

# Effect of fertilization on *Pimpinella anisum* L. in different locations in Serbia

Milica Aćimović, Željko Dolijanović, Snežana Oljača, Dušan Kovačević, Mićo Oljača

*Corresponding author email:* [acimovicbabićmilica@gmail.com](mailto:acimovicbabićmilica@gmail.com)

**ABSTRACT:** The study is focused on morphological and productive traits of anise in three locations in Serbia, with different microclimatic conditions. Also, experiment has included application of different types of fertilizers approved for organic production system, as well as chemical fertilizer used in conventional agriculture. Plots where no fertilizer was applied were used for control purposes. The results showed that location had a great influence on plant height, number of umbel per plant, number of seeds in umbel, 1000 seed mass and whole plant mass in both investigated years. In 2011, locations had influence on umbel diameter and, in 2012, on yield of essential oil per hectare. Different fertilizer types had influence only in 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 Apiaceae family, widely cultivated for fruit and essential oil, used in medicine and perfumery, and for flavoring. Medicinal applications include use as an appetizer, carminative and sedative agent, or for stimulating milk production in breast feeding 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).

It is known that organic fertilizers compared to the chemical 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 couple papers on the application of chemical and organic fertilizers 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 fertilizers 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 fertilization with different types of organic and microbiological fertilizers on yield of anise in an organic farming system.

## Materials and Methods

### Plant material and field trial

Field experiments were carried out during the growing season of 2011 and 2012, at the three research stations: 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 randomized block system and treatments included 6 different fertilizers: control (F1), Slavol (F2), Bactofil B-10 (F3), Royal Ofert granules (F4), vermicompost (F5) and chemical fertilizer (F6).

Slavol and Bactofil B-10 are microbiological fertilizers. Slavol contain *Azotobacter chroococcum*, *A. vinelandi*, *Dermia* sp. *Bacillus megaterium*, *B. lichenformis*, *B. subtilis*. Bactofil B-10 contain *Azotobacter vinelandi*, *Azospirillum brasilense*, *A. lipoferum*, *Bacillus megaterium*, *B. subtilis*, *B. circulans*, *B. polymixa*, *Pseudomonas fluorescens*. Beside bacterias, this fertilizers contain natural vitamins and growth stimulator.

Royal Ofert granules are organic waste from poultry and pig farms inoculated with larval domestic fly, and vermicompost are modified cattle manure with *Lumbricus terrestris*.

The requested quantities of fertilizers except Slavol, were applied and incorporated to the 5 cm layer of soil before the sowing of anise seeds. Dose of application investigated fertilizers are: Slavol (7 l ha<sup>-1</sup> by watering twice during vegetation), Bactofil B-10 (1.5 l ha<sup>-1</sup>), Royal Ofert granules (3 t ha<sup>-1</sup>), vermicompost (5 t ha<sup>-1</sup>), and chemical fertilizer NPK (400 kg ha<sup>-1</sup> 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 central row from each fertilized plot.

In time of full flowering (June), was measured the plant height (PH). Harvest was performed when the seed turned color to brownish yellow (August), and after dried in shadow for couple days to obtain average weight, and the whole plant mass was measured (WPM). Also, were measured: umbel diameter (UD), number of umbels per plant (No UP), number of seeds in umbel (No SU). After that plants were manually harvested in order to determine seeds weight per plant (SWP). Also, we calculate 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 seed was performed on Faculty of Chemistry (Belgrade) by distillation on Clevenger-type apparatus. In seed testing laboratory (Sremska Mitrovica) the weight of 1000 seeds was measured (TSM).

### Soil and weather conditions

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

Table 1. Agrochemical analysis of soil

	pH (KCl)	CaCO <sub>3</sub> (%)	Humus (%)	Total nitrogen (%)	AlP <sub>2</sub> O <sub>5</sub> (mg 100 g <sup>-1</sup> )	AlK <sub>2</sub> O (mg 100 g <sup>-1</sup> )
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 pH soil was used potentiometric method, for CaCO<sub>3</sub> molar volume of carbon dioxide, humus content was determine by Turin method, total nitrogen by Kjeldahl method, available phosphorous and potassium with Al-method, Egner-Riehem.

### Computation and data analysis

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

### Morphological and productive traits

As it can be seen from table 2, in first investigated year applying chemical NPK type of fertilizer had significant influence only in case of the whole plant mass. On control plot and on plot where the biofertilizer Slavol was applied, it achieved the lowest values comparing to other fertilizers 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: <sup>ns</sup>Not significantly different.

Location had significant influence in case: PH, UD, No UP, No SU, TSM and WPM. The highest PH, No UP and WPM was recorded at L1. On L2 was recorded the highest UD and No SU, 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 application of different sources of fertilizers had no effect on investigation parameters. Location in this year, as in previous, had influence on PH, No UP, No SU, TSM, WPM, but also on EOH. In this investigated year, the location wasn't a significant influence on UD compared to the previous experimental year. Like in previous year, the highest PH and No UP was on L1. The highest No UP and WPM was 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: <sup>ns</sup>Not significantly different

Morphological characteristic of anise (PH, No UP, No SU) relates to plant genetic structure, growing conditions and agricultural practices. Most investigated agricultural practices are plant densities (Tuncurk and Yildirim 2006), sowing dates (Zehtab-Salmasi et al. 2001), irrigation (Zehtab-Salmasi et al. 2001, Aloghareh et al. 2013) and fertilization (Jevdović and Maletić 2006; Yassen et al. 2010; Nabizadeh et al. 2012; Darzi et al. 2012). Also location has a great influence, but only couple authors investigate 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 wasn't 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 had significant influence, and in the second year interaction F\*L was significant. PH varied between 40.92 and 53.18 cm what is similar to findings Curioni et al. (2003) who reported that the plant height varied between 52.3 and 45.17 cm, on average 49.57 cm. Results Nabizadeh et al. (2012) showed that different levels of chemical nitrogen (46% urea nitrogen) and biological nitrogen (Azotobacter) had no significant influence on PH, what supported our experiments. UD in our experiment was from 5.90 to 6.62 cm, and No UP 14.58 to 19.17. In experiment conducted by Zehtab-Salmasi et al. (2001) this parameter was from 8.23 to 17.57, and Tuncurk and Yildirim (2006) achieve 9.26–12.20 umbels per plant. The total number of umbels in investigations of Curioni et al. (2003) in 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. In our experiment, application of different fertilizers had no influence on this parameter, as the results Darzi et al. (2012) indicated that TSM was not affected by vermicompost and phosphate solubilizing bacterium.

On SWP in our experiment have no influence fertilizer or location and in first year in average was 8.17 g, and in second 7.34 g. Yassen et al. (2010) refer that this parameter in case of anise varied in high range – between 2.11 and 9.80 g depending on nitrogen fertilizers and growth tryptophan stimulants. WPM i.e. biological yield per plant in first growing season was in average 17.67 g and in 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 wasn't influenced by locality and fertilization in both investigated years. In experiments of Jevdović and Maletić (2006), the application of fertilizers has a significant influence on yield, and the best results were showed by biological fertilizer Bactofil. EOH was in average in the first year (64.29 kg ha<sup>-1</sup>) and it was not depending of fertilization and location. In second investigated year, average value of EOH was lower – 51.87 kg ha<sup>-1</sup>, and depend on 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 fertilization 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 granules resulted in highest diameter of umbel and content of essential oil. The application of microbiological fertilizer Bactofil B-10 in the pre-sowing phase enabled maximum weight of 1000 seeds. By application of chemical fertilizers recorded the highest value for the following parameters: 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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Table 4.

Abbreviations and Symbols	
PH	Plant Height (cm)
UD	Umbel Diameter (cm)
No UP	Number of Umbel per Plant
No SU	Number of Seeds per Umbel
TSM	Thousand Seed Mass (g)
SWP	Seed Weight per Plant (g)
WPM	Whole Plant Mass (g)
HI	Harvest Index (%)
SYH	Seed Yield per Hectare (kg ha <sup>-1</sup> )
EOH	Essential Oil Yield per Hectare (kg ha <sup>-1</sup> )

## References

- Al-Awak R.H. 2010. The effect of sowing dates and potassium fertilization on the yield and the quality of the produced essential oil of anise plant (*Pimpinella anisum* L.). Master thesis, Faculty of Agriculture, Damascus University.
- Aloghareh R.R., Tahmasebi B.K., Safari A., Armand R. and Odivi A.G. 2013. Changes in essential oil content and yield components of anise (*Pimpinella anisum* L.) under different irrigation regimes. *International Journal of Agriculture and Crop Sciences (IJACS)* 2013/6-7/364-369).
- Curioni, A.O., Arizio O.P., Garcia M. and Alfonso W. 2003. Preharvest phenometric characteristics of anise *Pimpinella anisum* L. plants under various agroecoclimatic conditions. *Revista Brasileira de Plantas Medicinalis*, 5: 17-22.
- Darzi M.T., Sayedhadi M.H. and Rejali F. 2012. Effects of the application of vermicompost and phosphate solubilizing bacterium on the morphological traits and seed yield of anise (*Pimpinella anisum* L.). *Journal of Medicinal Plants Research*, 6: 215-219.
- Ipek A., Demirayak S. and Gurbuz B. 2004. A study on the adaptation of some anise (*Pimpinella anisum* L.) population to Ankara conditions. *Journal of Agricultural Sciences*, 10: 202-205.
- Jevdović R. and Maletić R. 2006. Effects of application of certain types of fertilizers on anise seed yield and quality. *Journal of Agricultural Sciences, Belgrade*, 51: 117-122.
- Jevdović, R., Todorović, G., Marković, T., Kostić, M., Sivčev, I., Stanković, S. 2012. Effect of fertilization on yield, seed quality and content of essential oil of anise (*Pimpinella anisum* L.) and dill (*Anethum graveolens* L.). *Proceedings of the 7<sup>th</sup> CMAPSEEC*, 27<sup>th</sup>-31<sup>st</sup> May, 2012, Subotica, Republic of Serbia.
- Kosalec I., Pepeljnjak S. and Kutrak D. 2005. Antifungal activity of fluid extract and essential oil from anise fruits (*Pimpinella anisum* L., Apiaceae). *Acta Pharmaceutica*, 55: 377-385.
- Kubo I. and Himejima M. 1991. Anethole, a synergist of polygodial against filamentous microorganisms. *Journal of Agriculture and Food Chemistry*, 39: 2290-2292.
- Nabizadeh E., Habibi H. and Hosainpour M. 2012. The effect of fertilizers and biological nitrogen and planting density on yield quality and quantity *Pimpinella anisum* L. *European Journal of Experimental Biology*, 2: 1326-1336.
- Naguib, N.Y.M. 2011. Organic vs chemical fertilization of medicinal plants: a concise review of researches. *Advances in Environmental Biology* 5(2): 394-400.
- Ozcan M.M. and Chalchat J.C. 2006. Chemical composition and antifungal effect of anise (*Pimpinella anisum* L.) fruit oil at ripening stage. *Annals of Microbiology*, 56: 353-358.
- Ozel A. (2009). Anise (*Pimpinella anisum*): changes in yields and component composition on harvesting at different stages of plant maturity. *Experimental Agriculture*, 45: 117-126.
- Rajeshwari C.U., Abirami M. and Andallu B. 2011. In vitro and in vivo antioxidant potential of aniseeds (*Pimpinella anisum*). *Asian Journal of Experimental Biological Sciences*, 2: 80-89.
- Tunc I. and Erler F. 2000. Fumigant activity of anethole, a major component of essential oil of anise *Pimpinella anisum* L. *Integrated Protection of Stored Products IOBC Bulletin*, 23: 221-225.
- Tuncturk M. and Yildirim B. 2006. Effect of seed rates on yield and yield components of anise (*Pimpinella anisum*). *Indian Journal of Agricultural Sciences*, 76: 679-681.
- Ullah H. 2012. Fruit yield and quality of anise (*Pimpinella anisum* L.) in relation to agronomic and environmental factors. Doctoral thesis, Faculty of Agricultural and Nutritional Sciences, and Environmental Management Justus Liebig University Giessen, Germany.

- Yassen A.A, Mazher A.A.M. and Zaghloul S.M. 2010. Response of anise plants to nitrogen fertilizer and foliar spray of tryptophan under agricultural drainage water. *New York Science Journal*, 3: 120-127.
- Yazdani D., Rezazadeh S., Amin G., Zainal Abidin M.A., Shahnazi S. and Jamalifar H. 2009. Antifungal activity of dried extracts of anise (*Pimpinella anisum* L.) and star anise (*Illicium verum* Hook, f.) against dermatophyte and saprophyte fungi. *Journal of Medicinal Plants*, 8: 24–29.
- Zehtab-Salmasi S., Javanshir A., Omidbaigi R., Alyari H. and Ghassemi-Golezani K. 2001. Effects of water supply and sowing date on performance and essential oil production of anise (*Pimpinella anisum* L.). *Acta Agronomica Hungarica*, 49: 75-81.