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INFLUENCE OF EFFECTIVE MICROORGANISMS ON THE DIAMETER OF THE CAP AND MASS OF MUSHROOMS (*Agaricus bisporus*)

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Abstract

Mushrooms, due to their nutritional value, are increasingly included in people's diets. A small number of species are cultivated commercially in the world. There is also not a lot of research related to mushroom cultivation technology. The goal of the work was to determine the application of effective microorganisms in the production of champignon mushrooms in briquettes. EM Aktiv preparation is used in two treatments. In treatment EM1, compost was sprayed in the briquettes after mycelium sowing. Peat used as mulch was also sprayed twice. In the course of cultivation, spraying with water mist with the addition of preparations was carried out. In the EM2 treatment, only the initial treatment of compost and peat was carried out. The diameter of the champignon cap and the weight of the champignon were measured in each harvest (5 harvests in total). The diameter of the cap showed statistically significant differences only in dependence on the harvest term. With the length of the growing time, the diameter of the mushroom cap also increased. Mushroom mass was statistically highly dependent on treatment and harvest time. In treatment EM 1, an increase in the mass of champignons was determined by 19.97% compared to the control, and in treatment EM 2 by 0.04%.

Key words: Mushrooms, effective microorganisms, diameter of the mushroom cap, mass of mushrooms.

Introduction

Mushroom cultivation is one of the fastest growing and most promising agricultural sectors. Mushroom cultivation has advantages due to low production costs, and return value in a short period (Ahmad Zakil et al., 2020). Due to the increase in world population, the demand for fresh mushrooms has also increased. Although about 2,000 species are known to be true edible mushrooms of superior quality, only 35 species are commercially grown (Rathore et al., 2017). In addition to nutrition, mushrooms are used as dietary products that can help in the treatment of various degenerative disorders (Ma et al., 2018). Mushroom-based products are used in traditional Chinese medicine in acupuncture and for chiropractic purposes. The types of mushrooms that are grown the most are champignons (*Agaricus biisporus*), oyster mushrooms (*Pleurotus sp.*), then the Japanese shiitake mushroom (*Lentinus edodes*), and the rice straw mushroom (*Volvariella volvaceae*).

Agaricus bisporus is a type of mushroom - champignons that are most represented in commercial production. The largest producers of champignons in the world are China, Japan, the USA and the Netherlands, and in Europe Poland and Spain are significant (www.fao.org/faostat/en/).

The level of champignon production in Serbia is increasing year by year. The average production of champignons in the period 2006-2020 was 5,050.70 tons with an increase of 37.1%. The chemical composition of mushrooms determines their nutritional value and organoleptic properties, and it differs depending on the type of mushroom, the substrate on which it grows, atmospheric conditions, age and stage of collection.

According to the chemical composition of champignons, it can be said that they are a dietary product. In 100 g of fresh mushrooms, there are 3.09 g of protein, 3.26 g of carbohydrates, 0.34 g of fat and 1 g of fiber. In addition to essential amino acids, mushrooms also contain significant amounts of lysine, alanine, arginine, glycine, histidine, glutamic acid, aspartic acid, acids, proline and serine. In addition, champignons have an antioxidant effect because they are carriers of phenol derivatives, β-carotene, tocopherol, and ascorbic acid.

For the successful production of champignons, a good source of nitrogen, pH, temperature and humidity of the environment must be provided (Bellettini et al., 2019). Limiting factors are the limited selection and supply of biological pest control products that can be applied throughout the growing process (Rosmiza et al., 2016).

With the development of awareness about health-safe food, research on the possibility of mushroom production using effective microorganisms as growth stimulators and preventive protection against diseases is very important.

Effective microorganisms (EM) are an example of a supplemental compound that can be used in mushroom production. EM is defined as mixed cultures of microorganisms that provide natural benefits when applied as an inoculant in the soil ecosystem, while in mushroom cultivation it is an additive to increase microbial diversity in the substrate (Joshi et al., 2019). This group of microorganisms has an appropriate role in preventing the occurrence of green mold disease (Potočnik et al., 2015; Joshi et al., 2019). The application of EM in the agricultural industry is no longer new. However, it is still relatively new in mushroom cultivation applications.

The aim of the work was to determine the influence of preparations with EM on the diameter of the cap and the weight of the mushroom *Agaricus bisporus*.

Material and method of work

Research was carried out in 2022 in the chambers of Delta Danube d.o.o. Kovin, which is engaged in the production of champignons *Agaricus biisporus.* In the premises, the average air temperature was maintained at 21.8°C; average compost temperature 25.1°C; air humidity about 80%. The peat briquettes were seeded with fungal spores. After 17 days of incubation, the briquettes were opened and covered with a 5 cm layer of peat.

The experiment was set up as a two-factorial (factor A harvest time; factor B treatments). The treatments were applied according to the schedule in Table 1. For the spraying treatments, a microbiological preparation with effective microorganisms EM Aktiv (commercial name) was used.

Control briquettes are filled with standard technology used in production. Three briquettes were prepared for each treatment.

Table 1. Method of application of the tested factors

Treatments	28. 04. 2022.	29 .04. 2022.	01. 05. 2022.	03. 05. 2022.	04. 05. 2022.
EM 1	Compost: 50 ml EM+0.5 l H_2O Peat: 2 l H_2O/m^2	1 l H_2O/m^2	1.5 l H_2O/m^2	1 l H_2O/m^2	Compost: 100 ml EM+ 1.5 l H_2O
EM 2	Compost: 100 ml EM+1 l H_2O Peat: 2 l H_2O/m^2	1 l H_2O /m^2	1.5l H_2O/m^2	1 l H_2O / m^2	2.5 l H_2O/m^2
Control	2 l H_2O/m^2	2 l H_2O /m^2	0.5 l H_2O/m^2		2 l H_2O/m^2

During the cultivation, the briquettes were sprinkled with a fine mist of water to prevent them from drying out. During dewing, when the mushrooms were the size of peas in EM 1 treatment, 30 ml of EM Aktiva was added (Tabela 2).

Table 2. Scheduling for briquette sprinkled

Treatments	11 .05. 2022.	12. 05. 2022.	13. 05. 2022.	14. 05. 2022.	15. 05. 2022.	16. 05. 2022.
EM 1			0,5 l H_2O /m^2		EM 30 ml + 0.5 l H_2O	1 l H_2O/m^2
EM 2			0.5 l H_2O /m^2		0.5 l H_2O/m^2	1 l H_2O/m^2
Control	0.5 l H_2O/m^2	1 l H_2O /m^2	0.5 l H_2O/m^2	1 l H_2O/m^2	1 l H_2O /m^2	1 l H_2O/m^2

The harvest took place in five dates from May 18. 2022. until May 27. 2022 (Figure 1). At each harvest, the diameter of the cap and the weight of the champignons were measured. Samples for measurements were taken along an imaginary diagonal line per briquette (Figure 2).

Figure 1. Day zero of mushroom picking

Figure 2. An imaginary sample line

The first harvest is 20 days after covering, and the larger harvest is between the 22nd and 25th day after covering. Harvesting takes 4 - 5 days and is done manually. In that period, ventilation is very important, since mushrooms "breathe", which means that they produce large amounts of $CO2$, which must be expelled through the ventilation system. Otherwise, the color of the hat changes and the mushroom ripens faster, which results in a loss of quality and quantity. Mushrooms (champignons) are harvested at the stage of technological maturity, i.e. when the membrane is tight, while the fruits are still firm and closed. Maturity is judged by how tight the skin under the hat is, not by the size of the mushroom. Ripe champignons are both large and small, and the best size for placing them fresh is 3 to 5 cm. When about 70% of the wave is harvested, you should start watering for the second wave. If it is known that 90% of the champignon fruit is water, by picking 100 kg of mushrooms, 90 liters of water are taken from the substrate, and that much must be compensated before the second wave, within 2 - 3 days. Before the start of the formation of the second wave, cleaning is carried out, which involves removing the remains from the stalks, as well as from small, damaged, rotten and dead mushrooms. On such a detailed surface of briquettes, new individuals can be expected in 2 - 3 days, and in 5 - 6 days a new wave of harvesting. The cycle repeats itself and champignons can be harvested as long as they give birth, i.e., as long as there are nutrients in the compost.

Results and discussion

Successful production depends on external factors (temperature, moisture, light, substrate, air) and internal factors (genetic production potential). Requirements for water: During the incubation phase, it is necessary to maintain the air humidity at about 80%, the compost is not watered during this time, because it already contains the necessary moisture (about 70%). When the cover is reached, the peat should then have a relative humidity of about 75%, which should be maintained until the end of the cycle. In the cooling phase, the relative air humidity should be increased to about 88%, which should be maintained until the end of the cycle.

Temperature requirements: The temperature requirements of mushrooms (mycelium) are different, depending on the stage of development in which they are. Thus, while it is in the stage of incubation and covering, the temperature of the compost should be 25-26°C, and it should never exceed 27°C, because this leads to a decrease in fertility and the appearance of some diseases, and at temperatures of 30°C and above, the mycelia dies. As for the air temperature in these phases, it should be maintained at the level of 19 °C to 23°C, which depends on the activity of the compost, the season and the conditions in the nursery. It is a condition for the formation of nodules, ie. future fruits. This temperature is maintained until the end of the cycle.

Light requirements**:** Direct light does not interfere with the growth of mushrooms, but to a certain extent it can cause increased drying of the production areas, it can affect a certain change in the temperature regime in the nursery, then it can cause changes in the color of the fruiting body and thus reduce the quality, and it can also accelerate the development the causative agent of some diseases and pests. With all that in mind, mushroom production should be organized in dark rooms, because that's where the best yields and quality are achieved.

The diameter of the cap defines the category of champignons and significantly affects the total mass. The average diameter of the cap was 47.03 mm (Table 3). Factor A had a highly significant influence on the diameter of the cap, because with the increase in the number of days, the diameter of the cap also increased. The largest diameter of the cap was determined on the last day of harvest in the EM 1 treatment, and the smallest in the control on the first day of harvest. By applying treatment with EM, differences in the diameter of the cap were determined, but they were not statistically significant compared to the control.

Table 3. The diameter of the mushroom cap depending on the treatment (mm)

Dates of picking (A)	**Treatments (B)**			**Factor A**
	EM 1	**EM 2**	**Control**	
18.05.2022.	46.49	47.67	41.06	**45,07**
20.05.2022.	45.88	45.28	45.55	**45.57**
22.05.2022.	47.64	46.76	47.55	**47.32**
24.05.2022.	48.29	47.07	47.84	**47.73**
26.05.2022.	50.25	48.62	49.55	**49.48**
Factor B	**47.71**	**47.08**	**46.31**	
Average				**47.03**

	A **	B	A x B
F test	0.00	0.36	0.37
LSD $_{0.01}$	2.71	2.37	5.30
LSD $_{0.05}$	1.86	1.74	3.89

At each harvest, the weight of the mushrooms was measured. Based on the results, a highly significant influence of the examined variables and their mutual relationship on the mass of champignons can be observed. The average weight per harvest day was 1,507 kg (Table 4). In both treatments, a difference in the yield of mushrooms was determined, which is statistically highly significant compared to the control. In the EM 1 treatment, the mass of harvested champignons on a daily basis was 83.92% higher than in the control, and in the EM 2 treatment, it was 53.30% higher. The mass of champignons increased with the number of days. The highest mushroom mass was in the first treatment on the last day of harvest, and the lowest in the control on the first day of harvest. The obtained results are compatible with the mushroom cap diameter results. According to the mass and width of the cap, it was observed that the champignons in the EM 1 treatment were ready for harvesting earlier than in the EM 2 and control treatments. The obtained results are compatible with the researches of others. Wang et al., (2021) analyzed the influence of mushroom growth on the microbial activity of the substrate and determined that with mushroom growth there are changes in the composition and activity of the microbial community in the substrate, which leads to more intensive mushroom growth. According to Familoni et al., (2018) during the growing period, mushrooms interact with different groups of the microbial community that accelerate the compost mineralization process leading to a change in the composition of the substrate.

Table 4. Average yield of harvested champignons (kg)

Dates of picking (A)	**Treatments (B)**			**Factor A**
	EM 1	**EM 2**	**Control**	
18.05.2022.	0.068	0.072	0.052	**0.064**
20.05.2022.	0.744	0.780	0.665	**0.730**
22.05.2022.	2.159	1.944	1.884	**1.996**
24.05.2022.	3.026	2.226	2.154	**2.469**
26.05.2022.	3.043	2.515	2.779	**2.779**
Factor B	**1.808**	**1.507**	**0.983**	
Average				**1.507**

	A**	B**	A x B**
F test	0.00	0.00	0.00
$LSD_{0.01}$	0.36	0.20	0.44
$LSD_{0.05}$	0.25	0.14	0.32

The total mass of champignons for all five harvests was 24,144 kg (Table 5).

Table 5. Total yield of champignons (kg)

Yield	**Treatments**			**Total**
	EM 1	**EM 2**	**Control**	
In total	9.039	7.537	7.534	24.144
Deviation %	19.97	0.04	100	

The highest mass of harvested champignons was 9,039 kg in the EM 1 treatment, which was 19.97% more than in the control. In the EM 2 treatment, the total mass of harvested mushrooms was 0.04% higher than in the control.

Mushroom cultivation is important for sustainable production systems. According to Zhang et al., (2014) after mushroom harvesting, a large mixture of mycelial residues, carbohydrates and nutrients (bacterial proteins and minerals) secreted by microbes remains in the substrates. which is a valuable agricultural renewable resource. Some studies have shown that mushroom residues are not only a by-product, but also a compostable resource that can be applied as an organic fertilizer (Tangjuan et al., 2016).

Conclusion

Based on the research results, it can be concluded that the application of effective microorganisms had a significant impact on the growth and yield of champignons. The industrial production of champignons with the application of effective microorganisms has an advantage from the ecological aspect of the production of health-safe food.

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