The effect of humic acid and nano-biostimulant on the growth, yield and some secondary metabolites of watercress plant

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Abstract
A field experiment was conducted during the 2023-2024 agricultural season in the agricultural fields of the Horticulture and Landscape Engineering Department at the College of Agriculture, Al-Qasim Green University, located in the city of Hillah, Babil Governorate. The experiment was conducted to study the effect of humic acid and some micro-nano elements on the growth and yield of the Egyptian watercress variety. This experiment was conducted in a (3x3) factorial manner, using a completely randomized block design (RCBD) with three replicates, each containing nine treatments. The first treatment was distributed with three levels of humic acid, and the second treatment was a nano-stimulant.
The results showed that the humic H3 treatment, 20 ml/liter of water, had a significant effect on the chlorophyll content and total plant yield, 41.59 and 44.8 g/plant, respectively. The biostimulant S3 treatment, S20 ml/liter of water, had a significant effect on the chlorophyll content and total plant yield. The use of humic acid at concentrations of 10 or 20 ml/liter of water, It had no significant effect on secondary metabolites and dry matter

Introduction

Rocket (Eruca sativa) is a plant of medicinal and nutritional importance. It is an annual winter herb belonging to the Brassicaceae family. It is widely cultivated in temperate regions year-round, with the exception of the hot and very cold months (Mohamed Rafid, 2009). Abu Zeid (1986) found that rocket is a vegetable crop of medicinal and nutritional importance. It is used as a spice or salad. It is an ornamental plant in many Mediterranean countries. Its leaves are rich in vitamins (ascorbic acid), C, B, and A. Elements such as phosphorus, calcium, sulfur, iron, iodine, and fiber are also present. The presence of these compounds gives the plant its distinctive flavor and pungent taste. However, high concentrations of these compounds are toxic to humans because they lead to iodine deficiency in the human body and consequently goiter (Boras et al., 2011; Al-Dajwi, 1996). Botanical Description:

Arugula has several local names, totaling approximately 103, which vary across languages and countries. It belongs to the Angiosperm family of the Dicotyledons, in the Brassicales order, in the Cruciferae family. Its old scientific name is Brassica eruca L., and its modern scientific name is Eruca sativa Mill (Gray and Blame Wilson, 1989).

Arugula has two rows of seeds, each 1.5-2 mm in diameter, yellow to brown in color, and characterized by its high oil content (Franco, 1971).

Humic acid (polymeric polyhydroxy acid), an organic acid produced naturally and a humic compound resulting from the decomposition of the material, increases the absorption of nutrients by the plant, especially when exposed to drought. It also increases the plant's protein content and the number of beneficial microorganisms in the soil (Hatwigsen and Evans, 2000). Humic acid contributes to the development of chlorophyll, the synthesis of sugars, amino acids and enzymes, and helps in the process of photosynthesis (Chen et al.). 2004 (It also leads to increased root growth

strength through increased dry and wet weight and increased lateral branching of roots (Sernella et al., 2002).

Nano is a nanotechnology term that refers to materials whose particles are one-billionth of a meter in size. This unit is used to express the diameter dimensions and scale of microscopic particles and their atoms, or the particles of composite materials (Raab et al., 2011). Secondary fertilizers have special advantages that differ from traditional fertilizers. They have proven effective when used with a number of plants in terms of growth and productivity due to their small size, high absorption, and increased surface area, which leads to an increased absorption surface. They also excel in their rapid impact and access to areas that normal molecules cannot reach, which improves the efficiency of nutrient use and makes elements more available to the leaves, thus increasing photosynthesis and increasing nutrient production in the plant. They also improve plant resistance to drought, diseases, and insects due to their high permeability, which leads to increased growth, improved production, and reduced loss of nutrients when using nano fertilizers compared to traditional fertilizers (Sohair). EL-Ramady et al., 2018; Husen et al., 2017; Siddiqi et al., 2018; and others. Seaweed extracts are natural extracts from wild plants, seaweeds, and algae. Seaweed extracts are an important source of macro- and micronutrients, and are easily absorbed by plants. They also contain amino acids, auxins, gibberellins, and cytokines, which stimulate root growth and antioxidants, improve the yield characteristics of many plants, and increase their resistance to stress, water stress, and insect pests (Verkleij, 1992; Thomas and Li, 2004).

Materials and Methods

A field experiment was conducted during the 2023-2024 agricultural season in the agricultural fields of the Department of Horticulture and Landscape Engineering at the College of Agriculture, Al-Qasim Green University, located in the city of Hillah, Babil Governorate. The experiment was conducted to study the effect of humic acid and some micro-nano elements on the growth and yield of the Egyptian watercress variety. The experiment was conducted in a (3x3) factorial experiment, using a completely randomized block design (RCBD) with three replicates, each containing nine treatments. The first factor was distributed with three levels of humic acid, and the second factor was distributed with three levels of nano-stimulants. The averages were compared using the LSD test at the level of 5 (Al-Rawi 2000). Study factors:

First factor: Adding humic acid to the soil

1- Comparative treatment without addition, symbolized as H0

2- Adding humic acid at a concentration of 10 ml L-1, symbolized as H2

3- Adding humic acid at a concentration of 20 ml L-1, symbolized as H4

Second factor: Spraying nano-stimulant on leaves

1- Comparative treatment without addition, symbolized as S0

2- Spraying nano-stimulant at a concentration of 2 ml L-1, symbolized as S2

2- Spraying nano-stimulant at a concentration of 4 ml L-1, symbolized as S4

Seeds were planted on October 19, 2023, in terraces with three rows for each experimental unit. They were planted on either side of the row, alternating between rows with a distance of 20 cm between rows and 20 cm between plants. Irrigation, pest control, and weed control were carried out as recommended (Al-Sabayleh and Al-Tarawn, 2007). An irrigation system was used. By dripping if it was twice a week in the

early stages of cultivation and then according to the plant's need and environmental conditions, humic acid was added to the soil surface in three batches, the first batch was on 11/19/2023, the second batch was on 12/7/2023 and the third batch was on 12/21/2023. The plant was sprayed with the nano stimulant in three sprays as well, the first batch was on 11/21/2023 and the second spray was on 12/7/2023 and the third spray was on 12/21/2023 sprayed on the plant.

Traits Studied

Relative Chlorophyll Content in Leaves:

1. The chlorophyll content in rocket leaves was estimated at flowering time using a SPAD-502 Chlorophyll Meter, taking readings from five plants per experimental unit and then averaging them (Minnotti et al., 1994). The readings were measured in SPAD units, as mentioned in (Jemison and Williams, 2006).

2. Percentage of Dry Matter in Leaves (%):

The percentage of dry matter in mature leaves was calculated by weighing 100 grams of fresh leaves, placing them in paper bags, and air-drying them indoors. The samples were then placed in an oven at 68°C until the weight stabilized, and the percentage was calculated. Percentage of Dry Matter = (Dry Weight of Sample x Wet Weight of Sample) x 100 (Al-Sahaf, 1989).

Carbohydrate content of leaves (mg/gm-1 dry weight -3)

The amount of carbohydrates in the leaves was estimated according to the method adopted by Herbert et al. (1971). 250 mg of the dried sample was crushed with 10 ml of water, then centrifuged. The filtrate was taken and the volume was made up to 10 ml with distilled water. 1 ml of the filtrate was taken and 1 ml of 5% phenol reagent and 5 ml of 80% sulfuric acid were added. The sample was then left to stand for 25 minutes at room temperature. The absorption intensity was measured at a wavelength of 490 nm. A blank was prepared in the same way without adding the sample. The standard curve was prepared using glucose.

Extraction of active compounds

The method used by Gregorio et al. (2020) was adopted, which involved taking 2 grams of rocket leaf powder and adding 40 ml of 80% (v/v) ethanol. This was placed in a 100 ml glass beaker, then placed in a shaking water bath at 32°C at 180 rpm for 72 hours. 4 - Estimation of total phenols (mg.g-1 dry weight)

The method described by Singleton and Rossi (1965) and modified by Gregorio et al., 2020, was followed. 1 ml of the extract was taken, 1 ml of distilled water was added, and 5 ml of Folin Ciocalteu's reagent at a concentration of 10% (v/v) was added. The sample was left for 8 minutes, then 4 ml of 7.5% sodium carbonate was added. The samples were left for 90 minutes at 25°C, after which the optical absorbance reading was taken at a wavelength of 765 nm. A calibration curve was created using gallic acid at concentrations ranging from 10 to 100 µg/cm3. After calibration, the readings were compared to mg.g-1 dry weight. 5- Estimation of Total Flavonoids (mg.g-1 dry weight)

The method used by Zhishen et al. (1999) was adopted. 0.5 ml of the extract was taken, 1.5 ml of methanol was added, and the mixture was well mixed. 0.1 ml of 10% aluminum chloride (AlCl3) solution was added, followed by 0.1 ml of 1M potassium acetate (CH3CO2K), followed by 2.8 ml of distilled water. The samples were left at room temperature for 30 minutes, after which the optical absorbance readings were taken at a wavelength of 510 nm. A calibration curve was created using catechin at concentrations ranging from 5 to 45 µg/cm3. After calibration, the readings were compared to mg.g-1 dry weight.

6- Total Yield (g/plant)

The total yield was calculated based on the yield of each experimental unit. The equation was then applied to calculate the total yield.

Total yield = Yield per plant * Number of plants

Results and discussion

1-Content relative Chlorophyll in papers Watercress Spade^{-1}

from during results table1We notice that addition The catalyst nano effect morally in rate chlorophyll Plant Watercress where She gave Treatment S3manure Nano higher value And it reached 41.77Spade^{-1}.as and that addition sour Humic I influenced morally in rate Chlorophyll and appeared in treatment H3And she gave41.59Spade^{-1}.But with regards To interfere And table same We notice that addition sour Humic The catalyst Vital effect morally in rate Chlorophyll where She gave Treatment H2S2higher value And it reached 43.73Spade^{-1}

table(1) impact sour Humic The catalyst Vital in growth And the result Classify Watercress(Egyptian) For class Content relative For chlorophyll in papers Spade^{-1}

an average sour Humic H	Motivator Vital nano Sml liter-1			sour Your day ml liter^{-1} H
	S3	S2	S1	
36.90	41.57	32.40	36.73	H1
41.22	40.27	43.73	39.67	H2
41.59	43.47	40.57	40.73	H3
	41.77	38.90	39.04	an average The catalyst Vital nano S
S= 2.661	H*S= 4.610		H= 2.661	0.05 LSD

2/acid effect Humic The catalyst nano in content Papers from Phenols

Show table2that addition The catalyst Vital nano did not Influence morally in content plant from Phenols where.like that It was noted also that addition sour Humic did not Influence morally. Also Found that Interference between The catalyst Vital nano And sour Humic did not It affects morally on content Papers from Phenols.

table2 impact sour Humic The catalyst Vital nano and interference Between them in content Papers from Phenols Plant Watercress(amalgam.sadness^{-1} weight dry)

an average Humic H	Motivator Vital nano Sml liter-1			Your day Hsadness liter^{-1}
	S3	S2	S1	
0.789	0.778	0.780	0.810	H1
0.795	0.779	0.802	0.804	H2
0.793	0.792	0.786	0.800	H3
	0.783	0.789	0.805	an average The catalyst Vital nano S
S= NS	H*S= NS		H= NS	0.05 LSD

3/acid effect Humic The catalyst nano in content Papers from flavonoids

table Below son that addition The catalyst Vital nano did not affects morally on content Papers from flavonoids.like that that addition sour Humic did not Influence morally in content Papers from flavonoids.with regards To interfere between The catalyst Vital And sour Humic We notice presence Differences Moral And effect morally in content Papers from flavonoids where She gave Treatment H2S2higher value And it reached36.21amalgam.sadness^{-1} weight dry

table3 impact sour Humic The catalyst Vital nano and interference Between them in content Papers from flavonoids Plant Watercress(amalgam.sadness^{-1} weight dry)

an average Humic H	Motivator Vital nano Sml liter-1			Your day Hsadness liter^{-1}
	S3	S2	S1	
35.28	35.55	35.14	35.13	H1
35.30	34.58	36.21	35.10	H2
35.18	35.39	35.26	34.90	H3
	35.17	35.54	35.04	an average The catalyst Vital nano S
S= NS	H*S= 1.147		H=NS	0.05 LSD

4-Carbohydrates

between from table Below that addition sour Humic did not Influence morally in rate Carbohydrates For plants.like that with regards To add The catalyst Vital We notice non presence Differences Moral between Transactions.And table We notice Ban Interference between sour Humic The catalyst Vital nano did not Influence morally in content Papers from Carbohydrates.

table4 impact sour Humic The catalyst Vital nano and interference Between them in rate Carbohydrates Plant Watercress amalgam sadness^{-1} weight dry

an average Humic H	Motivator Vital nano Sml liter-1			Your day Hsadness liter^{-1}
	S3	S2	S1	
3.55	2.50	3.50	4.65	H1
3.25	4.50	2.97	2.30	H2
3.65	2.92	3.55	4.49	H3
	3.31	3.34	3.81	an average The catalyst Vital nano S
S= NS	H*S= NS		H= NS	0.05 LSD

5/impact sour Humic biocatalyst nano The youngest in growth And the result plants Watercress For the description ratio Centennial For the material dry%

from during table5showed Results that addition sour Humic did not Influence morally on The material dry.But with regards To add The catalyst Vital showed Results Excellence treatment CONTROLon rest Transactions And it reached17.38%.When showed treatment Interference Tuff Treatment H3S1higher value And it reached17.57%.

table5 impact sour Humic biocatalyst nano The youngest in Growth and outcome plants Watercress For the description ratio Centennial For the material dry%

an average Humic H	Motivator Vital nano Sml liter-1			Your day Hsadness liter^{-1}
	S3	S2	S1	
15.83	15.73	14.83	16.91	H1
15.68	13.90	15.57	17.57	H2
16.49	14.97	16.83	17.57	H3
	14.87	15.74	17.38	an average The catalyst Vital nano S
S= NS	H*S= 2.968		H=NS	0.05 LSD

6-The result kidney

between table6that addition sour Humic I influenced morally in Result plant kidney where She gave Treatment H3higher value And it reached44.8sadness/plants.But with regards To add The catalyst Vital She gave Treatment S2higher value And it differed morally on rest Transactions And it reached46.0sadness/plants.like that with regards To interfere We notice that Treatment H3S2 I influenced morally And she gave higher value And it reached55.7

sadness/plants

table(6)impact sour Humic And some Elements nano The youngest in growth And the result Classify Watercress(Egyptian) For class The result kidney For plants sadness/plants

an average Humic H	Motivator Vital nano Sml liter-1			Your day Hsadness liter^{-1}
	S3	S2	S1	
39.9	35.9	43.5	40.3	0 H1
32.3	35.2	39.0	22.6	10 H2 ml
44.8	47.4	55.7	31.5	H3 20ml
	39.5	46.0	31.5	an average The catalyst Vital nano S
S= 5.52	H*S= 9.56		H= 5.52	0.05 LSD

that addition Motivator Vital nano may Hassan in a lot from Attributes studied From it Area Paper and chlorophyll like that The result kidney as in table number1And a table number6And Condolences that to entrance molecules nano For the abstract algae nano to papers plant via The gaps quickly Super And penetrate it easily And it is done Convert it to Vessels The carrier In a way more efficiency(Wang and others , 2013)

may Returns to containment Abstract algae nano on Elements Food And auxins, Gibberellins and Cytokines Which she has role big in incentivize split cells For tissues Vegetarianism And its extension And its expansion Which Leads to events balance in Operations Vitality and physiological inside tissues Vegetarianism Which Cause more efficiency construction Photoelectric) Strikand others , 2003and DonandCurry,2003)Which mirror on more Area Paper and chlorophyll table1

that addition sour Humic to soil Leads to more absorption Elements Food from before plant especially in condition Expose it For drought as And more from content plant from proteins And more number Neighborhoods microscopic Useful in soilHatwigsenand 2000, Evans,),And more sour Humic from development chlorophyll table1And gather sugars and acids The trustee and enzymes And helps in practical Composition Photoelectric Chen)and others.2004(as Leads to more power growth The group root from during more the weight dry And dates And more Branches Side For the roots more Result plant kidney table6 (Sernella,and others2002)

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