The Effect of Spraying with kinetin and the Organic Fertilizer POT_MIN on the growth and Active Substance of Watercress (*Eruca sativa* Mill.)

S.S. Abdulhussein[1]*

1College of Biotechnology, Al-Qasim Green University, Babylon, Iraq

Corresponding author: sarh.s@biotech.uoqasim.edu.iq

Abstract

A field experiment was carried out during the fall agricultural season 2023-2024 in Al-Qadisiyah city, Iraq. The aim of this study was response of watercress (*Eruca sativa* Mill.) to spraying three levels of kinetin (0, 150, and 250 mg/ L^{-1}) and three levels of organic fertilizer (0, 3, or 5 mg/L^{-1}), and the interaction between them in growth, yield, and phenolic compounds of the watercress. The results showed that adding kinetin led to a significant increase in the studied traits, as it exceeded the second level B_1 (150 mg/L^{-1}) by giving it the highest values in the number of leaves (32.65 leaves^{-1}) and the yield of the experimental unit of leaves (5.35 kg. 4m^{-2}). Spraying of organic fertilizer had a significant effect on the studied characteristics, as the second level, F1 (3 ml/L^{-1}), was superior, as it gave the highest values in plant height (37.12 cm) and the number of leaves (30.95 leaves $^{-1}$). As for the interference treatments, the treatment achieved B1F1 (150 mg/L^{-1} + 3 mL/L^{-1}), a significant increase in plant height (43.85 cm), the number of leaves (37.2 leaves^{-1}), and the yield of the experimental unit of leaves (6.36 kg. 4m^{-2}). The results of the analysis were shown by a liquid chromatography High-performance. The B0F2 treatment 0+5 ml/L^{-1} gave the highest average values for the concentrations of phenolic compounds, which reached the concentration of gallic acid (0.11 mg/g^{-1}) and ferulic acid (1.52 mg/g^{-1}).

Keywords: Watercress seeds, organic fertilizer, kinetin, HPLC.

1-Introduction

Eruca sativa Mill. makes a comeback. The Brassicaceae family is a diverse group of plants believed to be native to Central Asia and Eastern Europe, and is well cultivated in Mediterranean countries [1]. Watercress leaves have good nutritional value, as each (100 grams) contains 2.6 grams of protein, 0.7 grams of fat, 3.6 grams of carbohydrates, 1.6 grams of fiber, in addition to vitamins, including vitamins A and C, and the elements sodium and potassium. Calcium, iodine, phosphorus, iron, zinc, sulfurous substances, vegetable oils, thiamine, riboflavin, niacin, peroxidine, and the spicy taste of watercress are due to the presence of the pungent mustard substance (sinigrin) [2]. Recent studies indicate that rocket oil reduces the percentage of total fat and cholesterol in the body [3]. Given the importance of the active compounds found in rocket from a medical perspective, there has been a trend towards producing these compounds and working to increase them. Hence, a necessity to study the factors that affect the amount of active compounds in rocket plants. Many agricultural treatments aim to enhance the

productivity of medicinal plants for active substances. The most important of their methods is the use of organic, biological, and chemical fertilizers [4] .

As [5](Al- Jarjari, 2015) indicated that when humic acid was added to watercress at a concentration of (4 gm L^{-1}) to a significant increase in some of the studied characteristics: plant height, number of leaves, and Leaves area, the percentage of dry matter of the second and third branches, the dry weight of the roots, percentage of flowering plants, product of the experimental unit, amount of vitamin C, percentage of sugars in the seeds, amount of alkaloids in the seeds, and the amount of phenols. Growth regulators play an important role in plant growth and improving its natural products. Many studies indicate that treating Plants with growth regulators improves plant growth, crop quality, seed production, and stimulates plant branching. This encourages cell division, increases their size, delays senescence, increases flowering, and improves fruit setting [6]. This study focused on studying the watercress, and treating it with the growth regulator and organic fertilizer POT-MIN, and observing the changes that occur in the watercress and the active compounds in the plant. Watercress is also affected by growth regulators, including Kinetin and a type of cytokinin, which is a type of plant hormone that encourages cell division. Kinetin was originally isolated by [7, 8].

Kinetin is part of a group of compounds known as cytokinins, a class of growth regulators in plants. Kinetin promotes cell division and is active in the processes of cellular growth and differentiation in plants. It also acts as an antioxidant, preventing oxidative damage caused by free radicals (Drugs.com). Watercress yield is also affected by growth regulators, as [9] showed that spraying with kinetin at a concentration of 50 mg/L had a significant effect on the fresh and dry weights, leaf area, and sugar content of the leaves. Organic fertilizers, which come mainly from crop residues such as rice bran, various oilseed cakes, and animal by-products such as meat bone meal, blood meal, fish meal, and crab meal, are sometimes distinguished from animal manure or animal waste-based compost. They contain high levels of nutrients, specifically, eg, Nitrogen in oilseed cake and blood meal, and P in rice bran and meat bone meal, which are also high in organic matter and a variety of micronutrients in general [10, 11].

This study aims to investigate the effect of spraying with kinetin on the content of active compounds in plant leaves, and to compare the effect of organic fertilizer and spraying with kinetin on the yield and growth of watercress, and the content of active compounds.

2-Materials and Methods

The experiment was carried out in the fall season of 2023-2024 in Al-Qadisiyah city, Iraq, to study the effect of spraying with kinetin and the organic fertilizer Butman on growth, yield, and active compounds in watercress. Samples were taken from a field experiment at a depth of 0_30 cm before planting to conduct physical and chemical analyses. Table No. 1 shows the results of the analyses conducted in the laboratories of the Soil and Water Department at the College of Agriculture, Al-Qasim Green University. Watercress seeds were brought, and the germination rate reached 90% in the test.

2-1 Average Plant Height (cm)

Plant height was measured using a graduated ruler.

2-2 Average Number of Leaves per Plant^{-1}

The leaves of all plants in one experimental unit were counted, and the average number of leaves per plant was calculated by dividing the total number of leaves in one experimental unit by the number of plants.

2-3 Total Yield per Experimental Unit (kg):

The dry weight of all plant leaves was measured using a sensitive balance, and an average was taken from each experimental unit.

2-4 Separation of phenolic compounds:

Take 5 leaves of each experimental unit and dry them on filter paper for (20) days at a temperature room with constant stirring to avoid rotting of theleaves and after drying grinded by the mill device until it turned To a finepowder then weigh (1) g of dried sample and put in a capacity of 200 ml, then add 50 ml of absolute ethanol solvent (99.8%) on the samples and soakthem for 48 hours and then filter the solution by Filter paper and thenconcentrate the filter using the rotary evaporator down to about (20) Raw mlfor all samples Hydrolysis Acid was performed by taking 5 ml of the extract Raw ethanolic and add 100 ml of HCL (1N) to it and put on an electric heaterto boil for half hour, then cool the solution and add to it) (30 ml of ethyl acetatein two stages and separated by separation funnel, Then he took the top layer,which is the total phenols [14]. Five phenolic compounds were measured from the extract of rocket leaves to provide standard samples, all of which are gallic acid and ferulic acid. The samples that were subjected to acid hydrolysis were determined by Shimadzu LC-20 AD HPLC after purification with diaphragm filters (0.1µM) using a wavelength of 210 nm and a flow rate of 1ml. min^{-1}, and the mobile phase (acetonitrile 85 + % H_2O 15%) using a column (C18) with dimensions of (250×4.6 mm). The concentration of each compound was calculated in the models according to[14]. The measurements were carried out under the separation conditions as shown in the equation:

$$Compound\ conc. = \frac{Area\ under\ the\ curve\ of\ the\ comp.}{Area\ under\ the\ curve\ of\ the\ comp.standard} \times Standardmaterial\ conc. \times \frac{Extract\ size}{Wt.of\ the\ milled\ samp.}$$

2.5 Statistical design

The experiment included 27 experimental units divided into three replicates of 9 units each. Then, the seeds were planted on October 26, 2023. The planted seeds germinated after 2-5 days of planting. Then, the seedlings were transferred to the field after the appearance of four true leaves on November 1, 2023 [12]. The experiment was carried out within the Randomized Complete Block Design (R.C.B.D) in three replicates, and the results were analyzed for the studied traits and compared the significant differences between Averages according to Duncan's Multiple Range Test and at the level of Probability 5% [13]. The first factor included the growth regulator kinetin and was symbolized by the letter B, and was distributed randomly on the repeaters, and included the following levels: (0, 150, or 250 mg/L^{-1}). The second factor included liquid organic fertilizer, POT-MIN, and was randomly distributed to repeaters. It is

denoted by the letter F and includes the following levels: (0, 3, and 5 ml/L^{-1}). Use POT-MIN, a commercial product processed by TroAgri Kimya San, and illustrate the table of (2) its basic components: The plants were sprayed twice, the first month after planting seedlings on October 26, 2023. The second spraying was a month after the first spraying.

Table (1): Some physical and chemical properties of field soil.

Value	Soil components
720	**Sand (gm.kg^{-1})**
220	**Silt (gm.kg^{-1})**
60	**Clay (gm.kg^{-1})**
Mixed sandy	**Soil texture**
7.37	**Degree of soil reaction (PH)**
1.52	**Electrical conductivity (dS.m^{-1})**
32	**Nitrogen (mg.kg^{-1})**
8.4	**Phosphorus (mg.kg^{-1})**
80	**Potassium (mg/kg^{-1})**
3	**Organic matter (g. kg^{-1})**
59	**Gypsum**

Table (2) Basic components of organic fertilizer POT-MIN under study

Concentration	Substance
25% w/w	humic acid
3%	Potassium
4—6	PH
30%	organic carbon
0 - 5 %	organic nitrogen
48%	organic matter

Table (3): Separation conditions of HPLC high performance liquid chromatography for extracted phenolic compounds from the leaves of the watercress

Specifications	Vocabulary of class conditions
4.6×250 mm c18	Separation column
85% Acetone +15%water	Mobile Phase
1 ml.min-1	Speed of Mobile phase molecules
5 ml	Cell size
210 nm	Wavelength
40 C	Separation temperature

3 - Results and discussion

3-1 Plant height (cm) :

Table 4 shows the effect of spraying kinetin and organic fertilizer, and their interaction on plant height. The table also indicates that the second level F_1 (3 ml. L-1) and the third level F2 (5 ml. L^{-1}) of organic fertilizer were significantly superior, reaching (41.12 cm) and (41.48 cm), respectively, compared to the comparison treatment (0 ml. L-1), as the plant height reached (37.12 cm). The same table also indicates that the plant height increased with increasing kinetin levels. The second level B1 was (41.38 cm), and the third level B2 was (39.59 cm), but it

Was not significant, while the comparison treatment reached (38.75 cm). While the interaction between organic fertilizer levels and quinine played a role in the superiority of the second level F1 (3 ml.L-1) of organic fertilizer and the second level of quinine B1 (150 $mg.L^{-1}$), which reached (43.85 cm^2), compared to the comparison treatment (37.60 cm^2), which did not differ significantly with most other treatments. The reason for the increase in plant height may be because humic acid increases the strength of root growth and thus increases the absorption of nutrients, which increases the process of photosynthesis and leads to pushing the upper growth upwards and thus leads to the elongation of the plant and works to activate some hormones in the plant such as cytokinin which stimulates cell division and auxin which helps cells to elongate and thus increases the height of the plant [15].

Table (4): Effect of kinetin Spraying and POT-MIN Organic Fertilizerand Their interaction on Plant Altitude Watercress (cm.plant $^{-1}$).

AVERAGE	250 mg.L	150 mg.L	0 mg.L		
AVERAGE	B2	B1	B0		
	b 36.52	b 37.25	b 37.60	F0	0 ml.L
b 37.12	ab 39.6	a 43.85	ab 39.93	F1	3 ml.L
a 41.12	ab 42.66	ab 43.06	ab 38.73	F2	5 ml.L
a 41.48	a 39.59	a 41.38	a 38.75	AVERAGE	

* Similar letters have no significant differences according to the Dunkin'polynomial test below level 5 %

3-2 Number of leaves.Plant^{-1}

Table 5 shows the effect of spraying kinetin and organic fertilizer andthe interaction between them on the characteristics of the number of leaves plant. The results of adding levels of organic fertilizer did not show any significant differences between the levels, either for kinetin levels, concentrations II and III (150 mg.l-1 and 250 mg.l.l-1) Significantly) (32.65)and (28.32) leaf.plant^{-1} on the comparison treatment (0 $mg.l^{-1}$), which amounted to (26.28) leaves.Plant^{-1}. As for the interaction between the levels oforganic

fertilizer and kinetin, it appears to be superior to the second level of the two treatments of organic fertilizer and kinetin B1F1, which amounted to (37.2) leaves.Plant^{-1}, which did not differ significantly from mostother transactions. The reason for the increase in the number of leaves may be attributed to the increase in kinetin, according to [12]. kinetin stimulates cell division and dilation as it leads to the formation of leafy buds(vegetative) by stimulating Tissue differentiation and development into leaves, as well as kinetin terminates apical dominance and thus stimulates the emergence of Leafy side shoots

Table (5): Effect of kinetin Spraying and POT-MIN Organic Fertilizerand Interaction between them on leaf count Watercress leaf plant. Plant^{-1}

AVERAGE	B2	B1	B0	level of kinetin
a 26.31	b 24.05	ab 30.05	b 24.84	F0
a 30.95	ab 30.40	a 37.2	b 25.25	F1
a 30.70	ab 30.53	ab 30.72	ab 30.85	F2
	ab 28.32	a 32.65	b 26.28	AVERAGE

* Similar letters have no significant differences according to the Dunkin'polynomial test below level 5 %

3-4 the yield of the experimental unit of leaves (kg4.pm^{-2}):

The results of Table 6 indicate the effect of kinetin and organic fertilizer and the interaction between them on the characteristics of the yield.The experimental unit of the plant notes that the organic fertilizer significantly affected the yield of the experimental unit of leaves, This increase was directly proportional to the increase in the level of fertilizer, and the F2 treatment (5 ml. L $^{-1}$) the highest increase it amounted to(4.59 kg.4m^{-2}) compared to the comparison treatment F0 (without fertilization) which gave the lowest rate of (3.59 kg). As for the levels of kinetin, the results showed that the second level B1 (150 Mg.L^{-1}), which amounted to (5.35 kg.4m^{-2}), compared to the comparison coefficient B0, which was (3.7 kg.4m^{-2}). For the interaction, the B1F1 treatment showed significantly superior to all coefficients at concentration (150 mg.L.) kinetin(3 ml/liter^{-1}) organic fertilizer. The reason for thein crease in yield in the experimental unit is the increase in the level of Organic fertilizer may be due to contains growth-stimulating substances such as hormones and vitamins, and this leads to increased plant weight [16]. As well as organic fertilizer works to form a total Good root and then increases the absorption of nutrients and their transfer to the parts of the plant, and this reflects positively on wet total Vegetative Weight [17]. Potassium has an important role in cell division. It activates enzymes that are involved in photosynthesis and protein formation, in addition to its important role in the mechanism of Opening and closing the stomata, which in turn increases the absorption of nutrients and thus increases the vegetative total [18]. The reason for the increase in vegetative qualities may be attributed to the role of growth regulators

Plants that encourage the action of plant nutrients, especially organic ones, which physiologically affect the increase in nutrient work [19]. as well as that cytokinin leads to an increase in the size of cells in the leaves of plants and, Thus, its amplitude, which in turn increases the wet weight of the vegetative system [20].

Table (6): Effect of kinetin and Organic Fertilizer POT-MIN and the interaction between them on the Yield of Experimental Unit of Leaves of Watercress Plant (4 m^{-2}.kg)

AVERAGE	B2	B1	B0	levels of kinetin levels pot-min
b3.59	cd3.40	bcd4.35	d3.03	F0
a5.05	abcd4.47	a6.36	bcd4.31	F1
a4.59	ab4.64	a5.36	b3.76	F2
	ab4.17	a5.35	b3.7	AVERAGE

* Similar letters have no significant differences according to the Dunkin polynomial test below level 5 %

Effect of Spraying Kinetin and Organic Fertilizer - POT-MIN and Their Interaction in the Concentration of Phenolic Compounds in Watercress Diagnosed by High Performance Liquid Chromatography (HPLC), It is noted from the results of Table (7) that the effect of spraying kinetin and organic fertilizer and the interaction between them on the concentration of phenolic compounds, all treatments were affected by the study factors, asthey appeared galic acid and ferulic acid in all treatments.

The results of Table (7) showed the appearance of the highest average of the galic acid, the highest average was obtained at the treatment (B0F2) 0.11 $mg.g^{-1}$ dry weight (compared to the lowest rate at the treatment rate) B0F1 (whose concentration was 0.001 $mg.g^{-1}$ dry weight), ferulic acid the highest average was obtained at the treatment (B0F2) 1.52 $mg.g^{-1}$ dry weight (compared to the lowest rate at the treatment rate) B0F0 (whose concentration was 0.007 $mg.g^{-1}$ dry weight).

The increase in phenolic concentrations may be attributed to the roleof organic matter in equipping the plant with the microelements N, P, K, which increased vegetative growth by increasing meristem cell division, growth and increase leaf surface area and the increase of processed nutrients in the leaves represented by carbohydrates and proteins necessary to build plant tissues, which is reflected in the increase in the proportion of phenolic compounds in the plant, and organic fertilizers and humic materials have an effective and vital effect in increasing the physiological activities of the plant and its reflection on growth and chemicalcomposition [21].

Also, the increase in phenol concentrations by increasing organic matter canbe due to the role of organic acids in increasing photosynthesis, which resultsin the compound (PEP) Phosphoenol pyruvate formed in the process of glycolysis, as well as Erythrose-4-phosphate formed in photosynthesis or Pentose phosphate pathway, and when these two compounds combine, they result in (Shikimic acid) and thus leads to an increase in the concentrations of phenolic compounds

in the plant as products of secondary metabolism [22].

Table (7) Effect of Spraying kinetin and Organic Fertilizer - POT-MINand Their Interaction in the Concentration of Phenolic Compounds in watercress Diagnosed with High Performance Liquid Chromatography (HPLC)

ferulic acid	galic acid	comound 1	
2.64min	3.23 min	standard R1 min	
Con. mg/g	Con. mg/g	Treatment	N
0.007	*	B0 F0	1
0.01	0.001	B0 F1	2
1.52	0.11	B0 F2	3
0.13	0.6	B1 F0	4
0.01	0.5	B1 F1	5
0.02	0.4	B1 F2	6
0.03	0.005	B2 F0	7
0.04	0.006	B2 F1	8
0.05	0.07	B2 F2	9

4-Conclusion

Spraying with the growth regulator kinetin at a concentration of (150 mg/L^{-1}) achieved the best significant increases in most of the experimental indicators. The B0F2 treatment (5 + 0 ml/L $^{-1}$) has the best results in phenolic compounds Spraying at three levels produced significant differences in all vegetative growth indicators. Spraying kinetin at a concentration of (150 mg/L $^{-1}$) has a positive effect in increasing the productivity of watercress leaves. Conducting further studies on other concentrations and growth regulators and using organic fertilizers can increase the growth and active compounds of watercress.

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