.56b	3.13b	1.65ab	0.89b	1.22b	1.90bc
20g/L LC	32	33	29	31.49	7.21a	7.34a	4.19	2.06c	3.09ab	3.56ab	1.90a	0.96ab	1.78c	2.11c
25g/L LC	32	33	29	31.98	6.93a	7.12a	4.78	1.37d	3.68a	3.89a	2.04a	1.11a	2.09d	2.47d
p-value	-	-	-	ns	*	*	ns	**	**	**	**	**	**	**
SEM	-	-	-	0.14	0.18	0.12	0.09	0.35	0.48	0.66	0.14	0.06	0.28	0.17
%C.V.	-	-	-	1.09	5.87	3.92	5.20	30.87	54.04	68.09	21.57	18.16	55.38	22.95

Note: LC: liquid compost fertilizer, T: temperature, DO: dissolve oxygen, EC: electrical conductivity, P_2O_5: phosphate content, NH_3: ammonia content, B: before azolla cultivation, A: after 10 days of azolla cultivation, ns: not significant ($p > 0.05$), *: significant at $p < 0.05$, **: extremely significant at $p < 0.01$, a-d: significant differences, SEM: standard error of mean and %C.V.: coefficients of variance (%).

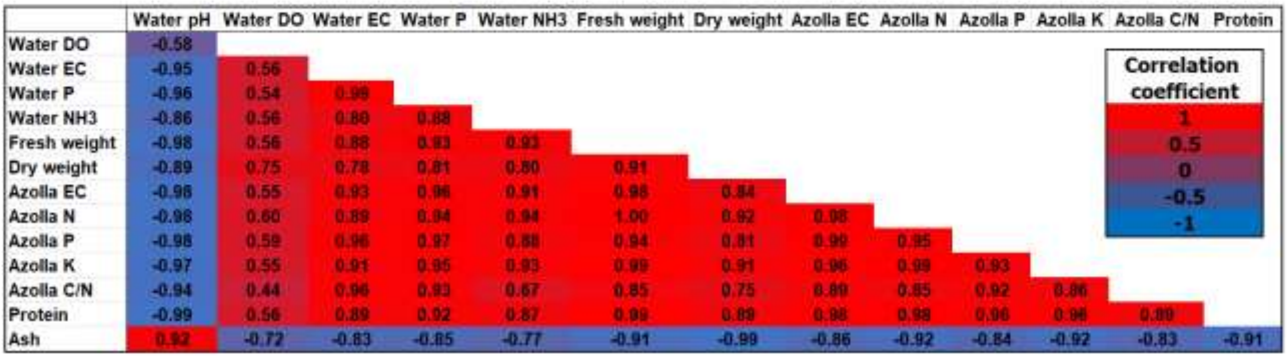

Figure 1: Pearson correlation coefficients between dissolved oxygen, electrical conductivity, phosphate content and ammonia content of water before cultivation and fresh and dry weight, electrical conductivity, nitrogen content, phosphate content, potash content, carbon-to-nitrogen ratio, protein content and ash content of azolla.

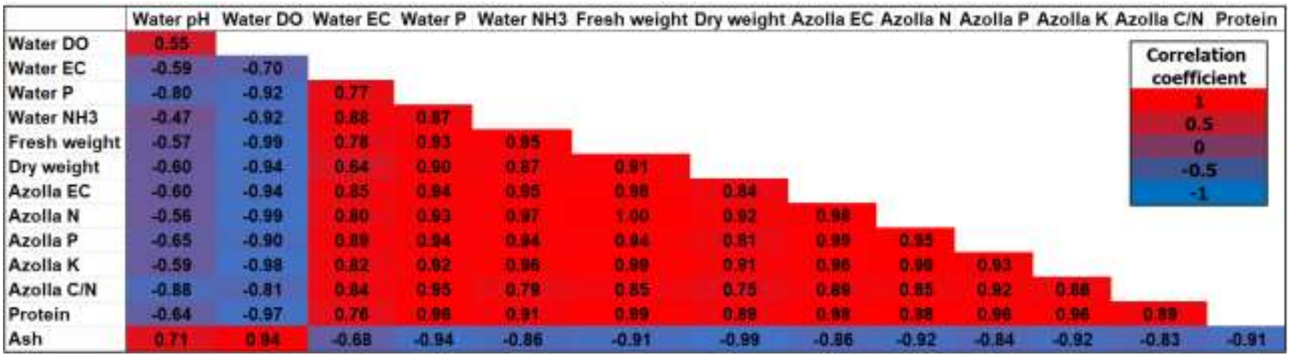

Figure 2: Pearson correlation coefficients between dissolved oxygen, electrical conductivity, phosphate content and ammonia content of water after cultivation and fresh and dry weight, electrical conductivity, nitrogen content, phosphate content, potash content, carbon-to-nitrogen ratio, protein content and ash content of azolla

Azolla nutritional profiles have significant implications for their use as agricultural feeds and biofuels. The nutrient contents of azolla after 10 days of growth are shown in table 4 and their correlations with other variables are displayed in figures 1 and 2. The values of moisture, protein, fat, fiber and ash contents were consistent with previously reported results.[1,3,16,21] The treatments had no significant effects on moisture, fat, fiber, calcium, magnesium, manganese, or iron content in dried azolla. Nevertheless, the use of urea fertilizer and LC had impacts on protein and ash contents.

Protein contents of azolla subjected to the control, urea and 10 g/L LC treatments were similar and the lowest, but protein contents were significantly augmented by higher levels of LC applications.

On the contrary, ash contents were highest under the control, urea, 10 g/L LC and 15 g/L LC treatments and decreased at higher LC levels. Higher protein contents were positively correlated with water ammonia and phosphate contents and thus azolla fresh and dry weight.

Table 4
Nutritional contents of azollas after 10 days of cultivation.

Treatment	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Ca (ppm)	Mg (ppm)	Mn (ppm)	Fe (ppm)
1, control	6.21	19.23c	3.03	10.29	18.33c	0.81	0.12	0.73	0.15
2, urea	6.30	19.56c	3.07	10.11	18.49c	0.83	0.19	0.78	0.19
3, 10g LC	6.27	19.30c	3.09	10.20	18.28c	0.89	0.16	0.73	0.16
4, 15g LC	6.34	21.21b	2.98	10.27	18.03c	0.81	0.16	0.75	0.16
5, 20g LC	6.48	23.02ab	3.06	10.30	17.56b	0.86	0.17	0.75	0.18
6, 25g LC	6.39	24.18a	3.00	10.32	16.11a	0.86	0.19	0.79	0.19
p-value	ns	**	ns	ns	*	ns	ns	ns	ns
SEM	0.04	0.86	0.02	0.03	0.36	0.01	0.01	0.01	0.01
%C.V.	1.50	10.01	1.40	0.77	5.00	3.80	15.69	3.32	10.03

Note: ns: not significant ($p > 0.05$), *: significant at $p < 0.05$, **: extremely significant at $p < 0.01$, a-c: significant differences, SEM: standard error of mean and %C.V.: coefficients of variance (%).

The results suggest that the LC treatments are effective at enhancing not only the yield but also the protein content of azolla. Because proteins are essential to livestock growth, the LC treatment might be beneficial to the use of azolla for livestock feed and supplements. Since fat is the core component of azolla used for biodiesel production[4,15], the use of LC to directly promote azolla use for this purpose may not be appropriate because the LC treatments did not increase the fat content. Nevertheless, azolla may still be effectively integrated into biofuel refineries, thanks to its high raw biomass, which was improved by the LC applications. Thus, the use of liquid compost fertilizer remains an accessible way to enhance azolla growth and yield for both livestock feed and biofuel applications.

Conclusion

The liquid compost fertilizer enhanced the growth and yield of azolla. When used in a higher amount, the liquid compost was more effective than the urea fertilizer. The liquid compost application also increased nitrogen content, phosphate content, potash content and carbon-to-nitrogen ratios. This azolla growth enhancement was due to the rich phosphorous and nitrogen profiles of the liquid compost fertilizer. While azolla could effectively remove phosphate from water, higher application rates of the liquid compost resulted in higher phosphate and ammonia levels and lower dissolved oxygen in the water.

Compared to the control and the urea treatment, the use of the liquid compost improved protein contents and reduced ash content in azolla. Thus, the liquid compost can be used to promote the application of azolla as livestock feed and in biofuel production. To evaluate this potential use, further work should explore the integration of azolla, subjected to liquid compost fertilizer treatment into livestock farms and biorefineries.

Acknowledgement

The authors appreciate Valaya Alongkorn Rajabhat University under the Royal Patronage for financially supporting this work and allowing the use of an open greenhouse for the experiment.

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(Received 22nd April 2024, accepted 26th June 2024)