
Aseptic cultivation of *Coprinus comatus* (O. F. Mull.) Gray on various pulp and paper wastes

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The chemical components of pulp and paper wastes from industrial paper mill were analyzed prior to aseptic cultivation. Mycelial running, primordium initiation, fruiting body development, yield and bioefficiency of *Coprinus comatus* on pulp and paper waste supplemented with rice bran were studied. Chemical analysis revealed that brown paper waste contains 48 ppm of Pb. The fastest mycelia colonization (8 days), primordium initiation (12 days) and fruiting body development (14 days) were realized in substrate composed of pure coarse gray paper waste. However, the highest yield (9.53 g) and biological efficiency (23.96%) were recorded in the formulation containing light blue paper waste + 10% rice bran. The fruiting bodies produced in contaminated paper wastes were detected to consist of 16.15 ppm of Pb. In general, we have successfully demonstrated the cultivation of *C. comatus* on pulp and paper wastes enriched with rice bran and its ability to absorb Pb from contaminated substrates.

Key words – biological efficiency – cultivation phases – heavy metals – mycoremediation

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Introduction

Coprinus comatus (O. F. Mull.) Gray, which is known worldwide as shaggy ink cap, lawyer's wig, and shaggy mane and locally known in the Philippines as *kabuteng hapon* and *kabuteng demonyo* due to its inky black basidiocarps (Reyes et al., 2009)-, is a member of Agaricales family, and frequently inhabit lawns, gardens and piles of rice straw. Its basidiocarps have autolytic characteristics which digest upwards from the bottom of the pileus, eventually becoming inky to liberate its basidiospores. The *Volvariella* grows in the country particularly in Luzon considered it as a weed fungus due to its aggressive behaviour of growing and dominating the substrates

intended for *V. volvacea* cultivation, as a result of limitation in space and scarcity of nutrients (Reyes et al., 2009). Being a weed fungus in mushroom cultivation but due to its desirable nutraceutical profile, Reyes et al (2009) successfully shifted its ecological niche from being a weed fungus to an economic crop. They initiated the aseptic cultivation of *C. comatus* on sawdust-rice grit (8 parts: 2 parts v/v) based formulation in a miniaturized bottle under laboratory conditions. This agaric mushroom is a delicious and nutritious food source (Luo et al., 1991). It contains 8 essential amino acids namely valine, leucine, lysine, isoleucine, threonine, phenylalanine, tryptophan and methionine. The presence of γ -

amino butyric acid (GABA) and ornithine, the ability to inhibit the angiotensin converting enzyme confirmed its importance as a natural source of nutraceutical (Reyes et al., 2009). Additionally, it can topple blood sugar and fat, inhibit growth of tumors (Liu and Zhang, 2003) and has an antioxidative potential (Popovic et al., 2010). Although this mushroom is popular as culinary ingredient, its availability in the local market becomes limited due to its seasonal occurrence in the wild. Local mushroom hunters collect them from decomposing piles of rice straw. Cultivation of *C. comatus* was reported on different agricultural residues such as rice straw, cotton waste, corn cobs, urea, manure, lime (Yang and Xue, 2000), chicken manure supplemented by solid sisal waste (Lyantagaye et al., 2010). However, no attempts have been made on the cultivation on pulp and paper waste as its basal substrate especially under aseptic condition.

In the Philippines, one paper mill alone generates 100-120 tons of paper sludge per day (Fernandez et al. 2001), thus, creating major problem of waste disposal. This material mainly consists of cellulose fibre and inorganic materials. Being cellulosic, cultivation of edible mushrooms on pulp and paper waste represents one of the most efficient biological ways to convert this industrial residue into additional profit. Baysal et al (2003) successfully demonstrated the utilization of pulp and paper waste as the base material supplemented with rice husk for the yield improvement of oyster mushroom (*Pleurotus ostreatus*).

In the present study, we developed new production technology for *C. comatus* using pulp and paper waste as a basal medium enriched with rice bran which served as a strong foundation for industrial waste utilization.

Methods

Source of strain and revival of culture

A pure culture of *C. comatus* was obtained from the Center for Tropical Mushroom Research and Development. Agar blocks of approximately 10 mm² x 3 mm from the pure stock culture of *C. comatus* were aseptically transferred into sterilized potato sucrose *gulaman* (PSG) plate. Culture plates

were incubated at room temperature to allow growth of the secondary mycelia.

Fruiting body performance of *C. comatus* on paper pulp formulations

The production of fruiting bodies of *C. comatus* was evaluated on pulp and paper waste from an industrial paper mill as a base substrate. Initially, four samples of pulp and paper wastes (fine gray, coarse gray, light blue and brown) were brought to the Analytical Services Laboratory of the Philippine Rice Research Institute for chemical characterization of the pulp and paper waste ingredients. The protocol of Reyes et al (2009) on aseptic cultivation was adopted with minor modifications. Pulp and paper wastes were moistened to gain 65% moisture content (MC). Following the formulations (pure pulp and paper waste and an addition of rice bran as supplement) of the different pulp and paper wastes, 40 grams of each formulated material was placed in a miniaturized glass bottle occupying approximately half to give space for the appearance of fruiting bodies. Bottled substrates were individually covered with polypropylene sheets, secured with a rubber band and sterilized at 15 psi, 121 °C for 45 minutes. Each was inoculated with a 10 mm diameter mycelia disc of the revived culture of *C. comatus* and subsequently incubated at room temperature 30±2 °C under alternating light and dark condition. After mycelial colonization, the bottled cultures were placed into a growing chamber with 80-90% RH to allow the primordium initiation and fruiting body development.

Each treatment was replicated 3 times in 2 experimentation set-ups. The number of days was recorded after the total mycelial colonization of the substrate, primordium initiation and fruiting body development were observed. The weight of non-mature fruiting bodies was determined and percentage biological efficiency was computed. Data were analyzed using analysis of variance (ANOVA) in one way classification analysis. Duncan Multiple Range Test (DMRT) was used to determine the significant treatment comparison at 5% level of significance. The Sirichai Statistics 6.07 computer program was used for analysis.



Fig. 1 – Fruiting bodies of *C. comatus* grown on LBPP + RB medium in a miniaturized glass bottle.

Results and Discussion

Chemical contents of pulp and paper wastes

A leading industrial paper mill in the country that generates a huge pulp and paper sludge daily provides pulp and paper wastes. Disposal of this waste is becoming a major problem for the industry. We intervened to create efficient means of its utilization, i.e. through mushroom cultivation. But prior to that, the four types of pulp and paper waste were analyzed to determine the chemical contents and to check their safety to humans. The organic carbon, nitrogen, pH and lead (Pb) content of the different pulp and paper wastes are presented in Table 1. Among the samples, fine and coarse gray pulp and paper waste had the highest organic carbon and the lowest amount of nitrogen. Light blue pulp and paper waste had the lowest organic carbon but higher amount of nitrogen when compared with the nitrogen content of the fine and coarse gray pulp and paper waste. In terms of pH, all samples were basic with a pH range of 8-10. Though it is imperative to reformulate these substrates with nutritional supplements to adjust the pH condition and improve the nutrients, obviously, pulp and paper wastes

hold promising nutritional value for excellent growth performance of *C. comatus*. Paper sludge is a mixture of solid chemical residues in the process of manufacturing and bleaching the pulp and paper. It is also known to contain heavy metals (Ismail et al, 2010). In this study, it is revealed that among the four samples, brown pulp and paper waste contained 48 ppm of lead. It is therefore, not recommended as a substrate for mushroom cultivation.

Mycelial colonization, primordial initiation and fruiting body development

The time periods of the different phases of cultivation of *C. comatus* are given in Table 2. The formulation containing pure CGPP had the shortest period of mycelial colonization (8 days), primordium initiation (12 days), and fruiting body development (14 days). It was followed by FGPP + RB with 9.33 days, 12.67 days, 14.33 days, in respective phases of cultivation, which was statistically comparable with pure FGPP. Despite of the relatively long time periods of fructification in these formulations compared to 8 days when grown in sawdust-rice grit (8:2 v/v) formulation, it is better than 17 days when spawned in sawdust-rice bran (8:2 v/v) formulation (Reyes et al.,

Table 1 Organic carbon, nitrogen, pH and lead (Pb) content of four types of pulp and paper wastes.

Pure Pulp/Paper Waste	Organic Carbon (%)	Nitrogen (%)	pH	Lead (ppm)
Fine gray paper pulp (FGPP)	90.40 ± 0.40	0.14 ± 0.01	10 ± 0	ND
Coarse gray paper pulp (CGPP)	90.00 ± 0.20	0.11 ± 0.01	9 ± 0	ND
Light blue paper pulp (LBPP)	32.80 ± 0.10	0.29 ± 0.00	9 ± 0	ND
Brown paper pulp (BPP)	52.60 ± 0.30	0.51 ± 0.01	8 ± 0	48

(In Table: Values are the mean ± SD of chemical contents of paper waste. ND: not detected, lead detection limit is 2 ppm)

2009). Findings of the present study suggest that among the pure pulp and paper wastes evaluated, CGPP is the most suitable substrate for the various phases of *C. comatus* cultivation due to high amount organic carbon and low amount of nitrogen. Though CGPP and FGPP contain almost the same amount of nutrients (C:N in Table 1), FGPP had higher pH (9.6), which was excessively higher with the optimum pH range of 6.0-8.0 of *C. comatus* (Jang et al., 2009). This relatively high pH triggers the slow mycelial running. But addition of rice bran to FGPP stimulates cultivation, which might be attributed to the enrichment of the formulation and the adjustment of the pH. However, pure LBPP recorded the most extensive period in all phases of cultivation most likely due to very low organic carbon and relatively high amount of nitrogen, which is known to inhibit mushroom growth in excessive amounts (Demirci, 1998). Addition of rice bran to LBPP shortens the time periods in all phases of cultivation by 33%.

Yield and Biological Efficiency

After determining the most favorable substrate for the various phases of cultivation, the yield and biological efficiency of *C. comatus* cultivation were also evaluated. There were significant differences found in the mean yield within three to four flushes among the different substrate formulations (Table 2). It was revealed that addition of 10% rice bran to the pulp and paper waste substrates significantly increased the mean yield compared to pure pulp and paper waste substrates. The LBPP + RB formulation produced the highest mean yield of 9.58 g with a biological efficiency of 23.96%. Moreover,

the largest fruiting bodies were also observed on this substrate (Figure 1). The data gathered in this study are better than the previous report of 14% bio-efficiency when the mushroom was grown in formulated sawdust-rice bran substrates and 18% bio-efficiency in sawdust-rice grit combination (Reyes et al., 2009). However, pure CGPP which provided the efficient cultivation properties recorded the lowest bio-efficiency of 0.12%. Thus, addition of rice bran to pulp and paper waste as substrate for cultivation of *C. comatus* amends the chemical contents which positively affected the mushroom yield.

Since fungi are known to accumulate heavy metals (Tyler, 1980; Bano et al., 1981), we have investigated the ability of *C. comatus* to accumulate lead (Pb), which was detected in BRPP. The dried fruiting bodies harvested on this substrate contained 16.15 ppm of lead. This significant result indicates that this mushroom can be an agent for Pb-mycoremediation. Also, this implies that substrates must be lead free before utilizing them for the mushroom cultivation.

Industrial pulp and paper wastes enriched with 10% rice bran can be used for the cultivation of *C. comatus*. Pure paper pulps stimulate the cultivation properties but resulted to low yield. However, addition of rice bran to pulp and paper waste significantly increased the mushroom yield and enhanced bio-efficiency. The present study also revealed that *C. comatus* accumulates lead (Pb) from the contaminated pulp and paper waste substrate. Furthermore, cultivation of other wild edible mushrooms on clean and safe pulp and paper waste as basal medium enriched with rice bran and other supplements is recommended.

Table 2 Mycelial colonization, primordium initiation, fruiting body development, mushroom yield and biological efficiency of *C. comatus*.

Substrate	Mycelial colonization (day)	Primordium initiation (day)	Fruiting body development (day)	Yield (gram)	BE ^b (%)
FGPP (pure) ^a	9.67 ± 0.58 ^d	13.67 ± 0.58 ^d	14.67 ± 0.58 ^d	0.14 ± 0.07 ^d	0.35
CGPP (pure)	8.00 ± 0.00 ^e	12.00 ± 0.00 ^e	14.00 ± 0.00 ^d	0.05 ± 0.01 ^d	0.12
LBPP (pure)	18.00 ± 1.00 ^a	21.00 ± 1.00 ^a	23.33 ± 0.58 ^a	0.67 ± 0.14 ^d	1.67
BRPP (pure)	13.67 ± 0.58 ^b	16.33 ± 0.58 ^{bc}	18.67 ± 0.58 ^{bc}	0.55 ± 0.18 ^d	1.37
FGPP + RB	9.33 ± 0.58 ^d	12.67 ± 0.58 ^{de}	14.33 ± 0.58 ^d	5.70 ± 0.21 ^b	14.26
CGPP + RB	12.67 ± 0.58 ^{bc}	16.67 ± 0.58 ^{bc}	18.33 ± 1.15 ^c	6.16 ± 0.43 ^b	15.40
LBPP + RB	12.00 ± 0.00 ^c	15.67 ± 1.53 ^c	19.33 ± 2.89 ^{bc}	9.58 ± 2.23 ^a	23.96
BRPP + RB	12.67 ± 0.58 ^{bc}	17.33 ± 0.58 ^b	20.67 ± 0.58 ^b	4.25 ± 0.22 ^c	10.62

(In Table: Values are the mean ± SD of time periods of cultivation phases (days) and yield (grams), and means having the same letter of superscript in the same column are insignificantly different from each other at 5% level of significance. ^a FGPP: fine gray, CGPP: coarse gray, LBPP: light blue, and BRPP: brown pulp and paper waste, RB: 10% rice bran. ^b Biological Efficiency (%) = (mean yield / 40 g wt of substrate) x 100.)

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