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STUDIES ON APPLICATION OF DIFFERENT SUBSTRATES ON CULTIVATION OF PLEUROTUS SAJOR-CAJU (FR.) (SYN. LENTINUS SAJOR-CAJU (FR.))

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ABSTRACT

Mushrooms consumption has generated interest in man from early civilization. They have a unique texture and flavour that are not found in other food crops. Cultivation of edible Mushrooms currently economical, biotechnology for lignocellulose organic waste recycling that combines the production of protein rich food with the reduction of environmental pollution. Therefore the research was carried out to investigate the cultivation of oyster mushroom (Pleurotus sajor-caju) on different substrates such as paddy straw, black gram waste, sugarcane bagasse ,coir waste and detritus of algae. The paddy straw considered as control. Among all aspects, paddy straw (control) was found as a best substrate with yield (±598.33gms) followed by detritus of algae, black gram waste, sugar cane bagasse and coir waste. So the paddy straw investigated at different intervals of soaking time (2, 4, 6 & 8 hrs). The paddy straw soaked for 8 hrs found as a best result when compared with other substrates soaked for different time intervals. The effects of various agro-waste on yield, biological efficiency (BE), protein content and minerals were analyzed. Mycocompost also prepared by using different bio-wastes including detritus of algae. NPK content , micronutrients and C:N ratio were analysed and the results were found. The detritus of algae yield a good phycocompost.

KEY WORDS:

Mycocompost, Phycocompost, detritus of algae and biological efficiency.

INTRODUCTION:

Mushroom cultivation is a profitable agribusiness and Oyster mushroom is an edible mushroom having an excellent taste and flavour. It grows wild in the forest and is cultivated in the temperate and sub tropical regions of the world[1] (Shah *et al.*, 1981).Oyster mushroom (*Pleurotus spp.*) is commonly called as Dhingri in India because of its oyster like shape. Genus *Pleurotus* belongs to family Tricholomataceae and has about 40 well-recognized species, out of which 12 species are cultivated in different parts of country. *Pleurotus* is an efficient lignin-degrading mushroom and can grow well on different types of lignocellulolosic materials[2]. (Poonam Dehariya and Deepak Vyas, 2013). Substrate is one of the most important parameter in mushroom production, as mushrooms depend on substrates for nutrition and the substrate is normally a source of lingocellulose material which supports growth, development and fruiting of mushroom[3&4] (Miles and Chang, 2004., Shah *et al.*, 1981). Different species of *Pleurotus* can grow well in variable temperature conditions; hence they are ideally suited for cultivation throughout the year in various regions of tropical country like India[5] (Ahmed *et al.,*2009) .Cultivation of edible mushrooms might be the only current process that combines the production of protein-rich food with the reduction of environmental pollution .It represents one of the most efficient biotechnological processes for lignocellulosic organic waste recycling oyster mushroom has been widely cultivated in many different parts of the world. It has abilities to grow at a wide range of temperatures utilizing various lignocelluloses .Unlike other mushroom species, oyster mushrooms can be easily grown on varieties of natural substrates like rice husk, wheat bran, leaf litters, straw and are the easiest, fastest and cheapest to grow, require less preparation time and production technology. Scarcity and high cost of these substrates have necessitated a search for alternative substrate. The objective of this work was to study the effect of various substrates on the linear mycelia growth and yield of paddy straw mushroom. The following objectives were followed for the present study

- Evaluate substrate formulas based on phycocompost and other alternative materials in the production of oyster mushrooms (*Pleurotus sajor caju.*).
- Evaluate detritus of algae as the enriched layer in the production of oyster mushrooms (*Pleurotus sajor caju.*)
- The detritus substances of algae dumped in shoreline areas due to tidal properties. They would lead to contaminate the seashore areas. Hence that are must be recycled without making pollution. The composting is an efficient way to reused it such a valuable way.

MATERIALS AND METHODS:

Collection of samples

The present experiments were conducted on cultivation aspect of *Pleurotus sajor-caju* using various substrates collected from local areas. The pure culture (Mother spawn) was obtained from Tamilnadu, Agriculture College, TNAU, Madurai, and used for mass production. The spawn were sub cultured for further use.

Classification : (Alexopoules and Mims, 1979) [6]

Scientific Name - *Pleurotus spp.*
Phylum - *Basidiomycotina*
Class - Basidiomycetes
Subclass - Hollobasidiomycetidae
Order - Agricales
Family -Polyporaceae
Genus - *Pleurotus*

Cultivation studies

Substrates used for *Pleurotus sajor-caju* cultivation was listed in **Table-1.** The substrates chopped in small pieces and steeped in water. The sterilized polyethylene bag of 100 gauge thickness and 30x45 cm size was used. The polyethylene bag perforated with 2mm diameter holes. In polyethylene bag first layer of pasteurized respective agro wastes was laid to a thickness of 5-6 cm and pressed gently with palm to assume cylindrical shape. Grain spawn was added at the ratio of 20g: 2 Kg. (Spawn: Substrate). Subsequently second, third and fourth layer of straw was spread over each layer and pressed gently. The bag mouth closed and tied with thread and perforation was given all over the bag for exchange of gases and maintains temperature inside the bag with sterilized needle. The bags were kept in incubation room for spawn running in darkness for a period of 15 days. The spawn running was determined by observing the white growth of mycelium all over the straw completion of spawn run the bag was cut longitudinally with sharp blade and polyethylene bag was removed.

The mushroom bed was then kept in cropping room for emergence of sporophore primordial emergence was noticed after 3 to 6 days of removal of polyethylene bag and attained desirable size within 5 to 6 days. The fruiting bodies were harvested by gently twisting with thumb and fingers. Each substrate replicated in thrice.

Effect of Different intervals of soaking time

The chopped paddy straw (control) soaked for different intervals **(Tables-2).** This paddy straw soaked at different intervals used as substrates. The procedure was as same as effect of different agro wastes.

Biological Efficiency (Chang et.al,1981):

The conversion percentage from dry substrate to fresh mushroom is indicated by a term "Biological efficiency". The biological efficiency was calculated using the formula.

$$\text{Biological efficiency} = \frac{Weight\ of\ fresh\ mushroom\ harvested}{Weight\ of\ dry\ substrate\ used}$$

Biochemical Analysis

Biochemical parameters such as protein content[7] **(Lowrys et.al., 1951)** and mineral analysis (P, K, Mg, Mn, Zn, Ca and S) also done. **Protein, fat, ash and total carbohydrate were determined with the procedure recommended by**[8& 9] **AOAC (1995) and Wankhede *et al.,* (1976). The crude fiber was determined with procedure recommended by**[10] **Ranganna (1986)**

Mycocompost with the use of detritus substances of algae:

The algal specimen *Gracilaria crassa* was collected from seashore during low tide of full moon and half moon days. This was brought to laboratory and the cleaned material by repeated washing with 5% formaldehyde and water to remove the surface micro flora and other contaminants. This was dried under shade and chopped into pieces. This was used for enriched substratum for oyster mushroom cultivation. The spent mushroom was used for the compost preparation. The compost bed prepared by subsequent layers of soil, brick, fine sand, red soil and spent mushroom were added. Finally garden soil added as cashing material. The diluted butter milk is spray in to each layer to activate the microbial consortia in the environment. Frequent water spray done regularly to reduce the heat emission. 80-90% degraded material converted in to compost at the intervals

Sample collection

The algae specimens are collected from Mandapam and Thonithurai coast line of Rameswaram District. , Tamil Nadu, India. The seaweed was taxonomically identified and voucher specimens were deposited at the PG Research Department of Botany, Sri Parasakthi college for women, Courtallam.

Gracilaria crassa[11] Harvey ex J.Agardh, 1876

Kingdom : Plantae
Phylum : Rhodophyta
Class : Florideophyceae
Order : Gracilariales
Family : Gracilariaceae
Genus : *Gracilaria*
Species : *crassa*

Key to Species[13]: (Boergeson,1938)

1.Thallus terete .....

2. Thallus compressed to foliose . . . . . .

3. Axes irregularly constricted, branching di- to trichotomously.

4. Axes not constricted (except at the base of the side branches), branching subdichotomous .....*G. canaliculata.(* Syn: *Gracilaria crassa*)

Sample preparation

The live wet specimens collected and bring to the laboratory. Repeated washing done to remove the slit, debris, and other shells and other epiphytic flora and fauna. The material cleaned with 5% formaldehyde to remove the surface micro flora this to be avoid contamination. The sample were dried under shade and powdered. The powder sample used for further assay.

Compost preparation

The compost of algal samples was prepared in earthern pot-1, control pot (sand alone)-2 and garden soil pot-3. The each pot was filled with Brick 50 g' , fine sand 50 and together layer of red soil 50 g were added. Then sample was added with 10 ml of butter milk . Finally garden soil added as a casing layer. The dilution of water was spray into each layer. It can be continued into 5 layers. Each layer dilution of buttermilk was sprayed. The procedure was followed for each algae sample in compost preparation. At 46 days intervals 80-90% degraded into digested compost. The NPK content of compost was analyzed in the nearby soil testing centre and the different concentrations of compost is used to grow plants.

RESULTS AND DISCUSSION

The result of the present study on the evaluation of different agro wastes for cultivation of *Pleurotus sajor-caju* are presented and discussed follows.

Five different types of substrates were investigated to determine the spawn running, pinhead formation and fruit body formation of *P.sajor caju.* Mycelia extension on different substrates is shown in **Table. 1.** The highest mycelial growth, pinhead formation and fruit body formation was observed in paddy straw substrate (535g) followed by a mixture of a detritus substances of algae and paddy straw, black gram wastes,sugarcane bagasse, coir wastes respectively. Mycelial growth is a preliminary step that creates suitable internal conditions for fruiting. Thus, outstanding growth of mycelium is a vital factor in mushroom

.cultivation[12] (Pokhrel *et al.,* 2009). The increase in the yield of mushroom in paddy straw is due to easier way of getting sugars from the cellulosic substances[13].(Ponmurugan *et al.* 2007)

The different types of substrates were investigated to determine the growth and yield of *P.sajor caju.* Growth and Biological efficiency on different substrates is shown in **Table. 1I.** The highest yield and biological efficiency was observed in paddy straw substrate (535g) followed by a mixture of a detritus substances of algae and paddy straw, black gram wastes ,sugarcane bagasse, coir wastes respectively. The paddy straw with detritus substances of algae wastes shows higher results because of the influence of paddy straw. Das and Mukherjee (2007) [14]It was found that when weed plants were mixed with rice straw in the ratio 1:1, there is increase in the yield than when used individually.

Sivaprakasam and Kandasamy (1980) and Rama sundara lakshmi (2005) [15&16]The investigators reported that the coir waste (coconut fiber) was high lignin content which influences the growth of *P.sajor caju.* Because of its high lignin content it takes more time for mycelium development.

Among the different intervals of soaking time the paddy straw soaked for maximum hours (8 Hrs) given higher yield. The present results were confirmed with the previous results [17] of Indira *et al.* (2010)

The minerals potassium, Iron, phosphorous, calcium, sulphur and magnesium are abundantly found in mushrooms (**Table-III).** Mustafa[18] et al., (1991) Similar results[18] reported that the content of minerals like phosphorous, potassium, calcium, sulphur, magnesium and Iron essential for human nutrition was higher in oyster mushrooms than in many fruits and vegetables.

Compared to cereals, pulses, fruits and vegetables, the protein content of mushroom is much higher on dry weight basis. Usually the protein content of cultivated mushrooms ranges between 2.5-3% on fresh weight basis and 20-35% on dry weight basis. This study indicates that the fruit bodies are quite rich in protein 26.5%on dry weight basis. The present results were confirmed with the previous results[19] of Rama sundara lakshmi (2005).

Compost is ready to use when it is dark brown and crumbly with an worthy compost to a point of being the compost pile should no longer be recognizable in finished compost except for some woody pieces. The present study reveals the following results

The physico chemical parameters of compost such as temperature, P^H, moisture content, electrical conductivity (EC) amount of N,P,K and other nutrients (Ca,Mg,So_4,Zn, Fe, Mn,Cu,and B). The results were given in **Table IV.** All the Parameters are good condition to enhance the growth of seedlings. This would leads to balance the interrelationship between the microbes and nutrient uptake by the plants. The yield of digested compost was prepared by *Gracilaria crassa* and spent mushroom. To enhance the partial anaerobic digestion 10 ml of

buttermilk was added to the composting materials. 25-40 days phyco compost and Mycocompost was ready to use when it is dark brown or black in colour **Table -2** . Similar results was done by Prema[20] (2008) in the application of panchakavya on the growth of bhendi and tomato plants[21&22] . Aziz shiralipour et. al.,(1992) Venkataraman kumar et. al.,(2003).

The degradation time composting materials was obtained up to 40 days. The colour, Texture, TSS, P^H, Temperature and Bacterial count population also. The number of bacterial population was high up to the dilution of 10^4. In 10^{10} dilution also the number of colonies were high in all the three compost and control also. These bacterial population play a vital role in the positive consoritium. The results was tabulated by in **Table-3.**These were confirmed by earlier report of [23 & 24] . Kanopa et.al., (1996) and Beffa et. al., (1996).Presences of numerous colonies indicate the microbial consoritium which performed the beneficial aspects of plant growth. This would helpful to enrich the agricultural practices[25,26&27] . Macauley et. al., (1993); Kane and Mullis (1973)and Hallmann et.al., (1997). Compost energizes the soil feed where, which is made up to microscopic bacteria and fungi from symbiotic or mutually rewarding partnerships with plants roots making with possible for vegetables to feed themselves or more efficiently. Research shows that compost enhances the ability of tomatoes and other vegetables to stand up to common discussion and may improve their flours and nutrition too compost also helps the soil retain moisture through composting you enhances your gardens ability to grow healthy plant while reducing your volume of trash.

Conclusion:

Mushroom cultivation is one of the best ways by which the residues of many things can be recycled. *P. sajor caju* well grown on different substrates are nutritious with high protein fibre and low fat. It may also offer economic incentives for agribusiness to examine these residues as valuable resources and develop new ways to use them to produce nutritious mushroom products. Therefore, the mushroom cultivation may become one of the most profitable agribusiness that could produce food products from different substrates and help to dispose them in an environment friendly manner. Availability of different substrates at different localities would be applicable for integrated cultivation practices and increase the employability skills among people. Large scale cultivation of *P. sajor-caju* using suitable substrates can help people in rural areas improve their income. Mushroom cultivation is not just an agribusiness but also a noteworthy means for restoration, replenishment and remediation of earths over burden ecosphere, thereby benefiting all the inhabitants of the planet earth.The residues of many things can be

- recycled- environment friendly manner
- Offer economic incentives for agribusiness

- Produce nutritious products and Improve income of rural people

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Table-1: Days for completion of spawn running, pinhead formation and fruiting body formation of different phases of *P. sajor-caju* production on different substrates

SL. No	Substrate Used	Spawn running	Pin head formation	Fruit body formation
1.	Paddy straw	18	22	26
2.	Paddy straw+ algal residue (10:1)	19	24	28
3.	Black gram waste	28	33	42
4.	Sugarcane bagasse	34	44	49
5.	Coir waste	37	45	49

Table-2:Effect of Various Agrowastes on Yield and Biological Efficiency of *Pleurotus sajor-caju*

SL. No	Substrate Used	Yield g/ Kg dry substrate				Yield g/500g	Biological Efficiency %
		I Harvest	II Harvest	III Harvest	Total		
1.	Paddy straw	340	115	40	535	266	88.65
2.	Paddy straw+ algal residue (10:1)	320	100	70	490	245	81.32
3.	Black gram waste	310	90	50	460	230	76
4.	Sugarcane bagasse	125	80	40	245	122.5	40.66
5.	Coir waste	90	40	10	140	70	23.32

Table-3:Effect of Various Agrowastes on Yield of *Pleurotus sajor-caju* and amount of protein present in dry mushrooms cultivated by paddy straw soaked for 8 Hrs.

Table-4:Mineral analysis

Sl.No	Reagents	Observation	Inference
1.	Filtrate + Picric acid	Yellow precipitate is formed	Presence of Potassium
2.	Potassium ferrocyanide	Blue precipitate	Presence of Iron
3.	Ammonium molybdate	Bluish green precipitate	Presence of Phosphorous
4.	Dilute sulphuric acid	Needle shaped crystals	Presence of Calcium
5.	Barium chloride	White Precipitate	Presence of Sulphur
6.	Dipotassium hydrogen ortho phosphate	Needle shaped crystals	Presence of Magnesium

Table-5: Biochemical analysis

Substrates used	Moisture(%)	Total carbohydrate (%)	Protein(%)	Fat (%)	Crude fibre (%)	Ash (%)
Paddy straw	89.30	55.50	25.33	2.60	7.15	6.35
Paddy straw+ detritus of algae	88.50	52.20	23.60	2.80	6.78	6.68
Black gram waste	87.95	56.00	22.10	2.75	6.90	6.15
Sugarcane bagasse	88.75	51.30	23.00	2.85	7.50	7.00
Coir pith waste	89.35	52.80	20.33	2.60	7.82	5.90

Table-6.Physico Chemical Parameters of Phycocompost

Name of the Samples	Temperature (^{o}c) at three different depth (cm)			TSS (mg /l) in different (nm)				Moisture g / wt	P^{H}	Ec dsm^{1}	N kg/ ha	P kg/ha	K mg/kg	o. c %
	5	10	15	420	540	620	680							
C (1)	28	26	27	0.58	0.68	0.6	0.64	3.0	5.3	0.64	134.4	112	310.5	61
S-1	28	26	29	0.70	0.72	0.70	0.70	2.5	6.5	0.49	159.6	137	163.9	67
S-2	20	18	17	0.58	0.68	0.60	0.64	2.1	6.7	0.53	169.3	132	168.8	59

S-1: *Gracilaria crassa* ; S-2: *Gracilaria crassa*+ Spent mushroom of detritus of algae

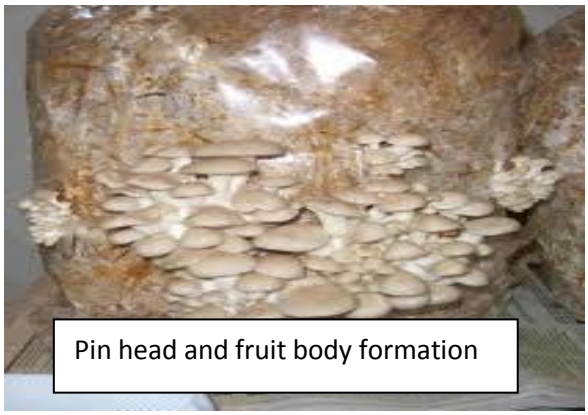
Table-7. Nutrient Availability

Nutrient	Soil Test level
N	High
P	Optimum
K	High
Ca	Low

Days / Yield (g) of Compost			
Days	C-1	S-1	S-2
25	120	130	110
30	160	160	160

Mg	Low		35	180	170	190
So_4	Low					
Zn	Low		40	210	240	240
Fe	High		45	245	285	285
Mn	Low		50	365	375	395
Cu	Low					
B	Low		55	500	500	500

PLATE-I




Sugarcane Bagasse