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EFFECT OF FERTILIZATION ON *Pimpinella Anisum L.* IN DIFFERENT LOCATIONS IN SERBIA

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Abstract

Aniseed (*Pimpinella anisum* L.) is a plant from parsley family, widely cultivated for fruit and essential oil. Aniseed is an important raw natural material mostly used in medicine, pharmaceutics, perfumery and cosmetic industries. It is also used as functional food; it is added to confectionary products and alcoholic beverages. Recent research found that aniseed has an antimicrobial and antifungal properties, as well as antioxidant effect on human health. Also, it is found that aniseed has insecticidal activity against storage pests and mosquitoes. The study of this plant is focused on its morphological and productive traits in three locations in Serbia (Mošorin, Veliki Radinci and Ostojićevo), with different microclimatic and soil conditions. Field trials were conducted during two successive years (2011/12) by randomised block system. The experiment has included the application of different types of fertilisers approved for organic production system (Slavol, Bactofil B-10, Royal Ofert biohumus, vermicompost), as well as a chemical fertiliser (NPK) used in conventional agriculture. Plots where no fertiliser was applied were used for control purposes. The results showed that the location had a great influence on plant height, number of umbels per plant, number of seeds in an umbel, 1000 seed mass and whole plant mass in both investigated years. In 2011, the locations had an influence on the umbel diameter and, in 2012, on yield of essential oil per hectare. Different fertiliser types had an influence only in the first investigated year in case of whole plant mass.

Keywords: *aniseed, morphological features, productive traits, yield*

Introduction

Anise (*Pimpinella anisum* L.) is a plant from parsley family, widely cultivated for fruit and essential oil. Anise is an important raw natural material mostly used in medicine, pharmaceutics, perfumery and cosmetic industries. It is also used as functional food; it is added to confectionary products (honey cookies, candies) and alcoholic beverages (liqueurs and sweet flavored wines) (Acimovic et al. 2013). Medicinal applications include use as an appetizer, carminative and sedative agent, or for stimulating milk production in breastfeeding mothers (Ozel, 2009). Recent research found that anise essential oil poses antioxidant potential (Rajeshwari et al., 2011), antimicrobial activity (Kubo and Himejima, 1991; Kosalec et al., 2005; Ozcan and Chalchat, 2006; Yazdani et al., 2009) and insecticidal activity against storage pests (Tunc and Erler, 2000) and mosquitoes (Prajapati et al., 2005; Erler et al., 2006). It is known that organic fertilisers compared to the chemical ones have a lower content of nutrients and act more slowly, but they are more effective than chemical in continuous use, and have a complex chemical composition (Naguib, 2011). From available literature, there are a of couple papers on the application of chemical and organic fertilisers in growing practices of anise (Jevđović and Maletić, 2006; Darzi et al., 2012; Nabizadeh et al., 2012; Jevđović et al., 2012). For this reason, the aim of our study was to investigate the application of various types of fertilisers available in our country, whose application is permitted in organic

production system according to Law on Organic Production of Serbia. The scientific objective of this paper was to examine the influence of fertilisation with different types of organic and microbiological fertilisers on yield and morphological traits of anise in an organic farming system.

Materials and Methods

Field experiments were carried out during the growing season of 2011 and 2012, at the three research localities: L1 (Mošorin, 45°18’N, 20°09’E), L2 (Veliki Radinci, 45°02’N, 19°40’E), and L3 (Ostojićevo, 45° 54’N, 20° 09’E).

The four-replicate trial was set up according to the randomised block system and treatments included six different fertilisers: control (F1), Slavol (F2), Bactofil B-10 (F3), Royal Ofert granules (F4), vermicompost (F5) and chemical fertiliser (F6).

Slavol and Bactofil B-10 are microbiological fertilisers. Slavol contains *Azotobacter chroococcum, A. vinelandi, Derxia sp*. *Bacillus megaterium, B. lichenformis, B. subtilis*. Bactofil B-10 contain *Azotobacter vınelandı, Azospırıllum brasılense, A. lıpoferum, Bacıllus megaterıum, B. suptılıs, B. cırkulans, B. polymıxa*, *Pseudomonas fluorescens*. Apart from bacteria, these fertilisers contain natural vitamins and growth stimulator.

Royal Ofert biohumus is made from organic waste from poultry and pig farms inoculated with domestic fly larvae. Vermicompost is modified cattle manure with *Lumbricus terrestris*.

The requested quantities of fertilisers, except Slavol, were applied and incorporated in the 5 cm layer of soil before the sowing of anise seeds. The doses of application investigated fertilisers are: Slavol (7 l ha^{-1} by watering twice during vegetation), Bactofil B-10 (1.5 l ha^{-1}), Royal Ofert biohumus (3 t ha^{-1}), vermicompost (5 t ha^{-1}), and chemical fertiliser NPK (400 kg ha^{-1} in formulation 15:15:15).

Aniseed (local cultivar) was sown during April, in continuous rows 35 cm apart, and with 200 plants per square meter. The plots were kept weed-free by hand weeding and hoeing. One sample is presented by 10 randomly selected plants from the central row from each fertilised plot.

In time of full flowering (June), the plant height (PH) was measured. Harvest was performed when the seed turned colour to brownish-yellow (August), and after it had dried in the shadow for a couple of days to obtain constant weight, after which the whole plant mass was measured (WPM). Umbel diameter (UD), number of umbels per plant (No UP), number of seeds in the umbel (No SU) were also measured. The plants were manually harvested in order to determine seed weight per plant (SWP). Also, we calculated the harvest index (HI=SWP/WPM*100), seed yield per hectare (SYH=SWP*2000000) and essential oil yield per hectare (EOH=SYH*% of essential oil in seeds). Determination of essential oil in seeds was performed on Faculty of Chemistry (Belgrade) by distillation with Clevenger-type apparatus. The weight of 1000 seeds was measured (TSM) in Seed-testing laboratory (Sremska Mitrovica).

Soil samples were taken from 0-30 cm, and analysed in Soil-testing laboratory of Agricultural Extension Service, Sremska Mitrovica, and are shown in table 1.

Table 1. Agrochemical analysis of soil

	pH (KCl)	$CaCO_3$ (%)	Humus (%)	Total nitrogen (%)	AlP_2O_5 (mg 100 g^{-1})	AlK_2O (mg 100 g^{-1})
L1	7.3	8.4	2.7	0.18	81.6	75.1
L2	7.1	2.0	2.5	0.16	22.4	21.7
L3	7.3	8.8	2.2	0.14	17.6	30.3

For determination of soil pH potentiometric method was used, for $CaCO_3$ molar volume of carbon dioxide was used, humus content was determined by Turin method, total nitrogen by Kjeldahl method, available phosphorous and potassium with Al-method, Egner-Riehem.
The obtained experimental data was processed by a mathematical statistical procedure using the statistical package STATISTICA 8.0 for Windows (Analytical software, Faculty of Agriculture, Novi Sad, Serbia), while the least significant difference (LSD) test was used for individual comparison of differences between means. Correlation analysis was performed by Statistica 8.0 package to determine the relationship among the characters according to Pearson method.

Results and Discussion

As it can be seen from table 2, in the first investigated year, applying chemical NPK type of fertiliser had a significant influence only in case of the whole plant mass. On the control plot and on plot where the biofertiliser Slavol was applied, the lowest values were achieved in comparison with other fertilised plots.

Table 2. Morphological and productive traits of anise in 2011

F	L	PH	UD	No UP	No SU	TSM	SWP	WPM	HI	SYH	EOH
-	**1**	53.18	6.28	19.17	104.95	4.39	9.01	19.36	47.62	1801.40	70.53
-	**2**	48.84	6.61	17.50	114.88	4.08	8.29	17.76	47.13	1657.39	67.62
-	**3**	41.19	5.90	15.21	98.42	4.77	7.22	15.90	45.51	1444.89	54.72
1	**-**	46.16	6.11	15.67	100.19	4.38	6.85	15.46	47.15	1371.04	52.17
2	**-**	47.47	6.05	16.50	104.15	4.36	7.44	16.21	46.63	1488.70	56.95
3	**-**	47.56	6.19	17.50	105.03	4.51	8.21	17.41	46.95	1641.16	63.85
4	**-**	47.21	6.38	17.50	106.19	4.36	8.27	17.88	46.50	1654.62	68.87
5	**-**	48.97	6.51	18.25	105.90	4.40	8.70	18.49	46.66	1740.07	67.07
6	**-**	49.03	6.34	18.33	115.03	4.46	9.56	20.60	46.63	1911.78	76.84
F		ns	ns	ns	ns	ns	Ns	3.54	ns	ns	ns
L		1.52	0.45	2.42	13.71	0.31	Ns	1.44	ns	ns	ns
F*L		ns	ns	ns	ns	0.76	Ns	ns	ns	ns	ns

LSD at 5% level: ^{ns}Not significantly different.

Location had a significant influence in case: PH, UD, No UP, No SU, TSM and WPM The highest PH, No UP and WPM were recorded at L1. On L2, the highest UD and No SU were recorded, but the smallest TSM. The highest value of TSM was recorded on L3 (4.77 g), and interaction F*L was significant only in case of this parameter.
According to table 3, in 2012, the application of different sources of fertilisers had no effect on investigation parameters. Location in this year, as in previous, had an influence on PH, No UP, No SU, TSM, WPM, but also on EOH. In this investigated year, the location was not a significant influence on UD compared to the previous experimental year. Like in the previous year, the highest PH and No UP were on L1. The highest No UP and WPM were on L2, and the highest TSM was on L3.

Table 3. Morphological and productive traits of anise in 2012

F	L	PH	UD	No UP	No SU	TSM	SWP	WPM	HI	SYH	EOH
-	**1**	51.36	6.62	18.17	111.47	3.66	7.39	17.52	42.26	1478.08	52.27
-	**2**	46.70	6.47	16.46	127.77	3.71	7.76	18.36	42.76	1552.18	59.01
-	**3**	40.92	6.23	14.58	107.87	4.36	6.87	15.82	43.32	1373.48	44.33
1	**-**	46.50	6.22	15.50	111.59	3.92	6.46	15.72	40.99	1292.99	44.94
2	**-**	47.02	6.62	16.25	113.53	3.88	7.13	16.58	43.21	1426.64	50.15
3	**-**	45.61	6.42	16.33	115.04	3.92	7.39	16.80	43.30	1477.85	50.64
4	**-**	46.72	6.52	16.67	116.39	3.95	7.54	17.79	43.74	1509.02	55.08
5	**-**	46.39	6.35	16.67	117.74	3.93	7.68	18.05	42.60	1535.50	52.45
6	**-**	45.71	6.53	17.00	119.94	3.87	7.83	18.46	42.82	1565.49	57.93
F		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
L		1.46	ns	2.64	13.37	0.10	ns	1.54	ns	ns	10.10

F*L	3.58	ns	ns	ns	0.12	ns	ns	ns	ns	ns

LSD at 5% level: [ns]Not significantly different

Morphological traits of anise (PH, No UP, No SU) relate to plant's genetic structure, growing conditions and agricultural practices. Most investigated agricultural practices are plant densities (Tuncturk and Yildirim, 2006), sowing dates (Zehtab-Salmasi et al., 2001), irrigation (Zehtab-Salmasi et al., 2001; Aloghareh et al., 2013) and fertilisation (Jevđović and Maletić, 2006; Yassen et al., 2010; Nabizadeh et al., 2012; Darzi et al., 2012). Location was also very influential, but only a couple of authors investigated this factor. Ullah (2012) investigated fruit yield and quality of anise in relation to agronomic and environmental factors in two different locations in Germany, but it was not a comparison between locations. Results of Al-Awak (2010) showed that there were significant differences between two locations in Syria regarding to production, essential oil percentage and anethole content.

In our experiment on PH in both investigated years, locations were of considerable influence and in the second year F*L interaction was significant. PH varied between 40.92 and 53.18 cm which is similar to Curioni et al. (2003) findings, who reported that the plant height varied between 52.3 and 45.17 cm, on average 49.57 cm. Results obtained by Nabizadeh et al. (2012) showed that different levels of chemical nitrogen (46% urea nitrogen) and biological nitrogen (*Azotobacter*) had no significant influence on PH, which supported our data. UD in our experiment ranged from 5.90 to 6.62 cm, and No UP 14.58 to 19.17. In an experiment conducted by Zehtab-Salmasi et al. (2001) this parameter was from 8.23 to 17.57, and Tuncturk and Yildirim (2006) achieved 9.26–12.20 umbels per plant. The total number of umbels in the investigations of Curioni et al. (2003) on average was 23.96 per plant. No SU was between 98.42 and 127.77, and TSM varied between 3.66 and 4.77 g. Ipek et al. (2004) reported that the TSM was from 4.01–5.46 g, which is a higher value than in our results. The application of different fertilisers had no influence on this parameter in our experiment, as the results of Darzi et al. (2012) indicated, TSM was not affected by vermicompost and phosphate solubilizing bacterium.

In our experiment, fertiliser or location had no influence on SWP and in the first year on average it was 8.17 g, and in the second 7.34 g. Yassen et al. (2010) reached a conclusion that this parameter in case of anise greatly varied – between 2.11 and 9.80 g depending on nitrogen fertilisers and growth tryptophan stimulants. WPM i.e. biological yield per plant in the first growing season was on average 17.67 g and in the second 17.23 g.

The HI was from 42.78 to 46.75 depending on the investigated year. As Zehtab-Salmasi et al. (2001) report, HI increased at the latest sowing date (from 39.67 to 40.31%) and in the water deficit (from 37.58 to 42.67%).

SYH was not influenced by locality or fertilisation in both investigated years. In experiments of Jevđović and Maletić (2006), the application of fertilisers had a significant influence on the yield, and the best results were achieved by biological fertilizer Bactofil. EOH was in average in the first year (64.29 kg ha^{-1}) and it did not depend on fertilisation or location. In the second investigated year, average value of EOH was lower – 51.87 kg ha^{-1}, and it depended on the growing location.

Conclusion

The weather conditions greatly affected the following parameters: plant height, quantity of seeds per umbel, weight of 1000 seeds and yield of essential oil per hectare. The influence of locality was notable in all tested parameters, except in harvest index, whereas fertilisation had significantly influenced seed yield per hectare and yield of essential oil per hectare. The application of vermicompost contributed to maximum plant height and number of seeds per umbel. The application of Royal Ofert biohumus resulted in the highest diameter of umbel

and content of essential oil. The application of microbiological fertiliser Bactofil B-10 in the pre-sowing phase enabled maximum weight of 1000 seeds. The highest value for the following parameters achieved by application of chemical fertiliser was recorded in: number of umbels per plant, seed yield per hectare, as well as whole-plant mass and yield of essential oil per hectare.

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