ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 6, Issue 4, 2013

ISSN - 0974-2441

Research Article

IN VIVO UTILIZATION OF SEAFOOD PROCESSING WASTES FOR CULTIVATION OF THE MEDICINAL MUSHROOM (*GANODERMA LUCIDUM*) USING AGRO-INDUSTRIAL WASTE

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Received: 19 July 2013, Revised and Accepted: 11 August 2013

ABSTRACT

Objective: To examine the utilization of seafood processing wastes for artificial cultivation of medicinal mushroom and composting in laboratory condition.

Method: The selected agro-industrial wastes (e.g., coir pith, woodchips, sugarcane bagasse) were mixed with fishery waste in specific ratio (1:1).The substrates which are not mixed with fishery waste are regarded as control. All the above materials (1:1,control) were allowed to decompose about 15 days. The composted materials were placed in heat resistant transparent polyethylene bags. Each sterile bag was then aseptically inoculated with *G.lucidum*. The bags were then incubated under ambient temperature and controlled humidity.

Results: The maximum biological yield per bed was obtained with sugarcane bagasse control bed 64.78 g/bed.The lowest yield was observed in woodchips (1:1) 4.6 g/bed. Based on mass obtained of *G.lucidum* the best substrates were ordered of like woodchips>coir>sugarcane.

Conclusion: The scope of this work is sea food wastes could be used to cultivate a medicinal mushroom while at the same time promoting environmental sustainability.

Keywords: Mushroom, fishery waste, solid substrates, biological yield.

INTRODUCTION

Fish waste can also be utilized in the production of organic fertilizers and composts, which have significant benefits over chemical based products. Fish waste can result in water quality problems at marinas with large numbers of fish landings or at marinas that have limited fish landing but poor flushing. The amount of fish disposed of in to a small area such as a marina can exceed that existing naturally in the water at any one time. Fish waste decomposes, which requires oxygen. In sufficient quantity, disposal of fish waste can thus be a cause of dissolved oxygen depression as well as odor problems [8, 18]. The catching and processing of fish generates a significant amount of waste. Of a total UK fish and shellfish resource of 851,984 tones, it is estimated that 43% (359,964 tones) ends up as products for human consumption and the remainder (492,020 tones) is classed as waste. The majority of waste is produced in the on-shore processing sector (35% of the resource) whereas discards and processing waste at sea produce smaller quantities (17% and 5% respectively of the resource).The fish industry developed around fishing ports when landings were plentiful and there was little concern about environmental impacts. Fish waste is rich in valuable minerals, enzymes, pigments and flavors that are required by many industries including food, agriculture, aquaculture and pharmaceuticals. Possible alternatives include silage production, which has potential in livestock feeding. Basidiomycetous fungi (mushrooms) can be defined as "macro fungi" with distinctive fruiting bodies that are large enough to be seen by the naked eye and to be picked by hand. It is estimated that there are approximately 1.5 million species of mushrooms in the world of which approximately 70,000 species are described. About 10,000 of the known species belong to the macro fungi of which about 5,000 species are edible and over 1,800 species are considered to have medicinal properties[3].The fruiting body, mycelia and spores of *G. lucidum* contain approximately 400 different bio active compounds which mainly include triterpenoids, polysaccharides, nucleotides, sterols, steroids, fatty acids, proteins/peptides & trace elements [19,12,17,16,26].A successful artificial cultivation of *Ganoderma lucidum* has been reported on most broad-leaf hardwood trees and commonly used species include oak, pecan, elder, choke cherry, and plum[4,6].It is normally cultivated on solid substrates or other lignocelluloses materials such as straw, sawdust and log [24,25].*Ganoderma* species can be cultivated on the sawdust which may originate from different kinds of trees[30,20].Hardwood sawdust is the basic substrate for the cultivation of most medicinal mushrooms [5] and the growth and development of them varied from one tree species to another[1].Hardwood sawdust and wheat bran mixture may be used in production of *Ganoderma lucidum* [21,35].*Ganoderma lucidum* has been reported to have a number of pharmacological effects including immunomodulating, anti-atherosclerotic, anti-inflammatory, analgesic, chemopreventive, antitumor, radio protective, sleep promoting, antibacterial, antiviral (including anti –HIV) , hypolipidemic, anti fibrotic, hepatoprotective, diabetic, anti–oxidative and radial scavenging, anti– aging , hypoglycemic and anti-ulcer properties.[34,4,15,13,19,12,32,33,26].Recently more studies demonstrated that *G. lucidum* contained anti bacterial constituents that are able to inhibit gram–positive and / or gram negative bacteria [34,25,13,19,11,26,27].*Ganoderma lucidum* may also have hypoglycemic activity, anti-inflammatory effects, and cytotoxicity toward hepatoma cells[9,2]. Bioactive polysaccharides in mushrooms can often be extracted from mycelia of the species without waiting for a full fruiting body to develop [28,14].The aim of the study is to reduce environmental pollution while producing medicinal mushroom.

MATERIALS AND METHODS

Tissue Culture Technique

A large healthy and fresh mushroom *G.lucidum* collected from the Chozhavanthan at Madurai district, Tamil Nadu and India. It should be cleaned with 75% alcohol. The mushroom should be split in half by hand longitudinally and some inside tissue taken from the upper part of the stripe. It should be placed centrally on the surface of the sterilized Rose Bengal agar with a sterilized needle and kept at room temperatures (28°C ± 2) for 8 days. Within two or three days some white, delicate mycelia will be produced from the small piece of the tissue. About eight days later the mycelium will grow rapidly and cover the surface of the agar medium. Then it will be ready to transfer to spawn substrate to make spawn. This spawn is used as inoculums for cultivation of mushroom.

Spawn production

The sorghum was cleaned manually to remove inert matter, stubble and debris. The cleaned grain was soaked in 0.5% Cu SO_4 for 10 min and the soaked grain was thoroughly washed and soaked in tap water for 2 hours. Thereafter, the soaked grain was drained and the excess water removed. Spawn containers should be made out of heat resistant material: mostly glass and polypropylene (PP).About 250 g of grain medium was filled in to polypropylene bag. It was sealed by using cotton wool plugged with poly vinyl chloride pipe ring and covered by a piece of paper by tying a rubber band around the neck. Autoclaved at 121°C, 15 psi, for 30 min and the sterilized bag was allowed to cool for 24 hours. Then immediately inoculated with mycelial culture of *G.lucidum* maintained on Rose Bengal agar.

Fish Compost preparation

Fishery wastes were collected from the food processing unit at Thoothukudi which contains head, tail, shells, intestine, fins, dead fishes and so on. The wastes obtained were brought to the laboratory and the un compostable materials such as shells and large bones were removed and the remaining wastes were cut into small uniform size pieces. Disease-free agro-industrial wastes were collected from Thoothukudi district which were cut in to small pieces (2-3cm) and sun-dried in order to achieve proper drying.The selected agro-industrial wastes were mixed with fishery wastes in specific ratio 1:1(500:500) which are not mixed with fishery wastes are regarded as control. All the above materials (1:1,control) were heaped in a separate plastic container and allowed to decompose for about 15 days. During the decomposition, water was sprayed over the materials with turning and restacking for every day to produce homogenize composed. Finally, the Composted materials were used for mushroom cultivation.

Mushroom bed preparation

Polypropylene bags of 14×18; 14×12 size were taken and the bottom of the bag was tied with a thread to provide a flat circular bottom of the mushroom beds. Composted materials were placed in polypropylene bags were sterilized at 121°C for 2 hrs, after cooling, the spawning was done 3-5 layers. The neck of the bag was prepared by using heat resistant PVC tube and plugged with cotton, then transferred to the culture room at 25-32°C temperature, 85-95% relative humidity. Water was sprayed 3-4 times per day and proper aeration was maintained in culture house to develop the fruiting bodies. After 15-20 days, the pinheads of *G.lucidum* emerged from the mouth of polypropylene bags.The cap formation of *Ganoderma lucidum* initiated in 2-3 days after opening the mouths of bags. Fruiting bodies were harvested when the caps become completely red and the white margin disappeared [22]. Experimental design was a Completely Randomized Block with three replicates.Data on period of after completion of mycelium running, biological yield, days of first harvest, number of fruiting bodies, length, diameter, biological efficiency, moisture content of mushroom and dry yield were recorded.The biological efficiency (BE) percentage [fresh weight of harvested mushrooms/dry matter content of the substrate] x100 [23], productivity (P)percentage (Mushroom dry weight / Compost initial dry weight) x 100 and percentage of consumption (Before cultivation –After cultivation) /100 was calculated. Compared the means with one-way ANOVA. The difference was statistically significant when $p < 0.05$.

Determination of moisture content

The moisture content of the mushroom was estimated by drying in an oven at 80°C for three consecutive days. It was cooled in a desiccator and weighed. The moisture content was calculated by the following formula.

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

RESULT AND DISCUSSION

Development of fruiting body

The reproductive growth of *Ganoderma lucidum* varied from one substrate to another. The first pin head formation within 16 days, was recorded on woodchips control. The highest time required for pin head formation (47.66 days) was recorded in sugar cane waste 1:1. The lowest period of fruiting body formation (64.00 days) was observed in woodchips control .The highest period of fruiting body formation (102.33 days) was observed in sugar cane waste 1:1. [10] demonstrated that Polypore mushroom was found to be rich in protein, fiber and cellulose. Polypore mushroom is found to grow on rotting hard wood logs. So, attempt was made to cultivate the species in laboratory condition. [31] also showed that *G.lucidum* grown on a wide variety of dead or dying trees, commercial production in the wood.

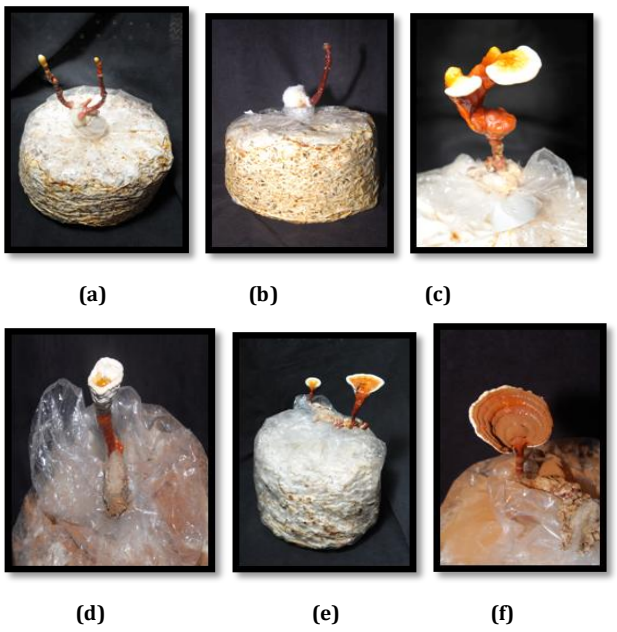


Fig.1:Growth of Ganoderma lucidum in (a)Woodchips control(b) Woodchips 1:1 (c) Coir pith control (d) Coir pith 1:1 (e) sugarcane control (f) sugarcane 1:1

Size of fruiting body

Length and diameter of fruiting bodies produced on agro-industrial wastes and fishery wastes ranged from 5.80 to 12.00 cm and 1.16 to 9.40 cm respectively (Fig 2). The highest length of fruiting body (12.00 cm) was recorded on coir pith control. While highest diameter of fruiting body (9.40 cm) was found on coir pith control. The least length of fruiting body in sugarcane waste (1:1) (5.80 cm) and diameter of fruiting body (1.16 cm) were recorded in woodchips (1:1)respectively.[31] reported that *G.lucidum* is traditionally cultivated in solid cultures. Solid cultures are used to obtain basidiocarps for tonic or tea. *G.lucidum* mycelium or spawn have normally been produced in solid cultures using substrates such as grain, sawdust or wood. The advantage of solid stage fermentation (SSF) over other technique is that a concentrated product can be obtained from a cheap substrate such as an agricultural residue with little pre-treatment or enrichment.

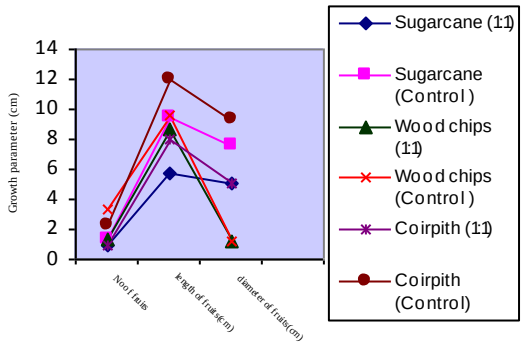
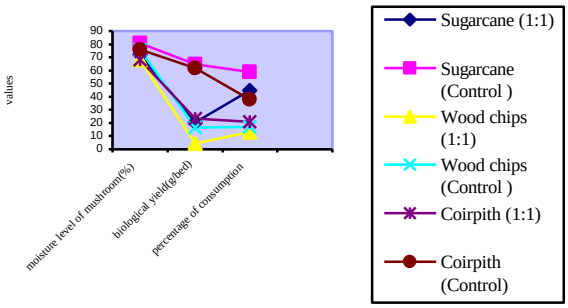


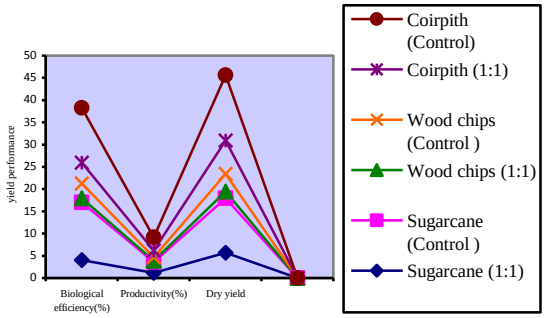
Fig.2: Effect of agro-industrial wastes on the development and size of fruiting bodies of *G.lucidum*

Biological yield, dry yield and biological efficiency

Significant difference was observed in biological yield, dry yield and biological efficiency of *Ganoderma* mushroom on agro-industrial wastes (Fig 3a and Fig 3b). The highest biological yield (64.78g/bed) was obtained from sugarcane waste control (Fig 1e) followed by coir pith control (61.73g/bed) (Fig 1c), coir pith 1:1 (23.15 g/ bed) (Fig 1d),sugarcane waste 1:1 (20.33g/bed) (Fig 1f) and woodchips control (16.7g/bed) (Fig 1a).woodchips 1:1 gave poorest yield (4.6g/bed) (Fig 1b).Dry yield was observed in coir pith control(14.73g/bed) followed by Sugarcane control (12.25g/bed),Coirpith1:1(7.45g/bed), Sugarcane 1:1 (5.70g/bed), Woodchips Control (4.0g/bed) and Wood chips 1:1 (1.49g/bed).The biological efficiency was highest (12.95%) sugarcane waste control in which was followed by coir pith control (12.34%),coir pith 1:1(4.63%) ,sugarcane waste 1:1 (4.066%)and woodchips control (3.34%) while lowest biological efficiency (0.92%) was observed in woodchips 1:1.[29] also suggested that various substrates for *G.lucidum* cultivation have been investigated as a supplement for substrate mixer. A successful artificial cultivation has been reported on solid substrates, utilizing saw dust and agricultural waste as the main components. The suitability of rice bran, rice husks, coconut fiber, peanut hulls, corn, sorghum and sugarcane bagasse as supplements for the substrate mixture and rice bran, ground corn and ground sorghum were found good supplements compared to some other agricultural residues such as rice husk, coconut fiber, peanut hull and sugar cane bagasse. Fig 3 shows effect of agro-industrial waste on percentage of food consumption, moisture level and productivity of *Ganoderma lucidum*.



[a]



[b[

Fig.3: Effect of agro-industrial wastes on yield of *Ganoderma lucidum* (a)moisture level of mushroom, biological yield and percentage of consumption (b) biological efficiency, productivity and dry yield.

CONCLUSION

This research is to looking at the possible use of fish waste and agro-industrial wastes for mushroom cultivation, which would provide medicinal food and encourage the biological conversion processes of agro-industrial wastes. Composting fish waste is a relatively new, practical and an environmental sound alternative to disposing of fish waste. It is economical, fairly odorless and a biologically beneficial practice for seafood operations. The study has shown that composting is safe, Practical and a cost effective solution for the seafood industry.

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