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Use of different agricultural wastes in *Ganoderma carnosum* Pat. cultivation

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Abstract: In this study, we aimed to determine the best agricultural waste media mixtures for *Ganoderma carnosum* Pat. cultivation, which is almost unknown in Turkey. Six different growing mixtures were prepared using oak sawdust, peanut shell, and corn cob: C (oak sawdust), M1 (2 oak sawdust + 1 wheat bran), M2 (2 peanut shells + 1 wheat bran), M3 (1 oak sawdust + 1 peanut shell + 1 wheat bran), M4 (2 corn cobs + 1 wheat bran), M5 (1 oak sawdust + 1 corn cob + 1 wheat bran). During the study, mycelial growth time, biological efficiency (BE), mushroom weight, total yield, cap diameter, cap thickness, dry matter of mushroom samples, and pH and moisture content of the mixtures were determined at different periods (after sterilization, after mycelia development, and after harvest). Mycelial development time was detected between 26.00 and 41.00 days. The highest and the lowest yield values were obtained from M2 (53.90 g kg^{-1}) and C (14.63 g kg^{-1}), respectively. The highest amount of dry matter was recorded in C (20.29%) while the lowest amount was obtained from M2 (17.97%). It has been determined that the average weight of the mushrooms varied between 6.46 and 23.00 g. The pH was the highest after sterilization (5.62) and the lowest after harvest (4.21) periods. In terms of moisture content, the highest content was observed after harvest period as 66.17%. When the results were evaluated based on yield and biological efficiency, which are the most important criteria for producers, the use of oak sawdust alone or in combination seems to be disadvantageous.

Key words: *Ganoderma*, mushroom production, plant wastes

1. Introduction

In recent years there has been an increasing consumer demand for functional foods with high nutritional and human health-promoting value. In this aspect horticultural crops gained more importance because they contain abundant bioactive compounds, including phenolic acids, flavonoids, flavonols, anthocyanins, macronutrients, vitamins, minerals, and volatile aromatic compounds (Colak et al., 2019; Gecer et al., 2020; Balci 2021; Juric et al., 2021).

Mushrooms not only provide nutritious and protein-rich food, but also some species produce medicinally effective compounds (Chang and Miles, 2004; Kibar, 2021). Some species of the genus *Ganoderma*, which cannot be consumed directly as food but is known to support the immune system, can also be evaluated in this group. The use of *Ganoderma lucidum* for this purpose is common in the genus *Ganoderma*. Studies on the culture of *G. lucidum* using different agricultural wastes have been carried out until today (Yakupoğlu and Pekşen, 2011; Veena and Pandey, 2011; Gurung et al., 2012; Mehta et al., 2014; Roy et al., 2015; Jeewanthi et al., 2017; Ozcariz-Fermoselle et al., 2018; Atila, 2020). On the other hand, studies on *Ganoderma carnosum* are very scarce. In a study carried out by Yalcin et al. (2020), its phenolic content and biological activity were determined, and its medicinal value was emphasized. In their study, wild *G. carnosum* samples collected from rotten *Abies* sp. stump were used. As far as we search there is no study related to the culture of *G. carnosum* in literature.

One of the most important advantages of mushroom cultivation is the opportunity to evaluate agricultural wastes. Nowadays, zero waste projects have become a priority all over the world. Cultivation of mushroom species also has important contributions in this respect. Besides, the used mushroom compost can be assessed for different purposes in agricultural production.

The Covid-19 epidemic, which has been experienced all over the world for the last two years, has once again revealed the benefits of nutrition that strengthens the

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immune system. There are many factors that affect the immune system, such as genetics, stress, regular sleep, and some unhealthy habits. Healthy nutrition also has an important place among these factors. Numerous studies have been published on the contribution of some species of the genus *Ganoderma* to the immune system (Zhang et al., 2019; Wang and Lin, 2019; Yang et al., 2020; Yalcin et al., 2020; Ren et al., 2021). Therefore, it is necessary to increase the studies on the cultivation of these species. In order to make mushroom cultivation more economical, it is necessary to investigate the usability of agricultural wastes of countries and even regions of countries.

Therefore, we aimed to investigate the effect of some agricultural wastes together with oak sawdust on the performance of *G. carnosum*. Peanut shell and corn cob were preferred because they are one of the main agricultural products of the Çukurova Region (Eastern Mediterranean, Turkey). The study also investigates which of the wastes used in the cultivation of *G. carnosum* increase yield and quality.

2. Material and methods

This study was carried out at Prof. Dr. Saadet BÜYÜKALACA Tissue Culture Laboratory and in a fully climate-controlled mushroom growing room of Çukurova University (Adana, Turkey). Spawn of *G. carnosum* was obtained from the Republic of Turkey Ministry of Agriculture and Forestry, Atatürk Horticultural Central Research Institute (Yalova, Turkey). Peanut shell, corn cob, and oak sawdust were used as agricultural wastes by adding wheat bran and 5% soy flour at different proportions (Table 1). During the preparation of the growing mixtures, measurements were performed with a pH meter to adjust the pH, and plaster and lime were added to the mixtures when needed. During the adjustment of the mixture moisture, it was soaked with tap water at regular intervals. At the end of the soaking, the excess water of the material was filtered and 1% lime, bran, and soy flour were added. The prepared substrate mixtures were filled as 1 kg for each high-temperature resistant polypropylene bag.

Table 1. Content of the growing mixtures used in cultivation of *G. carnosum*.

Code	Growing mixtures
C	Oak sawdust (control)
M1	2 Oak sawdust + 1 Wheat bran
M2	2 Peanut shells + 1 Wheat bran
M3	1 Oak sawdust + 1 Peanut shell + 1 Wheat bran
M4	2 Corn cobs + 1 Wheat bran
M5	1 Oak sawdust + 1 Corn cob + 1 Wheat bran

pH and moisture levels were determined at three different periods: after sterilization, after mycelia development, and after harvest. Yield and quality (mycelia development time, biological efficiency, total yield, mushroom weight, cap diameter, cap thickness, and dry matter content) measurements were carried out. Mycelia development time was calculated as days from mycelia inoculation to the end of the mycelia development in the bags. For obtaining a total yield, amount of daily harvests from each bag for each application and repetition was assessed (three repetitions and three bags in each repetition). The weight of each mushroom was detected on a scale as g in five mushroom samples and their averages were presented. Cap thickness and diameter were measured with a caliper as mm in five mushroom samples randomly selected and their averages were calculated. The mushroom samples harvested were weighed and then dried in food driers adjusted to 65 °C for obtaining dry matter content (Figures 1–2). After weighing of dried samples, the dry matter content was detected as %. The biological efficiency rate was determined as follows (Royse, 1985):

$$BE = \frac{\text{Fresh mushroom weight harvested}}{\text{Dry growing mixture weight}} \times 100$$

This study was planned according to the randomized block experimental with three replications. Three growing bags were used for each repetition. The data obtained were analyzed in the JMP statistical package program according to the randomized plot design. Percentage values were converted to angle values and statistical analysis was

Figure 1. *G. carnosum* samples at harvest stage.

Figure 2. Dried *G. carnosum* samples in food drier adjusted to 65 °C.

applied. Statistically significant was given as letters by applying the LSD test.

3. Results and discussion

The mycelia development time of *G. carnosum* in different growing mixtures was presented in Table 2. While differences among the mixtures were not found statistically significant, mycelia development varied between 26.00 and 41.00 days. There is almost two weeks difference between these two results, and this will be important for producers as it will provide earliness. While the fastest development was obtained from M4 with 26.00 days, the slowest development was observed in C with 41.00 days. The growing mixtures containing corn cob seem more successful in terms of mycelia colonization time. We could not find any study on the effect of different agricultural wastes on yield and quality in *G. carnosum*. Therefore, *G. lucidum*, one of the closest species and the most cultivated *Ganoderma* species, was assessed for the comparison. Mycelia development time was reported between 18.8 and 22.8 days in *G. lucidum* by Veena and Pandey (2011) and was not influenced by tested five different substrate combinations (22.50 sawdust : 67.50 paddy straw : 10.00 rice bran, 45.00 sawdust : 45.00 paddy straw : 10.00 rice bran, 67.50 sawdust : 22.50 paddy straw : 10.00 rice bran, 90.00 sawdust : 10.00 rice bran; 90.00 paddy straw : 10.00 rice bran). It was found to be 30.00–35.00 days by Gurung et al. (2012) in the substrate mixtures including *Shorea robusta* sawdust (35.00 days) and *Alnus nepalensis* sawdust (30.00 days) with gram flour, wheat bran, corn flour, and rice bran. Mycelial colonization period changed between 25.40 (rubber) and 34.20 (jack) days in different sawdust materials and mixtures such as rubber, mango, jack,

Table 2. Mycelial development time, biological efficiency, and yield values of *G. carnosum* cultured in the different growing mixtures.

Growing mixtures	Mycelia development time (day)	Biological efficiency (%)	Yield (g kg^{-1})
C	41.00	3.98 e	14.63 e
M1	33.00	7.51 d	28.93 d
M2	35.00	15.05 a	53.90 a
M3	33.00	9.68 c	37.48 c
M4	26.00	13.57 b	42.85 b
M5	29.00	10.76 c	41.81 bc
LSD	N.I.	1.31***	4.83***

[1] Statistical differences between the averages shown in separate letters in the same column were found to be significant
[2] N.I. Not important; *. $p < 0.05$. **$p \leq 0.01$. ***$p \leq 0.001$

lunumidella, rubber : mango, rubber : jack, and rubber : lunumidella studied by Jeewanthi et al. (2017). This varied from 14.20 (wheat straw) to 18.20 days (cottonseed meal) in different substrate materials such as wheat straw, sunflower meal, cottonseed meal, soybean straw, bean straw, oak sawdust, and poplar sawdust in the study performed by Atila (2020). Corn cob was not used in all these studies carried out in *G. lucidum*. If corn cob was used, maybe earlier mycelia colonization could be observed.

Percentages of biological efficiency (BE) obtained from different growing mixtures are given in Table 2. Differences among mixtures were found to be statistically significant. Biological efficiency rates varied from 3.98% to 15.05%. The highest biological efficiency rate was observed in M2 with 15.05% and it was followed by M4 (13.57%) and M5 (10.76%). The lowest value was obtained from C with 3.98%. Based on these results, we can say that the use of only sawdust without agricultural wastes resulted with low biological efficiency rate. Veena and Pandey (2011) found that biological efficiency varied from 25.7% (90 paddy straw : 10 rice bran) to 29.9% (22.50 sawdust : 67.50 paddy straw : 10 rice bran). BE was calculated between 0% (*Tectone grandis* with rice and wheat bran, *Gmelina arborea* with rice and wheat bran, *Michelia chambaca* with rice and wheat bran) and 7.60% (*Swietenia mahagoni* with wheat bran) in different sawdust materials by Roy et al. (2015). Wheat bran resulted with higher yield and BE than rice bran. Results of Jeewanthi et al. (2017) for BE were between 2.50% (rubber : jack) and 5.70% (rubber : lunumidella). In their study, jack caused lower BE. Atila (2020) detected that BE changed between 8.90% (cottonseed meal) and 24.70% (oak sawdust). In our study, the opposite was observed, and the lowest BE was obtained from the use of oak sawdust alone. Probably, different agricultural waste preferences resulted in different yield BE values.

Yield values of *G. carnosum* in different growing mixtures were determined and the difference between the mixtures was found to be statistically significant (Table 2). It was determined that the average yield values of *G. carnosum* in different growing mixtures ranged between 14.63 and 53.90 g kg^{-1}. Accordingly, the highest yield was obtained in M2 (53.90 g kg^{-1}), and it was followed by M4 (42.85 g kg^{-1}), and M5 (41.81 g kg^{-1}), which were in the same group statistically, respectively. Among the mixtures, the lowest value was observed in the C (14.63 g kg^{-1}). Yakupoğlu and Pekşen (2011) determined that the highest yield in *G. lucidum* was 73.07 g kg^{-1} in oak wood chips. Roy et al. (2015) found the highest yield as 235.20 g kg^{-1} in *S. mahagoni* sawdust including wheat bran in *G. lucidum*. It was 132.90 g kg^{-1} in the same sawdust including rice bran. This result shows how effective the supplements are. They observed similar results in *Dipterocarpur turbinatus*. Jeewanthi et al. (2017) recorded the highest yield in mango sawdust (49.30 g), and it was followed by rubber : mango (45.70 g) and rubber : sawdust (42.50 g) in *G. lucidum*. The lowest yield was detected in rubber : jack sawdust with 26.30 g. Mango sawdust seems to be more effective than the other sawdust materials in their study. The yield was between 28.60 and 86.10 g kg^{-1} in the study carried out by Atila (2020).

The average mushroom weight of *G. carnosum* in different growing mixtures is presented in Table 3, and the differences between the mixtures are found to be statistically significant. When the highest mushroom weight among the mixtures was determined in the M2 (23.00 g) and M1 (21.96 g), which were statistically in the same group, the lowest value was obtained from C (6.46 g). Yen (2008) determined that the average mushroom weight of *G. lucidum* strains in different sawdust mixtures was between 11.38 and 15.16 g. Yakupoğlu and Pekşen (2011) reported that the average mushroom weight in *G. lucidum* varied between 7.99 and 31.19 g. When evaluating such data, we should not forget that mushroom sizes and weights vary depending on whether the strain used was developed for bottle or bag culture.

The cap diameter of the *G. carnosum* in the different growing mixtures was determined, and the difference between the mixtures was found to be statistically significant (Table 3, Figure 3). Average cap diameter ranged from 37.43 (C) to 57.32 (M1) mm. Yen (2008) determined the mushroom diameter values of *G. lucidum* strains in different sawdust mixtures between 6.92 and 9.12 cm. Veena and Pandey (2011) found that the average cap diameter of *G. lucidum* varied between 73.00 and 93.00 mm. Atila (2020) determined it between 58.00 and 92.40 mm in *G. lucidum*. Compared to the literature, it is clear that *G. carnosum* has smaller fruiting bodies than *G. lucidum*.

The cap thickness of the *G. carnosum* in different growing mixtures was determined, and the difference between the mixtures was found to be statistically significant (Table 3). The average cap thickness was between 7.79 (C) and 16.23 (M3) mm. Veena and Pandey (2011) reported that the average cap thickness of *G. lucidum* varied from 6.90 to 8.10 mm. Atila (2020) recorded it between 6.00 and 8.10 mm in *G. lucidum*.

The average dry matter content of *G. carnosum* is presented in Table 3, and the difference between the mixtures is not statistically significant. The average dry matter content varied between 17.97% and 20.29%. Accordingly, the highest and the lowest dry matter content were obtained in C (20.29%) and M2 (17.97%), respectively.

The pH values of different growing mixtures used in *G. carnosum* at different periods are given in Table 4. Accordingly, the differences between the average of periods and the mixture x period interaction were found to be statistically significant, while the average of the mixtures

Table 3. Average mushroom weight, cap diameter, cap thickness, and dry matter content of *G. carnosum* cultured in the different growing mixtures.

Growing mixtures	Average mushroom weight (g kg^{-1})	Cap diameter (mm)	Cap thickness (mm)	Dry matter content (%)
C	6.46 c	37.43 c	7.79 c	20.29
M1	21.96 a	57.32 a	12.11 b	19.99
M2	23.00 a	46.82 b	10.34 bc	17.97
M3	15.96 b	53.78 ab	16.23 a	18.27
M4	16.41 b	55.25 a	12.39 b	18.62
M5	14.65 b	49.52 ab	10.57 bc	18.83
LSD	2.76***	8.08***	3.39**	N.I.

[1]Statistical differences between the averages shown in separate letters in the same column were found to be significant
[2]N.I. Not important; *. $p < 0.05$. **$p \leq 0.01$. ***$p \leq 0.001$

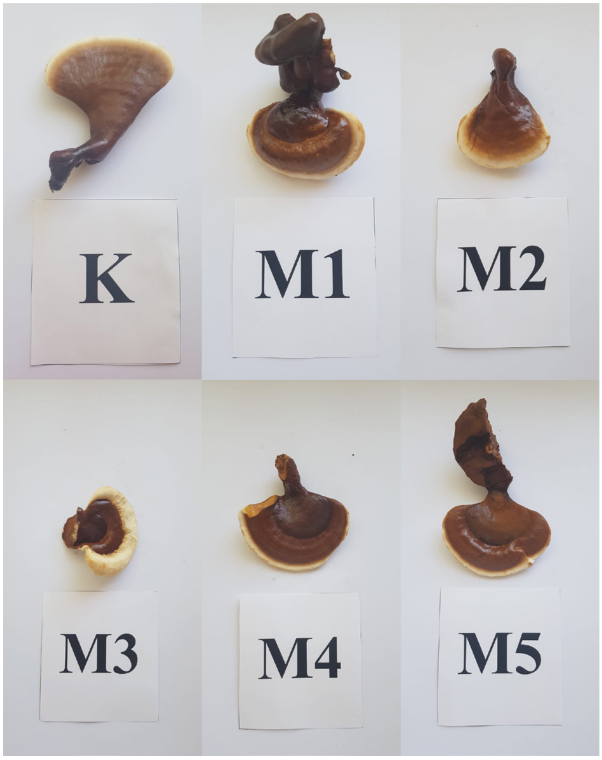

Figure 3. Harvested *G. carnosum* samples from different growing mixtures (K: Control) (The sampling for the photograph was carried out at random. The largest specimens were not selected).

Table 4. pH value of *G. carnosum* cultured in the different growing mixtures.

	Different periods			
Mixtures	AS	AMG	AH	Mean
C	5.72 b	4.97 d	4.73 e	5.14
M1	5.32 c	4.45 fg	4.19 hı	4.65
M2	5.25 c	4.60 ef	4.30 gh	4.72
M3	5.58 b	4.21 hı	4.26 ghı	4.69
M4	6.13 a	3.97 j	4.07 ıj	4.73
M5	5.73 b	3.94 j	3.71 k	4.46
Mean	5.62 A	4.36 B	4.21 B	

LSD period*** = 0.09, LSD mean = N.I., LSD period x substrate*** = 0.20
[1] Statistical differences between the averages shown in separate letters in the same column were found to be significant
[2] N.I. Not important; *. $p < 0.05$. **$p \leq 0.01$. ***$p \leq 0.001$
AS: After sterilization, AMG: After mycelia growth, AH: After harvest

was not statistically significant. When the effects of the mixtures were examined during the period, the highest pH value was obtained after the sterilization period (5.62) and this period was followed by after mycelia growth (4.36) and after harvest (4.21) periods, which were in the same group statistically, respectively. In the mixture x period interaction, the pH values varied from 3.71 to 6.13. The interaction was the highest in M4 (6.13) after sterilization, followed by M5 (5.73), C (5.72), and M3 (5.58) after sterilization, which was statistically similar. The lowest pH value in the interaction was determined in M5 after harvest (3.71). The average pH of the mixtures was found to be between 4.65 (M1) and 5.14 (C). Yakupoğlu and Pekşen (2011) reported that the pH values of the substrates prepared with wood chips and sawdust varied from 5.80 to 7.35 and from 5.70 to 7.05 in *G. lucidum*, respectively. Similar to our results, Atila (2020) determined pH values between 4.43 and 6.42 in *G. lucidum*.

In Table 5, the moisture content of the *G. carnosum* in different growing mixtures was determined, and the differences between the mixture average, and the mixture x period interaction were found to be statistically significant, while the period average was not found to be significant. In terms of the average moisture content of the mixtures, the highest and the lowest data were found in M4 (67.35%) and C (61.64%), respectively. The other mixtures were distributed between these values. When the mixture x period interaction was examined, the highest moisture content was recorded in M5 (69.18%) after sterilization. This was followed by after sterilization M2 (68.67%), after harvest M4 (68.42%), and after sterilization M4 (68.27%), respectively, which were in the same statistical group. The lowest amount of moisture in the mentioned interaction occurred in C (60.46%) after sterilization. Among periods, the moisture amount was the highest after sterilization (66.17%), and the lowest after harvest (63.47%) periods. Yakupoğlu and Pekşen (2011) reported that the moisture contents of the mixtures prepared with wood chips and sawdust varied from 50.26% to 60.09%, and from 55.24% to 65.92%, respectively in *G. lucidum*. Atila (2020) determined the moisture content of *G. lucidum* between 63.12% and 69.3% similar to our results.

4. Conclusion

In this study, the effects of different agricultural wastes on the yield and quality of *G. carnosum* were determined. During the study, pH and moisture content were analyzed in the growing mixtures at three different periods including after sterilization, after mycelia development, and after harvest. Mycelial development time, biological efficiency, total yield, mushroom weight, mushroom cap diameter, and thickness were also detected. As growing mixtures, oak sawdust, peanut shell, and corn cob were mixed at different proportions, and six different growing mixtures were prepared. The cultivation of this mushroom species is almost nonexistent in the world and does not exist in Turkey. However, its medicinal effects are known. In recent years, the addition of pandemic diseases such as Covid-19, as well as diseases such as cancer that cannot be fully cured, emphasizes the importance of keeping the immune system strong.

The result of the study showed that mycelia development time was the fastest in M4 (26.00 days) and M5 (29.00 days). Yield and biological efficiency parameters were found to be highest in M2 (53.90 g kg^{-1}, 15.05%), M4 (42.85 g kg^{-1}, 13.57%), and M5 (41.81 g kg^{-1}, 10.76%). These three parameters are very important among farmers. In this case, M4 seems to be successful in terms of earliness, and M2 and M4 are better in yield and biological efficiency. M2 and M4 growing mixtures do not contain any oak sawdust substrate. The fact that peanut and corn, which are widely grown in the Mediterranean Region, especially in Adana and the surrounding provinces, can be used as alternative wastes for the cultivation of *G. carnosum*, and this may cause a decrease in demand for oak sawdust. In other words, in our experiment, mixtures containing oak sawdust do not seem to be successful in terms of yield and biological efficiency. In fact, this is the desired result. Considering the goal of reducing the need for sawdust in mushroom cultivation in the world, this is a very important finding. The main objective of mushroom cultivation is to contribute to zero waste by evaluating agricultural wastes.

Table 5. The moisture content of *G. carnosum* cultured in the different growing mixtures.

	Different periods			
Mixtures	AS	AMG	AH	Mean
C	60.46 h	61.19 gh	63.26 e-h	61.64 c
M1	65.61 b-e	63.40 e-h	61.51 gh	63.51 bc
M2	68.67 ab	65.08 c-f	64.19 efg	65.98 ab
M3	64.94 def	63.15 e-h	61.28 gh	63.12 bc
M4	68.27 a-d	65.35 b-f	68.42 abc	67.35 a
M5	69.18 a	63.55 e-h	62.17 fgh	64.96 abc
Mean	66.17	63.62	63.47	

LSD period = N.I., LSD mean* = 2.13, LSD period x substrate* = 3.35

[1]Statistical differences between the averages shown in separate letters in the same column were found to be significant

[2]N.I. Not important; *. P < 0.05. **p ≤ 0.01. ***p ≤ 0.001

AS: After sterilization, AMG: After mycelia growth, AH: After harvest

Contribution of authors

MKS prepared *G. carnosum* spawn. GB and HT designed and managed experiments. GB, EK, and MY carried out all experiments and analyses. GB and HT wrote the manuscript. All authors read and approved the manuscript.

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Conflicts of interest

The authors declare no conflicts of interest.

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