DOI: https://doi.org/10.36036/MR.32.1.2023.136300
***Mushroom Research* 32 (1) : 35-40, 2023**

Mycelial growth of oyster mushroom in spawn made using different cereal grains, sawdust and vermiculite as substrate

Bonaventure Ufitinema[1*], Modeste Hakizimana[1, 2] and Jean De Dieu Niyomugabo[1]

[1]Department of Agricultural Engineering, Rwanda Polytechnic- IPRC Karongi,P.Box. 85 Karongi, Rwanda,
[2]Department of Crops, Horticulture and Soils, Egerton University, P.O. Box 536-20115, Nakuru, Kenya
**Corresponding author,* E-mail: ufbona@gmail.com

ABSTRACT

Lack of sufficient and high-quality spawn is the problem affecting mushroom production. Mushroom spawn can be made using different substrates. To identify suitable substrate for oyster mushroom spawn production, study on mycelial growth on different substrates in spawn bottles was undertaken. The experiment was conducted in Completely Randomized Design (CRD) with 6 different substrates (wheat, maize/popcorn, rice and millet grains, sawdust and vermiculite). Data were recorded after every 2 days of inoculation until full colonization of the substrates. The results showed that there were significant differences (Pd" 0.05) in mycelial growth on cereal grains (rice, millet, maize and wheat), sawdust, and vermiculite as observed on 2, 4, 6, 8, 10, 12 and 14 days after inoculation. The results indicated that shortest time for full colonization was taken in maize (~9.4 days) while rice was the second (10.1 days) followed by wheat (13.1 days), while vermiculite took 15 days for full colonization. Therefore, maize and rice grains can be recommended for preparation of oyster mushroom spawn.

Key words: Mushroom, spawn, grains, sawdust, vermiculite, mycelium, growth rate

Spawn is pure mushroom mycelia (the vegetative part of the fungus) grown on a sterilized grain or other medium (Maheshwari, 2013). One of the primary requirements in mushroom farming is the availability of high-quality mushroom spawn. Spawn preparation needs a pure mycelium culture with high vigour. Pure culture can be raised either by spore culture and mycelium culture method, which are suitable from the viewpoint of quality and quantity for preparation and propagation of vigourous pure stocks (Ukoima *et al*., 2009). Mycelium culture is the mostly used technique in spawn production (Kashi, 1996). Mushroom spawn is prepared in a sterilized medium with a substrate to grow around (Dulay *et al*., 2017).

Cereal grains such as maize, millet and rye are used to produce spawn due to their faster ramification of the substrate and their ease of planting (Stanley and Awi-Waadu, 2010). The first step in the production of the spawn is the culturing of the mushroom mycelia on nitrified agar media (Miah *et al*., 2017). Different cereal grains can be used as substrate for spawn production. The shelf life of grain spawn is considered better than sawdust or other materials while some types of grains support better mycelia growth of mushrooms (Mottaghi, 2004). Nwanze *et al*. (2005) showed that in *Lentinus squarrosulus* cultivation, corn spawn induced the highest yield and dry weight of fruiting compared to

Recieved on 12-05-2023; Accepted on 31-07-2023
https://epubs.icar.org.in/index.php/MR/article/view/136300

sorghum and wheat spawn. The environmental factors such as temperature, light, oxygen, carbon dioxide, humidity and pH have also been reported to affect mycelial growth during spawn preparation. In addition, many others factors such as nutrients, mushroom cultivar and sanitation also determine mycelial growth in spawn (Royse, 2014). Nutrient and environmental factors such as nitrogen content, moisture, lipid content, temperature, humidity and salt concentration affect the quality and the spawn running period (Wuest and Bengston, 1982; Royse, 2014). The inoculated bags must be kept in the semi-dark or dark until the mycelium completely colonizes the substrate (Horn, 2004).

Considering the current growth of the mushroom sector to address the problem of nutritional security worldwide, the production of quality mushroom planting materials (spawn) is the one of the key factor. The objective of this study was to evaluate different substrates like wheat, maize, rice, and millet grains, sawdust and vermiculite for oyster spawn production.

MATERIALS AND METHODS

Media preparation and oyster mother culture preparation

Culture media was prepared according to Miah *et al.* (2017). Readymade Potato Dextrose Agar (PDA) powder (39 g) was added into 1 litre flask containing distilled water and then boiled. After that the flask containing PDA media was plugged and autoclaved at 121°C for 15 minutes. The media was aseptically poured (before laminar flow) into sterile disposable Petri plates and quickly covered using Petri plate lids and was allowed to cool and solidify.

A fresh healthy oyster mushroom was selected and a small tissue section (about 2×2 mm) was cut using sterile surgical blade from the inner surface of the cap (often between the stipe and cap joint) and cleaned with 70% ethanol. Cut fragments were placed in the middle surface of the media, then covered with a plate lid, labelled and tightly sealed with a parafilm. Cultured plates were incubated in dark sterile cabinets at 25°C to enable mycelia establishment. The freshly growing mycelia in Petri plates were sub-cultured to obtain pure culture.

Spawn production

All the grains were ordered through from a local grower and the sawdust was collected from a saw miller while vermiculite was bought from a shop in Nakuru, Kenya. The grains were cleaned to remove any broken, shrivelled grains by handpicking undesired grains. After this, the grains were soaked overnight in clean water and then washed. They were boiled for 15 minutes taking care that grains should not split but remain slightly hard after boiling. The boiled grains were spread in a thin layer over a wire net to remove excessive water and enable them to cool to 27°C. The cooled grains were then mixed with 1.2 % by weight dry grains of commercial-grade gypsum ($CaSO_4 \cdot 2H_2O$.) and 0.3 % of calcium carbonate ($CaCO_3$). Gypsum prevents the sticking of grains together and calcium carbonate maintains the pH. The grains were filled in a clean mayonnaise bottle up to 2/3 third of its capacity. The bottles were plugged with non-absorbent cotton and covered with aluminium foil. These bottles were then sterilized at 121°C at 15 psi (103,435 Pa) for 2 hours on two consecutive days. Sterilized bottles were taken out from the autoclave, while still hot and were shaken to avoid clumping of grains and then allowed to cool for at least 3.5 hours before inoculation.

Colonized PDA medium in the Petri plates was cut into small sections which were used to inoculate the grains in mayonnaise bottles. The sections from one Petri plate were used to inoculate 3 bottles of grains. The inoculated grains were incubated at approximately 25°C and shaken frequently to prevent aggregation. The spawn was ready for use after 10-14 days depending on the type of substrate.

Mycelium running rate (MRR) for each type of grain was measured at days 2,4, 6, 8, 10 and 14 after inoculation. Inoculation was done by laying 4 small portions of mother culture on the top of substrate (cereal grains, vermiculite and sawdust) and downward mycelial growth was marked and measured at 2 day intervals. The mycelium running rate was then calculated (Stanley & Awi-Waadu, 2010). The number of days at which the mycelia fully colonized the bottles after the day of spawning was recorded.

Before analysis, Shapiro Wilk test and Probability Plot at 5% were conducted in SAS Software 9.4 M6 (SAS Institute Inc, Cary, NC 2017) to test the normality of the data. Collected data were checked for the assumption of equal variances (homogeneity) using residual plots.

Analysis of variance (ANOVA) was conducted using proc GLM for the following statistical model. Mean separation was done using Tukey's test at 95% confidence interval.

$$Y_{ij} = \mu + R_i + T_j + \varepsilon_{ij}$$

Where: Y_{ij}= Overall observations, μ= Overall mean, R_i= Replication of treatment,Tj= effect due to j^{th} treatment and ε_{ij}= Random error.

RESULTS

Mycelial growth of oyster mushroom on cereal grains, sawdust and vermiculite substrates on different days are presented in Table 1. The result showed that there was a significant ($P \leq 0.05$) difference among the grains, vermiculite and sawdust substrates. These substrates significantly ($P \leq 0.01$) influenced the mycelium running rate (growth) as observed on day 2, 4, 6, 8, 10, 12 and 14 days after inoculation.

There were no significant differences in the mycelial growth in maize (popcorn) and rice after two days and also on other days till complete colonization (Table 1). Similarly, wheat, millet and sawdust were not significantly different from each other on 2 days after inoculation (0.57cm, 0.51cm and 0.49 cm), respectively (Table 1). Vermiculite, popcorn and wheat were significantly different from each other. After two days, the highest rate of mycelium extension was obtained on popcorn grain (0.92cm) whereas the least were found on vermiculite substrate (0.31cm).

On the 4^{th} day after inoculation, the maximum and minimum mycelium running rate was observed on popcorn and millet grains substrates with 4.35 and 1.54cm, respectively. Mean separation showed that

Table 1. Effect of substrates on mycelia running rate

	Mean mycelial growth (in cm) after					
Substrates	**day2**	**day4**	**day6**	**day8**	**day10**	**day12**
Popcorn	0.92a	4.35a	8.12a	9.94a	10a	10a
Rice	0.88a	4.27a	8.07a	9.47a	10a	10a
Sawdust	0.49b	3.24b	5.96b	7.8b	8.83b	9.8ab
Vermiculite	0.31c	3.47b	6.17b	7.39b	8.31c	9.37c
Wheat	0.57b	1.68c	3.08c	5.47c	7.55d	9.59bc
Millet	0.51b	1.54c	3.01c	5.21c	7.37d	9.30c

The means followed by the same letters are not significantly different using Tukeys' honest significant difference (HSD) test at 5% level of significance.

popcorn, vermiculite and wheat were significantly different from each other while popcorn was not significantly different from rice. Similarly, vermiculite & sawdust, and wheat & millet were not significantly different from each other. Similar trend was there after 6 and 8 days.

On the 10th day, popcorn and rice substrates were already fully colonized by the mycelium, whereas sawdust and vermiculite were about three- quarter of the bottles (8.83 cm and 8.31 cm of the length of mycelium, respectively). On the 12th day, all the substrates were about to complete the colonization. On the last day of data collection (14th day) vermiculite was still not fully colonized (9.7 cm), whereas all other substrates were fully colonized.

Among all treatments used in the study, the results indicated that popcorn took the shortest period with 9.42 days to get fully colonized by mycelium. Rice was the second followed by wheat with 10.33 and 13.17 days, respectively, while vermiculite and sawdust were the last to get fully colonized, with 15.23 and 13.40 days, respectively. Even though all the substrates differed significantly for total days for colonization, sawdust, millet and wheat were not significantly different from each other. Additionally, rice, popcorn, wheat and vermiculite were significantly different from each other (Fig 1).

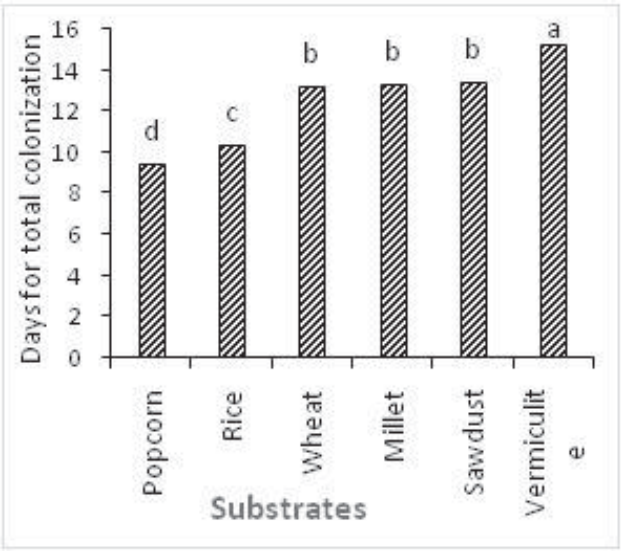

Fig 1. Days taken for complete colonization of Millet, Rice, Wheat and Popcorn grains, sawdust and vermiculite substrates by mycelium. The means followed by the same letters are not significantly different using Tukeys' honest significant difference (HSD) test at 5% level of significance

DISCUSSION

There was variation in the spawn running rate on the grains, sawdust and vermiculite. This indicates that mycelium growth is affected by different nutritional sources. Popcorn grains had the fastest running rate and vigorously growing mycelium after the inoculation.

This is in agreement with the study done by Stanley and Awi-Waadu (2010) who reported that the mycelia growth of *P. tuber-regium* and *P. pulmoniarius* were faster on white popcorn followed by red sorghum and millet that were used as substrates. Elhami and Ansari (2008), Sofi *et al*. (2014) demonstrated that among various substrates they used, maximum and minimum growth rate of *Pleurotus ostreatus* was recorded for popcorn and millet. Adebayo *et al*. (2014) also reported that the highest ramification rate and spawn productivity were obtained in white popcorn with a ramification rate of 0.378 cm/day followed by millet with a ramification rate of 0.31 cm/day, while mustard seeds had the lowest ramification rate of 0.056 cm/day. Sahu *et al*. (2014) also found that the minimum days required for spawn development of *P. flabellatus* in sorghum grains, popcorn and wheat were 7.75 days, 9days and 12.5 days respectively. Cereal grains are generally used as spawn substrates more than other materials such as sawdust and vermiculite. They contain enough carbohydrates which provide sufficient nutrition for mycelia growth (Ibekwe et al., 2008).

Smaller particles such as vermiculite, fine sawdust and millet grains are generally more compact than larger particles such as popcorn, rice and wheat grains which influence air circulation within the substrates. Popcorn substrate contains larger air spaces than rice,

wheat, millet, sawdust and vermiculite substrates. This increases ventilation within the popcorn and rice treatments resulting in improved respiration by the mycelia. Respiration rate is related to oxygen concentration of substrate (Sofi *et al*., 2014). Hence, the significantly higher growth rate of mycelia in the popcorn treatment compared to the other treatments. The Popcorn grains are larger and contain carbohydrates which play a key role in food reserves for mycelium growth (Dahlberg and Stenlid, 1990).

The period required for total colonization would be decreased significantly if the bottles were shaken after some days of inoculation, shaking break down the mycelia into fragments which disperse all over within the bottle and create various points of growth of the mycelia within the substrate, resulting in full substrate colonization within a shorter period (Scrase, 1995).The duration of the full substrate colonization phase has direct economic and yield importance in mushroom growth and production (Hultberg and Golovko, 2020). Popcorn substrate took lesser and approximately 9.4 days to be fully colonized by the mycelium. Rice was the second for the mycelium to fully colonize. Thus these are suitable substrates for spawn production of oyster mushroom.

DECLARATION OF COMPETING INTEREST

The authors have no competing interests to declare.

ACKNOWLEDGMENTS

The authors are grateful for the funding by World Bank through Centre of Excellent for Sustainable Agriculture and Agribusiness Management (CESAAM) at Egerton University.

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