

Original Research Article

Growth, Photosynthesis and Quality of Water Spinach (*Ipomoea aquatica*) as influenced by Magnetic nanoparticles (MNP) application

ABSTRACT

Aims: To characterize the growth, carbon assimilation and quality of *Ipomoea aquatica* as influenced by magnetic nanoparticles (MNP) application as well as to determine the best rates of iron oxide nanoparticles (~~0, 50, 100, and 150 mg/L~~) that give high growth, carbon assimilation and quality of *Ipomoea aquatica*.

Study design: *Ipomoea aquatica* plants were exposed to four different treatments of magnetic iron oxide nanoparticles (Fe_3O_4) (0, 50, 100 and 150 mg/L). The experiment was conducted in a randomized complete block design (RCBD) with 3 replications. One unit of experiment consisted of 8 plants and there were 96 plants ~~that have been utilized~~ used in the experiment.

Place and Duration of Study: Department of Biology, Faculty of Science, Universiti Putra Malaysia, between March 2018 and July 2018.

Methodology: The growth parameters measured included: plant height, basal diameter, total leaf number, leaf temperature, total chlorophyll content and plant biomass. The carbon assimilation parameters were measured using LICOR 6400 XT Portable Photosynthesis System i.e. transpiration rate (E), stomatal conductance and water use efficiency (WUE). Total phenolics and flavonoids contents ~~from their~~ leaves ~~extracts~~ were measured using Folin-Ciocalteu ~~reagents~~ method.

Results: It was observed that plant height, shoot length, plant temperature, total biomass, and total chlorophyll content were significantly influenced ($P \leq 0.05$) by the different concentrations of magnetic nanoparticles. The net photosynthesis rate (A), transpiration rate (E), stomata conductance (gs), maximum efficiency of photosystem II (Fv/fm), maximum quantum yield of phytochemical and non-photochemical process in photosystem II (Fv/fo), performance index and the density of reaction centers per PSII antenna chlorophyll of *Ipomoea aquatica* were significantly reduced at higher concentration of magnetic nanoparticles. However, water use efficiency and minimal fluorescence value (Fo) of *Ipomoea aquatica* increased with ~~the~~ increased of MNP concentration. In addition, the application of magnetic nanoparticles ~~had~~ significantly influenced ($P \leq 0.05$) the total flavonoids and total phenolics content in water spinach. Both of these parameters were increased when higher concentration of magnetic nanoparticles was applied to *Ipomoea aquatica*. This study showed that ~~application of~~ MNP ~~had~~ affected the growth, carbon assimilation and secondary metabolites production of *Ipomoea aquatica*.

Conclusion: In conclusion, the higher concentration of magnetic nanoparticles ~~can~~ reduced the growth rate and carbon assimilation of water spinach and enhanced the production of secondary metabolites.

Keywords: [*Ipomoea aquatica*, magnetic nanoparticles, growth, carbon assimilation, secondary metabolites]

1. INTRODUCTION

Based on the data from Department of Statistics Malaysia, agriculture sector was operated at 11,628 establishments in the year 2015 with an annual growth of 5.7 percent [1] and vegetables were contributing to agricultural commodities in which spinach, long bean, mustard, brinjal, tomato and cucumber were selected by which more than 100 percent were recorded in self-sufficiency ratio [2]. Malaysia population in 2018 is estimated at 32.4 million compared to 32.0 million in 2017 by which 1.1 per cent as the growth rate [3]. As the population increases, the demand for food and commercial energy is accelerating to fulfill the population requirements. According to Department of Agriculture Malaysia, Kangkung (*Ipomoea aquatica*) is one of the vegetables suggested by Malaysia government to be consumed widely due to its low price and Asia had consumed water spinach at the highest rate compared to other vegetables [4].

Ipomoea aquatica, which is also known as Kangkung, is a popular vegetable in countries like Malaysia, Hong Kong, Taiwan and other Asia countries. This edible vegetable is classified into the family Convolvulaceae. Kangkung is an aquatic or semi-aquatic yearly herb which usually creeps on moist soil or sand besides floating in water [5]. Countries like Southern Asia, Bangladesh, China and India had been using *Ipomoea aquatica* in folk medicine against different diseases including diabetes, malfunction of liver, constipation and Arsenic poisoning as it is known for its high nutritive values and consumable leafy vegetables [5]. Water spinach is also rich in minerals like flavonoids, phenolics and carotenes. As water spinach is easily grown plant, has short time of harvesting period and well adapted to environment changes, this makes the plant favorable to be cultivated. However, water spinach could accumulate foreign minerals like Cadmium, Zinc and Copper which enables it to be used as the sample plant for research [6].

Metals such as nickel, cobalt, and iron are used to demonstrate magnetic properties in which their magnetic particle size are within nanoscale [7]. Currently, magnetic nanoparticles have attracted researchers from different background like biotechnology, biomedicine, agriculture, magnetic fluids and data storage in which different ways are used in synthesizing those magnetic nanoparticles. However, the application of magnetic nanoparticles relies on the particle's condition on its steadiness [8]. The application of magnetic nanoparticles in the study of plant currently has attracted interest of the researchers for its ability to permit a particular localization to discharge their load as conveyed to the plants. Recent studies being applied on pumpkin plants to test on the specific localization, take-up, and translocation of magnetic nanoparticles (under 50 nm) [9]. Besides, gas exchange is also being influenced by the use of magnetic nanoparticles in which they act on photo-synthetic surface causing foliar warming and changes physiological process and cell elements of plants as the leaves are faced with stomatal obstacle [10].

The application of magnetic nanoparticles may enhance or retard the growth of *Ipomoea aquatica* through carbon nanotubes that increased the leaf gas trades properties of the plant. It was seen in *Arabidopsis thaliana* that treated with single wall carbon nanotubes (SWCNT) the plant had higher photosynthetic, photoabsorption and higher electron transport rates contrasted with the plant that was not treated with the materials. This was expected to a higher productivity of chloroplast when cooperated with the nanomaterials [11].

Secondary metabolites are also an important factors being influenced by the magnetic nanoparticle application. Secondary metabolites are the natural products present in low amounts in which its and whose production rely on different species, genera and families. Secondary metabolites are important in protecting plants from insects and

68 | pathogens, besides shaping imperative UV-radiation absorbing compounds that eventually
69 | reduce the chances for the plant to die [12]. The production of flavonoids and phenolics are
70 | the determinants for the production of secondary metabolite in plant. It is oftenly ~~to~~-observed
71 | that plants undergoing stress have^s higher total flavonoid and phenolics contents due to
72 | defensive mechanism in plant~~them~~. ~~The signal~~Biosynthesis in plant to produce secondary
73 | metabolites is usually stimulated by the accumulation of heavy metals like zinc, iron, and
74 | nickel which generates Reactive Oxygen Species (ROS) and induces oxidative stress in
75 | plant. Consequently, the induction of oxidative stress causes changes in signal transduction
76 | for the mechanism of gene coding and enzyme [13]. Besides, the production of ROS may
77 | cause damages to cell membrane, cell structure and photosynthetic site and thus the
78 | production of flavonoid at the generation site act as defensive mechanism due to its high
79 | antioxidant properties. In addition, induced phenolics are produced when the plant faces
80 | physical injury, infection or environmental stresses due to heavy metal irradiation or
81 | temperature [14].

82 |
83 | The low toxicity level of magnetic nanoparticles such as super paramagnetic nanoparticles
84 | (SPION) has been the reason for growing studies on the application of magnetic
85 | nanoparticles on organisms. A study shows that iron oxide nanoparticles is safe and non-
86 | cytotoxic at the level of 100 µg/ml when being compared with few metal oxide
87 | nanoparticles *in vitro* [15]. In one of the studies related to iron oxide nanoparticles exposure
88 | on sunflower plant, *Helianthus annuus*, it was seen that at the concentration of 50-100 mg/L
89 | the exposure resulted in reduction of root water pressure of the plant [16]. Besides, a study
90 | by Liu, Zhang and Lal [17] showed^a that the iron oxide nanoparticles were^s less toxic,
91 | besides stimulating root elongation during the germination of lettuce, *Lactuca sativa* at the
92 | concentration of 5–20 mg L⁻¹ while inhibited root elongation at 50 mg L⁻¹ [18].

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93 |
94 | Magnetic nanoparticles application is currently recognized in biomedicine to treat various
95 | diseases but ~~is~~ still far behind in plant biology. Magnetic-based materials can be used in
96 | production of certain chemicals to protect plant systematically from diseases besides
97 | controlling externally the movement of nanocarriers in the plant by using high power external
98 | magnet [19]. As water spinach is well known for its importance in culinary as vegetable and
99 | in traditional medicine, this study would help the plant to be continuously used in studies.

100 |
101 | The study of *Ipomoea aquatica* in the aspects of biochemical and physical towards magnetic
102 | nanoparticles is still far behind in current research. Besides, the research on the impacts of
103 | magnetic nanoparticles on growth, carbon assimilation and quality of plant in form of
104 | secondary metabolites production is still few in science studies. Hence, this research was
105 | conducted with few objectives which are to relate the growth, carbon assimilation and quality
106 | of *Ipomoea aquatica* as influenced by magnetic nanoparticles application, to determine the
107 | best concentration of magnetic nanoparticles that can promote the optimum growth and
108 | quality of *Ipomoea aquatica* and to infer the relationship between growth and secondary
109 | metabolites of *Ipomoea aquatica* as affected by magnetic nanoparticles application. ~~The
110 | study hypothesized that the application of magnetic nanoparticles influence growth, carbon
111 | assimilation and secondary metabolites of Ipomoea aquatica while the alternative hypothesis
112 | stated that the application of magnetic nanoparticles does not influence growth, carbon
113 | assimilation and secondary metabolites of Ipomoea aquatica.~~

114 | 115 | 116 | 2. MATERIAL AND METHODS

117 | 118 | 2.1 Synthesis of Iron Oxide Nanoparticles (Fe₃O₄) 119 |

120 The iron oxide nanoparticles (Fe_3O_4) were synthesized using co-precipitation method [20].
121 Iron (III) chloride powder and Iron (II) chloride powder were mixed with-in the ratio of 2:1 in a
122 250 ml conical flask and were dissolved in 150 ml of deionized water. The solution was
123 heated at 45°C bubbled with nitrogen gas for 15 minutes. The solution was then added with
124 20 ml of 25-30 % ammonia solution and stirred at 800 rpm for an hour. Then, the Iron (III)
125 oxide nanoparticles produced was collected by magnetic decantation. The Fe_3O_4 product
126 was then washed 3 times with acetone and centrifuged. The product was left to dry in
127 furnace one night and grinded after drying process to obtain the powder form. Fe_3O_4
128 produced were in black colored fine powder.

129

130 **2.2 Plant Materials and Maintenance**

131

132 The experiment was conducted at the Vegetables Field plot for Teaching and Research,
133 Taman Pertanian Universiti, Universiti Putra Malaysia. The source of planting materials in
134 the study was the seeds of *Ipomoea aquatica*. Seeds of *Ipomoea aquatica* were propagated
135 for 14 days in a tray and transplant to polybags containing a mixture of top soil and sand
136 (ratio 3:1). After 1 month, *Ipomoea aquatica* plants were exposed to four different treatments
137 of Fe_3O_4 (0, 50, 100 and 150 mg/L). The magnetic nanoparticles were conveyed to the
138 plants through watering. The magnetic nanoparticles were diluted in distilled water before
139 being applied to the plants. Each plant was watered with 40 mL of magnetic nanoparticle
140 solution. To maintain the plant growth and avoid plant wilting or attacked by any major plant
141 disease that can make taffectthe plants die, maintenance was done time to time.

142

143

144 **2.3 Experimental Design**

145

146 The polybags were arranged accordingly to Completely Randomized Block Design (RCBD)
147 with replication of 3 blocks. One unit of experiment consisted of 8 plants, and there were
148 total of 96 plants utilized in the experiment.

149

150 **2.4 Data Collection**

151

152 Data collection of plant growth were donecollected on Week 3, 6, 9 and 12 while the
153 destructive analysis and leaf gas exchange measurement were conducted on the 12th week.

154

155 **2.4.1 Plant Growth Measurement**

156

157 The plant growth measurement was done to obtain the Water Spinach plant height, number
158 of leaf, diameter of stem, root to shoot ratio, plant temperature and the chlorophyll content.

159

160 **2.4.1.1 Plant height**

161

162 Measuring tape was used to measure the plant height which starting from stem at soil
163 surface up to the highest shoot growth or at its tip.

164

165 **2.4.1.2 Plant basal diameter**

166

167 The basal tips of the plant were measured by using vernier caliper.

168

169

170 **2.4.1.3 Plant leaves number**

171

172 The whole leaves of the plants were counted manually (one by one piece) and then recorded
in every 3 weeks.

173

174 **2.4.1.4 Plant leaf temperature determination**

175

176 Infrared (IR) Thermometer was used to measure plant leaf temperature.

177

178 **2.4.1.5 Chlorophyll content measurement**

179

180 Total chlorophyll content in the leaves was measured using SPAD 502 chlorophyll meter
181 (Spectrum Tech Inc; Aurora, IL; USA). The leaf was clipped under the chlorophyll meter
182 clipper to obtain the reading for every treatment in each of the replication. The data was
183 collected every week from week 0 until week 12 of the experiment.

184

185 **2.4.1.6 Plant fresh weight measurement**

186

187 The plants were removed from the soil carefully and the dirt from the soil was rinsed with tap
188 water. The shoot, root and the leaf parts were separated and weighed using analytical
189 balance.

190

191 **2.4.1.7 Plant biomass (Dry weight) measurement**

192

193 All the plants were dried for 2 days in the oven at 60°C. The dry weight of root, stem and leaf
194 per seedling were recorded as plant biomass.

195

196 **2.4.1.8 Root to shoot ratio**

197

198 The root to shoot ratio was determined by dividing the dry weight of the root with the dry
199 weight of the shoot.

200

201 **2.4.2 Leaf Gas Exchange Measurement**

202

203 A LI-6400XT (Li-COR Inc; Nebraska; USA) portable photosynthesis system gave the reading
204 of leaf gas exchange. The instrument was warmed and calibrated for 30 minutes with the
205 ZERO IRGA mode. The measurements used optimal conditions set at 400 $\mu\text{mol mol}^{-1} \text{CO}_2$,
206 30°C cuvette temperature, 60% relative humidity with air flow rate set at 500 $\text{cm}^3 \text{min}^{-1}$ and
207 modified cuvette condition of 800 $\mu\text{molm}^{-2}\text{s}^{-1}$ photosynthetically photon flux density (PPFD).
208 The fully expanded young leaves were used to measure net photosynthesis (A),
209 transpiration rate (E) and stomata conductance (g_s) which also gave the measurement of
210 gas exchange. It was an automatic operation therefore the data was recorded in the LI-
211 6400XT console and further analyzed by Photosyn Assistant Software (Dundee Scientific,
212 Dundee, UK). Precautions step were considered while taking the measurements to avoid
213 errors.

214

215 **2.4.3 Chlorophyll Fluorescence Determination**

216

217 Mature leaf tissue of water spinach was collected from plants which ~~were~~ grown in a
218 greenhouse at 20° C with the supply of artificial light which provide a minimum photon flux
219 density of 550 $\mu\text{mol m}^{-2} \text{S}^{-1}$ for a 16 h photoperiod (Fi- totron H600, Fisons pic,
220 Loughborough, U.K.) and photosynthetically active radiation will be supplied at 250 $\mu\text{mol m}^{-2} \text{S}^{-1}$
221 during 16 h photoperiods.

222 **2.4.4 Total Phenolics and Flavonoids Quantification**

223

224 The total phenolics and flavonoid measurement follow methods from Ibrahim, Jaafar,
225 Rahmat, and Zaharah [21]. Plant tissue samples (0.1 g) that was initially grounded were

Comment [U1]: Ibrahim et al

226 extracted with 80% ethanol (10 mL) on an orbital shaker at 50°C for 120 minutes. The
227 mixture was filtered and the filtrate used for the quantification of total flavanoids and total
228 phenolics. Total phenolics content of the leaf samples was determined by using Follin-
229 Ciocalteu reagent (Sigma Aldrich, Missouri, USA; diluted 10-fold). The absorbance
230 measured at 725 nm. The results ~~were~~^{will be} expressed as mg g⁻¹ gallic acid equivalent (mg
231 GAE g⁻¹ dry sample). For total flavonoids determination, a sample (1 mL) mixed with NaNO₃
232 (Sigma Aldrich, Missouri, USA; 0.3 mL) in a test tube which covered with aluminum foil, and
233 left for 5 min. Then 10% AlCl₃ (Wako Pure Chemical Industries Ltd; Tokyo, Japan; 0.3 mL)
234 added followed by addition of 1 M NaOH (Kanto Chemical Co. Inc.; Hokkaido, Japan; 2 mL).
235 Later, the absorbance was measured at 510 nm using a spectrophotometer with rutin as a
236 standard (results expressed as mg g⁻¹ rutin dry sample).
237

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238 2.5 Statistical Analysis

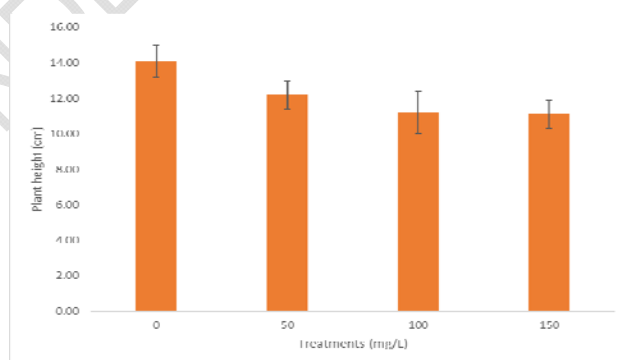
239
240 The recorded data was analyzed by using statistic software known as Statistical Package for
241 Social Sciences (SPSS) with the version 24. A two-way ANOVA Test was carried out for
242 data analysis for all the parameters in the experiment. Data was significant if the *p*-value
243 level ≤ 0.05.
244

Comment [U2]: This is used mainly for social work not scientific work....Are you sure this is what was used??

245 3. RESULTS AND DISCUSSION

246 3.1 Plant Height

247
248 Fig.1 showed that, different concentrations of magnetic nanoparticles application influenced
249 significantly the plant height of *Ipomoea aquatica* at *P* ≤ 0.05 in week 3. The highest plant
250 height was observed significantly in 0 mg/L (14.10 cm), followed by 50 mg/L (12.2 cm), 10
251 mg/L (11.2 cm) and lowest in 150 mg/L (11.1 cm), these differences were significantly
252 different. The current result indicates that the application of magnetic nanoparticles at higher
253 concentration would reduced the plant height of *Ipomoea aquatica*. Research had
254 shown that higher concentration of Fe₃O₄ with pro-longed exposure may lead to iron toxicity
255 in the plant. The plants that faced toxicity during growth stage may initiate stress and
256 causes reduction in plant height due to consequences effects of iron toxicity like production
257 of free radicals, root break down and bronzing of leaves which lead to yield loss [22]. The
258 application of Fe₃O₄ stimulate the production of Reactive Oxygen Species (ROS) under the
259 condition of excess or deficiency of Fe₃O₄ that responsible of signaling molecule that
260 stimulate or inhibit plant growth [23].
261
262



264 Fig. 1. The impact of magnetic nanoparticles on plant height of *Ipomoea aquatica*
265 Data are mean \pm standard error of mean (SEM). N= 24. Mean with different superscript
266 showed the significantly different at $P \leq 0.05$ using DMRT at 95% confidence levels.

Comment [U3]: Not letter shown in the figure...???

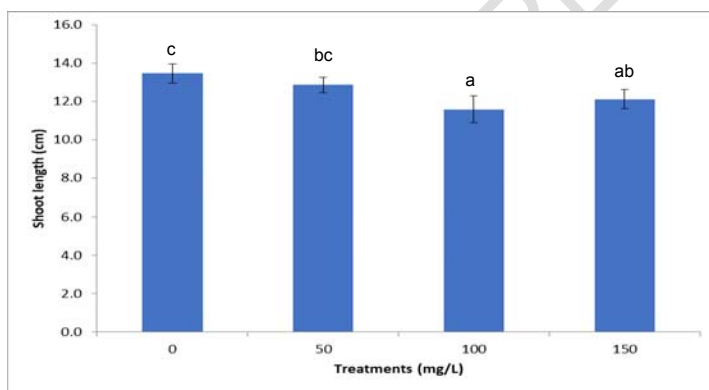
Comment [U4]:

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267 3.2 Shoot Length

269 In this study, the shoot length of *Ipomoea aquatica* has been influenced significantly by the
270 application of magnetic nanoparticle ($P \leq 0.05$; Fig. 2) in week 3. The longest shoot length
271 was observed in 0 mg/L (13.5 cm) followed by 50 mg/L (12.9 cm), 150 mg/L (12.1 cm) and
272 shortest for 100 mg/L (11.6 cm). From the result, we could observe that the shoot length of
273 *Ipomoea aquatica* increased initially with the application of magnetic nanoparticles at both
274 lower and higher concentration than 100 mg/L treatment. However, water spinach treated
275 with 100 mg/L reduces the shoot elongation. Overall, the application of magnetic
276 nanoparticles increased the shoot length of water spinach. From other researches, Fe_3O_4
277 between 100-200 mg kg^{-1} had shown positive effect on plant like *Spinacea oleracea* and no
278 any effect at lower concentration which is lower than 100 mg/L on *Helianthus annus*. The
279 stem length of *Spinacea oleracea* increased with the application of Fe_3O_4 while when there is
280 no oxidative stress induced in the plant, no positive or negative effect is shown on shoot
281 length like in *Helianthus annus* [18]. The shoot length of *Vigna radiata* applied with Fe_3O_4
282 had also shown positive effect [24].

Comment [U6]: What is the difference between this and section 3.1 above??? – Either use shoot length or plant height- look at the values in Fig 1 and 2. They are the same only that Fig 2 is better because it has letters



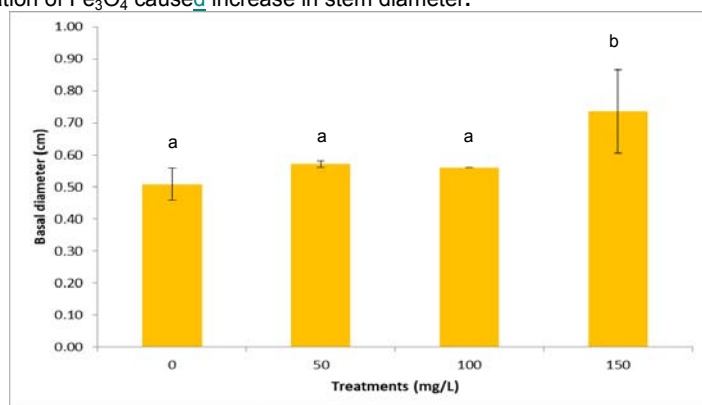
284

285 Fig. 2. The impact of magnetic nanoparticles on shoot length of *Ipomoea aquatica*
286 Data are mean \pm standard error of mean (SEM). N= 24. Mean with different superscript
287 showed the significantly different at $P \leq 0.05$ using DMRT at 95% confidence levels.

289 3.3 Basal Diameter

291 Different concentration of magnetic nanoparticles application has significantly influenced the
292 basal diameter of *Ipomoea aquatica* ($P \leq 0.05$; Fig. 3) in week 12. At 12th week, the
293 application of magnetic nanoparticles has shown a significant increase significantly their
294 basal diameter of *Ipomoea aquatica* at higher concentration which is at 150 mg/L. The
295 smallest basal diameter was observed in 0 mg/L (0.51 cm), followed by 100 mg/L (0.56 cm),
296 50 mg/L (0.571 cm) and greatest in 150 mg/L (0.74 cm). The current result indicates that the
297 application of magnetic would promote the stem diameter of *Ipomoea aquatica*. Based on
298 research, aquaporins are found on tonoplast or also known as vacuolar membrane which
299 allows water to be freely moves across the cells in the symplastic route. Therefore, the
300 abundance of aquaporins found on the membrane depends on the regulation of water flow.

301 During water stress due to high concentration of nanoparticles may lead to induction of
302 turgor-responsive aquaporins to maintain turgor pressure. This would cause the vacuole size
303 to increase that brings to cell expansion and increased of stem diameter [25]. Besides, a
304 research done on radish cotyledon shows that increase in turgor pressure increase the cell
305 expansion and growth. However, the study claimed that under unfavorable condition, cell
306 would not divide ~~but~~ [26]. Therefore, ~~it is proposed we could conclude~~ that higher
307 concentration of Fe₃O₄ caused increase in stem diameter.



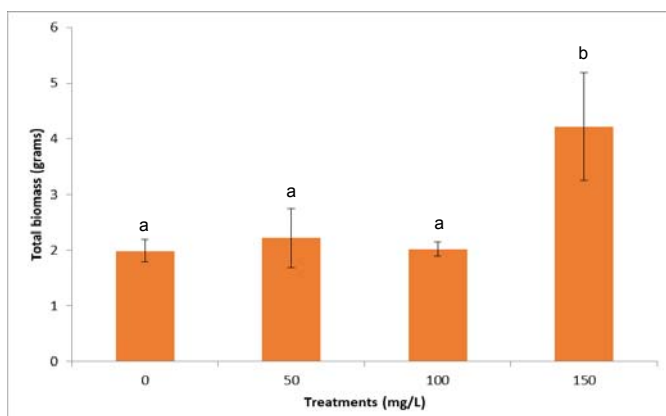
308
309 **Fig. 3. The impact of magnetic nanoparticles on basal diameter of *Ipomoea aquatica***
310 **Data are mean \pm standard error of mean (SEM). N= 24. Mean with different superscript**
311 **showed the significantly different at $P \leq 0.05$ using DMRT at 95% confidence levels.**
312
313

314 3.4 Total Biomass

315 Based on Fig. 4, different concentration of magnetic nanoparticles ~~significantly had~~
316 influenced ~~significantly~~ the total biomass of *Ipomoea aquatica* ~~as ($P \leq 0.05$)~~ in week 12. In
317 week 12, a significant effect was observed by which the application of Fe₃O₄ with the
318 concentration of 150 mg/L increased ~~significantly~~ the biomass of water spinach. The highest
319 total biomass was observed in 150 mg/L (4.2179 gram), followed by 50 mg/L (2.2189 gram),
320 100 mg/L (2.0213 gram), and 0 mg/L (1.9824 gram). The result obtained showed that iron
321 oxide nanoparticles with highest concentration would increase the total biomass of *Ipomoea*
322 *aquatica*. This result was in agreement with the finding by [Sheykhbaglou and Sedghi](#) [27].
323 From their research, it was found that the use of iron oxide nanoparticles increased the dry
324 weight of pod and peanut plant. They found that the application of Fe₃O₄ helped in
325 transferring iron and photosynthate particle in the leaves of plants. Besides, the total
326 biomass of *Vigna radiata* applied with Fe₃O₄ showed a positive result compared to the
327 application of ferum ions. This was ~~elaimed~~ due to the increase of α -amylase activity in the
328 seeds exposed to Fe₃O₄. The increase of total biomass was also observed in *Spinacea*
329 *oleracea* applied with magnetic nanoparticles [18]. However, study by Jeyasubramanian et
330 al. [28] showed that higher concentration of Fe₃O₄ ~~which is~~ (200 mg/L) cause decrease in
331 both wet and dry weight of spinach [29].
332

Comment [U7]:

Comment [U8R7]: Maintain same decimal points

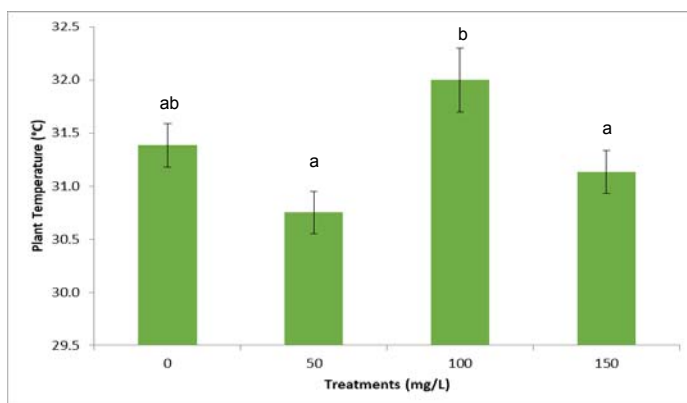


333 **Fig. 4. The impact of magnetic nanoparticles on total biomass of *Ipomoea aquatica***
 334 **Data are mean \pm standard error of mean (SEM). N= 24. Mean with different superscript showed**
 336 **the significantly different at $P \leq 0.05$ using DMRT at 95% confidence levels.**
 337

3.5 Plant Leaf Temperature

338
 339
 340 Fig. 5 showed that magnetic nanoparticle ~~had influenced~~ significantly influenced the plant
 341 temperature of *Ipomoea aquatica* ~~as~~ ($P \leq 0.05$) in week 12. The highest plant temperature
 342 was observed ~~at for~~ 100 mg/L, 32.0 °C followed by 0 mg/L (31.4 °C), 150 mg/L (31.1 °C) and
 343 50 mg/L (30.8 °C) in week 12. The result shows that a higher concentration of magnetic
 344 nanoparticles shows higher leaf temperature of water spinach. Plant temperature is often
 345 related to transpiration process. Transpiration is a cooling process taken by the plant to
 346 release water vapor from the plant through stomata and cuticle. Through transpiration,
 347 thermal energy is balanced by the loss of heat to its surrounding. Therefore, when the
 348 transpiration rate is decreased due to the accumulation of nanoparticles at the root surface
 349 and inhibits the water intake capacity, the plant temperature would increase. Besides, the
 350 stomata conductance also plays a major role in maintaining the leaf plant temperature. The
 351 closing and opening of stomata do give impact on the plant temperature [30]. Thus, higher
 352 concentration of Fe_3O_4 result higher plant temperature due to its relation with transpiration
 353 rate and stomata conductance.
 354

Comment [U9]: No- note that 0mg/L was second- which was higher than 150 and 50!!!

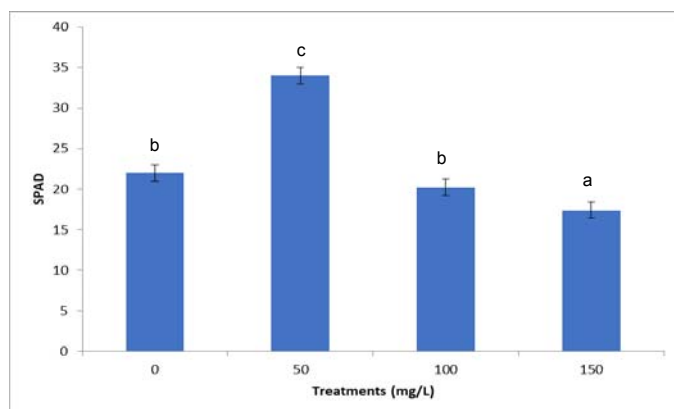


See that 0 and 100 mg/L were not statistically different!!! While 0, 50 and 150 were also same..

Fig. 5. The impact of magnetic nanoparticles on plant temperature of *Ipomoea aquatica*. Data are mean \pm standard error of mean (SEM). N= 24. Mean with different superscript showed the significantly different at $P \leq 0.05$ using DMRT at 95% confidence levels.

3.6 Total Chlorophyll Content

Fig. 6 shows the impact of magnetic nanoparticle on total chlorophyll content of water spinach. There was significant effect of the application of magnetic nanoparticles on the water spinach chlorophyll content at $P \leq 0.05$. After 12 weeks of treatment, the highest chlorophyll content was observed in *Ipomoea aquatica* treated with 50 mg/L Fe_3O_4 (34), followed by 0 mg/L (22) and 100 mg/L (20) and lowest in 150 mg/L (17). This result indicates that higher concentration of magnetic nanoparticles lead to decrease of chlorophyll content in water spinach which was supported by several studies. Based on research by Racuciu et al. [31], *Zea mays* plant treated with 20, 40, 60, 80, and 100 μ l/l of Fe_3O_4 suspension concentration showed decreased in chlorophyll content. The magnetic nanoparticles may showed both chemical and magnetic influence on the water spinach enzymatic structure that eventually influence the photosynthetic system of the plant at higher concentration. Besides, the application of nanoparticles would induce oxidative stress in plant that reduced the chlorophyll content in plant leaf. For example, zinc oxide nanoparticles had proven to reduce the chlorophyll content in wheat plant due to the formation of free radical [32]. Another research done on watermelon showed that the application of magnetic nanoparticles at higher concentration loss the content of chlorophyll. Due to the toxic substance exposure to the plant, the Malondialdehyde (MDA) production could be observed as a result of lipid peroxidation. Low level of MDA is important in protecting the structure and function of cell membranes. As the increase of MDA in plant with the presence of Fe_3O_4 , the penetration of large particles into the cell is disturbed that result in less efficiency of cell. Thus, iron is claimed to be the cause for enzymatic activity inhibition that reduce chlorophyll synthesis [33].

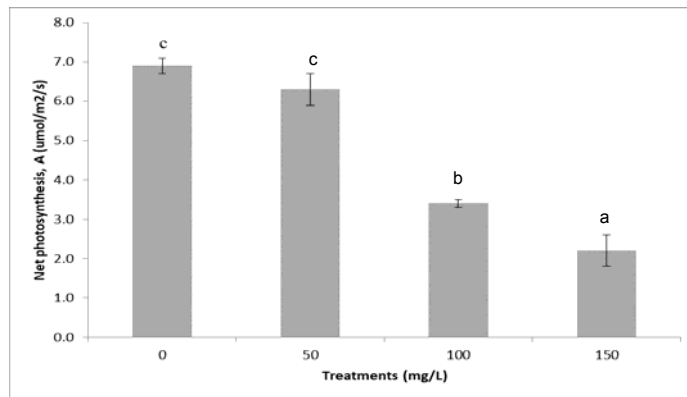


387

388 **Fig. 6. The impact of magnetic nanoparticles on total chlorophyll content of *Ipomoea***
 389 ***aquatica*. Data are mean ± standard error of mean (SEM). N= 5. Mean with different**
 390 **superscript showed the significantly different at $P \leq 0.05$ using DMRT at 95%**
 391 **confidence levels.**

392 3.7 Net Photosynthesis Rate

393 Fig. 7 showed the net photosynthesis rate of water spinach after 12 weeks of planting. The
 394 results shown that the net photosynthesis rate was significantly influenced significantly
 395 ($P \leq 0.05$) by the application of Fe_3O_4 during the planting period. The higher concentration of
 396 magnetic nanoparticles resulted in lower net photosynthesis rate of water spinach. The net
 397 photosynthesis of control treatment (0 mg/L) was higher than other treatments; which are
 398 50 mg/L, 100 mg/L and 150 mg/L that recorded 6.9 $\mu\text{mol}/\text{m}^2/\text{s}$, 6.3 $\mu\text{mol}/\text{m}^2/\text{s}$, 3.4 $\mu\text{mol}/\text{m}^2/\text{s}$,
 399 and 2.2 $\mu\text{mol}/\text{m}^2/\text{s}$ respectively. The photosynthesis rate could be have been altered due to
 400 the application of magnetic nanoparticles by which these nanoparticles could block the
 401 pathway and caused stress to the water spinach. Although nanoparticles are in the size of
 402 nanometer but their entry into the plant may cause some changes either by enhancing or
 403 inhibiting the photosynthesis rate. In this case, the Electron Transport Chain may be blocked
 404 by the nanoparticles and enhance stress to the plant manipulating and changing the
 405 normality of genes and enzymes like Rubisco. Rubisco is one of the enzymes that play an
 406 important role in the conversion of carbon dioxide into biological substances [34]. Therefore,
 407 the changes in production of Rubisco may lead to lower net photosynthesis rate with the
 408 application of magnetic nanoparticles.

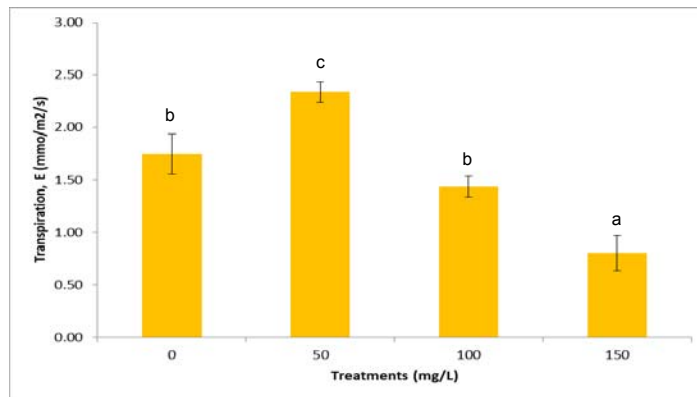


409

410 **Fig. 7. The impact of magnetic nanoparticles on net photosynthesis rate of *Ipomoea***
 411 ***aquatica*. Data are mean \pm standard error of mean (SEM). N= 5. Mean with different**
 412 **superscript showed the significantly different at $P \leq 0.05$ using DMRT at 95%**
 413 **confidence levels.**

414 3.8 Transpiration Rate

415 Based on Fig. 8, the transpiration rate of *Ipomoea aquatica* was significantly influenced by
 416 the application of magnetic nanoparticles after 3 months of experimental period as $P \leq 0.05$.
 417 The transpiration rate was highest observed in 50 mg/L treatment followed by the control
 418 treatment, 100 mg/L and 150 mg/L of Fe_3O_4 by 2.34 $mmol/m^2/s$, 1.75 $mmol/m^2/s$, 1.44
 419 $mmol/m^2/s$, and 0.80 $mmol/m^2/s$ respectively. This indicates that higher concentration of
 420 Fe_3O_4 would decrease the transpiration rate while an optimum concentration ~~did not~~
 421 inhibit the transpiration rate in which in this case is 50 mg/L of iron oxide nanoparticles.
 422 Transpiration rate is linked up by photosynthesis rate by means if there is reduction in
 423 photosynthesis rate, the transpiration rate would decrease too. Besides, nanoparticles
 424 applied to the plant may cover the root surface of the plant and causes water stress in water
 425 spinach. This is supported by a research using Titanium oxide nanoparticle in maize in which
 426 the water transport capacity of the primary cell wall was reduced due to accumulation of
 427 nanoparticles at the plant leaf [35]. Besides, the research done on watermelon to study the
 428 application of magnetic nanoparticles on the root activity showed that the Fe_3O_4 accumulated
 429 at the root surface that prevent the transmission of water and also other nutritional
 430 components by the plant [33].
 431

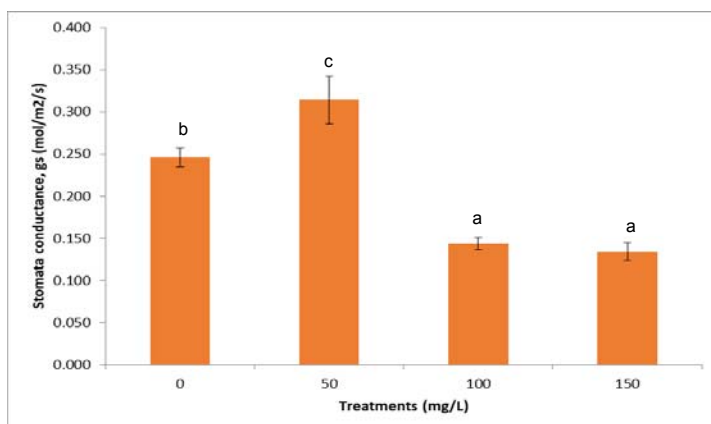


432

433 **Fig. 8. The impact of magnetic nanoparticles on transpiration rate of *Ipomoea***
 434 ***aquatica*. Data are mean \pm standard error of mean (SEM). N= 5. Mean with different**
 435 **superscript showed the significantly different at $P \leq 0.05$ using DMRT at 95%**
 436 **confidence levels.**

437 3.9 Stomata Conductance

438 The stomata conductance of water spinach was significantly affected by the application of
 439 magnetic nanoparticles as showed in Fig. 9 as $P \leq 0.05$. After 12 weeks of treatment
 440 application, the stomata conductance for 50 mg/L Fe_3O_4 was the significantly highest
 441 compared to 0 mg/L, 100 mg/L and 150 mg/L with 0.314 mol/m²/s, 0.246 mol/m²/s, 0.144
 442 mol/m²/s, and 0.134 mol/m²/s respectively. From the result, we could it was observed that
 443 higher concentration of Fe_3O_4 would decrease the stomata conductance of water spinach.
 444 Stomata conductance is determined by the degree of stomata aperture which estimates the
 445 rate of gas exchange and transpiration rate. A greater conductance is shown when the
 446 degree of stomata opening and its function in term of density and size is greater. When the
 447 plant undergoes greater photosynthesis and transpiration rate, the stomata conductance is
 448 greater. This statement is proven by the result of this experiment by which when the
 449 photosynthesis and transpiration rate of water spinach is lower for higher concentration of
 450 Fe_3O_4 , the stomata conductance follows the same pattern. The application of magnetic
 451 nanoparticles may cause leakage of electrolyte in the plant which alters the mechanism of
 452 potassium pump that controls stomata opening. The accumulation of magnetic nanoparticles
 453 may cause the stomata to be closed or partially closed which directly reduce the stomata
 454 conductance [14].

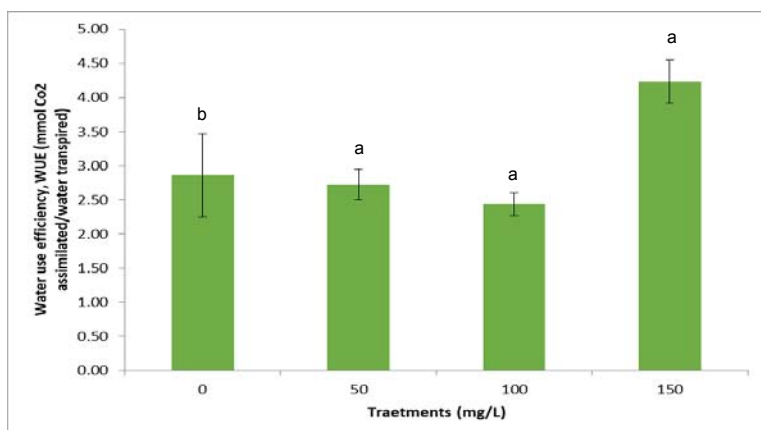


455

456 **Fig. 9. The impact of magnetic nanoparticles on stomatal conductance of *Ipomoea***
 457 ***aquatica*. Data are mean \pm standard error of mean (SEM). N= 5. Mean with different**
 458 **superscript showed the significantly different at $P \leq 0.05$ using DMRT at 95%**
 459 **confidence levels.**

460 3.10 Water Use Efficiency

461 From the Fig. 10, the result showed that the water use efficiency of water spinach was
 462 significantly affected by the application of Fe_3O_4 as $P \leq 0.05$ by which the significantly-highest
 463 water use efficiency was recorded for 150 mg/L treatment, followed by other treatments with
 464 0 mg/L, 50 mg/L and 100 mg/L at 4.23, 2.86, 2.72, and 2.44 $\mu\text{mol}/\text{CO}_2/\text{mmol}/\text{H}_2\text{O}$
 465 respectively. From the result, ~~we could it was~~ observed that the water spinach with the
 466 highest concentration of Fe_3O_4 showed greater water use efficiency. Water use efficiency is
 467 one of the important determinants of plant growth which is under stress as the plant
 468 maximize the capturing of soil moisture when there is limitation of water supply or lower
 469 stomata conductance in order to increase yield production [36]. Water use efficiency is
 470 related with transpiration rate and defined as the ratio of moles CO_2 assimilated per moles of
 471 water transpired [14]. Therefore, with the application of magnetic nanoparticles at higher
 472 concentration increase the transpiration rate of *Ipomoea aquatica*, the water use efficiency is
 473 also increased.

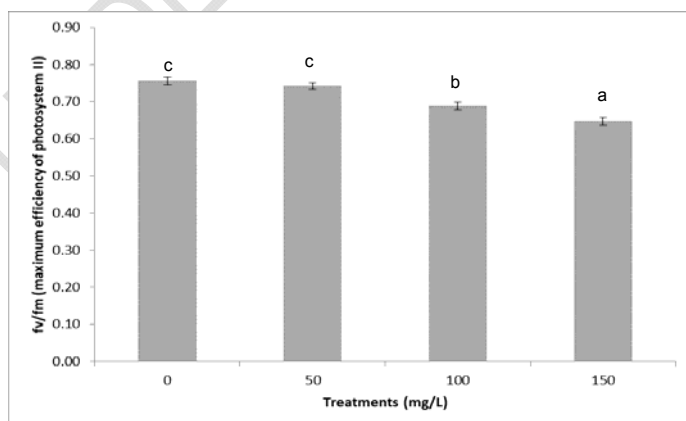


474

475 **Fig. 10. The impact of magnetic nanoparticles on water use efficiency of *Ipomoea***
 476 ***aquatica*. Data are mean ± standard error of mean (SEM). N= 5. Mean with different**
 477 **superscript showed the significantly different at P≤0.05 using DMRT at 95%**
 478 **confidence levels.**

479 3.11 Fv/Fm (Maximum Efficiency of Photosystem II)

480 Figure 11 shows the effect of different concentration of Fe₃O₄ on maximum efficiency of
 481 photosystem II or known as fv/fm ratio. The result of the application on water spinach was
 482 significant as P≤0.05 by which higher concentration of Fe₃O₄ showed lower fv/fm value. The
 483 ratio of fv/fm for 150 mg/L (0.65) was significantly lower than 100 mg/L (0.69) and followed
 484 by 50 mg/L (0.74) and 0 mg/L (0.76). The lowest fv/fm ratio showed by water spinach
 485 treated with 150 mg/l clearly shows that higher concentration of Fe₃O₄ would reduce the
 486 efficiency of photosystem II. Fv/fm is the main parameter used to detect any injury in
 487 photosystem II or photon inhibitory process as it is related to quantum yield of
 488 photosynthesis. The induction of stress on photosynthetic surface in plant would limit the
 489 transformation of light energy in photosystem II. Thus, the decrease in photosynthetic in
 490 plant would reduce the reduction of phytochemical activity in photosystem II [37].

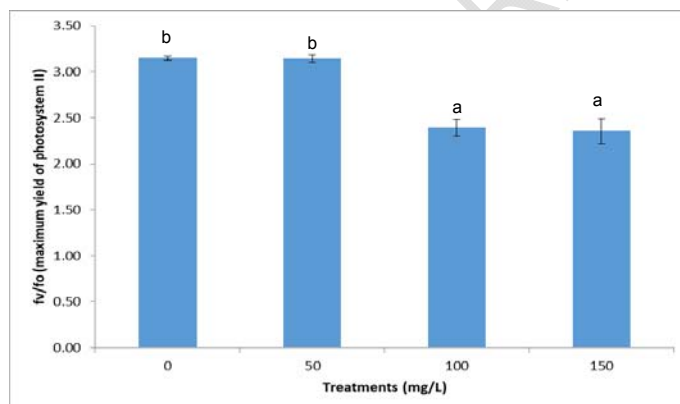


491

492 Fig. 11. The impact of magnetic nanoparticles on f_v/f_m (maximum efficiency of
493 photosystem II) of *Ipomoea aquatica*. Data are mean \pm standard error of mean (SEM).
494 N= 5. Mean with different superscript showed the significantly different at $P \leq 0.05$
495 using DMRT at 95% confidence levels.

496 3.12 F_v/F_o (Maximum Yield of Photosystem II)

497 Fig. 12 shows the effect of different concentration of Fe_3O_4 on maximum yield of
498 photosystem II or known as f_v/f_o ratio in 3 months planting periods. The result of the
499 application on water spinach was significant as $P \leq 0.05$ by which higher concentration of iron
500 oxide nanoparticles showed lower f_v/f_m value. The ratio of f_v/f_m for 0 mg/L (3.15) and 50
501 mg/L (3.14) was significantly higher than 100 mg/L (2.40) and 150 mg/L (2.35). As the two
502 parameters f_v/f_m ratio and f_v/f_o ratio showed the similar trend, we could it can be speculated
503 say that both parameters are related to each other. The F_v/f_o is more sensitive towards
504 changes to efficiency of photosystem II compared to f_v/f_m as it shows quantum yield of
505 phytochemical and non-photochemical process. This ratio indicates the state of photosystem
506 II on the energy absorbed and damaged occurred due to plant stress in leaf. This ratio is
507 also affected by the alteration of stomatal closure and carbon fixation process due to water
508 and temperature stress [38]. Besides, the accumulation of magnetic nanoparticles in the
509 photosystem I and II cause obstruction to photosynthesis in donor part of both photosystems
510 that reduce the f_v/f_o ratio [37].
511



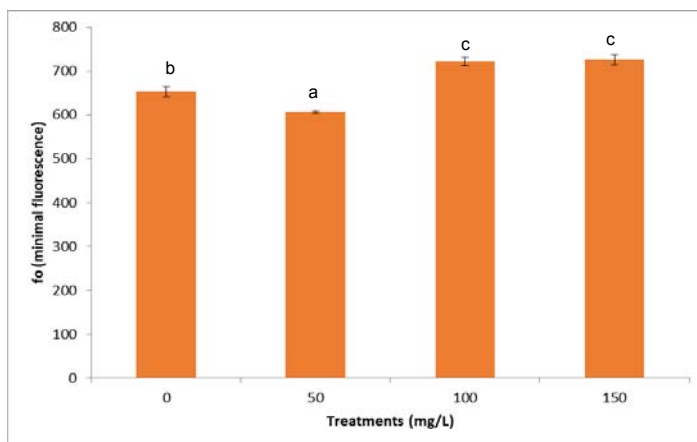
512

513 Fig. 12. The impact of magnetic nanoparticles on f_v/f_o (maximum yield of
514 photosystem II) of *Ipomoea aquatica*. Data are mean \pm standard error of mean (SEM).
515 N= 5. Mean with different superscript showed the significantly different at $P \leq 0.05$
516 using DMRT at 95% confidence levels.

517 3.13 F_o (Minimal Fluorescence)

518 Based on Fig. 13, the application of Fe_3O_4 had significantly influenced the minimal
519 chlorophyll fluorescence yield of water spinach as $P \leq 0.05$. The F_o of water spinach treated
520 with 100 mg/L and 150 mg/L Fe_3O_4 were significantly higher compared to 0 mg/L and 50
521 mg/L, 722, 726, 653 and 607 respectively. From the result, it is shown that the F_o value
522 increased as the concentration of Fe_3O_4 increased. The application of nanoparticles like
523 titanium oxide showed that with the increase of its concentration, the minimal fluorescence
524 value decreased as titanium oxide nanoparticles do protect the photosynthetic structure of

525 the tomato plant under mild heat stress. However, the study by Gao et al. [39] showed that
526 at high light intensity the minimal fluorescence in *Ulmus elongata* seedlings decreased.
527 Therefore, the best possible explanation for this would be the impact of magnetic
528 nanoparticles on water spinach is complex and it depends both on the concentration of
529 Fe_3O_4 and the environmental condition that would affect the activity of nanoparticles [40].
530 Besides, the application of magnetic nanoparticles increased the heat dissipation in water
531 spinach as the absorbed light energy could not be used for photosynthesis [37].
532

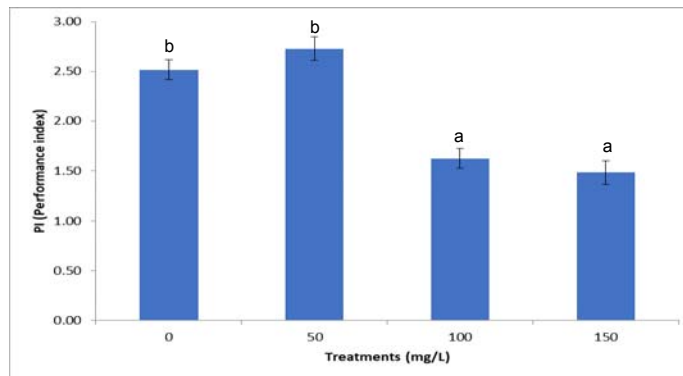


533

534 **Fig. 13. The impact of magnetic nanoparticles on fo (minimal fluorescence) of**
535 ***Ipomoea aquatica*. Data are mean \pm standard error of mean (SEM). N= 5. Mean with**
536 **different superscript showed the significantly different at $P \leq 0.05$ using DMRT at 95%**
537 **confidence levels.**

538 3.14 PI (Performance Index)

539 From the Fig. 14, the result showed that the performance index of water spinach was
540 significantly affected by the application of Fe_3O_4 as $P \leq 0.05$ by which the significantly highest
541 performance index was recorded for 50 mg/L treatment, followed by other treatments with 0
542 mg/L, 100 mg/L and 150 mg/L at 2.73, 2.51, 1.62, and 1.48 respectively. From the result, we
543 it waseould observed that the water spinach with lower concentration of iron oxide
544 nanoparticles showed greater performance index. Performance index is the one of the
545 parameters which is sensitive to environmental conditions in a plant as it is being used as a
546 suitable tool to reflect water deficit in a plant system. Based on research, performance index
547 had been used as a sensitive indicator of water stress in *Triticum aestivum*. Performance
548 index is a combination formula that takes into account the measurement of RC/ABS,
549 maximal energy reflux that reaches photosystem II and electron transport [41]. Therefore,
550 the application of magnetic nanoparticles that changes the net photosynthetic ticsis
551 rate and transpiration rate due to the accumulation of iron oxide nanoparticles at the root surface and
552 the blockage of Electron Transport Chain had affected the performance index of water
553 spinach. Besides, the carbon assimilation process and stomata conductance had correlated
554 with the performance index of water spinach which concluded that higher concentration of
555 Fe_3O_4 showed lower performance index in water spinach [42].



556

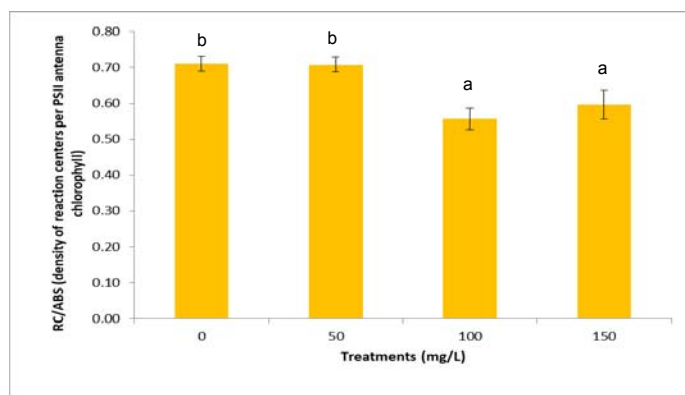
557 **Fig. 14. The impact of magnetic nanoparticles on PI (performance index) of *Ipomoea***
 558 ***aquatica*. Data are mean \pm standard error of mean (SEM). N= 5. Mean with different**
 559 **superscript showed the significantly different at $P \leq 0.05$ using DMRT at 95%**
 560 **confidence levels.**

561 **3.15 RC/ABS (Density of Reaction Centers Per PSII Antenna Chlorophyll)**

562 Fig. 15 shows the effect of different concentration of Fe_3O_4 on density of reaction centers per
 563 Photosystem II antenna chlorophyll in 3 month planting periods. The result of the application
 564 on water spinach was significant as $P \leq 0.05$ by which higher concentration of Fe_3O_4 showed
 565 lower density of reaction centers per Photosystem II antenna chlorophyll. The density of
 566 reaction centers per Photosystem II antenna chlorophyll for 0 mg/L and 50 mg/L is 0.71
 567 which was significantly higher than 100 mg/L (0.56) and 150 mg/L (0.60). RC indicates the
 568 number of active reaction center in photosystem II while ABS shows the quantity of light
 569 absorbed by the antenna chlorophyll. Thus, RC/ABS indicates the total number of active
 570 radiation per light absorption [43]. Due to the exposure of water spinach to Fe_3O_4 , free
 571 radicals production would induce stress to the plant and cause injury to the photosystem II
 572 that limits the active reaction center and light absorption in photosystem II. This parameter
 573 gives the same trend as the performance index as both shows the efficiency of
 574 photosynthesis process in the plant. Therefore, the application of Fe_3O_4 at higher
 575 concentration leads to plant stress and decrease the density of active reaction centers per
 576 photosystem II antenna chlorophyll.

577

578

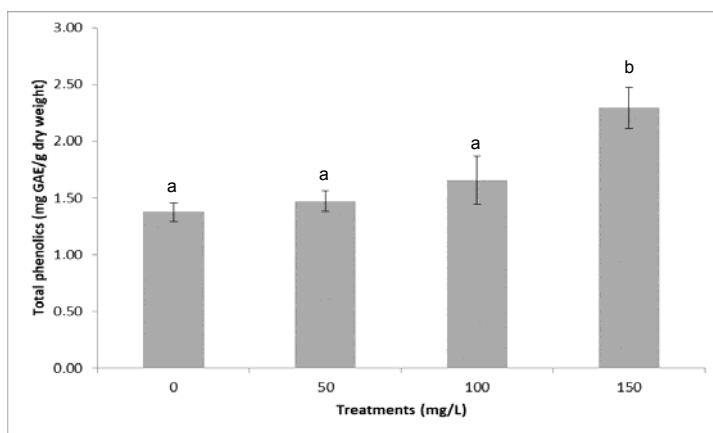


579

580 **Fig. 15. The impact of magnetic nanoparticles on RC/ABS (density of reaction centers**
 581 **per PSII antenna chlorophyll) of *Ipomoea aquatica*. Data are mean \pm standard error of**
 582 **mean (SEM). N= 5. Mean with different superscript showed the significantly different**
 583 **at $P \leq 0.05$ using DMRT at 95% confidence levels.**

584 3.16 Total Phenolics Content

585 Based on Fig. 16, different concentration of Fe_3O_4 had influenced significantly on the
 586 phenolics content of water spinach ($P \leq 0.05$). As the concentration of magnetic nanoparticles
 587 increases, the total phenolics content in the plant increased too. The lowest total phenolics
 588 content was observed in 0 mg/L iron oxide nanoparticles at 1.38 mg GAE/g dry weight and
 589 the highest for 150 mg/L treatment at 2.29 mg GAE/g dry weight. The greater production of
 590 phenolics at higher concentration of magnetic nanoparticles may be a sign of the plant under
 591 stress. The production of secondary metabolites is an effort of the plant to defend itself from
 592 the further damage of plant cell and ensure the survival of plant. The higher production of
 593 phenolic stimulates antioxidant activity in the plant [44]. In order to the response of a plant
 594 towards environmental stresses and protecting itself from damages in plant cell, the
 595 production of phenolic compounds is essential to maintain the plant growth and reproduction
 596 [45]. Based on the study by previous researchers, phenolics compounds do contain
 597 antibiotic and anti-nutritional properties that help in the defense system of a plant. Phenolics
 598 compound usually stored in the epidermal cells of leaves and shoots besides central vacuole
 599 of guard cells. Phenolics content can be divided into two groups which are preformed
 600 phenolics and induced phenolics by which preformed phenolics are being synthesized by the
 601 plant during development of plant tissues under normal condition. While, induced phenolics
 602 are produced when the plant faces physical injury, infection or environmental stresses due to
 603 heavy metal irradiation or temperature [14].

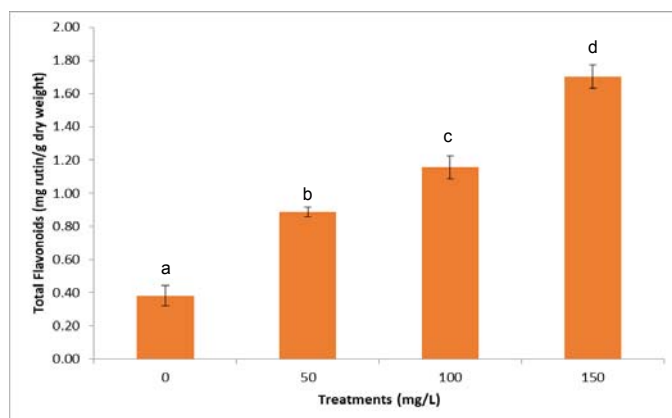


604

605 **Fig. 16. The impact of magnetic nanoparticles on total phenolics content of *Ipomoea***
 606 ***aquatica*. Data are mean \pm standard error of mean (SEM). N= 5. Mean with different**
 607 **superscript showed the significantly different at $P \leq 0.05$ using DMRT at 95%**
 608 **confidence levels.**

609 3.17 Total Flavonoids Content

610 Based on Fig. 17, the production of total flavonoid content ~~washad~~ significantly influenced by
 611 the application of magnetic nanoparticles at different concentrations of Fe_3O_4 ~~as~~ ($P \leq 0.05$). At
 612 the end of experiment of 12th week, the total flavonoid content in water spinach treated with
 613 150 mg/L Fe_3O_4 showed the highest reading at 1.70 mg rutin/g dry weight followed by 100
 614 mg/l treatment at 1.16 mg rutin/g dry weight, 50 mg/L treatment at 0.89 mg rutin/g dry weight
 615 and lastly 0 mg/L treatment at 0.38 mg rutin/g dry weight. From the result, ~~we-it could-was~~
 616 ~~observed~~ that higher concentration of Fe_3O_4 increase the production of flavonoid in water
 617 spinach. The production of flavonoid is one of the determinants for the production of
 618 secondary metabolite in plant. It is oftenly ~~to~~ ~~observed~~ that plant undergoing stress has
 619 higher total flavonoid content due to defensive mechanism in plant. Biosynthesis-Signaling in
 620 plant to produce secondary metabolites is usually stimulated by the accumulation of heavy
 621 metals like zinc, iron, and nickel which generates Reactive Oxygen Species (ROS) and
 622 induces oxidative stress in plant. Consequently, the induction of oxidative stress causes
 623 changes in signal transduction for the mechanism of gene coding and enzyme [13]. Besides,
 624 the production of ROS may cause damages to cell membrane, cell structure and
 625 photosynthetic site and thus the production of flavonoid at the generation site act as
 626 defensive mechanism due to its high antioxidant properties [14].



627

628 **Fig. 17. The impact of magnetic nanoparticles on total flavonoids content of *Ipomoea***
 629 ***aquatica*. Data are mean \pm standard error of mean (SEM). N= 5. Mean with different**
 630 **superscript showed the significantly different at $P \leq 0.05$ using DMRT at 95%**
 631 **confidence levels.**

632 4. CONCLUSION

633

634 Overall, it was found that the application of magnetic nanoparticles which was in the form of
 635 iron oxide nanoparticles had influence ond the growth of *Ipomoea aquatica*. The plant height,
 636 shoot elongation, plant temperature, total biomass and total chlorophyll content were
 637 significantly affected by the application of magnetic nanoparticles at higher concentration.
 638 Besides that, the leaf gas exchange characteristics were also influenced by the different
 639 concentrations of iron oxide nanoparticles, as $P \leq 0.05$. The net photosynthesis rate,
 640 transpiration rate, stomata conductance, maximum efficiency of photosystem II (Fv/fm),
 641 maximum quantum yield of phytochemical and non-photochemical process in photosystem II
 642 (Fv/fo), performance index and the density of reaction centers per PSII antenna chlorophyll
 643 of *I. aquatica* were significantly reduced at higher concentration of magnetic nanoparticles.
 644 While, water use efficiency and minimal fluorescence value of *I. aquatica* were increased
 645 with the increased of iron oxide nanoparticles concentration. In addition, the application of
 646 magnetic nanoparticles had significantly influenced the total flavonoids and total phenolics
 647 content in water spinach

Comment [U10]: These two are the same

648 5. ACKNOWLEDGMENT?

649

650

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652

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