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Effect of Three Different Culture Media on Mycelial Growth of Oyster and Chinese Mushrooms

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Abstract: The sporocarps obtained from NARC, Islamabad and local market were utilized as a source of tissue culturing. Initial culturing was done on malt extract agar plates. The discs (0.5 cm in diameter) from actively growing culture plates of three different mushrooms were planted on the fresh media plates of Malt extract agar medium (MEA), Murashige and Skoog's (MS) medium and potato dextrose agar (PDA) medium. Three replicates were taken in each case. An increase in diameter of the culture discs was recorded daily. The results indicted that the mycelial growth of *Pleorotus ostreatus*, varieties sajar, caju , citydeosus and *Volvariella volvacea* were maximum in medium plates containing MEA medium. The results were significant at P=0.05 level of significance. While potato dextrose agar medium (PDA), ended up with slowest growth.

Key words: Culture media, oyster mushroom, Chinese mushrooms

Introduction

Fungi inhabit every possible environment, including many unlikely ones, utilizing the organic materials from plants and animals and even other fungi for their nutrition and energy source. Unlike the chlorophyll containing plants, which convert solar energy into chemical energy, fungi, like animals, are totally dependent on the available organic material for all their nourishment. Unlike the animals, most fungi are stationary and can't pursue their food. (Kendrick, 1985; Alexopolus and Mims, 1996).

The fungi responsible for producing grand fruiting bodies are called mushrooms. These belong to two of the large classes of fungi i.e., Basidiomycetes and Ascomycetes (Webster, 1980; Dix and Webster, 1995).

Out of 250,000 species of fungi known as yet about 2000 are included in the list of edible fungi. While only 5-6 are grown on large scale (Ford and Clark, 1914). In different countries of Europe, at holiday, people of different communities are wandering in the forest having baskets in their hands in search of mushrooms. They enjoy by hunting different types of mushrooms, (Rehman and Shakir, 1997). Well one has to be extremely careful in deciding what to eat and what not to eat as regards mycophagy because some of the mushrooms are deadly poisonous (Benjamin, 1995).

Food stuffs of plants origin such as cereals, vegetables, potatoes and pulses constitute an important dietary source of protein for many segments of world's population particularly where animal protein is not only in short supply (Kadada, 1974) but are beyond the reach of middle and poor classes. Among unconventional sources of protein, higher fungi particularly mushroom stand out as a distinct class (Falanghe, 1967). The protein contents of these food stuffs i.e., vegetables, cereals etc. is low as compared to mushroom (Hayes and Haddad, 1976; Jandaik and Kapoor, 1975; Bano *et al*., 1980). For overall nutrition, mushroom falls between the best vegetables and animal protein sources, (Benjamin, 1995).

From the historical point of view, mushroom have prominent status in every era. Sometimes it had prominent religious status and sometimes it had regarded as spiritually important. It has been regarded as a symbol of glory and prestige in the past (Kausar, 1988). Today, mushrooms are loved the world over as delicacy to embellish taste and flavour of other dishes. The researchers have revealed that in addition to delicate flavour and snobvalue, mushrooms are rich source of proteins, amino acids, minerals (Jandaik & Kapoor, 1975; Hayes and Haddad, 1976) and vitamins (Kezeli and Dzabaridee, 1984).

Cultivation of mushroom is widely practiced in Europe, North America, Taiwan and China. The Oyster mushrooms (*Pleurotus* spp.) have been cultivated in large quantities in Japan for several years. Commercial production and consumption of mushroom has increased dramatically during the past few years in Europe, Asia and United States (Edward, 1977; Lelley, 1982).

In Pakistan, the industry of mushroom is newly borne and is progressing slowly as compared to other countries of the world. The causes of this slow progress of mushroom industry are shortage of technically trained people, presence of no research laboratories and ignorance of the people to advantages and importance of mushroom. Mushroom has a lot of production potential and due to its rapid growth it gives so large amount of crop which could not be compared with any other crop (Robinson and Davidson, 1959). Pakistan is rich in all type of natural resources and it has territories with different temperature and weathers which are suitable for mushroom cultivation. Suitable temperature and humidity are required for mushroom cultivation (Singh, 1981) which are available naturally in northern areas of Pakistan. These conditions could be attained at low cost in plain areas of Punjab, Sind and Balochistan. Climatic conditions in Pakistan for successful cultivation of all types of edible mushroom. (Rehman and Shakir, 1997).

In the present study, three types of mushrooms were cultured on different media i.e., MEA, PDA and MS and their mycelial growth rate was determined The purpose of present study was to:

1) Signify a medium for the best growth of mushroom.
2) Reduce the duration of the growth period.

Materials and Methods

Experiments were conducted with two varieties of oyster mushroom (*Pleorotus ostreatus*) and one of Chinese (*Volvariella volvacea*) mushrooms. The two varieties of oyster mushroom were sajar caju and citydeosus.

Preparation of the starter culture: Two methods were followed

to raise the starter culture. These were tissue culture and spore culture techniques.

Tissue culture technique: For this purpose fresh mushrooms from the market and NARC (National Agriculture Research Council) were obtained. The specimens were then carefully brought back to the biocontrol lab. in a carton. The petriplates containing malt extract agar medium (MEA) were inoculated aseptically by taking deep tissue of freshly harvested mushroom, with the help of a sharp aseptic razor. The petriplates were incubated at 25 ± 1 °C for 2-3 weeks. By this time the petriplates become covered with white mycelium.

Spore culture technique: A sterilized filter paper was taken and put into a sterilized petriplate aseptically. The pileus of each of the three varieties were placed aseptically on the filter paper. The plate was immediately covered with a lid. These plates were kept undisturbed for 24 hours. The spore print thus obtained on the filter paper was used to prepare mushroom culture on the medium plates. The spores were transferred from the spore print to the fresh medium (MAE) plates aseptically with the help of a sterilized razor. The patriplates were incubated at 25 ± 1°C for 2-3 weeks untill the spores started to grow.

Steps involved in the spore/tissue culturing

Washing and sterilization of the apparatus: The apparatus was washed properly and dried. The apparatus was then sterilized in the autoclave at 15 lb/sq. inch pressure for 15 minutes. The sterilization can also be done in an oven at 120°C temperature for 2 hrs.

Media preparation:

Following media were used for the purpose:

PDA – potato oextrose agar medium

Potato	250g
Dextrose or white sugar	20g
Agar-Agar	20g
Water	1000ml

MEA – malt extract agar medium

Malt Extract	20g
Agar-Agar	20g
Water	1000ml

MS – murashige and skoog's medium

Major Salts

Macronutrients – 10 X g/l

KNO_3	19
NH_4NO_3	16.5
$MgSO_4.7H_2O$	3.7
KH_2PO_4	1.7
$CaCl_2.6H_2O$	4.4

Micronutrients – 100 X mg/l

H_3BO_3	620
$MnSO_4.4H_2O$	223
$ZnSO_4.7H_2O$	860
$Na_2MnO_4.2H_2O$	25
$CuSO_4.5H_2O$	2.5 mg/100ml
$CaCl_2.6H_2O$	2.5 mg/100ml

Final volume – 100ml

Vitamins – 100X mg/100ml

Nicotianic acid	50
Thiamine Hcl	50
Pyridoxin Hcl	10
Myoinisitol	10,000

Fe – EDTA – 10 X 1 liter

Dissolved 5.57g of FeSO4 in 350 ml of water (heat if required). Dissolved 7.54g of Na_2EDTA in 350 ml of water (heat if required).

All three media were prepared according to above-mentioned composition by dissolving the chemicals in water, taken in 1000ml flask.

Sterilization of media: The flasks having media were sterilized in the autoclave at 15lb/sq inch pressure and 121 °C temperature for 15 minutes.

Pouring of media: The sterilized media were poured aseptically into the petriplates (11cm in diameter) which were previously sterilized. The petriplates were left to cool and the media to solidify.

Comparison of growth rates of mushroom mycelia on culture media: Mycelia of the oyster and chinese mushrooms raised through tissue /spore culturing techniques were processed further. Pieces (0.5 cm in diameter) from the culture plates of each of the mushroom variety were removed with the help of a cork borer aseptically. These were then planted on fresh media plates of each of the three replicates of each treatment viz., MEA, MS and PDA. These plates were incubated at 25 ± 1 °C temperature. The growth of mushroom mycelia on these replica plates was recorded daily with the help of a transparent ruler.

Results and Discussion

Considerable literature is now available regarding the cultivation of edible mushrooms. For example, Zadrazil (1997), has recently worked on changes *in vitro* digestibility of wheat straw during fungal growth and after harvest of oyster mushroom on laboratory and industrial scale. His results indicated that some strains of *Pleurotus* increased *in vitro* digestibility of cereal straw by 63% under laboratory

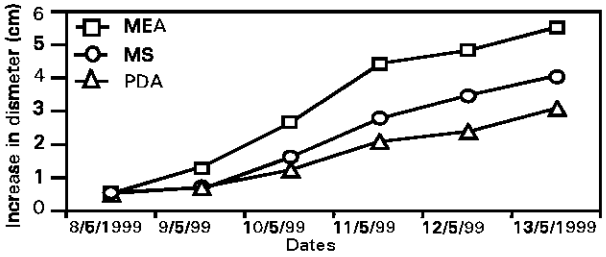

Fig. 1: Mycelial growth of *Pleurotus ostreatus* var.sajar caju in terms of increase in diameter on different culture media.

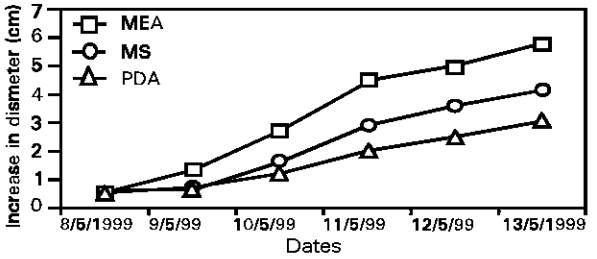

Fig. 2: Mycelial growth of *Pleurotus ostreatus* var.citydeosus in terms of increase in diameter on different culture media.

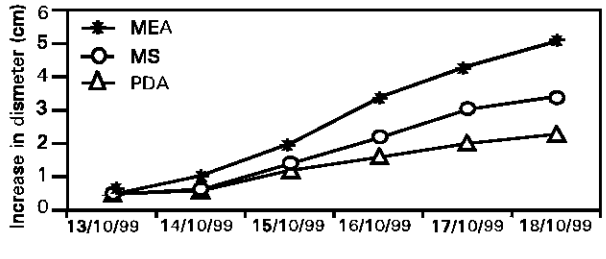

Fig. 3: Mycelial growth of *Volvariella volvacea* in terms of increase in diameter on different culture media.

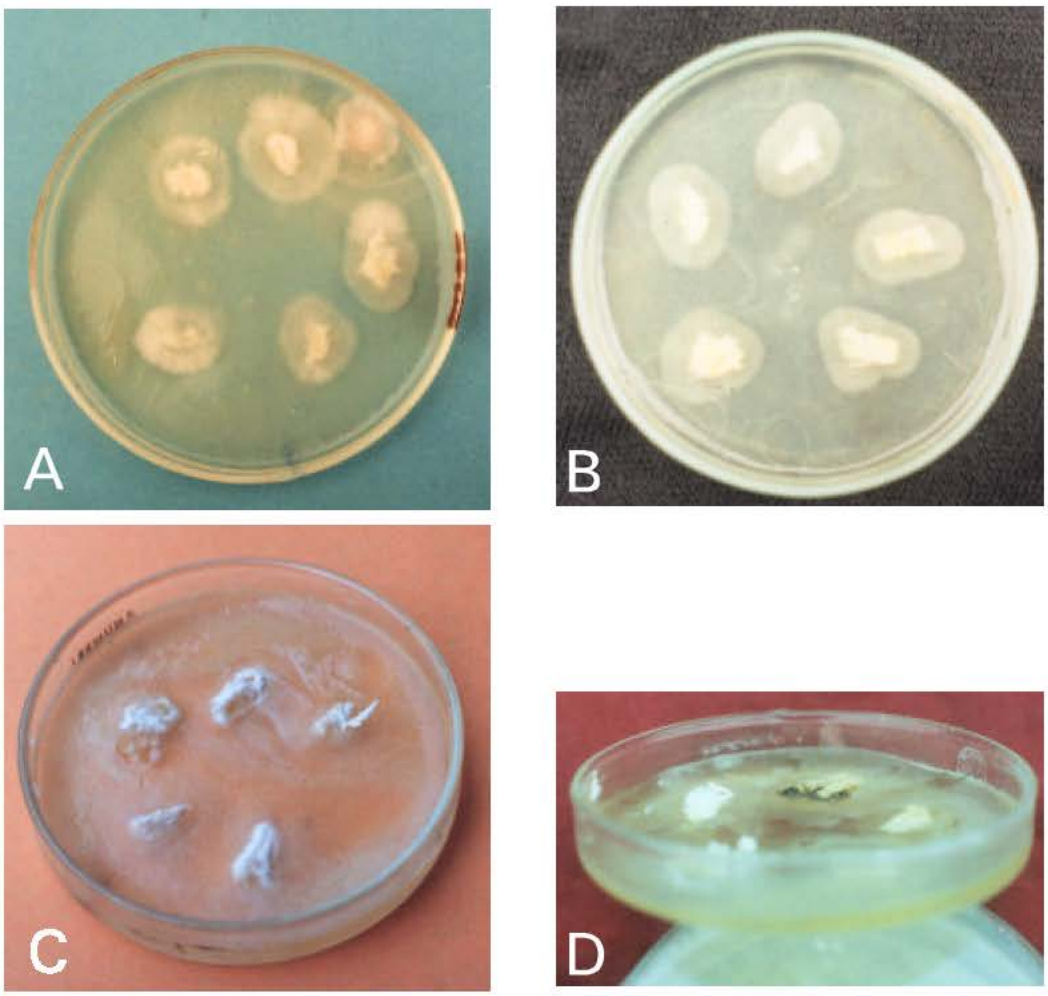

Plate 1: A: Oyster mushroom culture growing in malt extract agar medium
B: Oyster mushroom culture growing in MS medium.
C & D: Miniature mushrooms developing from the over ripe cultures of *Pleurotus ostreatus* var. sajar caju in malt extract agar medium

conditions. On the other hand, Sharma *et al.* (1998) and Worrall and Yang (1992), worked on the effect of spawn age and substrate amendments on yield of oyster mushrooms in Nagaland, which are in line with the result of present study that amending the substrate can effect the yield of mushroom. Another report by Rao (1995), emphasizes the effect of heat and inoculation with *Pleurotus ostreatus* on degradation of wheat straw. Some scientific work is also being carried out to improve the bioconversion and biotransformation efficiensies of the edible mushrooms. Bano *et al.* (1993). Much research has been done on varieties of oyster mushroom, Bano *et al.* (1993). In the present investigation as well, varieties of oyster mushrooms are preferred as these were the simplest as regards their substrate requirement is concerned and easily grown. They are also high yielding varieties. Bano *et al.* (1993). The growth of some species of *Pleurotus* (oyster mushroom) in different liquid media has been studied by some scientists. Pani and Patra (1994). In their work the growth of *Pleurotus* species was compared on ten different liquid media. Their results indicated that in all cases the growth of *Pleurotus* spp. in terms of dry weight was greatest on wheat seed extract. areas by tissue culturing in special lab. environment. For example, *Morchella* spp. (edible ascomycetous macromycetes) which are highly priced and appreciated in Europe and America, grow naturally only in Northern areas of Pakistan in specific soil and atmospheric conditions. It does not grow in plains of Punjab. It may be suggested that taking help from tissue culture technology, experiments can be done with various species of *Morchella*. Further studies would be needed to establish conditions for the cultivation of these macrofungi and others. In the present study oyster and chinese mushrooms were cultured successfully in the lab. environment. During the present investigation, different media i.e., MEA, MS and PDA were used for tissue culturing of oyster and chinese mushrooms. The results revealed that the mycelial growth rate of both types of mushrooms was high on MEA medium as compared to MS and PDA media but mycelial growth was slow on PDA medium as compared to MS and MEA media. These findings are very much in line with the observation of Suharban *et al.* (1996), who worked on suitability of different tuber crops on mycelial growth of *Pleurotus ostreatus* variety sajar caju in liquid and solid media. The results of present research showed that the mycelium of oyster mushroom took 6,8 and 10 days

but in the case of chinese mushroom, the mycelium took 7, 9 and10 days for complete growth in the media plates of MEA, MS and PDA media, respectively during the experimental period, (Fig.1, 2 & 3: Plate 1 A-D). These findings are very much in line with those of Kausar (1988). In the case of oyster mushroom, variety sajar caju, the miniature mushroom developed on MEA medium when culture plates were over-matured, (Plate 1C). The microscopic observation of sporocarp anatomy revealed their similarity to normal sized mushroom. It may be due to availability of required nutrients for mushroom in the media plates of MEA medium. These miniature mushrooms can be used for extra pure culturing. The results of present study are highly promising and it is suggested that whenever experiments as regards tissue culturing of these varieties are planned, their media preferences should be kept in mind.

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