THE EFFECT OF APPLYING WITH IMMUNOCYTOPHYTE ON THE CONTENT AND CHEMICAL COMPOSITION OF THE ESSENTIAL OIL FROM COMMON BASIL OF 'TRAKIA' CULTIVAR

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Abstract: The aim of the study was to establish the effect of the leaf treatments with Immunocytophyte on the content, yield and chemical composition of the essential oil distilled from dry leaf and stems biomass and flower spikes of common basil, 'Trakia' cultivar. The experiment was carried out in the period 2014-2016 on the Training-and-Experimental fields of the Agricultural University – Plovdiv, set by the block plot design in four replications, the plot size being 20 m2. The following variants were studied: 1) Control; 2) 0.5 tablet per 50 m^2, 3) 1 tablet per 50 m^2, 4) 1.5 tablet per 50 m^2, applied three times during the vegetation period before flowering of the second-order branches after basic fertilization with 16 kg/da of nitrogen (applied three times) and irrigation to 80-100% of water holding capacity. The results showed that the application of Immunocytophyte had a negative effect on the essential oil content. Nevertheless, higher essential oil yields were obtained in the treated variants thanks to the higher yield of dry matter.

The major components of the essential oil distilled from dry leaf and stem biomass and flower spikes are linalool, limonene, methyl chavicol and methyl cinnamate.

Key words: basil, immunocytophyte, composition, content, oil

Introduction

The sweet basil (*Ocimum basilicum* L.) is one of the most ancient medicinal plants and herbs. It is usually grown for the production of ethereal oil and also for obtaining fresh and dry leaves. The most important component of the basil is its ethereal oil. Its content varies from 0.1 to 0.45% depending on the ecological and agro-economic conditions (Arabaci and Bayram, 2004). The economically significant components of the ethereal oil that are applied in food industry, perfumery and cosmetics are methyl chavicol, methyl cinnamate and linalool. Growth regulators are chemicals applied in small quantities and lead to changes in plant growth, as evidenced by stimulation or inhibition of natural growth. Many researchers establish positive influence of different growth regulators on the vegetative development and extraction of essential oils for different types of basil. According Mahmod et al., (1996) the application of IAA (indole 3- acetic acid), and kinetin at basil increases the content of the essential oil .

Gupta, et al. (1992, 1995) reported that foliar application of triacontanol 10 mcg dose has had a positive effect on the yield of fresh herbs and extraction of essential oils in basil.

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All these facts allow us to consider that the application of biostimulators stimulates the vegetative growth and the yield of ethereal oil of basil. Considering the aforementioned, the purpose of this survey is to establish the influence of leaf treatments with Immunocytophyte on the content, the yield and the chemical composition of the essential oil distilled from dry leaf-and-stem mass and flower spikes of ordinary basil of the kind Trakiya.

Material and methods

The study was conducted during the period 2014-2016 in the experimental and integration base of the Plant Growing Department at the Agricultural University, town of Plovdiv. We made a field experiment whose main direction was to grow basil as a postharvest crop. The growing of basil starts with the preliminary production of seedlings. The period from the sowing of the seeds to the formation of the seedlings during the years of the survey lasted for 48-54 days. The experiment in the main field was made using the block method in four repetitions as the experimental lot covered an area of 20m^2 located on alluvial (previously marshy) soil. The planting of the seedlings was performed manually using standard seedlings at a distance of 70 cm between the rows and 15 cm within the row itself (96200 plants/ha). It was done on 16.07 during the three years of the survey. The gathering was conducted during the full blooming stage on 06.10 on the 82nd day after planting the seedlings during the years of the experiment. Variants of the factor:

1. Immunocytophyte 0.5 tablet per 50 m^2
2. Immunocytophyte 1 tablet per 50 m^2
3. Immunocytophyte 1.5 tablet per 50 m^2

4- no foliar fertilization (control sample).

*The treatment Immunocytophyte was conducted three times until the blossoming of the offshoots of the second order, using a small sprayer consuming 300 l/ha of solution during background fertilization with N-160 kg/ha (three times until the blossoming of the offshoots from the second order) and also irrigation in order to maintain the limit water absorption of the field – 80-95% in a layer of 0-30 cm.

When conducting the experiment, we examined the following indicators:

- yield of dry mass, content, yield and the chemical composition of the essential oil has been established using the gas chromatograph PYE UNICAM series 204.

The obtained data has been mathematically processed using the program SPSS.

Results and discussion

During all three years of the experiment, the combination of climatic factors was favorable for the growth of basil. The experimental year 2014 was characterized by the highest average monthly temperature for July and August, which combined with the high relative humidity of the air in August (70%) contributed to the better development of the plants, which resulted in higher yield of dry biomass.

The lowest values of the average monthly relative humidity of the air over the years of the survey were registered in August and early September of the year 2015, which combined with the relatively high temperatures had a negative effect on the growth of the plants, which resulted in lower yield of dry biomass compared to the other two years of the survey.

The variation of the temperature is the main factor affecting the content of essential oil. The registered lower content of oil in the dry leaf-and-stem mass and flower spikes in the year 2014 was due to the sudden drop of the temperature in early September from 28.1 ^{o}C during the third ten days of August to an average temperature of 17.9^{o}C during the first ten days of September. In 2016 the higher temperatures in September and the first ten days of October contributed to the accumulation of a larger quantity of oil in the dry leaf-and-stem mass and flower spikes.

1. Influence of the leaf treatments with Immunocytophyte on the yield of dry mass.

As regards the yield of dry mass, the obtained results show that the tested the leaf treatments with Immunocytophyte increase the yield of dry mass during each of the experimental years (Table 1). The maximum yield of dry mass during the years of the survey has been registered for the treatments with Immunocytophyte - 1 tablet per 50 m^2 with values and a relative increase compared to the control sample during the period of the survey - 8120.2kg/ha (119.4%). The tested foliar fertilizers do not influence the proportion of fresh and dry mass. There are some insignificant differences during the separate years. For the variants included in the survey, it was within 1: 5.55- 5.83 on average for the period.

Table 1. The effect of the leaf treatments with Immunocytophyte on the productivity of dry biomass

indicators	version	2014 y.	%	2015 y.	%	2016 y.	%	Average for the period	% compared to controlledlat
Yield of dry matter kg/ha	0- control 0.5 tablet 1 tablet 1.5 tablet	6820,31a 8130,85b 8080,11b 7230,35ab	100,0 119,3 118,4 106,0	6580,36a 7690,42b 7800,88b 6900,57a	100,0 116,9 118,6 104,9	7000,22a 8410,02b 8630,62b 7550,9ab	100,0 120,1 123,3 108,0	6800,29a 8040,76b 8120,2b 7330,61ab	100,0 118,3 119,4 107,8
LSD 5%		101.98		86.76		77.00		78.10	
Ratio Fresh to dry weight	0- control 0.5 tablet 1 tablet 1.5 tablet	1: 6,00 1: 5,77 1: 5,92 1: 6,09		1: 5,53 1: 5,55 1: 5,57 1: 6,02		1: 5,45 1: 5,36 1: 5,33 1: 5,65		1: 5,66 1: 5,55 1: 5,64 1: 5,83	

2. Evaluation of the leaf treatments with Immunocytophyte on the content of essential oil in the dry leaf-and-stem mass and flower spikes.

The aromatic and medicinal features of basil (*Ocimum basilicum*) are related to the presence of essential oil contained in glandular trichomes located on all overground parts.

The data in Table 2 shows that the foliar fertilizers have a negative influence on the content of essential oil. The obtained results are unidirectional during the separate years of the survey and there are proven differences from the control sample for the treatments with Immunocytophyte. On average for the period, the dry leaf-and-stem mass decreases maximally for Immunocytophyte – to 0.49% compared to the control sample of 0.53% and in the dry flower spikes it decreases most significantly for Immunocytophyte to 2.80% compared to the control sample of 2.89%.

Table 2. The effect of the the leaf treatments with Immunocytophyte on the content of essential oil in the dry leafs and stems biomass and flower spikes.

indicators	version	2014 y.	2015 y.	2016 y.	Average for the period	% compared to controlledlat-0
dry leaf and stem biomass						
content of essential oil %	0- control	0.46b	0.55b	0.59b	0.53a	100.0
	0.5 tablet	0.38a	0.52a	0.55a	0.48a	90.6
	1 tablet	0.38a	0.52a	0.56a	0.49a	92.5
	1.5 tablet	0.40ab	0.54b	0.57ab	0.50a	94.3
LSD 5%		0.04	0.02	0.035	0.29	
dry flower spikes						
content of essential oil %	0- control	2.59b	2.95c	3.14b	2.89a	100.0
	0.5 tablet	2.49a	2.83a	3.01a	2.78a	96.2
	1 tablet	2.47a	2.87b	3.06a	2.80a	96.9
	1.5 tablet	2.51a	2.86ab	3.06a	2.81a	97.2
LSD 5%		0.08	0.05	0.08	0.64	

3. Evaluation of the leaf treatments with Immunocytophyte on the yield of oil

The yield of ethereal oil is a function of the yield of plant mass and the percentage of essential oil. The analysis of experimental data (Table 3) shows that the highest yield of essential oil from the leaf-and-stem mass and the flower spikes during the separate years has been obtained when applying leaf treatments with Immunocytophyte. The maximum yield of oil from the dry leaf-and-stem mass over the years has been registered for the variant1 tablet per 50 m^2 with an average value for the period for 23 kg/ha (17%) and dry flower spikes- 78.5 kg/ha (120%). On average for the period, the maximum total yield of oil was registered for leaf treatments with Immunocytophyte - 1 tablet per 50 m^2 - 101.5 kg/ ha (119%).

Although the applied leaf treatments with Immunocytophyte decreases the content of essential oil in the dry leaf-and-stem mass and flower spikes, the obtained higher yield of oil is due to the registered higher yield of dry leaf-and-stem mass and flower spikes. Thus, the plants compensate for the lower levels of secondary compounds by means of a higher production of biomass, which leads to a higher yield of oil from unit area.

The output is an indicator showing the necessary quantity of raw material for obtaining 1 kg of essential oil. The data about it (Table 3) shows that the values for the variants with leaf treatments with Immunocytophyte are higher owing to the lower content of essential oil found in them. The largest quantity of leaf-and-stem mass for the yield of 1kg of essential oil during the years of the experiment was registered for Immunocytophyte - 1 tablet per 50 m^2 - with an average value for the period of 211.34 kg and dry flower spikes for the variant-36.11kg.

Table 3. The effect of the leaf treatments with Immunocytophyte on the yield of essential oil distilled from dry leafs and stems biomass and flower spikes

version	2014 y.			2015 y.			2016 y.		
	yield of the essential oil кg/ha	%	output	yield of the essential oil кg/ha	%	output	yield of the essential oil кg/ha	%	output
from the dry leaf and stem biomass									
0- kontrol	14.1a	100	217.39	20.0a	100	181.82	24.8a	100	169.49
0.5 tablet	17.9c	127	263.15	22.1b	111	192.31	27.7b	112	181.82
1 tablet	17.7c	126	263.15	22.3b	112	192.31	28.9b	111	178.57
1.5 tablet	16.6b	118	250.00	20.8ab	104	185.19	25.7ab	104	175.44
LSD 5%	0.13			0.11			0.14		
from the dry flower spikes									
0- kontrol	5.00a	100	38.61	74.1a	100	33.90	73.1a	100	31.84
0.5 tablet	58.2b	116	40.46	84.4c	114	35.64	85.2b	117	33.52
1 tablet	57.7b	115	40.60	88.0c	119	34.95	89.9c	123	32.79
1.5 tablet	51.9ab	104	39.90	74.3a	100	35.01	74.7a	102	33.09
LSD 5%	0.20			0.36			0.41		
total yield									
0- kontrol	64.1a	100		94.1a	100		92.9a	100	
0.5 tablet	76.1c	119		106.5b	113		112.9b	115	
1 tablet	75.4c	118		110.3b	117		118.8b	121	
1.5 tablet	68.5b	107		95.1ab	101		100.4 ab	103	
LSD 5%	0.43			0.62			1.58		

4. Influence of the leaf treatments with Immunocytophyte on the chemical composition of the essential oil obtained from dry leaf-and-stem mass and flower spikes

Essential oils are characterized by two or three comparatively high concentrations (20-70%) of some components compared to the other whose quantity is very small (Burt, 2004).

Table 4 shows that the following components have been identified: linalool, limonene, methyl chavicol and methyl cinnamate. The component whose content constitutes the largest percentage in the essential oil distilled from the dry leaf-and-stem mass and the flower spikes is linalool and the other constituents rank as follows in descending order: limonene, methyl chavicol and methyl cinnamate.

The applied leaf treatments with Immunocytophyte does not influence the content of linalool in the oil obtained from the dry leaf-and-stem mass. Its average quantity for the period varies from 55.24 to 57.04% in the individual variants and in the oil obtained from dry flower spikes, its quantity varies from 66.86 to 68.48%.

The content of limonene in the oil distilled from dry racemes varies from 4.91 to 5.88% and is almost twice as large in the oil obtained from dry leaf-and-stem mass – 9.57-10.87%. It has been proven that its content increases in the oil obtained from dry leaf-and-stem mass for the period of the survey as its maximum was registered for 1 tablet per 50 m^2 - 10.87%.

Table 4. The effect of the leaf treatments with Immunocytophyte on the chemical composition of the essential oil distilled from dry leafs and stems biomass and flower spikes

chemical ingredients %	version	2014y.	2015 y.	2016 y.	Average for the period	% compared to controlledlat	chemical ingredients %	2014y.	2015 y.	2016 y.	Average for the period	% compared to controlledlat
		essential oil distilled from dry leaf and stem						essential oil distilled from dry flower spikes				
linalool **%**	0- kontrol 0.5 tablet 1 tablet 1.5 tablet	58.19a 57.06a 57.19a 57.92a	56.53a 55.41a 55.62a 55.99a	56.41a 53.26a 54.98a 56.27a	57.04a 55.24a 55.96a 56.73a	100,0 96,8 98,1 99,5	linalool **%**	72.58a 71.15a 70.18a 72.47a	68.35a 67.58a 67.20a 68.32a	64.35a 63.02a 63.20a 64.43a	68.48a 67.25a 66.86a 68.41a	100,0 98,2 97,6 99,9
LSD 5%		4.70	4.45	5.56	3.05		LSD 5%	3.21	3.56	3.71	9.07	
lemonol **%**	0- kontrol 0.5 tablet 1 tablet 1.5 tablet	10.04a 11.11b 10.99b 10.08a	9.48a 10.26b 11.58c 9.68a	9.20a 9.85b 10.03c 9.73b	9.57a 10.41ab 10.87b 9.83a	100,0 108,8 113,6 102,7	Lemonol **%**	4.38a 5.23b 5.45b 4.55a	5.10a 5.35b 5.42b 5.29ab	5.24a 6.86b 6.78b 5.24a	4.91a 5.81a 5.88a 5.03a	100,0 118,3 119,8 102,4
LSD 5%		0.66	0.32	0.28	1.29		LSD 5%	0.24	0.22	0.36	1.55	
methyl chavicol - **%**	0- kontrol 0.5 tablet 1 tablet 1.5 tablet	2.73c 2.40b 1.98a 2.40b	2.11b 2.01ab 1.90a 1.93a	2.06b 1.99ab 1.90a 2.02ab	2.30a 2.13a 1.93a 2.12a	100,0 92,6 83,9 92,2	methyl chavicol - **%**	0.9a 0.10ab 0.11b 0.08a	0.10a 0.11a 0.13b 0.10a	0.10a 0.12ab 0.14b 0.11a	0.10a 0.11ab 0.13b 0.10a	100,0 110,0 130,0 100,0
LSD 5%		0.15	0.14	0.14	0.58		LSD 5%	0.019	0.017	0.028	0.028	
methyl cinnamate - **%**	0- kontrol 0.5 tablet 1 tablet 1.5 tablet	0.42b 0.37a 0.38a 0.41b	0.40b 0.38ab 0.37a 0.40b	0.53b 0.48a 0.49a 0.51ab	0.45a 0.41a 0.41a 0.44a	100,0 93,2 93,2 100,0	methyl cinnamate- **%**	0.40b 0.37ab 0.36a 0.36a	0.52c 0.45b 0.42a 0.50c	0.64b 0.56a 0.55a 0.59ab	0.52a 0.46a 0.44a 0.49a	100,0 88,5 84,6 94,2
LSD 5%		0.030	0.028	0.032	0.15		LSD 5%	0.033	0.028	0.046	0.24	

There is a tendency to an increase of its content in the oil obtained from dry flower spikes for the leaf treatments with Immunocytophyte. Under the influence of treatments with Immunocytophyte, the content of methyl chavicol in the oil obtained from dry leaf-and-stem mass decreases. On the contrary, in the oil obtained from dry flower spikes it has been proven to increase during the years, reaching its maximum after applying per 1 tablet per 50 m^2. The treatments with Immunocytophyte reduce the content of methyl cinnamate in the oil obtained from dry leaf-and-stem mass and flower spikes.

Conclusions

The threefold application of leaf treatments with Immunocytophyte leads to an increase in the yield of dry mass from unit area (kg/ha) in all treated variants. The strongest effect has been registered for Immunocytophyte1 tablet per 50 m^2.

After the threefold treatment with Immunocytophyte, the percentage of the essential oil in the dry leaf-and-stem mass and the flower spikes decreases. This decrease is the largest after applying Immunocytophyte1 tablet per 50 m^2 with proven difference compared to the control sample over the years.

The leaf treatments with Immunocytophyte has a positive effect on the yield of essential oil from dry leaf-and-stem mass and flower spikes from unit area (kg/ha), which increases the total yield. The maximum yield was registered for Immunocytophyte1 tablet per 50 m^2, which increases the yield of essential oil by 19% on average for the period compared to the control sample.

The leaf treatments with Immunocytophyte with have a positive effect on the content of limonene in the essential oil distilled from dry leaf-and-stem mass and flower spikes and also on the content of methyl chavicol in the essential oil obtained from dry flower spikes. The leaf treatments with Immunocytophyte do not influence the content of linalool in the oil obtained from the respective organs.

The leaf treatments with Immunocytophyte have a negative effect on the content of methyl cinnamate in the oil obtained from dry leaf-and-stem mass and flower spikes and the content of methyl chavicol in the oil obtained from dry leaf-and-stem mass.

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