Suitability of Different Supplements for the Growth and Yield of Oyster Mushroom Cultivation

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Abstract—**There are several species of edible mushrooms grown around the world and among them, particularly *Pleurotus ostreatus*, are highly nutritious and in high demand worldwide. In Sri Lanka, *P. ostreatus* is widely cultivated commercially and the nutrient requirement is supplied with different supplementations. This experiment was conducted to study the effects of different nutrient supplements on the growth and yield of *P. ostreatus*. The study tested different treatments, including a supplement mixture, corn flour, green gram flour, rice flour, and chickpea flour. The results showed that the mushrooms supplemented with chickpea flour showed a higher mycelial growth rate compared to other treatments. However, corn flour showed better results in terms of fruiting bodies per bag in both flushes. In the first flush, the yield (g/bag) was higher with green gram flour (137.80±17.48 g), followed by corn flour (126.95±20.16g). In the second flush, the mixture of supplements (144.95±14.99g) and corn flour (119.80±9.57 g) showed higher yields. Lastly, corn flour (246.75±22.64 g), the mixture of supplements (245.70±23.36 g), and green gram flour (244.05±24.01 g) resulted in a higher total yield. Since attention should be given to improving the total yield of a mushroom cultivation use of, corn flour, the mixture of supplements and green gram flour as the supplements can be recognized as more beneficial compared to the others.**

Keywords—Pleurotus ostreatus, nutrient supplementation, mycelial growth rate, yield

I. INTRODUCTION

Edible mushrooms can serve as a source of nutritious food or medicinal supplements, with some types providing both benefits. It is widely recognized that mushrooms possess these properties [1], which may have resulted in increased production and consumption worldwide [2]. Among the number of edible mushrooms, the *Pleurotus* genus attains considerable attention among growers and consumers. The genus *Pleurotus* has approximately 40 species, which are often known as "oyster mushrooms" [3], [4]. Most of the time, they can be seen under tropical and subtropical climates such as Asia, Europe, and Africa [2], [5] while they can be easily artificially farmed [3]. The majority of these species are produced in China, accounting for 87% of the world's total production [2]. *Pleurotus ostreatus* belongs to the family Pleurotaceae and it has a broad, fan, or oyster-shared cap with a length between 5 to 25 cm, while the color can range from white to grey or tan to dark brown. The margin is rolled inwards when young and smooth but is often lobed and wavy [3].

Many studies have been conducted to discover mushrooms' chemical composition, nutritional value, and health benefits. *P. ostreatus* is a good source of proteins and amino acids. They can provide all nine essential amino acids for humans, and they are especially high in lysine and leucine, which are deficient in most cereal diets [6]. It is a good source of carbohydrates, vitamins such as thiamine, riboflavin, niacin, and folates and a good source of minerals such as K, P, Zn, Cu, Mg, B, Mn, Ca, Na, and Fe [7, 8, 9]. Additionally, this diet can be considered well-balanced due to its low fat and energy levels and its higher amounts of dietary fiber and other beneficial compounds [10]. Meanwhile, several studies have recorded their health benefits such as antioxidant effects [4, 11] antibacterial effects [12], antiviral effects [13], anti-diabetic effects [14], anti-hyperlipidaemic effects [15, 16] and many more.

The ideal temperature for the growth of oyster mushrooms is 20° to 30° C, with humidity ranging from 55-70% during 6 to 8 months of the year [17]. For the growth of mushrooms usually as a nutritional source C (Carbon), N (Nitrogen), and other inorganic compounds are required. However, oyster mushrooms require a higher amount of C and a lesser amount of N. Since cellulose, hemicellulose, and lignin can act as major sources that can provide C, materials such as straw (rice, wheat), leaves (banana), cotton seed hulls, corncobs, sugarcane bagasse, sawdust, and waste paper can be used as the substrate for the growth of mushrooms [18], [19]. The oyster mushroom can use a wider range of waste materials as a substrate than any other fungus, and as a result, transform them into valuable vegetable food that is comparable in nutritional value to non-vegetarian food [20]. These naturally occurring cellulose, hemicellulose, and lignins only contain a certain amount of their nutrient elements, thus they need to be supplemented with chemical and biological supplements. The introduction of nutritional additions to the substrates used for mushroom production constitutes the agronomic procedure known as mushroom supplementation. Adding external nutrients can enhance the productivity of mushroom cultivation. However, there is debate surrounding the nutritional needs of mushrooms and the usefulness of new commercial additives [20, 21].

Though there are several commercially available supplements for the producers in the market, huge attention has been gained by the low-cost supplements which are considered waste materials for some other processes [21]. Such as peanut waste [22], cotton seed cake, soybean meal, urea, ammonium sulfate [23], olive mill waste [24], rice husk [25], and grapeseed meal [26]. In Sri Lanka, sawdust is a material that is widely utilized and favored by the commercial sector. To cultivate oyster mushrooms, it is supplemented with rice bran and a protein supplement. In the manufacture of substrates, protein supplements like soy and green gram powder are usually used. However, the cost of production can be reduced by substituting less expensive N sources, such as animal dung, which can provide a significant amount of secondary and main plant nutrients [27]. Currently, the Department of Agriculture's recommendation is to use sawdust (20 kg), rice bran (2 kg), CaO (400 g), soya flour (200 g), and $MgSO_4$ (40 g) for 50 packets of mushrooms [28]. In Sri Lanka, farmers commonly use a commercially available mixture of nutrient supplements which includes corn, chickpea, rice, green gram, and soya flour. When cultivating mushrooms with varying levels of supplementation, there were significant differences in growth, yield, and quality parameters [29]. Therefore, this experiment was conducted to study the suitability of using chickpea flour, green gram flour, corn flour, and rice flour for the growth and yield of oyster mushrooms as nutrient supplementations.

II. Methodology

The experiment was conducted at the "Green Core Techno Park", SAERC, SLTC Research University (6.8557° N, 80.0926° E, and 27 m above mean sea level), Ingiriya Road, Padukka, Sri Lanka from January 2023 to May 2023.

For this experiment as the substrate, sawdust (100 kg) supplemented with dolomite (2 kg), rice bran (10 kg), $MgSO_4$ (0.2 kg), gypsum (1 kg), and nutrient supplement (1.5 kg) were used to prepare 250 mushroom bags. To assess the impact of nutrient supplements, five different supplements were used as treatments within a Complete Randomized Design (CRD). These treatments included a supplement mixture (mixture of chickpea flour, green gram flour, corn flour, rice flour, and soya flour mixed in similar amounts) which most of the mushroom cultivators use as the nutrient supplement (T1), corn flour (T2), green gram flour (T3), rice flour (T4), and chickpea flour (T5), which were mixed separately. To add $MgSO_4$, it was dissolved in water and applied to the mixture, after which a moist mixture was created. Approximately 500-600 g of the moist mixture was then filled into a transparent polythene bag that measured 33 × 18 cm. The bags were sterilized for 2-3 hours by steaming them in a metal barrel while providing constant heat throughout the period. After the bags had cooled down completely, they were inoculated under a sterilized environment with *Pleurotus ostreatus* spawns purchased from the commercial market. The bags were then transferred to a dark room at a temperature of 28 °C while maintaining a relative humidity of 90 – 95 %. For three weeks, the vertical length (cm) of the mycelium was measured from four faces, once a week [18]. Once the mycelium had grown completely, one end of the packet was cut open to facilitate the mushroom growth out of the bag. The relative humidity was maintained at 85-90% and water was applied twice a day, while the temperature was kept around 25 – 28 °C within the growing chamber. The number of fruiting bodies and their fresh weight (g) were measured daily. These measurements were taken in two separate flushes, and the total measurements were calculated.

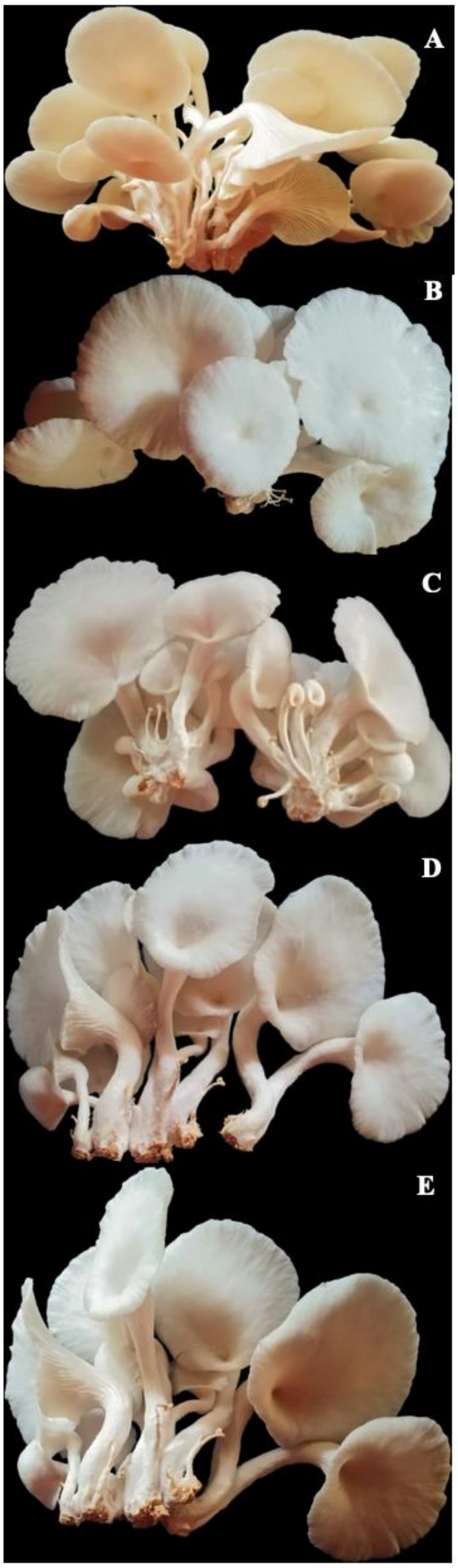

Fig.1. Harvested mushrooms from growing bags supplemented with Supplement mixture (A), Corn Flour (B), Green Gram Flour (C), Rice Flour (D) and Chickpea Flour (E)

Data were analyzed using the ANOVA test and the mean separation was done by using Fisher's least significant difference (LSD). As the statistical analysis software Minitab Version 19 was used.

III. Results and Discussion

A. Mycelial Growth Rate

According to the current study, there were significant differences among treatments for the mycelial growth rate during the three consecutive weeks studied (p<0.05). A comparatively higher mycelial growth rate compared to the other treatments, could be observed with the mushrooms supplemented with chickpea flour (T5) in all three weeks (Fig.2.). Several other studies have shown that chickpea flour can be used as a supplement for the growth of mushrooms in different substrates such as corn cobs, grasses, and cotton waste [23], [30], [31]. Meantime, reference [32] found that with the increment in the application rate of chickpea flour, the time taken to the completion of spawn running had reduced [32]. They found that when 2.5% chickpea powder was amended, spawn running was shown to be completed earlier. However, some opposite results have been found by [33] revealing that with the increased supplementation percentage of corn flour and wheat bran, the rate of mycelial growth has reduced gradually and such rapid mycelial development suggested that these levels of supplementation contained a greater C to N ratio (C/N), which favored rapid mycelial growth. Also, this was most likely owing to the high N level, which has been shown to hinder mushroom growth when present in excess in the substrate [33].

B. Number of Fruiting Bodies

There were significant differences among the supplements in both flushes separately and together for the number of fruiting bodies (p<0.05). Comparatively, a higher number of fruiting bodies were given with the corn flour supplement (T2). However, in the first flush other than corn flour, green gram flour (T3) also resulted in a higher number of fruiting bodies. Since the main focus should be given to the total number of fruiting bodies it can be said that corn flour supplement has a better effect in increasing the number of fruiting bodies compared to other supplements (Table I). The lignocellulosic substrate of wood components' poor availability of N is frequently seen as a barrier to its usage as a mushroom substrate therefore, supplemental N is crucial for the growth of fruiting bodies in mushrooms grown in sawdust [34].

On the other hand, chickpea flour had some concerns in terms of the number of fruiting bodies because it had a lower number compared to other treatments though its mycelial growth rate was higher. This shows the inability of mycelia to utilize the substrate fully therefore a yield reduction had resulted. This demonstrates that mycelial growth and mushroom output have different necessities [35]. On the other hand, [31] showed that *P. ostreatus* grown in cotton waste substrate supplemented with chickpea flour had better results, however, the results were lower compared to rice bran and wheat bran supplementations [31].

Except for the chickpea flour supplementation (T5) with all the other treatments a slight reduction in the number of fruiting bodies could be observed with the second flush compared to the first flush (Table I). Similar findings were shown by [35], and they have shown that the fact that fewer fruit bodies were recorded for each flush on average shows that the type and amount of N present in a substrate after each flush impact how much cellulose degrades, which in turn affects the yield [35].

C. Mushroom Yield

Mushroom yield in both flushes and the final total yield was significantly different in the tested supplements ($p<0.05$) (Table II). In the first flush, a higher yield could be observed with mushroom bags supplemented with green gram flour (T3), followed by corn flour (T2). The mixture of supplements (T1) was given a higher yield during the second flush, followed by corn flour (T2). Most importantly, corn flour (T2), the mixture of supplements (T1), and green gram flour (T3) gave a higher total yield. Similar findings have been shown, where 30% corn powder supplemented with rice straw as a substrate to cultivate milky white mushrooms showed better growth and yield compared to other supplements [36] and 20 and 8 % corn flour supplemented with the substrate of maize stalk residues to cultivate *P. ostreatus* [33].

When protein-rich additives are added to mushroom substrates, it can increase both the quality and quantity of the yield, according to reference [21]. In this study, all the supplements used are good sources of proteins Mixture of supplements, corn, green gram, rice, and chickpea flours have protein contents of 20.3%, 9.42%, 23.86%, 6.61%, and 19.30% respectively [37]. Although corn flour has a relatively lower amount of protein content, it showed better results in terms of yield in the current experiment. The composition and amount of supplement added to the growing bag can significantly affect the final yield [34]. Reference [33] concluded that the growth of *Pleurotus* species is favored by a substrate low in N content, which may explain why they obtained the best yield using 8% and 20% corn flour [33].

It is recommended to conduct further investigations on the mixing rates of current supplements to determine if a reduction in mixing rate would impact yield quality and quantity. Also conducting a cost-benefit analysis can help identify the feasibility of using these substrates among farmers.

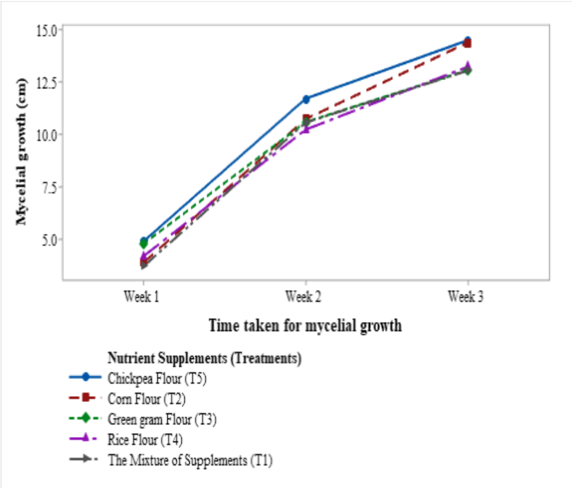

Fig.2. Mycelial growth in mushrooms with different nutrient supplements

TABLE I. NUMBER OF FRUITING BODIES (NFB) PER BAG IN FIRST FLUSH, SECOND FLUSH, AND TOTAL NFB

Nutrient Supplement	First Flush	Second Flush	Total NFB
The Mixture of Supplements (T1)	20.75 ± 3.24^{ab}	20.25 ± 2.82^{ab}	41.00 ± 4.15^{abc}
Corn Flour (T2)	25.50 ± 4.42^{a}	21.55 ± 2.31^{a}	47.05 ± 5.37^{a}
Green gram Flour (T3)	25.40 ± 3.16^{a}	17.45 ± 2.23^{ab}	42.85 ± 3.39^{ab}
Rice Flour (T4)	19.25 ± 3.52^{ab}	13.85 ± 2.03^{b}	33.10 ± 3.67^{bc}
Chickpea Flour (T5)	14.30 ± 1.70^{b}	16.10 ± 2.32^{ab}	30.40 ± 3.11^{c}

Values in each column represent the means of 20 replicates ± SE (Standard Error). The mean followed by the same letter within each column is not significantly different at $p<0.05$.

TABLE II. MUSHROOM YIELD PER BAG IN THE FIRST AND SECOND FLUSHES AND THE TOTAL YIELD

Nutrient Supplement	First flush (g)	Second flush (g)	Total yield (g)
The Mixture of Supplements (T1)	100.75 ± 14.56^{abc}	144.95 ± 14.99^{a}	245.70 ± 23.36^{a}
Corn Flour (T2)	126.95 ± 20.16^{ab}	119.80 ± 9.57^{ab}	246.75 ± 22.64^{a}
Green gram Flour (T3)	137.80 ± 17.48^{a}	106.25 ± 13.85^{b}	244.05 ± 24.01^{ab}
Rice Flour (T4)	93.80 ± 12.06^{bc}	92.45 ± 15.69^{b}	186.25 ± 17.58^{bc}
Chickpea Flour (T5)	80.60 ± 8.21^{c}	93.25 ± 12.37^{b}	173.85 ± 15.31^{c}

Values in each column represent the means of 20 replicates ± SE (Standard Error). The mean followed by the same letter within each column is not significantly different at $p<0.05$.

IV. CONCLUSION

In commercial mushroom cultivation, nutrient supplementation significantly impacts yield and quality. The current experiment explored alternative supplements to the common mixture used by farmers. Among corn flour, green gram flour, rice flour, and chickpea flour tested with chickpea flour showed the fastest mycelial growth but gave concerns about yield. Corn flour and green gram flour, along with the supplement mixture, performed well in terms of yield, indicating their potential as effective alternatives. Further research is needed to determine optimal mixing ratios for these supplements, to reduce ratios without compromising final yield. This study contributes valuable insights for improving mushroom cultivation practices in Sri Lanka.

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