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Research Article

PINK OYSTER MUSHROOM (PLEUROTUS DJAMOR) AND ITS EFFICACY AGAINST HUMAN PATHOGEN

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ABSTRACT

A variety of Pink oyster mushroom (*Pleurotus djamor*) is economically effective due to highest yield and abundant availability throughout the year and it can be cultivable through agro wastes. The pure culture preparation, mother spawn production running periods of spawn, pinhead formation and fruit body formation, yield evaluation, substrate preparation for spawn cultivation were clearly studied. The biological activity of the *Pleurotus djamor* were also evaluated by extracting with methanolic extract which shows maximum activity against *Vibrio cholera* and *Pseudomonas putida* and minimum activity observed in *Klebsiella pneumonia.* The spawn and biological efficiency were also measured. Hence pink oyster mushroom cultivation can be recommended for rural people and oyster mushroom growers.

Keyword: *Pleurotus djamor* , Cultivation, Antibacterial activity,

INTRODUCTION

Nowadays increased utilization of mushrooms for its nutritive potential and are recognized as an important food items from ancient periods. Mushrooms are macro fungus, with distinct fruiting body, can either be hypogenous or epigenous (Chang and Miles, 1992). Economic importance and nutritional importance of mushrooms were increased as edible food this may be due to its high protein and fiber content and rich in antioxidants which play a major role in human health and nutrition. *Pleurotus* spp. belongs to Oyster mushrooms are nowadays very popular (Adejoye *et al.,* 2006). Edible mushrooms can be cultivated with agricultural and agro-industrial wastes as the substrates which is efficient and socio-economically reliable technology for converting wastes materials into a valuable protein-rich food and a cash crop of commercial interest (Zhang *et al.,* 2002). An attractive feature of oyster mushrooms is that they can utilize a large variety of agricultural waste products and transform the lignocelluloses biomass into high-quality food, flavor and nutritive value (Quimio.,1978), Dehariya and Vyas.,(2013). Microbial technology help in large-scale recycling of agro waste as an alternative way to use agricultural residues / wastes use of organic material in mushroom production (Chang and miles. 2004; Khare *et al.,* 2010). Thus, the present study was mainly to evaluate the effect of agro waste as substrate for the growth and yield of Oyster mushroom and its antimicrobial efficacy of pink oyster mushroom (*Pleurotus djamor*).

MATERIALS METHODS

Collection of materials:

Pure culture of *Pleurotus djamor* (oyster mushroom) were purchased from the Mushroom society of India (ICAR) Directorate of mushroom research in Sloan, Himachal Pradesh, India. The wheat grains were collected from the local market.

Preparation of Mother spawn:

The mother spawn culture were prepared by using good cereals grains like wheat as substrate. Selection of the substrate to be free from diseases and damage by insect . Wheat grain thoroughly washed in sufficient water to remove solid debris. Washed grains are soaked first, for 16 hours, and then it was boiled for about (30-45 min) until the grains became good odor and looked like a succulent in nature. Excess water from the boiling grains is removed by spreading boiling grains on sieve made of fine wire mesh or muslin cloth. The grains are left as such for few hours on the sieve so that water on surface water get evaporates after that shadow dry (4-6) hours were spread out on the sterile polythene sheet placed on the floor. 250 g Wheat grains were mixed with (calcium carbonate) $CaCO_3$ (0.5%) gypsum $CaSO_4$ 2% (calcium sulphate) and chalk powder (calcium carbonate) the pH of grains pack should be around 7 to 7.8 and aware of clumps formation and packed tightly in 12×8 inch polythene bag plugged the neck with cotton. Then the bags were

sterilized, in an autoclave for 1 hour at 121°C. After sterilization the bags were allowed to cool for a day. Hence the bags were gently agitated. Then a piece of pure culture was placed aseptically through the hole of the mother culture packet and again the packets were plugged with cotton and covered with brown paper. It was placed into the growth chamber at 25°C in dark place. After 15 to 21 days the packets of the mother culture became white due to the completion of the mycelium running and then it was ready for inoculation in the spawn packets.

Figure 1: Cultivation of *Pleurotus djamor* mother spawns production

Oyster mushroom cultivation:

Multi-layered technique (Bano and Srivastava, 1962) was adopted for spawning. The Paddy straw were collected the nearby local farmers and chopped into small pieces of 2-3 inches. The paddy straw were soaked in water for16 hours to moisten and drained the excess water and autoclaved separately for15 -20 min at 121ºC after sterilization the straw were dried in a polythene sheet holding the moisture capacity about 70%. The polythene bags of size 24 x12 cm were filled with sterilized paddy straw. Each bag were filled with an alternate layer of raw material and oyster mushroom spawn (*P.djamor*) which helps to grow uniform fructifications of mushrooms and uptake of nutrients from the substrates. The mouth of polythene bag were sealed and 12 holes of about 2 cm were made on each polythene bags for aeration. After inoculating the spawn in the polythene bags, the bags were kept in cropping house where the temperature maintained at 25ºC and humidity around 80 to 90 % respectively. Moreover the cunny bags are hanged over the hut with sufficient light and ventilation and maintained for about 18- 20 days. The crop house were monitored regularly so as to analyze the growth characteristics and to reduce the microbial contamination as well as pest infestation.

Figure 2: Growth characteristics of Pleurotus djamor on paddy straw substrates; A.primordia formation, B. fruit body formation, C. maturation of fruit body harvesting stage.

Pin head were formed in 21st day. First primordial formation in the cunny bags were observed in 22nd day mushroom matured within 48 hours after primordial appearance. Depends upon the substrates used for the cultivation, and scratching techniques fruiting bodies of the mushroom and maturation increases. Matured mushroom are identified by curled margin of the cap by twisting to uproot from the base. For 100 gm seeds 450 gm of mushroom were harvested. The oyster mushroom seems to be pink in initial stage and change to pale on maturation. Depending on the substrates the colour of the mushrooms retains pinkish.

Preparation of mushroom extract:

10 g of dried finely milled mushrooms were soaked in 100 ml methanol for 24 hours, at room temperature under dark condition. The procedure were repeated twice and extracted with whatmann No.1 filter paper. All the two extracts were combined together and concentrated and stored in air tight container. Further antimicrobial studies were analysed.

Antibacterial assay:

Human pathogens *Escherichia coli, Staphylococcus aureus, pseudomonas putida*, *Vibrio cholera*, *Klebsiella pneumonia,* were collected from the Department of microbiology, Raja Muthiah Medical Collage and Hospital, Annamalai University, Annamalai Nagar. The pathogens were maintained on Nutrient Agar (NA) Medium studied for further analysis.

Antibacterial assay were determined by Agar well - diffusion by the following method Kirbuy and Baurer, 1967. Muller Hinton agar medium were prepared and poured into sterile Petri plates and allowed to solidify. The bacterial pathogens were swabbed on the plate using sterile cotton swabs. 0.5cm well were punctured in the medium. The different concentrations of the mushroom extract (25, 50, 75, 100µl) were

loaded in to separate wells and antibiotic ampicillin were used as a positive control. All the plates were incubated at 37˚C for 24h. Zone of inhibition were measured.

RESULT AND DISCUSSION

The study revealed the efficiency of the growth parameters and yield of *Pleurotus djamor*. Substrates are one of the most important parameters in mushroom cultivation which depends on the nutritional availability to support the mycelia growth and to develop mushroom fruiting bodies. Also, constructing the substrate in the cunny bags is an important factor for the growth of the mycelia as it should be suitable for the penetration of the mycelium in the basal substrates. Which ultimately influence the fruiting of the spawn running, pinhead formation and fruit body formation are three phases in the cultivation of mushroom.

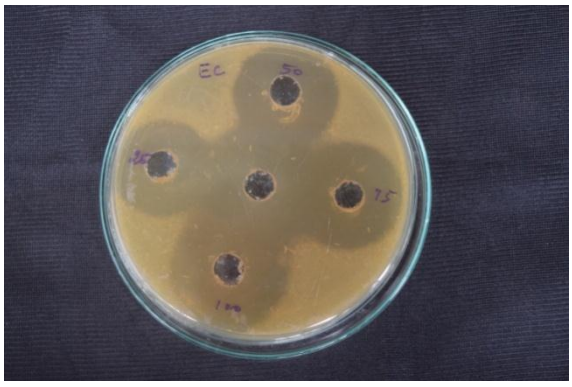
. In the present study, the result indicates that spawn running periods (12.33 ± 1.15) the results were found in accordance with the findings of (Satpal *et al.,* 2017). In the Pleurotus spp mycelium growing day was generally observed on 10-15 days (Ragunathan and Swaminathan, 2003). The pinhead formation is the second stage of mycelial growth during cultivation of mushroom. Pinhead formation (42 ± 5).The results of this study concur with the finding of (Rangaswami *et al.,* 1975) who reported that pinhead formation of *P.sajor-caju*was in 20-25day of incubation. A number of investigators have reported those different timing periods were taken for the fruiting body formation. In our study Fruit body formation (35.66 ± 7.09) and the result was support the finding of (Satpal *et al.,* 2017) obtained a crop from wheat straw + paddy straw 20- 23 days. Khanna and Garcha (1981) found the crop in 104 days on paddy straw and Tan (1981) reported that *P.ostreatus* and other species on cotton waste took 2-3 weeks for fruit body formation after spawn running. The number of primordia and number of effective fruiting bodies initiation had a linear relationship. In this study, the maximum number of Primordia initiation (47.33± 5.50) this was more are less similar with the results (Hasan *et al.,* 2015) stated a higher number of primordial initiation in *Pleurotus djamor* growing on wheat bran and supplement with sugarcane. Number total primordia per packet were found in Mahogany sawdust reported by (Islam *et al.,* 2010). Pileus diameter were recorded as (5.26 ± 0.90), Pileus thickness were measured as (0.6 ± 0.1), Stalk length were (2.56 ±0.32) and the Stalk diameters (0.6 ± 0.1) were recorded in our study. Hasan *et al.,(*2015) also highest Pileus diameter and thickness in wheat bran and supplement with sugarcane bags. This was more are less similar study. In this present study experiments results indicated that maximum yield (430.5 ± 0.42) was recorded in the paddy straw *Pleurotus djamor*. (Arish, 2010) was reported rice bran supplements the organic nitrogen which helps in the production of high yield. Cereal straw used for cultivation of oyster mushroom is poor source of nitrogen (0.8%) and at the time of fructification when most of the nitrogen is utilized for the mycelial growth, the depleted nitrogen in the substrates became inadequate and limits mushroom yield (Victor and Ifeanyi,2013).in this study paddy straw cultivation biological efficiency 43.00% BE were observed. (Satpal *et al.,* 2017) recorded the highest

biological efficiency in paddy straw + wheat straw these result more are less similar to the results.

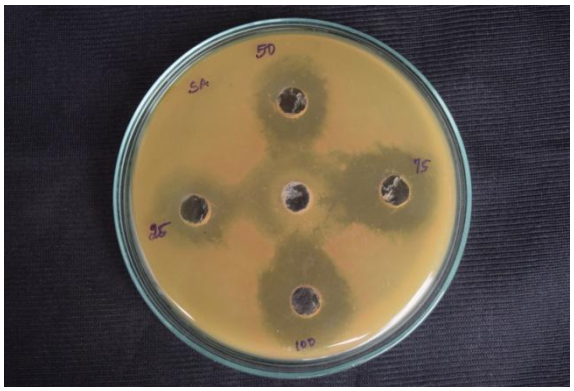
The methanolic extract of *Pleurotus djamor* were examined against both gram positive and gram negative human pathogens such as *Escherichia coli, Staphylococcus aureus, pseudomonas putida, Vibrio cholera and Klebsiella pneumonia.* The extract showed maximum inhibition in gram negative organisms such as *Vibrio cholera* (12mm) *Escherichia coli* (12mm) and *Pseudomonas putida*(11mm) whereas *Escherichia coli* and *Staphylococcus aureus* (11mm) .The maximum activity may due to the elution of polar compounds in Methanol extract Petrovi´c et al., 2014. The result was supported by Jegadeesh *et al.,* 2014 in which methanol extract of *P. djamor* showed maximum activity when compared with hexane extract in *E. coli* and *S.aureus*. *Klebsiella pneumonia* shows very less activity when compared with others.

S.No	TEST ORGANISM	Control (mm)	25µL(mm)	50 µL(mm)	75 µL(mm)	100µL (mm)
1	*Escherichia coli*	13	8	10	11	12
2	*Staphylococcus aurous*	12	5	7	9	11
3	*pseudomonas putida*	12	11	11	11	11
4	*Vibrio cholera*	13	10	10	11	12
5	*Klebsiella pneumonia*	12	2	4	7	9

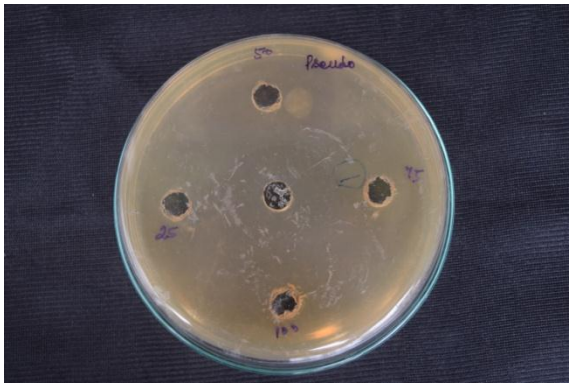
The zone of inhibition for all pathogens shown in table 1

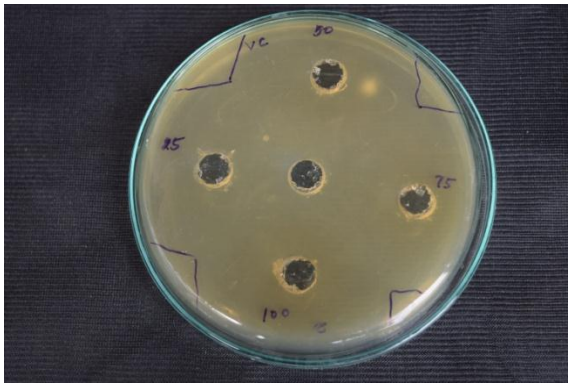


1. *Escherichia coli*,

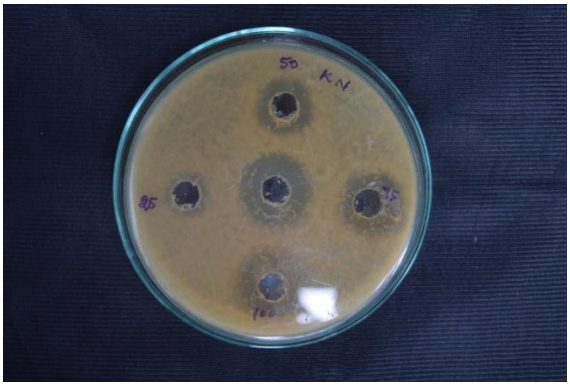


2 .*Staphylococcus aureus*





3. Pseudomonas putida, *4.Vibrio cholera*



4. *Klebsiella pneumonia,*

3. Antibacterial activity of *P.djamor* from methanolic extract

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