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THE EFFECT OF THE INTERACTION OF VARIOUS SPAWN GRAINS AND OIL TYPES ON CARPOPHORE DRY WEIGHT, STIPE LENGTH AND STIPE AND PILEUS DIAMETERS OF *LENTINUS SQUARROSULUS* (MONT.) SINGER

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ABSTRACT

Lentinus squarrosulus, an indigenous Nigerian mushroom species, was cultured on six different media that were inoculated separately with three different spawn grains and amended with six different oils at five different rates. The results revealed that the interaction of the different spawn grains with the various oil types produced a highly significant effect ($p<0.01$) on the stipe length, dry weight, and stipe and pileus diameters of *Lentinus squarrosulus*. The interactions of corn x coconut and corn x butterfat, respectively produced stipe lengths that were statistically longer than the comparable stipe lengths produced by the interaction of corn x groundnut and wheat x cotton, respectively. The shortest stipe lengths were, however, produced by the interaction of palm kernel oil with wheat and corn grains, respectively. The interaction of millet x groundnut produced stipe diameters, which were statistically wider than those produced by the interaction of corn x coconut that was statistically wider than the comparable diameters induced by the interaction of wheat x cotton and corn x butterfat, respectively. The poorest stipe diameters were produced by the interaction of wheat x palm kernel, wheat x palm, and corn x palm kernel oil. The carpophore dry weight produced by the interaction of corn x coconut was statistically heavier than the comparable dry weights produced by the interaction of corn x groundnut, corn x butterfat, and millet x palm oil. The comparable mean carpophore dry weights produced by the interaction of wheat x coconut, millet x coconut, wheat x cotton, millet x cotton, and wheat x groundnut oil, respectively were statistically heavier than the mean dry weights produced by the interaction of wheat x palm kernel, which was the poorest. The mean pileus diameters produced by the interaction of corn x coconut was statistically comparable to those produced by the interaction of wheat x coconut and corn x butterfat, but statistically heavier than the comparable mean diameters produced by the interaction of wheat x cotton and corn x groundnut oil. The comparable pileus diameters induced by the interaction of palm kernel oil with wheat and corn grain, respectively produced the poorest results. The current findings illustrate the importance of oil amendment in mushroom culture.

Key words: *Lentinus squarrosulus*, spawn grain, carpophore production.

INTRODUCTION

The culturing of mushrooms is environmentally friendly. The problem of air pollution may be avoided by using mushrooms to bioprocess the lignocellulosic waste materials, which may later be used as highly proteinaceous feed for livestock [1]. The mushrooms that are harvested have a high commercial and nutritive value and may be consumed by the grower or sold in the open market for profit [2].

Optimization of industrial mushroom production depends on improving the culture process [3]. A range of parameters including temperature, light, carbon dioxide concentration, humidity and pH have been shown to influence carpophore production [4]. Fruiting may also be stimulated by mechanical injury and chemical treatments [5].

There are also various additives that are known to stimulate fruiting. They include rice bran, cassava peels, carbohydrates such as glycogen, natural extracts like yeast and malt extract, as well as cell-free extracts [6]. Highly proteinaceous materials such as ground pigeon pea and soybean have been reported to stimulate high fruit yield. Wheat, rye and millet that are used in making spawn also belong to this genre [7]. In addition, lipids such as crude and refined vegetable oils, as well as fish oil may also be used to stimulate fruiting [8,9,10].

The effect of factors such as spawn grain, culture medium, oil type and rate on the culture of *Psathyrella atroumbonata* and *Lentinus squarrosulus* has been reported [11,12,13]. The current investigation is interested in the interaction of two of the factors, spawn grains and oil type, on the culture of *L. squarrosulus.*

MATERIALS AND METHODS

The effect of various spawn grains, culture media, oil types and rates on carpophore production of *Lentinus squarrosulus*

Various non-composted media including sawdust [14], animal bedding and rice, formulated [15, 16] and lime were used for these studies. To distinguish among three lime media, they were arbitrarily named as lime 1, lime 2 and lime 3 [17, 18] (Table 1).These six different media were supplemented with different rates (0.007, 0.014, 0.021 and 0.028 ml/g) of different lipid sources for example groundnut, coconut, palm kernel, butterfat, palm and cotton oils, respectively in order to study the effect of lipids on carpophore production. Two hundred and fifty gram of dry substrate from each of the above six different supplemented and non-supplemented media were placed in separate polypropylene heat resistant bags [19]. After thoroughly wetting the substrates, the bags were autoclaved for 15 minutes at 121°C and allowed to cool [20]. The substrates were then separately inoculated with 10 g (4% on dry weight basis) of three different types of spawn separately (wheat, corn and millet) [21]. All the bags were incubated in total darkness at 30 $\pm$ 2°C for three weeks after which the bags were aerated and exposed to light [19,22].

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Experimental design

The experiment was conducted in a split-split plot design, triplicated with medium as the main plot, oil type and rate as the sub-plot and spawn grain as the sub-sub-plot treatment [23]. The fruiting bodies from different flushes (1-3) in the different experiments were collected and the pileus and stipe diameters as well as the stipe lengths measured [20,24]. In addition, fresh and dry weights were also taken [25].

Statistics

In order to test the main and interactive effects of spawn grain, medium, oil type and rate of amendment, pileus and stipe diameter, stipe length and wet and dry weights of fruiting bodies were recorded and the data subjected to factorial analysis of variance [26]. When significant differences were determined for the main effects or their interactions (a p value of 0.05 or less), comparisons among means were made using Duncan's multiple range test [27]. The values 0.01, 0.1 and 1.0 were added to dry weights, stipe and pileus diameters and wet weight and stipe length values, respectively prior to analysis [28].

Spawn preparation

Three different types of grain, including corn, wheat and millet were used to produce spawn in order to determine which spawn produces the best crop yield. The spawns were prepared as shown in Table 2 and kept inside a water bath at 37°C and 70% relative humidity for two weeks in order for the spawn to run [29].

RESULTS

Spawn grain x oil type interaction

Mean stipe length, stipe and pileus diameter and carpophore dry weight of *L. squarrosulus* as affected by the interaction of spawn grain and oil type is shown in Table 3. Analysis of the data showed that wheat spawn interacted with cotton oil to produce a mean stipe length that was statistically longer than the one induced by coconut, which was significantly longer than that of groundnut. The interaction of wheat spawn with groundnut oil induced a mean stipe length that was longer than the comparable stipe lengths it induced with butterfat and palm, which were superior to palm kernel oil. Corn spawn induced similar mean stipe diameters in coconut and butterfat that were significantly longer than that of groundnut, which was statistically longer than the comparable stipe lengths induced with cotton and palm oils, respectively. The interaction of millet spawn with groundnut oil induced a stipe length that was longer than the similar stipe lengths it induced with coconut, cotton, butterfat or palm kernel, which were statistically longer than that of palm oil.

Wheat spawn grain interacted with the various oils to induce mean stipe diameters in the order of cotton or coconut, groundnut, butterfat, palm or palm kernel oil. However, corn spawn induced mean stipe diameters in the order of coconut, butterfat, groundnut, palm, cotton and palm kernel oil, respectively. The interaction of millet spawn with groundnut oil produced a mean stipe diameter that was statistically wider than the one induced by its interaction with butterfat, which was superior to the stipe

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diameter produced by it's interaction with coconut, cotton, palm kernel or palm oil, which were at par.

The interaction of wheat and millet spawn, respectively with the various oils produced similar mean carpophore dry weights. In contrast, the interaction of corn spawn with coconut oil produced a mean dry weight, which was statistically heavier than the ones produced by its interaction with groundnut and butterfat that were at par, but nonetheless statistically heavier than the comparable weights induced by cotton, palm kernel or palm oil.

The interaction of wheat spawn with coconut and cotton oils, respectively produced mean pileus diameters which were at par, but statistically wider than the one induced by its interaction with groundnut oil, which was superior to the comparable mean diameters induced by butterfat and palm oil. The interaction of corn spawn grain with coconut, groundnut or butterfat induced pileus diameters that were at par, but statistically wider than the comparable mean diameters induced with cotton and palm oil. In contrast, the interaction of millet spawn grain with coconut and groundnut oils, respectively produced mean pileus diameters that were at par, but statistically wider than the comparable diameters induced by its interaction with butterfat, palm kernel or palm oil.

DISCUSSION

Spawn grain x oil type interaction

It has been observed that the interaction of corn spawn x coconut oil produces the widest pileus diameters in *Psathyrella atroumbonata* as well as *L. squarrosulus* [30]. The same interaction induces the heaviest carpophore wet weight in *P. atroumbonata*, but the heaviest carpophore dry weight in *L. squarrosulus*. Although the interaction induces the widest stipe diameter in *P. atroumbonata*, it is the interaction of millet spawn x groundnut oil that produces the best result with *L. squarrosulus*. The interaction of spawn grain x oil type did not produce any significant effects on the stipe length of *P. atroumbonata*, but the interaction of corn spawn x butterfat induced the longest stipe lengths in *L. squarrosulus* [30]. Corn is thus the grain of choice to interact with coconut oil in order to produce the longest stipe length, heaviest dry weight and widest pileus diameter of *L. squarrosulus*. These results agree with the finding that corn induced better yields of *A. bisporus* than rye or wheat [9]. The effectiveness of the oils in carpophore production, however, is due to their high contents of fatty acids such as lauric and myristic acids [11].

CONCLUSION

The results thus far obtained reveal that *L. squarrosulus* can easily be cultured on readily available lignocellulosic wastes inoculated with various spawn grains and amended with different locally sourced lipids. Attempts should be made to explore this specie commercially in Nigeria.

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Table 1: Different carpophore production media

Media	Components	Method of preparation
Sawdust	62.5g sawdust 62.5g wood chips 125.0g brown rice	All the components were thoroughly mixed, moistened and sterilized for 15 minutes at 121°C
Animal bedding and rice	125.0g wood chips 125.0g brown rice	Same as above
Lime 1	195.0g sawdust 50.0g rice bran 2.5g $CaSO_4$ 2.5g $CaCO_3$	Same as above
Lime 2	235.0g sawdust 10.0g rice bran 2.5g corn meal 2.5g $CaCO_3$	Same as above
Lime 3	182.5g sawdust 62.5g corn cobs 5.0g $CaCO_3$	Same as above
Formulated	175.0g sawdust 70.0g rice bran 2.5g $CaCO_3$ 2.5g oatmeal	Same as above

Table 2: Spawn preparation

Spawn	Components	Method of preparation
Wheat	1.0kg wheat grains 12.0g $CaSO_4 \cdot 2H_2O$ 3.0g $CaCO_3$ 1.5 litre distilled water	1.0kg of wheat grains was boiled in 1.5 litre of water for 15 minutes and left to cool for an additional 15 minutes. The water was poured off and 900.0g of the cooked grains was mixed with 12.0g gypsum and 3.0g $CaCO_3$. The grains were then filled into bottles and sterilized for 20 minutes at 121°C. After cooling, the bottles were inoculated with pieces of agar medium colonized with mycelium and incubated for 2 weeks in total darkness.
Corn	Same as above except for use of corn as grain	Same as above
Millet	Same as above except for the use of millet as grain	Same as above except that the grains were boiled for 5 minutes

Table 3: Stipe length (cm), stipe diameter (cm), dry weight (g) and pileus diameter (cm) of *Lentinus squarrosulus* as affected by the interaction of spawn grain and oil type

Treatments	Oil					
Stipe length	Coconut	Cotton	Groundnut	Butterfat	Palm kernel	Palm
Grain						
Wheat	4.13cd	4.53b	3.88e	3.34g	2.62h	3.12g
Corn	5.06a	3.77e	4.48b	5.04a	2.52h	3.70e
Millet	3.94de	3.71e	4.18c	3.73e	3.71e	3.32g
SE± 0.079						
Stipe diameter						
Grain						
Wheat	0.31de	0.32cd	0.28gh	0.26i	0.22k	0.23jk
Corn	0.35b	0.24j	0.30ef	0.33c	0.19l	0.26i
Millet	0.27hi	0.27hi	0.49a	0.29fg	0.26i	0.26i
SE± 0.006						
Dry weight						
Grain						
Wheat	0.19bcd	0.18bcd	0.17bcd	0.14cde	0.06f	0.11def
Corn	0.51a	0.15cde	0.24b	0.23b	0.09ef	0.15cde
Millet	0.17bcd	0.18bcd	0.20bc	0.19bcd	0.14cde	0.20bc
SE± 0.024						
Pileus diameter						
Grain						
Wheat	2.66ab	2.58b	2.04cd	1.69fg	1.20h	1.71efg
Corn	2.81a	1.57g	2.50b	2.69ab	1.06h	1.64fg
Millet	2.18c	1.81ef	2.15c	1.92de	1.92de	1.91de
SE± 0.069						

Means followed by the same letter(s) within the same row or column in a treatment group are not significantly different statistically at 5% level of probability using DMRT.

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