

3
4 **Effect of Nitrogen Rates on Growth and Quality of**
5 **Water Spinach (*Ipomea aquatica*)**
6

7 **ABSTRACT**
8

Aims: The study was conducted to investigate the impact of nitrogen fertilization on growth, leaf gas exchange and bio-metabolite accumulation in *Ipomea aquatica*.

Treatment and experimental design: *Ipomea aquatica* plants were exposed to four different rates of nitrogen (0, 30, 60 and 90 N kg/ha) using Urea (46% N) as a nitrogen source. The experiment was laid out in Complete Randomize Design (CRD).

Place and Duration of Study: Department of Biology, Faculty of Science, Universiti Putra Malaysia between September to November 2016.

Methodology: Four nitrogen rates were applied (0, 30, 60 and 90 N Kg/ha) using NPK green fertilizer as a nitrogen source. The growth data collections were conducted once a week after the application of the treatments for the plant growth parameter. The total chlorophyll content in the leaves was measured using a Soil Plant Analytical Device (SPAD-502) chlorophyll meter. The leaf gas exchange was determined using a LI-6400XT portable photosynthesis system. Total phenolics and flavonoid were determined using Folin-Ciocalteu reagent.

Results: It was found that the growth parameters which are plant height, leaf numbers, branches numbers, total biomass and chlorophyll content recorded the highest measurement at 90 kg N/ha and the lowest at 0 kg N/ha. As for the leaf gas exchange, the positive effect of nitrogen fertilization on kangkung was shown by the increased in photosynthesis rate (A) and stomatal conductance (gs) where the highest measurement recorded at 90 kg N/ha, and the lowest at 0 kg N/ha. However, the water use efficiency (WUE) decreased as the nitrogen rates increased. At lower rates of nitrogen fertilization (30 kg N/ha) produced the highest production of secondary metabolites, where the total phenolics and flavonoids production were enhanced compared to other nitrogen treatments.

Conclusion: In conclusion, as the nitrogen rates increased, the growth and leaf gas exchange properties was enhanced however the production of total phenolics and flavonoids were reduced and get the highest accumulation at 30 kg N/ha.

9
10 *Keywords:* [Nitrogen, *Ipomea aquatica*, growth, leaf gas exchange, biometabolites production]

11
12 **1. INTRODUCTION**
13

14 In Malaysia, agricultural sector contributed about 8.5% to Gross Domestic Products (GDP). About
15 39% of the contributions originated from the production of food crops, fruits, and vegetables. It is
16 estimated that about 44, 000 hectares of the total area in Malaysia were used for vegetable cultivation
17 [1]. According to Department of Agriculture Malaysia in 2011, *Ipomea Aquatica* is one out of ten types
18 of vegetables that occupied the largest area for vegetable production. This plant is among the most
19 consumed vegetable in Asia. This is because of its low price compared to other types of vegetable.
20 Kangkung air or it's scientific name, *Ipomea aquatica*, is a widely known leafy vegetable, especially in
21 the Asian country. The plant is also commonly known by different local names, such as water spinach,
22 swamp cabbage, or water convolvulus. From its scientific classification, kangkung has been classified
23 under the family Convolvulaceae [2]. According to [3], Convolvulaceae family consists of primarily 1650
24 of tropical species. Moreover, the genus of kangkung which is *Ipomea* has about 500 to 600 different
25 species and it has been the most number of containing species in Convolvulaceae family [4]. This
26 species of the family can nicely be grown at almost anywhere at the higher or lower altitudes. *Ipomea*
27 *aquatica* is one of the species that is cultivated on the higher land. Besides easy to be grown,
28 Kangkung cultivation is favored due to its quick maturity period and it does not take long time to
29 mature and harvest. It can easily adapt towards it's growing environment and usually unsusceptible to
30 disease. Almost all parts of kangkung plant are edible [3].
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33 According to Susila et al. [5], nitrogen is the primary nutrient that **involved** in producing a high yield of
34 vegetables. Nitrogen is one of the macro-nutrients that is very crucial especially for a plant to have a
35 proper growth and development [6] such as that required in constructing the matter of the plant cell
36 and tissue [7]. The amount of nitrogen in the soil could be insufficient for the plant to grow. Therefore,
37 the source of nitrogen for plant especially in agriculture field is often found in the form of a fertilizer.
38 Both organic and inorganic nitrogen fertilizer is widely used in agriculture especially in cultivating
39 green crops to keep the source of nutrients for the plant being for **supplied** [6]. Practically, an
40 appropriate and suitable amount of nitrogen to be given to plant will affect its crop yield. Nitrogen **is**
41 **also** very important especially to promote the growth of the plant leaf [8]. Nitrogen is a crucial element
42 not only to promote the growth and plant development, also increase yield and quality in vegetable
43 crops. Increasing level of nitrogen resulting in a number of leaves, leaf length and plant body [8].
44 Nitrogen also enhancing the size of fruits and vegetables where at an optimum application of N will
45 result in a better size. The metabolic process which stimulated by N by enhance the vegetative and
46 also the reproductive growth in the plant. Besides, high plant biomass can be obtained when there is
47 high N accumulated in a shoot, along with the increasing of root growth in a plant if there is sufficient
48 amount of N supply [9]. However, the lack of N in a plant would **cause the reduced in plant**
49 **development and eventually will lower the crop yield. Plants can take up nitrogen (N) either as**
50 **inorganic ions (NH⁴⁺ or NO³⁻), or as organic N. In leafy vegetables, high uptake of NO³⁻ can cause**
51 **serious health problems to the consumers [7]. Nitrate has been attributed to have negative effects to**
52 **human health. Toxicity of nitrate to human can be manifested by headaches, syncope, vertigo and**
53 **discoloration that manifest in fingers or lips [6].**

54
55 Nitrogen **had** been proven to have a strong relationship with photosynthesis process in the plant.
56 Increasing N level leads to higher N content in leaf **vesf**. N also enhances the leaf chlorophyll and CO₂
57 assimilation which increase in the Rubisco activity [10]. Therefore, increase in the rate of
58 photosynthesis is the most vital biochemical process in plants [11]. According to [12,13], rate of
59 photosynthesis (A) depends on the growth **and** development of the plant's leaf. The leaf development
60 includes the increase in leaf area, leaf thickness, the surface volume of mesophyll cells, and leaf
61 chloroplast. The photosynthesis rate will be increased as the leaf development also increased [14].
62 Nitrogen is an element that has a significance role in photosynthesis which involves in the opening of
63 the stomata. The stomatal vent will decrease following the nitrogen deficit which then will decrease
64 the transpiration rate [15].

65
66 Secondary metabolites such as phenolic **acid compounds** in **plants are** usually associated with **the**
67 **survival of the plant survival** and health benefits for those who consume the plant. Low nitrogen level
68 in the plant has been reported to have more secondary metabolites compare to plant that has high N
69 level [16]. Application of more N level resulting in a decrease of phenolic concentrations based on
70 carbon/nutrients balance (CNB) hypothesis [17]. Flavanoids, also a secondary metabolite, **which**
71 **isare** widely distributed with different functions in plants. The biological functions of flavonoids include
72 defense against UV-B radiation, pathogen infection, nodulation and pollen fertility [18]. A study was
73 done by [8] on leaf mustard where the total phenolics concentration was observed to be decreased as
74 the level **of N** increased. It is well known that nitrogen application can directly affect the morphological
75 growth and yield of this plant, however, little work has been carried out to look on the impact of
76 nitrogen of the leaf gas exchange properties and previous work have not comprehensively considered
77 the production of secondary metabolites of *I. aquatica* under nitrogen fertilization. The main aim of the
78 research was to investigate the effect of nitrogen fertilization on the growth, leaf gas exchange and
79 production of secondary metabolites of *I. aquatica* and to determine the best nitrogen rates for growth
80 and development of *I. aquatica*. This research will provide the important information for vegetable
81 growers that involved in the cultivation of vegetables in Malaysia.

82 83 84 **2. MATERIAL AND METHODS**

85 86 **2.1. Plant material and maintenance**

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88 The experiment was conducted at the Department of Biology, Universiti Putra Malaysia, Serdang
89 (UPM), Selangor The seeds were pre-germinated in the nursery for two weeks after which there were
90 transplanted into the polybags filled with a mixture of topsoil, organic matter and sand with the ratio of
91 3:2:1. The nitrogen sources used was single fertilizer Urea (46% N). The polybags were arranged
92 according to Completely Randomized Design (CRD) with five replications. There were four nitrogen

93 rates were applied (0, 30, 60 and 90 Kg N/ha) with overall 160 of *I. aquatica* plants were used. The
94 growth data collections were conducted once a week for four weeks after the application of the
95 treatments for the plant growth parameter. Whereas the destructive analysis and leaf gas exchange of
96 the experiment was conducted at the end of the experiment.

98 **2.2. Plant height, leaf and branch numbers**

99
100 As for plant height, it was measured starting from the stem that was at the soil surface up until the
101 highest shoot grow or at tip using measuring tape. The leaf and branches number were counted
102 manually per plant basis

104 **2.3 Plant total dry weight measurement**

105
106 The plants were first removed from the soil carefully and the dirt from the soil were washed with tap
107 water. After that, the shoot and the root parts were separated. All the plants were dried in an oven for
108 48 hours at temperature of 60°C until constant weight reached.

110 **2.4 Total Chlorophyll content**

111
112 SPAD-502 chlorophyll meter was used to measure the total chlorophyll content of the leaves. Three
113 readings were taken at three spot on a leaf of each plant and the average readings were recorded.
114 Time interval between 9.00 a.m and 12.00 p.m was used to measure the chlorophyll content.

116 **2.5 Leaf gas exchange measurement**

117
118 The leaf gas exchange measurement was obtained after week 4 the treatment was given. The result
119 then was obtained by using the Portable Photosynthesis System machine (LICOR 6400 XT). The
120 IRGA was firstly warm up for at least 30 minutes before the leaf gas exchange was collected with
121 Zero IRGA mode. The optimal condition was set to 400 $\mu\text{mol mol}^{-1}$ carbon dioxide (CO_2), 30 °C
122 cuvette temperature, 60% relative humidity with air flow rate set at 500 $\text{cm}^3 \text{min}^{-1}$, and 800 $\mu\text{molm}^{-2}\text{s}^{-1}$
123 of cuvette condition of photosynthetic photon flux density (PPFD). The time for the measurement were
124 done at the morning of a day. The measurement of photosynthesis rate was taken from the first
125 kangkung leaves starting from the plant apex. The data then were recorded and stored in a console of
126 the system and analyse with Photosyn Assistant Software. The photosynthesis (A), transpiration rate
127 (E), stomata conductance (gs) and water use efficiency (WUE) data was recorded during the
128 measurement.

130 **2.7 Total phenolics and flavonoids quantification**

131
132 The methods used for extraction and quantification of total phenolics and flavonoids contents followed
133 that described in Ibrahim et al. [19]. A fixed amount of ground tissue samples (0.1 g) was extracted
134 with 80% ethanol (10 mL) on an orbital shaker for 120 min at 50 °C. The mixture was subsequently
135 filtered (Whatman™ No.1), and the filtrate was used for the quantification of total phenolics and total
136 flavonoids. Folin–Ciocalteu reagent (diluted 10-fold) was used to determine total phenolics content of
137 the leaf samples. The sample extract at 200 μL was mixed with Folin–Ciocalteu reagent (1.5 mL) and
138 allowed to stand at 22 °C for 5 min before adding NaNO_3 solution (1.5 mL, 60 g L^{-1}). After two hours
139 at 22 °C, absorbance was measured at 725 nm. The results were expressed as mg g^{-1} gallic acid
140 equivalent (mg GAE g^{-1} dry sample). For total flavonoids determination, samples (1 mL) were mixed
141 with NaNO_3 (0.3 mL) in a test tube covered with aluminium foil, and left for 5 min. Then 10% AlCl_3 (0.3
142 mL) was added followed by addition of 1 M NaOH (2 mL). The absorbance was measured at 510 nm
143 using a spectrophotometer with rutin as a standard (results expressed as mg/g rutin dry sample).

145 **2.8 Statistical analysis**

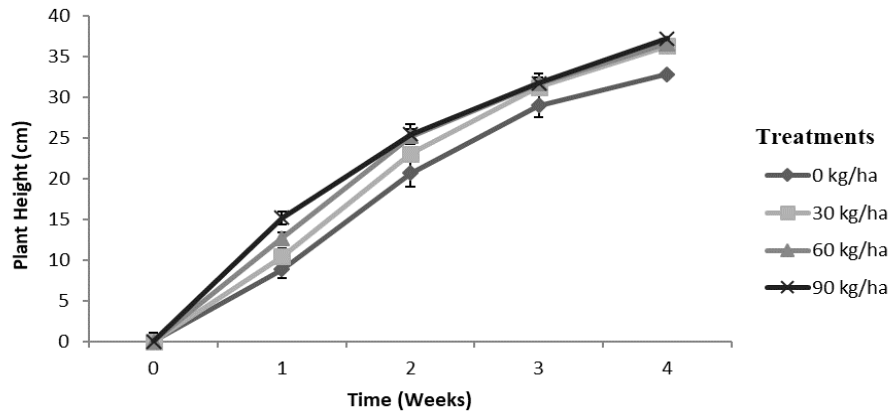
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147 Data were analysed using the analysis of variance procedure in SAS version 17. Means separation
148 between treatments was performed using Duncan multiple range test and the standard error of
149 differences between means was calculated with the assumption that data were normally distributed
150 and equally replicated.

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153 **3. RESULTS AND DISCUSSION**

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155 **3.1 Plant height**

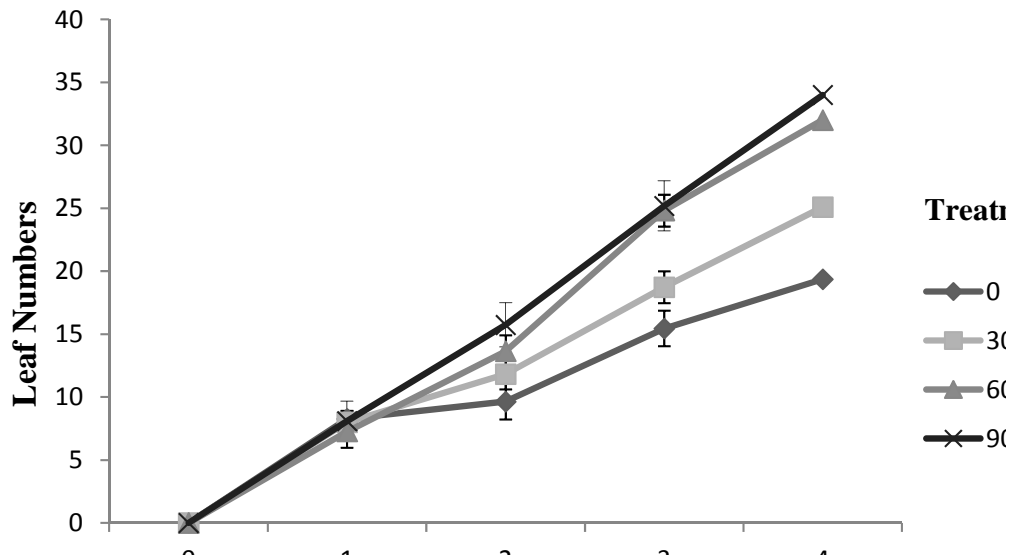
156
157 Figure 1 shows the plant height of *I. aquatica* as influenced by differing nitrogen treatments. The plant
158 height of kangkung was mostly affected by different rates of nitrogen treatment in all week of
159 measurement ($P \leq 0.05$). In view of the result obtained, as nitrogen levels increased from 0 to 90 kg
160 N/ha the plant height was enhanced in all weeks of measurement. In four weeks after treatment (4
161 WAT), plant at 0 kg/ha have the average height of 31.02 cm compared to 32.17 cm by 30 kg/ha,
162 35.61 cm by 60 kg/ha and 37.24 cm in 90 kg/ha. Clearly, as expected, applying higher rates of
163 nitrogen levels would enhance the plant height of *I. aquatica*. The positive effects on plant height
164 caused by the increase of nitrogen rates application may be due to the natural role of nitrogen on
165 vegetative growth performance of plants [6]. The increase in plant height under nitrogen fertilization
166 might be due to well-developed stem under high nitrogen fertilization that resulted in taller plant [20].
167 Besides that, increase in plant height might be associated with the increased of number and length of
168 the internodes by nitrogen [21]. The result obtained agreed with the previous work carried out by [4]
169 and [6] where the increment of nitrogen fertilization rates applied towards *I. aquatica* had significantly
170 increased the plant height at end of the harvesting period. It can be concluded, that in the present
171 study, that high application of nitrogen has shown to enhance the height of *I. aquatica*.



189 **Fig 1. The impact of different nitrogen rates on the height of *Ipomea aquatica*. Mean**
190 **with the same letter indicates that all of the groups were not significantly different**
191 **according to Duncan multiple range test ($P \geq 0.05$) N=10.**

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193 **3.2 Leaves numbers**

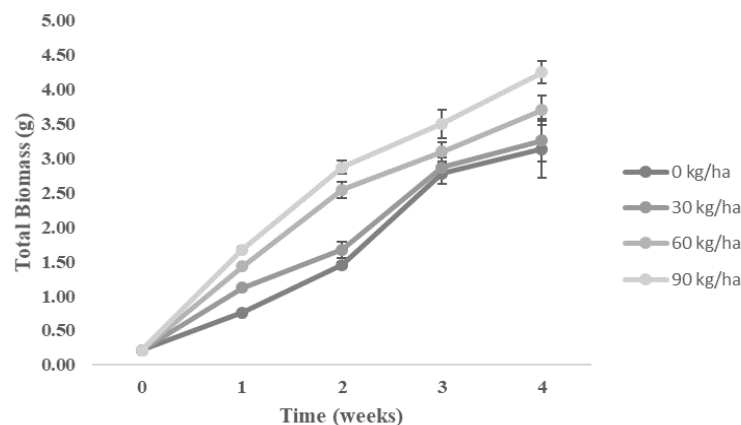
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195 The variation of leaf numbers with different nitrogen fertilization is in *I. aquatica* is depicted in Figure 2.
196 Generally, leaf number of *I. aquatica* was found to be influenced by the different rates of nitrogen
197 treatments (0, 30, 60 and 90 kg/ha; $P \leq 0.05$). Based on Figure 2, it shows that there were significant
198 effects of nitrogen fertilization rates on the number of leaves in every week of measurements. Overall
199 at 90 N kg/ha as the highest treatments of nitrogen applied, lead to the drastic production in the
200 number of leaves from 1 to 4 WAT. An increase in number of leaf age in plants indicates better plant
201 growth and development. Eventually, the plant production also will increase. Similar trends were
202 observed in [6] and [20] where they found that as the rate of nitrogen increases the *I. aquatica* leaf
203 numbers were also enhances. The increase in leaf number in *I. aquatica* might be due to increase in
204 internodes number with the high application of nitrogen [21]. The high application of nitrogen usually
205 would reduce the apical dominance and stimulated the development of lateral buds that eventually
206 increase the production of plant leaf and simultaneously enhanced the leaf numbers [22].



209
210 **Fig 2. The impact of different nitrogen rates on the leaves numbers of *Ipomea aquatica*. Mean**
211 **with the same letter indicates that all of the groups were not significantly different according**
212 **to Duncan multiple range test ($P \geq 0.05$) $N=10$.**
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215 3.3 Plant Total dry biomass

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217 Nitrogen application significantly influenced on the total plant dry weight of *I. aquatica* plant as shown
218 in Figure 3. The graph pattern shows increased in production in total biomass with the higher
219 application of nitrogen fertilization rates. At end of the treatments, It was observed that the highest
220 total biomass of kangkung was obtained in 90 kg N/ha, followed by 60 kg N/ha and 30 N kg N/ha that
221 recorded at 3.7g and 3.26g respectively. The lowest total biomass was recorded in control treatment 0
222 kg N/ha that just recorded 3.13g. The increase of total plant biomass with increasing nitrogen levels
223 can be explained by the increase in plant sink strength with increasing nitrogen levels. As nitrogen
224 uptake increased, more of accumulation of dry biomass will be expected due to increase in plant sink
225 strength that can accommodate initiation of new plant sink There were no significant different
226 occurred in between 0 and 30 N kg/ha treatment ($p \geq 0.05$). The result of the present study was in
227 agreement with the research conducted by [23] where, they found that the dry weight of shoot
228 increased with the increase of nitrogen supplied in *I. aquatica*. This justifies that high availability of
229 nitrogen was important in increasing the dry biomass of *I. aquatica* that was observed in the present
230 study [24,25].
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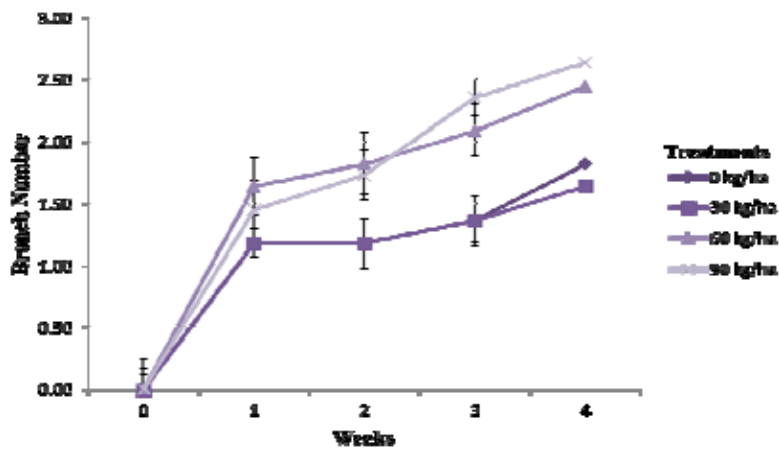
248 **Fig. 3. The impact of different nitrogen rates on total biomass of *Ipomea aquatica*. Mean with**
 249 **the same letter indicates that all of the groups were not significantly different according to**
 250 **Duncan multiple range test ($p \geq 0.05$) N=10.**

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253 3.4 Number of branches

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255 Figure 4 below shows the branches number of kangkung plant as affected by nitrogen treatments in
 256 all four weeks of treatment. As the higher rate of nitrogen treatments, the branching of plants was
 257 enhanced. At the first 2 weeks after the treatments were applied, the number of branches at 60 N
 258 kg/ha was higher than plants that were applied with 90 N kg/ha. But then, at week 3 and 4, the
 259 opposite results were obtained where the highest number of branches occurred at 90 N kg/ha. The
 260 study was in agreement with findings by Nashrin et al. [6] on *I. aquatica*, where the highest branching
 261 was obtained under highest nitrogen fertilization. Also, Osman and Abo Hassan [26], observed
 262 increased branching of Mangrove as nitrogen rate was increased to 100 kg N/ha. The increased in
 263 branching of the plant under high nitrogen fertilization might be due to increase in apical branches
 264 with higher nitrogen fertilization. This was due to enhanced vegetative growth under high nitrogen
 265 fertilization that enhanced the branching abilities of the plant [27].
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269 **Fig. 4. The impact of different nitrogen rates on the branch number of *Ipomea aquatica*. Mean**
 270 **with the same letter indicates that all of the groups were not significantly different according**
 271 **to Duncan multiple range test ($p \geq 0.05$) N=10.**

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273 3.5 Total Chlorophyll Content

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275 Figure 5 showed the impact of nitrogen fertilization on total chlorophyll content (TCC) of *I. aquatica* in
 276 4 weeks of treatments. There were significant differences were observed for TCC in every week of
 277 measurement ($P \leq 0.05$). The chlorophyll content increased after week 1 and reached it's maximum
 278 WAT content at week 3 as shown in Figure 5. In 1 WAT to 4 WAT, As the rate increased from 0 to 90
 279 kg/ha, The TCC was steadily enhanced with the increasing nitrogen rates. In 2 -4 WAT there was no
 280 significant difference observed between 60 and 90 kg/ha in TCC. The study was in agreement with
 281 findings of According to Bojović and Marković [28] where the higher application of nitrogen increased
 282 the TCC in wheat, where establishes a linear relationship between the rates of nitrogen and the
 283 chlorophyll content in plants. The plant that has been treated with high N level will result in higher
 284 chlorophyll content where this might be due to the immediate absorbance of nitrogen in plant [29].
 285 Since N is important for the structural element of chlorophyll and protein molecules, low N level will
 286 affect the formation of chloroplasts and the accumulation of chlorophyll in the plant [22]. Furthermore,
 287 as the plant age increased or getting mature, the N level tend to decrease and get mobilized to
 288 another part of the plant [29]. It can be concluded that in the present study, the higher rates of
 289 nitrogen application have increases the TCC in *I. aquatica*.

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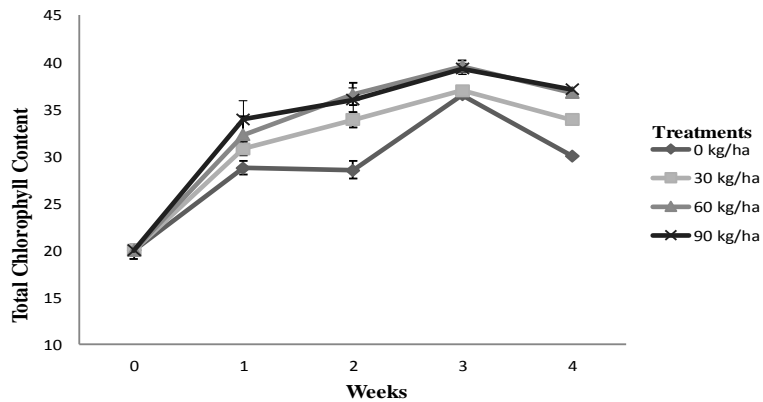
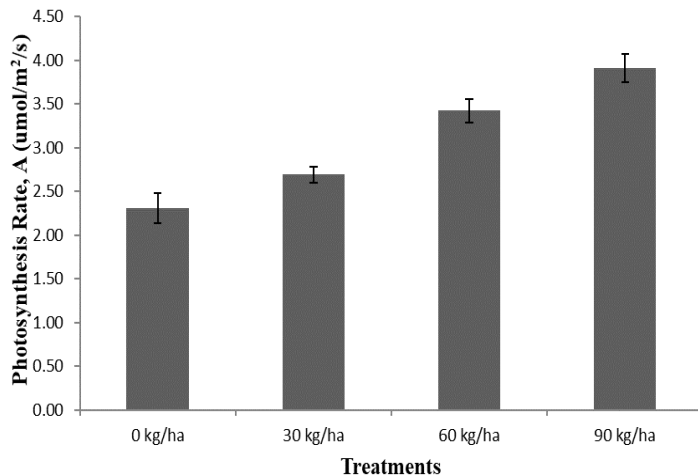


Fig. 5. The impact of different nitrogen rates on the total chlorophyll content of *Ipomea aquatica*. Mean with the same letter indicates that all of the groups were not significantly different according to Duncan multiple range test ($P \geq 0.05$) $N=10$.

3.6 Photosynthesis rate (A)

The photosynthesis rate of *I. aquatica* was affected by four different nitrogen treatments. It is clearly observed that from the graph pattern, as the nitrogen rate fertilization become higher ($0 > 90$ kg/ha), the rate of photosynthesis also enhances (Figure 6). The highest A was observed in 90 kg/ha nitrogen, followed by 60 and 30 kg/ha, with the means of 3.91, 3.42, and 2.69 $\mu\text{mol}/\text{m}^2/\text{s}$ respectively. The lowest A was observed in 0 kg/ha where it just recorded 2.31 $\mu\text{mol}/\text{m}^2/\text{s}$. The increase in A under high nitrogen level might be due to increases in leaf area that correspondingly enhanced photosynthetic activity per plant [30]. The result was also in agreement with Boussadia et al. [31] where higher nitrogen content has shown to enhance the photosynthesis rate in olive plants. The nitrogen and photosynthesis activity is linked together because of the Calvin Cycle protein which represents the nitrogen in leaf [32]. At lower N level, the rate of photosynthesis was low. This might be due to the greater resistance and low biochemical of chloroplast [33]. According to Makino et al. [34], the increase in the rate of nitrogen leads to a greater N allocation to Rubisco. Rubisco is the primary CO_2 for enzyme fixation where the amount of this enzyme can drastically affect the photosynthesis rate. Besides, high N is needed in Rubisco protein due to the low rate of catalysis in Rubisco. It can be concluded that, enhanced application of nitrogen would enhance rubisco production that enhanced the net photosynthesis of *Ipomea aquatica* that was observed in the present study.



335

336 Fig. 6. The impact of different nitrogen rates on the photosynthesis rate of *Ipomea*
337 *aquatica*. Mean with the same letter indicates that all of the groups were not
338 significantly different according to Duncan multiple range test ($P \geq 0.05$) N=10.
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342 3.7 Stomatal conductance (gs)

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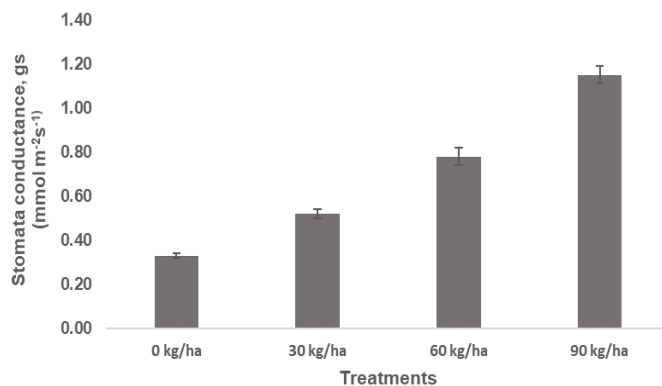
344 Stomatal conductance can be defined as the rate of carbon dioxide uptake and the water loss through
345 stomatal leaves [35]. Based on Figure 7 below, it is distinctly observed that different rates of nitrogen
346 had greatly affected the measurement of stomatal conductance. The higher the treatment
347 concentrations (0,30,60,90 kg/ha), the rate of stomatal conductance have shown to increase. The
348 stomatal conductance measurement was the highest at 90 N kg/ha ($1.15 \text{ mmol m}^{-2} \text{ s}^{-1}$), while the
349 lowest rate of stomatal conductance was measured at 0 kg/ha nitrogen treatment that recorded 0.33
350 $\text{mmol m}^{-2} \text{ s}^{-1}$. The present result was in agreement with the findings of [36], where they found that the
351 increase in photosynthesis rate and stomatal conductance are correlated to increase in nitrogen
352 application to the plants. Despite nitrogen, the size of the leaf can be important for certain plant
353 species as it helps for greater conductance through the high number opening of the stomata [37]. This
354 indicates that stomata conductance was enhanced with high levels of nitrogen applied to *I. aquatica*.

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362 Fig. 7. The impact of different nitrogen rates on the stomatal conductance of *Ipomea aquatica*.
363 Mean with the same letter indicates that all of the groups were not significantly different
364 according to Duncan multiple range test ($P \geq 0.05$) N=10.
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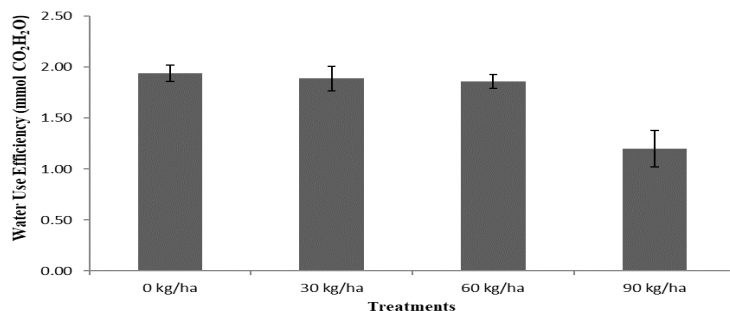
368 3.8 Water use efficiency (WUE)

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370 Water use efficiency (WUE) was illustrated in Figure 8 as it was influenced by the nitrogen treatments
($P \leq 0.05$). Plant with the highest concentration of nitrogen (90 kg/ha) has the lowest measurement
371 recorded in water use efficiency with the mean of $1.46 \mu\text{mol CO}_2/\text{H}_2\text{O}$ transpired. While the highest
372 measurement in water use efficiency was recorded in the plant that was applied with 0 Kg/ha nitrogen
373 with a mean of $1.97 \mu\text{mol CO}_2/\text{H}_2\text{O}$ transpired. The current result was contradicting with the findings
374 of Stewart [38] in cotton where the highest nitrogen application has shown to enhance the WUE in the
375 plant. The increased of WUE is usually, attributed to the increase of the transpiration rate and showed
376 plant under water stress condition. The current result showed that higher application of nitrogen rates
377 in *I. aquatica* can reduce the plant stress by having lower WUE. [22]. A similar result was obtained by
378 Artur et al. [39] where the increase of N has reduced the WUE in Marandu grass that showed a high
379 application of nitrogen can reduce stress in *I. aquatica*.

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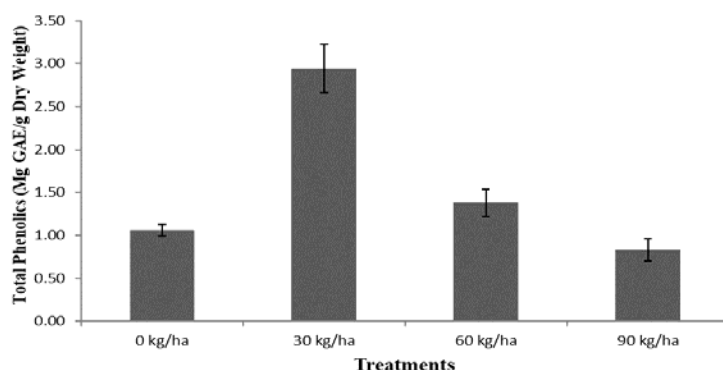
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385 **Fig. 8. The impact of different nitrogen rates on the water use efficiency of *Ipomea aquatica*.**
386 **Mean with the same letter indicates that all of the groups were not significantly different**
387 **according to Duncan multiple range test ($P \geq 0.05$) N=10.**

390 3.9 Total phenolics

391
392 Total plant phenolics contents were influenced by nitrogen fertilization ($P \leq 0,05$; Figure 9). As levels
393 of nitrogen enhanced, the total phenolics content was seemed to be reduced. Total phenolics was
394 203%, 41% and 13% higher in 30 kg/ha, 60 kg/ha and 0 kg/ha respectively compared to 90 kg/ha
395 treatments. The previous study had shown that when the level of nitrogen decreased, the phenolic
396 compound increased in Broccoli [40]. Another result obtained by Stewart et al. [41], also prove that the
397 phenolic content increased as the plant faced deficiency in nitrogen level. The result obtained in this
398 study suggested that at lower nitrogen fertilization i.e. 30 kg N/ha the production of total phenolics in
399 *Ipomea aquatica* was enhanced. According [42], when a plant undergoes N deficiency, the process of
400 distributing carbon-based secondary compounds will increase, thus, decreasing the synthesis of
401 nitrogen-based secondary compounds. Besides, Ibrahim et al. [19] stated that the increase in total
402 phenolics production under low N level also might be due to the increase of total carbohydrate
403 structural production that enhanced the production of carbon- based secondary metabolites.
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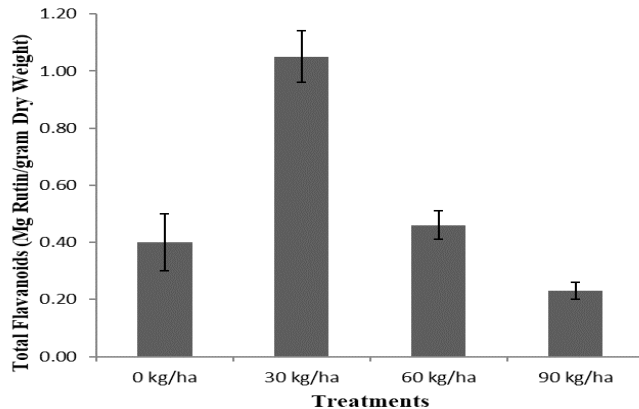


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407 **Fig. 9. The impact of different nitrogen rates on total phenolics of *Ipomea aquatica*.**
408 **Mean with the same letter indicates that all of the groups were not significantly different**
409 **according to Duncan multiple range test ($P \geq 0.05$) N=4.**

412 3.10 Total flavanoids

413
414 The total flavonoids of *Ipomea aquatica* were observed to be affected by the different rates of nitrogen
415 treatments (Figure 10; $P \leq 0.05$). The production of total flavonoids has the same trends with total
416 phenolics production content where plants which applied with 30 N kg/ha treatments has the highest
417 total flavonoids content (1.05 mg Rutin/g dry weight) compared to 90 kg/ha that only recorded 0.27

418 mg rutin/ g dry weight. The same observation was obtained by [43] (2012) in Yaupon where the
 419 flavonoid content reduces when applied with high N rate. According to [44] the flavonoids content in
 420 plant tissues can be increased when having lower nitrogen content in the plant tissues. The increases
 421 in synthesis of flavonoid at lower nitrogen level might be due to increases in phenylalanine availability
 422 that enhances the phenylalanine lyase (PAL) activity that simultaneously enhanced the production of
 423 secondary metabolites [45]. It can be concluded in the present study, that under high nitrogen level
 424 the production of total phenolics and flavonoids was reduced in *I. aquatica*.
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 430 **Fig.10.The impact of different nitrogen rates on total flavonoids of *Ipomea***
 431 ***aquatica*. Mean with the same letter indicates that all of the groups were not**
 432 **significantly different according to Duncan multiple range test ($P \geq 0.05$) N=4.**
 433

434 435 4. CONCLUSION

436
 437 In this work, four levels of nitrogen rates (0, 30, 60 and 90 kg/ha) was applied to *I. aquatica* to assess
 438 the growth, leaf gas exchange and production of secondary metabolites characteristics. It was found
 439 that as the nitrogen rates increased, the growth and leaf gas exchange properties of *I. aquatica* was
 440 enhanced. However, the production of phenolics and flavonoids of kangkung was reduced with high
 441 levels of nitrogen application as both total phenolics and flavonoid reached the highest content at 30
 442 kg N /ha. This work gives support that high nitrogen fertilization to *I. aquatica* can reduce the
 443 production of secondary metabolites although the growth parameters were enhanced with high
 444 nitrogen fertilization.
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