bottle + spawn grains + weight of fresh mycelia= (X+Y+Z) g; and fresh mycelial weight = (X+Y+Z)-(X+Y) g = Z g.

Percent mycelium coverage 20 DAI was scored visually by three data collectors and the average of the three was taken as final score for each of treatment replicates.

2.5 Data analysis

CRD factorial data analyses had been employed and the data analysis was performed using SAS version 9 statistical software package. Mean comparison among treatment groups was also conducted using least significant difference (LSD) method and differences between means at p value ≤ 0.05 were considered as significant.

3. Results

Based on the previous 5 years experience of the lead investigator in spawn making and available literature references, five spawn substrate media combinations had been tested to study the effect of the media mixes on fresh mycelial weight change of *P. ostreatus* 5, 10, 15, and 20 DAI and the percent mycelium coverage (PMC) at the end of incubation period; 20 DAI.

Urea substrate additive increased mycelial fresh weight gain

The result of the study indicated that the sorghum base substrate supplemented with wheat bran, $CaCO_3$, $CaSO_4$ and urea significantly ($P < 0.05$) increased the mean mycelial fresh weight gain (-2.375 ± 0.506 g) compared to other treatment groups (Table 2) and thus, this spawn substrate was found to be the most favorable media for mycelial weight gain and expansion of *P. ostreatus*. However, further supplementation of this substrate media with sucrose and citric acid did not affect the mean mycelium weight gain of *P. ostreatus.*

Table 2: Effects of substrates on *P. ostreatus* spawn mycelial weight gain

Trt	pH	Mean ± SE
1	5.63	0.450 ± 0.506^{a}
2	6.19	-0.800 ± 0.506^{a}
3	5.39	-0.383 ± 0.506^{a}
4	6.21	-2.375 ± 0.506^{b}
5	6.09	0.008 ± 0.506^{a}

Means with different superscripts are significantly ($P < 0.05$) different.

Substrate weight loss across incubation period explains mycelial weight gain

To understand whether the incubation period influences the mycelial establishment or not, the mycelia fresh weight change among treatments was evaluated at 5, 10, 15 and 20 DAI. The result has shown that with the exception of treatment 4 (sorghum + wheat bran + $CaCO_3$ + $CaSO_4$ + urea), all treatments showed weight gain during the first 10 days of incubation. The highest mean weight gain was observed during the initial period of mycelial establishment; 5 DAI in treatment 5 (sorghum + wheat bran + $CaCO_3$ + $CaSO_4$) followed by treatment 1 (sorghum + wheat bran + $CaCO_3$ + $CaSO_4$ + urea + sucrose + citric acid) and 3 (sorghum + wheat bran + $CaCO_3$ + $CaSO_4$ + urea + citric acid). As the time of incubation period increases, decrease in weight of colonized substrate media became evident and mycelial growth further continued with a negative weight gain 15 & 20 DAI across treatments. At 15 DAI, the highest weight loss was observed in treatment 4 followed by treatment 2 and 5 (Fig. 1). Furthermore, the weight loss increasingly continued across treatments and at the end of the incubation period (20 DAI) the highest weight loss was also observed in treatment 4. This significant weight loss difference may indirectly explain the use of citric acid in treatment 1 and 3 and the addition of sucrose in treatment 1 and 2 had no significant effect on mycelial proliferation during the incubation periods. On the other hand, the use of urea as nitrogen source additive in treatment 4 (a treatment without citric acid and sucrose) showed significant difference at $P < 0.05$ when compared with the change observed in the remaining treatments.

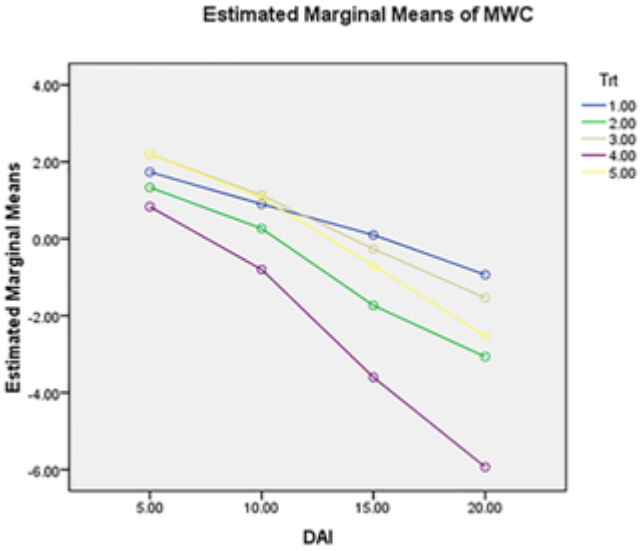

Figure 1: Mean mycelial weight change 5, 10, 15, and 20 DAI

The figure indicating weight change differences among treatments across the incubation period. In addition to analyzing the effect of incubation period on mycelial weight gain in each media combination, the data obtained in each incubation period was merged irrespective of the media used. The analysis has revealed the presence of significant difference in mycelia fresh weight during 5, 10, 15, and 20 DAI (Table 3). During the first 10 days of incubation, though the trend was decreasing, the mycelial weight gain was showed to be positive. However, during 15 and 20 DAI, the trend of weight gain was negative, with the highest weight reduction recorded -2.800 ± 0.452 g 20 DAI and -1.240 ± 0.452 g 15 DAI (Table 3).

Table 3: Effects of days after inoculation on *P. ostreatus* spawn mycelial weight gain

Days after inoculation (DAI)	Mean ± SE
5 DAI	1.660 ± 0.452^{a}
10 DAI	0.513 ± 0.452^{b}
15 DAI	-1.240 ± 0.452^{c}
20 DAI	-2.800 ± 0.452^{d}

Means with different superscripts are significantly ($P < 0.05$) different.

PMC is affected by substrate combination

Apart from analyzing the quantitative data on mycelial weight change, qualitative analysis indicating the live mycelial expansion had also been recorded. Likewise, percent colonization of the substrate with expanding mycelium was found to be in support of the quantitative data obtained through weight measurement during the experimental period (Fig. 2 and 3).

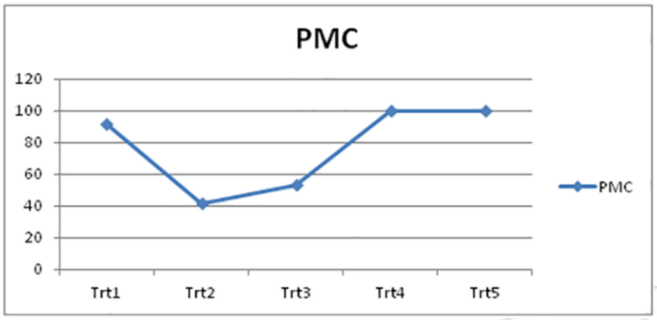

Figure 2: Line chart indicating mean percentage mycelial coverage 20 DAI

The line chart indicates, the highest coverage of mycelial colony, 100%, was observed in both treatment 4 & 5 followed by treatment 1 (91.67%). The lowest coverage was observed in treatment 2 (41.67%) followed by treatment 3 (53.33%), indicating sucrose and citric acid had no effect on mycelial expansion.

Furthermore, as indicated in Table 4, there was significant difference among treatment means in percent mycelia coverage 20 DAI at $P < 0.05$.

Table 4: Effect of various substrate combinations on PMC 20 DAI

Trt	Mean %age
1	91.67^{ba}
2	41.67^{b}
3	53.33^{ba}
4	100.00^{a}
5	100.00^{a}

Means with different superscript are significantly ($P < 0.05$) different.

Figure 3: Mycelial coverage 20 DAI

Figure 3 indicates from left to right in order of their arrangement the extent of mycelium coverage 20 DAI in treatment 1, 2, 3, 4, and 5. The picture for treatment 4 appears to be more vigorous and covered with white bright mass of mycelium suggesting the noticeable quality difference between treatment 4 and 5 regardless of the equivalent score they share in PMC.

4. Discussion

In spawn production, mycelia growth, rate and extent of mycelia expansion are affected by the type of base substrate, substrate combination and proportion of additives supplemented. This is supported by studies demonstrating the existing variation in mycelium proliferation among cereal grain types [12,13], mixed grain media[25], sawdust[16,27,28] and cereal grain[24] supplemented with various types of cereal bran, and the use of various additives to enrich the media with minerals, vitamins, and protein[18]. Moreover, $CaCO_3$ [20,23,29], $MgSO_4$ and $CaSO_4$ are added to improve the structure of the media and offset the pH. Apart from the media type, there is also response variation among mushroom species to the level of the pH of the media [17,29,30]. Values of pH are affected by differences in media, growth conditions, and strains or stocks used. Generally, the pH range for optimal mycelial growth of *P. ostreatus* is between 5.4 and 6.0[4].

Similarly, this study demonstrated treatment 4; substrate media combined from base substrate sorghum supplemented with wheat bran and with $CaCO_3$, $CaSO_4$ and urea at pH of 6.21 as the best media for the production of spawn for *P. ostreatus*. In this treatment, faster growth of mycelium was observed as evidenced by progressive weight loss beginning from 5 DAI and continuing until the end of the incubation period; 20 DAI (Fig. 1). Weight losses are associated with high rate of energy utilization by the invading mushroom mycelium resulting decrease in the overall weight of the substrate as the fungus feeds it. In the same way, Jiechi et al.[31] observed weight loss of substrates when *Auricularia auricula-judae* was cultured on sawdust substrates. Their result indicated that regularity in growth rate, weight loss and high rate of respiration correlates with the state of mycelial growth and substrate consumption. Thus, high rate of weight loss in the colonized substrate of treatment 4 may indicate that the proliferation of the mycelium and its aggressive utilization of the carbohydrate in the substrate mix and more importantly the relative favorability of the media for faster mycelial colonization and rapid nutrient utilization. The result of this study has also been supported with the qualitative data taken as PMC at 20 DAI (Fig. 2 and Table 4).

The insignificant differences observed in weight change of the remaining treatments suggest lower pH values in treatment 1 and 3 (Table 2) that was brought due to the addition of citric acid additive may have reduced the favorability of the medial for mycelial proliferation while the addition of sucrose in treatment 2 may have changed the C:N ratio of the media.

5. Conclusions

Spawn substrate media combined from sorghum, wheat bran, $CaCO_3$, $CaSO_4$, and urea was found to be significantly different from other media combinations in terms of mean fresh mycelia weight change and mean percent mycelia coverage and found to be the most favorable media mix in the production of spawn for *P. ostreatus* mushroom. Therefore, substrate combination of treatment 4, i.e., sorghum (87.2%) + wheat bran (10%) + $CaCO_3$ (1.5%) + $CaSO_4$ (0.5%) + urea (0.8%) is recommended as the best alternative spawn production media for *P. ostreatus* mushroom. Moreover, weight change in mycelial fresh weight showed significant difference at 5, 10, 15 and 20 DAI at $p<0.05$ with the highest weight reduction of -2.800 ± 0.452 g 20 DAI and -1.240 ± 0.452 g 15 DAI. Accordingly, 20 DAI was found to be favorable incubation period in this substrate media combination for better spawn mycelial growth of *P. ostreatus*.

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