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Evaluation of Turkish oregano (*Origanum onites* L.) under organic farming system

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Abstract

Spice production is crucial for the world economy. Today, the farming of spices faces significant challenges under climate change, such as heat stress, rising salinity, water stress, low soil nutrients, and increased pests, which lead to reduced yield and quality. The present study was conducted in Egypt on Turkish oregano (*Origanum onites* L.) plants. The trial was achieved under organic farming conditions. The purpose was to investigate the responses of the plant towards two natural stimulators, chitosan and biofertilizer. The design of the work was a split plot. Main plots were assigned to the foliar spray of chitosan at three concentrations (0, 5, and 10 liters/hectare) and sub-plots to biofertilizer EM (with and without). The collected data comprised plant height, fresh and dry herb weights per plant, fresh and dry herb yields per hectare, essential oil percentage, oil yield per plant and hectare, and GC-MS analysis of oils. The significant maximum growth and yield characteristics were obtained by spraying with chitosan at the highest concentration of 10 liters/hectare combined with soil drenching by biofertilizer EM. The major essential oil component was carvacrol, which is responsible for its pungent flavour. This previous treatment also recorded the maximum carvacrol percent in oil of all cuts. So, this treatment could be used to increase the quantity and quality of the organic product.

Keywords

Origanum onites, chitosan, biofertilizer, yield, carvacrol

Introduction

Organic farming is a system of agriculture that protects the environment. It uses natural procedures in which industrial products such as genetically modified organisms and external agricultural inputs such as chemical fertilizers and pesticides are banned (1). In contrast, organic farmers depend on natural methods and stylish environmental knowledge to achieve maximum health. In the long term, it means maintaining the ecosystem's productivity to become more sustainable. The importance of organic production is keeping pace with the European Union's decision to import organic agriculture products only from countries with organic agriculture law and Egypt follows this. Therefore, Egypt issued Organic Agriculture Law No. 12 of 2020. Currently, organic agriculture faces considerable challenges under climate change conditions, such as abiotic and biotic stresses for plants. Biostimulants, like chitosan and biofertilizer materials, are widely used in organic farming to make crops grow better (2-3).

Chitosan is a natural polymer created by deacetylating chitin, which can be found in crustaceans, insects, fungi, and other organisms. Chitosan is biodegradable, environmentally friendly, and a strong candidate for sustainable agriculture. Chitosan has been found to favor plants' development, productivity, and secondary metabolites. Hormones are present in chitosan. It also has a plentiful natural supply of amino acids. Chitosan boosts the plant's resistance to pests that infect crops by encouraging the production of numerous enzymes involved in the defence response. Chitosan helps plants in withstanding abiotic and biotic stresses also (4).

Biofertilizer EM is a group of 80 types of valuable microorganisms found in nature and not genetically modified. Professor Dr. Teruo Higa produced microbial inoculant as an effective microorganism (EM) at the University of Ryukyus, Japan, in the 1980s (5). The significant constituents of EM are microorganisms producing lactic acid, photosynthetic bacteria, yeast, and ray fungi. These microorganisms are integrated into the manufacturing process and can prevail in the inoculant liquid at pH 3.5 or lower. It can be added as an inoculant to maximise the microbial diversity of soils to improve soil properties and the development, yield, and quality of plants, specifically in organic farming. The biofertilizer EM can enhance photosynthesis in crops. It secretes antibiotics that eliminate microbes risky to the soil (5).

Spices are vital to the global economy, besides their importance for human health. The Lamiaceae family includes the Turkish oregano condiment, also known as Turkish thyme, and pot marjoram (scientific name: *Origanum onites* L.). It is a perennial herbaceous plant indigenous to the eastern Mediterranean basin, where it grows in the Greek islands, extending to western Turkey where it is cultivated. Because of its aromatic leaves, this herb adds flavour and a pungent aroma to a variety of dishes. Various preparations are used in pharmaceuticals in addition to their culinary applications. The herb has volatile oil and mainly contains carvacrol. Pharmacological studies have shown that this plant has a wide range of biological effects, including effects on the gastrointestinal tract, antimicrobial, antioxidant, lower cholesterol, anticancer, hepatoprotective, antidiabetic, antiviral and cardiorespiratory functions (6-8).

However, Egypt cultivates other types of oregano, such as Syrian, Egyptian, and Greek oregano (9, 10). Turkish oregano is not grown in the country, so it is imported from abroad due to its distinct pleasant flavour. On account of the high prices of its imports in foreign currency, it was necessary to think about cultivating it. The climate conditions in Egypt are suitable for the growth of oregano as a result of the diversity of the existing climate. A package of technical guidance must be provided on the various agricultural operations of this species, especially under organic farming.

This investigation aims to study the effects of spraying different concentrations of chitosan, adding biofertilier EM, and the interaction between these factors on the crop to make the highest organic production possible.

Materials and Methods

This investigation was carried out during the two seasons of 2019/2020 and 2020/2021 at a private farm in the Oraby Association, Al-Eubour (30° 14′ 8.3" N, 31° 31′ 34.1" E). The farm was certified for organic production. The area's soil analyses and water irrigation properties are displayed in Tables (1-3). Compost manure at 48 m^3/hectare was put through soil preparation. The properties of compost are shown in Table (4). *O. onites* seeds were imported and sown in the nursery on the 15th of October inside a greenhouse. The uniform seedlings were transplanted to the open field on the 1st of March. The agriculture was done using a drip irrigation system. The spaces between 'hills' were 30 cm and the rows were 75 cm apart (44444 plants/hectare) (Fig. 1). Monthly applications of potassium humate were conducted at the rate of 10 kg/hectare through irrigation. Biopesticides achieved pest management.

The experiment was designed as a split plot and included three replicates with six treatments. The main plots involved the foliar spray of chitosan at three concentrations (0, 5, and 10 liters/hectare) and the subplots had biofertilizer EM (with and without). Chitosan was obtained from Chitosan Egypt Company under the commercial name Chito Green. The solution ingredients were magnesium at 15%, total nitrogen at 15%, chitosan and carboxylic acids at 5% (11). Spraying with chitosan was performed one month after transplanting and repeated after each cut. Spraying was done on the leaves until run-off. Untreated plants were sprayed with distilled water. The biofertilizer EM (0.6×10^7/ml microorganisms) was obtained from the Egyptian Ministry of Environment. The essential composition of EM was photosynthetic and lactic acid bacteria, yeast, and actinomycetes. The biofertilizer was added to plants one month after cultivation and repeated as a soil drench after each harvest.

The herb was harvested three times per season in June (spring cut), August (summer cut), and October (autumn cut). It was harvested at a 10 cm level, leaving some branches for regrowth. The two-way ANOVA tested all the data (12), and L.S.D examined the differences at 5 % level of probability. The following data were measured at each harvest. Growth and yield attributes included:

Plant height (cm).

Fresh herb weight (g/plant).

Dry herb weight (g/plant).

Fresh herb yield (ton/hectare).

Dry herb yield (ton/hectare).

Any foreign materials were removed immediately from the harvested herb. They were dried at 70°C to a dry matter content determined by consistency. The sampling ensured it was representative of much of the raw material.

Some quality aspects were recorded as essential oil percentage in the air-dried herb by hydro-distillation (13).

Essential oil yield per plant (ml) was calculated as follows: oil percentage × herb dry weight/100.

Essential oil yield per hectare (l) was calculated as follows: essential oil yield per plant × number of plants per hectare.

GC-MS analysis of essential oil was performed by Gas Chromatography-Mass Spectrometry instrument at the Laboratory of Medicinal and Aromatic Plants, National Research Center, Egypt with the following specifications.

Device: a TRACE GC Ultra Gas Chromatograph (THERMO Scientific Corp., USA) coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system had a TR-5MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness). Analyses were conducted using helium as carrier gas at a flow rate of 1.3 ml/min at a split ratio of 1:10 and the following temperature program: 80°C for 1 min; rising at 4°C/min to 300°C and held for 1min. The injector and detector were held at 220 and 200°C, respectively. Diluted samples (1:10 hexane, v/v) of 1 µL of the mixtures were continuously injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Identification of the compounds depended on both comparison of the retention times with those of authentic samples and computer matching against commercial and library mass spectra built up from pure substances (14-16).

Table 1. Physical properties of the soil

Sand (%)	Silt (%)	Clay (%)	Soil texture
65.50	31.00	3.50	Sandy loam

Table 2. Chemical properties of the soil

pH	O.M. (%)	E.C. (ppm)	Soluble anions (meq/l)				Soluble cations (meq/l)			
			CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
8.61	0.40	588.80	-	0.50	5.50	3.17	2.50	1.50	4.85	0.32

Table 3. Chemical properties of the water

pH	E.C. (ppm)	Soluble anions (meq/l)				Soluble cations (meq/l)			
		CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
7.20	316.00	-	1.46	1.19	2.68	3.10	1.32	0.82	0.09

Table 4. Chemical properties of the compost

pH	EC (ppm)	O.M. (%)	C/N ratio (%)	N (%)	P (%)	K (%)	Ash (%)	Humidity (%)	Weight m^3 (kg)
6.38	3571.00	41.58	1 : 20.97	1.15	1.20	0.48	58.42	27.00	655.00

Fig. 1. Organic farming methods were used to cultivate Turkish oregano.

Results and Discussion

I- Effect of biofertilizer EM

Data presented in Tables (5-7) showed the influence of biofertilizer EM on growth attributes. The biofertilizer application recorded the significant best traits of plant height, fresh herb weight per plant, and dry herb weight per plant. These values were 19.17, 21.67, and 22.22 cm; 38.08, 56.58, and 118.38 g; and 14.91, 24.25, and 38.13 g for the first, second, and third cuts, respectively. Conversely, the uninoculated plants detected the lowest growth parameters in this regard.

Data from Tables (8 and 9) revealed how bio fertilizer EM significantly improved yield characteristics. The biofertilizer treatment showed the best results regarding fresh herb yield per hectare and dry herb yield per hectare. In that order, these measurements were 1.69, 2.52, and 5.26 tons; and 0.66, 1.08, and 1.70 tons for the first, second, and third cuts. On the other hand, the unfertilized oregano plants gave the lowest yield metrics.

The information in Tables (10-12) indicated that Biofertilizer significantly enhanced quality parameters such as essential oil percentage, oil yield per plant, and hectare. These records were 1.49, 1.62, and 2.29 %; 0.22, 0.40, and 0.90 ml; and 9.92, 17.63, and 40.00 l for the first, second, and third cuts sequence. In comparison, control had the lowest parameters of this concern.

For several reasons, the promoting influence of biofertilizer EM on the growth and yield of oregano crops is that it contains naturally effective microorganisms with numerous roles. They can increase the value of organic matter. They produce hormones and antioxidants, increase root growth and nitrogen fixation, and increase the dissolution of phosphorous in the soil. Their strains make CO_2, lower the pH of the soil, improve plant health by getting rid of pathogens, and make it easier for plants to take in nutrients, all of which help photosynthesis (5).

Studies on gladiolus (17), marjoram and peppermint (18), *Viola odorata* (19), chicory (20) and *Mentha piperita* (21) noticed that using biofertilizer EM could increase the yield.

II- Effect of chitosan concentrations

The growth parameters of plant height, fresh herb weight, and dry herb weight per plant were significantly increased with increasing chitosan concentration. The maximum values were from 10 liters/hectare. The given data for the first, second, and third cuts were 19.50, 22.84, and 22.17 cm; 42.09, 62.57, and 135.27 g; and 16.64, 26.66, and 43.67 g, respectively. In contrast, plants unsprayed with chitosan recorded the lowest growth characteristics (Tables 5-7).

Higher chitosan concentrations significantly enhanced yield indices such as fresh herb yield per hectare and dry herb yield per hectare. The highest values were at 10 liters/hectare. Its recorded measurements for the first, second, and third cuts were 1.87, 2.79, and 6.01 tons; and 0.74, 1.19, and 1.94 tons. Contrarily, plants that weren't sprayed with chitosan had the poorest yield traits (Tables 8 and 9).

The quality components of essential oil percentage, oil yield per plant, and hectare increased significantly with maximum chitosan concentrations. Ten liters/hectare represented the highest values. Given data for the first, second, and third cuts were 1.50, 1.65, and 2.36 %; 0.26, 0.44, and 1.05 ml; and 11.33, 19.56, and 46.45 l, respectively. On the other hand, plants that were not chitosan-sprayed had minor attributes (Tables 10-12).

The superior results for using chitosan at the highest concentration could be attributed to chitosan naturally containing a high percentage of gibberellin, auxin, and cytokinin. It is an excellent source of nitrogen and amino acids. Chitosan increases the plant's immunity against fungi and bacteria that infect plants (22); it stimulates the plant to produce many enzymes associated with this defense response. Treatment with chitosan increases proline levels, allowing the plant to tolerate abiotic and biotic stresses. Therefore, it works to improve vegetative growth and yield (23).

The increase in yield and quality of production under various stresses as a result of using chitosan was mentioned in various studies on basil (24), on marjoram (25), on stevia (26), on rosemary (27), on summer savory (28), on German chamomile (29), on fennel (30), on hyssop (31), on oregano plants (32, 33), as well as in another work where it was mentioned that the use of chitosan increased the percentage and yield of essential oil in thyme due to the role of chitosan in up-regulation new genes and different biosynthetic pathways for secondary metabolite production (34).

III- Effect of interaction

Data from Tables (5-7) revealed that the interaction between chitosan and biofertilizer improved growth performance positively. The foliar spray with chitosan at 10 liters/hectare and the application of biofertilizer EM showed the most significant increases in plant height, fresh herb weight per plant, and dry herb weight per plant. These first, second, and third cut detections were 22.00, 25.67, and 25.00 cm; 49.00, 71.47, and 155.99 g; and 19.33, 30.45, and 50.33 g, respectively. However, the control showed lower growth estimates.

The interaction between chitosan and biofertilizer boosted efficiency yield, according to Tables 8 and 9. Fresh herb yield per hectare and dry herb yield per hectare was significantly increased by the foliar application of chitosan at a concentration of 10 liters/hectare and the addition of biofertilizer. The fresh yield data for the first, second, and third cuts were 2.18, 3.18, and 6.93 tons, respectively, and the dry yield data were 0.86, 1.35, and 2.24 tons.

Table 5. Effect of chitosan concentrations, Biofertilizer EM, and their interaction on plant height (cm) (mean values of the two successive seasons)

Treatments	1st cut			2nd cut			3rd cut		
Chitosan	Biofertilizer EM			Biofertilizer EM			Biofertilizer EM		
	Without	With	Mean	Without	With	Mean	Without	With	Mean
0 liter / hectare	13.00^{f}	15.00^{g}	14.00^{a}	15.00^{f}	16.33^{g}	15.67^{a}	16.33^{f}	19.67^{g}	18.00^{a}
5 liter / hectare	16.00^{gh}	20.50^{i}	18.25^{b}	17.67^{h}	23.00^{j}	20.34^{b}	18.67^{g}	22.00^{h}	20.34^{b}
10 liter / hectare	17.00^{h}	22.00^{j}	19.50^{c}	20.00^{i}	25.67^{k}	22.84^{c}	19.33^{g}	25.00 i	22.17^{c}
Mean	15.33^{d}	19.17^{e}		17.56^{d}	21.67^{e}		18.11^{d}	22.22 e	
LSD 5 %									
Chitosan		1.01			1.00			0.84	
Biofertilizer EM		0.83			0.59			0.69	
Interaction		1.43			1.03			1.19	

Means with the same letter are not significantly different at 5% level of probability

Table 6. Effect of chitosan concentrations, Biofertilizer EM, and their interaction on fresh herb weight (g/plant) (mean values of the two successive seasons)

Treatments	1st cut			2nd cut			3rd cut		
Chitosan	Biofertilizer EM			Biofertilizer EM			Biofertilizer EM		
	Without	With	Mean	Without	With	Mean	Without	With	Mean
0 liter / hectare	19.67^{f}	25.23^{g}	22.45^{a}	30.17^{f}	39.33^{g}	34.75^{a}	53.65^{f}	67.06^{g}	60.36^{a}
5 liter / hectare	29.27^{h}	40.00^{j}	34.64^{b}	45.21^{h}	59.23^{j}	52.22^{b}	94.06^{h}	132.10^{j}	113.08^{b}
10 liter / hectare	35.17^{i}	49.00^{k}	42.09^{c}	53.67^{i}	71.47^{k}	62.57^{c}	114.54^{i}	155.99^{k}	135.27^{c}
Mean	28.04^{d}	38.08^{e}		43.02^{d}	56.68^{e}		87.42^{d}	118.38^{e}	
LSD 5 %									
Chitosan		0.79			0.84			5.82	
Biofertilizer EM		0.64			0.68			4.75	
Interaction		1.11			1.18			8.22	

Means with the same letter are not significantly different at 5% level of probability

Table 7. Effect of chitosan concentrations, Biofertilizer EM, and their interaction on dry herb weight (g/plant) (mean values of the two successive seasons)

Treatments	1st cut			2nd cut			3rd cut		
Chitosan	Biofertilizer EM			Biofertilizer EM			Biofertilizer EM		
	Without	With	Mean	Without	With	Mean	Without	With	Mean
0 liter / hectare	7.64^{f}	10.00^{g}	8.82^{a}	12.81^{f}	17.00^{g}	14.91^{a}	17.11^{f}	21.40^{g}	19.26^{a}
5 liter / hectare	11.49^{h}	15.40^{j}	13.45^{b}	19.43^{h}	25.29^{j}	22.36^{b}	30.33^{h}	42.67^{j}	36.50^{b}
10 liter / hectare	13.95^{i}	19.33^{k}	16.64^{c}	22.86^{i}	30.45^{k}	26.66^{c}	37.00^{i}	50.33^{k}	43.67^{c}
Mean	11.03^{d}	14.91^{e}		18.37^{d}	24.25^{e}		28.15^{d}	38.13^{e}	
LSD 5 %									
Chitosan		0.43			0.60			2.11	
Biofertilizer EM		0.35			0.48			1.72	
Interaction		0.61			0.84			2.98	

Means with the same letter are not significantly different at 5% level of probability

Table 8. Effect of chitosan concentrations, Biofertilizer EM, and their interaction on fresh herb yield (ton/hectare) (mean values of the two successive seasons)

Treatments	1st cut			2nd cut			3rd cut		
Chitosan	Biofertilizer EM			Biofertilizer EM			Biofertilizer EM		
	Without	With	Mean	Without	With	Mean	Without	With	Mean
0 liter / hectare	0.87^{f}	1.12^{g}	1.00^{a}	1.34^{f}	1.75^{g}	1.55^{a}	2.38^{f}	2.98^{g}	2.68^{a}
5 liter / hectare	1.30^{h}	1.78^{j}	1.54^{b}	2.00^{h}	2.63^{j}	2.32^{b}	4.18^{h}	5.87^{j}	5.03^{b}
10 liter / hectare	1.56^{i}	2.18^{k}	1.87^{c}	2.39^{i}	3.18^{k}	2.79^{c}	5.09^{i}	6.93^{k}	6.01^{c}
Mean	1.24^{d}	1.69^{e}		1.91^{d}	2.52^{e}		3.88^{d}	5.26^{e}	
LSD 5 %									
Chitosan		0.04			0.12			0.26	
Biofertilizer EM		0.03			0.09			0.21	
Interaction		0.09			0.17			0.37	

Means with the same letter are not significantly different at 5% level of probability

Table 9. Effect of chitosan concentrations, Biofertilizer EM, and their interaction on dry herb yield (ton/hectare) (mean values of the two successive seasons)

Treatments	1st cut			2nd cut			3rd cut		
Chitosan	Biofertilizer EM			Biofertilizer EM			Biofertilizer EM		
	Without	With	Mean	Without	With	Mean	Without	With	Mean
0 liter / hectare	0.34^{f}	0.44^{g}	0.39^{a}	0.57^{f}	0.76^{g}	0.67^{a}	0.76^{f}	0.95^{g}	0.86^{a}
5 liter / hectare	0.51^{h}	0.68^{j}	0.60^{b}	0.86^{h}	1.12^{j}	0.99^{b}	1.35^{h}	1.90^{j}	1.63^{b}
10 liter / hectare	0.62^{i}	0.86^{k}	0.74^{c}	1.02^{i}	1.35^{k}	1.19^{c}	1.64^{i}	2.24^{k}	1.94^{c}
Mean	0.49^{d}	0.66^{e}		0.82^{d}	1.08^{e}		1.25^{d}	1.70^{e}	
LSD 5 %									
Chitosan		0.02			0.03			0.09	
Biofertilizer EM		0.02			0.03			0.08	
Interaction		0.03			0.04			0.13	

Means with the same letter are not significantly different at 5% level of probability

Table 10. Effect of chitosan concentrations, Biofertilizer EM, and their interaction on essential oil percentage (mean values of the two successive seasons)

Treatments	1st cut			2nd cut			3rd cut		
Chitosan	Biofertilizer EM			Biofertilizer EM			Biofertilizer EM		
	Without	With	Mean	Without	With	Mean	Without	With	Mean
0 liter / hectare	1.40^{f}	1.43^{g}	1.42^{a}	1.43^{f}	1.48^{g}	1.46^{a}	1.90^{f}	1.97^{g}	1.94^{a}
5 liter / hectare	1.45^{h}	1.50^{j}	1.48^{b}	1.52^{h}	1.66^{j}	1.59^{b}	2.02^{h}	2.35^{j}	2.19^{b}
10 liter / hectare	1.47^{i}	1.53^{k}	1.50^{c}	1.58^{i}	1.71^{k}	1.65^{c}	2.18^{i}	2.54^{k}	2.36^{c}
Mean	1.44^{d}	1.49^{e}		1.51^{d}	1.62^{e}		2.03^{d}	2.29^{e}	
LSD 5 %									
Chitosan		0.01			0.02			0.02	
Biofertilizer EM		0.01			0.02			0.01	
Interaction		0.01			0.03			0.02	

Means with the same letter are not significantly different at 5% level of probability

Table 11. Effect of chitosan concentrations, Biofertilizer EM, and their interaction on essential oil yield/plant (ml) (mean values of the two successive seasons)

Treatments	1st cut			2nd cut			3rd cut		
Chitosan	Biofertilizer EM			Biofertilizer EM			Biofertilizer EM		
	Without	With	Mean	Without	With	Mean	Without	With	Mean
0 liter / hectare	0.11^{f}	0.14^{h}	0.13^{a}	0.18^{f}	0.25^{g}	0.22^{a}	0.33^{f}	0.42^{g}	0.38^{a}
5 liter / hectare	0.12^{g}	0.23^{j}	0.18^{b}	0.30^{h}	0.42^{j}	0.36^{b}	0.61^{h}	1.00^{j}	0.81^{b}
10 liter / hectare	0.21^{i}	0.30^{k}	0.26^{c}	0.36^{i}	0.52^{k}	0.44^{c}	0.81^{i}	1.28^{k}	1.05^{c}
Mean	0.15^{d}	0.22^{e}		0.28^{d}	0.40^{e}		0.58^{d}	0.90^{e}	
LSD 5 %									
Chitosan		0.01			0.01			0.06	
Biofertilizer EM		0.01			0.01			0.05	
Interaction		0.01			0.02			0.08	

Means with the same letter are not significantly different at 5% level of probability

Table 12. Effect of chitosan concentrations, Biofertilizer EM, and their interaction on essential oil yield/hectare (l) (mean values of the two successive seasons)

Treatments	1st cut			2nd cut			3rd cut		
Chitosan	Biofertilizer EM			Biofertilizer EM			Biofertilizer EM		
	Without	With	Mean	Without	With	Mean	Without	With	Mean
0 liter / hectare	4.89^{f}	6.22^{h}	5.56^{a}	8.00^{f}	11.11^{g}	9.56^{a}	14.67^{f}	18.67^{g}	16.67^{a}
5 liter / hectare	5.33^{g}	10.22^{j}	7.78^{b}	13.33^{h}	18.67^{j}	16.00^{b}	27.11^{h}	44.44^{j}	35.78^{b}
10 liter / hectare	9.33^{i}	13.33^{k}	11.33^{c}	16.00^{i}	23.11^{k}	19.56^{c}	36.00^{i}	56.89^{k}	46.45^{c}
Mean	6.52^{d}	9.92^{e}		12.44^{d}	17.63^{e}		25.93^{d}	40.00^{e}	
LSD 5 %									
Chitosan		0.27			0.40			2.30	
Biofertilizer EM		0.22			0.33			1.88	
Interaction		0.37			0.56			3.25	

Means with the same letter are not significantly different at 5% level of probability

Table 13. Effect of interaction within treatments on essential oil composition

R.T.	Compound	Spring cut		Summer cut		Autumn cut	
		Control	Chitosan 10 liter/hectare + biofertilizer EM	Control	Chitosan 10 liter/hectare + biofertilizer EM	Control	Chitosan 10 liter/hectare + biofertilizer EM
3.60	Thujene	2.55	1.99	2.85	2.18	2.49	2.08
3.73	α-Pinene	0.92	0.68	1.53	1.12	0.80	0.61
4.03	Camphene	0.47	0.39	1.07	0.67	0.35	0.23
4.49	2-α-Pinene	0.15	0.13	0.26	0.17	0.12	0.10
4.62	α-Myrcene	2.43	1.89	2.55	1.94	2.30	1.63
5.00	l-Phellandrene	0.48	0.38	0.54	0.42	0.51	0.38
5.17	α-Humulene	2.67	2.27	3.19	2.35	3.37	2.26
5.37	Cymene	6.10	6.93	13.40	10.78	8.71	6.00
5.47	Sabinene	0.38	0.38	0.59	-	0.40	0.37
5.67	trans-α-Ocimene	0.08	-	-	-	-	0.05
5.97	Terpinene	11.30	10.14	12.08	8.55	10.69	8.67
6.36	4-Thujanol	0.64	0.67	0.68	0.60	0.22	0.34
6.51	p-Mentha-1,4(8)-diene	0.24	0.19	0.38	0.31	0.31	0.26
6.89	Linalool	-	-	-	-	0.07	-
7.58	α-Campholenal	0.14	0.17	0.20	0.17	0.12	0.15
7.91	trans-Pinocarveol	-	0.07	0.31	0.11	0.07	-
8.57	Borneol	2.57	3.02	5.05	3.99	2.08	1.58
8.67	4-Terpineol	1.56	1.74	3.13	2.31	2.13	1.69
8.95	Carane, 4,5-epoxy-	0.10	0.07	-	-	-	-
9.07	p-Menth-1-en-8-ol	0.18	0.14	0.33	0.23	0.20	0.12
9.90	Vetiverol		0.07	-	-	-	0.10
9.92	Linalyl acetate	0.08	-	0.28	0.16	0.07	-
10.25	Carvone	0.12	0.07	0.12	0.13	0.07	0.07
11.18	Thymol	0.86	0.59	0.38	0.43	25.65	1.30
11.35	Carvacrol	64.03	66.63	46.65	57.73	35.13	69.20
13.31	trans-Caryophyllene	0.74	0.47	1.30	1.41	1.38	0.81
14.46	Germacrene-D	0.10	-	0.52	0.49	0.19	0.17
14.95	α-Bisabolene	0.87	0.53	2.41	2.85	2.20	1.31
16.52	Caryophyllene oxide	0.22	0.23	0.20	0.33	0.28	0.15
Total identified components		99.98	99.84	100.00	99.43	99.91	99.63
Total hydrocarbon compounds		31.04	28.11	45.8	35.55	35.95	26.62
Total oxygenated compounds		68.94	71.73	54.2	63.88	63.96	73.01

R.T. = Retention time

Furthermore, combining chitosan and biofertilizer improved flavor (Tables 10-12). Essential oil percentage, oil yield per plant, and hectare were significantly enhanced with chitosan at the rate of 10 liters/hectare and biofertilizer EM. These findings were 1.53, 1.71, and 2.54%; 0.30, 0.52, and 1.28 ml; and 13.33, 23.11, and 56.89 l regarding the first, second, and third cut for each. On the other hand, the control treatment displayed the lowest quality projections.

Another quality factor is essential oil composition. The provided data in Table (13) explained that carvacrol was the principal constituent of oil as a factor responsible for spicy taste and health benefits, while oil contained little thymol. Carvacrol content was affected by treatments. The highest carvacrol percent in oil was recorded from plants that received chitosan at 10 liters/ hectare and biofertilizer. In that order, these concentrations were 66.63, 57.73, and 69.20% for the first, second, and third cuts. In comparison, the oil under control had the poorest carvacrol concentration.

The effectiveness of the treatment (spraying with chitosan at a concentration of 10 l/hectare and applying biofertilizer EM) was attributed to their combined enhancement role in the plant's physiological processes, as described previously, which promoted the growth, yield, and secondary metabolites. These findings coincided with (28) on summer savory (*Satureja hortensis),* (35) on peppermint, (36) on vinca, (31) on hyssop, and (32, 33) on oregano.

Conclusion

Present studies on *O. onites* under organic culture conditions, concluded that, to produce profitably yielding attributes such as the highest herbage yield with a good spicy flavor. The plants should be foliar sprayed with chitosan (Chito Green) at 10 liters/hectare concentration and inoculated with biofertilizer EM as a soil drench. This treatment should be done a month after transplanting and each time of plant cut.

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Authors contributions

All authors contributed equally. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical issues: None.

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