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Influence of Magnetic Field on the Mycelial Growth Rate of *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. and *Lentinula edodes* (Berk.) Pegler

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Abstract: The effects of magnetic field application on the mycelial growth rate of oyster (*Pleurotus ostreatus*) and shiitake (*Lentinula edodes*) mushrooms were studied. 30 ml, %2 Malt Extract Agar (MEA) which autoclaved at 121°C for 30 minutes was placed in 9 cm diameter sterilized Petri dishes. Then, the mycelia of both fungi were planted in the Petri dishes under sterilized conditions, and magnetically exposed to one of seven magnetic field strengths, 0.25, 0.5, 1, 5, 10, 50, and 100 mT for different periods of time (5, 10, 15, and 30 min) using a solenoid., The cultures were conserved at room temperature (approximately 23°C). Once every two days, the growth rates of the mycelia were measured for a period of 20 days. A two-way analysis of variance was performed with SPSS18 for different magnetic field strengths and intervals of application. The LSD test was performed to show the differences. The following results were found: 1) Magnetic field application had a significant effect on the mycelial growth rate of shiitake mushroom, but exposure time and magnetic field-exposure time interaction had not any influence on the growth. In this study, it was concluded that 5 mT and 10 mT magnetic field exposure may be efficient for the development of shiitake mushroom mycelia. 2) Mycelial growth rate of oyster mushroom was not significantly affected by a magnetic field, exposure time, and magnetic field-exposure time interaction.

Keywords: *Pleurotus ostreatus, Lentinula edodes*, Magnetic field, Mycelial growth rate.

Manyetik Alanın *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. ve *Lentinula edodes* (Berk.) Pegler 'in Misel Büyüme Hızına Etkisi

Öz: Bu çalışmada, manyetik alan uygulamasının istiridye (*Pleurotus ostreatus*) ve şitake (*Lentinula edodes*) mantarları misel büyüme hızına etkileri incelenmiştir. 121°C'de 30 dakika otoklavlanan 30 ml, %2 Malt Extract Agar (MEA), 9 cm çapında sterilize edilmiş petri kaplarına dökülmüştür. Daha sonra, her iki mantarın miselleri steril koşullar altında petri kaplarına ekilmiş ve bir solenoid kullanılarak farklı sürelerde (5, 10, 15 ve 30 dak.) 0.25, 0.5, 1, 5, 10, 50 ve 100 mT olmak üzere yedi manyetik alan kuvvetinden birine maruz bırakılmıştır. Kültürler oda sıcaklığında (yaklaşık 23°C) muhafaza edilmiştir. Her iki günde bir, misellerin büyüme oranları 20 günlük bir süre boyunca ölçülmüştür. Farklı manyetik alan güçleri ve uygulama aralıkları için SPSS18 ile iki yönlü varyans analizi yapılmıştır. Manyetik alan ve uygulama süreleri arasındaki farkı göstermek için LSD testi kullanılmıştır. Yapılan analizler sonucunda şu sonuçlar bulunmuştur: 1) Manyetik alan uygulaması, şitake mantarının misel büyüme hızı üzerinde önemli bir etkiye sahip olmuştur, ancak maruz kalma süresi ve manyetik alan-maruz kalma süresi etkileşiminin büyüme üzerinde herhangi bir etkisi görülmemiştir. Bu çalışmada, şitake mantarı

misellerinin gelişimi için 5 mT ve 10 mT manyetik alan maruziyetinin etkili olabileceği sonucuna varılmıştır. 2) İstiridye mantarının misel büyüme hızı, manyetik alan, maruz kalma süresi ve manyetik alan-maruz kalma süresi etkileşiminden önemli ölçüde etkilenmemiştir.

Anahtar kelimeler: *Pleurotus ostreatus, Lentinula edodes*, Manyetik alan, Misel büyüme oranı.

Introduction

In recent years, there has been a growing concern for food production due to the increasing population (Fujimaki and Kikuchi, 2010). For this reason, scientists are trying to find production methods that are effective, environmentally friendly, and inexpensive. Biological, chemical, and physical methods are being used to achieve higher growth and yield. However, although chemical methods are effective in increasing growth and yield, it has been observed that they can be harmful in the later stages of development. Researchers working on magnetic field applications have reported high performance in terms of growth and yield in many vegetables, fruits, and mushrooms (Jamil et al., 2012).

Mushrooms are one of the oldest known foods in the world. Mushrooms are important because they are low in calories, high in nutritional value, rich in protein and vitamins. Also, mushrooms are easy to produce and their production costs are low (Ali et al., 2007).

Nowadays oyster and shiitake mushrooms are among the popular mushrooms produced in the world.

Shiitake (*Lentinula edodes* (Berk.) Pegler) are an edible mushroom species that can be found naturally in the forest or being done culture on logs in China, Korea, Japan, Singapore, Thailand, and other Asian countries. Not only these countries, but it is also now grown in many other countries, such as Brazil, Canada, the Netherlands, the United Kingdom and the United States (Slee 1991; Ciesla,2002; Aji,2009, Sesli et al., 2020). Chittaragi et al., (2018) pointed out that shiitake mushroom is cultivated throughout the world and contributes around 25 percent of the aggregate yearly generation of mushrooms. Shiitake mushroom is a nonpathogenic white-rot fungus. It secretes a class of lignocellulolytic enzymes, which permit it to grow on lignocellulosic substrates rich in lignin (Leatham, 1986).

Zembron-Lacny et al. (2013) indicated that compounds produced by shiitake which are attributed to have antioxidant, antimicrobial, antilipidemic, anticancer, anticarcinogenic, and immunoregulatory activities include lentinan, eritadenine, ergothioneine as well as vitamins, especially provitamin D2 (ergosterol and calciferol), vitamins B (thiamine, riboflavin, and niacin) and pantothenic acid. Magnesium, nickel, copper, phosphorus, strontium, and zinc are the minerals found at high concentrations.

Pleurotus ostreatus (Jacq. ex Fr.) P. Kumm.called oyster mushroom , is edible fungi cultivated throughout the world, particularly in Southeast Asia, India, Europe, and Africa (Eşitken, 2003; Tesfaw et al., 2015; Sesli et al., 2020). Oyster mushrooms are the second-largest (Debeaujon, 2000) next to *Agaricus bisporus* (J.E.Lange) Imbach, Mitt. Naturf. Ges. Luzern 15:15 (1946) or the third-largest (Bhatnagar and Deb, 1977) commercially produced mushroom in the world (Tesfaw et al., 2015). Yıldız et al. (2002) addressed that recently some species of *Pleurotus* are cultivated commercially because of their rich mineral contents and medicinal properties, short life cycle, reproducibility in the recycling of certain agricultural and industrial wastes, and low demand on resources and technology.

Improving the performance of mushroom mycelia is very important in terms of cost reduction and time-saving. Magnetic field application is one of the important factors that affect mycelial growth (Sarıtaş, 2015). The influence of magnetic field application on living organisms has been studied by some researchers focusing on germination, vigour as well as growth at later stages of development (Flórez et al., 2007; Marks and Szecówka, 2010; Jamil et al. 2012). Each living organisms have a particular reaction to the electromagnetic field (Ružič et al., 1997) including mushroom mycelia. Shams et al. (2013) indicated that the effects of electromagnetic energy on living tissues are the reason why they are used for agricultural development. Effects of such energy depend on the type, seasonal life spans, field intensity, and duration of treatment (Piacentini et al., 2001).

There are several studies on the effects of magnetic field applications on the mycelia of *Pleurotus* species (Peláez et al., 2013; Mosa et al., 2018). However, there are no previous studies in the literature about the effects of magnetic field application on the mycelia of shiitake. In this study, we examined the impact of various magnetic field strengths (0.25, 0.5, 1, 5, 10, 50, and 100

mT) and exposure durations (5, 10, 15, and 30 min) on the development of the mycelia of oyster and shiitake mushrooms.

Material and method

Shiitake and oyster mushroom mycelia, and the medium for mycelial growth, malt extract agar (MEA), were used in this study. The mycelia of both kinds of mushroom were obtained from a company

in Bursa. A solenoid and strong magnets were used to expose the fungal mycelia to the magnetic fields. A Gauss/Teslameter magnetic field measuring device (Me Magnet-Phy Dr. FH 51 Steingroev GmbH, Art No: 2000510 and serial number: 113 592 CE) was used to determine the effect of the magnetic field on the mycelial development of shiitake and oyster mushrooms. Measurements were performed with a digital multimeter.

The MEA medium (2%), which was used for the mycelial growth, was autoclaved at 121°C for 30 min. Then approximately 30 ml of sterilized medium was poured into plastic Petri dishes with a diameter of 9 cm. A total of 96 shiitake mycelium pieces and 96 oyster mushroom mycelium pieces were placed on the MEA plates (Figure 1). Powerful magnets and a solenoid for low magnetic field strengths were used to generate the necessary magnetic fields for the experiment. The magnetic field was applied to the mycelia at various doses (0.25, 0.5, 1, 5, 10, 50, and 100 mT) and durations (5, 10, 15, and 30 min). The control groups were not subjected to magnetic field treatment. Three replicates were done for each treatment. The growth rates of fungal mycelia were measured once every two days for 20 days. Then, variance analysis (two-way ANOVA) was performed to determine significance. LSD (Least significance difference) was used for multiple comparisons of means. The significance level was set at $p < 0.05$ for all analyses. All statistical analyses were performed using SPSS22.

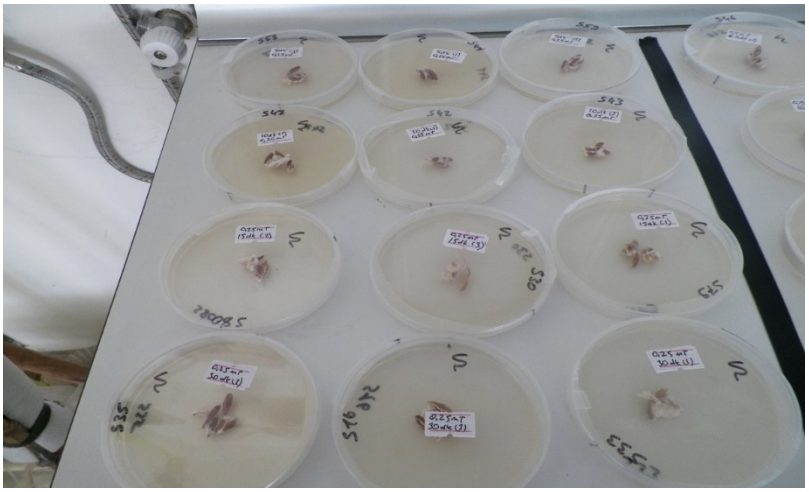

Figure 1. A magnetic field applied shiitake mushroom mycelia

Results and Discussions

Shapiro-Wilk test 'test of normality test' was done whether there was a normal distribution for two types of mushroom mycelium (Table1 and Table 2). Analysis of data showed that the variables were not normally distributed ($p<0.05$). A logarithmic transformation was applied to eliminate the skewness of the original data distributions.

Table 1. Kolmogorov-Smirnov and Shapiro-Wilk tests of normality for shiitake mushroom mycelium

Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Statistic	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
0.193	96	0.000	0.756	96	0.000

Table 2. Kolmogorov-Smirnov and Shapiro-Wilk tests of normality for oyster mushroom mycelium

Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Statistic	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
0.187	96	0.000	0.843	96	0.000

Two-way ANOVA was performed to determine the effect of density and exposure duration of the magnetic field on mycelial growth. Table 3 showed that various magnetic field strengths significantly affected the development of shiitake mycelium ($p < 0.05$), whereas no effect of exposure durations on the mycelial growth ($p > 0.05$) was observed.

Table 3. Two-way ANOVA test results of mycelial growth mean between magnetic field strengths and exposure time for shiitake mushroom mycelium.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	0.021[a]	31	0.001	1.260	0.216
Intercept	81.660	1	81.660	151236.246	0.000
MA	0.009	7	0.001	2.470	**0.026***
Time	0.001	3	0.000	0.548	0.651
MA * Time	0.011	21	0.001	0.958	0.524
Error	0.035	64	0.001		
Total	81.716	96			
Corrected Total	0.056	95			

LSD (Least significance difference) multiple comparison tests were performed to compare the mean values of mycelial growth of shitake mushroom according to the various magnetic field strengths ($p < 0.05$) (Table 4). The magnetic field treatment of 5 mT resulted in a significant increase in mycelial growth in comparison with the 50 mT, 100 mT, and control group. In the same way, mycelia exposed to a magnetic field of 10 mT grew higher than 50 mT and the control group (Table 4). It was reported that static and pulsed magnetic fields affected the growth and enzymatic activity of fungi (Owen, 1998; Nagy and Fischl, 2002).

Table 4. Effect of magnetic field exposure on the mycelial growth rate of shiitake mushroom

(I) MA	(J) MA	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.25mT	0.5mT	.0004	.00949	.966	-.0185	.0194
	1mT	.0027	.00949	.775	-.0162	.0217
	5mT	-.0121	.00949	.207	-.0310	.0069
	10mT	-.0062	.00949	.517	-.0251	.0128
	50mT	**.0208***	**.00949**	**.032**	.0018	.0397
	100mT	.0085	.00949	.373	-.0104	.0275
	Control	.0133	.00949	.165	-.0056	.0323
0.5mT	0.25mT	-.0004	.00949	.966	-.0194	.0185
	1mT	.0023	.00949	.808	-.0166	.0213
	5mT	-.0125	.00949	.193	-.0314	.0065
	10mT	-.0066	.00949	.490	-.0255	.0124
	50mT	**.0204***	**.00949**	**.036**	.0014	.0393
	100mT	.0081	.00949	.396	-.0109	.0271
	Control	.0129	.00949	.179	-.0061	.0319
1mT	0.25mT	-.0027	.00949	.775	-.0217	.0162
	0.5mT	-.0023	.00949	.808	-.0213	.0166
	5mT	-.0148	.00949	.124	-.0338	.0041
	10mT	-.0089	.00949	.352	-.0279	.0100
	50mT	.0180	.00949	.062	-.0009	.0370

	100mT	.0058	.00949	.544	-.0132	.0247
	Control	.0106	.00949	.269	-.0084	.0295
5mT	0.25mT	.0121	.00949	.207	-.0069	.0310
	0.5mT	.0125	.00949	.193	-.0065	.0314
	1mT	.0148	.00949	.124	-.0041	.0338
	10mT	.0059	.00949	.536	-.0130	.0249
	50mT	**.0328***	**.00949**	**.001**	.0139	.0518
	100mT	**.0206***	**.00949**	**.034**	.0016	.0395
	Control	**.0254***	**.00949**	**.009**	.0064	.0443
10mT	0,25mT	.0062	.00949	.517	-.0128	.0251
	0,5mT	.0066	.00949	.490	-.0124	.0255
	1mT	.0089	.00949	.352	-.0100	.0279
	5mT	-.0059	.00949	.536	-.0249	.0130
	50mT	**.0269***	**.00949**	**.006**	.0080	.0459
	100mT	.0147	.00949	.126	-.0043	.0336
	Control	**.0195***	**.00949**	**.044**	.0005	.0384
50mT	**0,25mT**	**-.0208***	**.00949**	**.032**	-.0397	-.0018
	0,5mT	**-.0204***	**.00949**	**.036**	-.0393	-.0014
	1mT	-.0180	.00949	.062	-.0370	.0009
	5mT	**-.0328***	**.00949**	**.001**	-.0518	-.0139
	10mT	**-.0269***	**.00949**	**.006**	-.0459	-.0080
	100mT	-.0123	.00949	.201	-.0312	.0067
	Control	-.0075	.00949	.435	-.0264	.0115
100mT	0,25mT	-.0085	.00949	.373	-.0275	.0104
	0,5mT	-.0081	.00949	.396	-.0271	.0109
	1mT	-.0058	.00949	.544	-.0247	.0132
	5mT	**-.0206***	**.00949**	**.034**	-.0395	-.0016
	10mT	-.0147	.00949	.126	-.0336	.0043
	50mT	.0123	.00949	.201	-.0067	.0312
	Control	.0048	.00949	.615	-.0142	.0238
Control group	0,25mT	-.0133	,00949	,165	-,0323	,0056
	0,5mT	-.0129	.00949	.179	-.0319	.0061
	1mT	-.0106	.00949	.269	-.0295	.0084
	5mT	**-.0254***	**.00949**	**.009**	-.0443	-.0064
	10mT	**-.0195***	**.00949**	**.044**	-.0384	-.0005
	50mT	.0075	.00949	.435	-.0115	.0264
	100mT	-.0048	.00949	.615	-.0238	.0142

* indicates significant difference at p<0.05 level (LSD multiple comparison test

The growth rate was characterized with respect to the magnitude of the applied magnetic field, ignoring the time interval of application. At first (between days 2 and 8), the control showed a good growth rate but the growth rate was later (between days 10 and 20) found to be best at 5 and 10 mT (Figure 2). The mycelial growth rate was highest under the magnetic field of 5 mT and 10 mT, respectively, and least at the control group and 50 mT (Figure 2).

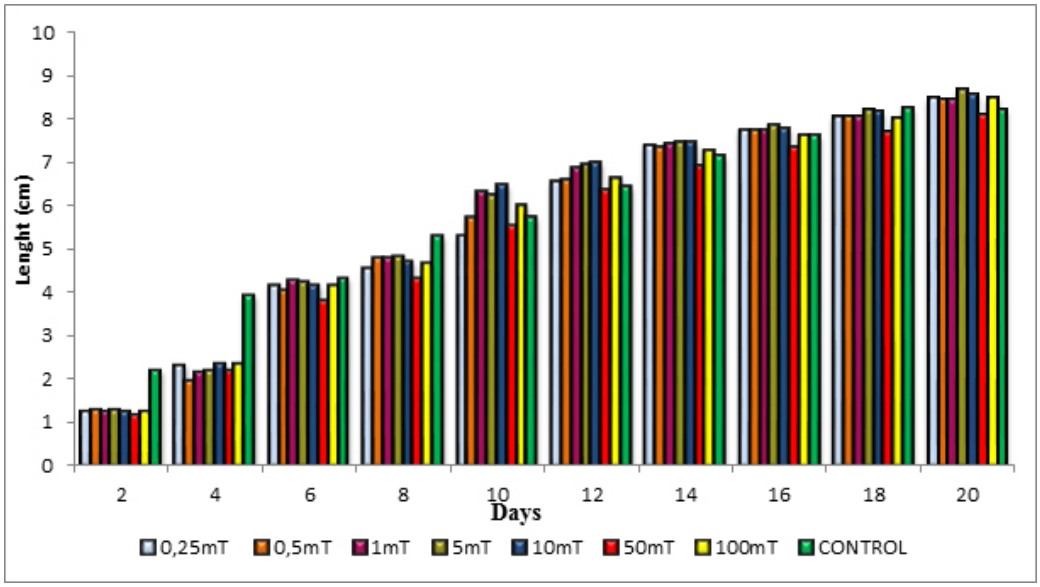

Figure 2. The developmental course of shiitake mushroom mycelium in the different magnetic field strengths

Table 5 showed that the magnetic field strength and exposure durations had no effect on the development of the oyster mushroom mycelium (p>0.05) (Table 5).

The magnetic field applied *Pleurotus* mycelia and non-exposed control group was examined in terms of mycelial growth rate ignoring the exposure time (Figure 3). No significant differences (p>0.05) were found among the magnetic field strengths. At the end of the 20th day, the best mycelial growth rate result was however obtained with the 0.5 mT and the worst was the 100 Mt (Figure 3).

Table 5. Two-way ANOVA test results of mycelial growth mean between magnetic field strengths and exposure time for oyster mushroom mycelium

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	.111[a]	31	.004	.733	.828
Intercept	74.352	1	74.352	15278.158	.000
MA	.039	7	.006	1.142	.349
Time	.021	3	.007	1.469	.231
MA * Time	.050	21	.002	.491	.964
Error	.311	64	.005		
Total	74.774	96			
Corrected Total	.422	95			

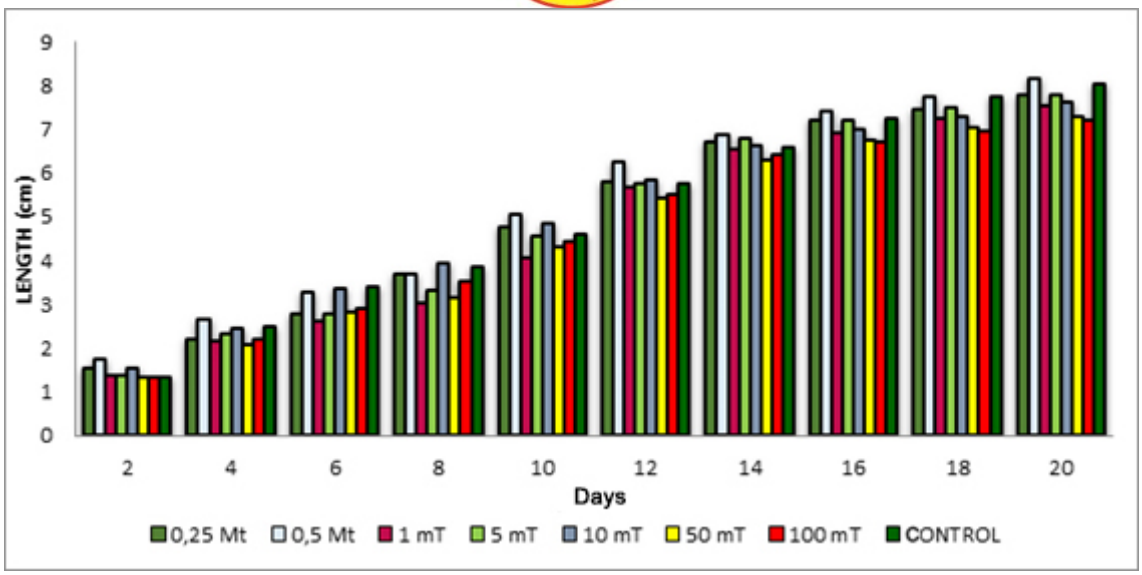

Figure 3. The developmental course of oyster mushroom mycelium in the different magnetic field strengths

Mycelial growth data were taken on 7th day and it was observed that the magnet treatment of 250 mT with the exposure period of 7 days showed the greatest mycelial diameter (8.8 cm), with the indication that this treatment showed the lowest variation compared to the others. In the Dunnett post-Anova test, the treatment of 125 mT with 7 days of exposure was the only one that showed significant differences compared with the control group. It was concluded that the application of magnetic fields has a positive growth effect on the fungus (Pelaez *et al.*, 2013).

In a similar study conducted by Mosa *et al.* (2018), different magnetic field strengths (2, 25, 50, 100, and control) were applied on *P. ostreatus* mycelia. In this study, there was no significant difference in mycelial growth rate under the 50 and 100 mT magnetic fields. It was consistent with our study.

Conclusion

In this study, the development of the mycelia of shiitake and oyster mushrooms was examined under various magnetic field strengths and application intervals. Shiitake mushroom mycelia showed better development under magnetic fields of 5 and 10 mT as compared with the control group. However, no effect was observed for the application interval. According to the results obtained from this study, it can be concluded that the magnetic field exposure of 5 mT and 10 mT may be beneficial for the development of shiitake mushroom mycelia. The magnetic field strength and exposure durations had no effect on the development of the oyster mushroom mycelium.

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