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Characterization of environmental conditions required for production of livestock and fish fodder from duckweed (*Lemna gibba* L.)

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Abstract

The present study was conducted to evaluate and characterize the environmental conditions required for the cultivation and production of duckweed (*Lemna gibba* L.) growing in natural drainage water system, and cultivated in a polyethylene mesocosm pond for the determination of its potential as aquatic fodder rich in protein for both fishes and livestock. Ten plants and water samples were collected from ten sites of a natural drainage water system and analyzed for their physico-chemical characteristics and nutrients contents. The collected fresh frond colonies of duckweed were washed carefully and cultivated in two polyethylene mesocosms (2×4×0.4 m). One of them was filled with drainage water (0.3 m deep) and the other filled with underground water enriched by NPK source for one year.The optimum water depth was (30 cm) with transparency (20 cm), while the ideal pH for the best growth ranged between (6.5-8) and the optimum water temperature ranged between (25-35ºC). The optimum electrical conductivity ranged between 4000-14800 mmhos/cm, the dissolved oxygen ranged from 1 to 4 mg/l and the biological oxygen demand ranged from 11 to 33mg/l. The ideal mean PO_4 and NH_4 were 10-12mg/l respectively. The mean concentration of Ca, K, Mg, Na and HCO_3 were 50, 120, 36, 220 and 135 mg/l. The mean ash contents was 16, 16.4 and 17% for the three samples type, and the mean total fat contents were 10, 8.5 and 7.5% respectively, while the mean total fresh weight production was 290, 325 and 353ton/hectare/year in the natural conditions, cultivated on drainage water and cultivated on underground water respectively, that gave mean total dry weight 29, 31.5 and 32 ton/hectare/year. The results of analysis of the dry matter showed that the mean % of crude protein contents at the duckweed were 40, 41 and 44% for plants collected in nature, cultivated on drainage water and cultivated on underground water respectively. The mean protein concentration was higher in the cultivated plant than in the natural condition where it was 44%. On the other hand, the mean carbohydrates content was 12, 13 and 12% plants collected in nature, cultivated on drainage water and cultivated on underground water respectively, while the mean crude fiber content was lower in the cultivated plant on the underground water (7%) and higher in the natural growing plants (10%). The ability of duckweed to propagate rapidly by consuming nutrients makes it an excellent candidate for nutrients uptake from water and a good source of protein rich biomass suitable for feeding a wide range of animals including fishes.

Introduction

With the high cost of the protein required for feeding domestic animals and fishes to produce meat, it is important to find cheaper fodder. Duckweed (*Lemna gibba* L.) is a small vascular free floating flowering aquatic macrophytes belonging to family of Lemnaceae, which can be found worldwide on the surface of or slow flowing eutrophic fresh and brackish waters, forming a familiar green mats. Duckweeds can reproduce vegetatively by producing a daughter fronds which stay attached to the mother frond to form a colony until the maturation. A low temperature duckweds produce special fronds called turions which are rich in starch and serve as an overwintering form.

Duckweeds may also reproduce by forming flowers and setting fertile seeds (Hillman 1961) it can tolerate a wide range of pH (4.5-8.5) and high salts concentrations (up to 4000 mg/liter total dissolved solids) (Zimmo, 2003; Leng et al. 1995). Mkandawire et al. (2005) stated that when levels of nitrogen in the water are high, duckweeds store nitrogen as protein and ammonium ions as a useful N source. Ammonia was found toxic to duckweed when pH of water rises to the point that allows to the formation of free ammonia. The plants can tolerate very high ionized ammonia (NH_4-N) concentrations. Urea is the best fertilizer for the plant, and is rapidly converted to ammonia under normal conditions. Duckweeds tolerate concentrations of elemental N up to 375 mg/l (Rejmánková 1979**)**. The nutrients taken up by duckweeds are assimilated into plant protein. Under ideal growth conditions more than 40% protein content on dry weight is determined (Skillikorn et al. 1993). It is distributed naturally in water with decaying organic matter that supply the plant with growth nutrients (Smith and Moelyowati 2001). Duckweed species have an inherent capability to exploit favorable ecological conditions by growing more rapidly. Their wide geographic distribution indicates a high probability of sample genetic diversity and good potential to improve their agronomic characteristics through selective breeding (Skillicorn et al. 1993). It is usually used as a source for domestic animals and fish feeds where it has been fed to domestic animals and fishes as complement diets. Duckweeds are used as fish feeding in fresh and drying form (Hassan and Edwards 1992).

The main goal of this study is to evaluate and characterize the suitable environmental conditions needed for successful growth and biomass production of duckweeds in vitro and vivo and to assess of its nutrient composition as well as to determinate its potential as aquatic fodder rich in protein for fishes and livestock.

Materials and methods

Water and plant analysis

Ten plants and watersamples were collected from different sites at drainage water system where duckweed is the dominant species and analyzed for both physico-chemical characteristics and plant nutrients contents, according to standard methods for analysis of water and waste water (APHA 1992). Water pH, temperature, depth, transparency, electric conductivity and dissolved oxygen were measured *in situ* using the multi-probe system (model Hydra lab-Surveyor), and remeasured in the laboratory using bench-top. The biological oxygen demand (BOD), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na) and carbonate contents (HCO_3) of water samples were measured according to the standard method (APHA 1985, 1989 and 1992). The results were tabulated as minimum, maximum and mean values with standard deviation. The ten collected plant samples were weighed to determine the fresh weight (kg/m^2) then oven-dried at 135 °C for 2 hours where the dry weight of the samples were determined regard to its fresh weight (kg/m$_2$), then ground using plastic milling apparatus to appraise the total protein contents, total carbohydrates, crude fibre, lipid contents and total ash contents, according to the standard methods (APHA 1985, 1989 and 1992).

Experimental layout

The frond colonies of fresh duckweed collected from the drainage water system, were washed by clean water and cultivated in two polyethylene mesocosms (2×4×0.4 m), as the bottom covered with thick polyethylene material to prevent the seepage of water. One of the mesocosms was filled with drainage water (0.3 m deep) while the other was filled with underground water enriched by a continuous source of NPK as follow: triple super phosphate (TSP) was added as a source of phosphorus and calcium (Chokchai and Supachai 2013). TSP and urea was added (1:5 w/w) to the mesocosme. Urea as a source for N was applied to the duckweed at the rate of 2g^{-1} m^{-2} day^{-1}, which is equivalent to 0.9g^{-1} m^{-2} day of nitrogen. Assuming a 50 percent loss before the plant was able to utilize the nitrogen 0.45g^{-1} m^{-2} day^{-1} (Skillikorn et al.1993), where the harvesting extends for one year. The fresh plant sample was collected after three days of cultivation by using a circular mesh machine. The growth rate of the cultivated plants was determined, then, it is oven-dried at 135°C, for 2 hours and weighed as well as ground to a powder where the dry matter was used for determination of the total crude protein, total fate, total carbohydrate, the crude fiber and the total ash contents. Organic nitrogen content was estimated by using Micro-Kjeldahl method, then data were multiplied by 6.25 to obtain protein content values. The continues harvesting and weighed to determine the total biomass production at the large scale according to hectare and expressed as fresh weight per ton/hectare/year and the total plant production was expressed as dry weight per ton/ hectare/year.

Results

Water analysis

The results of water analysis of ten samples collected from different sites at drainage water system as shown in Table 1, revealed that the water depth ranged between 20-40cm with mean value of 30cm, while the transparency ranged between 15-25 cm with mean value of 20 cm and the water temperature ranged between 18-35 ºC with optimum temperature range of 25-30 ºC, the minimum recorded pH was 5, while the maximum recorded pH was 9 with mean value of 7, and the optimum range resulted 6.5-8. On the other side the electrical conductivity (EC) ranged between 4000-14800mmhos/cm, the mean value was 9200mmhos/cm. The minimum dissolved oxygen concentration for the duckweed growth is 1mg/l while the maximum concentration was 4mg/l with mean value of 2.5 mg/l, where the minimum concentration of the biological oxygen demand (BOD) was 11 mg/l and the maximum concentration of the BOD is 33mg/l, with mean value of 22mg/l. The phosphorus concentration (PO_4) at ten sites ranged between 8 and 12mg/l, with mean value10mg/l, and the NH_4 concentration ranged between between 9 and 15 mg/l with the mean value of 12mg/l. The concentration of calcium ion ranged between 30 and 70mg/l, with mean value of 50 mg/l and optimum range of 35-54mg/l, while the potassium concentration showed flocculation from 75 to 165mg/l, with mean value of 120mg/l. On the other hand the magnesium show minimum concentration of 28 mg/l and maximum concentration was 44 mg/l, with mean value of 36mg/l, the sodium concentrationin water samples ranged between 150 and 270mg/l, while the mean value was 220mg/l, and the minimum concentration of HCO_3 was of 95 mg/l, where the maximum concentration was 175 mg/l, with optimum range from 120-140mg/l.

Plant analysis

The analysis of ten samples of duckweed, collected from natural drainage water system, cultivated on drainage water and cultivated on underground water as shown in Table 2, revealed that, the mean total fresh weight production was of 290, 325 and 353ton/hectare/yea (in the natural, cultivated on drainage water and cultivated on underground water respectively), that gave mean total dry weight of 29, 31.5 and 32ton/hectare/year, respectively. On the other hand the mean water contents in the fresh plants are 91, 90 and 89%

Table (1): The physicochemical analysis of ten water samples collected from natural drainage water system dominated by *Lemna gibba L.* species.

Physicochemical Characteristics of water samples	Minimum concentration	Maximum concentration	Mean Value	Optimum range
Water depth (cm)	20	40	30 cm	25-30
Transparency (cm)	15	25	20 cm	20-25
Temperature (ºC)	18	35	32 ºC	25-30
pH	5	9	7	6.5-8
E.C. (mmhos/cm)	4000	14800	92000	5000-8000
DO (Dissolved oxygen) mg/l.	1	4	2.5mg/l	2-3
BOD (Biological oxygen demand) mg/l	11	33	22 mg/l	16-24
K (potassium) mg/l	75	165	120 mg/l	95-125
Mg (magnesium) mg/l	28	44	36 mg/l	30-37
Ca (Calcium) mg/l	30	70	50 mg/l	35-54
Na (sodium) mg/l	150	270	220 mg/l	180-210
Hco3(carbonate) mg/l	95	175	135mg/l	120-140
PO4 (phosphorus)mg/l	8	12	10 mg/l	7-9
NH4(TKN)mg/l	9	15	12 mg/l	10-12

in natural collected plant, cultivated on drainage water and cultivated on underground water respectively. The results of analysis of the dry matter showed that the mean % of crude protein contents of the duckweed were 40, 41 and 44% for natural collected plants, first cultivated on drainage water and cultivated on underground water respectively. The mean protein concentration was higher in the cultivated plants. However, the mean carbohydrates contents was 12, 13 and 12% for plants collected in nature, cultivated on drainage water and cultivated on underground water respectively, while the mean crude fiber content was lower in the cultivated plant with the underground water (7%) and higher in the natural growing plants (10%). The mean ash contents were 16, 16.4 and 17% for the three types respectively. The mean total fat content was 10, 8.5 and 7.5%, respectively.

Discussion

Duckweed is a small free floating aquatic macrophytes, monocotyledons belonging to family Lemnaceae that presents in slow flowing and still standing fresh and brackish water habitat across the world. The results of the study on this plant collected in ten sites of drainage water system, revealed that duckweeds can grow at water depth ranged between 20 and 40 cm with mean value 30 cm and the optimum water depth for best growth was 30 cm, while the measured water transparency ranged between 15 and 25 cm with mean value of 20 cm as well as water temperature ranged between 18 and 35ºC with optimum temperature range between 25 and 30 ºC. The minimum recorded pH was 5, while the maximum recorded pH was 9, with a mean value of 7 and the optimum pH ranged from 6.5 to 8. According to Leng et al. (1995) and Zimmo (2003), *L. gibba* can grow at water temperature between 6 and 33°C, where many species of duckweed tolerate low temperatures by forming a turions and the plant sinks to the bottom of a lagoon where it remains dormant until warmer water brings about a resumption of normal growth.

Also Abou EL-Kheir et al. (2007) found that *L. gibba* can use the nutrients and give best growth on drainage waste water habitat leading to decrease in nutrients concentration in water and this agrees with Nafea and Zyada (2015) that found that *L. gibba* can absorb nutrients from water and forms biomass rich in nutrients specially protein. Also Goopy and Murray (2003) confirmed that duckweeds can absorb nutrients from the waste and drained water forming biomass rich in protein, carbohydrates and pigments suitable for feeding domestic animals and fishes. On the other side, the electrical conductivity of the water samples collected from drainage system ranged between 4000 and 14800 mmhos/cm, and according to Leng et al. (1995) the plants can tolerate a relatively high concentrations of salts (up to 4000 mg/liter total dissolved solids). The mean value of dissolved oxygen concentration in the water dominated by *L. gibba* is 2.5mg/l, while the mean concentration of the biological oxygen demand (BOD) was 22mg/l. The phosphorus concentration (PO_4) at drainage water samples collected from ten sites ranged between 8 and 12 mg/l, with a mean value 10 mg/l, and the NH_4 concentrations ranged between 9 and 15 mg/l, with a mean value of 12 mg/l. Mkandawire et al. (2005) stated that when the level of nitrogen in the water is high, duckweeds store nitrogen as protein and ammonium ions as a useful N source. Ammonia was found toxic to duckweeds when pH of water rises to the point that allow to the formation of free ammonia. These plants can tolerate very high ionized ammonia (NH_4-N) concentrations where urea is the best fertilizer for the plant, and is rapidly converted

Table (2): Nutrients composition of *Lemna gibba* L. expressed as g/ 100 g dry mater

Parameters	Plant from natural drainage water			Plant cultivated on drainage water			Plant cultivated on underground water		
	min.	max.	mean	min.	max.	mean	min.	max.	mean
Total Fresh weight ton/hectare/year))	280	300	290	320	330	325	320	350	335
Total dry weight (ton/ hectare /year)	28	30	29	31	32	31.5	31	33	32
Water contents in fresh plant %	89	93	91	88	92	90	87	91	89
Dry matter %	7	11	9	8	12	10	9	13	11
Crude Protein %	35	45	40	37	45	41	40	48	44
Carbohydrates	11	13	12	12	14	13	10	14	12
Crude Fat %	8	12	10	7	10	8.5	6	9	7.5
Crude Fiber %	8	12	10	7.5	11	9.25	5	9	7
Ash contents %	15	17	16	15	18	16.5	16	18	17

to ammonia under normal conditions. Duckweeds tolerate concentration of elemental N up to 375 mg/l (Rejmánková 1979). The optimal nutrient profile of growth of duckweed doesn't necessarily produce the best quality of plant material in terms of protein content and digestibility. Leng (1999) has suggested that optimal protein content will be obtained where nitrogen is present at 60 mg N/l or greater. The concentration of calcium ion ranged between 30-70mg/l, with mean value of 50 mg/l, and optimum range of 35-54mg/l, while the potassium concentration shows flocculation from 75 and 165mg/l, with mean value of 120mg/l. The magnesium showed minimum concentration of 28 mg/l and maximum concentration was 44 mg/l, with a mean value of 36mg/l. The sodium concentration in water samples ranged between 150 and 270mg/l, while the mean value was 220mg/l and the minimum concentration of HCO_3 was 95 mg/l, where the maximum concentration was 175 mg/l, with optimum range from 120 to 140mg/l.

The results of analysis for ten plants of *L. gibba* collected from natural drainage water system, cultivated on drainage and underground water enriched by nutrients revealed that, the mean total fresh weight production was 290, 325 and 353ton/hectare/year in the natural, cultivated on drainage water and whose cultivated on underground water, respectively, that gave mean total dry weight 29, 31.5 and 32 ton/hectare/year. The mean water content in the fresh plants was 91, 90 and 89% plants collected in nature, cultivated on drainage water and cultivated on underground water respectively. The results of analysis of the dry matter showed that the mean % of crude protein contents at the *L. gibba* L. were 40, 41 and 44% for natural collected plants, cultivated on drainage water and cultivated on underground water respectively.

The mean protein concentration was higher in the cultivated plants than the natural growing ones where it was 44%. Leng et al. (1995) stated that the crude protein level of duckweeds depends primarily on the level of the nutrients concentrations in the water during the course of feeding program specially nitrogen and phosphorus where its level varied between 24 and 45%. On the other hand, the mean carbohydrates contents was 12, 13 and 12% for plants collected in nature, cultivated on drainage water and cultivated on underground water respectively, while the mean crude fiber content was lower in the cultivated plant on the underground water (7%) and higher in the natural growing plants (10%).

The mean ash contents were 16, 16 and 4.17% for the three types respectively. The mean total fat contents were 10, 8.5 and 7.5%. The ability of duckweeds to propagate rapidly by consuming nutrients makes it an excellent candidate for nutrients uptake from water and a good source of protein rich biomass suitable for feeding a wide range of domestic animals including fishes and a promising source of protein used as fodder for livestock, this is in the same trend of the data obtained by Skillikorn et al. (1993) that analyzed the duckweed plants which growing on a waste water pond and in a culture pond enriched with fertilizers where the fresh duckweed had from 92 to 94 % water content and dry matter contain high fiber and ash contents from 10 to 16.7% respectively. The protein contents ranged between 35 and 45%, with higher concentrations of essential amino acids (lysine and methionine) than most plant proteins, as well as, it contain high concentrations of trace minerals and pigments, particularly beta carotene and xanthophylls, that make duckweed meal an especially valuable supplement for domestic animals (Porath et al. 1979).

Gaigheret et al. (1984) and Chokchai and Supachai (2013) stated that the protein content in the cultivated duckweeds at a beef cattle feedlot reached 44% while those cultivated on piggery effluent, had a crude protein level of 39% and this protein could be used as an effective protein supplement in fish and domestic animals feeding.

The biomass production from the culture pond for duckweeds ranged between 280 and 350 ton / hectare/ year, which give about 29-32 ton/hectare/ year of dry matter.

Under the optimum conditions of available sun light, temperature and nutrients, duckweeds can double their mass between 16 to 48 hours,which is faster than almost any other higher plant (Leng et al. 1995). So to obtain an optimum growth rate of duckweeds, this plant needs to be managed, protected from wind, maintained at an optimal density by judicious and regular harvesting and fertilized to balance nutrient concentrations in water. Leng et al. (1995), stated that the good managed culture of duckweeds will give from 25 to 35 ton dry matter/ha/year, containing up to 45% crude protein and a highly digestible dry matter.

A total of seven feeding trials using duckweeds were applied to fishes and livestock by Rural Industries Research and Development Corporation by Bio-Tech Waste Management Pty Ltd (1998), confirming that these plants can be used as protein sources. Also it can be used in fresh or in dry forms for both fishes and domestic animals, this is assured also by findings

Fig. 1. Cultivation of *L. gibba* L. in mesocosms

of Haustein et al. (1988,1992) and Mkandawire et al. (2005), where they used duckweeds to feed domestic animals by different forms: as fresh diet for cheeps and pigs, mixed with corn and maize for feeding chickens that leading to reduction of amounts of soy bean used in diet, increasing of eggs production and improved hatching rate. On the other hand they reported that the growth rate of carps increased in ponds supplemented with fresh duckweeds. Hassan and Edwards (1992) mentioned that the growth rate of *Tilapia* and feed conversion to supplementation with duckweeds at the rate of 30 g duckweed DM per 1 kg fish live weight is in linear relation. Younis and Nafea (2015) stated that the aquatic plants have high concentrations of nutrients and are a promising source for animal feed including fishes. Robinson et al. (1980) used duckweeds in the diet of Catfish giving high growth rate and high conversion values.

Conclusions

The Egyptian environmental conditions are more suitable for the cultivation of duckweeds as it can be used to feed fishes and livestock in both dry and fresh forms in a safe way when collected from unpolluted water bodies and produced using clean, enriched and good managed underground water.

The application of duckweeds in the field of fish culture and livestock feeding will have a positive impact on the national economy in several ways as it considered as a cheap source of fodder with high quality protein, low fiber contents, high minerals which save and reduce the uses of the other expensive sources of protein like soy bean and fish protein.

The study recommended to cultivate duckweeds in the drainage water system in the small canals and drains for nutrients removing and biomass production as a new fodder for domestic animals and fishes.

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