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Effect of Nitrogen Fertilization and Foliar Spraying of Iron and Zinc on Growth, Yield and Chemical Composition of Basil (*Ocimum basilicum* Var. Genovase) Plant

Badawy M. Y. M. and Wafaa H.A. Abd-Allah

Medicinal and Aromatic Plants Department, Desert Research Center, El-Mataria, Cairo, Egypt

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ABSTRACT

This study was carried out to study the effects of nitrogen fertilization and foliar applications of iron (Fe) and zinc (Zn) on *Ocimum basilicum* var. genovase plant. Field experiment was conducted during two successive seasons of 2016 and 2017 at El-Maghara Research Station - Desert Research Center, North Sinai Governorate. The experiment was arranged as completely randomized design with 7 treatments and three replications. Fe and Zn treatments in single and double combinations plus N fertilization at two levels (150 and 200 kg ammonium nitrate/fed/season), In addition to the control (75 kg ammonium nitrate (33.5 % N)/fed/season). The results revealed that, in the first cut, the plant height, number of branches/plant, fresh and dry weights/plant and fresh and dry yields of herb/fed, volatile oil (percentage and L /fed), nitrogen, phosphorus and total carbohydrates contents were significantly increased by using 200 kg ammonium nitrate/fed/season + Fe and Zn as a foliar spray (0.5 g/l) (N_2FZ) followed by 150 kg ammonium nitrate/fed/season + Fe and Zn as a foliar spray (0.5 g/l) (N_1FZ) compared with other treatments. Meanwhile, in the second cut treating the basil plants with 200 kg ammonium nitrate/fed/season + Fe and Zn as a foliar spray (0.5 g/l) (N_2FZ) caused a significant increment in the same parameters. This treatment (N_2FZ) caused the highest volatile oil percentage and oil yield/fed (L), especially in the second cuts in both seasons. The main components of the resulting volatile oil from this treatment were Linalool (44.01%), 1,8-Cineole (17.99%), α-Bergamoten (5.12%), α-Cadinol (5.07%), Bornyl acetate (4.43%), γ-Muurolene (3.15%) and Eugenol (0.43%). While. the highest content of α-Cadinol (6.28%) and Bornyl acetate (6.06%) resulted from adding 150 kg ammonium nitrate + Fe and Zn as a foliar spray treatment.

Keywords: *Ocimum basilicum* var. genovase, Ammonium nitrate, Fe, Zn, foliar spray, Volatile oil, Linalool.

1. Introduction

Basil is one of the most important medicinal plants which grows well under Egyptian conditions and plays a vital role for the export of the country. Sweet basil (*Ocimum basilicum* L.) is an annual plant belonging to family Lamiaceae. the leaves of this plant can be used as fresh or dried for use as a spice FAO (2017). Essential oils extracted from leaves and flowering tops can be used as aroma additives in food and pharmaceuticals industry. Basil oil was reported to have antimicrobial and antioxidant. In this respect, Beatović *et al.,* (2015) showed that, a greater ability to inhibit fungal growth with essential oil of *Ocimum basilicum* compared to commercial antifungal agents, and also, there was a very high antioxidant capacity of the basil oil, (Gulcin *et al.*, 2007) reported that, extracts of the leaves displayed powerful antioxidant activity in various assay models

Nitrogen is one of the most important nutrients for crop production because it affects dry matter production by influencing leaf area development and maintenance as well as photosynthetic efficiency. Nitrogen deficiency causes a decrement in growth and yield, which was mentioned by Argyropoulou *et al.,* (2015) on *Ocimum basilicum* plant, who reported that, dry biomass production, plants height, roots length and number of leaves were dramatically decreased as a result of nitrogen deficient plants. In

Corresponding Author: Badawy M. Y. M., Medicinal and Aromatic Plants Department, Desert Research Center, El-Mataria, Cairo, Egypt. E-mail: moustafabadawy@hotmail.com

addition, net photosynthesis and transpiration rates and stomatal conductance were also restricted, indicating that the primary metabolism was severely limited by low nitrogen availability. Moreover, there was positive relations between nitrogen percentage with leaf area, chlorophyll a and b density (Larimi *et al.,* 2014).

Also, iron (Fe) is an important micronutrient that is a cofactor for approximately140 enzymes that catalyze unique biochemical reactions (Brittenham 1994). Hence, iron fills many essential roles in plant growth and development, including chlorophyll synthesis and chloroplast development (Miller *et al*., 1995 and Nikolic and Kastori, 2000). Meanwhile, zinc is another micronutrient that is closely involved in the metabolism of RNA and ribosomal content in plant cells, leading to stimulation of carbohydrates, proteins and the DNA formation. It is also, required for the synthesis of tryptophan, a precursor of IAA which acts as a growth promoting substance (Amberger, 1982).

Thus, good nutrition from N, Fe and Zn elements for sweet basil plants help to get good growth and thus working to increase the yield and had a high quality. That was the aim of this study, in order to try to provide some of the elements necessary to obtain a higher yield and a higher quality (the highest volatile oil) of basil plant.

The reason for choosing Genovese basil in this study is that it is of great importance, as it is one of the most important varieties of sweet basil that is well known by its diversity as a source of essential oils. In addition to, it gives a high yield of fresh and dry herb yield (Maggio *et al*., 2016 and Reham *et al*., 2019).

Therefore, this study aimed to investigate the effect of Fe and Zn treatments in single and double combinations plus N fertilization at two levels on the growth, yield and some chemical constituents as well as volatile oil production of *Ocimum basilicum* var. genovase plants.

2. Materials and Methods

2.1. Location and plant material

This study was conducted in El-Maghara, Experimental Station of Desert Research Center (DRC), during two successive seasons 2016 and 2017, to study the effects of nitrogen fertilization and micronutrient (Fe and Zn) on the growth, yield, volatile oil, nutrient content and carbohydrates of sweet basil plants grown in sandy soil under Middle Sinai conditions.

Certified seeds of sweet basil (*Ocimum basilicum* L. var. genovese) were obtained commercially from Enza Zaden (Enza Zaden Deutschland GmbH & Co. KG). Seeds were sown in the plastic house on the 8th and the 10th of March 2016 and 2017, respectively, in plastic trays of agriculture formulated with 1:1 peat moss and sand soil. After 60 days, basil seedlings (app. 12 - 15 cm height) were transplanted in the field at distances of 30 cm between hills (one plant/hill) and 70 cm between rows (at 18650 plant/fed.).

During land preparation, compost manure at 5 m^3/fed and calcium super phosphate (16 % P_2O_5) at a rate of 200 kg/fed were mixed with the soil before transplanting. N fertilizer was divided into two equal doses. The first addition was implemented after one month from transplanting, while the second one was applied after the first cut directly, the same thing with potassium was added at a rate of 50 kg potassium sulfate (48 % K_2O) with each cut, the whole experiment using drip irrigation with 4 l/h in two added times in the morning and afternoon every three days.

Fertilization treatments:

The fertilization treatments included the following:

1- Control (75 kg ammonium nitrate (33.5 % N)/fed/season). (recommended dose).
2- 150 kg ammonium nitrate (33.5 % N)/fed/season + Fe as a foliar spray (N_1F).
3- 150 kg ammonium nitrate (33.5 % N)/fed/season + Zn as a foliar spray (N_1Z).
4- 150 kg ammonium nitrate (33.5 % N)/fed/season + Fe and Zn as a foliar spray (N_1FZ).
5- 200 kg ammonium nitrate (33.5 % N)/fed/season + Fe as a foliar spray (N_2F).
6- 200 kg ammonium nitrate (33.5 % N)/fed/season + Zn as a foliar spray (N_2Z).
7- 200 kg ammonium nitrate (33.5 % N)/fed/season + Fe and Zn as a foliar spray (N_2FZ).

Foliar spray of Fe and Zn

Iron (Iron 12 % EDTA) and Zinc (Zinc 13 % EDTA) which were used in this study obtained from Misr El-Dawliya for Agricultural and Industrial Development Company. Treating the basil plants with Iron (0.5 g/l) and Zinc (0.5 g/l) as a foliar spray over plant two times in each cut, the first application after 30 days from transplanting, the second one after 15 days from the first one and was repeated the same thing with the second cut, i.e. the first application after 7 days from the first cut and the second one after 15 days from the first one.

2.2. Harvested and data recorded

Plants were harvested on both 8th and 10th August (90 days after transplanting, in the first and the second seasons, respectively) and 15th and 25th October (67 and 75 days after the first cut, in the first and the second seasons, respectively) both at full bloom, when the essential oil would be optimal (Topalov, 1962). Basil plants were harvested by cutting at 10 cm above soil surface, the fresh herb weight was taken.

The basil herb were dried in an oven at 60° C until constant weight, then the dry weight was taken. Data were recorded for the following parameters: plant height, number of branches/plant, fresh and dry weights/plant, fresh and dry yield of herb/fed.

2.3. Experiment design and statistical analysis

The complete randomized block design (CRBD) was used in this experiment with three replicates. The data obtained were subjected to the statistical analysis of variance using Mstate Statistical Software. L. S. D. test at 0.05 was used to compare the means of treatments, according to Snedecor and Cochran (1982).

2.4. Chemical analysis

2.4.1. Determination of essential oil percentage

The essential oil percentage in basil leaves was determined according to the British Pharmacopoeia (1963).

2.4.2. GC/ Mass analysis of volatile oil

The chemical compositions of the essential oil were determined using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30 mm, 0.251 mm, 0.1 mm film thickness). The quantification of all the identified components was determined using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system according to Adams (2007).

2.4.3. Determination of nitrogen, phosphorus, potassium and total carbohydrates

Element contents were determined in the acid digested solution, which was prepared according to Hach *et al.* (1985). Nitrogen content was determined by the modified micro- Kjeldahl method, potassium was estimated using flame photometer method and phosphorus content was estimated according to Page *et al.* (1982). Total carbohydrates percentages in leaves were determined according to Chaplin and Kennedy (1994).

Soil and water analysis

Soil analyses are shown in Table (1). Soil samples representing the experimental area were taken at 0-30 cm depth. Water analysis is shown in Table (2) taken from the irrigation water used from El-Maghara Station.

Table 1: Chemical analysis of the soil.

pH	E.C (mmhos/cm)	Soluble cations (mg /L)				Soluble anions (mg /L)			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}
7.70	2.80	114.10	36.77	440	12	0	34.07	728.7	340.07

(TDS), mg/L	Total nitrogen (%)	Phosphate, mg/L
1792	0.42	85.5

Table 2: Water analysis of the irrigation water.

pH	(TDS), mg/L	E.C mmhos/cm	Soluble cations (mg /L)				Soluble anions (mg /L)			
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}
7.5	2688	4.20	188.40	79.79	560	66	0	238.48	923.02	580

3. Results and Discussion

3.1. Vegetative growth:

3.1.1. Plant height (cm)

In the first cut of both seasons data in Table (3) clear that that, N_2FZ gave the highest mean values of plant height (43.44 and 44.67 cm) followed by N_1FZ treatment then N_1F which recorded 42.78 and 42.67 cm during 1st and 2nd seasons, respectively. Meanwhile, in the second cut in both seasons, the tallest plants (51.56, 48.78 and 48.44 in the first season and 55.22, 54.45 and 52.89 cm in the second season) resulted from using, N_1FZ, N_2FZ and N_2Z, respectively where, there was a significant difference between these treatments and other treatments. But, the shortest plants were obtained with control plants in both seasons.

3.1.2. Number of branches/plant

Data in Table (3) showed that, in the first cut during both seasons, the highest number of branches/plant resulted from using N_2FZ, N_1FZ and N_2F treatments, without a significant differences between them, the values were 12.71, 11.51 and 11.44 in the first season and 14.45, 13.40 and 12.83 branches/plant in the second one, respectively. Meanwhile, in the second cut in both seasons, using N_2FZ treatment led to a significant increment in number of branches/plant compared with other treatments. The lowest values were 8.67, 17.11 in the first season and 9.85, 23.44 branches/plant in the second one (in the first and second cuts, respectively) achieved with control treatment.

Table 3: Effect of nitrogen fertilization and foliar spray of iron and zinc on plant heightand number of branches/plant of basil (*Ocimum basilicum* var genovase) plants, during 2016 and 2017 seasons.

Treatments	Plant height (cm)				Number of branches/plant			
	First season		Second season		First season		Second season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	35.33	31.67	39.09	42.00	8.67	17.11	9.85	23.44
N_1F	37.00	44.11	42.67	48.45	10.51	21.11	12.17	32.56
N_1Z	36.00	46.67	39.22	51.89	10.00	29.78	10.67	37.00
N_1FZ	42.78	51.56	42.33	55.22	11.51	32.11	13.40	41.78
N_2F	40.56	44.89	42.00	50.00	11.44	27.67	12.83	35.89
N_2Z	38.56	48.44	41.44	52.89	10.54	30.44	12.00	37.67
N_2FZ	43.44	48.78	44.67	54.45	12.71	34.00	14.45	47.33
L.S.D at 0.05	2.86	3.53	2.50	2.49	1.61	4.96	2.39	3.50

3.1.3. Fresh and dry weights/plant and fresh and dry yields of herb/fed

Data represented in Tables (4 and 5) showed that, in the first cut in both seasons, treating the sweet basil plant with 200 or 150 kg ammonium nitrate/fed/season + foliar spray with Fe + Zn led to the heaviest fresh and dry weights/plant and fresh and dry yields of herb/fed, meanwhile, in the second cut in both seasons, the highest values from these parameters resulted from adding 200 kg ammonium nitrate/fed/season + foliar spray with Fe plus Zn to sweet basil plant, there was a significant difference between this treatment and other treatments. whereas, the control treatment resulted in the lowest values.

Table 4: Effect of nitrogen fertilization and foliar spray of iron and zinc on fresh weight/plant and fresh yield of herb/fed of basil (*Ocimum basilicum* var. genovase) plants, during 2016 and 2017 seasons.

Treatments	Fresh weight/plant (g)				Fresh yield of herb/fed (kg)			
	First season		Second season		First season		Second season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	67.56	120.00	75.64	153.333	1259.96	2238.00	1410.69	2859.67.
N_1F	93.52	223.33	98.82	243.33	1744.15	4165.17	1842.95	4538.17
N_1Z	85.51	256.67	91.42	280.00	1594.76	4786.83	1704.94	5222.00
N_1FZ	112.50	276.67	118.32	306.67	2098.13	5159.83	2206.63	5719.33
N_2F	103.04	276.67	105.05	320.00	1921.70	5159.83	1959.18	5968.00
N_2Z	96.66	273.33	104.21	316.67	1802.71	5097.67	1943.60	5905.83
N_2FZ	117.87	336.67	136.56	395.33	2198.21	6278.83	2546.93	7372.97
L.S.D at 0.05	20.08	53.31	21.38	20.61	374.5	994.2	398.8	384.4

Table 5: Effect of nitrogen fertilization and foliar spray of iron and zinc on dry weight/plant and dry yield of herb/fed of basil (*Ocimum basilicum* var. genovase) plants, during 2016 and 2017 seasons.

Treatments	Dry weight/plant (g)				Dry yield of herb/fed (kg)			
	First season		Second season		First season		Second season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	19.38	43.33	27.08	50.00	361.44	808.17	505.04	932.50
N_1F	27.05	86.67	28.78	90.00	504.48	1616.33	536.71	1678.50
N_1Z	22.85	90.00	26.37	100.00	426.09	1678.50	491.76	1865.00
N_1FZ	29.10	90.00	34.36	110.00	542.72	1678.50	640.79	2051.50
N_2F	28.43	93.33	30.97	110.00	530.13	1740.67	577.53	2051.50
N_2Z	26.07	93.33	27.50	110.00	486.14	1740.67	512.88	2051.50
N_2FZ	32.25	130.00	37.12	153.33	601.46	2424.50	692.25	2859.67
L.S.D at 0.05	4.53	9.22	6.14	20.17	84.59	172.3	114.6	376.2

Generally evident from the previous results that with increasing nitrogen fertilization caused the increase in vegetative parameters and yield due to the important role of nitrogen in many biological processes in basil plant. In this connection, the nitrogen fertilizer encourages vegetative growth and increases oil yield in aromatic plants by enhancing leaf area development and photosynthetic rate (Frabboni *et al.,* 2011). Many studies indicated that increasing in nitrogen fertilization led to increase in all growth parameters such as Omer *et al.* (2008) which showed that,the application of ammonium sulfate at the rate of 60 kg N/fed/season at two equal portions in each cut could be recommended for maximizing herb and leaves yields of *Ocimum amercianum*,L. var. pilosum plants. Biesiada and Kus (2010) found that, the highest yields of sweet basil were recorded at the dose of 150–250 kg N/ha. Frabboni*et al.* (2011) found that, the best results from total fresh weight, fresh weight of the leaves and total dry weight of *Ocimum basilicum* plant resulted from 160 kg N/ha. On sweet basil, Bufalo *et al.,* (2015) demonstrated that the highest fresh weight was obtained from the plants grown with conventional fertilizer at a rate of 250 kg N/ha. Also, using Fe, Zn as a foliar application caused increasing in all vegetative parameters and yield of many plants such as spraying *Matricaria chamomilla* plants with micronutrients (Fe, Zn and Mn) in all applied treatments increased growth characters (fresh and dry weights of herb plant, percentage of dry weight of herb plant and number of branches on main stem plant), floral traits (number of flowers/plant and fresh and dry weights of flowers/plant) as mentioned by Hassanain *et al.* (2006). The above results are in agreement with those obtained by. Senthil Kumar *et al.* (2009) on Davana *(Artemisia pallens* Wall.) and Hellal *et al.,* (2011) on dill *(Anethum graveolens* L) plants, where, they revealed that N at 93.75 kg ha^{-1} and 100 kg N ha^{-1} respectively gave the highest values of number of laterals. Also, Said-Al Ahl and Mahmoud (2010) on sweet basil, found that addition of micronutrients (Zn and / or Fe) had an active effect comparing with control, the highest plant height and number of branches being with iron application and zinc gave the highest value of fresh weight, whereas a mixture of iron + zinc gave the highest values of dry matter and essential oil yield. Said-Al Ahl *et al.* (2010) showed that, treating dragonhead plants with 90 kg N/fed and foliar treatments (Zn, Fe, and Mn) produced the highest plant height, branches number and fresh herb. Saeideh *et al.* (2011) on *Plantago psyllium* plant, showed that the seed yield, seed swelling and mucilage percentage were increased by foliar application of Fe and Zn, compared with control (untreated). AL-Salmani (2013) using Fe plus Zn (50 mg/l) as a foliar application with wheat plant gave the highest growth parameters andyield of grains. In contrast of our results, Reham *et al.* (2019) performance of eight cultivars of sweet basil grown under Egyptian conditions, they reported that Genovese cultivar produced the most vigorous plants with the highest fresh and dry herb. The increment of plant height as a result of spraying with mixture of N and microelements may be due to the effect of them on cell division and elongation, enhancing activation of photosynthesis and metabolism of organic compounds that encourage plant development. N is concede a constituent of fundamental cellular components such as amino acids, proteins and nucleic acids. It is also the officer of P, K and other nutrients, promotes photosynthesis because the N increases the amount of chlorophyll, these results are in harmony with those obtained by Aziz and El-Sherbeny (2004) on *Sideritis montana*; Abd El-Wahab (2008) on *Trachyspermum ammi*, they reported that, the positive effect of nitrogen and microelements on number of branches per plant can be attributed to their role in encouraging the meristematic activity i.e., and thus increasing. the division and elongation of cells and then increasing the vegetative parameters and yield.

3.2. Volatile oil production

3.2.1. Volatile oil percentage and oil yield/fed

Data obtained in Table (6) showed that, in the first cut in both seasons, the highest volatile oil percentage (0.93 and 0.80 % in first season and 0.95 and 0.84 % in second one) and oil yield/fed (5.09 and 4.73 l/fed in first season and 6.13 and 5.88 l/fed in second one) resulted from using 150 kg ammonium nitrate/fed/season + foliar spray with Fe plus Zn and 200 kg ammonium nitrate/fed/season + foliar spray with Fe plus Zn, respectively. The lowest volatile oil percentages were 0.27 and 0.35 %, which obtained with using 200 kg ammonium nitrate/fed/season + foliar spray with Fe treatment in the first and second seasons, respectively. Meanwhile, in the second cut in both seasons, treating basil plants with 200 kg ammonium nitrate/fed/season + foliar spray with Fe plus Zn recorded the highest volatile oil percentage (1.00 % in the first season and 1.10 % in the second season) and oil yield/fed (24.25 l/fed in the first season and 31.46 l/fed in the second season). The lowest volatile oil percentage were 0.50 and 0.55 % in the first and second seasons, respectively, which obtained with control treatment.

Table 6: Effect of nitrogen fertilization and foliar spray of iron and zinc on volatile oil percentage and oil yield/fed of basil (*Ocimum basilicum* var .genovase) plants, during 2016 and 2017 seasons.

Treatments	Volatile oil %				Oil yield/fed (L)			
	First season		Second season		First season		Second season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	0.53	0.50	0.51	0.55	1.93	4.04	2.58	5.13
N_1F	0.47	0.50	0.60	0.63	2.37	8.08	3.24	10.68
N_1Z	0.53	0.70	0.64	0.80	2.21	11.73	3.15	14.92
N_1FZ	0.93	0.70	0.95	0.83	5.09	11.75	6.13	16.94
N_2F	0.27	0.60	0.35	0.75	1.41	10.61	2.08	15.39
N_2Z	0.53	0.60	0.73	0.78	2.59	10.44	3.76	15.91
N_2FZ	0.80	1.00	0.84	1.10	4.73	24.25	5.88	31.46
L.S.D at 0.05	0.19	0.07	0.19	0.08	1.26	2.25	1.36	3.09

The increasing in oil yield in the second cut in both seasons due to high weights of herb in second cuts compared with the first cuts, in addition to the relative increase in the oil percentage in most treatments in second cuts. These results agree with many researchers such as Hassanain and Abdella (2003) found that, adding ammonium nitrate to sweet basil plant significantly increased essential oil percentage than both of ammonium sulphate and urea. Omer *et al.,* (2008) showed that, the application of ammonium sulfate at the rate of 60 kg N/fed/season at two equal portions in each cut could be recommended for maximizing oil yield of *Ocimum amercianum* L. var. pilosum plants. On sweet basil Said-Al Ahl and Mahmoud (2010), found that application a mixture of iron + zinc gave the highest essential oil % under soil salinity condition. Said-Al Ahl *et al.* (2010) on dragonhead plant, showed that, treating dragonhead plants with 90 kg N/fed and foliar treatments (Zn, Fe, and Mn) produced the highest essential oil production. The present results of manganese are in harmony with those of Rania and Wafaa (2017) on sweet basil (*Ocimum basilicum* L.) plant. They found that treatment of Fe+Zn+Mn, at 50 ppm each, caused a significant increment in the volatile oil percentage and oil yield / fed.

3.2.2. Analysis of *Ocimum basilicum* var. genovase volatile oil components by GC-MS:

Selected three volatile oil samples to discretion of the components, it is a representing three treatments (150 kg ammonium nitrate + Fe and Zn as a foliar spray (N_1FZ) and 200 kg ammonium nitrate + Fe and Zn as a foliar spray (N_2FZ)) as well as control treatment). The samples of the essential oil during the second cut of the second season were subjected to (GC-MS) analysis. The main compounds are shown in Table (7) and Figures (1, 2 and 3).

Table 7: Chemical composition of the essential oil for *Ocimum basilicum* var. *genovase* plant using GC-MS:

No.	Compound name	Control	(N_1FZ)	(N_2FZ)
1	Thujene	--	--	0.13
2	α-Pinene	1.34	1.38	1.28
3	Camphene	0.30	0.34	0.39
4	Sabinene	0.82	0.71	0.38
5	β-Pinene	2.41	2.48	1.93
6	α-Myrcene	1.87	1.32	2.06
7	α-Phellandrene	0.12	0.15	0.24
8	α-Terpinene	0.31	0.30	0.39
9	D-Limonene	0.18	--	0.16
10	1,8-Cineole	19.65	19.11	17.99
11	α-Ocimene	1.40	0.63	0.57
12	γ-Terpinene	0.36	0.36	0.42
13	2-Carene	--	0.57	--
14	α-Terpinolene	0.63	--	--
15	Linalool	35.38	34.28	44.01
16	Camphor	1.31	1.23	1.57
17	Endo-Borneol	--	--	0.20
18	α-Terpineol	1.54	1.07	0.63
19	Terpinen-4-ol	--	0.14	--
20	4-Terpinyl acetate	0.23	0.22	0.88
21	Carvone	0.30	--	--
22	Pulegone	--	2.16	--
23	Bornyl acetate	4.32	6.06	4.43
24	1,2-Cyclohexanediol,1methyl4(1methylethenyl)	0.22	0.15	--
25	Eugenol	6.73	5.29	0.43
26	Trans Caryophyllene	0.17	--	--
27	α -Bergamoten	5.80	4.73	5.12
28	α-Cubebene	0.31	0.33	0.24
29	α-Elemene	--	--	1.49
30	Humulene	0.60	0.90	0.90
31	Germacrene-D	1.92	1.50	0.88
32	γ-Gurjunene	0.17	--	--
33	α-Selinene	--	--	0.43
34	α-Guaiene	1.01	1.35	1.73
35	γ-Muurolene	2.59	3.14	3.15
36	Trans-Calamenene	0.21	--	--
37	α-Muurolene	0.15	0.25	0.33
38	Veridiflorol	0.65	1.02	0.62

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39	Spathulenol	0.23	0.23	0.15
40	Cubenol	0.83	0.94	0.71
41	α-Cadinol	5.42	6.28	5.07
42	tau.Muurolol	0.25	0.25	0.20
	Total identified compounds	99.97	98.87	99.11
	Unknown	0.03	1.13	0.89

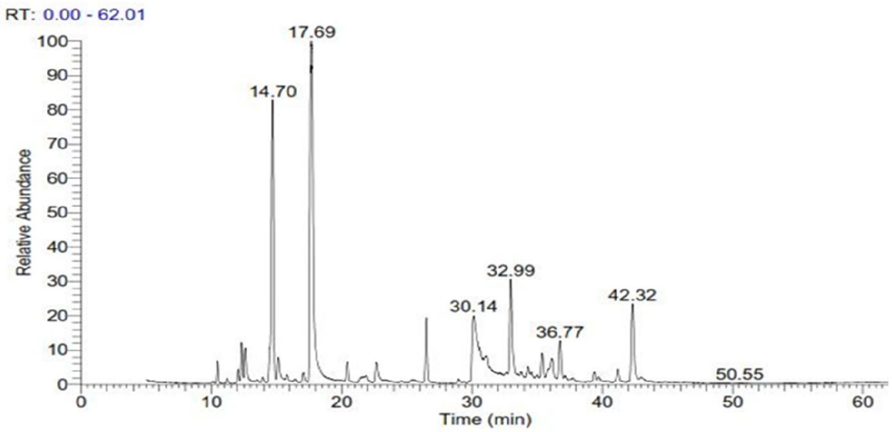

Fig. 1: Chromatogram of *Ocimum basilicum* var. genovase plant oil extracted from control plants.

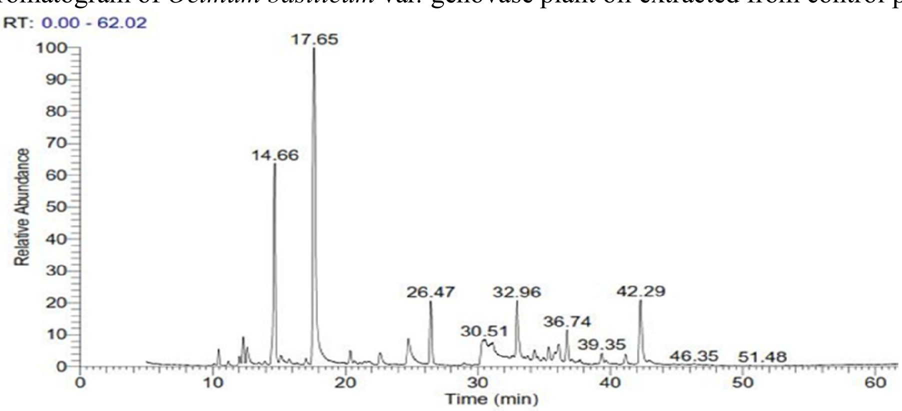

Fig. 2: Chromatogram of *Ocimum basilicum* var. genovase plant oil extracted from plants treated with (150 kg ammonium nitrate + Fe and Zn as a foliar spray (N_1FZ), during the second season.

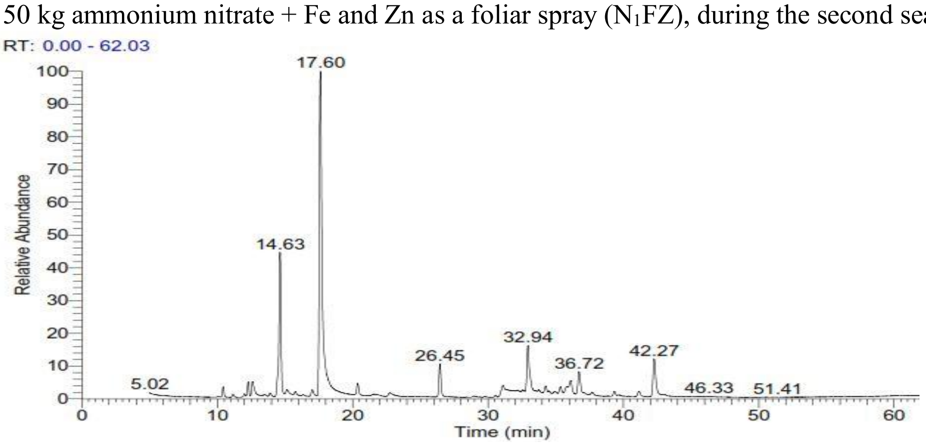

Fig. 3: Chromatogram of *Ocimum basilicum* var.genovase plant oil extracted from plants treated with (200 kg ammonium nitrate + Fe and Zn as a foliar spray (N_2FZ), during the second season.

Fourty two compounds were identified, the main compounds of the essential oil were Linalool, 1,8-Cineole, Eugenol, α-Cadinol, Bornyl acetate, α-Bergamoten and γ-Muurolene. It can be seen from (N_2FZ) treatment (200 kg ammonium nitrate + Fe and Zn as a foliar spray), the major components were Linalool (44.01%), 1,8-Cineole (17.99%), α-Bergamoten (5.12%), α-Cadinol (5.07%), Bornyl acetate

(4.43%), γ-Muurolene (3.15%) and Eugenol (0.43%). Meanwhile, when using (N_1FZ) treatment (150 kg ammonium nitrate + Fe and Zn as a foliar spray), the major components were Linalool (34.28%), 1,8-Cineole (19.11%), α-Cadinol (6.28%), Bornyl acetate (6.06%), Eugenol (5.29%), α-Bergamoten (4.73%) and γ-Muurolene (3.14%). Whereas, in control plants ((Zero NFZ), the major components were Linalool (35.38%), 1,8-Cineole (19.65%), Eugenol (6.73%), α-Bergamoten (5.80%), α-Cadinol (5.42%), Bornyl acetate (4.32%) and γ-Muurolene (2.59 %).

From the previous results it can be noted that, there was the highest Linalool content (44.01%), when using the high level of ammonium nitrate (200 kg ammonium nitrate + Fe and Zn as a foliar spray) with basil plants, but the lowest content of Linalool (34.28 %) was recorded with treated plants with (150 kg ammonium nitrate + Fe and Zn as a foliar spray). Meanwhile, the volatile oil resulted from control treatment had the highest content of 1,8-Cineole (19.65 %) and Eugenol (6.73%), but, when using (N_2FZ) treatment (200 kg ammonium nitrate + Fe and Zn as a foliar spray) gave the lowest content of 1,8-Cineole (17.99 %) and Eugenol (0.43%). The highest content of α-Cadinol (6.28%) and Bornyl acetate (6.06%) resulted from adding 150 kg ammonium nitrate + Fe and Zn as a foliar spray treatment.

3.4. Chemical constituents

Data represented in Table (8) mentioned that, the highest total carbohydrates content resulted from treating the basil plants with 200 kg ammonium nitrate/fed/season + foliar spray with Fe plus Zn (N_2FZ) followed by150 kg ammonium nitrate/fed/season + foliar spray with Fe plus Zn (N_1FZ), with noted that these treatments, which gave the highest volatile oil percentage in the same order, which explained the reason for the high volatile oil percentage with treating the basil plants with these treatments. Also, noticed that, in most treatments, nitrogen and total carbohydrates content of basil plants in the first cut in both seasons were higher than the second cut, including explains the role of nitrogen in the synthesis of carbohydrates in plants.

Table 8: Effect of nitrogen fertilization and foliar spray of iron and zinc on nitrogen, phosphorus, potassium and total carbohydrates content of basil (*Ocimum basilicum* var genovase) plants, during 2016 and 2017 seasons.

Treatments	N %				P %			
	1st season		2nd season		1st season		2nd season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	1.47	0.98	1.35	1.23	0.32	0.37	0.38	0.35
N_1F	1.84	1.53	1.59	1.84	0.36	0.37	0.43	0.48
N_1Z	1.47	1.35	1.45	1.59	0.39	0.64	0.41	0.66
N_1FZ	1.96	1.23	2.57	1.23	0.47	0.70	0.48	0.76
N_2F	1.96	1.55	2.21	1.84	0.39	0.70	0.44	0.68
N_2Z	1.74	1.47	1.84	1.59	0.39	0.63	0.42	0.63
N_2FZ	2.70	1.96	2.70	2.21	0.61	0.76	0.63	0.78
Treatments	**K %**				**Total carbohydrates %**			
	1st season		2nd season		1st season		2nd season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	1.06	0.80	1.13	0.96	13.02	11.02	14.33	13.62
N_1F	1.14	0.93	1.27	1.05	14.60	12.71	17.22	14.25
N_1Z	1.08	0.92	1.13	1.12	13.86	16.77	15.64	18.06
N_1FZ	1.19	1.16	1.46	1.20	22.21	17.28	24.79	19.63
N_2F	1.25	1.05	1.65	1.20	19.44	14.13	19.64	16.15
N_2Z	1.22	0.99	1.38	1.31	18.38	15.10	19.50	16.91
N_2FZ	1.36	1.34	1.65	1.36	26.40	18.50	26.85	20.59

Also, using (N_2FZ) treatment recorded the highest content of potassium, followed by N_2F, N_2Z or N_1FZ treatments. Meanwhile, the highest nitrogen and phosphorus content resulted from treating the basil plants with 200 kg ammonium nitrate/fed/season + foliar spray with Fe plus Zn followed by150 kg ammonium nitrate/fed/season + foliar spray with Fe plus Zn and using 200 kg ammonium nitrate/fed/season + foliar spray with Fe. These treatments recorded the highest fresh and dry weights/plant, this may be due to the role of nitrogen and iron in increasing the biological processes such as photosynthesis in plant, which reflected an increase in vegetative growth. These results confirmed by many researchers such as Evans (1989) showed that, the photosynthetic capacity of leaves is related to the nitrogen content primarily because the proteins of the Calvin cycle and thylakoids represent the majority of leaf nitrogen. Larimi *et al.* (2014) on sweet basil showed that, positive relations was shown between nitrogen percentage with leaf area, chlorophyll a and b density, that with increased nitrogen percentage caused increases leaf area and chlorophyll a and b density, also the role of iron, Hassanain*et al.* (2006) showed that, spraying *Matricaria chamomilla* plants with micronutrients (Fe, Zn and Mn) in all applied treatments increased chemical constituents [concentrations of chlorophylls (a and b), carotenoids and total free amino acids in fresh herb, percentages of N, P, K, total carbohydrates and protein, and concentrations of Fe, Mn, and Zn in dry matter of herb]. on sweet basil (*Ocimum basilicum* L.). Rania and Wafaa (2017), found that treatment of Fe+Zn+Mn, at 50 ppm each, caused an increment in the volatile oil yield, also, this treatment increased the concentrations of Linalool to 52.69% and 1,8 cineole to 25.66%.

4. Conclusion

In this study, it was found that Fe and Zn had a beneficial effect on yield and essential oil production of *Ocimum basilicum* var. genovase plant. The obtained results also showed that the application of these two elements in combination with 200 or 150 kg ammonium nitrate/fed/season (100 or 75 kg/fed/cut) had more positive and significantly effects on yield and essential oil of basil compared to their individual applications in the first cut. Meanwhile, in the second cut, it was better in addition ammonium nitrate to basil plants at a rate 200 kg/fed/season (100 kg/fed/cut) in combination with Fe (0.5 g/l) plus Zn (0.5 g/l) as a foliar spray.

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