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Culturing of Duckweed (*Lemna minor*) under Different Chicken Manure Concentration in the Lab

J. O. Ed-Idoko [a*], J. O. Apochi [b], M. O. Onah [a] and U. O. Ibrahim [c]

[a] Fisheries and Aquaculture Department, Federal University of Agriculture, Makurdi (Joseph Sarwuan Tarka University Makurdi), Benue State, Nigeria.
[b] Mediatrix Integrated Agricultural Limited, Abuja, Nigeria.
[c] Department of Fisheries and Aquaculture, Usman Danfodiyo University Sokoto, Sokoto State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The research on the Culturing of Duckweed (*Lemna minor*) plants under different chicken manure concentrations in the laboratory was conducted to determine relative growth rate of duckweed and to determine the physicochemical parameters of the experimental water. The experimental design was based on an assumption that duckweed spores are contained in the bottom of flood plain stagnant pools. The sprouting of duckweed (*Lemna minor*) was monitored under media chicken manure concentrations of 5g per 10 litre for treatment one. Treatment two was 7.5g per 10 litre of water. Treatment three, 10g per 10 litre of water, treatment four, 12.5g per 10lit of water, and for

**Corresponding author: Email: edidoko.john@gmail.com;*

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treatment five 15g per 10 litre of water was used. 75 litre plastic bowls were used for this experiment. Each bowl were inoculated with 100 pods of duckweed. Water quality in tanks treated with chicken manure and inoculated with duckweed shows that there was no significant difference in water quality across the treatments ($p>0.05$). However, the pH varied over the weeks of experimentation with increase in pH being observed from an initial value of 7.92 to a final value of 10.25 in week 4. There was a high percentage increase of 45% in DO for T2 while all other treatments recorded declines in DO. Each treatment was replicated, giving a total of 12 experimental containers including the control treatment. To every plastic container 100g of wet soil that was collected was introduced along with 10 liters of domestic water supply. The various chicken manure levels were weighed and randomly assigned to the experimental containers in replicate. Under favorable climatic conditions and nutrient balance in growth media, *Lemna minor* can double its biomass within seven days. The plot of numerical abundance of duckweed stems in the culture media revealed that duckweed mean levels in T2 (M=213.8000) was more than other treatments with significant difference at $P<0.05$. T5 (M= 83.6000) was least, and Control (M= 95.0000), during the experiment.

Keywords: Culture, numerical abundance; inoculate; biomass; flood plain; physiochemical parameters.

1. INTRODUCTION

1.1 Background of the Study

Water is one of the most important and abundant compounds of the ecosystem. All living organisms on the earth need water for their survival and growth. As of now only earth is the planet having about 70% of water. But due to increased human population, industrialization, use of fertilizers in agriculture and man-made activity, it is highly polluted with different harmful contaminants. Natural water contains different types of impurities which are introduced into aquatic system by different ways such as weathering of rocks and leaching of soils, dissolution of aerosol particles from the atmosphere and from several human activities, including mining, processing and the use of metal based materials. Industrial development (either new or existing), results in the generation of industrial effluents. And if untreated, results in water, sediment, and soil pollution (Fakayode and Oniawanwa, 2002).

Duckweed species are small floating aquatic plants found worldwide and often seen growing in thick, blanket-like mats on still, nutrient-rich fresh and brackish waters (Adhikari et al., 2015). They are monocotyledons belonging to the botanical family Lernaceae and are classified as higher plants or macrophytes, although they are often mistaken for algae. The family consists of four genera; Lenumn, Spirodela, Woiffia, and Wolffiella, among which about 40 species have been identified so far (Buddhavarapu and Hancock, 1991). These species are important for aquaculture as live phytoplankton food for fish and for fish feed production. All species occasionally produce tiny, almost invisible flowers and seed but what triggers flowering is unknown. Many species of duckweed cope with low temperatures by forming a special starchy "survival" frond known as a turion. With cold weather, the turion forms and sinks to the bottom of the pond where it remains dormant until rising temperatures in the spring trigger resumption of normal growth (Zhao et al., 2014).

1.2 Justification

Duckweed because of their increasing importance in animal feeds are sought out from the wild. As yield from the wild is of unpredictable quality and may not meet the requirements of animal, the surest and most reliable source of supply, therefore, it is to establish their growth condition right from spore stage to mature colonies.

This work is further justified by the need to grow the plant all the year round using cheap locally available materials without necessary waiting on the yearly emergence of wild colonies.

1.3 Objectives of the Study

i. To determine relative growth rate of duckweed.
il. To determine the physicochemical parameters of the water.

2. MATERIALS AND METHODS

2.1 Experimental Condition

The experiment involving the influence of varying chicken manure level on the growth of duckweed

was carried out in the Fisheries Laboratory of the Joseph Sarwuan Tarka University Makurdi.

2.2 Source of Manure

The chicken manure used was obtained from a near by poultry house for use. It was then sun dried and air dried, for 1week and stored in a sealed bag until when required.

2.3 Source of Duckweed

Soil collected from a flood plain area along Ankpa ward, Makurdi, which previously supports a wild duckweed culture was used as source of duckweed spore. The flood plain contains a stagnant pool which is usually filled with water duckweed every year during the rainy season and dries up completely between February to April of every year.

2.4 Methodology

The experimental design was based on an assumption that Duckweed spores are contained in the bottom of the flood plain stagnant pool. The sprouting of Duckweed (*Lemna minor*), was monitored in 12 experimental containers of 75 litres Plastic bowls, under media chicken manure concentrations of 5g per 10litres of water, for Treatment One. Treatment Two was 7.5g per 1Olitres of water was used. For Treatment Three, 10g per 10 litres of water was used. For Treatment Four, 12.5g per 1Olitres of water. For Treatment Five, 15g per 10litres of water was used. Each bowl was inoculated with 100 pods of Duckweed.

To every plastic container, 100g of wet loamy-clay soil that was collected and 10liters each of domestic water supply were introduced. The various Chicken manure levels was weighed and randomly assigned to the experimental containers in replicate. The Control Treatment was also acarried in replicates, which was similar to those at treatment levels but without manure. The cultures were examined at about 12-1pm daily and visible Duckweed colonies were removed and counted for all treatments. The water temperature was determined at both Control and Treatment levels using a thermometer (Jenway 9015) and pH meter (C14WPA). The various manure concentration of treatments were replenished on the seventh day and the experiment were terminated after four weeks (28 days), when no further increase in Duckweed sprouting was observed.

2.5 Data Analysis

The data collected and analyzed for ANOVA with an aid of statistical analytical programme (SPSS) and treatment means were separated with Duncan Multiple Range Test and Post- hoc comparisons using the Turkey HSD test. P values at <0.05 was considered significant.

3. RESULTS

3.1 Duckweed Biomass and Growth Rate

A one-way ANOVA was conducted to compare the effect of treatment on the outcome variable across different treatment groups for four Weeks. There was a significant effect of treatment on the outcome at the p<.005 level for the five conditions, F (5, X) = 41.881, p=.000.

Post- hoc comparisons using the Turkey HSD test indicated that the mean score for the highest treatment value T2 (M=213.8000) was significantly different from the lowest treatment value T5 (M= 83.6000), with Control (M= 95.0000), and other treatment groups specifically:

T2 vs. Control: MD = -147.67, SE = 12.65, p<.005.
T2 vs. T3: MD = 84.67, SE = 12.65, p<.005.
T2 vs. T4: MD = 131.33, SE =12.65, p<.005.
T2 vs. T5: MD = 157.33, SE = 12.65, p<.005.

Treatment 2 both in Week 3 and Week 4 perform significantly well than the other treatments.

List 1. ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Week0	.000	5	.000	.	.
Week1	8770.000	5	1754.000	.	.
Week2	35920.500	5	7184.100	.	.
Week3	50303.833	5	10060.767	41.881	.000
Week4	60110.278	5	12022.056	19.712	.000

List 2a. Multiple Comparisons

Dependent Variable	(I) Treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
week3	Control	T1	-33.66667	12.65497	.155	-76.1737	8.8404
		T2	-147.66667*	12.65497	.000	-190.1737	-105.1596
		T3	-63.00000*	12.65497	.003	-105.5070	-20.4930
		T4	-16.33333	12.65497	.785	-58.8404	26.1737
		T5	9.66667	12.65497	.969	-32.8404	52.1737
	T1	T2	-114.00000*	12.65497	.000	-156.5070	-71.4930
		T3	-29.33333	12.65497	.259	-71.8404	13.1737
		T4	17.33333	12.65497	.743	-25.1737	59.8404
		T5	43.33333*	12.65497	.045	.8263	85.8404
	T2	T3	84.66667*	12.65497	.000	42.1596	127.1737
		T4	131.33333*	12.65497	.000	88.8263	173.8404
		T5	157.33333*	12.65497	.000	114.8263	199.8404
	T3	T4	46.66667*	12.65497	.029	4.1596	89.1737
		T5	72.66667*	12.65497	.001	30.1596	115.1737
	T4	T5	26.00000	12.65497	.369	-16.5070	68.5070

List 2b. Multiple Comparisons

Dependent Variable	(I) Treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Week4	Control	T1	-30.33333	20.16414	.668	-98.0631	37.3964
		T2	-157.33333	20.16414	.000	-225.0631	-89.6036
		T3	-74.66667	20.16414	.028	-142.3964	-6.9369
		T4	-14.33333	20.16414	.997	-82.0631	53.3964
		T5	13.00000	20.16414	.985	-54.7298	80.7298
	T1	T2	-127.00000	20.16414	.000	-194.7298	-59.2702
		T3	-44.33333	20.16414	.305	-112.0631	23.3964
		T4	16.00000	20.16414	.963	-51.7298	83.7298
		T5	43.33333	20.16414	.326	-24.3964	111.0631
	T2	T3	82.66667	20.16414	.014	14.9369	150.3964
		T4	143.00000	20.16414	.000	75.2702	210.7298

Dependent Variable	(I) Treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
		T5	170.33333	20.16414	.000	102.6036	238.0631
	T3	T4	60.33333	20.16414	.092	-7.3964	128.0631
		T5	87.66667	20.16414	.009	19.9369	155.3964
	T4	T5	27.33333	20.16414	.751	-40.3964	95.0631

Table 1. Water quality parameters of Chicken manure treated water with Duckweed as phytoremediation agent to improve water quality

Treatment	pH	DO (Ml/L)	Temp (°C)
Control	8.62±0.74	3.96±0.22	26.60±0.31
T1	8.65±0.73	3.80±0.30	27.20±0.21
T2	9.27±0.40	4.52±0.33	27.30±0.08
T3	9.89±0.45	4.60±0.42	26.90 ± 0.29
T4	9.28±0.51	3.94±0.16	27.50 ± 0.07
T5	8.93±0.20	4.50±0.55	27.70 ± 0.35
P- value	**0.068**	**0.510**	**0.076**
Week			
Initial	7.92±0.29[a]	4.42±0.44	27.47 ± 0.25
Week 1	8.61±0.40[a]	4.33±0.24	27.02 ± 0.34
Week 2	8.63±0.45[a]	4.27±0.25	27.20 ± 0.23
Week 3	10.13±0.15[b]	3.97±0.35	27.05 ± 0.27
Week 4	10.25±0.18[b]	4.12±0.38	27.33 ± 0.17
P –value	**1.56×10^{-5}**	**0.895**	**0.614**

Means in the same column followed by different superscripts differ significantly (p<0.05)

Table 2. percentage Change in Water Quality Parameters following phytoremediation

Treatment	pH	DO	Temp
Control	48.62	16.67	-1.12
T1	41.85	-11.76	0.74
T2	14.40	45.00	-1.09
T3	29.31	-42.59	1.48
T4	35.18	-7.50	-0.72
T5	10.06	-29.51	-3.15

3.2 Water Quality

Water quality in tanks treated with chicken manure and phyto-remediated using duckweed (Table 1) shows that there was no significant difference in water quality across the treatments ($p>0.05$). However the pH varied over the weeks of experimentation with increase in pH being observed from an initial value of 7.92 to a final value of 10.25 in week 4.

Table 2 shows that pH increased generally across the treatments with the highest percentage increase of 48.62% being observed in the control, and the lowest percentage increase 10.06% recorded in T5. There was a high percentage increase of 45% in DO for T2, while all other treatments recorded declines in DO. Temperature change was marginal across all treatments with the highest increase of 1.48% being observed in T3, while the highest reduction of 3.15% was observed in T5.

4. DISCUSSION

Primary productivity is the basis of whole metabolic cycle in natural ecosystems. Productivity studies are of paramount interest in understanding the effect of pollution on an aquatic ecosystem. In the tanks utilized in this experiment, productivity fluctuated greatly among treatments but increased as the days increased. The biomass of duckweed also increased after treatment. The work done by (Alkhateeb and Asker, 2005) showed that *Lemna minor* is very effective in phytoremediation of industrial wastewater (Carvalho and Martin, 2001), their study reveal that four aquatic plants *Typha domogenas*, *Lemna obscura* (duckweed), *Hydrilla verticillata* and Swamp lily can be used as Phyto removal agents for Selenium in aqueous solutions.

In this study, duckweed spore in the treatment 3 increased with significant difference especially at week 3 and 4.

As shown in the graph (Fig. 1 below) over the four weeks, the highest average performance is Treatment 2 (M=213.80) followed by Treatment 3 (M=149.80), Treatment 1 (M=118.80), Treatment 4 (M=103.6), Control (M=95.00), and Treatment 5 (M=83.6) respectively. This suggests that the conditions were most favourable for the growth

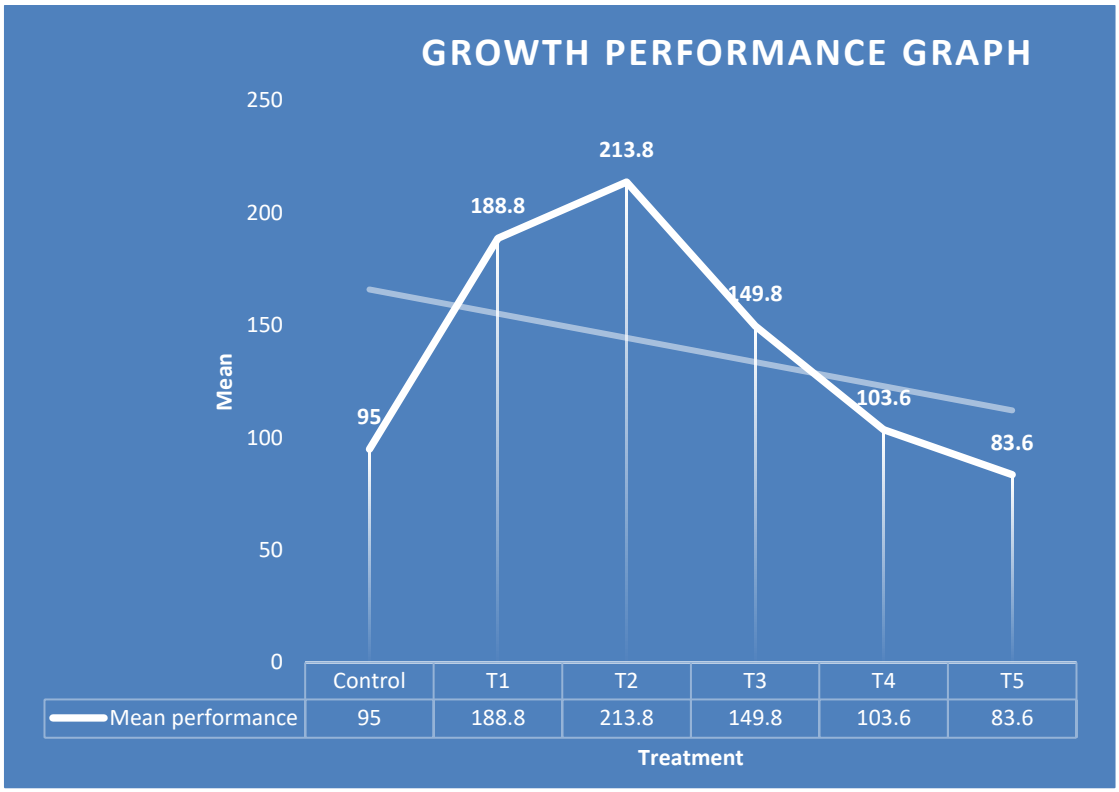

Fig. 1. Graph showing mean number of Duckweed stems in culture tanks per each treatment in experimental period

and multiplication of duckweed (*Lemna minor*) spores. The decline in spore value in the treatments 4 and 5 could be due to the high concentration and toxicity which may have affected the survival of the spores negatively. One procedural factor to note is that the experiment was conducted in the lab, and not outdoor which could have affected the result since the substantially increased accumulation of Nitrogen, Phosphates, and other fertilizers in water and in the presence of sunlight, results in dramatic water eutrophication, and aquatic plants blooming.

Many studies had shown that duckweed has been employed to treat agricultural, municipal, and even industrial wastewater streams into clean non-potable water (Ekperusi et al., 2019, Yu et al., 2014). The advantages of using Duckweed for the ecological restoration of eutrophic water have been highlighted: rapid growth and high biomass production, high photosynthesis efficiency, enormous nutrient uptake capacity, wide adaptation to various aquatic ecosystems, and effortless harvesting (Liu et al., 2020). The results of the current experiment indicated that Duckweed reduced Nitrogen content in water. Duckweed can efficiently utilize Nitrogen, Phosphate, and other inorganic nutrients in water, and ameliorate the physicochemical properties and micro-environment of water (Liu et al., 2020).

Generally, Duckweed grows well at a concentration of Nitrogen ranging from 7 to 84 mg/L (Sarkar et al., 2017). The optimum Nitrogen concentration for prosperous Duckweed growth is 28 mg/L (Cedergreen and Madsen, 2002) while Nitrogen concentration exceeding 60 mg/L exerts substantial toxicity to water body (Priya et al., 2012).

Under favorable climatic conditions and nutrient balance in growth media, *Lemna minor* can double its biomass within two days (Driever et al., 2005, Cheng and Stomp, 2009) reported a growth rate of L. minor close to 29$gm^{-2}day^{-1}$ in high strength swine wastewater, while the total Kjeldahl Nitrogen (TKN) and Total Phosphorous (TP) absorbed by Duckweed were 90% and 88.6%, respectively.

(Obek and Hasar, 2002) analysed the role of Duck weed (*Lemna minor)* harvesting in

biological Phosphate removal from secondarily treated effluents. Orthophosphate can be efficiently removed if Duck weed is frequently harvested. The Phosphate concentration decreased from the initial value in T1, but increases were observed in the other treatments. (Allinson et al., 2000) observed, during the treatment of alkaline industrial wastewater by *Azolla filiculoides* and *Lemna minor* that alkalinity and fluoride concentration decreased.

5. CONCLUSION

Under favourable climatic conditions and nutrient balance in growth media, *Lemna minor* can double its biomass within seven days. pH rose from an initial value of 7.92 to a final value of 10.25 in week 4. There was a high percentage increase of 45% in DO for T2 while all other treatments recorded declines in DO.

6. RECOMMENDATION

From the study, it is recommended that:

1. The best condition for use to grow optimum quantity of duckweed (*Lemna minor*) spores using chicken manure for aquaculture use is the mixture of 100g loam-clay soil with 7.5g chicken manure concentrate and 100l water.
2. *Lemna minor* multiplicity at right values can be used to increase DO content of water

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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