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SPENT MUSHROOM SUBSTRATE AS BIOFERTILIZER IN AGROECOSYSTEMS OF BLUEBERRY

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Abstract: *Blueberries (Vaccinium spp.) are one of the most commercially significant berry crops. Of all the fruit crops, blueberries are perhaps the most amenable to organic production: pest problems are fewer than with most other fruits, and they preferentially use ammonium nitrogen which is a direct breakdown product of organic nitrogen sources. Spent mushroom substrate (SMS) is a by-product of mushroom growing. It is often classified as waste despite that it is high in organic matter and mineral micronutrient and can be reused as a bioadditive or fertilizer. Spent mushroom substrate from Lentinula edodes was tested as a fertilizer in comparison with peat. Determination of the chemical composition, number of microorganisms and the content of total microbial biomass, direction of microbiological processes in soil fertilized by SMS and peat were conducted. The initial data for the analysis, calculations and mathematical analysis were the results of the first year studies of the multi-year experiment. The actual content of elements showed that SMS contains a high content of all nutrients except N ammonium. The salt content is within acceptable limits. High content of N nitrate indicates a possible risk of excessive vegetation. Analysis of the cationic-anionic composition of the aqueous extract from SMS showed, that the content of all salts is within the permissible norm, except for a slight excess of the norm by Na+. Toxic water-soluble salts do not exceed acceptable value. Microbiological studies of the structure of soil microbial communities showed positive effects of SMS on microbial activity. Basing on these results assumptions can be made that spent mushroom substrate from Lentinula edodes has all possibilities to be used as a biofertilizer in agricultural sector.*

Keywords: *spent mushroom substrate, fertilizer, blueberry, chemical composition, soil microorganisms, microbial biomass.*

INTRODUCTION

Blueberry: Morphology and cultivation. Blueberry plants (*Vaccinium spp.*) are perennial woody shrubs that mature in 5- to 7 years. Canes emerge from a central crown, and each plant typically produces 15 to 18 upright canes by maturity. Growth habit is usually upright, although some sorts have a spreading form. Blueberry plants are deciduous, reaching full dormancy during the winter months.
Root growth begins in spring when soil temperatures reach 6-7°C, usually the time buds begin to swell. Root growth stops when fruit reach maturity and resumes again after harvest. In fact, during late summer and autumn, after harvest and when soil temperatures drop below 15-16°C again, blueberry roots make most of their growth. Blueberries have extremely fibrous root systems. Moreover, blueberry root systems are shallow. Roots are contained primarily (90 percent) within the dripline. Because they lack root hairs, blueberry roots are sensitive to drought and changes to soil-water conditions.

Blueberry flower buds form in autumn, with 5 to 8 flower buds on each shoot. Each bud has 5 to 10 potential flowers. Flowering can last 1 to 2 weeks, although early flowering sorts usually bloom for a longer period due in part to genetics and to cooler temperatures. Buds on shoot tips open first, and then flowering occurs sequentially down shoots. Buds on thin canes open before those on thick, woody canes.
Blueberries have urn-shaped, inverted flowers that shield them from wind and rain. As a result, self-pollination often cannot occur and pollination by insects is required. lower sweetness readily attracts insects such as bees; bees vibrate/sonicate and loosen pollen, thereby spreading pollen from flower to flower or self-pollinating flowers. Stigmas are only receptive to pollen for 3 to 6 days after bloom.
Pollinated flowers turn red colored, while unpollinated flowers remain white. Each of several ovaries per flower requires pollination; the more that are pollinated, the larger the fruit will develop. Some sorts are self-unfruitful, so cross-pollination increases fruit set for these sorts, also resulting in earlier fruit production and larger berries. Overall, open flowers with exposed stigmas are susceptible to a number of potential problems. They are the least cold hardy flower stage and can be easily damaged during extreme cold weather. Early blooming sorts also risk poor pollination as cool spring weather limits bee activity
Blueberry fruit develop approximately from 2 to 3 months after bloom, dependent upon cultivar, weather conditions, and plant vigor. Overall, thicker canes produce larger berries. Fruit is initially tart, but as fruit-acids are broken down during ripening, tartness is lost. Sugar is manufactured by leaves and transported to berries. Sugar levels increase for several days after fruit turns blue. For the highest quality fruit, berries should be allowed to ripen on plants; fruit flavor and sugar content will not improve after harvest. Green fruit contain approximately 7% sugar while mature fruit contain approximately 15%. Additionally, fruit size increases up to 35% after fruit turns blue culminating in about 85% water. Consequently, drought reduces fruit size, lowers starch content, and ultimately results in lower sugar content (Gautheir Ward N., Kaiser C., eds., 2013).
Blueberry is one of the most commercially significant berry crops. It is mainly cultivated in the United States and Canada, but also in Europe, Australia, Chile and New Zealand.. European cultivation of blueberry began after 1920, in the Netherlands. In the following years, cultivation spread to Poland and Germany, where the first European blueberry breeding products, crosses of North American genotypes, were introduced. However, blueberry cultivation did not expand into southwestern Europe until the 1980s. Today, European blueberry production is mainly concentrated in Germany, Poland, France, the Netherlands, Lithuania, Romania, Italy and Spain. For the last year's production of this crop is increasing in response to increased consumer demand for healthy foods.
Blueberry requires acidic, well-drained soils, with optimum acidity ranging from pH 4.5 to 4.8. As blueberry has a shallow root system (located mainly at depths smaller than 60 cm) the soil should be mulched with a deep layer (at least 10 cm) of organic mulch, such as bark, sawdust or leaves. Mulching increases the amount of organic matter in the soil, keeps moisture in the soil, protects roots from heat and helps to control weeds.
The blueberry root system has a limited water uptake capacity. Therefore, the amount of water applied and irrigation scheduling and distribution have a significant impact on its cultivation. Microjet and drip irrigation systems are currently the most commonly used in blueberry plantings, while sprinkler irrigation is used mainly for frost protection and cooling.
Most blueberry plantings require nitrogen applications each year, while other nutrients are generally applied only as needed. Ammonium sulfate is the preferred nitrogen source for blueberry, especially if the soil pH is relatively high (above 5.0), because it tends to decrease soil pH levels. Nitrogen is usually split in multiple soil applications during the spring, using granular formulation on the surface of the mulch, in order to increase nitrogen efficiency (Daniele Prodorutti et al., 2007).
Of all the fruit crops, blueberries are perhaps the most amenable to organic production. Pest problems are fewer than with most other fruits, and they preferentially use ammonium nitrogen which is a direct breakdown product of organic nitrogen sources. Even with these advantages, more research on growing blueberries organically is needed, especially in the area of pest management, organic bioadditives and fertilizers (Carroll, J., Pritts, M.P., and Heidenreich, C., eds., 2016).

Spent mushroom substrate. From waste to fertilizer. The end result of mushroom cultivation is not only the mushroom itself but also a huge amount of spent mushroom substrate (SMS). Traditionally SMS is discarded as wastes, creating an environmental nuisance, despite the fact that it is high in organic matter and mineral micronutrient. In last 10 years it became an increasing challenge for mushroom production industry and the emergence of a new line of research aimed at identifying the most environmentally and economically sustainable way to reuse it. The first thing that drew attention was the physical properties and nutritional

value of SMS.
In 2007 Fangliang Lia together with Qingbo Kong, Qing Zhang, Huangping Wang, Limin Wang, and Tao Luoc started a 10 year experiment to find out the how SMS affect soil humus composition, microbial biomass and functional diversity in paddy fields. In 2016 they collected soil from 6 treatments. The results showed that application of SMS could significantly increase the contents of (contents of soil organic carbon) SOC, soil total alkali-soluble humic carbon (HEC), humic acid carbon (HAC), especially the treatment of with high quantity of SMS, but not significantly increase the content of fulvic acid carbon (FAC). This indicated that the application of SMS could be considered as a good strategy for improving soil quality by reducing the input of chemical fertilizer. The appropriate application of SMS showed somewhat positive impact on the microbial biomass and microbial functional diversity. Sufficient but not excessive application of SMS was beneficial to the carbon source utilization of rapid growing microorganisms (r-strategy). Basing on these results authors made a conclusion that, application of SMS is an effective measure to improve paddy soil productivity. However, they also mentioned that subsequent studies are needed to further elucidate the changes in soil environment, soil nutrient cycling and soil microbial properties after the application of SMS (Fangliang et al., 2020).

In 2009 E. Medina, C. Paredes, M.D. Pérez-Murcia, M.A. Bustamante and R. Moral shared results of their experiment – spent mushroom substrates as component of growing media for germination and growth of horticultural plants. The results showed that the use of spent mushroom substrates in professional horticulture contributes to their disposal in an environment-friendly way and reduces the need for peat simultaneously (Medina et al., 2009).
In 2010 E. Herrero-Hernández, M.S. Andrades, M.S. Rodríguez-Cruz and M.J. Sánchez-Martín represented results of their studding of effect of spent mushroom substrate applied to vineyard soil on the behavior of copper-based fungicide residues. The results obtained show the influence of the solid organic carbon from the SMS organic residue in the retention of Cu from a Cu-based fungicide immediately after its application to the soil. The decrease in the concentration of the metal in the topsoil of the amended soils with time occurred parallel to the decrease and/or loss of the organic carbon in the soil. This effect, together with the ability of Cu to form soluble complexes with the dissolved organic carbon derived from SMS, facilitates the leaching of Cu and the decrease in total Cu content in the topsoil. In the case of the unamend soil, the decrease in the total content of Cu in the topsoil cannot be linked to the decrease in the OC content, since the degree of mineralization of the OC was low (Herrero-Hernández et al., 2011).
In 2017 Xingyao Meng together with group of scientists were experimenting with growth medium for tomato and pepper seedlings based on Composted biogas residue and spent mushroom substrate. The results showed that it is feasible to use biogas residues and SMS compost as a growth medium to replace peat in growing tomato and pepper seedlings. Compost can provide nutrition for plant growth comparable to that in chemical fertilizers. Though the addition of compost resulted in a slightly higher pH and electrical conductivity than the optimal range, amendment of the growth medium with compost yielded a better or comparable seedling quality (Meng et al., 2017).
In Ukraine mushroom growing, especial exotic mushroom is now on its rise and no researches connected with question of SMS were done (Ravlikovsky, Symochko, 2019). Therefore, it's needed to be studied in more detail.

MATERIALS AND METHODS

Experimental site and investigation design. The experiment was conducted on blueberry farm «Green FE» where spent mushroom substrate from shiitake was tested as a fertilizer in comparison with peat. Blueberry sorts «Duke» and «Bluecrop» were chosen for test. Determination of the chemical composition, number of microorganisms and the content of total microbial biomass, direction of microbiological processes in soil fertilized by SMS and peat were conducted in the private laboratory «FitoLab» and at the Institute of Agroecology and Environmental Management of National Academy of Agrarian Sciences of Ukraine. Data and requirements of national standards for blueberry cultivation were used as reference values. The initial data for the analysis, calculations and mathematical analysis were the results of the first year studies of the multi-year experiment. We determined the mean values (x) and their standard deviations (SD). The level of significance in the study was $P < 0.05$. Dispersion analysis and the Tukey test were used to compare the averages of the independent samples. The logistic transformation was applied to the data expressed as a percentage.

Description of blueberry sorts. «Duke» - early season sort. Concentrated harvest, suitable for mechanical harvest. Blooms late. Needs very good growing site. Large, firm, mild, sweet fruits, holds up well on bush. Consistently large yields. Moderately resistant to twig blight, susceptible to *Botryosphaeria* stem blight (dieback), moderately susceptible to mummy berry fruit phase.
«Bluecrop» - early and middle season sort. High, adapts to variety of sites. Consistent yields. Long harvest period. Large and very large firm, high quality fruits, resistant to cracking, limited shelf-life. Drought resistant. Very resistant to shoestring virus and moderately resistant to red ringspot virus, mummy berry, and powdery mildew. Very susceptible to anthracnose (Figure 1).

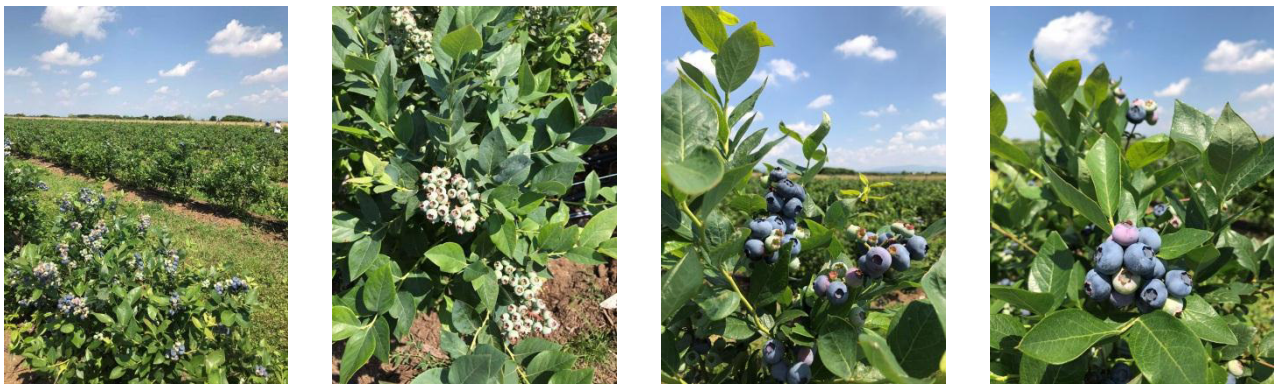

Figure (1) Blueberry sorts «Duke» and «Bluecrop»

Description of substrate preparation, SMS and soil sampling. Substrate for shiitake mushroom consists of next raw components – beech sawdust (aged for 2-3 month before use, moister level – 24-28%, particle size – 2-4 mm), barley, corn, sunflower seeds of fine grinding and wheat bran. All of components are mixed together. During mixing water is added. The optimal humidity of the substrate during and after mixing is between 56-63%. After mixing substrate is packed in special bags with microfilters and sterilized. Temperature during sterilization – 112-121°C, overpressure – 0.5-1 atm. This is needed to kill bacteria and molds before inoculation. After sterilization, substrate is cooled to 18-20°C. After cooling, the substrate is inoculated. The amount of mycelium for inoculation is usually 1-2% of the weight of the substrate. After inoculation, bags are sealed and moved to the incubation chambers where it is kept under controlled climate conditions (temperature – 21-25°C, air humidity – 60-80%, concentration of CO_2 – 2000-10000 ppm (0.2-1%)). During incubation substrate is passing through next stages: mycelium running, mycelium coat and bumps formation, pigmentation phase, coat hardening phase. Depending on type of substrate and conditions in chamber incubation period can vary from 16 to 20 weeks. After pigmentation and coat hardening phase substrate is moved from incubation chambers to fruiting. In first two-three days air temperature in fruiting chamber should be low – 12-14°C, and humidity high – up to 95%. This period is called temperature shock and is needed to stimulate the formation of primordial. Bags are cut off the substrate after first primordial appear. During or shortly after cutting substrate receives a water bath. The temperature in fruiting chamber is rising up to 18-20°C, concentration of CO_2 is kept in rang 1700-2000 ppm (0.17-0.2%). Mushroom is picked in dry conditions. On average, the yield of the first wave is 15-25% of weight of the substrate. Up to 5 waves can be received from one substrate but their yield is much lower – 10-12% (Ravlikovsky, Symochko, 2020). After harvesting SMS is received.
Spent mushroom substrate was crushed to small particles and stored n heaps for 6-8 months. During this period it was periodically mixed. The method and the amount which was used for fertilizing the soil with SMS was the same as it is used for peat (Figure 2).

Figure (2) Fertilized soil with peach (on the left) and SMS (on the right)

The soil sampling was carried out by standard methods (ISO 10381-6: 1993). The soil samples were selected from the 0−20 cm layer of the plants over the period when the system reached its climax - stable, balanced state. All samples were prepared using the unified procedure: they were air dried and grounded to < 3 mm in size; visible plant and mesofauna residues were removed. Experiments were performed in 4-fold repetition.

Chemical composition study. Chemical composition was carried out by standard methods as are used for peat (GOST 27984.6-88; GOST 27984.5-88; GOST 27984.4-88; GOST 27984.10-88; GOST 26483-85; GOST 26423-85; GOST 26715-85; GOST 7079-2009; GOST 7882-2015).

Microbiological study of soil microorganisms. For the microbiological analyses, soil samples were selected from each variant in 4-fold replication and prepared an average sample. Batches of 10 g each were put on sterile mortar and then the microorganisms were separated from the soil particles using the method of D. Zviahyntsev (1991). The quantitative compound of the microorganisms of the main ecological-trophic and taxonomic groups in soil was determined using the methods of inoculating the soil suspension to standard growth medias, which are generally accepted in soil microbiology (Zviahyntsev, 1991): streptomyces and bacteria which use mineral nitrogen (N_{min}) – to starch-and-ammonia agar (SAA), the number of pedotrophs – to soil agar (SA), oligotrophs on purified agar (PA), micromycetes – to Czapek-Dox agar, bacteria which use organic nitrogen (N_{org}) – to meat infusion agar (MIA) After the inoculation to the media, the bacteria were incubated at the temperature of 28ºC during 5–14 days. The colonies which grew in these media were calculated assuming that one colony is formed from one vital cell. The results of assessments of the number of microorganisms grown on the nutrient media were expressed in Colony Forming Units (CFU) per 1 g of dry soil. For this purpose, the moisture of the soil samples was determined for the experiments using the thermostat-gravimetric analysis, and recalculated the obtained number of colonies taking into consideration the coefficient of moisture and solution of the soil suspension. The inoculations were repeated three times, the obtained data were analyzed using mathematical statistics, calculating the confidence interval in the number of microorganisms.

Direction of soil microbiological processes. The direction of microbiological processes in the soil was determined by the appropriate coefficients (Andreyuk & Valagurova, 1992):

- coefficient of mineralization (K_{min}) was calculated by the ratio of the number of microorganisms immobilizing the mineral forms of nitrogen (C_{SAA}) to the number of organotrophs (C_{MIA}) by the formula: $K_{min} = C_{SAA} / C_{MIA}$;
- coefficient of oligotrophity (K_{ol}) was calculated by the ratio of the number of microorganisms, which are able to absorb nutrients from very rarefied solutions to the total number of eutrophic microorganisms by the formula: $K_{ol} = C_{PA} / (C_{SAA} + C_{MIA})$;
- coefficient of pedotrophity (K_{ped}) were calculated as the ratio of the number of pedotrophic microorganisms (C_{SA}) to the number of microorganisms using organic nitrogen (C_{MIA}): $K_{ped} = C_{SA} / C_{MIA}$;
- coefficient of transformation of organic matter (K_{tom}) was calculated by formula: $K_{tom} = (C_{MIA} + C_{SAA}) \times (C_{MIA} / C_{SAA})$.

RESUTS AND DISCUSSION

The actual content of elements showed that SMS has a high content of all nutrients except N ammonium (Table 1). The salt content is within acceptable limits. High content of N nitrate indicates a possible risk of excessive vegetation, the formation of large leaves of dark green color, which can lead to shading and irrational use of water from the soil by the plant.

Table (1) The actual content of elements in terms of the initial humidity of the samples

№	Name of the sample	Ca^{2+} mg/100 g	Mg^{2+} mg/100 g	P_2O_5 mg/100 g	K_2O mg/100 g	N ammonium mg/100 g	N nitrate mg/100 g
1	Soil fertilized by peat	427.6	210.6	78.0	29.09	4.0	1.61
2	Soil fertilized by SMS	455.6	729.6	338.0	340.5	41.0	183.0

Analysis of the cationic-anionic composition of the aqueous extract from SMS showed, that the content of all

salts is within the permissible norm, except for a slight excess of the norm by Na^+ (Table 2). Toxic water-soluble salts do not exceed acceptable value.

Table (2) Cationic-anionic composition of the aqueous extract of the samples, mg-eq./100 g

№	Name of the sample	HCO_3^-	Cl^-	Ca^{2+}	Mg^{2+}	Na^+	K^+	SO_4^{2-}	Amount of salts, %	pH
1	Soil fertilized by peat	0.15	0.30	0.29	0.50	0.21	0.10	1.34	0.11	4.31
2	Soil fertilized by SMS	0.01	0.12	2.79	2.64	1.18	4.22	1.31	0.37	4.04
Toxicity threshold for plants		*0.80*	*0.30*	-	-	*1.00*	-	*1.70*	*no salinity*	*Acidic*

For both soil samples the number of microorganisms and the content of total microbial biomass was determined (Table 3). The enrichment of soil by SMS led to the growth of microbial biomass (406.34±38.10). In the soil fertilized by SMS decreased number of oligotrophs more than in 3 times and pedotrophic microorganisms in 2 times in compare with soil fertilized by peat. Fertilization of soil by SMS creates new favorable conditions for soil microorganisms and leads to changes in the structure of their community.

Table (3) The number of soil microorganisms and the content of total microbial biomass

	Soil fertilized by peat	Soil fertilized by SMS
Micromycetes, $\times 10^3$ CFU g^{-1}	11.65±0.18	1.48±0.04
Bacteria which use organic nitrogen, $\times 10^6$ CFU g^{-1}	47.41±5.82	17.53±0.43
Bacteria which use mineral nitrogen, $\times 10^6$ CFU g^{-1}	28.0±0.56	24.7±1.44
Oligotrophs, $\times 10^6$ CFU g^{-1}	147.64±32.42	41.21±0.36
Streptomyces, $\times 10^6$ CFU g^{-1}	19.52±0.28	9.41±0.07
Pedotrophs, $\times 10^6$ CFU g^{-1}	84.25±2.36	43.71±1.81
Total microbial biomass, mcg	357.53±13.48	406.34±38.10

Basing on the number of soil microorganisms coefficients of mineralization, oligotrophity, pedotrophity and transformation of organic matter were calculated (Table 4).

Table (4) Direction of soil microbiological processes

№	Name of the sample	Coefficient of mineralization K_{min}	Coefficient of oligotrophity K_{ol}	Coefficient of pedotrophity K_{ped}	Coefficient of transformation of organic matter K_{tom}
1	Soil fertilized by peat	1.0	1.56	1.78	94.61
2	Soil fertilized by SMS	1.95	1.21	2.49	26.31

For soil fertilized by peat values of the coefficients were next: mineralization – 1.0, oligotrophity – 1.56, pedotrophity – 1.78, transformation of organic matter – 94.61. For soil fertilized by SMS we got next values: coefficient of mineralization – 1.95, coefficient of oligotrophity – 1.21, coefficient of pedotrophity – 2.49 and coefficient of transformation of organic matter – 26.31.

According to the results of microbiological studies of the structure of soil microbial communities we can conclude about the positive effects of SMS on microbial activity. Therefore, it's needed to be studied in more detail impact of agricultural land use on the soil microbiome, the dependence of the number and structural diversity of microbiota on environmental and human factors that determine the stability of agricultural soils (Patyka & Symochko, 2013; Symochko et al, 2015; Symochko & Hamuda, 2015).

CONCLUSION

The actual content of elements showed that SMS contains a high content of all nutrients except N ammonium. The salt content is within acceptable limits. High content of N nitrate indicates a possible risk of excessive vegetation, the formation of large leaves of dark green color, which can lead to shading and

irrational use of water from the soil by the plant. Analysis of the cationic-anionic composition of the aqueous extract from SMS showed, that the content of all salts is within the permissible norm, except for a slight excess of the norm by Na+. Toxic water-soluble salts do not exceed acceptable value. According to the results of microbiological studies of the structure of soil microbial communities we can conclude about the positive effects of SMS on microbial activity. Therefore, it's needed to be studied in more detail impact of agricultural land use on the soil microbiome, the dependence of the number and structural diversity of microbiota on environmental and human factors that determine the stability of agricultural soils.
Basing on these results assumptions can be made that spent mushroom substrate from *Lentinula edodes* has all possibilities to be used as a biofertilizer in agricultural sector.

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