Iraqi Journal of Desert Studies 2023, 13 (2): 83-88 **Original Article**

Response of Some Vegetative Traits and Chemical Content of Jaafari Plant to Water Stress and Foliar Spraying with Extract of Ag NPs

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Abstract

A pot experiment was carried out to study the effect of treating the Jaafari plant, Tagetes erecta L., with the nano- and aqueous extract of the flowers of the Jaafari plant, prepared using silver nanoparticles and water stress, on some vegetative growth characteristics and the Jaafari plant's content of some secondary metabolites. A completely randomized block design was used to distribute the treatments. The experiment included Two treatments: treatment with four concentrations of water extract (0, 5, 10 and 15) mg L-1 and three levels of water stress, namely full watering every two, four and six days, based on the gravimetric method, with four replicates for each treatment. Treatment with plant extract at a concentration of 10 mg resulted. L-1 resulted in a significant increase in the rates of plant height, number of leaves, and flower diameter, recorded at 33.33 cm, 11.06 leaves per plant and 7.62 cm, respectively. Treatment with nano extract at a concentration of 10 mg resulted. L-1 significantly increased the carbohydrate and protein content of the leaves and the flowers, as these characteristics gave rates of 27.39% and 10.59%, respectively. The water stress treatment when watering every two days significantly increased plant height, recording 28.58 cm. The same treatment also significantly increased the average flower diameter, recording 6.71 cm. Watering every two days also significantly increased most chemical traits, including the carbohydrate and protein content of the leaves, amounting to 25.80% and 9.38%, respectively.

Keywords: *Tagetes erecta* L., Water stress, Foliar Nutrition, silver nitrate-extracted marigold.

استجابة بعض الصفات الخضرية والمحتوى الكيميائي للنبات الجعفري للإجهاد المائي والرش الورقي بالمستخلص النانوي للزهور الجعفرية.

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المستخلص

نفذت تجربة أصص لدراسة تأثير معاملة نبات الجعفري .*Tagetes erecta* L بالمستخلص النانوي المائي لأزهار نبات الجعفري المحضر باستخدام جزيئات الفضة النانوية والإجهاد المائي في بعض صفات النمو الخضري و محتوى نبات الجعفري من بعض مركبات ألأيض الثانوي. تم استخدام تصميم القطاعات العشوائية الكاملة لتوزيع المعاملات. تضمنت التجربة: معاملتين: المعاملة بأربعة تراكيز من المستخلص المائي (0، 5، 10، 15) ملغم لتر−1 وثلاثة مستويات من الإجهاد المائي وهي الري الكامل كل يومين وأربعة وستة أيام، اعتماداً على الطريقة الوزنية. بواقع أربع مكررات لكل معاملة. نتج عن المعاملة بالمستخلص النباتي بتركيز 10 ملغ. لتر−1 زيادة معنوية في معدلات ارتفاع النبات وعدد الأوراق وقطر الزهرة حيث بلغت 33.33 سم و11.06 ورقة للنبات و7.62 سم على التوالي. نتج عن المعاملة بمستخلص النانو بتركيز 10 ملغ. أدى لتر−1 إلى زيادة معنوية في المحتوى الكربوهيدراتي والبروتيني للأوراق والأزهار، حيث أعطت هذه الصفات نسبة 27.39% و10.59% على التوالي. أعطت معاملة الإجهاد المائي عند الري كل يومين زيادة معنوية في ارتفاع النبات حيث بلغ 28.58 سم. كما أعطت نفس المعاملة زيادة معنوية في متوسط قطر الزهرة حيث بلغ 6.71 سم. كما أدى الري كل يومين إلى زيادة معنوية في معظم الصفات الكيميائية بما في ذلك نسبة الكربوهيدرات والبروتين للأوراق بلغت 25.80% و9.38% على التوالي.

الكلمات المفتاحية: *Tagetes erecta* L ، الإجهاد المائي، التغذية الورقية، المستخلص المائي لنبات القطيفة، نترات الفضة النانوية.

Introduction

Jaafari plant (*Tagetes erecta* L.), which belongs to the composite family, is one of the ornamental plants with known medicinal uses because it contains a high percentage of carotenoid substances, including carotenoids, beta-carotene, and lutein, which are known as antioxidants that contribute to the prevention of cancerous diseases and the treatment of eye inflammation from the burning rays of the sun. So, these substances are used in coloring foods and drugs. The leaves and flowers of these plants contain mono-, di- and polyphenols (Hassan et al., 2020). Distinctive essential oils are extracted from the leaves and flowers as an antibacterial-fungal agent, lotion, and disinfectant. It also contains flavones (riboflavin) and heterogeneous tricyclic organic compounds. These substances have essential properties in pharmaceutical materials and are used to manufacture drugs and poisons. The crushed fibers are used externally to stop bleeding.

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The use of nanotechnology in the agricultural field is one of the effective and essential means for plants and also for protecting the environment from pollution resulting from using fertilizers, chemicals and pesticides and their negative impact not only on the environment but also goes beyond that to affect human
health. It also increases the plant's yield without affecting its chemical composition. On the other hand, the technology is not limited to agricultural technology only, as it is also used to manufacture chemicals such as pesticides and others (Khalid, 2006) .
The synthesis of silver nanoparticles (AgNPs) using plant extracts generally involves obtaining the plant extract and a solution of the silver salt. AgNPs are formed by mixing the two solutions in different quantities under certain intermediate conditions (pH, stirring time, temperature, etc.). In the final stage, the nanoparticles are separated and purified, followed by confirming the formation and synthesis of AgNPs through different chemical analysis methods. The appearance of AgNPs was observed with a color change. After the reaction, a color change from yellow to brown was observed, which, according to other studies, can be attributed to the reduction of Ag+ to the nanosilver metal, Ag0. (Osakabe et al, 2014; Paradikovi´c et al , 2019) .
The flowers contain carotenoid pigments, used as a coloring agent for foods and medicines. They include carotenoids, beta-carotene, and lutein. They are known as antioxidants that contribute to preventing cancerous diseases and treating eye inflammation from direct sunlight.
Water stress is explicitly the case in which the rate of transpiration is higher than the rate of water absorption, which causes a decrease in the water content of plant tissues from the normal level. Water stress may be temporary and reversible initially, which helps increase the active substances in the plant cell. Simple stress causes slight effects on plants when exposed to it, and the plant recovers when the danger of stress is gone. With increased stress, the plant deviates from its natural conditions and ages early or enters a latency stage (Lee et al., 2008; Osakabe et al, 2014) indicates that exposing a plant (*Ocimum* Sp.) For four levels of water stress (125%, 100%, 70%, and 50%), the field capacity significantly increased the percentage of total carbohydrates at levels 125 and 50%. The lowest percentage of nitrogen was at 125 and 50%. The highest percentage is at the level of 100%, the lowest percentage of phosphorus and potassium at the level of 125%, and the highest percentage is at the level of 100%, As mentioned by Osakabe and his team (Das et al., 2018) that drought is one of the essential non-vital factors affecting plant growth and development. Drought occurs in conditions of scarcity of irrigation water or rain for some time, Sufficient to deplete soil moisture to the extent that it affects all vital activities and plant growth and directly affects the process of photosynthesis, respiration, transpiration, and the manufacture of protein, proline, and oils. This research is conducted with the aim of Response to some vegetative growth traits and the percentage of active substances in the leaves and flowers of the Jaafari plant through treatment with nano-silver nitrate at different concentrations. Response of some vegetative growth traits and the proportion of active substances in the leaves and flowers of the Jaafari plant through treatment with water stress Response of some vegetative growth traits and the percentage of active substances in the leaves and flowers of the Jaafari plant through the interaction between the study factors.

Materials and Methods

A potting experiment was carried out in the wooden canopy of the Department of Life Sciences at the College of Education for Pure Sciences / Anbar University to study the effect of foliar spraying with the aqueous extract of Jaafari flowers prepared using silver nanoparticles at four levels (0, 5, 10 and 15 mg L-1) under the influence of four levels. Water stress includes full watering every two days, every four days, and every six days in some characteristics of vegetative and flowering growth and the concentration of some secondary metabolites in the leaves and flowers of the Jaafari plant during the spring season of 2023 and for the period from 1/2 to 1/8/2023. A randomized complete block design (RCBD) was used to distribute the experimental units for the factorial experiment. Foliar spray treatments were coded as E0, E1, E2, and E3, respectively, and water stress coefficients, measured based on the gravimetric method, were coded as W0, W1, W2, and W3, respectively. Also, with four replicates for each treatment, the experimental units were brought to 48 pots . The pots were prepared with dimensions of 26 x 30 cm and filled with soil mixed with peat moss in a ratio of 1:2. The field capacity of the pots' soil was measured. The seeds were planted on February 1, 2023 in moist pots, with ten seeds in one pot, with equal distances between the seeds. I sprayed the pots with a little water and left them in the greenhouse until the seeds sprouted completely after (2-4) days. When the plants reached the stage (4-5 true leaves), they were thinned out to one plant per pot. During the first month, irrigation was done daily with normal water. After a month of planting, water stress treatments were applied by weight. Then, the plants were sprayed with the nano extract of Jaafari flowers a week after using the stress treatments, with two sprays and an interval of 20 days between the two sprays. Preparation of nano-aqueous extract of Jaafari flowers:

The flowers of the Jaafari plant were washed and cleaned well with distilled water and then dried in the shade to preserve the secondary metabolites in them. After drying, they were ground using an electric grinder and sieved with a fine sieve. Dissolve 20 grams of the resulting powder after sifting in 200 ml of distilled water, heat it using a hot plate, and stir it with the magnetic stirrer for an hour and a half. The solution was then filtered using special filter paper, and the filtrate was dried in an electric oven at 40°C for 48 hours. After drying, an amount of 0.1 g is taken from the filtrate, then 100 ml of distilled water is added to it, then taken. Silver nitrate in 0.0785 grams in 200 ml of deionized water, each placed in a special cup. 3 ml of the mixture of Jaafari plant extract and 15 ml of dissolved silver nitrate are taken and placed in the heating device for half an hour with continuous stirring, after which the color changes from light yellow to golden, which is the color that indicates the formation of the nano extract.

Studied Traits

1. Plant Height (cm): Measured from the soil surface to the top of the plant using a standard ruler.
2. Leaf Count (leaves/plant): The total number of leaves was counted at the end of the experiment.
3. Flower Diameter (cm): The diameter of the flower was measured at two distant points using a micrometre.
4. Flowering Date (days): The flowering date was recorded when the first flower opened in each treatment.
5. Total Carbohydrates in Leaves (%): Assessed according to the method of Joslyn (1970).
6. Protein Concentration (%): Protein content was calculated by multiplying the nitrogen value by a constant factor of 6.25.

statistical analysis

After collecting and tabulating the data related to the study, it was statistically analyzed according to the system of factorial experiments with a randomized complete block design (R.C.B.D.) and using the 15-Gen Stat program, and the least significant difference (L.S.D.) test was used. To distinguish between the response of plants to different treatments statistically at the probability level of 0.5.

Results and Discussion

Plant Height (cm)

(Table 1) shows a substantial difference between the study parameters regarding plant height. A statistically superior plant height of 33.330 cm was seen in the treatment using jute plant extract with nano silver nitrate at 10 mg/L, exceeding other therapies. The lowest plant height, 21.130 cm, was achieved during the treatment with a 15 mg/L dosage. The plants that were watered for two days reached an average size of 28.586 cm, whereas the plants that were irrigated for six days exhibited an average elevation of 21.563 cm. This data relates to water stress treatments. In the same table, the combined treatment W1E2 (irrigation for two days + jute plant extract with nano silver nitrate at 10 mg/L) showed the highest average plant height of 37.317 cm.

Table 1. Effect of jute plant extract with Nano silver nitrate and hydroponic tension on the average height of jute plants (cm).

Nano Extract mg l^{-1}	Water stress (day)			
	W0 = 2	W1 = 4	W2 = 6	Means
E0 = 0	24.493	22.197	20.517	22.402
E 1 = 5	28.997	22.493	20.977	24.156
E 2 = 10	38.130	37.317	24.543	33.330
E 3 = 15	22.723	20.450	20.217	21.130
Means	28.586	25.614	21.563	
LSD 5%	E = 0.479		W = 0.414	E×W = 0.829

Leaf count (leaf/plant^{-1})

When assessing the average leaf count for the "Jafri" plant, the results of (Table 2) showed substantial changes across treatments. The table below demonstrates significant differences between when the plant was treated with a hydrothermal extract of the "Jafri" plant containing 10 mg/L silver nanoparticles. The highest leaf count was 11.067 leaves/plant-1, while 6.859 leaves/plant-1 were reported in the control treatment. The average number of leaves was unaffected by the water stress treatments, and there were no noteworthy interactions between the various research treatments.

Table 2. shows the impact of water stress and silver nanoparticles in "Jafri" plant hydrothermal extract on secondary branch rate (plant^{-1}).

Nano Extract mg l^{-1}	Water stress (day)			
	W0 = 2	W1 = 4	W2 = 6	Means
E0 = 0	7.267	7.120	6.190	6.859
E 1 = 5	8.303	7.443	6.990	7.579
E 2 = 10	12.110	9.497	11.593	11.067
E 3 = 15	7.440	7.173	6.643	7.086
Means	8.780	7.808	7.854	
LSD 5%	E = 1.679	W = NS	E×W =NS	

Flower Diameter (cm)

The findings of (Table 3) support the experiment on the Jafri plant's considerable differences between all treatments. With a flower diameter (E2) of 7.626 cm, the treatment using a plant extract of Jafri at a concentration of 10 mg/L stood out. The flower's diameter, measured at 5.513 cm at a dose of 5 mg/L (E1), was lower. With a floral diameter of 6.715 cm, the irrigation treatment lasting two days (W0) distinguished itself significantly from the other treatments. In contrast, the irrigation treatment lasting six days (W2) resulted in a smaller flower diameter of 5.148 cm.

The same table's results indicated the superiority of the combined treatment W0E2 (Jafri plant extract with the assistance of nano-silver nitrate at a concentration of 10 mg/L and irrigation after two days) as it recorded the highest rate in flower diameter, significantly differing from the other treatments, at 8.837 cm. On the other hand, the flower diameter was lower in treatment W2E3 (Jafri plant extract with assistance of nano-silver nitrate at a concentration of 15 mg/L and irrigation after six days), measuring 5.150 cm.

Table 3. Effect of aqueous extract of Jafri plant with nano-silver nitrate and irrigation on flower diameter (cm)

Nano Extract mg l^{-1}	Water stress (day)			
	W0 = 2	W1 = 4	W2 = 6	Means
E0 = 0	6.237	5.220	5.083	5.513
E 1 = 5	6.490	4.117	4.040	4.882
E 2 = 10	8.837	7.590	6.450	7.626
E 3 = 15	5.297	5.133	5.020	5.150
Means	6.715	5.515	5.148	
LSD 5%	E = 0.142	W = 0.123	E×W = 0.246	

Flowering Time (Days)

According to (Table 4) data, there was a significant difference solely between the study components themselves in pairwise interactions. With a length of 51.860 days, flowering was observed to be promoted by treatment W0E2 (Jafri plant extract with aid from nano-silver nitrate at a concentration of 10 mg/L and watering after two days). In contrast, the Jafri plant extract treatment W2E3 (with nano-silver nitrate at a concentration of 15 mg/L and irrigation after six days) postponed flowering for 67.725 days. The different factor treatments did not significantly differ from one another.

Table 4. Effect of aqueous extract of Jafri plant with Nano-silver nitrate and irrigation on flowering time (days).

Nano Extract mg l^{-1}	Water stress (day)			
	W0 = 2	W1 = 4	W2 = 6	Means
E0 = 0	60.137	61.765	65.230	62.377
E 1 = 5	62.513	64.223	66.220	64.319
E 2 = 10	51.860	56.280	60.220	56.120
E 3 = 15	61.693	64.127	67.725	64.515
Means	59.051	61.599	64.849	
LSD 5%	E = NS	W = NS	E×W = 15.644	

Carbohydrate Percentage in Leaves

The results of (Table 5) revealed significant differences in the study treatments. The treatment using nano silver-enhanced jute plant extract at a concentration of 10 mg/L exhibited the highest carbohydrate content in leaves, reaching 27.392%. On the other hand, the carbohydrate content decreased to 19.143% when treated with the jute plant extract at a concentration of 15 mg L^{-1}. As for the irrigation treatments, treatment W0 (irrigation after two days) showed a statistically significant increase in relative leaf carbohydrate content, reaching 25.805%, compared to a decrease after six days of irrigation.

The results of the same table indicated significant differences in the interaction treatment. The treatment W0E2 (a jute plant extract with Nano silver nitrate at 10 mg/L concentration, combined with hydraulic stress and irrigation after two days) outperformed others, with a carbohydrate content of 31.723% in leaves. Conversely, treatment W2E3 (jute plant extract with Nano silver nitrate at 15 mg L^{-1} concentration, combined with hydraulic stress and irrigation after six days) recorded a carbohydrate content of 18.043%.

Table 5. Effect of aqueous extract of Jaafari plant with nano silver nitrate and water stress on the percentage of carbohydrates in the leaves

Nano Extract mg l^{-1}	Water stress (day)			
	W0 = 2	W1 = 4	W2 = 6	Means
E0 = 0	27.090	25.230	25.087	25.802
E 1 = 5	24.210	24.257	22.260	23.576
E 2 = 10	31.723	28.183	22.270	27.392
E 3 = 15	20.197	19.190	18.043	19.143
Means	25.805	24.215	21.915	
LSD 5%	E = 0.407	W = 0.407	E×W = 0.814	

The content of protein in the leaves (%)

The results in (Table 6) reveal significant differences in the protein content of the leaves among various treatments. The treatment involving an aqueous extract of Jaafari plant with nano silver nitrate at a concentration of 10 mg/L stood out significantly, reaching a protein content of 10.590%. On the other hand, the potassium content in the leaves decreased when treated with an aqueous extract of Jaafari plant at a concentration of 15 mg/L, reaching 6.980%. As for the irrigation treatments, the treatment labeled as W0 (irrigation after two days) showed a significant increase in protein content, reaching 9.380%, while the protein content in the leaves decreased to 7.967% when subjected to irrigation after 6 days.

The results from the same table confirmed the significance of the interaction between the studied factors. The treatment W0E2 (which combines the application of the plant extract at a concentration of 10 mg/g with irrigation after two days) exhibited a significant increase in leaf protein content, reaching 12.010%. Conversely, the protein content in the leaves decreased in treatment W2E3, recording 6.363%.

Table 6. Effect of aqueous extract of Jaafari plant with silver nanoparticles and water stress on the protein content of leaves (%).

Nano Extract mg l^{-1}	Water stress (day)			
	W0 = 2	W1 = 4	W2 = 6	Means
E0 = 0	27.090	25.230	25.087	25.802
E 1 = 5	24.210	24.257	22.260	23.576
E 2 = 10	31.723	28.183	22.270	27.392
E 3 = 15	20.197	19.190	18.043	19.143
Means	25.805	21.915	24.215	
LSD 5%	E = 0.470	W = 0.407	E×W = 0.814	

The table results indicated significant differences in vegetative, floral, and chemical traits. These differences were represented by leaf area, number of secondary branches, flower diameter, flower weight, petals, and relative chlorophyll content. The increase in these traits can be attributed to the influence of the aqueous extract of the Jaafari plant using silver nanoparticles due to their ability to move in mineral-rich and organic-rich soil. Additionally, these nanoparticles can absorb and retain essential and secondary nutrients, thereby increasing the active components in plant parts. Silver nitrates directly affect the formation of essential oils in plants, such as monoterpenes, sesquiterpenes, ciscospterin, esters, alcohols, aldehydes, monophenols, diphenols, polyphenols, oxides, ketones, acids, lactones, sulfur compounds, and nitrogen compounds. These findings align with Singh – Sangwan et al. (2001).

Regarding the impact of water stress, stress conditions increased cell phenolic content (Table 14). Secondary metabolic processes and their products related to structure, production, secretion, and storage are influenced when exposed to water stress. This impact is often positive, affecting the products' quantity, quality, enzymatic activities, soluble substances, and positive accumulation due to water stress (Singh – Sangwan et al , 2001; Turtola et al , 2003). For instance, plants are prompted to produce higher amounts of terpenoids due to limited carbon allocation for growth, diverting more carbon toward stress resistance mechanisms, resulting in elevated essential oil content in plants (Turtola et al, 2003).

Conclusion

This study was conducted on the effect of foliar spraying with the nano-aqueous extract of Jaafari flowers on some vegetative growth characteristics and the plant's content of secondary metabolites. The nano-aqueous extract was prepared using silver nanoparticles and was sprayed at four levels (0, 5, 10, and 15 mg L^{-1}) under The effect of four levels of water stress: Water stress includes full irrigation every two days, every four days, and every six days. Most of the studied traits responded to spraying significantly differently from the control treatment. Therefore, we recommend using nanoflower extract to improve the vegetative growth traits of this plant.

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