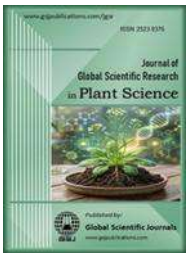
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Endogenous Growth Regulators (Auxin, Gibberellin, Kinetin, and Abscisic Acid) in *Nigella Sativa L.* Plants under the Influence of Humic Acid, Gibberellic Acid, and Their Interaction

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ABSTRACT

The experiment was carried out to find out the effect of foliar application of humic acid and gibberellin and their interaction on the internal growth hormones of the black seed plant with three increasing levels of humic acid (0, 10, 20) mg. L-1 and four levels of gibberellic acid (0, 25, 50, 100) mg. L-1 in some features (measurement of auxin hormone, gibberellin hormone, kinetin hormone, abscisic acid). The experiment was done according to the randomized complete block design with three replicates and 36 experimental units. The findings are as follows:

1. The effect of both humic and gibberellin acids had a positive effect on the studied traits.
2. The role of the high concentrations of each of them was in giving increased percentages for most of these traits.
3. The bilateral interaction between humic acid and gibberellin and spraying with high concentration had a positive effect.

1. Introduction

The black seed is carminative, stimulant and diuretic, and a poultice of the seeds cures cuts, scorpion stings, and eczema. The seeds have antiseptic, anti-parasitic property especially in children, menstruation, helpful in asthma and a tonic for the immune system (Thakur et al., 2021). Seeds are kept inside the folds of cloth as a moth repellent. Nigella sativa is effective in the diseases of prostate and colon, nervine tonic and sex or for the treatment of diabetes. The parts used from this plant are the black seeds; collected seeds when they are ripe. The seeds contain 40% fixed oil, 1% saponins (melantin), and 1.4% volatile oil (Shah, 2004).

Humic acid is one of these organic acids that occur naturally. Humic acid is one of the humic substances, which are formed through the decomposition of organic material. Its addition to the soil leads to increased nutrient uptake by plants because it helps transport nutrients from the soil to the plant, particularly in conditions of

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drought. Moreover, humic acid enhances root development and increases the protein level of the plant. Humic acid influences seed germination, seedling and root growth and nutrient uptake (Gerke, 2022), and improves all physiological and biochemical processes in plants (Canellas and Olivares, 2014). Humic acid is a natural biological organic acid and has a great influence on the growth of plants and their quality.

Nevertheless, the way through which humic acid promotes the volatile compounds composition is unknown (Noroozisharaf and Kaviani, 2018). Humic acid is a naturally occurring substance found in deposits of organic materials that have fossilized for thousands of years. It provides many physiological and essential advantages for the plant and also biological, chemical, and physical advantages to the soil. The extraction process involves the use of an alkaline solution of the potassium salt, resulting in potassium humate. Some of its advantages are promotion of seed germination and root cuttings formation. Also, it serves as a chelating agent, making it easy for chelation of elements such as calcium, magnesium, phosphorus, and micronutrients like iron, manganese, and copper.

It is effective in treating soil salinity through the mobilization of calcium and its chelation process, making it available and active. Calcium has a positive thermal and magnetic energy field, which disrupts the ionic bond of sodium chloride and makes it possible to displace and leach the sodium along with the drainage water. Calcium also stimulates enzymes and energy complexes. It causes the activation of cytokinins, which cause cell division and root stimulation as well as increase osmotic pressure in the plant, making it stress tolerant. It also helps in improving early ripening and the quality of the fruit. It is simple to apply, dissolves easily, and can be injected at any time. It is a chelating agent that requires no other agent to work. It is environmental friendly, has no odor, it is non-toxic and does not contaminate ground water. It is most preferred in farms (Abdel-Hafez, 2026).

Gibberellins are a class of growth-promoting plant hormones and they are the terpenoids of four isoprene units and consists of either 19 or 20 carbons (Ghosh and Halder, 2018). The gibberellins were isolated from a fungus called Gibberella fujikuroi, which has had much attention for a long time, beginning from the observations done by a group of Japanese scientists in a disease of the rice seedlings called at that time 'Foolish Seedling (Efati Lakeh and Padasht-Dehkaei, 2019), in which the symptoms of the disease are initiated by The stems of the plant are longitudinal, and it is one of the naturally-produced plant hormones within the plant tissue Endogenons, which have been proven to be produced by lower plants like mosses, ferns, algae, bacteria, fungi, and higher plants. It was found that GA3 is the most abundant, and it is called Gibberellic acid (Mohapatra and Sahu, 2022).

Their functions are controlled by their antagonistic interaction with abscisic acid since both hormones have different physiological impacts on one another (Castro-Camba et al., 2022). Application of gibberellin hormone on chrysanthemum plants promoted vegetative growth, leading to higher height of plants, leaves per plant, leaf area, chlorophyll concentration, and germination (Lafi et al., 2021). The use of gibberellic acid has shown positive impacts on promoting vegetative and flower growth, as well as planting density (Nassour et al., 2022).

2. Materials and Methods

Field experiment was carried out in winter growing season 2020-2021 at Latifiya area, in the governorate of Mahmoudiyah within Baghdad Governorate in order to study the effect of humic and gibberellin acids and their interactions on the growth regulators inside the black bean plant (Nigella sativa L.). The field was prepared by the use of automated plowing of the experimental field twice (orthogonal), followed by leveling and fertilizing with DAP (Diamino phosphate).

The experiment included two factors:

1. Humic acid concentrations (10 mg. L-1, 20 mg. L1- as well as the control treatment).
2. Concentrations of gibberellin acid (25 mg. L-1, 50 mg. L-1, 100 mg. L-1 - as well as the control treatment).

In this study, the design used was Randomized Complete Block Design (RCBD) with a factorial design (3 x 4 x 3). The experiment was done on three replicates where each replicate contained twelve experimental units.

Determination of the leaf content of the plant growth regulators auxin, gibberellin, kinetin and abscisic acid in a High-Performance Liquid Chromatography (H.P.L.C.).

The technique of Ünyayar et al (1996) was adopted to isolate plant hormones following the Fast Liquid Chromatography (FLC) technique, which entailed taking 10 mg of the sample (leaves of the plant) and grinding it thoroughly to make it into a paste-like form by mixing it with 60 ml of a combination of methanol (12V), ammonia (5V) and chloroform (3V). Afterward, filtration and centrifugation of the mixture were done at a speed of 6000 rpm for 15 seconds, after which the filtrate was mixed with 25 ml of non-ionic water and then the chloroform phase was discarded while the methanol phase was evaporated and dried using a rotary evaporator at 30°C. The obtained mixture was dissolved and the pH adjusted to 2.5 and then injected using the Japanese Shizmadzu LC 2010A H.P.L.C.

- Liquid phase: 1% acetic acid in methanol+ 1 mmol tetrabutyl ammonium phosphate + acetonitrile, particles size 3µm (50 x 2.1 mm lid), UV detector, wave length 280 nm, injection volume 20 ml. The temperature is 30 °C, since the retention time of the beam spaces were determined for standard samples

Unknown substance = (pattern packet area)/ (standard packet area) x standard solution concentration x number of dilutions.

Statistical analysis.

The data analysis has been conducted using the statistical software SAS-Statistical Analysis System (2012). The factorial experiment (3×4×3) and Randomized Complete Block Design (RCBD) have been used for the analysis of the effect of various factors on the characteristics under investigation. The significant differences among the means have been compared by using the LSD – Least Significant Difference test at (0.05) probability level.

3. Results and discussion

Auxin content (mg.100gm-1 fresh weight) in leaves.

The findings of Table (1) proved the significant effect of increasing concentrations of humic acid in the increasing of auxin content; the highest average was 15.90 mg. Humic acid, which reached 13.44 mg. 100 gm-1 of fresh weight. The same table indicated the significant effect of gibberellin acid in increasing the auxin content in increasing the element's concentration from zero to 100 mg. l-1 the average increased from 12.11 to 16.59 mg. 100 g-1 of fresh weight.

The findings of the table on the binary interaction between the concentrations of humic acid and gibberellin proved the existence of significant differences in the increase in auxin content, and the highest was at concentrations 20 and 100 mg. 1liter- of humic and gibberellin acids, which reached 17.78 mg. 100 g-1of fresh weight. The lowest was 8.41 mg. 100g-1 of fresh weight at zero concentration of humic and gibberellin acids.

Table (1): auxin in the leaves of black seed under the influence of humic acid, gibberellic acid, and their interaction.

Concentrations of Humic acid ($mg.L^{-1}$)	Concentrations of Giberellin acid ($mg.L^{-1}$)				Average
	0	25	50	100	
0	8.41	15.59	15.94	17.42	14.34
10	13.70	13.00	12.48	14.58	13.44
20	14.21	15.63	15.96	14.78	15.90
L.S.D (0.05)	Humic acid× Giberellin acid=1.020				Humic acid = 0.510
Average	12.11	14.74	14.79	16.59	
L.S.D (0.05)	Giberellin acid=0.625				

Gibberellin content (mg.100gm-1 fresh weight) in leaves.

The outcomes of Table (2) illustrated the significant role of increasing the concentrations of humic acid in increasing the amount of gibberellins, where the highest average was 20.40 mg. Humic acid, reaching 18.99 mg.100 g-1 fresh weight. Moreover, the same table indicated that there was a significant impact of gibberellin acid in increasing the amount of gibberellins by increasing the concentration of the element from zero to 100 mg. L1 - The average concentration increased from 17.16 to 22.99 mg. 100 g-1 fresh weight.

Furthermore, the outcomes of the table for the interaction effect between the concentrations of humic acid and gibberellin revealed that there were significant differences in increasing the amount of gibberellins. The highest value was at concentrations 10 and 100 mg. 1 liter - of humic and gibberellin acids, which reached 24.87 mg. 100 g-1 fresh weight. While the lowest value was 14.28 mg.100gm-1 fresh weight. At zero concentration of humic and gibberellin acids.

Table (2): gibberellin-in the leaves of black seed under the influence of humic acid, gibberellic acid, and their interaction.

Concentrations of Humic acid ($mg.L^{-1}$)	Concentrations of Giberellin acid ($mg.L^{-1}$)				Average
	0	25	50	100	
0	14.28	18.32	19.65	23.98	19.06
10	18.21	18.00	20.51	24.87	20.40
20	19.00	18.59	18.27	20.11	18.99
L.S.D (0.05)	Humic acid× Giberellin acid= 2.630				Humic acid= 1.315
Average	17.16	18.30	19.48	22.99	
L.S.D (0.05)	Giberellin acid =1.947				

Kinetin content (mg.100gm-1 fresh weight) in leaves.

The findings of Table (3) indicated a significant effect of raising the concentrations of humic acid in raising the concentration of kinetin, where the highest average is 19.25 mg.100gm-1 fresh weight at the concentration of 20 mg.l-1 of humic acid concentration, and the lowest average at zero concentration of humic acid, which equaled 18.50 mg.100gm-1 fresh weight. The same table indicates a significant effect of gibberellin acid in raising the concentration of kinetin by raising the concentration of the element from zero to 100 mg. L1 - The average concentration rises from 15.54 to 23.70 mg. 100 g-1 fresh weight.

The results of the table for the interaction of the concentrations of humic acid and gibberellin show that there are significant differences in raising the concentration of kinetin, where the highest value of concentrations 20 and 100 mg. 1 liter - of humic and gibberellin acids, as it equals 25.01 mg. 100 g-1 fresh weight. The lowest value is 14.52 mg.100gm-1 fresh weight. At zero concentration of humic and gibberellin acids.

Table (3): kinetin in the leaves of black seed under the influence of humic acid, gibberellic acid, and their interaction.

Concentrations of Humic acid ($mg.L^{-1}$)	Concentrations of Giberellin acid ($mg.L^{-1}$)				Average
	0	25	50	100	
0	14.52	16.81	20.57	22.10	18.50
10	16.91	18.32	17.49	24.00	19.18
20	15.19	17.38	19.41	25.01	19.25
L.S.D (0.05)	Humic acid× Giberellin acid= 0.958				Humic acid= 0.479
Average	15.54	17.50	19.16	23.70	
L.S.D (0.05)	Giberellin acid=0.518				

The content of abscisic acid (mg.100gm-1 fresh weight) in the leaves.

The outcomes of Table (4) have illustrated the important role played by the rise in concentrations of humic acid in enhancing the level of abscisic acid content, and the maximum average concentration was 13.60 mg. 11.30 mg.100gm-1 fresh weight. Also, the same table illustrated the important role played by the increase in the concentration of gibberellin acid in increasing the abscisic acid content, where the concentration has ranged between zero to 100 mg. L1 - The average concentration has ranged between 9.41 to 15.31 mg. 100 gm-1 fresh weight.

However, the outcomes of the table regarding the binary interaction of concentrations of humic acid and gibberellin have proved the existence of important differences concerning the rise in the levels of abscisic acid content, and the highest average was achieved through the use of concentrations 10 and 100 mg. 1 liter of humic and gibberellin acids with an average of 16.42 mg. 100 g-1 fresh weight. While the lowest value was 9.08 mg.100gm-1 fresh weight. At zero concentration of humic and gibberellin acids.

Table (4): abscisic acid in the leaves of black seed under the influence of humic acid, gibberellic acid, and their interaction.

Concentrations of Humic acid ($mg.L^{-1}$)	Concentrations of Giberellin acid ($mg.L^{-1}$)				Average
	0	25	50	100	
0	9.08	14.07	14.00	13.87	12.76
10	10.11	12.57	15.31	16.42	13.60
20	9.05	10.00	10.51	15.65	11.30
L.S.D (0.05)	Humic acid× Giberellin acid= 1.368				Humic acid= 0.684
Average	9.41	12.21	13.27	15.31	
L.S.D (0.05)	Giberellin acid= 0.796				

As a result of the increased use of humic acid, an increase in the above-mentioned characteristics was observed. The cause of this increase is because of the role played by humic acid in the increase in the amount of IAA, needed for cell elongation and cell division (Mora et al., 2014), or the effect of humic acid in increasing the number of lateral radicals, leading to the increase in the number of lateral radicals, cellular differentiation, and cell elongation (Tahiri et al., 2015), while the cause of this increase is also because of the effect of humic acid in the increase of gibberellin acid (Elmongy et al., 2018) and its role in stimulating cell division and elongation, which causes the enlargement of cells by increasing the softness of their walls (Rai et al., 2017), or by activating some of the genes of cell chromosomes, resulting in the activation of DNA formation on which the formation of m-RNA depends, and then the formation of hydrolysis enzymes to provide cell growth and expansion.

Humic acid plays a key function in the formation of plant hormones to facilitate the synthesis of plant pigments, where the gibberellin hormone leads to an increase in chlorophyll content in plants through the internal effect on plastids (Mindari et al., 2014), and humic acids help in improving vital activities and cell balance and occur at a very high growth rate under ideal conditions for cell division and nutrient absorption by the roots due to increased root branching (Pettit, 2004).

The effect may be ascribed to the ability of humic acid to enhance the permeability of cell membranes, which impacts the cell membrane directly and increases its permeability, resulting in the facilitation of nutrient transport to places where their presence is required (Kumar and Singh, 2017), and the effect is due to the action of the active groups of hydroxyl and carboxylic acids in acids. Humic (Wang et al., 2017); moreover, the acid has an effect comparable to the hormone indoleacetic acid.

The process of activation of these enzymes is due to the functions of humic acid being an antioxidant and plays a role in controlling the expression of genes (Cordeiro et al., 2011) and helps increase the membrane permeability, osmotic regulation and intra-cellular regulation (Porfirio et al., 2016). The presence of humic acid as a complementary source of polyphenols helps function as the chemical and respiratory mediator and consequently leads to the increased life

functions of the plant since the enzyme system functions effectively (Schellekens et al., 2017).

The humic acids also increase the concentration of the proteins and amino acids and help activate the enzymes functioning in the Krebs cycle (Vaccaro et al., 2015), and the humic acids help increase the enzymatic activity such as catalase, superoxide dismutase, ascorbate and glutathione peroxidases (Nagasawa et al., 2016).

The above-mentioned factors increased as a result of application of gibberellic acid. This is caused by the physiological role of gibberellic acid in control of the activity of enzymes and activation of the metabolic process, for example, an increase in soluble carbohydrates due to the activation of the alpha enzyme (Shahzad et al., 2021) and production of the carboxylase enzyme after several hours of its addition, and also activation of formation of amino acids. The application of gibberellic acid leads to the fact that the processed food is shifted in the direction of growth points (Danziger and Bernstein, 2021), auxin along with gibberellic acid (GA) promotes cell division and elongation, and it regulates the growth of the plants and proves the important role of these hormones and their interaction in regulation of fruit initiation and development. In addition, recent researches have proved molecular and genetic evidence of the interaction of ARF/IAA and DELLA proteins in the regulation of auxin and GA signaling pathways in response to auxin and GA during the development of fruits (He and Yamamuro, 2022).

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