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Effect of Brassinolide and Salt Stress on some Vegetative Growth Characteristics and Yield of Active Substance of Marjoram

Mohammed Mahdi Saleh[1], Thamer A. Zahwan[2] and Nadhim Salim Ghanim[3]

[1-3] Department of Horticulture, College of Agriculture, Tikrit University, Tikrit, Iraq.

[1]E-mail: mohameed.m.salih@st.tu.edu.iq
[2]E-mail: thamir@tu.edu.iq
[3]E-mail: nadhimsalim@tu.edu.iq
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Abstract. The study was conducted in Department of Horticulture and Landscape, College of Agriculture - Kirkuk University during the spring season of 2022 to study the influence of foliar spray with Brassinolide and salt stress on some vegetative growth characters and yield of active substance of marjoram., the experiment which included two factors, the first is Brassinolide growth regulator at four concentration (0.1, 0.2, 0.3) g.L^{-1} while the second is adding NaCl (0,10, 5, 15) gm. L^{-1}. The experiment was designed according to the randomized complete block design (R.C.B.D), with three replications and three plants for experimental unite. The results showed that the superior Brassinolide with a concentration of 0.3 mg. L^{-1} in number of branches, total chlorophyll, fresh weight, and dry matter (15.96) 18.28 mg. $1gm^{-1}$, (71.19%) respectively, while NaCl did not show any significance for all traits compared to control treatment. As for the interaction, the treatment (0.3gm.l-1 Brassinolide + 0gm.l- 1 Nacl) gave a high significant in the number of branches, dry matter 15.96 and 71.19, respectively, while the treatment (0.2 g.l^{-1} Brassinolide + 5 g.l^{-1}) excelled in total chlorophyll fresh weight.

Keywords. Origanum marjoram, Brassinolide, Salt stress, Active substance.

1. Introduction

Marjoram, Origanum majorana, is a perennial and aromatic herbaceous plant belonging to the Lamiaceae family [1] majorana has many health properties, including anti-inflammatory, antimicrobial, and analgesic. It has many active compounds, flavonoids, glycosides, and Hydroquinone in addition to the triterpenes [2]. Brassinolide was discovered recently as a growth regulator, and many studies have proved its existence in the Brassicaceae family[3,4] in addition, the role of increasing the efficiency of the stress-resistant enzyme system. Salt stress, is the process of accumulating dissolved salts or collecting them to a degree that exceeds their natural rates in the medium growth[5]. investigation showed that exposing plants to harsh environmental conditions and stresses led to stimulating the activity of proteins in a group of proteins known as Late Embryogenesis (LEA) proteins. Abundant Protein [6].

2. Materials and Methods

The experiment was conducted in the Department of Horticulture and Landscape Engineering - College of Agriculture in the spring season of 2022 on marjoram plants grown in two anvils. A randomized complete block design (R.C.B.D) was used, with three replications for each treatment, and every 3 plants represented one experimental unit, where 144 plants of uniform size were prepared. And its distribution systemically. this study, two factors, the first is the effect of brassinolide with four concentrations (0, 0.1, 0.2, 0.3), while the second is the salt stress NaCl, with 4 concentrations (0, 5, 10, 15) g L^{-1} and the process of three irrigations Every week.

The brassinolide was sprayed twice, and the difference between one spray and another was two weeks. As for the salt stress treatment, NaCl was added twice every week with irrigation water. The process of adding and spraying was done in the morning. The statistical analysis adopted [7], and the means were compared to the Duncans Multiple Range Test under a probability of 5%. The aim of the study is to find out the effect of spraying with brassinolide and salt stress on vegetative traits and the yield of active substances. Treatments as follow:

2.1. Brassinolide

- B1 – control.
- B2 - 0.1 mg L^{-1}.
- B3 - 0.2 mg L^{-1}.
- B4 - 0.3 mg L^{-1}.

2.2. NACL

- N1- control.
- N2 - 5 g L^{-1}.
- N3 -10 g L^{-1}.
- N4 -15 g L^{-1}.

2.3. Statistical Analysis

2.3.1. Number of Branches

The number of branches is calculated, and in each experimental unit, the number of branches is calculated for three plants.

2.3.2. Dry matter (%) Leaves.

10 sheets of paper are taken from each experimental unit on 1/6/2021 and cleaned with distilled water to remove dirt and dust. They are air dried, then the wet weight is taken and placed in perforated bags in an electric oven at a temperature of 65 °C until the weight is stable. Then the samples were extracted and weighed. Using a sensitive electronic scale, the dry percentage was calculated according to what was mentioned by [8] in the following equation:

$$\text{Dry matter \%} = \frac{\text{dry weight}}{\text{wet weight}} \times 100$$

2.3.3. Total Chlorophyll (mg.gm^{-1} fresh weight)

It was estimated according to the method of Knudson et al. [9] when leaves were cut into small pieces by sterilized scissors. 0.25 g and weighed and placed in dark containers. 15 ml of concentration ethanol was added and stored in a dark place for 24 hours. Then the filtrated was collected and the process was repeated three times. Measurement of the solution by Spectrophotometer -EMC labv 1100 at wavelengths of 649 and 665 nm and according to each of chlorophyll a and b according to the following two equations

Chlorophyll a (mg g^{-1} fresh weight) = (13.70) (A665) _ (5.76) (A649)

Chlorophyll b (mg g^{-1}) = (25.80) (A649) _ (7.60) (A665)

Total chlorophyll (mg.g^{-1} fresh weight) = chlorophyll a + chlorophyll b

A = wavelength (nm)

2.3.4. Ultrasonic Extraction of Phenolic Compounds

The phenolic compounds were extracted from homogenized plant sample (3 g) using ethanol/water (70/30) solvent. Extraction process was carried out using Ultrasonic Bath (USA) at the room temperature for 1 hour . Quantification of individual phenolic compounds was performed by reversed phase HPLC analysis, using a SYKAMN HPLC chromatographic system equipped with a UV detector), Chemstation , a Zorbax Eclipse Plus-C18-OSD .25cm, 4.6mm column. The column temperature was 30ºC the gradient elution

method with eluent A (methanol) and eluent B (1% formic acid in water (v/v)) was performed, as follows: initial 0-4 min, 40 % B ; 4-10 min, 50 % B; . and flow-rate of 0.7 mL/min. The injected volume of samples 100 µL and standards was 100 µL and it was done automatically using autosampler. The spectra were acquired in the 280 nm [10].

3. Results and Discussion

3.1. Number of Branches

Table (1) data showed a treatment B4 gave significant increases at(0.3), where gave the highest value at 13.02, compared to control. followed by treatment B3 and B2, which recorded 7.74 , 7.10, while compered to control treatment of 5.51 respectively. As the effect of salt stress, the control N1 gave a highest significant value of all treatment which gave value of10.66, followed by N2 , N3,tretments which recorded 8.97 , 7.70, respectively, N4 recorded alowest value of6.04.

As for interaction B3N1 recorded the highest number of branches, which gave 15.96, while it was followed by B3N2 and B3N3, which recorded 14.56 , 12.53, respectively, while the lowest rate of number of branches was recorded, B1N4, which gave value of 4.36 .

Table 1. Effect of Brassinolide and salt stress and interaction on number of branches for grown season 2022.

Brassinolide/ Nacl	**B1 0 mg L $^{-1}$**	**B2 0.1 mg L $^{-1}$**	**B3 0.2 mg L $^{-1}$**	**B4 0.3 mg L $^{-1}$**	**Average Nacl**
Nacl 0 g L $^{-1}$	7.43 H	8.60 EF	10.66 I	15.96 A	10.66 A
Nacl 5 g L $^{-1}$	5.30 J	7.93 GH	8.10 FG	14.56 B	8.97 B
Nacl 10 g L $^{-1}$	4.96 J	6.63 I	6.70 I	12.53 C	7.70 C
Nacl 15 g L $^{-1}$	4.36 K	5.26 J	5.50 J	9.02 E	6.04 D
Average Brassinolide	5.51 D	7.74 C	7.74 B	13.02 A	

3.2. Total Chlorophyll (mg.gm^{-1} f w.t)

Table (2) data showed B4 recorded a high significant differences at value 18.28 mg.g^{-1} chlorophyll, while B1 recorded a lowest value at 7.88 mg. g-1 while the salt stress did not show any significant difference expected the control was gave the highest value amount 17.13 mg.g^{-1}.while N4 gave the lowest value 8.36 mg.g^{-1} chlorophyll. As for the interaction B4N2 was significantly superior to the rest of the treatments. The treatment of chlorophyll, which was amount 23.14 mg.g^{-1} chlorophyll, while B1N4 gave 7.88 mg.g^{-1} gave a lowestvaluein chlorophyll

Table 2. Effect of Brassinolide and salt stress and interaction on total chlorophyll for grown season 2022.

Brassinolide/ Nacl	**B1 0 mg L $^{-1}$**	**B2 0.1 mg L $^{-1}$**	**B3 0.2 mg L $^{-1}$**	**B4 0.3 mg L $^{-1}$**	**Average Nacl**
N1 0 g L $^{-1}$	10.38 H	16.39 E	19.63 C	22.12 B	17.13 A
N2 5 g L $^{-1}$	8.10 J	11.57 G	17.90 D	23.14 A	15.18 B
N3 10 g L $^{-1}$	7.38 K	9.18 I	13.08 F	16.36 E	11.50 C
N4 15 g L $^{-1}$	5.66 M	6.56 L	9.71 I	11.49 G	8.36 D
Average Brassinolide	7.88 D	10.92 C	15.06 B	18.28 A	

3.3. Dry Matter %

Table (3) showed that a significant differences between Brassinolide cncentration, treatment of B4 was significantly increases to all treatments, recording 57.29 dry matter%, followed by B3 , B2, , which gave 57.41 , 40.64 respectively. As for effect of salt stress, the control superior all treatments significantly which recorded 55.19 dry matter% , followed by N3. And N2 47.23 ,42.01 respectively , while treatment N4 recorded the lowest value 33.87 of the percentage of dry matter in the leaves. The interaction between the treatments had significant differences, as the treatment B4N1 superior all treatments and gave the highest value for dry matter 71.19, followed by B3N1, which recorded 65.79, while the treatment B1N4 recorded the lowest value 16.07, as while as B1N4 gave the lowest value 20.34 dry matter %.

The results presented in above results indicate the stimulating effect of brassinolide a plant steroid, which has similarities to animal steroids, which are produced by plants at very low concentrations, despite its stimulating effects on cell division, especially in the meristem regions of the stem and root, which increases the growth of the vegetative and root system [11], which works to increase vital and metabolic activities, increase plant growth in general, and the accumulation of nutrients within plant tissues.

The decrease in the chlorophyll with the increase in the percentage of salinity is due to the high salt concentration solution, which reduces the absorption of nutrients or minerals that are necessary to build the chlorophyll molecule, such as nitrogen, magnesium, and iron, through the phenomenon of the antagonism (antagonism) for these nutrients through the process of photosynthesis. Thus, the negative effect extends to the percentage of dry matter in the leaves, and plant size. Salts work on changing the composition of chloroplasts, which works to inhibit the action of enzymes that participate in the building chlorophyll molecule, and the efficiency of the photosynthesis process decreases [12] the effect of salinity that works to inhibit the effectiveness of chlorophyllase enzyme that causes the breakdown of chlorophyll molecules[13].

Thus, it is negatively reflected in the process of photosynthesis, which is the basis for providing what is necessary for the plant and accumulation in plant tissues, limiting plant growth and thus its small size.

3.4. Rosemaric ppm

Table No. (4) data showed that B4 was significantly superior at rest of treatments, followed by B3,B2,B1 which recorded 23.44 ppm, 22.74 , 21.96 ppm, respectively, while salt stress had no significant effected. The comparison treatment had the lowest percentage of the active substance Rosemaric, while treatment N4 recorded the lowest of the active substance in leaves, followed by treatments N3 and N2, which were 21.67 and 22.02 ppm. As for interaction treatment of B4N1, was gave a highest percentage value 23.94 ppm, followed by B3N1 , B4N2, which recorded 23.71 , 23. .44 ppm, respectively.

Table 3. Effect of Brassinolide and salt stress and interaction on dry matter for grown season 2022

Brassinolide/ Nacl	**B1 0 mg L $^{-1}$**	**B2 0.1 mg L $^{-1}$**	**B3 0.2 mg L $^{-1}$**	**B4 0.3 mg L $^{-1}$**	**Average Nacl**
N1 0 g L $^{-1}$	29.24 GH	54.52 CDE	65.79 AB	71.19 A	55.19 A
N2 5 g L $^{-1}$	25.96 GH	45.44 EF	60.62 BC	56.92 BC	47.23 B
N3 10 g L $^{-1}$	20.56 IH	33.97 G	58.36 BC	55.14 CD	42.01 C
N4 15 g L $^{-1}$	16.07 I	28.62 GH	44.86 F	45.92 DEF	33.87 D
Average Brassinolide	22.96 C	40.64 B	57.41 A	57.29 A	

Table 4. Effect of Brassinolide and salt stress and interaction on Rosemaric for grown season 2022.

Brassinolide/ Nacl	B1 0 mg L $^{-1}$	B2 0.1 mg L $^{-1}$	B3 0.2 mg L $^{-1}$	B4 0.3 mg L $^{-1}$	Average Nacl
N1	21.48	22.94	23.71	23.94	23.02
0 g L $^{-1}$	K	E	B	A	A
N2	21.28	22.82	23.19	23.44	22.68
5 g L $^{-1}$	L	F	D	C	B
N3	20.51	21.17	22.38	22.60	21.67
10 g L $^{-1}$	O	M	H	G	C
N4	20.34	20.90	21.69	21.94	21.21
15 g L $^{-1}$	F	N	J	I	D
Average Brassinolide	20.90	21.96	22.74	22.98	
	D	C	B	A	

3.5. Hydroquinoue ppm

Table (5) as a result of spraying brassinolides gave a highest increases on the vegetative system of the plant, with treatment B4, which amount 13.19 ppm,. However, salt stress did not show any significant. Compared to control which recorded the highest value over all treatments with value of 13.08, while the lowest value was recorded by N4, which amount 8.38 ppm. the interactionof treatment B3N1 gave the highest value of all treatments which recorded 15.12 ppm, followed by B3N1,B4N2, which gave 14.89 , 14.60 ppm, respectively, as well as B1N4 gave the lowest active ingredient Hydroquinoue at 5.83ppm

Table 5. Effect of spraying marjoram plant with Brassinolide and Hydroquinoue salt stress.

Brassinolide/ Nacl	B1 0 mg L $^{-1}$	B2 0.1 mg L $^{-1}$	B3 0.2 mg L $^{-1}$	B4 0.3 mg L $^{-1}$	Average Nacl
N1	8.29	14.03	14.89	15.12	13.08
0 g L $^{-1}$	L	E	B	A	A
N2	7.79	13.32	14.25	14.60	12.49
5 g L $^{-1}$	M	F	D	C	B
N3	6.27	11.08	11.94	12.48	10.44
10 g L $^{-1}$	O	I	H	G	C
N4	5.83	7.18	9.93	10.58	8.38
15 g L $^{-1}$	F	N	K	J	D
Average	7.04	11.40	12.75	13.19	
Brassinolide	D	C	B	A	

3.6. Hecperetin ppm

Table (6) data showed that brassinolide significant differences between treatments, as the treatment of B4, recorded the highest significant difference by giving 10.68PPM, followed by B3, and B2, while the control gave the lowest value of 7.97. the salt stress did not record any significant difference. as well as control was superior to all treatments with the highest percentage which was recorded at 10.76PPM. The lowest value was recorded by N4 which recorded 8.32 PPM. the interaction treatment amount B1N4 gave a value of 7.50 Table (6).

The reason for stimulating the production of the active substance as a result of the use of brassinolide is due to the basic ability of brassinolide to stimulate cells, differentiate vascular tissues, and develop lateral roots, as well as the reason for this is due to the increase in division and elongation of parenchyma cells [14] Excess levels of sodium chloride in the growth medium are followed by an increase in the plant's uptake of it and at the same time it works to inhibit the uptake of other ions [15] and an increase in the concentration of the ion in the growth medium works to reduce the selective ability to absorb mineral elements, which causes an increase in Sodium-ion absorption in their tissues [16] and thus the lack of all the necessary and necessary vital activities such as divisions and plant growth in a necessary manner as a result of cell plasmacytosis within plant tissues.

Table 6. Effect of spraying marjoram plant with brassinolide and Hesperetin salt stress.

Brassinolide/ Nacl	B1 0 mg L^{-1}	B2 0.1 mg L^{-1}	B3 0.2 mg L^{-1}	B4 0.3 mg L^{-1}	Average Nacl
N1	8.50	10.97	11.72	11.87	10.76
0 g L^{-1}	L	E	B	A	A
N2	8.18	10.71	11.30	11.50	10.42
5 g L^{-1}	M	F	D	C	B
N3	7.69	9.39	9.90	10.30	9.32
10 g L^{-1}	O	I	H	G	C
N4	7.50	7.92	8.82	9.04	8.32
15 g L^{-1}	F	N	K	G	D
Average Brassinolide	7.97 D	9.75 C	10.43 B	10.68 A	

References

[1] Al-Shamaa, A.li Abdel-Hussein Al-Shamaa .1989. Drugs and chemistry of medicinal plants, Dar Al-Kutub for printing and publishing, Beirut, Iraq.

[2] A.O.A.C(2006) Oficial methods of analysis . Horwitz, W. L., George, W. and Latimer, Jr(18th ed). Wasthington, DC: Assoctiation of official Agricultural chemsists

[3] Al-Sahhaf, Fadel Hussein. 1989. Applied plant nutrition. House of Wisdom for publishing. Baghdad University. Ministry of Higher Education and Scientific Research.

[4] Azzam, Hassan (1977) Fundamentals of Field Crop Production, Grain Crops and Fields, Damascus

[5] Dure lll L.; Greenway, S.C. and Galau G.A. (1981). Development biochemistry of cotton seed embryogenesis andin vivo protin synthesis. J. Biochem., 20:4162-4168

[6] Hayat, S. and Ahmed, A. (2011) Brassinosteroids: A class of plant hormones. Springer London. UK.

[7] Islam, T.M.(2014) Mammalaian hormones in plant and their roles in plant peronosporomycetes interaction. Current Topics in Phyto chemistry, (12):89-106

[8] Knudson ,L.L.; Tibbitts , T.W and Edwards G.E.,(1977). Measurement of Ozone injury by determination of leaf chloro phyll concentration.Plant physiol.,Go:606_Go8.

[9] SAS. 2001. Sas / stat User Guide for Personal Compulrrs., Sas lnstitute lnc Cary, N.C.S.A.

[10] Blaga R. ,Jelena M. , Aleksandra R. ,Rados P. and Vesna N. .2015.Phenolic Composition Antioxant, Antimicrobial andCytotoxic Activites of Allium porrumL.(Serbia) Extracts. Vol. 3,No. 9,564-569 , DOI: 10.12691 /jfnr-3-9-1.

[11] Change , C . W. and Dregne, H. E. (1955). Effect of exchangeable sodium on soil properties and on growth and cation of alfalfa and cotton. Soil Sci. Soc. Amer. Proc., 19 : 29 – 39.

[12] Torres, B. C. (1972). The effect of nitrate and temperature on seed germination. Ohi. J. Sci., 67 (2) : 120 – 123.

[13] Zandi ,M.(2012). The role of corticosteroids in today's oral and maxillofacial surgery .Intech., 21:539-556.

[14] Al-Khafaji, Amani, Amman (2014). Growth regulators, their applications and their horticultural uses. College of Agriculture, University of Baghdad, Ministry of Agriculture.Higher education and scientific research, Al Madar University Press

[15] Seeman, R.J. and Sharky, T.D. (1986) Salinity and nitrogen effects on photosynthesis Ribulose.1-5 biphosphate carboxylase in(phoseolus vulgaris L.) plant physiol, 82:555-560.

[16] Balsamo, R.A.and Tbomso W.W.(1995) Salt effect on membrane of the hypodermis and mesophyll cells of agricennia germinant