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SHORT COMMUNICATION

Effect of substrate pre-treatment methods on the fruit body production of *Pleurotus sajor-caju* (Fr.) Singer

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Abstract

Oyster mushroom cultivation is a profitable agri-business utilizing various lignocellulosic agro-forest waste materials. Among all the species, *Pleurotus sajor-caju* (Fr.) Singer is reportedly the best species showing maximum productivity in a very short cultivation period of time on paddy straw in subtropical agro climate. However, substrate treatment is a pre-requisite for elimination of various competitor moulds and bacteria to allow efficient colonization of fungal mycelia for obtaining high yield of oyster mushrooms. In the present study, different methods of substrate treatment, viz: Bavistin+Formalin, cold lime treatment, autoclaving, and hot water treatment were evaluated for their efficacy on the growth and productivity of *P. sajor-caju* on paddy straw. Substrate treatment by autoclaving proved to be the best method in terms of spawn run, pinhead initiation, maturation of fruiting bodies, cropping duration, mushroom yield (286.66g) and biological efficiency (94.61%). However, it produced lighter fruiting bodies though more in number. On the other hand, cold lime treatment gave a heavier but lesser number of fruit bodies with total yield (235.33g) and BE (77.67%). Treatment with Bavistin+Formalin gave a yield of 238.00g with 78.67% BE. Hot water treatment provided the lowest yield (232.33g) with 76.68% BE.

Keywords: *Pleurotus sajor-caju*, Substrate Treatment method, Mushroom Yield, Biological efficiency

1. Introduction

Oyster mushrooms (*Pleurotus* spp.) are a group of edible mushrooms having an exceptional ability to degrade and utilize a wide variety of lignocellulosic raw materials (Oladipo et al., 2020). Due to high protein content ranging from about 25-50%, they are considered as a suitable substitute for meat (Stanley, 2011; Kakon et al., 2012). Besides proteins, they are rich in vitamins and minerals and have a low calorific value (Syed et al., 2009; Thakur and Singh, 2013). Furthermore, these mushrooms are also known to possess various medicinal properties such as anticancer, anti-inflammatory and antiviral properties (Chang, 2007; Alam et al., 2008). Cultivation of oyster mushrooms has gained popularity throughout the world due to the simple and cost-efficient technology involved in its production (Hossain, 2017). With the growing demand and high-profit returns, taking up mushroom cultivation could profit farmers, young entrepreneurs and especially the womenfolk in rural areas. *Pleurotus sajor-caju* is one of the high-yielding popular oyster mushrooms that shows maximum productivity in a very short period of time (Singh et al., 2019). It can efficiently degrade paddy straw and a wide variety of other agricultural waste and lignocellulosic substrates to produce high-quality food of great nutritive and medicinal values. For optimally productive oyster mushrooms cultivation, pretreatment of substrate prior to spawning either by complete sterilization or pasteurization is a critical step to eliminate the potential competitor moulds and bacteria, prevent diseases, accelerate spawn run and enhance the growth and fruit body yield (Diana et al., 2006; Chang, 2008; Gowda and Manvi, 2019). Among all the methods of substrate treatment adapted for ideal mushroom cultivation, the chemical treatment method of substrate sterilization has gained popularity over other methods due to its low-cost investment and less preparation time. Depending upon the physicochemical parameters of the substrate, different methods of substrate treatment have been reported effective by different workers for preventing competitor moulds and also resulting in highest mushroom yields (Saritha and Pandey, 2010; Hernandez and Sánchez, 2003; Lakshmipathy et al., 2012; Senthilnambi et al., 2011). Therefore, in the present study, four different substrate pre-treatment methods were studied to evaluate their effect on the growth and productivity of *Pleurotus sajor-caju*.

2. Materials and methods

The experiment was conducted in an outdoor mushroom house in the Department of Botany, Rajiv Gandhi University, Arunachal Pradesh from October to December, 2022. The pure culture of the test organism, *Pleurotus sajor-caju* (DMRP-112) was procured from ICAR- Directorate of Mushroom Research, Solan, India and maintained on Potato Dextrose Agar medium at 25±5°C in a B.O.D. incubator. Mushroom spawn was prepared on whole rice grains. Grains were first boiled for 15 min. and mixed with 2% Calcium Sulphate and 0.5% Calcium Carbonate. The mixture was filled into polypropylene bags, plugged and autoclaved at 15 lb p.s.i. at 121°C for 1hr. The autoclaved bags were inoculated with two weeks old culture of *P. sajor-caju* and incubated at 25±5°C in a B.O.D. incubator for 3-4 weeks. Fresh paddy straw collected from local farmers was sun dried for a week and chopped to a size of 3-5 cm. Substrate sterilization was done by following four different treatment methods (Table 1):

(i) Autoclaving: Substrate soaked overnight in plain water, drenched next day to remove excess water, packed into 12·16 inch polypropylene bags, and then autoclaved at 15 lb p.s.i. at 121°C for 1hr.
(ii) Cold Lime treatment: Overnight soaking in 2% lime ($CaCO_3$) solution.
(iii) Hot water treatment: Boiling of pre-soaked substrate at 80±5°C for 1hr.
(iv) Bavistin + Formalin: Overnight soaking in Bavistin + Formalin solution (0.075mg/L and 1.25 ml/L, respectively) in a container covered with polythene sheet.

Table 1. Different substrate Pre-treatment methods for cultivation of *P. sajor-caju*

SN	Substrate pre-treatment method	Description
1	Bavistin+Formalin	Substrate soaked overnight in a solution of Bavistin+Formalin (@75ppm +500ppm)
2	Cold Lime Treatment	Substrate soaked overnight in 2% lime solution
3	Autoclaving	Pre-soaked substrate autoclaved at 15 lb p.s.i.at121°C for 1hr
4	Hot Water Treatment	Pre-soaked substrate boiled at 80±5°C for 1hr

Table 2. Effect of substrate pre-treatment methods on various growth parameters of *P. sajor-caju*

Substrate pre-treatment method	**Days taken**			
	Substrate colonization	Pinhead formation	Maturation of fruiting bodies	Cropping period of 2 flushes
Bavistin+ Formalin	16.0±0.7b	21.0±0.3b	26.0±0.6c	36.0±0.6c
Lime	15.0±0.7b	21.0±0.7b	26.0±0.6c	37.0±0.6c
Autoclaving	9.0±0.9a	14.0±0.6a	20.0±0.6a	31.0±0.6a
Hot Water	10.0±0.3a	16.0±0.0a	21.0±0.0b	32.0±0.6b

*Data presented as mean±SEM of 3 replicates. Values in the same column not sharing common superscript letter(s) are significantly different at p≤0.05 by using Tukey's HSD test.

Table 3. Effect of substrate pre-treatment methods on average number and average weight of fruiting bodies, yield and biological efficiency of *P. sajor-caju*

Substrate pre-treatment method	Average number of fruiting bodies	Average weight of fruiting bodies (g)	Total yield (g)	Biological efficiency (%)
Bavistin+ Formalin	42.00 ± 6.42^{ab}	3.89 ± 0.38^{ab}	238.00 ± 7.85^{a}	78.67 ± 2.66^{a}
Lime	27.00 ± 4.48^{a}	5.20 ± 0.54^{c}	235.33 ± 9.01^{a}	77.67 ± 2.95^{a}
Autoclaving	68.00 ± 8.25^{c}	3.05 ± 0.33^{a}	286.66 ± 15.07^{b}	94.61 ± 1.91^{b}
Hot Water	43.00 ± 4.93^{ab}	3.75 ± 0.30^{ab}	232.33 ± 2.90^{a}	76.68 ± 2.66^{a}

*Data presented as mean ± SEM of 3 replicates. Values in the same column not sharing common superscript letter (s) are significantly different at p≤0.05 by using Tukey's HSD test.

In case of each of the liquid treatments, excess water present in the substrate was removed by spreading it on clean poly sheets for about 1 hour, until 60% moisture was retained.
About 1.0 kg wet substrate treated with either of the methods was packed into 12x16 inch plastic bags and inoculated with mushroom spawn @3% (w/w). Ten replicates were kept for each treatment group. Inoculated bags were transferred to a cropping room and incubated under dark conditions at a relative humidity of 75-85% for a proper mycelial run. After completion of the mycelial colonization, the bags were opened to expose the colonized substrate and watered twice daily to prevent the substrate from drying. The mature fruit bodies were harvested when the caps of the basidiocarp attained a diameter of about 5-11 cm or began to fold inward.

Observations were recorded for number of days for complete substrate colonization, days for pinhead initiation, days for maturation of fruiting bodies, number of fruiting bodies, average weight of fruiting bodies (g), total mushroom yield (g), and cropping period of two flushes. Biological efficiency (BE) of the substrate was calculated as:

$$Biological\ efficiency\ (\%) = \frac{Fresh\ weight\ of\ mushroom\ (g)}{Dry\ Weight\ of\ Substrate\ (g)}\ x\ 100$$

2.1. Statistical analysis

The data were statistically analyzed using Analysis of Variance (ANOVA) at 0.05 significance level followed by Tukey's HSD test in IBM SPSS Statistics 21. The results were expressed as mean values and standard error (SEM).

3. Results

Substrate pre-treatment by autoclaving took significantly the shortest time to complete substrate colonization (9 days), pinhead formation (14 days), and maturation of fruiting bodies (20 days). It also had the shortest cropping period of 31 days (Table 2). Hot water treatment showed statistically similar results to the treatment by autoclaving with 10 days taken to complete substrate colonization and 14 days for pinhead formation. It took 21 days for the maturation of fruiting bodies and completed the cropping period in 33 days. This was followed by treatment with lime, and Bavistin+Formalin, which showed statistically similar results with both taking maximum time to complete substrate colonization (15 and 16 days respectively), pinhead initiation (21 days each), maturation of fruiting bodies (26 days each). The longest cropping period of 37 days was observed for cold lime treatment while treatment with Bavistin+Formalin showed a significantly similar but shorter duration of 36 days.

Kinds of substrate pre-treatment methods significantly affected the number and the average weight of fruiting bodies produced (Table 3). It was also observed that the average weight of fruiting bodies varied inversely with the number of fruiting bodies produced. The highest number of fruiting bodies was produced on paddy straw treated by autoclaving (68.0), followed by treatment with hot water (43.0) and Bavistin+Formalin (42.0). Cold lime treatment resulted in the lowest number of fruiting bodies (27.0), but with the highest average weight (5.20g). The average weight of fruiting bodies in other three treatments was recorded to be 3.89g for Bavistin+Formalin, 3.75g for hot water treatment and the lowest 3.05g for autoclaving.

Mushroom yield and biological efficiency

Mushroom yield and biological efficiency varied significantly with the methods of substrate pre-treatment methods (Table 3). A statistically higher yield (286.7g) and biological efficiency (94.6%) were recorded for autoclaving. It was followed by Bavistin+Formalin (238g and 78.7%) and cold lime treatment (235.3g and 77.7%). The lowest yield of 232.3g with 76.7% biological efficiency was recorded for hot water treatment. The yields and biological efficiency recorded for Bavistin+Formalin, cold lime and hot water treatments were statistically similar.

4. Discussion

It is evident from the study that the type of substrate pre-treatment methods affected the various growth characteristics, yield and biological efficiency of *P. sajor-caju* on paddy straw. The fastest complete substrate colonization in 9 days was recorded on paddy straw treated by autoclaving Girmay et al. (2016) reported complete colonization by mushroom spawn in 14-19.67 days for autoclaved substrates. However, a more delayed substrate colonization of 21-35 days for *Pleurotus* spp. was observed by Alvarez and Bautista (2021). Hot water treatment also exhibited comparatively faster substrate colonization in 10 days. The earliest pinhead formation in 14 days was observed for autoclaving and the longest duration of 21 days was recorded for both Bavistin+Formalin and lime. These results of the present study vary from the other studies which recorded 17-33 days duration for pinhead formation in case of treatment by autoclaving (Girmay et al., 2016), and 22-32 days for chemically treated substrates (Dehariya and Vyas, 2013; Kalita, 2015; Raghav et al., 2016; Hossain, 2017; Singh et al., 2018).

The fast substrate colonization and early appearance of pinheads on substrates treated by autoclaving and hot water methods could be attributed to the extractive removal, hydrolysis and alteration the physical structure of ligno-cellulosic substrate (Lamptey et al., 1985) as the effect of hydrothermal activity greatly helps to loosen the complex polymer of lignin in the substrate material and renders easy access to lignocellulolytic enzymes to release a good amount of fermentable sugars for the feeding mushroom mycelia (Mosier et al., 2005).

The minimum and maximum time taken for transformations of pinheads into mature fruiting bodies were recorded in case of substrates prepared by autoclaving (20 days), and Bavistin+Formalin treatment (26 days). The days recorded were found to be relatively shorter in comparison to the duration recorded by Singh et al. (2018), who demonstrated that cultivating *P. sajor-caju* on paddy straw treated with hot water required 38.9 days to form mature fruiting bodies. For chemically treated paddy straw, Hossain (2017) reported that maturation of fruiting bodies required 27 days. This is on par with the recorded 26 days taken by *P. sajor-caju* to completely transform into mature fruiting bodies in Bavistin+Formalin treated substrate in the present study.

The cropping period of *P. sajor-caju* showed varied results with the shortest duration recorded for autoclaving (31 days) followed by hot water treatment (32 days), Bavistin+Formalin (36 days), and the longest duration for cold lime treatment (37 days). Kathiravan and Krishnakumari (2020) treated paddy straw using three treatment methods namely, chemical, boiling water, and steam sterilization and found the duration of *P. sajor-caju* cropping period to range from 23.17-26.17 days for chemical sterilization, 21.67-24.33 days for boiling water treatment and 20.17-22.33 days for steam treatment.

In this study, the number and the average weight of fruiting bodies were found to be significantly influenced by the type of pre-treatment methods. Autoclaved substrate produced the highest number of fruiting bodies (68.00) but with the lowest average weight (3.05g). The lowest fruiting bodies were obtained for lime treatment (27.00) and this result resembles the study by Khan et al. (2013), who recorded 26.8 fruiting bodies for substrate treated with 2% lime.

Paddy straw treated by autoclaving reportedly gave the highest significant yield (286.7g) with biological efficiency of 94.6% followed by Bavistin+Formalin (238g) with BE 78.67% and cold lime treatment (235.33g) with BE 77.67%. The lowest yield of 232.3 g with BE 76.7% obtained for hot water treatment in the study differs from the results reported by Akhter et al. (2017), who recorded a lower yield of 156.8g and BE 32.56% on paddy straw treated by boiling in hot water at 80±5˚C for 1hr. Various studies have also recorded different yields and biological efficiency for *P. sajor-caju*. Alvarez and Bautista (2021) reported mushroom yield ranging from 18.26-132.82g for different substrates autoclaved at 121˚C for 1 hr. Patil (2012) reported a yield of 836.66g with BE 83.66% on paddy straw treated by autoclaving at 121˚C for 20 min. Chemically treated substrates have been documented to give mushroom yields ranging from 425.30-694.38g by Singh et al. (2018). Hossain (2017) recorded *P. sajor-caju* yield ranging from 260-803g on various substrates which were treated using a solution of 10g Carbendazim and 120ml formalin in 100L water. The results of the present study are at par with the findings of Kalita (2015), who compared the yield and biological efficiency of five different treatment methods and concluded autoclaving as the most ideal method of mushroom substrate sterilization.

5. Conclusion

The current study demonstrated that pre-treating the lignocellulosic substrate using various techniques has a substantial impact on the various growth traits and yield of *P. sajor-caju*. Autoclaving the paddy straw at 121°C for 1 hr exhibited the most desirable results, and therefore, concluded as the most effective method of substrate pre-treatment for cultivation of *P. sajor-caju*. Treatment with either Bavistin+Formalin or 2% lime also gave satisfactory results with statistically similar yield and biological efficiency. The simple and cost-effective methods of substrate treatment with Bavistin+Formalin or 2% lime could be easily adopted by researchers and growers. Among all the substrate pre-treatment methods tried, hot water treatment of paddy straw resulted in low yields and biological efficiency of *P. sajor-caju*.

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Author's contribution

Tenya Rina designed and supervised the experiment, and edited the manuscript. Litnya Tangjang conducted the experiment, analyzed the data and drafted the manuscript. Titel Megu assisted in conducting the experiment and drafting the manuscript.

Conflict of interests

Authors have no conflict of interests

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