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Original Article

Effect of Humic Acid Concentrations and Methods of Applications on Growth and Development of Arugula vegetables (Eruca sativa Miller)

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Abstract

Eruca sativa commonly is known as arugula, which is one of the plants that have a nutritional and health value. However, arugula vegetables require nutrient-rich, well-drained soil, as poor or poorly drained soil can cause root rot and yield loss. Therefore, this study investigates the effects of various humic acid concentrations (HAC) and application methods (HAM) on the growth of Eruca sativa. Plants were cultivated in plastic pots under semi-field conditions at a farm in the Jouddam area, which is located around Zawia city, Libya. The plants were treated either by foliar spray or by irrigating with different humic acid (HA) concentrations (0, 0.25 and 0.5) %. The treatments were arranged in a factorial experiment following a completely randomized design with four replicates. Humic acid was applied three times during the growing season (at 25, 35, and 45) days after sowing. The findings showed that all evaluated traits were significantly affected by the applied treatments. Growth and yield traits demonstrated significant responses to both humic acid concentrations and application methods ($P< 0.001– 0.01$). The highest values of growth and yield traits were recorded for plants irrigated with a 0.5% humic acid compared to the control and other treatments. Whereas, the control recorded the lowest trait values. An increase in humic acid concentration consistently enhanced all studied traits. Besides, both application methods (spraying and irrigation) improved growth traits compared to the control, with irrigation proving more effective than foliar spraying. This study concludes that humic acid is highly beneficial for Eruca sativa, enhancing growth conditions and supporting robust plant production.

Keywords: Eruca sativa, Humic acid, Foliar spray, Irrigation .

Introduction

Eruca sativa Mill is an annual multi-purpose vegetable plant of Brassicaceae family, that has attracted attentions due to various medicinal and valuable traits such as high vegetative growth, excellent resistance to the biotic and abiotic stresses (Siahmarguee and Ghaderifar, 2017). The plant leaves have a high nutritional value and contain carbohydrates, fats, vitamins A, B1, B2, B3, and C, elements, glucosinolates and phenolic (El-Dabaa *et al.*, 2019; Amran and Abbass, 2024). In addition, studies found that there are some plant extracts include Eruca sativa plant that could be used as plant growth stimulator (Ehtaiwesh and Qarimidah, 2021). In

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addition, studies found that both Eruca sativa seed powder and Eruca sativa plant extracts could be used as bioherbicide to reduce the infection of some bacterial, fungal, and viral diseases (El-Dabaa *et al*., 2019; Hussein, 2021).

Some soils in North-Western region of Libya are characterized as an unfertile soil, therefore most of crops and vegetables cannot grow well, abiotic environmental factors induce pressures on crop growth and productivity (Abd El-Aziz, 2018, Ehtaiwesh, 2019). This stress negatively influences crops productivity. Therefore, finding integrated and compatible approaches should be adopted for obtaining healthy crops. Various approaches have been proposed to overcome some of the stresses that hinder plant production, including the use of eco-friendly methods. Some studies indicate the use of some substances, such as algal biochar, abscisic acid, salicylic acid, yeasts, as growth promoters (Abohbell *et al*., 2024; Ehtaiwesh, 2022; Ehtaiwesh, 2023; Boorboori and Li, 2024; Ehtaiwesh and Abuiflayjah. 2024; Xiang *et al*., 2024).

Humic acid is defined as complex organic substance that occurs naturally in soil as it forms through the decomposition of plant residues, seaweed and animal residue (Mahler *et al*., 2021). Humic acid structure includes mainly carbon, hydrogen, oxygen, nitrogen, and sulfur (Rupiasih, and Vidyasagar, 2005). Humic acid fertilizer refers to a fertilizer that can be used to provide nutrients to crops based on humic acid, humin, fulvic acid (De Melo *et al*., 2016; Jarukas *et al.,* 2021). The studies have indicated that humic acid had positive effects in stress mitigation (Cha *et al*., 2020; Qin and Leskovar, 2020; Shen *et al*., 2020; Ibrahim *et al*., 2024; Khaled and Fawy, 2011; Taha and Osman, 2018; Ali *et al*., 2020). Humic acid plays a critical role in supporting plant by enhancing soil conditions by boosting aeration, creating a looser and fluffier texture. It also enhances water retention, improve cation exchange capacity and remove salts from soil and benefiting plant health (Yang *et al*., 2021). Humic acid helps improve nutrient uptake by plant roots, ensuring improved growth and yield traits (Selladurai and Purakayastha, 2016; Ampong *et al*., 2022). Moreover, humic acid stimulates root system growth and branching, which allow plants to explore a greater soil volume, uptake more water and nutrients, and anchor plants securely (Canellas *et al*., 2008; Tavares *et al.,* 2021).

The current study hypothesizes focus on both the growth and yield of arugula vegetables (Eruca sativa) by using different concentrations of humic acid and its addition methods.

Materials and Methods

The experiment was conducted in the fall of 2022 at Jouddam area, Zawia city western region of Libya. Seeds of arugula were sown at a depth of 2-3 cm in 4kg plastic pots filled with Peat moss. In randomized complete design and four replications were maintained for each treatment. The experiment included three application methods, HA levels and sessions in days 25, 35 and 45. Before sowing, 0.25g/kg of urea (46% N) was added to each pot. After sowing, irrigation was applied at the appropriate times with tap water to maintain soil moisture near maximum water-holding capacity. Two weeks after sowing seedlings were thinned to two intact plants per pot and fertilized with (20:20:20 NPK) and micronutrient. Plants were treated with different humic acid concentrations as mentioned above by foliar spray or irrigation with different levels of humic acid solution (0, 0.25, and 0.5%). Twenty-five days later, pots were divided into three groups, each group represent Humic acid (HA) treatment. First group no HA was added (represent the control group), second group included two different concentration of HA (0.25 and 0.5%) were applied. At each HA concentration level, plants were treated either with HA foliar sprayed or HA added with irrigation water. Humic acid application was applied three times (25, 35, and 45 days of sowing by spraying the plants until complete covering of the plant or irrigating the plants by designed HA levels solution. Control was treated with water until complete covering of the plant or irrigated with water. 60 days of sowing, plants of Eruca sativa were harvested and plant samples from each treatment were collected for growth and yield traits data.

Data Collection

Sixty days of sowing, a plant was randomly harvested from each replicate for data collection. The plants were removed from peat moss carefully to not damage the root system, and then plants were carefully cleaned to remove peat moss particles that adhere to root system. Plant's shoots and roots were separated from each other, then the root system was washed thoroughly by deionized water to avoid any contaminations. Roots and stem diameter were measured (mm) using a meter scale. The shoot fresh weight was estimated in (g) per plant using a balance. The numbers of leaves plant−1 were counted, and then leaf area (cm2) was measured by Image program. Leaf samples were photographed with a ruler, and then leaf areas were calculated as cm2 using the Image program as described by Ahmad *et al.*, (2015) and Zabaleta *et al.*, (2024).

The relative growth rate (RGR) was calculated during two periods of plant growth at 25 and 60 days after planting using the equation of Meganid *et al.*, (2015).

RGR = (W2 – W1/t2-t1)* 100 ....(1)

Where, W is plant dry weight (g), t is the time (days), and the subscripts 1 and 2 are initial and the second sampling of plant weight.

Relative water content (RWC) was calculated according to Rady *et al.*, (2016), where leaf samples were weighed immediately after collection to obtain fresh weight (FW), and then leaf samples were placed in fresh water and kept in the dark. After 24 h, the turgid weight (TW) was obtained. For the dry weight (DW), the leaf samples were oven-dried for 24 h at 60°C until constant weight (Rady *et al.*, 2016), and then the RWC was then calculated following the formula :

RWC%=(FW– DW)/(TW-DW)* 100 ....(2)

The plants were then placed in an oven at 50 °C until constant weight to record plant dry weight (g). Weights were estimated in g per plant using a balance.

Statistical analysis

The experiment was conducted in four replications and obtained values were expressed as mean ± SE. Statistical analysis was performed with SPSS for Windows Software v. 27. A two-way analysis of variance test (ANOVA) was conducted in order to test the significance of humic acid application on plant growth parameters. The means were compared by Duncan's multiple range test at $p \leq 0.05$.

Results and Discussion

The results presented in Table 1, illustrate the effect of both humic acid concentration (HAC) and humic acid application methods (HAM) on various growth and yield traits of arugula plants. The effect of humic acid concentration (HAC) was extremely significant ($P<0.001$) on all studied traits. The results also showed that humic acid application methods (HAM) into the plant had a highly significant ($P<0.001$- <0.01) effect on all studied traits. In addition, the results demonstrated that the interaction between humic acid concentration and humic acid application methods (HAC x HAM) had a significant effect ($P<0.05$) on growth and yield traits. Numerous studies have suggested that humic acid had direct beneficial effect in enhancing overall plant biomass, as well as its indirect beneficial effects, such as improving fertilizer efficiency and reducing soil compaction (Zhou *et al.*, 2019; Kandra *et al.*, 2024). Other studies have concluded that low molecular mass of humic substances is readily absorbed by plants and actively modified plant metabolism by acting as hormone-like substances, influencing plant signaling pathways to enhance root architecture and nutrient acquisition, leading to improved plant growth (Canellas *et al.*, 2022; Iwaniuk *et al.*, 2023; Maffia *et al.*, 2025).

Table1. Probability values of the effects of Humic Acid Concentration (HAC), Humic Acid Application Methods (HAM), and HAC x HAM interaction on various growth traits of arugula.

Traits	HAC	HAM	HAC x HAM
Shoot length (cm)	<.001	0.004	0.046
Root length (cm)	<.001	0.002	0.035
Stem diameter (cm)	<.001	0.004	0.042
Leaf number plant^{-1}	<.001	0.002	0.028
Leaf area (cm^{2})	<.001	<.001	0.047
Fresh weight plant^{-1} (g)	<.001	<.001	0.049
Dry weight plant^{-1} (g)	<.001	<.001	0.040
Relative water content	<.001	0.004	0.038
Relative growth rate	<.001	0.001	0.036

Table 2 demonstrates the main effects of humic acid concentration (HAC) on the growth and productivity traits of arugula. The results indicate that humic acid significantly improved growth and productivity traits compared to the control. Particularly, these increases were more pronounced in Eruca sativa plants treated with a higher humic acid concentration (0.5%). The results also suggest that both humic acid concentration and application methods influence the growth of arugula plants compared to the control. The study revealed that the level of humic acid has a significant impact on growth characteristics. As shown in Table 2, all studied traits increased as the humic acid concentration increased from 0.25% to 0.5%. The observed improvement in growth and yield traits may be attributed to the stimulatory effects of humic acid. Evidence suggests that the effects of humic substances depend on their concentration. Researchers have noted that the stimulating effects of humic acid are linked to the presence of certain micronutrients (Wang *et al.*, 2021).

Table 2. Effect of humic acid concentrations (HAC) on various growth traits of arugula.

Traits	HA 0 %	HA 0.25 %	HA 0.5%
Shoot length (cm)	18.8^{c}*	20.0^{b}	21.9^{a}
Root length (cm)	12.1^{c}	12.9^{b}	14.5^{a}
Stem diameter (cm)	0.21^{c}	0.24^{b}	0.29^{a}
Leaf number $plant^{-1}$	4.88^{c}	5.63^{b}	6.63^{a}
Leaf area (cm^2)	127^{c}	133^{b}	144^{a}
Fresh weight $plant^{-1}$ (g)	13.1^{c}	15.4^{b}	18.9^{a}
Dry weight $plant^{-1}$ (g)	1.99^{c}	2.08^{b}	2.36^{a}
Relative water content	84.8^{c}	86.5^{b}	87.5^{a}
Relative growth rate	2.50^{c}	2.90^{b}	3.40^{a}

*Values of some traits followed by different letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$

The study revealed that all growth and yield traits of arugula plants treated with humic acid showed significant improvements compared to untreated plants (Table 3). In particular, applying of humic acid with irrigation water led to greater improvements in these traits compared to both the control and other application methods. The response of plants to irrigation with a humic acid solution was clearly noticed, it was more effective than foliar application, consistent with the findings of a previous study (De Hita *et al.*, 2020). This could be attributed to the role of humic substances in improving soil structure and texture, as well as its ability to increase the availability of certain nutrients for absorption (Tavares *et al.*, 2019; Tahoun *et al.*, 2022). Additionally, several studies have demonstrated that humic substances enhance root growth, allowing roots to absorb nutrients more efficiently. However, when humic acid is applied to leaves, this effect may be less pronounced, likely because leaves are less efficient at nutrient absorption compared to root (De Hita et al., 2020).

Table 3. Effect of humic acid application methods (HAM) on various growths of arugula plants.

Traits	Control	HA Spraying	HA Irrigation
Shoot length (cm)	18.8[c]*	20.6[b]	21.3[a]
Root length (cm)	12.1[c]	13.4[b]	14.0[a]
Stem diameter (cm)	0.21[c]	0.25[b]	0.29[c]
Leaf number $plant^{-1}$	4.88[c]	5.75[b]	6.50[a]
Leaf area (cm^2)	127[c]	136[b]	141[a]
Fresh weight $plant^{-1}$ (g)	13.0[c]	16.2[b]	18.1[a]
Dry weight $plant^{-1}$ (g)	1.99[c]	2.14[b]	2.30[a]
Relative water content	84.8[c]	86.7[b]	87.3[a]
Relative growth rate	2.50[c]	3.00[b]	3.40[a]

*Values of some traits followed by different letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

Figures 1, 2, and 3 illustrate the effects of humic acid concentration and application methods on various growth and yield of arugula, including shoot length, root length, stem diameter, number of leaves, leaf area, relative growth rate, shoot fresh and dry weights, and relative water content. The results revealed a significant improvement in these traits due to the application of humic substances. Furthermore, the findings indicated that applying humic acid through irrigation was more effective than using the foliar spray method. Plants irrigated with a 0.5% humic acid solution achieved the highest values across all growth and yield traits when compared to those treated with foliar spraying at the same concentration.

Figure (1a) demonstrates that the average shoot length (cm) increased by 6% and 7% when plants were foliar sprayed or irrigated with a 0.25% humic acid, respectively, compared to the control. Furthermore, shoot length increased by 14% and 20% when plants were foliar sprayed or irrigated with a 0.5% humic acid, respectively, relative to the control. Similarly, root length (cm) was affected by both humic acid concentration and application method, as shown in Figure 1b. Root length increased by 3% and 10% when plants were foliar sprayed or irrigated with a 0.25% humic acid, respectively, compared to the control. In addition, root length increased by 19% and 21% when plants were foliar sprayed or irrigated with a 0.5% humic acid, respectively, compared to the control.

A comparable trend was observed in Figure (1c), which illustrates the improvement in plant diameter (cm) under humic treatments. Both humic level and its application method significantly increased plant diameter. Plant diameter increased by 10% and 19% when plants were foliar sprayed or irrigated with a 0.25% humic acid, respectively, compared to the control. Remarkably, stem diameter increased by 24% and 52% when plants were foliar sprayed or irrigated with a 0.5% humic acid, respectively, compared to the control. This observed increase can likely be attributed to enhanced cell division and elongation. Humic acid improves soil structure and increases its water retention capacity, making water more readily available for plant root absorption and facilitating cell elongation. Similar findings have been reported in previous studies (Man-Hong *et al.*, 2020; Ampong *et al.,* 2022).

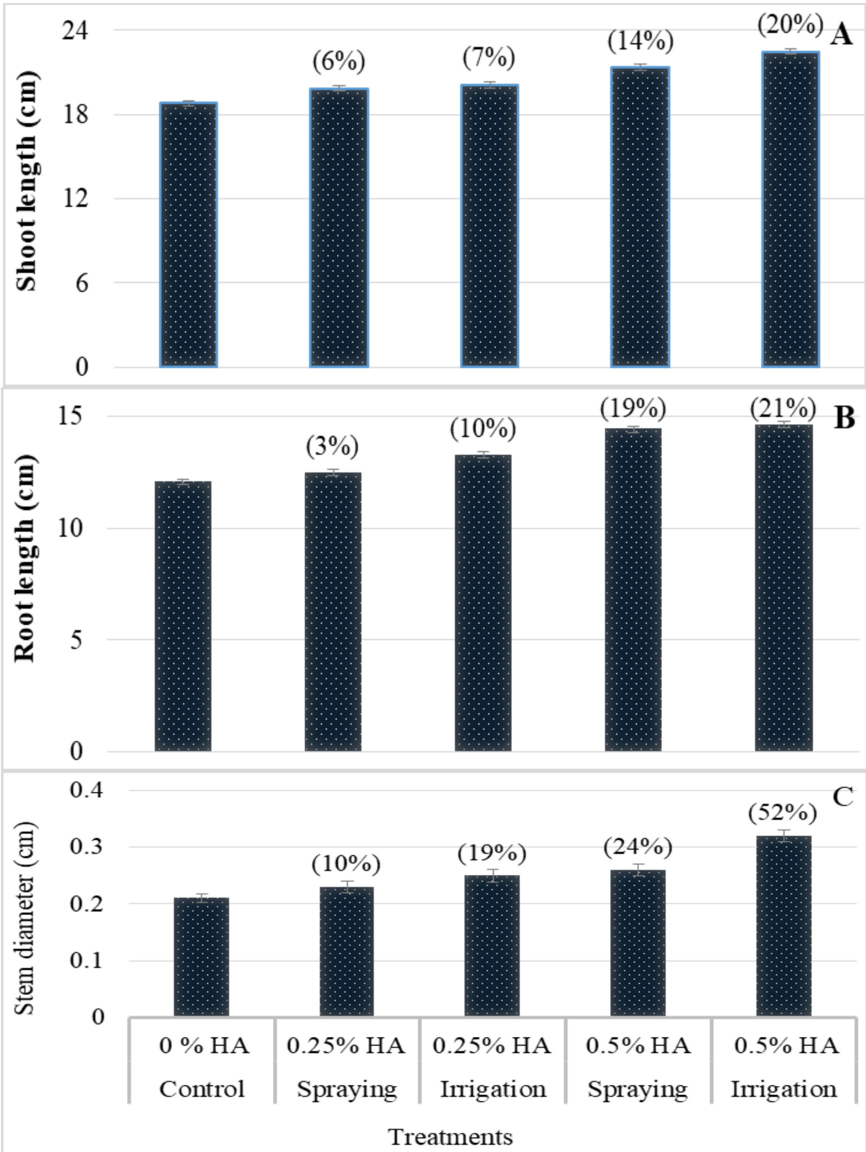

Figure 1: Effects of humic acid concentrations and application methods on (a) shoot lenght (cm), (b) root lenght (cm) and (c) stem diameter of arugula plants.

Results in Figure (2a) indicate that the number of leaves per plant increased significantly with humic acid treatments. Specifically, when plants were treated with a 0.25% humic acid solution, foliar spraying and irrigation led to increases of 13% and 18% in leaf count, respectively, relative to the control. Likewise, a 0.5% humic acid application enhanced the leaf number by 23% via foliar spraying and by 49% through irrigation. In addition, the application of humic acids positively affected leaf development. Plants treated with 2.5 ml/L of humic acid exhibited increases in average leaf area of 3% (foliar spraying) and 6% (irrigation) over the control. The maximum effect was observed at 5 ml/L, where foliar spraying and irrigation resulted in leaf area increases of 11% and 17%, respectively (Figure, 2b). Similarly, humic acid applications significantly boosted the plant's relative growth rate as shown in Figure 2c. A 0.25% treatment increased the relative growth rate by 12% when foliar sprayed and by 20% with irrigation. Moreover, a 0.5% treatment further enhanced the growth rate by 24% via foliar spraying and 48% via irrigation compared to the control. The observed improvements in growth are likely due to humic substances enhancing the plant's uptake of essential nutrients (Ampong *et al.*, 2022). Owing to their low molecular weight, these substances can be partially absorbed by the plant, where they may also improve cell membrane permeability and exert hormone-like activity (Nardi *et al.*, 2021). This hormone-like activity stimulates root development and improves plant vigor.

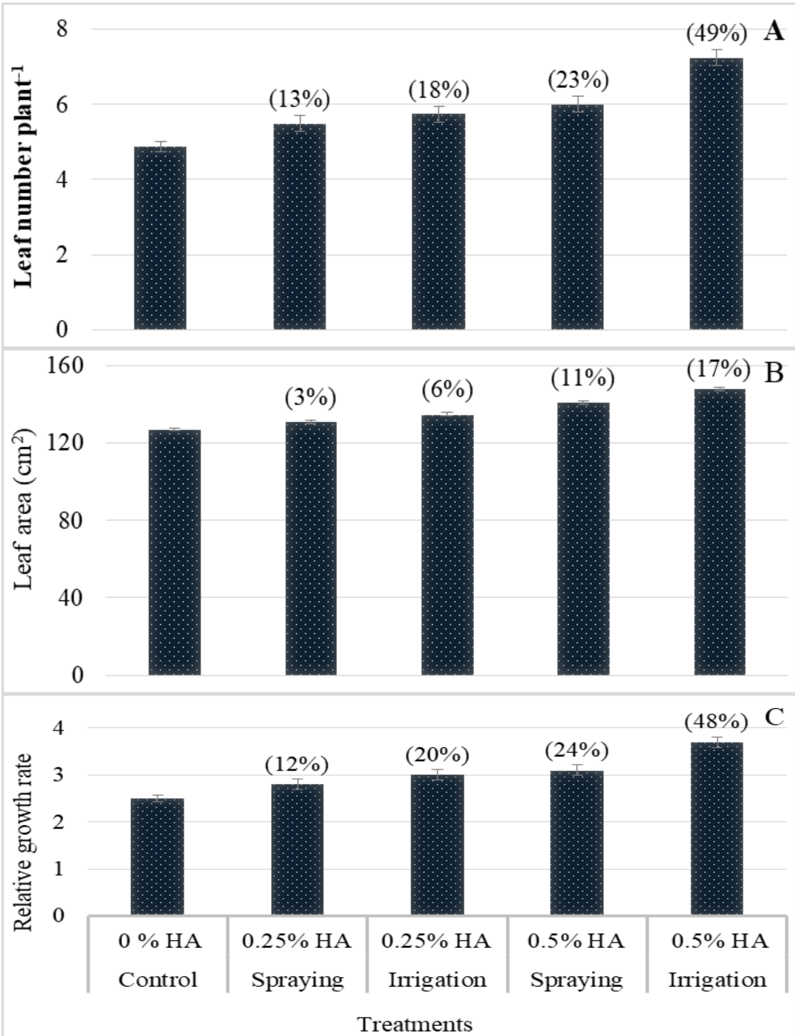

Figure 2: Effects of humic acid concentration and pplication methods on (a) leaf number plant-1, (b) leaf area and (c) relative growth rate of arugula plants.

Figure (3) illustrates the interactive effects of humic acid concentration and application method on the fresh biomass, dry biomass (g per plant), and relative water content (%) of arugula plants. As shown in Figure (3a), increasing humic acid concentrations led to a progressive rise in plant fresh weight, with the irrigation method producing a more pronounced increase compared to foliar spraying. Figure (3b) demonstrates that plant dry weight improved significantly under different treatments. Specifically, compared to the control, plants treated with 0.25% HA by foliar spraying, 0.25% HA by irrigation, 0.5% HA by spraying, and 0.5% HA by irrigation exhibited increases of 2%, 7%, 13%, and 24%, respectively. Similarly, relative water content was positively affected by the interaction between humic acid concentration and application method. As showed in Figure 3c, maximum increments of 1.4%, 2.6%, 3%, and 3.3% were recorded for the treatments of 0.25% HA spray, 0.25% HA irrigation, 0.5% HA spray, and 0.5% HA irrigation, respectively, relative to the control.

These enhancements in arugula plants may be attributed to humic acid's role in making nutrients more available by promoting root growth and facilitating micronutrient transfer (Nardi *et al.*, 2017). Other studies have noted that humic substances stimulate protein synthesis, thereby influencing plant nutritional mechanisms (Vaccaro *et al.*, 2015). Moreover, the observed increase in water content aligns with earlier findings, which suggest that humic acid improves soil structure and enhances water retention, ultimately boosting plant productivity (García *et al.*, 2012; Chen *et al.*, 2022). Other studies reported that the organic nature of humic substances creates a conducive environment for beneficial soil microbes. These microorganisms contribute to the breakdown of organic matter, further releasing nutrients and maintaining soil health. Enhanced microbial activity can promote symbioses, such as those with soil beneficial bacterial and beneficial fungi, which further assist plants in nutrient and water absorption. (Lumactud *et al.*, 2022; Ai *et al.*, 2023). Due to improved nutrient uptake and water retention, plants treated with humic acid often show increased biomass accumulation and improved relative growth rates. Also, better nutrient availability and improved soil structure contribute to more robust growth throughout the plant's life cycle, ultimately leading to higher crop yields and improved quality.

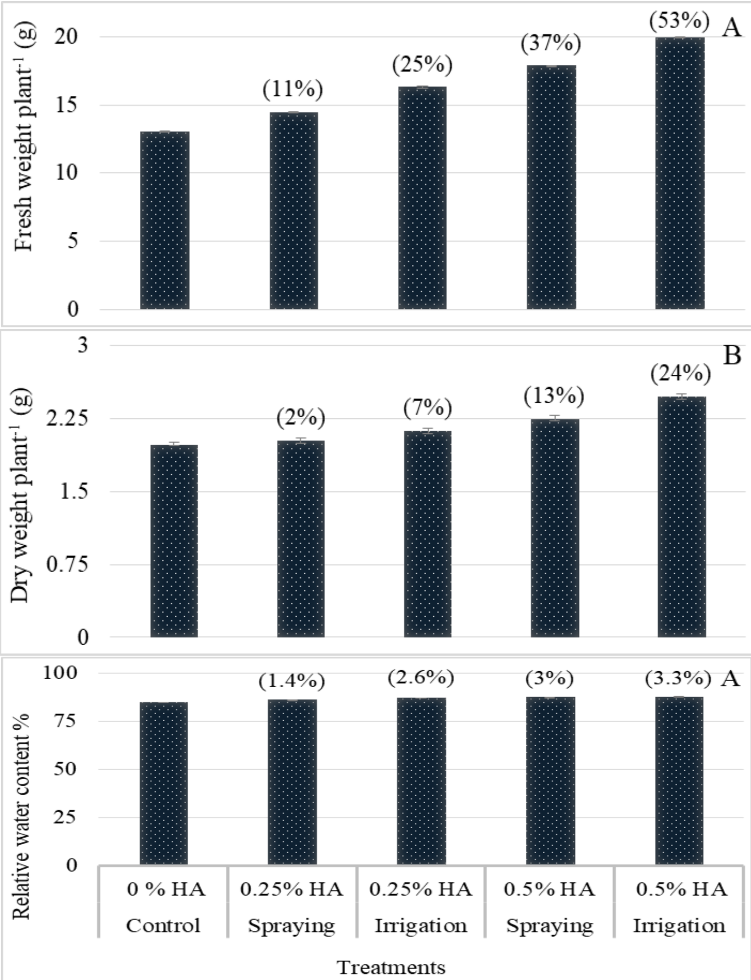

Figure3: Effects of humic acid concentration and application methods on (a) plant fresh weigh, (b) plant dry weight and (c) water content of arugula plants.

Conclusion

Overall, this study evaluated the impact of varying concentrations and methods of humic acid application on the vegetative growth and yield of arugula plants. The findings of the current study demonstrated that humic acid significantly enhances plant growth parameters, with irrigation using 0.5% yielding the most pronounced improvements. When comparing all measured growth and yield traits, plants treated with humic acid consistently outperformed the control group, underscoring its effectiveness. These results provide a practical reference for incorporating humic acid into irrigation water or soil management practices, ultimately promoting healthier plants, improved nutrient utilization, and increased productivity.

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تأثير حامض الهيومك على نمو إنتاجية نبات الجرجير Eruca sativa Mill

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المستخلص

يعتبر نبات الجرجير من النباتات ذات القيمة الغذائية والصحية، ولكن تتطلب زراعة نبات الجرجير تربة غنية بالمغذيات وجيدة التصريف؛ لأن التربة الفقيرة أو سيئة الصرف يمكن أن تسبب تعفن الجذور وفقدان في المحصول؛ ولهذا هدفت الدراسة إلى تقييم تأثير تراكيز وطرق مختلفة لإضافة حمض الهيومك على نمو نبات الجرجير Eruca sativa Mill. تمت زراعة النباتات في أوعية بلاستيكية، في بيئة شبه حقلية في مزرعة بمنطقة جوددائم بمدينة الزاوية، ليبيا. تم معاملة نباتات الجرجير بالرش الورقي أو بالري بتراكيز مختلفة من حامض الهيومك (0 و0.25 و0.5 %). رتبت هذه المعاملات في تجربة عاملية وفق التصميم العشوائي الكامل بأربعة مكررات، تم معاملت النباتات بحامض الهيومك ثلاث مرات (25، و35، و45 (يوماً من الزراعة خلال موسم النمو. أظهرت النتائج أن جميع الصفات المدروسة تأثرت معنوياً بالمعاملة بحامض الهيومك؛ حيث أظهرت النتائج أن تأثير تراكيز حامض الهيومك وطرق إضافته كانت معنوية P< 0.001-0.01)) على صفات النمو والإنتاجية. وقد تم الحصول على أعلى معدل لصفات النمو والحاصل عند ري النباتات بماء يحتوي على (0.5%) من حامض الهيومك مقارنة بمعاملة المقارنة والمعاملات الأخرى، وفي الوقت نفسه سجلت معاملة الشاهد أقل القيم لجميع الصفات المدروسة، كما أشارت النتائج إلى زيادة معنوية في جميع الصفات مع زيادة تركيز حامض الهيومك، بالإضافة إلى ذلك أشارت النتائج إلى أن إضافة تراكيز مختلفة من حامض الهيومك بطريقتي الرش والري أدت إلى زيادة في صفات النمو مقارنة بالشاهد، ومع ذلك فإن طريقة استخدام حمض الهيوميك مع الري كان أكثر فعالية مقارنة بطريقة الرش الورقي، وخلصت الدراسة إلى أن حمض الهيوميك مفيد لنباتات الجرجير؛ لأنه يحسن ظروف النمو التي تدعم إنتاج نباتات جيدة.

الكلمات الدالة: نبات الجرجير Eruca sativa، حامض الهيومك، الري، الرش الورقي.

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