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 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 include 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 the leaves extracts were measured using Folin-Ciocalteu reagents.
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 concentration 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 reduce 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 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 with environment changes, this enables 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 been interested by 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 face with stomatal obstacle [10].

The application of magnetic nanoparticles may enhance or retard the growth of *Ipomoea aquatica* somehow like 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 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 factor being influenced by the magnetic nanoparticle application. Secondary metabolites are the natural products presence in low amounts in which its production rely on different species, genera and families. Secondary metabolites are important to protect plants from insects and pathogens besides shaping imperative UV-radiation absorbing compounds that eventually reduce the chances for the plant to die [12]. The production of flavonoids and phenolics are the determinants for the production of secondary metabolite in plant. It is often to observe that plant undergoing stress has higher total flavonoid and phenolics content due to defensive mechanism in plant. Biosynthesis in plant to produce secondary metabolites is usually stimulated by the accumulation of heavy metals like zinc, iron, and nickel which generates Reactive Oxygen Species (ROS) and induces oxidative stress in plant. Consequently, the induction of oxidative stress causes changes in signal transduction for the mechanism of gene coding and enzyme [13]. Besides, the production of ROS may cause damages to cell membrane, cell structure and photosynthetic site and thus the production of flavonoid at the generation site act as defensive mechanism due to its high antioxidant properties. In addition, induced phenolics are produced when the plant faces physical injury, infection or environmental stresses due to heavy metal irradiation or temperature [14].

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

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

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

2. MATERIAL AND METHODS

2.1 Synthesis of Iron Oxide Nanoparticles (Fe_3O_4)

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

2.2 Plant Materials and Maintenance

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

2.3 Experimental Design

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

2.4 Data Collection

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

2.4.1 Plant Growth Measurement

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

2.4.1.1 Plant height

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

2.4.1.2 Plant basal diameter

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

2.4.1.3 Plant leaves number

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

2.4.1.4 Plant leaf temperature determination

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

2.4.1.5 Chlorophyll content measurement

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

2.4.1.6 Plant fresh weight measurement

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

2.4.1.7 Plant biomass (Dry weight) measurement

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

2.4.1.8 Root to shoot ratio

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

2.4.2 Leaf Gas Exchange Measurement

A LI-6400XT (Li-COR Inc; Nebraska; USA) portable photosynthesis system gave the reading of leaf gas exchange. The instrument was warmed and calibrated for 30 minutes with the ZERO IRGA mode. The measurements used optimal conditions set at 400 µmol mol-1 CO_2, 30°C cuvette temperature, 60% relative humidity with air flow rate set at 500 cm3 min-1 and modified cuvette condition of 800 µmolm-2s-1 photosynthetically photon flux density (PPFD). The fully expanded young leaves were used to measure net photosynthesis (*A*), transpiration rate (*E*) and stomata conductance (*gs*) which also gave the measurement of gas exchange. It was an automatic operation therefore the data was recorded in the LI-6400XT console and further analyzed by Photosyn Assistant Software (Dundee Scientific, Dundee, UK). Precautions step were considered while taking the measurements to avoid errors.

2.4.3 Chlorophyll Fluorescence Determination

Mature leaf tissue of water spinach was collected from plants which are grown in a greenhouse at 20º C with the supply of artificial light which provide a minimum photon flux density of 550 umol- m- 2 · S-1 for a 16 h photoperiod (Fi- totron H600, Fisons pic, Loughborough, U.K.) and photosynthetically active radiation will be supplied at 250 umol- m-2 · S-1 during 16 h photoperiods.

2.4.4 Total Phenolics and Flavonoids Quantification

The total phenolics and flavonoid measurement follow methods from Ibrahim, Jaafar, Rahmat, and Zaharah [21]. Plant tissue samples (0.1 g) that initially grounded were extracted with 80% ethanol (10 mL) on an orbital shaker at 50˚C for 120 minutes. The mixture was filtered and the filtrate used for the quantification of total flavanoids and total phenolics. Total phenolics content of the leaf samples was determined by using Follin-

Ciocalteu reagent (Sigma Aldrich, Missouri, USA; diluted 10-fold). The absorbance measured at 725 nm. The results will be expressed as mg g−1 gallic acid equivalent (mg GAE g−1 dry sample). For total flavonoids determination, a sample (1 mL) mixed with $NaNO_3$ (Sigma Aldrich, Missouri, USA; 0.3 mL) in a test tube which covered with aluminum foil, and left for 5 min. Then 10% AlCl3 (Wako Pure Chemical Industries Ltd; Tokyo, Japan; 0.3 mL) added followed by addition of 1 M NaOH (Kanto Chemical Co. Inc.; Hokkaido, Japan; 2 mL). Later, the absorbance was measured at 510 nm using a spectrophotometer with rutin as a standard (results expressed as mg g−1 rutin dry sample).

2.5 Statistical Analysis

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

3. RESULTS AND DISCUSSION

3.1 Plant Height

Fig.1 showed that, different concentration of magnetic nanoparticles application influenced significantly the plant height of *Ipomoea aquatica* as $P \leq 0.05$ in week 3. The highest plant height was observed significantly in 0 mg/L (14.10 cm), followed by 50 mg/L (12.2 cm), 10 mg/L (11.2 cm) and lowest in 150 mg/L (11.1 cm). The current result indicates that the application of magnetic nanoparticles at higher concentration would reduce the plant height of *Ipomoea aquatica*. Research had shown that higher concentration of Fe_3O_4 with pro-long exposure may lead to iron toxicity in the plant. The plants that faced toxicity during growing stage may initiate stress and causes reduction in plant height due to consequences effects of iron toxicity like production of free radicals, root break down and bronzing of leaves which lead to yield loss [22]. The application of Fe_3O_4 stimulate the production of Reactive Oxygen Species (ROS) under the condition of excess or deficiency of Fe_3O_4 that responsible of signaling molecule that stimulate or inhibit plant growth [23].

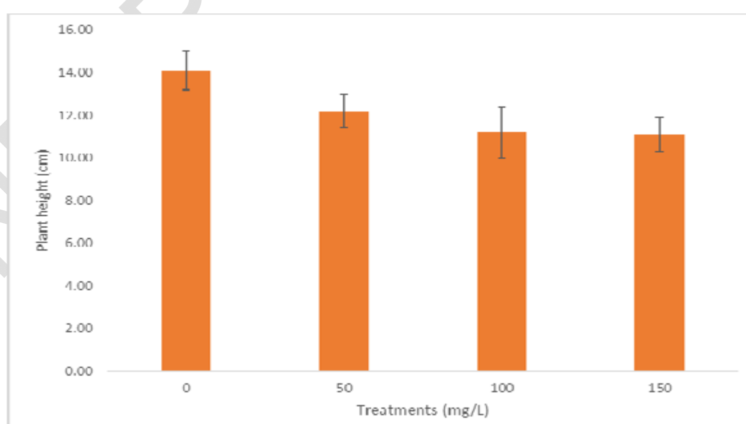

Fig. 1. The impact of magnetic nanoparticles on plant height of *Ipomoea aquatica* Data are mean ± standard error of mean (SEM). N= 24. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

3.2 Shoot Length

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

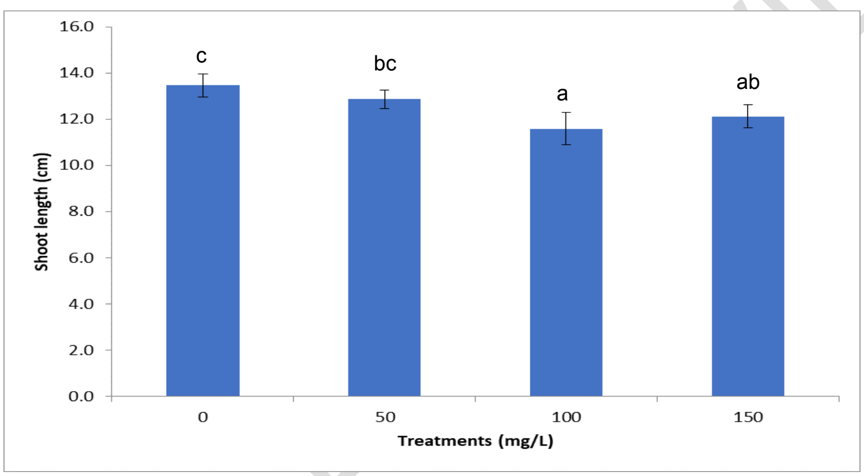

Fig. 2. The impact of magnetic nanoparticles on shoot length of *Ipomoea aquatica* Data are mean ± standard error of mean (SEM). N= 24. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

3.3 Basal Diameter

Different concentration of magnetic nanoparticles application has significantly influenced the basal diameter of *Ipomoea aquatica* ($P \leq 0.05$; Fig. 3) in week 12. At 12th week, the application of magnetic nanoparticles has shown to increase significantly the basal diameter of *Ipomoea aquatica* at higher concentration which is at 150 mg/L. The smallest basal diameter was observed in 0 mg/L (0.51 cm), followed by 100 mg/L (0.56 cm), 50 mg/L (0.571 cm) and greatest in 150 mg/L (0.74 cm). The current result indicates that the application of magnetic would promote the stem diameter of *Ipomoea aquatica*. Based on research, aquaporins are found on tonoplast or also known as vacuolar membrane which allows water to be freely moves across the cells in the symplastic route. Therefore, the abundance of aquaporins found on the membrane depends on the regulation of water flow. During water stress due to high concentration of nanoparticles may lead to induction of turgor-responsive aquaporins to maintain turgor pressure. This would cause the vacuole size to increase that brings to cell expansion and increased of stem diameter [25]. Besides, a research done on radish cotyledon shows that increase in turgor pressure increase the cell expansion and growth. However, the study claimed that under unfavorable condition, cell would not divide but [26]. Therefore, we could conclude that higher concentration of Fe_3O_4 cause increase in stem diameter**.**

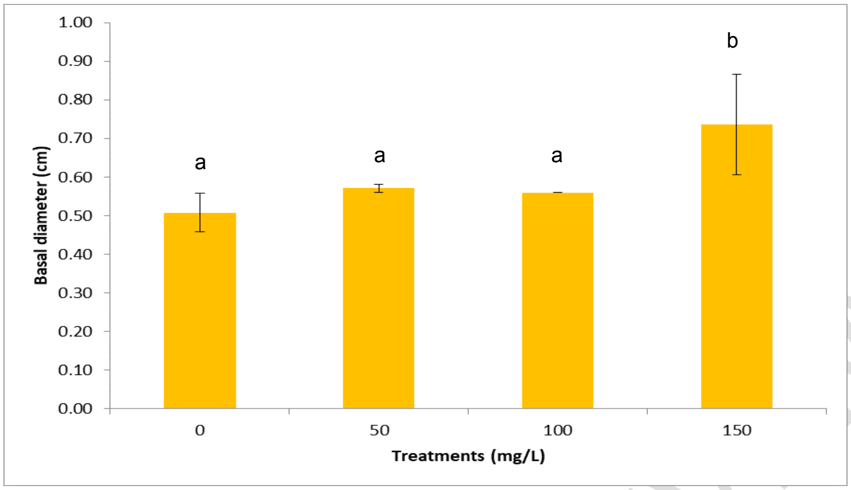

Fig. 3. The impact of magnetic nanoparticles on basal diameter of *Ipomoea aquatica* Data are mean ± standard error of mean (SEM). N= 24. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

3.4 Total Biomass

Based on Fig. 4, different concentration of magnetic nanoparticles had influenced significantly the total biomass of *Ipomoea aquatica* as *P*≤0.05 in week 12. In week 12, a significant effect was observed by which the application of Fe_3O_4 with the concentration of 150 mg/L increased significantly the biomass of water spinach. The highest total biomass was observed in 150 mg/L (4.2179 gram), followed by 50 mg/L (2.2189 gram), 100 mg/L (2.0213 gram), and 0 mg/L (1.9824 gram). The result obtained showed that iron oxide nanoparticles with highest concentration would increase the total biomass of *Ipomoea aquatica*. This result was in agreement with the finding by [27]. From their research, it was found that the use of iron oxide nanoparticles increased the dry weight of pod and peanut plant. They found that the application of Fe_3O_4 help in transferring iron and photosynthate particle in the leaves of plants. Besides, the total biomass of *Vigna radiata* applied with Fe_3O_4 showed a positive result compared to the application of ferum ions. This was claimed due to the increase of α-amylase activity in the seeds exposed to Fe_3O_4. The increase of total biomass was also observed in *Spinacea oleracea* applied with magnetic nanoparticles [18]. However, study by Jeyasubramanian et al. [28] showed that higher concentration of Fe_3O_4 which is 200 mg/L cause decrease in both wet and dry weight of spinach [29].

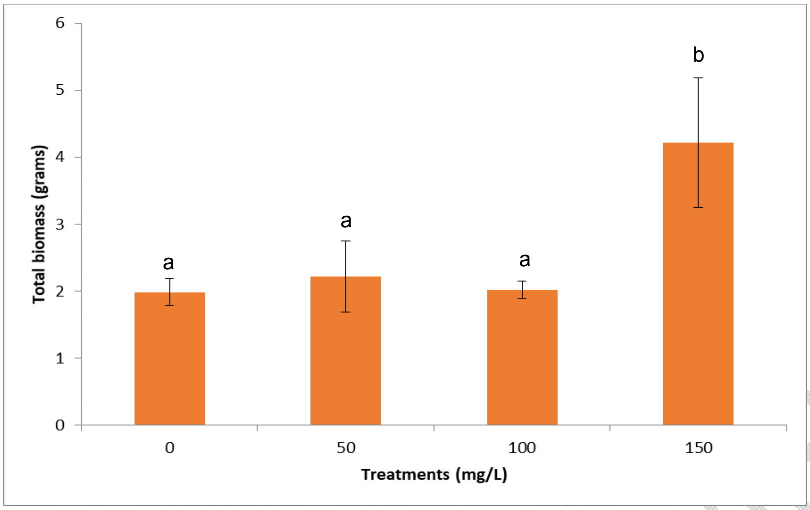

Fig. 4. The impact of magnetic nanoparticles on total biomass of *Ipomoea aquatica* Data are mean ± standard error of mean (SEM). N= 24. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

3.5 Plant Leaf Temperature

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

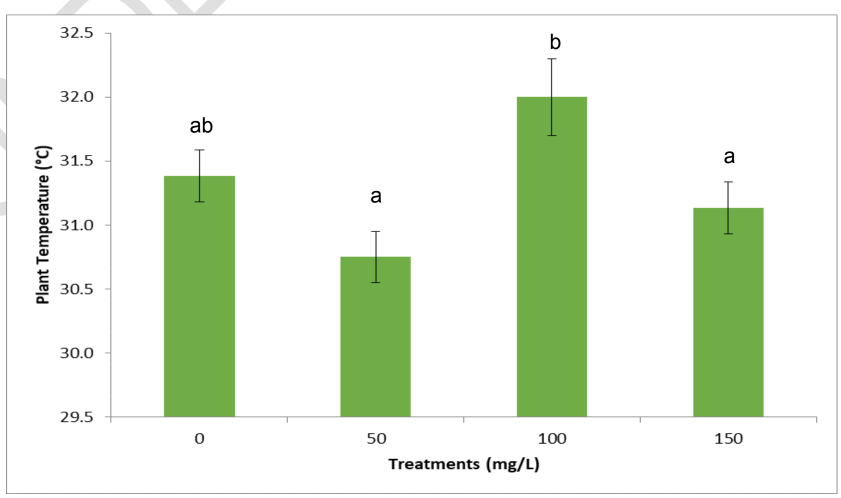

Fig. 5. The impact of magnetic nanoparticles on plant temperature of *Ipomoea aquatica*. Data are mean ± standard error of mean (SEM). N= 24. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

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 as $P \leq 0.05$. 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 µl/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].

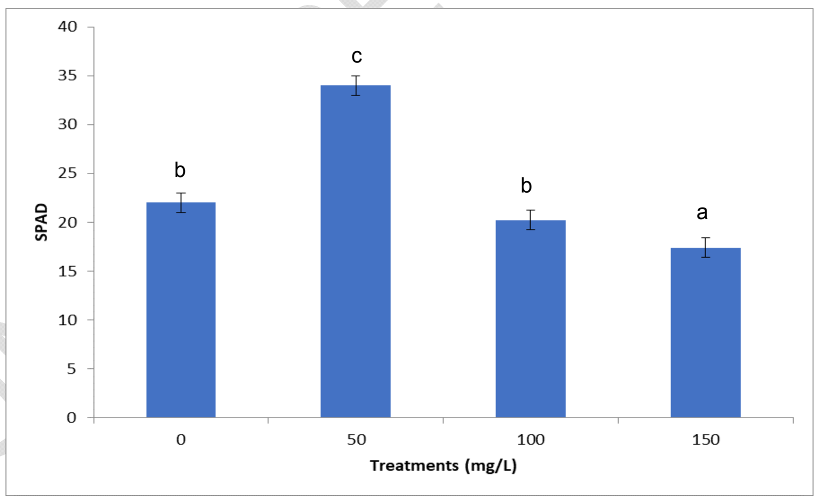

Fig. 6. The impact of magnetic nanoparticles on total chlorophyll content of *Ipomoea aquatica.* Data are mean ± standard error of mean (SEM). N= 5. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

3.7 Net Photosynthesis Rate

Fig. 7 showed the net photosynthesis rate of water spinach after 12 weeks of planting. The results shown that the net photosynthesis rate was influenced significantly (*P*≤0.05) by the application of Fe_3O_4 during the planting period. The higher concentration of magnetic nanoparticles resulted in lower net photosynthesis rate of water spinach. The net photosynthesis of control treatment (0 mg/L) is higher than other treatments which are 50 mg/L, 100 mg/L and 150 mg/L that recorded 6.9 umol/m^2/s, 6.3 umol/m^2/s, 3.4 umol/m^2/s, and 2.2 umol/m^2/s respectively. The photosynthesis rate could be altered due to the application of magnetic nanoparticles by which these nanoparticles could block the pathway and causes stress to the water spinach. Although nanoparticles are in the size of nanometer but their entry into the plant may cause some changes either by enhancing or inhibiting the photosynthesis rate. In this case, the Electron Transport Chain may be blocked by the nanoparticles and enhance stress to the plant manipulating and changing the normality of genes and enzymes like Rubisco. Rubisco is one of the enzymes that play an important role in the conversion of carbon dioxide into biological substances [34]. Therefore, the changes in production of Rubisco may lead to lower net photosynthesis rate with the application of magnetic nanoparticles.

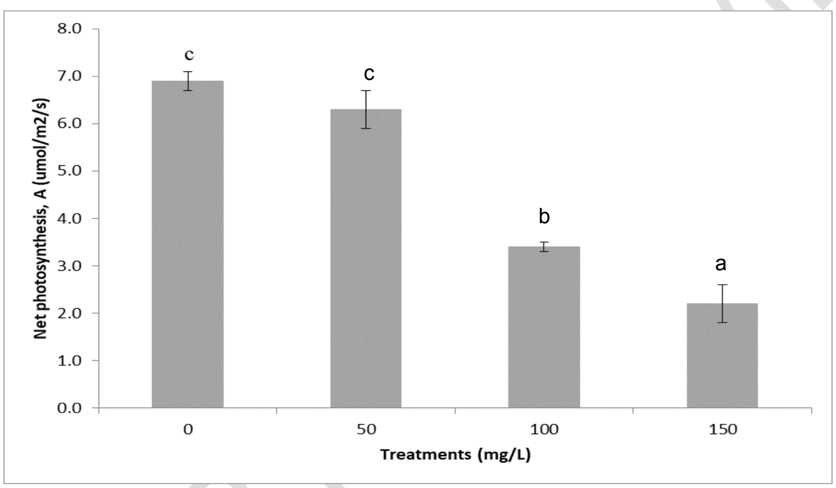

Fig. 7. The impact of magnetic nanoparticles on net photosynthesis rate of *Ipomoea aquatica*. Data are mean ± standard error of mean (SEM). N= 5. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

3.8 Transpiration Rate

Based on Fig. 8, the transpiration rate of *Ipomoea aquatica* was significantly influenced by the application of magnetic nanoparticles after 3 months of experimental period as *P*≤0.05. The transpiration rate was highest observed in 50 mg/L treatment followed by the control treatment, 100 mg/L and 150 mg/L of Fe_3O_4 by 2.34 mmo/m^2/s, 1.75 mmo/m^2/s, 1.44 mmo/m^2/s, and 0.80 mmo/m^2/s respectively. This indicates that higher concentration of Fe_3O_4 would decrease the transpiration rate while an optimum concentration could inhibit the transpiration rate in which in this case is 50 mg/L of iron oxide nanoparticles. Transpiration rate is linked up by photosynthesis rate by means if there is reduction in photosynthesis rate, the transpiration rate would decrease too. Besides, nanoparticles applied to the plant may cover the root surface of the plant and causes water stress in water spinach. This is supported by a research using Titanium oxide nanoparticle in maize in which the water transport capacity of the primary cell wall was reduced due to accumulation of nanoparticles at the plant leaf [35]. Besides, the research done on watermelon to study the application of magnetic nanoparticles on the root activity showed that the Fe_3O_4 accumulated at the root surface that prevent the transmission of water and also other nutritional components by the plant [33].

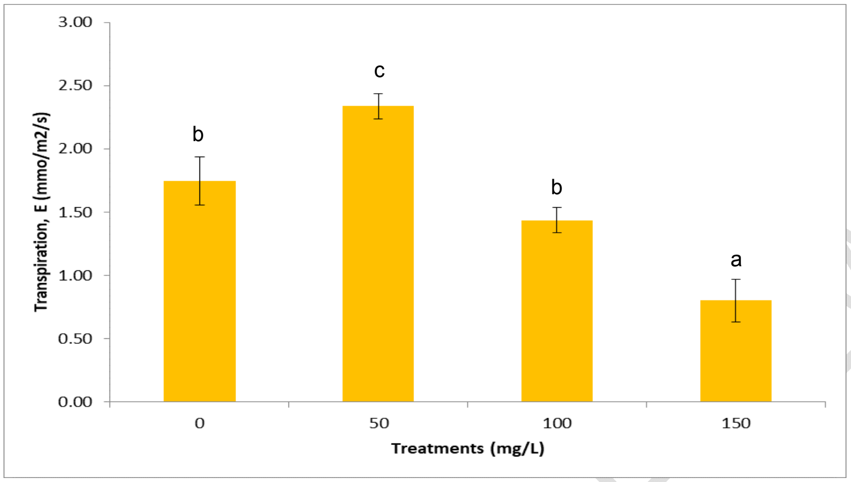

Fig. 8. The impact of magnetic nanoparticles on transpiration rate of *Ipomoea aquatica.* Data are mean ± standard error of mean (SEM). N= 5. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

3.9 Stomata Conductance

The stomata conductance of water spinach was significantly affected by the application of magnetic nanoparticles as showed in Fig. 9 as $P \leq 0.05$. After 12 weeks of treatment application, the stomata conductance for 50 mg/L Fe_3O_4 was the significantly highest compared to 0 mg/L, 100 mg/L and 150 mg/L with 0.314 mol/m^2/s, 0.246 mol/m^2/s, 0.144 mol/m^2/s, and 0.134 mol/m^2/s respectively. From the result, we could observe that higher concentration of Fe_3O_4 would decrease the stomata conductance of water spinach. Stomata conductance is determined by the degree of stomata aperture which estimates the rate of gas exchange and transpiration rate. A greater conductance is shown when the degree of stomata opening and its function in term of density and size is greater. When the plant undergoes greater photosynthesis and transpiration rate, the stomata conductance is greater. This statement is proven by the result of this experiment by which when the photosynthesis and transpiration rate of water spinach is lower for higher concentration of Fe_3O_4, the stomata conductance follows the same pattern. The application of magnetic nanoparticles may cause leakage of electrolyte in the plant which alters the mechanism of potassium pump that controls stomata opening. The accumulation of magnetic nanoparticles may cause the stomata to be closed or partially closed which directly reduce the stomata conductance [14].

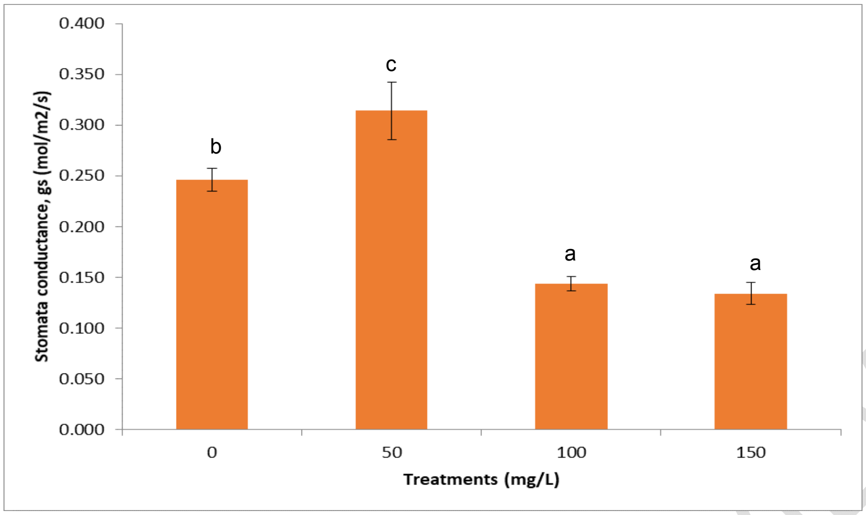

Fig. 9. The impact of magnetic nanoparticles on stomatal conductance of *Ipomoea aquatica.* Data are mean ± standard error of mean (SEM). N= 5. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

3.10 Water Use Efficiency

From the Fig. 10, the result showed that the water use efficiency of water spinach was significantly affected by the application of Fe_3O_4 as $P \leq 0.05$ by which the significantly highest water use efficiency was recorded for 150 mg/L treatment, followed by other treatments with 0 mg/L, 50 mg/L and 100 mg/L at 4.23, 2.86, 2.72, and 2.44 umol/CO_2/mmol/H_2O respectively. From the result, we could observe that the water spinach with the highest concentration of Fe_3O_4 showed greater water use efficiency.Water use efficiency is one of the important determinants of plant which is under stress as the plant maximize the capturing of soil moisture when there is limitation of water supply or lower stomata conductance in order to increase yield production [36]. Water use efficiency is related with transpiration rate and defined as the ratio of moles CO_2 assimilated per moles of water transpired [14]. Therefore, with the application of magnetic nanoparticles at higher concentration increase the transpiration rate of *Ipomoea aquatica*, the water use efficiency is also increased.

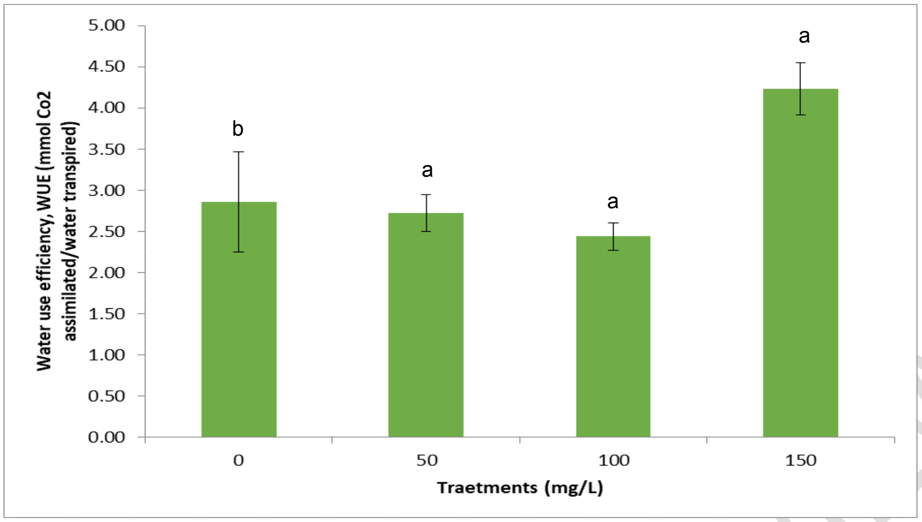

Fig. 10. The impact of magnetic nanoparticles on water use efficiency of *Ipomoea aquatica*. Data are mean ± standard error of mean (SEM). N= 5. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

3.11 Fv/Fm (Maximum Efficiency of Photosystem II)

Figure 11 shows the effect of different concentration of Fe_3O_4 on maximum efficiency of photosystem II or known as fv/fm ratio. The result of the application on water spinach was significant as $P \leq 0.05$ by which higher concentration of Fe_3O_4 showed lower fv/fm value. The ratio of fv/fm for 150 mg/L (0.65) was significantly lower than 100 mg/L (0.69) and followed by 50 mg/L (0.74) and 0 mg/L (0.76). The lowest fv/fm ratio shown by water spinach treated with 150 mg/l clearly shows that higher concentration of Fe_3O_4 would reduce the efficiency of photosystem II. Fv/fm is the main parameter used to detect any injury in photosystem II or photon inhibitory process as it is related to quantum yield of photosynthesis. The induction of stress on photosynthetic surface in plant would limit the transformation of light energy in photosystem II. Thus, the decrease in photosynthetic in plant would reduce the reduction of phytochemical activity in photosystem II [37].

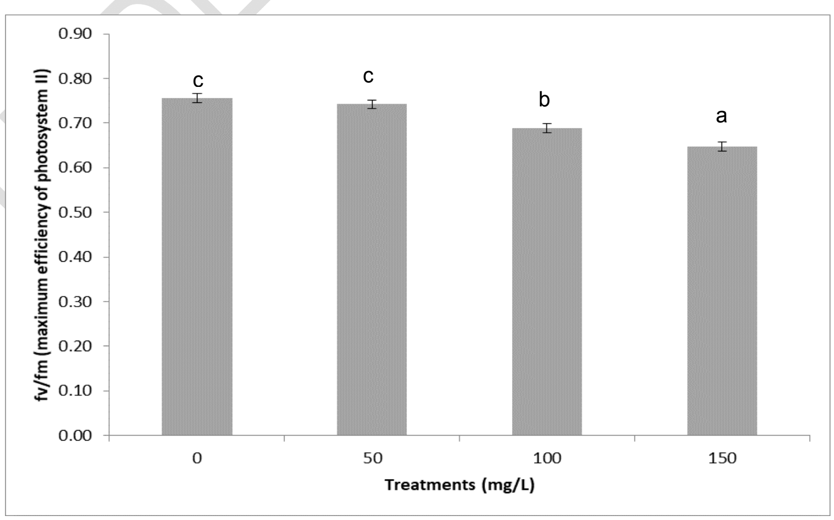

Fig. 11. The impact of magnetic nanoparticles on fv/fm (maximum efficiency of photosystem II) of *Ipomoea aquatica*. Data are mean ± standard error of mean (SEM). N= 5. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

3.12 Fv/Fo (Maximum Yield of Photosystem II)

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

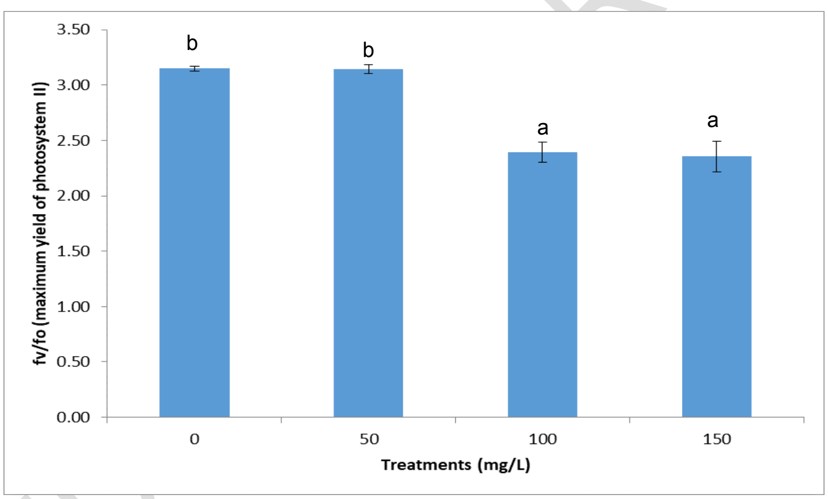

Fig. 12. The impact of magnetic nanoparticles on fv/fo (maximum yield of photosystem II) of *Ipomoea aquatica*. Data are mean ± standard error of mean (SEM). N= 5. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

3.13 Fo (Minimal Fluorescence)

Based on Fig. 13, the application of Fe_3O_4 had significantly influenced the minimal chlorophyll fluorescence yield of water spinach as $P \leq 0.05$. Fo of water spinach treated with 100 mg/L and 150 mg/L Fe_3O_4 were significantly higher compared to 0 mg/L and 50 mg/L, 722, 726, 653 and 607 respectively. From the result, it is shown that the Fo value increase as the concentration of Fe_3O_4 increase. The application of nanoparticles like titanium oxide showed that with the increase of its concentration, the minimal fluorescence value decreased as titanium oxide nanoparticles do protect the photosynthetic structure of the tomato plant under mild heat stress. However, the study by Gao et al. [39] showed that at high light intensity the minimal fluorescence in *Ulmus elongata* seedlings decreased. Therefore, the best explanation for this would be the impact of magnetic nanoparticles on water spinach is complex and it depends both on the concentration of Fe_3O_4 and the environmental condition that would affect the activity of nanoparticles [40]. Besides, the application of magnetic nanoparticles increased the heat dissipation in water spinach as the absorbed light energy could not be used for photosynthesis [37].

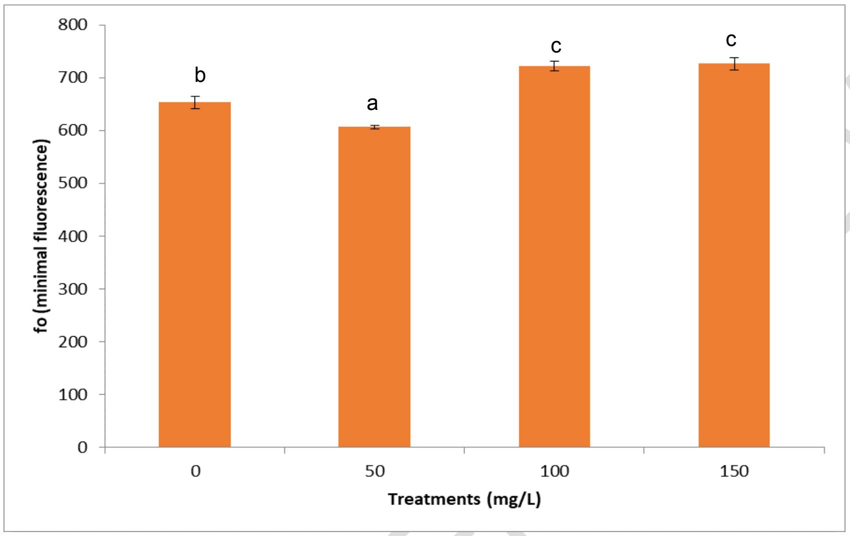

Fig. 13. The impact of magnetic nanoparticles on fo (minimal fluorescence) of *Ipomoea aquatica*. Data are mean ± standard error of mean (SEM). N= 5. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

3.14 PI (Performance Index)

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

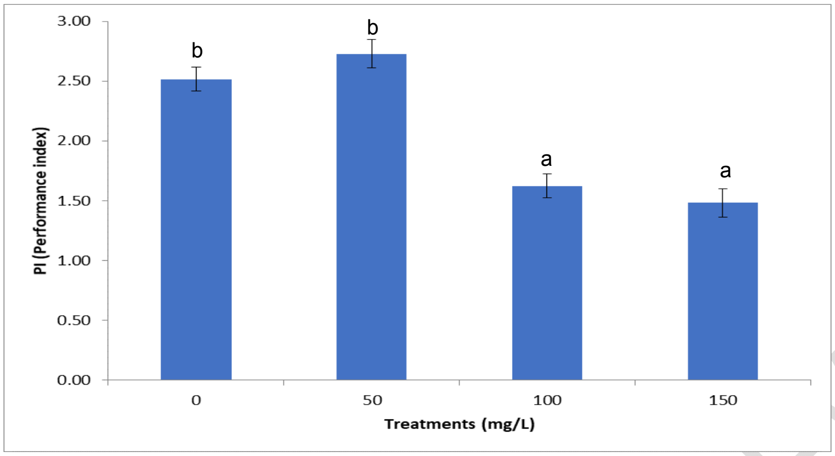

Fig. 14. The impact of magnetic nanoparticles on PI (performance index) of *Ipomoea aquatica*. Data are mean ± standard error of mean (SEM). N= 5. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

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

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

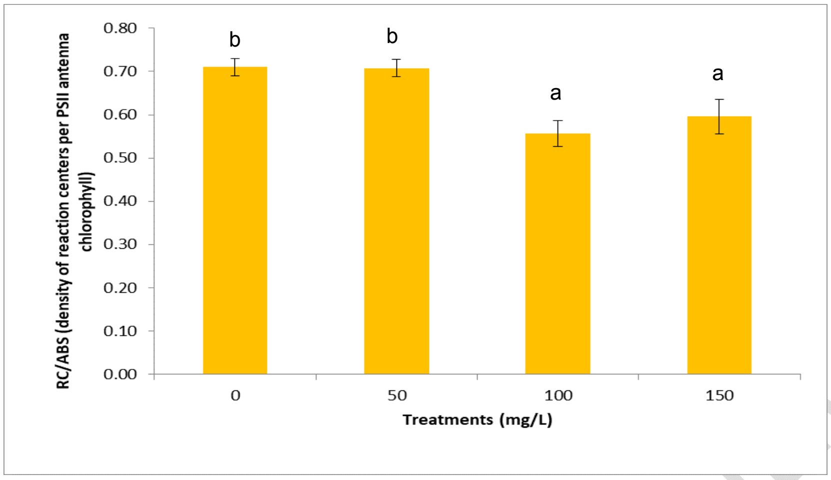

Fig. 15. The impact of magnetic nanoparticles on RC/ABS (density of reaction centers per PSII antenna chlorophyll) of *Ipomoea aquatica*. Data are mean ± standard error of mean (SEM). N= 5. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

3.16 Total Phenolics Content

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

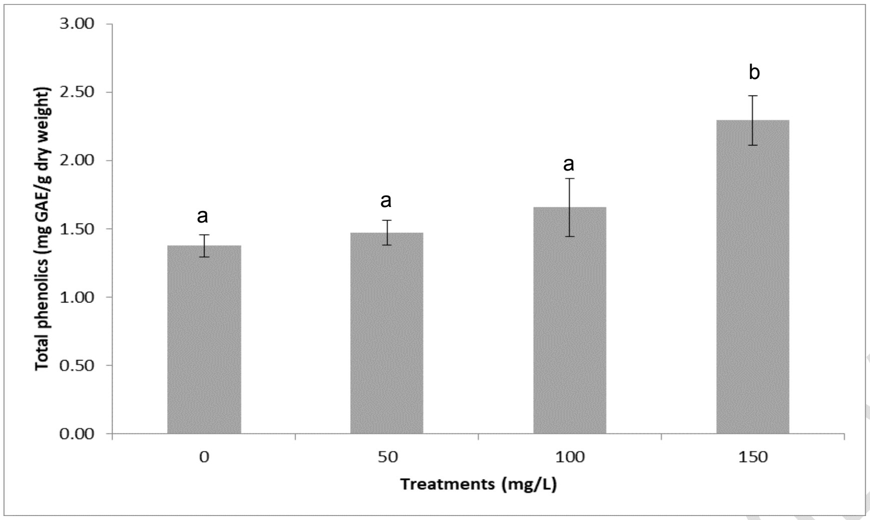

Fig. 16. The impact of magnetic nanoparticles on total phenolics content *of Ipomoea aquatica*. Data are mean ± standard error of mean (SEM). N= 5. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

3.17 Total Flavonoids Content

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

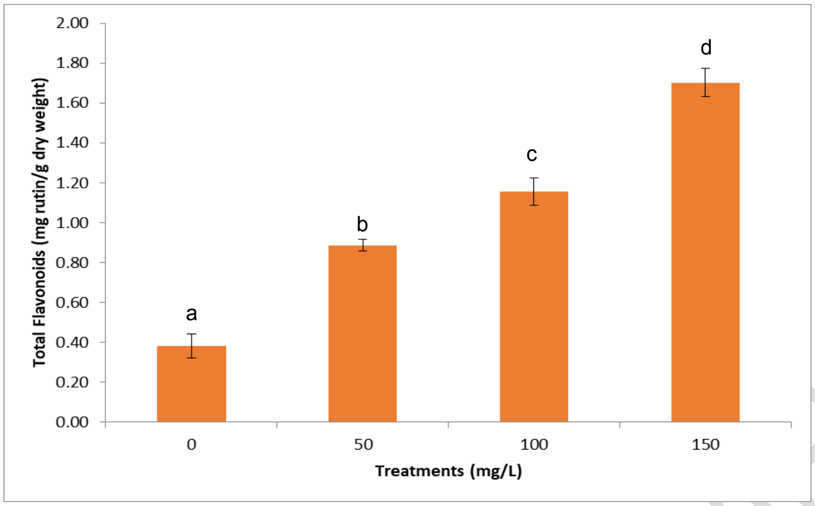

Fig. 17. The impact of magnetic nanoparticles on total flavonoids content of *Ipomoea aquatica*. Data are mean ± standard error of mean (SEM). N= 5. Mean with different superscript showed the significantly different at P≤0.05 using DMRT at 95% confidence levels.

4. CONCLUSION

Overall, it was found that the application of magnetic nanoparticles which was in the form of iron oxide nanoparticles had influenced the growth of *Ipomoea aquatica*. The plant height, shoot elongation, plant temperature, total biomass and total chlorophyll content were significantly affected by the application of magnetic nanoparticles at higher concentration. Besides that, the leaf gas exchange characteristics were also influenced by the different concentrations of iron oxide nanoparticles as *P*≤0.05. The net photosynthesis rate, transpiration rate, stomata conductance, 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 *I. aquatica* were significantly reduced at higher concentration of magnetic nanoparticles. While, water use efficiency and minimal fluorescence value of *I. aquatica* were increased with the increased of iron oxide nanoparticles concentration. In addition, the application of magnetic nanoparticles had significantly influenced the total flavonoids and total phenolics content in water spinach

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