PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 8 (7): 1052-1054, 2009
ISSN 1680-5194

Cultural Studies of Mycelia of *Volvariella volvacea, Pleurotus tuber-regium* and *Pleurotus sajor-caju* on Different Culture Media

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Abstract: Various agar culture media were investigated for culturing the mycelia of *Pie urotus. sq/or caju, P. tuber-re glum* and *V voivacea* edible mushrooms. The study was conducted at Cross River University of Technology, Calabar Campus, Nigeria. Selected mushroom species were cultured to source for low input, cheap and other method of growing active mycelia, for the production of viable mushroom spawn (seeds). The results revealed that *P. sajor caju* had the highest mycelia growth (7.8 cm) on rice bran/soil culture media while *P. tuber-regium* had the highest mycelia growth (5.8 cm) on cassava peels/soil culture media. *V. voivacea* had the highest mycelia growth (7.1 cm) on palm fibre culture medium. The least mycelia growth (1.5-4.4 cm) was observed on potato dextrose agar culture media and yeast agar culture media. Therefore natural supernatant extracts culture media stimulated higher mycelia growth than synthetic agar culture media employed in this study. Thus it is recommended that *P. sajor caju, P. tuber-ragium* and *Vvoivacea* mycelium can be grown culturally on rice bran/soil, cassava peels/soil and palm fibre culture media respectively.

Key words: Media, agar, supernatant, mycelia growth

INTRODUCTION

This study investigated into the performance of *Plerotus sajor caju* and *Volva volvaceae* on the different culture media in the Laboratory. Inability to culture edible mushrooms has prevented interested persons into the business of mushroom production. The techniques employed in this study will assist farmers cultivate mushrooms. It is an economic driven research especially for rural farmers. Also production of active mycelium, which this study deals with, is one of the most critical stages of mushroom cultivation, which has prevented a lot of interested and would be mushroom farmers from getting involved. Once people are sure of source and availability of mushroom seeds (Spawn) much more interest will be developed. It might even become a hobby or passion beside income generation. In Europe, North America, Japan, China, South East Asia and Australia where adequate technology are available mushrooms are cultivated for export trade and local consumption. Ugandan mushroom growers for instance are currently selling 44 tones per year to Japan, 40 tones to U.S.A and 2 tones to the Democratic Republic of Congo (Spore, 2006). Today China is the major producer of mushroom in the world (Qei, 1992).

In contrast to the cultivation of higher plants, the culture of fungi is relatively a recent innovation. Just as plants have seeds responsible for propagating the race, mushrooms like all fungi produces spores, which are minute and microscopic and are dispersed by wind. These spores germinate and develop mycelium as long as conditions are favourable, it continues to grow, ramify and absorb nutrient from the media on which it grows. In this manner the fungi race is perpetuated.

Culturing began in France about 1894, Fergusen (1902) and Duggan (1905) gave the method of preparation of pure culture through tissue culture or spore culture. Jenison (1948) and Kaul (1981) reported that when meals of grains, legumes, orange, banana, cetery, alfalfa, parsnip, corn steep and gluten extracts are incorporated in agar mycelia growth are accelerated. Excellent growth of mycelia due to incorporation of malt and yeast extracts in agar was observed with *Coprinusfimetarius* (Fries, 1948) *Agaricus blazei* (Block *et al*., 1953) *Fomes lignosus* (Riggenbach, 1957) and *Aurcularia Spp* (Quimio, 1981). Volz and Berneke (1991) ascribed the beneficial effect of these extracts on mycelia growth to the thiamine, amino acids and nutrient contents of the extracts.

Kadiri and Kehinde (1990) investigated the effect of glutamic and aspartic acids and their corresponding keto acids and half amides on mycelia growth of *Tricholoma* species. They found all the compounds to accelerate mycelia growth. According to Miles and Chang (1997) the active mycelia which is used to produce mushroom seeds (spawn) depends on agar medium for food substances necessary for its growth and ramification. Jablonski (1981), Ingold and Hudson (1993) and Poppe (2000) reported that good culture

media can influence mushroom growth and this also depends on available nutrients, pH, microbial activities, aeration, water content or free water activity. Edward (2000) also reported that the more easily accessible nutrients are, the more dense the mycelia ramification. All these influence on mycelia growth are due to the contents of the culture media, clean environment and procedure adopted.

MATERIALS AND METHODS

Palm fibre culture media: Spent palm fibre, being the pericap waste (discarded) after the oil extraction process was collected from a palm oil mill at Awi in Akamkpa Local Government Area of Cross River State, Nigeria.

Palm fibre culture media was made using 150 g of palm fibre waste, 1 litre of distilled water added to the waste in a 500 m1 glass beaker. This was placed in a steaming water bath for 2 h. It was allowed to cool to room temperature; filtered with a piece of muslin cloth and the supernatant retrieved. 7 g of pure agar (Agar technical) was added to the palm fibre extract (300 ml) to give approximately 20 g I-L boiled to dissolve and autoclaved at 121°C for 15 min. Palm fibre culture agar was then dispensed into Petri dishes (85 mm). The same process was adopted in obtaining rice bran; sawdust, cassava peels and garden soil culture media.

Palm fibre/garden soil culture media: Palm fibre extract and garden soil extract were equally combined to make a volume of 300 ml. 7 g of pure agar was added to this combination (300 ml) to give approximately 20 g boiled to dissolve and autoclaved at 121°C for 15 min and dispensed into Petri-dishes (85 mm). The same process was used in obtaining other combinations such as cassava peels/soil culture media and rice bran/soil culture media.

Potato Dextrose Agar (PDA) and Yeast Agar (YA) culture media: These agar media were prepared as described by Chang and Hayes (1978). 39 g of potato dextrose agar was added to 1 litre distilled water, placed in a boiling water bath to dissolve agar. It was autoclaved at 121°C for 15 min. After cooling, it was then dispensed into Petri dishes (85 mm). The same process was adopted in obtaining yeast agar culture media adding 23 g to 1 litre of distilled water.

Matured mushrooms of the selected species under study were collected from the wild and washed in 5% bleach and rinsed with distilled water. Spores were collected aseptically and inoculated on the different media in the Petri dishes.

RESULTS

The results on suitable culture media for *F. sajor caju, F. tuber-re gium* and *V. volvacea* are presented on (Fig. 1). The result showed that *P. sajor caju* had the highest

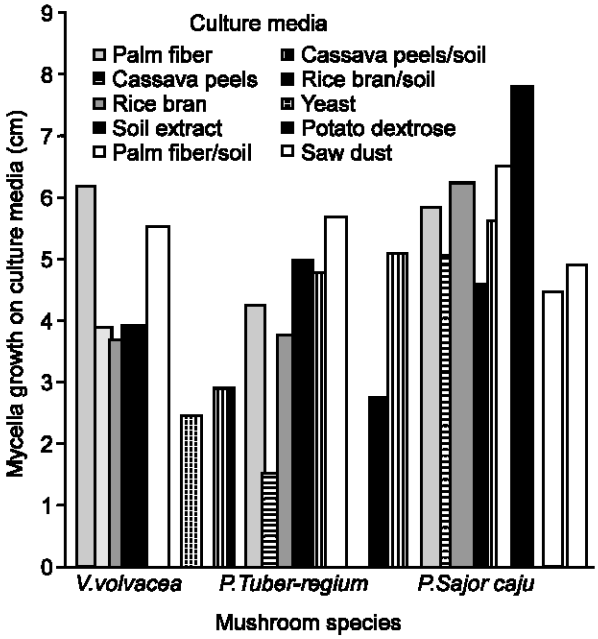

Fig. 1: Response of selected edible mushrooms species to different culture media

mycelia growth (7.8 cm) on rice branlsoil culture media. The result also revealed that *P. sajor cju* recorded (6.3 cm) and (6.1 cm) mycelia growth on cassava peels/soil culture media and rice bran culture media respectively. The least mycelia growth (4.2 cm) for this species was recorded from potato dextrose culture medium. There was no growth on yeast culture medium for this spp as indicated by this result. The result also showed that *P. tuber-regium* had the highest mycelia growth (5.8 cm) on cassava peels/soil culture media. This was followed by (5.0 cm) mycelia growth on sawdust culture media. The lowest mycelia growth (1.5 cm) for *P. tuberregiurn* was recorded from cassava peels culture media. No growth was observed on yeast and rice bran'soil culture media. The result also revealed that *V. volvacea* had the highest mycelia growth (7.1 cm) on palm fibre culture media. This was followed by (5.7 cm) mycelia growth on cassava peels/soil culture media. The least mycelia growth (2.3 cm) for this specie *(V. volvacea)* was observed on yeast culture media. There was no mycelia growth on potato dextrose culture media and rice bran/soil culture media for *V. Volvacea*.

DISCUSSION

All the supernatant culture media stimulated higher mycelia growth than synthetic agar culture media such as potato dextrose culture media (PDA) and yeast culture media (YEA) employed in this study. The result on culture media indicates that *V.volvacea, J'.tuber regium* and *P. sajor caju* can grow very well on cassava peels/soil and rice bran/soil culture media respectively.

These results compare favourably with the findings of Jenison (1948) and Kaul (1981) who obtained excellent mycelia growth due to incorporation of agar in legumes, molasses, gluten, meals of grains, corn steep, extracts of orange, banana, celery, alfalfa, parsnip etc. This result is further supported by Quimio (1981) who obtained accelerated mycelia growth of *Auriculari* Spp due to incorporation of malt and rice bran extracts in agar. The stimulatory substances suspected to be present in the culture media were amino acids, vitamins and essential nutrients, which combined to influence the growth of mycelia on these culture media. This view is also supported by Kadiri and Kehinde (1990) who investigated the effect of amino acids and aspartic acids and their corresponding keto acids and half amides on mycelia growth of *Tricholoma* species and found all the compounds to accelerate mycelia growth.

The purpose of culturing mushroom mycelia is to boost it to a state of vigour such that it will rapidly colonise the selected organic matrix for spawn (mushroom seeds) production.

Conclusion and recommendation: Mushroom farming is not just a rapidly expanding agribusiness, its also a significant tool for the restoration, replenishment and remediation of the earths overburden ecosphere. The rapid development and growth of the mushroom industry from a punitive cave culture into one using technical and controlled methods, ensures that interested farmers and those who are growers may not need to worry about spawn (mushroom seeds) sources. Palm fibre, rice bran/soil and cassava peels/soil culture media are suitable media for culturing *V. volvacea*, *P. sajor caju* and *P. tuber-re gium* mycelium respectively. They stimulated luxuriant mycelia growth and extension better than other media employed in this study. It is however recommended that the use of farm wastes supernatant extracts as culture media be encouraged in culturing and boosting the vigour of mushroom mycelia for spawn (seed) production. Farmers are also encouraged to make enquiries at any stage of their production. And always work in a clean, sterile and aseptic environment whether in the laboratory or in the mushroom house.

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