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Changes in the essential oil of *Lavandula officinalis* over boron application and harvest dates along with the response of oil to nanotechnology against *Sitotroga cerealella*

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Abstract

Environmental, agronomic and climatic conditions influence the growth and quality of plants. *Lavandula officinalis* is cultivated for its essential oil production. This study was conducted to investigate the effect of foliar application of boron (0, 2.5 and 5 mg B L^{-1}) and date of harvest (1st of July, 1st of August and 1st of September) on the production of *L. officinalis* plants and its essential oil as well as comparing the effect of the essential oil at different harvest dates and its nanoform against *Sitotroga cerealella.* Application of boron at 5 mg L^{-1} gave the highest increase in vegetative growth and essential oil yield in plants harvested in September. Eucalyptol (18.46–31.14%) was found to be the major compound of lavender oil, followed by (+)-2-Bornanone (21.30-24.41%), then α-pinene (12.76-14.99%) and β-pinene (11.86-13.59%). Eucalyptol and (+)-2-Bornanone increased when the plant age increased and reached their highest relative contents in the plants harvested in September and sprayed with boron at 0, 2.5 and 5 mg L^{-1}. Both α-Pinene and β-Pinene reached the maximum values in the plants harvested in September and sprayed with 5 and 2.5 mg B L^{-1}, respectively. Using nanoformof the essential oil improved the efficiency of lavender oil at different harvest dates against *S. cerealella,* and it was more effective than their normal formulations. Treatments with higher concentrations of nano or normal formulations resulted in higher percentage of mortality and reduction of adult emergence. The results can to be used for the improvement of *L. officinalis* production under drip irrigation system in arid and semi-arid conditions.

Keywords: Lavender, boron, cuts dates, volatile oil, natural insecticide, oil nanoform, Angoumois grain moth

Introduction

Lavandula officinalis L. belongs to Family Lamiaceae and commonly known as lavender. The essential oil of lavender is used in pharmacology, biological activities, cosmetics and the perfumes industry [1]. Its essential oil possesses antimutagenic, anticancer and insecticidal activities [2,3]. Anti-mutagenic activity has been associated with linalool antioxidant properties [4]. Various *L. officinalis* extracts reduced anxiety and depression, which are associated with Alzheimer's disease, and improved scopolamine-induced memory impairment [5,6]. Different monoterpenes, such as α-pinene, β-pinene, eucalyptol, linalool, α-terpineol and terpinen-4-ol exhibit anti-inflammatory activity, antiviral effect against influenza virus strains, antifungal activity [7,8] and antimicrobial activities [9,10]. Borneol and 1,8 cineole also showed anti-parasitic effect [11].

Depending on cultivation area and plant genotype, lavender oil's quantitative composition varies. Moreover,1,8 cineole and bisabolol changed in a contrary trend with seasonal variations [12]. The difference in genotype, development periods, ecological conditions, age, season, organ and extraction method may be the cause of the terpenoid chemical composition variations [13].

Harvest time, ecological and climatic conditions might impact the herb yield, volatile oil content and quantitative constituents of some medicinal and aromatic plants. Also, the content and composition of secondary metabolites of lavender species essential oil could be influenced by harvest time [14]. Terpenes concentration in aromatic plants varies during the season and day. Plants cultivated in semi-arid or arid conditions are frequently subjected to inadequate soil fertility and display numerous nutrition deficiencies as a result of the alkaline

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calcareous nature and the low organic matter that inhibit the productivity of crop [15]. The requirements for boron vary greatly between plant species and even between genotypes within the same species. As a result, boron levels in the soil that are insufficient for one crop may be toxic for another [16]. Boron is an essential nutritional element [17] and plays a crucial role in oxidative stress enzymes alteration [18] and in many metabolic reactions in plants grown in arid or semi-arid regions which display various nutritional deficiencies.

Because of stored products pests, stored product grains remain vulnerable to qualitative or quantitative damages with up to 88% germination loss and 29% weight loss [19]. In tropical and temperate regions, one of the field-to-store lepidopteran rice pests is the Angoumois grain moth (*Sitotroga cerealella*). If the control is neglected, one gravid female moth can devour a total of 50 grams of rice over the course of next three generations [20]. Although pesticides have enhanced agricultural production, they have also been shown to be worldwide pollutants [21]. In grain reserves, using antifeedants, natural deterrents and biopesticides may be preferable than using chemicals [22]. Plant secondary metabolites with an insecticidal nature can be utilized to control insect pests in addition to the traditional plant extracts that are used as pesticides. Nanotechnology can be used to enhance these biomolecules' efficiency. Delivery of crop protection chemicals could be enhanced by nanotechnology. Nanoencapsulation of pesticides improved shelf life and efficiency, dispersal, control releasing and protection from environmental conditions [23].

The purpose of this research was to study the impact of boron foliar application and harvest date on growth, yield and volatile oil constituents of *Lavandula officinalis* plants grown under newly reclaimed soil conditions as well as the biological efficacy of the essential oil along with its nanoform of lavender oil against *Sitotroga cerealella*.

Materials and Methods

The two-year field experiment was carried out at the Agricultural Experimental Station of the National Research Centre at Nubaria district, Beheira Governorate west of the Nile Delta, Egypt, under a drip irrigation system during the 2019 and 2020 seasons to evaluate the impact of three boron foliar treatments (0, 2.5 and 5 mg B L^{-1}) and three harvest dates (1st of July, 1st of August and 1st of September) on the production and essential oil of *L. officinalis* as well as the insecticidal effect of lavender essential oil and its nanoform against *S. cerealella*.

The soil was prepared two weeks before transplantation and divided into lines (75 cm in between). The physical and chemical properties of the used soil as shown in Table (1) were determined according to Jackson [24] at the special unit of Soils and Water Use Department, National Research Centre, Dokki, Giza, Egypt, from which it could be observed that the soil is sandy in texture. The normal agricultural practices for lavender cultivation were carried out as recommended. Plants were irrigated with a drip irrigation system (4 L $hour^{-1}$).

Cuttings of *L. officinalis* were obtained from Horticulture Research Institute, Agricultural Research Center, Egypt and were transplanted in the permanent field and adjusted to drippers (60 cm in between) on 5th and 3rd March 2019 and 2020, respectively. The metrological data of the experimental farm region during the growing periods are presented in Table (2) according to the data available on an international website of specialized in the metrological data (www.timeanddate.com).

The experiment was factorial in split plot design with 9 treatments and three replicates for each treatment; the main plots contain three levels of boric acid as foliar application (0, 2.5 and 5 mg B L^{-1}) and the subplots consist of three different harvest dates (1st of July, 1st of August and 1st of September). Application of boric acid treatments was performed twice; 40 days from transplantation and 40 days later in both seasons.

Plant height and fresh and dry weights of herb (g $plant^{-1}$and ton ha^{-1}) as well as essential oil percentage (%) and yield (ml $plant^{-1}$ and L ha^{-1}) were recorded at the three different harvest dates. Essential oil extraction was carried out by hydro-distillation using Clevenger-type apparatus according to Egyptian Pharmacopoeia [25]. The resulted essential oil was separately dried over anhydrous sodium sulfate and kept in the refrigerator till be used. To identify the main constituents and to determine their relative percentages, the essential oils were separately subjected for GC-MS analysis using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Centre following the conditions mentioned by Omer *et al*. [26].

Total soluble phenol (mg g^{-1} dry herb) was determined in dried herb according to Singleton *et al*. [27]. Antioxidant activity of dried herb (%) was determined depending on the ability of the extract to scavenge DPPH free radicals according to Tekao *et al*. [28] and the suitable modifications of Kumarasamy*et al*. [29].

The recorded data were analyzed as split plot design by analysis of variance (ANOVA) using the General Linear Models procedure of CoStat [30]. Least significant difference (LSD) test was applied at 0.05 probability level to compare the means of the treatments.

Table 1. The physical and chemical properties of the experimental soil during 2019 and 2020 seasons

Physical properties							
	Very coarse sand (2-1 mm)	Coarse sand (1-0.5 mm)	Medium sand (0.5-0.25 mm)	Fine sand (0.25-0.1 mm)	Very fine sand (0.1-0.05 mm)	Silt + Clay (0.5> mm)	Texture
2019	13.83	53.91	2.35	18.08	9.75	2.08	Sandy
2020	11.92	57.15	1.97	19.12	8.03	1.81	Sandy

Chemical properties										
	pH	E.C. (dSm^{-1})	Cations (meq l^{-1})				Anions (meq l^{-1})			
	(2.5:1)	(1:1)	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	So_4^{--}
2019	8.04	0.96	3.5	0.9	4.62	0.6	--	1.9	6.1	1.62
2020	8.07	1.23	4.5	0.75	6.38	0.75	--	3.0	7.4	1.98

Table 2. Monthly average of metrological data of the experimental area during 2019 and 2020 seasons

2019 season					2020 season				
Month	Air temperature ºC			R.H.	Month	Air temperature ºC			R.H.
	Max.	Min.	Aver.	%		Max.	Min.	Aver.	%
Mar. 2019	24	8	16	70	Mar. 2020	29	9	17	68
Apr. 2019	34	9	18	65	Apr. 2020	31	12	19	67
May 2019	44	13	23	61	May 2020	41	14	23	61
Jun. 2019	33	20	27	68	Jun. 2020	41	15	25	65
Jul. 2019	38	22	28	66	Jul. 2020	32	23	27	71
Aug. 2019	34	21	28	69	Aug. 2020	33	21	28	70
Sept. 2019	32	19	27	65	Sept. 2020	35	22	28	69

R.H.: Relative humidity

Preparation and characterization of nanoforms

Here, we aimed to compare the nano and bulk oil and their effect on *S. cerealella*, depending on the harvest dates only while boron levels were excluded. So, in each harvest date we chose the oil of the plants received boron at 0 mg L^{-1} to be used.

Oil in water (o/w) nanoemulsions were prepared using the miniemulsion polymerization method modified version of the method described by Zhang *et al.* [31]. Firstly, essential oil was emulsified in distilled water 1:1 (v/v) using Tween 80 by stirring for 10 minutes. The emulsion was dropsied in poly ethylene glycol 3% solution in a ratio of 1:1 (v/v) with continuous mechanical stirring. The emulsion was sonicated using ultrasonic cleaner set, modelWUC-DO3H 290W and 60 Hz for 60 min, and then was sonicated using a high energy ultra-sonication probe (model VCX750, 750W, 20 kHz) for 3min.The loaded nano-capsule suspension was equilibrated overnight. Nanoemulsions were obtained as dispersion in aqueous solution [32].

The morphological shape characterization of prepared nanoformulation was carried out using Transmission Electron Microscopy (TEM) (Jeol, JEM-2100). The nanocapsule suspension was diluted with distilled water and deposited onto a carbon-coated copper grid.

Bioassay tests

Bioassay tests were carried out with *S. cerealella* eggs. Wheat grain was sterilized at 60ºC for 4 hours. Samples of 20 g wheat grain were placed in plastic cups of 120 ml in capacity. Series concentrations of 100, 200, 400 and 800 ppm were prepared from each lavender formulation. Each concentration was added to a piece of cotton and transferred in each plastic cup. Ten eggs of *S. cerealella* were then transferred to each cup with three replicates, of 10 eggs/replicate. Those cups were incubated at 25±2ºC and 65-70% RH for 35 days, then containers were sieved out separately and newly emerged adults moth were totalled and documented as described by Odeyemi and Daramola [33].

Mortality of hatched larvae was evaluated.

$$\text{Corrected mortality }\% = \frac{\text{Observed mortality} - \text{Control mortality}}{100 - \text{Observed mortality}} \text{ x } 100$$

$$\%\text{ Adult emergence} = \frac{\text{Total number of adult emergence}}{\text{Total number of eggs}} \text{ x } 100$$

$$\text{Reduction \% in F1} - \text{progeny} = \frac{\text{No. of adult emerged in control} - \text{No. of adult emerged in treatment}}{\text{No. of adult emerged in control}} \text{x100}$$

Statistical analysis

All insecticidal data were subjected to analysis of variance (ANOVA), and means were evaluated using the new Duncan's multiple range test (*P*> 0.05). Log-probit model analysis was carried out on the percentage mortality of moth results to evaluate 50% (LC_{50}) and 90% (LC_{90}) lethal concentrations.

Toxicity index and Relative potency were calculated as followed:

$$\text{Toxicity Index} = \frac{\text{LC50 value of the highest effeciency formulation}}{\text{LC50 value of the other formulation}} x100$$

$$\text{Relative potency} = \frac{\text{LC50 value of the least effeciency formulation}}{\text{LC50 value of the other formulation}}$$

Results and discussion

Data presented in Table (3) showed that foliar application of boron and harvest date had affected significantly plant height, fresh and dry weights of herb (g plant^{-1} and ton ha^{-1}) in both seasons. Increasing the concentration of boron from 0 to 2.5 and 5 ppm increased the height, fresh and dry weight and yield (g plant^{-1} and ton ha^{-1}) of lavender plants. Concerning harvest date, plant height, fresh and dry weights of herb gradually increased with plant age increasing. In the first season, the maximum fresh weight (892.1 g plant^{-1} and 19.8 ton ha^{-1}) and dry weight (267.4g plant^{-1} and 5.9 ton ha^{-1}) were found in September (at the third harvest date). It might be concluded that boron foliar spraying at 5 ppm gave the greatest height, fresh and dry weight and yield (g plant^{-1} and ton ha^{-1}) of plants harvested in September in both seasons. The average temperatures in the experimental area were 27 and 28°C in the first and second seasons, respectively.

Long days and high temperature increase photosynthetic carbon and the application of B stimulates photosynthesis and chlorophyll production which ultimately results in an increase in the dry weight and a higher yield [34]. These findings agree with those obtained by Aziz*et al*. [35] who stated that the greatest values of *Achillea millefolium* flowering heads fresh and dry weights were obtained at the third harvest.

Essential oil content

Boron treatments and harvest date had a significant impact on the essential oil percentage (%) and yield (ml plant^{-1} and L ha^{-1}) (Table 4). Foliar application of boron increased the essential oil percentage and yield of lavender oil as compared with the untreated plants. The highest oil yield (175.1 and 167.2 L ha^{-1}in the first and second seasons, respectively) was obtained with the application of boron at 5 ppm and there was no significant difference between 2.5 and 5 ppm boron.

Concerning harvest date, the highest essential oil yield (223.3 and 211.7 L ha^{-1} in the first and second seasons, respectively) was recorded with plants harvested in September.It might be concluded that the highest oil yield was recorded with plants harvested in September and sprayed with boron at 5 ppm, while there was no significant difference between 2.5 and 5 ppm of boron. These results are in harmony with Choudhary *et al.*[36] who found that boron in moderate concentration (2.5 mg Kg^{-1} soil) increased essential oil production in *Mentha arvensis* and *Cymbopogon flexuosus* plants, whereas increasing boron concentrations led to a decrement in the essential oil content.

Essential oil constituents

The identified compounds and their relative concentration of lavender oil as affected by foliar application of boron and harvest date were presented in Table 5. Monoterpenes hydrocarbons represented the majority (93.25-96.87%) in the essential oil of all treatments and the oxygenated monoterpenes were dominated (51.03-61.66%), followed by non-oxygenated (38.35-49.66%), then sesquiterpene hydrocarbons (3.14-6.75%). Eucalyptol (18.46-31.14%) was found to be the major compound of lavender oil, followed by(+)-2-Bornanone (21.30-24.41%), then α-pinene (12.76-14.99 %) and β-pinene (11.86-13.59%). Other constituents were presented at a low concentration as Camphene (2.13-2.76%), Sabinene (4.02-5.35%), β-Myrcene (0.80-3.70%), D-Limonene (1.64-3.59%), endo-Borneol (2.65-4.59%), Terpinen-4-ol (0-1.33%), α-Terpineol (0-3.34%) and Linalool (0.15-1.04%). Eucalyptol content increased with increasing the plant age of lavender. Lavender plant harvested in September and sprayed with boron at 0, 2.5 and 5 ppm produce the highest relative concentration of Eucalyptol (31.14, 30.97, and 29.12 %, respectively) as compared with plant collected in July and August which gave the lowest content. These results agreed with Choudhary*et al*. [36] who noticed an increase in menthyl acetate and menthol content at 2.5 mg B Kg^{-1} soil in *M. arvensis* and in the content of citral and geraniol in *C. flexuosus*as compared to the control.

The component (+)-2-Bornanone was the second major constituent, reached 23.59% in plants harvested in August and sprayed with 2.5 ppm boron, while in the control plants harvested in September increased slightly (24.41%) as compared with other treatment. A slight increase was obtained in the relative concentration of α-Pinene (14.91and 14.99%) of plants harvested in September and sprayed with boron at 2.5 and 5 ppm, respectively. β-Pinene reached the maximum value (13.59%) with plants collected in September and sprayed with 2.5 ppm boron

While other constituents such as Sabinene, β-Myrcene, D-Limonene, Fenchone, Terpinen-4-ol, were increased in the plants collected in July and gradually decreased in August and reached the lowest concentration in September. The 4-Carene was absent in the essential oil of plants either harvested in August or in September. Plants treated with boron at 2.5 ppm and harvested in August increased their relative contents of Camphene, ɣ-Terpinene, cis-β-Terpineol, Linolool, α-Campholenal, while application of boron at 5 ppm increased Pinocarvone, endo-Borneol, α-Terpineol, Caryophyllene, and δ-EIemene. All these components decreased in the essential oil of plants harvested in September.

Table3. Effect of boron foliar application and harvest date on vegetative parameters of *lavandula officinalis* plants.

Treatment		Plant height (cm)		Herb fresh weight (g plant^{-1})		Herb Dry weight (g plant^{-1})		Herb fresh yield (ton hectar^{-1})		Herb Dry yield (ton hectar^{-1})	
Boron mg l^{-1}	Harvest date	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
0	Jul.	30.3	38.3	316.8	345.6	84.0	86.9	7.0	7.7	1.9	1.9
	Aug.	40.3	40.3	557.5	550.4	183.7	180.5	12.4	12.2	4.1	4.0
	Sept.	50.3	44.7	769.2	760.5	231.2	230.6	17.1	16.9	5.1	5.1
2.5	Jul.	31.0	39.3	341.4	356.2	95.6	97.5	7.6	7.9	2.1	2.2
	Aug.	45.0	42.0	604.0	631.2	192.0	198.4	13.4	14.0	4.3	4.4
	Sept.	50.3	49.7	927.9	883.6	274.3	260.5	20.6	19.6	6.1	5.8
5	Jul.	31.7	40.0	360.5	353.8	108.1	112.6	8.0	7.9	2.4	2.5
	Aug.	49.7	44.0	621.0	685.3	193.5	201.9	13.8	15.2	4.3	4.5
	Sept.	51.0	50.7	979.2	891.0	296.7	277.7	21.8	19.8	6.6	6.2
L.S.D. at 5%		2.38	Ns	22.11	14.74	8.78	6.70	0.50	0.32	0.21	0.17
Boron mg l^{-1}											
0		40.3	41.1	547.8	552.2	166.3	166.0	12.2	12.3	3.7	3.7
2.5		42.1	43.7	624.4	623.7	187.3	185.5	13.9	13.9	4.2	4.1
5		44.1	44.9	653.6	643.4	199.4	197.4	14.5	14.3	4.4	4.4
L.S.D. at 5%		1.40	0.62	21.68	10.60	9.74	2.23	0.50	0.21	0.21	0.09
Harvest date											
Jul.		31.0	39.2	339.5	351.9	95.9	99.0	7.56	7.8	2.1	2.2
Aug.		45.0	42.1	594.1	622.3	189.7	193.6	13.2	13.8	4.2	4.3
Sept.		50.6	48.3	892.1	845.0	267.4	256.3	19.8	18.8	5.9	5.7
L.S.D. at 5%		1.68	1.33	15.60	10.40	6.20	3.86	0.35	0.23	0.15	0.10

Table 4. Effect of boron foliar application and harvest date on essential oil production of *lavandula officinalis* plants

Treatment		Oil percentage (%)		Oil content (ml plant^{-1})		Oil yield (l hectar^{-1})	
Boron (mg l^{-1})	Harvest date	1st season	2nd season	1st season	2nd season	1st season	2nd season
0	Jul.	1.02	1.00	3.2	3.5	71.6	77.1
	Aug.	1.13	1.16	6.3	6.4	140.4	141.7
	Sept.	1.05	1.09	8.1	8.3	180.1	184.0
2.5	Jul.	1.14	1.12	3.9	4.0	86.8	88.6
	Aug.	1.33	1.28	8.1	8.0	179.0	178.9
	Sept.	1.19	1.16	11.0	10.2	244.6	226.8
5	Jul.	1.01	1.18	3.6	4.2	81.0	92.8
	Aug.	1.44	1.21	9.0	8.3	199.0	184.5
	Sept.	1.13	1.13	11.0	10.1	245.2	224.4
L.S.D. at 5%		0.038	0.074	0.48	0.55	10.64	11.65
Boron mg l^{-1}							
0		1.07	1.08	5.88	6.04	130.7	134.3
2.5		1.22	1.18	7.66	7.42	170.1	164.8
5		1.19	1.17	7.88	7.52	175.1	167.2
L.S.D. at 5%		0.033	0.034	0.43	0.33	9.46	6.73
Harvest date							
Jul.		1.06	1.10	3.59	3.88	79.8	86.2
Aug.		1.30	1.21	7.78	7.58	172.8	168.4
Sept.		1.12	1.13	10.05	9.53	223.3	211.7
L.S.D. at 5%		0.022	0.043	0.28	0.32	6.14	6.72

A previous study by Liao *et al*. [37] on the constituents of the essential oils in Lavandine showed a remarkable difference depending on plant age, harvest time, climatic conditions, the extraction method, plant species and soil conditions. El-Sayed [38] stated that *L. officinalis* plants treated with 400ppm paclobutrazol (PBZ) contained volatile oil with great oxygenated monoterpenes content. On the other hand, α-pinene, camphor and menthol were the main constituents of from *L. officinalis* essential oils from Iran [39].

Table 5. Effect of boron foliar application and harvest date on essential oil constituents of *Lavandula officinalis* during 2020 season

RT	KI	Compounds	Area % July mg B l^{-1}			August mg B l^{-1}			September mg B l^{-1}		
			0	2.5	5	0	2.5	5	0	2.5	5
3.52	912	Tricyclene	0.51	0.45	--	0.50	0.45	0.49	0.29	0.29	0.33
3.73	921	α-Pinene	14.86	14.27	14.42	14.04	12.76	13.46	14.12	14.91	14.99
4.10	936	Camphene	2.68	2.51	2.37	2.76	2.57	2.76	2.27	2.13	2.34
4.60	955	Sabinene	5.30	5.23	5.24	5.03	4.60	5.35	3.53	4.02	4.06
4.74	960	β-Pinene	13.34	13.15	13.35	12.77	11.86	12.51	12.56	13.59	13.33
4.98	968	β-Myrcene	3.70	3.47	3.35	2.72	2.50	2.85	0.80	0.95	0.94
6.15	1003	D-Limonene	3.59	3.50	3.72	2.81	2.48	2.60	1.64	2.10	2.13
6.29	1007	Eucalyptol	18.46	18.63	18.98	19.33	19.24	20.10	31.14	30.97	29.12
7.04	1031	ɣ-Terpinene	0.61	0.57	0.42	--	--	1.58	--	0.18	--
7.50	1044	cis-β-Terpineol	0.57	0.71	0.96	1.27	1.39	--	0.82	0.75	1.07
7.92	1055	4-Carene	0.49	0.48	0.42	--	--	--	--	--	--
8.17	1062	Fenchone	0.75	0.67	0.64	0.39	--	0.35	0.15	--	0.17
8.54	1071	Linolool	0.94	0.91	0.82	0.78	0.90	1.04	0.15	0.15	0.21
9.66	1096	α-Campholenal	--	--	--	--	0.42	0.43	0.14	--	0.17
10.07	1106	L-trans-Pinocarveol	--	--	--	0.49	0.59	0.50	0.27	0.22	0.31
10.45	1116	(+)-2-Bornanone	21.30	21.49	21.33	22.89	23.59	22.27	24.41	22.40	22.91
10.97	1130	Pinocarvone	0.46	0.43	0.44	0.59	0.69	0.70	0.38	0.32	0.44
11.31	1138	endo-Borneol	3.84	3.97	3.80	4.22	4.86	4.39	2.95	2.65	3.01
11.63	1146	Terpinen-4-ol	1.33	1.30	0.99	0.79	1.01	0.79	0.13	0.20	--
12.31	1161	α-Terpineol	2.69	2.92	2.87	2.71	3.34	2.99	--	--	--
12.36	1162	Myrtenal	--	--	--	--	--	--	1.12	0.88	0.89
16.01	1243	Menthol, acetate	--	--	--	0.40	--	--	--	--	--
21.04	1354	Caryophyllene	0.37	--	0.44	0.43	0.45	0.37	0.20	0.20	0.21
22.86	1393	(+)-epi-Bicyclosesquiphellandrene	--	0.43	--	--	--	--	--	0.16	0.15
24.84	1440	δ-EIemene	4.21	4.92	4.97	5.07	5.82	4.47	2.94	2.94	3.22
25.33	1451	trans-calamenene	--	--	0.45	--	0.48	--	--	--	--
Monoterpines			95.42	94.66	94.12	94.49	93.25	95.16	96.87	96.71	96.42
Sesquiterpenes			4.58	5.35	5.86	5.5	6.75	4.84	3.14	3.3	3.58
Non-oxygenated copmounds			49.66	48.98	49.15	46.13	43.97	46.44	38.35	41.47	41.7
Oxygenated copmounds			50.34	51.03	50.83	53.86	56.03	53.56	61.66	58.54	58.3
Total of identified compounds			100	100	99.98	99.99	100	100	100	100	100

RT: Retention time, KI: Kuvet index

Eucalyptol, linalool and linalyl acetate were the main components of the essential oil of the most studied species in Croatia [40]. In southeast Spain linalool, linalyl acetate, camphor and eucalyptol were the main components of *Lavandula×intermedia* [41], while the main components of *L. intermedia* essential oil in Western Iran were camphor, eucalyptol and borneol [42].

The main constituents of the natural and nanforms of the essential oil

The variation in the main constituents of the natural and nanoforms of the essential oil of *Lavandula officinalis* among different harvest dates were presented in Table (6). Monoterpene hydrocarbons reached the highest relative percentage (96.61%) with oil nanoform of plant harvested in August. The total of oxygenated monoterpenes increased from 50.34 to 87.65, from 53.86 to 91.18 and from 61.66 to 88.91% in normal and nanoforms of the volatile oil from plants collected in July, August and September, respectively. On contrary, non-oxygenated compounds decreased from 49.66 to 6.83, from 46.13 to 6.15 and from 38.35 to 5.56% then sesquiterpene hydrocarbons decreased with oil nanoform. It might be concluded that nanoform of oil increased monoterpene hydrocarbons and oxygenated monoterpenes and while non-oxygenated compounds and sesquiterpene hydrocarbons were decreased as compared with bulk form of oil.

Table 6. The main constituents of the essential oil of the natural and nano emulsions forms of *Lavandula officinalis* among different harvest dates at 2020 season

RT	KI	Compounds	Area % July Normal	July Nano	August Bulk	August Nano	September Bulk	September Nano
3.52	912	Tricyclene	0.51	--	0.50	--	0.29	--
3.73	921	α-Pinene	14.86	2.89	14.04	2.24	14.12	2.18
4.10	936	Camphene	2.68	0.48	2.76	0.48	2.27	0.44
4.60	955	Sabinene	5.30	0.22	5.03	0.58	3.53	0.33
4.74	960	β-Pinene	13.34	3.13	12.77	2.70	12.56	2.45
4.98	968	β-Myrcene	3.70	--	2.72	0.15	0.80	--
5.29	978	1-Octen-3-ol	--	0.19	--	0.38	--	0.17
6.15	1003	D-Limonene	3.59	--	2.81	--	1.64	--
6.29	1007	Eucalyptol	18.46	33.48	19.33	43.21	31.14	38.13
7.04	1031	ɣ-Terpinene	0.61	--	--	--	--	--
7.50	1044	cis-β-Terpineol	0.57	0.53	1.27	--	0.82	0.13
7.92	1055	4-Carene	0.49	--	--	--	--	--
8.17	1062	Fenchone	0.75	0.41	0.39	0.34	0.15	--
8.54	1071	Linolool	0.94	1.62	0.78	1.37	0.15	1.13
9.66	1096	α-Campholenal	--	--	--	--	0.14	--
10.07	1106	L-trans-Pinocarveol	--	--	0.49	--	0.27	--
10.12	1108	β-Pinone	--	--	--	--	--	0.14
10.45	1116	(+)-2-Bornanone	21.30	36.36	22.89	31.31	24.41	36.21
10.97	1130	Pinocarvone	0.46	0.30	0.59	0.61	0.38	0.57
11.31	1138	endo-Borneol	3.84	6.54	4.22	4.33	2.95	5.20
11.63	1146	Terpinen-4-ol	1.33	0.67	0.79	2.16	0.13	1.42
12.06	1155	Crypton	--	--	--	0.66	--	--
12.31	1161	α-Terpineol	2.69	5.49	2.71	4.09	--	4.05
12.36	1162	Myrtenal	--	0.51	--	0.62	1.12	0.85
12.58	1167	l-Verbenone	--	--	--	0.33	--	--
12.81	1172	cis-Piperitol	--	0.09	--	0.12	--	--
13.46	1185	cis-Carveol	--	--	--	0.19	--	--
13.81	1192	β-Citral	--	0.13	--	0.15	--	--
14.23	1201	Carvone	--	--	--	0.38	--	0.17
14.65	1211	Geraniol	--	0.12	--	--	--	--
15.09	1222	Cadina-1,3,5-triene	--	0.11	--	--	--	0.16
15.17	1224	Citral	--	--	--	0.21	--	
16.01	1243	Menthol, acetate	--	--	0.40	--	--	----
21.04	1354	Caryophyllene	0.37	--	0.43	--	0.20	--
24.84	1440	δ-EIemene	4.21	--	5.07	--	2.94	--
27.79	1507	Caryophyllene oxide	--	0.73	--	0.58	--	0.63
31.08	1587	tau.-Muurolol	--	0.48	--	0.14	--	0.11
Monoterpines			95.42	93.16	94.49	96.61	96.87	93.57
Sesquiterpenes			4.58	1.32	5.5	0.72	3.14	0.90
Total of non-oxygenated compounds			49.66	6.83	46.13	6.15	38.35	5.56
Total of oxygenated copmounds			50.34	87.65	53.86	91.18	61.66	88.91
Total of identified compounds			100	94.48	99.99	97.33	100	94.47

RT: Retention time, KI: Kuvet index

Eucalyptol was found to be the major compound of lavender oil and reached to 43.21% in oil nanoforms of plants harvested in August. The component (+) -2-Bornanone recorded 36.36% with oil nanoform of plants harvested in July. On the contrary, α-pinene decreased from 14.86 to2.89, from14.04 to 2.24 and from 14.12 to 2.18% and β-pinene decreased from 13.34 to 3.13, from 12.77 to 2.70 and from 12.56 to 2.45% in normal and nanoform of plant harvested in July, August and September, respectively.

Other constituents which were presented at a low concentrations, endo-Borneol, Linolool, Terpinen-4-ol, α-Terpineolwere increased with nanoforms, while cis-β-Terpineol, Fenchone were decreased.Myrtenal, l-Verbenone, lcis-Piperitol, β-Citral, Carvonewere presented in nanoforms and were notdetected in bulk oil in plants, in the contrary, Caryophyllene and δ-EIemene were not detected in nanoforms.

Phytochemical constituents

The effect of boron foliar application and harvest date on total phenols, antioxidant activity and boron concentration of *L. officinalis* plants were presented in Table 7. Foliar application of boron at 2.5 mg L^{-1} showed the maximum increase in total phenolic content and antioxidant activity in herb of lavender plants while the concentration of boron increased with plant sprayed with boron at 5 mg L^{-1}. Choudhary*et al.* [36] suggested that increasing concentrations of B (2.5, 5, 10, 20 and 30 mg B Kg^{-1} soil) increased oxidative damage and antioxidant enzymes activity. Boron is an essential nutritional element and plays a main role in numerous metabolic processes, including RNA metabolism, the water use regulation and transport, metabolism of carbohydrate, protein, phenol and oxine, germination of pollen, pollen tube growth, ripening of fruits, roots growth, the protection of conductive tissues and pectin, ATP and of nucleic acid synthesis [17].

Table 7. Effect of boron foliar application and harvest date on total phenols, antioxidant activity and boron concentration of *lavandula officinalis* plants

Treatment		Total phenlols (mg g^{-1})		Antioxidant activity (%)		Boron (ppm)	
Boron mg l^{-1}	Harvest date	1st season	2nd season	1st season	2nd season	1st season	2nd season
0	Jul.	27.7	32.9	41.6	47.5	53.2	51.31
	Aug.	34.8	42.7	51.7	71.4	54.1	51.34
	Sept.	40.3	51.5	60.8	83.0	53.3	51.71
2.5	Jul.	36.5	36.1	57.1	54.3	56.8	56.67
	Aug.	43.1	53.3	65.4	83.8	60.6	63.16
	Sept.	51.2	59.8	75.1	85.2	65.5	65.52
5	Jul.	31.6	30.5	50.2	42.0	61.6	61.85
	Aug.	35.5	45.0	54.0	77.1	64.1	65.57
	Sept.	44.9	46.1	67.6	75.9	72.8	70.00
L.S.D. at 5%		4.66	3.71	2.52	3.25	2.69	1.95
Boron mg l^{-1}							
0		34.3	42.4	51.4	67.3	53.5	51.5
2.5		43.6	49.7	65.8	74.4	61.0	61.8
5		37.3	40.5	57.3	65.0	66.1	65.8
L.S.D. at 5%		4.03	2.23	2.82	2.06	1.51	1.96
Harvest date							
Jul.		31.9	33.2	49.6	47.9	57.2	56.6
Aug.		37.8	47.0	57.0	77.4	59.6	60.0
Sept.		45.5	52.5	67.9	81.4	63.9	62.4
L.S.D. at 5%		2.69	2.14	1.45	1.88	1.55	1.13

The high content of total phenols may be because of the role of boron in enhancing the phenolic compounds that utilized in the synthesis of the cell wall. Also, Mengel and Kirkby [43] stated that boron may adjust the 6-phosphogluconate dehydrogenase enzyme activity by complexing with its substrate 6-phosphogluconate. The initial stage of the pentose phosphate pathway is regulated by this enzyme. Therefore, it is said that the activity of the pentose phosphate pathway is lowered in the presence of boron in favour of glycolysis. On the other hand, a lack of boron favours the pentose phosphate pathway, which causes phenolics accumulation.

Plants treated with 400ppm paclobutrazol (PBZ) contained volatile oil with great oxygenated monoterpenes content, which enhance the biological, antioxidant and antibacterial properties (38). Also, many other previous studies showed that volatile oils terpenes such as β–terpinolene, Eucalyptoland linalool can contribute to the antioxidant activity [44-46].

Concerning harvest date, plants harvested in September recorded the highest total phenols, antioxidant activity and boron concentration of *L. officinalis* plants.

Transmission Electron Microscopy (TEM)

The prepared Lavender nanoforms, i.e. July, August and September nano forms, were examined using a Transmission Electron Microscope (TEM). Data from TEM showed that the nanoparticles diameters ranged between 20-50 nm and almost all the prepared nanoparticles were spherical with a smooth surface Figure (1). All samples showed a multi-core and shell encapsulation. The core expresses the active ingredient (lavender oil at different three cuts dates) and the shell expresses the loading material (polyethylene glycol).

Effect of bulk and nanoform of lavender oil against *Sitotroga cerealella*

Data in Tables (8-11) showed that the insecticidal efficacy of lavender essential oil at different harvest dates was found significant against the tested insect *Sitotroga cerealella*.

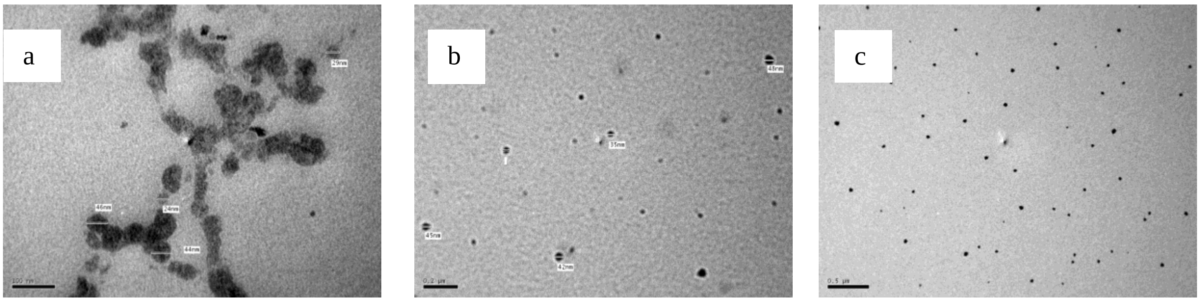

Figure 1. Transmission Electron Microscopy (TEM) images of lavender oil nano-emulsion at a)July, b)August and c)September

The results revealed that the maximum toxicity was depicted by the nanoformulation of July oil (LC_{50} was 139.49 ppm) while the bulk formulation of August oil was the least effective formulation (LC_{50} was 489.92 ppm). Whereas, the other formulations were arranged in a descending order according to their toxicity index to be a bulk form of July oil (55.40), nanoform of September oil (51.76), nanoform of August oil and bulk form of September oil (40.63) and finally bulk form of August oil (28.47).The LC_{50} values were 139.49, 251.51, 269.468, 343.30, 343.33and 489.92 ppm for the nanoform of July oil, bulk form of July oil, nanoform of September oil, nanoform of August oil, bulk form of September oil and bulk form of August oil, respectively. Adult emerge% and reduction% in F1-progeny were also affected gradually by different concentrations of oils at different cuts dates. The highest concentration of all treatments (nano or bulk) was the most effective on both emergence and reduction percentages in F1-progeny. Nano-formulations were more significantly effective than the bulk ones for the three cuts dates of lavender oil.

Nanotechnology pioneering has opened a new way for the nano-sized formulations development that are environmentally friendly, as it is very effective for pest control with less residual toxicity [47]. Different shapes of nanoparticles can be formed, such as a capsule that serves as a physical shell and is better able to survive environmental deterioration. Polyethylene glycol (PEG) is a polyether compound which is flexible and water-soluble polymer, considered biologically inert and safe by the Food and Drug Administration (FDA). In this study, PEG was used as a loading material to act as a shell in the prepared nano capsules. It plays a rule in control releasing of active ingredient, i.e. lavender oil, by this way it improve and elongate persistence of biological pesticides, protecting them from environmental factors i.e. temperature, humidity and evaporation. As a result, nanoparticles provide longer-lasting protection than traditional insecticides [48]. Contrary to traditional pesticides formulations, nano-formulations were formed with the specific purpose of increasing the solubility of weakly soluble or insoluble active components and releasing the biocide in a targeted and controlled manner [49]. This means that a lesser amount of active ingredient per area is needed for application and that it can distribute active ingredients over time in a sustained manner, potentially maintaining its effectiveness for a long time [50]. Therefore, the application cost is also lowered as a result of the lower required dosage. The controlled-release formulations must also be inert until the active ingredient is released, which is crucial [51]. Recently, a lot of research has been concerned with nanoparticles that developed through diverse synthesis techniques as new insecticides.

Table 8. LC_{50}, LC_{90} values of the lavender oils nano and bulk formulations at different harvest dates against *Sitotroga cerealella* eggs

Harvest dates	Oil type	LC50 (ppm) (Confidence limit)	LC90 (ppm) (Confidence limit)	Toxicity Index	Relative potency
Jul.	Nano	139.49 (13.39-224.07)	654.35 (504.40-1056.80)	100	3.51
	Bulk	251.51 (205.74-309.40)	509.50 (423.07-672.43)	55.40	1.95
Aug.	Nano	343.30 (271.25-488.24)	713.193 (544.557-1158.471)	40.63	1.43
	Bulk	489.92 (347.11-1179.64)	1053.877 (694.82-3018.81)	28.47	1.00
Sept.	Nano	269.468 (104.704-373.338)	649.673 521.335-955.020	51.76	1.82
	Bulk	343.33 (278.56-454.39)	623.78 (496.71-937.88)	40.63	1.43

Table 9. Percentage of mortality, adult emerge and reduction in F1- progeny of *Sitotroga cerealella* against different concentrations of July lavender oil in its nano and bulk forms.

Oil type	Concentration (ppm)	Mortality % (Confidence limit)	Adult Emerge % (Confidence limit)	Reduction % in F1- progeny
Nano	100	41.98±2.38g (31.73-52.22)	54.76±2.38c (44.52-65.01)	40.18±2.46g (29.59-50.77)
	200	53.98±0.60e (51.38-56.58)	42.76±0.60e (40.16-45.35)	52.58±0.62e (49.90-55.27)
	400	60.79±2.61d (49.54-72.03)	35.95±2.61f (24.70-47.20)	59.62±2.70d (48.00-71.25)
	800	90.84±0.20a (89.98-91.70)	5.90±0.20i (5.03-6.76)	90.68±0.21a (89.79-91.57)
Bulk	100	21.21±1.40h (15.20-27.23)	75.52±1.40b (69.51-81.54)	18.72±1.45h (12.51-24.94)
	200	48.25±1.52f (41.73-54.77)	48.48±1.52d (41.97-55.00)	46.67±1.57f (39.93-53.40)
	400	67.89±1.92c (59.62-76.17)	28.85±1.92g (20.57-37.12)	66.96±1.99c (58.41-75.51)
	800	80.93±0.43b (79.09-82.76)	15.81±0.43h (13.97-17.65)	80.43±0.44b (47.69-66.27)
Control	0.00	3.23±0.06i (2.98-3.48)	96.77±0.58a (94.29-99.25)	-
F value		327.16**	339.40**	186.34**

Means within the same column followed by the same letter are not significantly different. ANOVA, Dunken (P > 0.05)

Although numerous studies have been carried out to assess their toxicity against a variety of insect pests, it is still unclear exactly how their mode of action against insects [52].

This study explored the potent insecticidal activity of lavender oil at different harvest dates against *S. cerealella,* which is one of the harmful pests of warehouse commodities all over the world. It was concluded that using nanotechnology improved the efficiency of lavender oil at different harvest dates against *S. cerealella.* This may be due to monoterpenes hydrocarbons in lavender essential oil reached to the highest relative concentration 96.61% with oil nanoform of plant harvested in August and the oxygenated monoterpenes increased. Eucalyptol was the major compound of lavender oil and reached to 43.21% with oil nanoforms of plant harvested in August and the (+) -2-Bornanone was the second compound recorded 36.36 with oil nanoforms of plants harvested in July.

Table 10. Percentage of mortality, adult emerge and reduction in F1- progenyof *Sitotroga cerealella* against different concentrations of August lavender oil in its nano and bulk forms.

Oil type	Concentration (ppm)	Mortality % (Confidence limit)	Adult Emerge % (Confidence limit)	Reduction % in F1- progeny
Nano	100	24.24±1.44f (18.03-30.45)	75.61±3.43b (60.87-90.34)	18.64±3.54e (3.41-33.87)
	200	38.91±0.36d (37.39-40.44)	60.71±3.00c (47.82-53.22)	34.03±3.10d (20.71-47.35)
	400	49.36±1.36c (43.52-55.21)	47.37±1.36d (41.53-53.22)	47.82±1.40c (41.77-53.86)
	800	96.74±0.00a (96.74-96.74)	0.00±0.00f (0.00-0.00)	96.77±0.00a (96.77-96.77)
Bulk	100	23.12±1.83f (15.23-31.01)	73.61±1.83b (65.72-81.51)	20.70±1.90e (12.54-28.85)
	200	28.48±1.59e,f (21.65-35.31)	68.25±1.59b,c (61.74-75.08)	26.24±1.64d,e (19.18-33.30)
	400	34.00±2.02d,e (25.33-42.67)	62.74±2.02c (54.06-71.42)	31.94±2.97d (22.98-40.90)
	800	66.74±5.77b (41.90-91.58)	30.00±5.77e (5.16-54.84)	65.77±5.29b (40.10-91.44)
Control	0.00	3.23±0.06g (2.98-51.09)	96.77±0.58a (5.16-54.84)	-
F value		144.36**	109.63**	81.39**

Means within the same column followed by the same letter are not significantly different. ANOVA, Dunken (P > 0.05)

Table 11. Percentage of mortality, adult emerge and reduction in F1- progeny of *Sitotroga cerealella* against different concentrations of September lavender oil in its nanoand bulk forms.

Oil type	Concentration (ppm)	Mortality % (Confidence limit)	Adult Emerge % (Confidence limit)	Reduction % in F1- progeny
Nano	100	43.44±5.70d (18.89-67.98)	53.30±5.70c (28.76-77.85)	41.69±5.89f (16.33-67.05)
	200	72.09±2.33c (62.07-94.41)	24.65±2.33e (14.62-34.67)	71.30±2.41d (60.94-81.66)
	400	80.07±3.33b (65.73-94.41)	16.67±3.33f (2.32-31.01)	79.55±3.44c (64.73-94.37)
	800	88.34±1.80a (80.60-96.07)	8.40±1.80g (0.67-16.41)	88.09±1.86b (80.10-96.08)
Bulk	100	7.35±0.56f (4.96-9.74)	92.48±1.26a (87.05-97.91)	2.38±0.83h (1.20-5.97)
	200	21.07±0.43e (19.21-22.93)	71.86±0.43b (97.00-73.72)	20.13±0.46g (18.15-22.12)
	400	50.34±0.93d (46.35-54.32)	42.59±0.93d (38.61-46.58)	51.35±0.99e (47.10-55.60)
	800	92.93±0.00a (92.93-92.93)	0.00±0.00h (0.00-0.00)	96.77±0.00a (96.77-96.77)
Control	0.00	3.23±0.06f (2.98-3.48)	96.77±0.56a (94.29-99.25)	-
F value		200.88**	209.86**	155.70**

Means within the same column followed by the same letter are not significantly different. ANOVA, Dunken (P > 0.05)

It was clear that all nanoforms were more effective than their bulk formulations. All the results revealed that the mean toxicity was concentration dependent. Treatment with a higher concentration of nano or bulk formulations resulted in a higher percentage of mortality and a reduction in adult emergence. Our results are agreed with Batool *et al*. [53], who stated that nanoencapsulation of *Albizia procera* (ApCP) with graphene oxide (GQDs) resulted in no population. The LC_{50} of protein with GQDs and without GQDs assured the promising control of insect pest of the study as treatments showed valuable decreases in LC_{50} when treated to ApCP encapsulated with GQDs. Different studies witness for nanoformulations as an emerging trend for pest management strategies which support the results of increasing insecticidal efficacy of ApCP after nanoencapsulation. Kumar *et al*. [23] reported that nanoencapsulation of a biopesticide could make it more potent by providing protection through environmental aggressions and improved/controlled release to the target site. Mohammed and Nasr (54) reported that anise essential oil–based nanoform containing 81.2% of (E)-anethole revealed toxicity of (LC_{50} = 9.3% v/v) and reduced rust-red flour beetle progeny significantly. They added that eucalyptus volatile oil nanoform with 8.57 nm particle size showed higher insecticidal activity against *T. castaneum*. The same findings were obtained by Soudy *et al*. [32], Youssef and Ali [55] and Abd-El Wahed *et al*. [56] who stated that nanoforms have more toxic effects than their bulk forms against both insects and mites' pests and they added that this method could dramatically reduce production costs of pesticides compared to the commonly used wet-milling and high advances of a new philosophy of converting technical material (TC) into a normal formulation.

Conclusion

It might be concluded that boron and harvest date affect herb yield and phytochemical content which affect the regulation of the biosynthesis of essential oil of *Lavandula officinalis* grown in newly reclaimed land. From our results it was noticed that, boron application at 5 mg L^{-1} gave the highest values of vegetative growth parameters and essential oil yield in plants harvested in September. Eucalyptol the major compound of lavender oil, followed by (+)-2-Bornanone, α-pinene and β-pinene.It was concluded that using nanotechnology improved the efficiency of lavender oil at different harvest dates against *S. cerealella*, as it was clear that all nanoformulations were more effective than their bulk formulations.

Conflict of interest

All authors declare that they have no conflict of interest

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