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Emad Saleh Hamed
Medicinal and Aromatic Plants Department, Desert Research Center, Cairo, Egypt

Correspondence
Emad Saleh Hamed
Medicinal and Aromatic Plants Department, Desert Research Center, Cairo, Egypt

Effect of nitrogenous fertilization and spraying with nano-fertilizer on *Origanum syriacum* L. var. *syriacum* plants under North Sinai conditions

Emad Saleh Hamed

Abstract
This work was carried out during the two successive seasons of 2016 and 2017 at North Sinai Governorate to study the possibility to decrease quantity of applied chemical nitrogen fertilizers to *Origanum syriacum* var. *syriacum* plants by using of foliar CO_2 nano-fertilizer "lithovit". The experiment included five treatments:- application of the full recommended N dose, 3/4 N dose + foliar spray with lithovit, 1/2 N dose + foliar spray with lithovit, 1/4 N dose + foliar spray with lithovit and foliar spray with lithovit only. The work was planned as a complete randomized block design. Results showed that application of 3/4 N dose followed by 1/2 N dose + foliar spray with lithovit significantly super passed full N dose in growth, herb and oil yield characters while in other cuts these variations were non-significant. 1/2 N dose + foliar spray with lithovit could be recommended as the best treatment. The identified components of volatile oil were α-pinene, β-pinene, myrcene, ρ-cymene, γ-terpinene, α-phellandrene, terpineol, thymol and carvacrol.

Keywords: oregano, nitrogen, lithovit, yield, volatile oil

Introduction
Oregano (*Origanum syriacum*, Family: Lamiaceae) is an important spice commercially cultivated in North Sinai Governorate for culinary and medical purposes. The two common oregano varieties which are cultivated there are *Origanum syriacum* var. *synaicum* (*Aegyptiacum*) which is endemic to Sinai and *Origanum syriacum* var. *syriacum* which is introduced to North Sinai farmers from Palestine. The research paper herein will deal with the second oregano variety.

Origanum syriacum var. *syriacum* (syn. *Majorana syriaca*) "locally known as zaatar in Arabic", also called as: Syrian oregano, Lebanese oregano, Syrian marjoram, wild marjoram and Palestinian thyme. The plant is a chemaephyte subshrub herb native to Palestine, Israel, Syria, Lebanon and Jordan where it is widely cultivated and grown naturally. In Egypt, cultivation of the *syriacum* variety is introduced to North Sinai from Palestine as the plant has drawn attention for its faster growth and higher productivity than the local oregano variety. Stems of the plant are ascending or erect, up to 90 cm tall, usually brown. Leaves are petiolate to sessile or entire, oval dark green. Floral leaves are obovate, as long as the calyx. Spikes are oblong, the calyx slit on the outer side, corolla white. The plant leaves are used as main ingredient of ''zaatar'' a traditional condiment prepared by mixing dried and crushed plant leaves, roasted sesame seeds, crushed sumac fruit epicarp and olive oil, a very good mixture that is used almost daily in the Middle East as a food additive in salads and spice for pastry and meat. In traditional medicine, a herbal tea is prepared from oregano to treat cold, flu, cough anxiety and gastrointestinal complaints. Furthermore, modern medicine proved that it has antioxidant, antimicrobial and antiproliferative properties [1-7].

In North Sinai Governorate, one of the main problems facing farmers there that they have to use high amounts of chemical nitrogen fertilizers to increase productivity of their cultivated crops under poor desert sandy soil as nitrogen is a necessary and major element for plant growth and yield increase. With regard to the cultivation of medicinal and aromatic plants, leafy herbs tend to accumulate nitrate in their leaves and thus could pose a risk to human health [8]. Recently, there is a new world trend to reduce amounts of applied chemical nitrogen fertilizers in agriculture and to search for new alternatives to increase plants productivity such as nano-fertilizers.

Lithovit is a natural CO_2 foliar fertilizer, it is a compound made of limestone (calcium carbonate $CaCO_3$) and dolomite of $CaMg(CO_3)_2$ which are found in abundance in nature. According to nanotechnology, the movement of solids within a fine motor friction mill is accelerated at a rate of 20,000 rpm. This leads to successive collisions of the particles.

This result in a very fine form in the form of fine granules with a diameter of less than 10 micrometers and the ease to be released into the active carbon dioxide needed for plant metabolism. So, it supplies plants with CO_2 in much higher concentration than that in the atmosphere and so increases their photosynthesis. Furthermore, it contains a big number of micronutrients important for plant physiology [9-12].

So, the aim of this study is trying to reduce the amount of applied chemical nitrogen fertilizers to *Origanum syriacum* var. *syriacum* plants by using nano-fertilization technique in newly reclaimed lands of North Sinai.

Materials and Methods

This work was carried out at Baloza Agricultural Experimental Station of the Desert Research Center, Baloza region, North Sinai Governorate during the two successive seasons of 2016 and 2017. The experimental area represents a newly reclaimed sand dune soil. Mechanical and chemical analyses of the soil are presented in Tables (A) and (B). Analysis of irrigation water is shown in Table (C). The work was conducted on one year old *Origanum syriacum* var. *syriacum* plants cultivated under drip irrigation system in rows 75 cm apart and 50 cm within hills (11200 plants/fed.).

Nano-fertilizer "lithovit standard" was imported from Zeovita GmbH Company, Germany via Agrolink Company, Egypt. Lithovit was used at a concentration of 0.5%. Foliar application of lithovit was monthly conducted. The chemical analysis of lithovit is displayed in Table (D). Full recommended dose of nitrogenous fertilization for oregano plants in North Sinai was added after each cut as 200 kg ammonium sulphate (20.5% N) /fed./cut [13]. All other agriculture practices were performed according to the recommendations of Ministry of Agriculture and Land Reclamation.

The experiment involved five treatments as follows:- application full recommended N dose, 3/4 N dose + foliar spray with lithovit, 1/2 N dose + foliar spray with lithovit, 1/4 N dose + foliar spray with lithovit and foliar spray with lithovit only. The statistical design of the experiment was a complete randomized block design. Means of all data were compared by L.S.D. method according to [14]. In the two seasons the plants were harvested at flowering three times per season i.e. on spring, summer and autumn by cutting the vegetative parts of plants 15 cm above the soil surface leaving several branches for regrowth. For each cut, the following measurements were detected:-

A. Growth and yield characters

Plant height (cm), herb fresh weight (g/plant), herb fresh weight (ton/fed.), herb dry weight (g/plant) and herb dry weight (ton/fed.).

B. Quality characters

1. Essential oil percentage

Essential oil percentage was determined in the air dried herb by hydrodistillation for 3 hours using a Clevenger type apparatus [15].

2. Essential oil yield/plant (ml)

$$\text{This was calculated as follows : } \frac{\text{Oil percentage} \times \text{herb dry weight / plant}}{100}$$

3. Essential oil yield per fed. (l)

This was calculated as follows: Oil yield per plant × number of plants/fed.

4. Essential oil chemical composition

The essential oil samples of the second season were analyzed by using gas liquid chromatography apparatus at the Central Laboratory of National Research Center, Egypt. The specifications of GLC apparatus were as follows: HP 6890 Series GC System. Oven: initial temperature 70ºC, initial time 1.00 min. Ramps: rate 8.00, final temperature 220ºC, final time 20.00. Injector temperature: 220ºC. Capillary column: HP-5 5% phenyl methyl siloxane, length 30.0 m, diameter 320.00 µm, film thickness 0.25 µm, flow 1.5 ml/min. Front detector: temperature 250 ºC, flow rate of gas N_2 flow 20.0 ml/min, hydrogen flow 20.0 ml/min, air flow 200.0 ml/min.

Table A: Mechanical analysis of the experimental soil area.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
0-30	90	5	5	Sandy

Table B: Chemical analysis of the experimental soil area.

pH	E.C. (ds/m)	O.M. (%)	Soluble anions (meq/l)				Soluble cations (meq/l)			
			CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
8.17	5.99	0.17	-	2.46	40.71	17.17	19.53	13.25	26.91	0.65

Table C: Chemical analysis of the irrigation water.

pH	E.C. (ppm)	Soluble anions (meq/l)				Soluble cations (meq/l)			
		CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
7.76	2672.75	0.52	1.96	28.86	7.59	4.51	11.75	22.08	0.59

Table D: Chemical analysis of the nano-fertilizer "lithovit".

$CaCO_3$ "micron" (%)	$MgCO_3$ "micron" (%)	N (%)	P (%)	K_2O (%)	Ca (%)	Mg (%)	Mn (mg/l)	Zn (mg/l)
24	41	0.4	0.7	0.98	3	2	18.10	1.60

Results and Discussions

I. Growth and herb yield characters

Data of the effect of nitrogen fertilization and foliar spraying with nano fertilizer "lithovit" on plant height, herb fresh weight per plant and feddan as well as herb dry weight per plant and feddan are presented in Tables (1, 2, 3, 4 and 5) and illustrated in Figs. (1 and 2).

In the two seasons it was remarkable that foliage production was varied among the different cuts and both treatments of 3/4 N dose and 1/2 N dose + foliar spray with lithovit which recorded either insignificant or significant increments over control treatment (application of full recommended N dose only) whereas, foliar spray with lithovit only recorded the lowest parameters. These results proved that, nano fertilizer "lithovit" induced high effect on reducing the amount of applied chemical nitrogen fertilizer as it could save between 25 to 50% of nitrogen quantity. This positive influence may be attributed to the role of lithovit which supplies plants with CO_2 in much higher concentration than that in the atmosphere and so increases their photosynthesis and yield. Moreover, using of lithovit increases plant resistance to physiological stress as well as increases tolerance to frost, drought and

resistance to fungal infections [16].

The enhancing effect of lithovit on productivity of medicinal and aromatic plants was reported by several researchers i.e. [17] on *Allium cepa* who found that foliar application with lithovit showed significant effects for most of studied characters such as earliness of bolting, bolting period, number of umbel scapes/plot, height of umbel scape, diameter of umbel, seed yield/plot and average weight of 100 seeds. Also, [18] on *Moringa olifera* found that foliar spray with lithovit enhanced vegetative growth of treated plants and [19] on *Cymbopogon citratus* observed that foliar spray of lithovit produced heaviest fresh and dry weights of herb and highest number of tiller/plant. Also, lithovit improved growth and yield of rose plants [16].

Table 1: Effect of nitrogenous fertilization and foliar spray with lithovit on plant height (cm) of *Origanum syriacum* var. *syriacum* during the two successive seasons of 2016 and 2017.

Fertilization treatments	First season			Second season		
	Spring cut	Summer cut	Autumn cut	Spring cut	Summer cut	Autumn cut
Full recommended N dose	43.61	41.67	26.83	65.67	32.67	30.33
3/4 N dose + foliar spray with lithovit	47.17	47.00	33.67	67.47	37.50	35.17
1/2 N dose + foliar spray with lithovit	38.39	44.11	32.17	65.67	34.50	31.43
1/4 N dose + foliar spray with lithovit	37.44	41.50	32.50	63.67	31.50	28.17
Foliar spray with lithovit only	35.33	38.75	20.17	62.50	30.93	29.50
LSD at 0.05	4.53	4.60	3.88	3.17	3.60	1.83

Table 2: Effect of nitrogenous fertilization and foliar spray with lithovit on herb fresh weight (g/plant) of *Origanum syriacum* var. *syriacum* during the two successive seasons of 2016 and 2017.

Fertilization treatments	First season			Second season		
	Spring cut	Summer cut	Autumn cut	Spring cut	Summer cut	Autumn cut
Full recommended N dose	41.88	190.89	160.61	505.00	203.00	171.00
3/4 N dose + foliar spray with lithovit	48.10	234.50	229.03	543.00	254.33	232.33
1/2 N dose + foliar spray with lithovit	30.46	217.57	191.75	507.33	208.50	170.50
1/4 N dose + foliar spray with lithovit	17.10	131.96	117.34	436.67	173.17	140.17
Foliar spray with lithovit only	12.64	126.10	105.66	367.00	160.67	135.67
LSD at 0.05	4.95	30.54	30.00	56.22	9.41	8.57

Table 3: Effect of nitrogenous fertilization and foliar spray with lithovit on herb fresh weight (ton/feddan) of *Origanum syriacum* var. *syriacum* during the two successive seasons of 2016 and 2017.

Fertilization treatments	First season				Second season			
	Spring cut	Summer cut	Autumn cut	Total	Spring cut	Summer cut	Autumn cut	Total
Full recommended N dose	0.47	2.14	1.80	4.41	5.66	2.27	1.92	9.85
3/4 N dose + foliar spray with lithovit	0.54	2.63	2.57	5.74	6.08	2.85	2.6	11.53
1/2 N dose + foliar spray with lithovit	0.34	2.44	2.15	4.93	5.68	2.34	1.91	9.93
1/4 N dose + foliar spray with lithovit	0.19	1.48	1.31	2.98	4.89	1.94	1.57	8.4
Foliar spray with lithovit only	0.14	1.41	1.18	2.73	4.11	1.8	1.52	7.43
LSD at 0.05	0.06	0.22	0.32	0.46	0.63	0.11	0.10	1.53

Table 4: Effect of nitrogenous fertilization and foliar spray with lithovit on herb dry weight (g/plant) of *Origanum syriacum* var. *syriacum* during the two successive seasons of 2016 and 2017.

Fertilization treatments	First season			Second season		
	Spring cut	Summer cut	Autumn cut	Spring cut	Summer cut	Autumn cut
Full recommended N dose	14.06	77.82	73.30	189.04	93.13	82.41
3/4 N dose + foliar spray with lithovit	16.80	95.95	85.76	203.28	117.56	100.55
1/2 N dose + foliar spray with lithovit	10.84	87.79	80.19	190.11	95.06	82.14
1/4 N dose + foliar spray with lithovit	5.51	57.38	55.76	163.32	79.33	65.25
Foliar spray with lithovit only	4.65	52.07	42.78	137.00	73.12	63.11
LSD at 0.05	1.61	13.04	12.33	11.33	9.92	11.41

Table 5: Effect of nitrogenous fertilization and foliar spray with lithovit on herb dry weight (ton/feddan) of *Origanum syriacum* var. *syriacum* during the two successive seasons of 2016 and 2017.

Fertilization treatments	First season				Second season			
	Spring cut	Summer cut	Autumn cut	Total	Spring cut	Summer cut	Autumn cut	Total
Full recommended N dose	0.16	0.87	0.82	1.85	2.12	1.04	0.92	4.08
3/4 N dose + foliar spray with lithovit	0.19	1.08	0.96	2.23	2.28	1.32	1.13	4.73
1/2 N dose + foliar spray with lithovit	0.12	0.98	0.90	2.00	2.13	1.07	0.92	4.12
1/4 N dose + foliar spray with lithovit	0.06	0.64	0.63	1.33	1.83	0.89	0.73	3.45
Foliar spray with lithovit only	0.05	0.58	0.48	1.11	1.53	0.82	0.71	3.06
LSD at 0.05	0.02	0.15	0.14	0.48	0.13	0.11	0.13	0.16

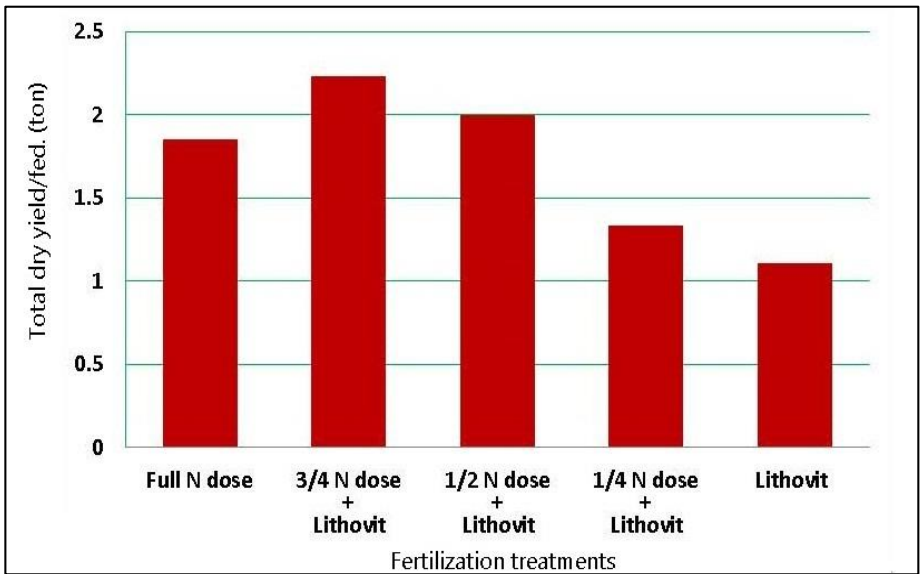

Fig 1: Effect of nitrogenous fertilization and spraying with lithovit on total herb dry yield/fed. for the first season.

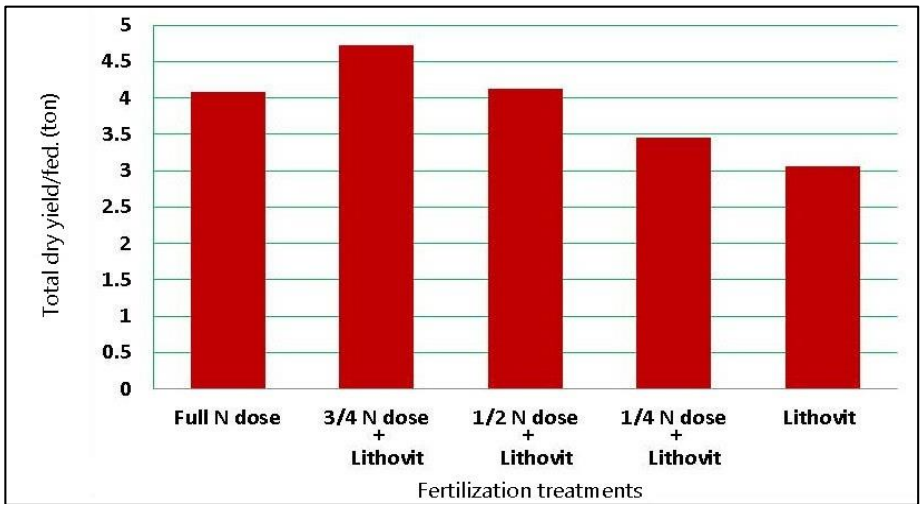

Fig 2: Effect of nitrogenous fertilization and spraying with lithovit on total herb dry yield/fed. for the second season.

II. Quality characters

A. Essential oil percentage and yield

Data presented in Tables (5, 6 and 7) show the effect of nitrogen fertilization and foliar spray with lithovit on essential oil percentage and yield. The obtained results indicated that zaatar herb has a rich volatile oil content which is responsible for its strong flavor and aroma as well as its pharmacological effect. Moreover, variations were existed within cuts in oil content. These findings were in harmony with [7]. As for the influence of fertilization treatments, in both seasons, it was observed that 3/4 N dose and 1/2 N dose + spraying with lithovit gave insignificant and significant variations over control treatment (application full recommended N dose) while, using of lithovit only recorded the least values. The stimulatory effect of lithovit on essential oil productivity may be attributed to its positive role in improving photosynthesis of plants to build up more metabolites necessary for inducing the volatile oil synthesis. These observations were in a parallel line with those of [19] who recommended spraying *Cymbopogon citratus* plants with lithovit to enhance essential oil yield. Furthermore, [20] on soybean plant demonstrated that foliar application with lithovit increased seeds fixed oil percentage over control treatment.

Table 5: Effect of nitrogenous fertilization and foliar spray with lithovit on essential oil percentage of *Origanum syriacum* var. *syriacum* during the two successive seasons of 2016 and 2017.

Fertilization treatments	First season			Second season		
	Spring cut	Summer cut	Autumn cut	Spring cut	Summer cut	Autumn cut
Full recommended N dose	2.03	2.32	1.03	1.46	1.54	1.02
3/4 N dose + foliar spray with lithovit	2.15	2.60	1.18	1.64	2.36	1.20
1/2 N dose + foliar spray with lithovit	1.99	2.34	1.15	1.47	2.23	1.06
1/4 N dose + foliar spray with lithovit	1.99	2.34	0.96	1.40	1.49	1.00
Foliar spray with lithovit only	1.57	2.30	0.89	1.40	1.43	0.95
LSD at 0.05	0.12	0.02	0.09	0.06	0.07	0.06

Table 6: Effect of nitrogenous fertilization and foliar spray with lithovit on essential oil yield / plant (ml) of *Origanum syriacum* var. *syriacum* during the two successive seasons of 2016 and 2017.

Fertilization treatments	First season			Second season		
	Spring cut	Summer cut	Autumn cut	Spring cut	Summer cut	Autumn cut
Full recommended N dose	0.29	1.81	0.76	2.76	1.43	0.84
3/4 N dose + foliar spray with lithovit	0.36	2.50	1.01	3.33	2.77	1.21
1/2 N dose + foliar spray with lithovit	0.22	2.05	0.92	2.80	2.12	0.87
1/4 N dose + foliar spray with lithovit	0.11	1.34	0.54	2.29	1.18	0.65
Foliar spray with lithovit only	0.07	1.20	0.38	1.92	1.05	0.60
LSD at 0.05	0.04	0.34	0.17	0.06	0.09	0.09

Table 7: Effect of nitrogenous fertilization and foliar spray with lithovit on essential oil yield / fed. (l) of *Origanum syriacum* var. *syriacum* during the two successive seasons of 2016 and 2017.

Fertilization treatments	First season				Second season			
	Spring cut	Summer cut	Autumn cut	Total	Spring cut	Summer cut	Autumn cut	Total
Full recommended N dose	3.25	20.27	8.51	32.03	30.91	16.02	9.41	56.34
3/4 N dose + foliar spray with lithovit	4.03	28.00	11.31	43.34	37.30	31.02	13.55	81.87
1/2 N dose + foliar spray with lithovit	2.46	22.96	10.30	35.72	31.36	23.74	9.74	64.84
1/4 N dose + foliar spray with lithovit	1.23	15.01	6.05	22.29	25.65	13.22	7.28	46.15
Foliar spray with lithovit only	0.78	13.44	4.26	18.48	21.50	11.76	6.72	39.98
LSD at 0.05	0.46	3.77	1.89	3.97	0.68	1.05	0.97	7.25

B. Essential oil composition

Data given in Tables (8, 9 and 10) show the effect of nitrogen fertilization and foliar spray with lithovit on essential oil omponents. The GLC analysis identified nine dominant components in the oil as follows: α-pinene (0.18-4.81%), β-pinene (0.24-5.42%), myrcene (0.30-3.42%), ρ-cymene (1.57-19.48%), γ-terpinene (0.78-10.26%), α-phellandrene (8.70-26.36%), terpineol (1.18-22.10%), thymol (3.39-28.78%) and carvacrol (0.68-4.83%). The total phenolic compounds (total thymol and carvacrol) ranged from 4.07 to 33.03%. These monoterpenes are responsible for the antibacterial and antiphlogistic activities of the herbal drug. As well as thymol has a characteristic phenol-like aromatic odor and a spicy flavor and carvacrol has a characteristic pungent warm taste and odor [21-22]. Parallel compounds were identified by [23] who demonstrated that volatile oil of *Majorana syriaca* grown under Palestine conditions was affected by location and contained α-phellandrene (1.62-8.13%), α-pinene (1.22-4.61%) β-myrcene (0.5-11%), m-myrcene (1.86-8.61%), γ-terpinene (11.96-30.80%), thymol (0.26-11.60%) and carvacrol (0.65-21.70%).

Also, the present analysis indicated that the volatile oil composition was varied by harvesting time and fertilization treatments as the nitrogen fertilization increased thymol content. These findings were agreed with [23] who observed variability in the major terpenoid components of the oil at different harvesting times and [7] who mentioned that volatile oil components were affected by agricultural treatments.

Table 8: Effect of fertilization treatments on constituents of volatile oil (spring cut).

Compound (%)	Foliar spray with lithovit only	1/4 N dose + foliar spray with lithovit	1/2 N dose + foliar spray with lithovit	3/4 N dose + foliar spray with lithovit	Full recommended N dose
α-Pinene	4.81	2.68	2.38	3.00	3.07
β-Pinene	4.21	2.79	2.43	2.95	2.49
Myrcene	3.38	2.12	1.86	2.25	2.23
ρ-Cymene	19.48	9.86	8.77	10.15	11.19
γ-Terpinene	3.63	2.40	2.19	2.30	2.28
α -Phellandrene	21.10	10.18	8.70	11.01	13.89
Terpineol	22.10	10.35	9.27	11.18	12.09
Thymol	3.39	21.63	27.10	24.96	26.14
Carvacrol	0.68	4.83	2.95	2.74	2.88
Total identified compounds	82.78	66.84	65.65	70.54	76.26

Table 9: Effect of fertilization treatments on constituents of volatile oil (summer cut).

Compound (%)	Foliar spray with lithovit only	1/4 N dose + foliar spray with lithovit	1/2 N dose + foliar spray with lithovit	3/4 N dose + foliar spray with lithovit	Full recommended N dose
α-Pinene	1.48	1.16	2.09	2.26	0.18
β-Pinene	4.86	2.61	4.85	5.42	0.24
Myrcene	2.41	1.28	2.37	2.62	0.30
ρ-Cymene	13.47	8.08	8.14	7.14	1.57
γ-Terpinene	2.18	8.88	8.85	9.18	0.78
α -Phellandrene	20.39	11.93	26.36	19.21	13.73
Terpineol	9.84	9.58	10.16	8.92	1.18
Thymol	16.08	21.88	14.93	15.84	28.78
Carvacrol	4.06	4.38	0.80	0.83	4.25
Total identified compounds	74.77	69.78	78.55	71.42	51.01

Table 10: Effect of fertilization treatments on constituents of volatile oil (autumn cut).

Compound (%)	Foliar spray with lithovit only	1/4 N dose + foliar spray with lithovit	1/2 N dose + foliar spray with lithovit	3/4 N dose + foliar spray with lithovit	Full recommended N dose
α-Pinene	0.81	0.95	1.39	1.73	1.14
β-Pinene	4.03	4.00	4.38	3.89	-
Myrcene	3.42	2.90	2.01	1.80	0.83
ρ-Cymene	16.52	10.33	5.34	5.02	2.04
γ-Terpinene	3.41	5.67	10.26	10.20	0.72
α -Phellandrene	17.81	20.15	20.62	26.07	21.47
Terpineol	7.67	8.11	8.30	8.57	6.44
Thymol	17.04	17.56	18.10	16.86	22.78
Carvacrol	0.92	0.77	0.95	0.79	1.14
Total identified compounds	71.63	70.44	71.35	74.93	56.56

From the abovementioned data, non-significant variations were found within the treatments of full N dose and 1/2 N dose + foliar spraying with lithovit in most cases. So, the treatment of 1/2 N dose + foliar spraying with lithovit could be chosen as best eco-friendly and economically fertilization treatment.

Finally, it was notable by this work that, using of nano-fertilizer can aid farmers to gradual convert from using of chemical-based agriculture to using of good agricultural practices under desert environments.

Conclusion

It could be concluded that cultivated zaatar "*Origanum syriacum* var. *syriacum*" plants at Baloza region, North Sinai should be foliar sprayed monthly with nano-fertilizer "lithovit standard" at a concentration of 0.5% beside applying 100 kg/fed. of ammonium sulphate after each cut.

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