



Effect of Baraceinolide and Sodium Chloride on the Proportion of some Volatile Oils and Proline in majorana Leaves

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Abstract

This research was carried out in collage of Agriculture, Kirkuk University in season 2022, to study the effect of bracinolide and sodium chloride in proportion of aviator oil and proline in leaves Marjoram plant. The experiment designed by t two factors. fires factor baraceanolide (0, 0.1, 0.2, 0.3) g. L⁻¹ while second factor sodium chloride (0, 5, 10, 15) g. L⁻¹. Using (R.C.B.D) and according Duncan's under probability 5%. Each experimental unit includes three pots, one sector contains sixteen experimental units, and the experiment consists of three sectors. Results showed positive effects as a result of using a growth regulator, that spraying 0.2g. L⁻¹ baraceanolid gave the highest number of leaves 510.21, while concentration at 0.3 g. L⁻¹ recorded the highest in nitrogen reached 2.52%, percentage of volatile oil carvone at 55.47%, Limonene at 14.96% and Linalool 6.48%, as well proline reached 26.91 at concentration of 15 g. L⁻¹ salts.

Keywords: Origanum majorana, Brassinolide, Sodium Chloride, Volatile Oils and Proline .

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1. Introduction

Medicinal and aromatic plants are the main source of many medical drugs that are used for different therapeutic purposes, being the main source of effective medical compounds that enter the process of preparing a large number of pharmaceutical compounds [1]. Recently it was observed spread of chronic diseases and many pathological infections that lead to death, as the therapeutic (pharmacological) effectiveness of antibiotics is threatened as a result of the emergence of pathogens resistant to these treatments and drugs [2]. *Origanum majorana* L. perennial aromatic herbaceous plants belonging Lamiaceae. This family includes a large number of widespread medicinal plants and includes about 200 genera, 300 species, 31 genera and 155 species found in Iraq [3] [4]. The Mediterranean region is the original home of Barcasch and its cultivation is widespread in southern France, America, England, Germany, India and China, in addition to some of the Arab countries such as Syria and Egypt [5] [6]. Many countries of the world have conducted studies and shown the importance of marjorams medically, which served as a medicinal treasure in nature. It was used by ancestors in the treatment of deadly diseases and epidemics that have infected humans for thousands of years [7].

2. Materials and methods

The study was conducted in collage of Agriculture in Kirkuk university. The plants were cut and propagated after obtaining the plant from a nursery in Baghdad Governorate, increasing to 6-8 tallest on 1/15/2022. The plant was prepared in the greenhouse until mid-February for acclimatization, and then the plant was transferred to larger anvils, 24 cm in size. It has a diameter of 22 cm and a capacity of 7 liters. The agricultural medium consists of peat moss and peat moss in a ratio of 1:1, with three pots for each purchase unit.

3. Experiment was design by R.C.B.D.

The study aimed to find effect of spraying baracenolide and sodium chloride in some

vegetative qualities and the proportion of flying oil and prolin in marjoram leaves. Treatments as follow:

Brassinolide

- B₁ - control.
- B₂ - 0.1 g L⁻¹.
- B₃ - 0.2 g L⁻¹.
- B₄ - 0.3 g L⁻¹.

Sodium Chloride

- N₁- control.
- N₂ - 5 g L⁻¹.
- N₃ -10 g L⁻¹.
- N₄ -15 g L⁻¹.

3.1 leaves number (leaf. Plant⁻¹): leaves are calculated and average was taken in the end of season at date 17/5/2022 and took its rates.

3.2 Total Nitrogen in leaves: Were taken from Petioles of leaves [8]. Leaves were washed with distilled water and dried in an electrolyte oven at 70 c so they digestion by H₂SO₄ and perchloric acid [9]. Nitrogen was measured by Micro Kjeldahl according to the method [10].

3.3 Extracting oil: Take (20 g) from fresh sample volume was completed in 100 ml of distilled water and put it in the clavinger for 3 hours, the oil was collected and add 20 ml of hexane to separate, and putted in the refrigerator and measured:

4.1 Estimation of active Volatile oil:

The inspection was carried out in the lab. Ministry of Science and Technology / Dep. Environment and Water, using a Japanese model Shimadzu 2010 gas, using an ionic flame detector (FID) and capillary separation column type (DM-5Ms) lengths (30m * 0.25 um * 0.25 mm), where the temperature of the injection and reagent area, was: (280, 340 C), while the temperature of the separation column was starting from 100) – 300 c), an average of 10 degrees / minute. Using inert nitrogen gas as a carrier gas. At a rate of 100 KPa, the retention time and package area were determined for

each of the standard model after it was injected [11]. As for the concentration for each compound, it was determined by comparing the package area of the standard material with the area of the model package. The compounds have been measured for Carvon, Linalool and Limonene.

$$\text{Percentage of Volatile Oils} = \frac{\text{Standard material} \times \text{sample area} \backslash \text{Standard material area} \times \text{Dilution} \backslash \text{Sample weight}}$$

4.2 percentage of proline in the leaves:

Amino acids were extracted according to the method provided by the scientist [12]. where (3 g) was taken from the sample and placed in a volumetric bottle with a capacity of (25 ml) and added (25 ml) of hydrochloric acid (6M) at a temperature of (15 m) for 3 hours, then the sample is dried by rotary evaporator and (5 ml) of sodium citrate pH 2.2 is added to it. The sample was nominated using a plastic filter (0.45um) and taken to the device to perform the injection process. For derivation process (1 ml) was taken from the extracted sample and added to it (200 microliters) of Ortho-phthalic aldehyde (5%) (OPA) and the sample was added for (2 min) after which (100 microliters of the last mixture was taken and injected in the Amino acid analyses). The examination was conducted in the laboratories of the Ministry of Science and Technology / Department of Environment and Water using an amino acid analyzer (of Korean origin). The method presented by the scientist [13]. was used, where

Table (1) shows the effect of spraying baracinolide on marjoram leaves and salt stress on the number of leaves (leaf. plant⁻¹)

BR	B ₁	B ₂	B ₃	B ₄	Average
N	0 g L ⁻¹	0.1 g L ⁻¹	0.2g L ⁻¹	0.3g L ⁻¹	Nacl
N1	490.30	616.67	651.20	564.33	580.63
0 g L ⁻¹	cde	ab	a	abc	a
N2	401.40	436.30	513.43	477.63	457.19
5 g L ⁻¹	efg	ede	cd	ced	b
N3	361.80	308.10	475.67	554.10	424.92
10 g L ⁻¹	fga	gh	ced	bc	b
N4	272.57	304.63	400.53	421.33	349.77
15 g L ⁻¹	h	h	efg	def	c
Average	381.52	416.43	510.21	504.35	

he used the carrier phase consisting of (methanol: acetonitrile: 5% acid). Formic (ratios) 20:60:20 (flow rate (1 ml/min). Use a separation column. (ZORBAX Eclipse-AAA; 3.5µm; L x i.d.=150 x 4.6 mm) To separate amino acids, while a fluorescence detector was used to detect amino acids at different wavelengths

(Ex = 445 nm, Em = 465 nm) The Clarity 2015 program was used to analyze amino acids.

$$\text{Percentage of Proline} = \frac{\text{Standard material} \times \text{sample area} \backslash \text{Standard material area} \times \text{Dilution} \backslash \text{Sample weight}}$$

3.Results

1.3 Leaves Number:

Table (1) results showed that treatment of baracinolide (B₄, B₃), gave the highest number of leaves and reached (510.21, 504.35). while control treatment (B₁, B₂) recorded the lowest 381.52 leaf. As a result of salt stress, the treatment of control (N₁) caused the highest number of leaves and reached 580.63 at the control, then followed by the treatment (B₃, B₂), which gave 457.19, 424.92, respectively. (N₄) gave the lowest leaves number with high concentration of Nacl which recorded 349.77 sheets. Another hand interaction of treatment of (N₁+B₃) superior all treatments excepted (N₁+B₂) and (N₁+B₄), with leaves number 651.20, 616.67, 564.33, while the treatment of (N₄+B₁) gave the lowest at 272.57, which did not make a significant difference with the transactions (N₄+B₂) and (N₃+B₂).

Brassinolide	b	b	a	a
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Means followed by the same letter (s) within each column during each season are not significantly different at 0.05.

2.3 Total of nitrogen (%):

Table (2) showed that treatment of B4(0.3 g.L⁻¹) superior on all treatments and gave nitrogen at 2.52742%, while B1 (control) recorded the lowest percentage of nitrogen and reached 2.04% As a result of salt stress on the plant, differences were found between the treatments used, and the highest percentage of

nitrogen recorded under the control was 2.46%, which exceeded the rest of the Nacl concentrations, while the stress concentrations recorded a lower percentage of nitrogen (N4, N3, N2) and was (2.39%, 2.19%, 2.06). %) and respectively. While the interaction B40.3 g. L⁻¹ + N1 0 g. L⁻¹ gave a highly results at 24.97%, while total of nitrogen gave lowest at B1 0 g. + N4 15 g. L⁻¹, which recorded at 1.71 %.

Table (2) the effect of precinolide spraying the marjoram and salt stress on the percentage of nitrogen leaves

BR N	B1 0g L ⁻¹	B2 g L ⁻¹ 0.1	B3 0.2 g L ⁻¹	B4 0.3 g L ⁻¹	Average Nacl
N1 0 g L ⁻¹	2.28 f	2.36 de	2.52 b	2.69 a	2.46 a
N2 5 g L ⁻¹	2.26 f	2.27 f	2.39 d	2.64 a	2.39 b
N3 10 g L ⁻¹	1.93 j	2.09 h	2.30 f	2.45 c	2.19 c
N4 15 g L ⁻¹	1.71 j	2.03 i	2.19 g	2.30 ef	2.06 d
Average Brassinolide	2.04 d	2.18 c	2.35 b	2.52 a	

Means followed by the same letter (s) within each column during each season are not significantly different at 0.05.

3.3 Carvone%

Table (3) showed the highest value of Carvone at 55.47% concentration of B₄ 0.3 mg l-1, but the control gave the lowest value of 54.10%. Also the salt stress, no significant are showed between all treatment, excepted the

control treatment which gave the highest value at 55.46%. as well as treatment of) B₄ 0.3 g. L⁻¹ + N₁ 0 g. L⁻¹) superior on all treatments that gave a value of 55.83%, but the lowest was recorded with(B₁ 0 g. + N₄ 15 g. L⁻¹) at value 49.33%.

Table (3) the effect of spraying the with baracinolide and salt stress on the proportion of the active substance Carvone in the peruscruash leaves

BR N	B1 0g L ⁻¹	B2 g L ⁻¹ 0.1	B3 0.2 g L ⁻¹	B4 0.3 g L ⁻¹	Average Nacl
N1 0 g L ⁻¹	52.80 h	54.11 e	56.11 c	58.83 a	55.46 a
N2 5 g L ⁻¹	52.03 j	52.97 g	54.86 d	56.86 b	54.18 b
N3 10 g L ⁻¹	51.44 k	52.28 i	52.95 g	53.49 f	52.54 c
N4 15 g L ⁻¹	49.33 m	50.84 l	51.38 k	52.71 h	51.06 d
Average Brassinolide	51.40 d	52.55 c	53.83 b	55.47 a	

Means followed by the same letter (s) within each column during each season are not significantly different at 0.05.

4.3 Limonene (%):

Table (4) showed a positive effect of brassinolide, as a treatment B₄ 0.3 g. L⁻¹ in percentage of Limonene was recorded at 14.96%, control treatment resulted the lowest value at 12.61% of the active substance. As a result, NaCl, the control treatment gave the

highest percentage of the active substance at 15.15083%, while the lowest at 12.17%, at 15 g. L⁻¹. As for the treatment of) B₄0.3 g. + N₁ 0 g. L⁻¹) recorded a largest percentage of Limonene at 166.78667, while the lowest percentage was recorded at transaction) B₁ 0 g. + N₄ 15 g. L⁻¹) which gave 11.32000%.

Table (4) the effect of spraying brassinolide and salt stress on the proportion of the active substance Limonene in e marjoram leaves

BR N	B1 0g L ⁻¹	B2 g L ⁻¹ 0.1	B3 0.2 g L ⁻¹	B4 0.3 g L ⁻¹	Average NaCl
N1 0 g L ⁻¹	13.77 h	14.80 c	15.19 b	16.78 a	15.15 a
N2 5 g L ⁻¹	13.00 j	14.26 f	14.69 d	15.19 b	14.28 b
N3 10 g L ⁻¹	12.36 k	13.00 j	13.90 g	14.51 e	13.44 c
N4 15 g L ⁻¹	11.32 m	11.63 l	12.39 k	13.34 i	12.17 d
Average	12.61	13.42	14.05	14.96	
Brassinolide	d	c	b	a	

Means followed by the same letter (s) within each column during each season are not significantly different at 0.05.

5.3 Linalool (%):

From Table (5) we conclude that B₄ (0.3 g. L⁻¹), which gave the highest concentration of brilled substance Linalool at 6.48, while the lowest value recorded in control treatment, which gave 4.61% of the active substance. As well as NaCl showed an excellent ratios of active substance, control treatment where gave

the highest value of 6.45%, while the lowest value recorded at 4.8417%. in the treatment of N₄(15 g. L⁻¹). The numbers showed the highest percentage of active substance Linalool, where (B₄ 0.3 g. L⁻¹ + N₁ 0 g. L⁻¹) superior all treatments in contrast (B₁ 0 g. L⁻¹ + N₄ 15 g. L⁻¹) recorded caused lowest value at 3.2000%.

Table (5) the effect of spraying brassinolide and brine on the proportion of the active substance Linalool in the marjoram leaves

BR N	B1 0g L ⁻¹	B2 g L ⁻¹ 0.1	B3 0.2 g L ⁻¹	B4 0.3 g L ⁻¹	Average NaCl
N1 0 g L ⁻¹	5.50 f	6.02 e	6.89 c	7.38 a	6.45 a
N2 5 g L ⁻¹	4.90 h	5.60 f	5.94 e	7.17 b	5.90 b
N3 10 g L ⁻¹	4.35 j	4.70 i	5.55 f	6.13 d	5.18 c
N4 15 g L ⁻¹	3.72 l	4.17 k	4.79 i	5.25 g	4.48
Average	4.61	5.12	5.79	6.48	
Brassinolide	d	c	b	a	

Means followed by the same letter (s) within each column during each season are not significantly different at 0.05.

6.3 Proline ratio

Table (6) showed that used baracinolide, caused the highest percentage of proline with B₁ treatment that reached, 29.95 in contrast (B₂), which recorded (20.16). From result salt stress, N₄ treatment with concentration, 15g. L⁻¹ gave at 26.91 in the proportion of proline with decrease in the percentage of salt, so it was followed by treatments (N₂, N₃) which

recorded (23.60, 21.92) respectively, while the lowest percentage of proline was recorded at the control and it was 20.99. So we note that treatment of (B₁ 0 g. L⁻¹ + N₄ 15 g.L⁻¹) caused The highest proline number at 33.69., while the lowest proline caused by treatment of (B₄ 0.3 g. L⁻¹ + N₄ 15 g. L⁻¹) which reached 19.25.

Table (6) the effect of baracinolide spraying and salt stress in the proportion of proline in the baraceous leaves

BR N	B1 0 g L ⁻¹	B2 0.1 g L ⁻¹	B3 0.2 g L ⁻¹	B4 0.3 g L ⁻¹	Average Nacl
N1 0 g L ⁻¹	25.59 e	19.83 l	19.30 l	19.25 o	20.99 d
N2 5 g L ⁻¹	28.50 d	20.07 k	19.64 m	19.47 n	21.92 c
N3 10 g L ⁻¹	32.08 b	21.08 h	20.81 i	20.45 j	23.60 b
N4 15 g L ⁻¹	33.63 a	30.25 c	22.27 f	21.49 g	26.91 a
Average Brassinolide	29.95 a	22.81 b	20.50 c	20.16 d	

Means followed by the same letter (s) within each column during each season are not significantly different at 0.05.

Discussion

The results contained in the above results indicate the catalytic effect of baracinolide, which is a botanical steroid, as it has a similar role to animal steroids, which are produced by plants in very low concentrations, despite its stimulating effects to cell division, especially in the ceremonial areas in the stem and root, which increases the growth of the vegetative and radical total [14]. which works to increase biological and metabolic activities and increase plant growth in general and the accumulation of nutrients within plant tissue. baracinolide works to synthesize carbohydrates in the plant [15]. as well as a cycle in getting rid of harmful effects by increasing chlorophyllous content and reducing racial maturation of membranes [16]. which works on Increasing the number of leaves and increasing the photosynthesis process and then increasing the proportion of nitrogen in the leaves of the marjoram plant .The increase in the proportion of active substances as a result of Brasinolide spraying can be explained to the role played by the

growth regulator in the increase of vegetative and reproductive growth by increasing the processes of cellular division and coding to build nuclear acids and cyclins, which are proteins with high volaism in increasing cell division, especially the enzyme (CDK) Cyclin Dependent Kinas enzymes. The growth regulator (baracinolide) also has an effective role in achieving maximum levels of utilization of growth factors (light, water) in addition to other important elements where they increase photosynthesis and primary and secondary basic products, as increasing the concentration of medically active substances in the plant [17]also The results may be explained by the role played by baraceinolide in the increase in vegetative growth and the construction of nucleic acids, proteins and carbohydrates, which led to the accumulation and increase of active substances within the plant.Secondary compounds play an important role in the adaptation of different plants and overcoming the damage of salt stress and phenols are one of the main groups of plant secondary compounds

that possess a wide range of biological functions in plants [18] . among [19] in a study of them that the low concentrations of sodium chloride on slow growth and yellowing of cells and some case

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تأثير البراسينولايد وكلوريد الصوديوم في نسبة بعض الزيوت الطيارة والبرولين في أوراق نبات البردقوش

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- البحث مسئل من اطروحة دكتوراه للباحث الاول.
- تاريخ استلام البحث 2023/09/18 وتاريخ قبوله 2023/10/24.

الملخص:

نفذت الدراسة في الظلة الخشبية التابعة لقسم البستنة وهندسة الحدائق كلية الزراعة جامعة كركوك لدراسة في الموسم 2022 تأثير البراسينولايد وكلوريد الصوديوم في نسبة الزيت الطيار والبرولين في أوراق نبات البردقوش. تضمنت التجربة عاملين الأول البراسينولايد بالمستويات التالية (0 ، 0.1 ، 0.2 ، 0.3) غم .لتر⁻¹ اما العامل الثاني كلوريد الصوديوم بالمستويات التالية (0 ، 5 ، 10 ، 15) غم.لتر⁻¹ . تم تنفيذ التجربة باستخدام تصميم القطاعات العشوائية الكاملة (R.C.B.D) والبيانات حلت باستخدام برنامج SAS وحسب اختبار دنكن متعدد الحدود. اذ ان كل وحدة تجريبية تضم ثلاث اصص والقطاع الواحد يحوي ستة عشر وحدة تجريبية والتجربة تتكون من ثلاث قطاعات . والنتائج أظهرت ان الرش بالمستوى 0.2غم .لتر⁻¹ من البراسينولايد اعطى اعلى عدد من الأوراق بلغ 510.21 بينما سجل التركيز 0.3 غم.لتر⁻¹ اعلى نسبة من النتروجين 2.52 % واعلى نسبة من الزيت الطيار Carvone 55.47% و Limonene 14.96% و Linalool 6.48% ، في حين اعلى نسبة من البرولين بلغت 26.91 عند تركيز الاملاح 15 غم.لتر⁻¹

الكلمات المفتاحية: البردقوش ، براسينولايد ، كلوريد الصوديوم ، الزيوت الطيار ، البرولين: