Influence of Cold and Heat Substrate Pre-Treatment Methods on the Growth and Yield of three Oyster Mushrooms (*Pleurotus pulmonarius, P. ostreatus,* and *P. florida)*

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Abstract
Pleurotus species, (oyster mushrooms) are cultivated worldwide and are one of the most widely cultivated mushrooms. Substrate preparation for these mushrooms is usually done by heat (pasteurization or sterilization using autoclave).This study was aimed at evaluating other alternative and easy procedure of substrate pretreatment for these mushrooms in comparison to the conventional heat treatment method. Three cold sterilization methods, using calcium hydroxide, hydrogen peroxide and sodium hypochlorite(bleach) baths were studied where heat treatment served as the control. Three species of oysters (Pleurotuspulmonarius, P. ostreatusand p. florida) were investigated andthe results obtained showed that the oysters took significantly less time (11 days on the average) to race through the substrate compared to the heat pre-treatment method (20 days). Days to primodial initiation were significantly (P< 0..05) shorter(18.82) on the cold treated substrates than the control (29.83). Daily mycelial extension was also highest (1.18cm) on the control and least (0.79) on the cold treated ones. The biological efficiency (BE)was significantly highest on the control (57.25%) compared to the cold treated substrate as well as the production efficiency. This findings substantiate the efficacy of substrate heat pretreatment method, however, cold substrate pretreatment method is equally viable, cheaper and less laborious.
Key word: *Oyster mushrooms, substrate pretreatment, Mycelia growth, Biological efficiency*

INTRODUCTION

Mushrooms are fungi. They are saprophytes living on dead organic matter such as plant residues and other wastes containing lignin, cellulose and hemi cellulose. They secrete extra cellular enzymes which help in digesting the complex organic matter on which they grow (Oie, 1996).

Oyster mushrooms are the second largest commercially cultivated mushrooms in the world (Royse, 2013). They have high culinary value with exotic taste and are rich in quality proteins, vitamins and minerals. Their low content of fat and sodium made them suitable for people with heart related diseases (Quimio et al, 1990).

Mushrooms are conventionally grown on treated lignocellulosic wastes, the wastes are subjected to heat treatments of various types such as autoclaving, pasteurization by steam or hot water by immersion (Stamets, 2000). This study aimed at comparing heat treatment substrate pretreatment methodwith the use of different chemical pretreatment methods rice straw as basal substrate testing its effects on growth and yield attributes of some oyster nushrooms.

MATERIALS AND METHOD

This experiment was conducted at the Mushroom research/ production section of the Vegetable Research Programme of National Horticultural Research Institute, Ibadan, Nigeria. The cultures of the oyster mushrooms (*P. pulmonarius, P. ostreatus*and *P. florida)* used in this study were generated by tissue culture of young fruiting bodies on potatoto dextrose agar. The cultures were refrigerated (4^0C) until needed.The spawn was prepared according to the method of Quimio*et al,*(1990).

Guinea corn(*Sorghum bicolor)*was washed soaked in water overnight, drained, parboiled for 15 minutes, drained again and bottled in 200ml bottles with each bottle containing 150g of seeds (wet weight). The bottled seeds were autoclaved at 121^0C for

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15 minutes. After cooling, the bottles were then seeded with the freshly prepared cultures above and were kept for 2 weeks for the mycelia of the various mushrooms to ramify the seeds to give the mother spawn. The planting spawn was generated by preparing *sorghum* seeds as outlined above, the seeds were then inoculated with the freshly prepared mother spawn following the same procedure.The fruiting substrate was prepared with rice straw chopped into sizes of 2-3cm and wereseparately soaked in solutions ofhydrated lime, (calcium hydroxide) prepared at a concentration of 10g/liter, household bleach (Jik, Reckit Benckiser Nigeria Limited, Agbara, Ogun State, Nigeria.) at 5ml/liter and hydrogen peroxide (Analar by BDH Chemical Limited, Poole, England.) at 5ml/ liter.

Three 15liter capacity plastic buckets were filled with 10 liter of water each to which calcium hydroxide, hydrogen peroxide and sodium hypochlorite(household bleach) of the above concentrations were individually added to each bucket. Equal volume of the chopped rice straw was added to each bucket, submerged in the solutionand held down with weights. The buckets were separately covered, left overnight and drained the following morning.

The drained rice straw from the different treatments was filled in sterile test tubes in triplicates, spawned and covered with aluminum foil to monitor mycelia growth of all the test mushrooms. Polyethylene bags were also stuffed with the drained rice straw from each of the treatment buckets at 300g/bag. Each bag was inoculated with 30g of spawn of the three mushrooms. The bags and test tubes were then moved to the incubation room for further growth. Mycelia growth were measured every other day from the day of spawning. The substrate bags were left undisturbed throughout the period of incubation, at the end of which they were cropped and the mushrooms were harvested and weighed as they appeared.

RESULTS AND DISCUSSION

Absence of contaminants after mushroom substrate pretreatment, spawning and spawn run is indicative of the ability of the substrate pretreatment method applied to prevent the growth of other contaminant microorganisms, Oseni*et al*, (2012). In this study, the different pretreatment methods used had varied effect on the days to substrate colonization ranging from 7.5 to15.5daysfor *P. pulmonarius,* 0 – 10.5 on *P. ostreatus*, 10 – 12 days on *P. florida* and average of 19.5 days for the three mushrooms on the control. This report is contrary to the findings of Atila (2016) who reported spawn run time of 16.8 and 19.9days on hot water and chemical (formaldehide) treatment respectively. Oei, (1991) reported that time taken by mushroom mycelium to ramify its substrate are influenced by growth media type, spawn quantity and spawning method and the prevailing climatic condition of the growing environment.

Number of days to appearance of primodia (mushroom initials) of *P. ostreatus* was shortest on substrates treated with calcium hydroxide and with bleach. *Pleurotuspulmonarius* was observed to have the shortest days to primodia initiation on the control and the longest on the calcium hydroxide bath. *P. florida* initiated shortest on hydrogen peroxide and the longest on the control (Fig 2). Shorter time taken by substrate pretreated by chemicals against those treated by heat may suggest that the mushrooms ceased the window of opportunity to get established in the substrate before the reactivation of the other

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contaminant microorganisms in the substrate. All the mushrooms recorded their shortest daily mycelia extension on substrates treated with calcium hydroxide and the longest on the control. *P. ostreatus* recording no growth at all (Fig3).

Biological efficiency was found to be highest on the control for all the tested mushrooms and lowest in others. *P. pulmonarius* had the highest biological efficiency and the lowest was recorded on the others (Fig 4).These findings implied that the various chemicals evaluated were able to make the competitor microorganisms in the substrate inactive only for some time which gave the mushroom a window of opportunity to race through the substrate. The metabolic activities of the growing mushroom might have changed the properties of the substrate which made the environment conducive for the inactive competitors to become active, hence the low biological efficiency obtained compared to the control in which the competitors were killed by the heat treatment applied. This agrees with the finding of Ali *et al* (2007) who reported that higher yield and BE of fresh fruiting bodies of *Pleurotusspp* were obtained on heat treated cotton wastes than formalin treated ones. Vinoltkumar and Babu, (2013) obtained and reported higher mushroom yield on autoclaved substrate than on cold pretreated substrate.

In conclusion, heat substrate pretreatment method for oyster mushroom cultivation still appeared to be the best, but the cold sterilization methods (calcium hydroxide, hydrogen peroxide and bleach baths) can still be employed as it is cheaper and less cumbersome. It can be used in areas where facilities for heat pretreatment are not readily available.

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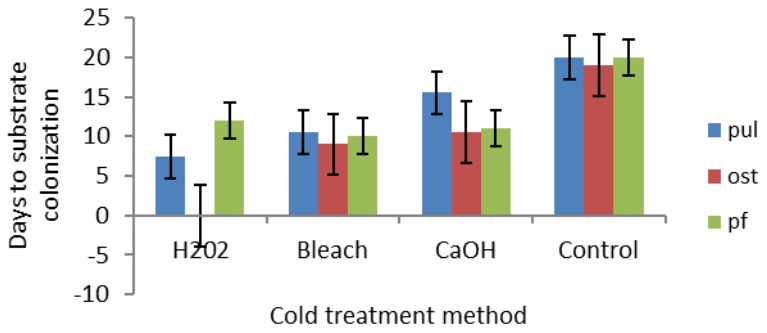

Fig 1: Effect of chemically treated substrates on number of days to mycelia colonization of*P. pulmonarius, P. ostreatus,*and *P. florida*
H_2O_2 = hydrogen peroxide, bleach = sodium hypochlorite, CaOH = calcium hydroxide.
pul= *P. pulmonarius* ,ost= *P. ostreatus*, pf= *P. florida,*

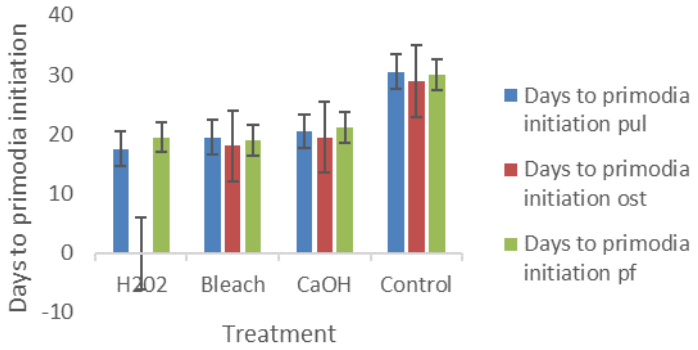

Fig 2: Effect of chemically treated substrate on number of days to primodia initiation of*P. pulmonarius, P. ostreatus*and *P. florida*
H_2O_2 = hydrogen peroxide, bleach = sodium hypochlorite, CaOH = calcium hydroxide.
pul= *P. pulmonarius* ,ost= *P. ostreatus*, pf= *P. florida*

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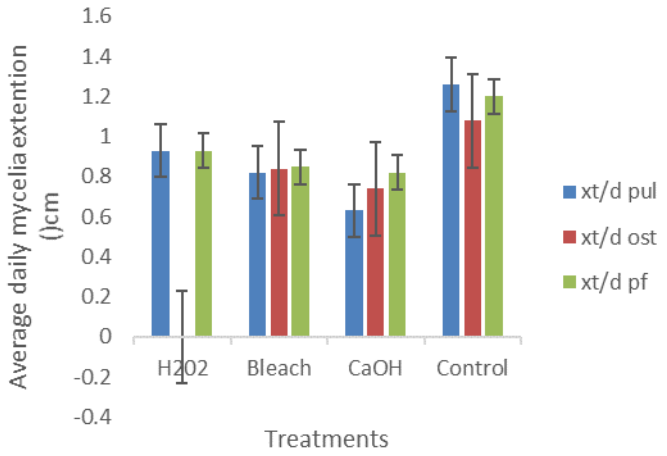

Fig 3: Effect of chemically treated substrate on daily growth of the mycelia of*P.pulmonarius, P.ostreatus*and *P.florida.*
H_2O_2 = hydrogen peroxide, bleach = sodium hypochlorite, CaOH = calcium hydroxide.
xt/d pul= *P. pulmonarius*, xt/d ost= *P. ostreatus*, Xt/d pf= *P. florida*

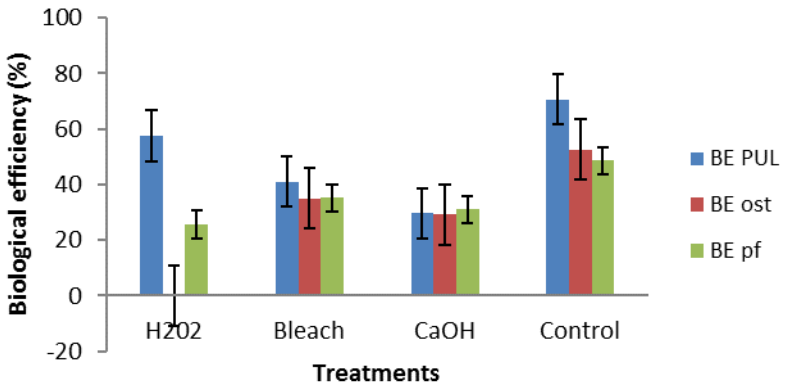

Fig 4: Effect of chemically treated substrates on the biological efficiency of*P. pulmonarius, P. ostreatus*and *P. florida.*
H_2O_2 = hydrogen peroxide, bleach = sodium hypochlorite, CaOH = calcium hydroxide.
BE PUL= *P. pulmonarius*, BE ost= *P. ostreatus*, BE pf= *P. florida,*

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Table 1: Effects of chemically treated substrates on the yield of*P pulmonarius, P.ostreatus*and *P florida*

Treatment	Fruit number			Total fruit weight (g)			Average fruit size (g)			Production efficiency (%)		
	pul	Ost	pf	pul	Ost	pf	pul	ost	Pf	Pul	ost	pf
Hydrogen peroxide	8.50	0.00	5.50	60.39	0.00	26.89	7.13	0.00	4.93	23.09	0.00	9.47
Bleach	9.00	8.00	7.00	42.95	36.82	36.90	4.77	4.68	4.95	16.06	13.72	13.98
Calcium hydroxide	7.50	6.50	7.00	31.11	30.60	32.57	4.16	4.74	4.65	12.32	11.66	11.82
Control	9.30	10.00	9.00	140.19	55.16	51.00	7.83	5.51	5.67	27.08	18.21	17.31
LSD	1.70	2.20	1.39	1.98	0.71	0.96	1.20	1.42	1.22	0.83	0.29	0.48

*Pul = Pleurotuspulmonarius, ost = Pleurotusostreatus*and *pf = Pleurotusflorida*

Table 2: Effects of chemically treated substrates on the growth of*P pulmonarius, P ostreatus*and *P florida*

Trt	Width of pileus			Length of stipe			Mycelia extension (cm)			Mycelia density		
	pul	Ost	pf	pul	ost	pf	pul	ost	pf	pul	ost	pf
H202	7.00	0.00	6.2o	6.10	0	4.05	9.25	0	9.25	4.70	0	3.90
Bleach	6.55	7.40	6.55	5.55	5.35	5.26	8.20	8.40	8.50	4.58	4.11	4.31
CaOH	6.20	5.65	6.55	5.60	4.10	4.75	6.25	7.40	8.15	3.37	4.36	4.42
Control	8.12	7.50	7.25	7.50	6.30	6.30	12.6	10.8	11.95	4.95	4.98	4.85
LSD	0.14	0.29	0.38	0.22	0.35	0.50	0.37	0.44	0.55	0.10	0.37	0.41

*Pul = Pleurotuspulmonarius, ost = Pleurotusostreatus*and *pf = Pleurotusflorida*

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