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Effect of Planting Dates and ZnO Nanoparticles Spraying on the Some Growth Indicators, Aromatic Oil and Its Content of Active Compounds of *Pimpinella anisum* L. Plant

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Abstract: A field experiment was conducted during a winter season of 2023 in the botanical garden of the College of Basic Education - Al-Mustansiriya University to knowing the effect of planting dates and ZnO nanoparticles spraying on the some growth indicators, aromatic oil and its content of active compounds of anise plant. A split-plots arrangement according to randomized complete block design (RCBD) was used with three replications. The main plots contained two planting dates (1/11 and 15/11), whereas the sub plots contained spraying of two concentrations of ZnO nanoparticles (5 and 10 mg L⁻¹) in addition to spraying of distilled water only (0 mg nano-Zn L⁻¹). The results revealed that second planting date (15/11) was significantly superiority in the plant content of nitrogen (15.65 mg N g⁻¹), plant content of phosphorous (9.72 mg P g⁻¹), plant height (73.33 cm), main branches number (20.56 branch plant⁻¹) and fruits number (20.33 fruit inflorescence⁻¹). Otherwise the first date (1/11) was significantly superiority in the 1000-fruits weight (3.89 g) and aromatic oil percentage (5.26%). As well, the spraying of ZnO nanoparticles at a concentration of 10 mg L⁻¹ was significantly superiority in plant height (73.17 cm) and 1000-fruits weight (4.21 g) and aromatic oil percentage (5.66%), while the spraying of ZnO nanoparticles at a concentration of 5 mg L⁻¹ was significantly superiority and gave a highest average of the plant content of nitrogen (14.98 mg N g⁻¹), plant content of phosphorous (9.66 mg P g⁻¹), main branches number (19.67 branch plant⁻¹), fruits number (19.33 fruit inflorescence⁻¹). Further, the results of HPLC analysis showed that the percentages of active compounds of anise aromatic oil were varied by the effect of planting date and spraying of ZnO nanoparticles.

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1. Introduction

In recent years, the importance of medicinal plants has emerged due to the medically important active compounds they contain and their lack of side effects, which makes them safer than medicines and more sustainable, in addition to their cheap price [1]. Anise is one of these plants because the plant contains active compounds of medicinal and industrial importance. Anise fruits contain aromatic oil that has therapeutic effects such as anti-[2], anti-bacterial, digestive and anti-flatulence as well as other medicinal uses [3].

Environmental factors have a major effect on the growth, yield and aromatic oil content of fruits of medicinal plants belonging to the

Apiaceae family [4]. Therefore, following the correct method of crop management, especially planting at the appropriate date, is the key for good crop performance, as the planting at the appropriate date will clarify the performance of the crop under varying environmental conditions, which will lead to knowledge of its behavior in this change, i.e. warranty Sync the growth of plant parts in suitable environmental conditions that will reflect on increased productivity, because planting at the appropriate time will provide elements environmental that the plant needs in its optimal form during its growth stages, which positively affects the plant growth and their economic yield [5].

Alteration the planting date alone isn't sufficient unless accompanied by nutrition with the nutrients necessary for growth, especially the low-availability elements in Iraqi soil, including the zinc [6]. So, the spraying of zinc is one of the factors that can be invested in improving plant productivity. Zinc plays many biochemical roles in plant cells, as it is included in the formation of the tryptophan, which is responsible for the biosynthesis of auxin hormone[7]. It is also a structural component and a cofactor and regulator for a wide range of enzymes, including dehydrogenase, alkaline phosphatase and phospholipidase, in addition to its role in nucleic acid metabolism, protecting proteins and producing high-viability pollen [8]. Recently, attention has focused on introducing some modern technologies in agriculture, including the use of nano-fertilizers to an increase the plant's benefit from the mineral element as a result of the properties that characterize its particles due to their small diameter, which increases their chemical activity and provides a larger area for metabolic [9]. In addition to its rapid absorption and spread within the plant tissues, it is considered a suitable mechanism for transferring nutrients to the targeted parts of the plant by investing the porous surfaces of the plant parts, which may increase the efficiency of its use by the plant [10]. So, spraying of zinc as its nano-form may increase the plant's benefit from it at the different stages of growth, and the positive effect of this is in stimulating vegetative growth, fruits yield and its quality. This research was implemented to find out the effect of planting dates and ZnO nanoparticles spraying on the some growth indicators, aromatic oil and its content of active compounds of anise plant.

2. Materials and Methods

A field experiment was conducted during a winter season of 2023 in the Botanical Garden of the College of Basic Education - Al-Mustansiriya University in a silt clay loam, which some of its physical and chemical properties are shown in (Table 1), and climatic conditions are shown in (Table 2), to find out the effect of planting dates and ZnO nanoparticles spraying on the some growth indicators, aromatic oil and its content of active compounds of anise plant.

A split-plots arrangement according to randomized complete block design (RCBD) was used with three replications. The main plots contained two planting dates (1/11 and 15/11), whereas the sub plots contained spraying of two concentrations of ZnO nanoparticles (5 and 10 mg L⁻¹) in addition to spraying of distilled water only (0 mg nano-Zn L⁻¹). The spraying was carried out in the early morning until the leaves were completely wet at twice, the first at a 4 true leaves appearance and the second after two weeks of the first. Soil management operators were implemented, and then the experiment area was splitted into 18 plots, the area of each plot was 1 m² (1 m × 1 m), which contains 6 lines, 20 cm apart. The seeds of anise plant were planted as a drill at a 80 Kg ha⁻¹. Crop management operators were implemented as needed.

The traits of plant content of nitrogen (mg N g⁻¹) and phosphorous(mg P g⁻¹), plant height (cm), main branches number per plant, fruits number per inflorescence, 1000-fruits weight (g) and the percentage of aromatic oil (%) were measured.

Table 1: Physical and chemical soil properties of soil

Trait	Value	Unit
Sand	185	g Kg ⁻¹ Soil
Loam	510	
Clay	305	
Ec	3.45	ds m ⁻¹
pH	7.3	-----
O.M	8.5	g Kg ⁻¹ Soil
Total CaCO ₃	5.46	
Available N	32.0	mg Kg ⁻¹ Soil
Available P	14.0	

2.1 Estimation and diagnosis of the active compounds in anise aromatic oil Using HPLC

The qualitative detection of active ingredients was performed by comparing the retention time of each compounds in the crude with the retention time in the standard compound, and then the concentrations of the active ingredients were calculated according to the equation below [11]:

$$\text{Concentration of active compounds} = (A_c / A_s) \times C \times D$$

As:

A_c = Area of the active ingredient in the crude

A_s = Area of the active ingredient in the standard compound

C = Concentration of standard compound

D = Number of dilution times

The data were statistically analysis using Genstat program, and LSD test was utilized to comparison between averages at a probability level of 0.05[12].

Table 2. Climatic conditions at the study location

Month	Max. Temp. (C°)	Min. Temp. (C°)	Humidity (%)
November	29.4	11.7	51
December	19.6	10.1	72
January	16.7	7.4	72
February	10.8	5.0	66
March	21.6	12.0	54
April	31.5	12.9	48
May	38.7	19.2	42

3. Results and Discussion

3.1 Plant content of nitrogen (mg N g⁻¹)

The results of (Table 3) explain that the second date (15/11) was significantly excelled in this trait (15.65 mg N g⁻¹) compared with the first date (1/11) which achieved a lowest (11.38 mg N g⁻¹). The exceed of the second date in this trait could be attributed to the climatic factors suitability during the plant's growing season (Table 2), which was positively reflected on the improving plant's growth and increasing cell division, and then increasing its absorption of necessary nutrients for growth [13].

The finding of (Table 3) show that the plant that sprayed with ZnO nanoparticles at a 5 mg L⁻¹ was significantly excelled in this trait (14.98 mg N g⁻¹) compared with the plant that sprayed with distilled water which was a lowest (11.36 mg N g⁻¹). The increment of plant content of nitrogen could be due to the biochemical effectiveness of zinc nanoparticles as a result of the small size of its molecules and the presence of its molecules at a large numbers on the leaves surface as well as providing a larger area for metabolic reactions, which led

to stimulating plant development and increasing its uptake of nutrients [9].

The di-interaction had significant effect on this trait (Table 3), as the plants that were planted in the mid-November date (15/11) and sprayed with ZnO nanoparticles at a 10 mg L⁻¹ achieved a highest value (17.49 mg N g⁻¹) with non-significant difference with the plants that were planted at same date and sprayed with ZnO nanoparticles at a 5 mg L⁻¹ which achieved 16.98 mg N g⁻¹, whereas the plants that were planted in the beginning of November (1/11) and sprayed with distilled water achieved a lowest (9.50 mg N g⁻¹).

Table 3. Effect of planting date, spraying of ZnO nanoparticles and di-interaction on the anise plant content of nitrogen (mg N g⁻¹)

Planting date	ZnO nanoparticles conc. (mg L ⁻¹)			Average
	0	5	10	
1/11	9.50	13.70	10.93	11.38
15/11	13.22	16.98	17.49	15.65
Lsd	2.04*			1.18*
Average	11.36	14.98	14.21	
Lsd	1.44*			

*There were significant differences at the level of $p \leq 0.05$

3.2 Plant content of phosphorous (mg P g⁻¹)

The finding of (Table 4) reveal that the second date (15/11) was significantly superiority in this trait (9.72 mg P g⁻¹) compared with the first date (1/11) which gave a lowest (6.94 mg P g⁻¹). The suitable climatic conditions at the second date (Table 2) may have encouraged plant growth and increased its absorption of nutrients from the soil solution, including phosphorus[13].

The finding of (Table 4) explain that the ZnO nanoparticles spraying at a 5 mg L⁻¹ was significantly superiority in this trait (9.66 mg P g⁻¹) compared with distilled water spraying which was a lowest (6.89 mg P g⁻¹). The reason of increment of the plant content of phosphorous could be due to the chemical activity of zinc nanoparticles in stimulating the growth of the plant and increasing its nutrients uptake [9].

The di-interaction had significant effect on this trait (Table 4), as the plants that were planted in the late date (15/11) with sprayed of ZnO nanoparticles at a 5 mg L⁻¹ achieved a highest value (11.88 mg P g⁻¹), while the plants that were planted in the early date (1/11) and sprayed with distilled water achieved a lowest (5.91 mg P g⁻¹).

Table 4. Effect of planting date, spraying of ZnO nanoparticles and di-interaction on the anise plant content of phosphorous (mg P g⁻¹)

Planting date	ZnO nanoparticles conc. (mg L ⁻¹)			Average
	0	5	10	
1/11	5.91	8.44	7.46	6.94
15/11	7.86	11.88	7.94	9.72
Lsd	1.32*			0.76*
Average	6.89	9.66	8.44	
Lsd	0.93*			

*There were significant differences at the level of $p \leq 0.05$

3.3 Plant height (cm)

The results of (Table 5) show that there was a significant effect of planting dates (1/11 and 15/11) on the plant height, as the late date (15/11) gave a tallest plants (73.33 cm) compared with the early date (1/11) which gave a shortest plants (54.56 cm). The increment of plant height in the late date could be attributed to the suitability of climatic factors during the plant's growing season (Table 2), which was positively reflected on the plant's growth and its absorption for necessary nutrients of growth, especially nitrogen and phosphorous (Tables 3 and 4).

The results of (Table 5) reveal that the ZnO nanoparticles spraying at a 10 mg L⁻¹ was significantly superiority in the plant height (73.17 cm) compared with distilled water spraying which was a lowest (55.17 cm). The superiority of plants which sprayed with zinc nanoparticles in this trait could be attributed to its chemical effectiveness as a result of the small size of its particles, which will increase the number of particles present on the leaves surface and provide a larger area for metabolic reactions, which may have stimulated the zinc nanoparticles in the formation of tryptophan, which is the basic substance for the synthesis of IAA, which is responsible for cell elongation [8].

The di-interaction had significant effect on this trait (Table 5), as the plants that were planted in the late date (15/11) with sprayed of ZnO nanoparticles at a 10 mg L⁻¹ achieved a highest value (84.33 cm), while the plants that were planted in the early date (1/11) and sprayed with distilled water achieved a lowest (49.00 cm).

Table 5. Effect of planting date, spraying of ZnO nanoparticles and di-interaction on the anise plant height (cm)

Planting date	ZnO nanoparticles conc. (mg L ⁻¹)			Average
	0	5	10	
1/11	49.00	52.67	62.00	54.56
15/11	61.33	74.33	84.33	73.33
Lsd	3.18*			1.84*
Average	55.17	63.50	73.17	

Lsd	2.25*
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*There were significant differences at the level of $p \leq 0.05$

3.4 branches number per plant

The finding of (Table 6) explain that the second date (15/11) was significantly exceeded in this trait (20.56 branch plant⁻¹) compared with the first date (1/11) which achieved a lowest (12.89 branch plant⁻¹). The increment of main branches number may be due to the rapid growth of the plant during its early stages of growth as a result of suitable climatic conditions (Table 2), which may have stimulated the growth of dormant lateral buds as a result of increasing the photosynthesis process activity and increasing the transfer of dry mater to lateral buds [14, 15].

The finding of (Table 6) clarify that the ZnO nanoparticles spraying at a 5 mg L⁻¹ was significantly superiority in this trait (19.67 branch plant⁻¹) compared with spraying of distilled water which gave a lowest (12.67 branch plant⁻¹). The increment of main branches number may be due to the zinc nanoparticles fertilizer properties, which may have led to its rapid absorption and transfer, as well as providing a larger area for various physiological processes in the plant as a result of the small diameter of its particles [9], as well as the roles of zinc in regulator of wide range of enzymes, including dehydrogenase, alkaline phosphatase and phospholipidase and proteins protection [8].

The di-interaction had significant effect on this trait (Table 6), as the plants that were planted in the late date (15/11) and sprayed with ZnO nanoparticles at a 5 mg L⁻¹ had a highest value (24.67 branch plant⁻¹), whereas the plants that were planted in the early date (1/11) and sprayed with distilled water had a lowest (11.00 branch plant⁻¹).

Table 6. Effect of planting date, spraying of ZnO nanoparticles and di-interaction on the main branches number per anise plant

Planting date	ZnO nanoparticles conc. (mg L ⁻¹)			Average
	0	5	10	
1/11	11.00	13.00	14.67	12.89
15/11	14.33	22.67	24.67	20.56
Lsd	2.14*			1.24*
Average	12.67	17.83	19.67	
Lsd	1.52*			

*There were significant differences at the level of $p \leq 0.05$

3.5 Fruits number per inflorescence

It is observe from the finding of (Table 7) that the second date (15/11) was significantly exceed in this trait (20.33 fruit inflorescence⁻¹) compared with the first date (1/11) which recorded a lowest (13.33 fruit inflorescence⁻¹). The reason of increase could be due to planting at the appropriate time and exposing the plants to appropriate climatic conditions during their life cycle, which helped the plant to absorb nitrogen and phosphorous (Tables 3 and 4) and produce branches (Table 6), which was positively reflected on increase the photosynthesis process activity and transfer dry mater to the sinks (fruits), and then increment of fruits number.

As for ZnO nanoparticles, The finding explain that the ZnO nanoparticles spraying at a 5 mg L⁻¹ was significantly excelled in this trait (19.33 fruit inflorescence⁻¹) compared with distilled water spraying which was a lowest (14.00 fruit inflorescence⁻¹). The superiority of the plants that sprayed with ZnO nanoparticles at a 5 mg L⁻¹ in their nitrogen and phosphorus content (Tables 3 and 4) and number of main branches per plant (Table 6) may have contributed to raising the photosynthesis process activity and increasing its metabolites transported to the fruiting sites (sinks) in the plant, and then increasing their number per inflorescence, or the increment of fruits number could be due to the appropriate concentration of zinc motivating the development of buds into reproductive branches as a result of its role in protein synthesis and protection. The di-interaction had non-significant effect on this trait (Table 7).

Table 7. Effect of planting date, spraying of ZnO nanoparticles and di-interaction on the fruits number per anise plant.

Planting date	ZnO nanoparticles conc. (mg L ⁻¹)			Average
	0	5	10	
1/11	11.00	15.67	13.33	13.33
15/11	17.00	23.00	21.00	20.33
Lsd .05	2.22 ^{N, S}			1.29
Average	14.00	19.33	17.17	
Lsd .05	1.58			

*There were significant differences at the level of $p \leq 0.05$

N.S= Not significant

3.6 1000-fruits weight (g)

The finding in (Table 8) explain that the early date (1/11) was significantly exceed in this trait (3.89 g) compared with the late date (15/11) which was a lowest (2.97 g). The increment of the 1000-fruits

weight at an early planting could be attributed to a decrease the number of fruits per inflorescence at same planting date (Table 7), which led to distribute the dry matter into a smaller number of fruits, thus increasing their weight.

The results of (Table 8) clarify that the ZnO nanoparticles spraying at a 10 mg L⁻¹ was significantly superiority in this trait (4.21 g) compared with ZnO nanoparticles spraying at a 5 mg L⁻¹ and distilled water spraying which was a lowest (2.97 and 3.12 g) respectively. The decrease of 1000-fruits weight of the plants sprayed with ZnO nanoparticles at a 5 mg L⁻¹ may be due to their superiority in the number of fruits per inflorescence (Table 7). This explains that the plants sprayed with at a of 5 mg L⁻¹ of ZnO nanoparticles have invested the obtainable growth inputs towards increasing the number of sinks at the expense of sink size.

The di-interaction had significant effect on this trait (Table 8), as the plants that were planted in the first date (1/11) and sprayed with ZnO nanoparticles at a 10 mg L⁻¹ had a highest (4.65 g), while the plants that were planted in the second date (15/11) and sprayed with ZnO nanoparticles at a 5 mg L⁻¹ had a lowest or sprayed with distilled water (2.33 and 2.81 g) respectively.

Table 8. Effect of planting date, spraying of ZnO nanoparticles and their di-interaction on the 1000-fruits weight (g) of anise plant.

Planting date	ZnO nanoparticles conc. (mg L ⁻¹)			Average
	0	5	10	
1/11	3.42	3.60	4.65	3.89
15/11	2.81	2.33	3.76	2.97
Lsd	0.97*			0.56*
Average	3.12	2.97	4.21	
Lsd	0.68*			

*There were significant differences at the level of $p \leq 0.05$

3.7 Aromatic oil percentage (%)

The finding in (Table 9) reveal that the second date (15/11) was significantly excelled in this trait (5.26%) compared with the first date (1/11) which was a lowest (4.18%). The superiority of the second date may be attributed to the growth of the plants in appropriate climatic conditions (Table 2), which resulted in an increase the photosynthesis process activity and then an increase its metabolites, including aromatic oil, as aromatic oils are by-products of the photosynthesis process, so the environmental factors affecting photosynthesis metabolism it will

also affect the production of these compounds [16,14] or the increase may be due to superior the second date (15/11) in the 1000-fruits weight (Table 8).

The finding in (Table 9) explain that the ZnO nanoparticles spraying at a 10 mg L⁻¹ was significantly excelled in this trait (5.66%) compared with control treatment (distilled water spraying) which was a lowest (3.67%). The role of ZnO nanoparticles when sprayed at a concentration of 10 mg L⁻¹ in increasing the weight of the fruit (Table 8) may explain the reason its superiority in the percentage of aromatic oil.

The di-interaction had significant effect on this trait (Table 9), as the plants that were planted in the late date (15/11) and sprayed with ZnO nanoparticles at a 10 mg L⁻¹ achieved a highest value (6.47%), whereas the plants that were planted in the early date (1/11) and sprayed with distilled water achieved a lowest (3.33%).

Table 9. Effect of planting date, spraying of ZnO nanoparticles and di-interaction on the aromatic oil percentage (%) of anise plant.

Planting date	ZnO nanoparticles conc. (mg L ⁻¹)			Average
	0	5	10	
1/11	3.33	4.37	4.84	4.18
15/11	4.01	5.29	6.47	5.26
Lsd	0.56*			0.32*
Average	3.67	4.83	5.66	
Lsd 05	0.39*			

*There were significant differences at the level of $p \leq 0.05$

3.7 Active compounds of anise aromatic oil

The results of (Table 10) reveal that the percentages of active compounds of anise aromatic oil were varied by the effect of interaction between planting date and spraying of ZnO nanoparticles. The second planting date (15/11) with spraying of ZnO nanoparticles at a 10 and 5 mg L⁻¹ as well as the spraying of distilled water had the highest percentages of anise-aldehyde (16.33, 14.77 and 14.11%) respectively compared with the first planting date (1/11) with spraying of distilled water which had a lowest (10.25%).

The finding of (Table 10) also show that the second date (15/11) with spraying of ZnO nanoparticles at a 5 mg L⁻¹ recorded a highest percentage of methyl chavcol (12.90%) followed by same planting date with spraying of ZnO nanoparticles at a 10 mg L⁻¹ and distilled water (11.60 and 11.45%) respectively compared with the first planting date (15/11) with spraying of ZnO nanoparticles at a 10 mg L⁻¹ which recorded a lowest percentage of this compound (8.22%).

Regarding of trans-anethole, the finding of (Table 10) clarify that the second planting date (15/11) with spraying of ZnO nanoparticles at a 5 mg L⁻¹ gave a highest percentage of this compound, reached to 28.56%, followed by the second planting date (15/11) with spraying of ZnO nanoparticles at a 10 mg L⁻¹, first planting date (15/11) with spraying of ZnO nanoparticles at a 5 mg L⁻¹, second planting date (15/11) with spraying of spraying of distilled water and first planting date (15/11) with spraying of ZnO nanoparticles at a 10 mg L⁻¹ which gave (25.89, 24.56, 24.26 and 22.33%) respectively compared with the first planting date (1/11) with spraying of distilled water which was a lowest (21.58%).

As for estrogen hormone compound, the finding of (Table 10) explain that the second planting date (15/11) with spraying of ZnO nanoparticles at a 10 mg L⁻¹ gave a highest percentage of this compound, reached to 18.12%, followed by the first planting date (1/11) with spraying of ZnO nanoparticles at a 10 mg L⁻¹ and second planting date (15/11) with spraying of ZnO nanoparticles at a 5 mg L⁻¹ which were 14.23 and 13.22% respectively compared with the first planting date (1/11) with spraying of distilled water which was a lowest (8.65%).

These results indicate that the delaying of planting date until mid-November with spraying of ZnO nanoparticles at a concentration of 10 mg L⁻¹ achieved the best results of anise-aldehyde and estrogen hormone active compounds. Likewise, the delaying of planting date with spraying ZnO nanoparticles at a 5 mg L⁻¹ gave the best results of the methyl chavcol and Trans-anethole active compounds. The suitable climatic conditions at the second date (Table 2), which were accompanied with preparing the plant with nano-Zno, which is known for its physiological roles within plant tissues, including stimulating the activity of a number of enzymes, may have led to raising the efficiency of the photosynthesis process, increasing by-products, including aromatic oil, and increasing its content of active compounds [17].

Table 10. Effect of interaction between planting date and spraying of ZnO nanoparticles on the active compounds of anise aromatic oil.

Planting date	ZnO nanoparticles conc. (mg L ⁻¹)	Anise-aldehyde	Methyl chavcol	Trans-anethole	Estrogen hormone
1/11	0	10.25	10.12	21.58	8.65
	5	11.89	9.66	24.56	9.88
	10	11.55	8.22	22.33	14.23
15/11	0	14.11	11.45	24.26	9.01

	5	14.77	12.90	28.56	13.22
	10	16.33	11.60	25.89	18.12

4. Conclusion

According to the research results, we conclude that planting anise in mid-November achieved the best results for most of the studied indicators, and the spraying of zinc oxide nanoparticles at a 5 mg L⁻¹ led to improving the plant's growth and its content of nutrients. However, the spraying of zinc oxide nanoparticles at a 10 mg L⁻¹ led to an increase the aromatic oil percentage in anise fruits.

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