Biological Forum – An International Journal **14(1): 1683-1687(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Optimizing the Substrates and Container for Enhancing the Bio Efficiency of *Pleurotus florida*

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(Received 16 December 2021, Accepted 22 February, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Biological efficiency of mushroom cultivation is very important one. The problematic issue in mushroom cultivation is selection of suitable substrate and container, which make a profitable biological efficiency. Mushroom is an important diet food. Which cultivated all over the world. For getting more output many substrates used commercially. Many lingocellulosic substrates used for the cultivation of the mushroom and also paddy straw required the least no of days for spawn running and pinhead initiation with the maximum yield and biological efficiency, followed by banana leaves in combination with rice straw at (1:3) ratio. Adding urea @ 0. 1% conc. to the substrate was found to slightly improve the yield and biological efficiency of the substrate. Different containers *viz.*, Plastic bottles 200g, plastic containers 300 g, mud pots 400g, perforated dustbins and polythene bags of 500 g. capacity of paddy straw (dry weight) were tried to evaluate the biological efficiency of *P. florida* and to avoid the use of polythene bags for mushroom production. Among them polythene bags followed by reusable semi transparent plastic containers recorded a B.E. of 131.02% and 124.89% respectively, which is the appropriate substrate and type container made a high biological efficiency of mushroom.

Keywords: Paddy straw, containers, Urea, *P. florida*, Inorganic nitrogen source.

INTRODUCTION

A substrate is any material or substance that serves as a medium of growth for a living thing in which enzymes can act upon and break it to release nutrients for the growing organism. *Pleurotus* species is a wood digesting fungi, which was first cultivated on logs (Ingale and Ramteke 2010). During the year 2017, 10 million tonnes of mushrooms were produced globally with major contributions from China (77%), Europe (12%), United States of America (4%), and India (1%) (FAOSTAT 2017). The poor management of the waste and effluents from households, industries, and agricultural fields is further deteriorating the already crippling ecosystem (Akhtar and Amin-ul Mannan, 2020). The protein content of mushrooms varies from 4-44% according to the species (Okoro and Achuba, 2012). Mushrooms can utilize a large variety of agricultural waste products and transform the lignocellulosic biomass into food of high quality, flavor and nutritive value. It can also decrease air pollution and environmental pollution by utilizing agriculture wastes (Karunakaran *et al.*, 2017). *Pleurotus* species belong to white-rot fungi that are known to produce a wide variety of polysaccharide and lignin degrading enzymes which are capable of degrading different lignocellulose materials (Daniel, 2016). Jaiganesh (2019) cultivated on the different substrates *viz.*, banana leaves, casuarina needle, coir pith, groundnut shell, paddy straw, sugarcane trash, sugarcane bagasse, saw dust and water hyacinth of *H. ulmarius* of which paddy straw substrate produced the highest yield and biological efficiency. A promising technique with the replacement of polyethylene bags was with reusable and durable perforated plastic buckets (Panjikkaran and Mathew 2013**).** Nguyen *et al.* (2021) ammonium chloride (NH_4 Cl), ammonium nitrate (NH_4 NO_3) and ammonium sulfate $(NH_4)_2$ SO_4 produce the good mycelial growth of *T. versicolor*. Jegadeesh *et al.* (2018) studied different substrates *viz*., paddy straw, sugarcane bagasse, coir pith, sorghum straw, ragi straw and mixed bed were used for the cultivation of pink oyster mushroom. Maximum yield and biological efficiency recorded (600.37 ± 27.02 g/bed, 120.07±5.40

per cent) of *P. djamor* var. *roseus* was obtained using paddy straw. This could be due to the presence and better availability of all the essential nutrients and minerals in this substrate. The containers play an important role in production of mushroom. Mushroom are saprophyte which utilize the various agrowastes and the current studies revealed paddy straw as the efficient substrate for increasing yield and good sporophore formation.

MATERIALS AND METHODS

Source and maintenance of culture. The pure culture of *Pleurotus florida* was procured from IIHR, Bangalore, and was sub cultured onto PDA plates. Subcultures were made periodically and maintained on potato dextrose agar (PDA) slants (Aneja, 2003) and stored at 25 ± 2°C for further investigations.

Preparation of spawn substrates of *P. florida.* This experiment comprised take sorghum grains for spawn production. The grains were boiled in water (300g/L) for 15 minutes, excess water was drained off, shade dried, then calcium carbonate and gypsum (1.5% of each) were added and mixed thoroughly. The grains were filled upto 2/3 levels in glucose bottles which were then plugged with cotton wool, covered with an aluminium foil cap and autoclaved at 121°C (15 psi) for half an hour. When cooled, the grains were inoculated with 1 cm plugs from agar cultures or grain spawn of *Pleurotus florida* and incubated at 25°C for two weeks.

Pasteurization of bed substrates. Here we take different agro wastes *viz.,* paddy straw, banana leaves, wood chips and sugarcane bagasse as a bed substrates then the substrate were pasteurized by soaking in (Carbendazim 10g + formalin 100ml) for 15 hrs. + autoclaving @ 121.6°C for 30 mins.

Efficacy of different bed substrates for bed preparation of *P. florida* cultivation

Seven different locally available agro wastes *viz.*, paddy straw, banana leaves, banana leaves + rice straw (1:3), banana leaves + rice straw(1:1), woodchips and sugarcane bagasse were tried to see the ability of the test fungi to colonize and form fruiting bodies. The substrates were soaked in water for different periods depending on their ability to retain water. Moisture content of 60-70% with 7.0 pH was maintained in each case. Sterilization was carried out and then spawning, bagging and hanging was done. Three replicates of each treatment were kept throughout the cultivation studies and the observations namely number of days for spawn run, number of days for pinhead initiation, average weight of sporophore, total yield/bag and biological efficiency (%) were recorded.

Efficacy of different containers for bed preparation of *Pleurotus florida* cultivation

Plastic bottles. Plastic bottles (2000 ml) capacity with small holes were used for filling the sterilized paddy straw. Each of the bottle contained 200 g of paddy straw on dry weight basis. Sorghum grain spawn was thoroughly mixed with the treated substrate before filling on to the bottles. Nine holes, were punched out in all the sides except at the bottom. After spawn running the caps placed at the top of the bottles were removed to induce fruiting bodies

Plastic container. Plastic container of capacity 300g were used to fill the processed paddy straw substrate. Spawn was mixed @ 20% (w/w) with the paddy straw. The containers were sealed on all sides. Nine to twelve holes were punched out in all the sides except at the bottom. After spawn running the lids placed at the top of the container were removed to induce fruiting bodies.

Mud pots. Mud pots of 400g capacity with 10-12 small holes were used to fill the processed paddy straw substrate. For each pot paddy straw with spawn was used for filling. The pots were sealed with polypropylene cover at top and transferred to the mushroom shed for further observation.

Perforated dustbins. Perforated dustbin of capacity 400g was used as container to fill the processed paddy straw substrate. Spawn was mixed @ 20% (w/w) with the paddy straw. The containers were sealed with polypropylene cover at top and transferred to the mushroom shed for further observation.

Polybags. Poly bags (80 G thickness) measuring 60 × 30 size with small holes was used for the preparation of mushroom beds. For each bag 500 g substrate (dry weight basis) was used. Layer method of spawning was done as suggested by Sivaprakasam (1980). The polybags were tied at both the end and transferred to the mushroom shed for further observation.

Evaluation of inorganic nitrogen supplementation on the growth and yield parameters of *P. florida*

Urea 0.1% & 0.2 per cent, Ammonium chloride 0.1 & 0.2 per cent, DAP 0.1 & 0.2 per cent (w/v) were used as the inorganic nitrogen supplements in the mushroom beds. These supplements were powdered well and thoroughly mixed with sterilized paddy straw before spawning. Paddy straw without any additives served as control. Parameters like Days for spawn run, days for pinhead initiation, average weight of the sporophore, yield and biological efficiency were calculated.

RESULTS AND DISCUSSION

A. Effect of various bed substrates on the growth and yield parameters of P. florida

The data from Table 1 revealed that all the locally available agro wastes used as a bed substrate for the cultivation of oyster mushroom, which produced the good yield. Among the different agro wastes tested *viz.*, Paddy straw, banana leaves, banana leaves + rice straw (1:1 ratio), banana leaves + rice straw (1:3), sugarcane bagasse and wood chips. The paddy straw was found to be a most efficient substrate in enhancing the yield with a biological efficiency of (649.67 g / bag, 129.93 per cent), followed by banana leaves + rice straw (1:3) ratio which recorded the second highest yield and biological efficiency (520.90 g/bed, 104.18 %), banana leaves + rice straw (1:1) ratio (495.89 g/bed, 99.17 per

cent), banana leaves (484.90 g/bed, 96.98 per cent) and Sugarcane baggase (476.34 g/bed, 95.26) respectively, in the decreasing order of merit. Woodchips produce least yield with a biological efficiency of (320.78 g/bed, 64.15 %). Similar results were observed by many previous researchers which lend support to our findings. Jatwa *et al*. (2016) reported paddy straw showed significantly produce the highest yield and biological efficiency of *P. florida*, *P. eous* and *P. sajor-caju*. Mondal *et al*. (2010) also reported that the combination of banana leaves with paddy straw @ (1:1 ratio) produced good yield.

Table 1: Evaluation of various bed substrates for cultivation of *P. florida.*

Sr. No.	Treatments	DFSR*	DFPI*	AWOS*	Yield (g/bed)*	BE (%)*
1.	Paddy straw	12.11[a]	15.36[a]	21.81[a]	649.67[a]	129.93[a]
2.	Banana leaves	16.65[d]	20.72[d]	18.21[c]	484.90[d]	96.98[d]
3.	Banana leaves + rice straw (1:1)	15.84[c]	20.10[c]	19.31[bc]	495.89[c]	99.17[c]
4.	banana leaves + rice straw(1:3)	15.20[b]	17.48[b]	19.63[b]	520.90[b]	104.18[b]
5.	Sugarcane bagasse	18.73[e]	21.36[e]	17.35[d]	476.34[e]	95.26[e]
6.	Woodchips	19.40[f]	22.01[f]	16.37[e]	320.78[f]	64.15[f]

*mean of three replication
*values not sharing a common superscript differ significantly at p<0.05 (DMRT)
DFSR : Days for spawn run
DFPI : Days for pinhead formation
AWOS : Average weight of sporophore
BE : Biological efficiency

B. Efficacy of different containers for bed preparation of P. florida cultivation

The five different containers *viz.*, mud pot, perforated dustbins, plastic bottles, plastic container and polypropylene bags used in the present study. Among them semi transparent plastic container had a positive impact on the growth and yield of the mushrooms. The substrate mixture in the polypropylene bag recorded minimum spawn run days (12.10) and the least pin head initiation days (15.32) and days for first flush (15.44) and also recorded an increase in the average weight of sporophores (21.78 g/bed), thereby increase in the yield (655.10 g bed) and biological efficiency (131.02 per cent), which was followed by plastic container in which *P. florida* recorded a yield of 374.65 g/bed and a biological efficiency of 124.89%. The minimum yield and biological efficiency was recorded (305.43 g/bed and 76.25 per cent) in mud pot (Table 2). The present study revealed that plastic container used as mushroom bed, produced the maximum yield and biological efficiency of *P. florida* which was followed by polypropylene bags. Sometimes opaque or semi-transparent containers with the limited light passage will be helpful to encourage quick spawn run (Panjikkaran and Mathew 2013). In the present study the least yield was recorded by mud pot. Mud pot showed the least bio efficiency recording 78.85% for *P. florida* because mud pots were found to be inferior which might be due to absence of light for the mycelial growth and primordial formation. Use of semi-transparent reusable containers will be environmentally benign, cost effective and may be helpful to design flexible automated oyster mushroom production systems under Indian conditions (Panjikkaran and Mathew 2013).

Table 2: Efficacy of different containers for bed preparation of *P. florida* cultivation.

Sr. No.	Different container	Quantity of substrates (g)	DFSR	DFPI	DFFF	AWOS	Yield (g)/bed	Biological efficiency (%)
1.	Plastic bottles	200	11.66[a]	14.53[a]	17.31[b]	18.45[c]	240.80[e]	120.40[c]
2.	plastic container	300	13.11[c]	15.81[c]	17.66[c]	18.72[b]	374.65[b]	124.89[b]
3.	mud pot	400	15.15[e]	18.90[e]	20.77[e]	17.30[e]	305.43[d]	76.25[e]
4.	perforated dustbins	400	14.19[d]	17.21[d]	20.02[d]	17.62[d]	350.62[c]	87.65[d]
5.	PP bags	500	12.10[b]	15.32[b]	15.44[a]	21.78[a]	655.10[a]	131.02[a]

*mean of three replication
*values not sharing a common superscript differ significantly at p<0.05 (DMRT)
DFSR : Days for spawn run
DFPI : Days for pinhead formation
DFFF : Days for first flush
AWOS : Average weight of sporophore
BE : Biological efficiency

C. Evaluation of inorganic nitrogen supplementation on the growth and yield parameters of P. florida

The different nitrogen supplements at different concentrations, urea @ 0.1 per cent recorded the least no. of days for spawn run and pinhead formation (12.03 & 15.36) and produced maximum weight of sporophore (25.83), maximum yield/bed with the biological efficiency (664.89 g/bed, 132.97 per cent) respectively. Among them Ammonium chloride @ 0.1 per cent was found to enhance the mycelial growth with days for spawn run and pin head initiation (12.95 & 16.14) respectively and also increased the yield and biological efficiency (638.60 g/bed & 127.72 per cent) respectively when compared to control (Table 3 and Fig. 1). Ammonium chloride 0.2 per cent and Urea 0.2 per cent significantly reduce yield and biological efficiency. In current studies the highest yield and biological efficiency was recorded Urea 0.1 per cent this results similarly with El- Kattan *et al.* (1991) reported. Ammonification process on the beds that leads to inhibit the growth of mycelium reported by (Bano and Srivastava 1962). Miller (1994) reported that ammonia evolved from the decomposition of nitrogen sources leads to significant rise in pH. Inorganic source of supplements was found to be poor counterparts of additives tested as reported by Eswaran and Thomas (2003); Senthilmurugan (2004). Hoa and Wang (2015) reported that ammonium chloride concentrations at 0.03~0.05% gave the highest mycelium growth of oyster mushroom of *P. ostreatus* and *P. cystidiosus*. When the inorganic nitrogen source concentration increases the mushroom yield and biological efficiency also reduced. The addition of nitrogen can increase oyster mushroom yield, which would otherwise decrease if the added nitrogen is in excess, given that nitrogen inhibits the fruiting process of the mushroom (Bellettini *et al*., 2019). A high level of N can repress the ligninolytic enzyme production, while at low availability of nitrogen, this metabolic pathway can be up-regulated. Mleczek *et al.* (2021) reported that ammonium and nitrate nitrogen may be too readily available, particularly if applied prior to spawning, to produce a beneficial effect on fruitifcation. Nguyen *et al*. (2021) ammonium chloride (NH_4Cl), ammonium nitrate (NH_4 NO_3) and ammonium sulfate $(NH_4)_2$ SO_4 produce the good mycelial growth of *T. versicolor.*

Table 3: Evaluation of inorganic nitrogen supplementation on the growth and yield of *P. florida.*

Sr. No.	Treatments	DFSR	DFPI	AWOS	Yield (g)/bed	BE(%)
1.	urea 0.1%	12.03^{a}	15.36^{a}	25.83^{a}	664.89^{a}	132.97^{a}
2.	urea 0.2 %	13.30^{bc}	16.79^{c}	22.91^{c}	630.23^{c}	126.04^{c}
3.	Ammonium chloride 0.1%	12.95^{b}	16.14^{b}	23.53^{b}	638.60^{b}	127.72^{b}
4.	Ammonium chloride 0.2%	13.82^{bc}	17.05^{cd}	20.20^{d}	620.67^{d}	124.13^{d}
5.	DAP 0.1%	14.15^{de}	17.66^{de}	19.22^{e}	600.78^{e}	120.15^{e}
6.	DAP 0.2%	14.47^{de}	18.04^{e}	18.65^{f}	590.56^{f}	118.11^{f}
7.	control	14.64^{e}	18.92^{f}	17.87^{g}	500.57^{g}	100.11^{g}

*mean of three replication

*values not sharing a common superscript differ significantly at p<0.05 (DMRT)

DFSR : Days for spawn run

DFPI : Days for pinhead formation

AWOS : Average weight of sporophore

BE : Biological efficiency

DAP 0.1%

Fig. 1. Evaluation of inorganic nitrogen supplementation on the growth and yield of *P. florida.*

CONCLUSION

From these research paddy straw required least no of days for spawn running and pinhead initiation with the maximum yield and biological efficiency followed by adding urea @ 0. 1% conc. to the substrate was found to slightly improve the yield and biological efficiency of the substrate and also semi transparent plastic containers recorded a biological efficiency of 131.02% and 124.89% respectively. Above the outputs based we have framed the cultivation of mushroom led to the highest biological efficiency.

FUTURE SCOPE

This research focused on the which substrate, container and amendment used for improve the yield and higher biological efficiency of mushroom. Beyond that which mushroom sample will analysed through GC-MS, NMR and FITR for quantify and qualify the specific nutrients. Which will help the substrate based enrichment of nutrients in the mushroom.

Conflict of Interest. None.

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How to cite this article: Ramesh Kumar R., Darwin Christdhas Henry L., Ayyandurai M. and Kokila A. (2022). Optimizing the Substrates and Container for Enhancing the Bio Efficiency of *Pleurotus florida*. *Biological Forum – An International Journal, 14*(1): 1683-1687.