Evaluating the Potential of Corn (Zea mays) Stalk Sugar as an Alternative Supplement on Yield and Growth Performance of Oyster Mushroom (Pleurotus ostreatus)

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Evaluating the Potential of Corn (*Zea mays*) Stalk Sugar as an Alternative Supplement on Yield and Growth Performance of Oyster Mushroom (*Pleurotus ostreatus*)

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Abstract: *The cultivation of oyster mushrooms (Pleurotus spp.) is recognized for its potential in sustainable agriculture yet achieving consistent and high-quality yields remains a challenge. Sugar additives in mushroom substrates have emerged as a promising approach to enhance cultivation outcomes, particularly for oyster mushrooms. The potential of corn stalk sugar to serve as a supplement in the composition of substrates to cultivate oyster mushroom was studied. Various concentrations of corn stalk sugar (0.5%, 1%, and 2%) were incorporated into a substrate composed of corncobs, rice bran, and lime. A control group supplemented with 2% molasses was included for comparison. Results demonstrated significant effects of corn stalk sugar concentrations on mushroom growth parameters. Notably, the substrate treatment containing 1% corn stalk sugar exhibited the shortest maturation period and highest yield performance compared to other concentrations and the control. Statistical analysis using Analysis of Variance (ANOVA) and Least Significant Difference (LSD) tests confirmed these observations.*

Keywords: Corn Stalk Sugar, Substrate, Oyster Mushroom, Biological Efficiency, Yield.

1. INTRODUCTION

Mushroom cultivation is an eco-friendly and profitable agribusiness opportunity, requiring minimal space and offering significant environmental and economic benefits [1]. White oyster mushroom (*Pleurotus ostreatus*) thrives on various substrates, including agricultural waste products [2], making it an adaptable and sustainable option for mushroom cultivation. Substrate supplementation, a technique employed to improve mushroom cultivation, plays a crucial role in enhancing growth and yield by providing essential nutrients, particularly in substrates with low protein content like corn cobs [3]. Gurung et al. [4] emphasize that the addition of suitable supplements promotes mycelial growth and ensures optimal fruiting body development. Sukra et al. [5] highlight that during fermentation, microorganisms utilize carbohydrates from the substrate as an energy source, producing water and carbon dioxide (CO2). The availability of glucose significantly influences bacterial activity and ecosystem functionality, underscoring the importance of maintaining sufficient nutrient levels in the substrate to support microbial communities [6]. Effective supplementation can enhance fermentation processes by increasing nutrient content, improving microbial protein synthesis, and supporting a beneficial microbial community for better fermentation quality [7][8]

In the Philippines, corn (*Zea mays* L.) stands as the second most important crop after rice, generating approximately 4 million tons of grain corn annually and producing an estimated 0.96 million tons of corn cobs [9]. Despite this substantial agricultural output, a large proportion of corn-related waste remains unutilized, often resulting in environmental pollution due to field burning [10]. Corn stalks, which share significant similarities of 91% with sugarcane in terms of structure and sugar content, hold immense potential as a renewable resource [11]. Corn stalks are typically converted into ethanol and livestock feed, while their potential for sugar extraction remains largely unexplored. Molasses, a sugarcane by-product, has been shown to improve substrate quality by increasing nutrient content and supporting microbial communities [12]. Corn stalk sugar offers a sustainable solution for waste management and supports resource-efficient agricultural practices in mushroom cultivation [13] [14]. Mushrooms are valued for their nutritional content, including essential proteins, dietary fiber, and bioactive compounds with immunostimulatory and anticancer properties [15]. Substrate formulation plays a critical role in cultivation, as variations in the stipe length and pileus diameter are influenced by substrate and additive combinations [16]. Thus, this study investigates the potential of corn stalk sugar as a supplement in oyster mushroom production to promote sustainability and enhance yield through improved substrate quality.

2. MATERIALS AND METHODS

2.1 Sample Preparation

The harvested sweet corn stalks from Agroforestry students at Caraga State University, Antongalon, Butuan City, Agusan del Norte, were manually cleaned of leaves and washed on-site before transportation to the school grounds. The corn stalks averaged 125.5 g each with a diameter of 18.1 mm and a length of 17.8 cm per node, with 5 to 6 nodes per stalk. Cutting and processing were completed on the same day. The cut stalks were transported to the Food Innovation Center (FIC) at Caraga State University for disinfection and processing. To remove dirt and residues, the stalks underwent parboiling before being transferred to containers for fermentation. Subsequently, the corn cobs for the mushroom substrate mixture were transported from the

province of Agusan del Sur and were shredded upon arrival.

2.2 Liquid Hot Water Extraction

Each container held chopped stalks, which were incorporated with hot water until fully submerged. During the HWE process, lignocellulosic biomass was immersed in hot distilled water [17, 18, 19]. HWE and LHW pretreatments offer advantages such as minimal chemical usage, lower environmental impact, and cost-effectiveness [20, 21]. By soaking corn stalks in hot water for 42 hours (h) without adding yeast, it ensures maximum sugar extraction without initiating fermentation. This method yielded a pure sugar solution since adding yeast would have converted sugars into alcohol and carbon dioxide [22, 23], reducing sugar content and confounding variables.

2.3 Preparation for Cornstalk Sugar

The fermented liquid was drained, poured into a wok pan for evaporation, during which initial foaming occurred due to entrapped air from fermentation but subsided as evaporation progressed, with impurity clarification being essential for enhancing sugar quality, recovery, and minimizing sucrose degradation during thermal processing [24]. Throughout the evaporation process, samples of the liquid were regularly taken to determine the appropriate amount of lime needed to achieve a pH level of 5-6. Maintaining an optimal pH range of 5-6 is essential for producing medium to high-quality brown sugar [25]. pH control is critical in sugar production processes, as it impacts product safety and edibility [26]. In the sugar industry, clarification or purification typically involved the use of lime [27]. The pH before adding the lime was about 4.04 and changed to 5.76 after gradually adding lime [27], which also resulted in a sugar content of 52% Brix. After 42 h, the liquid extracted from the container was tested and found to have a pH of 4-5 at a temperature of 28.9 °C. This indicated that the liquid underwent hydrothermal treatment, albeit at a lower temperature than that required for full liquid hot water (LHW) pretreatment [20, 28] using specialized equipment. Despite not achieving the higher temperatures associated with standard LHW pretreatment, the observed pH range still suggested some degree of hydrolysis and modification of the biomass components, particularly hemicellulose. The observed pH level indicated effective preservation of hemicellulose, suggesting potential biochemical transformations that could facilitate subsequent processing and production of the cornstalk sugar.

2.4 Mushroom Substrate Treatments

The extracted cornstalk sugar was incorporated into the mushroom substrate, along with lime, rice bran, and chopped corn cobs. Four treatment groups were established as presented in Table 1. Each treatment had 3 replications, with each replicate consisting of 15 samples.

Table 1. Mushroom Substrates Mixture under Different Treatment.

TREATMENT	COMPOSITION
T0 (Control)	80% Corn cobs + 16% Rice Bran + 2% Lime + 2% Molasses
T1	80% Corn cobs + 19% Rice Bran + 0.5% Lime + 0.5% Corn stalk Sugar
T2	80% Corn cobs + 18% Rice Bran + 1% Lime + 1% Corn stalk Sugar
T3	80% Corn cobs + 16% Rice Bran + 2% Lime + 2% Corn Stalk Sugar

Adding calcium carbonate ($CaCO_3$) regulated the substrate's pH, which was essential for optimal mushroom production and significantly impacted the growth and yield of oyster mushrooms [29]. The formulation was based on general recommendations and included a combination of 1% sugar or molasses, 1% lime [30], 20% rice bran, and 78% sawdust [31]. The control group (T0) comprised 80% corncobs, 16% rice bran, 2% lime, and 2% molasses, based on practices from Taguibo, Butuan City, with other treatments adjusting cornstalk sugar and rice bran concentrations while keeping corncob proportions constant; Xu et al. [32] reported a relative comparison with a substrate formula of 80% sawdust, 18% wheat bran, 1% gypsum, 0.5% lime, and 0.5% sugar, while [33] highlighted that lime, essential for pH neutralization, should not exceed 2% to avoid nutrient uptake impairment and inhibited mycelial growth. The mixture was moistened to achieve a moisture content of approximately 60% and piled into a heap, ensuring that nutrients were efficiently transported from the mycelium to the fruiting bodies [12, 34]. The substrate mixtures were fermented under tarpaulin and turned every three days to ensure even mixing, regulate temperature, and stabilize fermentation before cooling and bagging [5, 31].

2.5 Preparation of Mushroom Substrate Bags

Each bag weighed 350 g, compacted to remove air spaces and prevent moisture buildup and microbial growth, tightly wrapped with color-coded rubber bands for each treatment (T0 - Red, T1 - Blue, T2 - Yellow, T3 - Green), and arranged in layers in the pasteurizing chamber.

2.6 Pasteurization of Bagged Substrates

Fruiting bags were pasteurized in an improvised autoclave (drum) for over 10 h to eliminate fungal contamination, with temperatures between 75 and 100 °C effectively killing harmful microorganisms while keeping their reproductive phase inactive, as recommended by [35]. This process, achievable with conventional heating methods below 100 °C, which allowed the use of polypropylene or polyethylene bags [12]. Clean water was poured 4 to 6 deep inside the drum, with a perforated tin plate placed to hold the fruiting bags in layers, and the top covered with thick plastic. After pasteurization, the substrates were incubated to cool for at least 24 h.

2.7 Mushroom Spawning

An average of 22 g of spawn was poured into each bag, and the bag mouths were firmly sealed with rubber bands [36]. After spawning, the fruiting mushroom bags were inoculated for up to 21 days until fully colonized by mycelium, which was then carefully collected to compare

its performance across treatment groups.

2.8 Growing Mushroom Period

The bags were consistently monitored until pinheads formed, with the earliest signs of spawn run observed three days after spawning. Full colonization was first recorded at 10 days, with most bags achieving full colonization between 15 and 21 days [37]. Growth media beds were shielded with mosquito nets to minimize pests, in a cool space. Misting was regularly performed 2-3 times per day to maintain stable humidity and temperature levels, as recommended in the cultivation parameters [36].

2.9 Oyster Mushroom Harvest

Mushrooms were carefully picked, with harvesting recommended early in the morning when the caps were fully opened but had not yet flattened out or curled upwards, resembling oysters when mature [35]. Harvesting was done manually by gently twisting the mushroom base and removing tiny pinheads to prevent contamination [36]. Most mushrooms were harvested three days after pinhead emergence, with the second flush typically occurring 2 weeks later and the latest up to one month after the first flush.

2.10 Cultivation Parameters

2.10.1 Biomass

Each treatment bag was prepared with 350 g, pasteurized steam and inoculated with 22 g of spawn. The bags were arranged in a room maintained at 25-28°C and 75-90% relative humidity and weighed after cultivation.

2.10.2 Intrinsic Factors (N-P-K)

Nitrogen, Phosphorus, and Potassium (NPK) played a key role in ensuring the well-being and efficiency of plants and crops. The amount of NPK in the cornstalk sugar and substrate was determined by the analysis results from the Department of Agriculture – Regional Soils Laboratory.

2.10.3 Morphological Characteristics

This parameter included the period of colonization or spawn run, pinhead formation, fruiting body formation, and full maturity of oyster mushrooms, as described in the study by Elsisura & Figueroa [36].

a) Days from Spawning to Complete Spawn Run (S-CSR). This measured the number of days it took for the substrates to be fully colonized by the fungus from the day of inoculation.

b) Days from Spawning to Pinhead Formation (S-PHF). This recorded the number of days from inoculation to the formation of pinheads by the fungus.

c) Days from Pinhead Formation to Fruiting Body Formation (PHF-FBF). This was calculated by counting the days from the appearance of pinheads to the formation of the fruiting bodies.

d) Days from Fruiting Body Formation to Full Maturity (FBF-FM). This represented the number of days required for the fruiting bodies to reach full maturity and be ready for harvest after their initial formation.

2.10.4 Yield Performance

The yield performance was obtained by measuring the weight of freshly harvested mushrooms per treatment from the first flush to the second flush of mushroom harvest.

2.10.5 Biological Efficiency

Mushroom yields, or the harvested mushrooms, were weighed and recorded for calculation [38] to determine the efficiency of overall growth performance using the extracted cornstalk sugar.

$$\%BE = \frac{Fresh\ weight\ of\ mushroom(g)}{Dry\ weight\ substrate\ (g)} \times 100$$

2.11 Statistical Analysis

A statistical analysis was conducted on all the collected data using the computer software Statistical Tool for Agricultural Research (STAR). The study performed Analysis of Variance (ANOVA) to determine the appropriate cornstalk sugar treatment amount for oyster mushrooms. The difference between treatment means was then tested using the Least Significant Difference at $P< 0.05$. A Completely Randomized Design (CRD) was utilized in the study, with three treatments replicated three times.

3. RESULTS AND DISCUSSION

3.1 Biomass

The study revealed that substrate composition significantly impacts mushroom production. The four treatments tested (T0, T1, T2, T3) exhibited varying mass reductions, with T2 showing the highest loss, suggesting it was the most utilized by the mycelium. T2 and T3 were more efficient, yielding better productivity. The correlation between substrate decomposition and biomass highlights the importance of enzyme activity and substrate composition in optimizing mushroom yield and quality.

3.2 Intrinsic Factors (Nitrogen (N), Phosphorus (P), Potassium (K))

3.2.1 Cornstalk Sugar

The analysis of corn stalk sugar content shows that it meets the Philippine National Standard for Organic Soil Amendment (183:2016), which requires a total NPK percentage between 0.5% and 5%. The data indicates that the corn stalk sugar falls within this range, making it suitable for use in oyster mushroom cultivation. These results presented in Table 2 confirm that the corn stalk sugar provides essential nutrients necessary for oyster mushroom growth, in line with regulatory standards for organic soil amendments.

Table 2. Total NPK of the corn stalk sugar

	N (%)	P (%)	K (%)
Corn Stalk Sugar	0.695	0.63	3.64

N-Nitrogen; P-Phosphorus; K-Potassium.

3.2.2 Substrate Analysis

The study tested substrates with varying sugar concentrations to determine their total NPK levels. Nitrogen, essential for cell wall formation in fungi, significantly impacted growth, with fungi able to adapt to low nitrogen levels (0.03% to 1.0%), but excessive nitrogen could hinder fruiting [39]. Phosphorus played a key role in growth and yield, while potassium supported various physiological processes necessary for optimal mushroom cultivation. The N-P-K ratio in compost

varied from 1.5-0.5-1 to 3.5-1-2 [40]. Laboratory analyses presented in Figure 1 showed that T1 had the highest NPK content (0.86%, 1.00%, 1.02%), followed by T0 (0.86%, 0.75%, 0.96%), T3 (0.82%, 0.84%, 0.87%), and T2 (0.79%, 0.74%, 0.93%).

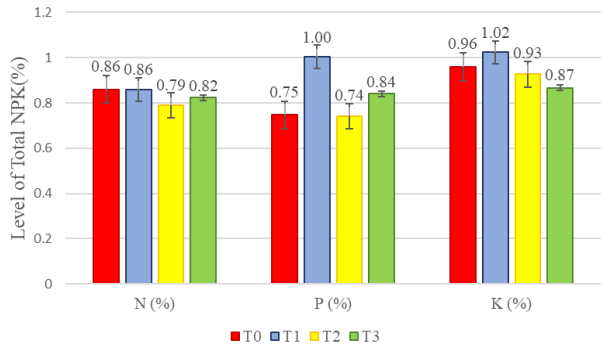

Fig 1. Graphical Representation of the Total NPK of the experimental substrate treatments

The pH data presented in Figure 2 for the various substrate treatments indicated that T0 had the highest pH level at 7.74, followed by T1 at 7.64. According to Choi [41], the ideal pH for mycelial growth ranges from 5 to 6.5, while Belletini et al. [12] determined that a pH range of 6.5 to 7.0 is optimal for fruiting. The pH of the substrate significantly influenced fungal growth and development. Nam et al. [42] emphasized the importance of incorporating lime powder to maintain a pH range of 6 to 8, as deviations outside this range—either below pH 6 or above pH 8—can negatively affect mycelial growth and reduce mushroom yields [33].

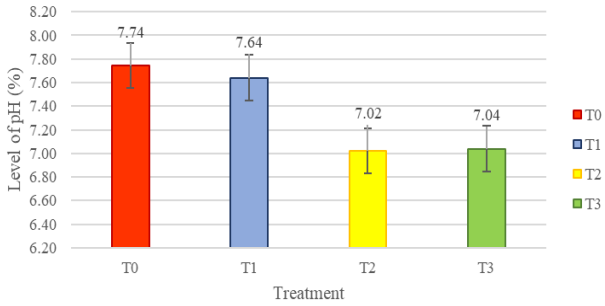

Fig 2. Graphical Representation of Potential Hydrogen across different treatments

The data presented in Figure 3 demonstrated the impact of substrate moisture content on oyster mushroom cultivation. According to Chang & Miles [43], the ideal moisture content for cultivating *Pleurotus* spp. ranges from 50% to 75%, which promotes optimal growth. Within this range, treatments T3 and T0 exhibited moisture contents of 55.86% and 51.43%, respectively. Treatment T2 showed a moisture content of 49.60%, while T1 had a lower moisture content of 47.69%. Maintaining appropriate moisture levels was crucial, as insufficient moisture could lead to the drying of fruiting bodies and a reduction in yield, while excessive moisture could decrease substrate porosity, hinder oxygen transfer, and suffocate mycelia, thereby impairing mushroom growth during the fruiting process [12].

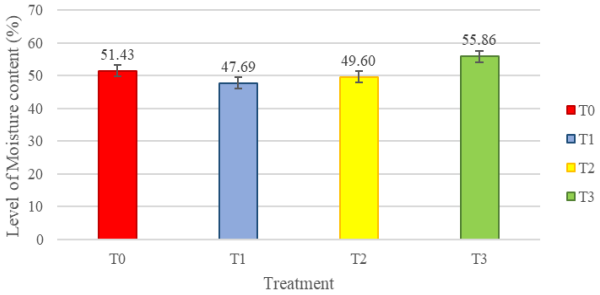

Fig 3. Graphical Representation of Moisture Content across different treatments

3.3 Morphological Characteristics

The analysis of the results presented in Table 3 showed no significant differences in the morphological parameters of the mushrooms across the treatments. However, T2, which consisted of 1% cornstalk sugar with suitable pH and moisture content, demonstrated the fastest spawn run, pinhead formation, fruiting body formation, and maturation period. The variations in growth stage durations were likely attributed to differences in substrate nutrient content.

Table 3. Effect of different treatments on morphological characteristics of oyster mushroom

Treatments	S-CSR (day)	S-PHF (day)	PHF-FBF (day)	FBF-FM (day)
T0	15.53[a]	59.51[a]	1.78[a]	2.78[a]
T1	14.29[b]	58.05[a]	1.58[b]	2.58[b]
T2	13.98[b]	45.73[c]	1.24[c]	2.24[c]
T3	14.04[b]	49.67[b]	1.25[c]	2.25[c]

Within the columns, the means denoted with the same superscript letters are not significantly different at ($P<0.05$) using the Least Significant Difference. S-CSR: spawning to complete spawn run; S-PHF: spawning to Pinhead formation; PHF-FBF: pinhead formation to fruiting body formation; FBF-FM: fruiting body formation to full maturity

3.3.1 Days from Spawning to Complete Spawn Run

As presented in Figure 4, T2 demonstrated the fastest colonization, achieving full substrate colonization in 13.98 days after inoculation. T3 followed closely, reaching complete mycelial colonization in 14.04 days, which was closely aligned with the findings of Girmay et al. [38] and Familoni et al. [44], who reported that spawn running was completed in 14 days. In contrast, T1 showed a slightly delayed mycelial initiation, occurring at 14.29 days. T0, the control group, exhibited the slowest mycelial development, taking 15.53 days to achieve full colonization.

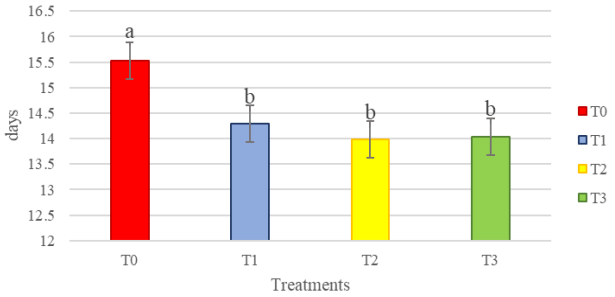

Fig 4. Graphical Representation of Days from Spawning to Complete Spawn Run across different treatments

3.3.2 Days from Spawning to Pinhead Formation

Figure 5 presents the duration of oyster mushroom pinhead formation starting from the day of spawning. Among the treatments, T2 exhibited the fastest pinhead initiation, occurring 45.73 days post-spawning. T3 followed with slightly delayed formation at 49.67 days, while T1 showed a longer duration of 58.05 days. The control group, T0, required 59.51 days for the initial pinhead appearance. These findings align with those of Pathmashini et al. [45], who reported a 45-day period for pinhead formation, like the results of T2, and Bhatti et al. [46], who observed a longer formation time of 61.33 days, comparable to T0. The variation in pinhead formation times across treatments may be attributed to factors such as room temperature and substrate nutrient availability, which influence mushroom growth and development [47].

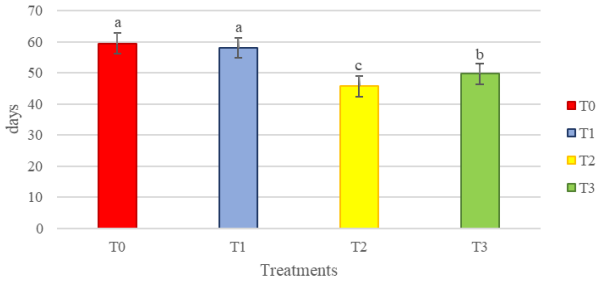

Fig 5. Graphical Representation of Days from Spawning to Pinhead Formation across different treatments.

3.3.3 Days from Pinhead Formation to Fruiting Body Formation

As presented in Figure 6, T2 exhibited the shortest period, taking only 1.24 days, followed closely by T3 with 1.25 days. T1 showed a moderate duration of 1.58 days, while T0 (control) had the longest period at 1.78 days. These findings align with the general understanding that fruiting body formation typically takes 2–3 days after pinhead formation, as reported by Ahmad Zakil et al. [48]. However, the results indicated faster development in some treatments, with T2 showing fruiting body appearance 1–2 days after pinhead formation, which contrasts with the broader range reported in the literature [52, 53]. These results were similar to those of Hassan et al. [49], who observed a 1–4-day period from primordial formation to fruiting body development in *Pleurotus* spp.

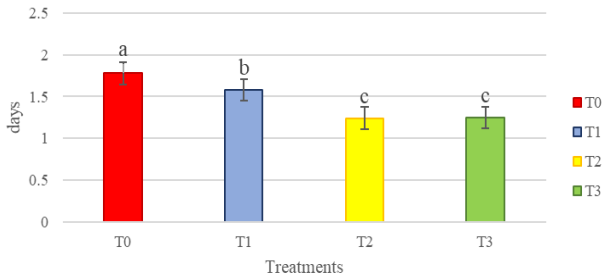

Fig 6. Graphical Representation of Days from Pinhead Formation to Fruiting Body Formation across different treatments

3.3.4 Days from Fruiting Body Formation to Full Maturity

Fig 7 presented the duration from fruiting body formation to harvest across different substrate treatments. T2 exhibited the shortest maturation period, taking only 2.24 days, followed closely by T3 at 2.25 days. T1 required 2.58 days, while T0, the control group, had the longest maturation period at 2.78 days. These results contrast with the general observation by Elsisura & Figueroa [36], which reported a consistent three-day maturation period for all substrate combinations.

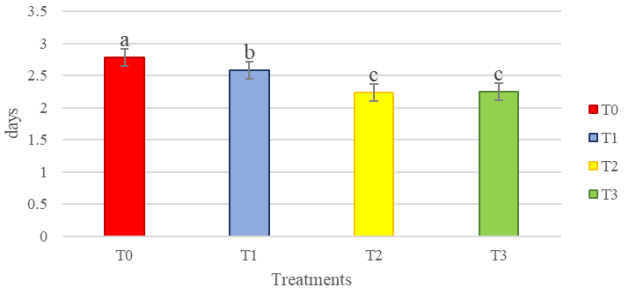

Fig 7. Graphical Representation of Fruiting Body Formation to Full Maturity across different treatments

3.4 Yield Performance

Table 4. Effect of different substrate treatment on yield (grams/bag) of oyster mushroom

Treatments	Flush Interval (days)	1st Flush (g/bag)	2nd Flush (g/bag)	Total Yield (g/bag)
T0	13.49^{a}	16.52^{c}	19.01^{b}	35.53^{c}
T1	12.73^{a}	21.50^{b}	23.03^{ab}	44.54^{b}
T2	10.28^{b}	26.96^{a}	26.74^{a}	53.71^{a}
T3	11.76^{ab}	25.05^{ab}	25.05^{a}	50.10^{ab}

Within the columns, the means denoted with the same superscript letters are not significantly different at ($P<0.05$) using the Least Significant Difference. g; grams.

The interval between the first and second flushes of oyster mushrooms, known as the mushroom flush, varied across the different treatments. T2 exhibited the shortest interval at 10.28 days, followed by T3 at 11.76 days, T1 at 12.73 days, and T0, which had the longest interval at 13.49 days. These findings align with those of Pal et al. [50], who reported intervals ranging from 10.20 to 14.20 days. In terms of yield, T2 produced the highest yield in

both flushes, with 26.96 g/bag in the first flash and 26.74 g/bag in the second flash. T0 yielded the least, with 16.52 g/bag in the first flush and 19.01 g/bag in the second. T2's total yield was 53.71 g/bag, significantly higher than the control group (T0) at 35.53 g/bag, and comparable to findings by Pathmashini et al. [45]. T3 and T1 also showed substantial yields of 50.10 g/bag and 44.54 g/bag, respectively. Overall, T2 proved to be the most effective treatment, yielding the highest total and having the shortest flush interval, likely due to favorable substrate conditions.

3.5 Biological Efficiency

The biological efficiency in this study varied across the different treatments as presented in Figure 8, ranging from 21.11% to 27.61%. T2 achieved the highest biological efficiency at 27.61%, followed by T3 (24.64%) and T1 (22.48%. T0 exhibited the lowest efficiency at 21.11%. These results are comparable with the study by Tavarwisa et al. [51], which reported biological efficiencies between 22.2% and 24.3%. T2 not only produced the highest yield but also demonstrated the best biological efficiency, suggesting that a 1% cornstalk sugar concentration significantly enhances mushroom growth and yield.

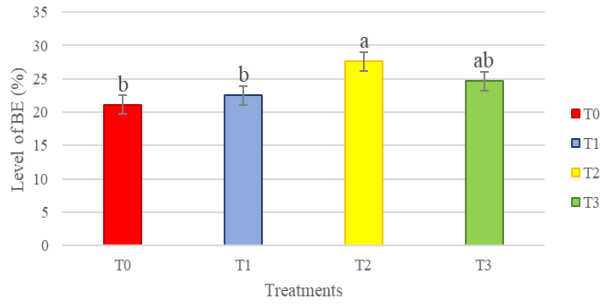

Fig 8. Graphical Representation of Biological Efficiency across different treatments

4. CONCLUSION

The results indicated that sugar concentrations significantly affected several aspects of mushroom growth, including colonization, pinhead formation, fruiting body development, and maturation. Treatment 2, which contained 1% cornstalk sugar, was the most effective, achieving a total NPK content of 0.79%, 0.74%, and 0.93%, a pH level of 7.02, and moisture content of 49.60%. It also demonstrated the fastest maturation time from pinning to fruiting body and achieved the highest yield of 53.71 g/bag. These findings suggested that Treatment 2 was a suitable substrate composition for oyster mushroom cultivation, enhancing yield and accelerating maturation. Further research must be undertaken to provide concrete data results on the utilization of these biomass wastes as part of the waste valorization strategies [52,53] for sustainability.

5. ACKNOWLEDGEMENTS

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