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Chemical response of chrysanthemum plants to organic materials and tryptophan

Nour Yar'ab Ahmad **Thamer Abdullah zahwan**

1, 2 Horticulture Department, College of Agriculture, Tikrit University

noor.y.a510@st.tu.edu.iq dr_thamirzahwan@yahoo.com

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ABSTRACT

A field experiment was carried out in the Horticultural Station at the department of Horticulture and Landscape, Tikrit University, during fall of 2023. The experiment studied the chemical response of chrysanthemum plants to organic materials consisting of 1:2 biochar and vermicompost (O) (0, 2, 4, or 6 %) and amino acid tryptophan (A) sprayed at levels (0, 0.25, 0.50, or 0.75 mg L^{-1}). The results showed that there were significant effects of the two factors on the active ingredient Kaempferol. There was significant superiority of treatments T_{13}, T_{14}, T_{15} and T_{16} (6% of the mix). These treatments recorded the highest values of Kaempferol (35.44, 35.60, 35.40 and 35.22 mg L^{-1} respectively). Active ingredient, apigenin was significantly superior under the treatments T_{13}, T_{14}, T_{15} and T_{16} which received 6% of the mix. These treatments recorded the highest values (18.40, 18.22, 18.60 and 18.32 mg L^{-1}, respectively) of this parameter. The plant height and number of branches were superior (20.08 cm, 15.75 branches) under the treatment O3 organic mix 6%.

Interaction of the two factors was significantly superior in Kaempferol at the treatment T_{14} (6% organic mix with 0.25 mg tryptophan). The treatment recorded the highest value (35.60 mg. L^{-1}). On the other hand, apigenin was significantly superior (18.60 mg. L^{-1}) at treatment T15 (6% organic mix with 0. 50 mg. L^{-1} of tryptophan). Plant height was the significantly superior (21.75 cm) under the interaction treatment of O3A1. However, number of branches was significantly superior at the interaction treatments O3A0, O3A1, O3A2 and O3A3. They recorded 16.33, 16.33, 15.33 and 15 branch plant-1 respectively.

الإستجابة الكيميائية لنبات الإقحوان للمواد العضوية والتربتوفان

نور يعرب أحمد[١] **ثامر عبد الله زهوان[٢]**

[١]و[٢] قسم البستنة وهندسة الحدائق، كلية الزراعة، جامعة تكريت، العراق

الخلاصة

نفذت التجربة زراعة مكشوفة في المنشآت البستنية التابعة لقسم البستنة وهندسة الحدائق/ جامعة تكريت اثناء الموسم الخريفي ٢٠٢٣، تضمنت التجربة الاستجابة الكيميائية لنبات الاقحوان دراسة عاملين، كان العامل الأول عبارة عن خليط المواد العضوية المتكون من ١: ٢ من الفحم الحيوي مع الديدان الحمراء (ويرمز له O) وبأربع مستويات مختلفة هي (0 ,2 ,4 ,6 %)، إما العامل الثاني فكان الحامض الاميني التربتوفان (يرمز له A) بأربع مستويات هي (٠، ٠.٢٥، ٠.٥٠ و٠.٧٥ ملغم. لتر[-١]). أظهرت النتائج وجود تأثير معنوي للعاملين على المادة الفعالة Kaempferol. كان هناك تفوق معنوي للمعاملات T١٥, T١٤, T١٣ وT16 (٦ % للخليط) سجلت هذه المعاملات أعلى قيم Kaempferol (٣٥.٤٤، ٣٥.٦٠، ٣٥.٤٠ و ٣٥.٢٢ ملغم لتر[-١] على التوالي). تفوقت المادة الفعالة apigenin معنوياً تحت المعاملات T١٥, T١٤, T١٣ وT16 والتي حصلت على ٦

% من الخليط. سجلت هذه المعاملات أعلى القيم (١٨.٤٠، ١٨.٢٢، ١٨.٦٠ و ١٨.٣٢ ملغم لتر$^{-1}$، على التوالي) لهذه المعاملات. كان ارتفاع النبات وعدد الأفرع متفوقاً (٢٠.٠٨ سم، ١٥.٧٥ فرعاً) تحت المعاملة ٣O (الخليط العضوي ٦%)، كان التداخل بين العاملين متفوقاً معنوياً في Kaempferol عند المعاملة ١٤T (خليط عضوي ٦% مع ٠.٢٥ ملغم تربتوفان). سجلت المعاملة أعلى قيمة (٣٥.٦٠ ملغم.لتر$^{-1}$). من ناحية أخرى، كان apigenin متفوقاً معنوياً (١٨.٦٠ ملغم.لتر$^{-1}$) عند المعاملة ١٥T (٦% خليط عضوي مع ٠.٠٥٠ ملغم.لتر$^{-1}$ تربتوفان) كان ارتفاع النبات أعلى معنوياً (٢١.٧٥ سم) تحت معاملة التفاعل مع ١A٣O ومع ذلك، كان عدد الفروع متفوقاً معنوياً في معاملات التفاعل ٠A٣O، ١A٣O، ٢A٣O و٣A٣O وسجلوا ١٦.٣٣، ١٦.٣٣، ١٥.٣٣ و١٥ فرع نبات$^{-1}$ على التوالي.

الكلمات المفتاحية: نبات الأقحوان، المادة الفعالة، التربتوفان، صنف محلي، الفحم الحيوي.

INTRODUCTION

Chrysanthemum is an annual plant grows between 20-60 cm height. It is belonging to the family of Asteraceae, and found near warm and humid areas (Ashwlayan *et al*., 2018). The cultivation of the plant has spread in Middle Eastern countries, specifically Cyprus, Turkey, and Iran. In addition, its cultivation has also been observed in India and China on a larger scale (Gonçalves *et al*., 2018). However, the chrysanthemum has many species and is widely distributed globally. There are approximately 25 species of the genus *Calendula*, including *C. officinalis*, *C. arvensis*, *C. tripterocarpa*, *C. stellata*, and *C. suffruticose*, which are the most common (Fallahi *et al*., 2020). *Calendula officinalis* L. is considered one of the most studied species and has been used medicinally since the 12th century (Gu *et al*., 2022). Chrysanthemum plant has been used in various therapeutic applications because it contains many effective biologically active compounds (Ossipov *et al*., 2024). The plant consists of terpenoids, steroids, phenolic compounds, carotenoids, triterpenoids, essential oils, quinones, fatty acids, minerals, and saponins. Also, it contains carbohydrates, tocopherols, with alpha-cadinol (a sesquiterpenoid) as the main component (Pedram Rad et al., 2019; Lima *et al*., 2022). Among the compounds studied, the active compound kaempferol, which is a flavonoid glycoside of the glycone-type with various pharmacological benefits (Jeon *et al*., 2024). In addition, It is antioxidant, anti-inflammatory, antimicrobial, anti-cancer, cardioprotective, and neuroprotective. Moreover, It has anti-diabetic, anti-osteoporotic, anxiolytic, analgesic, antioxidant and anti-allergic properties (Metwally *et al*., 2024). The active compound, Apigenin, is an edible flavonoid found abundantly in many vegetables and fruits (Lotfi and Rassouli, 2024).

Apigenin has a wide range of pharmacological properties and beneficial health effects. This suggests promising therapeutic potential in a wide range of human chronic diseases, especially cancer, and cardiovascular disorders. blood (Aprotosoaie and Miron, 2023). Biochar is produced from plant material that contains a high percentage of carbon. Many applications of biochar include food and mineral processing, agriculture, and soil and water conditioning. Biochar is composed of 70% carbon, and the remainder consists of nitrogen, oxygen, and hydrogen. Heating the total biomass in the absence of oxygen leads to the production of biochar. 70% is also used in the agricultural industry to increase soil fertility, as it is mixed with the soil. This has increased the efficiency of agricultural land production, and contains fine pores that act as water conservation devices. Consequently, this reduces irrigation frequency and water requirements for agriculture. Then produces positive effects to the environment, and increases economy of the countries (Maniraj *et al*., 2023). Vermicomposting is a natural, environmentally friendly and cost-effective technology that is a wise choice for the bioconversion of organic waste into value-added by-products (Chowdhury *et al*., 2023). It also has several organic nutrition sources, which leads to production of vermicompost. Due to increasing global need for food, vermicompost use and production are becoming increasingly popular in agri-food and animal waste management (Bellitürk and Sundari, 2024). Tryptophan is an essential aromatic amino acid derived primarily from the feeding system to be used by the host for protein synthesis (Grifka-Walk *et al*., 2021). Tryptophan is an aromatic amino acid that is a basic protein subunit essential for plant growth (Wu *et al*., 2024). This study aimed to increase the chemical content of active ingredients and determine their effect on vegetative growth.

MATERIALS AND METHODS

The experiment was carried out in the horticultural facilities of the department of Horticulture and Landscape, Tikrit University, during fall of 2023. Chrysanthemum seeds were brought from local markets in Baghdad. On 10/9/2023, 2-3 seeds were sown in each planting hole of foam trays supplied with peat moss substrate. At the stage of 2-3 true leaves, the seedlings were separated into plastic pots (24 cm diameter) containing 3:1 (v\v) loam: peat moss. The substrate was sterilized using sun exposure.

Foam plates were placed underneath the pots to prevent leaching and save the moisture. 1 g of NPK fertilizer was added as a first batch, according to the need of ornamental plants per acre. One month later, the second batch (1 g) of the fertilizer was added. All necessary operations and agricultural practices were carried out as needed. The experiment included a study of two factors. The first one was a mixture of organic materials consisting of 1:2 biochar with red worms (O) at four levels (0, 2, 4, 6%). This was added at one time before transplanting the seedlings. The second factor, amino acid of tryptophan (A), was sprayed at four levels (0, 0.25, 0.50, and 0.75 $mg.L^{-1}$) after a month of the planting (11/9/2023). The spraying was performed at three times with two week interval. All the involved were sprayed till run off. The experiment was designed based on the randomized complete block design (RCBD) (Al-Rawi and Khalfallah, 1980) with three replications. Each replication included 16 experimental unit. There were 48 experimental units with 4 pots of one plant each. The total number of the plants was 193. All data were analyzed according to the statistical software SAS (2003). Means were compared according to the Duncan's multiple rang test at the probability of 0.05.

Studied characteristics:

1- Measurement of polyphenols and monophenols content in the crude extract was determined by the colorimetric method of aluminum chloride. Briefly, 50 µL of crude extract (1 mg/mL ethanol) was made up to 1 mL of methanol, mixed with 4 mL of distilled water. Then 0.3 mL of 5% $NaNO_2$ solution; 0.3 mL of 10% $AlCl_3$ solution was added after 5 minutes of incubation. The mixture was left for 6 minutes. Next, 2 mL of 1 mol L^{-1} NaOH solution was added, bringing the final volume of the mixture to 10 mL using double distilled water. Left for 15 min, absorbance was measured at 510 nm. The total flavonoid content was calculated from the calibration curve. The result was expressed as mg rutin equivalent per g of dry weight, according to Habibatni *et al.* (2017). Lutein was determined after grounding 10 g and mix it 40 ml of ethanol and butanone. The solution was filtered using Whatman No.1 filter paper. The solution was placed in a centrifuge at 10,000 rpm for 10 minutes. The aqueous phase was collected and stored at 4°C. The absorbance was measured and recorded using a spectrophotometer at a wavelength of 446 nm for either the standard or the samples.

Lutein concentration (µg\g)= $\frac{A\ X\ V\ (ml)\ X\ dilution}{€\ X\ W\ (gm)}$

Where: A= absorbance reading at 446nm, V= extract volume (ml), €=Lutein absorbance factor (2589), W=dry weight of the sample based on Hajare *et. al.*, (2013).

2- Plant height (cm $plant^{-1}$)

This was measured from soil surface level to the highest point of the plant using tape measure.

3- Number of branches (plant $branch^{-1}$)

According to the number of branches present for each plant.

The treatments were all represented as follows: 1=T1(O0A0), 2=T2(O0A1), 3=T3(O0A2), 4=T4(O0A3)(O1A0), 5=T5(O1A0), 6=T6(O1A1), 7=T7(01A2), 8=T8(O1A3), 9=T9(O2A0), 10=T10(O2A1), 11=T11(O2A2), 12=T12(O2A3) 13=T13(O3A0), 14=T14(O3A1), 15=T15(O3A2) and 16=T16(O3A3).

RESULT AND DISCUSSION

1- Effect on Kaempferol

The results in Figure (1) indicated that organic mix and tryptophan had significant effect on kaempferol. The effect was superior under the treatments T13, T14, T15 and T16. They received 6% of organic mix and recorded the highest values of 35.44, 35.60. 35.40 and 35.22 mg. L^{-1}

respectively. The treatments without organic mix (T1, T2, T3 and T4) had the lowest values (20.14, 21.04, 20.98 and 20.78 mg. L^{-1} respectively) of this parameter.

There was significant superiority observed under interaction treatment T14 (6% organic mix with 0.025 mg.L^{-1} tryptophan). It showed the highest value (35.60 mg. L^{-1}), while treatment T1 (control) gave the lowest value (20.14 mg. L^{-1}).

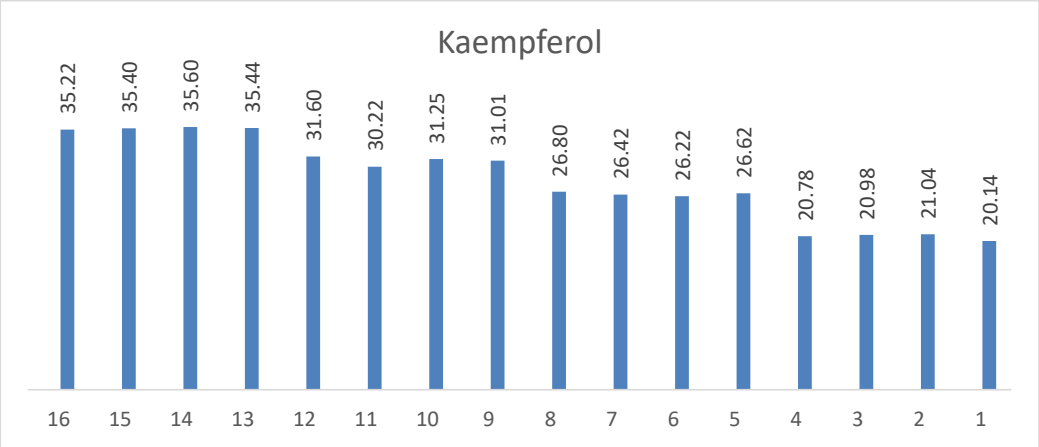

Figure 1. Effect of organic additives and tryptophan on Kaempferol in chrysanthemum plants.

2-Effect on Apigenin

Figure (2) showed the effect of organic additives and tryptophan on the active ingredient Apigenin. This parameter was significantly affected under the treatments T13, T14, T15 and T16 (18.40, 18.22. 18.60 and 18.32 mg L-1 respectively). On the hand, the treatments T1, T2, T3 and T4 (without organic additives) had the lowest values (6.15, 6.22, 6.12 and 6.22 mg. L-1 respectively) of this active ingredient.

The interaction between organic material and the amino acid had significant superiority. The treatment T15 (6% organic materials with 0.050 mg.L^{-1} tryptophan) recorded the highest value (18.60 mg.L^{-1}). However, the treatment T3 had the lowest value (6.12 mg L-1). The results in Figures (1 and 2) showed an increase in the active compounds in the plant. The reason of that may be because organic materials provided sufficient nutrients to the soil to meet the plant needs (Salehi *et al*., 2017). There was also a strong relationship between the levels of macronutrients in the soil (i.e., nitrogen, phosphorus, and potassium) and phenols, flavonoids, and active compounds (Aina *et al.*, 2019). Adding organic fertilizers enhanced the synthesis of phenols, flavonoids, and the content of active compounds to a greater level (Muscolo *et al.*, 2020). The reason for this may be attributed to biochar because biochar is an organic substance (Borthakur *et al*., 2019). This material improved the natural and chemical properties of the soil and increase ability to retain water and nutrients (Somerville *et al*., 2020). It may also be attributed to vermicompost, where it contains macro- and microelements necessary for plant growth. This fertilizer has a porous structure, good aeration, and high ability to retain water and microbial influence (Yılmaz *et al*., 2017). It improved plant growth because it is rich in potassium, sodium, chloride, sodium nitrate, calcium, and magnesium. Thus, the active compounds such as Kaempferol and Apigeni were increased. These results were consistent with Regmi *et. al.* (2023) on varieties of *viola cornuta*.

The results in Figures (1 and 2) showed an increase in active compounds of the plant under the addition of tryptophan. The reason of this may be attributed to stimulate physiological and chemical processes in the plant (Baqir *et al*., 2019). The amino acid used as plant stimulant, positively affected the quality of crops. Tryptophan is an important bio-stimulant that increased plant productivity (Rosa *et al.*, 2023). The reason for this may be attributed to the fact that tryptophan improved many biochemical procedures by influencing plant growth, differentiation. It also enhanced plant ability to absorb nutrients and water (Sadak and Ramadan, 2021). This amino acid can be applied to the soil, used as a foliar spray, and used in seed preparation (Mustafa *et al*., 2018). Recent studies indicated that foliar spraying of tryptophan improved growth and productivity of different plant species (Baqir and AL-Naqeeb, 2019).

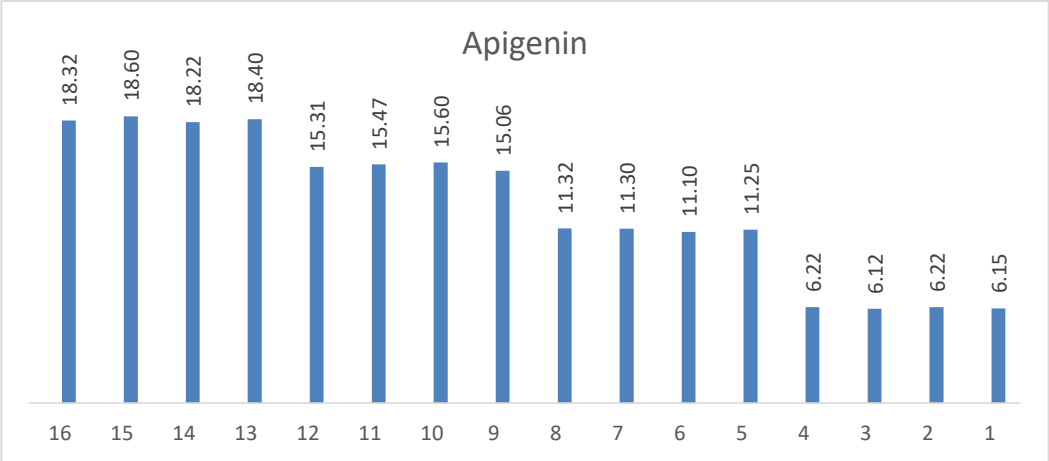

Figure 2. Effect of organic additives and tryptophan on Apigenin in chrysanthemum plants.

3- Plant height (cm plant^{-1})

The results of Table 1 showed that there were significant differences in the plant height under different treatments. There was significant superiority of O3 treatment in plant height (20.08 cm) compared to O_0 treatment (8.59 cm). In contrast, there was no significant difference of this characteristic between the treatments of the amino acid tryptophan.

Interaction between organic mixture and tryptophan had significant superiority under the treatment O_3A_1. This treatment recorded the highest value (21.75 cm), while the treatments O_0A_0, O_0A_1, O_0A_2 and O_0A_3 recorded the lowest (7.86, 8.93, 8.83 and 8.74 cm, respectively).

Table 1. Effect of organic additives and tryptophan on plant height (cm) in chrysanthemum plants.

treatments	A0 (0)	A1	A2	A3	Organic materials
O0	**7.86 e**	**8.93 e**	**8.83 e**	**8.74 e**	**8.59 d**
O1	**12.81 d**	**12.06 d**	**12.2 d**	**11.83 d**	**12.22 c**
O2	**15.07 c**	**13.63 cd**	**13.69 cd**	**15 c**	**14.35 b**
O3	**20.02 b**	**21.75 a**	**18.99 b**	**19.54 b**	**20.08 a**
Means of tryptophan	**13.94 a**	**14.09 a**	**13.43 a**	**13.78 a**	

* The averages, which take the same character for the two study factors and for the interaction between them, do not differ significantly from each other at the probability level (0.05) according to Duncan's multinomial test.

4-Number of branches (plant branch^{-1})

Results of Table 2. Indicated that there were significant differences in the number of branches between treatments. There was significant superiority of this parameter under O3 treatment. It gave the highest value (15.75 plant branches-1) compared to the lowest value (3.25 plant branches-1) under O_0 treatment. On the other hand, there were no significant differences under the treatments of tryptophan.

The interaction between organic materials and the amino acid was significant. There was superiority of the treatments O_3A_0, O_3A_1, O_3A_2 and O_3A_3, recording the highest values (16.33, 16.33, 15.33 and 15 plant-1, respectively). The treatments O_0A_1, O_0A_2 and O_0A_3 recorded the lowest values (2.67, 2.67 and 3.33 plant-1 branches, respectively).

Table 2. Effect of organic additives and tryptophan on number of branches (plant branch^{-1}) in chrysanthemum plants.

Treatments	A0	A1	A2	A3	organic materials
O0	4.33 ef	2.67 f	2.67 f	3.33 f	3.25 d
O1	6.33 cd	6 cde	5.67 de	6 cde	6.00 c
O2	8.67 b	7.67 bc	8.33 b	9 b	8.42 b
O3	16.33 a	16.33 a	15.33 a	15 a	15.75 a
Mean of tryptophan	8.92 a	8.17 a	8.00 a	8.33 a	

* The averages, which take the same character for the two study factors and for the interaction between them, do not differ significantly from each other at the probability level (0.05) according to Duncan's multinomial test.

The tables (1 and 2) indicated that the increase in vegetative growth characteristics can be because of the organic mix used. Plant growth and development depends on raw materials such as seeds, cuttings, water absorption and nutrients, photosynthesis in light, and required temperature. Applying biochar at specific levels may enhance seed germination and growth. It may also affect plant hormons, increase photosynthesis, and improve pigments averages. Consequently, properties of gas exchange through the leaves are improved (Zulfiqar *et al*. (2022). The results agreed with Yang and Zhang (2022) about increasing the chlorophyll content in *Centaurea cyanus* L. in current study, there was an improvement in the characteristics of plant height, number of branches. This was also consistant with Zulfiqar *et al*. (2021) who reported an improvement in growth characteristics of *Alpinia zerumbet*. Another reason can be the direct positive effect of vermicompost linked to increase nutrient availability and stimulate plant growth (Ievinsh, 2020). This induced plant growth, increased number of branches, leaf area, and chlorophyll content. This result was consistent with Alvarez *et. al*. (2019) in their study on petunia and pelargonium plants. It was also consistent with Yan *et al*. (2023) on types of ornamental plants.

Conclusions

The mixture of natural additives has achieved a significant increase in the vegetative growth qualities of the Chrysanthemum plant. The presence of an increase in the concentration of the active substance in the Chrysanthemum plant as a result of the mixture of natural additives. The presence of a moral improvement when treating tryptophan for most of the studied qualities. The overlap between natural additives and amino acid tryptophan gave high efficiency in most of the qualities of vegetative growth and all the qualities of the active substance of the Chrysanthemum plant.

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