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Assessment of the Production Potential of Three Lemnaceae Species Under Uniform Nutritional and Management Conditions

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ABSTRACT
Background and Objective: Due to the growing world population and the increase in demand for food, food security may be the greatest challenge of humanity in the future. Duckweeds have the potential to be a useful alternative for human food and animal feed due to their great nutritional content. This study evaluates the production potential of three Lemnaceae species under uniform nutritional and management conditions. **Materials and Methods:** To assess the production potential of available species of duckweed, the experiment was conducted at the University of Tehran. Three species of *Lemna gibba*, *Lemna minor* and *Spirodela polyrhiza*, were compared under the same nutritional conditions in a greenhouse. The 25% concentration of Standard Hoagland solution was used. **Results:** Results showed that there are significant differences among studied species in growth parameters. *Lemna minor and Lemna gibba* showed better results in dry weight, linear growth rate, relative growth rate and doubling time. The CP content of three species was more than 30% of DW. **Conclusion:** The biomass yield and nutritive value, of *Lemna minor*, were more promising to be grown for nutritional biomass production.

KEYWORDS
Duckweeds, growth rate, protein content, *Lemna gibba*, *Lemna minor*, *Spirodela polyrhiza*

INTRODUCTION
With the growing world population and limited agricultural lands, the global food shortage will become more critical in the future[1] and finding sustainable and economical raw materials for feed production is a major source of concern for the feed industry[2]. In recent years, the use of aquatic plants has attracted global attention due to many features compared to conventional cereal and grain crops[3,4]. Among aquatic weeds, duckweeds are potential candidates to be used as an economical source of human food and animal feed in developing countries[5,6].

Duckweeds (the world's smallest and fastest growing flowering plants) belong to the Lemnaceae family comprising 37 species grouped into five genera[7]. These tiny plants can produce huge biomass with a broad range of potential applications like the production of feed and food, biofuel and biogas. Besides productivity, duckweed's key advantage is its high crude protein content. Unlike many commonly

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cultivated protein or starch crops, duckweed is entirely edible due to its minimal support tissues. It has the potential to yield significantly more protein per unit of cultivation area compared to crops like soybeans, as the protein in duckweed is distributed throughout the plant, whereas soybeans primarily concentrate it in their seeds. These small aquatic plants offer a sustainable source of essential nutrients and could play a vital role in addressing food security challenges while minimizing land use, a critical factor in mitigating global climate change[8,10].

To improve the application of duckweeds for food and feed, many studies are needed in species selection and optimization of biomass production[11], since biomass production differs depending on duckweed species[12,13]. Studies on duckweeds were conducted to evaluate the phytoremediation ability or biomass production of single duckweed species. A few studies have been done to determine the biomass production potential and nutritive quality of duckweed species under hygienic and same nutritional conditions. Therefore the present experiment aimed to compare three local species of duckweeds on biomass production potential and especially protein content under the same nutritional and management conditions.

MATERIALS AND METHODS

Sample collection: Plant samples were collected from the natural habitats located in Gilan Province (a Coastal Province in the North of Iran) in September, 2022 and then were brought to a greenhouse at the University of Tehran for propagating. Species identified morphologically as *Lemna gibba*, *Lemna minor* and *Spirodela polyrhiza*.

Research methodology: For hygienic production, a trial was conducted from 22 December, 2022 to 5 January, 2023, under the semi-controlled condition of a Research Greenhouse of the Department of Horticultural Sciences, at the University of Tehran, Karaj, Iran (35°50'10", 51°00'41"). Then, 27 plastic pots (22 cm long×22 cm wide×20 cm depth) were allocated to three species in a Randomized Complete Block Design with three replications. The pots filled with 7 L of 25% concentration Hoagland standard solution[14] under natural light in the greenhouse. In each pot, 5 g of fresh biomass of species was transferred. Table 1, shows the situation where different duckweed species were grown. At the end of the experiment (15 days after incubating), whole duckweeds were harvested using a plastic net. After draining the excess solution and weighting of fresh biomass, the samples were dried at 65°C, for 3 days and were analyzed for dry weight (DW), linear growth rate (LGR), relative growth rate (RGR), doubling time (DT), nitrogen content (N) and crude protein content (CP). Pots surface area (0.05 m^2 was used as a production area when needed and then some values were calculated for 1 m^2.

Parameter calculation:
RGR was calculated in each group according to Hunt's equation[15,16]:

$$R=LnW_2-LnW_1/T_2-T_1$$

where, R is the relative growth rate (gg^{-1} day^{-1}), W_1 and W_2 are the initial and final plant fresh weights harvested from the pot area, respectively and (T_2-T_1) is the experimental period (15 days).

The biomass doubling time was calculated by the below equation[16]:

$$\text{Doubling time} = [\text{Ln}(2)]/\text{RGR}$$

Biomass productivity or linear growth rate (LGR) was calculated as follows[5]:

$$\text{LGR} = \frac{(\text{DW}(\%)\ \text{end}\times\text{FW end}) - (\text{DW}(\%)\ \text{start}\times\text{FW start})}{\text{Time}\times\text{Surface}}\ g^{-2}\ day^{-1}$$

Table 1: Media characteristics and environmental parameter

Parameter	Value	Parameter	Value
Light intensity at 1 p.m.	95 μmol/m^2/sec	Water temperature	15°C
Electrical conductivity (EC)	1 ds/m	Air temperature	22°C
Photoperiod	10:14 light/dark	Relative humidity	48%

Dry weight (DW): The dried plant samples were used for Kejldahl Nitrogen (KJ-N) measurement. Additionally, KJ-N content was used to calculate the protein content by multiplying it with the factor 6.25[17].

Statistical analysis: Statistical analysis of the obtained data was conducted using SAS software Version 9.4 (SAS Institute, Cary, North Carolina, USA). The Duncan's New Multiple Range Test was used to compare means at a 0.05 level of probability.

RESULTS AND DISCUSSION

Results showed that species influenced all the measured variables. Blocks showed a significant effect only on DW and LGR. The analysis revealed significant variations among species for most traits, including fresh weight (FW), dry weight (DW), leaf growth rate (LGR), relative growth rate (RGR), drought tolerance (DT), nitrogen content (N%) and crude protein (CP), as indicated by their respective mean square values. Blocks showed a significant effect only on DW and LGR. The coefficients of variation (CV) ranged from 0.92% to 18.65%, indicating moderate variability in the data.

Fresh weight accumulation differed among species, *Lemna gibba* and *Lemna minor* had significant differences with *Spirodela polyrhiza* (Fig. 1). Chowdhury *et al.*[18] and Chen *et al.*[19] also reported that fresh weight has significant differences among duckweed species. With comparison of three species observed that *Lemna minor* had higher biomass production per square meter than *Lanctuta punctuata* and *Spirodela polyrhiza*.

As shown in Fig. 2, the effect of species on dry weight (DW) accumulation was significant, *Lemna minor* had the highest and *Spirodela polyrhiza* had the lowest DW, but *Lemna minor* and *Lemna gibba* were similar in this trait. A similar trend was reported by Appenroth *et al.*[3].

As illustrated in Fig. 3, biomass productivity or linear growth rate (LGR) was significantly different ($p<0.01$) among studied species. *Lemna minor* growth rate was higher than the others. Zhao *et al.*[20] grew four different species of duckweeds in a mix of domestic and aquaculture wastewater and Chen *et al.*[19] state the comparison of three species of duckweed, observed the same results. Results of Chen *et al.*[19] studies showed that *Lemna minor* LGR have been higher than *Lanctuta punctata* and *Spirodela polyrhiza*.

Relative growth rate (RGR) was also different among species ($p<0.05$). *Lemna minor* and *Lemna gibba* had higher RGR than *Spirodela polyrhiza* (Fig. 4). Findings of Pagliuso *et al.*[10] and Peterson *et al.*[21] and accord with current results, but Paolacci *et al.*[22] and Li *et al.*[23] studying three species of duckweed observed different results.

Due to the difference in growth rates, the doubling time parameter (DT) was also significantly different at ($p<0.01$) As shown in Fig. 5. Species of *Lemna minor* and *Lemna gibba* showed lower DT compared to *Spirodela polyrhiza*. Other reports are stating that DT is different between *Lemna minor* and *Landoltia punctata*[24].

Plant tissue nitrogen content (N%) showed a difference among species ($p<0.01$). As shown in Fig. 6, *Lemna minor* had higher N% than the others, although all of them had considerable N%. Pagliuso *et al.*[10] and Toyoma *et al.*[25] with the comparison of four species of duckweed in three mediums showed similar N% with a range of 5.3-6.6% .Chowdhurey *et al.*[18] observed different results (N% was non-significant among studied species).

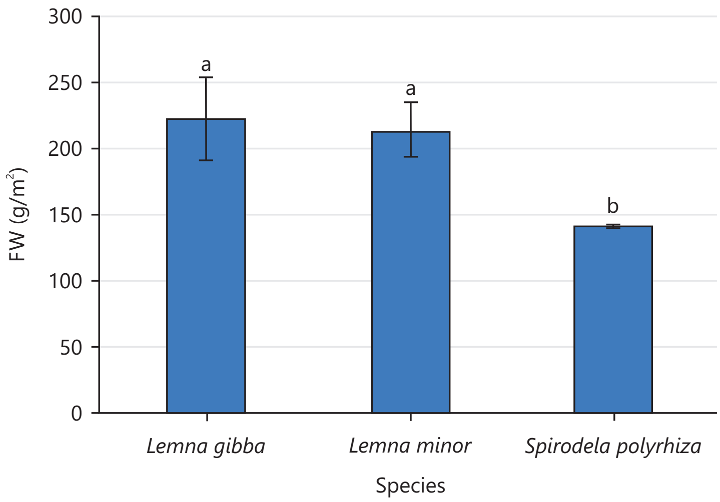

Fig. 1: Effect of species on fresh weight accumulation
Data are presented as Means±Standard Errors (SE) and [a,b]Indicated significantly different levels

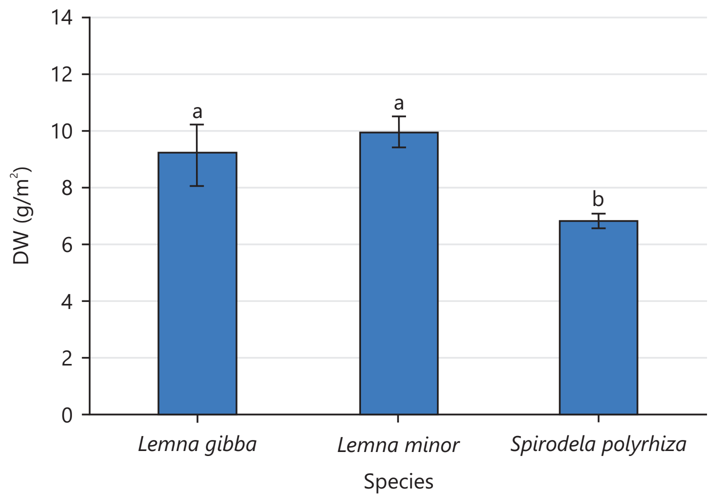

Fig. 2: Effect of species on dry weight accumulation
Data are presented as Means±Standard Errors (SE) and [a,b]Indicated significantly different levels

Table 2: Statistical analysis of species on measured parameter

		Mean squares						
Source of variations	DF	FW	DW	LGR	RGR	DT	N%	CP
Block	2	273.634^{ns}	3.524*	0.016*	0.0002^{ns}	20.4^{ns}	0.012^{ns}	0.46^{ns}
Species	2	616.546*	10.298**	0.059**	0.0008*	190.85**	0.514**	20.06**
Error	4	846.83	0.471	0.008	0.00007	3.64	0.003	0.11
CV		14.71	7.012	17.983	18.65	10.36	0.921	0.92

FW: Fresh weight per area, DW: Dry weight per area, LGR: Linear growth rate, RGR: Relative growth rate, DT: Doubling time, N: Nitrogen content and CP: Crude protein content. The givens are means of squares, degree of freedom and significance levels; **$p<0.01$, *$p<0.05$ and $^{ns}p\geq 0.05$ and ns: There are no significant effect

Current findings indicated that there is a significant difference among species on crude protein content (CP). *Lemna minor* and *Spirodela polyrhiza* reached the highest and lowest CP content, respectively (Fig. 7). This is consistent with Li *et al.*[23] reports in other duckweed species under different temperatures and light intensity, Zhao *et al.*[20] and Peterson *et al.*[21]. All of the duckweeds tested seem to be promising sources for protein production with more than 30% CP based on DW[26].

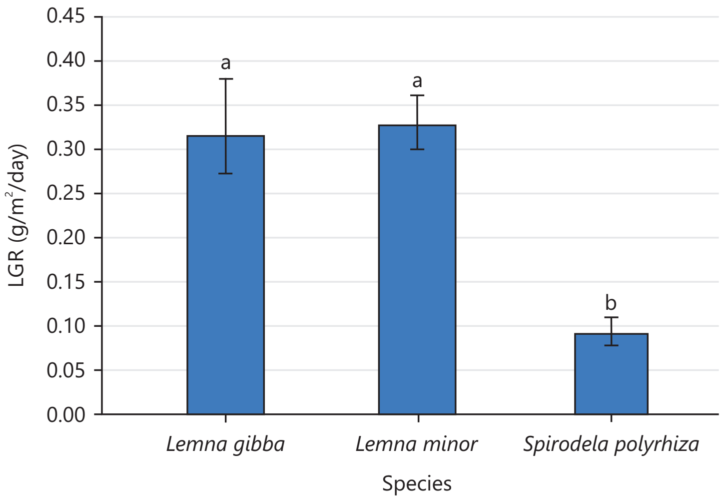

Fig. 3: Effect of species on linear growth rate (LGR)
Data are presented as Means±Standard Errors (SE) and [a,b]Indicated significantly different levels

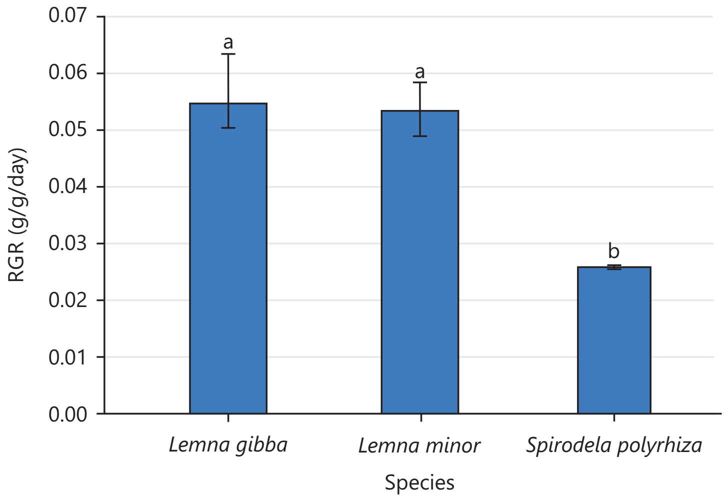

Fig. 4: Effect of species on relative growth rate (RGR)
Data are presented as Means±Standard Errors (SE) and [a,b]Indicated significantly different levels

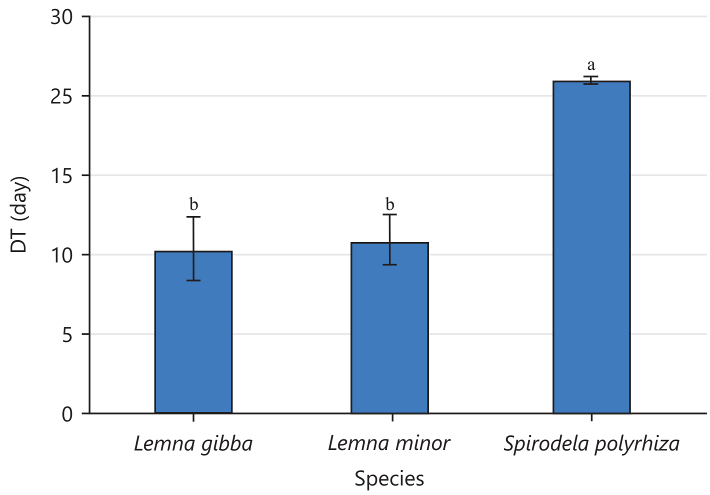

Fig. 5: Effect of species on doubling time (DT)
Data are presented as Means±Standard Errors (SE) and [a,b]Indicated significantly different levels

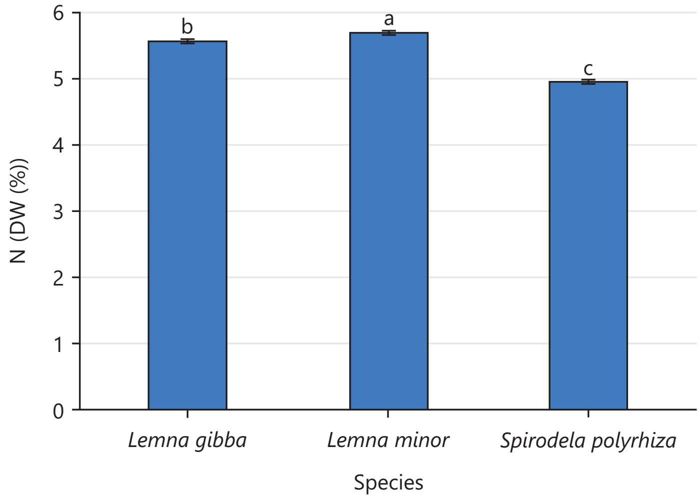

Fig. 6: Effect of species on nitrogen content (%)
Data are presented as Means±Standard Errors (SE) and [a,b]Indicated significantly different levels

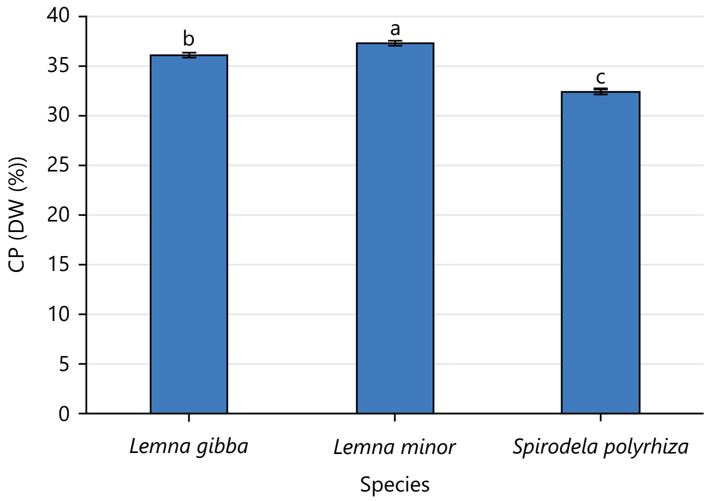

Fig. 7: Effect of species on crude protein content (CP)
Data are presented as Means±Standard Errors (SE) and [a,b]Indicated significantly different levels

CONCLUSION

The trial demonstrated significant effects of duckweed species on growth parameters and protein content. Among the tested species, Lemna minor exhibited the highest production performance, followed by Lemna gibba and Spirodela polyrhiza. While biomass production was lower compared to previous studies, likely due to suboptimal cultivation conditions, including a mean temperature of 15°C (below the optimal range of 20-30°C), protein content across all species was notable. Lemna minor, with a protein content exceeding 35%, qualifies as a protein-rich biomass, presenting a sustainable and cost-effective alternative to traditional high-protein sources such as fishmeal and soybean. The findings highlight the potential of Lemna minor for large-scale cultivation, especially in Iran, where such practices remain unexplored. Further research under varying conditions is essential to optimize biomass production and unlock the full potential of duckweed as a high-quality protein source.

SIGNIFICANCE STATEMENT

The trial revealed significant effects of duckweed species on growth and protein content, with *Lemna minor* showing the highest production and protein content exceeding 35%. Although biomass production was lower due to suboptimal conditions, *Lemna minor* qualifies as a sustainable, protein-rich alternative to traditional sources like fishmeal and soybean. The findings emphasize its potential for large-scale cultivation in Iran, warranting further research to optimize production under varying conditions.

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REFERENCES
1. Hasegawa, T., G. Sakurai, S. Fujimori, K. Takahashi, Y. Hijioka and T. Masui, 2021. Extreme climate events increase risk of global food insecurity and adaptation needs. Nat. Food, 2: 587-595.
2. Begho, T. and A.E. Irabor, 2024. Fish feed formulation: Does Nigerian farmers' risk and time preference play a part in January 2, 2025 choosing feed protein sources for intensively farmed fish? Aquaculture, Vol. 585. 10.1016/j.aquaculture.2024.740723.
3. Appenroth, K.J., K.S. Sree, V. Böhm, S. Hammann, W. Vetter, M. Leiterer and G. Jahreis, 2017. Nutritional value of duckweeds (Lemnaceae) as human food. Food Chem., 217: 266-273.
4. Appenroth, K.J., K.S. Sree, M. Bog, J. Ecker and C. Seeliger *et al*., 2018. Nutritional value of the duckweed species of the genus *Wolffia* (Lemnaceae) as human food. Front. Chem., Vol. 6. 10.3389/fchem.2018.00483.
5. Xu, Y.L., L. Tan, L. Guo, G.L. Yang and Q. Li *et al*., 2019. Increasing starch productivity of *Spirodela polyrhiza* by precisely control the spectral composition and nutrients status. Ind. Crops Prod., 134: 284-291.
6. Hafiz Ullah, B. Gul, H. Khan, N. Akhtar, Khushnood Ur Rehman and U. Zeb, 2022. Effect of growth medium nitrogen and phosphorus on nutritional composition of *Lemna minor* (an alternative fish and poultry feed). BMC Plant Biol., Vol. 22. 10.1186/s12870-022-03600-1.
7. Sree, K.S., M. Bog and K.J. Appenroth, 2016. Taxonomy of duckweeds (Lemnaceae), potential new crop plants. Emir. J. Food Agric., 28: 291-302.
8. Skillicorn, P., W. Spira, W. Journey and World Bank, 1993. Duckweed Aquaculture: A New Aquatic Farming System for Developing Countries. World Bank, Washington, DC, United States, ISBN: 9780821320679, Pages: 76.
9. Oron, G., 1994. Duckweed culture for wastewater renovation and biomass production. Agric. Water Manage., 26: 27-40.
10. Pagliuso, D., A. Grandis, J.S. Fortirer, P. Camargo, E.I.S. Floh and M.S. Buckeridge, 2022. Duckweeds as promising food feedstocks globally. Agronomy, Vol. 12. 10.3390/agronomy12040796.
11. Cui, W., J. Xu, J.J. Cheng and A.M. Stomp, 2011. Starch accumulation in duckweed for bioethanol production. Biol. Eng. Trans., 3: 187-197.
12. Zhao, Z., H. Shi, Y. Liu, H. Zhao, H. Su, M. Wang and Y. Zhao, 2014. The influence of duckweed species diversity on biomass productivity and nutrient removal efficiency in swine wastewater. Bioresour. Technol., 167: 383-389.
13. Ziegler, P., K. Adelmann, S. Zimmer, C. Schmidt and K.J. Appenroth, 2015. Relative *in vitro* growth rates of duckweeds (Lemnaceae)-The most rapidly growing higher plants. Plant Biol. J., 17: 33-41.
14. Rajaseger, G., K.L. Chan, K.Y. Tan, S. Ramasamy, M.C. Khin, A. Amaladoss and P.K. Haribhai, 2023. Hydroponics: Current trends in sustainable crop production. Bioinformation, 19: 925-938.
15. Beadle, C.L., 1985. Plant Growth Analysis. In: Techniques in Bioproductivity and Photosynthesis, Coombs, J., D.O. Hall, S.P. Long and J.M.O. Scurlock (Eds.), Pergamon, İzmir Province, Turkey, ISBN: 9780080319995, pp: 20-25.
16. Wendeou, S.P.H., M.P. Aina, M. Crapper, E. Adjovi and D. Mama, 2013. Influence of salinity on duckweed growth and duckweed based wastewater treatment system. J. Water Resour. Prot., 5: 993-999.

17. Casal, J.A., J.E. Vermaat and F. Wiegman, 2000. A test of two methods for plant protein determination using duckweed. Aquat. Bot., 67: 61-67.
18. Chowdhury, S.A., N. Sultana, K.S. Huque and Q.M.E. Huque, 2000. Manure based duckweed production in shallow sink: Effect of genera on biomass and nutrient yield of duckweed under the same nutritional and management conditions. Asian-Australas. J. Anim. Sci., 13: 686-693.
19. Chen, G., Y. Fang, J. Huang, Y. Zhao and Q. Li *et al*., 2018. Duckweed systems for eutrophic water purification through converting wastewater nutrients to high-starch biomass: Comparative evaluation of three different genera (*Spirodela polyrhiza*, *Lemna minor* and *Landoltia punctata*) in monoculture or polyculture. RSC Adv., 8: 17927-17937.
20. Zhao, Z., H. Shi, D. Duan, H. Li and T. Lei *et al*., 2015. The influence of duckweed species diversity on ecophysiological tolerance to copper exposure. Aquat. Toxicol., 164: 92-98.
21. Petersen, F., J. Demann, D. Restemeyer, H.W. Olfs, H. Westendarp, K.J. Appenroth and A. Ulbrich, 2022. Influence of light intensity and spectrum on duckweed growth and proteins in a small-scale, re-circulating indoor vertical farm. Plants, Vol. 11. 10.3390/plants11081010.
22. Paolacci, S., V. Stejskal, D. Toner and M.A.K. Jansen, 2022. Wastewater valorisation in an integrated multitrophic aquaculture system; assessing nutrient removal and biomass production by duckweed species. Environ. Pollut., Vol. 302. 10.1016/j.envpol.2022.119059.
23. Li, Y., F. Zhang, M. Daroch and J. Tang, 2016. Positive effects of duckweed polycultures on starch and protein accumulation. Biosci. Rep., Vol. 36. 10.1042/BSR20160158.
24. Kittiwongwattana, C. and S. Vuttipongchaikij, 2013. Effects of nutrient media on vegetative growth of *Lemna minor* and *Landoltia punctata* during *in vitro* and *ex vitro* cultivation. Maejo Int. J. Sci. Technol., 7: 60-69.
25. Toyama, T., T. Hanaoka, Y. Tanaka, M. Morikawa and K. Mori, 2018. Comprehensive evaluation of nitrogen removal rate and biomass, ethanol, and methane production yields by combination of four major duckweeds and three types of wastewat er effluent. Bioresour. Technol., 250: 464-473.
26. Leng, R.A., J.H. Stambolie and R. Bell, 1995. Duckweed-a potential high protein feed resource for domestic animals and fish. Livest. Res. Rural Dev., Vol. 7.