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Research Article

Evaluating the Efficiency of Different Substrates Used for Cultivating Oyster Mushroom

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Abstract

Oyster mushroom is a white fungus that uses lignin and cellulose found in most agro-wastes as its carbon source. A study was conducted at the University of Malawi to evaluate the efficiency of different substrates used for cultivating oyster mushroom namely banana leaves, maize stalks, beans pods, rice straw, ground nut shells, pea pods and bamboo leaves. The steps involved in preparation of spawn was collection and preparation of substrates, spawning of the substrates and incubation, management in the fruiting house, and harvesting. The selected substrates used for the project were collected from sites within Zomba district. Maize stalks were collected from Chinamwali, banana leaves from Kalimbuka, beans pods from Thondwe, rice straw from Likangala, bamboo leaves from Chikanda, ground nut shells were collected from New Road and finally pea pods were collected from Mpondabwino. Data was analyzed using Microsoft excel and was presented in tables and bar graphs, mean were used to interpret the data. The results showed that maize stalks had the fastest colonization, whilst bamboo leaves had the slowest colonization. However, banana leaves substrate had the highest average yield as compared to the rest of the average yields obtained from the other substrates. Ability of the particular substrate to promote different enzyme's (cellulase(s), hemicellulase(s), and laccase(s)) secretion which are important in the degradation of cellulose, hemicelluloses and lignin contributed much to mushroom fruiting. Contamination of the substrates was found to be much in peas pod substrates, bamboo leaves and rice straw due to fast decomposition. In conclusion, the study revealed that banana leaves substrate had the highest average yield as compared to the rest of the average yields, whilst peas pod substrate had the lowest average yield. The study also revealed that much interference with good performance of mushroom came as a result of poor conditions.

Keywords: Oyster; Mushroom; colonization; substrate; yield; contamination

Introduction

A mushroom is a fleshy, spore-bearing fruiting body of a fungus typically produced above ground on soil or on its food source [1]. Mushrooms are in the group of fungi called Basidiomycota. Mushroom can both grow wild or can be cultivated by farmers. In terms of food nutrients, mushrooms are virtually fat and have fewer calories. In addition to that, mushrooms are a good source of vitamins and minerals. They are also a very rich source of proteins and therefore can be used as a substitute for meat protein. Mushroom cultivation can help reduce vulnerability to poverty and strengthens livelihoods through the generation of a fast yielding and nutritious source of food and a reliable source of income. Thus, mushroom cultivation can directly improve livelihoods through economic, nutritional and medicinal contributions. The cultivation of mushrooms for food and medicine is increasingly popular across the world, including Africa. However, the pace of progress in Africa is slow. Never before in contemporary times has the potential of mushrooms been so widely known and advocated. Mushroom application has transcended food and medicine into bioremediation of oil spills. Mushroom cultivation techniques have been widely reported, but many prospective farmers in Africa who want to grow mushrooms do not have access to information Chang, 1993 as cited by [2]. Some mushroom farmers are aware of cultivation techniques using a range of substrates but, generally, they face a lack of information on the commercial potential of indigenous mushrooms. In addition to that, mushroom sector in Africa is characterized by lack of infrastructure, inadequate technical support, and scarcity of mushroom scientists and poor knowledge of mushroom diversity.

Malawi has been introduced to small commercial production of oyster and button mushrooms. The national aim of mushroom production is to increase mushroom production to satisfy domestic demand whilst broadening the source of protein, vitamins and minerals, and increasing smallholder farmer's income [2]. Oyster mushroom (Pleurotus spp.) cultivation is inexpensive and has readily available substrates like coffee residues, rice straw, banana leaves, cotton seed hulls, maize stalks and saw dust. Any type of organic matter containing lignin and cellulose can be used as oyster mushroom substrates, and this includes almost all agricultural wastes. Oyster mushroom is a white fungus that uses lignin and cellulose together as its carbon source and turns the host into white [3]. In terms of structure, oyster mushrooms have three distinct parts- a fleshy shell or spatula shaped cap (pileus), a short or long lateral or central stalk called stipe and long ridges and furrows underneath the pileus called gills or lamellae. The gills stretch from the edge of the cap down to the stalk and bear the spores. The spores are smooth, cylindrical and germinate very easily on any kind of mycological media within 48-96 hrs. The mycelium of Pleurotus is pure white in color. Environmental factors for mushroom cultivation include temperature, relative humidity, light, carbon dioxide and acidity of substrate [3]. Oyster mushroom require different environmental conditions at each growing stage. During incubation, appropriate relative humidity is 65-70% and water content of substrate is 65%. Optimal temperature for mycelial growth is 20-25oC, but some thermophilic strains reach optimal growth at 25-35oC. Mushroom mycelia are quite durable to high concentration of carbon dioxide during incubation. Carbon dioxide concentration should be less than 800ppm in its reproductive growth though the number differs according to strains. Fruiting body formation also requires high relative humidity of up to 80-95% and temperature should be lower than optimal mycelial growth by 10oC. Unlike most crops, mushroom should be cultivated on a small indoor area. As such, oyster mushroom can be grown on a small-scale with a moderate initial investment, but convert high amounts of substrate to fruiting bodies which increase potential profitability. In this way, oyster mushroom cultivation is the most economical way to utilize agricultural by-products which would otherwise be burned or left to rot in the field and resulting in environmental pollution. Choice of substrate to use depends mostly on availability of the substrate within the neighborhood which cuts transport costs. A secure supply of readily available substrates allows for sustainable mushroom production. The spent mushroom cultivation media can also be used as compost or livestock feed. Oyster mushroom cultivation is therefore a good environmental conservation method. This research therefore aimed at evaluating the efficiency of different locally available substrates used for cultivating oyster mushroom in Malawi

Materials and Methods

Location

The experiment was conducted at the University of Malawi, in microbiology laboratory. A pure culture of Pleurotus Sajor caju was used to inoculate sterilized rice grain packed in glass bottles of 700 ml to produce planting spawn. The bottles were then covered with cotton wool followed by aluminum foil.

Collection and preparation of substrates

The selected substrates were collected from sites within Zomba district. The substrates included banana leaves, maize stalks, beans pods, rice straw, ground nut shells, pea pods and bamboo leaves. Maize stalks were collected from Chinamwali, banana leaves from Kalimbuka, beans pods from Thondwe, rice straw from Likangala, bamboo leaves from Chikanda, ground nut shells were collected from New Road and finally pea pods were collected from Mpondabwino. Rice straw, banana leaves and maize stalk substrates were cut into two-inch-long pieces, whilst the other substrates were not cut into pieces because they were of good size already and they shrink when wet. Substrates (banana leaves, maize stalk, rice straw, and ground nut shell) were prepared by soaking them overnight in water to allow the substrates to absorb enough moisture and also this enhanced the settlement of dust particles in the substrate. The other substrates like peas pod, beans pods and bamboo leaves never require overnight soaking, hence these were soaked few minutes prior to sterilization. Soaked substrate were sterilized in the autoclave at 121˚C for 15 minutes. After sterilization substrate were allowed to cool to around 24oC under clean and sterile conditions and left for 3 to 4 hours to drain excess water off.

Spawning of substrates

After colonization was complete in the bottles, the spawn was then inoculated in different substrates packed in small sized bags (35cm by 25cm). Spawning of the substrates was done in a strictly protected room to avoid contamination. The spawn was being put in the bags after each layer of 3 to 4 inch of substrate. Some space was left unfilled at the tip of the bag for easy tying. Each substrate was in triplicates.

Incubation

The inoculated substrates were then incubated in the dark. The incubation was done at room temperature. The bags under incubation were observed after every two days to see colonization rate in the different bags. After full colonization the bags were transferred into the fruiting house. Full colonization in this case means when the mycelia had grown throughout the substrate. The transferring of the bags was dependent on the bag that had fully been colonized. Bags not fully colonized were left still under incubation till they are fully colonized.

Management in the fruiting house

The bags in the mushroom house were then hung. Openings were then cut through the bags using sterilized razor blades to allow fruiting bodies to develop. By this far, the mushroom was now ready for fruiting. Temperature and humidity levels in the mushroom house were controlled as desirable by oyster mushroom growing. Temperature in the house ranged from 18oC to 23oC. Relative humidity in the house ranged from 72 to 88%. The floor in the house was always kept wet to maintain humidity levels in the house. The bags were also checked for contamination each day of entry into the house.

Harvesting

Mushrooms from the bags were maturing differently. Harvesting of mature mushroom was done with sterile hands as well as with sterilized razor blades. Hands and sterilized razor blades were used to harvest mature mushroom cluster from the bags. The number of mushrooms on each cluster harvested were counted and then weighed on the triple beam balance. From each cluster at least three mushroom caps diameters were measured. The sizes of the mushroom from different substrates were also compared. The results obtained were then recorded.

Data Collection Methods

In this research performance of mushroom growth in the mushroom house was being observed after every two days and data was recorded. Temperature and humidity levels inside the mushroom house were measured using thermometer and hygrometer respectively and recorded as well. Duration of fruiting was recorded along with the yield of mushroom from different substrates that was measured by using a calibrated scale.

Data Analysis

In this research statistical approaches were used to organize and analyze the data. Data was analyzed in Microsoft excel and presented in tables, bar graphs. Means and correlation coefficients were used in the analysis of this data. Number of mushrooms per cluster from each of the bags was counted and the whole cluster was then weighed on a scale. The masses were recorded in a table. Then average mass on each substrate was calculated and compared using statistical approaches.

Results and Discussions

Mushrooms have been used as food and medicine in many parts of the world since time immemorial. Although mushrooms are often grouped with vegetables and fruits, they are actually fungi. They are macro-fungi which belong either to Basidiomycetes or Ascomycetes and they are very distinct from plants, animals and bacteria [4]. Table 1 shows part of the progress of colonization in the bags observed during incubation. Colonization occurred in maize stalk substrate (MAS), rice straw substrate (RSS), banana leaves substrate (BAN), ground nuts substrate (GNS), and bamboo leaves substrate (BAM), beans pods substrate (BES) and pea pods substrate (PPS) in order of their colonization levels from highest to the least. Maize stalk substrate was found to be the fastest in colonization, seconded by rice straw, then banana leaves, ground nuts substrate, bamboo leaves, beans pod substrate and peas pod substrate respectively. One of the factors contributing to the variations in speed of colonization among the substrates was found to be moisture content [5]. It was observed that during incubation maize stalk, rice straw, ground nuts and bamboo substrates had relatively right moisture content conducive for mycelial growth. As such mycelia grew faster in them. Full colonization in these four substrates was observed within 20 to 30 days. However, mycelial growth in peas pod substrate and beans pods substrate was far slower so much that the spawn running took 44 days and 43 days respectively. The slower spawn running in these substrates was due to excessive moisture content. The main nutritional sources for oyster mushroom are cellulose, hemicellulose and lignin which are available in most agricultural waste [3]. Among mushrooms, Pleurotus (oyster mushroom) can make use of the largest variety of waste substrates with its fast mycelial growth and its multilateral enzyme system that can biodegrade nearly all types of available wastes. However, studies of efficiency of different oyster mushroom substrates have revealed that some substrates are fast colonized by mycelia and produces high yield. The previous studies have focused on the establishing the best technique to be used for mushroom cultivation. This has been either comparing growth of different varieties of mushrooms, the nutritional value of the different cultivated mushroom or indeed finding out the effects of fertilizers applied to the substrate on the yield. In Malawi most investigations have just been comparing two or three substrates like that done by Lupapa & Gama (1995).

Table 1: Showing Progress of substrates colonization.

Substrate type and colonisation (%)							
DAYS	MAS	RSS	BAN	GNS	BAM	BES	PPS
4	20	18	15	10	8	5	5
8	40	36	30	20	16	10	10
12	60	54	45	30	24	15	12
16	80	72	60	40	30	18	16
20	100	90	75	50	35	22	18

In the study done by [6] to find out cheaper means of producing planting spawn and also to improve the yield of oyster mushroom, they found that yields from maize stalks + maize husks followed by cotton waste and maize stalks only had significantly higher yields than the other substrate combinations. Similarly, in a study done by [7], they reported to have found banana leaves to be high yielding. In most of these studies some substrates have been found to better support the growth of oyster mushroom. This study evaluated the efficiency of different substrates used for cultivating oyster mushroom namely: maize stalks, rice straw, banana leaves, ground nuts shells, bamboo leaves, pea pods and beans pods. Mushrooms are valuable health foods which are low in calories and provide essential minerals. The growing interest in the cultivation of mushrooms can therefore help in solving many problems of global importance such as protein shortage as well as improving the health and well-being of people. Oyster mushroom, Pleurotus ostreatus is a common edible mushroom. It belongs to the class Basidiomycetes, subclass Hollobasidiomycetidae and order Agricales [5]. In nature, oyster mushrooms appear in cluster on dead trees from late fall to spring, and are distributed almost all around the world.

Table 2 shows the results obtained during the fruiting period of the mushroom under study. It can be observed that all the substrates tested had produced some mushroom. This indicates that all these substrates had the conditions favoring the production of mushroom. However, it can be observed that there is much variation in the figures of the yields from each substrate. Average mass was highest in Maize stalk substrate and the average diameter was highest in banana leaves substrate. This shows that different substrates contribute differently to the mushroom body parameters hence there is need for better choice of substrate when choosing to grow mushroom. Table 2 results revealed that the substrates that were used in this study had some potential of enabling mushroom growth regardless of the variations of the yield obtained from them. One of the factors leading to such variations could be attributed to the ability of the particular substrate to promote different enzyme's (cellulase(s), hemicellulase(s), and laccases) secretion which are important in the degradation of cellulose, hemicelluloses and lignin, respectively. Pleurotus species have extensive enzyme systems capable of utilizing complex organic compounds that occur as agriculture wastes. These mushrooms are also found to be one of the most efficient lignocelluloses solid state decomposing types of white rot fungi [8]. Thus, many agricultural and industrial wastes can be utilized as substrates for production of Pleurotus species.

Table 2: Showing results obtained during the mushroom fruiting period.

Substrate	Average mass (g)	Average cluster number	Average mushroom cap diameter (mm)
Maize stalks	103.8	6	53
Rice straw	147	4	52
Banana leaves	167.9	6	54
Ground nuts pods	152.3	10	48

Oyster mushrooms are the third largest cultivated mushroom in the world. It was first cultivated in Germany as a subsistence measure during World War I, and is now grown commercially around the world for food (www.botany.hawaii.ed/faculty/wong/BOT135/lect16). China, the world leader in Oyster production, contributes nearly 85% of the total world production of about a million tones. The other countries producing oyster mushrooms include Korea, Japan, Italy, Taiwan, Thailand, India and Philippines. The present production of this crop in India is only around 1500 tones due to low domestic demand. Cultivated mushrooms have now become popular all over the world. There are over 200 genera of macro fungi which contain species of use to people. Twelve species are commonly grown for food and/or medicinal purposes, across tropical and temperate zones, including the Common mushroom (Agaricus), Shiitake (Lentinus), Oyster (Pleurotus), Straw (Volvariella), Lion's Head or Pom Pom (Hericium), Ear (Auricularis), Ganoderma (Reishi), Maitake (Grifola rondosa), Winter (Flammulina), White jelly (Tremella), Nameko (Pholiota), and Shaggy Mane mushrooms (Coprinus). Commercial markets are dominated by Agaricus bisporus, Lentinula edodes and Pleurotus spp, which represent three quarters of mushrooms cultivated globally (Marshall & Nair, 2009). The growing of mushrooms, either for consumption, sale or processing into high value medicinal products, is being promoted in southern Africa by NEPAD. Working in six countries, the SANBio Mushroom project works with farmers, supplying mushroom seed (or 'spawn') and training in production and processing methods (http://www.agfax.net). In Malawi, farmers have been introduced to commercial production of oyster and button mushrooms, while Namibia is focusing on growing mushrooms for the herbal medicine market. The focus in Malawi is more on the production technology for smallholder farmers so that they can produce the mushrooms for consumption but also for sale. Almost all the mushroom cultivators in the country are growing Pleurotus ostreatus. This species is most preferred because of its easiness to cultivate using the low-cost cultivation method being practiced in the country. On average, the annual P. ostreatus production is estimated at 240 kg per grower [7]. Mushroom cultivators are selling their produce at prices ranging from MK800 (USD2.04) to MK2000 (USD5.10) per kg. However, mushroom cultivation is not that popular in Malawi. This may be, partly, attributed to lack of technical know-how and awareness on the economic, nutritive and medicinal benefits of cultivated mushrooms. The results in Figure 1 show that banana leaves had the highest average yield (167.9g) as compared to the rest of the average yields obtained from the other substrates. Second highest yield was that from ground nuts pods (152.3g), then rice straw (147.0g), then beans pods (122), then maize stalks (103.8g), then bamboo leaves (64.2g), and finally peas pods (63.8g) respectively. This revealed the potential of banana leaves as a good substrate for the cultivation of Pleurotus sajor caju mushroom. [9] in their study found out that banana leaves had 62.07% holocellulose and 18% lignin. So, the high yield in banana leaves in the present study can be attributed to the fact that the mushroom has the ability to degrade lignocellulosic materials in the substrate. This agrees with the report of Visser 1996 as cited by [9] who reported that the extracellular enzymes secreted by the fungus contain amorphous homo and heteropolysaccharides which help in the degradation of carbohydrates in the substrates. The higher hemicellulose content recorded for the fungus in banana leaves indicate that it is a valuable product for the lignin degrading fungus. According to a study done by Poppe (2004), maize straw has 48% cellulose, 16% lignin; banana leaves have 31% cellulose, 18% lignin; beans pods substrate has 33% cellulose, 45.5% hemicellulose and 17% lignin; and rice straw has 41% (others 37%) cellulose, 13(others 14%) lignin. Whilst Bai et al. (2013) reported that bamboo leaves have 39% cellulose, 21% hemicellulose and 23% lignin. Tagwira et al. 1999 as cited by [10] report that ground nuts have 65.7% cellulose and 21. 2% other carbohydrate components. Whilst peas pod substrate has 61.35% holocellulose and 22.12% lignin. Such variations in carbohydrate percentages will determine how much the fungus has as its food source for energy, hence resulting in differences in yield production in the substrates. The increased level of nutrients available in spawn would provide more energy for mycelia growth and development. Past studies showed that certain agro wastes proved to give higher yields than others. For example, maize straw substrate was found to be one of the high yielding substrates in the study done by [6]. However, in this study maize substrate was only found to be superior over the other substrates in colonization rates but not high yielding as well (Table 1 and Figure 1).

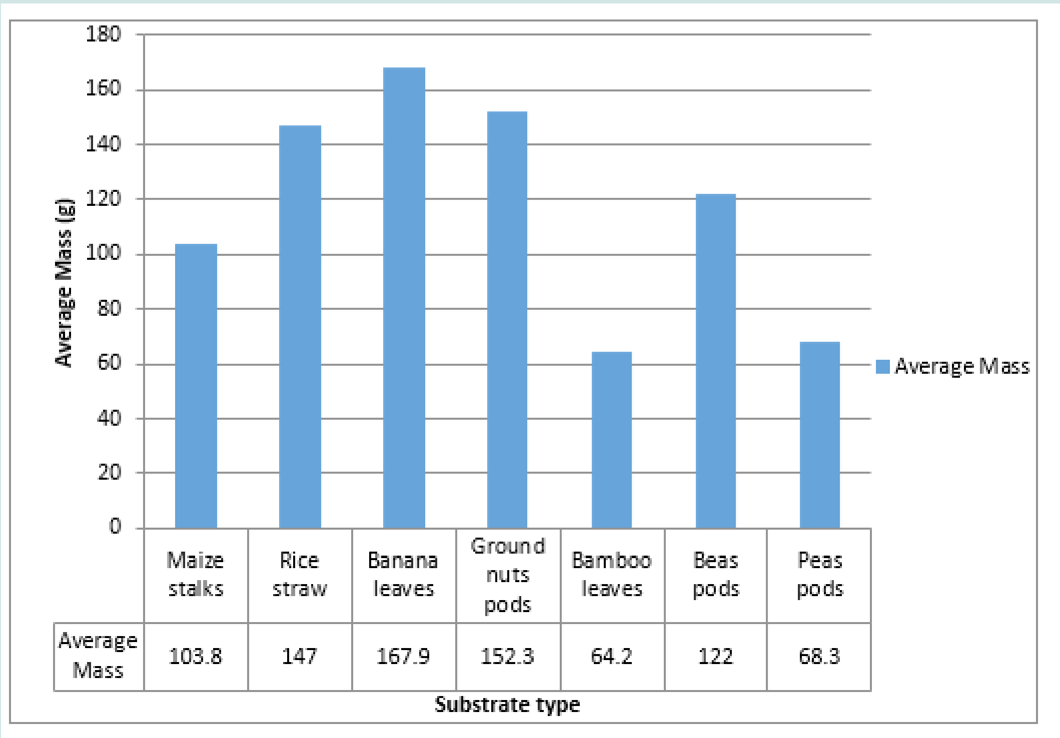

Figure 1: Shows the average mushroom yield (g) obtained from each substrate.

The variations in stipe length and mushroom cap diameter were observed in the substrates used in this study.

From Figure 2, it can be observed that the mushroom cap diameter ranged from 12mm to 54mm, peas pods substrate (Pisum sativum L) being far different from the rest of the substrates in that it had the smallest cap diameter but had longer stipe. [11] reported that smaller oyster mushroom cap diameter and longer mushroom stipe length often reveal poor conditions for mushroom growth and are undesirable characteristics as for market quality. The present study verifies this in that there was poor performance of mushroom observed in the pea pods substrates which had only one bag producing mushroom out of the three bags. Of course, peas pods substrate was found to have much moisture content which attributed to failure of mushroom to fruit in the other bags as well as poor performance in the bag which produced mushroom [12-18]. In the present study, it was found that banana substrates produced mushroom of relatively larger mushroom cap diameter than the rest of the substrates. The correlation coefficient for yield (weight) and mushroom cap diameter was found to be 0.678. This shows that there is a strong positive correlation between yield and mushroom cap diameter as shown also in Figure 2.

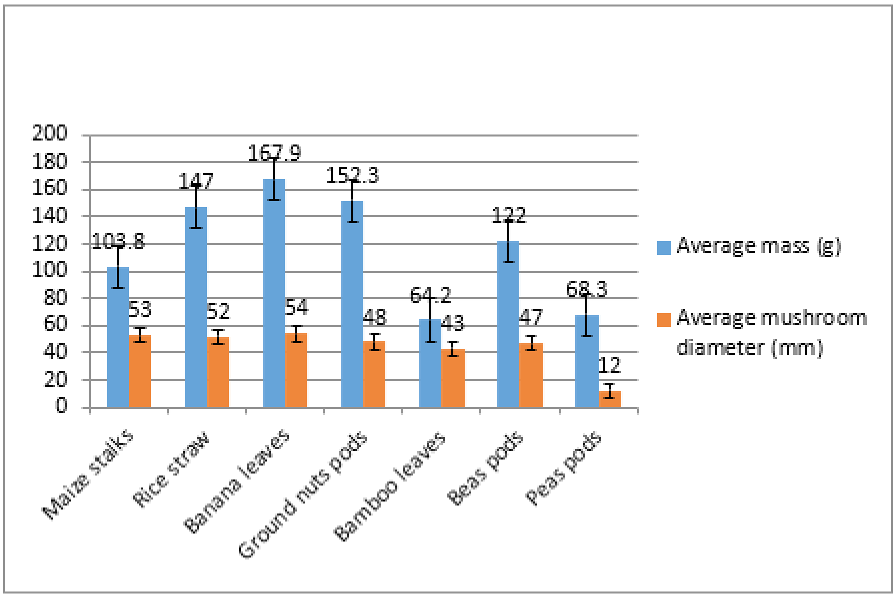

Figure 2: shows average mass (g) and average diameter (mm) against substrate.

Conclusion

The study has revealed that oyster mushroom can grow in most of the agro-wastes that are normally rendered useless after harvest. All the substrates tested in this study had produced some mushroom. Banana leaves substrate has proved to be the best and highest yielding in this study, whilst peas pod substrate has proved to be lowest yielding. However, given right conditions, oyster mushroom can perform better on the other substrates as well. The study has revealed that much interference in mushroom cultivation come as a result of poor conditions. For instance, peas pod substrate can produce more mushroom as per the results of the single bag of peas pod substrate obtained in this study if given right moisture content. The study has also exposed other substrates for oyster mushroom cultivation that were not being used. This ensures an all-season mushroom cultivation. Oyster farmers can therefore switch to different substrates depending on availability within vicinity.

Recommendations

Whilst these results may appear to be confounding, they demonstrate that other factors beyond the scope of this study should come into consideration. These factors include availability of nutrients in the substrates, rather than the total content, room size, room design and season.

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