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RESEARCH ARTICLE

Improving the growth of black cumin (*Nigella sativa L.*) by humic acid *and Trichodermia harzianum* as *biofertilizer*

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Abstract

A field experiment was carried out in one of the agricultural fields which belongs to the Khalis district in Diyala Governorate, Iraq, located at a distance of 16 km northeast of Baqubah, during the fall season of the year 2021-2022 in a soil with a clay texture. An experiment was carried out with the aim of studying the effect of organic fertilization with four levels of humic acid, namely 0, 2 $g.M^{-2}$ and 4,6 $g.M^{-2}$ and bio-fertilization with the Trichodermia harzianum fungus, which was added at a rate of 0.3 gm, affected some vegetative traits of *Nigella Sativa L.* The results showed that the treatment of adding humic acid at the level of 6 gm^{-2} was significantly superior in terms of plant height, number of branches and dry vegetative weight, which amounted to 53.51. cm and 16.01 branches. plant-1 and 31.89 gr. plant-1, respectively, and the treatment of adding humic acid at the level of 4 $g.m^{-2}$ was significantly superior, with the highest mean of the index of chlorophyll in the leaves reaching 32.02 SPAD, compared to the control treatment, which recorded the lowest averages for the mentioned traits, respectively. Plant height, number of branches, dry vegetative weight and chlorophyll index, which were 48.30 cm and 16.71 branches. plant -1 and 43.37 g. plant-1 and 34.31 SPAD, respectively, compared to the control treatment, which amounted to 29.61 cm and 6.58 branches. plant-1, 27.25 gm^{-1}, and 27.50 SPAD, respectively.

Keywords: Organic fertilization, bio-fertilization, black seed, vegetative traits

Introduction

Medicinal plants had a great importance in many fields, including therapeutic, complementary, preventive and nutritional medicine, the pharmaceutical and food industries, and biological control because of that they contain many effective compounds (Shakya, 2016). Among those medicinal plants is the black cumin plant, *Nigella sativa L.,* which is an annual herbaceous plant belonging to the Ranunculaceae family, which is considered as one of the most important medicinal plants used in direct medication or after extracting the active compounds. It was found that the organic and biological fertilizers are good alternatives that are cheap and environmentally friendly, and that they play an important role in fixing nitrogen and improving the nutritional status of plants, as well as increasing the absorption efficiency of phosphorus, potassium and other elements, which leads to an increase in plant size and productivity (Abd El-lattief, 2012). As organic fertilization is considered one of the most important agricultural processes and it better maintains soil fertility, preparation of nutrients and the ability to exchange cations and anions, furthermore, the organic fertilizers release nutrients quickly and thus provide nutrients continuously (Wan et al., 2021).

Humic acid is an important humic organic acid because it contains many nutrients, which prompted researchers to add it to agricultural crops (Berger and Gutjahr, 2021). Humic acid works to improve the chemical and physical properties of the soil and significantly increase the biomass as well as provide nutrients to the plant and reduce the volatilization of nitrogen in the form of ammonia gas and also increase the efficiency of ammonia representation (Muter et al., 2015 and Ehsan et al., 2016). Trichoderma harzianum is considered to be one of the imperfect fungi that live sparsely on organic matter. It is present in the rhizosphere and greatly affects plant growth because of its high ability to settle in the root zone and secrete many organic compounds that contribute to stimulating plant growth, as well as lowering the soil reaction number (pH) which leads to an increase in the readiness and absorption of some important micronutrients such as iron and manganese, as well as their secretion of many biochemical

compounds (Hermosa et al., 2012) that are involved in the metabolic processes, so the current study aimed to test the effect of organic fertilization with humic acid and bio-fertilization with Trichoderma harzianum fungus in Vegetative characteristics of black cumin plant which is grown under field conditions

Materials and Methods

A field experiment was carried out in one of the agricultural fields in Al-Tahweelah district/ Al-Khalis district/ Diyala governorate in a soil with a clay texture. Then and there, the experimental field was divided into three sectors, each sector includes eight experimental units. All of experimental units were fertilized with phosphate fertilizer at a rate of 120 kg ha^{-1} in the form of triple superphosphate P2O5 46% mixed with the targeted soil before planting. Nitrogen was added at a rate of 120 kg. ha^{-1} in the form of urea N% 46 in two batches, the first batch is one month after germination, and the second batch at the beginning of the flowering stage. Potassium fertilizer was added at a rate of 100 kg ha^{-1} in the form of potassium sulfate K_2O% 41.5, and humic organic fertilizer was added in two batches, the first is one month after germination and the second is two months after the first one, at a concentration of 0 g. m^{-2}, 2 g. m^{-2}, 4 g. m^{-2}, 6 g. m^{-2}. The seeds of the local black cumin were sown on 11/19/2021 by placing 3 seeds-5 seeds in each jar. As for the bio-fertilization, it was done by mixing the *Trichoderma harzianum* bio-fungi with seeds at the same time in the sowing stage, at the rate of 0.3 gm per jar. With a distance of 25 cm between one pot and another, the irrigation was according to the plant's need. The plants were thinned to one plant per pot, and the plant was harvested on 7/5/2022 for the middle lines of each experimental unit.

Measurement of some characteristics of vegetative growth

Ten plants were taken randomly from the two middle lines of each experimental unit for the purpose of studying the characteristics of vegetative growth.

Plant height (cm): The height of ten plants was measured randomly from the two middle lines using a measuring tape from the ground level to the top of the plant and the average height of one plant was taken.

Number of branches of the plant: The number of branches was calculated in ten randomly taken plants, according to the average number of branches for each plant.

Dry weight of total sprouts: The dry weight of the vegetative in total is calculated, if the vegetative parts were dried in the electric oven at a temperature of 80°C for 48 hours, and the dry weight were calculated by using a sensitive scale.

Determination of leaf chlorophyll index (SPAD): Chlorophyll in SPAD was estimated in the field using a 502 Chlorophyll-meter (Blackmer and Schapers, 2013).

Results and Discussion

The results in Tab.1 show that the organic fertilization with humic acid led to a significant increase in plant height, and the highest average plant height reached up to 53.51 cm when the fertilization treatment had a concentration of 6 gm. cm, with an increase of 63.09%. The reason may be due to the fact that organic fertilizers, especially the hydrolyzed ones such as humic acid fertilizer, improve the fertile and physical properties of the soil, which increases the readiness of the macro and micro elements necessary to build plant cells and to carry out vital processes (Dhiab, 2012) as well as its effective role in increasing cell division and cell elongation (Aziz and Al-Salman, 2021). The results of Tab. 1 also showed that there were significant differences in the increase of plant height rates when inoculated with the *Trichoderma harzianum* fungus, as the treatment with fungus fertilization gave the highest average plant height of 48.30 cm, compared to the non-fertilization treatment with fungus, which gave the lowest average plant height of 39.99 cm, with an increase rate which is amounted to 20.78%..The reason may be due to the ability of the fungus to increase the availability of nutrients in the soil such as phosphorus, potassium, iron, zinc and copper, and then increase the plant content of these elements (Singh, 2010).

The interaction between humic acid and fertilization with the *Trichoderma harzianum* fungus resulted in a significant increase in plant height, as the interaction treatment was given at the level of 6g. m^{-2} of humic acid and inoculated with the *Trichoderma harzianum* fungus at a concentration of 0.3 g. The highest average plant height reached 61.60 cm, with a percentage of an increase of 108.03% over the controlled treatment, which gave the lowest average of the plant height of 29.61 cm.

Table 1: The effect of organic and biological fertilization and the interaction between them on the height of the black cumin plant (cm)

fertilization with *T.harzianum* fungus	fertilization with humic acid ($g.m^{-2}$)				Organic fertilization
	6	4	2	0	Bio-fertilization
39.99 b	45.43 c	43.33 c	41.6 c	29.61 e	**Without fertilization**
48.3 a	61.6 a	50.2 b	45.41 c	36.01d	**fertilization with T.harzianum fungus**
	53.51 a	46.76 b	43.5 c	32.81d	**fertilization with humic acid**

Number of branches (plant branch 1-)

The results in Tab. 2 showed that the number of branches in the black seed plant increased significantly with the superiority of

the level 6 gm.m^2 over the rest of the levels of humic addition, as the average number of branches reached 16.01 branches. Plant-1 compared to the treatment without adding humic acid, which recorded the lowest number of 10.44 branches. Plant 2-, with an increase rate of 53.35%. The reason is attributed to the importance of humic acid in increasing cell division and cell elongation due to its direct effect on various vital processes in plants such as respiration, photosynthesis, protein synthesis and various enzymatic reactions, as its effect is almost similar to that of plant hormones and has a role in increasing the absorption of nutrients and thus increasing Synthetic substances accumulated in the plant. (Zhang et al., 2017; Muhammad Amin, 2022). It is also noted that there are significant differences in the number of branches of the black cumin plant when inoculated with the *Trichoderma harzianum* fungus, as the treatment of fertilization with the *Trichoderma harzianum* fungus at a concentration of 0.3 g gave a significant increase in the number of branches, reaching 16.71 branches. Plant 1 compared to the treatment of not adding the fungus, which recorded 9.33 branches. Plant 1 with an increase of 79.09%, and the reason is due to the role of bio-fertilizers in increasing the supply of the nitrogen element, which has an important role in the production of the auxin hormone, which encourages cell division and elongation, as well as the entry of the nitrogen element into the production of cytokinin, which stimulates the growth of lateral shoots and an increase in the number of branches (Vidakovic - PaPic, 2000; Jamir, 2021). The interaction between humic acid and fertilization with *Trichoderma harzianum* fungus led to a significant increase in the number of branches, as the overlap treatment was recorded at the level of 6 gm plant-2, and inoculation with *Trichoderma harzianum* fungus at a concentration of 0.3 gm had the highest average number of branches reaching 19.76 plant branches -1 compared to the control treatment, which amounted to 6.58. Branch Plant-1.

Table 2: **The effect of organic and biological fertilization and the interaction between them on the number of branches of the black cumin plant**

fertilization with *T.harzianum* fungus	fertilization with humic acid (g.m^{-2})				Organic fertilization
	6	4	2	0	Bio-fertilization
9.33 **b**	12.26 cd	10.98 ed	7.5 fe	6.58 f	**Without fertilization**
16.71 **a**	19.76 a	17.26 ab	15.53 bc	14.3 bcd	**fertilization with T.harzianum fungus**
	16.01 a	14.12 ab	11.51 bc	10.44 c	**fertilization with humic acid**

The dry weight of the sprout

The results in Tab. 3 showed that the dry weight of the sprout of the black cumin plant increased significantly when adding humic acid at the level of 6g. m^{-2}, as the average dry weight of the sprout was 41.56 g. plant-1, compared to the treatment without adding humic acid, which recorded 31.89 gm plant-1, with an increase of 30.32%. The reason for the increase may be attributed to the fact that the addition of humic acid improves the physical properties of the soil, increases the readiness of nutrients, provides the plant with the necessary nutrients because of the nutrients it contains, and lowers the pH in the soil, which contributed to the dissolution of some of the nutrient compounds and thus increased its readiness, which leads to increased vegetative growth and Plant dry weight gain (Nosir and Abdelkade, 2017; Mohamed, 2013).

We also notice from the results of Tab. 3 that there was a significant difference in the dry weight of the sprout when inoculated with the *T. harizanum* fungus, as the fertilization treatment with mushrooms gave a significant increase of 43.37 gm plant-1 compared to the treatment without adding mushrooms, which recorded 31.00 gm plant-1, an increase of 39.90%. This increase is due to the role of *Trichoderma* in elevating the availability of nutrients, especially phosphorus and nitrogen, as well as the secretion of growth-stimulating substances (Al-Saffar et al., 2016). Also, the treatment of interaction between humic acid and fertilization with *Trichoderma harzianum* resulted a significant increase in the dry weight of the shoot, as the The overlap treatment at the level of 6 gm^{-2} of humic acid and inoculation with T. harizanum fungus at a concentration of 0.3 g gave the highest mean of 48.86 gm^{-1}, compared to the control treatment that gave the lowest mean of 27.25 gm M^{-1} the effect of organic and biological fertilization and the interaction between them on the dry weight of sprouts of black cumin plant (gm plant-1) (Tab. 3)

Table 3: **The effect of organic and biological fertilization and the interaction between them on the dry weight of sprouts of black cumin plant (gm plant-1)**

fertilization with *T.harzianum* fungus	fertilization with humic acid (g.m^{-2})				Organic fertilization Bio-fertilization
	6	4	2	0	
39.99 **b**	34.26 cd	31.75 d	30.76 ed	27.25 e	**Without fertilization**
48.3 **a**	48.86 a	45.26 ab	42.83 b	36.53 c	**fertilization with *T.harzianum* fungus**
	41.56 a	38.5 b	36.8 b	31.89 c	**fertilization with humic acid**

Chlorophyll Index (SPAD)

The results in Tab.4 showed that the index of chlorophyll in the leaves of the black cumin plant increased significantly when adding

humic acid at the level of 4gm², as the average index of chlorophyll SPAD was 32.02, compared to the treatment without adding humic acid, which gave 29.98 SPAD, an increase of 6.80%. The reason is due to the fact that humic acid has a role in increasing the content of leaves of magnesium, which leads to an increase in the content of leaves of chlorophyll (Bhatla and Manju, 2018). The results also showed that there were significant differences in increasing the average index of chlorophyll when inoculated with the *Trichoderma harzianum* fungus, as it reached 34.31 SPAD, compared to With the treatment of not adding mushrooms, which gave 28.70 SPAD, with an increase of 19.54%, this is attributed to the role of mushrooms in increasing readiness and absorption of nutrients, which leads to an increase in leafy area and thus an increase in chlorophyll concentration in plant leaves (Cairney, 2000). The *Trichoderma harzianum* fungus resulted in a significant increase in the chlorophyll index of 34.73 SPAD, compared to the control treatment, which gave the lowest average of 27.50 SPAD, with an increase of 26.29%.

Table 4: The effect of organic and biological fertilization and the interaction between them on the average index of chlorophyll in the leaves of the black cumin plant (SPAD).

Fertilization with *T.harzianum* fungus	Fertilization with humic acid (g.m^{-2})				Organic fertilization
	6	4	2	0	Bio-fertilization
39.99	29.32	29.36	28.65	27.5	**Without fertilization**
b	bcd	bcd	cd	d	
48.3	34.73	36.25	33.8	32.46	**fertilization with T.harzianum fungus**
a	ab	a	abc	ad	
	32.02	32.8	31.22	29.98	**fertilization with humic acid**
	a	a	a	a	

References

Abd El-Lattief, E. A. (2012). Improving Bread Wheat Productivity and Reduce Use of Mineral Nitrogen by Inoculation with Azotobacter and Azospirillum Under Arid Environment in Upper Egypt.International Conference on Applied Life Sciences (ISALS).

Al-Saffar, Fawz Abd al-Salam, Abd al-Razzaq Khader, and Najwa Ibrahim A. Al-Barhawi (2016). The Capacity pneumonia Klebsiella Bacteria in fixing the atmospheric nitrogen. University of Al Mosul. *Tikrit J. Pure Sci.*, **5**.

Amin M., Emad A. (2022) The effect of adding agricultural sulfur and humic acid on the growth and yield of black seed. Master Thesis, College of Education for Pure Sciences, University of Diyala.

Aziz, Basman Wissam and Tawfiq Bashir Al-Salman. (2021). The effect of bioinoculation with Klebsiella pneumoniae and Glomus mosseae fungi, and the role of humic acid and the biocide Trichoderma harzianum on the yield and percentage of oil in Nigella sativa. Kirkuk University *J. Agric. Sci.*, **12**, 89-102.

Berger, Fand Gutjahr, C (2021) Factors affecting plant responsiveness to arbuscular mycorrhiza. *Curr. Opin. Plant Biol.*, **59**:1-9.

Bhatla, S C., Manju A. L. (2018). Plant Physiology, Development and Metabolism. *Springer*:1-1251.

Blackmer T.M., Schapers J.S. (2013). Use of a chlorophyll meter. *Production*.

Borges L. E., Baldotto M. A. (2015). Growth and production of ornamental sunflower grown in the field in response to application of humic acids. Ciencia Rural, *Santa Maria* **45**:1000-1005.

Cairney, J. W. (2000). Evolution of Mycorrhizal Systems. *Naturwissenscha Ften,* **87**:467-475.

Dhiab, N. S. (2012). Using phosphate rock and superphosphate and adding fertilizers fungal and bacterial growth and yield of potatoes. PhD thesis. Horticulture Department, College Agriculture Baghdad University.

Ehsan, S. Javed, Saleem I., Niazl A. (2016). Effect of Humic Acid on Micronutrient Availability and Grain Yield of Wheat *Aestivum riticum* L.). *J. Agric–Res,* **54**:173-184.

Hermosa, R., Viterbo, A., Chet, I., Monte, E. (2012) Plant-beneficial effects of Trichoderma and of its genes**.** *Microbiology***, 158,** 17-25**.**

Jamir, A. R., Chattopadhyay, N., & Momin, M. C. (2021) Effect of biofertilizers with various levels of inorganic nutrients on growth and yield of black cumin (Nigella sativa L.) var. Azad Kalonji. *Environ. Conserv. J.*, **22**, 73-78.

Amin M., Emad A. (2022) The effect of adding agricultural sulfur and humic acid on the growth and yield of black seed. Master Thesis, College of Education for Pure Sciences, University of Diyala.

Muhammad, Ibtisam Jassim. (2013) The Effect of organic fertilization on some soil characteristics, growth and production Phthalate option. Master thesis, College of Agriculture, University of Baghdad.

Muter O., B. Limane ; S. Strikauska., M. Klavins (2015) Effect of humic-rich peat extract on plant growth and microbial activity in contaminated soil. *Mater. Sci. Appl. Chem.* **doi: 10.1515/msac-2015-0012 2015/32.**

Nosir, W., & Abdelkader, M. A. (2017) Effect of natural additives on *Nigella sativa* growth in hydroponic system. *Future***.** 2: 11-30.

Papic – Vidakovic, T. (2000) An efficiency of Azotobacter soil. Novisad (Yugoslavia).

Shakya, A. K. (2016) Medicinal plant: Future source of new drugs. *International J. Herb. Med.* 4: 59-64.

Singh,V., Singh, P., Yadav, L., Awasthi, S., Joshi, B., Singh, R., Lal, R., and Duttamajumder,S. (2010) Increasing the efficacy of *Trichoderma harzianum* **fornutrient uptake and control of red rot in sugarcane.** *J. of Horticulture and Forestry***.** 2: 66-71.

Wan, L. J., Tian, Y., He, M., Zheng, Y. Q., Lyu, Q., Xie, R. J., Yi, S. L. (2021) Effects of Chemical Fertilizer Combined with Organic Fertilizer Application on Soil Properties, Citrus Growth hysiology, and Yield. *Agriculture***.**11: 1-15.

shui-qin Z., Liang Y., Zhi-an LIN., Yan-Ting LI, Shu-wen H., Bing-qiang Z. (2017) Advances in humic acid for promoting plant growth and its mechanism, *J.of plant.Nutri,and ferti***,** 23:1065-1076.