Original Research Article

Effect of Nitrogen Rates on Growth, Carbon Assimilation and Quality of Water Spinach (*Ipomea aquatica)*

ABSTRACT

Aims: This study was conducted to investigate the impact of the nitrogen fertilization on the growth, leaf gas exchange and bio-metabolite accumulation in *Ipomea aquatica*.
Study design: *Ipomea aquatica* plants were exposed to four different rates of nitrogen (0, 30, 60 and 90 N kg/ha) using NPK green fertilizer as a nitrogen source using. Complete Randomize Design (CRD) .
Place and Duration of Study: Department of Biology, Faculty of Science, Universiti Putra Malaysia between September to November 2016.
Methodology: There were 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 SPAD chlorophyll meter. The leaf gas exchange was determined using a LI-6400XT portable photosynthesis system. Total phenolics and flavonoid was 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, while 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 was reduced and get the highest accumulation at 30 kg N/ha.

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

1. INTRODUCTION

In Malaysia, agriculture sector has contributed about 8.5% to Gross Domestic Products (GDP). About 39% of the contributions originated from the production of food crops, fruits and vegetables. It is estimated that there are about 44, 000 hectares of the total area in Malaysia was used for vegetable cultivation [1]. According to Department of Agriculture Malaysia in 2011, *Ipomea Aquatica* is one out of ten types of vegetables that consumed the highest area for vegetable production. This plant is among the most consumed vegetable in Asia. This is because of it's low price compared to other types of vegetable. Kangkung air or it's scientific name, *Ipomea aquatica*, is a widely known leafy vegetable especially in Asian country. The plant is also commonly known with different local names, such as water spinach, swamp cabbage, or water convolvulus. From it's scientific classification, kangkung has been classified under a family of Convolvulaceae [2]. According to [3], Convolvulacea family consist of primarily 1650 of tropical species. Moreover, the genus of kangkung which is *Ipomea* has about 500 to 600 different species and it has been the most number of containing species in Convolvulaceae family [4]. This species of family can nicely grown at almost anywhere at the higher or even the lower land. *Ipomea aquatica* is one of the species that is cultivated on the higher land. Besides easy to be grown, kangkung often be the favorable plant to be cultivated because it does not take long time to mature and harvest. It can easily adapt towards it's grow environment and usually unsusceptible to disease. Almost all parts of kangkung plant are edible [3].

According to Susila et al. [5], nitrogen is the primary nutrient that involve in producing a high yield of vegetables. Nitrogen is one of the macro-nutrients that is very crucial especially for a plant to have a proper growth and development [6] such as that required in constructing the matter of the plant cell and tissue [7]. The amount of nitrogen in the soil could be insufficient for the plant to grow. Therefore, the source of nitrogen for plant especially in agriculture field often be found in the form of a fertilizer. Both organic and inorganic nitrogen fertilizer are widely used in agriculture especially in cultivating green crops to keep the source of nutrients for plant being supply [6]. Practically, an appropriate and suitable amount of nitrogen to be given to plant will affect it's crop yield. Nitrogen also is very important especially to promote the growth of the plant leaf [8]. Nitrogen is a crucial element not only to promote the growth and plant development, also increase yield and quality in vegetable crops. Increasing level of nitrogen resulting in a number of leaves, leaf length and plant body [8]. Nitrogen also enhanced the size of fruits and vegetables where at optimum application of N will resulting in a better size. The metabolic process which stimulated by N by enhance the vegetative and also the reproductive growth in plant. Besides, high plant biomass can be obtained when there is high N accumulated in shoot, along with the increasing of root growth in plant if there is sufficient amount of N supply [9]. However, the lack of N in plant would caused the reduced in plant development and eventually will lower the crop yield.

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

Secondary metabolites such as phenolic in plant usually associated with the plant survival and health benefits for those who consume the plant. Low nitrogen level in plant has been reported to have more secondary metabolites compare to plant that has high N level [16]. Application of more N level resulting in decrease of phenolic concentrations based on carbon/nutrients balance (CNB) hypothesis [17]. Flavanoids also a secondary metabolite which is widely distributed with different functions in plants. The biological functions of flavonoids include defense against UV-B radiation, pathogen infection, nodulation and pollen fertility [18]. A study done by [8] on leaf mustard where the total phenolics concentration was observed to be decreased as the level increased. It is well known that nitrogen application can directly affect the morphological growth and yield of this plant, however, little work has been carried out to look on the impact of nitrogen of the leaf gas exchange properties and previous work have not comprehensively considered the production of secondary metabolites of *I. aquatica* under nitrogen fertilization. there was no study about the production of secondary metabolites and leaf gas exchange properties under different nitrogen rates. The main aim of the research was to investigate the effect of nitrogen fertilization on the growth, leaf gas exchange and production of secondary metabolites of *I. aquatica* and to determine the best nitrogen rates for growth and development of *I. aquatica*. This research will provide the important information for vegetable growers that involved in cultivation of vegetables in Malaysia.

2. MATERIAL AND METHODS

2.1. Plant material and maintenance

The experiment was conducted at Department of Biology, Universiti Putra Malaysia, Serdang (UPM), Selangor where the area of the experiment was located at the site where the direct sunlight is always sufficient and available. These seeds were allowed to germinate for two weeks. After all the seeds has been germinated, all the plants then were transplanted into the polybags filled with a mixture of topsoil, organic matter and sand with the ratio of 3:2:1. The nitrogen sources used were from the NPK green fertilizer (15:15:15). The polybags were arranged according to Completely Randomized Design (CRD) with five replications. There were four nitrogen rates were applied (0, 30, 60 and 90 Kg N/ha) with overall 160 of *I. aquatica* plants were used. The growth data collections were conducted once a week for four weeks after the application of the treatments for the plant growth parameter. Whereas the destructive analysis and leaf gas exchange of the experiment was conducted at the end of the experiment.

2.2. Plant height, leaf and branch numbers

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

2.3 Plant total dry weight measurement

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

2.4 Total Chlorophyll content

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

2.5 Leaf gas exchange measurement

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

2.7 Total phenolics and flavonoids quantification

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

2.8 Statistical analysis

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

3. RESULTS AND DISCUSSION

3.1 Plant height

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

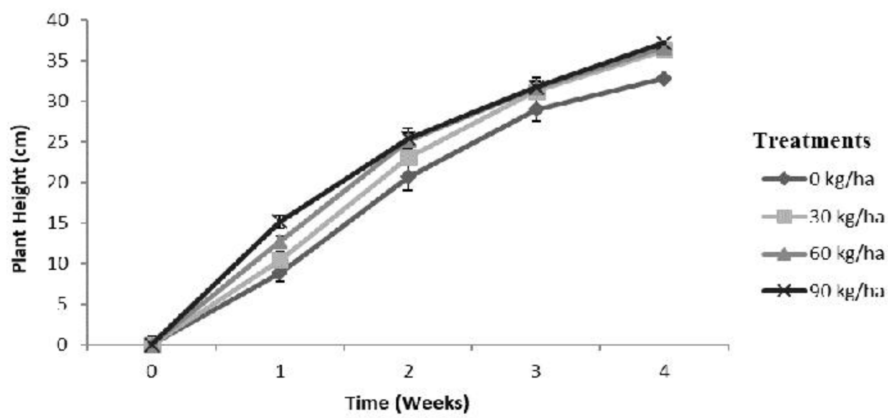

Fig 1. The impact of different nitrogen rates on the height of *Ipomea aquatica.* Mean with the same letter indicates that all of the groups were not significantly different according to Duncan multiple range test ($P \geq 0.05$) N=10.

3.2 Leaves numbers

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

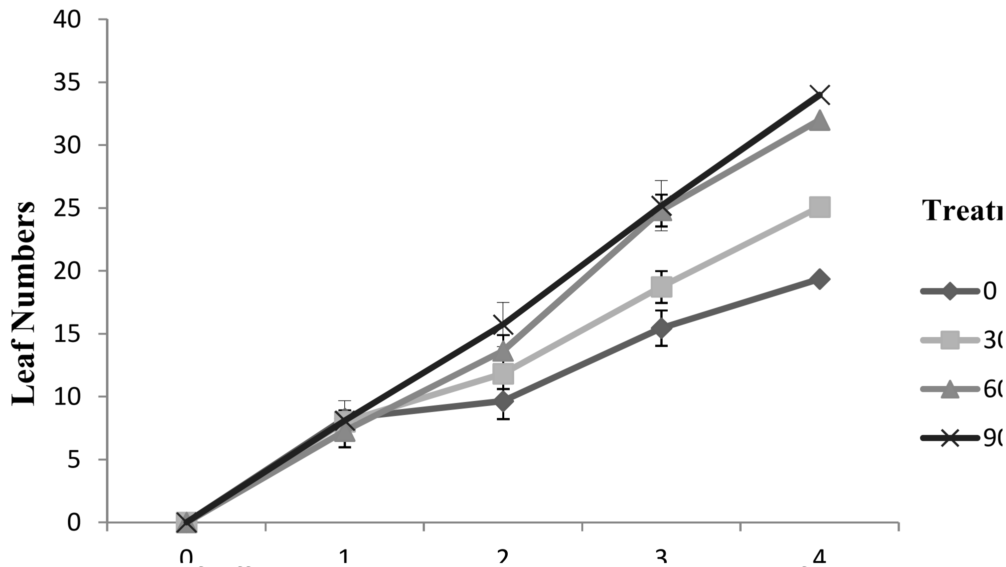

Fig 2. The impact of different nitrogen rates on the leaves numbers of *Ipomea aquatica.* Mean with the same letter indicates that all of the groups were not significantly different according to Duncan multiple range test (*P* ≥ 0.05) N=10.

3.3 Plant Total dry biomass

Nitrogen application had significantly influenced on the total plant dry weight of *I. aquatica* plant as shown in Figure 3. The graph pattern show increased in production in total biomass with higher application of nitrogen fertilization rates. At end of the treatments, It was observed that the highest total biomass of kangkung was obtained in 90 kg N/ha, followed by 60 kg N/ha and 30 N kg N/ha that recorded at 3.7g and 3.26g respectively. The lowest total biomass was recorded in control treatment 0 kg N/ha that just recorded 3.13g. The increase of total plant biomass with increasing nitrogen levels can be explained by the increase in plant sink strength with increasing nitrogen levels. As nitrogen uptake increased, more of accumulation of dry biomass will be expected due to increase in plant sink strength that can accommodate initiation of new plant sink There were no significant different occurred in between 0 and 30 N kg/ha treatment (p ≥ 0.05). The result of the present study was in agreement with the research conducted by [23] where, they found that the dry weight of shoot increased with the increase of nitrogen supplied in *Ipomea aquatica*. This justify that high availability of nitrogen was important in increasing the dry biomass of I. aquatica that was observed in the present study [24,25].

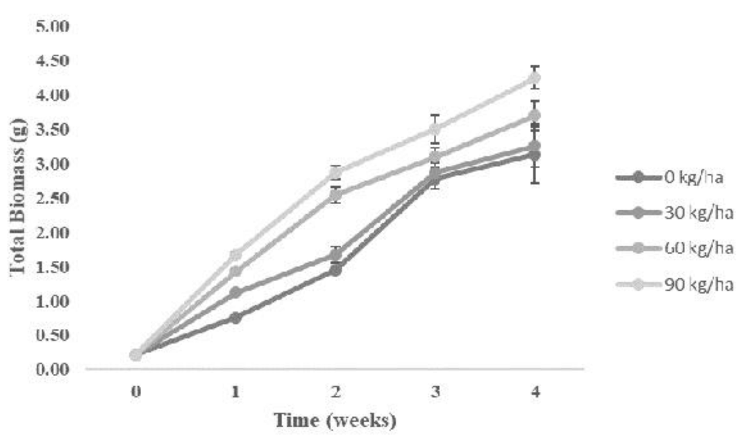

Fig. 3. The impact of different nitrogen rates on total biomass of *Ipomea aquatica*. Mean with the same letter indicates that all of the groups were not significantly different according to Duncan multiple range test ($p \geq 0.05$) N=10.

3.4 Number of branches

The Figure 4 below shows the branches number of kangkung plant as affected by nitrogen treatments in all four weeks of treatment. As the higher rate of nitrogen treatments, the branching of plants was enhanced. At the first 2 weeks after the treatments were applied, the number of branches at 60 N kg/ha were higher than plants that were applied with 90 N kg/ha. But then, at week 3 and 4, the opposite results were obtained where the highest number of branches occurred at 90 N kg/ha. The study was in agreement with findings by Nashrin et al. [6] on *Ipomea aquatica*, where the highest branching was obtained under highest nitrogen fertilization. Also, Osman and Abo Hassan [26], observed increased branching of Mangrove as nitrogen rate was increased to 100 kg N/ha. The increased in branching of the plant under high nitrogen fertilization might be due to increase in apical branches with higher nitrogen fertilization. This was due to enhanced vegetative growth under high nitrogen fertilization that enhanced the branching abilities of the plant [27].

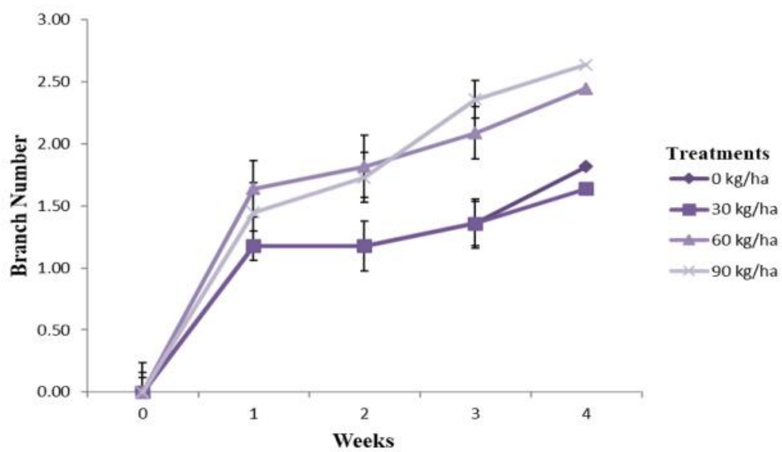

Fig. 4. The impact of different nitrogen rates on the brancg number of *Ipomea aquatica*. Mean with the same letter indicates that all of the groups were not significantly different according to Duncan multiple range test ($p \geq 0.05$) N=10.

3.5 Total Chlorophyll Content

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

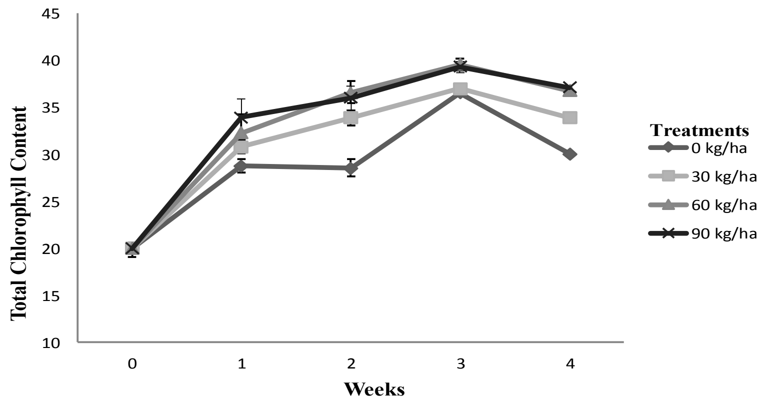

Fig. 5. The impact of different nitrogen rates on the total chlorophyll content of *Ipomea aquatica*. Mean with the same letter indicates that all of the groups were not significantly different according to Duncan multiple range test ($P \geq 0.05$) N=10.

3.6 Photosynthesis rate

The photosynthesis rate of *I. aquatic*a 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 umol/m^2/s respectively. The lowest A was observed in 0 kg/ha where it just recorded 2.31 umol/m^2/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 have shown to enhanced the photosynthesis rate in olive plants. The nitrogen and photosynthesis activity is linked together because of the Calvin Cycle protein which represent 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 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.

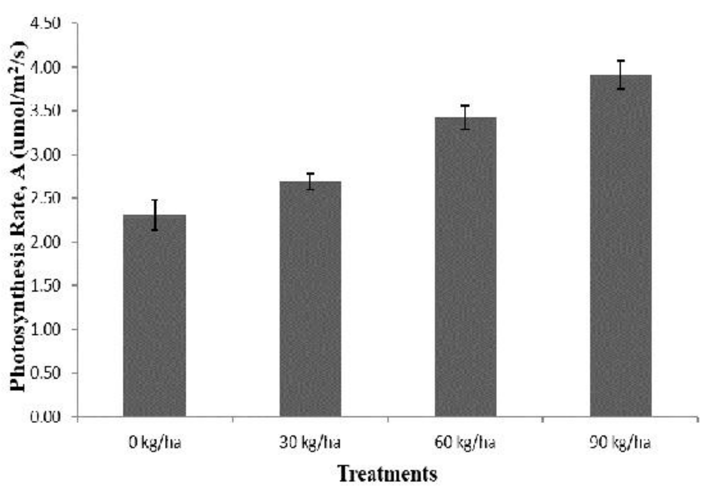

Fig. 6. The impact of different nitrogen rates on the photosynthesis rate of *Ipomea aquatica.* Mean with the same letter indicates that all of the groups were not significantly different according to Duncan multiple range test ($P \geq 0.05$) N=10.

3.7 Stomatal conductance

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

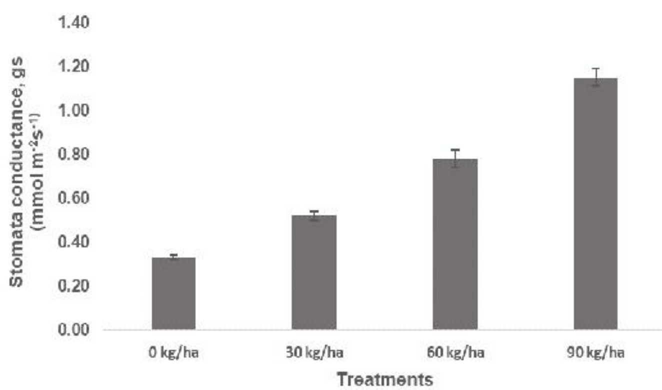

Fig. 7. The impact of different nitrogen rates on the stomatal conductance of *Ipomea aquatica*. Mean with the same letter indicates that all of the groups were not significantly different according to Duncan multiple range test ($P \geq 0.05$) N=10.

3.8 Water use efficiency (WUE)

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 recorded in water use efficiency with the mean of 1.46 µmol CO_2/H_2O transpired. While the highest measurement in water use efficiency was recorded in plant that was applied with 90 Kg/Ha nitrogen with mean of 1.97 µmol CO_2/H_2O transpired. The current result was contradicting with the findings of stewart [38] in cotton where the highest nitrogen application has shown to enhanced the WUE in the plant. The increased of WUE is usually, attributed to the increase of the transpiration rate and showed plant under water stress condition. The current result showed that higher application of nitrogen rates in I. aquatica can reduce the plant stress by having lower WUE. [22]. A similar result was obtained by Artur et al. [39] where the increase of N has reduced the WUE in Marandu grass that showed high application of nitrogen can reduce stress in *I. aquatica*.

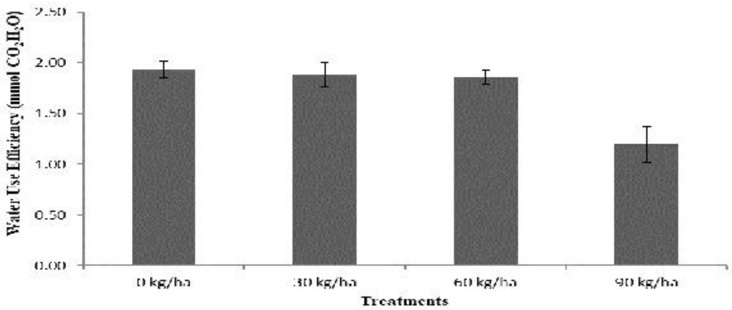

Fig. 8. The impact of different nitrogen rates on the water use efficiency of *Ipomea aquatica*. Mean with the same letter indicates that all of the groups were not significantly different according to Duncan multiple range test (P ≥ 0.05) N=10.

3.9 Total phenolics

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

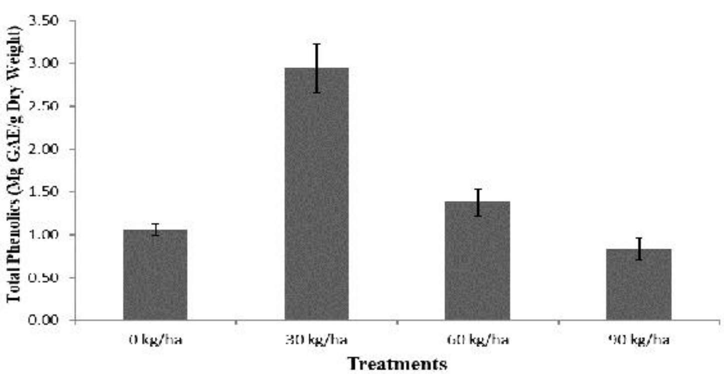

Fig. 9. The impact of different nitrogen rates on total phenolics of *Ipomea aquatica.* Mean with the same letter indicates that all of the groups were not significantly different according to Duncan multiple range test ($P \geq 0.05$) N=4.

3.10 Total flavanoids

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

mg rutin/ g dry weight. The same observation was obtained by [43] (2012) in Yaupon where the flavonoid content reduces when applied with high N rate. According to [44] the flavonoids content in plant tissues can be increased when having lower nitrogen content in the plant tissues. The increases in synthesis of flavonoid at lower nitrogen level might be due to increases in phenylalanine availability that enhances the pheyllaline lyase activity that simultaneously enhanced the production of secondary metabolites [45]. It can be concluded in the present study, that under high nitrogen level the production of total phenolics and flavonoids was reduced in *I. aquatica.*

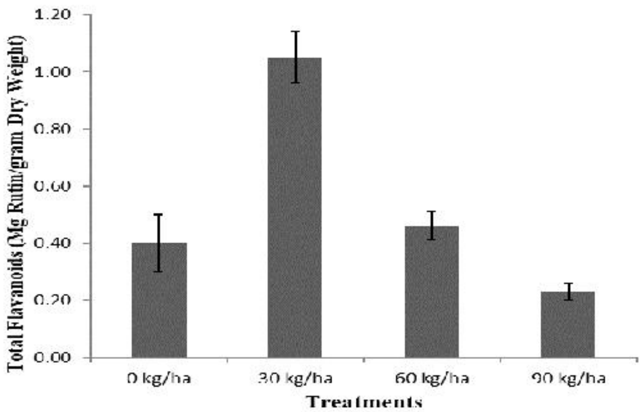

Fig.10.The impact of different nitrogen rates on total flavonoids of *Ipomea aquatica.* Mean with the same letter indicates that all of the groups were not significantly different according to Duncan multiple range test (*P* ≥ 0.05) N=4.

4. CONCLUSION

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

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