Research article

Hasan, H. and F.A. Manan, Total Phenolic and Flavonoid Content of Elaeis guineensis Leaves Treated with Different Amount of Nitrogen-Potassium Fertilizer. International Journal of Life Sciences and Biotechnology, 2020. 3(2): p. 156-163. DOI: 10.38001/ijlsb.699527

Total Phenolic and Flavonoid Content of *Elaeis guineensis* Leaves Treated with Different Amount of Nitrogen-Potassium Fertilizer

Hazrina Hasan[1], Fazilah Abd Manan[1]*

ABSTRACT
The amount of fertilizer used as source of nutrients play an important role for successful growth of oil palm. In this study, the effect of different amount of nitrogen (N) and potassium (K) fertilizer on the biochemical properties of oil palm were studied. Oil palm trees were supplied with fertilizer consist of constant amount of Bayovar rock phosphate (BRP), Kieserite (KS), and Foliar Boron as well as different levels of muriate of potash (MOP) and ammonium sulphate (AS). The treatments were labeled as T1, T2 and T3. Six planting materials indicated as A, B, C, D, E and F were tested. The total phenolic content (TPC) and total flavonoid content (TFC) in the leaves of the oil palm trees were analyzed using Folin-Ciocalteu method and Aluminium chloride method, respectively. From the results, the leaves of planting material A and F showed significant responses towards different levels of fertilizer where the TPC and TFC contents reduced at the highest fertilizer level. Planting material C and D responded quite similarly in terms of TPC content with planting material A and F, respectively. Overall, different planting materials showed different pattern of responses specifically for TPC and TFC towards fertilizer level.

ARTICLE HISTORY
Received
06 March 2020
Accepted
16 May 2020

KEYWORDS:
Phenolic, flavonoid, *Elaeis guineensis*, fertilizer

Introduction

Oil palm (*Elaeis guineensis*) is an important perennial crop in many countries such as Malaysia, Indonesia, some African and Columbia. The oil palm belongs to the species *Elaeis guineensis* and family Arecaceae. In 2016, the crude palm oil generated was 17.32 million tonnes, covering 5.74 million hectares land [1]. Suitable fertilizer application for oil palm trees is important due to the nature of plants being vulnerable to nutrient deficiencies. Unfortunately, most oil palm plantations have low soil fertility which is needed for oil palms to produce optimum fresh fruit bunch yield. Therefore, balancing fertilizers is necessary to maintain high yield and good nutritional status of oil palm and prevent over-fertilization that possibly brings negative impact to the environments.

[1] Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia.
*Corresponding Autor: Fazilah Abd Manan, e-mail: m-fazilah@utm.my

Nitrogen is one of the most important nutrient elements for plant growth and physiological health which make up chlorophyll, protoplasm, amino acids and proteins [2]. On the other hand, potassium is necessary for sustaining osmotic balance, photosynthesis, transports of phloem and fruit quality [3]. Generally, macro and micro nutrients are vital in various functions in plant cells including providing resistance to diseases [4]. Among the oil palm by-products are empty fruit bunches, palm pressed fibre, oil palm fronds, palm kernel cake, palm oil mill effluent and oil palm trunk [5]. Oil palm fronds can be used as ingredients for livestock feeding, an alternative way for by-product disposal [6]. Several studies have reported that oil palm leaf extracts are rich in antioxidant activity from carotenoid, tocopherol, and phenolic compounds [7, 8, 9]. Phenolic compounds are ubiquitous in plants. This compound act as antioxidants and involves in plant defense system [10]. Flavonoids and phenolic acids are the two majör classes of phenolic compounds. Phenolic compounds such as flavonoid being the main constituent that can be found in oil palm leaves alcoholic extract [11]. Flavonoid act as antioxidant to protect plants from various biotic and abiotic stresses [12, 13] and served as plant-derived foods and beverages. Depending on the progenies, oil palms may respond differently to nutrient inputs. Therefore, this study aimed to determine the total phenolic and flavonoid content in the leaves of oil palm from different planting materials when treated with different levels of NK fertilizer.

Materials and Methods

Plant samples

Elaeis guineensis leaves from frond-17 were harvested from four-year old oil palm trees planted in Johor, Malaysia. The leaves were taken from oil palm of different planting materials indicated as A, B, C, D, E, and F, treated with different levels of NK fertilizer (T1, T2, and T3). T1 (control) consist of 2 kg Bayovar rock phosphate (BRP), 1.25 kg Kieserite (KS) and 0.125 kg Foliar Boron. T2 consist of 2.7 kg ammonium sulphate (AS), 1.75 kg muriate of potash (MOP) + control and T3 consist of 5.4 kg AS, 3.5 kg MOP + control. After collection, samples were transported directly to the laboratory and kept in the freezer at -80°C before further analysis. Leaves were washed and dried overnight in the oven at 65°C for extraction process. The leaves were crushed into powder using mortar and pestle and extracted with distilled water at a 1:10 (dry weight: volume) ratio

for 1 hour at 90°C. The mixture was centrifuged at 9000 rpm for 10 min at 4°C. The supernatant obtained was used for determination of total phenolic and flavonoid content.

Analysis of total phenolic content (TPC)

The total phenolic content was analyzed following Folin-Ciocalteu method [14]. Standard curve of gallic acid solution at the concentration of 0, 10, 20, 30, 40, 50 and 60 μg/mL were prepared. 0.2 mL of the supernatant was mixed with 0.8 mL of distilled water. 0.1 mL of Folin-Ciocalteu reagent was added and left for 3 minutes at room temperature. Then, 0.8 mL of 20% (w/v) Na_2CO_3 was added into the mixture and incubated 2 hours in the dark. The absorbance was measured using UV-Vis spectrophotometer at 765 nm. The absorbance, y obtained after analysis for each *Elaeis guineensis* leaf sample was used in the equation y=0.0121x – 0.048 obtained from the standard curve. Then, the value obtained, x was substituted in C1 in the equation C=C1 x V/m where,

C= Total phenolic content in GAE in μg/g

C1= Concentration of gallic acid established from standard curve in μg/ml

V= Volume of extract in ml

m= Weight of plant extract in g

Analysis of total flavonoid content (TFC)

The total flavonoid content was assessed by aluminium chloride colorimetric method [15]. Standard curve of quercetin solution (in 80% (v/v) ethanol) at the concentration of 0, 20, 40, 60, 80, 100, 120, 140 and 160 μg/mL were prepared. 0.2 mL of the extract supernatant was mixed with 0.15 mL of 5% $NaNO_2$ and incubated in the dark for 6 minutes at room temperature. Then, 0.15 mL of 10% (w/v) $AlCl_3$ was added to the mixture and kept in dark for 6 min at room temperature. After that, 0.8 mL of 10% (w/v) NaOH was added into the mixture and incubated in dark for 15 min at room temperature. The absorbance was measured using UV-Vis spectrophotometer at 510 nm. The absorbance, y obtained after analysis for each *Elaeis guineensis* leaf sample was used in the equation y=0.0046x – 0.0187 obtained from the standard curve. Then, the value obtained, x was substituted in C1 in the equation C=C1 x V/m where,

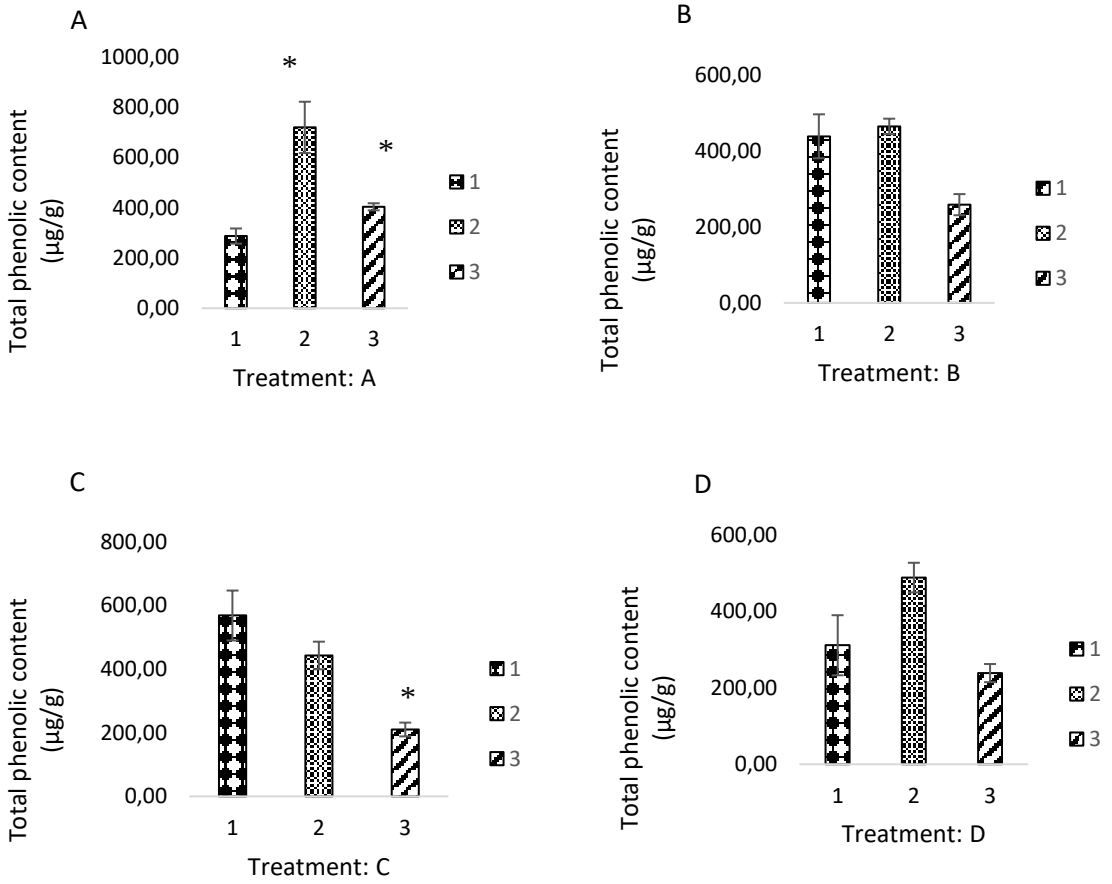
Statistical analysis

All experiments were conducted in triplicates. Data reported are mean ± standard error. Microsoft Excel 2013 was used for statistical analysis.

Results and Discussions

Total phenolic content in oil palm leaves

Fig 1 shows the total phenolic content (TPC) in oil palm leaves of planting material A-F. For planting material A, results showed that the highest TPC was obtained in the oil palm leaves at T2 fertilizer level, while the lowest TPC was recorded in the leaves of oil palm at T1 fertilizer level. Data analysis showed significant differences of TPC content in T2 and T3 when compared to T1. In planting material B, no significant differences between treatments were recorded. For planting material C, T3 treatment had significantly reduced the production of phenolic compounds. This reduction was also observed at T2. The trend was similar for planting material F. For planting material E, T2 significantly increased the production of TPC, although the TPC level slightly dropped for T3. For some plants, nitrogen deficiency will enhance the accumulation of phenolic compound in plant tissues [16]. However, in this study, this pattern was observed only for planting material C and F. Total phenolic content in plants may be influenced by different nitrogen source of the fertilizer, besides other environmental factors during plant growth and developmental stages.



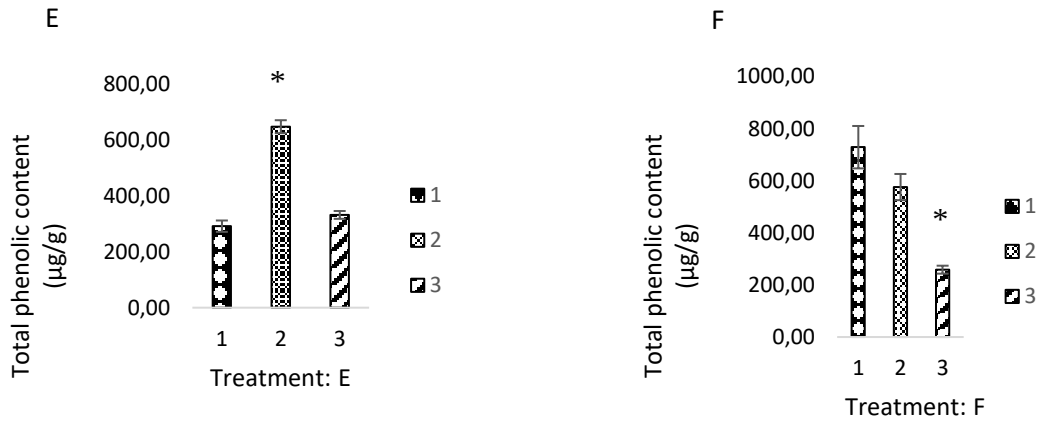
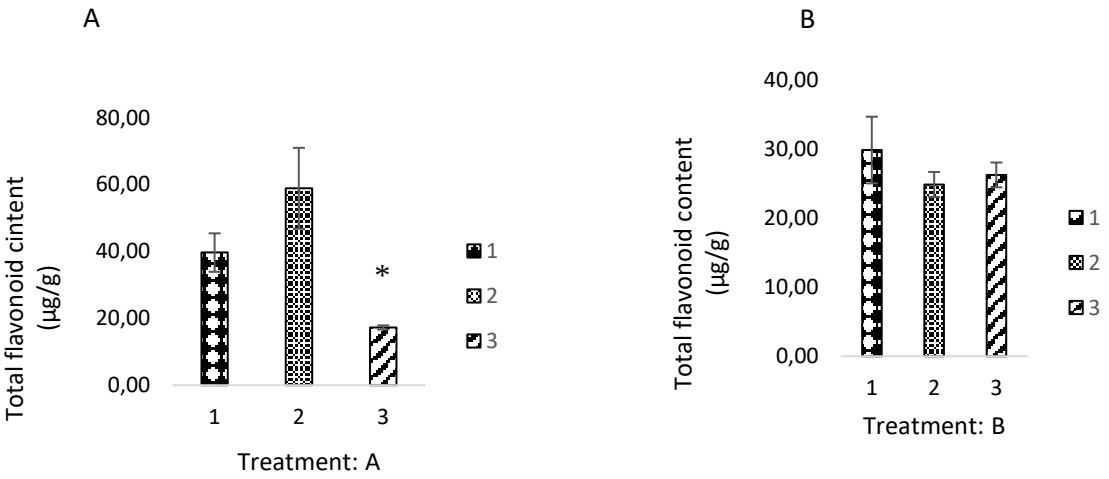


Fig 1 Total phenolic content in the leaves of oil palm. (A-F): Planting material A-F treated with different amount of fertilizer. Data are mean ± standard error, n=3. (* indicates significant difference in total phenolic content compared to control using t-test; $p<0.05$)

Total flavonoid contents of oil palm leaves

Fig 2 shows the total flavonoid content (TFC) of oil palm leaves from planting material A-F. The results showed that the highest TFC was obtained in the leaves of oil palm at T2 fertilizer level, and the lowest TFC was recorded for oil palm leaves treated at T3 fertilizer level in planting material A. TFC of T3 treated leaves differ significantly compared to T1. Another planting material that showed significant responses towards different levels of fertilizer was planting material F. The lowest TFC was recorded at T3 while the highest TFC was found in the leaves of plants supplied with fertilizer at T1. It can be concluded that the fertilizer at T2 and T3 had assisted the progeny to produce less flavonoid compound.



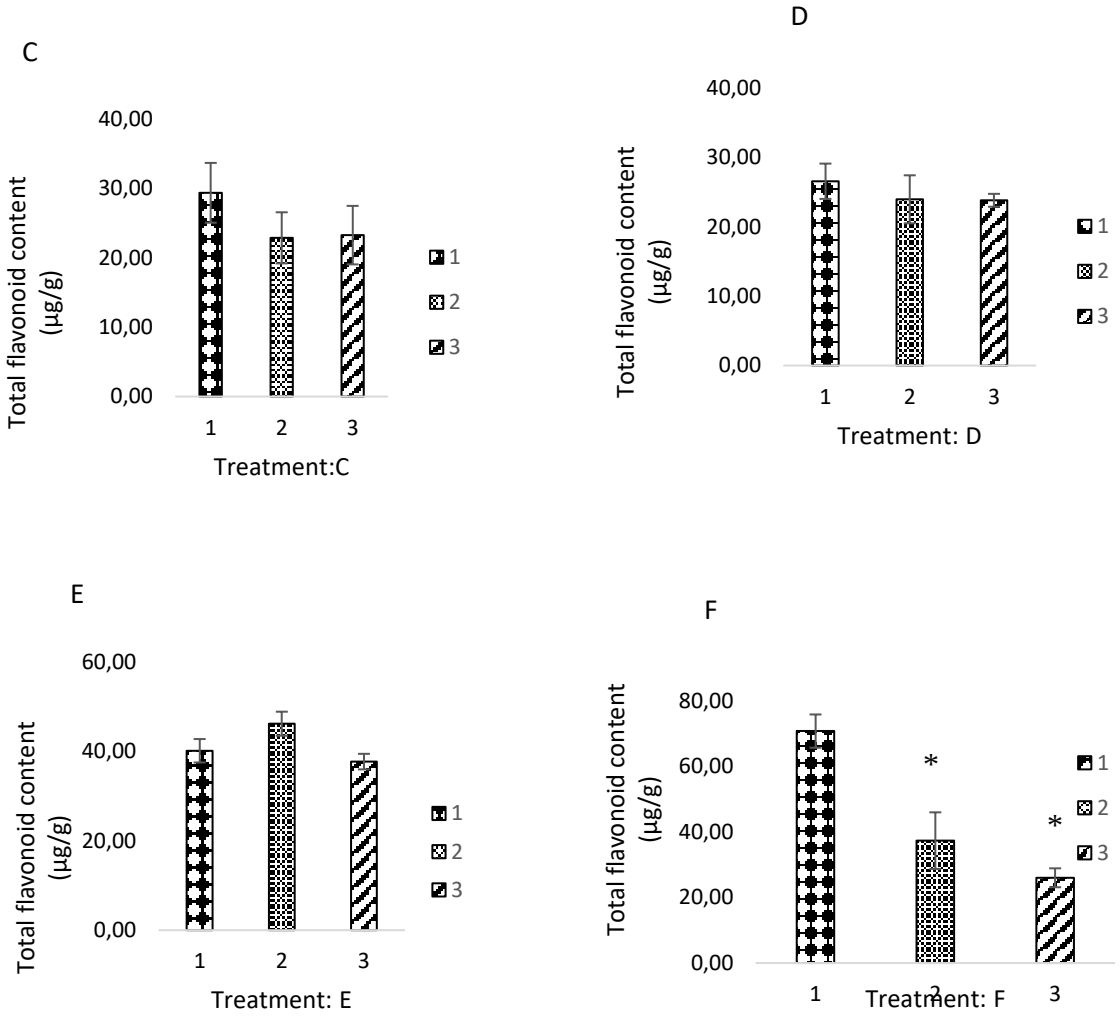


Fig 2 Total flavonoid content in the leaves of oil palm. (A-F): Planting material A-F treated with different amount of fertilizer. Data are mean ± standard error, n=3. (* indicates significant difference in total flavonoid content compared to control using t-test; $p<0.05$)

In this study, different amount of NK fertilizer supplied to oil palm trees had shown different responses in terms of total phenolic and flavonoid content of oil palm leaves, depending on the planting materials. Although nitrogen is an essential element for amino acids and construction of proteins, excessive amount of this nutrient in plant tissue can cause mineral toxicity and reduce physiological and phenological responses to the plant [17]. Similar to total phenolic content, several studies also reported that total flavonoid content decreased with excessive nitrogen uptake [18, 19, 20]. Lower flavonoid content

in plants that received high amount of nitrogen was observed previously in species such as apple trees and *Sesamum indicum* L. seeds [21, 22]. This trend was presence although not significantly observed in some planting materials with some non-synchronize trend at T2.

Conclusion

Different planting materials showed various responses in the total phenolic and flavonoid content when applied with different fertilizer treatments. For total phenolic content, planting material F showed the highest TPC at T1. Even T1 contained only basic amount of fertilizer, this treatment has led to high amount of phenolic content compared to T2 and T3. Similarly, T3 showed the lowest total phenolic content for planting material C. For total flavonoid content, T1 showed the highest total flavonoid content for planting material F, a similar trend to it's total phenolic content. Nonetheless, different trends of responses were recorded among the tested planting materials, hence total phenolic and flavonoid content in the leaves of different progenies vary greatly with NK fertilizer level.

Acknowledgements
The authors would like to thank Universiti Teknologi Malaysia for the support from Geran Universiti Penyelidikan (GUP Tier 1); Vote Number: Q.J130000.2545.13H92. We also thank Advance Agriecological Research (AAR) Sdn.Bhd for providing samples and advice related to the project.

Funding
This study is funded by Geran Universiti Penyelidikan (GUP Tier 1), Universiti Teknologi Malaysia (UTM): grant number: Q.J130000.2545.13H92.

References

1. Ahmad Kushairi, D, Malaysian Oil Palm Industry Performance 2016 and Prospects for 2017. Palm Oil Economic Review and Outlook Seminar 2017. http://www.mpob.gov.my/images/stories/pdf/2017/2017_Dr.KushairiPALMEROS2017.pdf. Retrieved on 31st October 2019.
2. Abass Almodares, R. T., C. Min, and F. Majid, The effect of nitrogen and potassium fertilizers on growth parameters and carbohydrate contents of sweet sorghum cultivars. Journal of Environmental Biology, 2008. 29(6): 849-852.
3. Tripler, C. E., S.S. Kaushal, G.E. Likens, and M. Todd Walter, Patterns in potassium dynamics in forest ecosystems. Ecology Letters, 2006. 9(4): 451-466.
4. Fageria, N. K., V.C. Baligar, and C.A. Jones, Growth and mineral nutrition of field crops. CRC Press, 2010.
5. Abdullah, N., and F. Sulaiman, The oil palm wastes in Malaysia. In Biomass now-sustainable growth and use. IntechOpen, 2013.

6. Mohamed, W. Z. and. M. Mohd Farid, Oil-palm by-products as feeds for livestock in Malaysia, In: International Palm Oil Congress (PIPOC 2011), 15 -17 Nov 2011, KLCC, Kuala Lumpur, Malaysia.
7. Han, N., and C. May, Determination of antioxidants in oil palm leaves (*Elaeis guineensis*). American Journal of Applied Sciences, 2010.7(9): 1243-1247.
8. Soundararajan, V. and S. Sreenivasan, Antioxidant activity of *Elaeis guineensis* leaf extract: An alternative nutraceutical approach in impeding aging. APCBEE Procedia, 2012. 2: 153-159.
9. Vijayarathna Soundararajan, S. S., Antioxidant activity of *Elaeis guineensis* leaf extract: An alternative nutraceutical approach in impeding aging. APCBEE Procedia, 2012.2: 153-159.
10. Lattanzio, V., Phenolic compounds: Introduction, In book: K.G. Ramawat and J.M. Me´rillon. Natural products, Chapter 50. Springer-Verlag Berlin Heidelberg. 2013. p.1543-1580.
11. Yin, N. G.S., S. Abdullah, and C.K. Phin, Phytochemical constituents from leaves of *Elaeis guineensis* and their antioxidant and antimicrobial activities. International Journal of Pharmacy and Pharmaceutical Science, 2013. 5(Suppl 4), 137-140.
12. Alothman M., R. Bhat, and A.A. Karim, Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry, 2009.115(3): 785-788.
13. Enujiugha, V. N., J.Y. Talabi, S.A. Malomo, and A.I, Olagunju, DPPH radical scavenging capacity of phenolic extracts from African yam bean (*Sphenostylis stenocarpa*). Food and Nutrition Sciences, 2012. 3(1): 7-13.
14. Waterhouse, A.L., Determination of total phenolics. In ERE Wrolstad (ed), Current Protocols in Food Analytical Chemistry. John Wiley & Sons, Inc., New York, 2001. pp. I1.1.1-I1.1.8.
15. Zou, Y., Y. Lu, D. Wei, Antioxidant activity of a flavonoid-rich extract of Hypericum perforatum L. *in vitro*. Journal of Agricultural Food Chemistry, 2004. 52: 5032-5039.
16. Argyropoulou, K., Salahas, G., Hela, D., and Papasavvas, A, Impact of nitrogen deficiency on biomass production, morphological and biochemical characteristics of sweet basil (Ocimum basilicum L.) plants, cultivated aeroponically. Agriculture and Food, 2015. 3: 32-42.
17. Sheikh, S., and Ishak, C. F., Effect of nitrogen fertilization on antioxidant activity of Mas cotek (Ficus deltoidea Jack). Journal of Medicinal Plants Studies, 2016. 4(4): 208-214.
18. Knobloch, K. H., Bast, G., and Berlin, J., Medium-and light-induced formation of serpentine and anthocyanins in cell suspension cultures of Catharanthus roseus. Phytochemistry, 1982. 21(3): 591-594.
19. Smoleń, S., and Sady, W., The effect of various nitrogen fertilization and foliar nutrition regimes on the concentrations of sugars, carotenoids and phenolic compounds in carrot (Daucus carota L.). Scientia Horticulturae, 2009. 120(3): 315-324.
20. Stewart, A. J., Chapman, W., Jenkins, G. I., Graham, I., Martin, T., and Crozier, A., The effect of nitrogen and phosphorus deficiency on flavonol accumulation in plant tissues. Plant, Cell and Environment, 2001. 24(11): 1189-1197.
21. Leser, C. and D. Treutter, Effect of nitrogen supply on growth content of phenolic compounds and pathogen (scab) resistance of apple trees. Physiologia Plantarum, 2005. 123: 49-56.
22. Elhanafi, L., M. Houhou, C. Rais, I. Mansouri, L. Elghadraoui, and H. Greche, Impact of excessive nitrogen fertilization on the biochemical quality, phenolic compounds, and antioxidant power of Sesamum indicum L seeds. Journal of Food Quality, 2019. Article ID 9428092, 1-6. https://doi.org/10.1155/2019/9428092.