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Effect of Waste Organic Substrates Supplemented with Mango Leaf Aqueous Extract on the Mycelial Growth of *Pleurotus sajor-caju* and *Pleurotus florida*

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Most of the *Pleurotus* sp. can use variety of substrate materials than any other mushroom. In this study, effect of dried mango leaves aqueous extract (MLAE) upon radial mycelial growth of *Pleurotus sajor-caju* and *Pleurotus florida* was evaluated on eight different waste substrate materials which included, mango leaves, wheat straw, sugarcane bagasse, waste cotton, waste paper, wood shavings, paddy straw, and coconut coir. Best growth was observed on dried mango leaves. Radial mycelial growth was significantly increased when sugarcane bagasse and coconut coir were supplemented with the extract of dried mango leaves. Notable increase in growth was observed in case of paddy straw and waste paper upon supplementation. Mango leaves extract based agar medium was found to be better than Sabouraud's dextrose agar (SDA) medium.

Keywords: *Pleurotus* sp., Organic substrates, Supplementation, Mycelial growth.

The mushroom cultivation is a socioeconomic activity with excellent perspectives for developing countries seeking diversification of its agricultural production. Four species of mushroom are cultivated commercially in India, namely *Agaricus bisporus, Pleurotus sp. Volvariella sp.* and *Calocybe indica*[1].

Mushrooms are attractive crop for developing countries where demand for protein is ever increasing. One of the fascinating features about mushrooms is their ability to utilize various types of agricultural wastes. Oyster mushrooms are one of the choice edible mushrooms cultivated in the tropics, and has gained much importance in the last one decade in many countries including India. In recent years the world production and consumption of this mushroom has increased tremendously[2].

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Species of *Pleurotus* are endowed with the capacity to degrade unfermented natural lignino-cellulosic wastes. From the time the substrate is spawned until the end of cropping, there occurs a spectrum of qualitative and quantitative changes in the various substrate constituents, viz., cellulose, hemi-cellulose, lignin, sugars, amino acids, phenols, ash, nitrogen, etc[3].

The genus *Pleurotus* comprises a group of edible ligninolytic mushrooms with medicinal properties and important biotechnological and environmental applications. The cultivation of *Pleurotus* sp. is an economically important food industry worldwide which has expanded in the past few years. One of the most important aspects of *Pleurotus* sp. is related to the use of their ligninolytic system for a variety of applications, such as the bioconversion of agricultural wastes into valuable products for animal feed and other food products and the use of their ligninolytic

enzymes for the biodegradation of organo - pollutants, xenobiotics and industrial contaminants[4].

The aim of the present study was to evaluate the effect of various organic wastes of agricultural and industrial origin supplemented with dried mango leaves aqueous extract upon the growth of *Pleurotus sajor-caju* and *P. florida*.

MATERIAL AND METHODS

The cultures of *Pleurotus* sp. were obtained from Fungal Biotechnology and Invertebrate Pathology Laboratory, Dept. of Biological Sciences, R.D. University, Jabalpur M.P. Cultures were maintained by sub culturing on Potato dextrose Agar slants. Spawn of the cultures were prepared on wheat grains[5]. Eight organic waste substrates were used in the experimentation viz., mango leaves, coconut coir, wood shavings, wheat straw, paddy straw, waste paper, waste cotton and sugarcane bagasse. All the substrates were briefly washed in water to remove the dirt and soil. Substrates were dried over night in an oven at 70°C and chopped to a size of around 1 cm. In the first experiment equal quantity (20 gm) of these substrate materials were taken in beakers and 100 ml of 2% $CaCO_3$ tap water solution was added for adjusting the pH, and kept overnight. Similarly, in another set 100ml tap water containing 2 gm of dried chopped mango leaves were boiled for 5 min to obtain mango leaves aqueous extract (MLAE). It was filtered and 2gm of $CaCO_3$ was added to the extract. Instead of 2% $CaCO_3$ solution, this brown colored mango leaves extract was used to soak the substrates as demonstrated above. After 24 hours at room temperature excess water was removed and four gm of each substrate was filled in petridishes so as to form two slots of eight substrates. First slot was not supplemented with MLAE and was treated as normal while, the second slot was supplemented with 2% MLAE. These petridishes were autoclaved at 121°C 15 psi for 30 min. Plates were cooled and inoculated in the center with a single grain of spawn, and incubated at 25±2°C in a BOD incubator. Average radial growth was measured as increase in diameter in centimeters on day-3, day-6 and day-9 with the help of transparent plastic ruler[6] (Fig. 1). Duplicates of both the sets were kept, and the experiment was repeated twice.

Agar medium based on MLAE (2% & 10%) was prepared and mycelial growth rates of both the *Pleurotus* species on this medium was compared with two common mycological media i.e., Sabouraud's dextrose agar (SDA) and Malt extract agar (MEA) by using a a vernier (Mitutoyo)[7].

RESULTS AND DISSCUSSION

For both the species best growth was obtained on mango leaves of all the substrates. Leaves were

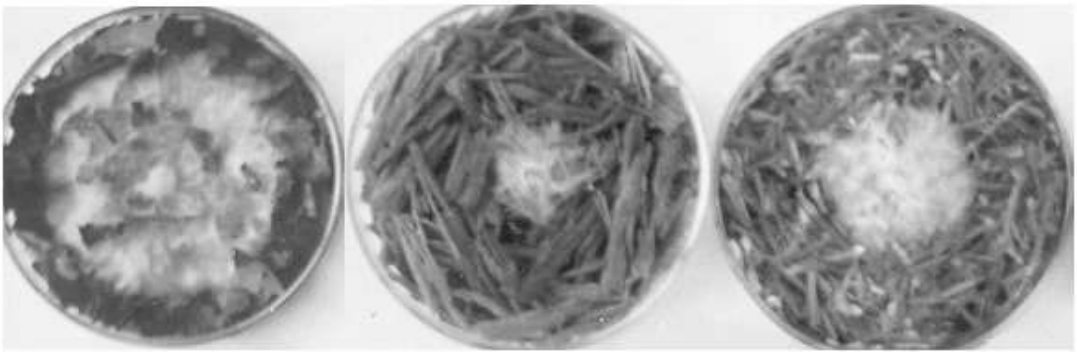
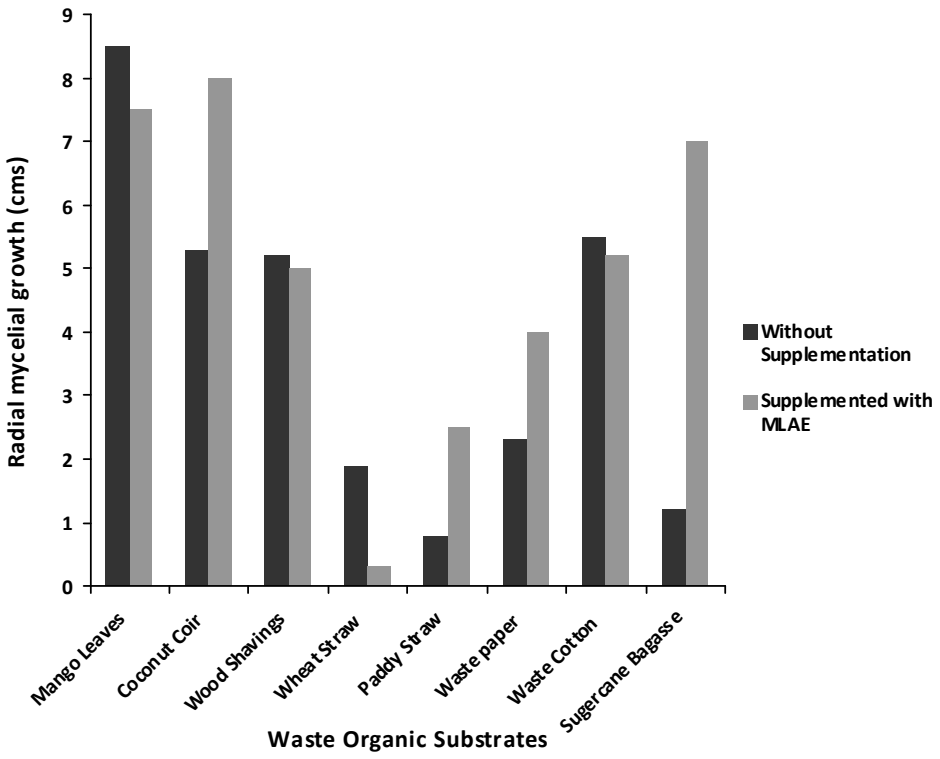


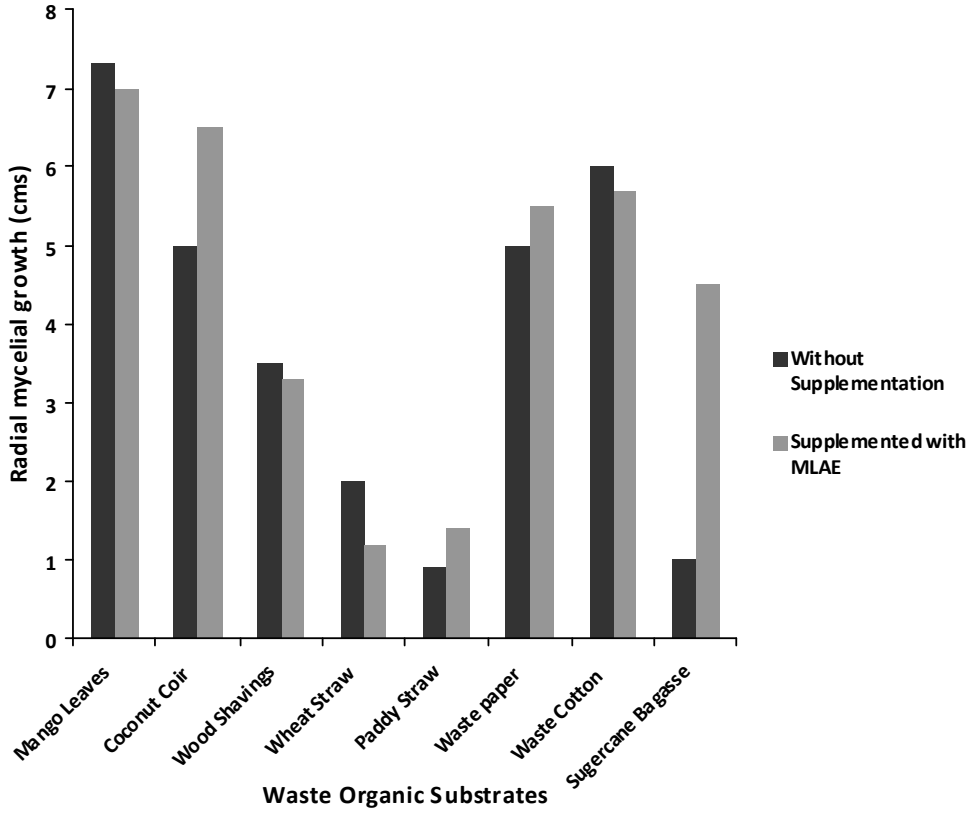
Fig. 1. Photographs showing growth of *Pleurotus sajor-caju* on mango leaves, bagasse and wheat straw

fully colonized on 7th day of incubation in case of *P. sajor-caju*. *Pleurotus sajor-caju* showed good growth was on wood scrapping, cotton and coconut coir. Besides mango leaves, *P. florida* showed good growth on coir, paper and cotton. Rate of colonization was least on sugarcane bagasse and wheat straw for both the fungal strains. While little growth was seen in paddy straw for both the fungal strains (Graph-1). For both the species the growth on waste paper and cotton was more as compare to wheat and paddy straw. Similar results were obtained by Patil *et al*.[8]. They reported a superiority of cotton stalks over paddy straw. Jadhav *et al*.[9] also observed relatively higher yields on cotton wastes.

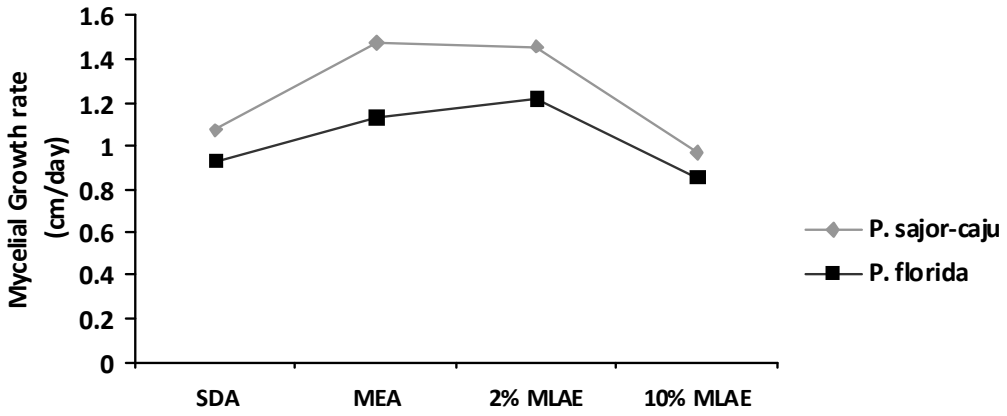
A wide variety of substrates have been successfully tested for the cultivation of oysters, important among them are straw of cereals and millets, hulled maize cobs, cotton wastes and banana pseudostem[10]. The degradation of lignocellulosic biomass of banana pseudostem was investigated by Ghosh *et al*.[11] during solid state fermentation (SSF) by *P. ostreatus* and *P. sajor-caju*. Baysal *et al*.[12] made use of waste paper supplemented with peat, chicken manure and husk rice for the production of oyster mushroom. Obodai *et. al*.[13] have evaluated eight lignocellulosic by-products as substrates for cultivation of the oyster mushroom, *P. ostreatus*. Shah *et al*.[14] investigated the cultivation of oyster mushroom on different substrates and found highest yield, biological efficiency and number of fruiting bodies on sawdust. Similarly, *Pleurotus sajor-caju* (Fr.) Singer was cultivated on selected agro wastes viz. cotton stalks, groundnut haulms, soybean straw, pigeon pea stalks and leaves and wheat straw, alone or in combinations. Cotton stalks, pigeon pea stalks and wheat straw alone



Graph 1. Comparative mean radial mycelial growth (cm) of *Pleurotus sajor-caju* on various substrates without supplemaentation and with MLAE supplementation upon six days of incubation



Graph 2. Comparative mean radial mycelial growth (cm) of *Pleurotus florida* on various substrates without supplementation and with MLAE supplementation upon six days of incubation



Graph 3. Mycelial growth rate (cm/day) studies of *Pleurotus* sp. on three different natural media

All the values are mean of three replicates.

or in combination were found to be more suitable than groundnut haulms and soybean straw for the cultivation. Organic supplements such as groundnut oilseed cake, gram powder and rice bran not only affected growth parameters but also increased yields[5]. Thomas *et al*.[15] evaluated lignocellulosic biomass from coconut palm such as bunch waste, leafstalk, leaflets and coir pith (by-product from coir processing industry) as substrates for cultivation of oyster mushroom, *Pleurotus sajor-caju* (Fr.) Singer.

When the substrates were supplemented with MLAE, some significant changes were observed in the radial mycelial growth. Best growth was again observed on mango leaves. The growth was increased in coir and bagasse. Upon supplementation slight increase in growth occurred on paddy straw. Besides its growth promoting effect, supplementation with MLAE exerted slight negative effect on growth as was observed in case of wood shavings and cotton. More pronounced negative effect was observed on wheat straw (Graph-2). Supplementation of main substrates with nutrient or combination of two or more substrates were reported to increase the yields of *P. sajor-caju*[16].

In case of *Pleurotus sajor-caju* a growth rate of 1.46 cm/day was observed on 2% mango leaves extract agar medium which was very close to the growth rate observed on malt extract agar (MEA). For *P. florida* 2% MLAE agar was found to be the better medium than others. High concentration of mango leaves extract exerted inhibitory effect on the fungal growth, as the least growth was observed on 10% MLAE agar (Graph-3).

From this study it can be concluded that sugarcane bagasse and coconut coir could be used with supplemented mango leaves extract for rapid production of fruiting bodies. Supplementation of MLAE to paddy straw and paper will also be beneficial to increase the mycelial growth and efficiently colonized of the substrate by the fungal mycelia. The extract is not suitable to be used with wood shavings, waste cotton and wheat straw. The 2% MLAE agar is better than SDA and is as good as MEA so, it could be used as a replacement to these expensive mycological media. For the further studies biological efficacy will be determined after cultivation. The growth promoting active ingredient(s) in the MLAE will be extracted through solvent extraction technique and purified for analysis.

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REFERENCES

1. Sharma, V.P., Sharma, S.R., Kumar, S. Physiological requirements and cultivation of *Agrocybe aegerita*. *Mush. research*., 2004; **13** (20): 66-70.
2. Balakrishnan, B., Nair, M.C.: Development on the biotechnology of oyster mushrooms. In: *Advances in Mushroom Biology and Production* (Rai RD, Dhar BL, Verma, RN eds). MSI, Solan, 1997; pp 83-89.
3. Rajarathnam, S., Bano, Z. *Pleurotus* mushrooms. Part III. Biotransformations of natural lignocellulosic wastes: commercial applications and implications. *Crit. Rev. Food Sci. Nutr*., 1989; **28**(1): 31-113.
4. Cohen, R., Persky, L., Hadar, Y. Biotechnological applications and potential of wood-degrading mushrooms of the genus *Pleurotus*. *Appl Microbiol Biotechnol*., 2002; **58**(5): 582-94.
5. Mane, V.P., Patil, S.S., Syed, A.A., Baig, M.M.V. Bioconversion of low quality lignocellulosic agricultural waste into edible protein by *Pleurotus sajor-caju* (Fr.) Singer. *J. Zhejiang Univ. Sci. B*., 2007; **8**(10): 745–751.
6. Adedokun, O.M., Ataga, A.E. Effects of Crude Oil and Oil Products on Growth of Some Edible Mushrooms. *J. Appl. Sci. Environ. Manage*., 2005; **10**(2): 91-93.
7. Sastre-Ahuatzi, M., Tellez-Tellez, M., Diaz-Godinez, G., Montiel-Gonzalez, A.M., Diaz, R., Sanchez, C. Mycelial growth of strains of *Pleurotus ostreatus* Developed on agar and its correlation with the productivity in pilot plant production farm. *Braz. J. Microbiol*., 2007; **38**: 568-572.
8. Patil, B.D., Jadhav, S.W., Kakade, S.S. Studies on cultivation of *Pleurotus sajor-caju* on different substrates. *J. Maharashtra Agric. Univ*., 1989; **14**(1): 156–158.

9. Jadhav, A.B., Bagal, P.K., Jadhav, S.W. Effect of different substrates on yield of oyster mushroom. *J. Maharashtra Agric. Univ*., 1996; **21**(4): 424–426.
10. Bano, Z., Rajarathnam, S., Nagaraja, N. Some important studies on *Pleurotus* mushroom technology. *Indian Mush. Sci.*, 1987; **12**(2): 67-71.
11. Ghosh, M., Mukherjee, R., Nandi, B. Production of extracellular enzymes by two *Pleurotus* species using banana pseudostem biomass. *Acta Biotechnol*., 2004; **18(3)**: 243-254.
12. Baysal, E., Peker, H., Yalinkilic, M.K., Temiz, A. Cultivation of oyster mushroom on waste paper with some added supplementary materials. *Bioresour technol*., 2003; **89**(1): 95-97.
13. Obadai, M., Cleland-Okine, J., Vowotor, K.A. Comparative study on the growth and yield of *P. ostreatus* mushroom on different lignocellulosic by-products. *J. Ind. Microbiol Biotechnol*., 2003; **30**(3): 146-149.
14. Shah, Z.A., Ashraf, M., Ishtiaq, M. Comparative Study on Cultivation and Yield Performance of Oyster Mushroom (*Pleurotus ostreatus*) on Different Substrates (Wheat Straw, Leaves, Saw Dust). *Pakistan J. Nutr*., 2004; **3**(3): 158-160.
15. Thomas, G.V., Prabhu, S.R., Reeny, M.Z., Bopaiah, B.M. Evaluation of lignocellulosic biomass from coconut palm as substrate for cultivation of *Pleurotus sajor-caju* (Fr.) Singer. *World J. Microbiol. Biotechnol*., 1998; **14**(6): 879-882.
16. Jadhav, A.B., Bagal, P.K., Jadhav, S.W. Biochemical changes in different agro residue to oyster mushroom cultivation. *J. Maharashtra Agric. Univ*., 1998; **23**(1): 22–24.