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RESEARCH PAPER

Comparative study of different spawn and bed substrates for the cultivation of *Pleurotus cystidiosus*

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Abstract: Spawn and bed production studies were carried out with isolate of *Pleurotus cystidiosus* having Genbank accession number KY887023. *P. cystidiosus* took the minimum time for spawn run in paddy grains amended with one per cent yeast extract (5.63 days), with thicker and fluffy mycelial growth and hence identified as the best amended substrate. Spraying of rubber wood sawdust with 2.5 per cent of 1 *M* Potassium Dihydrogen Phosphate (KH_2PO_4) resulted in production of the maximum BE (192.76 per cent) by *Pleurotus* sp. Multi locational trials with *P. cystidiosus* proved the species as the best oyster mushroom for low temperature areas of Kerala like Idukki and Wayanad.

Key words: Bed, Biological efficiency, *Pleurotus cystidiosus*, Spawn

Introduction

Pleurotus or oyster mushrooms are the most popular and widely cultivated mushrooms in Kerala. In this context, the exploitability of a promising isolate of *Pleurotus cystidiosus* for cooler (temperate) regions of Kerala *viz.*, Idukki district using locally available materials is the need of the hour. Isolate of *P. cystidiosus* registered at Genbank database with accession number KY887023 was used for the present study. The 6 days old culture was maintained on Potato dextrose agar medium at 25 ^{0} Celsius and was used for further spawn and bed production studies (Fig 1).

Material and methods

Evaluation of different substrates for spawn production

Different substracts *viz.,*grains (paddy grains, sorghum) and rubber sawdust were evaluated for the spawn production of *P. cystidiosus*. The procedure for the spawn production was followed according to the standard techniques (Sinden, 1934 and Deepa, 2016). Spawns were prepared to attain the final moisture content of 60 per cent.

Evaluation of different amendments for spawn production

Substrates used for production of spawns were separately amended with additives at different concentrations *viz*., yeast extract (0.5, 0.75 and 1%), iron in the form of ferrous sulphate (0.25, 0.5 and 0.75%) and thiamine (25, 50 and 75 ppm). Nine experiments with each substrate and respective nine amendments, were laid out separately in CRD, to find the best amendment in each substrate, with three replications for each treatment. Spawns prepared from the substrates alone (without amendments) were served as controls. Also, three experiments, with three substrates and respective nine amendments, were laid out separately in CRD, to find the best spawn substrate with the best amendment, with thirty treatments and three replications for each treatment.

Substrate-calcium carbonate mixture was prepared using grains and rubber sawdust, separately, as per the standard procedure. The amendments were separately dissolved in warm distilled water and mixed with the prepared mixtures, to attain the final moisture content of 60 per cent. The amended sawdust-calcium carbonate mixture was then filled in polypropylene bags, followed by sterilization, inoculation and incubation.

Parameters studied for deciding the efficacy of different substrates and amendments on spawn production

Parameters *viz.*, time taken for spawn run *i.e.,* number of days from inoculation to complete colonization of substrate by the mycelium, nature of mycelial growth, presence of fungal/ bacterial contaminants and shelf life of spawn were recorded.

Also, the following scale was used for assessing the myelial growth pattern of spawn (Priya *et al*., 2017):

++++ : Thicker and fluffy growth

+++ : Thick growth

++ : Poor growth

Cultivation trials of *P. cystidiosus* with different bed substrates

Locally available substrates *viz.*, paddy straw, sawdust of rubber tree and neopeat using polybag method of cultivation were evaluated for the production of *P. cystidiosus*, according to the procedure given by Baskaran *et al*. (1978).

Polybag method using paddy straw as substrate

Chemical sterilization of paddy straw was carried out, by soaking paddy straw in water containing 75 ppm carbendazim (bavistin) and 500 ppm formalin for 18 hr. Then, the excess water was drained off and straw was spread over a silpaulin sheet under sun. Straw was dried under sun, until the moisture content was reduced to 60 per cent. The mushroom beds were prepared using polypropylene covers of 60 x 30 cm size (thickness of 100 mm gauge), following the polybag method of cultivation.

Polybag method using saw dust as substrate

Saw dust of rubber tree was soaked in water for 24 hr and excess water was drained off the next day. The drained material was sun dried, mixed with 3 per cent calcium carbonate and

filled in polypropylene covers (60 x 30 cm size, with thickness of 100 mm gauge). The mixture was sterilized at 121 ^{0}C for 2 h, under 1.02 kg/cm^2 pressure, in an autoclave. Mushroom beds were prepared using the sterilized mixture following polybag method of cultivation.

Polybag method using neopeat as substrate

Neopeat blocks were soaked in water for 24 hr. Excess water was drained off the next day, followed by steam sterilization in order to prepare the mushroom beds using poly bag method.

For each bed, 1000 g (dry weight) of substrate and 150 g of spawn were used.

Evaluation of amendments for polybag method of mushroom production

Nitrogen supplements *viz.,* wheat bran (2 and 4%), rice bran (2 and 4%), neem cake (2 and 4%) and chemical sprays *viz.*, urea (0.5 and 1%), gypsum (1 and 2%), 1 *M* Potassium Dihydrogen Phosphate (KH_2PO_4) (2 and 2.5%) and gibberellic acid (GA) (10 and 20 ppm) were used as amendments for the different substrates. Nine experiments with each bed substrate and respective fourteen amendments, were laid out separately in CRD, to find the best amendment in each bed substrate, with three replications for each treatment. Here, beds prepared from the substrates alone (without amendments) served as the controls. Also, three separate experiments with three bed substrates and respective fourteen amendments were laid out separately in CRD, to find the best bed substrate with the best amendment, with fourty five treatments and three replications for each treatment.

Each of the bed substrate was processed according to the standard procedure and mixed separately with the nitrogen supplements (20 g and 40 g per kg of substrate). The mixture was filled in polypropylene cover (60 x 30 cm size, with thickness of 100 mm gauge) and steam sterilized for 1 hr. Mushroom beds were prepared in polypropylene covers of 60 x 30 cm size (thickness of 100 mm gauge) using the sterilized, amended substrate. Spawning was done layer by layer, up to four layers, by filling the polythene bag with spawn and amended substrates. The prepared polybags were made compact by tying at the top and few holes were provided for air circulation. The beds were then transferred to incubation chamber for initiation of spawn run. After the completion of spawn run, beds were transferred to cropping room for fruiting and one-inch slits (8-10 in number) were made in the polybags for the emergence of pinheads. Chemicals *viz*., urea (0.5 and 1%), gypsum (1 and 2 %) and 1 M KH_2PO_4 (2 and 2.5%) were dissolved in water, to prepare the required concentrations. Stock solution of GA (50 ppm) was prepared in 95 per cent ethanol and was used for the preparation of working standards *viz.*, 10 and 20 ppm. After completion of spawn run, the spawn bag was opened at the top and one inch slits (8-10 in number) were made around the beds, for spraying the required chemical solutions, at three stages of mycelial run (500 ml each) *viz.*, at complete spawn run, after first harvest and 15 days later.

Parameters deciding the efficacy of substrates and amendments on mushroom production

Parameters *viz.*, number of days taken for the development of mushroom sporocarps (from pinhead stage to maturity stage), total yield per bed from three harvests (kg/bed), total crop period (days), average weight of sporocarp (g), number of sporocarps, biological efficiency and the incidence of microbial contaminants/insect pests were recorded.

Formula mused for calculating Biological efficiency (BE) (per cent)

$$\text{B.E (\%)} = \frac{\text{Total weight of fresh mushrooms harvested per polybag(g)}}{\text{Dry weight of substrate per polybag (g)}} \times 100$$

The best bed substrate and amendment, was selected based on the above parameters, results were statistically analysed and interpreted.

Cropping conditions

The inoculated substrates were kept in a spawn running room at 25^{0}C and 85-90 per cent relative humidity under dark conditions. After the complete spawn run, polybags were moved to a cropping room, at 28 ^{0}C, 80 per cent or above relative humidity and light intensity of 100 lux. The walls and floor of cropping room was sprayed with water twice a day to maintain humidity during the cropping time.

Multilocation trials on seasonal variation in production of *P. cystidiosus*

Seasonal variation in production of *P. cystidiosus* was studied in two regions of Kerala *viz*., Vellayani of Trivandrum district (with tropical climate) and Thankamany of Idukki district (with temperate climate). Trials were carried out in three seasons *viz.*, February-May, June-September and October-January using sawdust of rubber tree as the bed substrate.

Results and discussion

Evaluation of substrates for spawn production

P. cystidiosus took the minimum time for spawn run (5.63 days) with maximum shelf life (81.58 days) and recorded absence of contamination with thicker and fluffy growth in paddy grains amended with yeast extract 1 per cent (Table 1). Also, *P. cystidiosus* took the minimum time for spawn run (6.30 days) with maximum shelf life (54.32 days) and recorded absence of contamination in sorghum grains, amended with yeast extract 1 per cent (Table 2). Similarly *P. cystidiosus* took the minimum time for spawn run (8.33 days) with maximum shelf life was (108.32 days) and recorded absence of contamination in rubber wood sawdust amended with yeast extract 1 per cent. (Table 3). Thus yeast extract 1% was identified as the best amendment for the three substrates used. *P. cystidiosus* took the minimum time for spawn run in paddy grains amended with one per cent yeast extract and hence identified as the best amended spawn substrate.

Madrupji (2017) recorded that *P. cystidiosus* took 3.25, 3.75, 4.00, 6.00 and 6.75 days for initiation of mycelium run on maize, bajra, finger millet, wheat and sorghum grain spawn respectively. They also identified maximum preferability for spawns prepared from sorghum and wheat in *P. cystidiosus*, as it showed the maximum reduction in weight due to loss of moisture from grain spawn and nutrients uptake.

Evaluation of amendments for mushroom production

P. cystidiosus recorded minimum time for spawn run, pinhead formation and first harvest in paddy straw sprayed with 1M KH_2PO_4 @ 2 per cent spray (35.62, 40.61 and 44.47 days). Total yield per bed from three harvests, biological efficiency, crop period and average weight of sporocarps were noted to be the highest with wheat bran amendment @ 4 per cent (2510.32 g, 125.52%, 124.32 days, 23.17 g). Maximum number of sporocarps was recorded with urea @ 0.5 per cent spray (253.42) (Table 4 and Fig 2).

P. cystidiosus recorded minimum time for spawn run, pinhead formation and first harvest was when cultivated in rubber wood sawdust sprayed with 1 M KH_2PO_4 @ 2.5 per cent spray (45.15, 51.30 and 56 days). Total yield from three harvests, biological efficiency and crop period was recorded highest for 1 M KH_2PO_4 @ 2.5 per cent spray (3855.12 g, 192.76%, 145.63 days). Average weight of sporocarp recorded was the maximum for wheat bran amendment @ 4 per cent (36.28 g). Maximum number of sporocarps was recorded with GA 20 ppm (289.37) (Table 5 and Fig 3).

Table 1. Evaluation of amended paddy grains for spawn production of *Pleurotus* sp.

Treatments	*Days for spawn run	Nature of growth	*Per cent spawns contaminated	*Shelf life (days)
Thiamine (25 ppm)	8.35^{e}	++	53.3	75.33^{f}
Thiamine (50 ppm)	11.30^{c}	++	60	72.37^{h}
Thiamine (75 ppm)	12.33^{b}	++	60	71.35^{i}
Yeast extract (0.5%)	7.33^{f}	+++	33.3	79.30^{c}
Yeast extract (0.75%)	6.17^{h}	++++	-	80.30^{b}
Yeast extract(1%)	5.63^{i}	++++	-	81.52^{a}
Ferrous sulphate(0.25%)	6.31^{h}	++++	-	75.65^{e}
Ferrous sulphate(0.50%)	9.07^{d}	++	60	74.32^{g}
Ferrous sulphate(0.75%)	12.63^{a}	++	60	71.33^{i}
Control	7.07^{g}	+++	33.3	76.42^{d}
S.Em. (±)	0.828			1.158
C.D (0.05)	0.184		0.175	0.175

*Average of three replications, each replication denotes 5 spawns, Means followed by similar superscripts do not differ significantly at 5% level

Table 2. Evaluation of amended sorghum grains for spawn production of *Pleurotus* sp.

Treatments	*Days for spawn run	Nature of growth	*Per cent spawns contaminated	*Shelf life (days)
Thiamine (25 ppm)	10.33^{e}	+++	33.3	48.37^{e}
Thiamine (50 ppm)	14.63^{b}	++	53.3	45.35^{h}
Thiamine (75 ppm)	17.07^{a}	++	53.3	42.32^{i}
Yeast extract (0.5%)	8.32^{g}	+++	33.3	51.33^{c}
Yeast extract (0.75%)	6.63^{h}	++++	-	53.33^{b}
Yeast extract (1%)	6.30^{i}	++++	-	54.32^{a}
Ferrous sulphate (0.25%)	6.62^{h}	++++	-	48.32^{e}
Ferrous sulphate (0.50%)	12.27^{d}	++	53.3	47.32^{f}
Ferrous sulphate (0.75%)	13.63^{c}	++	60.0	46.32^{g}
Control	10.07^{f}	+++	33.3	49.32^{d}
S.Em. (±)	1.182			1.155
C.D. (0.05)		0.103		0.069

*Average of three replications, each replication denotes 5 spawns, Means followed by similar superscripts do not differ significantly at 5% level

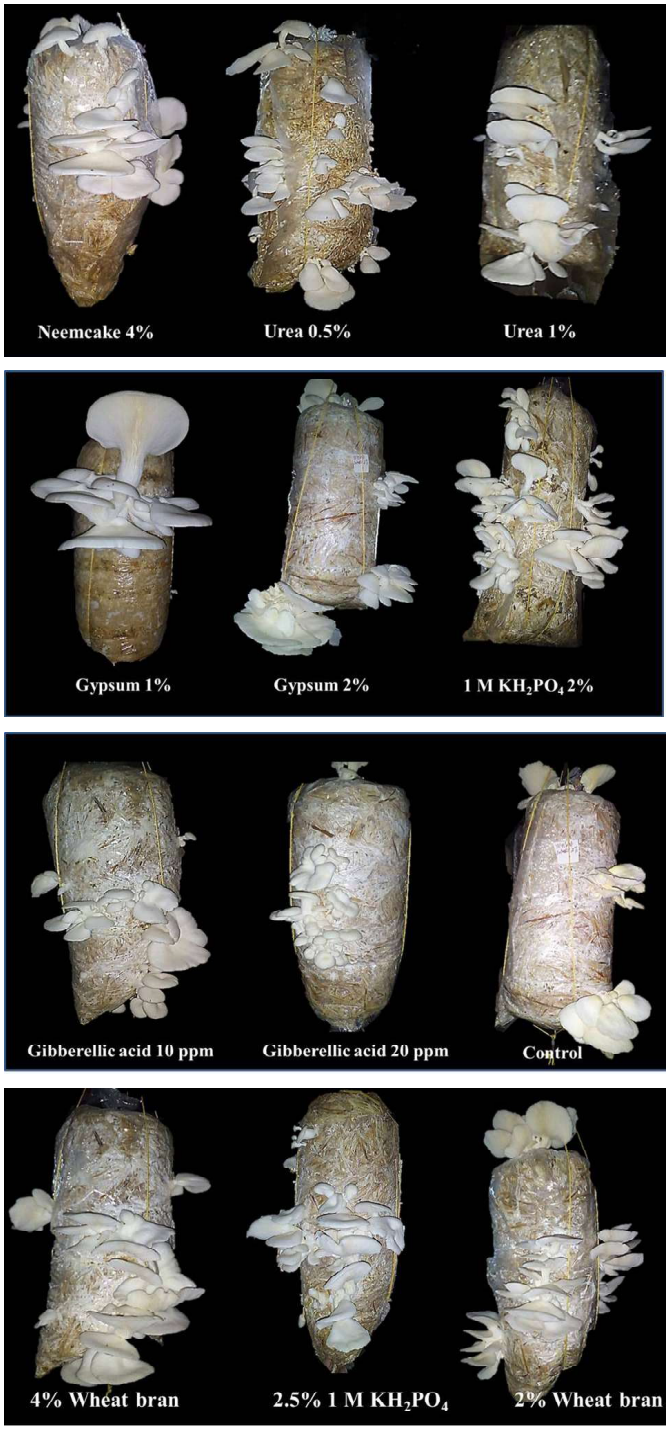

Fig. 2. Influence of amended paddy straw on yield parameters of *P. cystidiosus*

P. cystidiosus recorded minimum time for spawn run in neopeat sprayed with 1 M KH_2PO_4 @ 2.5 per cent spray (51.12 days) and 1 M KH_2PO_4 @ 2 per cent spray (51.25 days). Rice bran amendment @ 2 per cent took the minimum time for pinhead formation and first harvest (56.39 and 59.25 days). Total yield from three harvests, biological efficiency, crop period, average weight of sporocarp and maximum number of sporocarps were recorded in beds amended with wheat bran @ 4 per cent (751.38 g, 37.57 %, 111.29 days, 13.12 g, 57.32) (Table 6 and Fig 4).

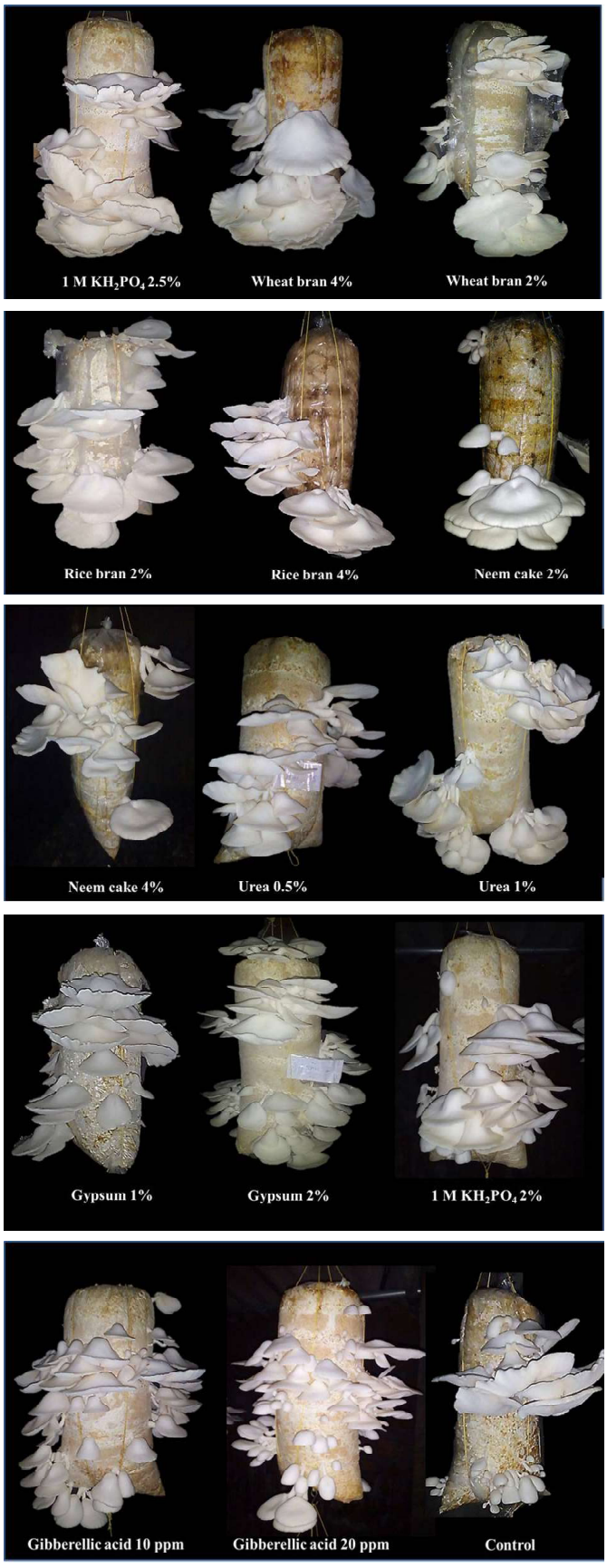

Fig. 3. Influence of amended sawdust on yield parameters *of P. cystidiosus*

Table 3. Evaluation of amended rubber wood sawdust for spawn production of *Pleurotus* sp.

Treatments	*Days for spawn run	Nature of growth	*Per cent * spawns contaminated	Shelf life (days)
Thiamine (25 ppm)	12.62^{c}	+++	33.3	103.30^{g}
Thiamine (50 ppm)	14.27^{b}	+++	33.3	101.30^{h}
Thiamine (75 ppm)	15.35^{a}	++	33.3	100.30^{j}
Yeast extract (0.5%)	11.35^{e}	++++	-	104.10^{e}
Yeast extract (0.75%)	8.62^{g}	++++	-	107.30^{b}
Yeast extract (1%)	8.33^{h}	++++	-	108.30^{a}
Ferrous sulphate (0.25%)	8.62^{g}	++++	-	104.30^{d}
Ferrous sulphate (0.5%)	10.63^{f}	++++	-	103.60^{f}
Ferrous sulphate (0.75%)	12.32^{d}	++++	33.3	101.10^{i}
Control	11.35^{e}	++	-	104.60^{e}
S.Em. (±)	0.757			0.811
C.D. (0.05)	0.147			0.111

*Average of three replications, each replication denotes 5 spawns, Means followed by similar superscripts do not differ significantly at 5% level

Table 4. Influence of amended paddy straw on yield parameters of *Pleurotus* sp.

Treatments	Days for spawn run*	Days for pinhead formation*	Days for first harvest*	Total yield per bed from three harvests (g)*	Total crop period (days)*	Average weight of sporocarp(g)*	No of sporocarps*	BE(%)*
2% WB	39.29^{b}	44.40^{d}	47.62^{d}	2361.32^{c}	123.42^{c}	12.72^{f}	186.37^{c}	118.07
4% WB	39.57^{a}	44.62^{c}	48.63^{c}	2510.32^{a}	124.32^{a}	23.17^{a}	140.33^{h}	125.52
2% RB	37.63^{e}	43.32^{f}	47.00^{f}	1220.12^{l}	120.32^{f}	15.37^{e}	65.33^{op}	61.01
4% RB	38.31^{d}	43.62^{e}	47.63^{d}	1611.42^{g}	121.33^{e}	17.83^{c}	90.33^{l}	80.57
2% NC	38.63^{c}	43.28^{f}	47.27^{e}	1202.38^{m}	119.33^{h}	15.91^{d}	76.33^{n}	60.12
4% NC	38.22^{d}	42.62^{g}	46.63^{g}	1660.32^{f}	119.67^{g}	12.75^{f}	130.33^{i}	83.02
0.5% U	36.33^{g}	41.30^{i}	45.31^{i}	1320.35^{j}	112.33^{l}	5.37^{k}	253.42^{a}	66.02
1% U	35.62^{h}	40.62^{j}	44.62^{k}	1239.38^{k}	112.63^{k}	10.83^{g}	114.33^{k}	61.97
1% Gy	35.62^{h}	41.62^{h}	45.62^{h}	1692.37^{e}	114.33^{j}	17.89^{c}	85.39^{m}	84.62
2% Gy	36.62^{f}	43.63^{e}	47.62^{d}	1570.32^{h}	115.33^{i}	9.63^{h}	162.33^{d}	78.52
2% KH	35.62^{h}	40.61^{j}	44.47^{l}	2273.35^{d}	122.33^{d}	9.32^{i}	244.33^{b}	113.67
2.5% KH	35.48^{h}	40.63^{j}	44.62^{k}	2495.31^{b}	123.62^{b}	18.12^{b}	141.63^{g}	124.77
10ppmG	36.59^{f}	45.62^{b}	49.62^{b}	960.33^{n}	119.33^{h}	7.65^{j}	126.33^{j}	48.02
20ppmG	36.33^{g}	46.32^{a}	50.39^{a}	820.33^{op}	119.68^{g}	5.11^{l}	160.62^{e}	41.02
Control	36.45^{fg}	41.39^{i}	45.05^{j}	1340.35^{i}	112.33^{l}	9.43^{i}	143.15^{f}	67.02
S.Em.(±)	0.362	0.477	0.479	142.535	1.095	1.348	14.280	
C.D.(0.05)	0.226	0.162	0.137	0.117	0.071	0.122	0.106	

WB: Wheat bran, RB: Rice bran, NC: Neem cake, U: Urea, Gy: Gypsum, KH: KH_2PO_4, G: Gibberellic acid

*Average of four replications, each replication denotes 5 beds, Means followed by similar superscripts do not differ significantly at 5 % level

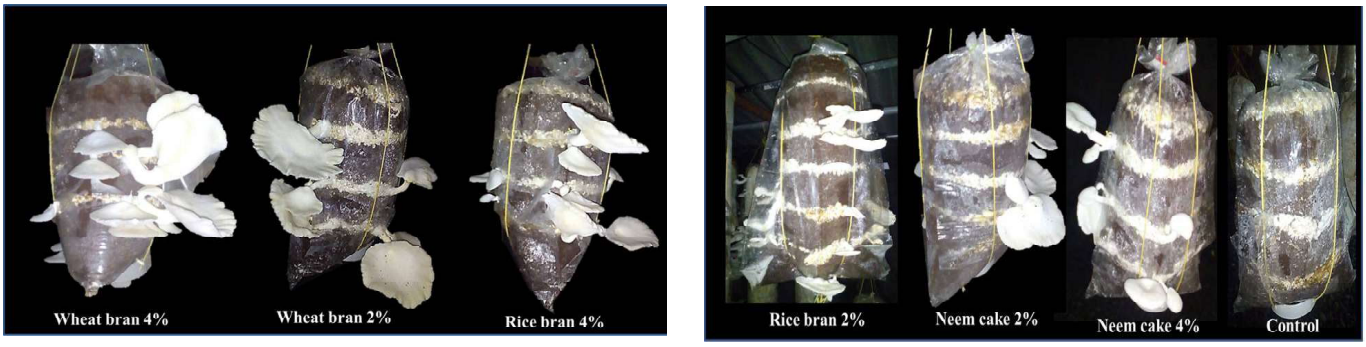

Fig.4. Influence of amended neopeat on yield parameters of *P. cystidiosus*

Thus 4 per cent wheat bran amendment was identified as the best amendment in paddy straw and neopeat whereas spraying with 2.5 per cent of 1 M KH_2PO_4 was the best treatment in rubber wood sawdust which recorded maximum biological efficiencies. This may be because, wheat bran consisted of protein (12%), carbohydrates (60%), fat (3.5-3.9%), minerals (2-8.1%), vitamins and bioactive compounds. It also contained compounds such as carotenoids, lignans, phenolic acids, phytosterols, flavonoids, phytic acid and á-tocopherol (Apprich *et al*., 2014).

Among all substrate-treatment combinations, spraying of rubber wood sawdust with 2.5 per cent of 1 M KH_2PO_4 gave the

Table 5. Influence of amended rubber wood sawdust on yield parameters of *Pleurotus* spp.

Trts.	Days for spawn run*	Days for pinhead formation*	Days for first harvest* three harvests (g)*	Total yield per bed from	Total crop period (days)*	Average weight of sporocarp (g)*	Number of sporocarps*	BE (%)*
2% WB	48.23^{b}	54.30^{d}	58.33^{d}	3802.32^{c}	145.31^{b}	21.85^{g}	173.33^{f}	190.1
4% WB	48.62^{a}	54.62^{c}	58.62^{c}	3815.41^{b}	145.31b	36.28^{a}	06.30^{m}	190.7
2% RB	47.30^{d}	53.32^{f}	57.32^{g}	2915.11^{g}	143.30^{e}	15.12^{j}	193.29^{e}	145.7
4% RB	47.62^{c}	53.62^{e}	57.62^{f}	2960.08^{f}	143.65^{d}	22.42^{f}	132.31^{j}	148.0
2% NC	46.30^{f}	52.35^{j}	56.62^{h}	2620.10^{j}	142.62^{f}	25.55^{d}	102.31^{n}	131.0
4% NC	46.68^{e}	53.00^{h}	57.63^{f}	2692.05^{h}	142.31^{g}	21.35^{h}	127.33^{k}	134.6
0.5%U	45.22^{ij}	51.63^{k}	56.30^{j}	2371.15^{l}	136.63^{j}	14.49^{k}	163.37^{g}	118.5
1%U	45.62^{h}	51.62^{k}	56.49^{i}	2171.32^{m}	136.12^{k}	10.25^{l}	213.35^{d}	108.5
1% Gy	46.10^{g}	52.62^{i}	57.63^{f}	2680.45^{i}	138.62^{i}	19.12^{i}	140.32^{h}	134.0
2% Gy	45.62^{h}	53.15^{g}	58.00^{e}	2442.30^{k}	139.62^{h}	9.25^{m}	271.32^{b}	122.1
2% KH	45.15^{j}	51.20^{l}	56.00^{k}	3172.08^{d}	144.33^{c}	23.62^{e}	134.32^{i}	158.6
2.5% KH	45.25^{ij}	51.30^{l}	56.00^{k}	3855.12^{a}	145.63^{a}	35.88^{b}	107.31^{l}	192.7
10ppmG	45.57^{h}	56.66^{b}	61.67^{b}	1930.40^{n}	142.31^{g}	7.48^{n}	257.33^{c}	96.5
20ppmG	45.53^{h}	57.15^{a}	62.00^{a}	1730.35^{op}	142.63^{f}	5.92^{op}	289.37^{a}	86.5
Control	45.30^{i}	51.31^{l}	56.31^{j}	3115.18^{e}	136.67^{j}	29.32^{c}	107.35^{l}	155.7
S.Em. (±)	0.298	0.479	0.478	170.243	0.854	2.468	16.394	
C.D. (0.05)	0.114	0.127	0.120	0.104	0.098	0.176	0.059	

WB: Wheat bran, RB: Rice bran, NC: Neem cake, U: Urea, Gy: Gypsum, KH: KH_2PO_4, G: Gibberellic acid

*Average of four replications, each replication denotes 5 beds, Means followed by similar superscripts do not differ significantly at 5 % level

Table 6. Influence of amended neopeat on yield parameters of *Pleurotus* spp.

Trts.	Days for spawn run*	Days for pinhead formation*	Days for first harvest* three harvests (g)*	Total yield per bed from (days)*	Total crop period sporocarp (g)*	Average weight of	Number of sporocarps*	BE (%)*
2% WB	55.30^{b}	59.31^{b}	62.30^{b}	463.28^{b}	110.29^{b}	11.20^{b}	42.25^{d}	23.16
4% WB	56.22^{a}	60.29^{a}	63.32^{a}	751.38^{a}	111.29^{a}	13.12^{a}	57.32^{a}	37.57
2% RB	52.45^{d}	56.39^{f}	59.25^{f}	410.38^{d}	108.30^{e}	9.51^{d}	48.40^{b}	20.52
4% RB	53.28^{c}	57.31^{d}	60.41^{d}	460.29^{c}	109.38^{c}	9.82^{c}	47.31^{c}	23.01
2% NC	52.30^{d}	56.67^{e}	59.63^{e}	241.32^{f}	108.62^{d}	7.37^{e}	33.22^{f}	12.07
4% NC	53.35^{c}	57.68^{c}	60.67^{c}	253.65^{e}	108.31^{e}	7.37^{e}	34.30^{e}	12.68
0.5% U	52.40^{d}	-	-	-	-	-	-	-
1% U	52.15^{d}	-	-	-	-	-	-	-
1% Gy	51.38^{efg}	-	-	-	-	-	-	-
2% Gy	51.62^{ef}	-	-	-	-	-	-	-
2% KH	51.25^{g}	-	-	-	-	-	-	-
2.5% KH	51.12^{g}	-	-	-	-	-	-	-
10 ppm G	51.65^{e}	-	-	-	-	-	-	-
20 ppm G	51.32^{fg}	-	-	-	-	-	-	-
Control	52.15^{d}	-	-	-	-	-	-	-

WB: Wheat bran, RB: Rice bran, NC: Neem cake, U: Urea, Gy: Gypsum, KH: KH_2PO_4, G: Gibberellic acid

*Average of four replications, each replication denotes 5 beds, Means followed by similar superscripts do not differ significantly at 5 % level

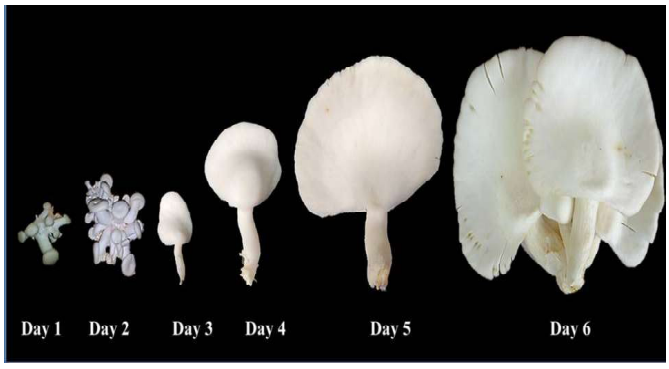

Figure 5. Developmental morphology of *P. cystidiosus*

maximum BE for the *Pleurotus* sp. (192.76 %). Sumi (2016) stated that nutrient status of rubber sawdust was higher than any other sawdust. It contained 1.68% nitrogen, 0.48% phosphorous, 1.18% potassium, 0.12% calcium and 0.04% magnesium. Also sawdust has uniform size and structure which facilitates enrichment of substrate.

Major insect pests identified from different substrates were phorid flies (*Megaselia* spp.), staphylinid beetles, black ants and mites. *Trichoderma* sp., *Aspergillus* sp., *Penicillium* sp., *Coprinus* sp. and *Bacillus* sp. were the major contaminants identified.

Neopeat is the registered trade name of degraded, washed and sterilized coirpith, obtained after processing coconut husk

with a pH of 5.7 to 6.5. In this work, neopeat gave the lowest yield for *P. cystidiosus*. Low yield of sporocarps with neopeat as substrate have also been reported in *H. ulmarius* (Sumi, 2016). This may be due to high content of fibrous material, lignin and tannin in coirpith (Priya *et al*., 2017).

Ho and Wang (2015) reported that sugarcane bagasse was the most suitable substrate for cultivation of *P. cystidiosus* which gave the highest values of cap diameter, stipe thickness, protein, fiber, ash, mineral content (Ca, K, and Mg) with biological efficiency of 84.68 %.

Developmental morphology of *Pleurotus* sp.

The sporocarps of *P. cystidiosus* took an average of 5 days from the day of pinhead formation to complete maturity. Pinheads were formed 41.25 days after spawning. The pileus of the pinhead was soft textured, white in colour, circular with clear margin of size 0.68 x 0.55 cm and white stipe of length 0.88 cm, with weight of 1.07 g. After 3 days of pin head formation, the white pileus attained regular margin and got bulged at the centre with size of 6.60 x 6.01 cm and curved white stipe of length 3.9 cm. After 45 days of spawning, the mushroom attained full maturity and fleshy, pure white, large sporocarps with regular margin and flattened pileus were formed. The mature sporocarps had pileus of size 14.10 x 11.08 cm and stipe of length 3.10 cm, with average weight of 31.09 g. At 46.25 days of spawning, mushroom turned overmature and dull white, large sporocarps with regular margin and flattened pileus of size 14.20 x 11.20 cm was formed, with short, curved stipe of length 2.89 cm (Fig 5). Sporocarps of *Pleurotus* spp. were normally produced in clusters and rarely seen as single sporocarp. Even within a bunch, the sporocarps differed in their size and morphology. The maturity of sporocarps of *Pleurotus* spp. were greatly influenced by factors such as temperature, humidity and aeration.

Seasonal variation in production of *P. cystidiosus*

In Trivandrum and Idukki, *P. cystidiosus* gave the maximum yield during June-September (361.35 and 1927.20 g), followed by October-January (355.32 and 1875.28 g) and February-May (187.28 and 1457.55 g). In Trivandrum and Idukki, *P. cystidiosus* took the mimimum time for harvest during October-January (68.20 and 54.18 days) followed by June-September (71.30 and 56.30 days) and February-May (84.38 and 69.25 days). Also the sporophores formed in Idukki were larger, white and more attractive, compared to those formed in Trivandrum.

Conclusion

The present study identified and standardized the cultivation of *Pleurotus cystidiosus*, a promising oyster mushroom which can revolutionize the mushroom farming in the temperate areas of Kerala.

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