

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

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20 **Running title:** Production of *Spirodela polyrhiza* and its biochemical composition study

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26 **ABSTRACT**

27 The culture technique of greater duckweed *Spirodela polyrhiza* (L. Schleiden) was  
28 standardized in outdoor tanks using three different manures: manure 1 - cattle manure, poultry  
29 droppings and mustard oil cake, manure 2 - urea, potash and triple superphosphate and manure  
30 3 - cattle manure, urea, potash and triple superphosphate. Significantly ( $p<0.05$ ) higher  
31 production was recorded in manure 1 compared to others. Manure 1 was subsequently selected  
32 for pond culture. In ponds, the production of duckweed was  $2020\pm150$  kg ha<sup>-1</sup> month<sup>-1</sup> dry  
33 weight basis. Protein content was significantly higher ( $p<0.05$ ) in duckweed cultured in manure  
34 1. The amino acid profile study showed the presence of essential (37.4%), non-essential  
35 (58.2%) and free (4.5%) amino acids. Leucine, isoleucine and valine contributed 51.4% of total  
36 essential amino acids. Duckweed contained 7% lipid and  $\alpha$ -linolenic acid (36-37%) was the  
37 major fatty acid. The study showed the nutritional value of duckweed as an animal feed  
38 ingredient.

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

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## 51 **1. Introduction**

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

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

76 digestibility of *S. polyrhiza* has been observed (Wee, 1991). Similarly, analysis of the  
77 proximate composition showed that *S. polyrhiza* are a rich source of protein, although content  
78 varies from 23.8 - 40.9% (Hasan and Edwards, 1992; Hillman and Culley, 1978). Amado et al.  
79 (1980) reported the amino acid composition of 94 different strains of duckweeds. They  
80 suggested that all essential amino acids (except methionine) are present in sufficient amount in  
81 all strains of duckweeds. Recently, Appenroth et al. (2017) found around 25% protein level in  
82 *S. polyrhiza* cultured in nutrient medium. They also suggested that the levels of critical amino  
83 acids in duckweeds are within the recommended range of World Health Organization (WHO)  
84 for human. It is also a rich source of pigments, especially carotene and xanthophylls (Leng et  
85 al., 1995). Notably the nutritional and biochemical value of such macrophytes is highly variable  
86 and depends largely on water quality of the culture system (Boyd, 1971). Therefore, there is an  
87 urgent requirement to develop large-scale culture techniques for the production of nutrient-rich  
88 duckweeds. There is immense scope for large scale production of duckweeds in tropical climate  
89 (Chakrabarti, 2017).

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

## 100 **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<sup>-3</sup> (Srivastava et al., 2006). Manure 2: urea, potash and triple superphosphate were  
107 used at the rate of 20, 4 and 4 kg ha<sup>-1</sup> day<sup>-1</sup>, 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<sup>-1</sup>day<sup>-1</sup>, 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<sup>-1</sup>, 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<sup>-1</sup> month<sup>-1</sup> (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<sup>2</sup> (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<sup>-1</sup> (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<sup>-1</sup> month<sup>-1</sup> (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:

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

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<sup>-1</sup> day<sup>-1</sup>.

### 150 2.5. *Biochemical assays*

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

159 The amino acid profile of greater duckweeds was estimated with an L-8900  
160 Automatic Amino Acid Analyser (Hitachi Co. Ltd., Tokyo, Japan). The powdered  
161 duckweed sample was first hydrolysed using 6 N HCl for 22 h at 110 °C. Then hydrolysed  
162 sample was dried in a Nitrogen Evaporator (PCi Analytics, EV PLUS 08, Maharashtra,  
163 India). In the sample, 0.02 N HCl was added and the concentration of protein was 0.5 mg  
164 mL<sup>-1</sup> of sample. The sample was kept in the Auto sampler and sample injection volume was  
165 20 µL. As methionine, cysteine and tryptophan are destroyed during hydrolysis of sample  
166 with 6 N HCl, specific reagents are used for the estimations of these amino acids. Performic  
167 acid and hydrobromic acid (48%) were used for methionine and cysteine. For tryptophan,  
168 the sample was hydrolysed with 4 N methanesulfonic acid and 3-(2-aminoethyl) indole. The  
169 remaining methodology was identical for all amino acids. The ninhydrin derivative of  
170 proline and hydroxyproline was monitored at 440 nm, and other amino acids were monitored  
171 at 570 nm. The amino acids (peak areas) were quantified using the supplied Amino Acids  
172 Mixture Standard Solutions, Type B and Type AN-2 (Wako Pure Chemical Industries,  
173 Limited, Japan). Standard solutions for glutamine and tryptophan (Sigma-Aldrich, USA)  
174 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

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

208         Significantly ( $p < 0.05$ ) higher dissolved oxygen levels were found with manure 2  
209 compared to the other two treatments throughout the study period (Fig. 2A). This group was  
210 followed by manure 3 and lowest dissolved oxygen level ( $<1 \text{ mg L}^{-1}$ ) was found in manure 1.  
211 Ammonia levels were significantly ( $p < 0.05$ ) higher in manure 1 compared to the other two  
212 treatments throughout the study period (Fig. 2B). In manure 1, ammonia levels ranged from  
213 1.34 - 30.65, 7.52 - 18.57 and 15.25 - 17.85  $\text{mg L}^{-1}$  in the first, second and third phases,  
214 respectively. In manure 2, ammonia levels ranged from 1.94 - 9.34, 0.03 - 7.71 and 1.44 - 3.33  
215  $\text{mg L}^{-1}$  in the first, second and third phases, respectively. In manure 3, ammonia level ranged  
216 from 0.17 - 10.97, 0.27 - 4.08 and 0.23 - 0.41  $\text{mg L}^{-1}$  in the first, second and third phases,  
217 respectively. The lowest range of ammonia levels were found in the third phase regardless of  
218 manures.

219         Nitrite level was significantly ( $p < 0.05$ ) higher in manure 2 and manure 3 in the first  
220 phase compared to manure 1 (Table 1). There was no significant ( $p > 0.05$ ) difference between  
221 these two former groups. In the second and third phases, nitrite levels were significantly ( $p <$   
222  $0.05$ ) higher in manure 2 compared to the other two treatments. Nitrate level was significantly  
223 ( $p < 0.05$ ) higher in manure 2 compared to the other two treatments throughout the study period.  
224 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  $\mu\text{S cm}^{-1}$ .

### 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  $\text{g g}^{-1} \text{day}^{-1}$ ) 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  $\text{g g}^{-1} \text{day}^{-1}$  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  $0.08 \pm 0.02$ ,  
239  $0.06 \pm 0.03$  and  $0.07 \pm 0.03$   $\text{g g}^{-1} \text{day}^{-1}$  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  $^{\circ}\text{C}$  and 7.76 - 8.30, respectively during the study period. Dissolved oxygen level  
246 ranged from 1.25 - 4.57  $\text{mg L}^{-1}$  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  $\text{mg L}^{-1}$ , respectively. Phosphate  
248 level ranged 1.15 - 2.0  $\text{mg L}^{-1}$  during the study period (Table 2). Conductivity ranged from  
249 1032 - 1251  $\mu\text{S cm}^{-1}$  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<sup>-1</sup> day<sup>-1</sup> in the first, second and third harvests, respectively. The average  
256 RGR value was 0.22 ± 0.13 g g<sup>-1</sup> day<sup>-1</sup>. Total production was 2020 ± 150 kg ha<sup>-1</sup> month<sup>-1</sup> on  
257 dry matter basis, equivalent to 24 tonnes ha<sup>-1</sup> yr<sup>-1</sup> (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  $\alpha$ -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

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

#### 281 **4. Discussion**

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

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

300 greater duckweed was  $0.08 \pm 0.02$  fronds  $\text{day}^{-1}$  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 °C in pond (Khondker et  
303 al., 1993), although *S. polyrrhiza* 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  $\mu\text{M}$   
306 phosphate level) was the dominant turion formation inducing factor. In laboratory axenic  
307 culture, *S. polyrrhiza* were exposed at 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  white light (Appenroth et al., 2017). In  
308 the present study, good growth of *S. polyrrhiza* was found at light intensity between 105 - 151  
309  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  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. polyrrhiza* that grown at low  
319 temperature compared to the plants grown at a higher temperature. *S. polyrrhiza* 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

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

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

350 <sup>1</sup> year<sup>-1</sup>) when the duration of culture period increased to 6 months. The yield of greater  
351 duckweeds in domestic wastewater (Reddy and Debusk, 1985), sewage effluent (Sutton and  
352 Ornes, 1975) and nutrient non-limited water (Reddy and DeBusk, 1985) were 17 - 32, 14.6 and  
353 11.3 tonnes ha<sup>-1</sup> yr<sup>-1</sup>, respectively. Based on the available data, an average harvest of 10 - 20  
354 tonnes duckweed ha<sup>-1</sup> year<sup>-1</sup> could be expected under optimum environmental conditions  
355 (Hasan and Chakrabarti, 2009). In a similar study, *Lemna minor* was produced in ponds using  
356 organic manures. The production was lower (702.5 kg ha<sup>-1</sup> month<sup>-1</sup>, DW) compared to *S.*  
357 *polyrhiza* (Chakrabarti et al., 2018). The initial amount of duckweed introduced for culture also  
358 influenced production. A seeding rate of 60 kg m<sup>-2</sup> for *S. polyrhiza* was recommended (DWRP,  
359 1998). In the pond culture, only 1 kg pond<sup>-1</sup> (200 m<sup>2</sup>) *S. polyrhiza* was introduced in the present  
360 study.

361         The proximate composition of greater duckweed varied with nutrient availability of the  
362 culture system. In the present study, the protein, lipid, ash and carbohydrate contents of greater  
363 duckweeds were influenced by the quality of the manures. The protein content of the  
364 duckweeds (30.5 ± 0.03 - 35.82 ± 0.14%) was higher in the present study compared to some  
365 previous studies. The duckweeds collected from Thailand showed 23.8 ± 0.8% protein content  
366 (Hasan and Edwards, 1992), whereas 25.6 ± 0.2% protein content was recorded in plants  
367 collected from a pond in Nigeria (Fasakin et al., 1999). In USA, 13.1% crude protein was found  
368 in greater duckweed collected from low-nutrient lagoon (Culley et al., 1981), whereas 40.9%  
369 crude protein was found in plants grown in a dairy cattle-waste lagoon (Hillman and Culley,  
370 1978). In the present study, lipid contents of duckweeds ranged from 7.11 - 7.2%, whereas lipid  
371 contents of 2.5 - 6.7% were reported in the earlier studies (Hasan and Chakrabarti, 2009).  
372 Appenroth et al. (2017) found around 5% lipid content in duckweed. Similarly, the ash content  
373 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  $15.2 \pm 0.4$  -  $18.3 \pm 1.0\%$  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  $\alpha$ , chlorophyll  $\beta$  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 *oligorhiza*) 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  $\alpha$ -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<sup>-1</sup>) 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).

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

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

623 **Fig. 5** Relative growth rate (RGR) and total production of *S. polyrhiza* in ponds. RGR was  
624 measured thrice at 10 days interval. Bars with different superscripts are significantly ( $p <$   
625  $0.05$ ) different ( $n = 3$ ).

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

627

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

628 **Table 2**

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

<b>Parameter</b>	<b>Range</b>	<b>Mean <math>\pm</math> SE</b>
Temperature ( $^{\circ}$ C)	30.5 - 33.0	32.00 $\pm$ 1.0
pH	7.76 - 8.30	---
Dissolved oxygen (mg L <sup>-1</sup> )	1.25 - 4.57	2.50 $\pm$ 0.25
Ammonia (mg L <sup>-1</sup> )	5.02 - 17.57	15.25 $\pm$ 0.7
Nitrite (mg L <sup>-1</sup> )	0.005 - 0.01	0.008 $\pm$ 0.002
Nitrate (mg L <sup>-1</sup> )	0.05 - 2.05	0.921 $\pm$ 0.3
Phosphate (mg L <sup>-1</sup> )	1.15 - 2.00	1.52 $\pm$ 0.07
Conductivity ( $\mu$ S cm <sup>-1</sup> )	1032 - 1251	1150 $\pm$ 37.0

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644 **Table 3**645 Proximate composition of *S. polyrhiza* (% of dry weight).

<b>Parameter</b>	<b>Manure 1 (Organic)</b>	<b>Manure 2 (Inorganic)</b>
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<sup>-1</sup> of dry weight) profile of *S. polyrhiza* cultured with organic manures in tanks.

<b>Amino acids</b>	<b>Concentration</b>
<b>Essential</b>	
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
<b>Non-essential</b>	
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 <sub>2</sub> )	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
<b>Free</b>	
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 <sub>2</sub> )	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
γ- Amino isobutyric acid (γ-AiBA)	0.478 ± 0.024

703 **Table 5**  
 704 Fatty acid composition of *S. polyrhiza* as percentage of total fatty acids (Percentage)  
 705 or as mg fatty acids per 100 g dry weight (Absolute).  
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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
<b>Total saturated</b>	<b>39.38 ± 3.12</b>	<b>661.0 ± 20.21</b>	<b>32.48 ± 0.76</b>	<b>696.70 ± 48.49</b>
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*
<b>Total monoenes</b>	<b>9.09 ± 6.37</b>	<b>159.2 ± 124.19</b>	<b>11.39 ± 0.53</b>	<b>244.57 ± 22.72</b>
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*
<b>Total n-6 PUFA</b>	<b>11.35 ± 0.76</b>	<b>190.7 ± 8.09</b>	<b>13.82 ± 0.24</b>	<b>296.11 ± 8.41*</b>
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
<b>Total n-3 PUFA</b>	<b>36.13 ± 0.30</b>	<b>606.9 ± 27.91</b>	<b>39.56 ± 1.00</b>	<b>847.61 ± 17.75*</b>
<b>Total DMA</b>	<b>4.04 ± 0.18</b>	<b>68.0 ± 4.35</b>	<b>2.76 ± 0.06*</b>	<b>59.09 ± 1.49</b>
<b>Total PUFA</b>	<b>47.48 ± 3.07</b>	<b>797.6 ± 35.99</b>	<b>53.37 ± 1.241</b>	<b>1143.72 ± 26.16*</b>
<b>Total Fatty acids</b>		<b>1685.8 ± 275.3</b>		<b>2144.1 ± 329.9</b>

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

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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).  
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Amino acids	<i>Glycine max</i> meal (g 100 g <sup>-1</sup> )	<i>Spirodela</i> <i>polyrhiza</i> (g 100 g <sup>-1</sup> )	<i>Cyprinus</i> <i>carpio</i> (g 100 g <sup>-1</sup> diet)	<i>Oreochromis</i> <i>niloticus</i> (g 100 g <sup>-1</sup> 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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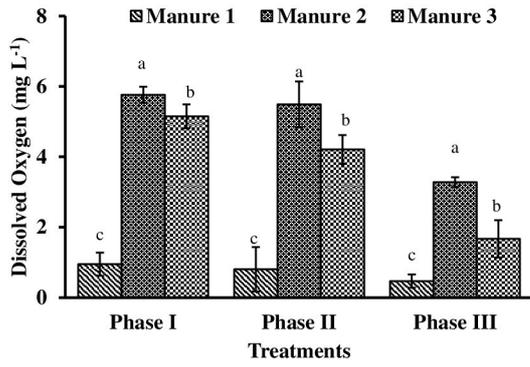


Fig. 2 (A)

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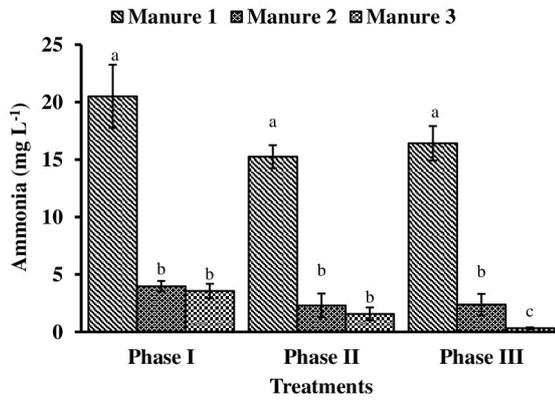


Fig. 2 (B)

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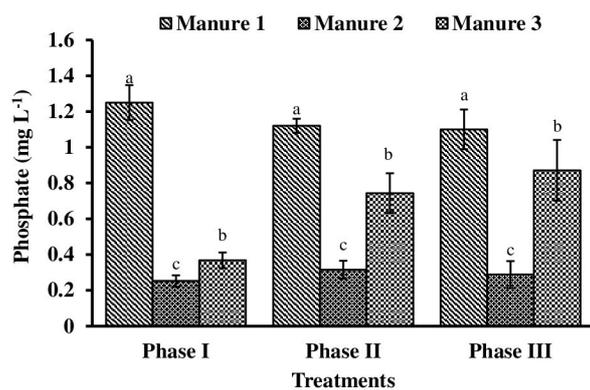


Fig. 2 (C)

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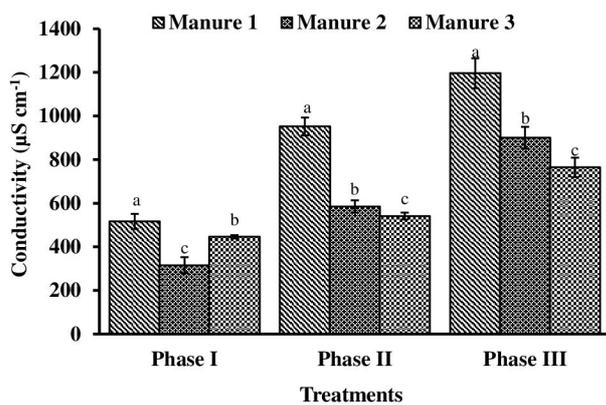


Fig. 2 (D)

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