Universiti Malaysia Terengganu Journal of Undergraduate Research *eISSN: 2637-1138*
Volume 5 Number 3, July 2023: 78-88

AMMONIUM-INDUCED CHANGES IN THE ASCORBIC ACID, CAROTENOID, PHENOLIC AND FLAVONOID CONTENT IN THE CULTURES OF *Aglaonema simplex*

MOHD-RIDWAN ABDULLAH ASRI[1], AMIRRUDIN AHMAD[1,2], MUHAMAD FAIRUS NOOR HASSIM[1] AND AZIZ AHMAD[1*]

[1]*Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.*
[2]*Institute of Tropical Biodiversity and Sustainable Development, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu. Malaysia.*

**Corresponding author: aaziz@umt.edu.my* *https://doi.org/10.46754/umtjur.v5i3.406*

Abstract: Malayan Sword or Borneo Sword (*Aglaonema simplex*) is a semi-aquatic plant that inhabits the swamp forest or adjacent to a river. The plant species belong to the Araceae family and are native to Southeast Asia. Ammonium is one source of nitrogen for plants. In higher concentrations, ammonium is toxic to plants. The present study examines the effects of ammonium concentrations (0 mM to 45.0 mM) on biomass, α-tocopherol, ascorbic acid, chlorophyll, carotenoid, phenolic, flavonoid, and flavones levels in an aquatic plant culture, *Aglaonema simplex*. Results showed that ammonium concentrations induced changes in the biomass and biochemicals of the plant. Ammonium at 36.0 mM produced the highest biomass at 1.5-fold dry weight, ascorbic acid at 1.2-fold and total phenolic produced 1.3-fold dry weight in biomass after 28 days of treatment. Carotenoid, chlorophyll, and flavonoid content varied among the ammonium concentrations and depend on the culture period. Ammonium concentrations did not significantly influence ($p > 0.05$) the α-tocopherol content in the treated plants. The finding suggested that ascorbic acid and phenolic might act as shields to diminish the effects of ammonium toxicity and can be induced by an appropriate ammonium concentration applied into the culture medium.

Keywords: Ascorbic acid, carotenoid, flavonoid, α-tocopherol, chlorophyll, phenolic.

Introduction

Malayan Sword or Borneo Sword (*Aglaonema simplex*) is a semi-aquatic plant species that belongs to the Araceae family. It is native to Southeast Asia and inhabits swamp forests or areas adjacent to a river. In nature, the plant is a hardy, long-lived species, and perfectly ideal for open-topped aquarium decoration but it has a short lifespan in completely submerged conditions. Previous studies showed that plant extracts containing alkaloids possess antibacterial activities and ligand inducers for high-density cholesterol uptake in the bloodstream (Zuriah *et al.,* 2017).

Plant growth and biochemical production depend highly on nitrogen sources supplied into the culture medium (Yamada & Sato, 2021). Ammonium (NH_4^+) and nitrate (NO_3^-) are the two major forms of dissolved nitrogen assimilated by higher plants. The availability and the ratio of these two nitrogen forms often influence plant growth and phytochemical biosynthesis (Kumar *et al.,* 2020; Naseri *et al.,* 2022). However, high ammonium concentrations cause toxicity and eliminate plant growth (Fang *et al.,* 2021; Hachiya *et al.,* 2021). In toxic ammonium conditions, plants change the phytohormone, photosynthetic pigments, and soluble carbohydrates to balance the hormonal homeostasis and oxidative stresses (Sun *et al*., 2022). Fang *et al.* (2021) stated that plants employed antioxidant compounds such as α-tocopherols, ascorbic acid, carotenoids, chlorophylls, flavonoids, phenolics, and oxidative enzymes including superoxide dismutase, guaiacol peroxidase, and glutathione reductase in a few plants.

Nonetheless, ammonium toxicity studies mainly focused on terrestrial plants as compared with aquatic plants. Aquatic plant responses to ammonium toxicity may differ from the terrestrial plants. Moreover, Naseri *et al.* (2022) mentioned that the hydroponic or plant tissue culture technique is a nature-friendly and effective system for plant proliferation and metabolite production from plants. The system also allows mineral nutrient manipulation for high biomass productivity pest and disease-free and notable secondary metabolite production compared to open or soil cultivation systems. Consequently, the in vitro culture or hydroponic cultivation of plants can be a sustainable resource for pharmaceutical production. Therefore, the current study objective is to determine the effect of ammonium on *Aglaonema simplex*, a type of aquatic plant using *in vitro* conditions.

Materials and Methods

The experiment was carried out in the plant biotechnology laboratory at the University of Malaysia Terengganu. An established in vitro culture of *Aglaonema simplex* plantlets was used as plant material. Before the treatment, plantlets were proliferated in a solid MS (Murashige & Skoog, 1962) basal medium added with B5 (Gamborg *et al.,* 1968) vitamins, 0.3% (w/v) sucrose, and 1.0 mg/l benzyl aminopurine as previously described (Zuriah *et al.,* 2017). Subsequently, two-month-old plantlets with almost similar fresh weight were aseptically transferred into the treatment medium. The treatment medium consisted of a modified liquid B5 (Gamborg *et al*., 1968) medium, to which an ammonium sulphate solution was applied as an ammonium source. The final ammonium concentrations in the treatment media were at 0, 9, 18, 27, 36, and 45 mM. All cultures were placed on an orbital shaker (80 rpm) and incubated in a culture room set at 28°C temperature and 16 hours photoperiods under white light provided by fluorescent lamps. Three cultures were randomly sampled at 7, 14, 21, and 28 days from each ammonium treatment. At harvest, plantlets' biomass was measured using an electronic balance and continued with the secondary metabolite's quantifications with 3 replications respectively.

Chlorophyll Content

Chlorophyll content was estimated based on the method described by Harborne (1984). Freshly harvested leaves (0.15 g) were homogenized in 10 ml of 80% (v/v) acetone using a mortar and a pestle. The mortar and pestle were rinsed with 5.0 ml of the acetone solution. The collected slurry was combined and centrifuged at 3000 x *g* for 10 minutes. The recovered supernatant was mixed with 25 ml of 80% (v/v) acetone and read for absorbance at 645 nm and 663 nm wavelengths using a spectrophotometer.

Carotenoid Content

Carotenoid content was measured based on the method espoused by Leichtenthaler (1987). The freshly harvested leaves (0.1 g) were homogenised using a mortar and a pestle in 3.0 ml of 80% (v/v) acetone added with clean sand. The homogenate was centrifuged at 10^3 x g for 10 minutes and collected supernatants were read for absorbance at 663.2 nm, 646.8 nm, and 470 nm wavelengths using a spectrophotometer.

Alpha-tocopherol Content

The content of α-tocopherol was quantified according to the method described by Hodges *et al*. (1996). The fresh leaf of the plant (0.1 g) was ground up using a mortar and a pestle in 1.5 ml acetone. Hexane (0.5 ml) was added to the slurry, vortexed for 30 seconds and centrifugated at 1000 x g for 10 minutes. The top layer solution was removed and repeated the extraction with hexane twice. The hexane extracts were combined. Subsequently, 0.5 ml extract was mixed with 0.4 ml of 0.1% (w/v) 3-(2-pyridyl)-5-6-diphenyl-1,2,4 triazine (DPT) dissolved in absolute ethanol and 0.4 M of 0.1% (w/v) ferric chloride dissolved in absolute ethanol. Absolute ethanol was added to the mixture to 3.0 ml, gently swirled and left at room temperature for 4 minutes. The mixture was added with 0.2 ml of 0.2 M orthophosphoric acid diluted in ethanol and left at room temperature for another 30 minutes. Absorbances were read at 554 nm

wavelength using a spectrophotometer and α-tocopherol content was estimated based on α-tocopherol (0 µg/ml to 1.4 µg/ml) standard curved.

Ascorbic Acid Content

Ascorbic acid content was estimated based on the method described by Jagota and Dani (1982). The fresh leaf of the plant (0.1g) was ground with 1.0 ml of 10% (v/v) trichloroacetic acid (TCA). Homogenate was centrifuged at 1000 x g for 10 minutes at 4^0C. Supernatant was recovered. Under the dim light, a 300 µl supernatant was mixed with 1700 µl distilled water and 200 µl of 10% (v/v) Folin reagent and allowed for a 10 minutes reaction. Absorbance was read at 760 nm wavelength using a spectrophotometer. Ascorbic acid in the sample was calculated based on ascorbic acid (0 µg/ml to 60 µg/ml) standard curved.

Phenolic and Flavonoid Content

The metabolites were extracted according to the method of Chang *et al.* (2002). The fresh leaf was harvested (1.0 g) and homogenized in 10.0 ml of 95% (v/v) ethanol. The supernatant obtained after centrifugation at 10^3 x g was used for total phenolic, flavonoid and flavone content quantifications. The replications were used for each treatment.

Total phenolic content was measured according to the method of Waterman & Mole (1994). One millilitre of plant extract was mixed with 15 ml distilled water, 4.0 ml Folin-Ciocalteu reagent and 6.0 ml of 20% (w/v) sodium carbonate solution. The reaction was allowed for 2 hrs. Subsequently, absorbance was read at 760 nm wavelength using a spectrophotometer. The total phenolic content was estimated based on standard curves of gallic acid in methanol in the range of 0 to 250 µg/ml.

The total flavonoid content was measured according to the method described by Chang *et al.* (2002). A half-millilitre of plant extract was mixed with 1.5 ml of 95% (v/v) ethanol, 10% (w/v) $AlCl_3$, 0.1 ml of 1.0 M potassium acetate solution and 2.8 ml distilled water. The mixture was left at room temperature for 30 min. Subsequently, absorbance was read at 415 nm wavelength using a spectrophotometer.

Flavone content was also determined based on the formation of the $AlCl_3$ chloride complex as described by Popova *et al.* (2003). Two millilitres of plant extract were mixed with 20 ml of absolute methanol, 1.0 ml of 5% (w/v) $AlCl_3$ solution and made up to 50 ml with distilled water. After 30 min of reaction, the absorbance was read at 425 nm wavelength using a spectrophotometer. Flavonoid and flavone content were estimated based on quercetin (0 µg/ml to 120 µg/ml) standard curved.

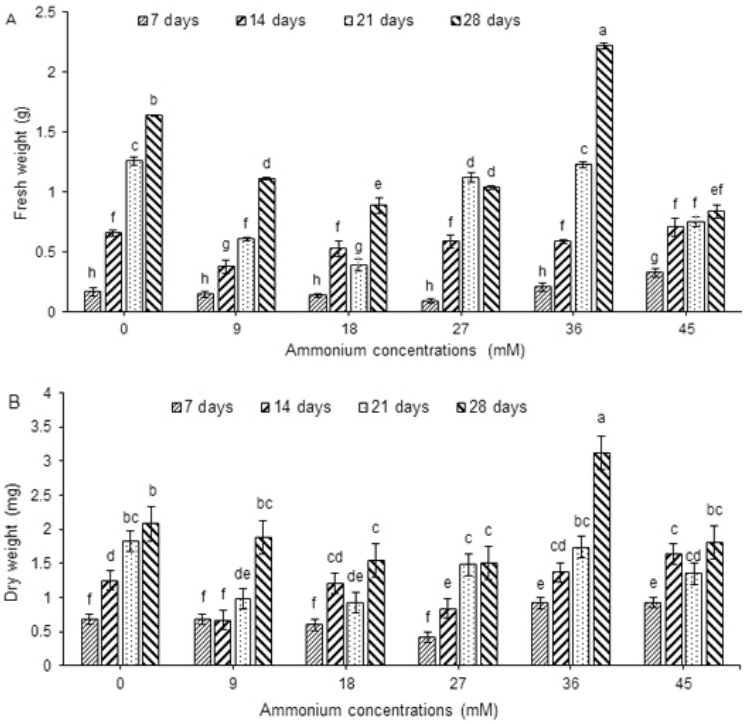
Statistical Analysis

Data were subjected to the One-way analysis variant (ANOVA) using the Statistical Package for Social Science (SPSS). The significant differences in mean values were determined by the Duncan Multiple Range (DMRT) tests at $p \leq 0.05$.

Results

Plant Weight Differences

Results in Figure 1 showed the effects of ammonium concentration on the fresh and dry weight of *Aglaonema simplex* plantlets after 28 days of treatments. Both fresh and dry biomass exhibits similar trends [Figures 1(A) and 1(B)]. The plant biomass increased as the culture period increased. The highest biomass was recorded in 36.0 mM ammonium (2.3 g fresh wt. and approximately 3.0 mg dry wt.). Nevertheless, the plant biomass decreased by the highest amount in an ammonium concentration (45.0 mM) treatment. In this ammonium concentration, the fresh and dry wt. were approximately 1.0 g and 1.5 mg respectively.



Figures 1: Effects of ammonium concentration on the fresh weight (A) and dry weight (B) of *Aglaonema simplex* cultures after 28 days of treatments. Bar with different small letters significantly different at $p \leq 0.05$ using a Duncan multiple range test

Chlorophyll Content

The results showed that chlorophyll content varied between ammonium concentrations and culture periods (Figure 2). Nonetheless, chlorophyll content in the *A. simplex* plantlets was almost consistent in 27.0 mM ammonium during the culture period. The chlorophyll content in this treatment was approximately 1.7 µg/g fresh wt. of the sample throughout the culture period.

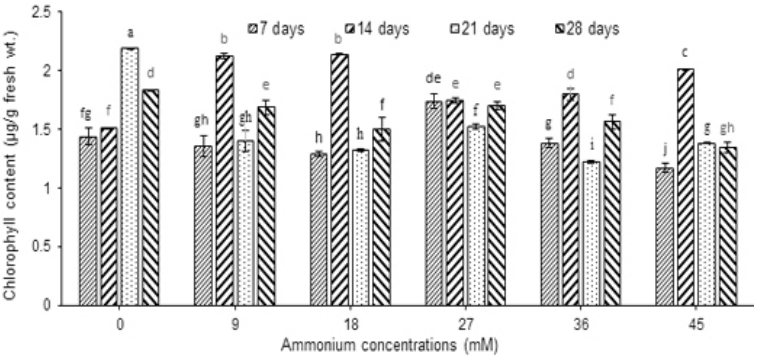


Figure 2: Effects of ammonium concentration on chlorophyll content in *Aglaonema simplex* cultures after 28 days of treatments. Bar with different small letters significantly different at $p \leq 0.05$ using the Duncan multiple range test

Carotenoid Content

The carotenoid content in the *A. simplex* plantlets was severely dropped in the ammonium-free medium (Figure 3), from 4.2 µg/g fresh wt. of the sample after the 7 days of culture to 1.0 µg/g fresh wt. of the sample after 21 and 28 days of culture. The highest carotenoid content was recorded in 18.0 ammonium treatment after 7 days of culture. However, the carotenoid in this treatment was dropped as the culture period increased. In general, carotenoid content fluctuated between .5 to 3.6 in 27 mM and 36 mM of ammonium treatments after 21 days of culture. Carotenoid content was decreased in all ammonium treatments after 28 days of the culture period.

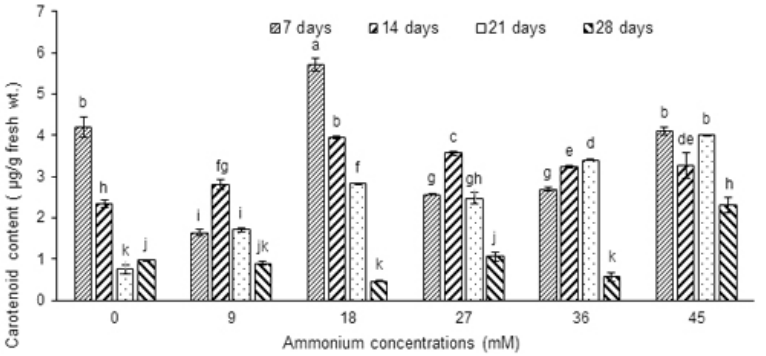


Figure 3: Effects of ammonium concentration on the carotenoid content in *Aglaonema simplex* cultures after 28 days of treatments. Bar with different small letters significantly different at $p \leq 0.05$ using the Duncan multiple range test

Alpha-tocopherol Content

The results in Figure 4 showed that α-tocopherol increased with the culture period. However, the α-tocopherol concentrations did not significantly differ between the treatments except for that pf the 18.0 mM and 27.0 mM ammonium concentrations. The α-tocopherol concentrations in these two treatments were slightly lower compared with the control and other ammonium concentrations. Nonetheless, the α-tocopherol content was higher than 5.0 µg/g fresh wt. of sample for both ammonium concentrations. Whereas the highest α-tocopherol content fluctuated between 7.1 to 7.3 0 µg/g fresh wt. of sample.

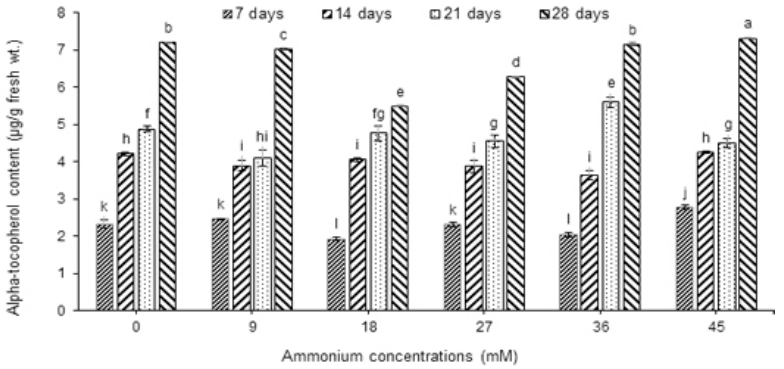


Figure 4: Effects of ammonium concentration on the α-tocopherol content in *Aglaonema simplex* cultures after 28 days of treatments. Bar with different small letters significantly different at $p \leq 0.05$ using the Duncan multiple range test

Ascorbic Acid Content

The ascorbic acid content varied among the ammonium treatments and culture period (Figure 5). Generally, the ascorbic acid content decreased as the ammonium concentration increased after seven days of treatment. The highest concentration of ascorbic acid was recorded in the 45.0 mM ammonium treatment after 14 days of culture. It reached up to approximately 90 µg/g fresh wt. of the sample. However, the ascorbic acid in this ammonium concentration dropped after 21 and 28 days of culture to 65 and 50 µg/g fresh wt. of the sample, respectively.

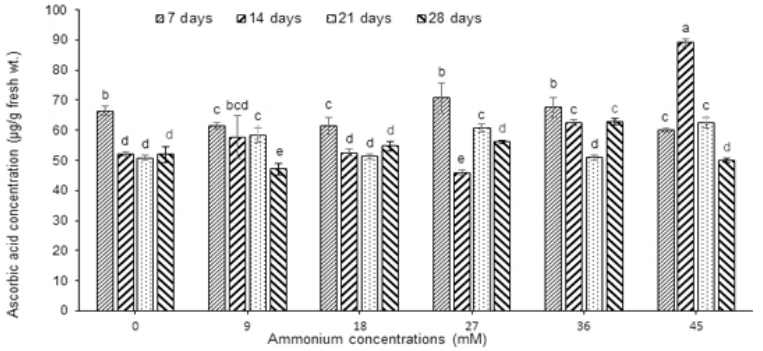


Figure 5: Effects of ammonium concentration on the ascorbic acid content in *Aglaonema simplex* cultures after 28 days of treatments. Bar with different small letters significantly different at $p \leq 0.05$ using the Duncan multiple range test

Phenolic Content

The phenolic content in the *A. simplex* varied between ammonium concentrations applied in the culture medium and the period of culture. The highest phenolic content was measured in plantlets treated with 27.0 mM of ammonium after seven days of culture. It reached up to 70 µg/g fresh wt. of the sample, however, dropped as the culture period increased. For ammonium concentrations from 0 mM to 36 mM, the high phenolic content was after 14 and 21 days of culture. However, in 45.0 mM ammonium, the highest phenolic content was after 28 days of culture (Figure 6).

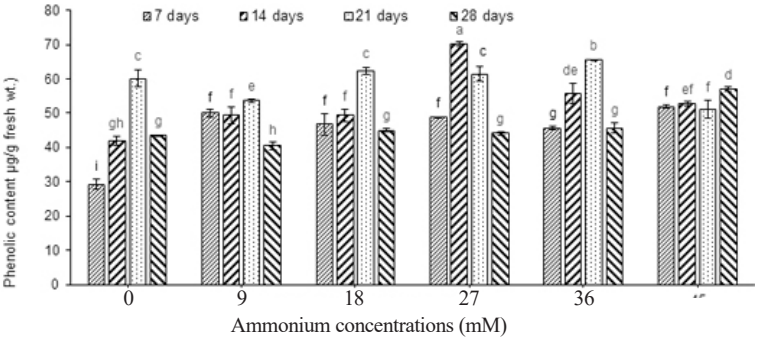


Figure 6: Effects of ammonium concentration on the phenolic content in *Aglaonema simplex* cultures after 28 days of treatments. Bar with different small letters significantly different at $p \leq 0.05$ using the Duncan multiple range test

Flavonoid Content

The results showed that ammonium concentration also induced changes in flavonoid content in *A. simplex* plantlets (Figure 7). The highest flavonoid content was measured in treatment with 27.0 mM ammonium after seven days of culture. In other ammonium concentrations, high flavonoid content was found after 21 days of the culture period. Throughout the culture period, constant flavonoid content was found in the treatment of 45.0 mM, around 40 µg/g fresh wt. of the sample.

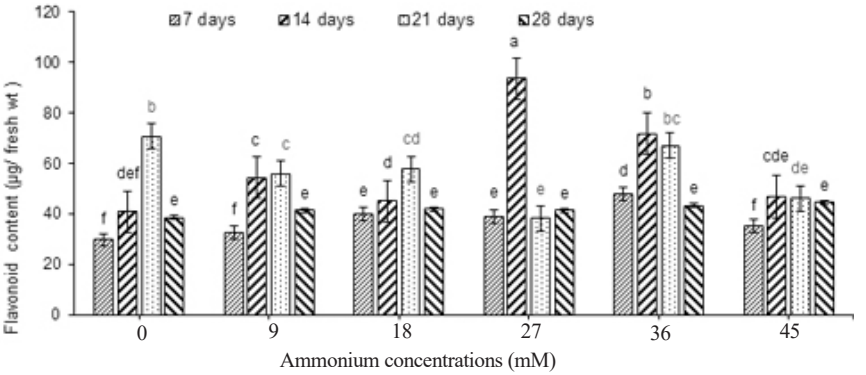


Figure 7: Effects of ammonium concentration on the flavonoid content in *Aglaonema simplex* cultures after 28 days of treatments. Bar with different small letters significantly different at $p \leq 0.05$ using the Duncan multiple range test

Flavone Content

Results in Figure 8 showed the effects of the ammonium concentrations on the flavone content in *A. simplex* culture. In the 9.0 mM and 36.0 mM ammonium treatments, the high flavone was recorded after seven days of culture. The highest amounts of flavones were found in the 36.0 mM ammonium treatment, up to 70 µg/g fresh wt. of the sample. On the other hand, in 18 mM and 27 mM of ammonium content produced the highest flavone count after 21 days of culture. In these conditions, the flavone content reached up to 60 µg/g fresh wt. of the sample.

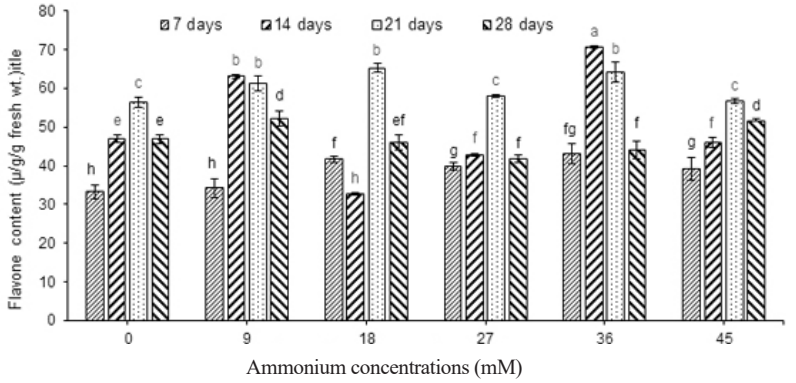


Figure 8: Effects of ammonium concentration on the flavone content in *Aglaonema simplex* cultures after 28 days of treatments. Bar with different small letters significantly different at $p \leq 0.05$ using the Duncan multiple range test

Discussion

Plant growth and biomass are influenced by available nutrients, particularly the major nutrient that involves in the majority of metabolite biosynthesis pathways. An appropriate amount of nitrogen sources such as ammonium supply to cells determined the biosynthesis of chlorophyll for photosynthesis activity, proteins, and enzymes utilised involved in plant growth and development. On the other hand, higher ammonium concentrations may cause toxicity and consequently poor growth (Naseri *et al.,* 2022). In this study, the highest biomass of *A. simplex* was found in a culture medium supplied with 36.0 mM ammonium (Figure 1). Meanwhile, in a medium consisting of 45.0 mM ammonium, the biomass was reduced. Various mechanisms for ammonium toxicity in various plant species have been reported. These include reduced protein glycosylation, acidification of the external environment and energy loss during the assimilation of excess ammonium in the cells (Liu *et al.,* 2017).

In some plant species, root growth is sensitive to excess ammonium and root development was reported as related to ethylene signalling and auxin transportation. More importantly, species supplied with ammonium as the main nitrogen source may suffer from decreased rates of net photosynthesis. Excessive concentration of ammonium in leaf tissue can cause the separation of electron transport reactions from phosphorylation in the chloroplast. Although a high biomass of *A. simplex* was obtained in 36.0 mM ammonium, the highest chlorophyll content was measured in lower ammonium levels, 27.0 mM (Figure 2). These results showed that the chlorophyll biosynthesis in this plant is active in moderate ammonium concentration. The assimilated may transport and be utilised in the biosynthesis of other metabolites such as amino acids and alkaloids. Chlorophyll plays a role in capturing photons as an energy source in photosynthesis processes and in photoprotective processes in plants that contribute to effective biomass accumulation (Pareek *et al.,* 2017).

Chlorophyll and carotenoids contribute to the pigmentation of plant organs. In normal conditions, dark green leaves are due to high chlorophyll content, while yellow to orange colours are due to lower chlorophyll but high carotenoid content. It was found that higher carotenoid content was in the medium supplied with lower ammonium concentration and at the early growth stages (Figure 3). Cheng *et al.* (2023) reported that ammonium ions reacted with other antioxidant compounds including carotenoids. The finding suggested that ammonium-free medium or lower ammonium concentration can be a possible strategy to enhance the high carotenoid accumulation in higher plants.

Results also showed that α-tocopherol content in the *A. simplex* did not significantly influence ($p > 0.05$) by the ammonium concentration (Figure 4). On the other hand, a higher α-tocopherol content was measured in the prolonged cultures, after 28 days of treatment. This result suggested that ammonium concentration may not function as a direct elicitor for carotenoid biosynthesis in *A. simplex*. Moreover, α-tocopherols are non-enzymatic lipid-soluble antioxidants synthesised only by photosynthetic organisms through the shikimate pathway and isoprenoid. Previous studies reported that c carotenoids and α-tocopherol inhibit lipid peroxidation and protect photosystem II from oxidative damage by scavenging lipid peroxyl radicals and singlet oxygen in many plants (Qadir *et al.*, 2017; Ali *et al.,* 2022). The current finding suggested that both metabolites, carotenoids, and α-tocopherol may act as a buffer to mitigate excess ammonium in the culture medium exposed to the root of *A. simplex* cultures. After a long culture period, the cell proliferation slows down; therefore, the nutrients might be used for the biosynthesis of storage metabolite as preparation for senescence.

Interestingly, the highest ascorbic acid content was detected in *A. simplex* culture in the highest ammonium treatment (45.0 mM) after 14 days of culture. This agrees with Viviani *et al.* (2022), where ascorbic acid is the major

hydrophilic antioxidant ad powerful inhibitor of lipid peroxidation and works in concert with α-tocopherol to reduce oxidative stress. However, this study showed that ascorbic acid in *A. simplex* culture was highly increased in high ammonium concentration in the early treatment period. At the same time, α-tocopherol was high toward the end of the experiment (Figure 4 and Figure 5).

The current finding also revealed that moderate ammonium concentration (27.0 mM) could trigger higher production of phenolic and flavonoids by *A. simplex* cultures. Nonetheless, Nasari *et al.* (2022) reported that high phenolic content was produced by Moldavian balm in a lower or ammonium-free medium. Ammonium availability was also reported to affect the phenolic composition in green and purple basil (Prinsi *et al.,* 2020). Differences in responses of these two plants might be due to the natural habits and genetics of the plants, where *A. simplex* is a semi-aquatic plant while Maldavian balm is a terrestrial plant. Meanwhile, flavone was highly increased in the plants treated with 36.0 mM ammonium. All of these three groups of metabolites, phenolic, flavonoid and flavone were highly produced after 14 days of treatment and sharply dropped after 28 days of culture (Figures 6, 7, and 8). The finding suggested that phenolic and flavonoids might work in concerted with ascorbic and α-tocopherol to reduce the toxicity effects of higher ammonium concentration. Moreover, results showed that a moderate concentration of ammonium ranging between 27.0 and 36.0 mM appeared to induce a high α-tocopherol, ascorbic acid, phenolic, flavonoids and flavones metabolite production by the *A. simplex* cultures. This indicated that in *A. simplex* culture, these metabolites might be involved in regulating the stress due to high ammonium concentrations. Foyer *et al.* (2020) suggested that under stress conditions such as particularly high ammonium concentrations, these metabolites act in a signalling network of hormonal and a series of acclimatization responses to prevent major perturbations in redox homeostasis and regulate the biochemical processes that allow survival of the under-stress plants (Akram *et al.,* 2017). Qadir *et al.* (2017) mentioned that plant responses to ammonium are profoundly influenced by the nutrient absorption status, particularly concerning nitrate availability. Since the culture media are supplied with similar nitrate concentrations and used by plants after ammonium in the nitrogen assimilation activity.

Conclusion

The *A. simplex* platelets biomass, ascorbic acid, carotenoid, chlorophyll, phenolic, flavonoid and flavone content in the plant was reliant on ammonium concentration applied in the culture medium and culture period. Ammonium at 27.0 to 36.0 mM was the most appropriate concentration for the highest biomass production as well as the metabolite contents. The highest ascorbic acid, carotenoid, chlorophyll, phenolic, flavonoid and flavone were obtained after 21 days the treatment period. The finding suggested that appropriate ammonium concentrations play a crucial role in determining the *A. simplex* productivity. Further studies should be conducted to identify the specific biochemical such as alkaloids and enzymes triggered by the ammonium concentrations. The effects of ammonium on plant growth and respective metabolites should also be determined.

Acknowledgements

The authors thank the University of Malaysia Terengganu for the platform for the research activities.

References

Akram, N. A., Shafig, F., & Ashraf, M. (2017). Ascorbic acid – A potential oxidant scavenger and its role in plant development and abiotic stress tolerance. *Frontiers in Plant Science*, *8*, 613.

Ali, E., Hussain, S., Hussain, N., Kakar, K. U., Shah, J. M., Zaidi, S. H. R., Jan, M., & Zhang, K. (2022). Tocopherol as plant protectors: An overview of tocopherol

biosynthesis enzymes and their role as antioxidant and signalling molecules. *Acta Physiologiae Plantarum*, *44*(20), 1-11. https://doi.org/10.1007/s11738-021-03350-x.

Chang, E. F., Yang, M. H., Wen, H., M. & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colourimetric methods. *Journal of Food and Drug Analysis*, *10*(3), 178-182.

Cheng, W., Xian, F., Zhou, Z, Hu, K., & Gao, J. (2023). Solubility and stability of carotenoids in ammonium- and phosphonium-based ionic liquids: Effect of solvent nature, temperature and water. *Molecules, 28*, 3618. https://doi.org/10.3390/molecules28083618.

Fang, G., Yang, J., Sun, T., Wang, X., & Li, Y. (2021). Evidence that synergism between potassium and nitrate enhances the alleviation of ammonium toxicity in rice seedling roots. *PLOS ONE, 16*(9), e0248796. https://doi.org/10.1371/journal.pone.0248796.

Foyer, C.H., Kyndt, T., & Hancock, R. D. (2020). Vitamin C in plants: Novel concepts, new perspectives and outstanding issues. *Antioxidant & Redox Signal*, *32*, 463-485.

Gamborg, O. L., Miller, R. A., & Kojima, K. (1968). Nutrient requirement of suspension cultures of soybean root cells. *Experimental Cell Research*, *50*, 151- 158.

Hachiya, T., Inaba, J., Wakazaki, M., Sato, M., Toyooka, K., Miyagi, A., Kawai-Yamada, M., Sugiura, D, Nagakawa, T, Kiba, T., Gojon, A., & Sakakibara, H. (2021). Excessive ammonium assimilation by plastidic glutamine synthetase causes ammonium toxicity in *Arabidopsis thaliana*. *Nature Communications, 12*, 4944. https://doi.org/10.1038/s41467-021-25238-7.

Harborne, J. B. (1984). *Phytochemical methods* (2nd ed., pp. 69-76). New York: Chapman and Hall.

Hodges, D. M., Andrew, C. J., Johnson, D. A. & Hamilton, R. J. (1996). Antioxidant compound responses to chilling stress in differently sensitive inbred maize lines. *Physiologiae Plantarum*, *98*, 685-692.

Jagota, S. K., & Dani, H. M. (1982). New colourimetric techniques for estimation of vitamin C using folin phenol reagent. *Analytical Biochemistry, 127*, 178-182.

Liu, Y., & Von Wirén, N. (2017). Ammonium as a signal for physiological and morphological responses in plants. *Journal of Experimental Botany, 68*, 2581–2592.

Leichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigment of photosynthetic biomembranes. In Packer I & Dauce R (Eds.). *A method in enzymology*. (Vol. 148, pp. 350-382). New York: Academic Press.

Kumar, V., Kim, S. H., Priatama, R. A., Jeong, J. H., Adnan, M. R., Saputra, B. A., *et al*. (2020). NH_4^+ suppresses NO_3^--dependent lateral root growth and alters gene expression and gravity response in OsAMT1 RNAi mutants of rice (*Oryza sativa*). *Journal of Plant Biology*. *63*, 391–407. https://doi.org/10.1007/s12374-020-09263-5.

Murashige T., & Skoog T. (1962). A revised medium for rapid growth and bioassays with tobacco. *Physiology Plantarum*, *15*, 43-425.

Naseri, A., Alirezalu, A., Noruzi, P., & Alirezalu, K. (2022). The effects of different ammonium to nitrate ratios on antioxidant activity, morpho-physiological and phytochemical traits of Moldavian balm (*Dracocephalum moldavica*). *Scientific Reports*, *12*, 16841, https://doi.org/10.1038/s41598-022-21338-6.

Pareek, S. Sagar, N. A., Sharma S., Kumar V., Agarwal T., González-Aguilar G. A., & Yahia E. M. (2017). Chlorophylls: Chemistry and biological functions. In Yahia, E. M. (Ed.) *Fruit and vegetable*

phytochemicals: Chemistry and human health. (2nd ed., pp. 269–284). Haryana, India.

Popova, M., Bankova, V., Butovska, D., Petkov, V, Damyanova, B., Sabatini, A. G., Marcazzan, G. L., & Bogdanov, S. (2003). Poplar type propolis and analysis of its biologically active component. *Honeybee Science, 24*(2), 61-66.

Prinsi, B., Negrini, N., Morgutti, S., & Espen, L. (2020). Nitrogen starvation and nitrate or ammonium availability affect phenolic composition in green and purple basil differently. *Agronomy*, *10*(4), 498.

Qadir, O., Siervo, M., Seal C. J., & Brandt, K. (2017). Manipulation of contents of nitrate, phenolic acids, chlorophylls, and carotenoids in lettuce (*Lactuca sativa* L.) via contrasting responses to nitrogen fertilizer when grown in a controlled environment. *Journal of Agriculture and Food Chemistry, 65*, 10003–10010.

Sun, T., Wang, T., Qiang Y., Zhao G., Yang J., Zhong H., Peng X., Yang J., & Li Y. (2022). CBL-interacting protein kinase OsCIPK18 regulates the response of ammonium toxicity in rice roots. *Frontiers in Plant Science*, *13*, 863283. https://doi.org/10.3389/fpls.2022.863283

Viviani, A., Fambrini, M., Giordani, T., & Pugliesi, C. (2021). L-ascorbic acid in plant: From biosynthesis to its role in plant development and stress response. *Agrochemical, 65*(2) 151-171. https://doi.org/10.12871/00021857202124

Waterman, P. G., & Mole, S. (1994). *Analysis of phenolic metabolites*. Blackwell Scientific Publication. Cambridge.

Yamada Y., & Sato F. (2021). Transcription factors in alkaloid engineering. *Biomolecules, 11*, 1719. https://doi.org/10.3390/biom11111719.

Zuriah, I., Aziz, A., & Sifzizul, T. T. M. (2017). Phytochemical screening of in vitro *Aglaonema simplex* plantlet extracts as inducers of sr-b1 ligand expression. *Journal of Sustainability Science and Management, 12*(2), 1823-8556.