Effect of spraying number and type of growth regulator on growth, leaves yield and Antioxidant Activity for Fenugreek leaves

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ABSTRACT

The experiment was conducted in one of a private field during the winter season (2017-2018) in Babylon province, Al-Taliaa district, to know the effect of spraying number (one, two, three spraying) and the type of growth regulators (GA3, NAA, GA3×NAA) as well as control treatment on growth Indicators (plant height, the number of secondary branches, the number of leaves, leaf area, leaf area index, total chlorophyll pigments in leaves), leaves yield (fresh weight, the dry weight, the yield of unit area from the fresh and dry leaves) and antioxidant activity of seeds (total phenolic compounds, total antioxidant capacity and The percentage of free radical scavenging). The treatments were distributed on factorial experiment according to randomized complete block design, with three replicates, the averages were compared by using least significant difference at probability level of 0.05. The results showed that there were significant differences in the number of sprayings and the type of growth regulator in most Indicators of growth and fresh and dry leaves yield. The treatment of twice spraying with NAA gave the highest values in the seeds content of total phenolic compounds and the total antioxidant capacity amounted of (257.88, 53.69 mg 100 g^{-1} dry weight). The treatment of Twice spraying with GA3×NAA gave the highest averages in the percentage of free radical scavenging in seeds amounted of (79.83 %).

Keywords: Number of Spraying, Growth regulators, Antioxidant Activity, Fenugreek.

تأثير عدد الرشات ونوع منظم النمو في النمو والحاصل الورقي والفعالية التأكسدية لأوراق الحلبة

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المستخلص:

نفذت التجربة في أحد الحقول الخاصة أثناء الموسم الزراعي الشتوي 2018/2017 في محافظة بابل/ ناحية الطليعة لمعرفة تأثير عدد الرشات (رشة ورشتان وثلاث رشات) ونوع منظم النمو (GA3 و NAA و NAA×GA3) بالإضافة الى معاملة المقارنة في مؤشرات النمو (ارتفاع النبات وعدد التفرعات الثانوية وعدد الأوراق والمساحة الورقية ودليل المساحة الورقية ومحتوى الأوراق من صبغات الكلوروفيل الكلي) والحاصل الورقي (الوزن الطري والوزن الجاف وحاصل وحدة المساحة من الأوراق الطرية والجافة) والفعالية التأكسدية للأوراق (الفينولات الكلية والسعة الكلية المضادة للأكسدة والنسبة المئوية لكبح الجذور الحرة), وزعت المعاملات في تجربة عاملية وفق تصميم القطاعات العشوائية الكاملة بثلاث مكررات، وتمت المقارنة بين المتوسطات حسب إختبار أقل فرق معنوي عند مستوى إحتمال 0.05. بينت النتائج وجود فروق معنوية لعدد الرشات ونوع منظم النمو في معظم مؤشرات النمو والحاصل الورقي الطري والجاف، واعطت معاملة الرش مرتان بمنظم النمو NAA أعلى القيم في محتوى البذور من الفينولات الكلية و السعة الكلية المضادة للأكسدة بلغت 257.88 و 53.69 ملغم.100غم وزن جاف على التتابع، وأعطت معاملة الرش مرتان مع منظمي النمو NAA×GA3 أعلى المتوسطات في النسبة المئوية لكبح الجذور الحرة بلغت 79.83 %.

الكلمات المفتاحية: عدد الرشات، منظمات النمو، مضادات الأكسدة، الحلبة.

1. INTRODUCTION

Fenugreek is one plant of the Fabaceae family and its scientific name (*Trigonella foenum-graecum* L.), belongs to Trigonella genus has 50 species. The Fenugreek is considered a multipurpose plant used in many countries of the world as food and medicine for humans and plant reforming and fertilizing for soil and animal feed, The spraying number of growth regulator is considered an important factor in the Foliar Spraying technique to

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increase or improve agricultural production, The use of growth regulators is considered an agricultural chemical biochemical methods that the plant invests nutrients efficiently and exploits its physiological and genetic abilities to the highest level, Growth regulators also contribute to understanding the processes of composition and its components for their influence on plant growth and development (Moes and Stoble, 1991). Gibberellic acid is used to manage the production of many field and horticulture crops, especially fenugreek plants, for the purpose of increasing vegetative growth, increasing the number of flowers and the length of pods, as well as improving their quality. Acetic acid naphthalene is one of the auxinic growth regulators that stimulate the plant to achieve the highest relative efficiency in utilization Of the lighting period, as it enhances the plant's Apical Dominance and stimulates vertical growth, thereby increasing the area of light-resistant vegetation, thereby increasing the production and accumulation of dry matter without significantly increasing the cost of production (Sumeriya et al., 2000). Bairva et al., (2012) found significant differences in their experiment to knowing the effect of spraying the Fenugreek plants with Gibberellic acid at concentration of (0, 50, 100 $mg.L^{-1}$) and naphthalene at concentrations of (0, 10, 50 $mg.L^{-1}$), With three spraying and without spraying in some vegetative growth indicators where the first spraying was conducted after 25 of the cultivation and the second spraying after flowering 50% of the plants and the third spraying after 20 days from the second spraying, there were significant differences of the spraying of the plants three times compared with the control treatment left without spraying, Shivran et al., (2016) showed that the spraying number of Fenugreek plants with the growth regulators (naphthalene acetic acid) at a concentration of (50 $mg.L^{-1}$) and triacotanol at a concentration of (500, 1000 $mg.L^{-1}$) has significant differences between the number of sprayings, Meena et al., (2014) also found significant differences in their experimentation to determine the effect of spraying Fenugreek plants with Gibberellic acid at the concentrations of (0, 50, 100 $mg.L^{-1}$) with the three spraying, where the first spraying was conducted after 25 of the cultivation and the second spraying after flowering 50% of the plants and the third spraying after 20 days from the second spraying. It found a significant superiority for the spraying treatment at concentration of (50 $mg.L^{-1}$), Antioxidant compounds and their standardization in medical and food plants are among the priorities that today's researchers in the field of alternative and complementary medicine are interested in therapeutic and prevention of a number of chronic diseases (Al-Mohammad, 2018). This experiment aims to:

1) To know the effect of the number of spraying of growth regulators on the indicators of vegetative growth for the Fenugreek plants and the productivity of the unit area from the fresh and dry leaves yield and its effect on the indicators of seed yield and its components.
2) To know the effect of single spraying for the Gibberellic acid and naphthalene acetic acid and the interaction between them with the number of sprayings in the indicators of vegetative growth for the Fenugreek plants and the productivity of the unit area from the fresh and dry leaves yield and their impact on the indicators of the seeds yield and its components.
3) Knowing the effect of the number of sprayings and the type of growth regulator in Antioxidant Activity for leaves and seeds.

2. MATERIALS AND METHODS

The experiment was conducted in one of a private field during the winter season (2017-2018) in Babylon province, Al-Taliaa district. The experimental design included two factors. First, the effect of the number of sprayings. This factor was conducted in three levels. First level,

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growth regulators were sprayed once after 30 days of cultivating. Second Level, Spraying growth regulators twice after 30 and 45 days of cultivating and third level spraying growth regulators Three times after 30, 45 and 60 days of cultivating, spraying was performed using a 16 L Backpack Sprayer in the early morning to give sufficient time to contact the spraying solution with the total vegetative. The second factor is the type of growth regulator, which included four levels: the first is spraying the Gibberellic acid (GA3), the second is spraying Naphthalene (NAA), and the third level is spraying the Gibberellic acid and Naphthalene together (GA × NAA). The growth regulators were sprayed individually or interacting at a concentration of (50 $mg.L^{-1}$) for each and the fourth level is spraying the control treatment with distilled water only, The treatments were distributed on factorial experiment according to Randomized Complete Block Design (RCBD), with three replicates, the averages were compared by using least significant difference at probability level of 0.05 (Al-Rawi and Khalaf Allah, 2000). The soil was analyzed in the high Graduate Laboratory, University of Al-Qasim green, College of Agriculture, Department of Soil and Water Resources to knowning how much fertilizer it needs. Nitrate and Diamoium Phosphate (DAP) (21% nitrogen and 48% phosphorus) were added at a level of (90 $kg.ha^{-1}$) to ensure mixing with soils (Abu Dahi and Al-Yunis, 1988). The seeds of the yellow local cultivar were cultivated on 15/10/2017 (Al-Hadwani, 2003). The percentage of urea fertilizer (46% nitrogen) was added at level (176 $kg.ha^{-1}$) in the two batches, the first one after 14 days of cultivating and the second after 30 days of cultivating (Al-Doori, 2002), the following indicators were measured:

1) Indicators of vegetative growth

It was measured after the transformation of vegetative buds into flowering buds by measuring the height of the plant, the number of secondary branches, the number of leaves, the leaf area, the leaf area index and the leaves content of total chlorophyll.

2) The leaves yield

The fresh and dry weight of the leaves and the productivity of the unit area from the fresh and dry leaves yield

3) Antioxidant activity of seeds

Total phenols were estimated using the Fulin-Ciocalteu Reagent reagent compared to the standard curve of Gallic acid (100 gm dry weight) and The percentage of free radical scavenging using method using by (Mashkor et al., 2014) using DPhenyl-1-picrylhy, and total antioxidant capacity (TAC) was estimated according to the method used by (Prieto et al., 1999) to measure the green color intensity of the complex compound of Molybdenum Phosphate in the acid media.

3. RESULTS AND DISCUSSION

1) Indicators of vegetative growth

Table (1) shows significant differences in the number of spraying and the type of growth regulator in vegetative growth Indicators (plant height, number of secondary branches, number of leaves, leaf area, leaf area index and the leaves content of total chlorophyll), where recorded the highest average when spraying three times, which amounted of (66.89 $cm.plants^{-1}$, 6.04 $branch.plant^{-1}$, 54.29 $leaf.plant^{-1}$, 2.171 $dm^2.plant^{-1}$, 3.475, 56.30 SPAD), respectively, compared to the lowest averages in the plants of spraying treatment once which amounted to (57.84 $cm.plant^{-1}$, 5.45 $branch.plant^{-1}$, 45.12 $leaf.plant^{-1}$, 1.805 $dm^2.plant^{-1}$, 2.887, 55.19 SPAD), respectively. These increases may be due to the fact that the repeated spraying process led to increase the accumulation of growth regulator in the plant, thus increase its physiological effects (Sahib, 2012). The synchronizing of the three spraying operations may be consistent with plant growth stages, which led to a significant increase in growth indicators (Singh, 2010;

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Bairva et al., 2012; Veni, 2014; Prajapat et al., 2015; Shivran et al., 2016). The table shows that there is a significant effect for the spraying with growth regulator in the above indicators, where the spraying with Gibberellic acid × naphthalene acetic acid was excelled by giving it the highest averages (excluding plant height), which amounted of (6.36 branch.plant^{-1}, 54.46 leaf.plant^{-1}, 2.178 dm^2.plant^{-1}, 3.485, 57.07 SPAD), respectively, compared to the lowest averages in the plants of the control treatment, which amounted of (5.01 branch.plant^{-1}, 43.26 leaf.plant^{-1}, 1.730 dm^2.plant^{-1}, 2.768 and 54.10 SPAD), respectively, The spraying of two growth regulators together makes it difficult to attribute the significant increases to one, but it is clear that each of them took its role in influencing the nature of the response of the indicators of growth, there is an increase in the rise of plants as a result of the effect of Gibberellic acid, At the same time, the effect of naphthalene acetic acid showed by promote Apical Dominance and stimulate vertical growth (Dutta et al., 2008), thereby increasing the vegetative area objecting to the light, increase the production, accumulation of dry matter, thus increasing the vegetative growth indicators (Gour et al., 2009; Veni et al., 2014). Naphthalene acetic acid is considered one of the auxinic growth regulators that increase the biomass and urge it to achieve the highest relative efficiency in the light period (Sumeriya et al., 2000). At the same time, the increase in fresh weight may be due to the effect of the Gibberellic acid in stimulating the flexibility of the cells walls and their expansion, thus increase their absorption to water, then increase their size and weight, their content of protoplasm, increase the life of the leaf, the table also shows significant differences in the interaction treatments between the two experiment factors, where the plants that were sprayed three times with Gibberellic acid × naphthalene acetic acid recorded the highest averages except for the plant height amounted of (6.86 branch.plant^{-1}, 60.17 leaf.plant^{-1}, 2.406 dm^2.plant^{-1}, 3.851, 58.07 SPAD) respectively, compared to the control plants that sprayed once, which amounted to (4.96 branch.plant^{-1}, 40.23 leaf.plant^{-1}, 1.609 dm^2.plant^{-1}, 2.574, 53.49 SPAD), respectively.

1) Indicators of leaves yield

Table (2) shows significant differences for the number of sprayings in the leaves yield indicators (fresh weight, dry weight, the yield of unit area from the fresh and dry leaves). where resulted from the spraying two times the highest averages amounted of (92.90 g.plant^{-1}, 10.54 g.plants^{-1}, 14.474 tons.ha^{-1}, 2.384 tons.ha^{-1}), respectively, compared to the once spraying, which amounted to (77.65 g.plant^{-1}, 8.51 g.plant^{-1}, 13.185 tons.ha^{-1}, 2.240 tons.ha^{-1}), respectively. This increase is due to the fact that these two traits are the result of all the accumulated effects of fresh and dry weights (Pariari et al., 2007). The increase in vegetative growth indicators lead to increase fresh leaves productivity which lead to increase the dry leaves productivity (Emongor, 2007; Amit et al., 2018; Krishnaveni et al., 2016). The same table shows a significant effect for the type of growth regulator in the above indicators, where the spraying with Gibberellic acid × naphthalene acetic acid was excelled by giving it the highest averages amounted of (94.47 g.plants^{-1}, 10.66 g.plants^{-1}, 14.624 tons.ha^{-1}, 2.420 tons.ha^{-1}), respectively, compared to the control plants, which gave the lowest averages amounted of (75.60 g.plant^{-1}, 8.27 g.plant^{-1}, 13.814 tons.ha^{-1}, 2.196 tons.ha^{-1}), respectively. The table also shows there were significant differences in the interaction treatments between the experimental factors, where the plants that were sprayed three times with Gibberellic acid × naphthalene acetic acid recorded the highest averages amounted of (105.47 g.plant^{-1}, 12.41 g.plant^{-1}, 14.624 tons.ha^{-1}, 2.479 tons.ha^{-1}), respectively, compared to the control plants that were sprayed once, which amounted of (75.20 g.plant-1, 8.15 g.plant^{-1}, 13.011 tons.ha^{-1}, 2.165 tons.ha^{-1}), respectively.

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Table 1: Effect of the number of sprayings and the type of growth regulator in the indicators of vegetative growth

Treatments			plant height (cm.plant^{-1})	number of secondary branches (branch.plant^{-1})	number of leaves (leaf.plant^{-1})	leaf area (dm^2.plant^{-1})	leaf area index	the leaves content of total chlorophyll (SPAD)
The number of spraying		**1**	57.84	5.45	45.12	1.805	2.887	55.19
		2	62.63	5.68	48.62	1.944	3.112	55.70
		3	66.89	6.04	54.29	2.171	3.475	56.30
LSD 0.05			**2.02**	**0.14**	**0.77**	**0.031**	**0.049**	**0.07**
Type of growth regulator		**Control**	55.61	5.01	43.26	1.730	2.768	54.10
		GA	72.71	6.28	48.77	1.950	3.121	55.55
		NAA	60.17	5.24	50.88	2.035	3.256	56.19
		GA×NAA	61.32	6.36	54.46	2.178	3.485	57.07
LSD 0.05			**2.34**	**0.16**	**0.89**	**0.036**	**0.057**	**0.08**
The number of spraying × Type of growth regulator	**1**	**Control**	53.16	4.96	40.23	1.609	2.574	53.49
		GA	64.78	5.83	44.28	1.771	2.833	55.12
		NAA	55.68	5.07	46.60	1.864	2.982	55.76
		GA×NAA	57.73	5.94	49.37	1.974	3.159	56.40
	2	**Control**	56.05	4.95	43.43	1.737	2.779	54.26
		GA	73.35	6.24	47.47	1.898	3.038	55.61
		NAA	60.33	5.27	49.74	1.989	3.183	56.15
		GA×NAA	60.79	6.27	53.85	2.153	3.446	56.76
	3	**Control**	57.63	5.13	46.12	1.844	2.951	54.56
		GA	80.00	6.78	54.56	2.182	3.492	55.92
		NAA	64.50	5.37	56.31	2.252	3.604	56.66
		GA×NAA	65.43	6.86	60.17	2.406	3.851	58.07
LSD 0.05			**4.05**	**0.27**	**1.54**	**0.062**	**0.989**	**0.14**

2) Indicators of leaves yield

Table (2) shows significant differences for the number of sprayings in the leaves yield indicators (fresh weight, dry weight, the yield of unit area from the fresh and dry leaves). where resulted from the spraying two times the highest averages amounted of (92.90 g.plant^{-1}, 10.54 g.plants^{-1}, 14.474 tons.ha^{-1}, 2.384 tons.ha^{-1}), respectively, compared to the once spraying, which amounted to (77.65 g.plant^{-1}, 8.51 g.plant^{-1}, 13.185 tons.ha^{-1}, 2.240 tons.ha^{-1}), respectively. This increase is due to the fact that these two traits are the result of all the accumulated effects of fresh and dry weights (Pariari et al., 2007). The increase in vegetative growth indicators lead to increase fresh leaves productivity which lead to increase the dry leaves productivity (Emongor, 2007; Amit et al., 2018; Krishnaveni et al., 2016). The same table shows a significant effect for the type of growth regulator in the above indicators, where the spraying with Gibberellic acid × naphthalene acetic acid was excelled by giving it the highest

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averages amounted of (94.47 g.plants^{-1}, 10.66 g.plants^{-1}, 14.624 tons.ha^{-1}, 2.420 tons.ha^{-1}), respectively, compared to the control plants, which gave the lowest averages amounted of (75.60 g.plant^{-1}, 8.27 g.plant^{-1}, 13.814 tons.ha^{-1}, 2.196 tons.ha^{-1}), respectively. The table also shows there were significant differences in the interaction treatments between the experimental factors, where the plants that were sprayed three times with Gibberellic acid × naphthalene acetic acid recorded the highest averages amounted of (105.47 g.plant^{-1}, 12.41 g.plant^{-1}, 14.624 tons.ha^{-1}, 2.479 tons.ha^{-1}), respectively, compared to the control plants that were sprayed once, which amounted of (75.20 g.plant-1, 8.15 g.plant^{-1}, 13.011 tons.ha^{-1}, 2.165 tons.ha^{-1}), respectively.

3) Antioxidant Activity

Table (2) shows significant differences for the number of sprayings in the Antioxidant Activity, where the twice spraying gave the highest averages in the leaves content from total phenol, total antioxidant capacity and the percentage of free radical scavenging amounted of (158.90, 47.90 mg.100 g-1 dry weight, 75.19%), respectively, compared to the lowest averages in the spraying treatment three times for the total phenols amounted of (133.18 mg.100 g^{-1} dry weight) and once spraying treatment for the other two indicators amounted of (40.61 mg.100 g^{-1} dry weight, 53.81%). This increase may be due to the fact that the plant's bio-activities and the accelerated rate of bio-building processes are increasing in the logarithmic stage for the plant (at second spraying), which increases the need for additional quantities of antioxidant compounds to protect the cell from free radicals, especially the electron transport chain electron which Coincided with the second spraying after 45 days from cultivating. Table (2) also shows significant differences in the type of growth regulator in 3) Antioxidant activity indicators. Spraying with the growth regulator NAA led to the highest averages in the leaves content of the total phenols, the total antioxidant capacity and the percentage of free radical scavenging amounted of (201.59, 49.20 mg.100 g^{-1} dry weight, 71.19%), respectively, compared to the lowest average in the control treatment amounted of (86.66, 36.72 mg,100 g^{-1} dry weight, 46.51%), respectively. This may be justified by the sensitivity of the Fenugreek plant to the type and concentration of non-plant compounds. This compound with auxinic effect may cause non-abiotic stress for plants. This has led to an increase in the production of antioxidant compounds, including total phenols, thus increasing the percentage of free radical scavenging in the leaves. The table also shows significant differences in the interaction treatments between the two experiment factors, where the plants that were sprayed twice with acetic acid naphthalene recorded the highest averages in the leaves content of the total phenols, the total antioxidant capacity amounted of (257.88, 53.69 mg.100 g-1 dry weight), the twice spray treatment with growth regulators (GA × NAA) gave the highest percentage of free radical scavenging amounted of (79.83%), The spraying treatment three times with growth regulators (GA × NAA) gave the lowest averages in the leaves content of the total phenols amounted of (48.26 mg, 100 g^{-1} dry weight), While the once spraying treatment with the distilled water gave the lowest averages in the total antioxidant capacity and the percentage of free radical scavenging amounted of (33.70 mg, 100 g^{-1} dry weight, 26.99%), respectively.

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Table 2: Effect of the number of sprayings and the type of growth regulator in indicators of yield and Antioxidant Activity.

Treatments			Leaves yield				Antioxidant Activity		
			Fresh weight (g.plant^{-1})	Dry weight (g.plant^{-1})	The yield of unit area		mg.100 g^{-1} dry weight		the percentage of free radical scavenging
					Fresh (tons.ha^{-1})	Dry (tons.ha^{-1})	Total phenolic	total antioxidant capacity	
The number of spraying		1	77.65	8.51	13.185	2.240	142.51	40.61	53.81
		2	85.41	9.39	14.060	2.294	158.90	47.29	75.19
		3	92.90	10.54	14.474	2.384	133.18	43.99	58.07
LSD 0.05			**0.52**	**0.18**	**0.048**	**0.015**	**11.23**	**0.55**	**0.59**
Type of growth regulator		Control	75.60	8.27	13.814	2.196	86.66	36.72	46.51
		GA	82.27	9.04	13.891	2.278	143.42	43.33	68.60
		NAA	88.93	9.94	14.295	2.331	201.59	49.20	71.19
		GA×NAA	94.47	10.66	14.624	2.420	147.79	46.60	63.12
LSD 0.05			**0.60**	**0.21**	**0.056**	**0.017**	**12.97**	**0.75**	**0.69**
The number of spraying × Type of growth regulator	1	Control	75.20	8.15	13.011	2.165	62.41	33.70	26.99
		GA	75.17	8.26	13.110	2.187	105.05	40.21	57.23
		NAA	78.96	8.68	13.215	2.252	179.26	45.26	66.05
		GA×NAA	81.25	8.93	13.403	2.355	223.32	43.25	64.98
	2	Control	74.57	8.20	13.107	2.203	89.84	39.87	68.35
		GA	80.38	8.84	13.871	2.246	116.09	45.91	77.13
		NAA	89.97	9.89	14.395	2.303	257.88	53.69	75.44
		GA×NAA	96.70	10.63	14.865	2.425	171.78	49.68	79.83
	3	Control	77.03	8.47	12.814	2.218	107.72	36.58	44.19
		GA	91.25	10.03	13.891	2.400	209.11	43.86	71.45
		NAA	97.86	11.25	14.295	2.438	167.63	48.65	72.07
		GA×NAA	105.47	12.41	14.624	2.479	48.26	46.87	44.55
LSD 0.05			**1.03**	**0.37**	**0.097**	**0.030**	**22.46**	**1.33**	**1.19**

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