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Research Article

Growth and Yield Performance of *Calocybe indica* (Purkayastha and A. Chandra) Milky Mushroom in Various Agricultural Wastes in the Philippines

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ABSTRACT

The Calocybe indica, commonly called the milky mushroom, originated in India and was known for its distinctive taste and health benefits. It was investigated for its potential to be cultivated in several substrate combinations from different agricultural wastes in the Philippines. The sampled substrates include rice straws, banana leaves, corn husks, and corn cobs. The rice hull served as the control substrate. The growth and yield performance of C. indica were assessed based on the rate of mycelial growth and colonization, duration of harvest time, number of fruiting bodies produced, the diameter of the cap, stalk length and width, size of fruiting bodies, and biological efficiency. Results revealed that the T10 (substrate containing 100% corn husks) demonstrated a rapid rate of mycelial growth and colonization, completing the substrate's full colonization in 20 days by week 4. The highest yield, largest cap, thickest stalks, and high biological efficiency were observed in the T14 substrate (containing 100% corn cobs). The poorest growth and performance were observed in the T17 substrate (containing 25% corn cobs + 75% rice hull). This research demonstrated the potential of the agricultural byproducts in the sustainable cultivation of C. indica as a nutritious and healthy food source.

Keywords: *Calocybe indica*, Rice straw, Banana leaves, Corn husk, Corn cobs.

INTRODUCTION

Mushrooms are placed under the kingdom Fungi, renowned for their distinctive fruiting bodies and their positive impact on health, delicacy, and unique flavor. Mushrooms are rich in protein, low in calories, and have a balanced nutritional profile compared to meat and vegetables [1], [2].

The *Calocybe indica*, also known as Milky Mushroom, originated in India [3], [4]. It is an edible mushroom famous for its considerable size, relatively high yield, and luscious taste. It is rich in protein, lipids, minerals, fibers, and essential amino acids [5], [6], [7]. Its ability to grow vigorously in the tropics offers a competitive advantage against the oyster mushroom and becomes the world's famous button mushroom's emerging contender [5].

The Philippines is a tropical agricultural country. Its increasing demand for food is equivalent to its increasing agricultural waste due to continuous population growth. Agricultural wastes like rice straws and corn husks, when not correctly managed, can contribute to pollution [8], [9], [10]. Other agricultural wastes, such as banana and corn farming, may produce considerable waste biomass, frequently disposed of in an unsustainable manner [11], [12].

Mushroom cultivation provides an environmentally friendly alternative to managing agricultural wastes, converting the lignocellulosic by-products into growth substrates of mushrooms [13], [14]. This procedure minimizes the repercussions of agricultural waste while furnishing beneficial sources of healthy foods. The tropical climate of the Philippines is remarkably favorable to mushroom farming; however, the cultivation of Milky mushrooms is still under research and remains hampered. This research tested the potential of the different agricultural wastes and their

combinations, such as rice hulls, rice straw, banana leaves, corn husks, and corn cobs, as substrate solutions to boost the growth and yield performance of *C. indica* in the Philippine setting [14].

METHODS AND METHODOLOGY

Collection of Pure Culture

The mother culture (F_o) of *C. indica* was obtained from the Mushroom Culture Division of the Bureau of Plant Industry (BPI) in Malate, Manila.

Calocybe indica Grain Spawn Preparation

The *C. indica* grain spawn was prepared using 1 kg of sorghum seeds. The seeds were cooked halfway in water for 20–25 minutes and were drained to remove the excess water. Cooked sorghum seeds were loaded into ketchup bottles, refilling up to three-quarters of the bottle's capacity. The individual bottle was securely enclosed with a cotton plug covered with paper securely tightened with a rubber bond. All bottles were autoclaved for 45 minutes at 121°C and 15 psi pressure following Oei and Nieuwenhuijzen [1] with modifications. All bottles were allowed to cool at room temperature for 24 hours after autoclaving. The agar block *C. indica* were aseptically inoculated in all bottles and were allowed to be incubated for 1-2 weeks at 30-35 °C.

Preparation of Mushroom Substrates

This research used 10 kilograms each of sun-dried agricultural wastes such as rice straw, banana leaves, corn husks, and corn cobs as substrate for growing *C. indica*. The 25-kilogram sun-dried rice hull was used as the control substrate. All substrates were ground and chopped into 1-2 inch pieces. Each agricultural waste was immersed in water for three hours and drained, removing excess water until the desired 60-70% moisture level was reached. The substrates were fermented for 1-2 weeks with continuous mixing every other day to prevent the build-up of foul odor.

Individual and different combinations of the substrates resulting in 17 treatments were prepared as experimental substrates: Treatment 1 (T1) - 100% rice hull (RH) (control substrate); T2 - 100% rice straw (RS); T3 - 25% RH + 75% RS; T4 - 50% RH + 50% RS; T5 - 75% RH + 25% RS; T6 - 100% banana leaves (BL); T7 - 25% RH + 75% BLs; T8 - 50% RH + 50% BL; T9 - 75% RH + 25% BL; T10 - 100% corn husk (CH); T11 - 25% RH + 75% CH; T12 - 50% RH + 50% CH; T13 - 75% RH + 25% CH; T14 - 100% CC; T15 - 25% RH + 75% CC; T16 50% RH + 50% CC; T17 - 75% RH + 25% CC. Each of the treatments was conducted twice with three replicates per trial, resulting in a total of six bags per treatment.

A total of 500 grams of substrate were packed with supplements such as 1.5% $CaCO_3$ Forty, 1.5% $CaSO_4$, 4.0% rice bran, and 3.0% maize powder for each treatment. All prepared substrates were loaded in 10 x 12 inches polypropylene bags securely sealed with PVC ring plugged with cotton, wrapped in autoclavable plastic and paper, and fastened with rubber bands. All fruiting bags were sterilized for 1 hour at 121°C and 15 psi following Amin et al. [14] with modifications.

After sterilization, all bags were allowed to cool for 24 hours. The grain spawn of *C. indica* was aseptically inoculated in all the treatment substrates for spawn running. All inoculated treatment substrates were incubated in a dark room for 1 to 3 weeks until *C. indica* mycelia fully colonized the substrates.

Casing

The casing was prepared using carbonized rice hull (CRH) sterilized for 30 minutes at 121°C and 15psi. The CRH was distributed evenly over each bag to form a uniform layer of 1 cm thickness. The bed surface was maintained with approximately 60% moisture by regular water spraying following the Bokaria et al. with some modifications [15].

Cultural Condition

All fruiting bags were maintained at a relative humidity of 60-80% at 30-35°C, which was accomplished by watering the bags with a sprinkler daily [16].

Growth and Yield Parameters

The growth and yield performance of C. indica in the different treatments were observed using the following parameters: 1) number of days for mycelial growth and colonization to complete after spawning; 2) primordial

formation and first harvest; 3) quantity of mushroom fruiting bodies produced, including the size of the cap, length and width of stalk; 4) mushroom yield (g) and size of fruiting; and 5) biological efficiency, which were calculated after the cropping period. All parameters used were adapted from Amin et al. [14] and Jambaro et al. [17].

Statistical Analysis

The resulting data were collected and analyzed for homogeneity using Levene's test [18]. In assessing the overall significance of the data, the analysis of variance (ANOVA) with the Kruskal-Wallis test was applied [19]. In comparing the mean differences among the various varieties, the Mann-Whitney U test was utilized [20].

RESULTS AND DISCUSSION

Rate of mycelial growth and colonization of *C. indica* on different treatment substrates

The observations for the mycelial growth on the different treatments were done one week after the inoculation of *C. indica* spawn in the fruiting bags. The percentage of mycelial growth was observed by visual inspection and was recorded weekly. The graphical illustration showing the percent mycelial growth of *C. indica* on the different treatment substrates weekly is shown in Figure 1. On the other hand, the number of days for complete colonization of *C. indica* on the various treatment substrates is shown in Figure 2. Results revealed significant differences in the percentage of mycelial growth between the treatment substrates based on Kurkal-Wallis H-tet at $p \leq 0.05$, except for the fourth week.

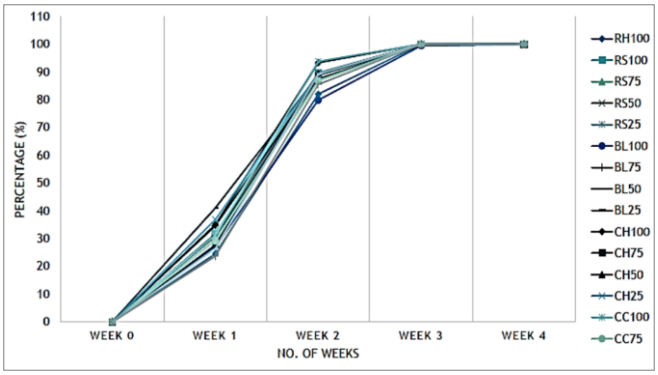

Figure 1. Graph representation of the weekly percentage of mycelial growth of *C. indica* on different treatment substrates.

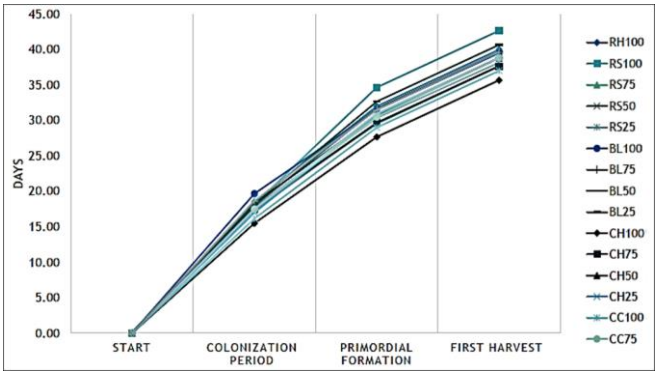

Figure 2. Graph representation of the rate of mycelial colonization, formation primordia, and first harvest of *C. indica* on different treatment substrates.

The T8 (50% BL + 50% RH) exhibited rapid mycelial colonization, with 41% colonization in its 1st week of incubation. The T16 (50% CC + 50% RH) showed the slowest mycelial colonization. In the second week, T14 (100% CC) performs mycelial colonization fastest with 93.83%. The T8 (50% BL + 50% RH) observed fastest in the first week declined, completing the colonization in the fourth week. The T13 (25% CH + 75% RH) and T10 (100% CH) completed the mycelial colonization in the third week. Mycelial growth and colonization of *C. indica* in most of the treatment substrates were completed in the third week. The most prolonged rate of growth and mycelial colonization was observed in treatment substrates T6 (100% BL), T7 (75% BL + 25% RH), T8 (50% BL + 50% RH), and T9 (25% BL +

75% RH). The rapid growth rate and mycelial colonization were observed in T10 (100% CH), completed in 16 days during the third week. The slowest rate, which took 20 days to colonize by the fourth week, was observed in T6 (100% BL),

The substrates corn husks and corn cobs were also found to exhibit the fastest mycelial colonization rate in the study by Elenwo and Okere [21] from their research Wastes Recycling using Edible Mushroom Cultivation. Cangy and Peerally [22], on the other hand, found that corn husk has a high carbon and low nitrogen content, which aligns with the nutritional requirements of mushroom mycelia for efficient growth. Gerrits [23] found that rapid lignin degradation occurs during the spawn run, while cellulose and hemicellulose degradation occurs slowly. Banana leaves are high in cellulose content and low lignin levels, which may be attributed to a more prolonged period of mycelial colonization for substrates that contain banana leaves [24]. The gradual decomposition of banana leaves can be associated with the substrate's limited bioconversion capacity, which hampers the efficient breakdown of nutrients for mycelial growth.

Formation of primordia and first harvest period of *C. indica* on different treatment substrates

The primordial formation is the next phase in mushroom cultivation after the mycelial growth and colonization. Oei and Nieuwenhuijzen [1] described the formation of primordial or tiny pinheads in mushroom cultivation as the initial fruiting body, which indicates the beginning of the reproductive phase. The appearance of the first pinheads was observed on the 28th day in T10 (100% CH). The T2 (100% RS) was revealed to be the slowest in the formation of primordial growth, with pinheads appearing on the 35th day. Figure 2 shows the data for each treatment substrate from the pinhead formation to full maturation and harvest of the fruiting bodies of *C. indica*. For all treatments, completing mycelial colonization to the first harvest of fruiting bodies took between 16 to 36 days. The first harvest of *C. indica* was obtained from T10 (100% CH) on its 36th day. The last harvest was obtained from T2 (100% RS) on its 43rd day. The Kruskal-Walis H-test showed significant differences in all treatments regarding the rate of mycelial growth and colonization, primordia initiation, and reap the first harvest at $p \leq 0.05$. The data revealed that the treatment that best performs in mycelial colonization, primordial formation, and first harvest was T10 (100% CH); the weakest performance was observed in T6 (100% BL). The T2 (100% RS) also took a prolonged time to form primordia and reap the first harvest.

Narain et al. [25] implied that the substrate's lignocellulosic and carbon: nitrogen (C: N) ratio influences mushrooms' mycelial growth and primordial development. The rapid mycelial colonization and the first to produce pinheads in T10 (100% CH) may mean that the substrate contains the necessary nutritional requirements for mycelial growth. The poor performance of T6 (100% BL) in mycelial colonization may be attributed to insufficient nutrients and the treatment substrate's slow degeneration of lignin and cellulose content. The weak performance of T2 (100% RS) for primordial formation could be attributed to environmental factors affecting fruiting bodies' growth and formation. Additionally, the varying nutritional requirements of the mushroom must be regarded as it transitions from the vegetative to the reproductive phase.

Quantity of *C. indica* fruiting bodies generated on the treatment substrate

This research used the fruiting body, the edible part of the mushroom, as the key indicator. Along with the well-developed fruiting bodies, this research measured and recorded the diameter of the mushroom cap, length and width, fruiting body size, total yield, and biological efficiency of each treatment substrate. The highest number of effective fruiting bodies were obtained from T14 (100% CC) and T2 (100% RS), with an average score of 1.33. The rest of the treatment substrates yielded the lowest quantity of effective fruiting bodies produced, with an average score of 1.00. The Kruskal-Wallis H-test [18] revealed no significant differences in the number of effective fruiting bodies across the various substrates and combinations at $p \geq 0.05$. Chang [26] stated in the journal Overview of Mushroom Cultivation and Utilization as Functional Foods that environmental conditions influence the number and quality of fruiting bodies.

The largest cap diameter, measuring 9.95 cm, was recorded in T14 (100% CC). The smallest diameter of the cap was acquired in T16 (50% CC + 50% RH) with an average of 2.17 cm and T17 (25% CC + 75% RH) at 2.74 cm. There is a significant difference between all the treatment substrates regarding cap diameter. This indicates that the mushrooms grown in different treatment substrates produced variations in the cap sizes. Onyango et al. [27] stated that the larger fruiting bodies are rated more favorably and considered high quality in mushroom cultivation.

The most extended stalk was obtained in T1 (100% RH) with an average of 6.23 cm, and the shortest stalk was recorded in T16 (59% CC + 50% RH). On the other hand, the largest stalk width was obtained in T2 (100% RS), with an average width of 8.12 cm, followed by T14 (100% CC), with an average width of 6.72 cm. The smallest stalk width was observed in T17 (25% CC + 75% RH), with an average of 2.52 cm. Results indicate that the components or ingredients of the treatment substrate affect the stalk width of *C. indica*.

Overall, results revealed that the cap diameter consistently outperformed the stalk length and width of the harvested C. indica fruiting bodies. Royes [28] demonstrated that this case is advantageous since small caps with longer stalks and narrower stalk widths are generally less desirable in the market.

Total yield of mushroom

Results showed that T14 (100% CC) yielded the heaviest fruiting bodies of approximately 80.3 g. The lowest yield was recorded in T1 (100% RH), averaging 25.50 g. Statistical analysis showed significant differences in the total yield of fruiting bodies among the treatment substrate at $p \leq 0.05$. Isikhuemhen and Okhuoya [29] emphasized that variations across different treatment substrates could be associated with the nutrient content of each treatment substrate, including their lignocellulosic composition.

Size of Mushroom Fruiting Bodies

The results revealed that T7 (75% BL + 25% RH) produced the largest fruiting bodies with an average length of 8.27cm. The most petite fruiting bodies were obtained in T17 (25% bCC + 75% RH) with an average length of 3.77 cm (Figure 3).

Figure 3

Figure 3. Harvested *C. indica* from the different treatment: T1 (100% RH (Control); T2 (100% RS; T3 (75% RS + 25% RH; T4 (50% RS + 50% RH); T5 (50% RS + 25% RH; T6 (100% BL); T7 (75% BL + 25% RH; T8 (50% BL + 50% RH; T9 (25% BL + 75% RHl; T10 (100% Corn Husk); T11 (75% CH + 25% RH; T12 (50% CH + 50%RH; T13 (25% CH + 75% RHl; T14 (100% CC;; T15 with (75% CC + 25% RH); T16 (50% CC + 50% RH); T17 (25% CC + 75% RH).

Biological efficiency of treatment substrates

The T14 (100% CC) exhibited the highest biological efficiency at an average of 14%, while the T17 (25% CC + 75% RH) revealed the lowest biological efficiency at an average of 5.83%. The biological efficiency in cultivating *C. indica* in the different treatment substrates demonstrated a highly significant difference $p \leq 0.05$. The T14 (100% CC) was revealed to be significantly different from the rest of the treatment substrates in terms of the size of the mushroom cap, stalk length and width, total mushroom yield, mushroom size, and biological efficiency based on Mann-Whitney U Test.

CONCLUSION

This study revealed that T10 (100% CH) substrate is the best choice for cultivating *C. indica* to achieve rapid mycelial growth colonization and a short harvest duration in the early growth stages of the mushroom. Moreover, T14 (100% CC) was confirmed to be a superior treatment substrate with the utmost biological efficiency (14%), colossal cap, broad stalk, and largest fruiting bodies. In contrast, the treatment substrates that performed poorly in producing more minor and less efficient yields is T17 (25% CC + 75% RH. These findings correspond to Maurya et al. [30] [31], who emphasized substrate quality and spawn purity as crucial factors for producing high-quality *C. indica*. This research stressed the use of typical agricultural wastes, especially corn cobs, in cultivating *C. indica* to support sustainable agriculture efficiently and, at the same time, provide a health-giving food source.

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Conflict of Interest:

The authors affirm that this research has **no conflict of interest.**

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