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EFFECT OF SPAWN STORAGE AT THE TEMPERATURE OF LIQUID NITROGEN OF FOUR STRAINS OF FIELD MUSHROOM *AGARICUS BISPORUS* (LANGE) IMBACH ON ITS GROWTH AND PINHEAD SETTING

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ABSTRACT. The spawn of *Agaricus bisporus* refrigerated in liquid nitrogen was tested. The results of the experiment showed that the spawn nitrogen refrigeration did not influence the mycelium growth as well as pinhead formation.

Key words: *Agaricus bisporus*, spawn, storage, liquid nitrogen, mycelium growth

Introduction

Spawn plays a significant role in the production process of the field mushroom and is considered as the most important yield-forming factor (**Gapiński** et **al.** 1989, **Overstijns** 1996). In 1996, the Polish standard PN-R-67060:1996 was established which specifies, among others, conditions for spawn storage. According to **Vedder** (1980), commercial spawn can be stored at the temperature of 0 to 2°C for several weeks without deterioration of its quality, whereas at room temperature, only for some days. **Szudyga** (2002) recommends storage of commercial mycelium at the temperature of up to 2°C paying attention to the cleanliness of the cold store. **Gapiński** et **al.** (1990) found that the significant physiological changes occurring in the spawn were influenced most by: the storage time, temperature and the presence of bacteria. The yields of the field mushroom depend, to a considerable extent, on the way of spawn storage (**Sobieralski** et **al.** 1994). The above-mentioned researchers maintain that the spawn quality was influenced, primarily, by the duration of storage and strain. On the other hand, **Sobieralski** et **al.** (2002) demonstrated that the growth of the field mushroom mycelium after storage was influenced by the following factors: strain, type of substrate, temperature and duration of storage.

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The objective of the performed investigations was to determine the impact of spawn storage at the temperature of liquid nitrogen of four strains of the field mushroom on its growth, setting of pinheads and yields in conditions of commercial cultivation.

Material and methods

Four strains of *Agaricus* which have been cultivated in our country i.e. 'Italspawn F56', 'Horst U3', 'Somycel 516' and 'Irish 501' were used in the experiment.

Grain spawn used in the experiment was produced on rye grain which was prepared in the following way: rye grain was boiled for 20 minutes and then filtered. After cooling down, 3 g of gypsum and 20 g of chalk was added to each kilogram of the boiled grain and then it was mixed thoroughly.

The grain prepared in the way described above was used to fill polypropylene bags containing biological filters. Each bag was filled with 0.5 kg of rye grain. After sealing, the polypropylene bags were sterilized for one hour in an autoclave at the temperature of 121°C and pressure of 0.12 MPa. After cooling, the grain was inoculated with the examined spawn and placed in an incubator at the temperature of 25°C and air relative humidity of 80-85% until the moment it was grown over by the mycelium completely.

Next, ten grains of the spawn were placed in polypropylene test tubes which were filled with sterile 10% glycerol solution. The test tubes were then sealed and placed in a refrigerator at the temperature of 3-4°C. Afterwards, they were put into a container with liquid nitrogen at the temperature of –196°C. The mycelium containing test tubes were stored in the liquid nitrogen for 24 months and then they were removed from it and placed in an incubator at the temperature of 24-25°C. They were taken out of the incubator after one hour and the mycelium was removed from test tubes and rinsed with sterile water. Next, two grains of the spawn were transferred onto Petri dishes of 10 cm diameter on which previously prepared solidified farmyard manure – peat medium was placed. Once the substrate was inoculated with the mycelium, it was incubated in the incubator at the temperature of 25°C and air relative humidity of 80-85%.

After 14 days of incubation, the diameter of the mycelium colony was measured. Measurements were made with the accuracy of 1 mm. Once the experiments on the impact of the storage of mycelium on its growth on agar substrates were terminated, the authors selected combinations which were studied with regard to the number of set pinheads of the carpophores. This experiment was carried out on two strains: 'Horst U3' and 'Somycel 516'.

The experiment was conducted in Styrofoam containers measuring 30 × 40 × 15 cm. Each container was filled with 6.5 kg of the cultivation substrate which was mixed with the spawn of the examined mushroom strains. The growing substrate was purchased at the "COMPOST COMPANY Ltd." production enterprise from Goździn. The experimental spawn made up 5% of the weight of the substrate. In order to maintain constant humidity, the top surface of the substrate was covered with newspaper paper. The incubation process was carried out at the temperature of 24-25°C until the mycelium covered the entire surface of the substrate. At the termination of the incubation process, the paper was removed and the substrate surface was covered by 6 cm thick peat layer. The cover was prepared from the mixture of highmoor and sand. The applied peat was neutralized with lime on the basis of the neutralization curve to pH of about 7.5, then it was

moistened and mixed with sand at the volume ratio of 1:3. The mycelium growth in the cover took place at the temperature of 25°C and air relative humidity of 90-95% until the mycelium grew into the cover to the height of 5.5 cm. Then the temperature was decreased to the temperature of 17-18°C. Pinheads appearing on the cover surface which were set between days 7 and 14 from the moment of the temperature reduction were counted. The number of the determined pinheads constituted a mean value from three randomly selected areas of 25 cm^2 for each container. The pinhead number was then calculated per 1 cm^2 of the cultivation area. The control was the number of pinheads obtained from a cultivation conducted in identical conditions but using fresh mycelium of tested strains, not one that was stored.

In order to ensure better clarity of the presented results, the following designations were adopted and used throughout the study:

1) fresh mycelium of tested strains was designated as g.ś.,
2) the 'Italspawn F56' strain was designated as F56,
3) the 'Horst U3' strain was designated as U3,
4) the 'Somycel 516' strain was designated as 516,
5) the 'Irish 501' strain was designated as 501.

The experiments in which the impact of the storage method on the growth of mycelium on the farmyard manure-peat substrate was investigated were established in six replications employing the random design, whereas the experiments on the effect of the storage method on the number of the set pinheads of the carpophores were established in four repetitions using the random design.

The results regarding the growth of mycelium on substrates as well as the amount of the set pinheads of the carpophores of the field mushroom were determined for the mean values from four replications.

All statistical analyses were carried out in accordance with the appropriate one and two factorial experiments with the LSD calculated at the level of $\alpha = 0.05$.

Results

The authors failed to find any influence of the length of the spawn storage in conditions of liquid nitrogen on the diameters of the mycelium colonies.

The diameters of colonies obtained after 14 days of incubation from the fresh mycelium of the examined strains and that stored for the period of 3, 6, 12 and 24 months did not differ significantly among another (Figs 1-4).

The authors also failed to observe any impact of the examined strains on the colony diameters of mycelium stored at the temperature of liquid nitrogen.

Colony diameters of the mycelium of the four strains of the field mushroom stored at the temperature of liquid nitrogen did not differ significantly from one another. In addition, the examined strains failed to show differences with regard to the diameters of colonies obtained from the fresh spawn. The above-mentioned correlations concerned all the examined strains.

It should be emphasised that all the examined strains of the field mushroom were characterized by very similar mycelium growth. After 14 days of incubation, the diameter of the mycelium colonies of the four examined strains was approximately 7 cm.

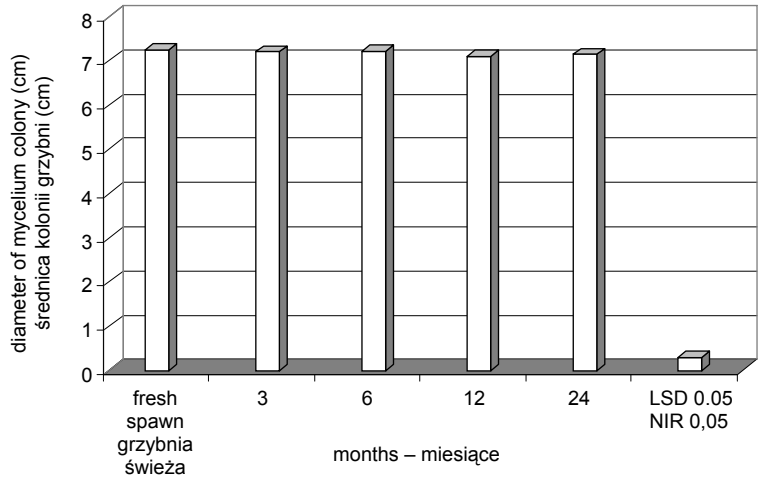

Fig. 1. Mycelium growth of the fresh 'Italspawn F56' strain and stored in liquid nitrogen
Ryc. 1. Wzrost grzybni odmiany 'Italspawn F56' nie przechowywanej i przechowywanej w temperaturze ciekłego azotu

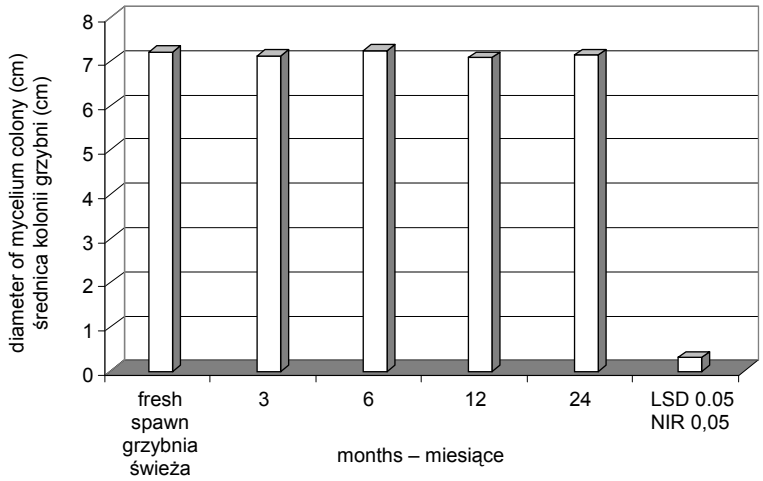

Fig. 2. Mycelium growth of the fresh 'Horst U_3' strain and stored in liquid nitrogen
Ryc. 2. Wzrost grzybni odmiany 'Horst U_3' nie przechowywanej i przechowywanej w temperaturze ciekłego azotu

It was found that the storage of mycelium of the examined mushroom strains in conditions of liquid nitrogen failed to exert a significant impact on the number of the set pinheads of the carpophores (Fig. 7). The fresh spawn of the 'Horst U3' strain developed 3.2 pinheads per 1 cm^2, while the spawn stored in liquid nitrogen – 3.0 pinheads per 1 cm^2.

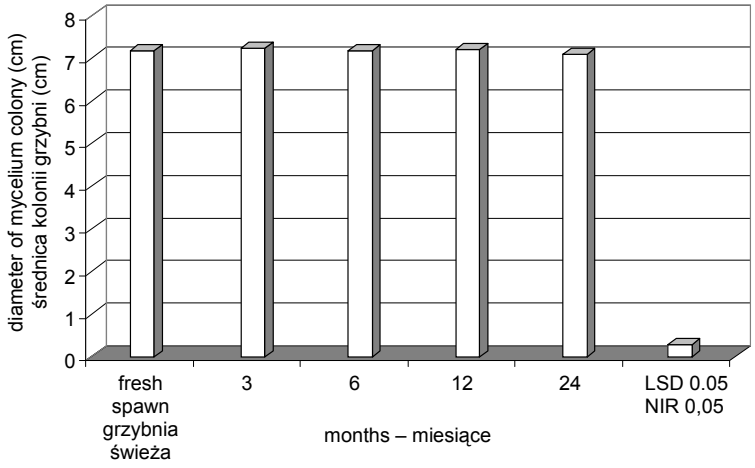

Fig. 3. Mycelium growth of the fresh 'Somycel 516' strain and stored in liquid nitrogen
Ryc. 3. Wzrost grzybni odmiany 'Somycel 516' nie przechowywanej i przechowywanej w temperaturze ciekłego azotu

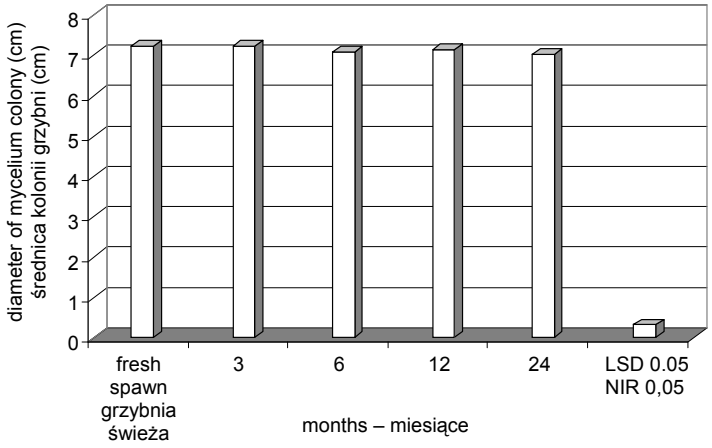

Fig. 4. Mycelium growth of the fresh 'Irish 501' strain and stored in liquid nitrogen
Ryc. 4. Wzrost grzybni odmiany 'Irlandzka 501' nie przechowywanej i przechowywanej w temperaturze ciekłego azotu

The fresh spawn of the 'Somycel 516' strain developed 3.3 pinheads of carpophores per 1 cm^2, whereas the spawn stored in liquid nitrogen – respectively, 3.4 and 3.2 pinheads per 1 cm^2.

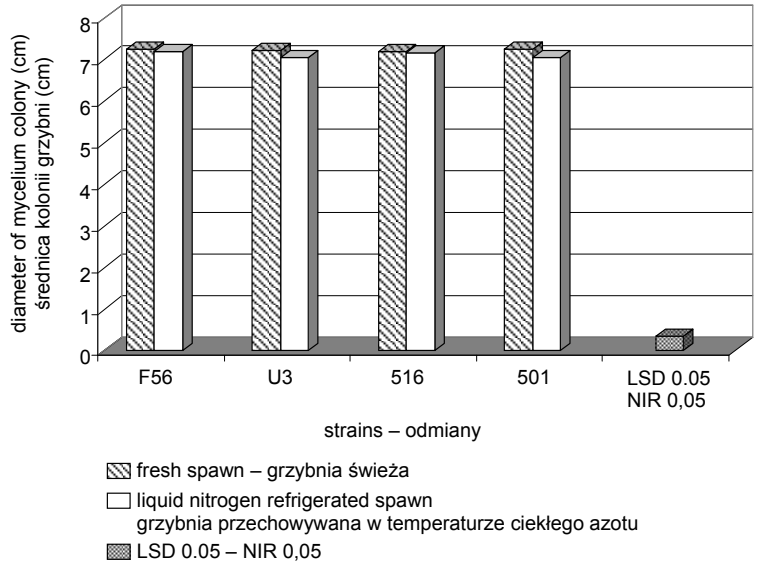

Fig. 5. Growth of fresh and nitrogen refrigerated spawn, independently of the period of storage
Ryc. 5. Wzrost grzybni świeżej badanych odmian i przechowywanej w temperaturze ciekłego azotu, niezależnie od długości okresu przechowywania

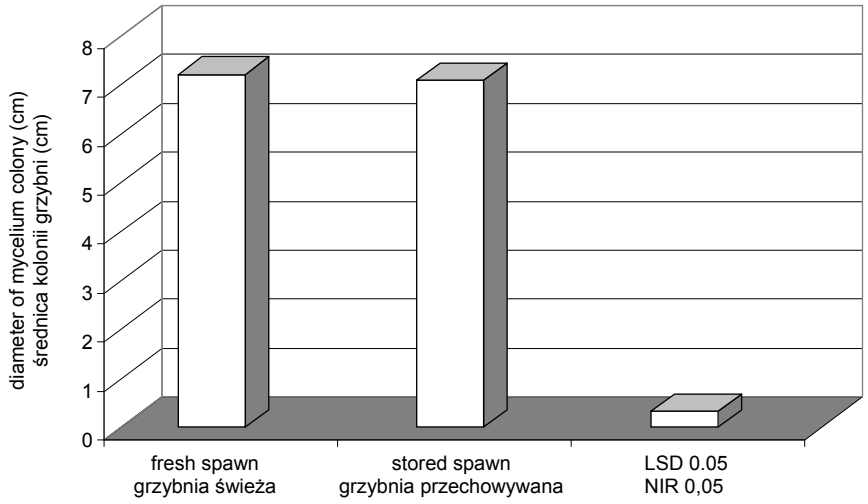

Fig. 6. Growth of fresh and nitrogen refrigerated spawn
Ryc. 6. Wzrost grzybni świeżej i przechowywanej w temperaturze ciekłego azotu

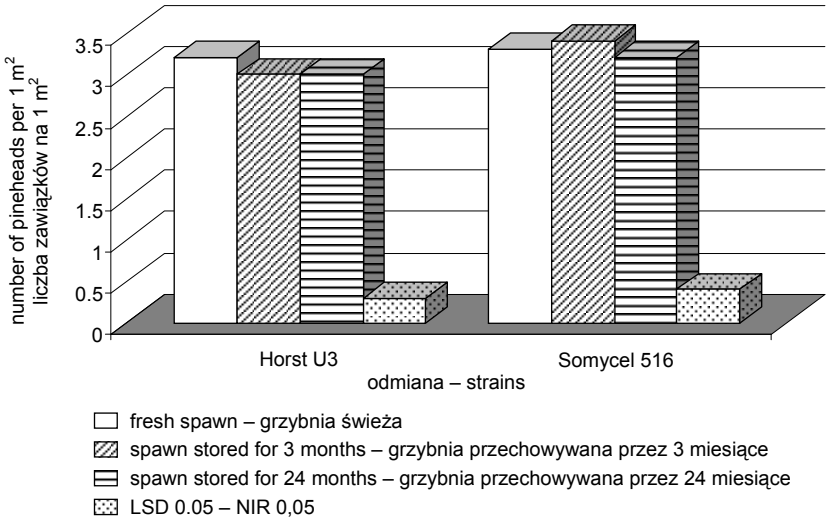

Fig. 7. Pinheads formation by the fresh and refrigerated (3 and 24 months) spawn of 'Horst U3' and 'Somycel 516' strains
Ryc. 7. Wiązanie zawiązków przez grzybnie odmian 'Horst U3' i 'Somycel 516', świeżą i przechowywaną przez 3 i 24 miesiące

Discussion

The performed experiments confirmed that the storage of the field mushroom spawn in conditions of liquid nitrogen is a very good method of storage of the reproduction material because it does not lead to the worsening of the rate of mycelium growth and does not influence the formation of pinheads of the carpophores. Many researchers consider the rapid and proper growth of the mycelium as the most important condition of securing a high yield of the field mushroom and one of the most important goals of cultivation (**Gapiński** and **Siwulski** 1984, 1985, **Makarova** and **Rybakova** 1990). **Szudyga** (2002) recommends organoleptic control of the spawn quality which must be characterized by a typical mushroom smell and must be free from infections. The culturing substrate quickly overtaken by mycelium hyphae provides a natural barrier limiting the occurrence of competitive mushrooms (**Gapiński** et **al.** 1988 b, **Sobieralski** 1998).

The performed experiments revealed that the storage of spawn in conditions of liquid nitrogen did not influence the growth of the mycelium. In addition, the investigations corroborated the results of experiments of other researchers who demonstrated that long-term (several years) storage of the spawn of the field mushroom at the temperature of –196°C did not result in changes of its productivity, while the quality of the obtained carpophores did not differ from the quality specified in appropriate standards (**Elliott** 1985, **Hwang** and **San Antonio** 1972, **Jodon** et **al.** 1982). According to **Bednarek** (1997), the best spawn quality was obtained from the incubation chambers which were not stored in a cold store. This kind of spawn inoculated into good quality substrate developed noticeably faster and, therefore, it was possible to speed up the appearance of the first flush even by up to three days.

The results of the presented investigations allowed the authors to conclude that spawns stored in conditions of liquid nitrogen are characterized by the growth similar to that observed in the case of fresh spawn. In addition, the obtained results are in keeping with the research results obtained by the authors earlier. In the case of the above-mentioned studies, the authors employed a different method of assessment of setting of the carpophores (beakers of 600 cm^3 volume) and mushroom strains with white and brown carpophores (**Siwulski** and **Sobieralski** 1995 a, b). On the basis of the obtained research results as well as the available literature data, it can be stated that the storage of the field mushroom spawn at the temperature of liquid nitrogen is a good method of storage of mushroom spawn which does not affect its growth and the setting of the pinheads.

Conclusions

1. The spawn storage of the examined strains of the field mushroom for 24 months in conditions of liquid nitrogen did not affect the growth and the setting of the pinheads of the mushrooms.

2. The examined strains showed very similar growth rate of the mycelium and setting of pinheads both before and after the period of storage.

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WPŁYW PRZECHOWYWANIA GRZYBNI CZTERECH ODMIAN PIECZARKI DWUZARODNIKOWEJ W TEMPERATURZE CIEKŁEGO AZOTU NA JEJ WZROST I WIĄZANIE OWOCNIKÓW

S t r e s z c z e n i e

Badano wpływ przechowywanej grzybni ziarnistej czterech odmian pieczarki dwuzarodnikowej na jej wzrost i wiązanie owocników. Grzybnię ziarnistą czterech odmian pieczarki przechowywano przez 24 miesiące w warunkach ciekłego azotu. Wzrost grzybni badano na pożywce obornikowo-torfowej. Uprawę prowadzono w pojemnikach wypełnionych 6 kg podłoża. Powstające zawiązki owocników liczono na powierzchni okrywy pomiędzy 7. a 14. dniem od obniżenia temperatury, a następnie przeliczono na 1 cm^2 powierzchni uprawy. Badania wykazały, że przechowywanie grzybni pieczarki w temperaturze ciekłego azotu nie miało wpływu na jej wzrost i wiązanie owocników. Badane odmiany pieczarki były bardzo zbliżone pod względem wzrostu grzybni i wiązania owocników.