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Effect of spraying of humic acid and nano iron on some chemical characteristics of Eggplant (*Solanum melongena* L)

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Abstract

Objective

Nowadays, improving the nutritional quality of eggplant leaves and fruits and increasing the efficiency of nutrients in different climatic conditions has attracted the attention of researchers. In addition, it is very important to study the effect of nutrition on the content of chlorophyll, protein, anthocyanin and alkaloids with simple and reproducible methods. Therefore, the aim of this study was to investigate the effect of different amounts of humic acid and nano-iron on some chemical properties of eggplant (Solanum melongena L., Barcelona cultivar).

Materials and methods

This research was performed during the 2025 growing season at the Research Station of the College of Agriculture and Marshlands, University of Thi Qar, Iraq. A factorial design with two main factors was used in this research. The first factor was foliar spraying with humic acid at three concentrations: 0, 2, and 4 g/L. while, foliar spraying with nano-iron at five concentrations: 0, 20, 40, 60, and 100 mg/L was considered as the second factor. Then, total leaf protein percentage, leaf nitrogen percentage, fruit anthocyanin content, leaf chlorophyll percentage, and fruit alkaloid content were measured. The results were analyzed using the Genstat statistical program and analysis of variance (ANOVA) for the studied traits. The means were tested according to the least significant difference (LSD) test ($p \leq 0.05$).

Results

The results showed that the greatest positive effect on chemical properties was caused by the concentration of 4 g/L humic acid. This treatment increased the percentage of leaf nitrogen (3.73%), total leaf protein (23.32%), leaf chlorophyll (17.68%), fruit anthocyanin (11.33%), and fruit alkaloids (0.45%). In addition, the best performance was obtained with a concentration of 60 mg/L nanoiron. For this treatment, the values of 3.56% leaf nitrogen, 22.28% protein, 16.51% chlorophyll, 10.82% anthocyanin, and 0.43% alkaloids were observed. Based on these results, it can be said that both treatments have a strengthening role in physiological processes and the accumulation of important chemical compounds in the plant.

Conclusion

According to the results obtained, treatments of 4 g/L humic acid and 60 mg/L nanoiron can be used as the best recommended treatments to improve the quality of eggplant leaves and fruits. Because these substances probably increase the absorption of nutrients and enhance metabolic activity, causing chlorophyll and secondary beneficial compounds to be produced more in the plant. Therefore, these treatments can be recommended for use in nutritional programs in areas that have similar conditions to the study area.

Keywords: anthocyanin accumulation, chlorophyll concentration, leaf nitrogen content, nutrient efficiency, plant biochemical responses

Paper Type: Research Paper.

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Introduction

One of the plants that is rich in essential nutrients, vitamins and organic acids and is very important for human health is eggplant (Solanum melongena L.) (Alhaithloul et al., 2019). The location of cultivation and widespread production of this vegetable includes subtropical regions such as India and Central America, throughout Asia and Africa, and warm temperate regions of the Mediterranean basin and South America (FAO, 2020). Antioxidant compounds, including ascorbic acid, phenolic compounds, and flavonoids, are found in high amounts in eggplant fruits (Lyu et al., 2024). Another reason for the increasing demand for eggplant is that it has been shown to help inhibit tumor growth and reduce inflammation (Matsubara et al., 2005). Like many horticultural crops and plants, eggplant also requires adequate nutrients for proper growth and production. Based on the results of various studies, it has been found that slow-release fertilizers can be used to improve yield and enhance fruit quality (Wang et al., 2015). Various sources, such as organic, synthetic or combined sources, can be used to provide optimal plant nutrition for growth and development. Therefore, many studies have focused on the development and application of organic fertilizers, biofertilizers and nanonutrients and report their advantages and disadvantages. (Yadav et al., 2019). The biological decomposition of plant and animal residues produces a complex organic material of variable molecular weight, called humus. This organic material is usually dark brown to black in color (Suh et al., 2014). Humus is classified into three groups; fulvic acid, humic acid, and humin, based on solubility and molecular characteristics (Souza et al., 2022). These groups, especially humic acids and amino acids, enhance plant physiological excellence and act as effective biostimulants. These are commonly used as safe and cost-effective alternatives to chemical fertilizers (Souri and Hatamian, 2019; Amiri Foroutaghi et al., 2022). Various research results have shown that humic acid plays an important role in increasing plant growth. For example, it plays a role in increasing salt stress tolerance in eggplant genotypes (Kiran et al., 2014), increases the concentration of volatile compounds in Thymus vulgaris (Noroozisharaf and Kaviani, 2018), improves fresh yield and leaf NPK content in eggplant (Mammadova, 2023), and increases the yield and quality of strawberry fruits (Zydlik and Zydlik, 2023). One of the powerful new technologies is nanotechnology. It is an interdisciplinary-multidisciplinary field that uses a set of tools and techniques from engineering, physics, chemistry, and biology (Mohammadabadi et al., 2009; Heidarpour et al. 2011; Mohammadabadi and Mozafari, 2018). With the advances made in nanoscience and nanotechnology, humanity has been able to achieve the fabrication and identification of submicron-sized bioactive carriers (Mortazavi et al., 2005; Zarrabi et al., 2020). Compared to micrometer-sized carriers, nanocarriers have higher surface area availability, greater potential for increased solubility, higher bioavailability, improved controlled release, and enable precise targeting of entrapped materials (Heidarpour et al. 2011; Mohammadabadi and Mozafari, 2019). In agriculture, nanotechnology has also become a reliable and promising tool for improving agricultural productivity and managing plant stresses (Rai and Ingle, 2012). Nanofertilizers have a high specific surface area and increased reactivity, so they can prevent nutrient loss, limit the accumulation of toxic elements in soil and water, and reduce the environmental damage associated with conventional fertilizers (Mohamed et al., 2023). Iron is an essential micronutrient that plays a key role in redox reactions and also serves as an important cofactor for key antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) (Tripathi et al., 2018). Various research results have shown that foliar application of iron nanoparticles can increase plant stress tolerance (Konate et al., 2017), increase free proline and total phenolic content, and stimulate carbohydrate and protein synthesis (Attia et al., 2022; Ashraf et al., 2022). Thus, the aim of this study was to assess the effects of different concentrations of humic acid and nano-iron on the growth and development of eggplant seedlings.

Materials and methods

This study was done at the Research Station of the Faculty of Agriculture and Natural Resources, Dhi Qar University, Dhi Qar Governorate, Iraq, during the 2024-2025 growing season. The study area has a hot and semi-arid climate.

A factorial design with two main factors was used in this research. The first factor was foliar spraying with humic acid at three concentrations: 0, 2, and 4 g/L. while, foliar spraying with nano-iron at five concentrations: 0, 20, 40, 60, and 100 mg/L was considered as the second factor. A total of 15 treatments were carried out with three replications, resulting in 45 experimental units.

Powdered humic acid with a purity of 90% was prepared and dissolved in distilled water at concentrations of 0, 2, and 4 g/L. The solutions were completely homogenized with a magnetic stirrer and prepared half an hour before foliar spraying. The solutions were sprayed with a hand sprayer in the early morning hours until the entire leaf and aerial parts were wetted (to the point of near-drip).

Iron oxide nanoparticles (Fe_3O_4) with particle sizes of 30-50 nm were prepared. Concentrations of 0, 20, 40, 60 and 100 mg/L were prepared using distilled water and a small amount of adhesive (Tween-20, 0.1%) to improve foliar uptake. Spraying was done in the same three stages as humic acid, and in each case humic acid was applied first and then nanoiron to avoid mixing of solutions. All agronomic operations including weeding, pest control, irrigation and uniform growth were carried out according to local recommendations. No other foliar chemical fertilizers were used to avoid interference with the results. Pest control was done with low-risk biological pesticides only when needed.

For nitrogen measurement, whole and developed leaves were sampled. The samples were dried at 70°C and then ground. Nitrogen percentage was measured by Kjeldahl method according to AOAC guidelines (Abrams et al., 2014).

Total protein content was calculated using the standard equation:

Protein % = N% × 6.25

To measure chlorophyll, 1 g of fresh leaf tissue was homogenized in 80% acetone solution and optical absorption was measured at wavelengths of 663 and 645 nm by spectrophotometer. The chlorophyll content was calculated based on the Arnon (1949) formula.

For measurement of anthocyanin, 2 g of fresh fruit peel was extracted with acidic methanol (methanol + 1% HCl) and optical absorbance was measured at 535 nm. Anthocyanin content was calculated according to the method of Giusti & Wrolstad (2001).

For the determination of alkaloids, 5 g of fresh sample was extracted with 70% ethanol and after filtration, the alkaloid content was determined using the titration method adopted from AOAC (Fazel et al., 2010). The results were reported in mg/100g fresh weight.

Data were analyzed using the Genstat statistical program (GenStat, 2008). Means were compared using the LSD test at a probability level of 5%. All data were checked for normality and corrected if necessary.

Results and discussion

Estimating the concentration of N in leaves: Results in Table 1 shows the effect of different concentrations of humic acid on the percentage of nitrogen in eggplant plant leaves. The concentration of 4 g/L showed the highest percentage of nitrogen (reaching 3.73), while the treatment without adding humic acid (0 g/L) recorded the lowest percentage (2.92). As for the effect of spraying with nano iron, the concentration 60 mg/L nano iron achieved the highest average percentage of nitrogen in the leaves (reaching 3.56), while the concentration of 0 mg/L recorded the lowest average (3.05). The Table 1 also indicates the presence of significant differences resulting from the interaction between humic acid concentrations and nano iron. The combined treatment with 4 g/L concentration humic acid and 60 mg/L nano iron recorded the highest percentage of nitrogen in eggplant plant leaves (reaching 3.83). In contrast, the control treatment (without additives) showed the lowest nitrogen content (2.13).

Estimating the concentration of protein in eggplant plant leaves: Results in thr Table 2 shows the effect of different concentrations of humic acid on the percentage of protein content in eggplant plant leaves. The results revealed the significant differences between used concentrations. The concentration of 4 g/L recorded the highest protein content (reaching 23.32), while the lowest percentage was recorded in the treatment without adding the acid (0 g/L) (18.34). Regarding the effect of nano-iron, the data showed that the concentration of 60 mg/L led to a significant increase in the protein percentage, (reaching 22.28), while the concentration of 0 mg/L recorded the lowest percentage (19.10). The interaction between humic acid and nano-iron concentrations showed a significant effect, with the combined treatment of 4 g/L humic acid and 60 mg/L nano-iron achieving the highest total protein content in leaves (reaching 23.94). In contrast, the control sample recorded the lowest protein content (13.35).

Table 1. Effect of spray concentrations of humic acid and nano-iron and their interaction on estimating the percentage of nitrogen in eggplant plant leaves

Humic Acid Con. (g/L)	Nano Iron Con. (mg/L)					Mean (Humic Acid)
	0	20	40	60	100	
0	2.13	2.84	3.15	3.27	3.24	2.92
2	3.36	3.47	3.52	3.59	3.52	3.49
4	3.66	3.70	3.72	3.83	3.74	3.73
Mean (Nano Iron)	3.05	3.33	3.46	3.56	3.50	
L.S.D.	Nano Iron	Humic Acid		Interaction		
0.05%	0.116	0.089		0.200		

Table 2. The effect of spraying with humic acid and nano iron and the interaction between them in estimating the percentage of protein in eggplant leaves

Humic Acid Con. (g/L)	Nano Iron Con. (mg/L)					Mean (Humic Acid)
	0	20	40	60	100	
0	13.35	17.77	19.69	20.44	20.46	18.34
2	21.02	21.71	22.25	22.48	22.10	21.91
4	22.92	23.17	23.21	23.94	23.40	23.32
Mean (Nano Iron)	19.10	20.88	21.71	22.28	21.99	
L.S.D.	Nano Iron	Humic Acid		Interaction		
0.05%	0.725	0.562		1.256		

Estimating the total chlorophyll in eggplant plant leaves (mg/100g): Results in Table 3 shows significant differences between the concentrations under study of humic acid. The concentration of 4 g/L recorded the highest total chlorophyll content, (reaching 17.68 mg/100g of fresh material), while the lowest value was recorded in the treatment without adding the acid (0 g/L) (14.09 mg/100g). Regarding the effect of nano-iron, it was found that the concentration of 60 mg/L led to a significant increase in chlorophyll concentration (reaching 16.51 mg/100g) compared to concentration of 0 g/L, which recorded 14.96 mg/100g. The interaction between humic acid and nano-iron showed a clear significant effect. The combined treatment at the concentration of 4 g/L humic acid and 60 mg/L nano-iron achieved the highest total chlorophyll content (reaching 18.50 mg/100 g). In contrast, the sample recorded the lowest concentration (13.54 mg/100 g).

Table 3. Effect of spraying with humic acid and nano-iron and their interaction on estimating the percentage of total chlorophyll (mg/100 g fresh matter) in eggplant plant leaves

Humic Acid Con. (g/L)	Nano Iron Con. (mg/L)					
	0	20	40	60	100	Mean (Humic Acid)
0	13.54	13.89	14.24	14.50	14.27	14.09
2	14.55	15.34	15.40	16.53	16.38	15.64
4	16.78	17.44	17.52	18.50	18.15	17.68
Mean (Nano Iron)	14.96	15.56	15.72	16.51	16.27	
L.S.D.	Nano Iron		Humic Acid		Interaction	
0.05%	0.340		0.263		0.589	

Estimating the anthocyanin pigment in fruit peel: Results in Table 4 shows significant differences between the used concentrations. The data showed that the concentration of 4 g/L of humic acid resulted in the highest anthocyanin pigment level (11.33 mg/100 g), while the concentration of 0 mg/L recorded the lowest value (9.26 mg/100 g). Regarding the effect of nano-iron, the concentration of 60 mg/L achieved the highest anthocyanin content (10.82 mg/100 g), while the lowest value was related to the concentration of 0 mg/L (9.86 mg/100 g). Regarding the interaction between humic acid and nano-iron, the results showed that the combined treatment with the concentration of 4 g/L humic acid and the concentration of 60 mg/L nano-iron showed statistically significant results and recorded the highest concentration of anthocyanin pigment (reaching 11.53 mg/100 g). In contrast, the control treatment (without additives) recorded the lowest value 8.35 mg/100 g).

Estimating the concentration of alkaloids in fruits: Results in Table 5 shows significant differences between the studied concentrations. When the concentration 4 g/L humic acid was used, the highest average alkaloid content of 0.43 mg/100g was achieved, while the concentration of 0 g/L recorded the lowest value (0.21 mg/100g). Results (Table 5) also represents that nano-iron different concentrations were significantly affected the level of alkaloids in eggplant fruits. The concentration of 60 mg/L recorded the highest rate (0.43 mg/100g), while the lowest value achieved in the treatment without adding iron (0 mg/L) (0.22 mg/100g). Regarding the interaction between nano-iron and humic acid Humic acid, a clear significant effect was found, as the combined treatment at the concentration of 4 g/L humic acid with the concentration of 60 mg/L nano-iron resulted in the highest concentration of alkaloids in the fruit (reaching 0.54 mg/100 g). In contrast, the control treatment (0 mg/L) recorded the lowest content (0.12 mg/100 g of fresh matter).

Table 4. Effect of spraying with humic acid, nano-iron, and their interaction on anthocyanin pigmentation in eggplant fruit peel (fresh weight)

Humic Acid Con. (g/L)	Nano Iron Con. (mg/L)					
	0	20	40	60	100	Mean (Humic Acid)
0	8.35	8.73	9.140	10.26	9.84	9.26
2	10.14	10.19	10.36	10.66	10.24	10.32
4	11.11	11.13	11.43	11.53	11.45	11.33
Mean (Nano Iron)	9.86	10.01	10.31	10.82	10.51	
L.S.D	Nano Iron		Humic Acid		Interaction	
0.05%	0.224		0.173		0.388	

Table 5. Effect of spraying with humic acid and nano-iron and their interaction on estimation of the percentage of alkaloids in the eggplant fruit mg/100 g (Fresh weight)

Humic Acid Con. (g/L)	Nano Iron Con. (mg/L)					
	0	20	40	60	100	Mean (Humic Acid)
0	0.12	0.15	0.21	0.33	0.25	0.21
2	0.24	0.31	0.39	0.42	0.37	0.34
4	0.31	0.41	0.44	0.54	0.46	0.43
Mean (Nano Iron)	0.22	0.29	0.35	0.43	0.36	
L.S.D.	Nano Iron		Humic Acid		Interaction	
0.05%	0.024		0.018		0.041	

Results of the current study (Tables 1, 2, 3, 4, and 5) confirm the effect of foliar spraying with humic acid and nano-iron on the chemical characteristics of eggplant. This is attributed to the role of humic acid on plant growth and productivity (Canellas et al., 2020; Nardi et al., 2021). The results of this study are in line with the results of their studies. In other studies (Al_Zubaidy and Hsabah, 2022; Sontakk et al., 2024; Haggag et al., 2024), researchers have shown that humus is responsible for dissolving and moving nutrients through root secretions. Humus also enhances the presence of nitric oxide in lateral roots, which promotes root growth. Also, studies indicate that humic acid is a source of the auxin indole acetic acid (IAA), which promotes cell division and expansion and acts as an exogenous auxin hormone, regulating root growth and shape (Zandonadi et al., 2010; Olaetxea et al., 2019; Nardi et al., 2021). Studies have found that spraying with iron nanoparticles increases proline and phenolic compounds in plant tissues, as proline plays a role in osmotic regulation (Nahar et al., 2017). Proline accumulation helps maintain cellular balance and reduce oxidative stress, while phenols help strengthen the plant's immune system. This is consistent with what many previous studies have confirmed, indicating increased levels of phenols and proline in infected plants (Attia et al., 2022; Mikulic-Petkovsek et al., 2013). The results of our study confirm the findings of Wang et al. (2011), who confirmed the role of iron in RNA synthesis and enzyme activity. They found that iron nanoparticles induce greater oxidative stress in plants and enhance the activity of antioxidant enzymes.

Conclusions: The results obtained in this study showed that the use of humic acid and nano-iron treatments had significant effects on eggplant characteristics. A concentration of 4 g/L of humic acid had the best effect on the amount of leaf chlorophyll, total protein, leaf nitrogen, anthocyanin, and fruit alkaloids. In addition, the best concentration for nano-iron used was 60 mg/L, which had the best effect on the amount of leaf chlorophyll, total protein, leaf nitrogen, anthocyanin, and fruit alkaloids. The best interaction effect of the two treatments was also obtained in the combination of 4 g/L of humic acid with 60 mg/L of nano-iron. These results indicate the important role of humic substances in increasing plant growth and quality, improving physiological activities, and absorbing nutrients, and can confirm their role as biostimulants. On the other hand, nanoiron treatment also stimulated the synthesis of important biochemical compounds in the plant, improved metabolic functions, and increased antioxidant activity. Therefore, it can be said that simultaneous treatment with humic acid and nanoiron can have positive effects on the nutritional value and chemical quality of eggplant. Therefore, it can be concluded that one of the ways to improve the quality of agricultural products can be the simultaneous use of organic stimulants and nanofertilizers. More research should be conducted on other varieties and at different growth stages and on a larger scale in order to have a general and final conclusion.

Author contributions

A. R.: Conceptualization, sample collection, experimental work, data analysis, manuscript drafting. N. A. G.: Supervision, methodology design, manuscript review and editing.

Data availability statement

The datasets generated through and/or analyzed in the current investigation are available from the corresponding author on reasonable request.

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Ethical approval

Not applicable.

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Conflict of interest

The authors declare no conflicts of interest related to this research. No financial, personal, or professional relationships exist that could have biased the design, conduct, or reporting of this investigation.

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تأثیر محلول‌پاشی اسید هیومیک و نانوآهن بر برخی ویژگی‌های شیمیایی بادمجان (Solanum melongena L.)

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چکیده

هدف: امروزه بهبود کیفیت تغذیه‌ای برگ و میوه بادمجان و افزایش کارایی عناصر غذایی در شرایط اقلیمی مختلف مورد توجه پژوهشگران قرار گرفته است. علاوه بر این، مطالعه تأثیر تغذیه بر میزان کلروفیل، پروتئین، آنتوسیانین و آلکالوئیدها با روش‌های ساده و قابل تکرار از اهمیت زیادی برخوردار است. بنابراین، هدف این پژوهش بررسی اثر مقادیر مختلف اسید هیومیک و نانوآهن بر برخی ویژگی‌های شیمیایی بادمجان (رقم بارسلونا) بود.

مواد و روش‌ها: این پژوهش در فصل رشد ۲۰۲۵ در ایستگاه تحقیقاتی دانشکده کشاورزی و تالاب‌ها، دانشگاه ذی‌قار، عراق انجام شد. آزمایش به‌صورت فاکتوریل با دو عامل اصلی طراحی شد. عامل اول، محلول‌پاشی اسید هیومیک در سه غلظت ۰، ۲ و ۴ گرم در لیتر بود. عامل دوم، محلول‌پاشی نانوآهن در پنج غلظت ۰، ۲۰، ۴۰، ۶۰ و ۱۰۰ میلی‌گرم در لیتر در نظر گرفته شد. سپس درصد پروتئین کل برگ، درصد نیتروژن برگ، محتوای آنتوسیانین میوه، درصد کلروفیل برگ و محتوای آلکالوئید میوه اندازه‌گیری شد. داده‌ها با استفاده از نرم‌افزار آماری Genstat و تحلیل واریانس (ANOVA) تجزیه و تحلیل شدند. میانگین‌ها بر اساس آزمون حداقل اختلاف معنی‌دار (LSD) در سطح احتمال ۰/۰۵ مقایسه شدند.

نتایج: نتایج نشان داد بیشترین تأثیر مثبت بر ویژگی‌های شیمیایی مربوط به غلظت ۴ گرم در لیتر اسید هیومیک بود. این تیمار موجب افزایش درصد نیتروژن برگ (۳/۷۳٪)، پروتئین کل برگ (۲۳/۳۲٪)، کلروفیل برگ (۱۷/۶۸٪)، آنتوسیانین میوه (۱۱/۳۳٪) و آلکالوئیدهای میوه (۰/۴۵٪) شد. همچنین بهترین عملکرد مربوط به غلظت ۶۰ میلی‌گرم در لیتر نانوآهن بود؛ به‌طوری‌که مقادیر

۳/۵۶٪ نیتروژن برگ، ۲۲/۲۸٪ پروتئین، ۱۶/۵۱٪ کلروفیل، ۱۰/۸۲٪ آنتوسیانین و ۰/۴۳٪ آلکالوئید مشاهده شد. بر اساس این نتایج می‌توان گفت هر دو تیمار نقش تقویتی در فرآیندهای فیزیولوژیکی و تجمع ترکیبات شیمیایی مهم در گیاه دارند.

نتیجه‌گیری: بر اساس نتایج به‌دست‌آمده، تیمارهای ۴ گرم در لیتر اسید هیومیک و ۶۰ میلی‌گرم در لیتر نانوآهن می‌توانند به‌عنوان بهترین تیمارهای پیشنهادی برای بهبود کیفیت برگ و میوه بادمجان مورد استفاده قرار گیرند. زیرا احتمالاً این مواد موجب افزایش جذب عناصر غذایی و تقویت فعالیت‌های متابولیکی شده و تولید کلروفیل و ترکیبات ثانویه مفید را در گیاه افزایش می‌دهند. بنابراین می‌توان این تیمارها را برای استفاده در برنامه‌های تغذیه‌ای در مناطقی با شرایط مشابه محل مطالعه توصیه کرد.

کلمات کلیدی: پاسخ‌های بیوشیمیایی گیاه، تجمع آنتوسیانین، غلظت کلروفیل، کارایی عناصر غذایی، محتوای نیتروژن برگ

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