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ORIGINAL PAPER

EFFECT OF THE SIZE OF CONTAINERS AND NUMBER OF PLANTS PER POT ON CONCENTRATIONS OF NITRATES(V), MERCURY AND LEAD IN THE HERBAGE OF SIX BOTANICAL VARIETIES OF BASIL*

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Abstract

A two-factorial greenhouse experiment was conducted in 2012-2014 in a greenhouse owned by the Department of Horticulture at the University of Warmia and Mazury in Olsztyn (NE Poland). The experiment had a randomized block design with three replications. The experimental factors were (1) six botanical varieties of basil: sweet basil, Thai 'Siam Queen' basil, Greek 'Minette' basil, purple basil, lemon basil and cinnamon basil, and (2) container size and the number of plants per pot: 0.7 dm^3 container with one plant per pot (control), 3.0 dm^3 container with one plant per pot, 3.0 dm^3 container with four plants per pot. In successive years of a three-year experiment, between 22 and 24 February, containers filled with organic substrate were placed on movable tables in the greenhouse, and arranged depending on a pot size (0.7 dm^3 and 3.0 dm^3). The organic substrate had salt concentration of 1.5 g dm^{-3}, pH 4.99, and the following chemical composition: N-NO_3 – 112 mg dm^{-3}, P – 257 mg dm^{-3}, K –143 mg dm^{-3}, Ca – 480 mg dm^{-3}, Mg – 383 mg dm^{-3}. Total yield and marketable yield were identical, and were expressed in kg m^2. Representative samples collected from the marketable yield were dried, ground and assayed for the content of nitrates(V) – by the colorimetric method with the use of salicylic acid, and heavy metals Hg and Pb – by atomic absorption spectrometry (AAS). Cinnamon basil and Greek 'Minette' basil were characterized by a significantly higher marketable yield of fresh herbage, compared with the remaining varieties. The botanical variety had no significant influence on the content of mercury and nitrates in herbage. The results of the three-year experiment indicate that growing basil plants in 3.0 dm^3 containers with four plants per pot significantly decreases herbage yield, but prevents excessive accumulation of nitrates(V) and mercury in the edible parts of plants.

Keywords: *Ocimum basilicum* L., greenhouse, heavy metals.

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INTRODUCTION

Basil is a popular culinary herb and ornamental plant, which is also used in herbal medicine. This herbaceous annual plant has high temperature requirements, and is therefore difficult to cultivate in field conditions in Poland. Alternatively, basil can be grown in a greenhouse, in containers under cover. Potted basil can be a source of extra income for vegetable growers, and greenhouse production can provide a year-round supply of fresh herbs (Jadczak et al. 2006, Kucharski, Mordalski 2009, Lee, Scagel 2009, Biesiada, Kuś 2010, Nurzyńska-Wierdak 2012). Basil grown in 500-750 cm^3 containers with 15 plants per pot are available on the market. However, insufficient moisture and nutrient supply suppress root growth, and potted basil plants eventually wilt and die. Studies investigating the correlation between the amount of substrate and plant characteristics show that excessively small containers decrease the yield and quality of basil herbage (Nurzyńska-Wierdak et.al. 2012).

Basil comes in many varieties that differ in yield and quality, therefore this study was undertaken to determine the herbage yield of six basil varieties and the concentrations of compounds exerting adverse health effects in basil leaves.

MATERIAL AND METHODS

A two-factorial experiment was conducted in 2012-2014 in a greenhouse owned by the Department of Horticulture at the University of Warmia and Mazury in Olsztyn (NE Poland). The experiment had a randomized block design with three replications. The first experimental factor consisted of six botanical varieties of basil: sweet basil Thai 'Siam Queen' basil, Greek 'Minette' basil, purple basil, lemon basil and cinnamon basil. The second experimental factor comprised the size of containers and the number of plants per pot: 0.7 dm^3 container with one plant per pot (control), 3.0 dm^3 container with one plant per pot, 3.0 dm^3 container with four plants per pot. In the consecutive years of a three-year experiment, between 22 and 24 February, containers filled with organic substrate were placed on movable tables in the greenhouse, depending on a pot size (0.7 dm^3 and 3.0 dm^3). The organic substrate made by company Hollas had a salt concentration of 1.5 g dm^{-3}, pH 4.99, and the following chemical composition: N-NO_3 – 112 mg dm^{-3}, P – 257 mg dm^{-3}, K –143 mg dm^{-3}, Ca – 480 mg dm^{-3}, Mg – 383 mg dm^{-3}.

Four basil seeds were sown, and one (strongest) seedling was left per pot at the two(three)-leaf stage. Thinning was noexcluded from the treatment with four basil plants per pot. An experimental unit area was 1 m^2, and each replicate consisted of 640.7 dm^3 containers with one plant per pot, and

253.0 dm^3 containers with one plant per pot or four plants per pot. Greenhouse conditions were adjusted to the growth stage of basil plants, and the recommended cultivation practices for basil were applied during the growing season. Individual weeds and moss buds were removed, and the organic substrate was aerated using a three-pronged cultivator. Chemical crop protection agents were not applied during the experiment. Yellow sticky traps were placed over the containers to control the number of pests (greenhouse whiteflies, Sciaridae). *Encarsia formosa* (3 insects per m^2) was used for biological control of greenhouse whiteflies. Basil herbage was harvested upon inflorescence emergence. Total yield and marketable yield were identical, and were expressed in kg m^{-2}. Representative samples collected from the marketable yield were dried, ground and assayed for the content of nitrates(V) – by the colorimetric method with the use of salicylic acid, and heavy metals Hg and Pb – by atomic absorption spectrometry (AAS). The concentrations of heavy metals in basil herbage were determined in the laboratory of the Chemical and Agricultural Station in Olsztyn, under Accreditation Certificate no. AB 277 issued by the Polish Center for Accreditation in Warsaw. Due to small differences in the analyzed values, the results were given as means for experimental years.

The results were processed statistically with analysis of variance (ANOVA). The significance of differences between means was estimated by the Tukey's range test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The experimental factors had a significant effect on the yield and nutritional value of basil herbage. Six botanical varieties of basil analyzed in this three-year experiment differ in leaf color, plant height, habit and aroma. The differences in yield characteristics of basil plants, observed in this study, were determined genetically and influenced by container size and the number of plants per pot (Table 1).

In 2012, cinnamon basil and Greek 'Minette' basil produced a significantly higher marketable yield of fresh herbage than purple basil did. In 2013, Greek 'Minette' basil was characterized by the highest yield, followed by cinnamon basil, whereas lemon basil produced the lowest marketable yield of fresh herbage. The marketable yield of purple basil and sweet basil was also low. In 2014, cinnamon basil and Greek 'Minette' basil were characterized by a significantly higher marketable yield of fresh herbage than the low-yielding Thai basil and purple basil. An analysis of mean values for the three-year experiment revealed that fresh herbage yield was highest in cinnamon basil and Greek 'Minette' basil, and significantly lowest in purple basil and lemon basil (1.00 kg m^{-2} and 1.06 kg m^{-2} respectively) as well as sweet basil (1.29 kg m^{-2}). The container size as a factor exerted varied effects on basil

Table 1

The effect of a container size and number of plants per pot on fresh herbage marketable yield of basil varieties (kg m^{-2})

Varieties of basil	Pot volume (dm^3) / number of plant in pot	Year			Means of 2012-2014
		2012	2013	2014	
Sweet basil	0.7 / 1 pcs.	1.93	1.24	1.30	1.49
	3.0 / 1 pcs.	1.21	1.08	2.03	1.44
	3.0 / 4 pcs.	0.95	0.58	1.31	0.95
Mean		1.36	0.97	1.55	1.29
Thai 'Siam Queen' basil	0.7 / 1 pcs.	2.28	0.50	1.38	1.39
	3.0 / 1 pcs.	0.87	2.12	2.84	1.94
	3.0 / 4 pcs.	0.69	1.19	0.92	0.93
Mean		1.28	1.27	1.71	1.42
Greek 'Minette' basil	0.7 / 1 pcs.	2.01	2.26	1.72	2.00
	3.0 / 1 pcs.	1.00	1.72	3.01	1.91
	3.0 / 4 pcs.	1.40	1.05	1.59	1.35
Mean		1.47	1.68	2.11	1.75
Purple basil	0.7 / 1 pcs.	1.31	0.66	0.65	0.87
	3.0 / 1 pcs.	0.60	1.39	1.58	1.19
	3.0 / 4 pcs.	0.95	0.68	1.11	0.92
Mean		0.96	0.91	1.12	1.00
Lemon basil	0.7 / 1 pcs.	1.57	1.55	0.76	1.29
	3.0 / 1 pcs.	1.35	0.72	1.80	1.29
	3.0 / 4 pcs.	0.69	0.26	0.86	0.60
Mean		1.20	0.84	1.14	1.06
Cinnamon basil	0.7 / 1 pcs.	2.46	1.40	1.82	1.89
	3.0 / 1 pcs.	1.65	1.57	3.04	2.09
	3.0 / 4 pcs.	1.20	1.22	1.51	1.31
Mean		1,77	1.40	2.12	1.76
Mean for pot volume (dm^3) / number of plant in pot	0.7 / 1 pcs.	1.93	1.27	1.27	1.49
	3.0 / 1 pcs.	1.11	1.44	2.38	1.64
	3.0 / 4 pcs.	0.95	0.83	1.22	1.01
LSD $_{\alpha = 0,05}$ for:					
varieties of basil (a)		0.49	0.50	0.59	0.32
pot volume (b)		0.23	0.37	0.32	0.23
interaction (axb)		0.17	0.17	0.17	0.10

yield. In 2012, the significantly highest fresh herbage yield was achieved when basil was grown in 0.7 dm^3 containers. In 2013, growing basil in 3.0 and 0.7 dm^3 containers with one plant per pot had a beneficial influence on fresh herbage yield. In 2014, an increase in yield was noted in basil grown in 3.0 dm^3 containers with one plant per pot. Over the three-year study, basil grown in 3.0 containers with four plants per pot produced the significantly lowest yield compared with the remaining treatments.

Fresh herbage yield was also affected by an interaction between the experimental factors, and it ranged from 0.60 kg m^{-2} to 2.46 kg m^{-2} in 2012, from 0.26 kg m^{-2} to 2.26 kg m^{-2} in 2013, and from 0.65 kg m^{-2} to 2.84 kg m^{-2} in 2014. An analysis of mean values for the three-year experiment revealed that the highest yield was achieved when cinnamon basil was grown in 3.0 dm^3 containers with one plant per pot (2.09 kg m^{-2}) and when Greek 'Minette' basil was grown in 0.7 dm^3 containers (2.00 kg m^{-2}). Lemon basil grown in 3.0 dm^3 containers with four plants per pot produced the lowest marketable yield of fresh herbage (0.60 kg m^{-2}).

The climate conditions in the vicinity of Olsztyn are not conducive to growing basil outdoors because this herb thrives in warm, sunny locations. The present experiment was conducted in a greenhouse, which eliminated the risk associated with adverse environmental conditions. According to Grabowski et.al. (2007), the onset of the growing season may be delayed by several weeks in the Masurian Lake District, where average daily air temperatures are lower than in the other regions of Poland. Basil is a thermophilous plant characterized by a long growing season. In its early stages of development, basil needs ambient temp. of 20 to 25°C and at least 12 h of daylight (Chang et al. 2005). Jadczak, Grzeszczuk (2005), Jadczak (2007) and Majkowska-Gadomska et al. (2014) demonstrated that basil seedlings grown in a greenhouse can be planted out in the field to achieve the desired yield and quality of fresh herbage, and to prevent supply delays. In a study by Majkowska-Gadomska et al. (2014) conducted in the Olsztyn Region, basil plants grown from seedlings were harvested at the end of July. Nurzyńska-Wierdak, Borowski (2011) harvested basil herbage at the end of July and at the beginning of August, when seedlings were grown in an unheated plastic tunnel in the Lublin Region. The demand for functional foods, including fresh herbs and spices that add flavor to dishes, is highest in the winter and spring. Basil is one of the most popular culinary herbs grown in pots and containers (Jadczak et al. 2006, Biesiada, Kuś 2010). Greenhouse growing of herbs and spices guarantees a steady supply in early spring Nurzyńska-Wierdak et al. (2012).

Poorter et al. (2012) carried out around 65 experiments investigating the effect of a container size on yield, and found that an increased pot size was always correlated with increased plant biomass. The positive effect of larger pots on the yield of culinary herbs and spices was also observed by other authors, e.g. Nurzyńska-Wierdak et. al. (2012). In our experiment, basil yield was lower than that reported by Nurzyńska-Wierdak, Borowski (2011) but higher than the yield of basil grown in the field in warmer regions of Poland (Jadczak 2005, Biesiada, Kuś 2010, Rosłon et al. 2011).

Spice plants have desirable chemical composition and provide numerous health benefits: they enhance appetite, stimulate digestion and facilitate nutrient absorption (Jadczak, grzeszczuk 2008, Kudełka et al. 2008).

Food products may contain undesirable compounds, such as heavy metals and nitrates(V) and (III). Heavy metals accumulate in the body, which exacerbates their toxic effects. They cause allergies, kidney and liver dysfunction. The maximum permissible levels of nitrates in foods have been laid down in Commission ... (2011), whereas the maximum limits for heavy metals and nitrates(V) in fresh herbs have not been established. Therefore, their concentrations in basil herbage were compared with the relevant standards for leaf vegetables.

The nitrate(V) content of fresh herbage is an important consideration in greenhouse growing of herbs, spices and leaf vegetables because a shorter production cycle may contribute to nitrate accumulation (Nurzyńska-Wierdak 2012). Nitrates(V) can be converted to toxic nitrates(III), carcinogenic compounds responsible for the breakdown of vitamins A and B, and carotenoids. Due to the absence of regulations relating to the maximum limits for nitrates(V) in herbs and spices, our results were compared with the nitrate(V) content of greenhouse-grown lettuce harvested in early spring where nitrate(V) concentrations should not exceed 4500 mg NO^-_3 kg^{-1} fresh weight. The comparison revealed that the nitrate(V) content of basil herbage was below this limit (Table 2). Nitrate(V) levels varied significantly across basil varieties in 2012 and 2014. In 2012, nitrate(V) content was highest in Greek 'Minette' basil, cinnamon basil and purple basil and lowest in lemon basil and Siam Queen'. In 2014, nitrate(V) concentrations were highest in lemon basil, purple basil and lowest in sweet basil, Greek basil and 'Siam Queen'. An analysis of the influence of the container size on the nitrate(V) content of basil herbage indicated that, in 2012, the latter was the highest in basil grown in 3.0 dm^3 containers with one plant per pot, whereas in 2013 and 2014 – in basil grown in 0.7 dm^3 containers. Each year, nitrate(V) accumulation was lower in basil grown in 3.0 dm^3 containers with four plants per pot, and the noted differences were statistically significant in 2012 and 2013. The average nitrate(V) content of basil herbage ranged from 645 to 3975 mg NO^-_3 kg^{-1} fresh weight in 2012, from 375 to 2701 mg NO^-_3 kg^{-1} fresh weight in 2013, and from 397 to 1243 mg NO^-_3 kg^{-1} fresh weight in 2014.

The mean values for the three-year experiment show that a basil ecotype had no significant effect on nitrate(V) accumulation, which was influenced by the container size. The nitrate(V) content of herbage was significantly higher in basil grown in 3.0 and 0.7 dm^3 containers with one plant per pot than in basil grown in 3.0 dm^3 containers with four plants per pot. Our results are consistent with the findings of other authors. In a study by Seidler-Łożykowska et al. (2007, 2008), the nitrate(V) content of basil herb-

Table 2

The effect of a container size and number of plants per pot on the nitrate(V) content of herbage in basil varieties (mg NO^{-}_{3} kg^{-1} fresh weight)

Varieties of basil	Pot volume (dm^3) / number of plant in pot	Year			Means of 2012-2014
		2012	2013	2014	
Sweet basil	0.7 / 1 pcs.	1281	2370	507	1386
	3.0 / 1 pcs.	2015	1645	491	1384
	3.0 / 4 pcs.	1200	1350	637	1062
Mean		1499	1788	545	1277
Thai 'Siam Queen' basil	0.7 / 1 pcs.	1182	1628	1088	1299
	3.0 / 1 pcs.	1800	1185	744	1243
	3.0 / 4 pcs.	811	784	422	672
Mean		1264	1199	751	1071
Greek 'Minette' basil	0.7 / 1 pcs.	1444	1689	810	1314
	3.0 / 1 pcs.	3975	1647	732	2118
	3.0 / 4 pcs.	645	375	397	472
Mean		2021	1237	646	1301
Purple basil	0.7 / 1 pcs.	2409	2283	1243	1645
	3.0 / 1 pcs.	2164	1697	1037	1633
	3.0 / 4 pcs.	1005	995	919	973
Mean		1859	1658	1066	1417
Lemon basil	0.7 / 1 pcs.	940	1823	1233	1332
	3.0 / 1 pcs.	981	2632	1219	1611
	3.0 / 4 pcs.	1259	1387	1128	1258
Mean		1060	1947	1193	1400
Cinnamon basil	0.7 / 1 pcs.	1896	1874	1014	1595
	3.0 / 1 pcs.	2650	2701	1137	2163
	3.0 / 4 pcs.	1155	1142	602	966
Mean		1900	1906	918	1575
Mean for pot volume (dm^3) / number of plant in pot	0.7 / 1 pcs.	1525	1945	983	1429
	3.0 / 1 pcs.	2264	1918	893	1692
	3.0 / 4 pcs.	1013	1006	684	901
$LSD_{\alpha = 0,05}$ for:					
varieties of basil (a)		735	n.s.	335	n.s.
pot volume (b)		436	723	n.s.	294
interaction (axb)		597	168	268	278

Table 3

The effect of a container size and number of plants per pot on the content of mercury and lead in herbage – means of 2013-2014 (mg kg^{-1} fresh weight)

Varieties of basil	Pot volume (dm^3) / number of plant in pot	Heavy metals (mg kg^{-1} DM)	
		Hg	Pb
Sweet basil	0.7 / 1 pcs.	0.00120	0.01200
	3.0 / 1 pcs.	0.00130	0.01480
	3.0 / 4 pcs.	0.00053	0.01600
Mean		0.00101	0.01430
Thai 'Siam Queen' basil	0.7 / 1 pcs.	0.00110	0.00830
	3.0 / 1 pcs.	0.00120	0.00840
	3.0 / 4 pcs.	0.00058	0.00760
Mean		0.00096	0.00810
Greek 'Minette' basil	0.7 / 1 pcs.	0.00110	0.02200
	3.0 / 1 pcs.	0.00120	0.02400
	3.0 / 4 pcs.	0.00049	0.01360
Mean		0.00093	0.01990
Purple basil	0.7 / 1 pcs.	0.00110	0.01390
	3.0 / 1 pcs.	0.00120	0.00470
	3.0 / 4 pcs.	0.00110	0.00960
Mean		0.00113	0.00940
Lemon basil	0.7 / 1 pcs.	0.00140	0.00640
	3.0 / 1 pcs.	0.00120	0.00480
	3.0 / 4 pcs.	0.00054	0.01080
Mean		0.00105	0.00730
Cinnamon basil	0.7 / 1 pcs.	0.00140	0.02340
	3.0 / 1 pcs.	0.00110	0.01240
	3.0 / 4 pcs.	0.00098	0.00840
Mean		0.00116	0.01470
Mean for pot volume (dm^3) / number of plant in pot	0.7 / 1 pcs.	0.00122	0.01430
	3.0 / 1 pcs.	0.00120	0.01150
	3.0 / 4 pcs.	0.00070	0.01100
LSD $_{\alpha = 0,05}$ for:			
varieties of basil (a)		n.s.	0.0038
pot volume (b)		0.0001	n.s.
interaction (axb)		0.0002	0.0017

age ranged from 306.2 to 5250.0 mg NO^-_3 kg^{-1} fresh weight. Biesiada, Kuś (2010) reported that nitrate(V) concentrations in field-grown purple basil ranged from 697 to 938 mg NO^-_3 kg^{-1} fresh weight depending on nitrogen fertilizer rates.

The mercury content of basil herbage did not exceed the maximum permissible levels (0.02 mg kg^{-1} fresh weight), and it ranged from 0.00049 mg kg^{-1} fresh weight in Greek 'Minette' basil grown in 3.0 dm^3 containers with four plants per pot to 0.00140 mg kg^{-1} fresh weight in cinnamon basil grown in 0.7 dm^3 containers with one plant per pot (Table 3). The maximum allowable concentration of lead in leaf vegetables is 0.300 mg kg^{-1} fresh weight. This limit was not exceeded in basil herbage, where lead content ranged from 0.0047 mg kg^{-1} fresh weight in purple basil grown in 3.0 dm^3 containers with one plant per pot to 0.0240 mg kg^{-1} fresh weight in Greek 'Minette' basil grown in 3.0 dm^3 containers with one plant per pot. Staniek, Krejpcio (2013), who analyzed lead concentrations in spice plants grown under organic and conventional systems, reported somewhat higher values at 0.033 - 0.037 mg kg^{-1} fresh weight.

CONCLUSIONS

1. Cinnamon basil and Greek 'Minette' basil were characterized by a significantly higher marketable yield of fresh herbage compared with the remaining varieties.

2. Basil varietes had no significant influence on the content of mercury and nitrates in herbage.

3. The results of a three-year experiment indicate that growing basil plants in 3.0 dm^3 containers with four plants per pot significantly decreases herbage yield, but prevents excessive accumulation of nitrates(V) and mercury in the edible parts of plants.

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