Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
https://www.plantsjournal.com
JMPS 2023; 11(2): 50-57

Received: 18-01-2023
Accepted: 24-02-2023

Wael Ismail Mohamed Toaima
Medicinal and Aromatic Plants Department, Desert Research Center, Cairo 11753, Egypt

Emad Saleh Hamed
Medicinal and Aromatic Plants Department, Desert Research Center, Cairo 11753, Egypt

Wafaa Hamed Abd El-Aleem
Medicinal and Aromatic Plants Department, Desert Research Center, Cairo 11753, Egypt

Corresponding Author:
Emad Saleh Hamed
Medicinal and Aromatic Plants Department, Desert Research Center, Cairo 11753, Egypt

Evaluation of savory (*Satureja hortensis* L.) under organic farming system

Wael Ismail Mohamed Toaima, Emad Saleh Hamed and Wafaa Hamed Abd El-Aleem

DOI: https://doi.org/10.22271/plants.2023.v11.i2a.1540

Abstract
Growing herbs and spices using organic methods are supported and encouraged in Egypt. This study was conducted on summer savory (*Satureja hortensis* L.) throughout the growing seasons of 2019/2020 and 2020/2021. The research was carried out on a plantation that adheres to organic farming practices to ascertain the impact of chitosan and bio-fertilizer EM on the crop. The study was designed with a split-plot as the layout. For the main plots, it was recommended to use rates for chitosan spray that were 0, 5, and 10 liters/hectare. For the subplots, it was recommended to use bio-fertilizer EM, either with or without. Our findings indicated that applying chitosan spray to plants at 10 liters/hectare while using bio-fertilizer produced the highest growth, herbage yield, and improved volatile oil characteristics. The volatile oil constituents were dominated by γ-terpinene and carvacrol. The highest levels of γ-terpinene and carvacrol were found in oils extracted from chitosan and biofertilizer-treated plants. These constituents were lower in control.

Keywords: *Satureja hortensis*, chitosan, bio-fertilizer EM, organic crop

Introduction
Farmers who practice organic agriculture do not use artificial growth regulators, antibiotics, chemical fertilizers, GMOs, or pesticides. Increasing soil production is important target of organic farming. Crop rotation and using organic fertilizers like compost, green manure, and bone meal are two basic tenets of organic farming. Both the diversification of cropping methods and the use of biological control of pests should be encouraged. Organic rules allow us to use substances that occur naturally. Organic farming accounts for 1.5 percent of farmland worldwide, which is insufficient for the growing global market [1, 2]. Organic farmers face several challenges, including lower yields compared to conventional agriculture, slow growth of plants, abiotic and biotic stresses, and high costs. As a result, they require new methods to increase yield while decreasing costs [3].

Various researchers are still working to develop organic farming techniques today. Biostimulants can boost plant growth and yield while also improving crop quality. They are a class of natural substances that have the possibility of lowering reliance on hazardous chemical fertilizers that degrade the environment. They promote physiological processes in plants that improve nutrient absorption, abiotic stress tolerance, and excellence indicators. They provide an opportunity to enhance organic fertilizer use and, as a result, contribute to more sustainable agricultural production. In biostimulants, numerous natural substances, such as chitosan and bio-fertilizer, are present [4, 5].

Chitin is a naturally occurring polymer found in crustaceans, insects, fungi, and other species. After cellulose, it is the most widespread present polysaccharide. Deacetylation is the process that transforms chitin into the naturally occurring polymer chitosan. Chitosan is biodegradable, non-toxic to the environment, and a promising alternative for environmentally responsible agriculture. It has been found that chitosan is beneficial to plants in terms of growth, productivity, and the production of active substances. There are hormones found in chitosan. In addition, it is biologically rich in nitrogen and amino acids. Chitosan strengthens the plant's fight against harmful insects and other organisms by stimulating the development of various enzymes involved in the plant's defensive mechanisms. Plants' defenses to biotic and abiotic stresses are boosted by chitosan [6].

Teruo Higa, a professor at the University of Ryukyus in Okinawa, Japan, created a technology that required isolating some helpful bacteria from the soil and called them effective microorganisms (EM). The EM Research Organization subsequently went on to sell this technology. Bio-fertilizer EM is packed with numerous microorganisms, many of which belong to essential categories of organisms commonly found in soil. These groups include photosynthetic bacteria, yeast, actinomyces, and lactic acid bacteria. All of these things can be discovered in liquid culture. They make more plant nutrients available, and the soil quality is improved [7].

The plant known as *Satureja hortensis* L. is related to Lamiaceae. It is an annual plant that is herbaceous and fragrant. Summer savory is another name for this herb in the Mediterranean region. It has a robust and spicy flavor but not as strong as winter savory. The delicious leaves well with a wide variety of different cuisines. The taste and the scent of summer savory are comparable to those of the mild herbs known as rosemary and thyme. It is usually served alongside vegetables, lamb, stuffing, and other sauces. Concerning the global summer savory market, the top export country of summer savory is China, with a share value of 22.53 percent. In comparison, Egypt has a stake of 2.69 percent in the export market regarding this crop. The herb has been utilized in treating digestive issues and for its antitussive and antispasmodic properties. The aerial parts have been used medicinally for a long time to prepare herbal tea, which is then used to cure a wide range of conditions, such as cramps, muscle soreness, nausea, indigestion, diarrhea, and infectious infections. The volatile oil can be extracted from savory leaves, and the major constituents of that oil are γ-terpinene and carvacrol. The volatile oil has characteristics that are effective against both bacteria and fungi [8-10].

The pharmaceutical and food industries place a lot of value on the secondary metabolites from medicinal and aromatic plants. Carvacrol, a spicy component, has significant antioxidant activity and has been utilized to improve animal antioxidant status. It acts on proapoptotic pathways in breast, liver, and lung cancer preclinical models. Furthermore, γ-terpinene has antifungal and antioxidant properties that aid in treating diabetes and Alzheimer's disease. It has an herbaceous-citrus scent [11-15].

In this study, the effect of spraying with different doses of chitosan, adding bio-fertilizer EM, and their combination on the savory crop are explored to find the most effective practices for organic farmers to use.

Materials and Methods

During the 2019/2020 and 2020/2021 growing seasons, this research was carried out at a plantation in the Oraby Association, Al-Eubour, which is located at 30˚ 14′ 8.3″ North and 31˚ 31′ 34.1″ East. The farm has earned certification for organic farming practices [16].

The soil physical parameters were sand = 65.50%, silt = 31.00%, clay = 3.50%, and sandy loam texture. The soil chemical properties were pH = 8.61, organic matter = 0.40%, E.C. = 588.80 ppm, HCO_3^- = 0.50 meq/l, Cl^- = 5.50 meq/l, SO_4^{--} = 3.17 meq/l, Ca^{++} = 2.50 meq/l, Mg^{++} = 1.50 meq/l, Na^+ = 4.85 meq/l, and K^+ = 0.32 meq/l. The water used for irrigation had the following chemical parameters pH = 7.20, E.C. = 316.00 ppm, HCO_3^- = 1.46 meq/l, Cl^- = 1.19 meq/l, SO_4^{--} = 2.68 meq/l, Ca^{++} = 3.10 meq/l, Mg^{++} = 1.32 meq/l, Na^+ = 0.82 meq/l, and K^+ = 0.09 meq/l. The Soil and water samples were analyzed according to [17].

The soil was improved with compost manure at a 48 m^3/hectare rate. Seeds of *Satureja hortensis* were sown on October 15th, and the planting took place in a greenhouse. The greenhouse's mean high and low temperatures were 28 and 23 ˚C, respectively, and the relative humidity was 65%. On February 1st, the uniform seedlings with 4-5 pairs of leaves were transplanted into the open field, where they will continue to grow. It was decided to employ drip irrigation. The spaces between rows were 75 cm and 30 cm within hills (44444 plants/hectare). The potassium humate was added at 10 kg per hectare each month by irrigation. The use of biopesticides proved successful in controlling the pest population.

In a split-plot design study, there were three replicates and six treatments. In the main plots, chitosan was sprayed on the plants in three different amounts (0, 5, and 10 liters/hectare). Still, in the subplots, bio-fertilizer EM was utilized (with addition and without addition). Chitosan was purchased from the Chitosan Egypt Company (marketed as Chito Green). The characteristics of this solution were as follows: 15% magnesium, 15% total nitrogen, chitosan, and carboxylic acids at a concentration of 5% each, and pH = 6. The first spraying of chitosan was conducted one month after the transplant, and subsequent sprayings were performed after each cut. It was spraying untreated plants with distilled water. The Egyptian Ministry of the Environment provided the bio-fertilizer EM (each ml includes 0.6 x 10^7 microorganisms). A month after planting, the bio-fertilizer was added, and then again, after harvesting, it was applied to the soil.

The herb was harvested when it was blossoming three times throughout the season: on April 20 (the spring harvest), July 8 (the summer harvest), and October 3 (the autumn harvest). It was cut down ten cm above the soil, but part of the branches was left to encourage new growth. The analysis of variance (ANOVA) statistically experienced all of the data [18], and the least significant difference (LSD) test compared the differences in means at 0.05. When the harvest was in progress, the following data were taken. Plant height in centimeters, weight of fresh herb in grams per square meter, weight of dry herb in grams per square meter, yield of fresh herb in tonnes per hectare, and yield of dry herb in tonnes per hectare are examples of growth and yield characteristics. The aerial parts were rapidly cleansed of any extraneous things that may have been present. The weight of dry herb was determined by drying the sample at 70˚C until it reached a consistent state.

Some quality parameters were detected as volatile oil percentage in the air-dried herb by hydrodistillation using a Clevenger apparatus [19], volatile oil yield per square meter (ml), and the following equation was used to determine this value as follows: oil percentage × herb dry weight per square meter/100, volatile oil yield per hectare (l) and this was estimated as follows: volatile oil yield per square meter × 10000 m^2, and GC-MS analysis of oil was achieved by Gas Chromatography-Mass Spectrometry instrument at the Laboratory of Medicinal and Aromatic Plants, National Research Center, Egypt. The compounds were identified by comparing their retention times to those of authentic samples and by computer matching against commercial and library mass spectra constructed from pure substances [20-24].

Results and Discussion

Growth and yield parameters

The effect of the bio-fertilizer EM on growth characteristics was analyzed and presented in Tables 1-3. Plant height and the weight of fresh and dry herbs per square meter were significantly improved due to using bio-fertilizer. These measurements were 34.72, 45.72, 40.81 cm, 663.33, 1102.22, 938.89 g, 237.78, 386.66, and 338.89 g, respectively, for the first, second, and third cuts. Oppositely, the uninoculated plants showed the lowest metrics in that respect. The data in Tables 4 and 5 demonstrated in what way the bio-fertilizer EM considerably improved yield characteristics. The treatment with biofertilizer produced the best findings in terms of the fresh yield per hectare and dry yield per hectare. Respectively, these measures were 6.63, 11.02, 9.39 tons, 2.38, 3.86, and 3.39 tons for the first, second, and third cuts. The yield from the plants that had not been fertilized was the lowest.

When the amount of chitosan was raised, the plants' height, the fresh herbs' weight, and the dry herbs' weight per square meter got better. The best results came from a concentration of 10 liters per hectare. The measurements for the first, second, and third cuts were 36.00, 46.84, and 42.33 cm; 713.34, 1203.33, and 996.67 g; and 251.67, 428.33, and 361.67 g. In comparison, plants without chitosan had the poorest growth characteristics (Tables 1-3). The yield of fresh and dry herbs per hectare increased as the chitosan concentration increased. The best results were obtained at a rate of 10 liters per hectare. The measures of its first, second, and third cuts were 7.14, 12.03, 9.97 tonnes, 2.52, 4.28, and 3.62 tonnes, respectively. In contrast, plants that had not been sprayed with chitosan exhibited yield characteristics that were the lowest (Tables 4 and 5).

According to Tables (1-3), combining bio-fertilizer with chitosan significantly increased plant development. Plant height and fresh and dry herb weights per square meter showed substantial increases after receiving a spray of chitosan at 10 liters per hectare and being treated with the bio-fertilizer EM. In this case, the first, second, and third cut detections came in at 38.00, 49.00, and 44.50 cm; 776.67, 1333.33, and 1120.00 g; and 276.67, 473.33, and 413.33 g, respectively. In contrast, the estimates were the least in control. According to Tables (4 and 5) and Figure (1), using chitosan in conjunction with bio-fertilizer led to a rise in yield. Chitosan applied as a foliar spray at a concentration of 10 liters per hectare in combination with bio-fertilizer resulted in a considerable increase in both the product of fresh herb per hectare and the yield of dry herb per hectare. Regarding the first, second, and third cuts, the fresh yield statistics were 7.77, 13.33, and 11.20 tonnes, while the dry yield data were 2.77, 4.73, and 4.13 tonnes.

Table 1: Influence of chitosan concentrations, bio-fertilizer EM, and their combination on plant height (cm) (average values of two consecutive seasons)

Treatments / Chitosan	**1st cut**			**2nd cut**			**3rd cut**		
	Bio-fertilizer EM			**Bio-fertilizer EM**			**Bio-fertilizer EM**		
	Without	**With**	**Mean**	**Without**	**With**	**Mean**	**Without**	**With**	**Mean**
0 liter / hectare	27.33	30.00	28.67	39.00	41.17	40.09	34.17	35.83	35.00
5 liter / hectare	32.00	36.17	34.09	43.50	47.00	45.25	37.05	42.11	39.58
10 liter / hectare	34.00	38.00	36.00	44.67	49.00	46.84	40.15	44.50	42.33
Mean	31.11	34.72		42.39	45.72		37.12	40.81	
LSD 5%									
Chitosan	0.44			0.60			0.71		
Bio-fertilizer EM	0.35			0.48			0.58		
Interaction	0.62			0.84			1.00		

Table 2: Influence of chitosan concentrations, bio-fertilizer EM, and their combination on weight of fresh herb per square meter (g) (average values of two consecutive seasons)

Treatments / Chitosan	**1st cut**			**2nd cut**			**3rd cut**		
	Bio-fertilizer EM			**Bio-fertilizer EM**			**Bio-fertilizer EM**		
	Without	**With**	**Mean**	**Without**	**With**	**Mean**	**Without**	**With**	**Mean**
0 liter / hectare	413.33	513.33	463.33	736.67	803.33	770.00	656.67 ^{f}	750.00	703.34
5 liter / hectare	580.00	700.00	640.00	920.00	1170.00	1045.00	800.00 ^{g}	946.67	873.34
10 liter / hectare	650.00	776.67	713.34	1073.33	1333.33	1203.33	873.33 ^{h}	1120.00	996.67
Mean	547.78	663.33		910.00	1102.22		776.67 ^{d}	938.89	
LSD 5%									
Chitosan	13.10			39.34			42.97		
Bio-fertilizer EM	10.69			32.12			35.09		
Interaction	18.52			55.63			60.77		

Table 3: Influence of chitosan concentrations, bio-fertilizer EM, and their combination on weight of dry herb per square meter (g) (average values of two consecutive seasons)

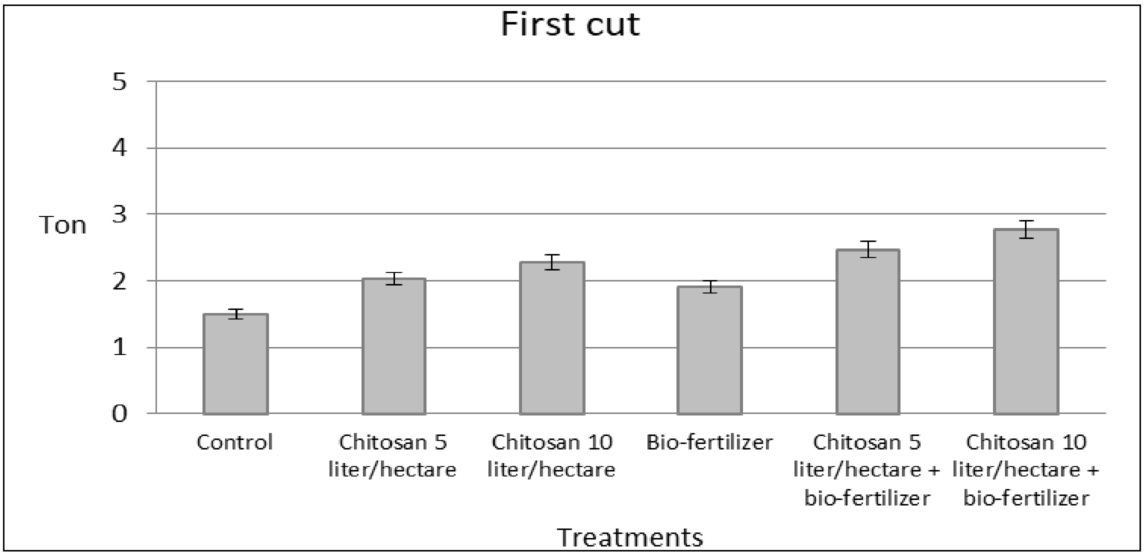
Treatments / Chitosan	**1st cut**			**2nd cut**			**3rd cut**		
	Bio-fertilizer EM			**Bio-fertilizer EM**			**Bio-fertilizer EM**		
	Without	**With**	**Mean**	**Without**	**With**	**Mean**	**Without**	**With**	**Mean**
0 liter / hectare	150.00	190.00	170.00	230.00	283.33	256.67	226.67	263.33	245.00
5 liter / hectare	203.33	246.67	225.00	326.67	403.33	365.00	283.33	340.00	311.67
10 liter / hectare	226.67	276.67	251.67	383.33	473.33	428.33	310.00	413.33	361.67
Mean	193.33	237.78		313.33	386.66		273.33	338.89	
LSD 5%									
Chitosan	6.63			27.10			5.55		
Bio-fertilizer EM	5.42			22.13			4.53		
Interaction	9.38			38.32			7.85		

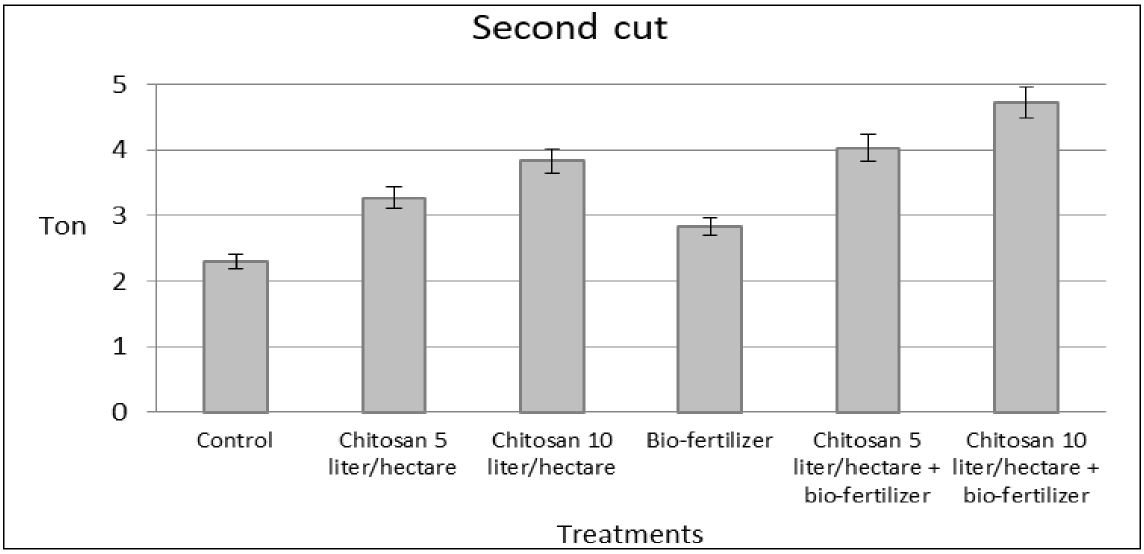
Table 4: Influence of chitosan concentrations, bio-fertilizer EM, and their combination on yield of fresh herb per hectare (ton) (average values of two consecutive seasons)

Treatments / Chitosan	1st cut			2nd cut			3rd cut		
	Bio-fertilizer EM			Bio-fertilizer EM			Bio-fertilizer EM		
	Without	**With**	**Mean**	**Without**	**With**	**Mean**	**Without**	**With**	**Mean**
0 liter / hectare	4.13	5.13	4.63	7.37	8.03	7.70	6.57	7.50	7.04
5 liter / hectare	5.80	7.00	6.40	9.20	11.70	10.45	8.00	9.47	8.74
10 liter / hectare	6.50	7.77	7.14	10.73	13.33	12.03	8.73	11.20	9.97
Mean	5.48	6.63		9.10	11.02		7.77	9.39	
LSD 5%									
Chitosan	0.13			0.39			0.43		
Bio-fertilizer EM	0.11			0.32			0.35		
Interaction	0.19			0.56			0.61		

Table 5: Influence of chitosan concentrations, bio-fertilizer EM, and their combination on yield of dry herb per hectare (ton) (average values of two consecutive seasons)

Treatments / Chitosan	1st cut			2nd cut			3rd cut		
	Bio-fertilizer EM			Bio-fertilizer EM			Bio-fertilizer EM		
	Without	**With**	**Mean**	**Without**	**With**	**Mean**	**Without**	**With**	**Mean**
0 liter / hectare	1.50	1.90	1.70	2.30	2.83	2.57	2.27	2.63	2.45
5 liter / hectare	2.03	2.47	2.25	3.27	4.03	3.65	2.83	3.40	3.12
10 liter / hectare	2.27	2.77	2.52	3.83	4.73	4.28	3.10	4.13	3.62
Mean	1.93	2.38		3.13	3.86		2.73	3.39	
LSD 5%									
Chitosan	0.07			0.27			0.06		
Bio-fertilizer EM	0.06			0.23			0.05		
Interaction	0.09			0.38			0.08		





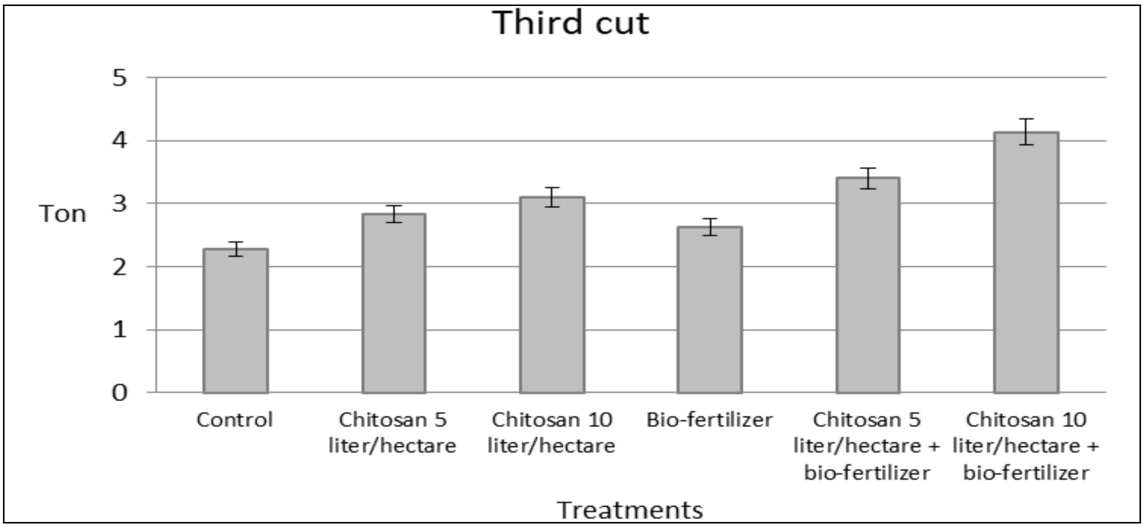


Fig 1: Effect of combination between treatments on dry yield per hectare

Quality parameters

According to the findings in Tables (6-8), bio-fertilizer considerably enhanced quality metrics such as the percentage of volatile oil, the amount of oil produced per square meter, and the total area. These records for the sequence's first, second, and third harvests were 1.42, 1.71, and 1.91%; 3.40, 6.66, and 6.58 ml; 33.97, 66.60, and 65.77 l, respectively. In contrast, the control treatment group had the least detections.

Additionally, the volatile oil percentage, oil production per square meter, and hectare significantly increased with maximal chitosan concentrations; 10 liters/hectare constituted the highest values possible. The first, second, and third cut values were 1.45, 1.74, and 1.97%; 3.65, 7.45, and 7.18 ml; 36.45, 74.50, and 71.80 l. Conversely, plants that had yet to be treated with chitosan displayed only minimally desirable characteristics (Tables 6-8).

Moreover, the combination of chitosan and bio-fertilizer improved flavor (Tables 6-8 and Figure 2). Increases in volatile oil percentage, oil output per square meter, and hectare were observed when chitosan was applied at a level of 10 liters per hectare in conjunction with bio-fertilizer. The first, second, and third cuts had the following results: 1.49, 1.77, and 2.11%; 4.12, 8.38, and 8.72 ml; 41.20, 83.80, and 87.20 l. On the other hand, the quality was deemed the poorest in control.

The primary components of the volatile oil responsible for the spicy flavor and health benefits, according to the data in Table 9, were γ-terpinene and carvacrol. The treatments affected the levels of γ-terpinene and carvacrol. The highest concentrations of γ-terpinene and carvacrol were found in oils from plants treated by chitosan at a level of 10 liters and bio-fertilizer. In that order, these contents were 35.11 and 36.33%; 33.00, 36.40, and 43.67% for the first, second, and third cuts, respectively. The control oil contained the least amount of γ-terpinene and carvacrol overall. According to [25], carvacrol and γ-terpinene are the major components of summer savory volatile oil, which agrees with the oil's chemical composition. Additionally, it meets the ISO requirements [26].

Table 6: Influence of chitosan concentrations, bio-fertilizer EM, and their combination on volatile oil percentage (average values of two consecutive seasons)

Treatments / Chitosan	**1st cut**			**2nd cut**			**3rd cut**		
	Bio-fertilizer EM			**Bio-fertilizer EM**			**Bio-fertilizer EM**		
	Without	With	Mean	Without	With	Mean	Without	With	Mean
0 liter / hectare	1.25	1.31	1.28	1.60	1.63	1.62	1.70	1.74	1.72
5 liter / hectare	1.35	1.45	1.40	1.66	1.73	1.70	1.77	1.89	1.83
10 liter / hectare	1.40	1.49	1.45	1.70	1.77	1.74	1.82	2.11	1.97
Mean	1.33	1.42		1.65	1.71		1.76	1.91	
LSD 5%									
Chitosan	0.01			0.01			0.01		
Bio-fertilizer EM	0.01			0.01			0.01		
Interaction	0.01			0.02			0.01		

Table 7: Influence of chitosan concentrations, bio-fertilizer EM, and their combination on volatile oil yield per square meter (ml) (average values of two consecutive seasons)

Treatments / Chitosan	**1st cut**			**2nd cut**			**3rd cut**		
	Bio-fertilizer EM			**Bio-fertilizer EM**			**Bio-fertilizer EM**		
	Without	With	Mean	Without	With	Mean	Without	With	Mean
0 liter / hectare	1.88	2.49	2.19	3.68	4.62	4.15	3.85	4.58	4.22
5 liter / hectare	2.75	3.58	3.17	5.42	6.98	6.20	5.01	6.43	5.72
10 liter / hectare	3.17	4.12	3.65	6.52	8.38	7.45	5.64	8.72	7.18
Mean	2.60	3.40		5.21	6.66		4.83	6.58	
LSD 5%									
Chitosan	0.09			0.45			0.10		
Bio-fertilizer EM	0.07			0.37			0.08		
Interaction	0.13			0.63			0.14		

Table 8: Influence of chitosan concentrations, bio-fertilizer EM, and their combination on volatile oil yield per hectare (l) (average values of two consecutive seasons)

Treatments / Chitosan	1st cut			2nd cut			3rd cut		
	Bio-fertilizer EM			Bio-fertilizer EM			Bio-fertilizer EM		
	Without	With	Mean	Without	With	Mean	Without	With	Mean
0 liter / hectare	18.80	24.90	21.85	36.80	46.20	41.50	38.50	45.80	42.15
5 liter / hectare	27.50	35.80	31.65	54.20	69.80	62.00	50.10	64.30	57.20
10 liter / hectare	31.70	41.20	36.45	65.20	83.80	74.50	56.40	87.20	71.80
Mean	26.00	33.97		52.07	66.60		48.33	65.77	
LSD 5%									
Chitosan	0.91			4.45			0.95		
Bio-fertilizer EM	0.74			3.63			0.77		
Interaction	1.27			6.29			1.35		

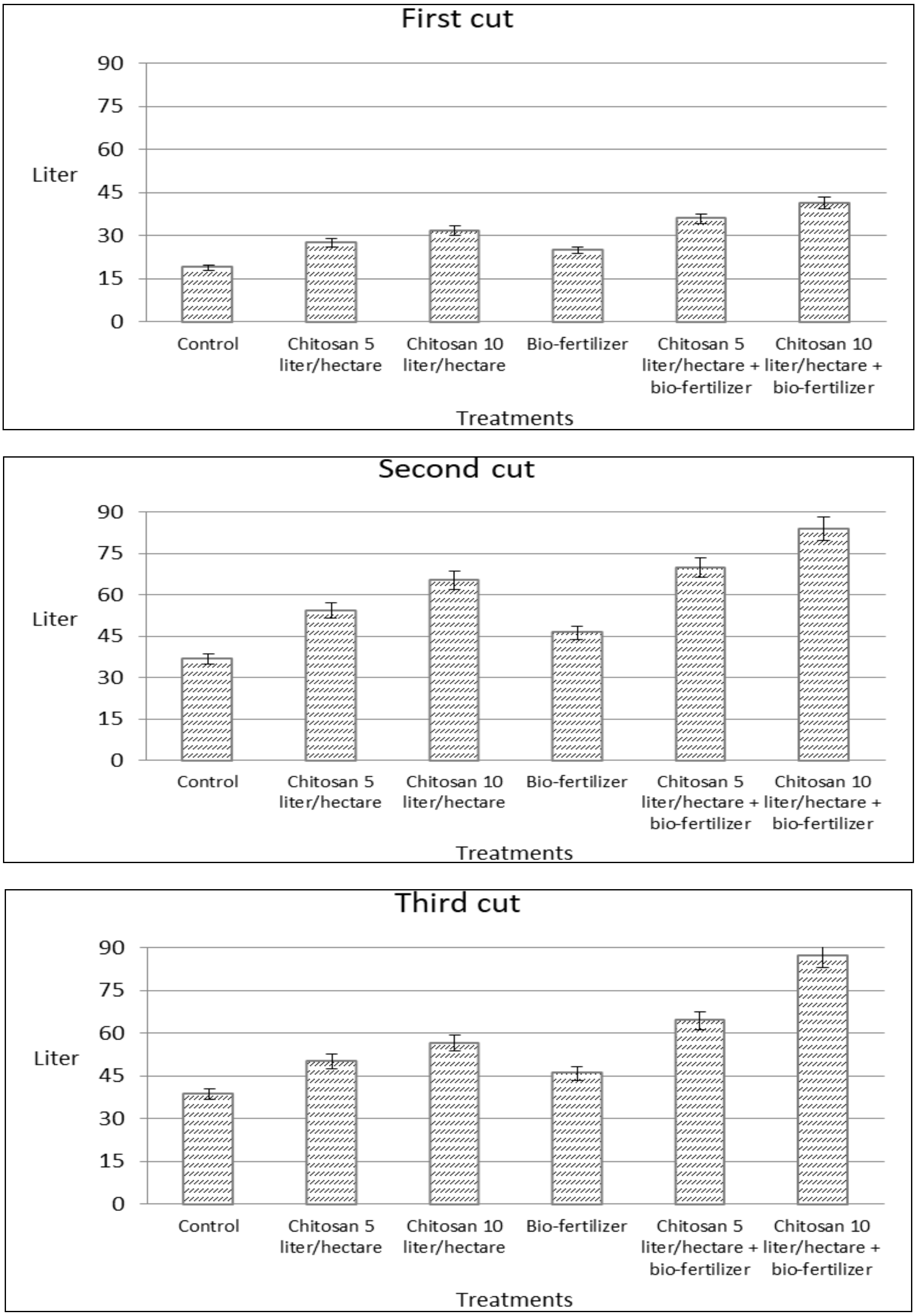


Fig 2: Data interaction's impact on volatile oil yield per hectare

Table 9: Effect of treatment combinations on volatile oil constituents

R.T.	Compound	Spring harvest		Summer harvest		Autumn harvest	
		Control	Chitosan 10 liter/hectare + bio-fertilizer EM	Control	Chitosan 10 liter/hectare + bio-fertilizer EM	Control	Chitosan 10 liter/hectare + bio-fertilizer EM
3.35	Thujene	3.99	4.22	2.86	2.50	2.26	2.53
3.47	α-pinene	2.14	2.16	3.13	2.39	1.45	1.51
3.76	Camphene	0.20	0.22	0.19	0.15	0.11	0.11
4.11	Sabinene	0.10	0.93	0.52	0.43	0.37	0.33
4.21	β-pinene	0.67	0.65	1.67	1.21	0.64	0.63
4.34	α-myrcene	4.62	4.59	3.38	2.71	2.20	2.25
4.88	α-terpinene	8.80	8.84	7.67	6.45	5.50	5.91
5.00	l-phellandrene	1.30	1.19	0.74	0.46	0.52	0.30
5.08	p-cymene	6.66	6.62	9.78	8.65	5.87	4.41
5.41	cis-ocimene	0.30	0.22	0.17	0.13	0.11	0.11
5.69	γ-terpinene	35.09	35.11	32.65	36.33	37.71	36.38
6.05	Trans-sabinine hydrate	0.18	0.05	0.18	0.16	0.12	0.10
6.20	p-mentha-1,4(8)-diene	0.10	0.05	0.14	0.10	0.06	0.08
6.72	Cis-sabinine hydrate	0.10	0.11	0.13	0.14	0.10	0.08
8.24	Endo-borneol	-	-	0.16	0.12	0.07	0.06
8.33	4-terpineol	0.20	0.10	0.50	0.37	0.28	0.22
8.73	Fenchyl alcohol	-	-	0.15	0.09	0.07	0.08
9.43	Carvacrol methyl ester	-	-	0.19	0.13	0.04	0.05
10.62	4-terpinenyl acetate	-	-	0.06	0.05	0.04	0.03
10.85	Thymol	0.14	0.11	0.13	0.10	0.10	0.10
11.04	Carvacrol	32.80	33.00	34.44	36.40	41.62	43.67
12.09	Carvacryl acetae	-	-	0.52	0.30	0.17	0.34
12.96	Trans-caryophyllene	0.10	0.17	0.39	0.27	0.24	0.32
13.32	Aromandendrene	-	-	0.02	0.04	0.07	0.04
14.32	Ledene	0.22	0.17	0.03	0.03	-	0.04
14.62	α-bisabolene	1.44	1.46	0.10	0.08	-	0.12
16.53	(-)-Caryophyllene oxide	-	-	-	-	0.02	-
23.42	p-Cymen-7-ol	-	-	-	-	0.02	-
Total identified components		99.15	99.97	99.90	99.79	99.76	99.80
Total hydrocarbon compounds		65.73	66.6	63.44	61.93	57.11	55.07
Total oxygenated compounds		33.42	33.37	36.46	37.86	42.65	44.73

R.T. = Retention time

The positive effect bio-fertilizer EM has on plants can be attributed to several factors. It possesses naturally beneficial bacteria that are capable of performing a variety of functions. They have the potential to increase the value of biological effects. They enhance root formation and the mechanism of nitrogen fixation and raise the total quantity of dissolved phosphorus in the soil. Additionally, they produce hormones and antioxidants. Their strains generate carbon dioxide (CO_2), which lowers the pH of the soil; they also promote herb immunity against pathogens; they raise the plant's ability to take in nutrients, which contributes to an increase in photosynthesis [7]. These results are in accord with the research of [27] on gladiolus, [28] on marjoram and peppermint, [29] on sweet violet, [30] on chicory, and [31] on peppermint, who mention that applied bio-fertilizer EM can raise production.

The high levels of gibberellin, auxin, and cytokinin found in chitosan are likely responsible for the remarkable outcomes of spraying high chitosan concentrations. It is a rich source of nutrients and amino acids. Chitosan strengthens the plant's resistance to pathogens like fungi and bacteria that can cause plant infection. It does this by stimulating the plant to produce many enzymes connected with the defense reaction. The treatment with chitosan results in an increase in proline levels, increasing the plant's ability to endure abiotic stressors. Last but not least, it works to promote vegetative development as well as yield and active components [4]. The usage of chitosan improved the yield and quality of crops under abiotic stresses, according to studies by [32] on basil, [33] on marjoram, [34] on stevia, [35] on rosemary, [36] on summer savory, [37] on German chamomile, [38] on fennel, and [39] on hyssop.

Conclusion

This organic agricultural program allowed us to harvest the highest summer savory yield, leading us to this conclusion. Chitosan should be sprayed onto the plants (Chito Green) at 10 liters per hectare, and the plants should be inoculated with bio-fertilizer EM. After 30 days of transplanting, we must perform this technique and do it again after every crop harvest.

References

1. Chandran S, Unni MR, Thomas S. Organic Farming: Global Perspectives and Methods. Woodhead Publishing; c2018. p. 1-13.
2. Batini N, Black S, Luca O, Parry I. A Comprehensive Greenhouse Gas Mitigation Strategy for the Netherlands. International Monetary Fund; c2021. p. 31.
3. Sangeetha J, Soytong K, Thangadurai D, Al-Tawaha ARM. Organic Farming for Sustainable Development. CRC Press; c2022. p. 27-31.
4. Rouphael Y, Jardin Pdu, Brown P, Pascale SDe, Colla G. Biostimulants for Sustainable Crop Production. Burleigh Dodds Science Publishing Limited; c2020. p. 1-20.
5. Gupta S, Staden GV. Biostimulants for Crops from Seed Germination to Plant Development: A Practical Approach. Academic Press; c2021. p. 1-3.
6. Ahmed S, Ikram S. Chitosan: Derivatives, Composites and Applications. Wiley; c2017. p. 1-10.
7. Khan BM. Environmental Biology. Lap Lambert Academic Publishing GmbH KG; c2012. p. 160.
8. Arrowsmith N. Essential Herbal Wisdom: A Complete Exploration of 50 Remarkable Herbs. Llewellyn

Worldwide, USA; c2009. p. 229-232.
9. Kutchin AV, Shishkina LN, Weisfeld LI. Chemistry and Technology of Plant Substances: Chemical and Biochemical Aspects. CRC Press; c2017. p. 213-214.
10. Tridge. https://www.tridge.com/intelligences/summer-savory.
11. Badr SEA, Sakr DM, Mahfouz SA, Abdelfattah MS. Licorice (*Glycyrrhiza glabra* L.): Chemical composition and biological impacts. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2013;4(3):606-21.
12. Wakuma Biratu, Derbew Belew, Edossa Ettissa. Evaluation of hot pepper (*Capsicum annuum* L.) cultivars for growth and dry pod yields against different blended fertilizer and nitrogen rates in raya Azebo, Southern Tigray. Int. J Res. Agron. 2021;4(2):15-22.
13. Sharifi-Rad M, Varoni EM, Iriti M, Martorell M, Setzer WN, Del Mar Contreras M, *et al*. Carvacrol and human health: A comprehensive review. Phytotherapy Research. 2018;32(9):1675-1687.
14. Bektašević M, Politeo O. Biological Application of Essential Oils and Essential Oils Components in Terms of Antioxidant Activity and Inhibition of Cholinesterase Enzymes. Advances in Extractions and Biological Applications; de Oliveira, M.A., de Aguiar Andrade, E.E., Eds.; IntechOpen: London, UK; c2022. p. 114-115.
15. TGSC Information System. http://www.thegoodscentscompany.com/data/rw1017031.html.
16. Agricert Organic Standard. http://www.agricert.pt
17. AOAC. Association of Official Analytical Chemists. Official Methods of Analysis. AOAC International, Washington, DC, 22nd ed., 2022.
18. Snedecor GW, Cochran WG. Statistical Methods. The Iowa State Univ. Press, Ames, Iowa, USA, 7th ed., 1982.
19. British Pharmacopoeia. Determination of Volatile Oil in Drugs. The Pharmaceutical Press; c1963. p. 112-125.
20. Stenhagen E, Abrahamsson S, McLafferty FW. Registry of Mass Spectral Data. 12th ed., Wiley & Sons; c1974, 4.
21. Massada Y. Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry. Illustrated, ed. Wiley & Sons; c1976. p. 251-255.
22. Jennings W, Shibamoto T. Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatograph. Academic Press, 1st ed; c1980.
23. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute, 29th ed; c2022.
24. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy, 4th ed., Allured Publishing Corporation, Carol Stream, USA, IL; c2007.
25. Babushok VI, Linstrom PJ, Zenkevich IG. Retention indices for frequently reported compounds of plant essential oils. Journal of Physical and Chemical Reference Data. 2011;40(4):043101-47.
26. ISO 7928-2. https://cdn.standards.iteh.ai/samples/14897/327c5b31e48443d3a087acb7fc9a7edd/ISO-7928-2-1991.pdf
27. Hassanein MM, El-Sayed SG. Effect of some organic and bio-fertilization treatments on gladiolus plants: 1-Vegetative growth and flowering. Journal of Plant Production. 2009;34(6):6567-6575.
28. Massoud GFA. Effect of Different Sources of Fertilizers on the Productivity of some Medicinal Plants. Ph.D. Thesis, Faculty of Science, Mansour University, Egypt; c2012.
29. Mohamed YFY., Ghatas YAA. Effect of mineral, biofertilizer (EM) and zeolite on growth, flowering, yield and composition of volatile oil of *Viola odorata* L. plants. Journal of Horticultural Science & Ornamental Plants. 2016;8(3):140-148.
30. Rezaienia N, Ramroudi M, Galavi M, Forouzandeh M. Study of agronomical characteristics, flower yield and root inulin percentage of chicory (*Chicorium intybus* L.) under soil fertilizers and drought stress. Journal of Plant Production Science. 2018;24(4):129-140.
31. Al-Amri SM. Response of growth, essential oil composition, endogenous hormones and microbial activity of *Mentha piperita* to some organic and biofertilizers agents. Saudi Journal of Biological Sciences. 2021;28(10):5435-5441.
32. Malekpoor F, Pirbalouti AG, Salimi A. Effect of foliar application of chitosan on morphological and physiological characteristics of basil under reduced irrigation. Crop Research. 2016;17(2):354-359.
33. El-Khateeb MA, El-Attar AB, Nour RM. Application of plant biostimulants to improve the biological responses and essential oil production of marjoram (*Majorana hortensis*, Moench) plants. Middle East Journal of Agriculture Research. 2017;6(4):928-941.
34. Mehregan M, Mehrafarin A, Labbafi MR, Naghdi BH. Effect of different concentrations of chitosan biostimulant on biochemical and morphophysiological traits of stevia plant (*Stevia rebaudiana* Bertoni). Journal of Medicinal Plants. 2017;16(62):169-181.
35. Waly AA, El-Fattah A, Hassan MAE, El-Ghadban EAE, Abd Alla AS. Effect of foliar spraying with seaweeds extract, chitosan and potassium silicate on *Rosmarinus officinalis* L. plants in sandy soil. Scientific Journal of Flowers and Ornamental Plants. 2019;6(3):191-209.
36. Alizadeh A, Moghaddam M, Asgharzade A, Sourestani MM. Phytochemical and physiological response of *Satureja hortensis* L. to different irrigation regimes and chitosan application. Industrial Crops and Products. 2020;158:112990.
37. Abdul-Hafeez EY, Ibrahim OHM. Effects of chitosan and BABA foliar application on flowering and chemical characteristics of German chamomile 'Bode-gold'. South African Journal of Botany. 2021;139:241-245.
38. El-Serafy RS, El-Sheshtawy ANA, Dahab AA, Al-Ashkar I. Can yeast extract and chitosan-oligosaccharide improve fruit yield and modify the pharmaceutical active ingredients of organic fennel?. Industrial Crops and Products. 2021;173:114130.
39. Samany SMA, Pirbalouti AG, Malekpoor F. Phytochemical and morpho-physiological changes of hyssop in response to chitosan-spraying under different levels of irrigation. Industrial Crops and Products. 2022;176:114330.
40. Farag MA, Abdelfattah MS, Badr SEA, Wessjohann LA. Profiling the chemical content of *Ficus lyrata* extracts via UPLC-PDA-qTOF-MS and chemometrics. Natural Product Research. 2014;28(19):1549-56.