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Research Article
(Araştırma Makalesi)

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Development of the non-grain spawn for edible mushroom (*Pleurotus ostreatus*): D-optimal mixture design approach

Yenilebilir mantar (*Pleurotus ostreatus*) için tahılsız misel geliştirilmesi: D-optimal karışım tasarımı yaklaşımı

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ABSTRACT

Objective: The objective of this study was to develop a non-grain spawn formulation for *Pleurotus ostreatus* with the D-optimal mixture design approach. The developed spawn was compared with the traditionally used grain spawn in terms of spawn running time and biological efficiency.

Material and Methods: Non-grain spawn was produced in 25 different formulations using wheat bran, poplar sawdust, perlite, $CaCO_3$, $CaSO_4$ components. In addition, oat spawn containing 1% $CaSO_4$ and 0.5% $CaCO_3$ from cereals was used as the control group. Spawn running times and the biological efficiencies of the samples were determined.

Results: The optimum mixing ratios for wheat bran, poplar sawdust, perlite, $CaCO_3$, and $CaSO_4$ used in the formulation of non-grain spawn production were determined as 11.44%, 28.87%, 50.74%, 5.89%, and 3.07%, respectively. The spawn running time of the improved non-grain spawn produced according to the above formulation was shortened by 20.16% as compared to the grain-derived spawn. This shows that the non-grain as one of the two different spawn production methods is technologically superior.

Conclusion: In the leading countries in mushroom production, new technologies and formulations are being developed day by day to shorten the spawn running time and increase biological efficiency and mushroom yield. It is thought that this study will contribute to the development of spawn technology, which is an essential input in mushroom cultivation.

ÖZ

Amaç: Bu çalışmada, D-optimal karışım tasarımı yaklaşımı ile *Pleurotus ostreatus* için tahılsız misel formülasyonu geliştirilmiştir. Geliştirilen misel, misel çalışma süresi ve biyolojik etkinlik açısından geleneksel olarak kullanılan tahıl misel ile karşılaştırılmıştır.

Materyal ve Yöntem: Buğday kepeği, kavak talaşı, perlit, $CaCO_3$, $CaSO_4$ bileşenleri kullanılarak 25 farklı formülasyonda tahılsız misel üretilmiştir. Ayrıca kontrol grubu olarak tahıl kaynaklı %1 $CaSO_4$ and %0.5 $CaCO_3$ içeren yulaf miseli kullanılmıştır. Örneklerin misel çalışma süreleri ve biyolojik verimlilikleri tespit edilmiştir.

Araştırma Bulguları: Tahılsız misel üretiminin formülasyonunda kullanılan buğday kepeği, kavak talaşı, perlit, $CaCO_3$ ve $CaSO_4$ için optimum karışım oranları sırasıyla %11.44, %28.87, %50.74, %5.89 ve %3.07 olarak belirlenmiştir. Yukarıdaki formülasyona göre üretilen geliştirilmiş tahılsız miselin sarım süresi tahıl kaynaklı misele kıyasla %20.16 kısalmıştır. Bu durum iki farklı misel üretim metotundan tahılsız olanının teknolojik açıdan daha üstün olduğunu göstermektedir.

Sonuç: Mantar üretiminde önde gelen ülkelerde misel sarim süresini kısaltmak, biyolojik etkinliği ve mantar verimini artırmak için her geçen gün yeni teknolojiler ve formülasyonlar geliştirilmektedir. Çalışmanın mantar yetiştiriciliğinde önemli bir girdi olan misel teknolojisinin geliştirilmesine katkı sağlayacağı düşünülmektedir.

INTRODUCTION

Mushrooms collected from nature have been used since ancient times, and in addition to this, the increase in the consumption of cultivated mushrooms is increasing day by day. The functionality of mushrooms may vary depending on the genus, species, subspecies, harvest time, storage, and substrates used in compost production (Bonatti et al., 2004; Beluhan & Ranogajec, 2011). Mushrooms are known as a food source with high protein, vitamin, dietary fiber, and mineral content (Sanmee et al., 2003), as well as their antiviral (Seo & Choi, 2021), antibacterial (Beltran-Garcia et al., 1997), anticholesterol (Khatun et al., 2007), anticarcinogenic (Vetter, 2019), antihypertensive (Ramakrishnan et al., 2017), antioxidant and antidiabetic properties (Doğan & Doğan, 2021).

Pleurotus ostreatus (Oyster mushroom) is the second popular exotic mushroom grown among cultivated mushrooms globally (Sánchez, 2010). *Pleurotus ostreatus* has the nutritional and bioactive properties mentioned above, and a gastronomic taste advantage (Waktola & Temesgen, 2020). The fact that *P. ostreatus* has high lignocellulosic enzyme systems and thus converting lignocellulosic agricultural residues such as forest and agricultural by-products into high value-added products makes the production of this mushroom attractive (Dubey et al., 2019; Ganash et al., 2021). Commercial spawn is the most crucial biotechnological input in mushroom production. Spawn type is vital in determining fruiting yield, biological efficiency, and fruiting body morphologies (Mamiro & Royse, 2008). Besides using various grains as a carrier in the production of spawn (Maurya et al., 2019), it has recently been used in different carriers and types (Wang et al., 2011; Liu et al., 2018; Zhang et al., 2019). High production costs and contamination risks can be counted among the most important disadvantages of using grain spawn (Rosado et al., 2002). Although grain spawn is used in commercial mushroom production, different techniques such as liquid spawn, sawdust spawn, stick spawn, stalk spawn, and block spawn have been developed due to their advantages. Liquid spawns have known quick preparation and fastly mycelial colonization (Ma et al., 2016). Sawdust spawn is one of the most popular choices because of its low cost, short running time, and high biological efficiency (Wang et al., 2011). The use of stick spawn reduced the spawn running time and improved the biological efficiency of the mushrooms (Zhang et al., 2014). It has been reported that stalk spawn has a similar spawn running time with stick spawn but with higher biological efficiency at a lower cost (Liu et al., 2018).

Experiment numbers and costs can be successfully reduced by optimizing the trial design. Mixture designs allow the interactions and relationships between components to be understood through modeling. The D-optimal design is an effective solution that performs closest to the target. The D-optimal design has been successfully applied in product formulations (Azarbad et al., 2019; Sahu & Patel, 2020). However, there is no study in the literature on the use of D-optimal design to identify and optimize the interactions of these components in novel spawn developed.

Spawn running time, biological efficiency, yield, and spawn type are important factors for the mushroom cultivation (Atila, 2019). Currently, the variety of spawns used is quite limited. Short running time, high biological efficiency, minimal risk of contamination, and low cost are important criteria in choosing spawn. The objective of this study was to develop a novel spawn with desirable features such as low cost, easy production, short running time, high biological efficiency, and converting forest and agricultural by-products into high value-added products. In this context, trial points were created with a D-optimal mixture design by using poplar sawdust, wheat bran, and perlite as the primary material source and calcium carbonate ($CaCO_3$) and calcium sulfate ($CaSO_4$) as an auxiliary source, and the mixture was optimized according to spawn running time and biological efficiency. It is thought that this study will contribute to the development of spawn technology, which is an essential input in mushroom cultivation.

MATERIAL and METHODS

Mushroom culture and other materials

Pleurotus ostreatus pure culture was obtained from Yozgat Bozok University, Bogazliyan Vocational High School culture collection. Malt-yeast extract medium (MYE) was used to reproduce the pure culture. MYE was obtained from Merck KGaA, Darmstadt, Germany. Used in liquid culture production, grain spawn production, non-grain spawn production, and mushroom production; malt extract, poplar sawdust, instant yeast powder, oat, wheat bran, $CaSO_4$, and $CaCO_3$ procured from local suppliers. Polyethylene microsac bag (Type 14A, Unicorn Bags, TX) purchased from the USA.

Liquid culture production

The modified liquid culture production by Stamets (2011) method was implemented. Firstly, 100 ml of distilled water was sterilized at 121°C for 2 hours and cooled to 25°C. Then two pieces of the section with 9 mm cork borer were taken into a waring blender and homogenized to prepare a mycelium-enriched liquid. Afterward, the broth, formulated with 40 g/L light-colored malt extract, 2 g/L yeast, 3 g/L sawdust with distilled water, was sterilized at 121°C for 2 hours. 10 mL of homogenized mycelium-enriched liquid was transferred into 90 mL of sterilized broth. The prepared mixture was incubated at 25°C in a shaking water bath to meet the oxygen demand of the hyphal knot. The process was completed when the transparent appearance disappeared (12 days).

Non-grain spawn production and D-optimal mixture design

The D-optimal mixture design was used for experimental non-grain spawn formulations. Components used in non-grain spawn production and their minimum-maximum ratios; A: wheat bran (5-80%), B: poplar sawdust (2-30%), C: perlite (5-60%), D: $CaCO_3$ (1-12%), and E: $CaSO_4$ (1-10%) as specified. The experimental design was tested at 25 trial points, as highlighted in Table 1. Oat grain spawn was used as the control group. Oat grains were boiled in random water for grain spawn production until they reached a moisture content of 60%. It was then mixed with 1% $CaSO_4$ and 0.5% $CaCO_3$. 2500 g oat grain was filled into filtered heat-resistant microbags and sterilized at 121°C for 2 hours. The sealed bags were cooled to 25°C and inoculated 30 mL of liquid culture. The bags were incubated at 25°C until full colonization was achieved visually.

According to the D-optimal design, each experimental point was created by mixing components of non-grain spawn formulations in the concrete mixer in different proportions (Table 1) and then water was added so that the total moisture content was 60%. The total moisture content was calculated by considering that the moisture content of sawdust and bran were 42.6% and 8.6%, respectively. The packages (Type 14A, Unicorn Bags, TX, USA) were filled in equal volume with 2500 g for grain spawn and 1440 g for non-grain spawn and sterilized for 3 hours at 121°C. The sealed bags were cooled to 25°C and inoculated 30 mL of liquid culture. The bags were incubated at 25°C until full colonization was achieved visually.

Substrate preparation, fruiting and harvesting

Finely chopped poplar sawdust, which was used as the primary substrate for cultivating *P. ostreatus*, and ¼ wheat bran were added to improve the protein ratio. In addition, 1% $CaCO_3$ was added to adjust the acidity of the substrate and mixed. The prepared 3000 g substrate was filled polyethylene package and sterilized at 121°C for 4 hours. The spawns were inoculated 5% into the substrate cooled to room temperature. It was incubated in a darkened room at 25°C in an air-conditioned room with 85% humidity. When the bag was visually full colonization by the mycelia, cross-shaped cuts of 3 cm in length were made to facilitate the fruit body exit. In this process, the temperature was reduced to 17°C, and the relative humidity was increased to 90-95%. The amount of CO_2 in the environment was ensured to be below 800 ppm. After the pinhead was seen, the photoperiod was applied 12 h per day. The mushroom was harvested as two flushes when the cap surface's diameter was 8 cm.

Spawn running time and biological efficiency

The day incubated bags were fully colonized by mycelia was determined as the spawn running time. Fruitbody harvested as two flushes were recorded as weight. Biological efficiency was calculated as the ratio of the total mushroom weight per bag to the dry weight of substrate and was expressed as a percentage (Estrada et al., 2009).

Table 1. Experimental trial points according to D-optimal mixture design and experimental values- predicted data

Çizelge 1. *D-optimal karışım tasarımına göre deneysel deneme noktaları ve deneysel değerler-tahmin edilen veriler*

Trial points	Non-grain spawn formulations (%)					Responses			
						Spawn running time (days)		Biological efficiency (%)	
	A: Wheat bran	B: Poplar sawdust	C: Perlite	D: $CaCO_3$	E: $CaSO_4$	Experimental value	Predicted data	Experimental value	Predicted data
1	57	16	5	12	10	19	19.33	81.32	80.47
2	5	30	43	12	10	17	16.17	89.78	90.08
3	40	18	35	6	1	15	15.73	85.55	85.08
4	36	2	60	1	1	16	15.38	81.71	80.10
5	58	2	38	1	1	17	16.92	80.00	81.21
6	8	30	60	1	1	15	13.96	86.60	87.92
7	5	22	60	12	1	15	14.90	84.93	85.31
8	16	13	60	1	10	16	16.63	83.27	83.25
9	24	30	24	12	10	16	16.24	88.00	88.63
10	44	17	33	1	6	15	16.25	84.46	84.71
11	80	13	5	1	1	21	20.92	72.00	73.54
12	52	30	5	12	1	18	17.71	80.00	79.65
13	80	3	6	1	10	21	20.94	76.41	75.45
14	22	19	43	9	8	15	16.15	89.94	86.93
15	16	2	60	12	10	18	17.72	79.15	79.80
16	44	2	33	12	10	19	17.95	82.49	83.62
17	54	30	5	1	10	18	17.89	79.10	79.43
18	54	30	5	1	10	18	17.89	78.65	79.43
19	52	30	5	12	1	18	17.71	80.36	79.65
20	59	9	21	3	8	19	18.21	80.28	81.47
21	80	2	5	8	6	21	21.60	76.77	75.26
22	8	30	60	1	1	14	13.96	89.23	87.92
23	5	22	60	12	1	14	14.90	84.56	85.31
24	80	2	5	12	1	22	22.01	73.98	75.07
25	80	13	5	1	1	21	20.92	74.30	73.54

Statistical analysis, model selection and optimization

In model selection, a reduced quadratic model was preferred, as suggested by Design Expert software (Version 13.0.5. Stat-Easy Co., Minneapolis, MN, USA), which includes all interactions of independent variables. However, the model was reduced by algorithmic selection of terms to obtain more consistent results. In this context, an auto select module of Design Expert software was used for model reduction based on Akaike's Information Criterion (AICc) (Akaike, 1978). To determine the reliability of the reduced quadratic model, it was demonstrated using Analysis of Variance (ANOVA), coefficient of determination (R^2), corrected R^2 (R^2_{adj}), predicted R^2 (R^2_{pred}), and lack of fit. SPSS 22.0 software (SPSS Inc., Chicago, IL) was used for all data analyses where $p<0.05$ was assumed to be statistically significant.

Numerical optimization was applied to determine the best mixture ratios in the spawn formulation. To determine the optimum points, mixture components (wheat bran, poplar sawdust, perlite, $CaCO_3$, $CaSO_4$) are marked as criteria in range. The responses, spawn running time and biological efficiency, were chosen to give minimum and maximum results, respectively. The optimum point was determined based on the highest desirability score.

RESULTS and DISCUSSION

Checking of the model fitting

As tabulated in Table 1, the results obtained from D-optimal mixture design were in good agreement with the experimental values and the predicted data on the responses of the experimental trial points. To increase model's effectiveness on the responses, the model reduction was applied based on Akaike's Information Criterion (AICc). In this situation, some components of responses are extremely important in determining (Friedman et al., 2001). The reduced quadratic model and statistical parameters for the experimental results are shown in Table 2. Statistical parameters such as R^2, R^2_{adj}, R^2_{pred} were used to determine the reliability of the actual equation produced from the model. R values close to 1 indicate a high correlation between experimental and predicted values (Pujari & Chandra, 2000). The R^2 values of the responses were determined as 0.933 and 0.948 for spawn running time and biological efficiency, respectively. Moreover, the difference between R^2_{pred} and R^2_{adj} is less than 0.2, and the difference between R^2 and R^2_{adj} values above 90% indicates the suitability of the model (Myers et al., 1995).

Table 2. Reduced quadratic model and statistical parameters

Çizelge 2. *İndirgenmiş ikinci dereceden model ve istatistiksel parametreler*

Responses	Reduced quadratic model actual equations	Regression (p-value)	R^2	R^2_{adj}	R^2_{pred}
Spawn running time (days)	=0.24xA+0.078xB+0.17xC+0.18xD+0.39xE-0.0019xAC-0.0038xAE	<0.0001	0.933	0.911	0.883
Biological efficiency (%)	=0.68xA+0.83xB+0.63xC+1.01xD+1.06xE+0.0061xAC+0.0082xBC	<0.0001	0.948	0.931	0.904

A:Wheat bran, B: Poplar sawdust, C: Perlite, D: $CaCO_3$, E: $CaSO_4$

According to the variance analysis results of spawn running time and biological efficiency responses, the F values were significant as 42.07 and 54.98, respectively. In addition, the lack of fit was found to be insignificant (Table 3). This shows that the model equations are sufficient to predict both responses for a different combination of components.

Table 3. Analysis of variance for responses

Çizelge 3. *Yanıtlar için varyans analizi*

Spawn running time (days)					Biological efficiency (%)				
Source	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**	**Source**	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**
Model	130.90	21.82	42.07	< 0.0001	**Model**	560.19	93.37	54.98	< 0.0001
AC	13.68	13.68	26.38	< 0.0001	**AC**	105.73	105.73	62.25	< 0.0001
AE	3.71	3.71	7.16	0.0154	**BC**	75.53	75.53	44.47	< 0.0001
Residual	9.34	0.5186			**Residual**	30.57	1.70		
Lack of Fit	8.34	0.6412	3.21	0.1029	**Lack of Fit**	24.23	1.86	1.47	0.3535
Pure Error	1.00	0.2000			**Pure Error**	6.34	1.27		
Cor Total	140.24				**Cor Total**	590.76			

A:Wheat bran, B: Poplar sawdust, C: Perlite, D: $CaCO_3$, E: $CaSO_4$

Effect of components on responses and evaluation

The effects of the components on spawn running time and biological efficiency are depicted in Figure 1 and Figure 2, respectively.

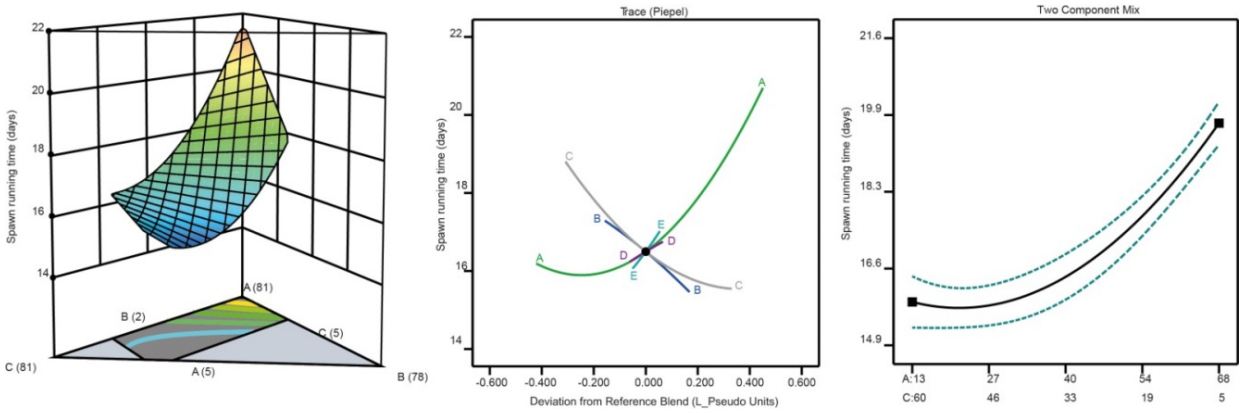

Figure 1. Representation of the effect of components on spawn running time with 3D surface, trace and two component mix plots; A: Wheat bran, B: Poplar sawdust, C: Perlite, D: $CaCO_3$, E: $CaSO_4$.

Şekil 1. *Bileşenlerin misel sarım süresi üzerindeki etkisinin 3 boyutlu yüzey, iz ve iki bileşenli karışım grafikleri ile gösterimi; A: Buğday kepeği, B: Kavak talaşı, C: Perlit, D: $CaCO_3$, E: $CaSO_4$.*

In general, spawn required more time for full colonization with an increase in the ratio of wheat bran, $CaSO_4$, and $CaCO_3$ in the formulation, but the process is shortened with an increase in the percentage of poplar sawdust and perlite. The carbon source, nitrogen compounds, macro and micro elements are effective compounds in mycelium growth (Fermor et al., 1985). A nitrogen source is essential for fungal hyphae to complete the substrat in a short time and for high biological efficiency (Kertesz & Thai, 2018). Moreover, *Pleurotus* spp. mycelium growth differed significantly in different nitrogen sources (Hoa & Wang, 2015). But as the mycelium biomass increases in the excess nitrogenous substrate like wheat bran, secondary metabolites such as urea and ammonia appear**.** The environment where bioactive reactions occur in mycelium's vegetative development in artificial environments is closed to external influences. This phenomenon causes the concentrations of toxin-effective components that suppress mycelial growth in the system to increase over time. It is also known that *P. ostreatus* does not require high nitrogen concentrations, especially during the vegetative phase (Yang, 2000).

In addition, $CaSO_4$ and $CaCO_3$ were used, respectively to prevent the formation of aerobic conditions by excess binding water and to regulate pH. High rates of these agents suppressed mycelial growth. This may be explained by the high rate of $CaSO_4$ bound by the water needed by the mycelium. Wheat bran was close to neutral (pH 6.79-7.04), while poplar sawdust was acidic (pH: 4.46). However, the increase in $CaCO_3$ in the formulation probably caused the pH to rise excessively with perlite and suppressed mycelial growth. Perlite is an aluminosilicate volcanic material with a high water-holding capacity and can be released when necessary. Although areas such as construction, agriculture, and horticulture are places where they are used extensively, it is also known to be used as mycelia carrier. According to study results, it can be stated that shortening of running time as perlite amount increases is due to better transmission of oxygen in the bag thanks to its amorphous structure and the easy progression of hyphae thanks to its porous structure (Homolka et al., 2001).

In this context, it is crucial to shorten the running time in spawn production. Due to the morphological structure of perlite, it is thought that the running time is shortened since it increases the inoculation point in spawn. The running speed of fungal hyphae is of great importance to accelerate production. Different techniques are being developed to shorten the spawn running time (Zhang et al., 2014).

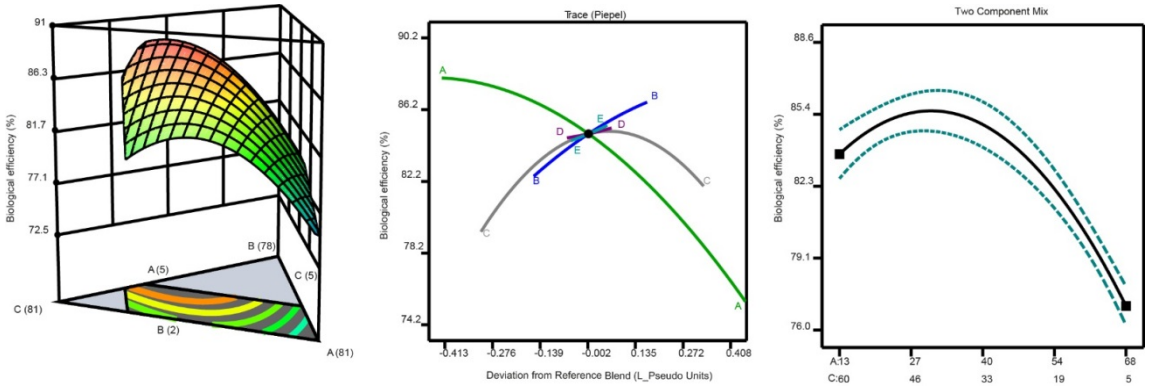

Figure 2. Representation of the effect of components on biological efficiency with 3D surface, trace and two component mix plots; A: Wheat bran, B: Poplar sawdust, C: Perlite, D: $CaCO_3$, E: $CaSO_4$.

Şekil 2. *Bileşenlerin biyolojik verimlilik üzerindeki etkisinin 3 boyutlu yüzey, iz ve iki bileşenli karışım grafikleri ile gösterimi; A: Buğday kepeği, B: Kavak talaşı, C: Perlit, D: $CaCO_3$, E: $CaSO_4$.*

The effect of sawdust showed a linear effect on both spawns running time and biological efficiency. An increase in the sawdust amount resulted in an increase, in biological efficiency while reducing the spawn running time. Sawdust, *Pleurotus* spp. The linear effect was also obtained in a study conducted by Khan et al. (2012). It is already known that sawdust is one of the best substrate sources for the growth of mushroom hyphae of the *Pleurotus* spp. Wheat bran-perlite interaction was found to be very important in both responses ($p<0.0001$). The effect of wheat bran on biological efficiency showed a curve effect just like spawn running time. Although the bran ratio is required at a certain level for mycelial growth, it caused a decrease in biological efficiency as the ratio increased. Similar to our results, It has been reported that nitrogen in the substrate provokes mycelium growth but slows or even stops the growth after a certain level (Curvetto et al., 2002). Therefore, it is vital to optimize the amount of protein source used in the spawn and substrate formulation. However, sawdust is low in protein content and therefore requires nitrogen as an additional source, especially in mushroom cultivation (de Carvalho et al., 2010). Protein-rich cereal bran is one of the methods generally applied to substrates in *P. ostreatus* to promote mycelial growth and increase mushroom yield (Oseni et al., 2012). But at the same time, the carbon-nitrogen balance is extremely important. Excessive nitrogen can degrade lignin and obstacle mycelial growth. This may slow down the spawn running time and delay the fruitbody formation time (Yang et al., 2013). Since it has known that the addition of nitrogen increases the yield of oyster mushrooms (Bellettini et al., 2019), the amount used in the formulation is important.

Biological efficiency, the perlite ratio used in the formulation increased until it reached the midpoints but then decreased by showing a curve effect. This situation is thought to be due to the decrease of wheat bran and poplar sawdust, which are the other major components, as the perlite ratio in the formulation increases. It was observed that perlite added to the substrate at a rate of 20% in *Agaricus bisporus* showed high biological efficiency (Colak, 2004).

Optimization and validation of experimental value and predicted data

Optimum mixture component ratio, values predicted by the model, values analyzed at optimum points, and control (grain spawn) are given in Table 4.

The desirability approach assigns "points" to a set of responses and a method that selects factor settings that maximize the score (Natrella, 2010). The desirability value of the optimum point solution is shown in Table 4. The optimum mixture point was determined as 11.44%, 28.87%, 50.74%, 5.89%, and 3.07% for wheat bran, poplar sawdust, perlite, $CaCO_3$, and $CaSO_4$, respectively. The experimental values analyzed according to the mixture prepared at the optimum point are given in Table 4. While the lowest spawn running time was seen in non-grain spawn with 15.33 days, the grain spawn running time was

longer with 19.20 days. The results showed that the difference between non-grain spawn and grain spawn was statistically significant ($p<0.05$). In terms of biological efficiency, although non-grain spawn is high numerically, no statistical difference was found between grain spawn and grain spawn ($p>0.05$). Also, the difference between the experimental and predicted data is insignificant for both responses ($p>0.05$). This is an indication of the reliability of the chosen model. In short, spawn produced without using grain in the optimum mixing ratio is superior to control group.

Table 4. Optimum point for non-grain spawn with experimental values and predicted data and grain spawn results

Çizelge 4. *Tahılsız misel için optimum noktalar, deneysel değerler ile tahmin edilen veriler ve tahıl misel sonuçları*

Optimum points (Mixture components %)					Desirability	Responses	Predicted data	Experimental value	Control (Grain spawn)
Wheat bran	Poplar sawdust	Perlite	$CaCO_3$	$CaSO_4$	0.981	Spawn running time (days)	14.29^{b}	15.33 ± 1.06^{b}	$19.20\pm0,96^{a}$
11.44	28.87	50.74	5.89	3.07		Biological efficiency (%)	89.94^{a}	$88.10\pm0,87^{a}$	$87.25\pm1,21^{a}$

Different letters in the same line indicate that the samples are statistically different ($p<0.05$)

CONCLUSIONS

In this study, non-grain spawn was developed for *P. ostreatus* with a D-optimal mix design approach using three main components as wheat bran, poplar sawdust, and perlite without using grain. Developed non-grain spawn shortened the spawn running time by 20.16% compared with grain spawn. Biological efficiency was not statistically different in both spawn usage. Spawn running time constitutes one of the most extended periods in the mushroom production process. Therefore, shortening this period will accelerate the circulation. So much some mushroom companies do not even wait for 2nd flush because it is not economical. As a result, the using non-grain spawn shortened the spawn running time required for full colonization to approximately four days. Especially in the leading countries in mushroom production, new technologies and formulations are being developed day by day to shorten the spawn running time and increase biological efficiency and mushroom yield.

In summary, using non-grain spawn provides advantages in time, energy, and installation costs in spawn production as it does not require pre-treatment (boiling, straining, cooling) as in grain spawn production. In addition, since it shortened the mushroom incubation period in mushroom enterprises, It also saves time, cost, and labor.

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