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THE EFFECT OF THE HUMIC ACID AND BRASSINOLIDE AND THEIR INTERACTION ON GROWTH, YIELD AND ACTIVE COMPOUNDS OF *TRIGONELLA FOENUM-GRAECUM* L. PLANT

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Abstract

The field experiment was conducted in the Botanical Garden of Department of Biology in College of Education for Pure sciences (Ibn Al-Haitham), University of Baghdad, for the growth season 2017-2018 to study the effect of application humic acid and brassinolide and their interaction on some morphological (stem diameter, number of branches) and chemical (proportion of carbohydrates and protein) characteristics and some active compounds (Vicenin 1, Apigenin) in the *Trigonella foenum-graecum* L. plant. Four concentrations of humic acid (0, 0.5, 1, 2) $mg.L^{-1}$ and brassinolide (0, 0.5, 1, 2, 4) $mg.L^{-1}$ were used. The experiment was designed by Randomized Complete Blocks Design (R.C.B.D) and with three replicates per concentrations and 60 experimental units. The experimental units will be fertilize based on their concentrations. The results showed: The effect of various concentrations of humic acid has led to a significant increase in all of the characteristics studied, a concentration of 2 $mg.L^{-1}$ of humic acid in each stem diameter, the number of branches, the proportion of carbohydrates and protein as well as the concentration of Vicenin 1 and the Apigenin compound. The spray of various concentrations from brassinolide led to a significant increase in all the characteristics studied and the highest increase at concentration of 2 $mg.L^{-1}$. The duel interaction between the humic acid and the brassinolide were significantly in all the characteristics studied, with exceeding treatment 2 $mg.L^{-1}$ acid and 2 $mg.L^{-1}$ofbrassinolide.

***Key words*:** Humic acid, Brassinolide, *Trigonella foenum-graecum* L., Active compounds, Carbohydrates, Protein.

Introduction

Medicinal plants are a major source of many medicinal drugs and have been used for thousands of years to treat health disorders and prevent disease (Singh, 2015). The fenugreek is a medicinal plant belonging to Leguminosae family and order Fabales (Basu, 2006). Fenugreek uses to reduce the proportion of fat and sugar in the blood due to the presence of effective compounds such as alkaloids, steroids, flavonoids and saponins (Kor *et al*., 2013). At present, the seeds of the fenugreek are used as a tonic, carminative, tranquilizer, diuretic, and sputum treatment used to treat mouth ulcers (Haji mehdipoor *et al*., 2010) and it is used for cancer treatment (Shirzad *et al*., 2013). Glycosides is an important part of the active material in the fenugreek plant, where it plays an important role in human life and the treatment of many diseases and is used as a booster and a heart stimulant (Al-Shamma, 1989; Mahmoud, 2008).

Humic acid is one of the compounds resulting from the decomposition of organic matter (Al-Nuaimi, 1999) and it is directly effects on the plant because it plays a similar role to plant hormones, stimulating plant growth, increasing the permeability of the cellular wall and increasing the breathing process and photosynthesis (Serenella *et al*., 2002). Humic acid improves plant growth by increasing nutrient element absorption and inhibitive activity of IAA oxidase enzyme, thus increases the activity of the hormone IAA and thereby improvement plant growth (Mahmoud *et al*., 1997).

Brassinolide is plant hormone that has a significant role in plant growth and development and has been defined as a crude fatty extract from the *Brassica napus* radish pollen. Brassinolide has a similar effect to auxin, gibberellins and cytokines, with its effect on physiological processes as elongation and division of cells, transporter vessels differentiation, flowering, aging resistance and manufacture of DNA, RNA and proteins (Al-Khafaji, 2014; Hayat and Ahmed, 2011).

Materials and methods

The experiment was carried out in the Botanical Garden of the Department of Biology inCollege of Education for Pure Science (Ibn Al-Haitham), University of Baghdad for the growth season 2017-2018

to study the effect of humic acid and brassinolide and their interaction in certain morphological and chemical characteristics and active compounds. The field was initialized by ploughing soil and sampling to estimate its chemical and physical characteristics according to the methods described (Page *et al*., 1982) as shown in table (1).

Table 1 : Some physical and chemical characteristics of the soil of the experiment prior to cultivation.

Characteristics	value	unit
Electrical condition (EC)	3.20	$dS.m^{-1}$
Degree of reactivity (pH)	7	-----
Sand	25	%
Clay	35	%
Silt	40	%
Soil texture	Clay-silt	-----
Nitrogen available	45	$mg.kg^{-1}$.soil
Phosphor available	17	$mg.kg^{-1}$.soil
Potassium available	248	$mg.kg^{-1}$.soil
Mg	413	$mg.kg^{-1}$.soil

The experiment was designed according to R.C.B.D. and three repeated each repeating unit containing 20 experimental units, the experimental unit area (60 × 60) cm^2 and the fertilizer (NPK) 20:20:20 for all experimental units and at the rate of 90 $kg.Ha^{-1}$. Seeds were planted on the date of 23-10-2017 after it was riddled and tested the germination ratio and culverted the plant at straight lines and the process of irrigation and jungle removal continued.

Characteristics studied

1. Stem diameter (cm): the stem diameter of three random plants per experimental unit is measured at 6 cm altitude from the soil surface using verner caliper.
2. Number of branches (branching. $Plant^{-1}$): The number of side branches of three random plants per experimental unit has been calculated.
3. Estimation of carbohydrates proportion in the vegetative total (%): The proportion of carbohydrates were estimated by Herber *et al.* (1971).
4. Estimation of protein proportion in the vegetative total (%): The protein proportion was calculated by multiplying the nitrogen proportion by a constant factor (6.25) according to the Vopyan (1984) method.
5. Method of diagnosis and estimation of Vicenin 1 and the Apigenin compound in the fenugreek plant using the high performance liquid chromatography (H.P.L.C.): The active ingredients were diagnosed using a H.P.L.C. device by Wen (2000) methods.

Results and Discussion

1. Stem diameter (mm)

The results of table 2 indicated that the effect of humic acid was significant, with a concentration exceeding 2 $mg.L^{-1}$ with the highest average of 3.221 mm and an increase of 7.22% compared to control. The cause of the effect of humic acid in the thickness of the epidermis and cortexby its effect on parenchyma and collenchyma cell size. This is due to the role of the nutrients contained in the humic acid in stimulating a large number of enzymes that are involved in the photosynthesis process, leading to increased chlorophyll concentration and thus increased carbohydrates accumulation that produced in plants. As well as an increase in the cambium activity in the formation of phloem outward and xylem to the inside and increase the number of vascular units. The nutrients that contained in the humic acid induce the cambium to form new vascular vessels in addition to increase the growth and differentiation of small vascular vessels that embedded within the pith rays cells. Thus leads to an increase in stem diameter (Azzaz *et al*., 2007), and these results correspond to Judy (2013) in Japanese pear seedlings.

The addition of brassinolide has a significant effect in increasing the mean stem diameter. At concentration of 2 $mg.L^{-1}$ the mean of this characteristic was 3.213 mm and the increase was 6.07% compared to control. The reason is that the brassinolide is involved in the expansion of the cell wall, so the increase in the characteristics of vegetative growth, including diameter of the stem from the addition of the brassinolide may be due to the elongation and division of the cells (Tabkwash, 2013).The reason for this is that the brassinolide is involved in many plant biotic processes such as cell elongation and cell expansion, thus increasing stem diameter (Al-Khafaji, 2014). These results correspond to the results of Al-Khattab (2017) in olive seedlings.

The results of the interaction between humic acid and brassinolide showed that there were significant differences in the mean of characteristic and the highest value of the interaction at concentration was 2 mg. L^{-1} of humic acid and 2, 1 and 0.5 mg. L^{-1} of brassinolide 3.299, 3.276 and 3.266 mm respectively and an increase rate of 14.23 and 13.43 and 13.09% respectively compared to control which gave the lowest value to the interaction of 2.888 mm.

Table 2 : The effect of the application of humic acid and brassinolide and their interaction on the mean stem diameter of the fenugreek (mm).

Concentrations of humic acid mg.L^{-1}	Concentrations of brassinolidemg.L^{-1}					Mean effect of humic acid
	0	0.5	1	2	4	
0	2.888	2.993	3.065	3.148	2.927	3.004
0.5	2.995	3.088	3.156	3.181	3.023	3.089
1	3.074	3.193	3.201	3.223	3.180	3.174
2	3.159	3.266	3.276	3.299	3.107	3.221
LSD 0.05	**0.068**					**0.030**
Mean effect of brassinolide	3.029	3.135	3.175	3.213	3.059	
LSD 0.05	**0.034**					

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

The results of table 3 showed that there were significant differences in the mean number of branches at the application of different humic acid, which gave a concentration of 2 mg. L^{-1} the highest mean of the characteristic is 10.01 branch. plant^{-1} compared to non-application of acid and an increase rate of 7.17%. The reason due to for the role of humic acid in increasing photosynthesis and that was positively reflected in the construction of a good root total and thus stimulated the increase in the formation of cytokines which is contradicting the auxins in its work. Subsequently negative affecting on the apical dominance and positively differentiating the vascular area connection between the lateral bud and the stem, which helps to grow the largest number of mean vegetative branches (Moore, 1982).

The increase in the number of branches by increasing the humic acid is due to the fact that these acids are increasing the biological activity of the plant by stimulating the enzymatic systems and increasing the composition of DNA, RNA and tRNA, which stimulate the formation of cytokines that stimulate the rapid division of cells. Thus, encourage the lateral growths (Jackson, 1993). These results are consistent with Hussein and Ramadan (2016), which indicated an increase in the number of mustard plant branches.

As for the effect of the brassinolide, it resulted in a significant increase in the meancharacteristic, with a concentration of 2 mg.L^{-1} in the treatment of brassinolide by gave the highest mean branch number was 9.99 branch.plant^{-1} with increase rate 6.16% in comparison to control. This due to the application of brassinolide help to increase absorption of elements of the soil and their utilization in the growth of the lateral branches (El-Khallal *et al*., 2009). Mandava *et al*. (1981) noted that growth at the application of brassinolide could be due to the interaction of the brassinolide with other internal hormones and thus lead to an increase in the characteristic of vegetative growth.

The duel interaction between the humic acid and brassinolide were significantly differences in the mean number of branches and the highest value of the interaction at concentration was 2 mg.L^{-1} of humic acid 2, 1 and 0.5 mg.L^{-1} from brassinolide 10.25, 10.18 and 10.15 branch.plant^{-1}respectively and an increase rate of 14.14 , 13.36 and 13.03% respectively compared to control, which gave the lowest value of the interaction value was 8.98 pranch.plant-1.

Table 3 : The effect of the application of humic acid and brassinolide and their interaction on the mean branches number of the fenugreek (branch. plant^{-1}).

Concentrations of humic acid mg.L^{-1}	Concentrations of brassinolidemg.L^{-1}					Mean effect of humic acid
	0	0.5	1	2	4	
0	8.98	9.30	9.53	9.78	9.10	9.34
0.5	9.31	9.60	9.81	9.89	9.40	9.60
1	9.55	9.92	9.95	10.02	9.88	9.87
2	9.82	10.15	10.18	10.25	9.66	10.01
LSD 0.05	**0.21**					**0.09**
Mean effect of brassinolide	9.41	9.74	9.87	9.99	9.51	
LSD 0.05	**0.11**					

3. Proportion of carbohydrates in vegetative total (%)

The results of the table (4) observed that the effect of humic acid was significant, as the mean proportion of carbohydrates in the vegetative total of the fenugreek plant from 16.21% at concentration 0 of the humic acid to 18.25% at concentration 2 mg. L^{-1} and the increase was 12.58%.This results due to the role of humic acid in increasing the content of chlorophyll in the leaves thus increasing the efficiency of photosynthesis and increasing its products as the carbohydrates (Abdul-Al-Hayani, 2016).Al-Ali and Abdul Majid (2013) explained that the increase in chlorophyll and the subsequent increase in the products of photosynthesis resulted in an increase in the proportion of synthesized carbohydrates.

The addition of brassinolide has a significant effect in increasing the mean of this characteristic at 2 mg.L^{-1}concentration the mean rate was 18.15% and the increasing rate was 10.33% compared with control that gave 16.45%. This result due to the increase in carbohydrates content in plants because the role of the brassinolide in increasing the efficiency of carbon representation, which has increased the net of CO_2, that is the final unit the structure of the carbohydrates (Mahgonb *et al*., 2006). The effect of brassinolide on fixation CO_2 at thephotosynthesis process by its effect

on the efficacy of carbonic anhydrase enzyme and this enzyme stimulates the environmental transformation between CO_2 and HCO_3^-. This in turn increases the availability of CO_2 for the Rubisco enzyme (a short name for the enzyme, which plays an important role in the first major step in carbon fixation, that is the process of converting carbon dioxide in the atmospheric air into high energy particles), resulting in a more efficient photosynthesis process (Sadeghi and Shekafandeh, 2014), which in turn increase the amount of carbohydrates in the vegetative total of plant.

As for the results of the duel interaction between humic acid and brassinolide, the results showed that there were significant differences in the meanof this characteristic and the highest value of interaction at concentration was 2 mg.L^{-1} of humic acid, 2, 1 and 0.5 mg.L^{-1} from brassinolide was 18.96, 18.74 and 18.64% respectively with increase of 25.65, 24.19 and 23.53% respectively compared to control which gave the lowest value of the interaction was 15.09%.

Table 4 : The effect of the application of humic acid and brassinolide and their interaction on the carbohydrates proportionin vegetative total of the fenugreek (%).

Concentrations of humic acid mg.L^{-1}	Concentrations of brassinolidemg.L^{-1}					Mean effect of humic acid
	0	0.5	1	2	4	
0	15.09	16.08	16.76	17.54	15.56	16.21
0.5	16.10	16.97	17.62	17.84	16.37	16.98
1	16.85	17.96	18.04	18.24	17.84	17.78
2	17.75	18.64	18.74	18.96	17.15	18.25
LSD 0.05	0.64					0.29
Mean effect of brassinolide	16.45	17.41	17.79	18.15	16.73	
LSD 0.05	0.32					

4. Protein proportion in vegetative total (%)

The results of the table (5) indicated the existence of significant differences in the mean protein proportion in the vegetative total of the fenugreek, with the effect of application different concentrations of humic acid. This application with concentration of 2 mg. L^{-1}gave the highest mean characteristic of 14.68% and an increase rate of 12.49% compared to control. This result due to humic has a high nitrogen intake, which is formation dense vegetative total in addition to increase nitrogen absorption, that in turn has been reflected in increased photosynthesis and protein content in the plant (Sadiq *et al*., 2009). As well as its role in activating the nitrate reductase enzyme which responsible of reduction nitrate to ammonia that is the basis for the formation of amino acids necessary for protein formation (Matloob *et al.*, 2002). The increase is due to protein proportion because of the increase in the nitrogen proportion.

As for the effect of the application progesterone hormone, the results showed that there were significant differences in the mean of this characteristic, with concentration of 2 mg. $L^{-1}$and the highest mean of 14.61% and an increase rate of 10.51% at control. The increase of protein in the vegetative total at the application of the brassinolide hormone due to increase nitrogen proportion in plants. These results are consistent with the findings of Al-Jubouri (2017) on the dill plant.

As for the duel interaction between humic acid and brassinolide, the results showed that there were significant differences in the mean nitrogen proportion in the vegetative total of plant. The highest mean at concentration of 2 mg.L^{-1} of humic acid 2, 1 and 0.5mg. L^{-1} of brassinolide was 15.27, 15.09 and 15.01% respectively, with an increase of 25.68, 24.20 and 23.54% respectively compared to control, which its mean was 12.15%.

Table 5 : The effect of the application of humic acid and brassinolide and their interaction on the protein proportion in the vegetative total of the fenugreek (%).

Concentrations of humic acid mg.L^{-1}	Concentrations of brassinolidemg.L^{-1}					Mean effect of humic acid
	0	0.5	1	2	4	
0	12.15	12.94	13.50	14.13	12.53	13.05
0.5	12.97	13.66	14.19	14.37	13.18	13.67
1	13.57	14.47	14.53	14.69	14.38	14.32
2	14.21	15.01	15.09	15.27	13.81	14.68
LSD 0.05	0.53					0.24
Mean effect of brassinolide	13.22	14.02	14.33	14.61	13.47	
LSD 0.05	0.26					

5. Proportion of active compounds Vicenin 1, Apigenin in seeds (%)

The results of the table (6, 7) showed that there were significant differences in the mean proportion of Vicenin 1and Apigenin compounds with the effect of different concentrations of humic acid. A concentration of 2 mg. L^{-1}gave highest value of 35.1% and 13.80% respectively, with an increase of 44.44% and 47.75% respectively compared to control. This is due to the fact that humic acid contains nutrients, including nitrogen, potassium and micronutrients. The potassium element is in the efficacy of 60 types of enzymes, which affects the metabolism of carbohydrates and the formation of fats. As well as the role of humic acid in stimulating biological processes in the plant especially photosynthesis and division which is the biocenter in the plant for the production of secondary compounds such as glycosides (Zahra *et al*., 1984; Bowes *et al*., 2004).

In addition to containing the humic acid the nutrients also contain balanced hormonal substances that increase the efficiency of the vegetative total in the synthesis and accumulation of carbohydrates materials and thus increase the metabolic processes leading to the production of these substances, with the result that effective compounds production has increased (Abdel-Amin, 2010).

As for the effect of application brassinolide, the results indicated that there were significant differences in the mean proportion of Vicenin1, Apigenin, which had a higher concentration of 2 mg. L^{-1} with the highest value for this characteristic was 34.5% and 13.55% respectively and the increase was 31.68% and 34.83% respectively compared to control. The role of the brassinolide hormone is due to increased vegetative and reproduction growth by increasing cellular divisions and encoded construction of nucleic acids and cyclins, which are highly active phosphoproteins increasing cellular division, especially cyclin dependent Kinase enzymes (CDK) (Hayat and Ahmad, 2011).

It is also attributed to the role of brassinolide in maximizing the use of growth factors such as light, water and important elements, as it leads to increased photosynthesis and its primary and secondary products, and increases the concentration of active compounds in plants (Youssef and Talaat, 1998).This may return to the role of hormone brassinolide increased vegetative growth and the construction of nucleic acids, carbohydrates and proteins (table 4, 5). Thus resulted in the accumulation and increase of medically effective compounds.

The duel interaction between humic acid and brassinolide was significantly inthe mean of this characteristic, the concentration was given 2 mg.L^{-1} of humic acid and 2 mg.L^{-1} from brassinolide, the highest interaction value of 40.1% and 15.76% respectively, with an increase of 78.22% and 101.02% respectively compared to control.

Table 6: The effect of the application of humic acid and brassinolide and their interaction on the mean Vicenin 1 of the fenugreek seeds (%).

Concentrations of humic acid mg.L^{-1}	Concentrations of brassinolidemg.L^{-1}					Mean effect of humic acid
	0	0.5	1	2	4	
0	22.5	23.9	24.9	26.8	23.3	24.3
0.5	24.8	28.7	34.1	34.5	26.7	29.8
1	27.9	35.1	35.6	36.4	31.1	33.2
2	29.6	38.2	39.3	40.1	28.6	35.1
LSD 0.05	2.1					1.0
Mean effect of brassinolide	26.2	31.4	33.5	34.5	27.3	
LSD 0.05	1.1					

Table 7: The effect of the application of humic acid and brassinolide and their interaction on the mean Apigenin of the fenugreek seeds (%).

Concentrations of humic acid mg.L^{-1}	Concentrations of brassinolidemg.L^{-1}					Mean effect of humic acid
	0	0.5	1	2	4	
0	7.84	9.38	9.79	10.53	9.14	9.34
0.5	9.75	11.28	13.40	13.57	10.50	11.70
1	10.95	13.79	13.98	14.32	12.22	13.05
2	11.64	15.02	15.44	15.76	11.14	13.80
LSD 0.05	0.84					0.38
Mean effect of brassinolide	10.05	12.37	13.15	13.55	10.75	
LSD 0.05	0.42					

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