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Effect of Nano zinc oxide and Acadian seaweed extract on some biochemical components and anatomical morphology of *Eruca sativa* Mill

Ruqaya Abdul munem Kadhim [1], Qasim Jasim athfua[2]
[1,2]Department of Life Sciences, College of Education, Qurna, University of Basrah. Iraq
Corresponding author: Qasim Jasim athfua (Ph.D.)
qasim.athfua@uobasrah.edu.iq

Abstract
Objectives. In the present study we set out to investigation the effect of concentration level of Nano zinc oxide and Acadian seaweed extract. on some chemical components and anatomical characteristics of *Eruca sativa* Milleaves.
Methods. The experiment involved examining the effects of two variables: one liter of zinc oxide nanoparticles and five fertilizer treatments with two concentrations of 75 and 150 g. Moreover, a liter of Nano zinc oxide in concentrations of 1 and 2 g A factorial experiment with three replications and a liter of Acadian marine plant was constructed using the design of randomized complete sectors. The three ways of use were seed soaking, plant spraying, and plant watering. Nano Zinc Oxide (Concentration 75%), Nano Zinc Oxide (Concentration150%), Acadian Extract (Concentration 1 g.L-1) and Acadian Extract (Concentration 2 g.L-1).
Results. Our results showed that the, plants treated with Acadian extract at a concentration of 2 g increased the content of total Chlorophyll, Chlorophyll a and Chlorophyll b associated with increased the Carotenoids compared to other fertilizer. When compared to other fertilizer treatments, the treatment with Acadian extract at a concentration of 1 g.l-1 led to the highest significant increase in the thickness of the epidermal layer and upper epidermis. The treatment with Acadian extract at a concentration of 2 g.l gave the highest thickness of the lower epidermal layer.
Conclusion. The compatibility of two levels of Nano zinc oxide with two levels of marine algae extract (Acadian) as well as the addition techniques increased the concentration of photosynthetic pigments in the plant's leaves and caused cells to expand, increasing the thickness of the spongy layer, columnar, upper, and lower epidermis. This, in turn, improved the plant's vegetative growth.

Keyword: Nano zinc oxide, Acadian extract, Rocket salad

Introduction

The plant known as Rocket salad (Eruca sativa Mill) is a member of the cruciferous (Brassicaceae) family. Central Asia is thought to have been its natural habitat, and from there, cultivation of the plant spread to other nations. It is grown in Italy, Portugal, Egypt, and Turkey[1]. The plant's leaves are high in organic compounds, carbohydrates, proteins, and tannins, as well as minerals including nitrogen, calcium, magnesium, potassium, copper, iron, sodium, manganese, and zinc [2] as well as vitamins, phenols, flavonoids, alkaloids, saponins, glycosides and alkaloids .
Watercress is regarded as a medical plant since it is utilized in the treatment of numerous ailments, including cancer [3] and it is a leafy vegetable crop because it is employed in human nourishment. It is the richest natural source of the chemical phenylethylisocyanate (PEITC) [1]. One of the contemporary technologies that has made its way into several industries, including agriculture, is nanotechnology [4]. Nanoparticles have been used as nano-fertilizers because of their impact on plant growth and development and being more effective than traditional fertilizers [5]. Zinc is one of the important elements in plants, as it is necessary in regulating sugar consumption, increasing energy, and thus producing chlorophyll and has a role in the synthesis of auxins [6].

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Zinc nanoparticles increased the efficiency of oxidizing enzymes and stimulated water absorption from the roots [7]. Mendez and colleagues [8] demonstrated that the treatment with zinc oxide nanoparticles increased vegetative and root growth and the accumulation of biomass.

Soliman study [9] showed that foliar spraying with Nano-zinc oxide on Moringa peregrina led to a significant increase in plant height, root length, stem diameter, leaf and stem dry weight, number of leaves, total chlorophyll content, protein percentage, carbohydrate percentage, and percentage of nutrients.

Marine algae extracts are characterized by providing the plant with a large natural stimulant such as cytokinins, auxins, gibberellins, proteins and amino acids that work on cell division and elongation and increase growth in them [10].

Acadian extract is a naturally occurring algal extract from the Ascophyllum nodosum species that is high in organic compounds, including plant hormones, as well as vitamins and amino acids. It also contains a variety of micro- and macronutrients [11].

The results of the previous study [12] showed that the effect of foliar spraying of two cultivars of sweet pepper with marine algae extract led to an improvement in vegetative and anatomical characteristics, as an increase in the thickness of the shell and vascular cylinder was observed. The previous study [13] showed that the use of Acadian extract on peppermint plant, at a concentration of 2 mg.L-1, led to an increase in the concentration of chemical components and chlorophyll pigments and improved vegetative growth characteristics.

In line, the results of earlier study [14] showed that the use of Acadian extract on two cultivars of zucchini squash led to significant results in vegetative and flowering growth characteristics.

Previous study [15] indicated that spraying with Acadian at a concentration of 2 g.L-1 on two cultivars of saffron led to a significant increase in the content of chlorophyll in leaves and an increase in the number of developing leaves and buds, the number of corms and the fresh weight.

Therefore, the aims of this study were to identify the effect of zinc oxide nanoparticles and Acadian extracts on the plant's chemical compounds content and their role in anatomical characteristics.

Material and methods

Study design

The experiment was conducted at the nursery of the Qurna Education College during the agricultural season of 2021–2022, in the Basra Governorate/Al-Qurna District. Before planting, random samples of the field's soil at a depth of 0–60 cm were gathered. Following the homogenous mixing of the samples and their exposure to sunlight for 24 hours, they were crushed and sieved through a sieve with 2 mm-sized pores, and some physical and chemical characteristics of the soil and irrigation water were assessed. Table.1.

Soil	Value
Phosphorous Mg/g	0.0028
Nitrogen	0.023
Potassium	0.56
Sand	13.62
Silt	35.73
Mud	50.65
Electrical conductivity ms/cm	1.923
pH	7.42
Salinity ppt	0.9

Tabile.1. Demonstrated the physical and chemical properties of field soil and irrigation water used in the experiment.

The experiment comprised a study of the effects of two factors. The first component included five fertilizer treatments, including the use of Nano zinc oxide at concentrations of 75 and 150 mg/l and Acadian seaweed extract at concentrations of 1 and 2 g/l, as well as distilled water (measurement treatment) and for the second element, three techniques were employed, including soaking seeds, misting paper and watering. There were 15 transactions, each of which had three replications, and 45 experimental units overall. wherein the seeds were given a 24-hour fertilizer soak.

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Spraying & Watering

After the plant developed its second genuine leaf, the additional operations foliage spraying and watering were performed three times over the course of two weeks, alternately between each spray and each watering. In a 5 x 3 x 3 factorial experiment employing the Randomized Complete Block Design, the workers were assigned the experimental transactions that comprised all possible combinations (RCBD).

Chlorophyll & Carotenoids measurement

The content of leaves in chlorophylls a and b, total chlorophyll, and carotenoids (mg. 100 gm-1 fresh weight) [16] as well as the thickness of the cuticle, the upper epidermis, the lower epidermis, the spongy layer, and the columnar layer were measured after the experiment. Method for the determination of photosynthetic pigments of total chlorophyll (mg. 100gm-1 fresh weight and carotene pigment (mg. 100gm-1 fresh weight).

Anatomical assessment

As previously described [17], sections of plant leaves including the middle vein, at a rate of 1 cm2, and the thickness of the cuticle layer, the Paliside tissue and spongy layer, the thickness of the upper and lower epidermis were measured.

Statistical analysis…….

Results & discussion

Photosynthesis pigments

Table.2. shows that the study factors had a significant effect on the chemical characteristics, as the plants treated with fertilizer treatments: Nano Zinc Oxide (Concentration 75%), Nano Zinc Oxide (Concentration150%), Acadian Extract (Concentration 1 g.L-1) and Acadian Extract (Concentration 2 g.L-1) significantly outperformed the plants in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids compared to the control treatment.Fertilizer treatments also differed significantly among themselves, as the plants treated with Acadian extract at a concentration of (2 g.L-1) showed the highest values for the above traits, which amounted to 21.275, 46.224, 67.167and 0.331 (mg. 100 gm-1 fresh weight), while less significant values were obtained from plants treated with Nano zinc oxide at a concentration of 75 mg. 13.636, 1 28.34, 8 41.97, 0.079 (mg. 100 gm-1 fresh weight).

The reason may be the superiority of plants treated with Acadian extract in photosynthesis traits to the Acadian marine algae which contain growth hormones, auxins, cytokines, nutrients, enzymes, and amino acids which have a role in encouraging physiological activities, which lead to an increase in photosynthetic pigments[18].Moreover , this is in line with the findings of Hegazi and co-worker [19] who used marine algae extracts to increase the chlorophyll content of an eggplant plant.

It also accords with previous results [20] when found that the Acadian grapes had the most significant rise in carotene pigment with a concentration of 3 g. L-1. Regarding the superiority of plants treated with Nano-zinc oxide over those treated with a comparison substance in the photosynthetic pigments, this might be because of its function in the formation of the amino acid tryptophan, which serves as the main building block for the production of IAA indole acetic acid, which is primarily responsible for cell division and elongation [17]or for a course in increasing the efficiency of antioxidant systems [21].

The same table shows that the various methods of use resulted in variations in the plants' traits, with the spraying approach being noticeably superior to the two other procedures (soaking seeds or watering), which varied greatly from one another. It functions by adding nutrients to areas of direct lack, ensuring their entry into plant tissues and subsequent direct involvement in their metabolism, hence reducing the energy necessary for the movement of ions of elements within the plant [22]. These features were significantly impacted by the interaction between the study's two contributing components.

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Methods	Spraying (mg. L-1)	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoids
Soak	Measurement treatment	8.640	16.966	25.607	0.038
	Nano zinc oxide 75	12.073	27.006	39.080	0.074
	Nano zinc oxide 150	14.386	36.036	50.423	0.110
	Acadian 1	16.986	40.976	57.963	0.242
	Acadian 2	19.970	45.066	65.037	0.319
Spraying	Measurement treatment	8.663	16.996	25.630	0.041
	Zinc 75	15.163	29.360	44.523	0.085
	Zinc150	17.083	38.623	55.707	0.127
	Acadian 1	21.360	43.560	64.800	0.265
	Acadian2	22.873	47.620	69.493	0.354
Watering	Measurement treatment	8.656	16.980	25.637	0.039
	Zinc 75	13.673	28.656	42.330	0.078
	Zinc150	15.990	37.453	53.443	0.123
	Acadian1	18.980	42.036	61.017	0.255
	Acadian2	20.983	45.986	66.970	0.321
L. S. D. 5%		0.062	0.0629	0.165	0.006
Methods	Soak	14.411	33.210	47.622	0.156
	Spraying	17.028	35.232	52.031	0.174
	Watering	15.656	34.222	49.879	0.163
L. S. D. 5 %		0.027	0.013	0.074	0.002
Composting	Measurement treatment	8.653	16.981	25.624	0.039
	Zinc 75	13.636	28.341	41.978	0.079
	Zinc150	15.820	37.371	53.191	0.120
	Acadian1	19.108	42.191	61.260	0.254
	Acadian2	21.275	46.224	67.167	0.331
L. S. D. 5%		0.035	0.017	0.095	0.003

Table.2. Demonstrated the adding Nano zinc oxide and Acadian extract on photosynthetic pigments.

Anatomical features:

Table.3. shows the main effects of the levels of Nano zinc oxide and marine algae extract (Acadian), methods of use and the interactions between them on some anatomical characteristics of plant leaves. In comparison to the other treatments, the Acadian extract treatment at a concentration of 1 g.l-1 resulted in the greatest increase in the thickness of the upper epidermis and columnar layer, which were 6.944 and 2.110, respectively. The treatment with Acadian extract at a concentration of 2g.l-1 resulted in the greatest increase in lower epidermis thickness, which was 2.054.

The plants treated with Nano-zinc oxide at a concentration of 75 mg. L-1 excelled in the thickness of the cuticle layer and the spongy layer, which reached 0.796 and 7.736. The results showed the superiority of the methods by spraying method significantly in the thickness of the cuticle layer, the vertical and spongy layer, the upper epidermis and the lower epidermis, which amounted to 0.683, 6.642, 7.214, 2.024 and 2.190,

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respectively. The interaction between the two study factors showed a significant effect on the anatomical characteristics under study.

Methods	Spraying (mg. L-1)	Cuticle thickness	Thickness of palisade Layer	Thickness of spongy layer	Thickness upper Epidermis	Thickness Lower Epidermis
Soak	Measurement treatment	0.310	4.810	5.370	1.476	1.093
	Nano zinc oxide 75	0.660	5.670	7.463	1.766	1.543
	Nano zinc oxide 150	0.586	5.140	7.180	1.516	1.277
	Acadian 1	0.503	6.000	6.216	1.966	1.417
	Acadian 2	0.403	5.750	5.840	1.823	1.390
Spraying	Measurement treatment	0.320	4.840	5.406	1.516	1.130
	Zinc 75	0.976	6.633	7.993	2.056	2.260
	Zinc150	0.813	6.127	7.853	1.993	2.050
	Acadian1	0.700	7.917	7.493	2.343	2.627
	Acadian2	0.606	7.693	7.326	2.210	2.883
watering	Measurement treatment	0.316	4.820	5.390	1.490	1.103
	Zinc 75	0.753	6.133	7.753	1.806	1.167
	Zinc150	0.630	5.643	7.436	1.716	1.300
	Acadian1	0.596	6.917	6.840	2.020	1.550
	Acadian2	0.533	6.750	6.556	1.840	1.890
L. S. D. 5%		0.034	0.273	0.109	0.084	0.226
Methods	Soak	0.492	5.474	6.414	1.710	1.344
	Spraying	0.683	6.642	7.214	2.024	2.190
	watering	0.566	6.053	6.795	1.774	1.402
L. S. D. 5%		0.015	0.122	0.048	0.037	0.101
Spraying	Measurement treatment	0.315	4.823	5.388	1.494	1.109
	Zinc 75	0.796	6.146	7.736	1.876	1.657
	Zing 150	0.676	5.637	7.490	1.742	1.542
	Acadian 1	0.600	6.944	6.850	2.110	1.864
	Acadian 2	0.514	6.731	6.574	1.957	2.054
L. S. D. 5%		0.019	0.157	0.062	0.048	0.130

Table.3. Demonstrated the adding of Nano zinc oxide and Acadian extract on photosynthetic pigments.

It is clear from the results of Table.3. the positive role of the extract on the anatomical measurements, which led to a significant increase in the thickness of the layers. The reason may be due to the fact that the extract contains natural hormones that stimulate biological processes, which include cell division and expansion [23]. Zubay study [12] confirmed that the action of auxins in increasing cell growth and differentiation is the result of their effect on the liberation of hydrogen ions and the increase in the softness of the cell wall to facilitate cell expansion and then build proteins and nucleic acids that stimulate cell division and increase the number of cells. The increase in these results is consistent with study of Boghdady [24] on chickpea plant.

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The cause of the use of seaweed extract changes in the anatomical structure of the stem and leaves of the plant. It is clear from the table the positive role of zinc oxide nanoparticles in increasing the thickness of the layers, and the reason for this may be due to the role of zinc, which is an important component for the formation of tryptophan, which enters the process of forming plant growth regulators, which includes IAA auxins, which has a role in increasing cell division and the process of differentiation in plants [25]. It is clear from the tables that foliar spraying gave the highest significant changes in the studied traits. The reason is that it is more efficient and effective, and that the absorption of nutrients increases by about (8-20) times if compared with the ground fertilization [26] or it may be the reason for the speed of absorption Nutrients by Vegetative Parts [27].

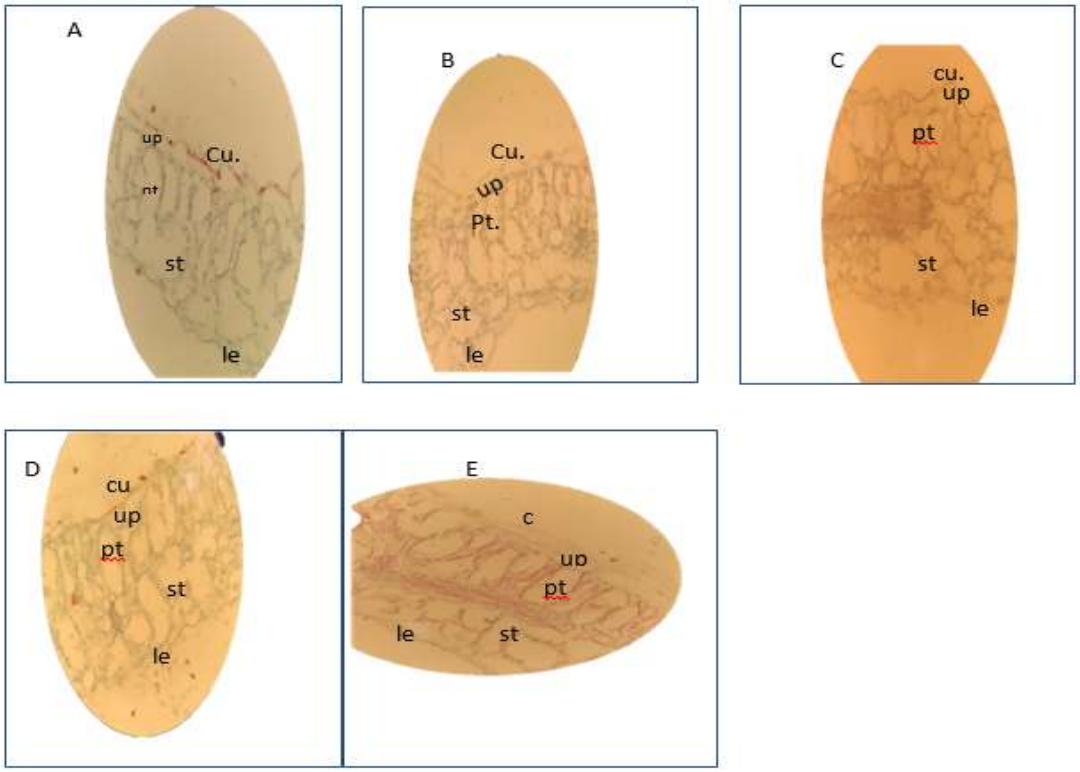

Fig.1. Cross-sections of *Eruca sativa* Mill leaves were subjected to foliar spraying for different treatments A, measurement treatment, B Nano zinc oxide 75 mg. l-1, C Nano zinc oxide 150 mg. L-1, D. Acadian 1 g. L-1, (E) Acadian 2 g. L-1. upper Epidermis, pt epidermal tissue, st spongy tissue, Lower Epidermis, cu cuticle

Conclusion

The compatibility between two levels of Nano zinc oxide and two levels of marine algae extract (Acadian) and the methods of addition led to an increase in the concentration of photosynthetic pigments in the leaves of the plant and led to the expansion of cells, which increased the thickness of the spongy layer, the columnar and the upper and lower epidermis, and this in turn led to an improvement in the vegetative growth of the plant.

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