



Wheat flour, an inexpensive medium for *in vitro* cultivation of coprophilous fungus *Coprinopsis cinerea*

Mohankumar S¹ and Savitha J^{1*}

¹ Department of Microbiology and Biotechnology, Bangalore University, Jnanabharathi campus Bangalore- 560056, Karnataka, India.

Mohankumar S and Savitha J. (2017) – Wheat flour, an inexpensive medium for *in vitro* cultivation of coprophilous fungus *Coprinopsis cinerea*. Current Research in Environmental & Applied Mycology 7(3): 144–154 (2017), Doi 10.5943/cream/7/3/1.

Abstract

Coprinopsis cinerea, a coprophilous basidiomycetous fungus generally called as inky cap mushroom is used as a model organism to study the evolution of fruiting bodies in higher fungi. Herbivorous animal dung is a major source of *Coprinopsis cinerea*, as it contains high carbon, nitrogen, and phosphorus elements. Due to the extensive application of *Coprinopsis cinerea* in Genetics, Molecular Biology and Microbial Biotechnology it is necessary to explore a suitable inexpensive medium for its *in vitro* cultivation. In our present study, we found that 2% wheat flour medium supported the vegetative growth and induced the fruiting body formation within 10 days at 30°C, pH 6 under dark compared to malt extract amended media. The number of fruiting bodies and biomass of fruiting bodies were also found higher in wheat flour medium compared to other media tested with similar cultural conditions

Key words – Basidiomycetes –Biomass –Fruiting body –Growth–Herbivores–dung–Inky cap

Introduction

Since ancient times mushrooms were widely used globally as food for their nutritional and medicinal value (Badalyan 2014). *Coprinopsis cinerea*, a coprophilous basidiomycete, belonging to the family Psathyrellaceae is a model organism for the study of fungal sex due to their short haploid period. Recent studies on *C. cinerea* has shown that it is a reservoir of many bioactive compounds such as antimicrobial peptides (AMPs), insecticidal peptides (Sabotic et al. 2016) and industrially important enzymes (Ruhl et al. 2013)

Cospin, a novel antimicrobial peptide (AMP) isolated from *Coprinopsis cinerea* showed antibacterial activity against several pathogenic bacteria including *Listeria monocytogenes* (Essig et al. 2014). Novel nematotoxic lectins were identified from *C. cinerea* which are toxic against many bacterivorous nematodes including *Aphelenchus avenae* (Plaza et al. 2016). Ethyl acetate extracts of *Coprinus cinereus* (Schaeff) S. Gray s. lat. cultivated on grasses supplemented with cow dung manure exhibited significant activity against *E. coli*, *Candida albicans* and *Aspergillus niger* (Ndyetabura et al. 2010). Cospin, a first fungal trypsin- specific protease inhibitor was identified by Sabotic et al. (2012) from *C. cinerea*, which showed toxicity against fungivorous insects. Methanolic extracts of a species of *Coprinopsis*, *C. atramentaria* revealed antioxidant activity and the compounds were identified as *p*-hydroxybenzoic acid (HA), *p*-coumaric (CoA) and cinnamic

acid (CA) (Heleno et al. 2014). Further, Heleno et al. (2014) identified methylated and glucuronated derivatives of phenolic acids of *C. atramentaria*, showed good activity against MCF-7-breastcarcinoma, NCI-H60-non-small cell lung carcinoma, HCT15-colon carcinoma cell lines. It also showed potent antimicrobial and demelanizing activities against *Aspergillus niger*, *Aspergillus fumigates*, *Penicillium verrucosum* var. *cyclopium*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus cereus* etc. *C. cinerea* also possess enzymes with novel characteristics (Kaur et al. 2011)

Beyond any doubt, herbivorous animal dung is a rich substratum for the growth and sporulation of coprophilous fungi, especially *Coprinopsis cinerea*. But, however, due to hygienic point of view, laboratory cultivation in dung or dung extract media are not recommended. Therefore, there is an immediate need to search for better and cheaper medium for vegetative growth, fructification and biomass production of *Coprinopsis cinerea*, which possess several significant metabolites of commercial value. Several reports are however, available on the growth of *Coprinopsis cinerea* on synthetic media and malt extract supplemented media is reported to be the best among them. The present study was aimed to identify a medium for faster growth, better biomass and fruit body production of *Coprinopsis cinerea* under laboratory conditions.

Materials & Methods

Isolation of *Coprinopsis cinerea*

Coprinopsis cinerea was isolated from fresh dung samples of various herbivorous animals such as cow, horse and buffalo. The samples were collected in a zip lock polythene bag from Hessarghatta, Bangalore during rainy season. The dung samples were brought to the laboratory and aseptically transferred to a sterilized flask (Richardson 2001). The flasks were incubated at room temperature (28°C) for 25 days for the formation of fruiting bodies. The fruiting bodies from each sample were removed aseptically and stored at -20°C for further use

Molecular Characterization of the isolates

The fungus was grown on malt extract agar medium and the mycelial mat was scrapped from the agar plates and used for genomic DNA isolation using Qiagen DNeasy Kit. The DNA was amplified by PCR amplification experiments using ITS primers with the forward sequence ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and reverse sequence ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). The amplified products were purified using geneO-spin PCR product purification kit (geneOmbio technologies Pune; India) and sequenced using an ABI PRISM Bigdye terminator V3.1 kit (Applied Biosystems USA). The sequences were analyzed using Sequencing Analysis 5.2 software. The sequence similarity was detected by Blastn (Altschul et al. 1997) against the NCBI GenBank database (<http://www.ncbi.nlm.nih.gov/BLAST/>). The sequences derived from these studies have been deposited in NCBI GenBank (Benson et al. 1999). Multiple sequence alignments were performed using Clustal W (Thompson et al. 2002). The evolutionary distance was calculated by the method of Jukes and Canter (1969). Phylogenetic tree was constructed by the neighbor-joining method and tree topologies were evaluated by bootstrap analysis using MEGA6 (Molecular Evolutionary Genetic Analysis) (Tamura 2013).

In vitro cultivation of *Coprinopsis cinerea*

The fruiting bodies of *C. cinerea* grown on cow, horse and buffalo dung were collected and washed thoroughly with distilled water (twice) and chopped into small pieces of approximately 1cm length using sterile scalpel and surface sterilized using sodium hypochlorite (4%), ethanol (70%) and sterile distilled water. The small pieces of sterilized fruiting bodies were inoculated on different agar media: Malt extract agar (MEA; 3% malt extract, 0.5% mycological peptone, 2% agar); Malt extract- yeast extract agar (MYA; 1% malt extract, 0.2% yeast extract, 2% agar); Glucose-peptone agar (GPA; 4% glucose, 1% peptone, 2% agar); Yeast extract-peptone agar (YPA; 1% yeast extract, 2% bacto-peptone, 2% agar); Yeast extract malt extract glucose/ Tryptophan agar

(YMG/T; 0.4% yeast extract, 1% malt extract, 0.4% glucose, 0.01% tryptophan, 2% agar); Malt extract yeast extract casamino acid agar (MYC; 1% malt extract, 0.2% yeast extract, 0.2% casamino acid, 2% agar); Dung extract agar (DEA; 0.2% Dung extract, 2% agar); mKjalke agar (1% yeast extract, 2% glucose, 0.05% CaCl₂ 2H₂O, 0.02% KH₂PO₄, 5 mg MgSO₄, 2% agar); Potato dextrose agar (PDA; 3.9% potato dextrose agar); YMG agar (0.4% yeast extract, 1% malt extract, 0.4% glucose, 2% agar) and the plates were incubated for 7 days at 30°C, pH 6 under dark conditions (Ruhl et al 2013). After the incubation period the plates were observed for morphological characteristics of the colonies.

Cultivation of *Coprinopsis cinerea* on wheat flour medium

Wheat flour medium was prepared with varying concentrations of wheat flour (1%, 2%, 3% of wheat flour for wheat flour broth and with addition of 1.5% agar for wheat flour agar) and inoculated with 5mm mycelial disc of *Coprinopsis cinerea* species for 7days for radial growth, 10 days for biomass production and 15 days for fruiting body production at 30°C, pH 6 under dark conditions. The colony diameter was measured at regular intervals and the numbers of fruiting bodies were also counted. Fresh and dry weight of the biomass was also noted as given above.

Statistical Analysis

All the experiments were done in triplicates and the mean values, standard deviation and standard error were calculated using the software IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp). The results were expressed as mean ± standard error.

Results

Identification of *Coprinopsis* spp.

Three fruiting bodies were isolated from three different dung samples and designated as C1, C2 and C3. The length of the stipe (Figs. 2A, 2B) and diameter of the pileus was measured (Table 1). The spore prints (Fig. 1) were dark brown and the basidiospores were black, ellipsoidal with a rounded apex and a pointy central germ pore (Fig. 2C). Based on these morphological data the fruiting bodies were identified as *Coprinopsis cinerea*.

Table 1 Morphological identification of *Coprinopsis cinerea* isolated from different dung samples.

Source of the dung	Code of the Isolate *	Length of the stipe (cm)*	Width of the pileus(cm)*	Diameter of the spore (µm)*
Cow	C1	8	1.5	8
Horse	C2	12	2.8	9
Buffalo	C3	11	2.3	8

Molecular characterization of the isolates

The molecular identification of the three isolates (C1, C2 and C3) was done by sequencing and characterizing the gene encoding for 18S rRNA. The sequence was queried in nucleotide BLAST search from NCBI to find the homology with the existing species of *Coprinopsis*. The first nucleotide sequence C1 isolated from cow dung was 389 bp, the second nucleotide sequence C2 isolated from horse dung was 620 bp and the third nucleotide sequence C3 isolated from buffalo dung was 466 bp. The nucleotide sequence showed 98% (C1) and 100% (C2 and C3) identity with *Coprinopsis cinerea*. The three nucleotide sequence data were deposited to GenBank and obtained the accession numbers *Coprinopsis cinerea* strain C1 (KX468976), *Coprinopsis cinerea* strain C2 (KX468976) and *Coprinopsis cinerea* strain C3 (KX468977). The phylogenetic trees were constructed for all the three isolates by obtaining the ITS sequence of closely related taxa (Fig. 3).

In vitro cultivation of *Coprinopsis cinerea* and selection of a suitable strain for further studies

The radial growth of the fungal colony on different media was measured for all the three strains of *Coprinopsis cinerea* (Fig. 4). Among all the media tested, successful growth was observed in only six media viz MEA, MYA, YMGTA, MYCA, YMGA, WFA and no growth was found in GPA, YPA, DEA, PDA and mKjalke media. Interestingly, we observed luxuriant and faster growth of *C. cinerea* strain C2 in MEA, YMGTA and WFA compared to other two strains of *C. cinerea* (C1 and C3) incubated for 7 days at 30° C, pH 6 under dark condition (best suited cultural condition for the cultivation of *C. cinerea* in laboratory). Based upon these results we selected *C. cinerea* strain C2 for further studies.

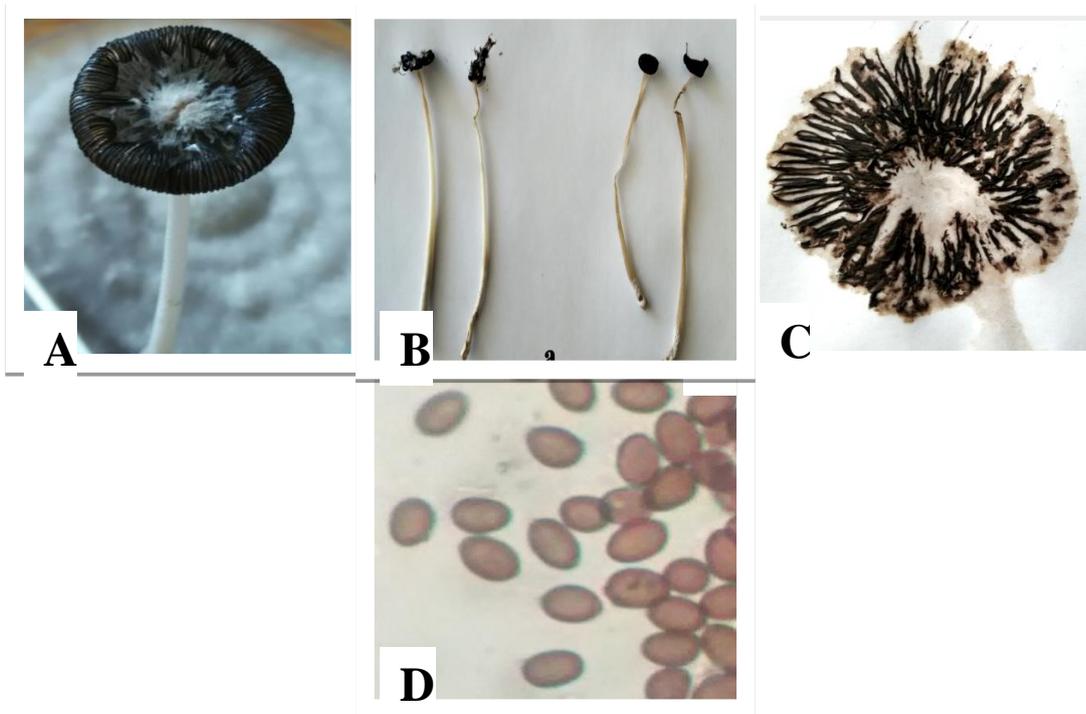


Fig 1 – Fruiting bodies (A, B), spore print (C) and spores (D) of *Coprinopsis cinerea*

Effect of solid and liquid media on *Coprinopsis cinerea* strain C2 growth

Coprinopsis cinerea strain C2 isolated from horse dung has showed growth in all the six media but significant growth was observed in wheat flour agar (WFA) medium (Fig. 4). Average radial growth of 9cm was achieved within 7 days of incubation in WFA compared to other media at 30° C, pH 6 under dark, whereas poor growth of 3cm and 5cm was observed in YMGA and MYA media respectively, incubated under similar cultural conditions. We have also observed production of fresh (84.96 grams/l) and dry (7.9grams/l) biomass was higher in WFB compared to other liquid media (Fig. 5) and the least fresh (2.2g/l) and dry (24.46g/l) biomass production was found in YMG media.’

concentrations (1 and 3%) (Figs. 5, 6). The maximum biomass (fresh, 85.22g/l and dry 7.7g/l) production was obtained at 2% wheat flour concentration compared to other two concentrations were tested (Fig. 7).

We also tested the suitable concentration of wheat flour for the growth of *C. cinerea* strain C2. Medium with 2% concentration of wheat flour showed faster radial growth of 9cm in solid medium within 7 days of incubation at 30° C, pH 6 under dark compared to other two

Discussion

Coprinopsis cinerea is commonly known as gray shag or inky cap mushroom due to its black colored pileus and deliquescence or autolysing property. It is particularly a suited organism to study meiosis in living system due to its synchronous meiotic development and prolonged prophase (Burns et al. 2010). It is also a model organism for studying fungal sex and evolution of fruiting

bodies in fungi. *C. cinerea* is a common flora of herbivores dung because of its rich organic matter. Due to hygienic concern of the dung and extensive application of *C. cinerea* in various fields, the present study was aimed at developing an inexpensive, hygienic medium suited for routine use for cultivation.

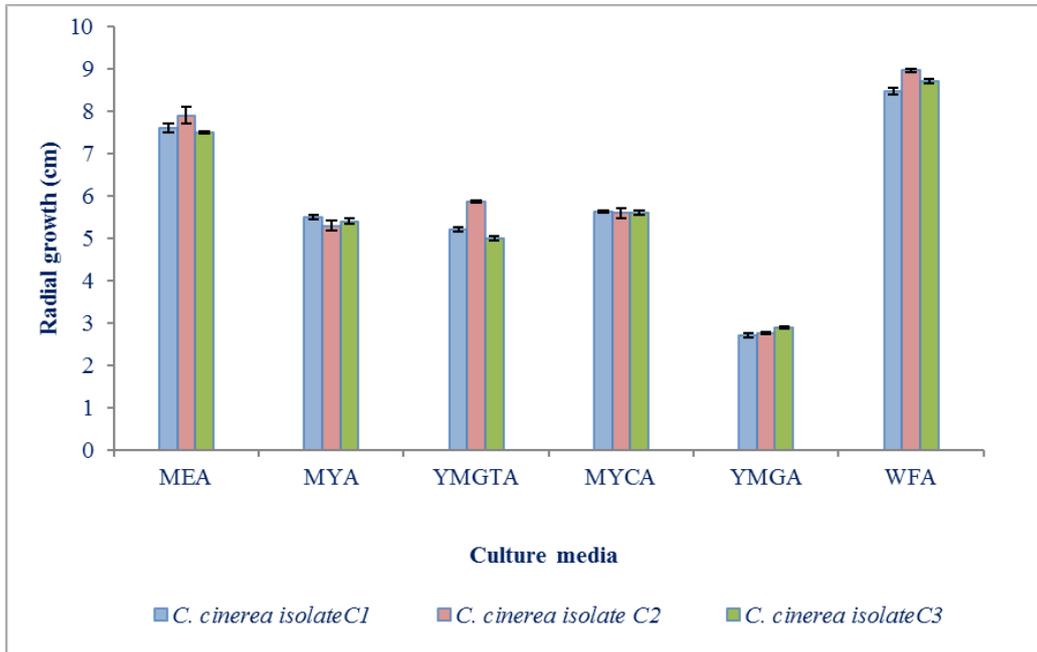


Fig 2 – Phylogenetic tree showing relationships of *Coprinopsis cinerea* strain C1, C2, C3 and related species of *Coprinopsis* identified on the basis of 18S rRNA gene sequences, using neighbor joining method in MEGA 6. Bootstrap values are shown at the branch points

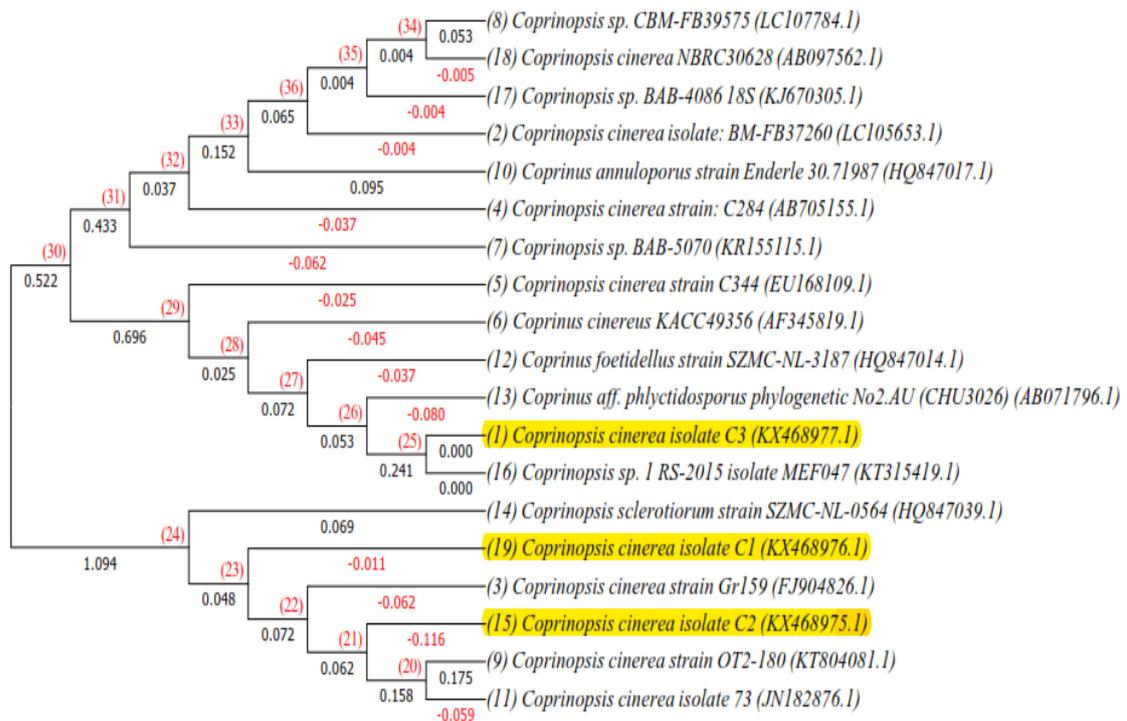


Fig 3 – Growth of *Coprinopsis cinerea* strain C1, C2 and C3 on different culture media incubated for 7 days at 30° C, pH 6 under dark: MEA- Malt extract agar, MYA- Malt extract yeast extract agar, YMGTA- Yeast extract malt extract glucose tryptophan agar, MYC-Malt extract yeast extract casamino acid agar, YMGA-Yeast extract malt extract glucose agar and WFA- Wheat flour agar.

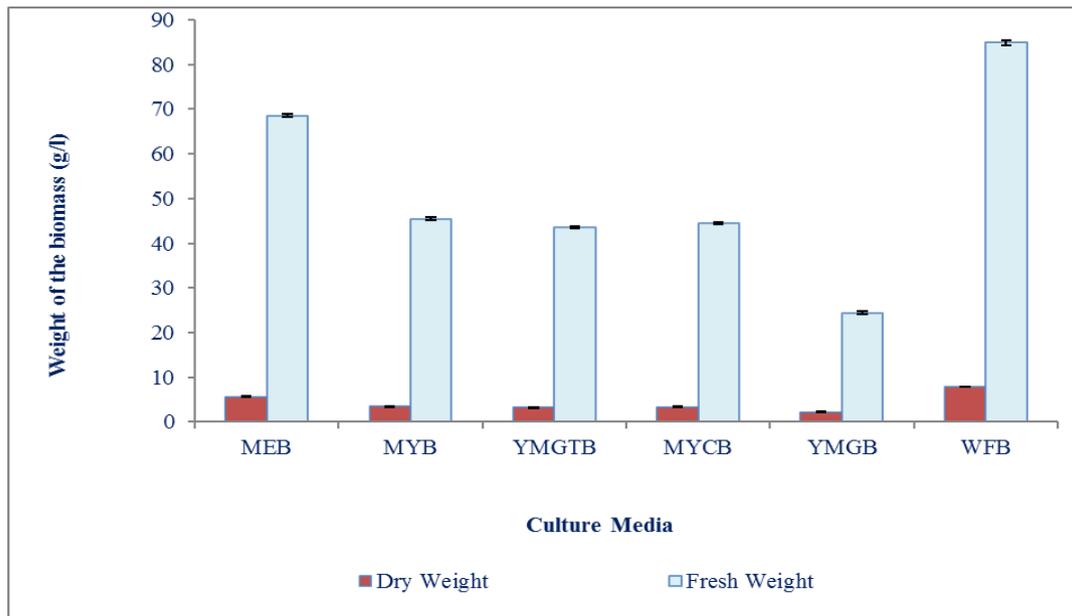
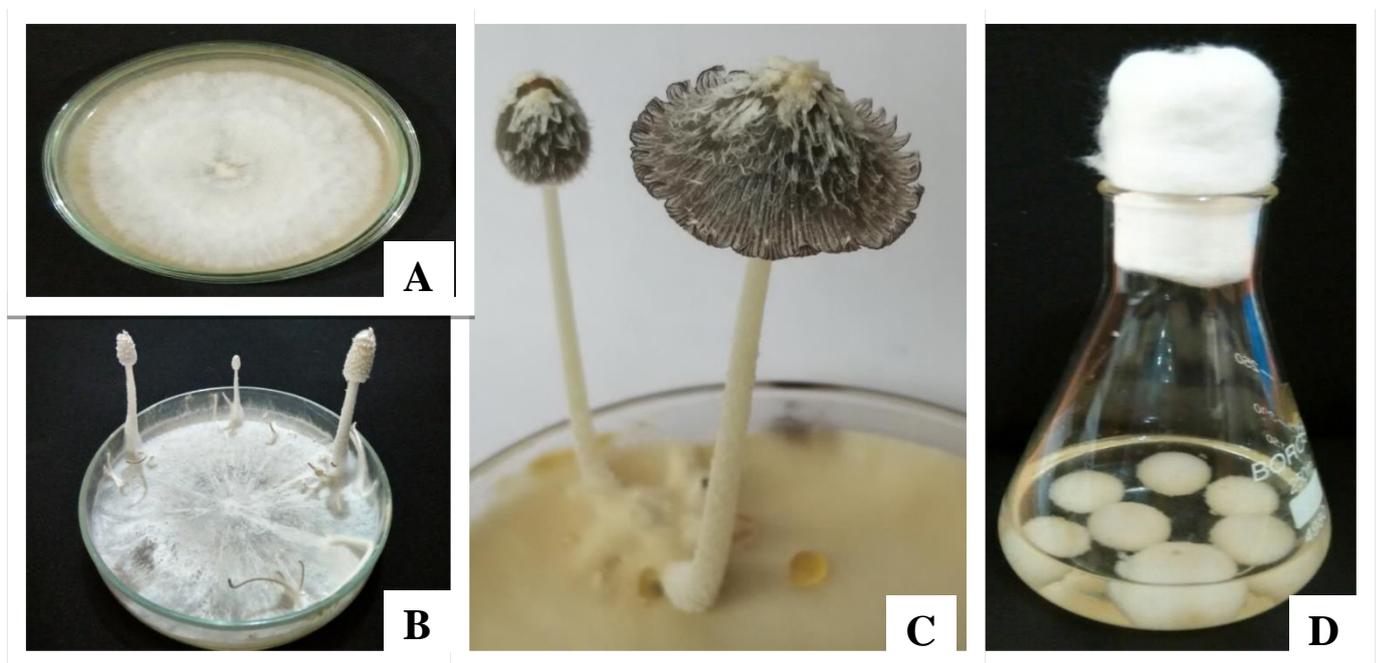


Fig 4 – Biomass production of *Coprinopsis cinerea* strain C2 on different culture media incubated for 10 days at 30° C, pH 6 under dark: MEA- Malt extract agar, MYA- Malt extract yeast extract agar, YMGA- Yeast extract malt extract glucose tryptophan agar, MEA- Malt extract yeast extract casamino acid agar, YMGA- Yeast extract malt extract glucose agar and WFA- Wheat flour agar. Six types of media induced fruiting body production of *C. cinerea* strain C2 incubated for 15 days at 37° C, pH 6 (Fig 8). However, wheat flour agar medium amended with 2% wheat flour supported faster growth and induced more number (4) of fruiting bodies compared to other media. In MYC medium we have observed only one fruiting body formation.



Figs 5 – Growth of *Coprinopsis cinerea* strain C2 on 2% wheat flour medium incubated for 10 days at 30° C, pH 6 under dark: A) Radial growth B) Fruiting bodies C) Mature fruit body D) Growth on wheat flour broth.

Coprophilous fungi are generally cultured on horse dung extract medium. But, however, the extract is not effective for induction of more number of fruiting bodies as it depends on the

freshness of the dung (Lange 1952). Cultures grown from stored dung failed to initiate fruiting body production although they supported normal vegetative growth (Eilers, 1972).

Media rich in disaccharides play a major role in mushroom growth and fruiting body induction of mushrooms compared to monosaccharide (Zhou et al. 2016; Cosgrove 1997). The nutrient profile of mushrooms influenced by the type of substrate on which they are cultivated (Kulshreshtha et al. 2014). Therefore, in our study, we searched for a better substrate providing rich source of disaccharides and other nutrients for the cultivation of *C. cinerea* under laboratory condition.

Several researchers have used synthetic media for growth and biomass production (Essig et al. 2014; Kaur et al. 2011; Dulay et al. 2016) of *C. cinerea*. Cultivation in combination with yeast extract, malt extract and peptone amended media (Sabotic et al. 2012) are although common, they failed to induce fruiting body and maximum biomass production. According to Jaekel et al. (2012) wheat flour has better nutritional and functional values, as it is a rich source of carbohydrates (80.76%), proteins (15.41%), lipids (2.12%) and ash (1.71%) and also contains fibers, minerals, vitamins, phenolic compounds, phytic acid, and phytoestrogens among other nutrients (Kulawinek et al. 2008). Carbohydrates in wheat flour are a mixture of disaccharide as well as monosaccharide such as sucrose (2.16 mg/g), maltose (0.57 mg/g), fructose (0.53 mg/g), and glucose (0.45 mg/g) (Codina et al. 2013).. We observed, the biomass and number of fruiting bodies increased significantly in wheat flour medium compared to malt extract amended media. Moreover, the wheat flour required for the growth of *Coprinopsis cinerea* was observed to be very low, just 2%, which enhanced the maximum growth and production of fruiting bodies.

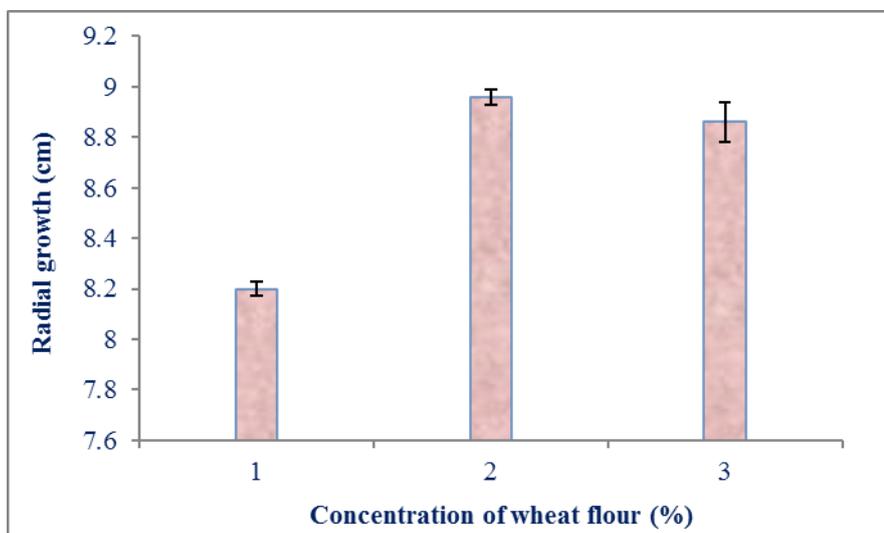


Fig 6 – Growth of *Coprinopsis cinerea* strain C2 on different concentrations of wheat flour media incubated for 7 days at 30° C, pH 6 under dark.

Our present study reports for the first time that wheat flour (2%) provides complete nutrient for maximum production of biomass and induction of fruiting bodies in *C. cinerea* without any additional supplement compared to other media reported earlier and tested by us. The preliminary work reported in this paper would certainly benefit the researchers working on mushrooms in general, and *C. cinerea*, in particular, as they have tremendous potential value in pharmaceutical and food industries.

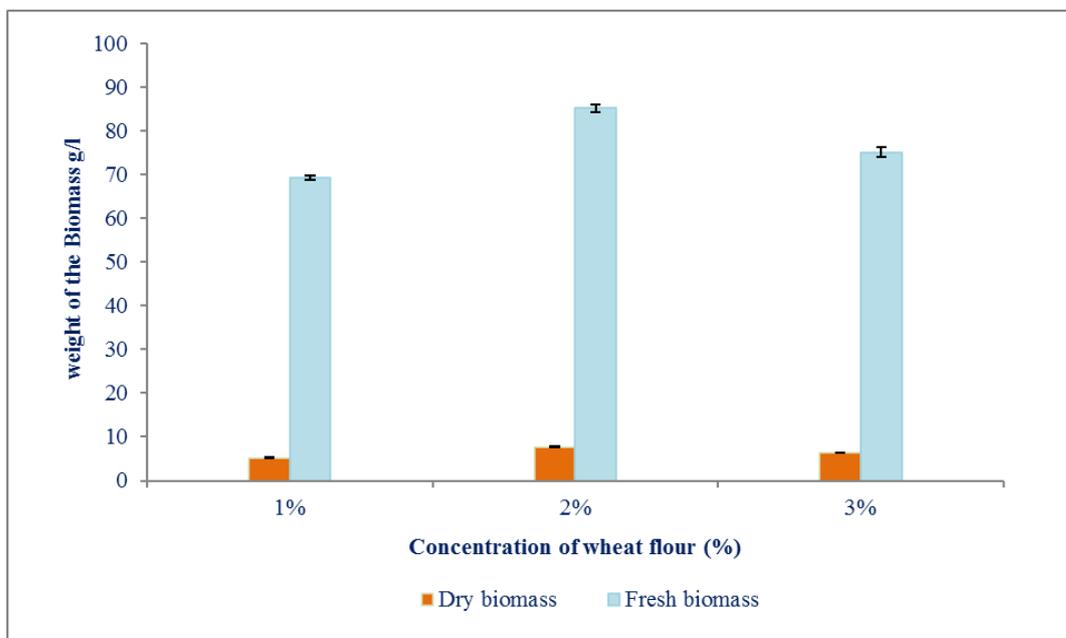
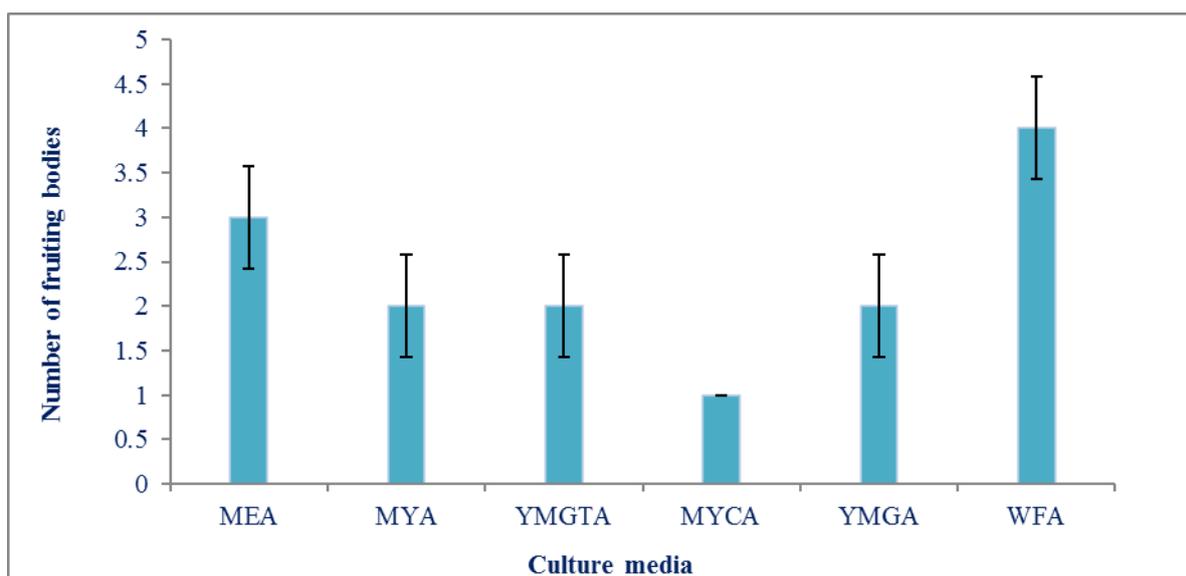


Fig 7 – Biomass production of *Coprinopsis cinerea* strain C2 on different concentrations of wheat flour broth incubated for 10 days at 30° C, pH 6 under dark



Figs 8 – Fruiting body production of *Coprinopsis cinerea* strain C2 on different culture media incubated for 15 days at 30° C, pH 6 under dark: MEA- Malt extract agar, MYA- Malt extract yeast extract agar, YMGTA- Yeast extract malt extract glucose tryptophan agar, MYCA- Malt extract yeast extract casamino acid agar, YMGA- Yeast extract malt extract glucose agar and WFA- Wheat flour agar

Acknowledgements

The authors would like to thank the Department of Microbiology and Biotechnology, Bangalore University, Bangalore for providing the facility to carry out this research work.

References

Altschul SF, Madden TL, Schaffer Aa, Zhang J et al. 1997 – Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research* 25, 3389–3402.

- Amandeep K, Atri NS, Munruchi K. 2014 – Taxonomic study on coprophilous species of *Coprinopsis* (Psathyrellaceae, Agaricales) from Punjab, India. *Journal of Fungal Biology* 5, 1–25.
- Badalyan SM. 2014 – Potential of mushroom bioactive molecules to develop healthcare biotech products. *Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Product* 13, 373–378.
- Barneche S, Jorcin G, Cecchetto G, Cerdeiras MP et al. 2016 – Screening for Antimicrobial Activity of Wood Rotting Higher Basidiomycetes Mushrooms from Uruguay against Phytopathogens. *International journal of medicinal mushroom* 18, 261–267.
- Barros L, Baptista P, Ferreira ICFR. 2007 – Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. *Food and Chemical Toxicology* 45, 1731–1737.
- Benson Da, Boguski MS, Lipman DJ, Ostell J et al. 1999 – GenBank. *Nucleic Acids Research* 27, 12–17.
- Boulianne RP, Liu Y, Aebi M, Lu BC, Kues U. 2000 – Fruiting body development in *Coprinus cinereus*: Regulated expression of two galectins secreted by a non-classical pathway. *Microbiology* 146, 1841–1853.
- Burns C, Stajich JE, Rechtsteiner A, Casselton L, Hanlon SE, et al. 2010 – Analysis of the basidiomycete *Coprinopsis cinerea* Reveals conservation of the core meiotic expression Program over half a billion years of evolution. *PLoS Genetics* 6.
- Codina GG, Mironeasa S, Voica DV. 2013 – Multivariate Analysis of Wheat Flour Dough Sugars , Gas Production , and Dough Development at Different Fermentation Times 31, 222–229.
- Cosgrove DJ. 1997 – Assembly and enlargement of the primary cell wall in plants. *Annual Review of Cell and Developmental Biology* 13, 171–201.
- Del Val AG, Plates G, Arenal F, Orihuela JC et al. 2003 – Novel illudins from from *Coprinopsis episcopalis* (Syn. *Coprinopsis episcopalis*) and the distribution of illudin-like compounds among filamentous fungi. *Mycological research* 107, 1201–1209.
- Donnell KO. 2016 – Mycological Society of America Molecular Phylogeny of the Nectria haematococca - *Fusarium solani* Species Complex Author (s): Kerry O ' Donnell Published by: Mycological Society of America Stable URL : <http://www.jstor.org/stable/3761588> REFERENCES Linked 92, 919–938.
- Doveri F, Sarrocco S, Pecchia S, Forti M, Vannacci G. 2010 – *Coprinellus mitrinodulisporus*, a new species from chamois dung. *Mycotaxon* 114, 351–360.
- Dulay RMR, Cardona EMG, Kalaw SP, Reyes RG et al. 2016 – CODEN (USA): PCHHAX Optimization of Liquid Culture Conditions of *Coprinopsis cinerea* as Natural Source of Bioactive Compounds 8, 313–319.
- Eilers FI. 1972 – An improved medium for coprophilous fungi. *Transactions of the British Mycological Society* 59, 347–349.
- Essig A, Hofmann D, Munch D, Gayathri S et al. 2014 – Copsin, a novel peptide-based fungal antibiotic interfering with the peptidoglycan synthesis. *Journal of Biological Chemistry* 289, 34953–34964. <http://doi.org/10.1074/jbc.M114.599878>
- Fukiharu T, Shimizu K, Nakajima A, Miyamoto T et al. 2015 – *Coprinopsis igarashii* sp. nov., a coprophilous agaric fungus from Hokkaido, northern Japan. *Mycoscience* 56, 413–418.
- Heleno SA, Ferreira IC, Calhella RC, Esteves AP et al. 2014 – Cytotoxicity of *Coprinopsis atramentaria* extract, organic acids and their synthesized methylated and glucuronate derivatives. *Food Research International* 55, 170–175.
- Heleno SA, Ferreira IC, Ciric A, Glamoclija J et al. 2014 – *Coprinopsis atramentaria* extract, its organic acids and synthesized glucuronated and methylated derivatives as antibacterial and antifungal agents. *Food and function* 5, 2521–2528.
- Heron JR. 1966 – Some Observations on Commercial Malt Extracts. *Journal of the Institute of Brewing* 72, 452–457.

- Hirasawa M, Shouji N, Neta T, Fukushima K, Takada K. 1999 – Three kinds of antibacterial substances from *Lentinus edodes* (Berk.) Sing. (Shiitake, an edible mushroom). *International Journal of Antimicrobial Agents* 11, 151–157.
- Huntley S, Hamann N, Wegener-Feldbrügge S, Treuner-Lange A et al. 2011 – Comparative genomic analysis of fruiting body formation in myxococcales. *Molecular Biology and Evolution* 28, 1083–1097.
- Jaekel LZ, Batista C, Steel CJ, Chang YK. 2012 – Influence of xylanase addition on the characteristics of loaf bread prepared with white flour or whole grain wheat flour. *Ciência e Tecnologia de Alimentos* 32, 844–849.
- Jukes TH, Cantor CR. 1969 – Evolution of protein molecules. *Mammalian protein metabolism* 3, 132.
- Kamada T, Sano H, Nakazawa T, Nakahori K. 2010 – Regulation of fruiting body photomorphogenesis in *Coprinopsis cinerea*. *Fungal Genetics and Biology* 47, 917–921.
- Kaur H, Dutt D, Tyagi CH. 2011 – Production of novel alkali-thermo-tolerant cellulase-poor xylanases from *Coprinopsis cinerea* HK-1 NFCCI-2032. *BioResources* 6, 1376–1391.
- Keirle MR, Hemmes DE, Desjardin DE. 2004 – Agaricales of the Hawaiian Islands . Agaricaceae : *Coprinus* and *Podaxis* ; Psathyrellaceae : *Coprinopsis* , *Coprinellus* and *Parasola*. *Fungal Diversity* 15, 33–124.
- Kirsch LDS, Macedo AJP, Teixeira MFS. 2016 – Production of mycelial biomass by the Amazonian edible mushroom *Pleurotus albidus*. *Brazilian Journal of Microbiology* 47, 658–664.
- Kitamoto Y, Gruen HE. 1976 – Distribution of Cellular Carbohydrates during Development of the Mycelium and Fruitbodies of *Flammulina velutipes*. *Plant Physiology* 58, 485–491.
- Kulawinek M, Jaromin A, Kozubek A, Zarnowski R. 2008 – Alkylresorcinols in selected polish rye and wheat cereals and whole-grain cereal products. *Journal of Agricultural and Food Chemistry* 56, 7236–7242.
- Kulshreshtha S, Mathur N, Bhatnagar P. 2014 – Mushroom as a product and their role in mycoremediation. *AMB Express* 4, 29.
- Kumhomkul T. 2015 – Lead accumulation in the inky cap mushroom (*Coprinopsis radiata*) and Bioaccumulation factors. *Thai Society of Higher Education Institutes on Environment*.
- Lange M. 1952 – Species concept in the genus *Coprinus*. *Dansk Botanisk Arkiv* 14: 7-162
- Martinez AT, Speranza M, Ruiz-Duenas FJ, Ferreira P et al. 2005 – Biodegradation of lignocellulosics: Microbial, chemical, and enzymatic aspects of the fungal attack of lignin. *International Microbiology* 8, 195–204.
- Money NP, Ravishankar JP. 2005 – Biomechanics of stipe elongation in the basidiomycete *Coprinopsis cinerea*. *Mycological Research* 109, 627–634.
- Naveed R, Hussain I, Tawab A, Tariq M et al. 2013 – Antimicrobial activity of the bioactive components of essential oils from Pakistani spices against *Salmonella* and other multi-drug resistant bacteria. *BMC Complementary and Alternative Medicine* 13, 265.
- Ndyetabura T, Lyantagaye SL, Mshandete AM. 2010 – Antimicrobial activity of ethyl acetate extracts from edible tanzanian *Coprinus cinereus* (schaeff) s. Gray s. lat. Cultivated on grasses supplemented with cow dung manure. *ARPJ. Agric. Biol. Sci* 5, 79–85.
- Plaza DF, Schmieder SS, Lipzen A, Lindaquist E, Kunzler M. 2016 – Identification of a novel nematotoxic protein by challenging the model mushroom *Coprinopsis cinerea* with a fungivorous nematode. *G3: Genes| Genomes| Genetics* 6, 87–98.
- Richardson MJ, 2001. Diversity and occurrence of coprophilous fungi. *Mycological Research* 105, 387–402.
- Rohmer L, Hocquet D, Miller SI. 2011 – Are pathogenic bacteria just looking for food? Metabolism and microbial pathogenesis. *Trends in Microbiology* 19, 341–348.
- Ruhl M, Majcherczyk A, Kues U. 2013 – Lcc1 and Lcc5 are the main laccases secreted in liquid cultures of *Coprinopsis cinerea* strains. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology* 103, 1029–1039.

- Sabotic J, Bleuler-Martinez S, Renko M, Caglic PA et al. 2012 – Structural basis of trypsin inhibition and entomotoxicity of cospin, serine protease inhibitor involved in defense of *Coprinopsis cinerea* fruiting bodies. *Journal of Biological Chemistry* 287, 3898–3907.
- Sabotic J, Ohm Ra, Kunzler M. 2016 – Entomotoxic and nematotoxic lectins and protease inhibitors from fungal fruiting bodies. *Applied Microbiology and Biotechnology* 100, 91–111.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013 – MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30, 2725–2729.
- Thompson JD, Gibson TJ, Higgins DG. 2002 – Multiple Sequence Alignment Using ClustalW and ClustalX. *Current Protocols in Bioinformatics*, Chapter 2, Unit 23.
- White TJ, Bruns S, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications*.
- Zhou S, Ma F, Zhang X, Zhang J. 2016 – Carbohydrate changes during growth and fruiting in *Pleurotus ostreatus*. *Fungal Biology* 120, 852–861.