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EFFECT OF FERTILIZATION RATES ON THE GROWTH PERFORMANCE AND PROXIMATE COMPOSITION OF DUCKWEED (*WOLFFIA GLOBOSA*) : AN ALTERNATIVE AQUA-FEED

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ABSTRACT

Growth performance and proximate composition of *Wolffia globosa* (Wolffia) were evaluated in a 12-d growth trial with six different NPK fertilization rates (single dose application) under natural sunlight. The intermediate fertilization rates of 43.4 mgl^{-1} N, 10.07 mgl^{-1} P & 25 mgl^{-1} K (represented by T_3) resulted in significantly higher net consolidated fresh biomass, dry matter yield and crude protein yield and hence was decided as the basal standard fertilization (BSF) rate. Notably, the yield appeared to peak during 3-6-d post fertilization and attained minima towards the end of the culture period. Further, a second 12-d growth trial was undertaken to evaluate impacts of re-fertilization on growth performance and protein content of wolffia. Higher consolidated fresh biomass yields were obtained at re-fertilization with 10% of BSF. Comparison of results of both experimental trials indicated that temperature has a more pronounced impact on protein content than on biomass yield.

Key words : *Wolffia globosa*, Fertilization rates, Growth performance, Nutritional value**.**

Introduction

In view of burgeoning human population (estimated to reach over 9 billion by 2050) (Pagliuso *et al.*, 2022) and shrinking per capita land as well as water availability, aquaculture productivity enhancement rather than horizontal expansion has become the major strategy for enhancing fish production in any country or region. Adoption of feed-based aquaculture systems has been one of the most common strategies to enhance aquaculture productivity and more than 2/3 of global aquaculture production now comes from feed-based culture systems.

Therefore, the availability of fish feed ingredients/ feed resource; especially suitable protein source has become integral to sustainable aquaculture development (Michael and Sogbesan, 2017).

The Lemnaceae family of aquatic plants, which includes duckweeds, has recently received more attention as a potential source of food for both humans and animals (Petersen *et al.*, 2021). According to Les *et al.* (2002), the Lemnaceae family consists of 37 species divided into five genera and the subfamilies Lemnoideae (Spirodela, Landoltia, and Lemna) and Wolffioideae (Wolffiella and Wolffia).

Wolffia is a rootless, free-floating, small plant with a length of around 0.5-0.8 mm, mostly found in lakes, ponds, and marshes (Sree *et al.*, 2016). The plant lacks a stem, roots, and leaves. The entire plant, known as a frond, has the potential to develop blooms and can be replicated by the budding process to boost daily yield by 50% (Yahaya *et al.*, 2022).

In an environment with suitable nutrients, light, and temperature, this plant grows rapidly (Chookhampaeng *et al.*, 2022). The culture media made from the chemicals or fertilizers added to the water provide the plant with a variety of nutrients (Chookhampaeng *et al.*, 2022). As additional nutrients, it needs nitrogen, phosphorus, and potassium (Hasan and Chakrabarti, 2009). Water with a pH of 5-5.5 and an ideal temperature range of 17.5–30°C were found to have the optimal growth rates for cultivation in water (Sree *et al*., 2015).

According to reports, duckweed has a protein content of 20 - 30%, which is higher than grain (de Beukelaaret *et al*., 2019). According to Appenroth *et al.* (2018), duckweed also contains 4–7% fat, 4–10% starch, carotenoids, and polyphenols including flavonoids and anthocyanins. Additionally, wolffia is traditionally used as a natural food source in Southeast Asia and is known to be a great source of protein. The Wolffia genus contains numerous subspecies, with Wolffia globosa being the most prevalent one.

In Thailand, *W. globosa* is known as Khai Nam or watermeal. For some dishes, such as salads, omelettes, or vegetable curries, raw wolffia is used (Yahaya *et al*., 2022). A greenhouse precision aquaculture was used to grow *W. globosa*, which has nine essential amino acids, iron, vitamins A, E, and B12, omega-3 fatty acids, zinc, potassium, and folate, as well as 7% minerals, 45% protein, 37% carbohydrates, 8% fats, and 3% water (Pagliuso *et al*., 2022).

The nutrient content and metabolite composition of duckweed have drawn a lot of interest, especially in the fields of animal feed, aquaculture, health supplements, biofertilizer, biofuel, and newly developed food products for humans (Naseem *et al*., 2020). According to Appenroth *et al.* (2017), ducks, swans and geese eat duckweed naturally. Since the 1960s, aquaculture has also been used to produce fish and other animals for use as food, including pigs, cattle, rams, sheep, horses, waterfowl and fish.

In Bangladesh, a project was financed by the World Bank to feed fish using duckweeds (Skillicorn *et al.*, 1993). Recent studies that go into greater detail on this issue include using Wolffia arrhiza meal to replace soy in the diet of Japanese quails (Suppadit *et al.*, 2012), using duckweed species in the feed of striped catfish (Da *et al*., 2013), rohu and carp (Sharma *et al*., 2016) and chicken (Shammout and Zakaria, 2015).

The aim of our research was to examine how different fertilization rates (N, P and K) in the culture medium affect the growth performance and nutritional value of *W. globosa*.

Materials and Methods

Experimental design

There were two experiments that were conducted in September and March month, respectively. The primary goals of the first experiment were to establish the optimal fertilization rate with respect to the growth characteristics and proximate composition of *W. globosa* (L.) and the primary goals of experiment two were to establish the impact of re-fertilization frequencies or rate following basal fertilization with respect to the biomass and protein content of *W. globosa* (L.). The experiments were conducted at the College of Fisheries, Central Agricultural University, Lembucherra, Tripura, India, in twenty-one thermocol fish iceboxes (58 cm x 39 cm x 30 cm) over the course of 12 days. The surface area of each box was 0.226 m^2. The boxes were cleaned and washed and were filled with groundwater to a 20 cm water depth, giving a volume of 50 liters. All boxes were set up under shade which made by using transparent polythene sheet and bamboo poles. A completely randomized design (CRD) with three replications was used. A modified Schenk-Hildebrandt medium (Appenroth *et al*., 2017) was used as reference fertilization (RF) to prepare different concentrations of N, P and K and of minerals. A single dose of fertilization [173.6 mg/liter nitrogen; 40.3 mg/liter phosphorous; 100 mg/liter potassium and 0.6 g/liter with vitamins and minerals mixture namely 'Agrimin Fort India' to fulfil the requirement of minerals for their growth] was done as a reference fertilization rate (RF) and five serially diluted (0-20 times) (RF/2; RF/4; RF/8; RF/16; RF/20) concentrations were prepared. Inoculums samples of Wolffia fronds were obtained from the College of Fisheries, Lembucherra and inoculated at a rate of 400 g /m^2 (90.4 g in each tank) in each treatment. Harvesting was done at two-day intervals.

Experiment 2 was carried out for another 12 days in March month. In this experiment the optimal basal fertilization emerged from Exp.1, was used as basal standard fertilization (BSF) rate and different re-fertilization (doses at 0-100% of BSF) were evaluated at 2-day intervals. For this experiment, to prepare different re-fertilization rates, T_3 treatment of our previous trial one was used as a BSF, because in T_3 the net biomass yield and protein yield were highest. On 0-day, basal dose of fertilization added in the system was same in all treatments but from 3rd day onward the re-fertilization of system was done as 0, 10, 20, 40, 60, 80 and 100% of BSF. The sample was analyzed for biomass yield, dry matter yield and protein content. The environmental temperature and light intensity were also recorded daily by using digital thermometer (YSI Pro ODO) and digital lux meter (model no. D. 33979).

Yield and biomass analysis

Every two days, the fresh biomass yield of wolffia was assessed. By deducting the quantity of inoculums from the total biomass, the yield was determined. The collected biomass samples were dried in an oven at 60 °C for 36 hours, weighed, and the increase in dry weight biomass over the course of the 2-day culture was computed (Dry matter = final value – Initial value). Additionally, powdered dried biomass was used for analysis.

Proximate composition analysis

For analysis, wolffia samples were taken at every harvest. Proximate composition of wolffia was performed using standard method (AOAC, 2005). The moisture content was determined by oven drying a weighed sample in porcelain crucibles at 105ºC for 24 h. The total volatile matter lost at this temperature was taken as the moisture content. The Ash content was determined by incinerating the dried samples in a Muffle furnace at 550ºC for 5-6 h. The percentage of protein (N × 6.25) was estimated in Kjeltec system after digestion with H_2SO_4 and catalyst (K_2SO_4 and $CuSO_4$ in 9:1), followed by distillation and titration. The percentage of lipid was determined using the petroleum ether method in soxtec system and crude fiber was estimated after weak acid and weak alkali digestion in fibertech system.

Statistical analysis

The data obtained was analyzed statistically and interpreted by using Statistical Package for Social Sciences (SPSS, version 16.0 for windows). Analysis of variance (one way - ANOVA) was performed to determine the differences between the mean values of different treatments. Differences in means were compared by Duncan's New Multiple Range test (multiple range test) at P< 0.05 level.

Results and Discussion

Effect of fertilization on growth performance of *W. globosa*

In accordance with the research on the impact of NPK fertilization rates on growth performance (Table 1), the highest mean consolidated fresh biomass in 12 days (1086.83±10.65 g m^{-2}) was obtained in T_3 and there were significant ($p<0.05$) differences between treatments. The first harvesting of wolffia produced the highest fresh biomass (218.5±1.95 g $m^{-2}d^{-1}$) in T3; however, the fresh biomass output declines in subsequent harvestings (second and third). Fresh biomass production in T_1 and T_2 was low during the entire trial period (with the exception of the control group). Dry matter (DM) yield of wolffia was higher (16.53±0.16 t DM $ha^{-1}y^{-1}$) in treatment T_3, but there was no significant difference between treatments T_3 and T_4 (Table 2). In the first trial, wolffia produced a net protein yield that ranged from 3.25±0.26 to 5.45±0.03 t ha^{-1} y^{-1} (Table 2) when the medium's N content was between 8.68 to 43.4 mg l^{-1}.

According to Zhang *et al*. (2014), productivity is directly correlated with the amount of nutrients present in the culture environment up to a point beyond which growth has been shown to slow. We found a rising growth trend as N: P: K (T_3) in the current investigation. According to Li *et al*. (2016), nutrient levels in the medium have a significant impact on the growth and yield performance of duckweeds. In the long-term experiment, it was found that wolffia productivity had decreased, which may have been caused by a number of growth-restraining factors. Wolffia fresh biomass and DM and CP output showed linear increases as the N, P and K concentration in the medium was raised ($P \leq 0.05$), however excessive concentrations have been shown to have harmful effects (Goopy and Murray, 2003). An

Table 1 : Mean values (±SE) for fresh biomass yield in trial 1 (Temperature- 31.5 ºC).

Treatments	Fresh biomass (g m^{-2} d^{-1})				
	3-d	6-d	9-d	12-d	Net fresh biomass (g m^{-2} in 12 days)
T_1	183.11±1.95^{b}	73.83±2.66^{b}	31.75±5.17^{ab}	11.08±6.39^{ab}	599.53±29.53^{b}
T_2	206.00±7.12^{c}	99.68±3.38^{c}	50.21±6.56^{bc}	25.11±7.71^{b}	761.96±41.87^{c}
T_3	218.55±1.95^{c}	141.02±7.04^{e}	97.46±4.61^{d}	86.39±4.61^{d}	1086.83±10.65^{f}
T_4	214.86±1.28^{c}	128.47±7.78^{de}	93.03±5.57^{d}	72.36±8.22^{cd}	1017.42±19.20^{ef}
T_5	214.12±3.69^{c}	109.27±1.95^{cd}	72.36±1.95^{cd}	65.71±1.95^{cd}	922.92±3.91^{de}
T_6	186.80±8.98^{b}	104.11±14.24^{c}	81.22±18.63^{d}	61.28±10.88^{c}	866.80±73.43^{cd}
Control (no fertilizers)	127.73±5.77^{a}	43.56 ±4.11^{a}	11.81±5.17^{a}	0.00 ±0.00	366.21±3.91^{a}

Different upper-case letters in the same column denote significant differences according to Duncan test (P<0.05) (d: day).

Table 2 : Mean values (±SE) for dry matter and crude protein yield of wolffia in trial 1.

Treatments	Parameters		
	DM, g m^{-2} (Consolidated)	DM, (t ha^{-1} y^{-1})	CP, (t ha^{-1} y^{-1})
T_1	29.97±1.48^{b}	9.12±0.45^{b}	2.80±0.17^{b}
T_2	38.10±2.09^{c}	11.59±0.64^{c}	3.54±0.21^{c}
T_3	54.34±0.53^{f}	16.53±0.16^{f}	5.45±0.03^{e}
T_4	50.87±0.96^{ef}	15.48±0.29^{ef}	4.44±0.26^{d}
T_5	46.15±0.20^{de}	14.04±0.06^{de}	3.59±0.04^{c}
T_6	43.34±3.67^{cd}	13.18±1.12^{cd}	3.25±0.26^{bc}
Control (no fertilizers)	18.31±0.20^{a}	5.57±0.06^{a}	0.83±0.01^{a}

Different upper-case letters in the same column denote significant differences according to Duncan test (P<0.05). (DM: dry matter, CP: crude protein).

our findings. In addition to micronutrients, temperature, light, algal bloom, insect infestation, wave action, species, and plant density, the availability of macronutrients like nitrogen, phosphorus, and potassium has a significant impact on plant growth and reproduction (Li *et al.*, 2016). As evidenced by higher chlorophyll concentrations, nutrients in the medium up-regulated the photosynthetic mechanism. Additionally, the rate of photosynthesis was accelerated, which led to the production of greater biomass (Zhang *et al.*, 2014).

Effect of re-fertilization on growth performance of *W. globosa*

Table 3 provides a summary of the wolffia mean fresh biomass yield. Re-fertilization system has a considerable impact on *W. globosa* growth

Table 3 : Mean values (± SE) for fresh biomass yield of wolffia in trial 2.

Treatments	Fresh biomass (g m^{-2} d^{-1})				
	3-d	6-d	9-d	12-d	Net fresh biomass (g m^{-2} in 12 days)
T_1	167.60±8.32^{a}	93.77±3.22^{a}	79.74±3.38^{a}	55.38±4.61^{a}	792.97±18.44^{a}
T_2	171.29±0.74^{a}	149.14±2.66^{c}	150.62±3.84^{bc}	154.31±22.53^{b}	1250.74±54.70^{b}
T_3	188.28±8.39^{a}	123.30±8.98^{b}	124.78±12.15^{b}	135.85±4.11^{b}	1144.42±56.40^{b}
T_4	174.98±5.57^{a}	149.14±12.87^{c}	159.48±5.57^{c}	132.16±28.06^{b}	1231.54±86.05^{b}
T_5	177.20±7.99^{a}	144.71±1.48^{bc}	144.71±0.74^{bc}	128.47±14.07^{b}	1190.19±29.64^{b}
T_6	173.51±8.98^{a}	150.62±5.86^{c}	134.38±12.62^{bc}	125.52±10.88^{b}	1168.04±54.03^{b}
T_7	181.63±12.79^{a}	145.45±7.04^{bc}	126.26±14.75^{b}	112.23±8.22^{b}	1131.13±74.14^{b}

Different upper-case letters in the same column denote significant differences according to Duncan test (P<0.05).
*d= day

abundance of unionized NH_3, which has greater toxic effects and ammonium accumulation is not well regulated by the plants, may have resulted from the use of urea as a primary source of nitrogen and higher temperature (30-31°C) as well as pH, particularly during later parts of the culture period up to 10 (Petersen *et al.*, 2021). Dry matter yields of duckweed were reported by Leng *et al.* (1995) to range from 10 - 30 tonnes/ha/year; in our experiment, the DM yield was found to be 16.53 tonnes/ha/year, which is comparable to this result. The CP yield of duckweed was between 6 and 10 tonnes/ha/year in the earlier study by Nguyen Duc Anh and Preston (1997) when the water's N level was between 10 and 30 mg/l. Li *et al.* (2016) showed 5.5 tonnes/ha/year protein yield at 3.5 mg/l N and 1.5 mg/l P, but 10.9 tonnes/ha/year CP yield at 35 mg/l N and 3.5 mg/l P. The CP yield in our study was tonnes/ha/year. These conclusions are supported by

and yield (p<0.05). The fresh biomass production gradually dropped over time at 0% re-fertilization (T_1) (no re-fertilization after basal fertilization), but it was largely steady in other treatments (T_2-T_7). In compared to control, the consolidated net biomass acquired during the culture period was significantly higher in all treatments (Table 3). However, differences in re-fertilization rates did not affect net biomass yield. Re-fertilization with 10% BSF was adequate to maintain the maximal growth rate (1250.74±54.70 g m^{-2} in 12 days) under natural climate conditions.

Re-fertilization with 10% BSF was adequate to maintain the maximal growth rate (1250.74±54.70 g m^{-2} in 12 days) under natural climate conditions. Up to a point beyond which growth has been shown to be stable, productivity is directly proportional to nutrient

Table 4 : Mean values (± SE) for DM yield of wolffia in trial 2.

Treatments	Parameters	
	DM, g m^{-2} (in 12 days)	DM yield (t ha^{-1} y^{-1})
T_1	39.65±0.92^a	12.06±0.28^a
T_2	62.54±2.73^b	19.02±0.83^b
T_3	57.22±2.82^b	17.41±0.86^b
T_4	61.58±4.30^b	18.73±1.31^b
T_5	59.51±1.48^b	18.10±0.45^b
T_6	58.40±2.70^b	17.76±0.82^b
T_7	56.56±3.71^b	17.20±1.13^b

*Different upper-case letters in the same column denote significant differences according to Duncan test (P<0.05).

concentrations in the culture environment (Li *et al.*, 2016). Similar trends were also seen in the DM yield, which was larger in T_2-T_7 than in control (Table 4), but there was no difference in re-fertilization rates (p>0.05). Increased growth and yield following re-fertilization with N, P, and K fertilizers may be attributed to the nutrients' role as essential components of nucleotides, proteins, chlorophyll, and enzymes as well as their involvement in a number of metabolic processes that directly affect the vegetative and reproductive phases of plants (Zhang *et al.*, 2014).

Effect of fertilization on proximate composition of *W. globasa*

Crude protein

Table 5 provides an overview of the mean values of wolffia crude protein (CP) content at various harvesting times. Over the course of the trial, the CP concentration at several harvests exhibits pronounced significant variation (p<0.05) within the treatments. The findings showed that when the N concentration increased from T_6 to T_3, correspondingly increased the CP content in *W. globasa* fronds. Notably, the first harvest should have had lower CP values for T_1 and T_2 since T_3 fertilization was excessively high. The third day following fertilization in the T_3 treatment, which is where the maximum crude protein content was found, was 33.95%. The CP content in the control group (which received no fertilizers) significantly dropped with time (going from 15.26 to 11.95%). On the seventh day of culture, the largest rise in CP in the T_1 and T_2 treatments was 32.14% and 32.44%, respectively.

The N concentration in the culture media was substantially correlated with the CP content of wolffia (p<0.05). According to Rodriguez and Preston (1996), as the N content of the water increased from 5 to 40 mg/l, the protein content in the dry matter increased from 20% to approximately 40%. Our findings support this conclusion. The outcome implies that fertilization causes the protein level in wolffia to increase. This might be the result of urea metabolism in wolffia, where urea was converted by urease into carbon dioxide and ammonium before being further absorbed into amino acids and proteins (Antia *et al.*, 1991). By enhancing the cultivation conditions, it can be inferred that the protein content of wolffia can be easily modified (Appenroth *et al.*, 2017).

Crude lipid

The mean values of the crude lipid content varied considerably (P<0.05) depending on the treatments (Table 5). The mean observed values of crude lipid in wolffia were higher (4.70±0.23%) for treatment T3 than for the other treatments, however on the third day of culture, there was no difference between treatments T3 and T4. On the third day of culture, no appreciable differences between the T_1, T_2, T_4, T_5 and T_6 treatments were found. A gradual decrease in lipid content was seen in all treatments on days 7 and 12 of the culture period (Table 5). In the control group (without fertilizers), the crude lipid content was lower (1.04±0.07 - 2.70±0.26%), whereas it typically ranged from 1.20±0.06 -4.70±0.23% for wolffia cultivated in nutrient-rich water.

In the present study, mean crude lipid concentrations of wolffia were found to be comparable to those found in reports by Appenroth *et al.* (2017) and Goopy and Murray (2003). But it must be emphasized that this information pertains to the entire biomass of the plant, not just the seeds, as is the case with most crop plants. According to published research, there is a positive association between lipid content and nitrate concentration (Borek *et al.*, 2009). We think that the mixo-trophic conditions of the cultivation may have contributed to this large variation (Yan *et al.*, 2013). The study's findings indicated that the application of fertilizers N, P and K causes increases in crude lipid content in the *W. globosa* plant.

Crude fiber

The mean values of *W. globasa* crude fiber content varied significantly between treatments (p<0.05) (Table 5). The control group's mean overall values of fiber in wolffia were greater (17%) than those of the other treatments. Notably, T_1 consistently displayed lower crude fiber concentrations. Further fiber contents showed an inversing trend with basal standard fertilization.

With a decline in the medium's nutrient concentration, *W. globosa* crude fiber content steadily rises (Table 5). The amount of crude fiber discovered in this investigation

Table 5 : Mean values (±SE) for proximate composition of wolffia in treatments one denotes significant differences according to Duncan test (P<0.05).

Parameters	Initial value	Days	Treatments						
			T_1	T_2	T_3	T_4	T_5	T_6	Control (no fertilizers)
Protein, (% in DM)	25.67±0.43	3rd	29.75±0.71^{d}	29.05±0.51^{cd}	33.95±1.06^{e}	29.81±1.73^{d}	26.86±0.19^{bc}	25.76±0.09^{b}	15.26±0.21^{a}
		7th	32.14±1.01^{d}	32.44±0.56^{d}	33.19±0.74^{d}	28.52±1.14^{c}	25.42±0.65^{b}	24.48±0.32^{b}	14.01±0.43^{a}
		12th	31.03±1.57^{d}	31.33±0.40^{d}	29.90±0.08^{d}	25.72±0.33^{c}	21.63±0.66^{b}	21.97±0.23^{b}	11.95±0.35^{a}
Crude Fat, (% in DM)	2.94±0.69	3rd	3.37±0.15^{b}	3.47±0.15^{b}	4.70±0.23^{c}	4.07±0.38^{bc}	3.90±0.12^{b}	3.40±0.12^{b}	2.70±0.26^{a}
		7th	2.53±0.09^{c}	2.40±0.06^{bc}	2.63±0.23^{c}	2.50±0.12^{c}	2.43±0.12^{bc}	2.03±0.18^{b}	1.33±0.09^{a}
		12th	1.90±0.12^{cd}	1.53±0.09^{bc}	1.67±0.12^{cd}	1.50±0.17^{bc}	1.30±0.06^{ab}	1.20±0.06^{ab}	1.04±0.07^{a}
Crude fiber, (% in DM)	10.29±0.17	3rd	9.78±0.27^{a}	10.28±0.14^{a}	10.34±0.26^{a}	10.39±0.28^{a}	10.56±0.37^{ab}	10.61±0.16^{ab}	11.22±0.20^{b}
		7th	9.92±0.25^{a}	10.94±0.18^{b}	11.05±0.08^{b}	11.13±0.18^{b}	11.20±0.17^{b}	11.42±0.29^{b}	13.04±0.30^{c}
		12th	10.10±0.35^{a}	11.03±0.31^{b}	11.20±0.18^{bc}	11.29±0.19^{bc}	11.97±0.25^{c}	14.01±0.20^{d}	14.99±0.33^{e}

was comparable to that found in studies by Goopy and Murray (2003) and Appenroth *et al.* (2017). The crude fiber content of duckweed was shown to decrease with an increase in the exchange rates of the medium with bio-digester effluent in a prior study by Nguyen Duc Anh and Preston (1998). According to Skillicorn and Journey (1993), the crude fiber content of duckweeds cultivated in nutrient-rich water is typically lower (7–10% DM) than that of duckweeds grown in nutrient-poor water (11–17% DM). Our outcome was consistent with this discovery.

Effect of re-fertilization on crude protein

Table 6 summarizes the average values of the CP content and net protein output from trial two. The CP content in *W. globosa* was significantly (p<0.05) affected by re-fertilization, and greatest values of CP content were found during T_2-T_7 in contrast to T_1 (control). With the exception of T_1, no treatment showed a significant difference (p>0.05), despite T_7 having a numerically slightly higher mean CP value (24.6%).

When the N concentration of the water was increased from 5 to 40 mg/l, Rodrguez and Preston (1996) found that the protein content in the dry matter increased from 20% to approximately 40%. The CP value found in our investigation falls somewhat outside of the range. It should be noted that the study was carried out in March, which is still spring (pre-summer), when the temperature ranged from 16.5-20.3°C in the morning (5-6 AM) to 26.5-29.6°C in the afternoon (2-3 PM). As a result, the early morning low water temperature may have contributed to the reduced protein concentration.

According to Li *et al*. (2016), *Landoltia punctata* and *Spirodela polyrhiza* have high protein content at 25°C (37.55 g m^2) and low protein content at lower temperatures. However, there was no discernible difference in the CP yield between the T_2, T_3, T_4, T_5, T_6 and T_7 treatments (Table 6). In contrast, the CP yield was at its highest (4.31±0.24 tonnes/ha/year) in the T_4 treatment at 40% re-fertilization of the system. In a prior study by Li *et al*. (2016), when the concentrations of N and P in the water were 3.5 mg/l and 1.5 mg/l, respectively, the CP output of duckweed was 5.5 tonnes/ha/year. Our findings support this conclusion.

Temperature and light intensity

Throughout trial 1, the water temperature in the test tanks was monitored every day in the afternoon throughout the culture phase. The water temperature fluctuated erratically were and generally fell within the usual range (31.21-31.59 °C), showing no discernible pattern. Wolffia grows more quickly as the water

Table 6 : Mean value (±SE) for CP % as well as CP yield on dry weight basis in treatments 2. At 0 days the protein content was 20.35% on dry weight basis.

Treatments	CP, % in DM	CP yield (t ha^{-1} y^{-1})
T_1	15.24±0.24^a	1.84±0.02^a
T_2	21.70±3.01^b	4.17±0.72^b
T_3	22.52±1.57^b	3.90±0.17^b
T_4	23.10±1.00^b	4.31±0.24^b
T_5	23.10±0.37^b	4.18±0.16^b
T_6	23.69±0.33^b	4.21±0.17^b
T_7	24.62±0.87^b	4.22±0.19^b

Different upper-case letters in the same column denote significant differences according to Duncan test (P<0.05).

temperature rises, although there is a maximum water temperature of 32°C at which growth slows and terminates at higher temperatures (>35°C) (Leng, 1999). During Trial 1, the ambient light intensity ranged from 4437 to 71000 lux.

Trial 2 was carried out in March, and daily readings of the water's temperature between 5:00 and 6:00 AM and 2:00 and 3:00 PM were taken. The protein concentration in wolffia is affected by water temperature, which ranged from 16.5 to 20.3 °C in the morning (Li *et al.*, 2016). However, in the afternoon, the temperature increased to 26.5-29.6 °C.

According to Landolt (1986), duckweed species generally thrive best at temperatures between 20 and 30 °C. During trial 2, the light output ranged from 16600 to 44950 lux. According to Landolt (1986), most species reach their growth potential at about 9,000 lux (at 24°C), and Landolt (1986) showed that light is favorably connected with temperature rises from 12°C up to 30°C. At a light intensity of 5670 lux (105 µmol.m^{-2} · s^{-1}), Li *et al*. (2016) recorded the highest relative growth rates and starch/protein in duckweed.

Conclusion

The use of N, P, and K fertilizers considerably impacts the growth and proximate composition of *W. globosa*, it is determined. Maximum biomass and dry matter yield can be maintained with basal standard fertilization of 43.4 mg l^{-1} N, 10.07 mg l^{-1} P, 25.0 mg l^{-1} K and 0.15g l^{-1} mineral mixture and 10% of basal standard fertilization, but maximum protein yield requires 100% re-fertilization of basal standard fertilization in the culture medium.

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