Journal of Horticultural Science & Ornamental Plants 14 (2): 84-97, 2022
ISSN 2079-2158

DOI: 10.5829/idosi.jhsop.2022.84.97

Growth, Yield and Essential Oil of Geranium (*Pelargonium graveolens* L.) Plants in Response to Zinc, Tryptophan and Ascorbic Acid

Mohamed Abd El-Kawy Abd El-Sayed, Salwa, S.S. Awad Alla and Sarah N. Abd El-Khalek

Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt

Abstract: This work was carried out during the two successive seasons of 2019/2020 and 2020/2021 at the farm of the Medicinal and Aromatic Plant Research Department in El-Kanater El- Khaireya, Qalubeia Governorate, Horticulture Research Institute, Agricultural Research Center, Dokki, Giza. The aim of the study was to investigate the effect of zinc, tryptophan and ascorbic acid in different concentrations on the growth, yield, essential oil productivity, antioxidant activity and antimicrobial properties of geranium plant. The results were summarized as follows, growth parameters of geranium plants showed a stimulation effect due to tested three factors, *i.e.* zinc, tryptophan and ascorbic acid at all concentrations compared to control plants. Ascorbic acid at rate 200 mg/l significantly increased each of plant height, number of branches, fresh and dry weights per plant and per feddan as well as essential oil percentage, essential oil yield (ml/plant and L/ feddan), photosynthetic pigments, antioxidant and antimicrobial activity of geranium oil.

Key words: *Pelargonium graveolens* · Zinc · Tryptophan · Ascorbic acid · Growth · Volatile oil production · Yield · Antioxidant · Antimicrobial activity

INTRODUCTION

Recently, the general public has become interested in the use of medicinal and aromatic plants instead of synthetic drugs, since they can provide natural compounds often without side effects and are less costly [1]. There are several types of natural substances, including secondary metabolites from plants, including essential oils and volatile substances, flavonoids and phenolic compounds are of particular interest because of their health benefits [2, 3]. According to a number of researchers, the production or chemical structure of essential oils, as well as their possible biological characteristics, have also been discovered to vary widely. A number of factors contribute to this variation, including geographical location, the environment conditions, cultivars, transplanting date, intercropping, distillation method, post-harvest and storage conditions [4].

Rose geranium (*Pelargonium graveolens* L.' Herit. ex Ait) is perennial aromatic herb belonging to Geraniaceae family. Its essential oil is highly valued, *Pelargonium graveolens* is widely cultivated in several countries, mainly in Egypt, Russia, Algeria, Morocco and Japan. The most common countries where geranium is cultivated commercially are Algeria, Egypt, Morocco, India and China. Rose geranium essential oil is used in cosmetics and fragrance industries, making it is one of the twenty most important oils in the world [5].

Because geranium oil has strong anti-inflammatory, immune-modulating, antibacterial and antifungal qualities, it is frequently used to treat haemorrhoids, dysentery, cancer and other inflammatory conditions [6, 7]. Many of these activities are attributed to its oxygenated monoterpenes content; including geraniol, citronellol, linalool and geranyl formate [8].

Zinc is a micronutrient that is important for numerous physiological functions, a deficiency of zinc will reduce crop yields. Zinc acts as a functional, structural, or regulatory cofactor of a large number of enzymes and is required for protein synthesis, photosynthesis and the synthesis of auxin. Additionally, it is required for the synthesis of tryptophan, which is a precursor for IAA and a growth-promoting compound, cell division and function and sexual fertilization [9]. In previous studies, Zn was found to have a strong impact on the essential oil biosynthesis of *Ocimum sanctum* [10]. Foliar application

Corresponding Author: Sarah N. Abd El-Khalek, Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.

of zinc increased essential oil of Peppermint plant [11]. Misra *et al*. [12] reported that Zinc deficiency had a significant effect on the production of geranium essential oil.

In plant tissues and organs, amino acids act as a source of energy, carbon and nitrogen [13]. L-tryptophan is an important amino acid. The common precursor of the plant hormone auxin is L-tryptophan which is a physiological precursor of indole acetic acid, its application at the right amounts may promote plant growth [14]. It may act as an ion transport regulator, osmolyte and modulates stomatal opening in plants [15].

Previous studies have indicated that amino acids have a positive impact on the production and components of essential oils in aromatic plants. For example, geranium, thyme, philodendron, navel orange, hyssop and lemon plants treated with amino acids have higher essential oil compositions than the untreated control [16-21].

Ascorbic acid is found in all living plant cells, but is most abundant in the leaves and flowers, where the plant is actively growing. AsA is an important molecule involved in cell division and osmotic regulation [22]. Ascorbic acid plays different roles in plant development, such as regulating the biological process, cell membranes growth, chemical change, flowering, senescence [23]. Undoubtedly, ascorbate in the leaves could control plant development by interacting with phytohormones [24]. It is an important co-factor in the formation of the many plant hormones such as gibberellin (GA) and abscisic acid (ABA), that effectively development growth and chemical compounds [25, 26].

According to available data on chemical composition of geranium essential oil, indicate that the main components were citronellol, geraniol and citronellyl formate [27]. Numerous researches have demonstrated the antibacterial and antifungal properties of *Pelargonium graveolens* essential oil and extracts [28]. The considerable cytotoxic effect that *P. graveolens* extracts gave and the probable positive contribution of flavonoid derivatives to this biological activity can be linked to the antimicrobial activity of these extracts. There have been reports of *P. graveolens'* antioxidant and antitermitic properties [29].

The main purposes of this study are investigation the effect of Zinc, Tryptophan and Ascorbic on yield and its components of geranium plants as essential oil productivity and studying the changes in chemical composition, antioxidants activity and antimicrobial induced of geranium oil by foliar application of Zinc, Tryptophan and Ascorbic acid on oil of geranium plants.

MATERIALS AND METHODS

This research was carried out in the farm of the Medicinal and Aromatic Plant Research Department in El-Kanater El- Khaireya, Qalubeia Governorate, Horticulture Research Institute, Agricultural Research Center, Dokki, Giza, during the two successive season 2019/2020 and 2020/2021. Cuttings of geranium [15-20 cm long] obtained from the same farm. In both seasons, cuttings were planted on 15th October. Cuttings were planted to the plots (2 × 2.5 m^2) that were prepared in the experimental field, with each plot containing three rows. The rows within each plot were 2 meters in length and 60 cm apart. Each plot contained 18 cuttings, at a spacing of 30 cm between plants. The physical and chemical characteristics of the soil of the experimental field are shown in Table (1).

The experiment design was a randomized complete blocks design, as described by Snedecor and Cochran [30], with 10 different treatments and three replicates (blocks). The plants were sprayed with zinc and tryptophan at rates (100, 150 and 200 mg/L) and Ascorbic acid at rates (50, 100 and 200 mg/L), in addition to the untreated plants (control). Six foliar spray treatments were applied, the first dose was four months after planting (on February 13th in both seasons) and were repeated 5 times at one-month intervals on 10th March, 14th April before the first cut and on 7th July, 4th August and 8th September before the second cut.

The recommended NPK fertilization consisted of calcium super phosphate (15.5% P_2O_5) at the rate of 300 kg/fed and potassium sulphate (48% K_2O) at the rate of 150 kg/fed. were added during soil preparation. But, Ammonium sulphate (20.5% N) at the rate of 450 kg/fed. was added as six equal portions (each 75 kg/fed.), *i.e* three portions were added before the first cut at 120, 150 and 180 days from transplanting date. While the other three portions were added after 30, 60 and 90 days from the first cut in July, August and September in both seasons.

Plants were harvested twice each season, on 1st June and 10th October. Plant shoots were cut to a height of 15 cm above the soil surface.

Data Recorded

Growth Characteristics: In both seasons, the following growth characteristics were recorded; plant height, shoots number, fresh weight and dry weight per plant.

Yield Productivity: Fresh yield per feddan (ton/fed.) for the both seasons was recorded.

Table 1: Physical and chemical analysis of the soil used for growing *Pelargonium graveolens* L. plants during the 2019/2020 and 2020/2021 seasons

Physical characteristics					Chemical characteristics								
First Season (2019)													
Clay%	Silt%	Fine sand%	Coarse sand%	Soil type	pH	N (ppm)	P2O5 (ppm)	K2O (ppm)	Zn (ppm)	Fe (ppm)	B (ppm)	Mn (ppm)	Cu (ppm)
41.65	26.74	29.17	2.44	Clay sand	7.22	27.3	108	180	5.54	3.77	1.24	0.75	0.51
Second Season (2020)													
Clay%	Silt%	Fine sand%	Coarse sand%	Soil type	pH	N (ppm)	P2O5 (ppm)	K2O (ppm)	Zn (ppm)	Fe (ppm)	B (ppm)	Mn (ppm)	Cu (ppm)
41.31	25.65	30.43	2.61	Clay sand	7.40	26.8	111	171	5.37	3.64	1.38	0.77	0.53

Essential Oil Productivity: The percentage of essential oil was determined in the fresh herb by hydrodistillation for 3 hours using a Clevenger type apparatus. The essential oil content was calculated as a relative percentage (v/w) [31]. Essential oil yield (ml/plant) and (L/fed.) were recorded.

Gas Chromatography Analysis: Samples taken from the essential oil obtained in the first cut of the first season were analysed using gas liquid chromatographic analysis (DsChrom 6200 Gas Chromatograph), to determine their main constituents [32].

Photosynthetic Pigments: Chlorophyll a, b and total carotenoids were measured in fresh green leaves according to Saric *et al.* [33].

Antioxidant Activity Using DPPH Radical Scavenging Activity: The hydrogen electron donating ability of the corresponding volatile oils was measured from the bleaching of the purple colored methanol solution of 2, 2-diphenyl – picrylhydrazyl (DPPH) [34]. 3 ml of oil was added to 1 ml of methanolic solution of DPPH and measured by spectrophotometer, the absorbance was read against blank at 517 nm. The antioxidant capacity to scavenge the DPPH radical for the oils was calculated by the following equation:

$$\text{Scavenging effect (\%)} = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$$

where A_{blank} is the absorbance of control reaction (containing each reagent except the sample) and A_{sample} is the absorbance of sample.

Test Bacteria and Fungi: The bacterial strains were *Escherichia. coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228 and *Salmonella enterica* subsp. ATCC 14028. The tested fungus was *Aspergillus brasiliensis* ATCC 16404. One strain of yeast was investigated in this study; *Candida albicans* ATCC 10231. The bacterial isolates were subcultured on Nutrient agar and yeast on potato dextrose agar and incubated aerobically at 37°C.

The Methods: Various concentrations (5, 10 and 20 µL) of *P. graveolens* essential oil samples extracted from plants treated Ascorbic acid, zinc and tryptophan at rate 200 mg/l were evaluated using agar well diffusion method [35]. The Mueller Hinton agar was used for testing of bacterial strains *Escherichia. coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228 and *Salmonella enterica* subsp. ATCC 14028 and potato dextrose agar was used for testing of fungal strains *Aspergillus brasiliensis* ATCC 16404 and *Candida albicans* ATCC 10231, for determination the Minimum Inhibitory Concentrations (MIC) where the agar plates were inoculated with bacterial and fungal strains under aseptic conditions and wells (diameter=8mm) were filled with 5, 10 and 20 µL of the test essential oil extracts. The plates were left for 90 minutes at 4°C to allow the diffusion of the essential oil extracts and then they were incubated at 37°C for 24 hours and at 25°C for 5 days, respectively. After the incubation period, the diameter of the growth inhibition zones was measured in millimeter (mm) according to Andrews [36]. Eighteen to 24 hrs single colonies on agar plates were used to prepare the bacterial suspension with the turbidity of 0.5 McFarland ($1\text{-}2\times10^8$) CFU/ml, the yeast suspension with the turbidity of 0.5 McFarland ($1\text{-}5\times10^6$) CFU/ml and the molds the yeast suspension with the turbidity of 0.5 McFarland ($0.4\text{-}5\times10^6$) CFU/ml [37-39].

RESULTS AND DISCUSSION

Growth Characteristics

Plant Height (cm): Data presented in Table (2) showed the effect of zinc, tryptophan and ascorbic acid on plant height of geranium plants. All tested factors significantly increased the plant height (cm) compared to the control in both seasons. Zinc treatment at rates (100, 150 and 200 mg/l) improved the plant growth in term of plant height as compared with the control. Values of plant height showed

Table 2: Effect of foliar application with Zinc, Tryptophan and Ascorbic acid on plant height (cm) and number of shoots/plant of *Pelargonium graveolens* plants during the 2019/2020 and 2020/2021 seasons

	Plant height (cm)				Number of shoots/plant			
	First Season		Second Season		First Season		Second Season	
Treatments	Cut1	Cut2	Cut1	Cut2	Cut1	Cut2	Cut1	Cut2
Control	56.00	54.00	63.67	61.33	13.00	17.33	12.33	15.00
Zn 1	58.00	57.33	66.67	63.33	14.33	22.00	16.00	19.00
Zn2	62.33	60.00	73.33	67.67	17.33	26.00	19.33	21.33
Zn 3	67.00	64.33	74.40	72.33	17.33	27.00	23.00	25.67
Try 1	70.67	65.33	74.00	72.33	22.67	24.00	21.67	25.33
Try 2	74.33	70.67	81.60	75.10	24.00	26.00	24.67	26.33
Try 3	81.67	75.00	86.53	77.33	26.33	29.67	28.33	30.67
ASA 1	74.33	70.67	78.33	75.33	19.33	23.00	25.33	26.00
ASA 2	82.00	78.33	86.33	83.00	20.33	29.33	27.67	29.33
ASA3	87.67	83.67	91.67	85.67	30.00	32.33	32.67	35.67
L.S.D 0.05	1.51	2.45	2.75	1.33	3.02	3.32	2.99	3.47

*Zn1= Zinc at rate 100 mg/l, Zn2= Zinc at rate 150 mg/l, Zn3= Zinc at rate 200 mg/L, Try1=Tryptophan at rate 100 mg/l, Try2=Tryptophan at rate 150 mg/l, Try3=200 mg/L, ASA1= Ascorbic acid at rate 50 mg/l, ASA2= Ascorbic acid at rate 100 mg/l and ASA3= Ascorbic acid at rate 200 mg/l

a gradual increase with increasing zinc levels, the tallest plants were obtained by the highest concentration of zinc in the two growing seasons. Many researchers discovered that Zn spraying led to an increase in plant height, such as Hendawy and Khalid [40] on *Salvia officinalis* plant, Pande *et al.* [41] on mint plant and Said-Al-Ahl and Omer [42] on *Coriandrum sativum*, they came to the conclusion that Zn spraying the plants considerably boosted their height. The fact that zinc accelerates cell division and regulates the metabolism of auxin may be the cause of zinc's beneficial influence on plant height [43]. Also, it could be observed that in the two seasons, three rates 100, 150 and 200 mg/L of tryptophan significantly increased plant height of geranium plants. The highest values of plant height were recorded for those plants sprayed with the highest concentration of tryptophan. Tryptophan-induced plant height increases may be ascribed to increased IAA availability, which promotes cell division and cell elongation. According to Mohite [44], treated plants' readily available tryptophan is transformed into IAA, which increases plant height. In addition, ascorbic acid at rates 200 mg/l in both seasons were sharp efficacy on increasing plant height (cm) than other treatments and control. Values of plant heights of geranium gradually increased with the increasing ascorbic acid levels, in the two seasons. The highest values were 87.67, 83.67, 91.67 and 85.67 cm in the first and second cuts for the first and second seasons respectively, in case of those plants sprayed with the highest concentration of ascorbic acid at rate 200 mg/l in comparison with other treatments. Ascorbic acid's stimulation of vegetative growth was observed in several medicinal and aromatic plants, like as, Reda *et al*. [45] on *Thymus vulgaris*, Hendawy and Ezz El-Din [46] on *Foeniculum vulgare,* Soltan *et al.* [47] on *Calendula officinalis*, Azoz *et al.* [48] on *Ocimum basilicum* and El-Moghazy and Al-Azzony [49] on *Pelargonium graveolens*. All of which are consistent with the current findings. It is important to note that the growth-promoting effects of ascorbic acid on various plant species depend on the tested concentration, the genotype used for the plant species, the time of application and the suggested procedures followed for the used genotype.

Number of Shoots/Plant: Data presented in Table (2) indicated that the number of shoots formed by geranium plants was significantly affected by the tested treatments. In both seasons, the highest concentration (200 mg/l) of zinc and tryptophan gave highly significant increase in number of shoots/plant compared to control. As well as, Ascorbic acid (ASA3) at rate of 200 mg/l gave the highest number of shoots/plant with values (30 and 32.33) in both cuts of the first season and 32.67 and 35.67 shoots/plant in the first and the second cuts of the second season, respectively. Due to the way ascorbic acid (ASA) affects cell division and differentiation, it is currently believed that ASA controls plant growth and development. The interaction of ascorbate with phytohormones may be the mechanism by which it controls plant growth and development and a cofactor for the manufacture of a number of phytohormones, including ethylene, gibberellins (GA) and abscisic acid, is ascorbate (ABA).

Table 3: Effect of foliar application with Zinc, Tryptophan and Ascorbic acid on herb fresh and dry weight/plant (g) of *Pelargonium graveolens* plants during the 2019/2020 and 2020/2021 seasons

	Herb fresh weight/plant (g)				Herb dry weight/plant (g)			
	First Season		Second Season		First Season		Second Season	
Treatments	Cut1	Cut2	Cut1	Cut2	Cut1	Cut2	Cut1	Cut2
Control	421.55	530.47	534.43	584.67	97.03	106.72	115.95	128.51
Zn 1	474.94	548.28	596.22	623.99	107.29	125.31	129.17	142.49
Zn2	522.50	622.76	611.02	643.69	119.82	134.86	130.39	139.35
Zn 3	566.58	641.25	627.50	692.11	134.42	147.37	142.30	159.06
Try 1	531.33	569.39	611.33	633.33	116.80	125.67	134.60	145.91
Try 2	555.14	659.69	638.79	680.48	125.94	136.51	144.73	156.51
Try 3	593.92	680.55	653.85	703.55	140.39	167.86	153.76	173.47
ASA 1	529.22	585.28	634.62	660.52	116.43	129.67	139.62	146.14
ASA 2	572.72	644.85	671.53	701.49	125.82	143.44	147.98	163.65
ASA3	694.50	762.14	721.93	779.83	152.89	183.02	161.19	192.34
L.S.D 0.05	21.41	18.03	12.42	10.71	9.69	11.29	8.51	12.28

*Zn1= Zinc at rate 100 mg/l, Zn2= Zinc at rate 150 mg/l, Zn3= Zinc at rate 200 mg/L, Try1=Tryptophan at rate 100 mg/l, Try2=Tryptophan at rate 150 mg/l, Try3=200 mg/L, ASA1= Ascorbic acid at rate 50 mg/l, ASA2= Ascorbic acid at rate 100 mg/l and ASA3= Ascorbic acid at rate 200 mg/l

Plants' ascorbic acid may control growth by interacting with phytohormones [24]. These findings were consistent with those reported by El-Gabas [50] who discovered that spraying ascorbic had a positive impact on the growth characteristics of sunflowers. Additionally, ascorbic acid has a considerable impact on the growth of German chamomile, according to Bahram *et al.* [51].

Fresh Herb/Plant (g): Data presented in (Table 3) indicated that foliar application with zinc, tryptophan and ascorbic acid were found to significantly increased fresh weighs (g/plant) when compared with the control treatment in both seasons. Plants sprayed with zinc (Zn3) and tryptophan (Try3) at rate 200 mg/l gave significantly heavier herb fresh weights, in comparison with control. Also, ascorbic acid at 200 mg/l in the two seasons was more effective on increasing fresh weights (g/plant) than other treatments. Values of herb fresh weight in the first season recorded 694.50 and 762.14 g/plant. while, in the second season the values were 721.93 and 779.83 g/plant for the first and the second cuts, respectively. These results agree with those of Ghazal [52], who demonstrated that ascorbic acid foliar spray at various concentrations considerably increased the fresh weight of the herb (*Thymus vulgaris* L.) compared to untreated plants. According to Zewail [53], ascorbic acid has a stimulatory effect on many physiological processes, including respiration, cell division and the activity of numerous enzymes. Helsper *et al.* [54] have also emphasized the importance of ascorbic acid's role in the regulation of photosynthetic carbon reduction. In general, it could be concluded that, geranium growth was stimulated by ascorbic acid. Therefore, geranium plants showed increments in height and branching consequently the fresh weight.

Herb Dry Weight (g)/Plant: Also, the results presented in Table (3) generally showed that zinc, tryptophan, ascorbic acid gave significantly heavier herb dry weights, in comparison to control. As for the effect of zinc, it was noticed that spraying geranium plants with Zn3 at rate 200 mg/l achieved significantly increases in dry weights in the two seasons. In the first season, values were 134.42 and 147.37 g/plant dry weight compared to untreated plants (control), in the first and second cuts, respectively. Also, in the second season, plant dry weight values were 142.30 and 159.06 g/plant dry weight compared to untreated plants (control) in the two cuts. Zinc application increased the photosynthetic activity which ultimately resulted in improving the growth [55].

The addition of tryptophan (Try3) at 200 mg/l had a significant effect on dry weight of geranium plants in both seasons. In the first season, Try3 showed an increase of dry weight (140.39 and 167.86 g/plant in the first and second cuts, respectively). The same trend was observed in the second season the values were (153.67 and 173.47 g/plant) compared to the control in the first and second cuts, respectively. Tryptophan's ability to stimulate activity by enhancing the production of phytohormones may also be responsible for this promoting impact [56]. Villareal *et al*. [57] also indicate that the higher observed increase in yield components by tryptophan application might be attributed to the greater enzymatic activities and rapid release of the nutrients in the soil.

Table 4: Effect of foliar application with Zinc, Tryptophan and Ascorbic acid on fresh herb yield/fed. (ton) of *Pelargonium graveolens* plants during the 2019/2020 and 2020/2021 seasons

	Yield of fresh herb/plant (ton/fed.)			
	First Season		Second Season	
Treatments	Cut1	Cut2	Cut1	Cut2
Control	9.27	11.67	11.76	12.86
Zn 1	10.45	12.06	13.12	13.73
Zn2	11.50	13.70	13.44	14.16
Zn 3	12.46	14.11	13.81	15.23
Try 1	11.69	12.53	13.45	13.93
Try 2	12.21	14.51	14.05	14.97
Try 3	13.07	14.97	14.38	15.48
ASA 1	11.64	12.88	13.96	14.53
ASA 2	12.60	14.19	14.77	15.43
ASA3	15.28	16.77	15.88	17.16
L.S.D 0.05	0.80	0.40	0.30	0.44

*Zn1= Zinc at rate 100 mg/l, Zn2= Zinc at rate 150 mg/l, Zn3= Zinc at rate 200 mg/L, Try1=Tryptophan at rate 100 mg/l, Try2=Tryptophan at rate 150 mg/l, Try3=200 mg/L, ASA1= Ascorbic acid at rate 50 mg/l, ASA2= Ascorbic acid at rate 100 mg/l and ASA3= Ascorbic acid at rate 200 mg/l

As previously shown in vegetative growth parameters, raising the application rate of ascorbic acid resulted in steady increases in the dry herb weight (in most cases). So, it was found that, ascorbic acid at rate 200 mg/l in the two seasons was more effective on increasing dry weights than other treatments. Values of herb dry weight in the first season recorded 152.89 and 183.02 g/plant and in the second season the values were 161.19 and 192.34 g/plant for the first and second cuts, respectively. Similar results were obtained by Balbaa and Talaat [58] who noted that with ascorbic acid application significantly increased dry herb weights of rosemary plants.

Yield of Fresh Herb (ton/fed.): The values of fresh herb yield per feddan as affected by foliar spray with various concentrations of zinc, tryptophan and ascorbic acid are presented in Table (4). the results showed the same trend that previously recorded in case of the effect of zinc, tryptophan and ascorbic acid on morphological characters of vegetative growth was also recorded in yield of fresh herb per feddan. In both seasons, zinc and tryptophan gave significantly higher values of herb fresh yield than control. Spraying of geranium plants with high level of zinc or tryptophan at rate 200 mg/l was more effective in increasing the yield of fresh herb per fed. in comparison to control.

In addition, the most effective treatment in this respect was ascorbic acid 200 mg/l which giving the highest yields in both seasons (15.28 and 16.77 tons/fed. in the first and second cut of the first season), while, in the second season, values were 15.88 and 17.16 tons/fed. in the first and second cuts, respectively). The increase in geranium plant biomass due to spraying with ascorbic acid confirms the role of vitamin C in regulation of photosynthesis processes and growth activation [59]. The results were consistent with those reported by El-Quesni *et al.* [60] on *Hibiscus rosa sineses*; Hendawy and Ezz El-Din [46] on *Foeniculum vulgare*; Khalil *et al.* [61] on *Ocimum basilicum* and Nikee *et al.* [62] on *Satureja hortensis*.

According to recent research findings, ascorbate's interaction with phytohormones may be the mechanism by which it controls plant growth and development. Ascorbate is cofactor for the synthesis of a number of phytohormones, including ethylene, gibberellins (GA) and abscisic acid. The endogenous ascorbate influences the biosynthesis of phytohormones, as well as the signal transduction pathway mediated by phytohormones [24].

Essential Oil Productivity

Essential Oil Percentage: Data recorded on the essential oil production of geranium plants in the two seasons (Table 5) showed that, significant increase of essential oil percentage of geranium plants were recorded in case of those plants sprayed with ascorbic acid, tryptophan and zinc at rate 200 mg/l compared to untreated plants (control) in both cuts in the first and the second seasons, respectively. In general, the most effective treatment in this regard was ASA3 at 200 mg/l which gave the highest oil percentage in two cuts of the two seasons.

Essential Oil Yield/Plant (ml): Data in Table, 5 illustrated the essential oil yield per plant in response to zinc, tryptophan and ascorbic acid, Generally, the yield of essential oil (ml/plant) was increased due to the tested factors. Spraying tryptophan increased the essential oil yield per plant comparing with spraying geranium plants with zinc in most cases. However, all tested ascorbic acid concentrations significantly increased essential oil yield/ plant comparing to the rest of the treatments and control. In both seasons, ascorbic acid at rate 200 mg/l gave the highest essential oil yield / plant with values of 0.97 and 0.99 ml/plant in the first and second cuts of the first season and 0.87 and 0.94 ml/plant in the first and second

Table 5: Effect of foliar application with Zinc, Tryptophan and Ascorbic acid on essential oil production of *Pelargonium graveolens* plants in the 2019/2020 and 2020/2021 seasons

	Essential oil %				Essential oil yield/plant (ml)				Essential oil yield/fed. (L)			
	First Season		Second Season		First Season		Second Season		First Season		Second Season	
Treatments	Cut1	Cut2	Cut1	Cut2	Cut1	Cut2	Cut1	Cut2	Cut1	Cut2	Cut1	Cut2
Control	0.10	0.09	0.07	0.06	0.42	0.48	0.37	0.35	9.27	10.50	8.23	7.72
Zn 1	0.09	0.09	0.09	0.07	0.43	0.49	0.54	0.44	9.40	10.86	11.81	9.61
Zn2	0.10	0.11	0.10	0.09	0.52	0.69	0.61	0.58	11.50	15.07	13.44	12.75
Zn 3	0.12	0.12	0.11	0.10	0.68	0.77	0.69	0.69	14.96	16.93	15.19	15.23
Try 1	0.09	0.10	0.10	0.08	0.48	0.57	0.61	0.51	10.52	12.53	13.45	11.15
Try 2	0.11	0.11	0.11	0.09	0.61	0.73	0.70	0.61	13.43	15.96	15.46	13.47
Try 3	0.12	0.12	0.11	0.10	0.71	0.82	0.72	0.70	15.68	17.97	15.82	15.48
ASA 1	0.10	0.11	0.10	0.09	0.53	0.64	0.63	0.59	11.64	14.16	13.96	13.08
ASA 2	0.13	0.11	0.11	0.10	0.74	0.71	0.74	0.70	16.38	15.61	16.25	15.43
ASA3	0.14	0.13	0.12	0.12	0.97	0.99	0.87	0.94	21.39	21.80	19.06	20.59
L.S.D 0.05	0.02	0.01	0.02	0.02	0.10	0.13	0.11	0.19	2.11	1.33	1.53	1.21

*Zn1= Zinc at rate 100 mg/l, Zn2= Zinc at rate 150 mg/l, Zn3= Zinc at rate 200 mg/L, Try1=Tryptophan at rate 100 mg/l, Try2=Tryptophan at rate 150 mg/l, Try3=200 mg/L, ASA1= Ascorbic acid at rate 50 mg/l, ASA2= Ascorbic acid at rate 100 mg/l and ASA3= Ascorbic acid at rate 200 mg/l

cut of the second season, respectively. These values were significantly higher than those obtained from control as well as the other treatments. Similar results were previously published by Noor El-deen [63] on *Majorana hortensis*, Said-Al Ahl *et al.* [64] on coriander and Nasiri *et al*. [65] on *Dracocephalum moldavi*ca.

Essential Oil Yield/fed. (L): Results also showed that the effect of various concentrations of zinc, tryptophan and ascorbic acid on essential oil yield /fed. The results recorded in the two seasons (Table 5) also showed that, an increments in oil yield/fed in response to these tested factors. The highest oil yields were obtained from the highest concentrations of zinc, tryptophan and ascorbic acid. On the other hand, untreated (control) plants gave significantly lower essential oil yield/fed. 9.27 and 10.50 L / fed. in both cuts of the first season and 8.23 and 7.72 in the first and second cuts of the second season, respectively, compared to all tested factors. In both seasons, the highest essential oil yield/fed. (21.39 and 21.80 L/fed.) in the two cuts in the first season and (19.06 and 20.59 L/fed.) in the two cuts in second season, respectively were produced by plants treated with ascorbic acid at rate 200 mg/l. It was worthy to note that these positive effects of ascorbic acid on geranium herb yield and essential oil percentage and its yield/ plant were reflected on results essential oil yield/fed. These results agree with those obtained by Taraf *et al.* [66] on lemongrass, Youssef and Talaat [67] on rosemary, Eid *et al.* [68] on *Jasminum grandiflorm,* as well as El-Lethy *et al.* [69] on geranium reported that foliar application of ascorbic acid caused pronounced increased in the yield of essential oil.

Ascorbic acid increases the ability of meristematic cells to synthesise the active substrate required for the biosynthesis of essential oils, which is one of the favourable impacts of ascorbic acid on essential oil production [70]. Ascorbic acid, which is widely used as a growth regulator, can enhance the production of EO either directly or indirectly by promoting plant growth and facilitating enzymatic processes [59].

Essential Oil Composition: The obtained results indicated that the chemical composition of *Pelargonium graveolens* volatile oil had been affected by the different concentrations of zinc, tryptophan and ascorbic acid (Table 6). These results can be summarized as follows:

Results of the chromatographic analysis of oil samples extracted from (*Pelargonium graveolens*) plants in the first cut of the first season (Table 6) showed that Citronellol was the most important essential oil component (with contents of 22.24-30.073%), followed by Geraniol (with contents of 6.34-17.07%), Citronellyl formate (with contents 6.84-13.26%), then linalool (with contents of 5.51-12.26%). The presence of these compounds in the essential oil of *Pelargonium graveolens* have been already reported in studies of Wang *et al.* [71] and Guenther [72].

Rose-like odour of geranium oil is a mixture of citronellol, geraniol and other alcohols. The quality of geranium oil is mainly determined by citronellol, geraniol contents and the ratio of citronellol: geraniol (Table 6). Citronellol content is a very important feature of the evaluation of geranium oil [72]. Citronellol is used in perfumes and insect repellent Taylor and Schreck [73].

Table 6: Effect of foliar application with Zinc, Tryptophan and Ascorbic acid on Essential oil composition of *Pelargonium graveolens* in first cut of the 2019/2020 season

	Treatments									
The components (%) of the essential oil	Control	Zn1	Zn2	Zn3	Try1	Try2	Try3	ASA1	ASA2	ASA3
α-pinene	1.54	0.8	0.46	1.86	1.67	1.55	0.69	0.89	0.7	1.23
β-pinene	1.95	0.68	1.29	1.21	1.17	1.88	0.98	0.79	0.95	0.73
Ɒ-Cymene	1.32	1.63	0.37	2.28	1.77	2.61	0.67	0.96	1.43	1.54
Limonene	8.31	7.76	6.44	6.67	6.83	5.49	6.38	5.7	4.99	5.93
Linalool	12.26	7.51	6.94	6.9	6.09	7.07	5.51	11.06	9.02	11.15
Isomenthone	7.47	9.26	10.47	9.98	9.03	9.54	9.37	7.89	6.74	7.81
α-Terpineol	3.17	5.51	5.43	3.64	8.9	5.34	6.9	5.51	5.53	5.92
Nerol	2.85	1.76	2.11	2.34	2.64	1.98	2.29	2.71	2.56	2.07
Citronellol	23.44	30.07	28.1	24.98	24.45	23.97	24.03	26.12	22.24	22.26
Citral	1.28	1.21	1.26	2.41	1.11	1.69	1.9	1.33	2.51	1.59
Geraniol	6.34	11.64	11.7	10.84	9.68	10.09	12.11	16.76	17.07	17.59
Geranyl butrate	1.28	1.89	1.41	1.53	1.93	2.45	1.18	1.02	0.9	1.16
Citronellyl formate	6.84	10.23	12.04	9.8	13.01	10.55	13.26	10.3	11.13	10.56
Geranyl acetate	3.37	4.17	3.22	4.34	3.92	5.42	5.37	3.87	4.2	4.16
β-Caryophyllene	2.56	3.6	1.72	3.67	2.1	2.18	4.4	3.28	2.19	4.49
Other Components	16.02	2.28	7.04	7.55	5.7	8.19	4.96	1.81	7.84	1.81
C:G ratio	3.70	2.58	2.40	2.30	2.53	2.38	1.98	1.56	1.30	1.27

*Zn1= Zinc at rate 100 mg/l, Zn2= Zinc at rate 150 mg/l, Zn3= Zinc at rate 200 mg/L, Try1=Tryptophan at rate 100 mg/l, Try2=Tryptophan at rate 150 mg/l, Try3=200 mg/L, ASA1= Ascorbic acid at rate 50 mg/l, ASA2= Ascorbic acid at rate 100 mg/l and ASA3= Ascorbic acid at rate 200 mg/l

The highest mean Citronellol content (30.07%) was found in the oil of those plants sprayed with Zn1 at 100 mg/l, followed by plants sprayed with Zn2 at rate 150 mg/l (with Citronellol content of 28.10%) and ASA1 at rate 50 mg/l (26.12%). A decrease in Citronellol content was observed with an increase in the concentration of zinc, tryptophan and ascorbic acid. While, the highest geraniol percentage (17.59%) was recorded in oil samples extracted from plants sprayed with ASA3 at 200 mg/l, followed by plants sprayed with ASA2 at 100 mg/l (17.07%) then ASA1 at 50 mg/l (16.76%). A C:G ratio of 1:1 – 3:1 is acceptable, while the most desirable ratio is 1:1. Oil with a C:G ratio of more than 3:1 is considered to be of poor quality for the perfume industry, but it can still be used by other industries for the manufacture of creams, soaps, toiletries and aromatherapy products [74].

The results showed that the C: G ratio was affected by different concentration of zinc, tryptophan and ascorbic acid. The citronellol: geraniol ratio was within the desired limit at (1.56, 1.30 and 1.27) with Ascorbic acid at rate 50, 100 and 200 mg/l, respectively), Tryptophan at rate 100, 150 and 200 mg/l gave (2.53 and 2.38 and 1.98, respectively) and Zinc at rate 100, 150 and 200 mg/l gave (2.58, 2.40 and 2.30, respectively). but control Exceed acceptable limits of C: G ratio (3.70). Best quality (low C:G ratio) was found in the oil of the plants treated with ascorbic acid ASA3 (200 mg/l). From the previous results the producers can choose spray geranium with Ascorbic acid at rate 200 mg/l to suit their desired oil composition.

Chemical Composition:

Photosynthetic Pigments: Chlorophyll a, b and carotenoids content of geranium plants (Table 7) demonstrated, in most cases, consistent and gradual increases in response to the various treatments. Zinc, tryptophan and ascorbic acid treatments provided a higher values of photosynthetic pigments in the first and second seasons compared to control plants. The highest level of ascorbic acid (200 mg/l) gave the highest values of leave pigments (chl. a, b and carotenoid) compared to control as well as the rest treatments in both cuts, during the two seasons, followed by ascorbic at rate 150 mg/l in most cases. Therefore, ascorbic treatments led to a significant increase in the plant fresh weight consequently this positive effect was reflected in herb fresh yield. The role of ascorbic acid in enhancing photosynthetic pigments in leaves of different medicinal and aromatic plants was reported by El-Quesni *et al.* [60]; Khalil *et al.* [61]; Nikee *et al.* [62] and Heikal and Helmy [75].

The activity of photosynthesis depends largely on photosynthetic pigments (chl. a, b and carotenoid). Changes in the content of photosynthetic pigments affect the level of metabolism and the intensity of plant growth and development [76].

Table 7: Effect of foliar application with Zinc, Tryptophan and Ascorbic acid on chlorophyll a, b and carotenoids (mg/g fresh weight) of *Pelargonium graveolens* plants during the 2019/2020 and 2020/2021 seasons

	Chlorophyll a (mg/g fresh weight)				Chlorophyll b (mg/g fresh weight)				Carotenoids (mg/g fresh weight)			
	First Season		Second Season		First Season		Second Season		First Season		Second Season	
Treatments	Cut1	Cut2	Cut1	Cut2	Cut1	Cut2	Cut1	Cut2	Cut1	Cut2	Cut1	Cut2
Control	0.57	0.64	0.64	0.56	0.23	0.14	0.15	0.13	0.75	0.71	0.72	0.75
Zn 1	0.71	0.75	0.70	0.61	0.25	0.16	0.19	0.16	0.77	0.95	0.79	0.83
Zn2	0.74	0.78	0.75	0.67	0.27	0.19	0.24	0.18	0.80	0.97	0.82	0.86
Zn 3	0.78	0.78	0.79	0.69	0.27	0.24	0.27	0.23	0.86	1.02	0.86	0.91
Try 1	0.76	0.81	0.75	0.64	0.28	0.26	0.20	0.21	0.90	0.98	0.82	0.86
Try 2	0.78	0.84	0.79	0.68	0.30	0.28	0.25	0.24	0.91	1.04	0.85	0.86
Try 3	0.80	0.85	0.83	0.76	0.33	0.32	0.27	0.26	0.95	1.12	0.90	0.92
ASA 1	0.86	0.86	0.80	0.67	0.29	0.29	0.22	0.26	0.95	1.00	0.83	0.95
ASA 2	0.88	0.90	0.82	0.71	0.31	0.33	0.28	0.29	0.97	1.12	0.87	1.00
ASA3	0.93	0.95	0.86	0.81	0.36	0.37	0.31	0.34	1.01	1.28	0.95	1.16
L.S.D 0.05	0.02	0.03	0.03	0.05	0.01	0.02	0.02	0.02	0.01	0.02	0.04	0.05

Zn1= Zinc at rate 100 mg/l, Zn2= Zinc at rate 150 mg/l, Zn3= Zinc at rate 200 mg/L, Try1=Tryptophan at rate 100 mg/l, Try2=Tryptophan at rate 150 mg/l, Try3=200 mg/L, ASA1= Ascorbic acid at rate 50 mg/l, ASA2= Ascorbic acid at rate 100 mg/l and ASA3= Ascorbic acid at rate 200 mg/l.

Table 8: Effect of foliar application with Zinc, Tryptophan and Ascorbic acid on Antioxidant activity of *Pelargonium graveolens* oil during the 2019/2020 and 2020/2021 seasons

	First Season		Second Season	
Treatments	First Cut	Second Cut	First Cut	Second Cut
Control	49.07	50.25	46.26	52.7
Zn 1	54.54	56.29	47.59	54.90
Zn 2	56.16	59.23	53.77	57.25
Zn 3	59.56	60.85	57.69	62.00
Try 1	57.68	61.55	53.91	63.00
Try 2	60.40	62.20	57.32	67.21
Try 3	62.68	63.22	62.77	69.69
ASA 1	59.34	61.86	58.14	66.90
ASA 2	62.30	63.56	63.99	69.54
ASA 3	66.27	67.25	66.77	74.08
L.S.D. at 0.05	2.54	3.18	2.39	3.41

*Zn1= Zinc at rate 100 mg/l, Zn2= Zinc at rate 150 mg/l, Zn3= Zinc at rate 200 mg/L, Try1=Tryptophan at rate 100 mg/l, Try2=Tryptophan at rate 150 mg/l, Try3=200 mg/L, ASA1= Ascorbic acid at rate 50 mg/l, ASA2= Ascorbic acid at rate 100 mg/l and ASA3= Ascorbic acid at rate 200 mg/l

Antioxidant Activity of *Pelargonium graveolens* Oil: Data in (Table 8) showed that foliar application of zinc, tryptophan or ascorbic acid led to significant increases in antioxidant activity (as DPPH- radical scavenging capacity). This positive effect in an antioxidant activity was found to be depend on concentration of zinc, tryptophan and ascorbic acid used. So, antioxidant activity was gradually increased with increasing concentrations of these treatments compared to control. The highest antioxidant activity of geranium oil (66.27 and 67.25 % in both cuts during first season) and (66.77 and 74.08% in both cuts during second season) were recorded in plants sprayed with ASA3 at rate 200 mg/l. On the other hand, the lowest antioxidant activity of geranium oil (49.07 and 50.25% in both cuts during first season) and (46.26 and 52.70% in both cuts during second season) were obtained in control plants. The increase in antioxidant activity can be regarded an advantage of Ascorbic acid used [77].

Anti-Microbial Activity: *P. graveolens* essential oil samples extracted from plants treated by Ascorbic acid, zinc and tryptophan at rate 200 mg/l exhibiting antibacterial activity on both Gram Positive and Negative bacterial strains with varied zone of inhibitions.

Data in Table (9) clarify the antimicrobial potency and its variance between essential oil semples extracted from *P. graveolens* plants treated with Ascorbic acid, zinc and tryptophan.

Whereas, the zone of inhibition of the essential oil extracted from plants treated with Ascorbic acid against *E. coli*, *B. subtilis*, *S. aureus*, *S. epidermidis*, *Salmonella enterica*, *C. albicans* and *A. brasiliensis* was 11 mm, 20 mm, 16 mm, 15 mm, 13 mm, 26 and 27 mm respectively. The MIC of the Ascorbic acid was 5 µL against *E. coli*, *B. subtilis*, *S. aureus*, *Salmonella enterica*, *C. albicans* and *A. brasiliensis* and 10 µL against *S. epidermidis*. However, of the essential oil extracted from plants treated with Zinc against *S. aureus*, *S. epidermidis*, *Salmonella enterica*, *C. albicans* and *A. brasiliensis* was 22 mm, 13 mm, 12 mm, 22 mm and 15 mm respectively. The MIC of the Zinc treatment was 5 µL against *S. aureus*, *S. epidermidis*, *Salmonella enterica*, *C. albicans* and *A. brasiliensis*.

Table 9: Antimicrobial activity of oil samples of *Pelargonium graveolens* L. against tested strains

	Treatments								
	Ascorbic acid (200 mg/l)			Zinc (200 mg/l)			Tryptophan (200 mg/l)		
	Oil concentrations (µL)								
	5	10	20	5	10	20	5	10	20
Tested Microorganism	Diameter of inhibition zone (mm)*								
E. coli	11	17	28	-	-	-	-	-	-
S. aureus	16	20	20	22	35	29	-	-	-
S. epidermidis	-	15	17	13	14	16	-	-	-
B. subtilis	20	24	25	-	-	-	-	-	-
S. enterica	13	14	21	12	13	15	-	-	12
C. albicans	26	31	35	22	29	26	0	15	37
A. brasiliensis	27	32	40	15	16	37	0	17	38

On the other hand, the zones of inhibition of the essential oil extracted from plants treated Tryptophan were 12 mm, 15 mm and 17 mm against *Salmonella enterica*, *C. albicans* and *A. brasiliensis* respectively. The MIC of the Tryptophan was 20 µL against *Salmonella enterica* and 10 µL against *C. albicans* and *A. brasiliensis*.

The above results in Table (9) concluded that the essential oil obtained from *P. graveolens* plants treated with Ascorbic acid consider the best treatment because of its antimicrobial effect *against E. coli, B. subtilis*, *S. epidermidis*, *Salmonella enterica* and *A. brasiliensis*. While that obtained from Zinc treatment had higher antibacterial activity against *S. aureus*, also it was indicated that the essential oil extracted from *P. graveolens* plants treated with Tryptophan had higher antifungal activity against *A. brasiliensis*.

The antimicrobial activities of the samples were measured in essential oils. The disk diffusion assay results for the growth inhibition zones of microorganisms considered are shown in (Table 9). The antimicrobial properties of essential oils were more efficient against bacteria than fungus and may be sources of novel antimicrobial agents in the future. The essential oils extracted from *P. graveolens* plants treated with Ascorbic acid exhibited the highest antibacterial activity against *Escherichia. coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228 and *Salmonella enterica* subsp. ATCC 14028. the more effective concentration of essential oil 20 mL, while that obtained by treatment with Zinc and tryptophan showed the highest antifungal activity against *Aspergillus brasiliensis* ATCC 16404 and *Candida albicans* ATCC 10231 with concentration 20 ml. Our results were similar to those reported by Silva and Fernandes [78], who investigated the antimicrobial activity of *P. graveolens* against pathogenic bacteria.

Previous researches would indicate that the bactericidal and fungicidal activities of the essential oil could be explained by the presence of high concentrations of oxygenated monoterpenes [79]. The two main monoterpenes in geranium oil, citronellol and geraniol, have been shown to have antibacterial properties [80-81]. Moreover, Zore *et al.* [82] have reported that the anti-Candida activity of geraniol, geranyl acetate and citronellol appears to be associated with their ability to damage membrane integrity. They inhibit germ tube induction at very low concentrations and arrest *C. albicans* cell cycle., geraniol and geranyl acetate have fungicidal activity at 0.064%v/v concentrations, i.e. MICs (561 mg/mL and 584 mg/mL, respectively) and killed 99.9% of inoculum within 15 and 30min of exposures, respectively.

CONCLUSION

This study suggests that geranium plants sprayed by 200 mg/l of ascorbic acid might be used by farmers to get the highest yield and essential oil. The use of ascorbic acid concentrations resulted in increases in enhancing growth, fresh herb yield, essential oil yield, antioxidant and antimicrobial properties of essential oil.

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