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RESEARCH ARTICLE Agricultural Science Digest

Utilization of Lignocellulosic Substrates on Oyster Mushroom (*Pleurotus citrinopileatus*) Cultivation

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ABSTRACT

Background: Mushroom cultivation increased throughout the world with the utilization of crop residues. Oyster mushroom (*Pleurotus* spp.) is suitable for most of the area and less expensive for cultivation with the availability of paddy straw. Increasing demand of paddy straw compels to search alternate source of substrate for mushroom production. Based on this information efforts were taken to compare many locally available leaf substrates for *Pleurotus citrinopileatus* production.

Methods: Leaf substrates *viz.,* teak, bamboo, maize, banana, coconut, mango, sugarcane bagasse and grass (*Dactyloctenium aegyptium*) were tested for the cultivation of *P. citrinopileatus.* Grain substrates *viz.,* paddy, cumbu, bengal gram, green gram, sorghum, black gram, ragi and the above leaf substrates were studied for their effect on radial mycelial growth and dry weight of *P. citrinopileatus*.

Result: All the leaf substrates were on par with each other for radial mycelial growth. Highest mycelial dry weight of 724.67 mg was recorded in paddy straw. Among the different leaf substrates used for mushroom bed preparation, banana leaf substrates showed highest yield of 115.00 g than paddy straw. Weight of mushroom spent bed was recorded to observe the utilization of substrates in which bamboo was decomposed at the maximum. Addition of grain substrates enhanced the growth of mycelium.

Key words: Grain substrates, Leaf substrates, Mycelial dry weight, *Pleurotus citrinopileatus*, Radial mycelial growth, Yield.

INTRODUCTION

Mushrooms are heterophytic and depend upon the organic matter for nutrient and live saprophytically or parasitically. *Pleurotus* sp. are commercially important edible mushroom and decomposes lignin and cellulose with high mycelial growth, enzymatic reaction and colonization of various agro wastes *viz.,* rice straw, corn, barley, wheat, coconut husks, and banana leaves and converted them into protein (Patel *et al*., 2012; Vega and Franco, 2013).

Oyster mushroom grew on residues like straw, saw dust, sugarcane bagasse, corn stalk, waste cotton, leaves and pseudostem of banana, banana leaves and used for cultivation (Kurt and Buyukalaca, 2010). Agro-wastes accumulation pollute air and soil, increases insect and pathogen proliferation, use of these wastes for *Pleurotus* cultivation is a low-cost/nutritionally important technique (Jhonatan Rafael Zárate-Salazar, 2020).

Production of agro wastes increased by 50 per cent (FAO) in the world (Cherubin *et al*., 2017) and re-use of wastes as raw material for the cultivation of mushrooms (Estrada and Pecchia, 2017) and used as organic fertilizer (Owaid *et al*., 2017). Corn stubble promoted greater mycelial growth and biological efficiency, reduced the harvest periods and productive capacity of *P. ostreatus* (Jhonatan Rafael Zárate-Salazar, 2020). Carbon-nitrogen ratio might be responsible for the higher mycelial growth in different grains and leaf substrates. Based on the above points this research work was taken to identify the locally available other sources of leaf and grain substrates for oyster mushroom productions.

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MATERIALS AND METHODS

Isolation of *Pleurotus citrinopileatus*

Pure culture of *Pleurotus citrinopileatus* was obtained by tissue culture technique and nucleus culture stored at 4°C in refrigerator. The same was used to prepare mother culture by growing on PDA medium and spawn production.

Leaf substrates used

Leaf substrates *viz*., teak leaf, paddy straw, sugarcane bagasse, grass (Kaka kal pul / Crow foot grass - *Dactyloctenium aegyptium*), maize, banana, coconut,

mango and bamboo were collected from the farm of Adhiparasakthi Agricultural College (APAC), G.B.Nagar, Kalavai . Leaf substrates were washed thoroughly with water, dried them and made as powder after proper drying. The powdered leaves were packed individually in a polythene bag, sealed and labelled them properly and stored at room temperature in the laboratory for further studies. Washed leaf substrates (whole leaves) were also stored properly in polythene bags for further experiment.

Grain substrates used

Quality grains of paddy, cumbu, bengal gram, green gram, sorghum, black gram and ragi were purchased from grain merchant, washed thoroughly with water, dried them and made as powder. The powdered grains were packed individually in a polythene bag, sealed and labelled them properly and stored at room temperature in the laboratory for further studies.

***In vitro* evaluation of leaf substrates for the growth of *P. citrinopileatus* on solid and liquid medium**

Solid medium

Potato Dextrose agar medium was prepared and distributed in to 250 ml conical flasks @ 50 ml per flask. Different leaf powders *viz.,* teak, bamboo, sugarcane bagasse, grass (Crow foot grass-*D. aegyptium*), maize, banana, coconut, mango and paddy straw were added individually in each flask @ five gram powder per flask, shake them well. Sterilized the flasks after plugging them with non- absorbent cotton at 15 lb. pressure for 20 min. in an autoclave. After sterilization under warm condition the molten PDA medium with substrates were transferred to sterilized Petri plates @ 15 ml per plate and allowed them to solidify. After solidification, nine mm disc of seven days old *P. citrinopileatus* culture was inoculated and plates were incubated at room temperature. PDA plates without substrates served as control. Growth of mushroom fungus was measured (in mm) from fourth day onwards and continued up to ninth day. Three replications were maintained. Per cent increase / decrease over control was calculated by the following formula:

$$\text{Per cent increase/decrease over control} = \frac{C - T}{C} \times 100$$

C- Control.
T- Treatment.

Liquid medium

Potato Dextrose broth (without agar) was prepared and distributed into 250 ml conical flasks @ 50 ml per flask. Above mentioned leaf powders were added individually in each flask @ five gram leaf powder per flask, shake them well. Sterilized the flasks after plugging them with non-absorbent cotton at 15 lb. pressure for 20 min. in an autoclave. After sterilization under cool condition the flasks were inoculated with nine mm disc of seven days old *P. citrinopileatus* culture and incubated at room temperature. Control was maintained as potato dextrose broth only without substrates. Growth of mushroom fungus was recorded nine days after incubation as mycelial dry weight (in mg) by transferring the mycelial mat only on pre weighed filter paper and then dried in a hot air oven at 60°C for three days. Final weight of the mycelium was obtained by detecting the filter paper weight and recorded the data. Three replications were maintained. Per cent increase / decrease over control was calculated as above.

***In vitro* evaluation of grain substrates for the growth of *P. citrinopileatus* on solid and liquid medium**

Solid medium

Potato Dextrose agar medium was prepared and distributed in to 250 ml conical flasks at the rate of 50 ml per flask. Different grain powders *viz.,* paddy, cumbu, bengal gram, green gram, sorghum, black gram and ragi were added individually in each flask @ five gram powder per flask, shake them well. Sterilized the flasks after plugging them with non- absorbent cotton at 15 lb. pressure for 20 min. in an autoclave. After sterilization under warm condition the molten PDA medium with substrates were transferred to sterilized Petri plates @ 15 ml per plate and allowed them to solidify. After solidification, nine mm disc of seven days old *P. citrinopileatus* culture was inoculated at the centre of the plate. Inoculated plates were incubated at room temperature. Control was maintained as PDA only without substrates. Growth of mushroom fungus was measured (in mm) from fourth day onwards and continued up to ninth day. Three replications were maintained. Per cent increase / decrease over control was calculated by the above formula.

Liquid medium

Potato Dextrose broth was prepared and distributed into 250 ml conical flasks @ 50 ml per flask. The above mentioned grain powder were added individually in each flask @ five gram per flask, shake them well and sterilized the flasks. After sterilization, under cool condition the flasks were inoculated with nine mm disc of seven days old *P. citrinopileatus* culture and incubated at room temperature. Control was maintained as potato dextrose broth only without substrates. Mycelial dry weight was recorded nine days after incubation by transferring the mycelial mat only on pre weighed filter paper and then dried in a hot air oven at 60°C for three days. By measuring the weight of the filter paper, the final weight of the mycelium was determined (in mg). Three replications were maintained. Per cent increase / decrease over control was calculated.

Preparation of mother spawn

Cleaned and half cooked sorghum grains were mixed with $CaCO_3$ @ 20 g/kg. The prepared grains were filled into polypropylene bags @ 250 g and mouth was inserted with PVC ring, plugged with non- absorbent cotton and tied them after covering with brown paper. Bags were sterilized at 15 lb. pressure for 1 hour. Allowed them to complete cooling and inoculated with nine mm disc of seven days old *P.*

citrinopileatus culture under aseptic condition. Inoculated bags were incubated at room temperature. The mycelium completely spread through the grains in about two weeks.

Preparation of bed spawn

Bed spawn was prepared as above and inoculated with fully grown mother spawn by transferring 10 g of mother spawn grains with mycelium of *P. citrinopileatus* in sterilized condition and incubated at room temperature. The mycelium completely spread through the grains in about two weeks.

Preparation of substrates

In this study, boiling method was used for sterilization of different substrates *viz.,* teak, bamboo, sugarcane bagasse, grass, maize, banana, coconut, mango and paddy straw. Washed and soaked the substrates in water for 6 h. The contents were boiled individually with fresh water over the flame for one hour and then spread over the polythene sheet for shade drying until 60-65 per cent of moisture content is retained.

Preparation of mushroom bed

The cultivation of oyster mushroom was usually carried out in transparent polythene bags (size of 60 × 30 cm and 80-gauge thickness). A well grown bed spawn was used @ 45 g/bed. The above substrates were individually used for bed preparation. Each sterilized substrate was staked as three layers by alternatively placing leaf substrates and bed spawn, Three replications were maintained. Total dry weight of each substrate was 350 g. The beds were arranged inside the spawn running room (mushroom shed) by hanging rope system and maintained at the room temperature of 22-25°C and relative humidity of 80-90 per cent inside the shed. Running of mycelium and initiation of sporocarp was observed in each substrate in the beds. Fully developed sporocarp (mushroom) was harvested from each bed, weighed individually and recorded the total yield after three harvests. Final weight of the bed also recorded to observe decomposition of substrates used.

Statistical analysis

Data of the experiments were analyzed by Completely randomized block design (CRD) using data entry module WASP - Web agri stat package for data entry and analysis.

RESULTS AND DISCUSSION

Mushroom is a good source of protein, dietary fibre, amino acids, vitamins and minerals and suitable for all age group of people. Mushroom is grown in paddy straw as a best organic substrate and demand is increasing in paper industries and packing material. Use of alternate source for paddy straw is necessary to mitigate the demand. After harvesting the economic part / produce of various crop, the residues were used as substrate source for mushroom cultivation by researchers. Based on this aspect various organic substrates were utilized in this experiment to substitute paddy straw.

Effect of leaf substrates on the radial mycelial growth of *P. citrinopileatus*

Influence of various leaf substrates on the radial mycelial growth of *P. citrinopileatus* was conducted and the results are shown in Fig 1. Among the leaf substrates, bamboo, grass, mango, coconut, sugarcane bagasse and paddy straw powder amended PDA medium recorded highest growth of 82.33 to 86.66 mm on eighth day after inoculation. On ninth day of observation all leaf substrates showed maximum growth of 87.00 to 90.00 mm.

The result of this study corroborated with the findings of Vajjaramatti *et al*. (1997). They reported that *P. sajor-caju* grew well on various substrates either sole or in combination with other substrates. The different stages of mycelial growth can be identified because the soluble sugars are consumed

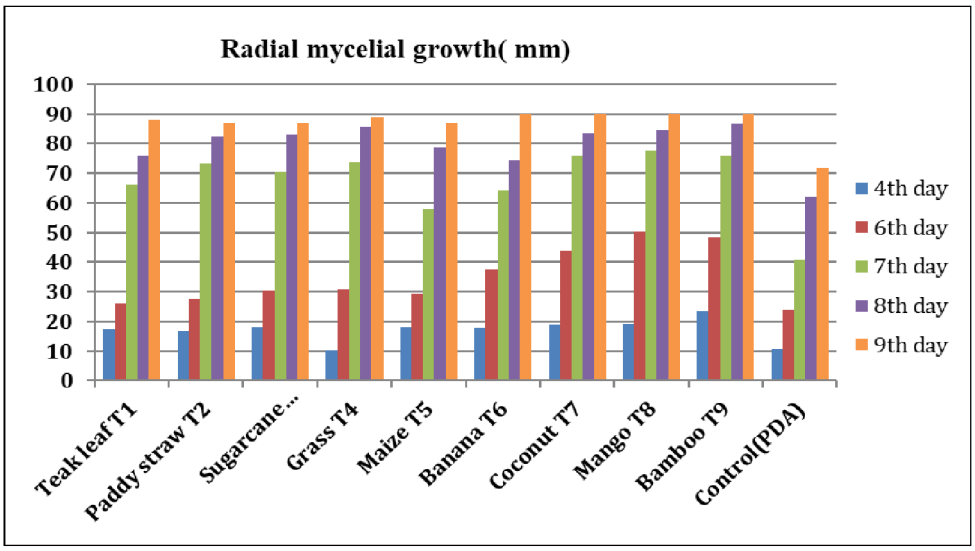

Fig 1: Effect of leaf substrates on radial mycelial growth of *P. citrinopileatus*.

during the stages of absorption, cell division, and mycelial growth, resulting in 4 to 10 mm of growth day^{-1}in substrate, and from 13 to 30 mm day^{1} in culture medium (Martínez *et al*., 2015).

Effect of leaf substrates on mycelial dry weight of P. *citrinopileatus*

Different leaf substrates were used for determining the mycelial dry weight of *P. citrinopileatus* and results are summarized in Fig 2. Among the leaf substrates, paddy straw recorded the highest mycelial dry weight of 724.67 mg followed by grass. The least growth of 452.00 mg was recorded in control (only potato dextrose broth). Generally, addition of leaf substrates increased the growth of *P. citrinopileatus* as indicated by increased mycelial dry weight.

This study varied with the report of Poonam and Deepak (2013). Among the substrates used for *P.sajor-caju*, soyabean straw showed highest yield (933.40 g) followed by wheat straw and paddy straw. They were able to colonize and degrade a large variety of ligno cellulosic residues, they require shorter growth time when compared to other edible mushrooms.

The results of de Carvalho *et al*. (2012) and Bernardi *et al*. (2013) also demonstrated that mycelial growth of less than 5 mm day^{-1} vary according to the nutrient medium and biological material. Mycelial growth can be influenced by the chemical composition, plant structure and particle size of the agronomic wastes used as substrate under controlled conditions of environment determined the productive performance of mushrooms.

Effect of various leaf substrates on mushroom yield

Mushroom yield was recorded from various bed substrates and the readings were depicted in Fig 3. Yield was recorded from 25th day after bed preparation and continued up to 40 days. Total yield of three harvest were taken for each substrate.

Highest yield of 115g was obtained from banana leaf substrate followed by paddy straw as (97.50 g) and grass (95.00 g). Least yield of 22.50 g was obtained from mango leaves. Leaf substrates of maize, bamboo and sugarcane bagasse also exhibited third ranking in yield.

This result is contradictory with the report of Kalita *et al*. (1997). They reported that paddy straw supported highest yield when compared to other substrates like water hyacinth, chopped banana leaves, betlenut husk and sugarcane bagasse. The lower performance of yield and biological efficiency of different agricultural wastes might be due to low lignolytic and cellulolytic activity (Pathak and Goel, 1988). Another report also contradictory with this result as the corn stubble promoted mycelial growth and biological efficiency, reduced the harvest periods and the daily productive capacity and also increased the organic matter loss in the substrates (Jhonatan Rafael Zárate-Salazar, 2020).

Effect of *P. citrinopileatus* growth on decomposition of leaf substrates

Harvested mushroom beds were weighed to determine the loss of weight of substrates, as a indication of utilization for the growth of mushroom. The results are portrayed in Fig 4. Initial weight of bed was recorded at the time of preparation with uniform dry weight of 350 g for each substrate. Sporocarp of mushrooms were harvested three times from each bed up to 40 days after preparation. Harvested beds were kept for another 15 days and then weighed which was considered as spent bed. *P. citrinopileatus* utilized the substrates (initial weight of 350 g) in different manner which

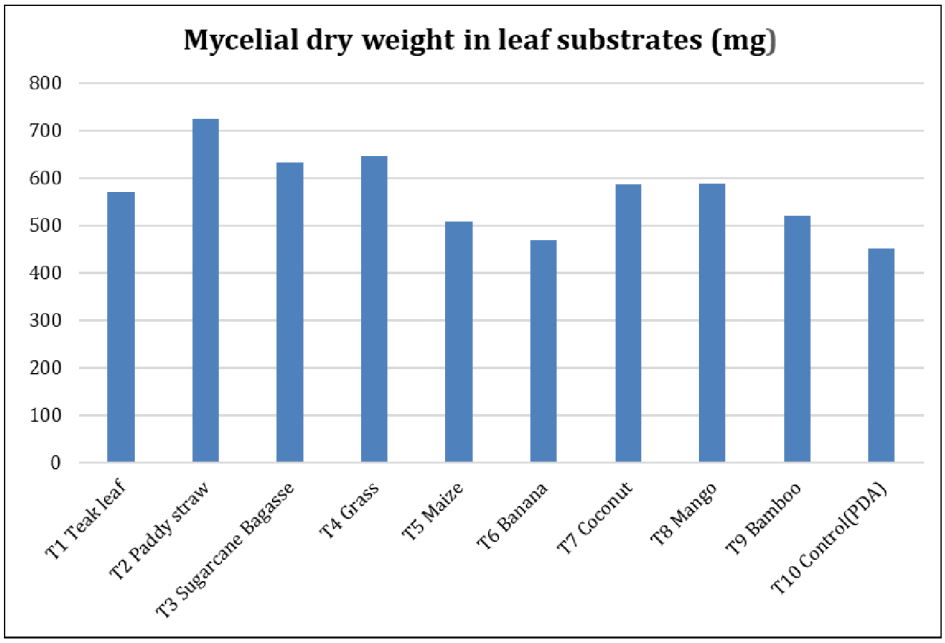

Fig 2: Effect of leaf substrates on mycelial dry weight of *P. citrinopileatus*.

was indicated by final weight of bed. Among the substrates bamboo was utilized at the maximum with the final weight of 70.00 g followed by maize (90.00 g) and banana (105.00 g). Paddy straw recorded 200 g. Correlated with yield, banana leaf was the best substrate and substitute for paddy straw.

Ramesh and Ansari (1987) used several locally available substrates *viz.*, rice straw, banana leaves, saw dust, oil palm refuse, oil palm bunch refuse and grass straw to study conversion efficiency of *Pleurotus sajor-caju*. Rice straw and banana leaves showed the conversion efficiency of 60 per cent and more. The mean of the fruiting body was high (71 g) on banana leaves compared with other substrates (21- 50 g). The balance between the C/N ratio in the substrate is important to promote the proper mycelial development of the mushrooms because the total carbon is composed of recalcitrant cellulose and hemicellulose (Ryu *et al*., 2015).

Effect of grain substrates on the radial mycelial growth of P. *citrinopileatus*

In vitro experiment was conducted to find out the effect of various grain substrates on the radial mycelial growth of *P. citrinopileatus* (Fig 5). The radial mycelial growth of *P. citrinopileatus* was increased by all the grain substrates amended in PDA medium. Mycelial growth was recorded from fourth day onwards up to 90.00 mm of growth occurred at any one of the substrates. The substrate black gram and ragi amended medium recorded 90.00 mm growth on ninth day of inoculation and others recorded 87.00 and 88.00 mm. Potato dextrose agar (PDA) medium only considered as a control recorded 72.00 mm growth. All other substrates were not significantly different on ninth day. Addition of grain substrates increased the growth from 20 to 25 per cent.

The results of this study coincided with the report of Sivaprakasam (1980) as sorghum and bajra grain spawn were found to give significantly higher yields than spawn

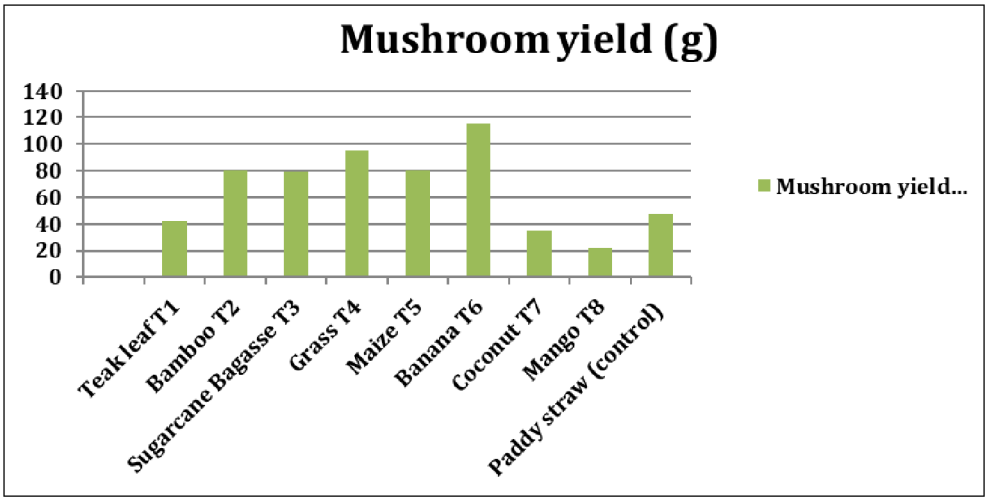

Fig 3: Effect of various leaf substrates on mushroom yield.

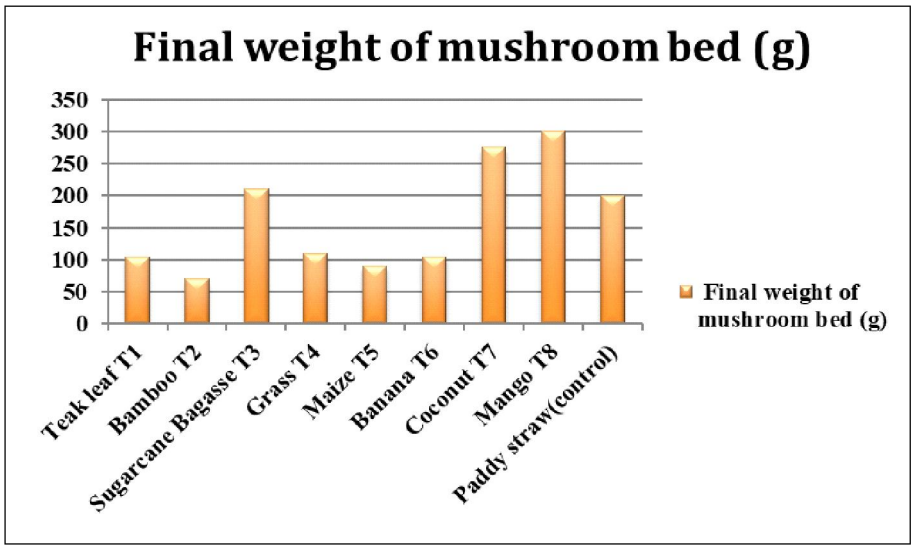

Fig 4: Effect of *P. citrinopileatus* growth on decomposition of leaf substrates.

from other grains. Pathmashini *et al.* (2008) also reported that ragi grain accelerated the spawn running, pin head formation, fruit body formation and increased the yield.

Effect of grain substrates on mycelial dry weight of P. *citrinopileatus*

Mycelial dry weight of *P. citrinopileatus* was determined by using different grain substrates and the results are given in Fig 6. Mycelial dry weight revealed the actual growth of fungus than radial mycelial growth. Dense or least growth of fungus in various substrates were clearly indicated in mycelial dry weight. Grain of black gram exhibited highest mycelial dry weight of 856.00 mg followed by bengal gram (816.00 mg). Green gram and paddy grains were next to black gram. Least dry weight of mycelium was recorded in ragi (429.00 mg) when compared to control (453.00 mg).

This result is in accordance with the report of Stanley and Awi-Waadu (2010). Among the various grains like wheat,

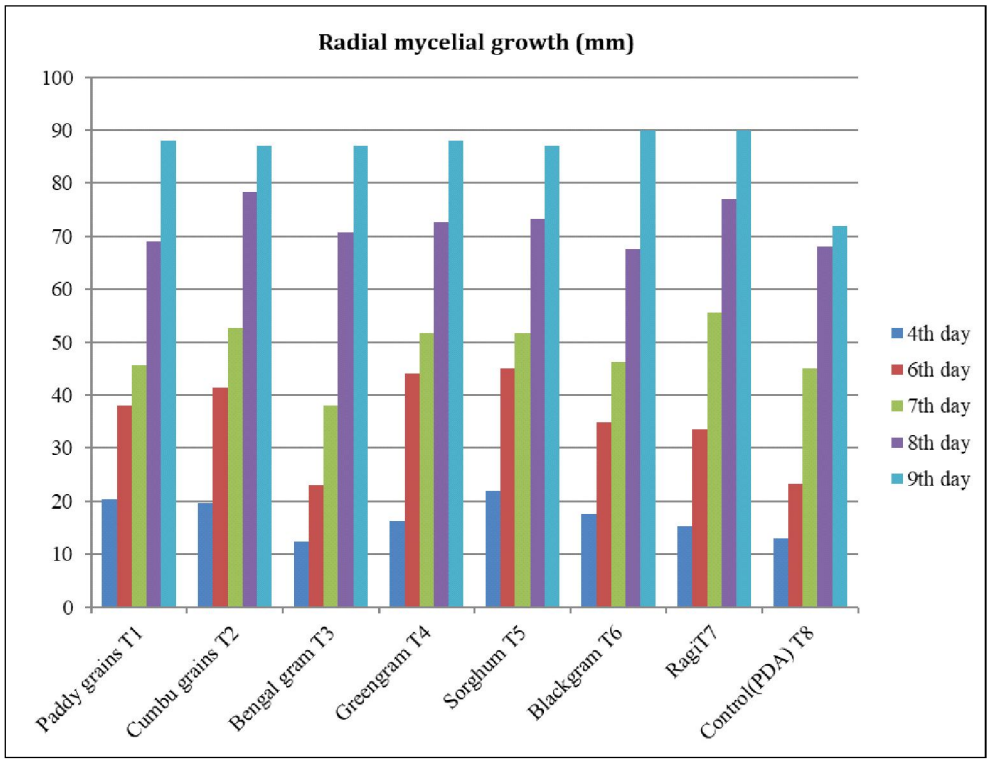

Fig 5: Effect of grain substrates on radial mycelial growth of *P. citrinopileatus*.

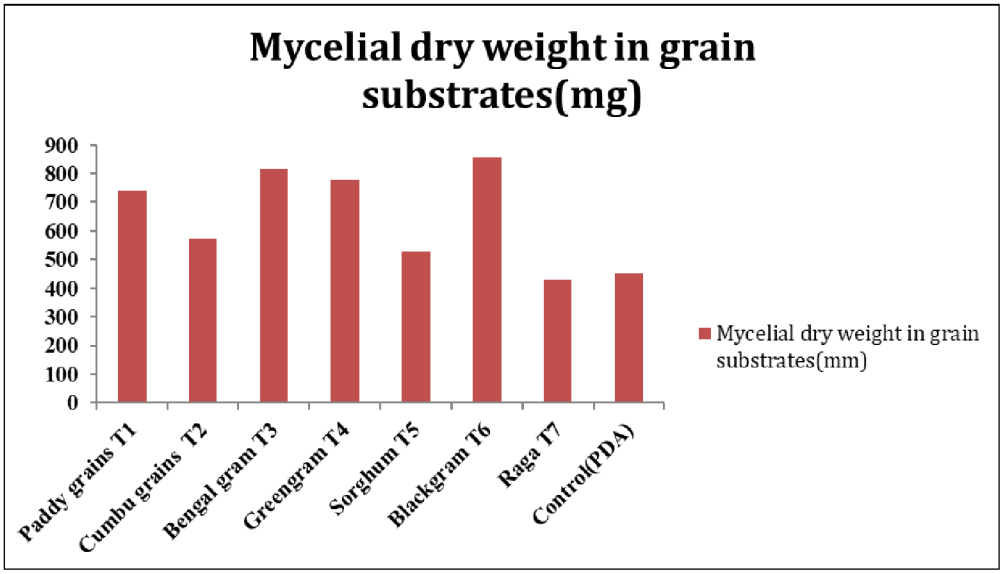

Fig 6: Effect of grain substrates on mycelial dry weight of *P. citrinopileatus*.

yellow maize, guina corn, millet, red sorghum and white maize used by the above workers on mycelial growth and mycelial dry weight of *P. tuberegium* and *P. pulmonarius,* white maize recorded 3.76 cm radial growth and 1.583 g of mycelial fresh weight followed by red sorghum.

CONCLUSION

The oyster mushroom *P. citrinopileatus* was cultivated on different leaf substrates *viz.,* teak, bamboo, maize, banana, coconut and mango and sugarcane bagasse and grass (*Dactyloctenium aegyptium*). Comparison of yield with weight of spent bed, banana leaf showed as best substrate with highest yield and suitable for cultivation of oyster mushroom *P. citrinopileatus*. Grain substrates on oyster mushroom showed that addition of grain powder in mushroom bed as organic additive may enhance the growth or utilization of above said whole grains for spawn preparation may also encourages the mushroom growth.

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Conflict of interest: None.

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