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Production potential of greater duckweed *Spirodela polyrhiza* (L. Schleiden) and its biochemical composition evaluation

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ABSTRACT

The culture technique of greater duckweed *Spirodela polyrhiza* (L. Schleiden) was standardized in outdoor tanks using three different manures: manure 1 - cattle manure, poultry droppings and mustard oil cake, manure 2 - urea, potash and triple superphosphate and manure 3 - cattle manure, urea, potash and triple superphosphate. Significantly (p<0.05) higher production was recorded in manure 1 compared to others. Manure 1 was subsequently selected for pond culture. In ponds, the production of duckweed was 2020±150 kg ha^{-1} $month^{-1}$ dry weight basis. Protein content was significantly higher (p<0.05) in duckweed cultured in manure 1. The amino acid profile study showed the presence of essential (37.4%), non-essential (58.2%) and free (4.5%) amino acids. Leucine, isoleucine and valine contributed 51.4% of total essential amino acids. Duckweed contained 7% lipid and α-linolenic acid (36-37%) was the major fatty acid. The study showed the nutritional value of duckweed as an animal feed ingredient.

Keywords: Spirodela polyrhiza, Organic manure, Proximate composition, Amino acids, Fatty acids

1. Introduction

The greater duckweed *Spirodela polyrhiza* (L. Schleiden) is a free-floating, fast growing aquatic plant, widely distributed in the still and slow-flowing water bodies globally. Morphologically, this monocotyledon plant is simple and lack specialised structures such as leaves or stems, but consist of flat ovoid leaf-like structures termed fronds with a rootlet for stabilisation. The bright green (upper part) and purple (lower side) colours of the fronds enhance its aesthetic value and make it suitable candidate for aquarium. Recent study shows the whole genome sequencing of *S. polyrhiza*, the most primitive member of Lemnaceae family (Michael et al., 2017; Hoang et al., 2018). It is a useful tool for further investigation with this duckweed. In accordance with other Lemnaceae, the usefulness and potential of *S. polyrhiza* has been recognized in recent days. It has utilisation for various purposes such as waste water remediation as it is able to remove nitrogen (particularly ammonia) with high efficiency (Culley and Epps, 1973; Sutton and Ornes, 1975, 1977), bio-fuel production (Jarvis et al., 1998; Zhao et al., 2012, 2014) and recombinant protein production (Khvatkov et al., 2018). It is also reported as a promising substrate for bio-hydrogen production, and recognised as an ideal plant in bioremediation and carbon cycle research (Kuehdorf et al., 2014; Olah et al., 2015; Tang et al., 2014; Wang et al., 2012, 2015; Xu and Deshusses, 2015; Xu et al., 2015). The copy number of the genes involved in the biosynthesis of two enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT) are amplified in greater duckweed. GS and GOGAT are the major biochemical module for ammonium assimilation (Wang et al., 2014). In recent year, duckweeds are also considered as rich protein source for human consumption (Appenroth et al., 2018; de Beukelaar et al., 2019).

S. polyrhiza is also gathering interest as a feed material/ingredient for fish, poultry and pigs (Cruz-Velásquez et al., 2014; FAO, 2001; Hasan and Chakrabarti, 2009). Less fibre content of the plant makes it easily digestible. In grass carp *Ctenopharyngodon idella*, a 75%

digestibility of *S. polyrhiza* has been observed (Wee, 1991). Similarly, analysis of the proximate composition showed that *S. polyrhiza* are a rich source of protein, although content varies from 23.8 - 40.9% (Hasan and Edwards, 1992; Hillman and Culley, 1978). Amado et al. (1980) reported the amino acid composition of 94 different strains of duckweeds. They suggested that all essential amino acids (except methionine) are present in sufficient amount in all strains of duckweeds. Recently, Appenroth et al. (2017) found around 25% protein level in *S. polyrhiza* cultured in nutrient medium. They also suggested that the levels of critical amino acids in duckweeds are within the recommended range of World Health Organization (WHO) for human. It is also a rich source of pigments, especially carotene and xanthophylls (Leng et al., 1995). Notably the nutritional and biochemical value of such macrophytes is highly variable and depends largely on water quality of the culture system (Boyd, 1971). Therefore, there is an urgent requirement to develop large-scale culture techniques for the production of nutrient-rich duckweeds. There is immense scope for large scale production of duckweeds in tropical climate (Chakrabarti, 2017).

In intensive management, supply of water and nutrient are essential for the continuous duckweed production of a predictable and useful biochemical composition (Hasan and Chakrabarti, 2009). Moreover, duckweeds are commonly cultured in wastewater which may contain unwanted components that are unsuitable for consumption by fish, other livestock, and ultimately human consumers. Inorganic and organic manures were successfully applied in Bangladesh for the production of duckweeds (BFRI, 1997; DWRP, 1998). The aim of the present study is to standardise the culture technique for the production of greater duckweed *Spirodela polyrhiza* in small tanks, and then large-scale production in ponds. The proximate, amino acid and fatty acid profiles of cultured *S. polyrhiza* are evaluated to establish its nutritional quality and suitability as an animal feed ingredient.

2. Materials and methods

101 *2.1. Tank culture*

102 *S. polyrhiza* were cultured in cemented outdoor tanks (1.2 m x 0.35 m x 0.30 m) using
103 both organic manures and inorganic fertilizers between December 2016 - March 2017. Three
104 different combinations of manures used for the production of duckweeds were as follows.
105 Manure 1: cattle manure, poultry droppings and mustard oil cake (1:1:1) were used at the rate
106 of 1.052 kg m^{-3} (Srivastava et al., 2006). Manure 2: urea, potash and triple superphosphate were
107 used at the rate of 20, 4 and 4 kg ha^{-1} day^{-1}, respectively (DWRP, 1998). Manure 3: cattle
108 manure, urea, potash and triple superphosphate were used at the rate of 750, 7.5, 1.5 and 1.5
109 kg $ha^{-1}day^{-1}$, respectively (BFRI, 1997). There were three replicates for each treatment. *S.*
110 *polyrhiza*, grown in the outdoor facility was introduced in the culture tanks (15 g $tank^{-1}$, fresh
111 weight) after 5 days of manure application. All tanks were re-manured at 10 day intervals for
112 sustainable duckweed production. In manure 1, organic manures were applied at a rate of one
113 fourth dose of the initial dose. In manure 2 and manure 3, the amount of manure was equal to
114 the initial dose. All manures were decomposed (5 days) before application. In each treatment,
115 when the surface was fully covered, harvesting was initiated, except the fifth harvest in manure
116 3. At the time of fifth harvest, growth of duckweeds was poor in this treatment; duckweeds are
117 totally harvested from all treatments. In all harvests (except the final), 50% of the total
118 duckweeds were collected; all duckweeds were collected after 118 days of culture and the
119 production was recorded as kg ha^{-1} $month^{-1}$ (dry weight, DW).

120 *2.2. Pond culture*

121 Three cemented ponds at the Central Institute of Fisheries Education (Indian Council
122 of Agricultural Research), located at Rohtak, Haryana were used for the production of *S.*
123 *polyrhiza* between July - August 2017. Each pond was 200 m^2 (20 m x 10 m) with water level
124 maintained as 50 cm. Among the three manures used in the tank culture of greater duckweeds,
125 highest production was obtained in manure 1, and so this treatment was selected for pond

126 production. All the organic manures, cattle manure, poultry dropping and mustard oil cake
127 (Srivastava et al., 2006) were decomposed for 5 days initially. *S. polyrhiza* cultures were
128 produced in a clean environment (outdoor tanks of Department of Zoology, University of
129 Delhi); then the plants were introduced in each pond at the rate of 1 kg pond^{-1} (fresh weight).
130 Initially, these greater duckweeds covered a small area of the water body (Fig. 1). In each pond,
131 after the initial dose, one fourth dose of manure was applied at intervals of 10 days. Greater
132 duckweeds were harvested thrice at 10 days intervals during 30 days of culture period. The
133 harvesting pattern was similar to tank production, i.e. duckweeds were harvested when the
134 whole water surface was covered. In first and second harvest, 50% duckweeds were harvested
135 and plants were totally collected during the third harvest. The production was expressed as kg
136 ha^{-1} month^{-1} (DW).

137 *2.3. Water quality*

138 Major water quality parameters were recorded at weekly intervals in both tanks and
139 ponds. A Solar Light lux meter (PMA 2100, USA) was used for the measurement of light
140 intensity in the outdoor systems at fixed time (10.00 a.m.) and it was expressed as an average
141 of replicates of individual treatment. A HACH Multi-meter (HQ 40d, USA) was used for
142 the estimation of temperature, pH, conductivity, dissolved oxygen, ammonia and nitrate
143 levels. Standard methods were followed for the estimation of phosphate and nitrite levels of
144 water (APHA, 2012).

145 *2.4. Relative Growth Rate (RGR)*

146 The RGR of *S. polyrhiza* was estimated with the formula:

147 $RGR = \ln (W_t/W_0)/t$

148 Where, W_t and W_0 were the weights of duckweeds at time t and zero reference time,
149 respectively; t was the time interval in days. RGR was expressed as g g^{-1} day^{-1}.

150 *2.5. Biochemical assays*

The proximate composition of *S. polyrhiza* was assayed following standard methods (AOAC, 2000). Briefly, samples were dried for 24 h at 110 ˚C in an oven for the estimation of moisture contents. Ash content was determined after incineration at 600 ˚C for 16 h. Crude protein content was assayed by Kjeldahl distillation and nitrogen content (N x 6.25) was determined using a Tecator Kjeltec Auto 1030 analyser (Foss, Warrington, UK). Crude lipid level was determined gravimetrically using a Tecator Soxtec 2050 (Foss, Warrington, UK) after Soxhlet extraction by Hydrotec 8000 digester (Foss, Warrington, UK). Carbohydrate content of sample was subsequently determined by subtraction of protein, lipid and ash values.

The amino acid profile of greater duckweeds was estimated with an L-8900 Automatic Amino Acid Analyser (Hitachi Co. Ltd., Tokyo, Japan). The powdered duckweed sample was first hydrolysed using 6 N HCl for 22 h at 110 °C. Then hydrolysed sample was dried in a Nitrogen Evaporator (PCi Analytics, EV PLUS 08, Maharashtra, India). In the sample, 0.02 N HCl was added and the concentration of protein was 0.5 mg mL^{-1} of sample. The sample was kept in the Auto sampler and sample injection volume was 20 µL. As methionine, cysteine and tryptophan are destroyed during hydrolysis of sample with 6 N HCl, specific reagents are used for the estimations of these amino acids. Performic acid and hydrobromic acid (48%) were used for methionine and cysteine. For tryptophan, the sample was hydrolysed with 4 N methanesulfonic acid and 3-(2-aminoethyl) indole. The remaining methodology was identical for all amino acids. The ninhydrin derivative of proline and hydroxyproline was monitored at 440 nm, and other amino acids were monitored at 570 nm. The amino acids (peak areas) were quantified using the supplied Amino Acids Mixture Standard Solutions, Type B and Type AN-2 (Wako Pure Chemical Industries, Limited, Japan). Standard solutions for glutamine and tryptophan (Sigma-Aldrich, USA) were prepared before analysis.

175 Further *S. polyrhiza* samples were dried at 40 °C and ground prior to extraction of
176 total lipid for fatty acid composition analysis. Total lipid was extracted from 1 g sample
177 (DW) by homogenising in chloroform/methanol (2:1, v/v) using an Ultra-Turrax tissue
178 disrupter (Fisher Scientific, Loughborough, UK), and content determined gravimetrically
179 (Folch et al., 1957). Fatty acid methyl esters (FAME) were prepared from total lipid by acid-
180 catalysed transesterification at 50 ˚C for 16 h (Christie, 2003), and FAME extracted and
181 purified (Tocher and Harvie, 1988). The FAME were separated and quantified by gas-liquid
182 chromatography using a Fisons GC-8160 (Thermo Scientific, Milan, Italy) equipped with a
183 30 m × 0.32 mm (i.d.) × 0.25 μm ZB-wax column (Phenomenex, Cheshire, UK), on-column
184 injector, and a flame ionisation detector. Data were collected and processed using
185 Chromcard software for Windows (version 2.01; Thermoquest Italia S.p.A., Milan, Italy).
186 Individual FAME was identified by comparison to known standards and published data
187 (Tocher and Harvie, 1988).

188 *2.6. Statistical analysis*

189 Data were presented as mean ± SE unless otherwise stated. One-way analysis of
190 variance, ANOVA, Duncan's multiple range test, DMR (Montgomery, 1984). Student's t-
191 test were used for the statistical analysis with significance accepted at $p < 0.05$ level.

192 **3. Results**

193 *3.1. Culture in tanks*

194 *3.1.1. Water quality*

195 Major water quality parameters were recorded in all treatments before the application
196 of manures. There was no significant ($p > 0.05$) difference in temperature, pH, dissolved
197 oxygen, ammonia, nitrite, nitrate and phosphate levels among treatments at the beginning of
198 the study. A wide range of water temperature 9.4 - 26.7 °C was recorded during the culture of
199 duckweed between December and March and this influenced the productivity (Table 1). The

whole culture period was broadly divided into three phases based on the temperature and light intensity in the culture tanks. In phase I (December 2016 - January 2017), water temperature and light intensity were 16.5 °C and 26.0 µmol photons m^{-2} s^{-1} at the beginning and then gradually decreased. The lowest temperature and light intensity were recorded in January. In phase II (February - March 2017) and phase III (March, 2017), water temperature and light intensity showed increasing trends. There was no significant ($p > 0.05$) difference in temperature and light intensity among the three different treatments during the culture period. Among these three different treatments, there was variation in pH in different phases.

Significantly ($p < 0.05$) higher dissolved oxygen levels were found with manure 2 compared to the other two treatments throughout the study period (Fig. 2A). This group was followed by manure 3 and lowest dissolved oxygen level (<1 mg L^{-1}) was found in manure 1. Ammonia levels were significantly ($p < 0.05$) higher in manure 1 compared to the other two treatments throughout the study period (Fig. 2B). In manure 1, ammonia levels ranged from 1.34 - 30.65, 7.52 - 18.57 and 15.25 - 17.85 mg L^{-1} in the first, second and third phases, respectively. In manure 2, ammonia levels ranged from 1.94 - 9.34, 0.03 - 7.71 and 1.44 - 3.33 mg L^{-1} in the first, second and third phases, respectively. In manure 3, ammonia level ranged from 0.17 - 10.97, 0.27 - 4.08 and 0.23 - 0.41 mg L^{-1} in the first, second and third phases, respectively. The lowest range of ammonia levels were found in the third phase regardless of manures.

Nitrite level was significantly ($p < 0.05$) higher in manure 2 and manure 3 in the first phase compared to manure 1 (Table 1). There was no significant ($p > 0.05$) difference between these two former groups. In the second and third phases, nitrite levels were significantly ($p < 0.05$) higher in manure 2 compared to the other two treatments. Nitrate level was significantly ($p < 0.05$) higher in manure 2 compared to the other two treatments throughout the study period. Phosphate level was significantly ($p < 0.05$) lower in manure 2 compared to the other two

225 treatments throughout the study period (Fig. 2C). Conductivity was significantly ($p < 0.05$)
226 higher in manure 1 compared to the other treatments throughout the study period (Fig. 2D). In
227 manure 1, conductivity ranged from 516 - 1196 μS cm^{-1}.
228 *3.1.2. Production and relative growth rate (RGR)*
229 The production of *S. polyrhiza* was affected by water temperature. The relative growth
230 rate of greater duckweeds was slow (0.02 - 0.04 g g^{-1} day^{-1}) at the beginning of the culture
231 period due to low temperature regardless of treatments. Greater duckweeds were first harvested
232 after 69 days of initial introduction in all three treatments. As water temperature increased, the
233 growth rate also increased and duckweeds were harvested another four times; second and fourth
234 harvests were performed after 10 days of the respective previous harvest and third and fifth
235 harvests were after 12 days of the respective previous harvest. The RGR values ranged from
236 0.021 - 0.158, 0.007 - 0.12 and -0.024 - 0.129 g g^{-1} day^{-1} in manures 1, 2 and 3, respectively
237 throughout the study period. In manure 3, poor growth of plant at fifth harvest compared to the
238 previous one resulted into negative RGR value. The average RGR values were 0.08 ± 0.02,
239 0.06 ± 0.03 and 0.07 ± 0.03 g g^{-1} day^{-1} in manures 1, 2 and 3, respectively. Total production of
240 duckweeds was significantly ($p < 0.05$) higher in manure 1 compared to the other manures (Fig.
241 3). This group was followed by manure 3 and minimum production was found in manure 2.
242 *3.2. Culture in ponds*
243 *3.2.1. Water quality*
244 In three different ponds at the Rohtak centre, water temperature and pH ranged from
245 32.4 - 30.5 °C and 7.76 - 8.30, respectively during the study period. Dissolved oxygen level
246 ranged from 1.25 - 4.57 mg L^{-1} on various days of study. Ammonia, nitrite and nitrate levels
247 of ponds ranged from 5.02 - 17.57, 0.003 - 0.12 and 0.23 - 2.44 mg L^{-1}, respectively. Phosphate
248 level ranged 1.15 - 2.0 mg L^{-1} during the study period (Table 2). Conductivity ranged from
249 1032 - 1251 μS cm^{-1} throughout the culture period of greater duckweed.

250 *3.2.2. Production and relative growth rate (RGR)*

251 *S. polyrhiza* was harvested three times from the ponds at 10 days intervals (Fig. 4A-B).
252 Greater duckweeds were harvested from the ponds and were cleaned thoroughly with tap water
253 to remove organic material, excess water was removed, air dried and then dried at 40 °C in an
254 oven. Dried duckweed was packed in airtight containers for further use. The RGR values were
255 0.48, 0.14 and 0.03 g g^{-1} day^{-1} in the first, second and third harvests, respectively. The average
256 RGR value was 0.22 ± 0.13 g g^{-1} day^{-1}. Total production was 2020 ± 150 kg ha^{-1} $month^{-1}$ on
257 dry matter basis, equivalent to 24 tonnes ha^{-1} yr^{-1} (Fig. 5).

258 *3.3. Biochemical composition*

259 There was a difference in the proximate composition of greater duckweed cultured with
260 organic manures (manure 1) and inorganic fertilizers (manure 2) in tanks. Protein content was
261 significantly ($p < 0.05$) higher, and carbohydrate and ash contents were significantly ($p < 0.05$)
262 lower, in duckweed cultured in manure 1 compared to manure 2 (Table 3). The amino acid
263 profile of greater duckweed cultured in organic manures showed the presence of essential
264 (37.4%), non-essential (58.2%) and free amino acids (4.5%). Among essential amino acids,
265 three branched chain amino acids, leucine, isoleucine and valine contributed 51.4%. Glutamic
266 acid and glutamine consisted 28.3% of the total non-essential amino acids in the greater
267 duckweed. The presence of taurine enhanced the nutritional value of greater duckweed (Table
268 4).

269 The fatty acid composition of *S. polyrhiza* was dominated by polyunsaturated fatty
270 acids (PUFA), which accounted for 47-53% of total fatty acids, primarily α-linolenic acid
271 (ALA, 18:3n-3) at around 36-39% (Table 5). Total saturated fatty acids accounted for 32-39%,
272 followed by linoleic acid (LA, 18:2n-6) at 11-14% and monoenes at 9-11%. As with proximate
273 composition, fatty acid profile was affected by manures. *S. polyrhiza* grown in inorganic
274 fertilizers (manure 2) having a higher proportion of ALA, LA and total PUFA, and lower

saturated and monounsaturated fatty acids. Due to the slightly higher (although not statistically significant) lipid content of *S. polyrhiza* grown in manure 2, all fatty acids were in higher absolute amounts (mg.100g^{-1} dry mass) in macrophytes grown in inorganic fertilizers. *S. polyrhiza* lipid contained no long-chain PUFA such as docosahexaenoic acid (22:6n-3), although there was a trace level of eicosapentaenoic acid (20:5n-3), most likely due to minor microalgal contamination within the macrophyte biomass.

4. Discussion

Water temperature and sunlight are major environmental factors that influence the growth of duckweed compared to the nutrient concentrations in the water (Hasan and Chakrabarti, 2009). In tank culture, *S. polyrhiza* was first harvested after 69 days of culture. The water temperature was generally below 15 °C during this period of culture, and lowest light intensity was also recorded during this period. Water temperature increased above 16 °C at the second phase of culture and only then duckweed grew well and harvested. Higher light intensity was also recorded at the second phase compared to the first one. In a comparative study, growth performance of *S. polyrhiza* was recorded at two temperature ranges of 10 - 12 and 26 - 28 °C (Song et al., 2006). It was found that cell growth, the synthesis, and absorption ability of duckweed decreased at low temperature compared to duckweed cultured at higher temperature. There was no change in frond number for 15 days at low temperature range.

In the present study, the relative growth rate (RGR) of greater duckweed was low during the first phase of tank culture and then increased regardless of treatments. In manure 3, RGR reduced in fifth harvest of phase three. Among the three manures, significantly ($p < 0.05$) higher production was found with manure 1 compared to the inorganic fertilizers. Therefore, organic manures were applied in pond culture of greater duckweed. In contrast to the tank culture, RGR value was maximum at first harvest in pond culture of greater duckweed and the average RGR value was higher in pond compared to tank production. The production rate of

300 greater duckweed was 0.08 ± 0.02 fronds day^{-1} in laboratory conditions (Lemon et al., 2001).
301 Higher temperature also resulted in enhanced growth rate in ponds in the present study. In
302 Bangladesh, highest growth of *S. polyrrhiza* was found at 22.2 - 22.5 ºC in pond (Khondker et
303 al., 1993), although *S. polyrhiza* survived at 10 - 12 °C, it could not grow well at a low
304 temperature (Song et al., 2006). The duckweed exposed to oxidative damage at low
305 temperature. Appenroth (2002) suggested that 15 °C temperature (combined with 30 µM
306 phosphate level) was the dominant turion formation inducing factor. In laboratory axenic
307 culture, *S. polyrhiza* were exposed at 100 µmol m^{-2} s^{-1} white light (Appenroth et al., 2017). In
308 the present study, good growth of *S. polyrhiza* was found at light intensity between 105 - 151
309 µmol photons m^{-2} s^{-1} in natural outdoor light.

310 In Bangladesh and India, a pH range from 6.5 - 7.5 (Islam and Khondkar, 1991) and
311 6.8 - 8.5 (Gopal and Chamanlal, 1991; Kaul and Bakaya, 1976) was found to be optimum for
312 the production of greater duckweed. In the present study, pH ranged from 6.98 - 7.86 and 7.76
313 - 8.30 in tank and pond culture systems, respectively. There was no direct effect of dissolved
314 oxygen on the production of greater duckweed as highest production was recorded in manure
315 1 with minimum dissolved oxygen level in tank culture. Leng et al. (1995) suggested that
316 maintenance of low dissolved oxygen with 6 - 7 pH should be the strategy for duckweed pond
317 management.

318 It was found that the root length was shorter in *S. polyrhiza* that grown at low
319 temperature compared to the plants grown at a higher temperature. *S. polyrhiza* with shorter
320 root length were inefficient in absorbing nitrogen, phosphorus and other nutrients from water
321 (Reddy and DeBusk, 1985). In tank culture, highest ammonia level was recorded in manure 1
322 at first phase and no production was recorded during this period. The ammonia level gradually
323 reduced in the second and third phases and the growth of duckweed enhanced. Even with the
324 same manure system (manure 1), lower levels of ammonia were found in ponds compared to

tanks. Absorption of nutrients helped in the higher production of duckweeds in ponds. The fluctuation of pH between 7.4 and 9.0 enhanced the ammonia toxicity in laboratory culture (Caicedo et al., 2000). In tank culture of duckweed, highest RGR was found in the second phase at 15.25 ± 1.0 mg L^{-1} ammonia concentration in manure 1. It is also interesting to see that in tank culture, poor growth of duckweeds in manure 3 during fifth harvest might be related to the low ammonia level in the culture tank. Leng et al. (1995) suggested that 7 - 12 mg N L^{-1} was optimum to maintain a protein content of 40% in duckweed. A TKN content of 20 - 30 mg L^{-1} was required for optimum growth (Culley et al., 1981) and maintenance of high protein content. In the present study, the ammonia level in the pond water also helped in the proper growth of the duckweed. Nitrification rate was slower in manure 1 compared to the other two treatments in the tank culture of duckweed. In manure 1, nitrate level was significantly higher in the second phase compared to the other phases. Phosphorus is a major limiting nutrient, although it is required in lesser amount. In the present study, the phosphate levels in manure 1 helped in the production of duckweed in both tanks and ponds. The optimum conductivity for maximum production of *S. polyrrhiza* was 650 - 1000 µS cm^{-1} (Gopal and Chamanlal, 1991). *S. polyrrhiza* completely disappeared in May due to reduced conductivity and alkalinity (Khondker et al., 1993). In the tank culture, the growth of greater duckweed was less in the first phase and the conductivity was minimum during this phase regardless of manures applied. Then conductivity increased with higher production of duckweed. In pond culture, the conductivity was always >1000 µS cm^{-1}.

In ponds, the production of greater duckweed was encouraging, 2020 ± 150 kg ha^{-1} $month^{-1}$ (24 tonnes ha^{-1} yr^{-1}) on dry matter basis. Literature showed a wide variation in the production of duckweed, with various climatic conditions and nutrient availability mostly being responsible for this variation. Edwards et al. (1990) reported ~20 tonnes ha^{-1} $year^{-1}$ (DM) production of *S. polyrhiza* during 1-3 months culture period; the yield decreased (~9 tonnes ha^{-}

^{1} year^{-1}) when the duration of culture period increased to 6 months. The yield of greater duckweeds in domestic wastewater (Reddy and Debusk, 1985), sewage effluent (Sutton and Ornes, 1975) and nutrient non-limited water (Reddy and DeBusk, 1985) were 17 - 32, 14.6 and 11.3 tonnes ha^{-1} yr^{-1}, respectively. Based on the available data, an average harvest of 10 - 20 tonnes duckweed ha^{-1} year^{-1} could be expected under optimum environmental conditions (Hasan and Chakrabarti, 2009). In a similar study, *Lemna minor* was produced in ponds using organic manures. The production was lower (702.5 kg ha^{-1} month^{-1}, DW) compared to *S. polyrhiza* (Chakrabarti et al., 2018). The initial amount of duckweed introduced for culture also influenced production. A seeding rate of 60 kg m^{-2} for *S. polyrhiza* was recommended (DWRP, 1998). In the pond culture, only 1 kg pond $^{-1}$ (200 m^{2}) *S. polyrhiza* was introduced in the present study.

The proximate composition of greater duckweed varied with nutrient availability of the culture system. In the present study, the protein, lipid, ash and carbohydrate contents of greater duckweeds were influenced by the quality of the manures. The protein content of the duckweeds (30.5 ± 0.03 - 35.82 ± 0.14%) was higher in the present study compared to some previous studies. The duckweeds collected from Thailand showed 23.8 ± 0.8% protein content (Hasan and Edwards, 1992), whereas 25.6 ± 0.2% protein content was recorded in plants collected from a pond in Nigeria (Fasakin et al., 1999). In USA, 13.1% crude protein was found in greater duckweed collected from low-nutrient lagoon (Culley et al., 1981), whereas 40.9% crude protein was found in plants grown in a dairy cattle-waste lagoon (Hillman and Culley, 1978). In the present study, lipid contents of duckweeds ranged from 7.11 - 7.2%, whereas lipid contents of 2.5 - 6.7% were reported in the earlier studies (Hasan and Chakrabarti, 2009). Appenroth et al. (2017) found around 5% lipid content in duckweed. Similarly, the ash content of the duckweed in the present study (18.51 ± 0.02 - 20.64 ± 0.26%) was comparable with

374 earlier studies, in which ash contents varied from 15.2 ± 0.4 - 18.3 ± 1.0% in greater duckweeds
375 collected from different geographical areas (Hasan and Edwards, 1992).

376 These data showed that culture of greater duckweed with a specific management
377 strategy helped in the production of valuable animal feed ingredients. *S. polyrhiza* is a new
378 generation sustainable crop (Hoang et al., 2018). Song et al (2006) reported that temperature
379 also influenced the soluble protein, chlorophyll α, chlorophyll β and carotenoid pigment of
380 duckweeds. The present study confirmed the earlier study. The presence of essential amino
381 acids viz. histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and
382 valine were documented in greater duckweeds (Ismail, 1998). The present study showed that
383 all the essential (including tryptophan) and non-essential amino acids were present in adequate
384 quantity in cultured duckweed. The present study also showed the presence of taurine in the
385 duckweeds. The presence of glutamic acid and glutamine confirmed the role of greater
386 duckweed in reducing nitrogenous materials in the water. Similar amino acids composition was
387 found in *L. minor* (Chakrabarti et al., 2018). The nutritional value of duckweed is comparable
388 with alfalfa, being a rich source of lysine and arginine (Guha, 1997). The composition of
389 essential amino acids in greater duckweed is comparable with soybean (NRC, 1998), the most
390 commonly used ingredient in the diet formulation of fish (Table 6). The amino acid
391 requirements of important cultivable species are documented (NRC, 2011). It is clear from the
392 present study that the amino acid profile of greater duckweed meets the nutritional
393 requirements of the cultivable species. The amino acid profiles of *Landoltia punctata* (= *S.*
394 *oligorrhiza*) and different clones of *Wolffia arrhiza* were sufficient to fulfilled the requirements
395 for human recommended by WHO ((Ismail, 1998; Appenroth et al., 2018).

396 In addition, *S. polyrhiza* demonstrated reasonable lipid content with ALA being the
397 major fatty acid component in present study. Inorganic fertilizers resulted in slightly higher
398 lipid content and relative percentage of ALA, which individually did not reach statistical

399 significance, but together had a significant effect, increasing the absolute content of ALA. The
400 PUFA content of *S. polyrhiza* grown in culture media was higher compared to the present study
401 though the total lipid level was higher in the latter (Appenroth et al., 2017). It was interesting
402 that in different species of *Wolffia* fat content was low, varied from 1-5%. PUFA levels were
403 above 60% of total fat. The n-3 PUFA level was higher compared to n-6 PUFA (Appenroth et
404 al., 2018). In the present study, the lipid and PUFA contents were higher in *S. polyrhiza*
405 compared to *Wolffia* spp. In *L. minor*, 60 - 63% of total fatty acid was PUFA; around 41-43%
406 α-linolenic acid and 17-18% linoleic acid (Chakrabarti et al., 2018).

407 **5. Conclusions**

408 The application of organic manures helped in the production of greater duckweed *S.*
409 *polyrhiza* in a sustainable manner. The temperature, light intensity, ammonia, phosphate
410 and conductivity significantly influenced the productivity of the water bodies. Proximate
411 composition, especially amino acid and fatty acid profiles confirmed the suitability of the
412 greater duckweed as a potential ingredient for the development of diets for fish and other
413 livestock.

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Figure legends

Fig. 1 Introduction of *S. polyrhiza* (1 kg pond^{-1}) in Rohtak, Haryana.

Fig. 2 Various water quality parameters (in parenthesis). (A) Dissolved oxygen, (B) ammonia, (C) phosphate and (D) conductivity of water found during three different phases of culture of *S. polyrhiza* in tanks. Phase I: December 2016 - January 2017, Phase II: February - March 2017 & Phase III: March 2017. Bars with different superscripts are significantly ($p < 0.05$) different (n = 3).

Fig. 3 Total production of *S. polyrhiza* cultured with three different organic manures and inorganic fertilizers in tanks. Bars with different superscripts are significantly ($p < 0.05$) different (n = 3).

Fig. 4 Production of *S. polyrhiza* (A) in ponds & (B) duckweeds after harvest.

Fig. 5 Relative growth rate (RGR) and total production of *S. polyrhiza* in ponds. RGR was measured thrice at 10 days interval. Bars with different superscripts are significantly ($p < 0.05$) different (n = 3).

626 **Table 1 Environmental parameters measured in tanks during the culture of *S. polyrhiza*.**

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Parameters	Manure 1		Manure 2		Manure 3	
	Range	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**
			Phase I (December 2016 - January 2017)			
Temperature (°C)	9.36 – 16.55	14.38 ± 0.34	9.36 – 16.55	14.38 ± 0.336	9.36 – 16.55	14.38 ± 0.34
Light intensity (µmol photons m^{-2} s^{-1})	14.56 – 49.43	27.29 ± 2.45	14.56 – 49.43	27.29 ± 2.45	14.56 – 49.43	27.29 ± 2.45
pH	7.20 – 7.91	---	7.04 – 7.86	---	6.98 – 7.85	---
Nitrite (mg L^{-1})	0.007 – 0.26	0.116 ± 0.01	0.13 – 1.01	0.47 ± 0.05	0.06 – 1.04	0.49 ± 0.07
Nitrate (mg L^{-1})	1.68 – 18.70	5.77 ± 1.13	6.58 – 43.66	30.76 ± 2.60	8.44 – 35.48	24.40 ± 2.01
			Phase II (February - March 2017)			
Temperature (°C)	15.70 – 19.33	17.80 ± 0.43	15.70 – 19.33	17.80 ± 0.43	15.70 – 19.33	17.80 ± 0.43
Light intensity (µmol photons m^{-2} s^{-1})	49.21 – 105.08	89.89 ± 3.25	49.21 – 105.08	89.89 ± 3.25	49.21 – 105.08	89.89 ± 3.25
pH	7.09 – 7.59	---	7.24 – 7.82	---	7.26 – 7.72	---
Nitrite (mg L^{-1})	0.02 – 0.12	0.055 ± 0.015	0.11 – 0.84	0.44 ± 0.08	0.006 – 0.12	0.09 ± 0.02
Nitrate (mg L^{-1})	5.95 – 44.73	25.77 ± 6.04	15.04 – 46.87	29.38 ± 4.58	16.15 – 34.94	24.42 ± 2.61
			Phase III (March 2017)			
Temperature (°C)	23.26 – 26.70	24.98 ± 1.72	23.26 – 26.70	24.98 ± 1.72	23.26 – 26.70	24.98 ± 1.72
Light intensity (µmol photons m^{-2} s^{-1})	137.41 – 151.16	143.79 ± 6.39	137.41 – 151.16	143.79 ± 6.39	137.41 – 151.16	143.79 ± 6.39
pH	7.27 – 7.56	-	7.18 – 7.43	-	7.28 – 7.39	-
Nitrite (mg L^{-1})	0.015 – 0.02	0.016 ± 0.00	0.37 – 0.07	0.52 ± 0.16	0.082 – 0.12	0.10 ± 0.02
Nitrate (mg L^{-1})	11.68 – 18.54	15.11 ± 3.44	33.51 – 36.95	35.23 ± 1.72	16.51- 34.94	24.41 ± 2.61

628 **Table 2**

629 **Environmental parameters measured** in *S. polyrhiza* culture ponds during the study period.

Parameter	**Range**	**Mean ± SE**
Temperature (°C)	30.5 - 33.0	32.00 ± 1.0
pH	7.76 - 8.30	---
Dissolved oxygen (mg L^{-1})	1.25 - 4.57	2.50 ± 0.25
Ammonia (mg L^{-1})	5.02 - 17.57	15.25 ± 0.7
Nitrite (mg L^{-1})	0.005 - 0.01	0.008 ± 0.002
Nitrate (mg L^{-1})	0.05 - 2.05	0.921 ± 0.3
Phosphate (mg L^{-1})	1.15 - 2.00	1.52 ± 0.07
Conductivity (µS cm^{-1})	1032 - 1251	1150 ± 37.0

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644 **Table 3**

645 Proximate composition of *S. polyrhiza* (% of dry weight).

Parameter	Manure 1 (Organic)	Manure 2 (Inorganic)
Protein	35.82 ± 0.14	30.50 ± 0.03*
Lipid	7.11 ± 0.11	7.19± 0.06
Ash	18.51 ± 0.02	20.64 ± 0.26*
Carbohydrate	38.38 ± 0.26	41.68± 0.17*

646 Data are presented as means ± SEM (n=3). *Denotes significant difference ($p < 0.05$)
647 between the two manures as determined by Student's t-test.
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Table 4

Amino acid (g 100 g^{-1} of dry weight) profile of *S. polyrhiza* cultured with organic manures in tanks.

Amino acids	Concentration
Essential	
Histidine (His)	0.771 ± 0.053
Isoleucine (Ile)	1.703 ± 0.150
Leucine (Lue)	3.322 ± 0.207
Lysine (Lys)	2.280 ± 0.129
Methionine (Met)	0.694 ± 0.059
Phenylalanine (Phe)	2.159 ± 0.144
Threonine (Thr)	1.502 ± 0.386
Tryptophan (Trp)	0.282 ± 0.018
Valine (Val)	2.383 ± 0.139
Non-essential	
Alanine (Ala)	2.384 ± 0.130
Arginine (Arg)	2.386 ± 0.120
Asparatate (Asp)	4.094 ± 0.212
Cysteine (Cys)	0.369 ± 0.039
Glutamic acid (Glu)	5.103 ± 0.380
Glutamine ($GluNH_2$)	1.250 ± 0.300
Glycine (Gly)	2.369 ± 0.110
Proline (Pro)	1.001 ± 0.110
Serine (Ser)	1.904 ± 0.120
Tyrosine (Tyr)	1.558 ± 0.050
Free	
Phosphoserine (p-Ser)	0.060 ± 0.002
Taurine (Tau)	0.023 ± 0.006
Phospho ethanol amine (PEA)	0.072 ± 0.001
α Amino adipic acid (α-AAA)	0.020 ± 0.001
α Amino-n- butaric acid (α-ABA)	0.141 ± 0.014
Cystathionine (Cysthi)	0.115 ± 0.001
β -Alanine (β-Ala)	0.072 ± 0.011
β -Amino isobutyric acid (β-AiBA)	0.354 ± 0.015
Ethanol amine ($EOHNH_2$)	0.112 ± 0.004
Ornithine (Orn)	0.027 ± 0.002
1 Methylhistidine (1 Mehis)	0.048 ± 0.003
Hydroxy proline (Hypro)	0.197 ± 0.010
ϒ- Amino isobutyric acid (ϒ-AiBA)	0.478 ± 0.024

Table 5

Fatty acid composition of *S. polyrhiza* as percentage of total fatty acids (Percentage) or as mg fatty acids per 100 g dry weight (Absolute).

Fatty acid	Manure 1		Manure 2	
	Percentage	**Absolute**	**Percentage**	**Absolute**
14:0	1.01 ± 0.22	16.9 ± 1.86	1.10 ± 0.30	23.65 ± 7.42
15:0	0.60 ± 0.04	10.1 ±0.46	0.40 ± 0.01*	8.56 ± 0.55
16:0	31.22 ± 2.33	524.1 ± 18.32	25.50 ± 0.40	547.04 ± 33.88
18:0	2.33 ± 0.23	39.1 ± 0.35	2.02 ± 0.13	43.39 ± 4.69
20:0	0.40 ± 0.04	6.6± 0.10	0.33 ± 0.01	7.04 ± 0.55
22:0	0.77 ± 0.10	12.9 ± 0.32	0.85 ± 0.03	18.17 ± 0.24*
24:0	3.05 ± 0.15	51.3 ± 3.16	2.28 ± 0.05*	48.85 ± 1.15
Total saturated	39.38 ± 3.12	661.0 ± 20.21	32.48 ± 0.76	696.70 ± 48.49
16:1n-9	4.76 ± 2.23	86.4 ± 27.76	6.75 ± 0.14	144.61 ± 3.60
17:1 n	0.00 ± 0.00	0.0 ± 0.00	0.30 ± 0.02*	6.34 ± 0.22*
18:1n-9	2.09 ± 0.13	35.2 ± 1.68	3.01 ± 0.64	64.93 ± 16.74
18:1n-7	2.24 ± 0.23	37.6 ± 0.25	1.34 ± 0.06*	28.68 ± 2.59*
Total monoenes	9.09 ± 6.37	159.2 ± 124.19	11.39 ± 0.53	244.57 ± 22.72
18:2n-6	11.35 ± 0.76	190.7 ± 8.09	13.49 ± 0.23	289.08 ± 8.33*
20:4n-6	0.00 ± 0.00	0.0 ± 0.00	0.33 ± 0.01*	7.03 ± 0.08*
Total n-6 PUFA	11.35 ± 0.76	190.7 ± 8.09	13.82 ± 0.24	296.11 ± 8.41*
18:3n-3	35.75 ± 2.18	600.6 ± 29.28	38.95 ± 1.08	834.63 ± 15.44*
20:5n-3	0.38 ± 0.12	6.3 ± 1.37	0.60 ± 0.08	12.98 ± 2.30
Total n-3 PUFA	36.13 ± 0.30	606.9 ± 27.91	39.56 ± 1.00	847.61 ± 17.75*
Total DMA	4.04 ± 0.18	68.0 ± 4.35	2.76 ± 0.06*	59.09 ± 1.49
Total PUFA	47.48 ± 3.07	797.6 ± 35.99	53.37 ± 1.241	1143.72 ± 26.16*
Total Fatty acids		1685.8 ± 275.3		2144.1 ± 329.9

Data are presented as means ± SEM (n=3). *Denotes significant difference ($p < 0.05$) between the two manures as determined by Student's t-test. DMA, dimethyl acetals; PUFA, polyunsaturated fatty acids.

724 **Table 6**
725 The essential amino acid profiles of soybean (*Glycine max*) meal and *S. polyrhiza* and their
726 requirement for *Cyprinus carpio* and *Oreochromis niloticus* (NRC, 1998, 2011).
727

Amino acids	***Glycine max* meal (g 100 g^{-1})**	***Spirodela polyrhiza* (g 100 g^{-1})**	***Cyprinus carpio* (g 100 g^{-1} diet)**	***Oreochromis niloticus* (g 100 g^{-1} diet)**
Histidine (His)	1.17	0.77	0.5	1.0
Isoleucine (Ile)	1.99	1.70	1.0	1.0
Leucine (Lue)	3.42	3.32	1.4	1.9
Lysine (Lys)	2.83	2.28	2.2	1.6
Methionine (Met)	0.61	0.7	0.7	0.7
Phenylalanine (Phe)	2.18	2.15	1.3	1.1
Threonine (Thr)	1.73	1.50	1.5	1.1
Tryptophan (Trp)	0.61	0.28	0.3	0.3
Valine (Val)	2.06	2.38	1.4	1.5
Arginine (Arg)	3.23	2.38	1.7	1.2
Cysteine (Cys)	-	0.36	-	-
Tyrosine (Tyr)	-	1.55	-	-
Methionine + Cysteine	1.31	1.07	1.0	1.0
Phenylalanine + Tyrosine	-	3.7	2.0	1.6

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Fig. 1.

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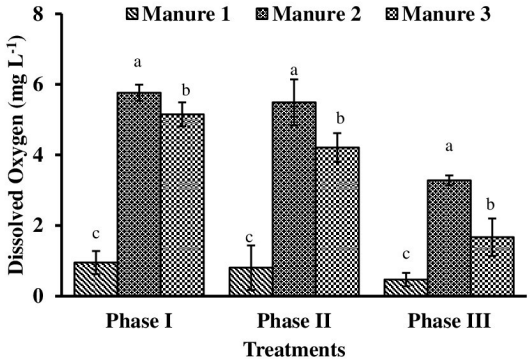

Fig. 2 (A)

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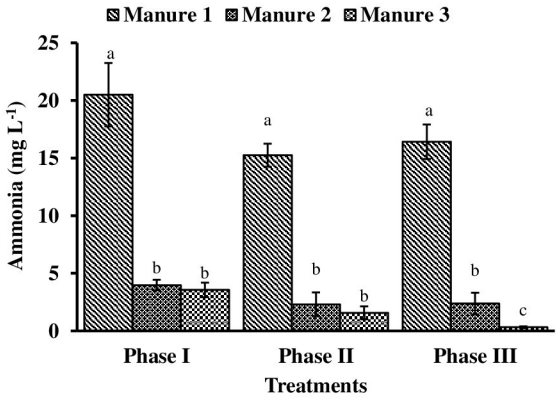

Fig. 2 (B)

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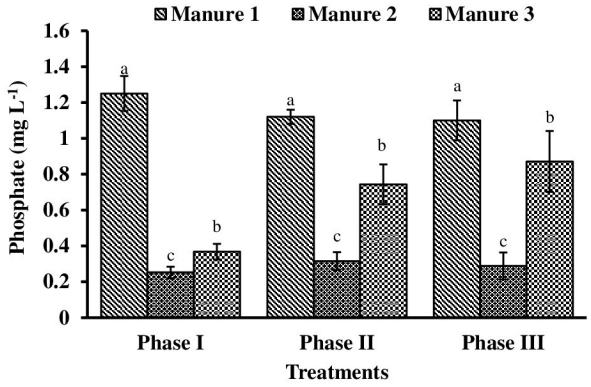

Fig. 2 (C)

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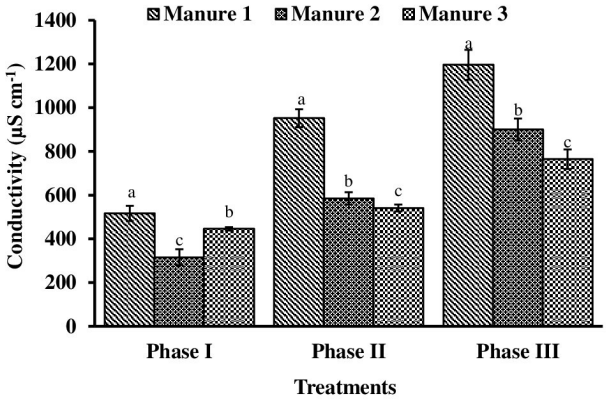

Fig. 2 (D)

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