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EFFECT OF ORGANIC AND MINERAL FERTILIZATION AND HUMIC ACID APPLICATION ON INCREASING THE FLAVONOID CONTENT IN *MORINGA OLEIFERA* LEAVES

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SUMMARY

The presented research carried out in the growing season of 2020 at the Department of Horticulture and Landscape, College of Agriculture, University of Anbar, Iraq, investigated the impact of mineral (chemical) and organic (birds) fertilization and foliar application of the humic acid on the active substances of the moringa (*Moringa oleifera)* plant leaves. The treatments included an organic fertilizer (300 g plant^{-1}), mineral fertilizer DAP (225 g plant^{-1}), and humic acid (3000 ppm). The experiment ran in a randomized complete block design (RCBD) with three replications. Results revealed that adding organic and mineral fertilizers and foliar application of humic acid affected and increased flavonoids significantly in moringa plant leaves, such as, six flavonoid components, i.e., catechin, quercetin, luteolin, rutin, apigenin, and kaempferol, amounting to 73.83, 21.51, 3.344, 20.55, 7.53, and 2.09 µg L^{-1}, respectively, compared with the control treatment (2.56, 3.22, 1.304, 2.25, 1.88, and 0.29 ppm, respectively).

Keyword: Moringa *Moringa oleifera* L., organic and mineral fertilizers, humic acid, flavonoids, leaf chemical composition

Key findings: The addition of organic and mineral fertilizer and foliar application of humic acid significantly affected the leaf chemical composition by increasing the active elements in the leaves of the moringa plant, such as various flavonoids, i.e., catechin, quercetin, luteolin, rutin, apigenin, and kaempferol.

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INTRODUCTION

The moringa (*Moringa oleifera* L.) tree is one of 13 species belonging to the genus, *Moringa* and the family Moringaceae. Mainly cultivated in African countries, especially Ethiopia, Kenya, and Sudan, it is also widely grown in the tropics. However, it is native to India but well known in Central Africa (Padayachee and Baijnath, 2012). *Moringa oleifera* is a fast-growing, drought-resistant tree, and its common names include moringa and drumstick tree. Moringa has many medical benefits, as its leaves are a source of antioxidants and a cancer treatment (Lamou *et al*., 2016). It also helps the treatment of asthma, bronchitis, eyes, and skin diseases because of its flavonoid contents (Ahmed *et al*., 2016).

The widespread cultivation of moringa is due to its young seed pods and leaves used as vegetables and for traditional herbal medicine; however, it lists an invasive species in several countries. The economic importance of moringa is mainly for its use as a food item because it contains high levels of carbohydrates, proteins, and minerals, such as, magnesium, potassium, iron, zinc, and phosphorus, which is rich, especially in its leaves (El-Sohaimy *et al*., 2015). Thus, it can benefit as a treatment for malnutrition diseases, especially in infants and pregnant women (Nalamwar *et al*., 2017). Moringa also serves in the production of biofuels because its seed oil has high levels of unsaturated fatty acids, such as, Omega3, which complies with all main specifications of the biofuel standards of the United States of America, Germany, and Europe (Padhi, 2016).

Other benefits of moringa also include water purification, as it works to pull pollutants from the water, such as, heavy metals and pesticides (Hendrawati *et al*., 2016; Jumaah *et al*., 2021). Decomposed organic fertilizers are among the primary factors that highly affect the growth of plants, as their addition improves vegetative growth and increases the yield significantly (Popov *et al*., 2022). Organic fertilizers also provide the plant with nutrients, build organic compounds, and enhance the progression of essential plant processes, which is evident in the growth of active substances (Verma *et al*., 2017; Mohammed *et al*., 2021).

In crop plants and fungi, the flavonoid contents are products of secondary metabolism. It has the distinct structure of polyphenols, and its biosynthesis occurs via the polypropanoid pathway, with the phenylalanine molecule it serves as its first initiator, as described in previous studies (Shanmugapriya *et al*., 2017). Findings of Sharayu and Asmita (2017) revealed that the flavonoids found in the moringa tree provided significant protection to the human body against environmental stresses resulting from heavy metal pollution, i.e., lead, which causes the dissolution of red blood cells.

With the medical significance of the active substances, in addition to the economic and environmental values of the moringa tree, with a few studies of it in Iraq, the timely research aimed to determine the effects of organic and mineral fertilizers and humic acid (HA) on the leaf content indicators of flavonoids in the moringa (*Moringa oleifera* L.) and to formulate the best fertilizer combination for better growth.

MATERIALS AND METHODS

Genetic material and procedure

The presented research sought to learn the effects of organic and mineral fertilization and foliar application of humic acid to increase the flavonoid contents in the *Moringa oleifera* plant leaves. The study began in the growing season of 2020 at the Department of Horticulture and Landscaping, College of Agriculture, University of Anbar, Iraq. The moringa seedlings came from one of the accredited private nurseries in Baghdad, Iraq, in the first week of January 2020, selecting 120 seedlings that grow homogeneously. Planting the seedlings in a plastic pot contained 15 kg of soil capacity. The added organic fertilizers measured 300 g plant^{-1}, while mineral fertilizer DAP was 225 g plant^{-1} and humic acid at 3000 ppm. Applying the treatments three times were as follows: the first after two weeks of planting, the second

Table 1. Details of the treatments used in the study.

No.	Treatments	Details
1	T1	Comparison transaction
2	T2	Organic fertilizer 40 Mgm ha^{-1}
3	T3	Nitrogen 400 kg ha^{-1} and phosphorus 450 kg ha^{-1} in the form of mineral fertilizer (DAP)
4	T4	Spray Humic acid at a concentration of 3000 mg lt^{-1}
5	T5	Organic fertilizer with mineral fertilizer
6	T6	Mineral fertilizer sprayed with Humic acid
7	T7	Organic fertilizer sprayed with Humic acid
8	T8	Organic fertilizer with mineral fertilizer and spraying with Humic acid

Studied Properties: Determination of some types of flavonoids using the HPLC device (mg L^{-1}).

after one month of the first incorporation, and the third application again after one month.

Experimental design and treatments

The experiment in a randomized complete block design (RCBD) had three blocks with 120 seedlings of the moringa plant. Fertilizer combinations (T1, T2, T3… T8) in the experiment consisted of each replication having eight treatments (Table 1). Each treatment included five seedlings (Al-Mohammad, 2013).

For various flavonoid content, the moringa leaf samples underwent assessment in the laboratory of the Department of Environment and Water, Ministry of Science and Technology Laboratories, Iraq, using a high-performance liquid chromatography (HPLC) instrument model (SYKAM) of German origin, as per the methodology by Zare *et al*. (2014). The carrier phase used methanol: distilled water: acetic acid (75:13:2), and a separation column of the type C18-ODS (25 cm*4.6 mm). Utilizing a UV detector was according to the UV wavelength - 360 nm. All the recorded data gained analysis and investigation according to the Genstat statistical program, with the arithmetic means further compared and separated using the least significant difference (LSD) test at the 5% probability.

RESULTS AND DISCUSSION

The various flavonoid contents examination in the moringa plant's leaf samples ran through HPLC, with the results detailed below.

Flavonoid Catechin content

In moringa leaf samples, the fertilizer and humic acid treatments revealed significant differences in the content of flavonoid catechin (Figure 1). The HPLC analysis indicated the superiority of treatment-T8 (organic and mineral fertilizers + humic acid) with the highest average content of flavonoid catechin (73.83 mg L^{-1}) compared with the control treatment-T1 (zero use of organic and mineral fertilizer + humic acid), which provided the least values of the said flavonoid content (2.56 mg L^{-1}).

Flavonoid Quercetin content

The HPLC analysis of the moringa leaf samples showed significant differences among all treatments (Figure 2). However, the highest content of quercetin flavonoid (21.51 mg L^{-1}) resulted in the treatment-T8 (use of organic and mineral fertilizer + humic acid) versus the control treatment-T1 (zero use of organic and mineral fertilizer + humic acid), which provided the lowest rate of the quercetin flavonoid (3.22 mg L^{-1}).

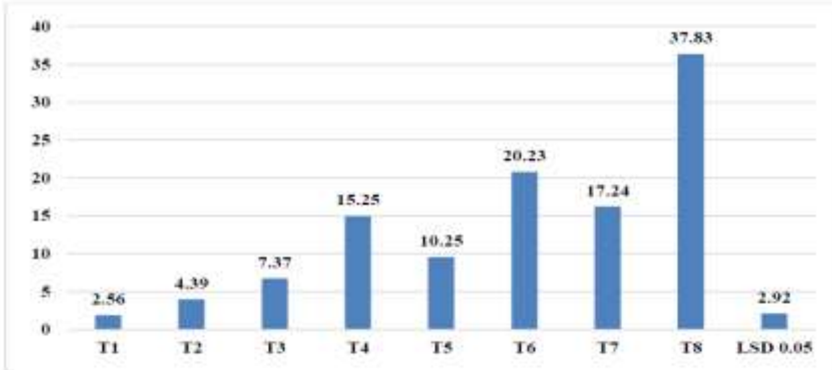

Figure 1. Influences of organic and mineral fertilization and foliar application of HA on the flavonoid Catechin content (mg L^{-1}) in the leaves of moringa plants.

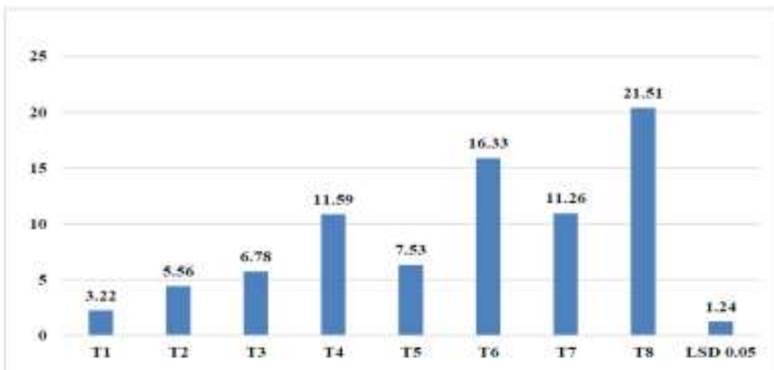

Figure 2. Influences of organic and mineral fertilization and foliar application of HA on the flavonoid Quercetin content (mg L^{-1}) in the leaves of moringa plants.

Flavonoid Luteolin content

The moringa leaf's sample analysis showed highly notable variances among the fertilizer treatments for flavonoid luteolin content (Figure 3). For flavonoid luteolin content in the Moringa leaves, the treatment-T8 (complete dose of organic and mineral fertilizer + humic acid) excelled over all other fertilizer treatments giving the maximum value (3.344 mg L^{-1}) compared with the control treatment-T1 with the lowest value (1.304 mg L-^{1}).

Flavonoid Rutin content

The flavonoid rutin content in moringa leaf had the analysis disclosing extremely substantial disparities due to various eight-fertilizer treatments (Figure 4). The T8 treatment (with a complete dose of organic and mineral fertilizers + humic acid) was significantly superior to all treatments giving the foremost value of flavonoid rutin (20.55 mg $^{-1}$) versus the control treatment-T1, having the minimum amount (2.25 mg L^{-1}).

Flavonoid Apigenin content

The analysis of moringa leaf samples with HPLC provided significant differences among the fertilizer treatments for flavonoid apigenin (Figure 5). The treatment-T8 led all treatments, with the highest rate for the flavonoid apigenin (7.53 mg L^{-1}) compared with the control treatment-T1, which gave the lowest value of the said flavonoid (1.88 mg L^{-1}).

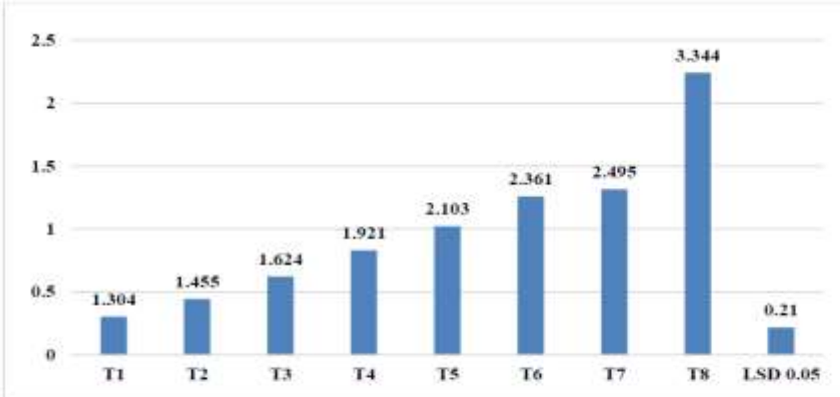

Figure 3. Influences of organic and mineral fertilization and foliar application of HA on the flavonoid Luteolin content (mg L^{-1}) in the leaves of moringa plants.

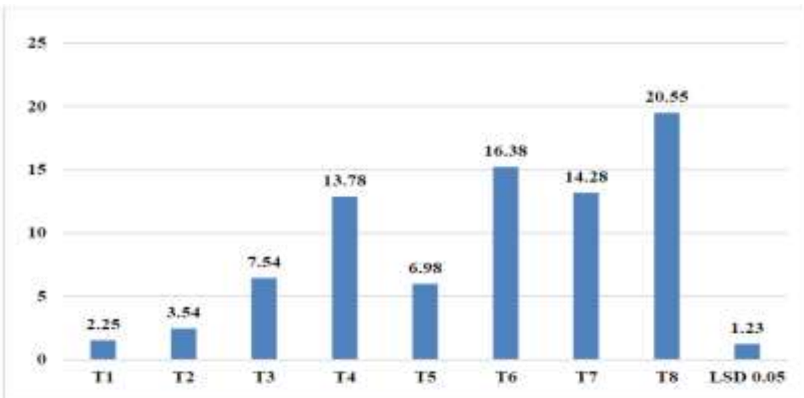

Figure 4. Influences of organic and mineral fertilization and foliar application of HA on the flavonoid Rutin content (mg L^{-1}) in the leaves of moringa plants.

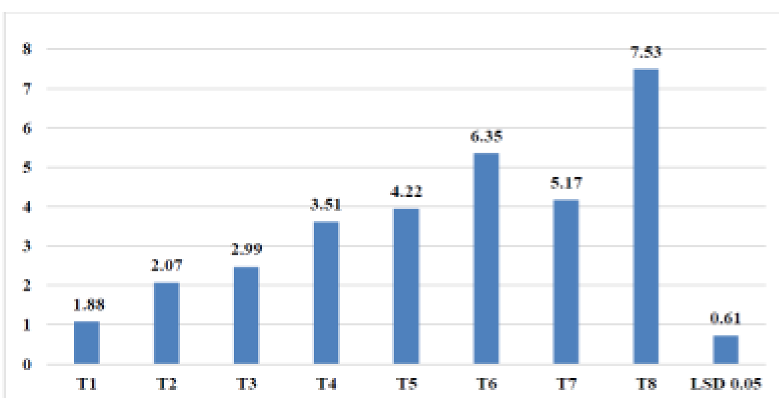

Figure 5. Influences of organic and mineral fertilization and foliar application of HA on the flavonoid Apigenin content (mg L^{-1}) in the leaves of moringa plants.

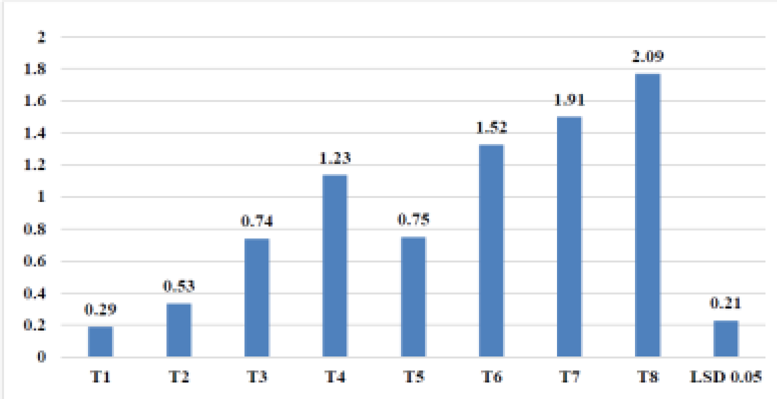

Figure 6. Influences of organic and mineral fertilization and foliar application of HA on the flavonoid Kaempferol content (mg L^{-1}) in the leaves of moringa plants.

Flavonoid Kaempferol content

The moringa leaf samples assessment recorded highly sizable dissimilarities among the fertilizer treatments for kaempferol flavonoid content (Figure 6). For leaf kaempferol flavonoid content, the treatment-T8 (complete dose of organic and mineral fertilizers + humic acid) resulted in the highest value (2.09 mg L^{-1}); however, it did not differ significantly from treatment-T7 (organic fertilizer + humic acid only) amounting to 1.91 mg L^{-1}. The control treatment provided the lowest value of kaempferol flavonoid (0.29 mg L^{-1}).

The HPLC analysis of the moringa leaf samples obtained from various fertilizer treatments revealed that the treatment-T8 (complete dose of organic and mineral fertilizers + humic acid) showed significant superiority for all six flavonoid content types. It may be due to the cumulative effect of the used fertilizers comprising organic and mineral fertilizers and humic acid, as mineral fertilizers are an indispensable source of foremost elements, i.e., nitrogen and phosphorus (IPNI, 2014). Nitrogen and phosphorus are vital and essential nutrients involved in the construction of proteins, nucleic acids, various membranes, and energy production, as the nitrogen element is one of the crucial elements in forming flowering branches and increasing the number of flowers and their knot percentage (Karim and Saad, 2015). The chief elements, viz., nitrogen and phosphate, obtained from mineral fertilizers, led to an increase in the content of the active substance and some mineral components in *Althae rose* L. plants (Al-Mohammadi, 2011).

In the celery plant, some mineral fertilizers enhanced the active substance, in addition to the significant increase in several crucial nutrients, such as, phosphorus and potassium, and the active substances of the leaves (Saeed, 2015). Al-Mohammed (2013) also reported that the nitrogen fertilizer use in the *Borago officinalis* L. plants significantly influenced the germination content of active substances and mineral elements. Hassan and Majeed's (2016) findings enunciated that adding mineral fertilizer to the *Salvia officinalis* plants showed the highest values of flavonoid contents at the level of 200 kg ha^{-1}. Past studies revealed that incorporating mineral fertilizers at different concentrations increased the leaf content of phenolic compounds, nitrogen, phosphorus, and potassium in the *Calotropis procera* plants (Al-Jawthari *et al*., 2016).

In Crataegus plants, the active substance increased by adding 500 g $seedlings^{-1}$ of organic fertilizers (Al-Hadethi, 2019). Al-Mohammadi and Ahmed (2016) found that the use of a mixture of some organic and mineral fertilizers escalated the leaf content of active substances and some mineral elements in the *Datura stramonium* L. Hassan (2010) also discovered that supplementing organic fertilizers enhanced the availability of some nutrients in the soil, yield, and quality of watermelon fruits.

Humic acid can act as a growth stimulant to improve plant productivity and quality standards under biotic and abiotic stress conditions. It can also promote plant growth and uptake of nutrients by modifying the soil environment. Humic acid also increases the uptake of calcium, potassium, nitrogen, magnesium, and phosphorus. By chelating various nutrients to be more accessible to the plant, several studies have shown that using humic acid enhanced growth and improved the quality in different cultured crops (Fahramand *et al*., 2014).

Past studies revealed that foliar application of humic acid had a vital effect on the growth and quality of cherry plants (Popescu and Popescu, 2018). Merwad (2017) also found that humic acid use showed significant results for vegetative characteristics in the Sudan herb plants. Ayoub's (2018) findings indicated that using humic acid gave positive results for most of the vegetative growth characteristics in the black seed plant. Likewise, results from Aminifard *et al*. (2012) exhibited an increase in the antioxidants and phenols content of the pepper plants after treatment with humic acid.

CONCLUSIONS

The research pursued to investigate the impact of mineral (chemical) and organic (animal) fertilization and foliar application of nutrients (humic acid) on the active substances of the moringa (*Moringa oleifera)* plant leaves. The organic fertilizer, mineral fertilizer DAP, and humic acid applications in three batches signified that adding organic and mineral fertilizers and foliar application of humic acid affected and increased the active elements in moringa plant leaves significantly compared with the control treatment.

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