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EXPERIMENTAL PAPER

Phytochemical and growth responses of *Mentha piperita* to foliar application of biostimulants under greenhouse and field conditions

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Summary

Introduction: The biostimulant products are able to improve quality and quantity of medicinal plants.

Objectives: The comparative effects of biostimulants foliar spraying on peppermint (*Mentha piperita* L.) were investigated.

Methods: These studies were done on the basis of randomized complete blocks design in 3 replicates during 2015.

Results: In field conditions, the highest leaves and stems dry weight by 400 mg/l chitosan (CH) + 400 mg/l citric acid (CA), essential oil content by 200 mg/l chitosan + 400 mg/l humic acid (HA) + 400 mg/l citric acid and menthol content in 200 mg/l chitosan + 800 mg/l humic acid + 400 mg/l citric acid were observed. In greenhouse conditions, the best results of those mentioned parameters were obtained by 400 mg/l chitosan + 800 mg/l humic acid + 400 mg/l citric acid, 800 mg/l humic acid and 400 mg/l chitosan + 400 mg/l humic acid + 400 mg/l citric acid, respectively.

Conclusions: The foliar application of effective biostimulants could improve the yield quality and quantity of *Mentha piperita*.

Key words: ***Mentha piperita L., chitosan, citric acid, humic acid, essential oil, menthol***

INTRODUCTION

Peppermint (*Mentha piperita* L.) from *Lamiaceae* family is a cultivated natural hybrid plant of *Mentha aquatica* L. (water mint) and *Mentha spicata* L. (spearmint). Its leaves are elliptic, jagged, cross, sharp, and slightly covered with fluff, and its height and width are about 4-7 cm and 2-3 cm, respectively [1-3]. This plant is cultivated widespread in all regions of the world. It is found wild occasionally with its parent species [4]. Its cultivation is of economic importance due to its essential oil content and menthol as the main ingredient in essential oil, used in oral hygiene products, pharmaceuticals, cosmetics, and food industry. Due to wide antifungal and antibacterial activities of essential oil in peppermint, it became one of the most demanded substances by the perfume and essences industries [5]. Various products of the aerial parts of *Mentha* species have been used for centuries as tonics, antispasmodic, anti-inflammatory agents, etc. in traditional medicine [6]. Because of these and other factors, essential oil of peppermint ranks high in the market [7].

A mixture of two or more PGRs (Plant Growth Regulators) or the combination with other substances (amino acids, nutrients, vitamins) is known as plant biostimulant, which improves the plant growth in small quantities application [8].

Chitosan is an abundant natural polysaccharide with low toxicity that is environmentally friendly and biodegradable. It is applied in different ways in horticulture and agriculture. Chitosan is obtained by deacetylation of chitin by the enzymatic method or alkaline hydrolysis. In agribusiness, chitosan has been utilized as a bioprotectant and biostimulant in the formulation of seed, fruit and vegetable coating. Chitosan is used to increase the plant productivity [9], control the release of agrochemicals, protect the plants against microorganisms and oxidative stress [10], and stimulates the plant growth [11-12]. In last investigations, an affirmative impact of chitosan has been seen on the growth and development of roots, shoots, and leaves of several plant species. Comparative outcomes were specified within radish (*Raphanus raphanistrum* L.) and sweet pepper (*Capsicum annuum* L.) [12-13]. Also, foliar application of chitosan increases vegetative growth and improves fruit quality of cucumber (*Cucumis sativus* L.) [11]. For other cultivated plants, Bittelli *et al.* [14] reported that foliar application of chitosan decreased transpiration in pepper plants, and reduced water use by 26–43% while maintaining biomass production and yield. Bittelli *et al.* [14] reported that foliar application of chitosan on the cultivated pepper (*Capsicum* sp.), decreased plant transpiration, and reduced water use by 26–43% maintaining biomass production and yield. Abdel-Mawgoud *et al.* [15] research on strawberry (*Fragaria ananassa L.*) demonstrated that foliar application of chitosan enhanced plant height, number of leaves, fresh and dry weights of the leaves, and yield components.

Humic materials are final products of microbial and chemical decomposition of dead biota in soils and are raised to be the most abundant naturally occurring organic substances on earth and the main components of soil organic matter [16].

Citric acid (2-hydroxy 2,3-propanetricarboxylic acid) is one of the most important organic acids produced by fermentation. Citric acid is a tricarboxylic acid and an intermediate product of plant and animal metabolism. It is a commodity chemical product consumed around the world [17], and the essential constituent in all living beings. It directly plays a role in the production of energy through tricarboxylic acid cycle, as well as in some processes related to the metabolism of carbohydrates, certain amino acids, and fatty acids [18].

The purpose of the study was to investigate the influence of biostimulants including chitosan, humic acid and citric acid on dry mass of raw materials and essential oil total content and composition of peppermint (*M. piperita* L.) cultivated in greenhouse and filed conditions.

MATERIAL AND METHODS

In order to evaluate the effects of biostimulants on the growth and phytochemical parameters of *Mentha piperita* L., field and greenhouse experiments were carried out in 2015 at research field and greenhouse of Medicinal Plants Institute, ACECR (56°35′ N and 50°58′ E; 1500 m of elevation). The soil was loam-silt with 0.071% nitrogen, 48.9 mg/kg phosphorous, 33.6 mg/kg potassium, EC 2.71 dS/m, and pH 8.3. The transplants were supplied by research greenhouse of the Medicinal Plants Institute,

ACECR. A voucher specimen (4580-MPIH) has been deposited in the Herbarium of Medicinal Plants Institute, ACECR. In greenhouse and field, the study was conducted on the basis of randomized complete blocks design with 10 treatments as described in table 1 for bio-stimulants with 3 replications. In the classic greenhouse, the duration of light and dark for growth was 16/8h. The average day and night temperatures during experiment were 22±3°C and 15±3°C, respectively. Also the average relative humidity was 55±5%. In each pot, five transplants of the same size were cultivated and all treatments were sprayed three times in three months after the establishment. Other crop operations were completed regularly during the growing season as needed (tab. 2). The average day/night temperatures and average relative humidity in field conditions are shown in table 3. In the field experiment the treatments were the same as bio-stimulants formulations mentioned in table 1. Soil samples were derived for analysis before field preparation and were fertilized on the basis of a soil experiment recommendation.

Table 1.

Treatments of bio-stimulants formulations on *Mentha piperita* L.

1-	Control treatment (distilled water)
2-	400 mg/l CA
3-	400 mg/l HA
4-	800 mg/l HA
5-	200 mg/l CH + 400 mg/l CA
6-	400 mg/l CH + 400 mg/l CA
7-	200 mg/l CH + 400 mg/l HA + 400 mg/l CA
8-	200 mg/l CH + 800 mg/l HA + 400 mg/l CA
9-	400 mg/l CH + 400 mg/l HA + 400 mg/l CA
10-	400 mg/l CH + 800 mg/l HA + 400 mg/l CA

CH: chitosan [Poly-(D)glucosamine], Poly[(1,4)-N-acetyl-D-glucose-2-amine)]; HA: humic acid [$(C_8H_{13}NO_5)n$, $C_{187}H_{186}O_{89}N_9S_1$]; CA: citric acid ($C_6H_8O_7$)

The transplants were transferred in rows 50 cm apart with the inter-row spacing of 20 cm. Each experimental plot contained of 5 rows. The replicates (blocks) with distance of 1.5 m one from another and plots with distance of 1 m from each side were considered. The irrigation and other field practices had been performed as needed (tab. 2). The studied traits per plant were leaves dry weight (g), stems dry weight (g), shoot dry weight (g), and content of essential oil (% w/v), menthol, menthone, α-terpinene, pulegone, menthyl acetate, and menthofuran (v/v%) per essential oil.

The plant materials were dried in the laboratory at a room temperature (26±2°C) away from sunlight to prevent changes in the nature of the plants' constituents until the constant weight was gained. The 50 g of dried shoots were used for essential oils extraction by hydro distillation method for 4 h using Clevenger-type apparatus [19]. The oils were dried over anhydrous sodium sulphate and kept at 4°C until analyzed.

The GC analysis was carried out on a Younglin Instrument Acme 6000M gas chromatograph equipped with Flame Ionization Detector (FID) and a HP-5 capillary column (30 m × 0.25 mm; 0.25 μm film thicknesses). The oven temperature was held at 50°C for 5 minutes, and then programmed at 3°C

Table 2

Planting, spraying and harvesting intervals of peppermint in field and greenhouse

	Operation	Date
1	Cultivation of transplants in greenhouse/field	13 April 2015
2	Foliar application of bio-stimulants (stage 1)	10 May 2015
3	Foliar application of bio-stimulants (stage 2)	07 June 2015
4	Foliar application of bio-stimulants (stage 3)	21 July 2015
5	Harvest time	06 August 2015

Table 3.

Monthly temperature and minimum average humidity in 2015

Month	Average temperature [°C]			Minimum average humidity [%]
	Minimum	Mean	Maximum	
April	6.3	12.8	19.3	48
May	12.7	20	27.3	43
June	15.9	23.7	31.5	34
July	18.1	25.8	33.6	35
August	19.5	27	34.5	34

per min to 240°C and after that programmed at 15°C per min to 300°C (held for 3 minutes). Other operating conditions were: carrier gas, He with a flow rate of 0.8 ml/min; injector and detector temperatures was 290°C, and split ratio, 1:10. GC/MS analysis was performed on a above mentioned GC coupled with an Agilent Technologies 5973 mass system. The other operating conditions were the same conditions as described above, mass spectra were taken at 70 eV. Mass range was from m/z 35–375 amu. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the essential oils were identified by comparison of their mass spectra and retention indices with those published in the literature [20-21] and presented in the MS computer library.

Analysis of variance (ANOVA) of the results was done using the SPSS software (ver. 24). The means in the results were compared by Duncan's multiple range test at $p \leq 0.01$.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

Greenhouse experiment

According to the results of variance analysis in greenhouse experiment, the effect of biostimulants was significant on stems dry weight, aerial parts dry weight, content of menthol, menthone, α-terpinene, pulegone, menthyl acetate, and menthofuran ($p \leq 0.01$), and also on leaves dry weight ($p \leq 0.05$) (tab. 4 and 5).

Growth traits

Based on the obtained results of mean comparisons, the highest amount of leaves dry weight was observed in treatment of 400 mg/l CH + 800 mg/l HA + 400 mg/l CA, while the lowest was attained in plants treated by 400 mg/l CA. The highest and the lowest stem dry weight was observed in 400 mg/l CH + 800 mg/l HA + 400 mg/l CA treatment and 400 mg/l CA, respectively. The highest shoot dry weight was related to treatment with 400 mg/l CH + 800 mg/l HA + 400 mg/l CA, however the lowest amount was attained in 400 mg/l CA (tab. 6).

Phytochemical traits

Regarding to mean comparison of phytochemical parameters, the highest and lowest amount of essential oil content was observed in 800 mg/l HA and 200 mg/l CH + 400 mg/l CA, respectively. The menthol content reached the highest value by treatment of 400 mg/l CH + 400 mg/l HA + 400 mg/l CA. The treatment with 400 mg/l CA showed the highest amount of menthone and menthofuran component.

Table 4

Analysis of variance for effects of biostimulants formulations on growth parameters in greenhouse condition

S.O.V	df	Mean square		
		Leaves dry weight	Stems dry weight	Shoot dry weight
Rep.(block)	2	0.0089	0.0116	0.0381
Treatment	9	0.1016*	0.0903**	0.353**
Error	18	0.0343	0.127	0.077
CV (%)		14.62	15.52	11.49

*,** – ns shows significant in 5%, 1%, and insignificant, respectively

Table 5

Analysis of variance for effects of biostimulants formulations on phytochemical parameters in greenhouse conditions

S.O.V	df	Mean square						
		Essential oil content [%]	Menthol	Menthone	α-Terpinene	Pulegone	Menthyl acetate	Menthofuran
Rep.(block)	2	0.0048	0.827	0.172	2.520	20.026	0.335	2.516
Treatment	9	0.217**	77.28**	18.24**	8.683**	2.477**	1.237**	5.750**
Error	18	0.0045	11.75	1.51	1.503	0.511	0.178	1.575
CV (%)		11.55	8.69	6.40	16.24	16.63	15.92	10.28

*,** – ns shows significant in 5%, 1%, and insignificant, respectively

The content of α-terpinene reached the highest level by foliar application of 400 mg/l CH + 800 mg/l HA + 400 mg/l CA. The maximum content of menthyl acetate was related to control treatment. The highest level of pulegone was recorded in 800 mg/l HA treatment (tab. 7).

Field experiment

Considering the variance analysis results in the field experiment, the biostimulants application had significant effect on leaves dry weight, essential oil component of menthyl acetate ($p \leq 0.05$), and also on content of menthol, menthone, α-terpinene, pulegone, menthofuran, stems dry weight and shoot dry weight ($p \leq 0.01$) (tab. 8 and 9).

Growth traits

The highest amount of leaves dry weight was obtained by application of 400 mg/l CH + 400 mg/l CA, while the lowest amount was observed in control treatment. The highest amount of stem dry weight was obtained in 400 mg/l CH + 400 mg/l CA and the lowest amount in control treatment. Shoot dry weight reached to the highest level by application of 400 mg/l CH + 400 mg/l CA, while the lowest level was obtained in control treatment (tab. 10).

Phytochemical traits

According to mean comparisons, the highest content of essential oil was related to both treatments of 400 mg/l CH + 400 mg/l CA and 200 mg/l CH + 400 mg/l HA + 400 mg/l CA. The highest menthol content was observed in treatment of 200 mg/l CH + 800 mg/l HA + 400 mg/l CA. The maximum amount of menthone and pulegon were recorded in 400 mg/l CH + 800 mg/l HA + 400 mg/l CA treatment. The maximum amount of menthafuran was obtained in foliar application of 400 mg/l CH + 400 mg/l HA + 400 mg/l CA, while the highest amount of α-terpinene was recorded in treatment of 400 mg/l CH + 800 mg/l HA + 400 mg/l CA. The plants treated with 800 mg/l HA showed the maximum value of menthyl acetate (tab. 11).

DISCUSSION

According to the obtained results, the application of biostimulants formulations had positive effects on growth and phytochemical parameters of *M. piperita* L. plants in greenhouse and field. In the field, the highest leaves dry weight was attained by 400 mg/l CH + 400 mg/l CA treatment in comparison with control, while in the greenhouse, the treatment of 400 mg/l CH + 800 mg/l HA + 400 mg/l CA increased the leaves dry weight significantly,

Table 6
Mean comparisons for the effects of biostimulants formulations on growth parameters in greenhouse conditions

Treatment*	Leaves dry weight per plant [g]	Stems dry weight per plant [g]	Shoot dry weight per plant [g]
1	1.04 ± 0.18	0.97 ± 0.08	2.01 ± 0.26
2	1.04 ± 0.20	0.91 ± 0.25	1.95 ± 0.42
3	1.15 ± 0.10	1.09 ± 0.06	2.24 ± 0.14
4	1.10 ± 0.15	1.15 ± 0.25	2.26 ± 0.38
5	1.31 ± 0.30	1.32 ± 0.19	2.63 ± 0.47
6	1.35 ± 0.06	1.26 ± 0.05	2.62 ± 0.01
7	1.34 ± 0.07	1.17 ± 0.08	2.51 ± 0.05
8	1.39 ± 0.13	1.24 ± 0.16	2.63 ± 0.06
9	1.29 ± 0.15	0.98 ± 0.20	2.28 ± 0.05
10	1.63 ± 0.26	1.47 ± 0.26	3.10 ± 0.28

*1: Control treatment (distilled water),
2: 400 mg/l CA
3: 400 mg/l HA
4: 800 mg/l HA
5: 200 mg/l CH + 400 mg/l CA
6: 400 mg/l CH + 400 mg/l CA
7: 200 mg/l CH + 400 mg/l HA + 400 mg/l CA
8: 200 mg/l CH + 800 mg/l HA + 400 mg/l CA
9: 400 mg/l CH + 400 mg/l HA + 400 mg/l CA
10: 400 mg/l CH + 800 mg/l HA + 400 mg/l CA

Table 7

Mean comparisons for the effects of biostimulants formulations on phytochemical parameters in greenhouse condition

Treatments	1		2		3		4		5		
	KI[b]	mean	KI[c]	mean	KI[c]	mean	KI[c]	mean	KI[c]	mean	KI[c]
Essential oil content [% w/v]	–	0.50±0.05	–	0.40±0.10	–	0.73±0.05	–	0.82±0.02	–	0.4±0.01	–
α-Terpinene [%]	1017	6.56±0.49	1027	7.72±1.08	1024	6.88±0.33	1024	6.79±1.38	1024	6.71±2.10	1024
Menthone [%]	1153	20.23±0.35	1155	23.77±2.13	1153	17.54±0.98	1153	18.49±0.50	1153	21.71±1.10	1153
Menthofuran [%]	1164	9.17±1.56	1173	14.16±0.93	1164	11.96±0.17	1163	12.05±0.25	1164	12.81±1.45	1164
Menthol [%]	1172	39.84±1.88	1189	30.21±4.64	1176	43.26±3.48	1177	38.70±2.08	1177	37.06±1.22	1177
Pulegone [%]	1237	3.80±1.27	1250	4.41±1.21	1242	2.97±1.43	1242	5.82±1.30	1243	5.24±1.82	1243
Menthyl acetate [%]	1295	4.25±0.22	1226	2.12±0.34	1298	3.05±0.125	1299	2.22±0.29	1299	2.59±0.10	1299

Treatments	6		7		8		9		10		
	mean	KI[c]	mean	KI[c]	mean	KI[c]	mean	KI[c]	mean	KI[c]	*p*-value
Essential oil content [% w/v]	0.40±0.10	–	0.60±0.10	–	1.01±0.07	–	0.70±0.05	–	0.61±0.02	–	$p \leq 0.01$
α-Terpinene [%]	6.25±0.65	1024	8.42±1.43	1025	7.44±1.05	1024	6.66±2.12	1024	12.03±0.24	1025	$p \leq 0.01$
Menthone [%]	17.33±1.08	1153	21.56±1.03	1154	16.32±1.59	1153	16.85±1.32	1153	18.59±0.45	1153	$p \leq 0.01$
Menthofuran [%]	12.42±1.06	1164	12.42±1.78	1165	11.44±1.37	1164	11.26±1.52	1164	13.57±1.63	1164	$p \leq 0.01$
Menthol [%]	43.35±3.92	1177	38.44±2.53	1178	45.05±2.77	1178	45.49±3.99	1177	33.01±4.24	1177	$p \leq 0.01$
Pulegone [%]	4.64±1.20	1243	4.94±1.80	1243	4.64±2.02	1243	4.00±2.08	1243	4.49±1.12	1243	$p \leq 0.01$
Menthyl acetate [%]	2.23±0.21	1299	2.18±0.31	1299	2.60±0.10	1299	2.60±0.06	1299	2.42±1.23	1299	$p \leq 0.01$

[a] 1: Control treatment (distilled water)
2: 400 mg/l CA
3: 400 mg/l HA
4: 800 mg/l HA
5: 200 mg/l CH + 400 mg/l CA
6: 400 mg/l CH + 400 mg/l CA
7: 200 mg/l CH + 400 mg/l HA + 400 mg/l CA
8: 200 mg/l CH + 800 mg/l HA + 400 mg/l CA
9: 400 mg/l CH + 400 mg/l HA + 400 mg/l CA
10: 400 mg/l CH + 800 mg/l HA + 400 mg/l CA
[b]: Kovats index in reference [18];
[c]: Calculated Kovats index relative to C_8–C_{24} n–alkanes on the HP–5 column.

Table 8

Analysis of variance for effects of biostimulants formulations on growth parameters in field conditions

S.O.V	df	Mean square		
		Leaves dry weight	Stems dry weight	Shoot dry weight
Rep.(block)	2	0.2809	2.486	4.049
Treatment	9	2.428*	17.709**	31.704**
Error	18	0.801	1.882	3.601
CV (%)		22.39	19.43	17.03

*,** – ns shows significant in 5%, 1%, and insignificant, respectively

Table 9

Analysis of variance for effects of biostimulants formulations on phytochemical parameters in field conditions

S.O.V	df	Mean square						
		Essential oil content [%]	Menthol	Menthone	α-Terpinene	Pulegone	Menthyl acetate	Menthofuran
Rep.(block)	2	0.004	0.217	0.147	0.014	0.0034	0.899	241.42
Treatment	9	0.881**	7.035**	2.910**	0.143**	1.289**	1.130*	2.180**
Error	18	0.073	0.586	0.242	0.0091	0.298	0.453	0.181
CV (%)		13.15	1.93	3.60	1.20	13.38	16.23	4.33

*,** – ns shows significant in 5%, 1%, and insignificant, respectively

Table 10
Mean comparisons for the effects of biostimulants formulations on growth parameters in field conditions

Treatment*	Leaves dry weight per plant [g]	Stems dry weight per plant [g]	Shoot dry weight per plant [g]
1	2.63±0.78	4.14±1.25	6.86±1.10
2	4.53±0.73	7.01±0.65	11.63±1.32
3	3.52±0.45	3.77±0.33	7.41±0.64
4	4.42±1.31	8.70±1.04	13.22±2.33
5	5.18±1.11	9.10±1.77	14.36±2.89
6	5.33±0.49	11.17±2.69	16.58±2.28
7	4.28±1.19	9.27±1.20	13.65±2.36
8	3.02±0.65	5.52±1.45	8.63±2.08
9	3.66±0.80	6.27±0.60	10.01±0.35
10	3.36±0.63	5.62±1.35	9.02±1.98

*1: Control treatment (distilled water),
2: 400 mg/l CA
3: 400 mg/l HA
4: 800 mg/l HA
5: 200 mg/l CH + 400 mg/l CA
6: 400 mg/l CH + 400 mg/l CA
7: 200 mg/l CH + 400 mg/l HA + 400 mg/l CA
8: 200 mg/l CH + 800 mg/l HA + 400 mg/l CA
9: 400 mg/l CH + 400 mg/l HA + 400 mg/l CA
10: 400 mg/l CH + 800 mg/l HA + 400 mg/l CA

Table 11
Mean comparisons for the effects of biostimulants formulations on phytochemical parameters in field conditions

Treatments	1		2		3		4		5			
	KI[b]	mean	KI[c]	mean	KI[c]	mean	KI[c]	mean	KI[c]	mean	KI[c]	*p*-value
Essential oil content [% w/v]	–	1.90±0.10	-	1.50±0.30	-	1.60±0.25	–	2.00±0.05	–	1.30±0.40	-	$p \leq 0.01$
α-Terpinene [%]	1017	8.10±0.10	1028	7.89±0.01	1024	7.86±0.03	1024	8.01±0.09	1024	7.97±0.04	1024	$p \leq 0.01$
Menthone [%]	1153	11.44±1.23	1157	12.90±0.50	1153	13.08±0.41	1152	14.10±0.10	1152	13.55±0.17	1153	$p \leq 0.01$
Menthofuran [%]	1164	8.72±4.36	1173	10.80±5.40	1164	10.09±5.04	1163	9.29±4.64	1163	10.22±5.11	1164	$p \leq 0.01$
Menthol [%]	1172	38.97±0.46	1188	39.94±0.02	1177	39.48±0.21	1172	40.58±0.34	1177	40.80±0.45	1177	$p \leq 0.01$
Pulegone [%]	1237	3.13±0.35	1250	3.13±0.91	1243	3.37±0.62	1242	3.99±0.22	1243	4.33±0.02	1243	$p \leq 0.01$
Menthyl acetate [%]	1295	4.34±0.54	1296	4.13±0.13	1299	4.48±1.02	1298	4.99±1.29	1299	4.37±0.01	1299	$p \leq 0.05$

Treatments	6		7		8		9		10		*p*-value
	mean	KI[c]	mean	KI[c]	mean	KI[c]	mean	KI[c]	mean	KI[c]	
Essential oil content [% w/v]	2.80±0.35	–	2.80±0.35	–	2.70±0.30	–	2.00±0.05	–	2.00±0.50	-	
α-Terpinene [%]	7.84±0.12	1024	7.95±0.08	1025	7.66±0.11	1023	7.45±0.11	1024	8.23±0.16	1025	
Menthone [%]	14.04±0.07	1153	14.14±0.12	1153	13.91±0.005	1152	14.54±0.32	1153	14.87±0.48	1153	
Menthofuran [%]	10.12±5.06	1163	9.73±4.86	1164	8.55±4.27	1162	11.25±5.62	1163	9.50±4.75	1164	
Menthol [%]	38.50±0.70	1177	40.16±0.13	1178	42.46±1.28	1176	37.18±1.36	1176	37.98±0.96	1177	
Pulegone [%]	4.44±0.22	1243	4.74±0.15	1244	4.41±0.07	1242	4.26±0.42	1243	4.97±1.01	1244	
Menthyl acetate [%]	3.84±0.28	1299	4.13±0.13	1299	2.79±0.69	1298	3.68±0.14	1298	4.72±1.16	1300	

[a] 1: Control treatment (distilled water)
2: 400 mg/l CA
3: 400 mg/l HA
4: 800 mg/l HA
5: 200 mg/l CH + 400 mg/l CA
6: 400 mg/l CH + 400 mg/l CA
7: 200 mg/l CH + 400 mg/l HA + 400 mg/l CA
8: 200 mg/l CH + 800 mg/l HA + 400 mg/l CA
9: 400 mg/l CH + 400 mg/l HA + 400 mg/l CA
10: 400 mg/l CH + 800 mg/l HA + 400 mg/l CA.
[b]: Kovats index in reference [18]; [c]: Calculated Kovats index relative to C_8-C_{24} n-alkanes on the HP-5 column.

as compared to control. The HA stimulates the quantity and quality of yield as well as the growth of the plants by the effect on complex mechanisms involved in photosynthesis, cell respiration, biosynthesis of protein, enzyme activities, uptake of water and nutrient, improvement of soil structure and increase of microbial populations. The results of this research are in line with results of Salwa [22] experiment. CH is a linear β-(1, 4)-glucosamine polymer produced by deacetylation of chitin [23]. The penetration of CH into cells causes the inhibition of interference with the synthesis of mRNA and proteins [24]. The maximum amount of stems dry weight in the greenhouse was observed in 400 mg/l CH + 800 mg/l HA + 400 mg/l CA in comparison with control, while the treatment of 200 mg/l CH + 400 mg/l CA increased the yield of stems dry weight in the field. The CH as a biostimulant and bioprotectant has biological effects such as plant growth promotion, the direct growth inhibition of some pathogenic microorganisms, generally fungi and elicits induced resistance in plants against their pathogens [25]. The mode of action for HA in plant growth and development can be broadly divided into direct and indirect properties as it affects the membranes resulting in enhanced transport of nutritional elements, improved protein synthesis, enriched photosynthesis and solubilization of micronutrients [26]. These results are in line with Sheikha and AL-Malki [27]. They showed the enhancement of bean shoot and root length, fresh and dry weights of shoots, root and leaf area as well as the level of chlorophyll in leaves by application of CH. According to results of Abu-Muriefah [28], foliar application of 200 mg/l CH in most cases resulted in a significant increase in plant growth parameters in common bean (*Phaseolus vulgaris* L.) under normal or stressed conditions. In the greenhouse 400 mg/l CH + 800 mg/l HA + 400 mg/l CA caused the highest yield of shoots dry weight compared to control. The maximum increase in yield of shoots dry weight happened by application of 200 mg/l CH + 400 mg/l CA in field. The valuable effects of HA and CH on plant growth and development may be attributed to the promoting effects on nutrients uptake and nutritional status especially nitrogen, potassium and phosphorous. According to Cho *et al* [29] results, CH treatments increased the total weight of sunflower plant, as compared to control. El-Nemr *et al.* [30] found that foliar application of HA with the highest concentration improved growth traits of Cucumber (*Cucumis sativus* L.) plants in comparison with control. The essential oil content was increased by two treatments of 400 mg/l CH + 400 mg/l CA and 200 mg/l CH + 400 mg/l HA + 400 mg/l CA in comparison with control treatment in filed, while in greenhouse the highest yield was occurred by 200 mg/l CH + 800 mg/l HA + 400 mg/l CA compared to control. HA has the positive effect on cell membrane functions by promoting nutrient uptake, respiration, biosynthesis of nucleic acid, ion absorption and enzyme activity as they are hormone-like substances [31]. According to results of Said-Al Ahl *et al.* [32], application of HA and indole acetic acid improved the essential oil content of dill (*Anethum graveolens* L.) plants in the field. These results are in line with those of Radmanesh *et al.* [33] on *Satureja hortensis* L. They reported that application of CA at 6 and 8 mM increased the content of essential oil to the highest level. Loschke *et al.* [34] reported that CH causes the expression of a variety of genes involved in the plant defense response that results in increased synthesis of plant secondary metabolites. The essential oil component of menthol showed the highest yield with the treatment of 200 mg/l CH + 800 mg/l HA + 400 mg/l CA compared to control in the field. In greenhouse the treatment of 400 mg/l CH + 400 mg/l HA + 400 mg/l CA increased in comparison with control treatment. According to results of El-Gohary *et al.* [35], application of HA treatments increased the component of menthol in peppermint plants. CH was found to enhance secondary metabolite production in cell suspensions and calli of various species [36]. Other essential oil components improved by application of biostimulants in field and greenhouse. These results are in agreement with results of Bagheri *et al.* [37] on *Mentha spicata* L. and Naeem *et al.* [38] on *M. arvensis* L.

This study indicated that the content and components of essential oil in *M. piperita* L. were significantly changed by the environmental conditions. The essential oil content in field conditions was higher than in greenhouse conditions, meanwhile, the total amount of menthol and menthone in the greenhouse was greater than in field conditions. This is due to cool night temperatures in greenhouse that it can accelerate the conversion to menthone [6]. However, these results were consistent with results of Morales *et al.* [4] which stated essential oil content of *Ocimum basilicum* L. was significantly higher in the field than in the greenhouse.

CONCLUSIONS

The results of this study showed that growth and phytochemical responses of *M. piperita* L. to various biostimulants and their concentrations were

different in the greenhouse and filed conditions. In general, the content of essential oil in leaves of *M. piperita* L. in the field conditions was significantly higher than that in the greenhouse. Although the menthol content in greenhouse and field was approximately similar, the menthone content in the greenhouse was higher than that in field conditions under different biostimulants treatments. The reason for different results by application of various biostimulants in greenhouse and field is the difference between the climates of both.

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Odpowiedź biochemiczna i wzrost *Mentha piperita* po dolistnym podaniu biostymulatorów w warunkach szklarniowych i polowych

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Streszczenie

Wstęp: Produkty biostymulujące mają właściwości podnoszące jakość i plon roślin leczniczych.

Cel: Porównanie efektów spryskiwania biosymulatorami liści mięty pieprzowej.

Metody: Badanie przeprowadzono w 2015 r. metodą wybranych losowo kompletnych bloków w trzech powtórzeniach.

Wyniki: W warunkach polowych najwyższy plon suchej masy łodyg i liści otrzymano przy zastosowaniu 400 mg/l chitosanu (CH) + 400 mg/l kwasu cytrynowego (CA), zawartość olejku eterycznego przy użyciu 200 mg/l chitosanu + 400 mg/l kwasu humusowego (HA) + 400 mg/l kwasu cytrynowego oraz zawartość mentolu przy zastosowaniu 200 mg/l chitosanu + 800 mg/l kwasu humusowego + 400 mg/l kwasu cytrynowego. W warunkach szklarniowych najlepsze wyniki powyższych parametrów uzyskano, stosując odpowiednio 400 mg/l chitosanu + 800 mg/l kwasu humusowego + 400 mg/l kwasu cytrynowego, 800 mg/l kwasu humusowego oraz 400 mg/l chitosanu + 400 mg/l kwasu humusowego + 400 mg/l kwasu cytrynowego.

Wniosek: Stosowanie dolistne efektywnych biostymulantów może podnieść jakość i plon mięty pieprzowej.

Słowa kluczowe: ***Mentha piperita L., chitosan, kwas cytrynowy, kwas humusowy, olejek eteryczny, mentol***