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Effect of different grain spawn materials on *Pleurotus ostreatus* (Jacq.) P. Kumm. mushroom cultivation under unregulated and regulated fruiting conditions

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Abstract: Quality spawn, which is also dependent on grain composition, is a critical factor that must be optimized to achieve successful and profitable mushroom farming. The characteristics of grain spawn composition (Factor A) and two microclimatic fruiting conditions (Factor B) were studied in the cultivation of *Pleurotus ostreatus*. Eight different grain material combinations (GMC1-8) made from wheat, barley, oat, and millet were used to prepare spawn and tested for mushroom cultivation under unregulated and regulated fruiting conditions. The physicochemical characteristics of the different grain spawn, substrate, time to attain the first flush, and BE (biological efficiency) in the different GMCs under the two fruiting conditions were determined. The differences in nutrient compositions of the GMCs tested did not result in a significant difference in the nutrient composition of the cultivation substrate. GMCs containing barley and oat gave BE values that were not significantly different under the two microclimatic conditions tested. GMCs containing 100 % wheat and millet resulted in the poorest BE recorded. However, equal combination of wheat, barley, and oat (GMC8) gave the best results among GMCs tested. Furthermore, it is more cost-efficient to use the GMC8 combinations since wheat is cheaper than all other grains tested.

Key words: biological efficiency; grain materials characteristics; microclimate in fruiting house; substrate colonization

Učinek različnih gojilnih substratov iz žitnih zrn na gojenje ostrigarja (*Pleurotus ostreatus* (Jacq.) P. Kumm.) v razmerah uravnavane in neuravnavane tvorbe trosnjakov

Izvleček: Kakovosten inokulacijski micelij in gojišče, ki je odvisno tudi od sestave žitnih zrn sta kritična dejavnika, ki morata biti optimizirana za doseganje uspešnega in donosnega gojenja gob. Preučevane so bile lastnosti in sestava žitnih zrn inokulacijskega micelija (Factor A) in dva režima mikroklimatskih razmer (Factor B) za tvorbo trosnjakov pri gojenju ostrigarja. Za pripravo inokulacijskega micelija in njegovega gojišča je bilo uporabljeno osem različnih kombinacij (GMC1-8) žitnih zrn, in sicer pšenice, ječmena, ovsa in prosa v razmerah uravnavane in neuravnavane tvorbe trosnjakov. Določene so bile fizikalno-kemijske lastnosti različnih žitnih zrn pri pripravi inokulacijskega micelija, njegovega gojišča, časa za doseganje prve tvorbe trosnjakov v dveh mikroklimatskih razmerah in različnih kombinacijah GMC. Preiskušene razlike v mineralni sestavi GMC niso dale značilnih razlik v sestavi mineralih hranil gojišča. GMC, ki so vsebovala ječmen in oves so imela biološko učinkovitost (BE), ki ni bila značilno različna v obeh preiskušenih mikroklimatskih razmerah. GMC, ki so vsebovala 100 % pšenico in proso so imela najslabšo biološko učinkovitost. Kombinacija enakih odmerkov pšenice, ječmena in ovsa (GMC8) je imela najboljši rezultat med preiskušenimi gojišči (GMCs). Cenovno je kombinacija GMC8 najboljša, ker je pšenica cenejša kot vsa ostala preiskušena žita.

Ključne besede: biološka učinkovitost; lastnosti žitnih zrn; mikroklima v prostoru tvorbe trosnjakov; kolonizacija substrata

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1 INTRODUCTION

The rapid development of industrial mushroom production around the world especially exotic mushrooms, is due to the growing interest in healthy eating. Today, more than 2,000 species of edible and / or medicinal mushrooms are known, many of them are widely used as a source of bioactive compounds (Friedman, 2016). Mushrooms of the genus *Pleurotus* (Fr.) P. Kumm., popularly called oyster mushrooms, are the second most consumed mushroom in the world (Sánchez, 2010). In Ukraine, the three industrially cultivated species are *P. ostreatus* (Jacq.) P. Kumm. (oyster mushroom), *P. pulmonarius* (Fr.) Quél (lung oyster), and *Pleurotus eryngii* (DC.) Quél. (popularly called king oyster), and each of them has valuable medicinal and nutritional properties (Bukhalo et al., 2011).

Spawn type and quality is a critical factor in the process of mushrooms cultivation. It is composed of a base, carrying the vegetative mycelia of the target fungi to be cultivated, which is used to inoculate the cultivation substrate. Various types of materials and technology for spawn production have been reported (Green, 1977). The most common technology, patented by James W. Sinden (Sinden, 1932), utilizes grain to make the spawn. Spawn quality is critical to successful mushrooms cultivation, and many factors must be considered and optimized to produce quality spawn. The main factors are:

1. Microbiological purity of the culture, i.e. the absence of foreign microbiota (bacteria, yeast, molds) in the mushroom culture (Dudka et al., 1978; Petrova, 2010);

2. Suitability of spawn base material (grains, sawdust, wood chips, etc.) for rapid colonization by target mushroom mycelia (Jhune et al., 2000; Sainos et al., 2006);

3. Grain-carrying potential, which is the quantity and quality of nutrients in grains that supports actively growing fungal cells during the transition to a cultivation substrate or during long-term storage (Mamiro & Royse, 2008; Zhang et al., 2014)

4. The number of inoculum units within a given quantity/mass of ready to-use spawn.

It is generally believed that the number of grains per unit of spawn quantity determines the number of inoculation points and rate of substrate colonization; hence the more the number of grains, the better and quicker colonization is expected (Subramanian et al., 2014; Sofi et al., 2014; Khonga et al., 2013). The importance of spawn material quality in successful mushrooms production was recognized as far back as 1905, by B. M. Duggar in one of the first books on industrial cultivation of mushrooms "The principles of mushroom growing and mushroom spawn making" (Duggar, B. M., 1905). The large number of scientific publications dedicated to improving the quality and methods of spawn production, and its application in different countries, indicate its importance in the mushroom industry (Alekseyenko et al., 2010; Bhatti et al., 2007; Hoa & Wang, 2015; Royse & Chalupa, 2009). The search for the best cereals grain for use in spawn production is complicated by factors related to the types of available local grains, as well as their moisture and nutritional contents, including the cultivation conditions i.e. soil and climate (Stanley & Awi-Waadu, 2010; Jiskani et al., 2007; Rosado et al., 2002; Ivanova & Kovalyshyna, 2018). In countries where there is a shortage of cereals, the use of wheat results in expensive spawn, hence they use sorghum (*Sorghum bicolor* (L.) Moench)) grains, cotton (*Gossypium hirsutum* L.) waste, and in some cases the leafy part of aquatic plants (Mahmoud, 2006).

The industrial production of mushroom spawn on cereal grains in developed countries is highly a technical process which continues to improve over the years. Quality spawn for commercial mushroom production is expensive. Hence, research to find affordable and effective ingredients, as well as low-cost technology, to lower the cost of commercial spawn is intense (Gregori et al., 2008). The mixture of different materials that should increase the nitrogen content in the ready spawn, and thus improve its colonization and speed of adaptation to cultivation substrates, is an area of active research (Hoa & Wang, 2015; Ivanova & Kovalyshyna, 2018). Kananen and McDaniel (2000) patented the formula of seed mycelium with mineral components (perlite and vermiculite), which increased the number of inoculation points in 100 grams of spawn to 20 thousand pieces. It is known that the properties of spawn materials used for inoculation have a decisive influence on the duration of the incubation period, biological efficiency (BE), and even the morphological features of the mushrooms cultivated (Soko et al., 2019)

The most available grain raw material useful in spawn production in Ukraine and most parts of Europe is wheat, and the least are oat and millet. Millet, which experienced significant reduction in production since 2017 (Petrenko, 2019). Also, the export and domestic demands on a particular grain crop can lead to significant price fluctuations. The nutrient content of grain depends on many physicochemical factors and cultivation conditions: the soil composition and climatic conditions under which they were grown, as well as inherent genetic characteristics and nutrient availability, can affect their performance as materials for spawn production (Shapovalenko et al., , 2017). These factors have made grains other than wheat expensive materials for making spawn. We believe that the use of local resources (grains) to produce quality spawn will reduce spawn cost and

boost mushrooms production and supply to the market at any particular location.

The biochemical composition of grain spawn material and microclimatic factors (temperature, humidity, air composition, and light) play significant roles in the vegetative mycelia adaptation to cultivation substrate after spawning, and these factors are readily optimized in large-scale industrial production situations (Bisko & Dudka, 1987; Zaikina et al., 2007; Vdovenko, 2015) However, in small-scale production, it is difficult to optimize factors like room temperature; sharp changes in outdoor temperature in winter and summer significantly affect the microclimate of the growing chambers. Such fluctuations could significantly reduce the productivity of cultivated mushrooms.

This study was done to assess the influence of grain-carrier composition on the productivity of *P. ostreatus* under two microclimatic conditions. The hypothesis is that different grains and their mixtures will have significant effects on fruit body yield during cultivation and that grain spawn material compositions can affect BE in oyster mushrooms differently under regulated and unregulated microclimatic conditions in *P. ostreatus*.

2 MATERIALS AND METHODS

2.1 GRAINS

The cereal grains used as spawn materials were wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), millet (*Panicum miliaceum* L.), and oat (*Avena sativa* L.). The general physical dimensions of the grains used in this experiment is reposted by Pilipyuk (2010) and shown in Table 1 below.

2.2 FUNGAL CULTURE

Pleurotus ostreatus, strain 2301 IBK (*P.o.* 2301), which is widely cultivated in commercial production during the winter in Ukraine, was selected and used for this study due to its performance reported in previous studies (Bisko et al., 2016; Myronycheva et al., 2017). The culture was obtained from the culture collection at the M. G. Kholodny Institute of Botany, National Academy of Sciences (NAS), Ukraine. Actively growing mycelia was inoculated onto 2 % malt extract agar and, incubated at 26 ± 1 °C for 8 days, and thereafter stored at 3 ± 1 °C until use. To prepare grain inoculum, stored culture from above was used to inoculate Petri dishes (90 mm) containing about 35 ml of 2 % PDA and incubated at 24 ± 1 °C for 8 days. Colonized medium in a plate was added to 250 ml sterile water in a blender and blend for 20 s to obtain mycelia suspension.

2.3 MOTHER SPAWN

A mixture of millet grain (95 %), rape seed (4 %), flax seed (0.5 %) and $CaCO_3$ (0.5 %) was used to prepare the mother spawn. First, the rape seed was soaked in water overnight (8-10 h) and drained before use. The millet was boiled in water for 35 ± 5 minutes, after which heating was discontinued, and the grains were allowed to remain in the hot water for additional 20 ± 3 minutes, and then drained for 15 ± 3 minutes. That treatment brought the millet grain moisture content to 44 ± 2 %.

The millet grain was then poured into a bath, and the pre-soaked rape seed, flax and $CaCO_3$ were added and mixed properly, before loading (5500 ± 53 g each) into heat-resistant polypropylene bags (580 x 490 mm), PP75/BEU6/X47-57 (from Sac02, Veldeken 29, 9850 Deinze, Belgium). The bags were sterilized at 129-132 °C, 1.1 bar (16 PSI) for three hours. After cooling to 26 ± 2 °C, the grain mixture was inoculated under aseptic conditions with the culture suspension from above, at the rate of 75 ml per bag, sealed and shaken to evenly distribute the suspension in the grain. The inoculated bags were placed on shelves in a clean room at a temperature of 22 ± 2 °C and relative humidity of 65 ± 5 %, to incubate for 10 days to achieve full colonization and matured mother spawn. The mother spawn was cooled to 11 ± 1 °C and stored at 0-2 °C until it was used (within 7 days).

Table 1: Physical characteristics and dimension of common grains

Type of grain	Length, mm	Width, mm	Thickness, mm	Weight/10 g
Wheat	4.0 – 11.2	1.6 – 4.0	1.6 – 3.4	2.5 – 3.5
Barley	7.0 – 14.6	2.0 – 5.0	1.4 – 4.5	3.0 – 4.6
Oat	8.0 – 18.0	1.4 – 4.0	1.2 – 3.5	2.2 – 4.2
Millet	1.9 – 3.2	1.5 – 2.9	1.3 – 2.0	0.4 – 0.7

Source: (Pilipyuk, 2010)

2.4 DIFFERENT TEST SPAWN

Wheat, millet, barley and oat combined in various proportions were used to prepare the various test spawn with different grain material compositions (GMCs). The combinations were: 1) wheat (1:0), control; 2) wheat-millet (2:1); 3) wheat-millet (1:2); 4) barley (1:0); 5) barley-millet (2:1); 6) oat (1:0); 7) oat-millet (2:1); and 8) millet-wheat-oat (1:1:1). From empirical observations, the various grains used require different cooking times to achieve desired moisture content, which is usually determined visually by the presence of a dry endosperm residue in the grain with a thickness of not more than 1 mm. Therefore, it was necessary to implement different cooking times for the grains used in the experiment.

To make GMC1, wheat (120 kg) was boiled in 150 l of water for 16 ± 2 minutes and allowed to remain in the hot water for additional 20 ± 2 minutes. For GMC2 or 3, millet (40 or 80 kg) was first boiled in 150 l of water for 20 ± 3 minutes, then wheat (80 or 40 kg), respectively was added and cooking continued for another 16 ± 2 minutes, and thereafter left in the hot water for additional 20± 1 minute. For GMC4, 120 kg barley was boiled in 150 l of water for 25 ±5 minutes and left in the hot water for additional 10 ± 2 minutes. For GMC5, millet (40 kg) was poured into 150 l of boiling water and cooked for 20 ±3 minutes, then 80 kg barley was added and cooked for additional 25 ±5 minutes and left in the hot water for additional 10 ± 3 minutes. For GMC6, oat grains (120 kg) was boiled in 150 l of water for 25 ± 5 minutes and allowed to stay an additional 10 ± 2 minutes in hot water before use. In GMC7, millet (40 kg) was boiled in 150 l water for 20 ± 2 minutes; 80 kg of oat grains was added and boiled further for another 25 ± 2 minutes and left in the hot water for 10± 5 minutes. GMC8 had three grains components. In 150 l of water, millet (40 kg) was boiled for 20 ± 5 minutes, oat (40 kg) was added and continued to cook for another 10 ± 3 minutes, and wheat (40 kg) was added to the rest grain in the pot and boiled for another 16 ± 2 minutes and allowed to stay in the hot water for 10 ± 1 minute.

The grains and mixtures made were drained of water and 1 % chalk (dry mass of grains) was added. The prepared mixture was packed (6095 ± 48 g each) into polypropylene bags (PP75/BEU6/X47-57) and sterilized as above. Upon cooling to room temperature, the bags were inoculated with mother spawn at the rate of 1.3 ± 0.2 % per wet mass of spawn material in the bag, sealed and mixed properly to distribute the inoculum evenly in the grains within each bag. Shaking was done on the 5th day after inoculation to aid quick and uniform colonization throughout the spawn bags. After 10 days, the spawn was cooled and stored in the same manner as mother spawn before use.

2.5 SUBSTRATES

Substrate for mushrooms cultivation was made from sunflower husk and barley straw at a ratio of 1:3 and pasteurized as previously reported (Holub et al., 2010). The characteristics of the substrate used were humidity 71.8 ± 4.1 %; pH 8.02 ± 0.31; the ratio C/N = 69.3 ± 6.4. After cooling, substrates were inoculated with spawn made from different GMCs above. The amount of spawn per bag was 4.8 ± 0.1 % (wet mass) and thoroughly mixed into the substrate in the common mechanical process for substrate inoculation in commercial production in Ukraine (Bisko & Dudka, 1987; Bandura et al., 2017). Each cylindrical substrate bag was made with plastic bags (size + 33×90 cm). A total of 55 ± 5 bags each was prepared for each of the 8 different spawn types (GMCs), with the following physical characteristics (average): diameter–22 cm, height–75 cm, mass–12430 ± 230 g, density 440 kg m^{-3}. 50 bags of each treatment were randomly distributed in growing chambers and 10-12 slit of 5-7 cm long at a distance of 10-15 cm from each other in a checkerboard pattern were punched onto each bag.

2.6 MICROCLIMATIC CONDITIONS

The influence of microclimatic conditions was studied in two different growing rooms. The cultivation rooms or grow room GC1 is the unregulated growing condition, which did not have equipment for regulating air mixture and recirculation system. Heated and humidified air was supplied to the chamber through a ventilation opening with an area of 0.45 m² in a volume that provided three times air changes per hour during the fruiting period. Air excess was removed through 3 windows under the roof (the total area of the opening was 1.3 m²). The microclimatic conditions of CO_2 and light in the grow room at the factory were unstable and depended on the parameters of the outside climate conditions.

Humidity and air composition (CO_2/O_2 ratio) in GC 2 were maintained by distributing humidified air mixture in the chamber through an air duct system.

The chamber where incubation was done for two weeks prior to fruiting was semi-dark, because light was turned on only during visual inspection (10-15 min per day) and the growing chambers windows allowed limited natural light with intensity from 50 to 70 lm m^{-2} into the chamber. The induction of fructification was initiated on the 14th day after inoculation by lighting from 150 to

200 lm m^{-2} up to 8 hours per day. The temperature in the chamber was gradually reduced from 20-22 °C to 16-18 °C over a period of 48 hours by using active ventilation with fresh air.

2.7 SAMPLES ANALYSES

Samples from substrates for analysis were taken before inoculation with spawn and on the 2nd day after inoculation and placement in the growing chambers. In the test spawn, samples were taken before inoculation with mother spawn and after incubation and the spawn was ready for use. Samples for spawn material analysis were taken prior to use for spawn preparation and after. Analysis for substrate bags and grain spawn materials were done in triplicate. Water content of spawn and substrates was determined by thermogravimetry at a temperature

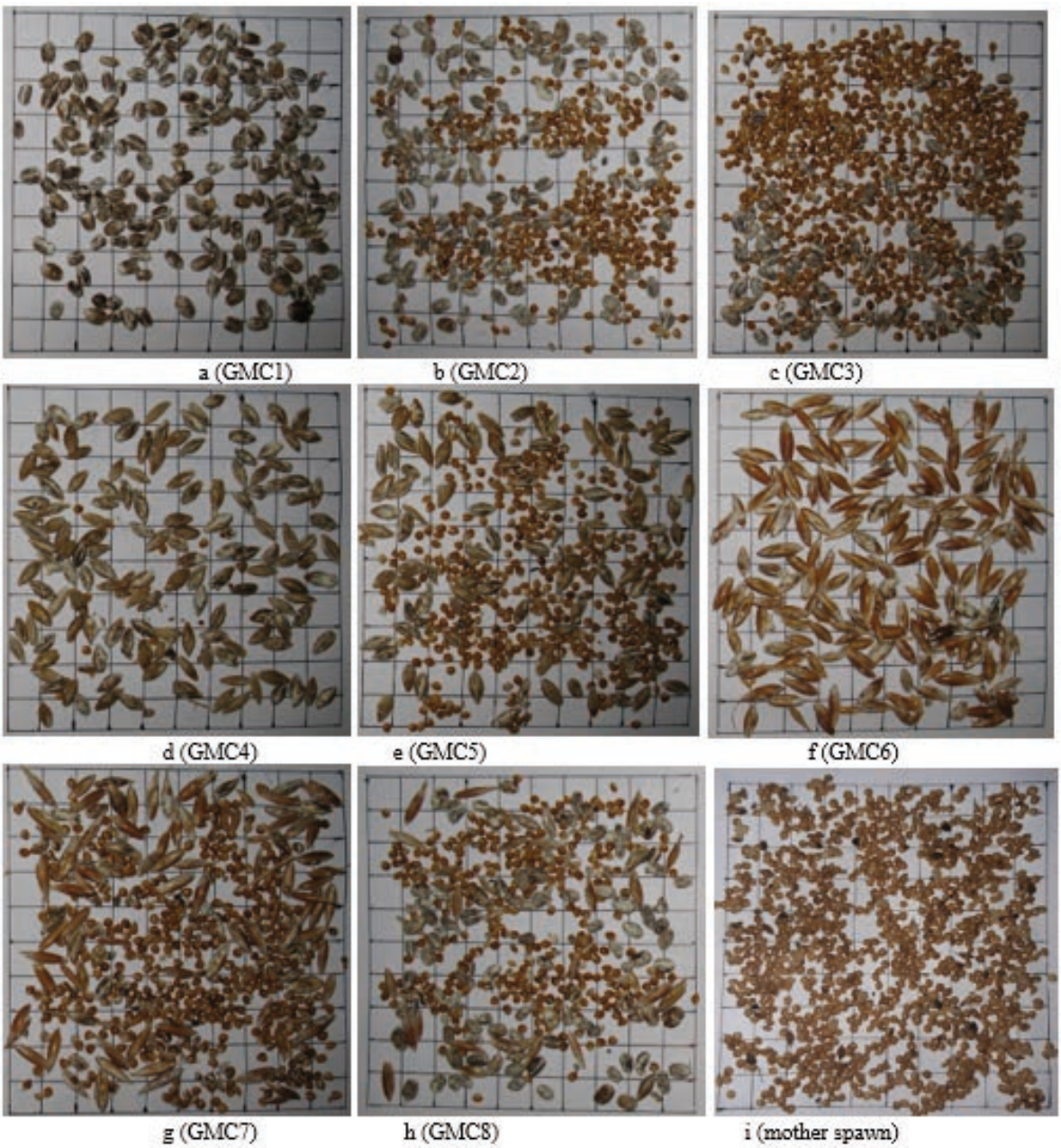

Figure 1: Appearance of the grains (10 g) in the eight different compositions of spawn materials spread on 100 cm^2 area: a) Wheat (1:0); b) Wheat-Millet (2:1); c) Wheat-Millet (1:2); d) Barley (1:0); e) Barley-Millet (2:1); f) Oat (1:0); g) Oat-Millet (2:1); h) Millet-Wheat-Oat (1:1:1); i) Millet-Rape-Flax (95:4:1)

of 102 ± 1 °C. The ash content was determined by the method of dry ashing at a temperature of 600 °C for 3 hours (Melent'eva et al., 2005).

To determine pH, 10 g of sample grain, spawn and substrate was added to 50 ml distilled and deionized water in a flask containing a magnetic rod. The flask was placed on a magnetic stirrer for 15 minutes, after which it was allowed to settle for 10 min and the supernatant was filtered into a new tube. The solution obtained was used for pH determination with pH meter (150 ME, LTD «Izmeritel`naya tekhnika», Russia) following the manufacturer's instructions.

The total nitrogen content was determined by the Kjeldahl method using the chloramine Pochynok's method. The C/N ratio was determined by the formula C/N = 0.52 × (100-a) / N, where *a* is the ash content (%); 0.52 is a coefficient of carbohydrate content, adjusted for biochemical characteristics of raw materials; N is the content of total nitrogen in the substrate (Zenova et al., 2002). The theoretical amount of total nitrogen in the substrate (*Ns*) after application of the spawn was calculated by the formula:

Ns = amount of nitrogen in the substrate × (1 - spawn application coefficient) + amount of nitrogen in spawn × spawn application coefficient (1).

The date of pinhead formation and the date of harvest at fruit bodies' maturity for each bags of substrate were recorded. The mass of mushrooms harvested from individual substrate bags was recorded. Biological efficiency (BE) was calculated by the ratio of the fruiting bodies' mass, collected during the first fruiting flush to the mass of dry solids in the substrate.

BE (%) = mass FB /dry mass of substrate × 100% (2).

Statistical analysis of data was performed using Microsoft Office Excel 2016 (license № HXV8M-8YJJ4-BCGR3-MRYX-8747Q) with QI Macros 2020 software and information complex "Agrostat New" (2013) (Ushkarenko et al, 2013).

3 RESULTS AND DISCUSSION

3.1 SPAWN

The analysis of mother spawn made from millet, rape and flax seed gave values of humidity 44,4 ± 0,83 %; total nitrogen 1.46 ± 0.08 %, ash 2.04 ± 0.12 %, C/N ratio 34.9 ± 0.75 / 1; the number of grains in 10 g was 980 ± 7 pieces, visual appearance is shown in Fig. 1-i. The physical appearance of the eight test (a-h) and mother (i) spawn compositions are shown in Figure 1.

Samples from each of the eight spawn compositions (GMC1-8) were taken twice: the first time after sterilization and the second, from matured spawn. They were analyzed for humidity, pH and the number of grains, indicated significant differences in the parameters measured. Data obtained from the analysis are shown in Table 2.

Statistical analyses indicate significant differences in moisture content, pH and the number of grains in 10 g of the spawn compositions tested. (Table 1). The lowest humidity was registered in GMC3 (40 %) and the highest in GMC6 (about 57 %). The pH in GMC1 was significantly different from the rest, despite the numerical differences in values recorded. The GMC3 gave the highest number

Table 2: Characteristics of grain spawn for growing *P. ostreatus* (average ± standard error s_e)

	Grain material composition (GMC)								
Indicators	1	2	3	4	5	6	7	8	LSD_{05}
Moisture content (%)	$48.4^{b} \pm 0.05$	$43.4^{bc} \pm 0.29$	$40.0^{c} \pm 0.26$	$45.7^{b} \pm 0.21$	$45.2^{b} \pm 0.63$	$57.2^{a} \pm 0.35$	$51.2^{b} \pm 0.19$	$43.8^{bc} \pm 1.05$	1.47
pH	$6.75^{a} \pm 0.02$	$6.03^{c} \pm 0.01$	$6.35^{bc} \pm 0.01$	$6.10^{c} \pm 0.01$	$6.41^{b} \pm 0.02$	$5.89^{c} \pm 0.03$	$6.02^{c} \pm 0.02$	$6.15^{c} \pm 0.01$	0.10
Total N content (%)	1.76 ± 0.02	1.77 ± 0.02	1.53 ± 0.03	1.98 ± 0.23	1.81 ± 0.02	1.87 ± 0.03	1.83 ± 0.02	1.69 ± 0.05	0.27
Ash content (%)	2.64 ± 0.02	3.26 ± 0.07	3.38 ± 0.18	4.04 ± 0.15	3.60 ± 0.26	4.96 ± 0.20	4.30 ± 0.07	3.99 ± 0.08	0.48
*C/N ratio	28.8	28.4	32.9	25.8	27.7	26.4	27.2	29.6	3.29
#Grains in 10 g GMC	189 ± 3^{c}	464 ± 16^{b}	712 ± 33^{a}	159 ± 2^{c}	395 ± 13^{b}	192 ± 4^{c}	481 ± 10^{b}	441 ± 20^{b}	56

*C/N = carbon : nitrogen ratio; N = Nitrogen; C = Carbon, GMC = Grain material composition

and was significantly different from the rest due to the size and abundance of millet. In fact, the results show that the GMCs can be grouped into three categories: Group a is represented by GMC3 (millet:wheat = 2:1), which had 712 ±33 grains. Group b is represented by GMC 2, 5, 7 and 8 where the number of grains ranged between 395 ±-13 and 481±10. Group ***c*** is represented by GMC1 and 6 where the number of grains ranged between 189 ±3 and 192 ± 4.

The highest total N was registered in GMC 4, the least was in GMC 3; apart from GMC3, the total N content in the rest of the GMCs were not significantly different. There was no statistical difference in the ash content and C/N ratio for all GMCs tested.

3.2 SUBSTRATE

The substrate used was produced by aerobic fermentation and the pasteurization method that is standard in oyster mushroom cultivation in Ukraine. [30]. After fermentation and pasteurization, the substrates during each repeated test were thoroughly mixed, bagged and analyzed for consistency before use in the cultivation. Analysis of the substrates gave results of humidity ranging from 68.5 ± 0.34 to 73.9 ± 0.12; pH from 7.95 ± 0.05 to 8.14 ± 0.07; ash content from 5.55 ± 0.23 to 7.06 ± 0.18 and C/N ratio from 59.0 ± 1.06 to 68.2 ± 1.84. A *t*-test comparison of total nitrogen (%) in the substrate indicated some differences, but did not result in significant difference in the N content among the substrates inoculated with the different GMCs and tested after two days of incubation (Table 3).

3.3 CLIMATIC CONDITIONS IN FRUITING ROOM

There were no statistical differences in the temperature, humidity and carbon dioxide parameters in both GC1 and GC2. However, the daily fluctuations in temperature (± 5.5 °C), humidity (± 7.7 %;) and carbon dioxide (± 155 ppm) in GC1 during fruiting were very high compared to GC2. The details of values obtained from three growing cycles for the parameters are shown in Table 4

3.4 EFFECT DIFFERENT GMCS ON TIME TO ATTAIN TOTAL SUBSTRATE COLONIZATION AND FIRST FLUSH

There was no significant difference in the number of days (8 ± 1 day) to achieve total substrate colonization in the different GMCs (spawn) tested. Among all the spawn types tested, GMC 7 significantly prolonged the time to

Table 3: Analysis of total nitrogen content in grains, spawn and substrates for the cultivation of *P. ostreatus* (average ± standard error s_e)

Grain material composition (GMC)	Total nitrogen, %						
	Mycelium (means with SE)			Substrate (means with SE)			Theoretical calculated with average data
	after sterilization (A)	In the ready spawn* (B)	Reach (B–A)*	before inoculation (C)	after inoculation (D)	Reach (D – C)**	
1	$1.15^{c} \pm 0.03$	$1.76^{abc} \pm 0.02$	0.61	$0.71^{c} \pm 0.02$	0.8 ± 0.03	0.09	0.76
2	$1.14^{c} \pm 0.04$	$1.77^{abc} \pm 0.02$	0.63	$0.79^{ab} \pm 0.02$	0.87 ± 0.04	0.08	0.83
3	$1.13^{c} \pm 0.05$	$1.53^{c} \pm 0.03$	0.40	$0.83^{a} \pm 0.01$	0.9 ± 0.02	0.07	0.86
4	$1.58^{a} \pm 0.03$	$1.98^{a} \pm 0.23$	0.40	$0.75^{bc} \pm 0.03$	0.85 ± 0.01	0.10	0.81
5	$1.43^{b} \pm 0.04$	$1.81^{ab} \pm 0.02$	0.38	$0.72^{bc} \pm 0,01$	0.82 ± 0.01	0.10	0.77
6	$1.48^{ab} \pm 0.04$	$1.87^{ab} \pm 0.03$	0.39	$0.74^{bc} \pm 0.01$	0.83 ± 0.04	0.09	0.79
7	$1.36^{b} \pm 0.03$	$1.83^{ab} \pm 0.05$	0.47	$0.78^{ab} \pm 0.02$	0.85 ± 0.01	0.07	0.83
8	$1.25^{bc} \pm 0.03$	$1.69^{bc} \pm 0.05$	0.44	$0.77^{abc} \pm 0.05$	0.85 ± 0.02	0.08	0.81
LSD	0.12	0.26		0.07	0.15		
p	0.000	0.069		0.051	0.52		

*(B-A) = Change GMCs N content before and after incubation
**(D-C) = Change substrate N content before and after incubation

Table 4: Technical parameters of microclimatic growing conditions (GC) (average ± standard error s_e)

Microclimatic conditions	Cultivation stage	Growing chamber	
		GC 1	GC 2
Temperature (°C)	Incubation	19.1 ± 6.0	22.0 ± 1.3
	Fruiting	18.7 ± 5.5	18.2 ± 0.3
Humidity (%)	Incubation	74.3 ± 7.1	78.7 ± 2.5
	Fruiting	92.1 ± 7.7	85.2 ± 3.4
CO_2 content (ppm)	Incubation	2039 ± 65	1245 ± 34
	Fruiting	961 ± 155	1222 ± 19

first flush harvest, 45 in GC1 and 34 days in GC2. There was no significate difference in the time to first harvest in all GMC´s tests under GC1 condition. It was the same observation under GC2 except GMC7. GMC5 and GMC6 were the fastest to attain total substrate colonization and first harvest. GMC7 inoculated substrate took the longest time to reach the harvesting stage, a similar trend was observed for time to attain total colonization in GC1. Duncan's test for comparison of averages indicated no significant difference ($p > 0.05$) in the number of days to reach first flush for a substrate inoculated with the same test GMC under GC1 or GC2, except in two treatments (GMC 6 and 7). Statistical analysis indicated 46 % interaction effect between spawn type (factor A) and microclimatic conditions (factor B) on the time to first harvest. However, the individual factor A and B had 10.5 % and 31.1 %, respectively, effect on time to first harvest. A statistical *t*-test comparison of means from cultivation under the two microclimatic conditions (GC1 and 2) indicated that the longest period to attain first flush was recorded in substrates inoculated with spawn made from a mixture of millet and oat, GMC7 (1:2) (Fig. 2), where it exceeded 45 days in GC1 and 34 days in GC2.

3.5 EFFECT OF GMCS AND MICROCLIMATIC CONDITIONS ON BIOLOGICAL EFFICIENCY

Under the controlled microclimate condition GC2, there was higher fruit body yield and BE compared to GC1 regardless of the GMC composition used (Fig. 3).

The highest BE (57 %) was obtained under GC2, when GMC6 was the spawn used, which was not significantly different from yield obtained in GMC5 and 8. The least was GMC7 (20 %). There was a slight difference in the GC1, because the highest BE was in GMC8 (41.1 %) and the least was in GMC7 (20 %). It should be noted that the grain mixtures used in GMC5 and 8 also gave higher yields even in unregulated microclimate conditions (GC1). The BE indicators for GMC1 (control), GMC3 (wheat-millet (1:2), and 7 (oat-millet (2:1) were lower and did not differ significantly under GC1 and 2 microclimate conditions.

The current global expansion of small-scale production of edible and medicinal mushrooms has necessitated the need to study, and optimize, all the factors that in-

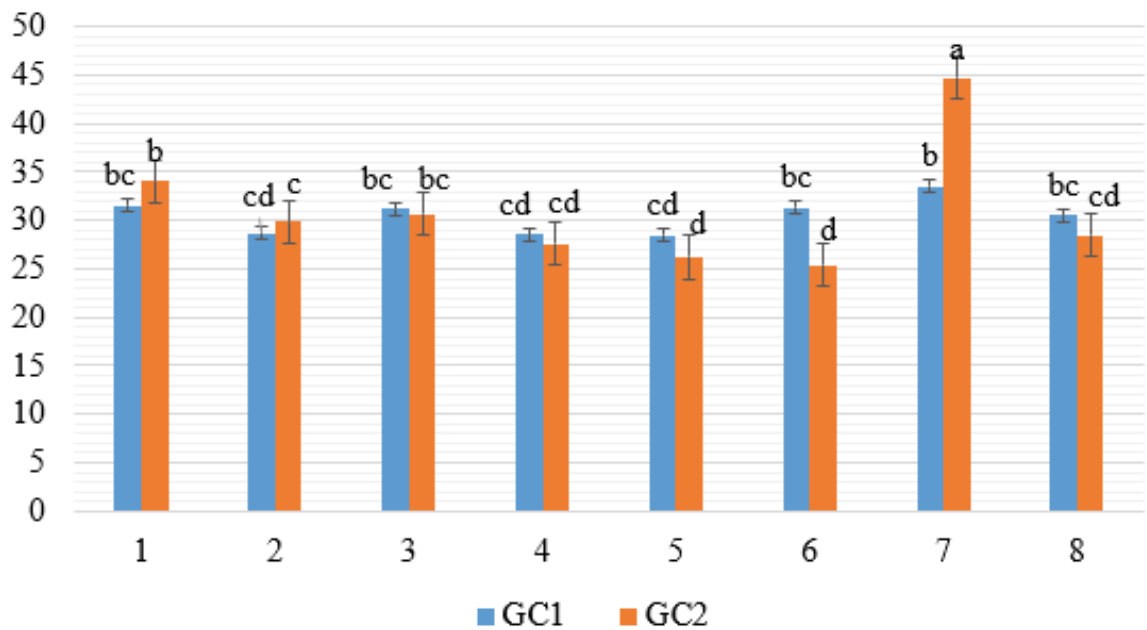

Fig. 2: Number of days to first harvest *P. ostreatus* in different GMCs under cultivation in unregulated (GC1) and regulated (GC2) microclimatic conditions

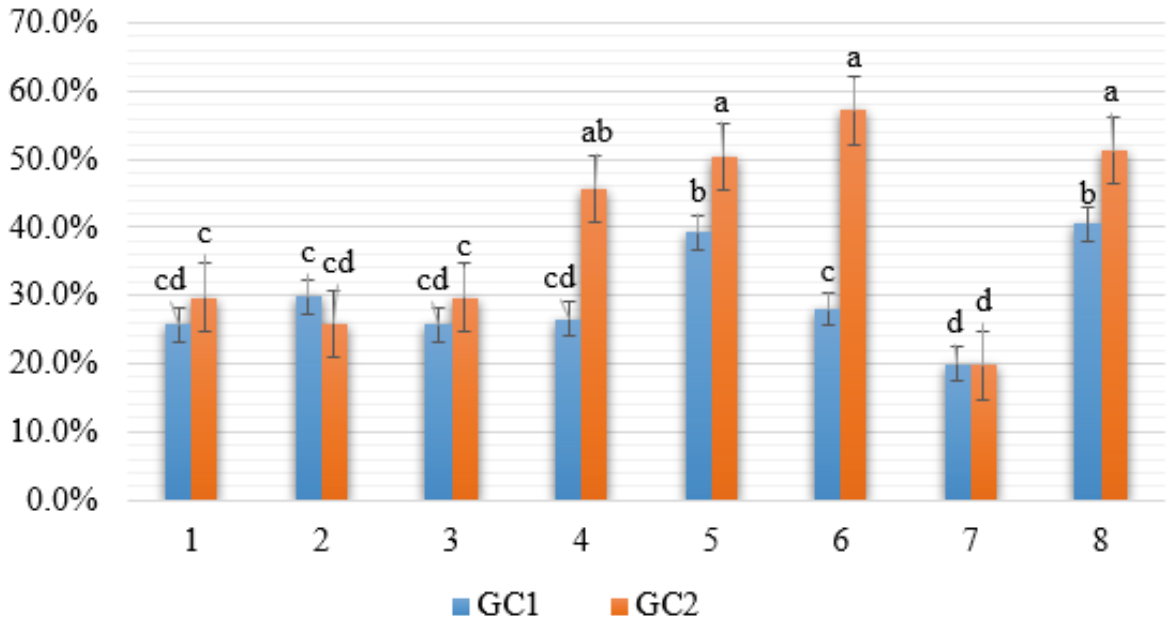

Fig. 3: Biological efficiency (%) in *P. ostreatus* with test spawn GMC1-8 during cultivation under unregulated (GC1) and regulated (GC2) microclimatic conditions

fluence efficient and profitable mushroom production, including the influence of spawn composition on yield outputs (Evdokimova et al., 2002). Although cereals are commonly used as spawn materials, their availability, nutritional content, and prices can affect the spawn quality and cost. In one report, grains were replaced with wood waste and obtained results that indicated significant reduction in the cost of spawn, although the incubation period was prolonged (Sofi et al., 2014). Other researchers used sorghum grains and found them to be suitable and cost effective for mushroom spawn (Jiskani et al., 2007; Stanley & Awi-Waadu, 2010; Willis et al., 2012). In Europe and America, highly effective additives (cereals bran, beer pellets, organic metals salts, etc.) have been added to wheat and barley to improve spawn quality (Jhune et al., 2000; Rosado et al., 2002; Mamiro & Royse, 2008; Gregori et al., 2008; Krupodorova & Barshteyn, 2015). Subramanian et al. (2014) tested different grains as spawn material and found oat and barley gave the best BE results during the cultivation of *P.ostreatus.*

It is also known that the type of soil and climatic conditions under which grains are grown can affect their physicochemical properties (Dubovik, 2007; Petrova, 2010; Gy`rka et al., 2015). However, the cooking process is critical for obtaining optimum water content in different grain materials, which allow rapid mycelia colonization and individual grain penetration during incubation. The sequence of cooking times during GMCs preparation was carefully calculated and followed in this experiment to achieve what was the best water content of grains in the test-spawn materials (wheat, millet, barley and oat). The significant differences in water content, pH and the number of grains in 10 g of the spawn compositions tested (Table 2) is due to the inherent nature of the grain materials, especially their water-holding capacity. Only the pH in GMC1 was significantly different from the rest, despite the numerical differences in values recorded for the others; we believe that this is entirely due to the inherent properties of wheat compared to the properties of the other grains tested. The grain material composition (GMC3) gave the highest number (712 ± 33) of inoculation points and was significantly different from the rest. It is obvious that this composition has the highest amount of millet, which had the least size and mass among the grains used. Millet's physical dimension (Table 1) vis-à-vis its presence and abundance in the GMCs is responsible for the group a, b and c of the GMCs tested. However, its presence did not seem to improve spawn quality.

The highest total N was registered in GMC4 (1.98 %), the least was in GMC3 (1.53 %); apart from GMC3, the total N content in the rest of the GMCs was not significantly different ($p = 0.051$). There were significant differences ($p > 0.001$) in the moisture content of grains used due to the grain material, prevailing climatic and soil conditions where the grain was cultivated, and the grain's postharvest processing (Horshchar V. & Horshchar O., 2011; Kaminskyi & Hliieva, 2015; Petrenko, 2017). However, the differences recorded did not seem to be critical

in the quality of the spawn produced because they did not affect yield. This is not surprising because prior to use, the grains were cooked and became hydrated to optimum grain water content for mycelia colonization and penetration.

There were significant differences in the pH of the grain materials, but that factor also did not affect the quality of the spawn. It is known that basidiomycetes, especially white rot fungi, are capable of adjusting the pH of its growth medium to its optimum growth condition (Bisko & Dudka, 1987; Bukhalo et al., 2011; Truhonovec et al., 2013). Therefore, the differences in the pH could be readily adjusted to optimum condition by *P. ostreatus* used in spawn preparation. The number of grains presented very interesting results and can be grouped into three significant categories, a, b and c. It appears that there was no correlation between the number of grains and the yield, especially in GC2, where the correlation coefficient of the number of grains with the BE from the substrates inoculated with different GMCs ranged from 0.37 – 0.42. This was a rather surprising finding, because it does not align with reports in literature. Many data reported indicate that the number of grains in spawn can affect their abundance and distribution in substrate and colonization rate, earliness to fruit body initiation and mushroom yield (Smetanina, 2013; Khonga et al., 2013, Subramanian et al., 2014). Though it is correct that the number of inoculation points are different, in this study as high as 712 in GMC3 and as low as 159 in GMC4, we did not observe that it was a critical factor that affected colonization rate and BE.

The different GMC tested gave different times to reach first harvest and BE from the cultivation studies (Figures 2 and 3). It was interesting to find that wheat, used universally for spawn production, appeared to not perform as well as other grains (except millet, in some cases). The nutritional content of wheat is not significantly different from that of other grains. However, it is possible the cover coat on millet, barley and oat may be responsible. During the inoculation of substrate in industrial production of oyster mushrooms, the mechanical process used to disentangle the compact spawn, for even distribution in the substrate, results in a grinding effect, which removes mycelia from the surfaces of the individual wheat grain in the spawn. This causes a delay in mycelia regeneration and initiation of substrate colonization. The seed coats that other grains carry protect the mycelia against that grinding effect, which could be responsible for the better performance observed in the colonization rate in the GMCs containing wheat and other grains.

Millet performed best when it was in combination with barley. It was not able to reverse the lag in the time of colonization when in combination with wheat (GMC1, 2 and 3) and it even performed worse when in combination with oat, as in the case of GMC7. However, when used in equal combinations, millet, wheat and oat produced colonization results that were not significantly different from the other spawn tested in this experiment.

The BE data (Fig. 3) indicate that the spawn made from grain mixtures of barley and millet (2:1) and millet, wheat and oat (1:1:1) are promising for improving the efficiency of industrial production of *P. ostreatus*. Oat's use as spawn carrier may be appropriate only for cultivation done in controlled microclimatic conditions, as in GC2.

The substrates inoculated with GMC1-3 had BE that were inferior to those without wheat, except for GMC8. It is possible the delayed effect on colonization is transient and resulted in the observed depressed BE, which were statistically significant in some cases (Figure 3). The substrates that contained oat, barley or millet (except GMC7) had better results, which were significantly different from others, especially under GC2 conditions. The BE in the other grains' composition was not significantly different from all the GMC containing wheat under GC1. The combination of millet and oat (GMC7) performed the worst among all spawn combinations tested; at this time, we have no explanation for why this is the case.

GMC8, which is a 1 : 1 : 1 ratio combination of millet, wheat and oat, showed BE results comparable to GMC 4-6, which were the best spawn and did not contain wheat. It appears that the combination of grains in GMC8 overcame the limiting effect that other wheat-containing GMCs had on colonization and BE. It was reported that different types of grains have different chemical contents that could affect their colonization by mycelia (Sainos et al., 2006). The combination of the millet, wheat and oat may have presented a suite of biochemical compounds that elicit different substrate degradation enzymes in *P. ostreatus* mycelia and made GMC8 to become the best among those tested. Furthermore, it is possible that the presence of millet and oat, which have seed coats, was sufficient to eliminate the mechanical damages of mycelia on wheat grains in GMC8 spawn composition. However, further research is needed to understand the biochemical composition and the interplay between the enzymes they induce for degradation and utilization. Microclimatic conditions are known to play key roles in determining mushroom production and biological efficiency (Dudka et al., 1978; Chang & Hayes, 2013; OECD, 2015; Bellettini, et al., 2019). Therefore, the influence of temperature, humidity, air composition and lighting is a constant topic of research, because as the range of cultivated mushrooms expands, the number of strains requiring different optimal microclimatic conditions increases, too. In our study, the microclimatic con-

ditions in GC1 and GC2 were similar (Table 4) but the fluctuations in the parameters of temperature, humidity and CO2 were enough to cause significant changes in the yield and BE. Furthermore, it is also evident that the GMC that was optimum for the two climatic conditions were different: GMC5 and 8 For GC1 and GMC6 and 8 for GC2. The fact that GMC8 the optimum spawn for GC1 and 2 makes it the prime combination for the mass production of spawn that could be used under unregulated (GC1) and regulated (GC2) cultivation conditions. This is important because there are currently many small mushroom production facilities across Europe and in many developing countries that do not have sophisticated growing chambers.

4 CONCLUSIONS

These experiments tested the suitability of different grain materials for spawn production. Results indicated that the use of wheat alone, or in combination with millet, is not the best among the tested grains in terms of time to first harvest and BE. Barley, oat and their combinations performed equally well as spawn materials for BE. It was obvious that the grow room condition, whether regulated or unregulated, had significant effect on BE and preference for spawn composition. Higher BE were obtained under regulated cultivation conditions for most spawn types tested. However, the combination that stood out the best was that of wheat, millet and oat (GMC8) because it out-performed all other substrate combinations under regulated and unregulated conditions. Furthermore, it will be more cost efficient to use GMC8 because it could represent significant savings on spawn materials and thereby lower the price of spawn in the mushrooms industry.

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