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 carried out during the growing season of 2023–2024 on the experimental fields of the Department of Horticulture and Landscape Engineering, College of Agriculture, Al-Qasim Green University, Hillah, Babil Governorate, to evaluate the effect of humic acid and some micro-nano elements on the growth and yield of Egyptian watercress. The experiment was conducted as a (3 × 3) factorial in a Randomized Complete Block Design (RCBD) with three replications (9 combinations). The first component factor was represented by three concentrations of humic acid and the second by a nano-biostimulant.

Studies has found that H3 (20 ml L^{-1}) treatment of humic acid significantly increased number of chlorophyll and total yield of plant (41.59 and 44.8 g $plant^{-1}$); and S3 (20 ml L^{-1}) treatment of nano-biostimulant is also significantly improved both traits. While, humic acid application at 10 and 20 ml L^{-1} did not alter secondary metabolite levels and dry matter significantly.

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 imparts the plant with its unique flavor and sharp taste. However, elevated levels of these compounds can be harmful to humans because they cause iodine deficiency in the body, which may result in 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, It enhances the plant's nutrient uptake, particularly during drought conditions. It also boosts the plant's protein levels and the population of beneficial microorganisms in the soil (Hatwigsen and Evans, 2000). Humic acid aids in chlorophyll production, the synthesis of sugars, amino acids, and enzymes, and supports 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 term in nanotechnology that describes materials with particles measuring one-billionth of a meter. This measurement is used to indicate the size and scale of microscopic particles, their atoms, or the particles within composite materials (Raab et al., 2011). Secondary fertilizers offer unique benefits that set them apart from traditional fertilizers. They have demonstrated effectiveness with various plants in promoting growth and productivity because of their small size, high absorption capacity, and larger surface area, which enhances their absorption surface. They also excel in their rapid impact and ability to access areas that normal molecules cannot reach. This enhances the efficiency of nutrient use and makes elements more available to the leaves, thereby increasing photosynthesis and boosting nutrient production in the plant. Additionally, they improve plant resistance to drought, diseases, and insects due to their high permeability. This results in increased growth, better production, and reduced nutrient loss 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 derived from wild plants, seaweeds, and algae. They serve as a vital source of macro- and micronutrients and are readily absorbed by plants. Additionally, they contain amino acids, auxins, gibberellins, and cytokines, which promote root development and contain antioxidants. These compounds enhance the yield traits of numerous plants and boost their resilience to stress, water scarcity, and insect pests (Verkleij, 1992; Thomas and Li, 2004).

Materials and Methods

A field study was performed during 2023-2024 growing seasons at the research fields of the Department of Horticulture and Landscape Engineering, College of Agriculture, Al-Qasim Green University, Hillah, Babil Governorate. The study aimed to investigate the effect of humic acid and different micro - nano elements on Egyptian watercress yield and growth. A factorial experiment (3x3) was used in a randomized complete block design (RCBD) with three replicates and nine treatment combinations, the first factor consisted of three concentrations of humic acid and the second factor consists of three levels of nano stimulants. The means were compared using the LSD test at the 5% level (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 proportion of dry matter in mature leaves was determined by weighing 100 grams of fresh leaves, putting them in paper bags, and air-drying them indoors. The samples were subsequently placed in an oven at 68°C until their weight remained constant, and the percentage was then calculated. Percentage of Dry Matter = (Dry Weight of Sample / Wet Weight of Sample) x 100 (Al-Sahaf, 1989).

Carbohydrate content of leaves (mg/gm^{-1} dry weight $^{-3}$)

Total carbohydrate content in leaf samples. This was determined by a colourimetric method based on the phenol–sulfuric acid reaction. 250 mg portions of oven-dried plant samples were homogenized with 10 ml of distilled water, and centrifuged to yield a clear extract. The supernatant was transferred to a separate dissolve in a final volume of 10 ml of distilled water. A 1 ml portion of this clear extract was mixed with 1 ml of 5% phenol solution, and 5 ml of 80% sulfuric acid was added dropwise, the mixture standing for 25 minutes at ambient temperature for the colour to develop. The absorbance was measured at 490 nm with a spectrophotometer. Blanks and standards (using pure glucose) were run simultaneously.

Extraction of active compounds

Gregorio et al. (2020) adopted the method involving taking 2 grams of rocket leaf powder and adding 40 ml of 80% (v/v) ethanol. This mixture was placed in a 100 ml glass beaker and then kept 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 total phenolic content was determined using a modified Folin–Ciocalteu colorimetric assay. An aliquot of 1 ml of the plant extract was mixed with 1 ml of distilled water and added to 5 ml of diluted Folin–Ciocalteu reagent (10%, v/v) and allowed to react for 8 minutes. Thereafter, 4 ml of sodium carbonate solution (7.5%) was added to the mixture. The reaction mixtures were then kept at 25 °C for 90 minutes to allow the color to develop fully. Absorbance was read at 765 nm on a spectrophotometer and the quantity of the total phenolic was quantified using a standard calibration curve of gallic acid of 10- 100 µg ml^{-1} and the result expressed as mg g^{-1} dry wt.

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 ($AlCl_3$) solution was added, followed by 0.1 ml of 1M potassium acetate (CH_3CO_2K), 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}

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

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}

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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