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### EVALUATION OF SPENT MUSHROOM SUBSTRATE, MINERAL NPK FERTILIZATION AND MANURE FERTILIZATION ON CHAMOMILE (*Chamomilla recutita* L. Rausch) YIELD AND RAW MATERIAL QUALITY

Cezary A. Kwiatkowski<sup>1</sup>, Elżbieta Harasim<sup>1</sup>⊠, Alena Yakimovich<sup>2</sup>, Barbara Kołodziej<sup>3</sup>, Marta Tomczyńska-Mleko<sup>4</sup>

<sup>1</sup>Department of Herbology and Plant Cultivation Techniques, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

<sup>2</sup>Institute of Plant Protection in Priluki, Minsk District, Mira 2, 223011 Belarus

<sup>3</sup>Department of Industrial and Medicinal Plants, University of Life Sciences in Lublin, Akademicka 15, 20-950 Lublin, Poland <sup>4</sup>Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Akademicka 15, 20-950 Lublin, Poland

### ABSTRACT

The aim of this experiment was to determine the effect of spent mushroom substrate (SMS), manure and mineral fertilization with nitrogen, phosphorus and potassium on chamomile (*Chamomilla recutita* L. Rausch) yield and raw material quality. Unfertilized chamomile plots were the control treatment. This study hypothesized that due to its high content of organic matter and macro- and micronutrients, SMS could be an alternative and innovative method of fertilization of this herbal plant. Given that the possibility of using organic fertilization is very limited, we should seek new methods to increase the organic matter content in cultivated soils. A three-year field experiment with a split-block design was conducted on podzolic soil under the climatic conditions of the central Lublin region (Poland). SMS used in this experiment was richer in dry matter and total nitrogen, but less rich in total organic carbon, phosphorus and potassium than farmyard manure. In each year of the study, the highest total yield of chamomile raw material was recorded in the treatment with SMS supplemented with mineral NPK fertilization in spring. The above-mentioned treatment also proved to be most beneficial for the majority of the analyzed quality parameters of the chamomile raw material. It was proved that due to fertilization of a chamomile plantation with SMS, herbal raw material characterized by the best health-promoting parameters (a high content of natural antioxidants) can be obtained.

Key words: chamomile, SMS, manure, mineral NPK fertilization, yielding, quality of herbal raw material

### INTRODUCTION

In recent years, SMS has been a substantial part of organic substrate materials due to the dynamic development of mushroom growing in European countries, in particular in Poland. An increase in the amount of this substrate is recorded especially in central-eastern Poland [Food and Agriculture Organization 2007, Kalembasa and Wiśniewska 2008a, Kalembasa and Majchrowska-Safaryan 2009]. According to the



<sup>&</sup>lt;sup>™</sup> elzbieta.harasim@up.lublin.pl

Regulation of the Minister of Environment of September 27, 2001 SMS is classified in the group of substrates from agriculture, horticulture, aquaculture, fishing, forestry, hunting, food preparation and processing as "Substrates not otherwise specified" [The Substrate Act... 2001].

SMS is characterized by a high content of organic matter, good nutrient availability in terms of total and available forms of macro- and micronutrients, neutral pH, a favorable narrow C/N ratio, and a low content of heavy metals [Kalembasa and Wiśniewska 2001, Jordan et al. 2008, Maszkiewicz 2010]. Due to the beneficial properties of SMS, it seems justified to utilize the organic matter and nutrients contained in it since this will allow us to obtain high crop yields with good economic traits. It is proposed that SMS can be used in agriculture to fertilize farmland and grassland, in horticulture, vegetable growing as well as in the establishment and maintenance of green spaces [Maher et al. 2000, Williams et al. 2001, Ciepiela et al. 2007, Rutkowska et al. 2009]. Some authors [Kalembasa and Wiśniewska 2008a, 2008b, Song and Siu-Wai 2007, Jordan and Mullen 2007, Wiśniewska-Kadżajan and Jankowski 2015] report a positive effect of spent mushroom substrate on the productivity of grasses, cereals, some vegetable crops and flowers, but experiments investigating the possibility of using this fertilizer in herb plantations are a "niche". In planning fertilization with SMS, we should take into account the fact that in the first year after application of SMS plants are able to use 20-25% of nitrogen contained in such substrate, 20-40% of phosphorus, and 65-85% of potassium. Therefore, it seems advisable to supplement SMS fertilization with mineral NPK fertilization, as noted by some authors [Wuest and Fahy 1991, Szudyga 2005, Wiśniewska-Kadżajan 2012].

German chamomile (*Chamomila recutita* (L.) Rausch.) is one of the oldest and most known medicinal plant. Chamomile herbal material comprises flower heads and crushed material that includes ligulate and disk florets as well as undeveloped or immature seeds. Due to its rich chemical composition and mild effect, chamomile finds application in many areas. It has an anti-inflammatory, carminative, spasmolytic and disinfectant effect. In combination with other herbs, it has a sedative and antipyretic effect [Rumińska 1981, Srivestava et al. 2010]. The essential oil content in chamomile raw material is 0.24–1.9%. The other important active ingredients include flavonoids, which are strong antioxidants, coumarins, proazulenes, choline, and phenolic acids [Polish Pharmacopoeia VI 2002]. Among the active ingredients in its essential oil,  $\alpha$ -bisabolol is of great importance (mainly its oxides). Research shows that this compound may induce apoptosis in leukemia-affected white blood cells [Cavalieri et al. 2011].

Taking into account the above considerations, it was hypothesized that fertilization with SMS (applied alone or supplemented with mineral NPK fertilization) would contribute to obtaining satisfactory yields of high-quality chamomile raw material, which would allow us to consider this fertilizer to be useful also in herbal cultures.

The aim of this study was to determine the effect of SMS as alternative organic fertilization on yield and some quality parameters of chamomile raw material compared to standard mineral NPK fertilization and manure fertilization.

### **MATERIAL AND METHODS**

### Site, field experiment and cultural practices

The experiment on growing chamomile (cv. 'Złoty Łan') was carried out over the period 2014–2016 in Fajsławice (the central Lublin region). This variety came from its breeder and producer – the Institute of Herbal Plants and Products in Poznań, Poland. This is a tetraploid multi-flowered variety with sparsely foliated even stems, characterized by high productivity as well as a high content of essential oil (1.5%) and  $\alpha$ -bisabolol (0.85%) [Seidler-Łożykowska 1999, 2000].

The field experiment was set up as a split-block design with 4 replicates in 9 m<sup>2</sup> plots (1.5 m  $\times$  6.0 m). Chamomile was grown on podzolic soil (soil class III). The soil content of major nutrients is shown in Table 1.

The data contained in Table 1 show that in each year of the experiment before the establishment of the experiment the soil had a slightly acidic pH, the content of available nutrients was at a medium level, while the humus content ranged 1.24–1.33%.

#### Table 1. Soil characteristics

	nЦ	Content of					
Year	1 M KCl	P (mg kg <sup>-1</sup> soil)	K (mg kg <sup>-1</sup> soil)	Mg (mg kg <sup>-1</sup> soil)	humus (%)		
2014	6.0	131	173	65	1.33		
2015	5.8	120	166	61	1.27		
2016	5.9	116	162	61	1.24		

Table 2. Characteristics of the organic materials used in the experiment (mean for 2014–2016)

Organic material	Air dry matter	Macronutrient content (g kg <sup>-1</sup> DM)			
	(70)	С	Ν	Р	К
Mushroom substrate	28.2	358.3	25.9	10.2	13.5
Farmyard manure	25.4	419.8	21.0	13.5	20.8

Each year, chamomile seeds were sown in the second 10 days of April at a seeding rate of 2.5 kg  $ha^{-1}$  and a row spacing of 35 cm. Chamomile crop protection involved mechanical weed control in the interrows (using a rotary weeder or hand hoe at the 3–5-leaf stage of chamomile). As part pest control (aphids, smut beetle, chamomile stem weevil), it was planned to apply the biological control product Spin Tor 240 S.C., but due to the lack of occurrence of the above-mentioned pathogens in the plantation this biological agent was not used.

The experiment included the following chamomile cultivation treatments:

- A. control treatment without fertilization
- M. mineral NPK fertilization,
- C. spent mushroom substrate (SMS),
- D. SMS + NPK,
- E. fermented farmyard manure (catlle).

Fertilization was applied at the following rates:

- B. NPK: N 50, P 40, K 60 kg ha<sup>-1</sup>,
- C. SMS: 15 t  $ha^{-1}$ ,

D. SMS + NPK: 15 t  $ha^{-1}$  + N - 30, P - 20, K - 50 kg  $ha^{-1}$ , E. manure: 15 t  $ha^{-1}$ .

Organic materials used in this experiment were as follows: SMS and fermented farmyard manure in which the dry matter content was respectively 28.2 and 25.4%, whereas the N content in dry matter was 25.9 and 21.0 g kg<sup>-1</sup> (tab. 2). SMS was poorer in carbon in organic compounds (358.3 g kg<sup>-1</sup> DM) as well as in phosphorus and potassium (respectively: 10.2 and 13.5 g kg<sup>-1</sup> DM) in relation to manure in which their contents were as follows: carbon 419.8 g kg<sup>-1</sup> DM, phosphorus – 13.5 g kg<sup>-1</sup> DM, and potassium – 20.8 g kg<sup>-1</sup> DM (tab. 2).

Tillage treatments were adjusted to the specific agronomy of chamomile, the previous crop and type of fertilization. After the previous crop (white mustard) was harvested, plough skimming and harrowing were done in all treatments. Subsequently, in the autumn (the first or second decade of October) organic fertilization (manure, spent mushroom substrate) was applied and the fertilizers were ploughed into the soil (treatments C–E). In the control treatment (A) and in treatment B, ridge ploughing was performed in the autumn and no other tillage operations were carried out until the spring. In the spring, the field was smoothed with a seedbed conditioner and prepared for application of mineral NPK fertilizers (treatment B) and sowing of chamomile seeds.

Mineral fertilization (both at the recommended rate – treatment B, and additional fertilization – treatment D) was applied in the spring before sowing chamomile. SMS and farmyard manure were applied in a single dose in the autumn (before autumn

ploughing during which they were ploughed into the soil). The applied rates and additional mineral NPK fertilization levels resulted from the initial soil nutrient availability and the hypothesized slower release of nutrients from the mushroom substrate and the related lower fertilizing effect of mushroom substrate alone (without NPK fertilization). White mustard grown for green manure was the previous crop in all treatments (A–E).

### Measurement and markings

Herbage, together with inflorescences (*Chamo-millae anthodium*) to be used for the production of crushed flowers, was harvested after about 10–15 days from the beginning of flowering. The raw material was collected from the entire area of each single plot (9 m<sup>2</sup>). The harvested herbage was dried at 35°C in an air circulation drying oven and subsequently threshed. The next operation was to separate crushed flowers. The crushed herbal material (flowers) was weighed and then divided into particular fractions using sieves with the following mesh sizes: 3.0, 1.0, 0.8, and 0.4 mm. The fractions obtained, i.e. disk florets, ray florets and seeds, were weighed, whereas the other plant parts, e.g. receptacles, ground stems and leaves, were rejected.

Samples were collected from the herbal material obtained to determine the chemical composition. The determinations included the following:

Determination of essential oil content by the pharmacopoeial method – steam distillation of the herbal material [Polish Pharmacopoeia IX 2011]. The volume of the extracted essential oil was converted into values per 100 g of raw material, expressing it as weight/volume percentages.

The determination of total phenolic acid content was performed by the modified VIS absorption spectrophotometry method using Arnov's reagent according to Polish Pharmacopoeia VI [2002]. The percentage content of phenolic acids was expressed as gallic acid equivalents.

Determination of flavonoid content using Christ-Müller's method [Polish Pharmacopoeia IX 2011]. Flavonoid content was determined spectrophotometrically, after extraction of flavonoids from the raw material, and expressed as quercetin equivalents.

Determination of anthocyanin content spectrophotometrically [Miłkowska and Strzelecka 1995].

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The determination involved colorimetric measurement of the level of reduction of a known amount of DPPH by the extract of the tested sample. Changes in the content of DPPH radicals were recorded spectrophotometrically after 10-minute incubation with the tested extracts at a wavelength of 517 nm. The determination was made using the method given by Yen and Chen [1995], while DPPH inhibition was calculated according to the formula proposed by Rossi et al. [2003].

Determination of antioxidant activity by the ABTS method given by Re et al. [1999]. Changes in the concentration of ABTS<sup>++</sup> cation radicals were determined spectrophotometrically after 6-minute incubation with the tested extracts. Compounds with antioxidant properties contained in these extracts reduced the concentrations of ABTS<sup>++</sup> cation radicals which were measured as a decrease in the absorbance of the solution at a wavelength of 734 nm.

### Weather conditions at the study site

To determine temporal and spatial variation in meteorological factors and to evaluate their effect on chamomile growth, Selyaninov's hydrothermal coefficient (K) was calculated [Bac et al. 1993] by dividing the monthly total rainfall by one tenth of the sum of average daily temperatures for a given month (tab. 3). The calculated values of the hydrothermal coefficient show that during the first months of the growing season chamomile plants were well supplied with water, while in May (in the years 2015-2016) we can even speak of an excess of water. As far as the summer months of 2015 and 2016 (VI-VIII) are concerned, on the other hand, slightly dry or dry periods were found to occur, whereas in July 2015 and June 2016 it was even a severe dry period. In this respect, the 2014 growing season is an exception because it should be considered to be favorable for the cultivation of chamomile in terms of water and thermal conditions.

Month –		Year	
	2014	2015	2016
IV	1.55	1.16	1.32
V	1.29	1.76	1.63
VI	1.51	0.57	0.26
VII	0.71	0.38	0.65
VIII	0.92	0.86	0.75

Table 3. The hydrothermal coefficient (K) during the growing seasons of 2014–2016

Hydrothermal coefficient value:  $K \le 0.5$  extremely dry, 0.51–0.69 very dry, 0.70–0.99 dry, K >1 no drought

#### Statistical analyses

The results were statistically analyzed and verified by Tukey's test at a significance level of  $\alpha = 0.05$ . The statistical analysis was presented using Statgraphics 5.0 software.

### RESULTS

# Total yield of chamomile raw material (t ha<sup>-1</sup>) and total yield structure (%)

In line with the expectations, the total yield of chamomile raw material in the control plots (unfertilized) was very low and ranged 0.37-0.53 t ha<sup>-1</sup>. Relative to the unfertilized plots, the best yield-increasing effect was produced by soil amendment with SMS

supplemented with mineral NPK fertilization – treatment D (a 2.5-fold increase in yield), followed by mineral NPK fertilization – treatment B, and amendment with SMS alone – treatment C (a 2-fold increase in yield). Manure fertilization (treatment E) contributed to a slightly lower increase in yield, by about 45%, compared to the control treatment (A). It should be noted that in treatments B–C and in treatment D the total yield of chamomile raw material was significantly higher, by respectively 15% and 29%, than the yields found in treatment E. This demonstrates that both alternative fertilization in the form of SMS and standard mineral NPK fertilization are more beneficial for chamomile yields than manure fertilization.

**Table 4.** Total yield of chamomile raw material (t ha<sup>-1</sup>)

Fertilization treatment –	Year			Moon		
	2014	2015	2016	Wiean		
А	0.53	0.37	0.42	0.44		
В	1.03	0.83	0.90	0.92		
С	0.99	0.81	0.92	0.90		
D	1.21	1.02	1.12	1.11		
E	0.84	0.76	0.79	0.79		
Mean	0.92	0.75	0.83	_		
LSD <sub>0.05</sub> for: fertilization (a) = 0.113, years (b) = 0.162, interaction (a $\times$ b) = 0.158						

A - control (without fertilization), B - mineral NPK fertilization, C - mushroom substrate, D - mushroom substrate + NPK, E - farmyard manure

	Specification				
Fertilization treatment	disk flowers	ligulate flowers	seeds		
A	85.4	11.8	2.8		
В	88.6	10.5	0.9		
С	88.0	10.8	1.2		
D	91.3	8.2	0.5		
E	87.5	10.9	1.6		
Mean	88.1	10.4	1.4		
LSD <sub>0.05</sub>	2.02	1.57	0.65		

**Table 5.** Dry weight of disk flowers, ligulate flowers and seeds in the total yield of chamomile raw material (%) – mean for 2014–2016

Explanation as in Table 4

Regardless of the fertilization treatments, chamomile produced the best yields in 2014, which was a year characterized by an even distribution of sunshine and thus the absence of major water deficits in the summer months during the growing season, whereas it gave significantly worse yields (treatments A, B and D) in 2015 when a severe drought in July reduced yields of many crops in Poland (tab. 4). Disk flowers by far dominated in the total yield of crushed herbal material of chamomile, accounting for about 88% of the raw material (tab. 5). Ligulate flowers (about 10.4%) and seeds (about 1.4%) had a lower percentage in the total yield structure. As regards fertilization used, SMS + NPK significantly affected a higher percentage of disk flowers in yield compared to treatments A-C and E.

### Essential oil and phenolic acid content in chamomile raw material

On average over the study period, all fertilization treatments included in the experiment contributed to a significant increase in the essential oil content in chamomile disk florets compared to the unfertilized control treatment (tab. 6). At the same time, no significant differences in the content of this component were found in treatments B–E. We can only say that SMS (treatments C and D) tended to have the most beneficial effect on the essential oil content in the chamomile raw material. The weather conditions in the 2014 growing season proved to be significantly more favorable for the essential oil content in the raw material relative to the 2015 growing season. More-

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Eastilization treatment	Year			Moon	
Fertilization treatment –	2014	2015	2016	Weall	
А	0.38	0.30	0.34	0.34	
В	0.57	0.49	0.50	0.52	
С	0.58	0.54	0.56	0.56	
D	0.60	0.55	0.57	0.57	
Е	0.55	0.47	0.49	0,50	
Mean	0.54	0.47	0.49	_	
$LSD_{0.05}$ for: fertilization (a) = 0.073, years (b) = 0.067, interaction (a × b) = 0.078					

Explanation as in Table 4

Fertilization treatment			Mean		
	2014	2015	2016		
А	192.44	178.67	182.96	184.69	
В	279.42	185.16	199.11	221.23	
С	317.21	280.13	292.42	296.58	
D	322.56	294.22	300.16	305.64	
Е	244.16	180.34	189.93	204.81	
Mean	271.15	223.70	232.91	-	
LSD <sub>0.05</sub> for: fertilization (a) = 14.221, years (b) = 18.318, interaction (a $\times$ b) = 18.451					

### **Table 7.** Total phenolic acids in disk florets of chamomile (mg GAE 100 $g^{-1}$ )

Explanation as in Table 4

### **Table 8.** Flavonoid content in disk florets of chamomile (mg CA 100 $g^{-1}$ )

Fertilization treatment		Mean			
	2014	2015	2016	Wedn	
А	29.17	28.21	28.04	28.47	
В	30.66	30.11	30.23	30.33	
С	34.95	33.03	33.22	33.73	
D	35.88	33.16	33.69	34.24	
Е	29.19	28.48	27.99	28.55	
Mean	31.97	30.59	30.63	—	
LSD <sub>0.05</sub> for: fertilization (a) = 1.698, years (b) = n.s., interaction ( $a \times b$ ) = n.s.					

Explanation as in Table 4

**Table 9.** Anthocyanin content in disk florets of chamomile (mg  $100 \text{ g}^{-1}$ )

Fertilization treatment		Mean				
	2014	2015	2016			
А	16.13	14.28	14.37	14.92		
В	18.22	16.61	16.81	17.21		
С	18.84	17.21	17.43	17.82		
D	18.92	17.63	17.59	18.04		
Е	18.05	16.48	16.76	17.09		
Mean	18.03	16.44	16.59	_		
$I_{s}$ SD <sub>0.05</sub> for: fertilization (a) = 1.272, years (b) = 1.246, interaction (a × b) = n.s.						

Explanation as in Table 4

over, during the 2015–2016 period which was less favorable for the cultivation of chamomile, significant differences in essential oil content were found between treatments D and E (under drought conditions, SMS showed a positive effect compared to manure).

The fertilization treatments applied in the experiment had a different effect on the phenolic acid content in disk florets of chamomile (tab. 7). On average over the study period, significantly the highest total phenolic acid content was found after application of SMS supplemented with mineral NPK fertilization and mushroom substrate alone. The content of this component found under the conditions of treatments D and C was significantly higher not only compared to the control (without fertilization) but also in relation to treatment E (manure fertilization) and B (mineral NPK fertilization). Interestingly, in the years with unfavorable weather conditions (2015-2016) the phenolic acid content in the chamomile raw material obtained from treatments B and E did not differ statistically from the determinations for this component in samples from the control treatment (without fertilization). To sum up, the year 2014 was significantly more favorable for the phenolic acid content in the chamomile raw material relative to the years 2015–2016.

## Flavonoid and anthocyanin content in chamomile raw material

Analyzing the data contained in Table 8, we note that the chamomile fertilization variants used in treatments B, C and D contributed to a significantly higher flavonoid content in the herbal raw material compared to the control (without fertilization) and treatment E (manure fertilization). At the same time, fertilization with SMS (treatments C and D) resulted in a significant increase in flavonoid content relative to treatment B (standard NPK fertilization). The particular study seasons did not affect significantly the flavonoid content in disk florets of chamomile. The study only found a trend toward greater accumulation of flavonoids in the year 2014 that was most favorable in terms of weather conditions. All fertilization treatments applied in this experiment were shown to have a statistically proven effect on a higher anthocyanin content (by about 13–17%) in relation to the control (without fertilization), but treatments C and D fertilized with SMS tended to show the most beneficial influence (tab. 9). When considering the content of the component in question in the chamomile raw material in the particular study seasons, we note that it was significantly higher in 2014 relative to the years 2015–2016 in which Selyaninov's hydrothermal coefficient (tab. 3) was found to be unfavorable in the summer months of the chamomile growing season.

### Antioxidant properties of chamomile raw material

SMS supplemented with mineral NPK fertilization in spring (treatment D) applied in chamomile fertilization had a significant positive effect on free radical scavenging activity determined in disk florets relative to treatments A, B and E (Table 10). Positive effects associated with free radical scavenging activity, relative to the unfertilized treatment and treatments B and E, were also found in the case of application of SMS alone in chamomile fertilization. The incorporation of SMS into the soil (treatments C, D) also reduced the adverse impact of the summer drought period in the months June-August 2015 and 2016 on free radical scavenging activity in the chamomile raw material compared to samples collected from treatments B and E. Regardless of the fertilization treatments, the year 2014 was more favorable for free radical scavenging activity in the chamomile raw material in relation to the years 2015–2016, but at a statistically insignificant level.

Application of organic fertilization of chamomile (SMS, manure – treatments C, E) and organicmineral fertilization (SMS + NPK – treatment D) resulted in higher antioxidant activity determined in disk florets of the herbal plant compared to the control (A) and standard mineral fertilization (B). However, a statistically proven difference related to an increase in this quality parameter was found only in the case of fertilization with SMS (both alone and supplemented with NPK fertilization in spring) –

Fertilization treatment		Mean			
	2014	2015	2016	Wiedli	
А	38.16	36.21	36.45	36.94	
В	39.68	36.33	36.57	37.52	
С	41.52	41.05	41.13	41.23	
D	42.37	41.66	41.78	41.93	
Е	39.15	36.19	36.08	37.14	
Mean	40.17	38.28	38.40	_	
LSD <sub>0.05</sub> for: fertilization (a) = 2.397, years (b) = n.s., interaction (a $\times$ b) = 2.875					

**Table 10.** DPPH free radical scavenging activity of disk florets of chamomile ( $\mu$ M TE g<sup>-1</sup>)

Explanation as in Table 4

**Table 11.** Oxygen radical absorbance capacity (ORAC) of disk florets of chamomile ( $\mu$ M TE g<sup>-1</sup>)

Fertilization treatment –	Year			Mean		
	2014	2015	2016	Wiedli		
А	10.74	9.58	9.44	9.92		
В	10.93	9.61	9.39	9.97		
С	11.92	11.74	11.81	11.82		
D	13.76	13.33	13.40	13.49		
Е	11.88	10.33	10.21	10.80		
Mean	11.84	10.91	10.85	-		
$LSD_{0.05}$ for: fertilization (a) = 0.897, years (b) = 0.884, interaction (a × b) = 0.955						

Explanation as in Table 4

Table 11. Similarly as in the case of free radical scavenging activity, the values of ORAC antioxidant activity of the chamomile raw material collected in the years 2015–2016, which were characterized by an unfavorable hydrothermal coefficient in the second part of the chamomile growing season, were similar to those determined in the favorable year 2014 when the soil was amended with SMS (treatments C and D). Under the conditions of the other treatments and regardless of the fertilization treatment, ORAC antioxidant activity was significantly higher in the raw material collected in 2014 than in the years 2015–2016.

### DISCUSSION

The obtained study results on the chemical composition of SMS relative to the composition of manure are similar to the determinations made by Wiśniewska-Kadżajan and Jankowski [2015]. The greatest similarity in the results relates to the narrow C/N ratio (which was 13.8 : 1.0). This is a very favorable ratio in terms of fertilizer value because it indicates a predominance of mineralization of organic nitrogen compounds over their synthesis. As a result, nutrients easily available to plants are released and the action of such organic material after its application to the soil is quick and short-lasting [Kalembasa and Wiśniewska 2001, 2006]. Gerrits [1994] noted that SMS contains on average 25-35% of dry matter, which is confirmed in the present study. Maszkiewicz [2010] reported that the macronutrient content in SMS is as follows (g kg<sup>-1</sup> DM): nitrogen 13.0–26.0, phosphorus 1.0-10.0, potassium 5.0-25.0, which is also in

agreement with the determinations reported in this paper. Nevertheless, in the literature of the subject we find large differences in the chemical composition of spent mushroom substrate, in particular as regards the N : P : K ratio in it. A study by Uzun [2004] shows that the N : P : K ratio in spent mushroom compost is 1.9 : 0.4 : 2.4 and it is strongly correlated to the duration of storage of spent mushroom compost. An Irish study [Jordan et al. 2008] proves that this ratio is 1.2 : 1.0 : 1.1. On the other hand, research conducted in Poland [Kalembasa and Wiśniewska 2001, Wiśniewska-Kadżajan 2012], including the present study, reveals that the N : P : K ratio in spent mushroom substrate is 1.0 : 0.4 : 0.5-0.8 and thus it is characterized by a clear deficiency of phosphorus and potassium. Some researchers draw attention to the high variation and imbalance in the chemical composition of SMS, which is an obvious drawback that should oblige us to continuously control their chemical composition and to make up the missing elements in order to improve their fertilizing qualities [Rao et al. 2007, Polat et al. 2009, Salomez et al. 2009].

The results of the present study demonstrate that from the point of view of yields obtained, SMS applied in autumn can be recommended as alternative fertilization of chamomile (in particular where additional mineral fertilization is used in spring). Uzun [2004] thinks that autumn application of spent mushroom compost is the most favorable time since in this way we eliminate the possible adverse effect of initial soil salinity, which sometimes occurs immediately after fertilizer application, on young growing plants. Polat et al. [2009] are also of opinion that the drawback of SMS can sometimes be a too high content of soluble salts and their negative impact on plant growth and development, though the abovementioned risk relates more to greenhouse crops than field crops.

In the literature, there is a lack of similar studies on the effect of SMS on the productivity of chamomile or other herbal plants. However, we find reports on the positive effect of this fertilizer on the productivity of cucumber, cauliflower, tomato, spinach, broccoli or bell pepper [Kryńska et al. 1983, Steffen et al. 1994, Martyniak-Przybyszewska and Wierzbicka 1996, Beyer 1999, Medina et al. 2009, Run-Hua et al. 2012], an increase in wheat grain yield [Song and Siu-Wai 2007] and in biomass of some grasses (annual ryegrass, orchard grass) [Kalembasa and Wiśniewska 2008a, Wiśniewska-Kadżajan and Jankowski 2015]. In their studies, Rak et al. [2001] and Jankowski et al. [2004] showed the beneficial effect of SMS supplemented with mineral fertilization on meadow sward yield, at the same time stressing that the use of this substrate as a soil amendment contributes to solving the problem of its management. The publications of Drzał et al. [1995], Maszkiewicz [2010] and Roy et al. [2015] also demonstrate that application of SMS as fertilizer produced beneficial effects in the form of significant increases in crop yields. Kwiatkowski [2015], Wang et al. [2008] and Bavec et al. [2012] note that the yieldincreasing effects of various types of fertilization depend, among others, on the type of crop plant and the type of soil and its initial nutrient availability. Relating this finding to the actual conditions of the present experiment, we note that soil amendment with SMS (but supplemented with mineral NPK fertilization in the spring) produced the most pronounced effect by increasing the chamomile raw material yield.

The present study reveals that SMS had a slightly lower impact on the quality of chamomile raw material than on yield. Nevertheless, it was found that such fertilization tended to have the most beneficial effect on essential oil and anthocyanin content and a statistically significant effect (SMS + NPK) on phenolic acid and flavonoid content as well as on DPPH free radical scavenging activity and ORAC (oxygen radical absorbance capacity) antioxidant activity determined in disk florets of chamomile.

Herbal raw material, which is an important source of compounds with antioxidant properties (flavonoids, anthocyanins, phenolic acids), treated as a natural additive to food products may have a great importance in neutralizing free radicals [Cao and Prior 1999]. In food, free radicals form as a result of processes such as frying and smoking, but also during storage. Oxidation processes take place not only in

food, but also in human organism. The interaction between free radicals and cellular macromolecules, such as nucleic acids, proteins, lipids and carbohydrates, leads to various damages: DNA strand breaking, point mutations, chromosomal aberrations, and ultimately cell death. Free radicals are also thought to be the beginning of development of many diseases of affluence, such as: atherosclerosis, diabetes, cataract, Parkinson's disease, and Alzheimer's disease [Rouseff and Nagy 1994, Kozłowska and Troszyńska 1999, Katsube et al. 2003].

In herbal plants such as: heartsease, field horsetail, chamomile and nettle, antioxidant activity is attributed primarily to flavonoids [Obidowska 1998]. Other authors attribute the main antioxidant role to essential oils and phenolic compounds [Zu et al. 2010, Nurzyńska-Wierdak 2011, Amorati et al. 2013]. When considering the above problems from the point of view of the agronomy of herbal plants (in particular as regards the impact of fertilization), it is important that agronomic practices should contribute to the possibly highest content of natural antioxidants and also other health-promoting compounds for humans and livestock in herbal raw material.

Ciepiela et al. [2007] showed SMS amendment to have an effect on increasing the sugar content in grasses compared to manure fertilization. Dunbar et al. [1996] found a beneficial effect of spent mushroom compost on the properties of tomatoes by improving their firmness and ascorbic acid content. Kalembasa and Wiśniewska [2008a], in turn, found an increase in the titanium and arsenic content in annual ryegrass biomass under the influence of soil amendment with spent mushroom compost (alone or combined with mineral fertilization), which increases the feeding value of grass.

Wiśniewska-Kadżajan and Jankowski [2015] also draw attention to the beneficial interaction of macronutrients contained in mineral fertilizers applied as a complement of fertilization with SMS on the example of orchard grass (an increase in total yield and a higher protein content in biomass).

In the opinion of some authors [Guo and Chorover 2004, Song and Siu-Wai 2007], fertilization of crops with SMS is related to weather condi-

tions, notably, spent mushroom substrate has a high soil water retention capacity and thereby a grown crop stands periodic drought conditions better. The present study also observed a trend toward better yields in the plots amended with spent mushroom substrate relative to the other fertilization treatments (NPK, manure) when a long-lasting drought occurred in the summer months (the years 2015 and 2016) during the growing season.

### CONSLUSIONS

In conclusion, in the light of the results of this study conducted under the soil and climatic conditions of central-eastern Poland (the Lublin region), SMS applied in autumn and supplemented in spring with mineral NPK fertilization at a reduced rate can be considered to be a fertilizer that is an alternative to standard mineral fertilization of chamomile. Application of SMS alone (without adding mineral fertilizers in spring) also results in a comparable yield and quality of chamomile raw material as in the case of application of standard NPK fertilization. Organic fertilization of chamomile with manure proved to be less beneficial for the total productivity of the herbal plant in question.

Comparing both organic fertilizers used in this experiment, SMS had a more favorable chemical composition and its greatest advantage was a narrow C : N ratio which provided a greater and faster availability of nutrients to plants. Moreover, in the years with unfavorable weather conditions (a drought in the summer months) SMS allowed water to be retained in the soil for a longer time. The positive effect of SMS on the content of natural antioxidants in chamomile raw material and on free radical scavenging activity should be considered to be particularly valuable because it is important from the health point of view.

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### REFERENCES

- Amorati, R., Foti, M.C., Valgimigli, L. (2013). Antioxidant activity of essential oils. J. Agric. Food Chem., 61, 10835–10847.
- Bac, S., Koźmiński, C., Rojek, M. (1993). Agrometeorology. PWN, Warszawa, 32–33.
- Bavec, M., Narodoslawsky, M., Bavec, F., Turinek, M. (2012). Ecological impact of wheat and spelt production under industrial and alternative farming systems. Renew Agric. Food Syst., 27, 242–250.
- Beyer, D. (1999). Spent mushroom substrate. Online. Mushroom spawn. cas.psu.edu/spent.htm.
- Cao, G., Prior, R.L. (1999). The measurement of oxygen radical absorbance capacity in biological samples. Methods Enzymol., 229, 50–62.
- Cavalieri, E., Rigo, A., Bonifacio, M., Carcereri de Prati, A., Guardalben, E., Bergamini, C., Fato, R., Pizzolo, G., Suzuki, H., Vinante, F. (2011). Pro-apoptotic activity of α-bisabolol in preclinical models of primary human acute leukemia cells. J. Transl. Med., 9, 45.
- Ciepiela, G.A., Jankowska, J., Jankowski, K., Kolczarek, R. (2007). Influence of unconventional organic manure on fodder quality from the meadow. Fragm. Agron., 1, 14–24.
- Dunbar, J., Wong, D.C.L., Yarus, M.J., Forney, L.J. (1996). Autoradiographic method for isolation of diverse microbial species with unique catabolic traits. Appl. Environ. Microbiol., 62, 4180–4185.
- Drzał, E., Kozak, E., Kucharski, B., Podgórski, L., Streb, M., Suchy, M., Synoś, A. (1995). Environmental microbiological and physicochemical risks from substrate. Biblioteka Monitoringu Środowiska, Warszawa, 138–143.
- Food and Agriculture Organisation (2007). FAOSTAT Database. http://www.fao.org>
- Gerrits, J.P.G. (1994). Composition, use and legislation of spent mushroom substrate in Netherlands. Compost Sci. Util., 2, 24–30.
- Guo, M., Chorover, J. (2004). Solute release from weathering of spent mushroom substrate under controlled conditions. Compost Sci. Util., 12, 225–234.

- Jankowski, K., Ciepiela, G., Jodełka, J., Kolczarek, R. (2004). Possibilites of mushroom compost utilization for fertilization of meadows. Ann. UMCS, E, 59, 4, 1763–1770.
- Jordan, S.N., Mullen, G.J. (2007). Spent mushroom legislation in Ireland. Proceedings of ESAI Environ, 37–41.
- Jordan, S.N., Mullen, G.J., Murphy, M.C. (2008). Composition variability of spent mushroom compost in Ireland. Bioresour. Technol., 99, 411–418.
- Kalembasa, D., Majchrowska-Safaryan, A. (2009). Affluence of the spent mushroom substrate from mushroom growing cellar. Zesz. Probl. Post. Nauk Roln., 535, 195–200.
- Kalembasa, D., Wiśniewska, B. (2001). Chemical composition of the beds after mushrooms production. Zesz. Probl. Post. Nauk Roln., 475, 295–300.
- Kalembasa, D., Wiśniewska, B. (2006). Changes of chemical composition of soil and perennial ryegrass *Lolium multiflorum* under the influence of the application of post-mushroom production bed. Zesz. Probl. Post. Nauk Roln., 512, 265–276.
- Kalembasa, D., Wiśniewska, B., (2008a). The influence of fertilization with mushroom production bed on the amount some macroelements in *Lolium multiflorum* LAM. Zesz. Probl. Post. Nauk Roln., 526, 191–196.
- Kalembasa, D., Wiśniewska, B. (2008b). The effect of fertilization with spent mushroom substrate on the content of phosphorus and its uptake and use by annual ryegrass. In: Phosphorus compounds in chemistry, agriculture, medicine and environmental protection. Pr. Nauk Uniw. Ekon. Wroc. Chem., 4, 109–114.
- Katsube, N., Iwashita, K., Tsushida, T., Yamaki, K., Kobori, M. (2003). Induction of apoptosis in cancer cells by bilberry (*Vaccinium myrtillus*) and the anthocyanins. J. Agric. Food Chem., 51, 68–75.
- Kozłowska, H., Troszyńska, A. (1999). The role of natural non-nutrients of plant origin as components of functional food. Żywn. Nauka Technol. Jakość Supl., 4(21) 63–73.
- Kryńska, W., Martyniak-Przybyszewska, B., Wierzbicka, B. (1983). An attempt to evaluate spent mushroom substrate as compost for growing greenhouse tomatoes and cucumbers. Materials Symposium ART Olsztyn, 110–125.
- Kwiatkowski, C.A. (2015). Yield and quality of chamomile (*Chamomila recutita* (L.) Rausch.) raw material depending on selected foliar sprays and plant spacing. Acta Sci. Pol. Hortorum Cultus, 14(1), 143–156.

- Maher, M.J., Smyth, S., Dodd, V.A., McCabe, T., Magette, W.L., Duggan, J., Hennerty, M.J. (2000). Managing spent mushroom compost. Teagasc, Dublin, 111–121.
- Martyniak-Przybyszewska, B., Wierzbicka, B. (1996). Effect of several substrates on yield of hotbed cucumber. Zesz. Probl. Post Nauk. Roln., 429, 237–240.
- Maszkiewicz, J. (2010). Spent mushroom substrate as fertilizer and fuel. In: White mushrooms. Biuletyn Producenta Pieczarek. Hortpress, 1, 59–60.
- Medina, E., Paredes, C., Pérez-Murcia, M.D., Bustamante, M.A., Moral, R. (2009). Spent mushroom substrates as component of growing media for germination and growth of horticultural plants. Bioresour. Technol., 100, 4227–4232.
- Miłkowska, K., Strzelecka, H. (1995). Flos Hibisci methods for identification and evaluation of raw material. Herba Pol., 41(1), 11–16.
- Nurzyńska-Wierdak, R. (2011). The essential oil of *Chamomilla recutita* (L.) Rausch. cultivated and wild growing in Poland. Ann. UMCS, sec. DDD, Pharmacia, 24, 25, 197–206.
- Obidowska, G. (1998). Substances of plant origin in cancer prevention. Przegl. Piek., 17, 2–4.
- Polat, E., Uzun, I.H., Topcuoglu, B., Önal, K., Onus, A.N., Karaca, M. (2009). Effects of spent mush-room compost on quality and productivity of cucumber (*Cucumis dativus* L.) grown in green-houses. Afr. J. Biotechnol., 8, 176–180.
- Polish Pharmacopoeia VI (2002). PZWL, Warszawa.
- Polish Pharmacopoeia IX (2011). PZWL, Warszawa.
- Rak, J., Koc, G., Jankowski, K. (2001). The application of post-mushroom compost in the regeneration of meadow sward destroyed by fire. Pam. Pul., 125, 401–408.
- Rao, J.R., Watabe, M., Stewart, T.A., Millar, B.C., Moore, J.E. (2007). Pelleted organomineral fertilizers from composted pig slurry solids, animal substrates and spent mushroom compost for amenity grasslands. Substrate Manage, 27, 1117–1128.
- Re, R., Pellgrini, N., Proteggente, A., Pannala, A., Yang, M. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assai. Free Rad. Biol. Med. 26, 9/10, 1231–1237.
- Rossi, M., Giussani, E., Morelli, R., Scalzo, R., Nani, R.C., Torreggiani, D. (2003). Effect of fruit blanching on phenolics and radical scavenging activity of highbush blueberry juice. Food Res. Int., 36, 999–1005.

- Rouseff, R.L., Nagy, S. (1994). Health and nutritional benefits of citrus fruit components. Food Technol., 11, 125–132.
- Roy, S., Barman, S., Chakraborty, U., Chakraborty, B. (2015). Evaluation of spent mushroom substrate as bio-fertilizer for growth improvement of *Capsicum annuum* L. J. App. Biol. Biotech., 3, 22–27.
- Rumińska, A. (1981). Rośliny lecznicze. Podstawy biologii i agrotechniki [Medicinal plants. Basics of biology and agronomy]. PWN, Warszawa.
- Run-Hua, Z., Zeng-Qiang, D., Zhi-Guo, L. (2012). Use of spent mushroom substrate as growing media for tomato and cucumber seedlings. Pedosphere, 22, 333–342.
- Rutkowska, B., Szulc, W., Stępień, W., Jobda, J. (2009). Possibility of agricultural utilization of spent mushroom substrates. Zesz. Probl. Post. Nauk Roln., 535, 349–356.
- Salomez, J., De Bolle, S., Sleutel, S., De Neve, S., Hofman, G. (2009). Nutrient legislation in Flanders (Belgium). Proceedings. More sustainability in agriculture. New fertilizers and fertilization management. Rome, 546–551.
- Seidler-Łożykowska, K. (1999). Comparison of some traits of chamomile strains and varieties with high content of  $\alpha$ -bisabolol. Part I. Herba Pol. 45, 312–317.
- Seidler-Łożykowska, K. (2000). Comparison of some traits of chamomile strains and varieties with high content of  $\alpha$ -bisabolol. Part II. Herba Pol. 46, 5–11.
- Song, L., Siu-Wai, Ch. (2007). Dual roles of spent mushroom substrate on soil improvement and enhanced drought tolerance of wheat *Triticum aestivum*. International Conferences, 3<sup>rd</sup> QLIF Congress. Crop production, soil management. 20-23 March, Germany.
- Srivastava, J.K., Shankar, E., Gupta, S. (2010). Chamomile. A herbal medicine of the past with bright future. Mol. Med. Report, 3(6), 895–901. https://doi.org/10.3892/mmr.2010.377
- Steffen, K.L., Dann, M.S., Fager, K., Fleisher, S.J., Harper, J.K. (1994). Short-term and long-term impact of an initial large scale SMS soil amendment on vegetable crop productivity and resource use efficiency. Compost Sci. Util., 24, 75–83.
- Szudyga, K. (2005). Mushroom substrates. In: Growing mushrooms. Hortpress, Warszawa, 73–74.
- Ustawa z dnia 27 kwietnia 2001 r. o odpadach [The Substrate Act of 27 April 2001 as amended]. Dz. U. 01.62.628.

- Uzun, I. (2004). Use of spent mushroom compost in sustainable fruit production. J. Fruit Ornam. Plant Res., 12, 157–165.
- Wang, Z.H., Li, S.X., Malhi, S. (2008). Effect of fertilization and other agronomic measures on nutritional quality of crops. J. Sci. Food Agr., 88, 7–23.
- Williams, B.C., McMullans, S., McCahey, S. (2001). An initial assessment of spent mushroom compostas a potential energy feedstock. Bioresour. Technol., 79, 227–230.
- Wiśniewska-Kadżajan, B. (2012). Assessment of usefulness of the substrate after mushrooms growing to plant fertilization. Ochr. Śr. Zasobów Nat., 54, 165–176.
- Wiśniewska-Kadżajan B., Jankowski, K. (2015). Effect of mushroom substrate supplemented with minerals on yield of biomass and protein of orchard grass. Acta Agrophys., 22(3), 335–344.
- Wuest, P.J., Fahy, H.K. (1991). Spent mushroom compost. Traits and Uses. Mushroom News, 39, 9–15.
- Yen, G.C., Chen H.Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agr. Food Chem., 43, 27–32.
- Zu, Y., Yu, H., Liang, L., Fu, Y., Efferth, T., Liu, X., Wu, N. (2010). Activities of ten essential oils towards *Propionibacterium acnes* and PC-3, A-549 and MCF-7 cancer cells. Molecules, 15, 3200–3210.