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Foliar Application of Glycine and/or Zinc Enhances Vegetative, Fruit and Essential Oil Characters of *Cuminum cyminum* L. Under Different Planting Methods

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Abstract

A field experiment was conducted during 2019/2020 and 2020/2021 seasons to investigate the impact of different concentrations of foliar application of glycine (200 and 400 ppm) and/or Zn (100 and 200 ppm) in addition to the control, and different planting lines (wide and narrow lines) on the growth, yield, chemical content as well as volatile oil and its active components of cumin (*Cuminum cyminum* L.). The recorded data showed that wide planting lines (terrace) significantly increased the vegetative (plant height, branch number/plant and fresh & dry weights of herb), yield (number of umbels/plant, weight of thousand seed and fruit yield per plant and per feddan), biochemical constituents (total phenolics, total flavonoids and antioxidant activity) and essential oil parameters (oil percentage, oil yield per plant and per feddan). Spraying cumin plants with glycine (200 and 400 ppm), chelated-zinc (100 and 200 ppm) and their combinations significantly reflected on most recorded parameters. The highest concentration of each substance was superior compared to lower concentration and control. Glycine at 400 ppm plus chelated-Zn at 200 ppm in combination with wide planting lines was the superior treatment and significantly increased all studied parameters compared with other combinations. So, we can recommend applying this treatment to produce clear, safe, and healthy cumin plants.

Keywords: *Cumin, Amino acids, Microelements, planting lines, MAPs*

Introduction

Cumin (*Cuminum cyminum* L.) is one of the important plants around the world (due to its food and medicinal features). It belongs to the *Apiaceae* family and is native to Egypt (Dhaliwal *et al.*, 2016). Cumin has various uses as a spice and medicine as toothache, epilepsy, dyspepsia, diarrhea and jaundice. It is also a natural antioxidant (Bettaieb Rebey *et al.*, 2012). Besides, this plant has antibacterial activity (Akrami *et al.*, 2015). Cumin is well recognized for its

aromatic and spicy nature. Granville *et al.* (1971) investigated the chemical and sensory features of cumin and pointed out that the flavor and odor of heated cumin differed from that of fresh fruits. *C. cyminum* possesses an antioxidant activity having an antioxidant index of 1.3, which is comparable with other spices, such as coriander (1.8), clove (1.3), chilly (1.5), and fenugreek (1.6) (Pruthi, 1980). The relative significance of volatile constituents to the odor and flavor contents were reported by Tassan and Russell (1975), who confirmed that the cumin odor properties are principally due to the aldehydes, such as cuminic aldehyde, p-mentha-1,3-dien-7-al, and p-mentha-3-en-7-al. Halim and Ross (1977) separated and identified the flavonoids (apigenin-7-o-glucoside, apigenin-5-0-glucoside, and luteolin-7-0-glucoside) of cumin fruits by column chromatography, TLC, acid hydrolysis, and spectrophotometry.

Density and distribution of plants during cultivating are important factors affecting plant growth and fruit yield. The best distribution of plants during planting allows the canopy to intercept light and hence increase growth and fruit yield. Intra spacing and competition for water as well as nutrients and light get optimum plant densities for all environmental factors (Karlen and Comp, 1985). Ibrahim and Abd El-Maksoud (2001) demonstrated that vegetative growth and productivity of the single maize plant were better with the wider planting of 40 cm. Moreover, yield characters followed the same trend while the seed yield/fed. was favored under narrow hill spacing of 20 cm compared with 70 cm row width. Plant density is one of the major factors affecting on plant growth and seed yield (Liu *et al.*, 2014). Normally, the best density for maximizing the seed yield of perennial plants is lower than that for maximizing biomass yield (Fulkerson, 1959). The lowest plant density recorded the highest seed yield per plant (SYP); nevertheless, to obtain the highest seed yield/ unit area, an appropriate moderate plant density is an important agronomic criterion (Rincker, 1976 and Li *et al.*, 2018). An appropriate plant density not only alleviates or optimizes plant-plant competition for resources, such as space, nutrients, moisture, and light (Raey and Ghassemi-Golezani, 2009; Khan *et al.*, 2017 and Jia *et al.*, 2018), also effectively decrease the occurrences of pests, weeds, as well as plant pathogens (Liu *et al.*, 2014). Optimum plant density would lead to vigorous vegetative growth and seed yield (Han *et al.*, 2013), leading to a considerable enhancement in fruit yield and quality (Askarian *et al.*, 1995).

Several previous studies cleared that foliar spray with amino acids or different bio-stimulants based on amino acids can increase seed yield and active components as well as their chemical composition of *Calendula officinalis* L. (Rafiee *et al.*, 2013), *Mentha piperita* L. (Hendawy *et al.*, 2015), *Ocimum basilicum* L. (Aghaye Noroozlo *et al.*, 2019), *Nigella sativa* L. (Ayyat *et al.*, 2021), *Coriandrum sativum* L. (Wafaa *et al.*, 2021), and *Foeniculum vulgare* Mill. (Elsayed *et al.*, 2022). According to Pratelli and Pilot (2014), amino acids play important roles in catalyzing the reactions of secondary metabolism in plants. So, foliar application of different amino acids can have beneficial features on vegetative growth and production (Sadak *et al.*, 2015; Shams *et al.*, 2016; Souri and Aslani, 2018 and Hussain *et al.*, 2018). Good growth and higher biomass

production via the application of different amino acid chelates of zinc or iron have also been recorded on some agronomic and horticultural crops (Khan *et al.*, 2012 and El Sayed *et al.*, 2014). Similarly, foliar amendments of a mixture of amino acids at 500 and 700 ppm increased plant height, fresh and dry weights, leaf N concentration, leaf yield and leaf soluble carbohydrates in celery (Shehata *et al.*, 2011). Glycine is the simplest amino acid and is mainly used for producing chelated fertilizers in amino chelates (Souri, 2016).

Zinc (Zn) is recognized as an important micronutrient, the shortage of zinc is a common issue in among several plants (Ojeda-Barríos *et al.*, 2014). It is important for the activity of certain enzymes such as dehydrogenase, isomerase, aldolase, trans phosphorylase, RNA and DNA polymerase. It also contributes to tryptophan synthesis, cell structure preservation, cell division and photosynthesis. As a cofactor, it gives increase to the synthesis of proteins because of its important role as a cofactor in several proteins (Marschner, 2012). Numerous studies have cleared that Zn nutrition considerably affected the number of umbels/ plant and fruit yield of many aromatic crops belonging to the *Apiaceae* family. Their results referred that foliar application of zinc element with specific sources presented a positive correlation between umbel number and fruit production. For example, the foliar application of Zn-EDTA achieved the highest fruit yield of caraway (Diab, 2007), fennel (Eid, 1983 and El-Sherbeny and Abou-Zied, 1986), coriander (Said-AlAhl and Omer, 2009), cumin (El-Sawi and Mohamed, 2002 and Akbari *et al.*, 2013) and Khella (Beshar and Mohamed, 1984).

This study investigated the impact of foliar application of glycine and/or zinc in combination with different lines width on the vegetative growth, yield, chemical components, volatile oil and its constituents of cumin.

Materials and Methods

This Field trial was carried out during 2019/2020 and 2020/2021 seasons at the Floriculture Experimental Farm (N – 27.252°; E – 31.09°) Assiut University, Egypt. The ambient temperature in this study location during the experimentation period was ranged between 11 to 32°C and the relative humidity was 27-50 %. Cumin seeds were obtained from Agricultural Research Center, El-Dokki, Giza, Egypt. Before sowing immediately, seeds were treated with fungicide (Micronized Soreil-KZ 70% W.P.) at 10 g/kg. The experiment was set up in clayey soil. The physical and chemical properties of experiential farm soil were analyzed before the application in compliance with the methods conducted by Jackson (1978) and Black *et al.* (1965), as shown in Table (1).

This investigation aimed to study the influence of the foliar application of glycine (NH₂CH₂COOH, produced in Germany) and/or zinc (Chelated Zinc: Nervanaid contains 14% Zn-EDTA; disodium Zn-chelated of ethylene diamine tetraacetic acid was used in the present work, it is produced in El-Motahda group Co., Egypt.) in combination with different lines width; narrow planting lines 30 cm wide and wide planting lines 80 cm wide (terrace) as well as their interactions

on vegetative growth, fruit yield and essential oil productivity and its constituents of cumin plants.

Table 1. Physical and chemical properties of experimental farm soil recorded as average of both seasons at the beginning of the experiment

Particle size distribution (%)				pH (1:2.5) soil suspension	EC. dS/m (1:5) soil extract	Total CaCO ₃ (%)	Organic matter (%)			
Sand	Silt	Clay	Texture grade							
23.5	27.0	49.5	Clay	7.71	1.13	1.85	1.87			
Soluble ions (meq/l, soil paste)										
Anions				Cations				Total N (%)	Total P (%)	Total K (%)
Cl ⁻	CO ₃ ⁼	HCO ₃ ⁻	SO ₄ ⁼	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺			
3.52	-	4.74	3.05	5.10	0.62	1.40	4.09	0.85	0.31	0.31

On October 15th, 2019 and 2020, seeds were sown in plots each was 2x1 m, including two different planting lines width; 1) narrow planting lines (two lines per plot), each line contained eight hills (2 plants per hill) spaced at 25 cm, and 2) wide planting lines (terrace) divided into four rows with 20 cm distance and each row contained 8 hills (one plant per hill) at 25 cm distance. A total number of cumin plants was approximately 60000 per feddan. All horticultural practices as irrigation, weeding and treating with fungicides were done whenever needed. The experiment consisted of 54 plots in three replicates (2 planting lines × 9 foliar applications × 3 replicates) in split-plot design. The main plots were planting lines and the sub-plots were the foliar application of glycine and/or chelated zinc.

Each experimental unit had the same chance of receiving on of the following foliar application treatments of glycine and zinc; tap water (control), glycine 200 ppm, glycine 400 ppm, zinc 100 ppm, zinc 200 ppm, glycine 200 ppm + zinc 100 ppm, glycine 200 ppm + zinc 200 ppm, glycine 400 ppm + zinc 100 ppm and glycine 400 ppm + zinc 200 ppm. The foliar application of the different treatments started 45 days after sowing at the rate of eight liters from the correspondent treatment per plot divided into four repeated times at two-weeks interval. Samples were selected randomly from plants of each plot for data recording. Data were recorded on the vegetative growth i.e., fruit yield and essential oil characteristics (percentage, yield and chemical constituents).

Essential oil extraction

The essential oil percentage of cumin fruits was estimated by the hydro-distillation method utilizing Clevenger's type apparatus using 50 g of crushed fruits (just before distillation) from each treatment and 500 ml of water placed in one liter-round-bottom flask. The distillation time was 3 h. Then, oil was left to stand undisturbed to ensure complete separation, according to U.S.P. (1995). Next, the essential oil was dried over anhydrous sodium sulfate to eliminate traces of moisture and stored in a refrigerator in the dark at 4°C until analysis. Finally, volatile oil percentage (ml/100 g) was estimated and further used for the calculation of oil yield (ml/plant) by multiplying the oil % by fruit yield per plant.

Essential oil yield per feddan (1/fed.) was calculated by multiplying the oil yield per plant by the number of plants per feddan.

GC-MS analysis of essential oil constituents

Random samples were selected and analyzed with GC-MS assay. The chemical components of each sample were executed with the Trace GC-ISQ mass spectrometer apparatus (Thermo Scientific, Austin, USA). Capillary column utilized was TG-5MS (30 m x 0.25 mm x 0.25 μ m film thickness). The oven temperature was initially held at 50°C and then extend by 5°C /min to 250 °C held for 2 min. and then extend to the final temperature of 310°C by 30°C/min and held for 2 min. The injector and MS transfer line temperatures were constants at 270 and 260°C, respectively; Helium as a carrier gas used at a constant flow rate of 1 ml/min. The solvent delay was 3 min and 1 μ l of diluted samples were injected using Autosampler AS1300 coupled with GC apparatus. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The temperature of the ion source was set at 250 °C. The active compounds were identified by comparison of their retention times (RT) and mass spectra with those of WILEY version 09 and NIST version 11 mass spectral database (Abdul-Hafeez *et al.*, 2020).

Determination of total phenolic content

The content of total phenolic compounds in the investigated essential oils was evaluated according to the method described by Taga *et al.* (1984). Briefly, each pure (100%) volatile oil (100 μ L) was dissolved in 80% ethanol (1 mL); 0.2 mL of this solution was made up of 0.3% HCl to 0.5 ml. An aliquot (100 μ L) of the resulting solution was added to 7% Na₂CO₃ (2 mL) and, after 2 min, the Folin-Ciocalteu reagent diluted with methanol 1:1 (100 μ L) was added and mixed well. After 30 min incubation, precisely 0.25 mL of the assayed sample was transferred into a 96-well plate and the absorbance recorded at 735 nm. The total phenolic calculated due to gallic acid equivalents from a calibration curve of gallic acid standard dilutions, and data was presented as μ g of gallic acid/100 μ L of volatile oil. Total phenolic value was calculated from the regression equation: $y = 0.00094x + 0.0482$ and expressed as μ g/ g GAE using the formula, $T = CV/M$, where T = total content of phenolic compounds (μ g/g GAE), C = concentration of gallic acid (μ g/mL), established from the calibration curve, V = volume of extract (0.25 mL) and m = the weight of plant extract (0.029 g).

Determination of total flavonoids content

The total flavonoid content in seed extract was spectrophotometrically determined by the aluminum chloride method (Marinova *et al.*, 2005). A 2 g sample was extracted with 10 ml methanol for 24 h. One ml of the extracts was added to distilled water (4 ml) in the flask. Then, 5% NaNO₂ (0.3 ml) was added. After 5 min, 10% AlCl₃ (0.3 ml) was added and after 6 min, 1 M NaOH (2 ml) was added. The mixture was diluted to 10 ml with distilled water. After incubation at room temperature, samples were measured at 510 nm and expressed as μ g catechin equivalents (CE/g D.M). Samples were analyzed in triplicates.

Antioxidant activity (DPPH radical scavenging assay)

The antioxidant activity of the seed samples was examined by employing the 1, 1-diphenyl-2-picryl hydrazyl (DPPH, Cal-Biochem, Germany) radical scavenging assay (Mensor *et al.*, 2001). The free radical of DPPH has an odd electron, which gives a maximum absorption at 517 nm (purple colour). 100 μ L of each volatile oil was diluted with 1 mL of 80% ethanol. The extract (10 μ L) was added to DPPH solution (100 μ L of 0.2 mM DPPH in ethanol) on a microtiter plate. The reaction mixture was incubated at 25°C for 5 min, after which the absorbance was recorded at 517 nm. When the antioxidants react with DPPH, the DPPH is reduced to DPPH-H and, consequently, the absorbance decreases. DPPH-H formation results in decolorization (yellow colour) concerning the number of electrons captured. The DPPH solution with corresponding solvents (i.e., without plant material) served as the control. Ethanol with the respective plant extracts served as the blank. The DPPH radical scavenging activity was then calculated as the percentage inhibition according to the following equation.

$$\% \text{ Inhibition of DPPH radical activity} = \frac{(\text{A control} - \text{A sample})}{\text{A control}} \times 100$$

Obtained Data were subjected to statistical analysis through "F" Test (Snedecor and Cochran, 1989) and means were compared according to L.S.D. (Steel and Torrie, 1982). Statistical analysis was conducted by Statistix 8.1 program.

Results and Discussion

Vegetative growth

It is clear that the treatment of wide planting lines (terrace) significantly increased the vegetative growth characteristics expressed as plant height, branch number and weight of herb (fresh & dry) in the first and second seasons, as compared to the narrow planting lines (Table 2). Data revealed that plants grown on wide planting lines reached 21.38 and 21.32 cm in height in the first and second seasons, respectively, and were characterized by more branches (7.10 and 7.11 during both seasons, respectively). In addition, fresh and dry weights of herb significantly improved by sowing cumin fruits on wide lines (54.32 and 6.78 g/plant in the first season and 54.72 and 6.78 g/plant in the second season) as compared to the narrow planting lines.

Data in Table 2 showed that spraying cumin plants with glycine at 200 and 400 ppm, chelated-zinc at 100 and 200 ppm and their combinations reflected significant effects on plant height, number of branches/plant, fresh and dry weights of herb in both seasons. The highest concentration of each substance was superior to those of the same substance at lower concentration or control. Glycine amino acid at a rate of 400 ppm plus chelated-Zn at a rate of 200 ppm was the superior treatment and significantly increased plant height (20.68 and 24.10 cm/plant for the first and second seasons, respectively), branch No./plant (7.00 and 8.47 for both seasons, respectively), herb fresh weight (52.26 and 70.49 g/plant for both

seasons, respectively) and herb dry weight (6.52 and 8.79 g/plant for the first and second seasons, respectively).

Table 2. Vegetative growth characteristics of *Cuminum cyminum* L. as affected by glycine and/or Zn in combination with different planting lines width treatments during 2019/2020 and 2020/2021 seasons

Planting Lines width (A)	Foliar application (B)	2019/2020				2020/2021			
		Plant height (cm)	Branch No./plant	Herb weight (g/plant)		Plant height (cm)	Branch No./plant	Herb weight (g/plant)	
				Fresh	Dry			Fresh	Dry
Narrow lines	Control	16.77	5.05	33.37	4.17	16.75	4.82	35.33	4.33
	G1 (200 ppm)	17.75	6.03	39.23	4.90	17.72	5.80	40.33	4.94
	G2 (400 ppm)	18.24	6.03	42.02	5.24	18.21	5.97	42.00	5.14
	Zn1 (100 ppm)	18.48	6.52	42.49	5.30	18.45	6.46	43.00	5.27
	Zn2 (200 ppm)	18.72	7.00	46.56	5.81	18.70	6.91	47.93	5.88
	G1+Zn1	18.72	6.52	44.12	5.50	18.70	6.44	45.17	5.53
	G1+Zn2	19.21	6.76	45.09	5.63	19.19	6.67	46.33	5.68
	G2+Zn1	19.70	7.00	49.00	6.11	19.68	6.91	50.50	6.19
	G2+Zn2	20.68	7.00	52.26	6.52	20.65	6.78	53.00	6.49
Mean		18.70	6.43	43.79	5.46	18.67	6.31	44.84	5.50
Wide lines (terrace)	Control	18.24	5.54	39.23	4.90	18.17	5.45	41.00	5.02
	G1 (200 ppm)	19.21	6.27	43.14	5.38	19.15	6.29	43.67	5.35
	G2 (400 ppm)	20.68	6.76	44.12	5.50	20.61	6.82	45.00	5.51
	Zn1 (100 ppm)	21.17	7.00	49.00	6.11	21.10	7.04	50.17	6.14
	Zn2 (200 ppm)	22.14	7.35	52.91	6.60	22.08	7.34	52.00	6.37
	G1+Zn1	21.17	7.00	58.77	7.33	21.10	6.91	60.33	7.39
	G1+Zn2	22.63	7.74	62.67	7.82	22.56	7.76	63.67	7.80
	G2+Zn1	23.12	7.74	68.53	8.55	23.05	7.89	67.33	8.25
	G2+Zn2	24.10	8.47	70.49	8.79	24.03	8.53	69.83	8.55
Mean		21.38	7.10	54.32	6.78	21.32	7.11	54.78	6.71
Mean of planting lines	Control	17.50	5.30	36.30	4.53	17.46	5.14	38.17	4.68
	G1 (200 ppm)	18.48	6.15	41.19	5.14	18.43	6.05	42.00	5.15
	G2 (400 ppm)	19.46	6.39	43.07	5.38	19.41	6.39	43.50	5.33
	Zn1 (100 ppm)	19.82	6.76	45.74	5.71	19.78	6.75	46.58	5.70
	Zn2 (200 ppm)	20.43	7.18	49.73	6.21	20.39	7.12	49.97	6.12
	G1+Zn1	19.96	6.76	51.44	6.42	19.90	6.68	52.75	6.46
	G1+Zn2	20.92	7.25	53.88	6.72	20.88	7.21	55.00	6.74
	G2+Zn1	21.41	7.37	58.77	7.33	21.36	7.40	58.92	7.22
	G2+Zn2	22.39	7.74	61.37	7.66	22.34	7.65	61.42	7.52
LSD 0.05	A	0.272	0.067	1.083	1.416	0.454	0.664	1.972	0.411
	B	0.075	0.037	0.436	0.569	0.075	0.168	1.091	0.226
	A*B	0.277	0.078	1.153	1.507	0.455	0.671	2.90	0.476

In a previous study, it was reported that wider row spacing and plant distance increased the biomass of the plant by producing healthy plant parts by receiving maximum sunlight for photosynthesis (Oad *et al.*, 2002). Our study results are in harmony with the previous findings of Oad *et al.* (2002) and Maghaddasi and Omidi (2016), who reported that growth parameters were significantly influenced by row spacing and plant density and increasing the plant density may be the primary reason to decrease light intensity around plants and diminished branching.

Amino acids are also a preferential source of nitrogen for plant nutrition. It has been shown that the partial replacement of nitrate by the amino acid application can have beneficial impacts on vegetative growth and production (Marschner, 2012; Sadak *et al.*, 2015 and Souri *et al.*, 2017). Amino acids are the intermediate constituents in nitrogen assimilation and play important roles in plant cell metabolism, as they are the main form of nitrogen translocation during the phloem to growing parts (Marschner, 2012 and Kolota *et al.*, 2013). Nitrate in the soil is very necessary to leaching and gaseous emissions (Kolota *et al.*, 2013); whereas the mandatory application of reduced forms of nitrogen under organic farming (Caruso *et al.*, 2012) or the advisable supply of ammonium and amino acids can prevent these effects and reduce nitrate accumulation in plant tissues (Cao *et al.*, 2010 and Souri *et al.*, 2017).

According to Pendas and Pendas (1984), the chelating ligands are most important in controlling plant cation translocation. Chemical forms of trace metals in phloem exudates differ for each element. It was found that zinc was almost all bound to organic compounds readily available to the plant, while the chemical form of zinc was partly complex. Zn is an important micronutrient element for vegetative growth and plays a vital role in plant processes. It is necessary for protein synthesis, photosynthesis, chlorophyll formation, auxin synthesis, cell division, pollen performance, fertility and germination, as well as for lipid metabolites, stability nucleic acid, RNA metabolism and DNA simulation and gene expression regulation (Hopkins, 1995).

Yield characters

Data cleared that number of umbels number/plant, thousand seed weight and fruit yield per plant and per feddan considerably responded to wide planting lines treatment resulting in significant increases except for fruit yield /plant and /fed. in the second season, as compared to narrow planting lines during both seasons (Table 3). In general, the results obtained from wide lines were markedly higher than those of narrow lines in both seasons. Accordingly, the highest values of those parameters resulted from wide planting lines that recorded the most apparent increase in umbel No. per plant reached 9.1 and 8.1 % over narrow lines in the first and second seasons, respectively. Such increases in umbels number corresponded to heavier fruit yield per plant and per feddan evaluated by 3.8 % higher than narrow lines in the first season only.

Cumin plants sprayed with glycine at 200 and 400 ppm, chelated-zinc at 100 and 200 ppm and their combinations led to significant increments in all fruit

characters during both seasons. The highest concentration of each substance was superior to those of the same substance at the lower concentration and the control. Glycine amino acid (400 ppm) + chelated-Zn (200 ppm) was recorded as the best treatment and significantly increased umbel No./plant (30.6 and 28.6 % over the control for the first season and 32.5 and 29.5 % for the second season).

Table 3. Fruit characteristics of *Cuminum cyminum* L. as affected by glycine and/or Zn in combination with different planting lines width treatments during 2019/2020 and 2020/2021 seasons

Planting Lines width (A)	Foliar application B))	2019/2020				2020/2021			
		Umbels No./plant	1000 seed weight (g)	Fruit yield		Umbels No./plant	1000 seed weight (g)	Fruit yield	
				g/plant	Kg/fed.			g/ plant	Kg/ fed.
Narrow lines	Control	24.58	3.05	6.03	351.77	24.81	3.02	6.17	370.00
	G1 (200 ppm)	26.54	3.24	7.49	439.67	26.17	3.28	7.67	460.00
	G2 (400 ppm)	28.49	3.36	8.54	502.45	28.67	3.43	8.74	524.29
	Zn1 (100 ppm)	29.47	3.44	8.31	488.50	29.32	3.49	8.50	510.00
	Zn2 (200 ppm)	31.42	3.78	9.93	586.17	30.80	3.85	10.17	610.00
	G1+Zn1	31.91	4.02	10.42	615.47	31.83	4.02	10.67	640.00
	G1+Zn2	32.40	4.32	10.91	644.77	32.77	4.36	11.17	670.00
	G2+Zn1	32.40	4.71	11.56	683.83	32.72	4.68	11.83	710.00
	G2+Zn2	33.37	5.10	12.21	722.90	33.60	5.01	12.50	750.00
Mean	30.06	3.89	9.48	559.50	30.08	3.90	9.71	582.70	
Wide lines (terrace)	Control	26.54	3.12	7.49	439.67	26.00	3.17	7.16	429.50
	G1 (200 ppm)	28.49	3.29	7.98	468.97	28.17	3.32	7.90	473.80
	G2 (400 ppm)	29.47	3.38	8.71	512.92	29.23	3.41	8.46	507.75
	Zn1 (100 ppm)	32.40	3.51	9.12	537.33	31.45	3.65	9.02	541.43
	Zn2 (200 ppm)	34.35	3.97	9.93	586.17	33.53	3.96	9.83	590.00
	G1+Zn1	34.35	4.13	10.91	644.77	34.18	4.15	10.81	648.60
	G1+Zn2	36.30	4.45	11.11	656.49	36.07	4.29	10.98	658.92
	G2+Zn1	37.28	4.94	11.56	683.83	37.35	4.74	11.47	688.33
	G2+Zn2	38.26	5.23	11.89	703.37	38.49	4.98	11.84	710.40
Mean	33.05	4.00	9.86	581.50	32.72	3.96	9.72	583.19	
Mean of planting lines	Control	25.56	3.08	6.76	395.72	25.40	3.10	6.66	399.75
	G1 (200 ppm)	27.51	3.27	7.74	454.32	27.17	3.30	7.78	466.90
	G2 (400 ppm)	29.00	3.37	8.63	507.69	28.95	3.42	8.60	516.02
	Zn1 (100 ppm)	30.93	3.47	8.71	512.92	30.38	3.57	8.76	525.72
	Zn2 (200 ppm)	32.89	3.87	9.93	586.17	32.17	3.91	10.00	600.00
	G1+Zn1	33.13	4.08	10.67	630.12	33.01	4.09	10.74	644.30
	G1+Zn2	34.35	4.39	11.01	650.63	34.42	4.33	11.07	664.46
	G2+Zn1	34.84	4.83	11.56	683.83	35.03	4.71	11.65	699.17
	G2+Zn2	35.82	5.16	12.05	713.13	36.05	5.00	12.17	730.20
LSD 0.05	A	0.308	0.007	0.038	2.261	1.883	0.070	N.S.	N.S.
	B	0.175	0.035	0.089	5.352	0.523	0.087	0.161	9.656
	A*B	0.361	0.048	0.124	7.392	1.913	N.S.	0.866	52.052

Established plant density or row spacing for seed production have been previously studied for tall fescue (*Festuca arundinacea* Schreber) (Fairey and Lefkovitch, 1999), sunflower (*Helianthus annuus* L.) (Barros *et al.*, 2004), Siberian wildrye (*Elymus sibiricus* L.) (Wang *et al.*, 2017) and many other plant species (Walton, 1977 and Momoh and Zhou, 2001).

As stated previously, the highest seed yield of cumin obtained from the best treatments could be attributed to the stimulative effects of glycine and/or Zn-EDTA due to their vital roles in promoting plant metabolism and activating the physiological processes leading to enhancing the growth and development which reflected on increases in thousand seed weight and fruit yield per plant and per feddan, and consequently the highest yield.

Considering this explanation, the most pronounced combination in this work proved that it was more sufficiently active to absorb the amount of nutrient elements at adequate levels and translocated them toward the leaves as a site of accumulation. This resulted in the highest contents, which were closely related to the best vegetative growth and higher increase in photosynthesis, carbohydrate metabolism, protein and metabolites synthesis, enzymes activity and regulation of auxin synthesis that stimulated fruit formation and consequently controlled seed yield. These findings were consistent with earlier observations on caraway supplied with Zn-EDTA by Diab (2007).

Active biochemical constituents

It is clear that wide planting lines treatment significantly increased total phenolics, flavonoids and antioxidant activity in cumin fruit content except for total phenolics in the second season, as compared to narrow planting lines in both seasons (Table 4). It was observed that all studied biochemical active constituent contents of cumin fruits cleared noticeable responses to glycine and chelated-zinc amendments and their combinations. All glycine and Zn treatments significantly increased total phenolics, flavonoids and antioxidant activity compared to control in the two seasons. However, the highest values were obtained by treating with glycine amino acid (200 ppm) + chelated-Zn (200 ppm), resulting in significant increases when compared with some other treatments (individual glycine or chelated-zinc treatments).

Many publications have been indicated that phenols reduction occurred in zinc-deficient plants since applying Zn can increase phenols excretion or interfere with zinc synergists (Cakmak, 2000 and Zheng and Wang, 2001). Numerous workers emphasized that phenolic compounds are essential in seeds' physiological and biochemical processes. Among the most significant functions of the phenolics are their accumulation in seeds may metabolically create an adaptive role by restricting the growth and development of seeds against DNA-damaging storage. In addition, phenolics may act as disease-resistance mechanisms in seeds and influence competition among plants by producing toxins such as cinnamate (Watts, 1988 and Vallee and Falchuk, 1993).

Table 4. Chemical constituents of *Cuminum cyminum* L. fruits as affected by glycine and/or Zn in combination with different planting lines width treatments during 2019/2020 and 2020/2021 seasons

Planting Lines width (A)	Foliar application (B)	2019/2020			2020/2021		
		Total soluble phenolic (µg GAE/g D.W)	Total flavonoids (µg CE/g D.W)	DPPH free radical scavenging activity EC ₅₀ (µg/ml)	Total soluble phenolic (µg GAE/g D.W)	Total flavonoids (µg CE/g D.W)	DPPH free radical scavenging activity EC ₅₀ (µg/ml)
Narrow lines	Control	4.19	1.78	2.00	4.91	1.63	2.04
	G1 (200 ppm)	6.35	2.77	1.79	6.50	2.61	1.99
	G2 (400 ppm)	6.97	2.99	1.85	7.14	2.85	1.92
	Zn1 (100 ppm)	5.66	2.10	1.92	5.79	2.12	1.85
	Zn2 (200 ppm)	6.13	2.19	1.89	6.28	2.22	1.83
	G1+Zn1	6.06	2.30	1.87	6.20	2.33	1.83
	G1+Zn2	6.29	2.56	1.83	6.44	2.46	1.78
	G2+Zn1	7.03	3.01	1.75	7.20	2.85	1.73
	G2+Zn2	7.18	3.13	1.65	7.35	3.03	1.68
	Mean	6.21	2.54	1.84	6.42	2.46	1.85
Wide lines (terrace)	Control	5.23	2.08	2.23	5.06	2.02	2.15
	G1 (200 ppm)	6.91	3.11	1.98	6.74	2.75	1.92
	G2 (400 ppm)	7.15	3.27	1.92	6.98	3.03	1.89
	Zn1 (100 ppm)	6.52	2.37	2.12	6.35	2.80	1.88
	Zn2 (200 ppm)	6.80	2.57	2.09	6.63	2.85	1.85
	G1+Zn1	7.55	3.35	1.86	7.38	3.21	1.89
	G1+Zn2	7.87	3.64	1.76	7.71	3.42	1.73
	G2+Zn1	6.57	2.62	2.05	6.41	2.56	1.72
	G2+Zn2	6.98	2.88	2.03	6.82	3.10	1.68
	Mean	6.84	2.88	2.00	6.68	2.86	1.86
Mean of planting lines	Control	4.71	1.93	2.12	4.99	1.83	2.10
	G1 (200 ppm)	6.63	2.94	1.89	6.62	2.68	1.96
	G2 (400 ppm)	7.06	3.13	1.89	7.06	2.94	1.91
	Zn1 (100 ppm)	6.09	2.24	2.02	6.07	2.46	1.86
	Zn2 (200 ppm)	6.47	2.38	1.99	6.45	2.54	1.84
	G1+Zn1	6.80	2.82	1.86	6.79	2.77	1.86
	G1+Zn2	7.08	3.10	1.79	7.07	2.94	1.75
	G2+Zn1	6.80	2.81	1.90	6.80	2.71	1.72
G2+Zn2	7.08	3.00	1.84	7.08	3.06	1.68	
LSD 0.05	A	0.065	0.037	0.018	N.S.	0.043	0.001
	B	0.041	0.023	0.006	0.010	0.120	0.006
	A*B	0.079	0.045	0.019	0.655	0.048	0.007

Essential oil characteristics

The data on essential oil characteristics revealed that oil percentage, oil yield per plant and per feddan showed considerable increases as response to wide lines except for oil yield /plant and /fed. in the second season, as compared to narrow lines in both seasons (Table 5). Clearly, all foliar applications of glycine and/or chelated-Zn treatments significantly increased oil %, oil yield per plant and per feddan compared to control in both seasons. Furthermore, the combination of the highest concentrations of glycine and Zn resulted in the highest oil percentage compared to the rest of treatments in both seasons.

Table 5. Essential oil yield and its constituents of *Cuminum cyminum* L. fruits as affected by glycine and/or Zn in combination with different planting lines width treatments during 2019/2020 and 2020/2021 seasons

Planting Lines width (A)	Foliar application (B)	2019/2020			2020/2021		
		Oil %	Essential oil yield		Oil %	Essential oil yield	
			ml/ plant	l/fed		ml/ plant	l/fed
Narrow lines	Control	1.63	0.10	5.76	1.77	0.11	6.57
	G1 (200 ppm)	1.73	0.13	7.63	1.87	0.14	8.62
	G2 (400 ppm)	1.83	0.16	9.21	1.99	0.17	10.45
	Zn1 (100 ppm)	1.83	0.15	8.95	2.02	0.17	10.32
	Zn2 (200 ppm)	1.92	0.19	11.32	2.07	0.21	12.64
	G1+Zn1	2.02	0.21	12.48	2.37	0.25	15.18
	G1+Zn2	2.12	0.23	13.71	2.57	0.29	17.23
	G2+Zn1	2.02	0.24	13.87	2.67	0.32	18.97
	G2+Zn2	2.22	0.27	16.08	2.67	0.33	20.03
	Mean	1.92	0.19	11.00	2.22	0.22	13.33
Wide lines (terrace)	Control	1.73	0.13	7.63	1.87	0.13	8.00
	G1 (200 ppm)	1.83	0.15	8.60	1.98	0.16	9.40
	G2 (400 ppm)	1.92	0.17	9.90	2.03	0.17	10.29
	Zn1 (100 ppm)	1.92	0.18	10.37	2.09	0.19	11.33
	Zn2 (200 ppm)	2.02	0.20	11.89	2.14	0.21	12.64
	G1+Zn1	2.02	0.22	13.08	2.18	0.24	14.16
	G1+Zn2	2.22	0.25	14.60	2.37	0.26	15.63
	G2+Zn1	2.32	0.27	15.88	2.52	0.29	17.37
	G2+Zn2	2.51	0.30	17.71	2.71	0.32	19.28
	Mean	2.05	0.21	12.18	2.21	0.22	13.12
Mean of planting lines	Control	1.68	0.12	6.70	1.82	0.12	7.29
	G1 (200 ppm)	1.78	0.14	8.11	1.92	0.15	9.01
	G2 (400 ppm)	1.88	0.17	9.56	2.01	0.17	10.37
	Zn1 (100 ppm)	1.88	0.16	9.66	2.05	0.18	10.83
	Zn2 (200 ppm)	1.97	0.20	11.60	2.10	0.21	12.64
	G1+Zn1	2.02	0.22	12.78	2.27	0.25	14.67
	G1+Zn2	2.17	0.24	14.16	2.47	0.27	16.43
	G2+Zn1	2.17	0.25	14.87	2.59	0.30	18.17
	G2+Zn2	2.36	0.29	16.89	2.69	0.33	19.66
	Mean	2.05	0.21	12.18	2.21	0.22	13.12
LSD 0.05	A	0.014	0.008	0.341	0.002	N.S.	N.S.
	B	0.011	0.011	0.596	0.001	0.012	0.583
	A*B	0.020	N.S.	N.S.	0.003	0.023	1.242

GC-MS analysis of cumin volatile oil detected more than 22 essential oil components, from which eight principal constituents were identified (Table 6). The total peak area of the eight compounds represents 77.08-86.97% of total detected constituents with different treatments. The other components represent 13.03-22.92% of the total detected constituents. The major constituents were cuminal (19.24 - 26.77%), β -Pinene (16.82-20.21%) and p -cymene (13.40-18.16%). There are many interpretations to the influence of amino acids on volatile oil content in plants. Firstly, amino acids influence the activity of enzymes and the metabolism of volatile oil (Talaat *et al.*, 2014). Carbohydrates, fatty acids, and amino acids represent the natural carbon pools for flavor compounds, which can also be liberated from their polymers. By amino acid degradation, phenyl propane benzenoids can be formed. From these, alcohols, aldehydes and esters can be obtained during hydrolysis of cyanogenic glycosides, from which aldehydes and ketones can be synthesized (Schwab *et al.*, 2008). Secondly, amino acids are a

source of energy, carbon, and nitrogen, which constitute plant tissues and organs (Gleadow and Moller, 2014).

Table 6. Essential oil yield and its constituents of *Cuminum cyminum* L. fruits as affected by glycine and/or Zn in combination with different planting lines width treatments during 2019/2020 and 2020/2021 seasons

Planting Lines width (A)	Foliar application B)	Essential oil constituents (%)								Percentage of total identified compounds
		Cuminal		γ -Terpinene	β -Pinene	ρ -Cymene	Desulphosinigrin	Linalool	Cumin alcohol	
		%	l/fed							
Narrow lines	Control	19.24	1.11	15.32	16.82	18.16	4.56	1.65	1.33	77.08
	G1 (200 ppm)	19.96	1.52	16.21	17.99	17.12	5.25	1.73	1.48	79.74
	G2 (400 ppm)	20.03	1.84	16.52	18.15	17.45	5.37	1.77	1.52	80.81
	Zn1 (100 ppm)	20.37	1.82	15.75	17.12	15.62	4.85	1.93	1.67	77.31
	Zn2 (200 ppm)	22.26	2.52	15.99	17.33	15.09	4.99	2.00	1.69	79.35
	G1+Zn1	22.58	2.82	16.66	18.25	14.96	5.77	1.85	1.61	81.68
	G1+Zn2	24.88	3.41	16.72	18.52	14.84	5.89	2.11	1.70	84.66
	G2+Zn1	23.06	3.20	16.85	19.22	14.65	6.09	1.94	1.65	83.46
	G2+Zn2	25.61	4.12	17.26	19.61	14.26	6.23	2.24	1.76	86.97
	Mean	22.00	2.48	16.36	18.11	15.79	5.44	1.91	1.60	
Wide lines (terrace)	Control	22.16	1.69	16.27	17.01	17.25	3.93	2.00	1.12	79.74
	G1 (200 ppm)	22.88	1.97	16.78	18.62	16.32	4.01	2.12	1.18	81.91
	G2 (400 ppm)	23.08	2.29	16.89	18.85	16.55	4.08	2.18	1.22	82.85
	Zn1 (100 ppm)	23.89	2.48	16.22	17.55	16.02	3.96	2.05	1.15	80.84
	Zn2 (200 ppm)	24.28	2.89	16.57	17.63	16.32	4.00	2.06	1.16	82.02
	G1+Zn1	25.03	3.27	17.09	19.16	13.85	4.16	2.25	1.32	82.86
	G1+Zn2	25.14	3.67	17.12	19.46	13.56	4.19	2.29	1.37	83.13
	G2+Zn1	26.08	4.14	17.55	19.85	13.44	4.22	2.30	1.39	84.83
	G2+Zn2	26.77	4.74	18.02	20.21	13.40	4.25	2.35	1.43	86.43
	Mean	24.37	3.02	16.95	18.70	15.19	4.09	2.18	1.26	
Mean of planting lines	Control	20.70	1.40	15.80	16.92	17.71	4.25	1.83	1.23	78.41
	G1 (200 ppm)	21.42	1.75	16.50	18.31	16.72	4.63	1.93	1.33	80.83
	G2 (400 ppm)	21.56	2.07	16.71	18.50	17.00	4.73	1.98	1.37	81.83
	Zn1 (100 ppm)	22.13	2.15	15.99	17.34	15.82	4.41	1.99	1.41	79.08
	Zn2 (200 ppm)	23.27	2.71	16.28	17.48	15.71	4.50	2.03	1.43	80.69
	G1+Zn1	23.81	3.05	16.88	18.71	14.41	4.97	2.05	1.47	82.27
	G1+Zn2	25.01	3.54	16.92	18.99	14.20	5.04	2.20	1.54	83.90
	G2+Zn1	24.57	3.67	17.20	19.54	14.05	5.16	2.12	1.52	84.15
	G2+Zn2	26.19	4.43	17.64	19.91	13.83	5.24	2.30	1.60	86.70

Conclusion

From the obtained results, it could be concluded that spraying cumin plants cultivated on wide planting lines with glycine amino acid at a rate of 400 ppm plus chelated-Zn at a rate of 200 ppm was the most effective treatment for improving all studied characters (growth, yield, chemical constituents and essential oil) as compared with other applied treatments.

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الرش الورقي بالجليسين و/ أو الزنك يزيد النمو والمحصول ومكونات الزيت العطري للكمون تحت طرق زراعة مختلفة

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الملخص

أجريت تجربة حقلية خلال موسمي 2020/2019 و2021/2020 لتقييم تأثيرات المعدلات المختلفة للرش الورقي للجليسين (200 و400 جزء في المليون) و/أو الزنك (100 و200 جزء في المليون) بالإضافة إلى الكنترول، وخطوط الزراعة المختلفة (خطوط عريضة وخطوط ضيقة) على النمو والمحصول والمحتوى الكيميائي وكذلك الزيت العطري ومكوناته في نباتات الكمون. سجلت البيانات أن خطوط الزراعة العريضة (المصاطب) أظهرت زيادة معنوية في قياسات النمو الخضري (طول النبات، عدد الفروع ووزن العشب الطازج والجاف)، المحصول (عدد النورات، وزن الألف ثمرة، محصول الثمار للنبات والقدان)، المحتوى الكيميائي (الفينولات الكلية، الفلافونيدات الكلية والنشاط المضاد للأكسدة) والزيوت العطرية (نسبة الزيت، محصول الزيت لكل نبات والقدان). أدى رش نباتات الكمون بالجليسين (200 و400 جزء في المليون) والزنك المخلّب (100 و200 جزء في المليون) وتوليفاتها إلى تأثيرات معنوية في أغلب المعاملات. كان التركيز الأعلى لكل مادة متفوقاً مقارنة بتلك الموجودة في نفس المادة عند التركيز الأقل وكذلك الكنترول. الجلايسين بمعدل 400 جزء في المليون بالإضافة إلى الزنك المخلبي بمعدل 200 جزء في المليون مع خطوط زراعة واسعة كان هو التوليفة الأفضل وأظهرت زيادة معنوية لجميع الصفات المدروسة مقارنة بالتوليفات الأخرى. لذلك، يمكننا أن نوصي بتطبيق التوليفة المثلى لإنتاج نباتات الكمون بجودة عالية وأمنة وصحية.