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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(4): 2604-2613. **OPEN ACCESS**

Effect of pH levels on duckweed's proximate composition for utilization as poultry and fish feed

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To investigate the effect of various levels of pH on the carbohydrate, minerals, and protein contents of duckweed (*L. minor*), an experiment was conducted in Department of Weed Science; the University of Agriculture Peshawar-Pakistan during 2016. The experiment was laid out in Completely Randomized Design (CRD) with 3 repeats. Data were taken on protein, lipid, carbohydrate and mineral contents. The laboratory trail consisted of pH levels from 4-10 as treatments. The highest protein, lipid and carbohydrate contents were recorded in control and pH 8 followed by pH 6 as compared to the lowest value in pH 4 and 10. Maximum mineral contents (Ca, Mg, Fe, Mn, and Zn combined) were recorded for pH 5, 8, 9, 10 and control, while lowest mineral contents were noted in pH 4 and 6. It is concluded from the results of the experiment that pH 7±1 was the favorable pH for maximum performance of the duckweed and beyond 8 and below 4 pH level the plant efficiency was negatively affected.

Keywords: Proximate analysis, Water pH, *Lemna minor* L., Complete Randomized Design, Peshawar.

INTRODUCTION

Agriculture, pisciculture, poultry and livestock share the same challenge of increasing food demand and consequently face increased competition in the use of feed. Shortage of major feedstuffs has increased today globally with expansion of poultry and livestock industry to meet the demand of rapidly growing population. Aquaculture industry is also finding it increasingly difficult to find a more economical source for essential feed ingredients (Fagbenro et al. 2003). Poultry production plays a key role in the economic uplift of the developing countries of the world (Alders and Pym, 2009; Kondombo, 2005). One of the major difficulties of poultry production is the insufficient supply of quality feed and high cost of feed ingredients particularly in developing countries. Poultry producers have to resort to domestic wastes and anything edible all around, which fail to contain the essential feed ingredients (Mehari, 2016).

In Pakistan indigenous feed resources are inadequate to meet livestock and poultry demand. The gulf between supply and demand for dry biomass, crude protein and metabolizable energy are 19.4%, 37.2% and 38.0%, respectively. Crop residues make the dominant portion of feed supply followed by fodder and grazing successively. Grains and by-products share 8.2% of the total supply of feed. In order to bridge the gulf between supply and demand large quantities of oil seed meal and other feed stuff is being imported to the country. With the rapid expansion in poultry, dairy and feedlot, the feed gap is widening with rapid pace. The trend of increasing feed demand has shifted the focus of animal nutritionists on efficient and sustainable utilization of the local nonconventional feed resources

(Habib et al. 2016).

The unavailability and rising cost of high quality protein feed constituents has captured the attention of animal nutritionists towards alternative source of protein in diet formulation. There are some unconventional sources of proteins, carbohydrates and minerals which can effectively be used as animal feed to get products with good nutritive value. Duckweed (*Lemna minor* L.) is one of them, which offers a cheaper source of feed in many developing countries like Pakistan. It is among the smallest angiosperms. It reproduces mostly vegetatively through budding in a short span of about two days if the environmental conditions are favourable (Journey et al. 1993). Duckweeds absorb and mitigate nutrient enrichment in eutrophic waters. They are effective in absorbing organic and inorganic nutrients from waste water of ponds, ditches, sewerage water and other water bodies. They effectively scavenge toxic metals and can be applied for the phytoremediation of waste waters. They are adapted to a wide range of climatic conditions but grow best in water with 06-33ºC (Leng et al. 1995). They are fairly tolerant to frost and can withstand stress conditions.

Duckweeds are good source of biomass production and yields of 10-30 tons dry biomass ha^{-1} year^{-1}, containing sufficient quantity of crude protein with good amino acid profile. Duckweed growth is affected by nutrients imbalance, toxic substances in medium; limits of pH and temperature; population density and competition with other plants. However, in favourable conditions duckweeds synthesize sufficient quantity of proteins, lipid, starch and minerals till the critical value nutrients is reached (Leng et al. 1995). In countries like Vietnam, poultry production relies on agricultural productivity, and accounts for 19% of the total livestock production (Vang et al. 2001). Poultry is owned by farmers on small scale, fed by agriculture by-products. *L. minor* feed resemble soyabean meal. Factors affecting nutritional composition of duckweeds are, water pH, temperature and nutrients. Duckweeds are rich in essential amino acids, which is deficient in other plant proteins (Porath et al. 1979). It is also a cheap source of minerals, beta carotene and xanthophylls (Journey et al. 1993). In comparison to terrestrial plants it contains 10x higher beta-carotenes and xanthophylls (Mbagwu and Adeniji 1988).

The use of duckweed as poultry feed has been established by many authors (Islam et al. 1997; Samnang, 1999). Its feed in poultry diet has shown remarkable outcomes in production of egg pigments and broiler skin (Haustein et al. 1990). Higher weight gain in chicks up to 3 week of age took place when fed with dehydrated duckweed up to 5 % of mixed feed (Truax et al.1972). Sewage grown duckweeds have been successfully used as substitute of Soyabean meal up to 15% in the diet of poultry for good egg and high yolk production (Haustein et al. 1990). High protein contents and amino acid balance along with vitamins and minerals make the duckweed an ideal candidate for animal feed (Landolt and Kandeler 1987; Men et al. 2001).

Being a reliable source of protein, carbohydrate and minerals, *L. minor* can be utilized for poultry as well as fish feed with a dual benefit of weed management, biomass disposal and protein supplement in the feed where it is easily available. The present study was conducted to evaluate the effect of pH levels on *L. minor*, and to know the favourable and inhibitory range of pH for the plant growth and to recommend the optimum pH range for management as well as production of *L. minor* under the climatic condition of (Khyber Pakhtunkhwa), Pakistan.

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MATERIALS AND METHODS

Experimental Site Description

An experiment was conducted to evaluate the effect of various levels of pH on the carbohydrate, minerals and protein contents of duckweed (*L. minor*). The experiment was carried out in Department of Weed Science, the University of Agriculture Peshawar, during April 2016, using Completely Randomized Design (CRD) with various treatments, replicated three times. Each pot was 21 inches in diameter and 10 inches deep. *L. minor* was collected from local fresh water bodies. A 10grams fresh biomass of the weed was transferred to each pot already amended and labeled with treatments i.e. pH 4, 5, 6, 7 (as control), 8, 9 and 10 in their respective replications. Data were recorded on the following parameters by the method given below for each parameter:

Protein contents were investigated by using Kjeldahl method. A known mass of oven dried sample was oxidized in sulphuric acid (H_2SO_4) by using a catalyst like ($CuSO_4$). All nitrogen present in the sample was converted into ammonium sulphate $(NH_4)_2SO_4$, the inorganic form. On reaction with NaOH, ammonium sulphate was released and distilled as ammonium hydroxide which was then accumulated in boric acid (H_3BO_3)

solution followed by titration with standard solution as shown by the following equation (Hach et al. 1985).

Powdered sample + H_2SO_4 $\rightarrow$ $(NH_4)_2 SO_4 + CO_2 + H_2O$

$(NH_4)_2SO_4 + 2NH_4OH + 2NaO \rightarrow NH_4OH + Na_2SO_4$

$2NH_4OH + H_3BO_3 \rightarrow (NH_4)_2BO_3 + 2H_2O$

$(NH_4)_2BO_3 + HCl \rightarrow 2NH_4Cl + H_3BO_3$

Calculations

The percent nitrogen and crude protein was calculated as follows

$$\% \text{ N} = \frac{(\text{S}-\text{B}) \times 0.014 \times \text{D} \times 100}{\text{Wt.of sample} \times \text{V}}$$

Where S = Volume of standard acid used for sample titration

B = Volume of standard acid used for blank titration

D = Sample dilution after digestion (volume ml)

*0.014 is the milli equivalent weight of nitrogen

Lipid content

Fat was extracted through Soxtec apparatus comprising an Extraction Unit and a Control Unit. The Extraction Unit was installed in a well-ventilated fume hood (air flow 0.5 ms^{-1}). A (0.5 grams) sample to be analyzed was weighed into thimbles and inserted in the Extraction Unit, containing extraction flasks. One-third of the flask was filled with petroleum ether which was used as solvent. The flasks were heated by the electrical heating plate. The three step extraction procedure consists of boiling, rinsing and recovery. First the solvent was heated. The heated solvent dissolved crude fat from the sample. Solvent was then heated to evaporate and recover by condensation. The flasks were dried in 100°C for one hour. The flasks were weighed again to calculate the difference in weight (Thiex et al*.* 2003).

Calculations

Weight (g) of sample used for extraction = W_1
Weight (g) of flask without fat = W_2
Weight (g) of flask with fat = W_3

$$\text{Extractable fat (\%)} = \frac{W3-W2}{W1} x\ 100$$

Mineral contents

Mineral contents were investigated by Atomic Absorption Spectrometry (AAS) laboratory of Department of Water Management Science, the University of Agriculture, Pakistan. Wet ashing /Acid digestion method was used for sample preparation. The digestion was made in Perchloric Acid. After digestion glass filter was used to filter the sample and 100ml volume was made with distilled water (Ghaedi et al. 2007).

Mineral Mg/100g sample = (AAS result) X volume of sample) X dilution factor/weight of sample Carbohydrate contents

Carbohydrate contents were investigated by sing the following formula:

% Carbohydrate content = 100 g Dry Sample - (Mineral content + Protein +Lipids) x 100

Analysis of the data

The data were analyzed by using computer packages Mstat-C GenStat software by using LSD test at 0.05 level of probability (Steel and Torrie, 1980).

RESULTS

The effect of pH levels was investigated on biochemical parameters of duckweed like, protein, lipid, carbohydrate and mineral composition of the plant. In most of the cases the pH significantly affected the parameters and it is clear to mention that variation in pH greatly changed the proximate composition of the plant. The overall effect is explained in the following paragraphs, for each parameter:

Protein contents (%):

Result obtained from experiment on effect of pH ranges on crude protein content revealed significant variation in protein content. Higher percentage of crude protein was observed between pH 6 and 8. The result also showed a maximum percentage of crude protein, (33 %) for plants grown in a medium maintained at pH 7. While (29.33 %) and (29.66 %) of crude protein were obtained from plants grown in pH 6 and pH 8, being the succeeding values as shown in (Table1, Fig. 1). Neutral pH favors nutrients availability to duckweed plants particularly nitrogen which is the key element in all amino acids. At the same time minimum protein contents (21.67 %) and (23.67 %) were recorded for pH4 and pH10 respectively, showing that protein content is affected by both acidic and basic pH. Crude protein content of duckweeds grown in different aquatic media ranges between (7- 45 %) depending upon the availability of nitrogen (Culley et al. 1981). According to FAO (2001) duckweed growth was best in the presence of high amount of nitrogen while Chakrabarti et al. (2018) reported protein content was high in *Lemna minor* which was cultured in organic matter.

Lipid Content (%)

Statistical analysis of the data discovered that pH variation in growth media alters lipid content of duckweed. Table 1 showed that maximum lipids content (8.33 %) were recorded for plants treated with pH 8 and pH 7 (7.66 %), while the lowest (2.67 %) were noted in pH 10. Lipids are synthesized through metabolic process which involves fatty acid synthesis and incorporation of fatty acid to triglycerides and other types of lipids (Fig. 2). The reason might be the favourable pH for synthesis lipid components. The whole metabolic process operates best at pH 8 (Mesocarp *et al.,* 1961) which is in accordance with the present study. Above pH 8 and below pH 5 fatty acid mobility decreases, resulting in low lipid metabolism by inhibiting lipid biosynthesis metabolic pathways.

Carbohydrate content (%)

Analysis of data regarding carbohydrate content indicated significant variation in carbohydrates percentage due to pH treatment effect. Maximum carbohydrate content (59.67 %) was noted in plants grown at pH 8, while minimum carbohydrate contents (50.67 %) were recorded in plants grown at pH 10, which is followed by pH 4 (52 %) (Table1, Fig 3). The data showed remarkable effect of pH on carbohydrate content of duckweed. Low carbohydrate content in pH4 might be because of decrease in quantum efficiency of photosystem II in acidic pH as recorded by Thornton (2009). Metabolic processes and carbon assimilation can best occur at neutral pH therefore more carbohydrates were observed in neutral or near to neutral pH.

Mineral content (%)

Mineral contents also varied with the variation in pH levels of growth media. Overall percentage of 5 elements, Ca, Mg, Fe, Mn, and Zn, is depicted in Tab. 2. A maximum of 0.102 % mineral contents was calculated for neutral pH (control). The result is because of more absorption of Ca and Mg in neutral pH. While lowest mineral accumulation (0.0437 %) was seen in pH4 which is strongly acidic. Our result is in agreement with those of Gambrell and Patrick (1988), who concluded highest absorption of macro and micronutrients in neutral pH.

Calcium (Ca) %

Results obtained from experiment data for the determination of % calcium content can be seen in Tab. 2, Fig. 4. Maximum calcium (0.029 %) was recorded for duckweeds grown in pH 7 (neutral), while minimum calcium (0.0038 %) was recorded for plants treated with pH 5 and pH 6 each. The result shows maximum calcium absorption in water having neutral pH. This absorption is attributed partly to the root length developed by duckweeds in water with neutral pH and partly the availability of calcium ions to the plant. Low pH for example, can cause phytotoxicity of some metals (Mn, Al) on one side whereas it minimizes the availability of dissolved carbon dioxide and other nutrients like (calcium, magnesium) while a high pH can reduce the availability of phosphate, sulphate, iron and manganese. Our results are in line with those of Gambrell and Patrick (1988) and Handreck and Black (2002) who also obtained similar results of (Ca) absorption.

Magnesium (Mg) %

Analysis of data about % Mg (Table 2, Fig. 5) reveals that maximum Mg (0.0293%) was recorded for plants cultured in pH 9 followed by pH 8 (0.0287%). Lowest value (0.0029%) for Mg percentage was recorded from treatments having pH 4. This shows an increased uptake of Mg in slightly alkaline pH in comparison to acidic. This result indicates maximum availability of Mg between control treatment and pH 10. Also a marked difference was observed in root length of the plant treated with different pH levels. Plants grown in neutral pH showed larger root length as compared to low pH as for uptake of minerals *L. minor* is entirely dependent on the tender roots.

Iron (Fe) %

The data presented in Table2 showed maximum Fe content (0.054%) for pH 5 treated plants, which indicates high Fe uptake in moderately acidic pH (Fig. 6). In low pH medium coupled with mild redox enhances the availability of micronutrients particularly metals. Gambrell and Patrick (1988) also recorded similar results for Fe uptake. Minimum Fe content (0.0257%) was recorded for pH 10, indicating a reduced Fe uptake at alkaline media. The optimum pH for iron availability is concluded to be moderately acidic as evident from iron contents at pH 5.

Table 1: Effect of pH levels on protein, lipid and carbohydrate content of duckweed (*Lemna minor*)

Treatments (pH)	Protein content (%)	Lipid content (%)	Carbohydrate (%)
4	23.67	6.50	52.00
5	27.00	6.80	53.00
6	29.33	7.93	56.00
7(Control)	33.00	7.66	59.00
8	29.67	8.33	59.67
9	26.33	5.67	55.00
10	21.67	2.67	50.67
LSD (0.05)	2.5349	0.8672	4.7114

Table 2: Effect of pH levels on mineral content of duckweed (*Lemna minor*)

Treatment (pH)	Ca (%)	Mg (%)	Fe (%)	Mn (%)	Zn (%)	Minerals Cumulative (%)
4	0.0115	0.0029	0.0290	0.0002	0.00007	0.0437
5	0.0038	0.0240	0.0540	0.0003	0.00005	0.0847
6	0.0038	0.0267	0.0273	0.0000	0.0001	0.0581
7(Control)	0.029	0.0280	0.0277	0.00001	0.00004	0.0852
8	0.0260	0.0287	0.0367	0.00007	0.00003	0.0914
9	0.0293	0.0293	0.0253	0.0003	0.00003	0.0843
10	0.0303	0.0283	0.0257	0.00008	0.00001	0.0844
LSD (0.05)	0.0018	0.0033	0.055	0.00008	0.00008	0.009

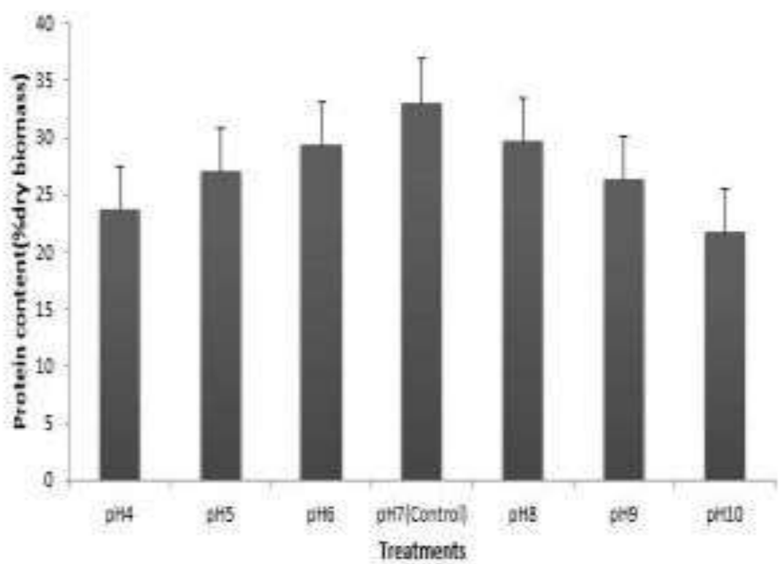

Fig. 1. Effect of pH levels on protein content (% dry biomass) of *Lemna minor*.

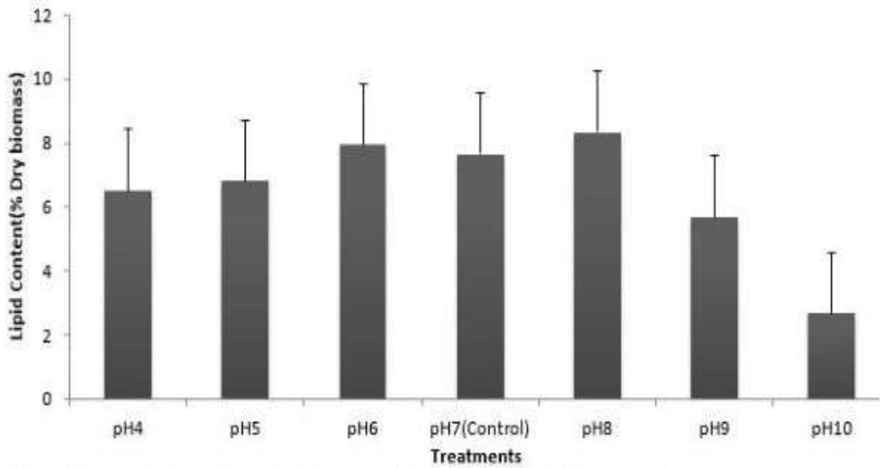

Fig. 2. Effect of pH levels on lipid content (% dry biomass) of *Lemna minor.*

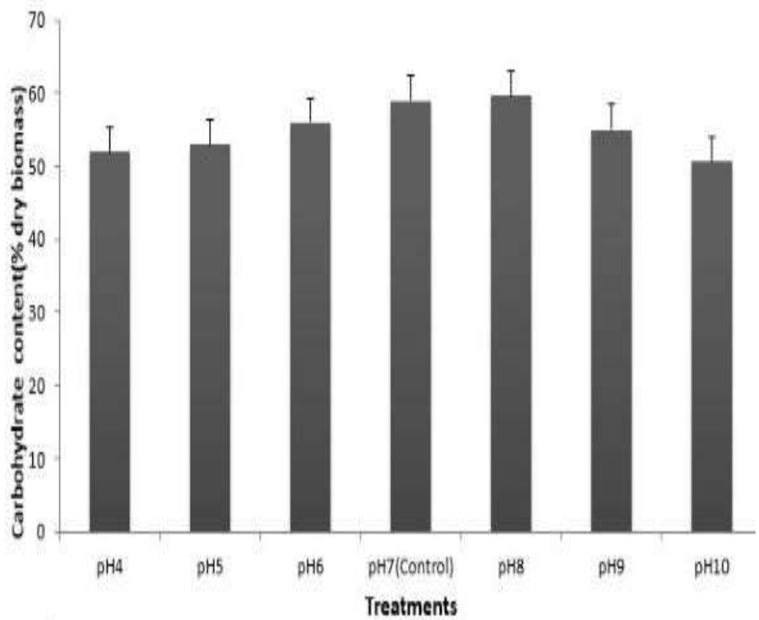

Fig. 3. Effect of pH levels on Carbohydrate content (% dry biomass) of *Lemna minor.*

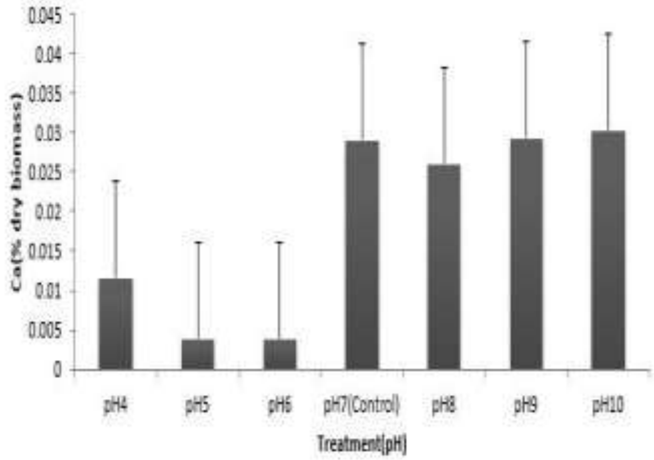

Fig. 4. Effect of pH levels on (Ca) content of *Lemna minor.*

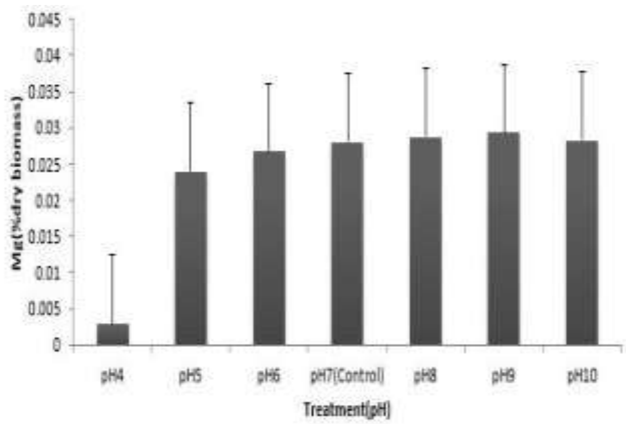

Fig. 5. Effect of pH levels on (Mg) content of *Lemna minor.*

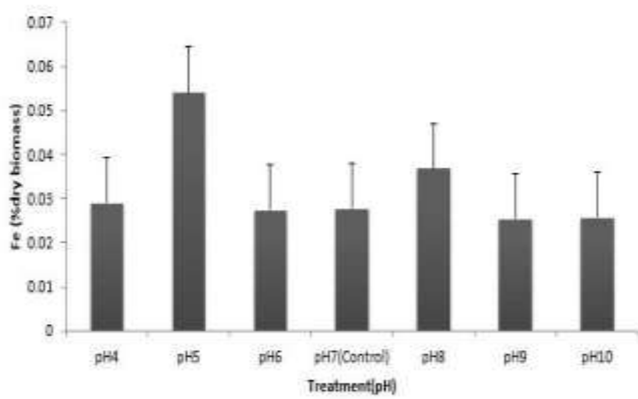

Fig. 6 Effect of pH levels on (Fe) content of *Lemna minor.*

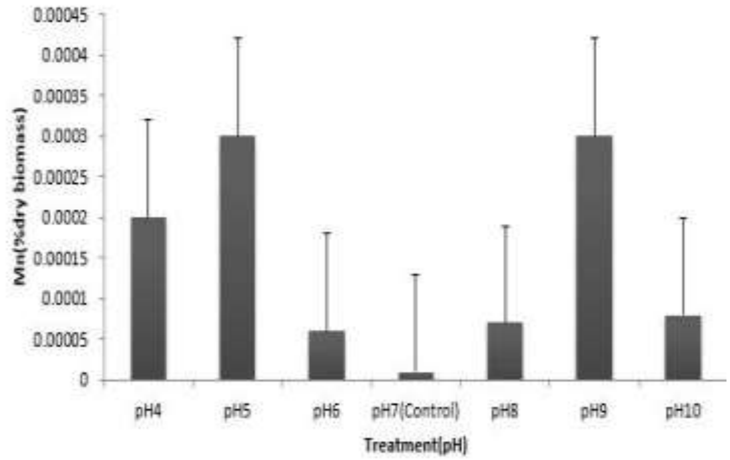

Fig. 7. Effect of pH levels on (Mn) content of *Lemna minor*.

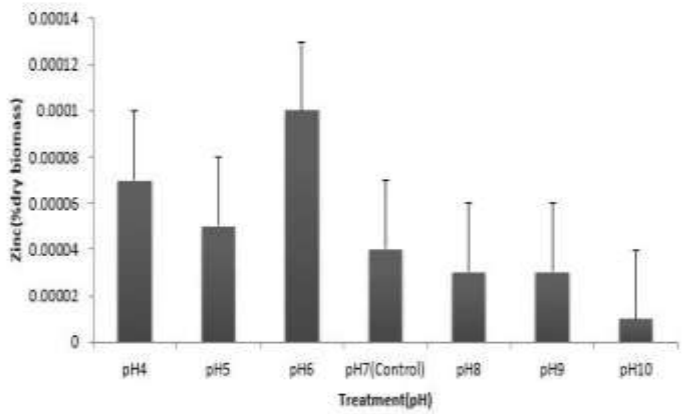

Fig. 8. Effect of pH levels on (Zn) content *L. minor*.

Manganese (Mn) %

Manganese is a trace element but its absorption at different pH level has shown a significant difference. A maximum value of (0.00287%) was noted (Tab. 2, Fig. 7) for plants grown at pH 5. Minimum values of Mn (0.00006%) and (0.00007%) were obtained for those plants grown in medium with pH 6 and pH 8, respectively. Trace element like (Mn) is available for biological absorption at moderately acidic pH (Gambrell and Patrick 1988) and they become less available for absorption at basic pH (Handreck and Black 1994). Trace elements also show specificity to pH regarding their solubility. This specificity differentiates absorption of micronutrients and macronutrients.

Zinc (Zn) %

Zinc is a trace element which occurs in very small/ traces concentration. The analysis of data for Zn contents after treatment of duckweed with different pH levels shows maximum percentage of zinc (0.00017%) for plants treated with pH 6. This shows more absorption of zinc at moderately acidic medium, which is true for most of the trace elements like Mn, Cu, and Fe. It has been observed that absorption of micronutrients increases with low pH Jackson and Caldwell (1993) concluded that low pH coupled with mild redox increases the availability of micronutrients. Therefore, it was concluded from the experimental results (Tab. 2, Fig. 8) that minimum Zn (0.000017%) was obtained for plants grown in high pH 10. This indicated less absorption of Zn at basic pH.

To investigate the effect of levels of pH on the

carbohydrate, minerals, and protein contents of duckweed (*L. minor* L.), the experiment was conducted in CR design with three replications. The experiment consisted of 7 pH levels (4-10). Data were recorded on protein, lipid, carbohydrate and mineral contents. The results showed that protein, lipid and carbohydrate contents were negatively affected by highly acidic (pH 4-5) as well as basic pH (9-10) medium. Mineral content analysis for the individual elements (Ca, Mg, Fe, Mn, and Zn) as well as their combined percentage showed that maximum mineral contents were found in control, which might be due to the best absorption of Ca and Mg in neutral pH. While lowest mineral content was noted at pH 4.

CONCLUSION

The effect of pH was significant on the protein, lipid, carbohydrate and mineral contents of *L. minor* L.

The proximate composition of the plant showed that it contains a fair quantity of all nutrients like proteins, essential amino acids, lipids, carbohydrates and minerals.

Due to its rapid growth it produces a lot of biomass and its short life cycle provide us an opportunity to be harvested twice a week.

Its chemical composition is sensitive to the growing conditions and its biochemical contents like proteins, minerals and carbohydrates can be manipulated by changing the pH of the growth media.

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CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

Higher Education Commission Pakistan (HEC) is highly acknowledged to support the research.

AUTHOR CONTRIBUTIONS

Hafiz Ullah performed the experiment. Bakhtiar Gul and Haroon Khan Designed and analyzed the experiment. Hafiz Ullah and Ishfaq Hameed wrote the manuscript. All authors read and approve the manuscript.

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