Manure Based Duckweed Production in Shallow Sink : Effect of Genera on Biomass and Nutrient Yield of Duckweed under the Same Nutritional and Management Conditions

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ABSTRACT : Biomass yield, nutritive value and nutrient utilization efficiency of different genera of duckweed (DW, *Lemnaceae*) under the same nutritional and management conditions were studied. Three genera of DW namely: *Lemna perpusilla*, *Spirodela polyrhiza* and *Wolffia arrhiza*, were cultivated in 18 plastic sinks each of $59\times54\times35$ cm^3 size. Each sink contained approximately 80 L of water and was charged once with 6.648 kg of anaerobically fermented cow dung effluent. The seed rate for *Spirodela*, *Lemna* and *Wolffia* were 600, 400 and 600 g/m^2 respectively. Duckweed were harvested at 48 hours intervals. Media total N concentration for *Lemna* and *Wolffia* gradually increased with time. Growth of duckweed was measured by subtracting the inoculum from the total biomass production. Growth of *Spirodela* ceased within 4 days but *Lemna* and *Wolffia* continue to grow up to 34 days. Bio-mass yield was significantly ($p<0.05$) higher in *Wolffia* (906 kg/ha/d) than *Lemna* (631 kg/ha/d). Dry matter (DM) yield was very similar in *Lemna* (14.80 kg/ha/d) and *Wolffia* (14.57 kg/ha/d). The N content was non-significant higher in *Lemna* (5.45%) than *Wolffia* (5.00%) and *Spirodela* (4.6%). The crude protein (CP, $N\times6.25$) yield was non-significantly higher in *Lemna* (4.83 kg/ha/d) than *Wolffia* (4.32 kg/ha/d). The acid detergent fibre (ADF) content was the highest in *Wolffia* (28.59%), followed by *Spirodela* (19.47%) and *Lemna* (12.39%). Utilization efficiency of CP was 273 and 314% respectively for *Wolffia* and *Lemna*. However, similar efficiency values for *Spirodela* was only 1.5%. Considering the bio-mass yield, nutritive value and nutrient utilization efficiency, production performance of DW were in the order of *Lemna*>*Wolffia*>*Spirodela* under the present experimental conditions. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 5 : 686-693*)

Key Words : Duckweed, *Lemna*, *Spirodela*, *Wolffia*, Bio-Mass Yield, Nutritive Value

INTRODUCTION

Duckweed (*Lemnaceae*) are small free floating aquatic plants found all over the world ranging from the cold temperate regions to the hot humid tropics and grows in different types of fresh water and brackish waters (Vries and Wal, 1998). Duckweed grows at temperatures between 6-33℃. Many species of duckweed can cope with low temperature by forming a turion and the plant sinks to the bottom of a lagoon where it remains dormant until warmer water brings about a resumption of normal growth (Leng et al. 1995). Duckweed can double their biomass within 16-48 hours under optimum nutrient availability, sunlight and water temperature. This is faster than any other higher plant. Under experimental conditions the extrapolated production rate can be 28 tons dry matter per ha/Y although yields are closer to 10-20 ton/ha/Y under real world conditions (Landolt and Kandler, 1987; Hassan and Edward, 1992). Duckweed grows in water with decaying organic material, providing it with a steady supply of nutrients. By efficient removal of mineralized nutrients, duckweed treats wastewater and enriches itself with highly digestible sources of nutrients. It has little fibre (about 5% of DM) with varying levels of protein (6-50%); as a result it has little or no indigestible material even with mono-gastric animals. This contrasts with many crops such as maize, rice or soya bean, where approximately 50% of the biomass are high fibre, low digestibility residues. Study on the natural habitat of duckweed in Bangladesh shows that it grows in all kinds of waste water nourished by household, industrial and farm wastes (BLRI, 1997). However, bio-mass yield and nutritive value of these duckweed often varies largely due to variation in the nutrient concentration of the media in which it grows (Gijzen and Khondker, 1997; BLRI, 1997; Sultana, 1998). In addition, variation in quality and quantity of biomass produced by a plant can partly be attributed to the genetic make up of the plant (Singh, 1994). Three genera (*Lemna*, *Spirodela* and *Wolffia*) of duckweed available in Bangladesh (Vries and Wal, 1998) have distinct morphological and botanical differences which might effect the bio-mass yield and nutritive value of duckweed. No systematic study has yet been done to determine the effect of genotype, i.e., genera of DW e.g., *Spirodela*, *Lemna* and *Wolffia* grown under the same nutritional and management conditions on their bio-mass yield and nutritive value. Therefore the present experiment was designed with the objectives of: determining the biomass yield, nutritive value and efficiency of utilization of nutrient by different genera of duckweed

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(DW) under the same nutritional and management conditions.

METHODOLOGY

Duckweed used

Three genera of DW, collected from the natural habitats located in and around the Bangladesh Livestock Research Institute (BLRI) were used for the trial. Dr. M. Zamman Khondker, (Professor, Department of Botany, Dhaka University), identified three genera of DW as *Lemna perpusilla* Torrey, *Spirodela polyrhiza* (L) Schleid and *Wolffia arrhiza* (L). Horkel ex Wimmer.

Sink and the bio-digester

Fermentation of cow dung was carried out in a Fixed Dome (Chinese) Bio-digester of $3.14\times(3.65)^2\times 2.84=29.7$ m^3 capacity which was charged daily with 50 kg cow dung mixed with water at a ratio of 50:50. Effluent collected from the displacement tank of the digester was used for fertilization of the sink for growing DW. The experiment was conducted in a series of 18 plastic sinks. Initially, eighteen $59\times54\times35$ cm^3 pits were dug in the soil which were then covered with a transparent polythene liner. Each sink had water-holding capacity of 80 L with a water depth of 35 cm and surface area of 320 cm^2. Each sink was fertilized with 6.648 kg of anaerobically fermented cow dung effluent (with average DM and N content of 7.4 and 2.46% respectively) to maintain 32 mg N/L of the media.

Experimental design and treatments

Eighteen plastic sinks were randomly allocated to three treatments in a completely randomized design. The three treatments were three species of DW namely, *Spirodela*, *Lemna* and *Wolffia*, each replicated six times in eighteen plastic sinks.

Seed rate, harvest and measurement of DW yield

The seed rate for *Spirodela*, *Lemna* and *Wolffia* were 600, 400 and 600 g/m^2 respectively as specified in earlier trails in this laboratory (BLRI, 1997). Media with DW was then stirred clockwise with hand, which gave a uniform DW cover on the surface of the media. DW generally established very quickly in the media and start multiplication within 8-14 hours. Duckweed were harvested with a nylon net in 48 hours interval by removing the whole DW from the media. After draining off the water, total weight of biomass was recorded and yield was calculated by subtracting the inoculum (seed amounts) from the total biomass. The DW seed were then put back into the media. The root length of *Lemna* was measured by extending the roots of five individual fronds and taking the average as the root length.

Chemical analysis

Samples of both DW and the media were stored at 4℃ (freezing at -20℃ found to cause autolysis of DW on thawing) for chemical analysis at every harvest during the 34 days of the experimental period. These samples were analyzed for dry matter (DM), organic matter (OM), nitrogen (N) and acid detergent fibre (ADF) according the method of AOAC (1984) within 2-3 days of harvest.

Statistical analysis

Data were analyzed manually by using ANOVA of completely randomized design using the computerized statistical programme. Linear regression of the model Y=a+bX, was also used where appropriate.

RESULTS

Media characteristics

Figure 1a, b and table 1 shows the characteristics of media where different DW species were grown. Total N concentration of the media for *Lemna* (figure 1a) and *Wolffia* (figure 1b) gradually (but not significantly) increased both with time. No relationship was observed between the N concentration of the media and the experimental period. Mean total N concentration of the media was 45, 42 and 32 mg/L respectively for *Lemna*, *Wolffia* and *Spirodela*. Mean media temperature were 28.37, 29.5 and 29.5℃ and pH were 7.92, 8.20 and 8.18 respectively for the *Lemna*, *Wolffia* and *Spirodela*.

Biomass yield

Figure 2 and table 2 shows the extrapolated biomass yield (fresh weight) of different species of DW. For *Wolffia* and *Lemna*, it was possible to have 17 harvests in 34 days until cessation of their growth (indicated from the negative growth rate). While for *Spirodela*, only 2 harvests in 4 days were possible before the cessation of the DW growth. For both *Wolffia* and *Lemna*, DW production gradually declined with time. Table 2 shows the bio-mass yield of *Lemna* and *Wolffia* in the 1st and 2nd harvest and also mean of 17 harvests. Yield of *Lemna* (952 kg/ha/d) and non-significantly higher in the 1st harvest as compared to that of the *Wolffia* (741 kg/ha/d) and *Spirodela* (759 kg/ha/d). However, in the 2nd harvest, *Wolffia* had significanlty ($p<0.01$) higher (3,323 kg/ha/d) biomass yield than the *Lemna* (1,807 kg/ha/d) and *Spirodeal* (1,609 kg/ha/d). Figure 2 shows production trend of *Wolffia* and *Lemna* which continued over the 34 days of experimental period, where biomass yield was significantly ($p<0.05$) higher in *Wolffia* (906 kg/ha/d) than *Lemna* (631 kg/ha/d). Linear regression

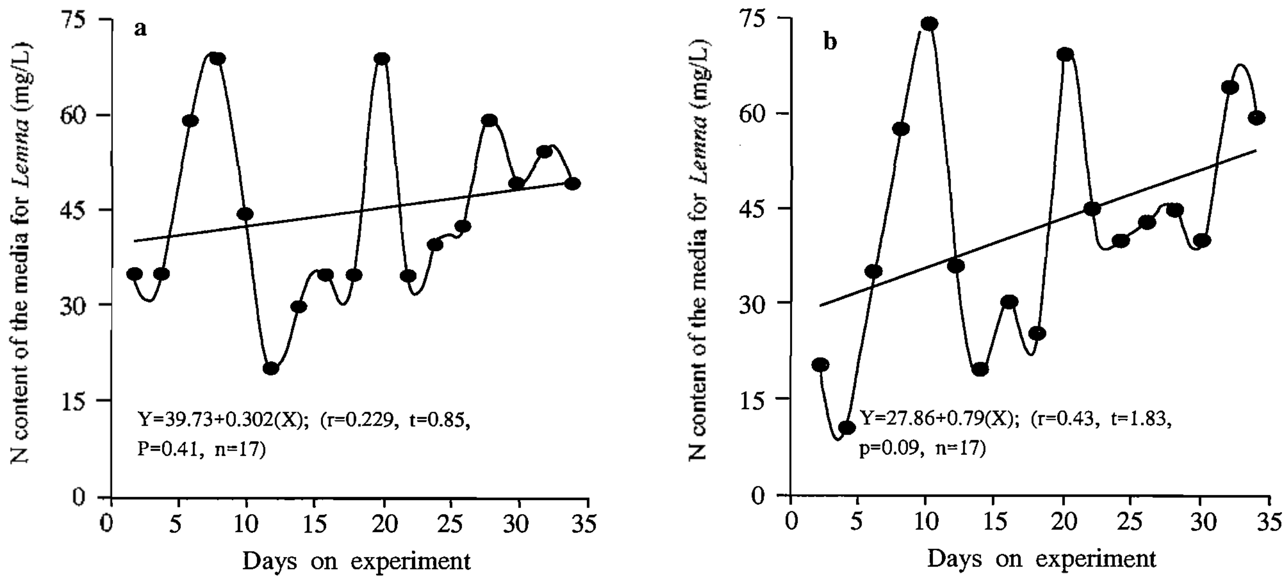

Figure 1. Media total N concentration (mg/l) for *Lemna* (a) and *Wolffia* (b) during 34 days of experimental period. Straight line represents the corresponding linear regression

Table 1. Characteristics of media containing 80 L water fertilized once with 6.648 kg of anaerobically fermented cattle manure

Parameter	*Lemna*	*Wolffia*	*Spirodela*	SED	Significance
Total N concentration (mg/L)[a]	45.18	42.00	32.50	5.42 (Resd. *df*=32)	NS
Temperature[b]	28.37	29.50	29.55	0.2189 (Resd. *df*=12)	NS
pH[b]	7.92	8.20	8.18	0.0616 (Resd. *df*=12)	NS

[a] Mean of 17 observation during the 34 days experimental period.
[b] Mean of 5 observation during the 34 days experimental period.

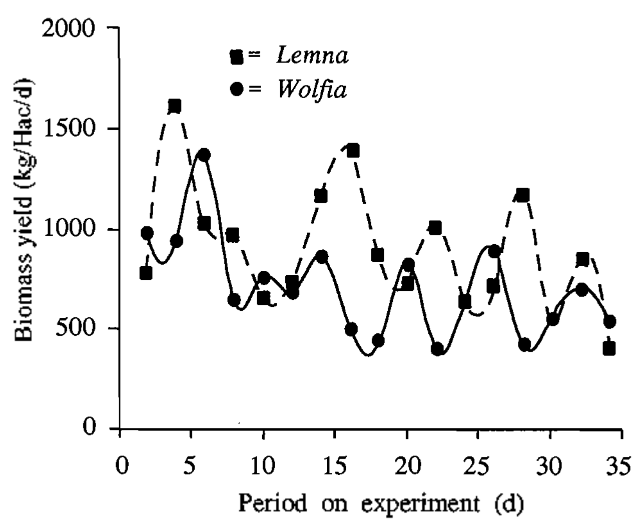

Figure 2. Biomass yield of *Lemna* and *Wolffia* (*Spirodela* is not included here) at different harvest during 34 days of experimental period. Each values represents the mean of six observations

(Eqn. 1) between the biomass yield (Y, kg/ha/d) and media N concentration (X, mg/L) was only significant ($p<0.05$) for the *Wolffia* but not for the *Lemna*.

$$Y=1260 - 9.31(\pm 3.632)X \quad \text{Eqn. 1.}$$
$$(r^2=0.304;\ t=2.56;\ n=17;\ p<0.05)$$

Here, biomass yield is negatively correlated with the media N concentration.

Chemical composition

Table 3 shows the chemical composition of different DW species. Dry matter content was significantly ($p<0.05$) higher in the *Spirodela* (8.5%) followed by *Lemna* (6.14%) and *Wolffia* (4.25%). Ash content was significantly ($p<0.05$) higher in *Wolffia* (23.37%) than *Lemna* (19.37%) and *Spirodela* (15.57%). N content was slightly (not significant) higher in *Lemna* (5.45%) than *Wolffia* (5.00%) and *Spirodela* (4.6%). The ADF content was the highest in *Wolffia* (28.59%), followed by *Spirodela* (19.47%) and *Lemna* (12.39%).

Table 2. Biomass yield (kg/ha/d) of different species of duckweed grown in media (80 L water) fertilized once with 6.648 kg of anaerobically fermented cattle manure

Parameter	*Lemna*	*Wolffia*	*Spirodela*	SED	Significance
1st harvest				Residual *df*=15	
Bio-mass yield	952	741	759	134.8	NS
2nd harvest				Residual *df*=15	
Bio-mass yield	1,807[b]	3,323[a]	1,609[b]	477.8	p<0.01
Mean of 17 harvest				Residual *df*=15	
Bio-mass yield	631[b]	906[a]	-	83.3	p<0.05

[a,b] Values with different superscripts in the same row differ significantly.

Table 3. Chemical composition (%) of different species of duckweed grown in media (80 L water) fertilized once with 6.648 kg of anaerobically fermented cattle manure

Parameter	*Lemna*	*Wolffia*	*Spirodela*	SED (Residual *df*=32)	Significance
Dry matter (g/100 g of fresh substances)	6.14	4.25	8.50	0.684	p<0.05
Ash (g/100 g of DM)	19.36	23.37	15.57	2.300	p<0.05
Organic matter (g/100 g of DM	80.65	76.63	84.84	6.150	NS
Crude protein (g/100 g DM)	34.06	31.25	28.75	4.581	NS
ADF[a] (g/100 g DM)	12.39	28.59	19.47	-	-

[a] ADF=Acid detergent fibre.

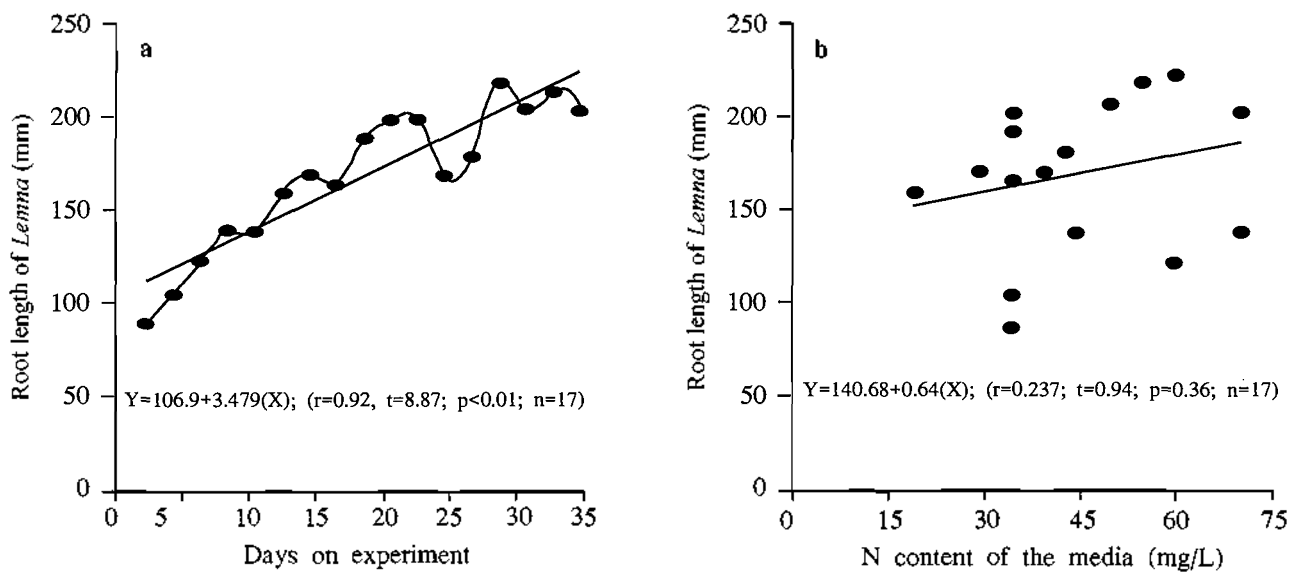

Figure 3. Root length of *Lemna* as affected by the experimental period (a) or media N concentration (b). Straight line represents the corresponding linear regression

Root length

Since *Wolffia* is devoid of any root and there were only two observation on *Spirodela*, only root length of *Lemna* are presented here in figure 3a. Root length increased linearly (p<0.01) with time at the rate of 3.5 mm/d (figure 3a). However, there was no significant at (p>0.05) correlation between the root length of *Lemna* and the total N concentration of the media (figure 3b).

Nutrient recovered

Table 5 shows the amounts of nutrients recovered during 34 days of experimental period. As expected, *Spirodela* had the lowest (p<0.01) amount of nutrients recovered (7.3, 6.1 and 3.7 g of DM, OM and CP, respectively) compared to that of the *Lemna* (670, 540

Table 4. Mean (of 34 days trial) dry matter (DM), organic matter (OM) and crude protein (CP) yield (kg/ha/d) of different species of duckweed grown in media (80 L water) fertilized once with 6.648 kg of anaerobically fermented cattle manure

Parameter	*Lemna*	*Wolffia*	*Spirodela*	SED	Significance
Mean of 17 harvest[a]				Residual *df*=32	
DM yield	14.80	14.57	-	2.29	NS
CP yield	4.83	4.32	-	0.79	NS
ADF yield[§]	1.83	4.17	-	-	-

[a] Value represents the mean of 17 harvest and values from Spirodela were not included in this comparison.
[§] ADF content was determined once, therefore it was not possible to have statistical analysis.

Table 5. Nutrient recovered (g) during 34 d of experimental period by different species of DW grown in media (80 L water) fertilized once with 6.648 kg of anaerobically fermented cattle manure (containing 492 g DM, 407 g OM and 73.8 g CP)

Parameter	*Lemna*	*Wolffia*	*Spirodela*	SED	Significance
Dry matter	670.0	650.0	7.3	39.0	$p<0.01$
Organic matter	540.0	500.0	6.1	31.0	$p<0.01$
Crude protein	230.0	200.0	3.7	13.6	$p<0.01$

Table 6. Efficiency of utilization (%) of different nutrients by different species of DW grown for 34 days in media (80 L water) fertilized once with 6.648 kg of anaerobically fermented cattle manure (containing 492 g DM, 407 g OM and 73.8 g CP)

Parameter	*Lemna*	*Wolffia*	*Spirodela*	SED	Significance
Dry matter	137	132	1.5	7.9	$p<0.01$
Organic matter	133	122	3.6	7.6	$p<0.01$
Crude protein	314	273	1.5	72.3	$p<0.01$

and 230 g of DM, OM and CP respectively) and *Wolffia* (650, 500 and 200 g of DM, OM and CP, respectively), which had recovered almost similar amounts of nutrients.

Efficiency of nutrient recovery

Nutrient recovery efficiency was measured as the proportion of nutrient that has been recovered from the supplied nutrient. Table 6 shows the efficiency of nutrient utilization by different DW species. Efficiency of utilization of DM, OM and CP of *Lemna* and *Wolffia* was more than unity. Utilization of CP was 273 and 314% respectively by *Wolffia* and *Lemna*. However, similar values for *Spirodela* were very low.

DISCUSSION

In this trail, three DW genera namely *Spirodela*, *Lemna* and *Wolffia* were grown in plastic sinks, containing 80 L of water, fertilized once with 6.648 kg of anaerobically fermented cattle manure (containing 492 g DM, 407 g OM and 73.8 g CP) during 34 days of experimental period.

Media characteristics

In the present trial, media temperature varies between 28-29.5℃ which is within the range (20-30℃) optimum for maximum DW growth (Length et al, 1992). However, pH of the media was alkaline (7.9-8.5). Although ammonia in ionized form (NH_4^+) is the preferred N substrate for duckweed, but alkaline pH shifts the $NH_4^+ \leftrightarrow NH_3$ balance towards un-ionized state (NH_3) and result in the liberation of free ammonia which, at higher concentrations (100 mg NH_3/mL) is toxic to DW (Leng et al., 1995).

Of the three macro-nutrients (N, P and K) essential for plant growth, concentration of N in the media was only measured. In this experimental system part of the N is converted into duckweed protein and the residual N should be lost or remain in the effluent, therefore, N concentration expected to decrease with the time. Whereas, N concentration found to increase at the rate of 0.3 mg/L/d (for *Lemna*, figure 1a) or 0.79 mg/L/d (for *Woffia*, figure 1b). Porath and Pollock (1982) suggested that N metabolism in such a DW based biotic system involve three different shunts in the N cycle. A microbes-driven shunt include at mospheric N fixation; a mixed plant and microbes driven shunt that causes reduction of N to organic nitrogenous compounds (e.g., bacterial protein, DW protein) and the third system that causes reduction of N to NH_4^+ also used by plant and microbes. It is probable that

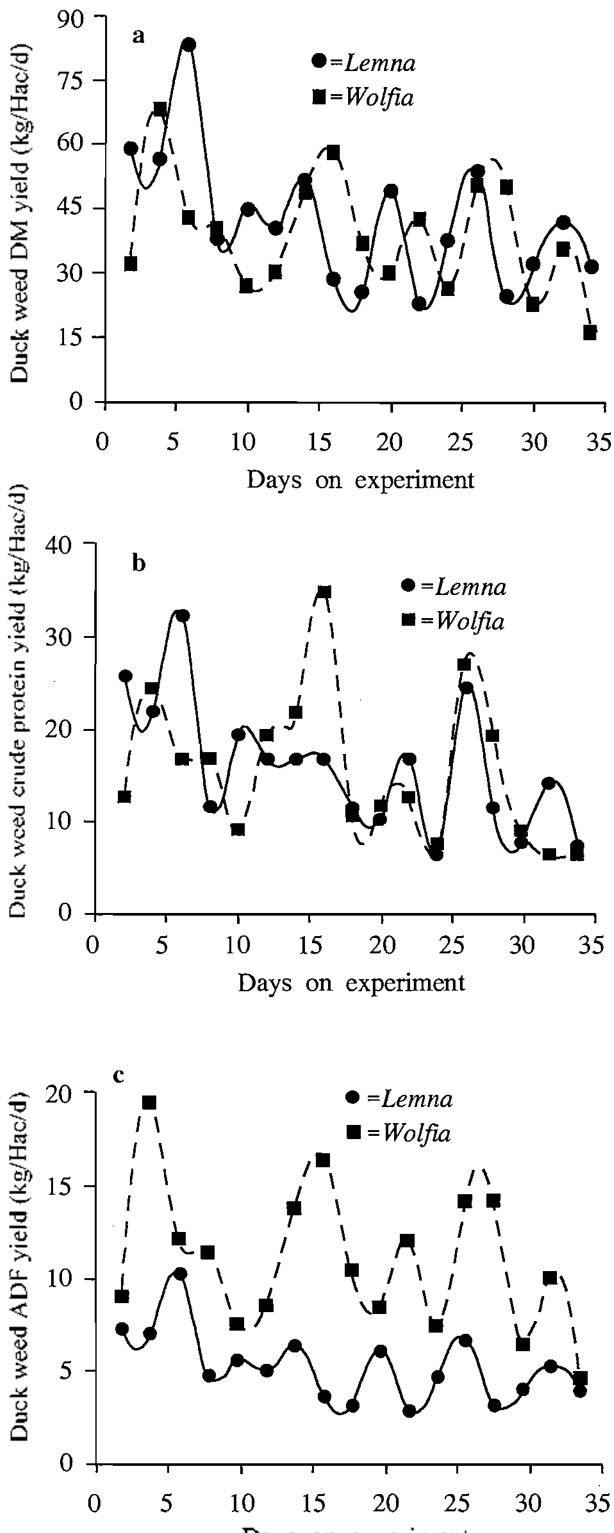

Figure 4. Dry matter (a), crude protein (b) and ADF yield (c) of *Lemna* and *Wolffia* (*Spirodela* is not included here) at different harvest during 34 days of experimental period. Each value represents the mean of six observations.

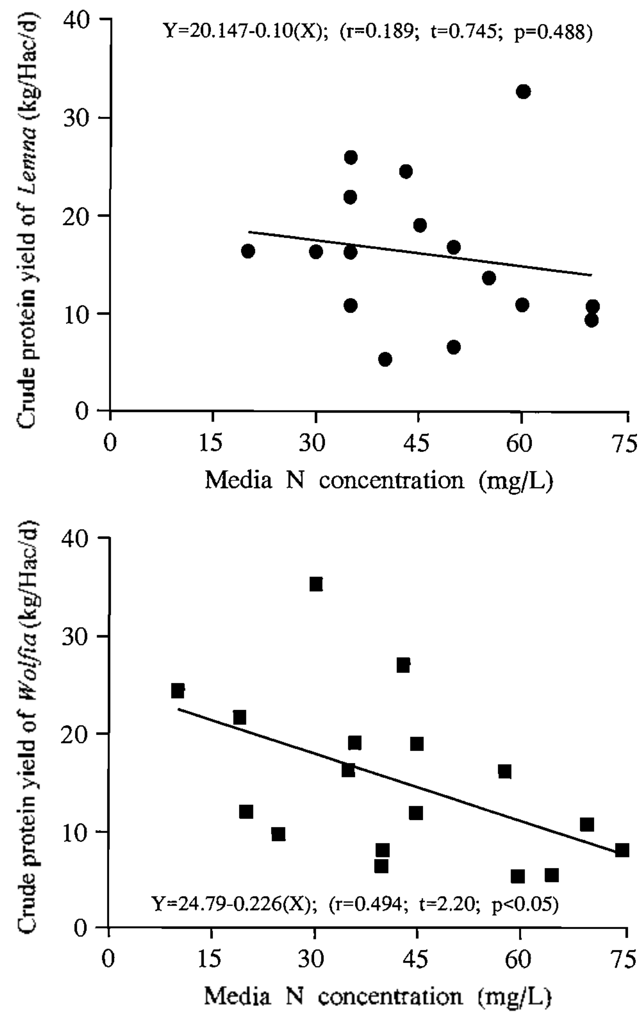

Figure 5. Relationship between the crude protein yield and media N concentration of *Lemna* (a) and *Wolffia* (b) observed during 34 days of experimental period. Straight line represents the corresponding linear regression.

during the course of the present experiment, the media was enriched with both mineralized (due to microbial decomposition) effluent N (i.e., $NH_4^+ \leftrightarrow NH_3$) and from atmospheric N fixation (also $NH_4^+ \leftrightarrow NH_3$). It is likely that rate of uptake of this NH_4^+ by the DW was probably less than its accumulation with the resultant gradual increase in total N concentration of the media. However, media N concentration (highest 75 mg/L) was still less than the optimum N concentration required for maximum growth of *Lemna*, *Wolffia* (75-150 mg/L) and *Spirodela* (150 mg/L; BLRI, 1997).

Biomass yield

Four points are apparent regarding the biomass yield of DW:

a) cessation of growth of *Spirodela* within four days (two harvest) while *Lemna* and *Wolffia* continued their growth up to 34 days (17 harvest) at the same nutritional and management conditions; b) extrapolated mean biomass yield was significantly higher in *Wolffia* than the *Lemna* or *Spirodela*; c) biomass yield declined with the progress of experimental period and d) biomass yield was negatively correlated with media N concentration.

Exact reason(s) for the cessation of *Spirodela* is not known. However, liberation of gases (CO_2, CH_4, etc.) from anaerobic fermentation of effluent resulted formation of excessive froth on the surface of the media which resulted floating of DW (Spirodela) over the front that may inhibited their nutrient absorption from the media with the consequent death. Another possibility is that under alkaline pH, as stated earlier, mineralized N was converted into un-ionized NH_3 state which at higher concentration (100 mg/L) is toxic to DW growth (Leng et al., 1995). Why *Spirodela* sinks had higher microbial fermentation than the others is not clear.

Higher biomass yield for *Wolffia* than *Lemna* or *Spirodela* has also been observed in a previous trial in the BLRI (BLRI, 1997), where biomass yield for *Wolffia*, *Lemna* and *Spirodela* were 1,750, 887 and 674 kg/ha/d respectively. Similarly, biomass yield of *Wolffia* was 81% higher than *Spirodela* and 52% higher than the *Lemna* (DRP, 1997).

Since nutrient was added to the sink once at the beginning of the trial, progressive decline in the biomass yield with time was expected due to the depletion of nutrients. In the present trial, only media N concentration was measured, which increased with time (probably due to the reasons stated earlier), but the concentration of other macro (K and P) and micro nutrients essential for DW growth, were not measured. These nutrients might have been depleted with time with the consequent decrease in DW production.

Over the normal range (15-150 mg N/L), media N concentration is positively correlated with the biomass yield (BLRI, 1997; Rodriguez and Preston, 1996). Whereas, in the present trial, this relationship was negative (see Eqn. 1). Media N concentration in this trial ranges between 20-75 mg/L with a mean of approximately 44 mg N/L, which is with in the optimum range. Therefore, negative relationship is probably an artifact of nutrient depletion other than N per se as stated earlier.

Nutritive value

Two points are apparent in the nutritive value of different DW species:

a) of the three DW species, *Wolffia* had the lowest DM and the highest ADF content and the CP content of *Wolffia* and *Lemna* was much higher than *Spirodela*; b) Mean DM and CP yield of *Wolffia* and *Lemna* were similar.

As a typical aquatic plant, three DW species studied here had very low DM (4-8%) but high CP (28-35%) content, which is similar to the previous observations in this laboratory (BLRI, 1997) and elsewhere (Rusoff et al., 1980). Due to relatively lower DM content, *Wolffia* yielded almost similar DM and CP compared to that of *Lemna* although biomass yield was higher in the former than the latter. Due to high fibre and low DM content, fresh *Wolffia* may not be an appropriate protein supplementation for chicken or similar mono-gastric animals but could be a good protein supplement for ruminants. On the other hand, relatively higher DM and lower ADF content of *Lemna* and *Spirodela* render them to be used in fresh condition as a protein supplement for all kinds of livestock including chicken and ducks. Nutrient yield of DW is quite comparable with that of conventional livestock fodder, e.g. maize, cowpea, napier grass. For example, annual DM and CP yield of maize (fodder) under Bangladesh conditions found to be 16 and 0.81 Ton/Hac respectively (Huque et al., 1995) while corresponding values for the Lemna in the present trial were approximately 15 and 5 ton/Hac respectively. Thus, recycling anaerobically fermented farm manure through DW production can ensure year round protein rich forage supply for livestock.

Nutrient recovery

Since yield of DM, OM and N of *Lemna* and *Wolffia* were more than that was given through cow dung effluent, the nutrient recovery efficiencies were more than 100%. Nitrogen recovery efficiency in this trial is particularly very high (273 and 314 per cent for *Wolffia* and *Lemna* respectively). These values were much higher than that observed in Mirzapur by PRISM where NH_3-N recovery efficiency ranged between 46-71% (Gijzen et al., 1996). This could due to incorporation of atmospheric N by the duckweed. To present date there is no report of direct incorporation of atmospheric N fixation by duckweed. One possible way could be fixation through N fixing organism into ammonia and then into the $NH_4^+ \leftrightarrow NH_3$ shunt (Porath and Pollock, 1982). Similarly, higher DM and OM recovery is also probably due to the incorporation of atmospheric CO_2 through photosynthesis.

CONCLUSION

Considering the bio-mass yield, nutritive value and nutrient utilization efficiency, production performance of DW were in the order of *Lemna*>*Wolffia*>*Spirodela* under the present experimental conditions.

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