Effect of Planting Date, Melatonin, and Ascorbic Acid on Growth, Yield, and Antioxidant Activity of Cumin (*Cuminum cyminum* L.)

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Abstract

The research was done in the winter period 2022-2023 on a private farm to study the effect of planting dates on 15th November, 1st December, and 15th December, and exogenous application of melatonin at (25 mg.L⁻¹), ascorbic acid (200 gm.L⁻¹), and their mixture on cumin plant. The experimental measures consisted of some indicators of the growth, yield, and antioxidant potential of cumin seeds, and used a factorial experiment was set up in a completely randomized blocks design with three replicates for the dissemination of experimental units, and the treatment means were separated depending on least significant difference test at probability level 0.05. The results showed significant superiority of planting on 1st December of cumin in all indicators of the study also foliar spraying of melatonin×ascorbic acid, but the treatment 1st December×melatonin×ascorbic acid proposed significant superiority in leaf and seed content of total flavonoids, total phenols, free radical scavenging activity, and antioxidant activity reached 18.3, 31.2 mg.QE/g⁻¹, 221.3, 58.6 mg.GAE/g⁻¹, 77.6, 44.4 mM.Trolox/kg⁻¹ DW and 94.2, 46.1 % leaves and seeds respectively.

Keywords: Cumin, Planting Date, Melatonin, Ascorbic Acid, Antioxidant Activity.



Introduction

Cumin (Cuminum cyminum L.) is an annual winter medicinal herb plant that belongs to the family Apiaceae, it is contained many active compounds in leaves and seeds, which gives a multiplicity of benefits of cumin plant, especially its distinguished content of phenols and flavonoids and their antioxidant activity, so it used as a spice, and medicinal plant, besides preventing cancer, detoxification of the liver. Cholesterol-lowering (1), acting as an antioxidant, antiseptic, antispasmodic, antidiabetic, aphrodisiac, bactericidal, carminative, digestive, depurative, diuretic, emmenagogue stimulant, and a tonic (2). Planting date is one of the most important

factors influencing the management of the production of medicinal plants in quantity and quality (3), especially in the last years in which the influence of many factors that changed the stability of the weather conditions increased in the climate of many parts of the world, for example, the effect of global warming, the expansion of cities and areas covered with cement and asphalt by buildings and transportation ways (4), in exchange for the decline of green vegetation that is soothing to the atmosphere and produces oxygen, as well as increasing desertification, water scarcity, and shrinking water bodies as a result of rainfall scarcity. All or some of these factors or their overlaps led to climate change and then decreased yield and quality of previous planting dates, which is giving reconsideration of planting dates very important (5). Many researchers noted that cumin is very sensitive to weather factors, especially length of photoperiod,

and temperature, which affected growth and yield indicators of cumin (6).

Melatonin is an amino acid compound found in plants in low concentrations (7). It regulates Biophysiochemical many activities, especially those related to transpiration, protein synthesis, and photosynthetic rate, and accumulation of proline, nitric oxide, and polyamines (8). It is also an antioxidant compound that increases the activity of antioxidant enzymes such as ascorbate peroxidase (APX) and glutathione reductase (9) and increases some endogenous antioxidant compounds such as ascorbic acid and glutathione (10). Ascorbic acid is an essential compound in the cell defense system, it is an important factor in inhibiting reactive oxygen species that are shaped from photosynthesis and the production of power compounds (11), whereas it is controlling to cell growth and division, in addition to acting as a cofactor for many enzymes (12). This research was carried out to enhance the content of leaves and seeds cumin of phenolics and flavonoid compounds which act as antioxidant compounds by testing the appropriate planting date and determining the effect of melatonin and ascorbic acid as acting increasing the growth and antioxidant compounds.

Material and Methods

The experiment was done during the winter season of 2022-2023 to determine the effect of the planting date (15th November, 1st December, and 15th December) and foliar application of melatonin (25 mg.L⁻¹) (7), ascorbic acid (200 mg.L⁻¹) (13), and their



mixture on cumin plants by measuring some parameters of growth, yield, and antioxidant activity, used a factorial experiment in a completely randomized blocks design with three replicates for the dissemination of experimental units, the treatment means were separated depending on least significant difference test at probability level 0.05.

The soil of the field was analyzed to test chemical and physical properties (Table 1), field was divided into three blocks, each block consists 12 plots (2.10×2.10 m), the amount of seed was 10 Kg.ha⁻¹, and seeds (Local variety) were sown according to the

above planting dates at a depth of 1-2 cm. Planting distance was 30 cm between the lines and between the plants (14), and fertilization was done with N⁸⁰+P⁵⁰+K⁶⁰ kg.ha⁻¹, a first batch of fertilizer (1/2 nitrogen+phosphorus+potassium) was added at the preparation of the field, and a second batch of nitrogen was added after 45 days of planting at the logarithmic growth phase of plants (15). The foliar spraying of melatonin and ascorbic acid was done twice at 45 and 75 days after planting respectively (7, 13).

Table 1. Chemical and physical properties of soil

рН	Organic Matter (%)	Ec dsm ⁻¹	Total N (%)		Available Potassium mgkg ⁻¹	Silt (%)	Clay (%)	Sand (%)	Soil Texture
7.1	2.13	3.2	3.5	8.4	253	46.1	28.4	25.5	Silt- Clay

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The evaluated the growth research parameters such as leaf number, plant height, branch number, total chlorophyll pigments, and dry weight. whereas yield parameters include the number of Umbels, number of Umbellates, the weight of 1000 seeds, seed yield, and oil percentage. While antioxidant activity consists of total flavonoids, total phenolics, antioxidant capacity, and radical scavenging activity for leaves and seeds. The leaf extract was prepared by taking 30 gm of the fully developed leaves at the beginning of the flowering stage for all treatments, the extraction was done by soaking the clean leave samples using 100 ml methanol overnight, then repeated three times, volume of extract was reduced to 5 gm.ml using a rotary evaporator, in the same way, extracted seeds after grinding. The content of total flavonoid compounds in dry and powdered leaves and seeds was measured using the method of aluminum chloride colorimetrical method by attractive optical fascination at wavelength 517 nm and standardizing evaluations on a Quercetin standard curve (16)with simple modification by (17), As for the total phenolics compounds, they were estimated by using a Folin-Ciocalteu reagent

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technique and recording visual absorption of a spectrophotometer at 710 nm then balances with a Gallic acid standard curve (18), the free radical scavenging activity was evaluated by the 2,2'-azino-bis(3ethylbenzothiazoline-6-sulphonic ABTS method and recording optical density at 734 nm then matched with the calibration curve of mM Trolox equivalents which was calculated as per kg dry weight of leaves and seeds (19), while antioxidant activity percentage was valued by 2, 2-diphenyl-1picrylhydrazyl (DPPH) free radical reagent then recording optical density of a spectrophotometer at 517 nm. calculated the percent scavenging effect by using the equation depending on (20).

Results and Discussion

Growth Parameters

Results of the analysis of variance in Table (2) present a significant effect of planting date on the growth parameters of cumin, planting on 1st December was significantly superior and recorded the highest averages on traits, leaves number, plant height, branches number, leaves content of total chlorophyll pigments, and plant percentage of dry weight reached 29.7 leaves.plant⁻¹, 36.4 cm, 10.12 branch.plant⁻¹, mg.100g⁻¹ FW, and 15.50 % respectively, compared with planting cumin on 15th December which noted the lowest averages reached 23.1 leaves.plant⁻¹, 29.8 cm, 8.94 branch.plant⁻¹, 60.5 mg.100g⁻¹ FW, and 10.98 % respectively. The reason for the superiority of the planting date on the first of December may be due to the availability of appropriate climatic conditions for the growth, development, and differentiation of cumin plants, especially when the plant passes through the logarithmic growth stage, which is the most important stage to be the best plant canopy. It entails determining the increase or decrease of morphological growth indicators, especially the number of leaves and branches, and the height of the plant promotes an increase in the leaf content of chlorophyll pigments when ready nutrients are available in the soil, and the result is an increase in the percentage of dry matter.

It is also noted from Table (2) that there is a significant superiority of spraying Melatonin ×Ascorbic Acid (Me×As) which gave the highest averages on leaves number, plant height, branches number, leaves content of total chlorophyll pigments, and dry weight percentage reached 29.1 leaves.plant⁻¹, 36.9 cm, 11.41 branch.plant⁻¹, 70.6 mg.100g⁻¹ FW. and 13.7 respectively, compared with control treatment (Con.) which spraying with distill noted the lowest averages water only reached 23.9 leaves.plant⁻¹, 29.6 cm, 7.85 branch.plant⁻¹, 58.6 mg.100g⁻¹ FW, and 11.11 % respectively. This action may be due to the role of melatonin as a plant hormone that promotes growth (21), photosynthesis, regulate transpiration, protein biosynthesis (22), and chlorophyll synthesis (23). In addition to the role of ascorbic acid in increasing cell division and development and extension of a cell wall (24), it also works to increase the level of auxins and glutathione in tissues, which vegetative period increases the and growth indicators (25), increases and

important roles as a cofactor in the metabolism of endogenous phytohormones such as cytokinins, gibberellins, and ethylene (26).

The table (2) show too there is a significant superiority of the treatment 15th Nov×Me×As that gave the highest means on leaves number, plant height, branches number, leaves content of total chlorophyll

pigments, and dry weight percentage reached 32.3 leaves.plant⁻¹, 41.8 cm, 12.22 branch.plant⁻¹, 81.5 mg.100g⁻¹ FW, and 14.59 % respectively, compared with treatment 15th Dec × Con. which noted the lowest averages reached 21.1 leaves.plant⁻¹, 26.2 cm, 7.37 branch.plant⁻¹, 55.9 mg.100g⁻¹ FW, and 10.33 % respectively.

Table 2. Effect of Planting Date, Melatonin, and Ascorbic Acid on Some Growth Parameters of Cumin

Treatments	Leaf No. (Leave. plant ¹⁻)	Plant Height (cm)	Branch No. (Branch. plant ¹⁻)	Total Chlorophyll (mg.100g FW)	Dry Weight (%)
15 th Nov	25.8	32.3	9.53	63.1	11.71
1 st Dec	29.7	36.4	10.12	71.8	13.50
15 th Dec	23.1	29.8	8.94	60.5	10.98
L.S.D $(P \le 0.05)$	1.02	1.22	0.33	1.91	0.12
Con.	23.9	28.6	7.85	58.6	11.11
Me	26.1	32.2	9.93	66.6	12.14
As	25.8	33.6	8.93	64.8	11.92
Me×As	29.1	36.9	11.41	70.6	13.07
L.S.D $(P \le 0.05)$	1.03	1.45	0.39	1.98	0.14
15 th Nov ×Con.	22.6	28.6	7.72	57.2	10.39
15 th Nov ×Me	25.4	31.7	9.85	65.2	11.85
15 th Nov ×As	24.8	32.9	9.23	63.4	11.38
15^{th} Nov × Me×As	30.5	35.9	11.33	66.5	13.22
1 st Dec ×Con.	28.1	31.1	8.46	62.6	12.61
1 st Dec ×Me	29.3	35.9	10.67	73.1	13.49
1^{st} Dec ×As	29.1	37.1	9.11	69.9	13.31
1^{st} Dec × Me×As	32.3	41.8	12.22	81.5	14.59
15 th Dec ×Con.	21.1	26.2	7.37	55.9	10.33
15 th Dec ×Me	23.5	29.1	9.26	61.4	11.08
15 th Dec ×As	23.4	30.9	8.45	61.1	11.08
15^{th} Dec × Me×As	24.5	33.2	10.68	63.7	11.41
L.S.D (<i>P</i> ≤0.05)	1.09	2.33	0.62	3.02	0.31

Yield Parameters

The Data in Table (3) extant a significant effect of planting date on the growth parameters of cumin, planting on 1st



December was a significant effect and documented the highest averages on number of umbels, number of umbellates, weight

1000 seeds, seed yield, and oil percentage umbels.plant⁻¹, reached 24.0 umbellates.umbels⁻¹, 7.31 g, 1.420 g.plant⁻¹, and 2.47 % respectively, compared with planting cumin on 15th December which noted the lowest means reached 22.7 umbels.plant⁻¹, 3.74 umbellates.umbels⁻¹, 5.96 g, 1.216 g.plant⁻¹, and 2.03 % respectively. In general, the superiority of planting date is often attributed to the appropriate environmental conditions for the growth stage of the medicinal crop, because the indicators of yield and its components are the outcomes of vegetative growth, and the results confirmed this f hypothesis.

The result in Table (2) display there is a significant superiority of exogenous application Me×As which offered the highest means on yield indicators reached 25.6 umbels.plant⁻¹, 4.75 umbellates.umbels⁻ ¹, 7.33 g, 1.505 g.plant⁻¹, and 2.51 % respectively, compared with exogenous application of cumin plants with distil water only (Con.) which noted the lowest means reached 21.2 umbels.plant⁻¹, umbellates.umbels⁻¹, 5.92 g, 1.110 g.plant⁻¹, and 1.91 % respectively. These results may be justified by the role of melatonin in regulating the production of basic organic substances, proteins, enzymes, amino and nucleic acids (23), and nitrogen metabolism in general (24), in addition, the role of ascorbic acid in regulating growth and protecting plants from abiotic environmental stresses (28).

Table 3. Effect of Planting Date, Melatonin, and Ascorbic Acid on Some Yield Parameters of Cumin

Treatments	Number of Umbels	Number of Umbellates	Weight 1000 seeds (g)	Seed Yield (g.Plant ⁻¹)	Oil (%)
15 th Nov	23.4	4.61	6.52	1.360	2.25
1 st Dec	24.0	4.81	7.31	1.420	2.47
15 th Dec	22.7	3.74	5.96	1.216	2.03
L.S.D ($P \le 0.05$)	0.09	0.03	0.02	0.025	0.07
Con.	21.2	3.86	5.92	1.110	1.91
Me	23.6	4.52	6.69	1.380	2.23
As	23.0	4.42	6.45	1.333	2.34
$Me \times As$	25.6	4.75	7.33	1.505	2.51
L.S.D ($P \le 0.05$)	0.11	0.04	0.02	0.031	0.07
15 th Nov ×Con.	21.2	3.81	5.81	1.120	1.91
15 th Nov ×Me	23.8	4.87	6.59	1.439	2.18
15 th Nov ×As	23.1	4.68	6.24	1.403	2.31
15^{th} Nov × Me×As	25.6	5.05	7.45	1.477	2.59
1 st Dec ×Con.	21.8	4.23	6.48	1.135	1.97

1st Dec ×Me	24.3	4.91	7.39	1.487	2.48	
1 st Dec ×As	23.5	4.88	7.25	1.405	2.65	seed
1^{st} Dec × Me×As	26.4	5.23	8.11	1.653	2.76	ling
15 th Dec ×Con.	20.6	3.54	5.46	1.076	1.85	s to
15 th Dec ×Me	22.8	3.77	6.09	1.213	2.02	
15 th Dec ×As	22.3	3.69	5.87	1.192	2.07	low
15^{th} Dec × Me×As	24.9	3.97	6.42	1.385	2.19	tem
L.S.D (<i>P</i> ≤0.05)	0.28	0.06	0.03	0.06	0.11	pera
Table (2) illustrates to	on there is a	cionificant			•	fure

Table (2) illustrates too there is a significant superiority of treatment the Nov×Me×As that provided the highest means on number of umbels, number of umbellates, weight 1000 seeds, seed yield, percentage reached and oil umbels.plant⁻¹, 5.23 umbellates.umbels⁻¹, 8.11 g, 1.653 g.plant⁻¹, and 2.76 % respectively, compared with planting cumin on 15th December which noted the lowest means reached 20.6 umbels.plant⁻¹, 3.54 umbellates.umbels⁻¹, 5.46 g, 1.076 g.plant⁻¹, and 1.85 % respectively.

Antioxidant Activity Parameters

The Statistics in Table (4) existing a significant effect of planting date on the antioxidant activity of cumin leaves and seeds, planting on 15th December was a significant effect and documented the highest averages total flavonoids on compounds, total phenolics compounds, free radical scavenging activity, antioxidant activity percentage reached 16.1, 27.6 mg.QE/g⁻¹, 203.8, 50.4 mg.GAE/g⁻¹, 75.9, 38.3 mM Trolox/kg⁻¹, and 90.9, 39.7 % leaves and seeds respectively, compared with planting cumin on 1st December which noted the lowest means reached 9.1, 23.8 mg.QE/g⁻¹, 179.7, 41.7 mg.GAE/g⁻¹, 74.6, 31.6 mM Trolox/kg⁻¹, and 83.5, 30.9 % leaves and seeds respectively. This may be due to the exposure of cumin plants to abiotic stress, especially the exposure of s, usually in the climatic location of the experimental field, which increased the rate of metabolism of antioxidant compounds represented by flavonoids and phenols, which is reflected positively in increasing indicators of oxidative activity.

The Data in Table (4) standing a significant effect of foliar application Me×As on the antioxidant activity of cumin leaves and seeds, and gave the highest means percentage reached 1, 27.6 mg.QE/g⁻¹, 203.8, 50.4 mg.GAE/g⁻¹, 75.9, 38.3 mM Trolox/kg⁻¹, and 90.9, 39.7 % leaves and seeds respectively, compared with planting cumin on 1st December which noted the lowest means reached 9.1, 23.8 mg.QE/g⁻¹, 179.7, 41.7 mg.GAE/g⁻¹, 74.6, 31.6 mM Trolox/kg⁻¹, and 83.5, 30.9 % leaves and seeds respectively.

Conclusion

Global warming resulting from the use of fossil fuels, the expansion of buildings and transportation routes, versus desertification and the decline of vegetation cover, has led to the necessity of reconsidering planting dates for each climate zone. The results of the statistical analysis of the research data indicate the truth of the above and the necessity of adopting planting dates that suit each climatic region to contribute to



increasing the indicators of medicinal crops in quantity and quality and choosing factors that enhance the improvement of the quality of natural products is extremely important.

Conflict of interest

The author has no conflict of interest.

Table 4. Effect of Planting Date, Melatonin, and Ascorbic Acid on Antioxidant Activity Parameters of Cumin

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Treatments	Total Flavonoids (mg.QE/g ⁻¹)		Total Phenolics (mg.GAE/g ⁻¹)		Free Radical Scavenging (mM Trolox /kg ⁻¹)		Antioxidant Activity (%)	
	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds
15 th Nov	11.8	23.8	190.8	46.6	75.3	34.7	85.1	35.8
1 st Dec	9.1	20.9	179.7	41.7	74.6	31.6	83.5	30.9
15 th Dec	16.1	27.6	203.8	50.4	75.9	38.3	90.9	39.7
L.S.D $(P \le 0.05)$	1.22	1.33	23.5	1.09	0.55	0.06	0.58	0.06
Con.	10.6	19.7	179.1	39.2	74.3	29.8	84.8	27.5
Me	12.0	23.0	187.5	44.2	74.8	34.0	85.3	34.7
As	12.6	25.7	193.1	47.7	75.3	36.2	86.7	36.9
$Me \times As$	14.0	28.0	205.8	53.8	76.6	39.4	89.0	42.7
L.S.D $(P \le 0.05)$	1.42	1.36	23.8	1.12	0.61	0.07	0.62	0.08
15 th Nov ×Con.	10.5	19.8	175.6	39.5	74.5	29.3	83.1	27.3
15 th Nov ×Me	11.8	22.1	186.4	44.7	74.6	33.9	83.6	34.6
15 th Nov ×As	12.1	25.4	191.2	48.4	75.1	36.1	85.4	36.7
15^{th} Nov × Me×As	12.6	27.8	209.8	53.6	76.8	39.5	88.3	44.6
1 st Dec ×Con.	7.9	16.3	174.2	35.4	73.6	28.4	82.5	24.1
1 st Dec ×Me	8.2	20.1	177.5	39.5	74.5	30.9	83.1	29.6
1^{st} Dec ×As	8.9	22.3	180.6	42.8	74.9	32.6	83.6	32.4
1^{st} Dec × Me×As	11.2	24.9	186.4	49.2	75.4	34.4	84.6	37.5
15 th Dec ×Con.	13.3	22.9	187.6	42.7	74.8	31.7	88.7	31.2
15 th Dec ×Me	15.9	26.8	198.5	48.3	75.2	37.2	89.3	39.8
15 th Dec ×As	16.7	29.4	207.6	51.8	75.8	39.7	91.2	41.7
15^{th} Dec × Me×As	18.3	31.2	221.3	58.6	77.6	44.4	94.2	46.1
L.S.D (<i>P</i> ≤0.05)	1.88	1.68	26.10	1.23	0.91	0.12	0.94	0.11

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