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Effect of Arginine and GA_3 on Some Qualitative Traits and Active Ingredients of *Matricaria chomomilla* L.

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Abstract

The experiment was carried out in the one fields of Saba Abcar area - north of Baghdad during winter season of 2022-2023 to study the effect of arginine and gibberellin acid on the some qualitative traits and active ingredients of chamomile plant. The experiment was designed according to randomized complete block design at three replications, the first factor included the spraying of three concentrations of arginine (0, 50 and 150 mg L^{-1}), whereas the second factor included the spraying of three concentrations of GA_3 (0, 100 and 200 mg L^{-1}). The results showed that the spraying of arginine at a 50 mg L^{-1} was significantly superiority in the chlorophyll index (26.63 SPAD), carbohydrates percentage (50.38%) and volatile oil yield (13.84 Kg ha^{-1}), while the spraying of arginine at a 150 mg L^{-1} was significantly superiority in the protein percentage (10.18%). Also, the results revealed that the spraying of GA_3 at 100 mg L^{-1} was significantly superiority in the chlorophyll index (24.90 SPAD), carbohydrates percentage (50.79%) and volatile oil yield (16.30 Kg ha^{-1}), whereas the spraying of arginine at a 200 mg L^{-1} was significantly superiority in the protein percentage (9.83%). The interaction between studied factors had significant effect on the all studied traits. Further, the results of HPLC analysis showed a difference in the percentages of the active ingredients of chamomile volatile oil.

Keywords: Chamomile, amino acids, growth stimulants, PGR, HPLC.

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تأثير الأرجنين وحامض الجبريلين في بعض الصفات النوعية والمركبات الفعالة لنبات البابونج *Matricaria chomomilla* L.

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الخلاصة

نفذت تجربة حقلية في أحد حقول منطقة سبع أبكار الواقعة شمال بغداد خلال الموسم الشتوي لعام 2022-2023 لمعرفة تأثير الأرجنين وحامض الجبريلين في بعض الصفات النوعية والمركبات الفعالة لنبات البابونج. نفذت التجربة بتصميم القطاعات الكاملة المعشاة وبثلاثة مكررات, تضمن العامل الأول رش ثلاثة تراكيز من الأرجنين (0 و50 و150 ملغم لتر$^{-1}$), بينما تضمن العامل الثاني رش ثلاثة تراكيز من حامض الجبريلين (0 و100 و200 ملغم لتر$^{-1}$). أظهرت النتائج أن رش الأرجنين بالتركيز 50 ملغم لتر$^{-1}$ تفوق معنويا بأعلى متوسط لدليل الكلوروفيل (26.63 سباد) ونسبة الكربوهيدرات (50.38%) حاصل الزيت الطيار (13.84 كغم ه$^{-1}$), في حين تفوق رش الارجنين بالتركيز 150 ملغم لتر$^{-1}$ معنويا بأعلى نسبة للبروتين (10.18%). كذلك أظهرت النتائج أن رش حامض الجبريلين بالتركيز 100 ملغم لتر$^{-1}$ تفوق معنويا بأعلى متوسط لدليل الكلوروفيل (24.90 سباد) ونسبة الكربوهيدرات (50.79%) حاصل الزيت الطيار (16.30 كغم ه$^{-1}$), في حين تفوق رش حامض الجبريلين بالتركيز 200 ملغم لتر$^{-1}$ معنويا بأعلى نسبة للبروتين (9.83%). كان للتداخل بين عاملي الدراسة تأثير معنويا في أغلب الصفات المدروسة. من جهة أخرى, أظهرت نتائج التحليل الكروماتوغرافي السائل ذي الأداء العالي (HPLC) وجود اختلاف في نسب المكونات الفعالة للزيت الطيار في البابونج.

الكلمات المفتاحية: البابونج، الأحماض الأمينية, محفزات النمو, منظمات النمو النباتية، HPLC.

Introduction

The world has witnessed a development in the field of medicines, which has been directed to the use of medicinal plants largely due to the lack of side effects, as well as the quality and quantity of active ingredients present in the plants [1].

Chamomile is one of the very important floral medicinal plants because it contains volatile oils[2].Also, it contains many antioxidants, so it was used in the treatment of cancerous tumors or at least reducing them [3]. Chamomile oil contains many active ingredients such as blue camazoline, which enters into pharmaceutical uses [4, 5] Also, chamomile contains flavonoids and indomethacin, which are anti-inflammatory and tumor growth inhibitory substances [6].

Arginine acid is one of the amino acids found in the plant proteins, which has a many roles in the physiological process which occur in plant tissue, as it stimulates cells division and delays aging [7]. it is also used in the oxidation of polyamines and the synthesis of nitric polyamines [8]. Also, its equip the plant with energy to perform vital functions [9, 10]. Arginine improves

the efficiency of water use and plays a role in carbohydrate metabolism and photosynthesis [11].

Gibberellin is one of the important and effective plant growth regulators in the growth of the plants. It has a role in the processes of cell division and elongation in the plant [12]. It is found naturally on the developing meristem tic tissues. Gibberellin is involved in the biosynthesis of proteins and enzymes, which has a role in the process of photosynthesis, and it turned out its effect on plants that contain oils because it improves the quality and quantity of oil in plants [13], as well as the effect of gibberellin on vegetative indicators [14]. Therefore, this research was carried out to study the effect of arginine and gibberellin acid on the some qualitative traits and active ingredients of chamomile plant.

Materials and Methods

The experiment was carried out in the one fields of Baghdad governorate during winter season of 2022-2023 to study the effect of arginine and gibberellin acid on the some qualitative traits and active ingredients of chamomile plant.

The experiment was designed according to randomized complete block design at three replications, the first factor included the spraying of three concentrations of arginine (0, 50 and 150 mg L^{-1}), whereas the second factor included the spraying of three concentrations of GA_3 (0, 100 and 200 mg L^{-1}). The spraying was carried out at a 4 leaves stage in the early morning using a 10 liter dorsal sprinkler until the leaves were completely wet.

Soil management were conducted, and then the experiment land was divided into 27 experimental units, each experimental unit included 5 rows, 25 cm apart. The seeds of chamomile were sown on the 5 November 2020 at a seeding rate of 12 Kg ha^{-1}. Crop management was carried out as needed.

Studied traits

Chlorophyll index (SPAD): It was calculated before flowering stage using a SPAD device.

The percentage of carbohydrates (%): It was calculated according to Herbert et al., (15) method.

The percentage of protein (%):It was calculated according to the equation below (16):

$$\textbf{Protein (\%) = Nitrogen (\%) × 6.25}$$

Volatile oil yield (Kg ha^{-1}): It was calculated by multiplying the percentage of volatile oil by the yield of dried flowers.

Estimation and diagnosis of the quantity and quality of active ingredients in chamomile volatile oil Using HPLC

The qualitative detection of active ingredients was performed by comparing the retention time of each compounds in the crude with the retention time in the standard compound, and then the concentrations of the active ingredients were calculated according to the equation below (17):

$$\textbf{Concentration of compounds in the crude} = \frac{\textbf{Ac}}{\textbf{As}} \times \textbf{C} \times \textbf{D}$$

As:

Ac = Area of the active ingredient in the crude

As = Area of the active ingredient in the standard compound

C = Concentration of standard compound

D = Number of dilution times

Results and Discussion

The results of (Table 1) show that there was significant effect of spraying of arginine on the chlorophyll index, as the spraying of arginine at a 50 mg L^{-1} gave a highest average (26.63 SPAD) compared with control treatment which gave a lowest average (17.28 SPAD).The reason of increase when spraying of arginine at a 50 mg L^{-1} may be due to its role in stimulating plant growth and increasing its ability to absorb nutrients, including those that enter into the synthesis of chlorophyll, as well as, the amino acids are a source of nitrogen, which is important element in the growth and construction [18].

Also, the results of (Table 1) reveal that there was significant effect of spraying of GA_3 on the chlorophyll index, as the spraying of GA_3 at a 100 mg L^{-1} achieved a highest average (24.90 SPAD) compared with control treatment which achieved a lowest average (19.24 SPAD). The reason of increase the chlorophyll index when spraying of GA_3 could be attributed due to its effect on cell divisions that occur in plastids [19].

The interaction between studied factors had significant effect on this traits (Table 1), as the spraying of arginine at a 50 mg L^{-1} with spraying of GA_3 at a 200 mg L^{-1} recorded a highest

value (27.56 SPAD) with non significant different with spraying of arginine at same concentration with spraying of GA_3 at a 100 mg L^{-1} (27.24 SPAD) and spraying of arginine at 150 mg L^{-1} with spraying of GA_3 at a 100 and 200 mg L^{-1} (25.66 and 26.19 SPAD) respectively, whereas the control treatment recorded a lowest value (10.45 SPAD).

Table 1: The effect of spraying of arginine and GA_3 and their interaction on the chlorophyll index (SPAD)

Arginine conc. (mg L^{-1})	GA_3 conc. (mg L^{-1})			Average
	0	100	200	
0	10.45	21.78	19.60	17.28
50	25.10	27.24	27.56	26.63
150	22.18	25.66	26.19	24.63
Average	19.24	24.90	24.54	
lsd .05	**Arginine conc. = 2.44**			
	GA_3 conc. = 2.44			
	Interaction = 4.88			

The results of (Table 2) indicate that the spraying of arginine at a 50 mg L^{-1} was significantly superiority and gave a highest carbohydrates percentage (50.38%) compared with control treatment which gave a lowest percentage (41.96%). This increase may be due to the direct or indirect effect of amino acids on the biochemical processes that occur in the plant which was positively reflected on increase the chlorophyll index (Table 1) and enhance the photosynthesis processes and their metabolic products, including carbohydrates [20].

Also, the results of (Table 2) show that the spraying of GA_3 at a 100 mg L^{-1} was significantly superiority and achieved a highest carbohydrates percentage (50.79%) compared with control treatment which achieved a lowest percentage (40.99%). The reason of increase the reason of increase could be due to the role of gibberellin acid in stimulating plant growth and increasing the photosynthesis rate, which led to an increase the chlorophyll index (Table 1) and then increase the accumulation of dry matter. The interaction between studied factors had significant effect on this traits (Table 2), as the spraying of arginine at a 50 mg L^{-1} with spraying of GA_3 at a 200 mg L^{-1} recorded a highest value (56.01%) with non significant different with spraying of arginine at same concentration with spraying of GA_3 at a 100 mg L^{-1} (52.76%) and spraying of arginine at 150 mg L^{-1} with spraying of GA_3 at a 100 mg L^{-1} (54.49%), while the control treatment recorded a lowest value (36.16%).

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Table 2: The effect of spraying of arginine and GA_3 and their interaction on the carbohydrates percentage (%)

Arginine conc. (mg L^{-1})	GA_3 conc. (mg L^{-1})			Average
	0	100	200	
0	36.16	45.14	44.57	41.96
50	42.36	52.76	56.01	50.38
150	44.46	54.49	46.63	48.52
Average	40.99	50.79	49.07	
lsd .05	**Arginine conc. = 2.82**			
	GA_3 conc. = 2.82			
	Interaction = 4.22			

The results of (Table 3) reveal that the spraying of arginine at a 150 mg L^{-1} was significantly superiority and gave a highest protein percentage (10.18%) compared with control treatment which gave a lowest percentage (7.44%). The reason of increase could be due to the role of amino acid in encourage plant growth, as amino acids are one of the important and essential sources of nitrogen, which in turn is mainly in the synthesis of protein and enzymes [18, 21].

Also, the results of (Table 3) show that the spraying of GA_3 at a 200 mg L^{-1} was significantly superiority and achieved a highest percentage (9.83%) compared with control treatment which achieved a lowest percentage (8.19%). The reason of increase may due to the role of gibberellin acid in stimulate of vegetative growth of the plant which led to an increase the percentage of protein [14, 22],

The interaction between studied factors had significant effect on this traits (Table 3), as the spraying of arginine at a 150 mg L^{-1} with spraying of GA_3 at a 200 mg L^{-1} recorded a highest value (11.13%), whereas the control treatment recorded a lowest value (6.39%).

Table 3: The effect of spraying of arginine and GA_3 and their interaction on the proteins percentage (%)

Arginine conc. (mg L^{-1})	GA_3 conc. (mg L^{-1})			Average
	0	100	200	
0	6.39	7.61	8.32	7.44
50	7.98	10.01	10.05	9.34
150	10.22	9.19	11.13	10.18
Average	8.19	8.93	9.83	
lsd .05	**Arginine conc. = 0.49**			
	GA_3 conc. = 0.49			
	Interaction = 0.86			

According to the results of (Table 4), the spraying of arginine at a 50 mg L^{-1} was significantly superiority and gave a highest volatile oil yield (13.84 Kg ha^{-1}) compared with control treatment which gave a lowest percentage (9.10 Kg ha^{-1}). The reason of increase may be due to the effect of arginine on the physiological processes that occur in the plant, as it is a source of carbon and energy that increases photosynthesis reactions and their metabolic products, including essential oil [23].

Regarding of GA_3 concentrations, the results of (Table 3) reveal that the spraying of GA_3 at a 100 mg L^{-1} was significantly superiority and achieved a highest volatile oil yield (16.30 Kg ha^{-1}) compared with control treatment which achieved a lowest percentage (6.36 Kg ha^{-1}).The reason of superiority in this trait when spraying of GA_3 may be due to its role in stimulating growth and then increasing the production of metabolic compounds, including the compounds that are involved in the synthesis of essential oil in plants [24].

The interaction between studied factors had significant effect on this traits (Table 4), as the spraying of arginine at a 150 mg L^{-1} with spraying of GA_3 at a 100 mg L^{-1} recorded a highest value (18.51 Kg ha^{-1}) with non significant different with spraying of arginine at a 50 mg L^{-1} with spraying of GA_3 at a 100 and 200 mg L^{-1} (16.41 and 17.59 Kg ha^{-1}) respectively, while the control treatment recorded a lowest value (4.40 Kg ha^{-1}).

Table 4: The effect of spraying of arginine and GA_3 and their interaction on the volatile oil yield (Kg ha^{-1})

Arginine conc. (mg L^{-1})	GA_3 conc. (mg L^{-1})			Average
	0	100	200	
0	4.40	13.98	8.91	9.10
50	7.15	16.41	17.59	13.84
150	7.17	18.51	11.03	12.24
Average	6.36	16.30	12.51	
lsd .05	Arginine conc. = 1.42			
	GA_3 conc. = 1.42			
	Interaction = 2.45			

The active Ingredients in the HPLC analysis (Table 5) revealed the presence of effective compounds in varying proportions between another compound, and the differences were obvious in the proportions of compounds when spraying arginine and GA_3, as the spraying of arginine at a 50 mg L^{-1} and GA_3 at a 100 mg.L^{-1} had the highest percentages of Chamazulene (12.96%), p-

Cymene (18.56%), α-Bisaboloxide A (10.23%) and α-Bisaboloxide B (10.12%),whereas the spraying of arginine at a 150 mg L^{-1} without spraying of GA_3 had the highest percentages of α-Bisabolene oxide A (13.22%) and Limonene (19.66%), while the spraying of GA_3 at a 100 and 200 mg L^{-1} without spraying of arginine had the highest percentages of α-Pinene and Camphene (10.75 and 18.12%) respectively. However, the spraying of arginine at a 150 mg L^{-1} and GA_3 at a 100 mg.L^{-1} had a highest percentage of Caryophylene (12.02%), and the spraying of arginine at a 50 mg L^{-1} and GA_3 at a 200 mg.L^{-1} had a highest percentage of Trans-B-farnesene (29.77%). The role of arginine in stimulating plant growth and increasing the nutrient absorption [23], in addition to the role of gibberellin in flowering and reducing aging [22] may have led to an increase the active ingredients in the chamomile flowers.

Table 5: Effect of Interaction between spraying of arginine acid and GA_3 on the active ingredients of volatile oil (%)

Active ingredients	Arginine conc. (mg L^{-1})								
	0			50			150		
	GA_3 conc. (mg L^{-1})								
	0	100	200	0	100	200	0	100	200
α-Pinene	4.63	10.75	6.33	7.11	6.88	5.98	6.22	5.02	4.74
Caryophylene	10.23	10.88	7.55	7.56	6.74	6.40	6.98	12.02	8.80
Chamazulene	5.56	5.92	7.00	11.12	12.96	10.22	7.45	1.00	7.00
p-Cymene	5.11	6.98	4.54	7.15	18.56	7.56	6.12	7.88	6.23
α-Bisaboloxide A	6.55	3.66	8.12	7.55	10.23	8.55	9.12	9.66	8.01
α-Bisaboloxide B	6.33	8.44	7.23	6.56	10.12	8.55	6.23	7.88	8.96
α-Bisabolene oxide A	8.22	12.88	11.47	10.12	9.66	11.56	13.22	10.55	12.01
Limonene	10.95	11.85	12.33	12.93	14.88	13.01	19.66	16.35	13.22
Trans-B-farnesene	19.66	21.55	20.88	22.33	24.56	29.77	20.43	21.67	25.01
Camphene	9.01	13.22	18.12	14.23	8.65	9.88	10.23	12.87	11.22

Conclusion

We can be concluded that the spraying of arginine at a 50 mg L^{-1} led to an improved the plant growth and increased its physiological effectiveness, which was positively reflected on increasing the qualitative traits of chamomile plants especially volatile oil and their content of active ingredients. Also, the spraying of GA_3 at a 100 mg L^{-1} showed a higher chemical and physiological effectiveness, which led to its superiority in most qualitative traits of chamomile plant.

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