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Eco-friendly and Cost-Effective Methods of Pasteurization of Substrates for Oyster Mushroom Cultivation

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ABSTRACT

The present study was conducted during October, 2020–March, 2021 at Mushroom Technology Laboratory, Department of Plant Pathology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India to find out the effects of solar heat and hot-water on pasteurization of substrates. A significant maximum temperature of 59.9°C was recorded in wheat substrate of 2.0' heap height covered with black polythene sheet for 6 h. A maximum temperature of 48.0°C was recorded in solar heat treatment of substrates for 6 h. The main aim of pasteurization is to reduce harmful microflora on substrates. The least bacterial colonies at 6.7×10^9 cfu g^{-1} substrate were recorded in wheat substrate of 2.0' heap height covered with black polythene sheet for 6 h and the highest at 9.4×10^9 cfu g^{-1} in paddy straw of 1.0' heap height uncovered for 2 h. The minimum fungal colonies at 4.7×10^4 cfu g^{-1} were recorded in wheat substrate of 2.0' heap height covered with black polythene sheet for 6 h followed by 4.9×10^4 cfu g^{-1} in paddy straw. A maximum number of fungal colonies at 6.9×10^4 cfu g^{-1} were seen in wheat straw of 1.0' heap height uncovered for 2 h. In hot-water treatment, the minimum bacterial colonies at 3.7×10^9 cfu g^{-1} substrate and fungal colonies at 1.9×10^4 cfu g^{-1} substrate were observed in wheat substrate treated at 70°C for 20 m followed by bacterial colonies at 4.3×10^9 and fungal colonies at 2.1×10^4 cfu g^{-1} in hot-water treated paddy substrate at 70°C for 20 m.

KEYWORDS: Hot-water, oyster, paddy, pasteurization, solarisation, substrates, wheat

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1. INTRODUCTION

India produces about 620 mt of crop residues every year, 234 mt is surplus and its 30% contributed by rice and wheat. An approximate of 16% of crop residue is being burnt and 62% is contributed by rice and wheat (Singh et al., 2020). Various works like Zhang et al. (2002), Ahmed et al. (2009), Mondal et al. (2010),Singh and Prasad (2012), Ashraf et al. (2013), Patel and Trivedi (2016), Pal et al. (2017) and Ejigu et al. (2022) have successfully cultivated *Pleurotus* species on wheat and paddy substrates. Therefore, the use of both crop residues for its cultivation is the best practical option. It belongs to the genus *Pleurotus* and known as '*Dhingri*' in India (Adejoye et al., 2006). It has many species suitable for round the year cultivation and also cultivated artificially (Randive, 2012). These mushrooms are currently in high demand for their nutritional worth (Chang, 1996). Salata et al. (2018) revealed that *P. ostreatus* has unique nutritional value. It has high fiber, low fat and a valuable element of an atherosclerosis diet. It has polysaccharides and polyphenols, which influence a human immune system and the presence of beta-D-glucans improves digestive system, lower blood cholesterol and triglycerides. It has a unique flavor and aroma that are rich in carbohydrates, protein, vitamins, minerals, ash and fiber (Lavelli et al., 2018, Raman et al., 2020). It has anti-bacterial, anti-fungal and anti-viral properties (Iwalokun et al., 2007, Thillaimaharani et al., 2013, Krupodorova et al., 2014).

The successful cultivation of oyster mushroom often requires substrate pasteurization. Saritha and Pandey (2010) tested various substrate pasteurization methods for *P. ostreatus* var. *florida* cultivation and revealed that steam pasteurization at 80°C for 2 h was most effective which gave highest BE at 82.8% followed by hot water treatment at 80°C for 1 h which gave BE at 77.6%. Jongman et al. (2013) reported that *P. ostreatus* × *P. florida* cultivated on steamed maize cobs had significantly higher BE of 69.4% than hot water treated (53.3% BE). Whereas, Ejigu and Kebede (2015) obtained the highest yield of oyster mushroom at 1.58 kg4 kg^{-1}on hot water treated maize stalk and the lowest at 0.50 kg4 kg^{-1} of substrate from saw dust. Vieira et al. (2016) observed that composting of different substrates for 7 days with conditioning showed higher yield and biological efficiency (BE) of *P. ostreatus* at 24.04 and 100.54%, respectively as compared to substrates without conditioning. Akhtar et al. (2017) revealed that hot water treatment of rice straw at 80°C for 3 h gave better yield of *P. ostreatus* (57.44% BE). In contrast, Kerketta et al. (2019) obtained highest yield of oyster mushroom on chemically treated wheat straw (123% BE) followed by hot water treatment (BE 119%). It is informed that paddy substrate pasteurized by parboiling method recorded fast spawn run (11.2 days) of oyster mushroom. Shrestha et al. (2021) and Alam and Singha (2020) evaluated that productivity of *P. ostreatus* is high in steam sterilized paddy straw (101.4% BE) and took the shortest time for pinhead (34.3 days), fruiting body (43.6 days) and crop duration (89.3 days).

The main objective of pasteurization is to reduce the maximum harmful microbial population mainly bacteria and fungi in the substrates. Generally, it is being done by chemicals, which is not an eco-friendly and cost-effective. Therefore, the present study was conducted to develop alternate methods of substrates pasteurization of two abundantly available wheat and paddy crop residues. The substrates pasteurized by different treatments of solar heat and hot water will be used to determine microbial load (cfu g^{-1} substrate) and a substrate having minimum microbial load after treatment may be used for oyster mushroom cultivation.

2. MATERIALS AND METHODS

2.1. Study area

The study was carried out during October, 2020–March, 2021at Mushroom Technology Laboratory, Department of Plant Pathology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India (29°08' N latitude,75°42' E longitude, altitude 215 m msl), situated in the semi-arid region of North-Western India. The wheat and paddy substrates were pasteurized by solar heat (solarisation) and hot-water treatments.

2.2. Solar heat treatments of substrates

The wheat and paddy substrates were soaked separately in plastic drums containing plain water for 12 h for softening, then excess water was drained out and 1.0', 1.5' and 2.0' heap heights of each water-soaked substrates were formed. These heaps were covered separately with black and transparent polythene sheets (50µ thickness) and left under natural sunlight at 10.0 am for 2, 4 and 6 h. The wheat and paddy substrates of 1.0, 1.5 and 2.0' heap height without polythene sheet covering served as check-1 and check-2. The temperature of each treatment was recorded after 2, 4, 6 h of solarisation from the centre of substrate heaps as per detail given below. The treated substrates were spread on cemented floor after required time of solarisation and waited till substrates had60%moisture and attained normal room temperature. The substrate samples were taken treatment wise as detailed below to find out the microbial load (bacterial and fungal) in each treatment.

2.2.1. Detail of solar heat treatments

T_1	Wheat straw of 1.0' height covered with black polythene sheet
T_2	Wheat straw of 1.5' height covered with black polythene sheet
T_3	Wheat straw of 2.0' height covered with black polythene sheet
T_4	Wheat straw of 1.0' height covered with transparent polythene sheet
T_5	Wheat straw of 1.5' height covered with transparent polythene sheet
T_6	Wheat straw of 2.0' height covered with transparent polythene sheet
T_7	Paddy straw of 1.0' height covered with black polythene sheet
T_8	Paddy straw of 1.5' height covered with black polythene sheet
T_9	Paddy straw of 2.0' height covered with black polythene sheet
T_{10}	Paddy straw of 1.0' height covered with transparent polythene sheet
T_{11}	Paddy straw of 1.5' height covered with transparent polythene sheet
T_{12}	Paddy straw of 2.0' height covered with transparent polythene sheet
(Check-1)	Wheat straw at 1.0' height uncovered
(Check-1)	Wheat straw at 1.5' height uncovered
(Check-1)	Wheat straw at 2.0' height uncovered
(Check-2)	Paddy straw at 1.0' height uncovered
(Check-2)	Paddy straw at 1.5' height uncovered
(Check-2)	Paddy straw at 2.0' height uncovered

2.3. Hot-water treatments of substrates

For this study, both the substrates were soaked in plain water, drained excess water as described earlier and then substrates were hot-water treated as per treatment's detail given below. The wheat and paddy substrates were soaked in plain water for 12 h, drained excess water and then treated in a solution of Bavistin (75 ppm) and Formalin (1250 ppm) for 20, 30 and 40 m which served as check-1 and check-2, respectively. The treated substrates were spread treatment wise separately on cemented floor till they attain required moisture and room temperature as described earlier. The substrate samples were taken treatment wise to check the microbial load (Bacterial and fungal).

2.3.1. Details of hot-water treatments

T_1 Wheat straw hot-water treated at 60°C temp; T_2 Wheat straw hot-water treated at 65°C temp; T_3 Wheat straw hot-water treated at 70°C temp; T_4 Paddy straw hot-water treated at 60°C temp; T_5 Paddy straw hot-water treated at 65°C temp; T_6 Paddy straw hot-water treated at 70°C temp; Check-1 Wheat straw chemically treated; Check-2 Paddy straw chemically treated

2.4. Microbial load determination of solar and hot-water treated substrates

The microbial load was determined treatment wise, using the viable plate count. The serial dilutions were prepared with sterile-distilled water using 1 g substrate sample as per standard method and plated onto the medium (spread plate method) in sterilized Petri Plates containing medium. The Nutrient Agar and Martin Rose Agar media were used for bacterial and fungal growth, respectively. To determine the bacterial colonies, the plates were incubated at 28±2°C for 48 h and for fungal colonies, plates were incubated at 23±2°C for 5 days. Each treatment was replicated thrice and factorial CRD was used for statistical analysis. The microbial colonies were calculated by using the following formula:

Microbial colonies (cfu g^{-1} substrate)=(Number of colonies × Dilution factor)/(Volume spread on the plate) ……..(1)

3. RESULTS AND DISCUSSION

3.1. Temperature of substrates after solar heat treatments

The maximum mean temperature of 55.8°C was recorded in wheat straw of 2.0' heap covered with black polythene sheet followed by 50.2°C in paddy substrate of 2.0' heap covered with black polythene sheet irrespective of time of treatment. The time of treatment also significantly influenced the rise in temperature of substrates. It was found maximum (48.0°C) in 6 h solarisation and minimum (41.5°C) in 2 h solarisation treatment irrespective of substrates and polythene sheets (Table 1).

If we take into consideration, the treatments as well as time of treatment, the highest temperature (59.9°C) was recorded in wheat substrate of 2.0' heap height covered with black polythene sheet for 6 h and was significantly higher as compared to all treatments×time of treatment. However, in paddy substrate a maximum temperature of 57.2°C was recorded in 2.0' heap height covered with black polythene for 6h. The significantly lowest temperature of 37.3°C and 37.1°C was measured in check-1 and check-2when solarised uncovered for 2h (Table 1).

3.2. Effect of solar heat treatments on bacterial colonies of substrates

The results indicated that the bacterial colonies significantly reduced to 7.2×10^9 cfu g^{-1} in wheat substrate of 2.0' heap height and covered with black polythene sheet irrespective of time of treatment. However, duration of solar heat treatment also significantly influenced the bacterial colonies with minimum 8.1×10^9 cfu g^{-1} substrate when solarised for 6

Table 1: Effect of solar heat treatments on rise in temperature of substrates

Treatments	Temperature (°C)			Mean Temp. (°C)
	2 h	4 h	6 h	
Wheat straw of 1.0' height covered with black polythene sheet	42.3	48.3	53.4	48.0
Wheat straw of 1.5' height covered with black polythene sheet	44.4	54.3	57.4	52.0
Wheat straw of 2.0' height covered with black polythene sheet	51.4	56.0	59.9	55.8
Wheat straw of 1.0' height covered with transparent polythene sheet	39.6	43.2	46.3	43.0
Wheat straw of 1.5' height covered with transparent polythene sheet	44.2	46.8	51.1	47.4
Wheat straw of 2.0' height covered with transparent polythene sheet	47.2	50.3	52.9	50.1
Paddy straw of 1.0' height covered with black polythene sheet	41.3	45.9	51.2	46.2
Paddy straw of 1.5' height covered with black polythene sheet	42.2	49.0	53.3	48.2
Paddy straw of 2.0' height covered with black polythene sheet	43.3	50.3	57.2	50.2
Paddy straw of 1.0' height covered with transparent polythene sheet	39.2	41.1	44.3	41.5
Paddy straw of 1.5' height covered with transparent polythene sheet	41.1	46.3	51.1	46.2
Paddy straw of 2.0' height covered with transparent polythene sheet	44.5	47.3	52.6	48.1
(Check-1) Wheat straw of 1.0' height uncovered	37.3	37.9	38.3	37.8
(Check-1) Wheat straw of 1.5' height uncovered	37.8	38.5	39.5	38.6
(Check-1) Wheat straw of 2.0' height uncovered	38.6	39.5	39.8	39.2
(Check-2) Paddy straw of 1.5' height uncovered	37.3	38.1	38.9	38.1
(Check-2) Paddy straw of 2.0' height uncovered	38.1	38.8	39.1	38.7
Mean	41.5	44.9	48.0	-
SEm±	Time = 0.034, Treatments = 0.080, Time × Treatments = 0.138			
CD (p=0.05)	Time = 0.040, Treatments = 0.100, Time × Treatments = 0.170			

h and significantly higher (8.7×10^9 cfu g^{-1}) in 2 h solarisation treatment. The least number of bacterial colonies (6.7×10^9 cfu g^{-1} wheat substrate)were recorded in 2.0' heap height covered with black polythene sheet for 6 h followed by bacterial colonies of 7.1×10^9 cfu g^{-1} wheat substrate in 1.5' heap height covered with black polythene sheet for 6 h (Table 2). However, in paddy 2.0' heap height covered with black polythene sheet for 6 h resulted significant reduction in bacterial colonies to 7.1×10^9 cfu g^{-1} substrate followed by 7.7×10^9 cfu g^{-1} paddy substrate of 1.5' heap height covered with black polythene sheet for 6 h. The significantly highest bacterial colonies of 9.2×10^9cfu g^{-1} wheat substrate and 9.4×10^9 cfu g^{-1} paddy substrate were recorded in 1.0' heap height and left uncovered for 2 h and these were significantly higher as compared to all other treatments and time of treatment (Table 2).

3.3. Effect of solar heat treatments on fungal colonies of substrates

The minimum mean fungal colonies of 5.2×10^4 cfu g^{-1} substrate were observed in wheat straw of 2.0' height covered with black polythene sheet followed by 5.5×10^4 cfu g^{-1} paddy substrate in 2.0' heap height covered with black polythene sheet irrespective of duration of treatment (Table 2). The time of solar heat treatment also influenced the fungal colonies count. The minimum fungal colony count was 6.0×10^4 cfu g^{-1} substrate in 6 h solarisation followed by fungal colonies of 6.3×10^4 cfu g^{-1} substrate in 4 h time of treatment. The wheat substrate of 2.0' heap height covered with black polythene sheet for 6 h resulted in significantly highest reduction of fungal colonies to 4.7×10^4 cfu g^{-1} followed by 4.9×10^4cfu g^{-1} paddy substrate of 2.0' heap height covered with black polythene sheet for 6 h. The maximum fungal colonies of 7.0×10^4 cfu g^{-1} paddy substrate were found in 1.0' heap height kept uncovered for 2 h (check-2) followed by 6.8×10^4 cfu g^{-1} wheat substrate of 1.0' heap height kept uncovered for 2 h (check-1).

The results revealed that the rise in temperature in wheat and paddy substrates was dependent on type of polythene used and time of solar heat treatment. It was observed that substrates of maximum 2.0' heap height covered with black polythene for maximum of 6 h resulted in more reduction in bacterial and fungal colonies of both substrates and the reason may be due to maximum temperature which had resulted in maximum killing of microflora. The rise in temperature was more in substrates covered with black

Table 2: Effect of solar heat treatments on bacterial and fungal colonies of substrates

Treatments	Bacterial colonies (cfu $g^{-1}\times10^9$)			Mean bacterial colonies (cfu $g^{-1}\times10^9$)	Fungal colonies (cfu $g^{-1}\times10^4$)			Mean fungal colonies (cfu $g^{-1}\times10^4$)
	2 h	4 h	6 h		2 h	4 h	6 h	
WS (1.0') covered with black polythene sheet	8.5	8.2	7.9	8.2	6.7	6.2	5.9	6.3
WS (1.5') covered with black polythene sheet	8.4	7.4	7.1	7.6	6.3	5.7	5.1	5.7
WS (2.0') covered with black polythene sheet	7.7	7.2	6.7	7.2	5.7	5.3	4.7	5.2
WS (1.0') covered with transparent polythene sheet	8.7	8.3	8.2	8.4	6.9	6.5	6.2	6.5
WS (1.5') covered with transparent polythene sheet	8.4	8.2	7.8	8.2	6.6	6.0	5.8	6.1
WS (2.0') covered with transparent polythene sheet	8.2	8.0	7.7	8.0	6.4	6.1	5.6	6.1
PS (1.0') covered with black polythene sheet	8.6	8.4	7.9	8.3	6.8	6.6	6.1	6.5
PS (1.5') covered with black polythene sheet	8.5	8.0	7.7	8.1	6.4	6.0	5.4	5.9
PS (2.0') covered with black polythene sheet	8.3	7.8	7.1	7.7	5.9	5.6	4.9	5.5
PS (1.0') covered with transparent polythene sheet	8.8	8.6	8.5	8.6	6.9	6.7	6.3	6.6
PS (1.5') covered with black polythene sheet	8.6	8.2	7.9	8.2	6.7	6.4	6.1	6.4
PS (2.0') covered with black polythene sheet	8.4	8.1	7.6	8.0	6.5	6.0	5.8	6.1
(Check-1) WS (1.0') uncovered	9.2	9.1	9.0	9.1	6.9	6.8	6.6	6.8
(Check-1) WS (1.5') uncovered	9.1	9.0	8.9	9.0	6.8	6.7	6.5	6.7
(Check-1) WS (2.0') uncovered	9.0	8.9	8.8	8.9	6.6	6.5	6.4	6.5
(Check-2) PS (1.0') uncovered	9.4	9.3	9.2	9.3	7.1	7.0	6.9	7.0
(Check-2) PS (1.5') uncovered	9.2	9.1	9.0	9.1	7.0	6.8	6.7	6.8
(Check-2) PS (2.0') uncovered	9.2	9.1	8.9	9.0	6.8	6.6	6.5	6.6
Mean	8.7	8.4	8.1	-	6.6	6.3	6.0	-
SEm±	Time = 0.017, Treatments = 0.042 Time × Treatments = 0.073				Time = 0.007, Treatments = 0.017 Time × Treatments = 0.030			
CD (p=0.05) Level	Time= 0.04, Treatments = 0.09 Time × Treatments = 0.15				Time = 0.05, Treatments = 0.09, Time × Treatments = 0.17			

WS: Wheat straw; PS: Paddy straw; cfu g^{-1}: colony forming unitsg^{-1}; g: gram; h = hours; ': feet; °C: degree celcius; poly.: Polythene; M: Minutes

polythene as compared to transparent polythene and it may be due to better absorption and retention of the light and heat in the black polythene sheet as compared to transparent polythene sheet and the present investigations are in agreement with the report of Alam and Singha (2020). The maximum reduction in microflora of wheat substrate was followed by paddy substrate of 2.0' heap height covered with black polythene sheet for 6 h. The slightly lower

temperature in paddy as compared with wheat substrate was observed which may be because of poor conductivity of paddy straw which is in conformity with the findings of Saritha and Pandey (2010). However, no literature is available on effect of solar heat treatments on microbial population of substrates before and after pasteurization treatments, therefore, it constitutes the first study.

3.4. Effect of hot-water treatments on bacterial colonies of substrates

The minimum bacterial colonies of 4.4×10^9cfu g^{-1} substrate were observed in wheat straw hot-water treated at 70°C followed by 4.7×10^9cfu g^{-1} at 65°C irrespective of time of hot-water treatment. Similarly, in paddy straw a minimum of bacterial colonies of 4.8×10^9 cfu g^{-1} substrate were observed at 70°C treatment followed by 65°C (5.1×10^9 cfu g^{-1}). The time of hot-water treatment of substrates also influenced the bacterial colonies and it was significantly reduced to 5.8×10^9 cfu g^{-1} at 40 m treatment followed by 6.0×10^9 cfu g^{-1} at 20 and 30 m of hot-water treatment irrespective of different treatments. The data presented in Table 3 also revealed different treatments with time of hot-water treatment greatly reduced the bacterial colonies in both substrates when treated at 70°C for 20 m i.e. 3.7×10^9 and 4.3×10^9 cfu g^{-1} substrate in wheat and paddy substrate, respectively. It further increased to 4.5×10^9 cfu g^{-1} and 4.9×10^9 cfu g^{-1} wheat substrate when hot-water treatment time increased to 30 and 40 m, respectively and a similar trend were observed in paddy substrate (Table 3).

Table 3: Effect of hot-water treatment on bacterial and fungal colonies of substrates

Treatments	Bacterial colonies (cfu $g^{-1}\times109$)			Mean bacterial colonies (cfu $g^{-1}\times109$)	Fungal colonies (cfu $g^{-1}\times104$)			Mean fungal colonies (cfu $g^{-1}\times104$)
	20 m	30 m	40 m		20 m	30 m	40 m	
WS hot-water treated at 60°C	5.8	5.4	5.1	5.5	4.2	3.9	3.4	3.8
WS hot-water treated at 65°C	4.9	4.8	4.4	4.7	3.0	2.6	2.0	2.6
WS hot-water treated at 70°C	3.7	4.5	4.9	4.4	1.9	2.5	2.7	2.4
PS hot-water treated at 60°C	6.2	6.1	5.8	6.0	4.8	4.3	3.9	4.3
PS hot-water treated at 65°C	5.4	5.1	4.9	5.1	3.3	3.1	2.8	3.1
PS hot-water treated at 70°C	4.3	4.9	5.2	4.8	2.1	2.7	3.0	2.6
WS chemically treated (check-1)	8.8	8.4	7.9	8.4	7.5	7.0	6.9	7.1
PS chemically treated (check-2)	9.0	8.7	8.1	8.6	7.9	7.2	7.0	7.3
Mean	6.0	6.0	5.8	-	4.3	4.2	4.0	-
SEm±	Time = 0.009, Treatments = 0.014 Time × Treatments = 0.025				Time = 0.011, Treatments = 0.017 Time × Treatments = 0.030			
CD (p=0.05) Level	Time = 0.02, Treatments = 0.03 Time × Treatments = 0.05				Time= 0.01, Treatments = 0.01 Time × Treatments = 0.02			

3.5. Effect of hot-water treatments on fungal colonies of substrates

The minimum mean fungal colonies of 2.4×10^4 cfu g^{-1} wheat substrate were observed when hot-water treated at 70°C followed by 2.6×10^4 cfu g^{-1} substrate when treated at 65°C irrespective of time of treatment. Similarly, paddy straw hot-water treated at 70°C have a minimum of fungal colonies of 2.6×10^4 cfu g^{-1} substrate irrespective of time of treatment and it differed significantly among all treatments. The duration of hot-water treatments of substrates also significantly influenced the fungal colonies which were minimum 4.0×10^4 cfu g^{-1} at 40 m treatment followed by 4.2×10^4 cfu g^{-1} substrate and 4.3×10^4 cfu g^{-1} substrate when treated for 30 and 20 m, respectively. It was also observed that hot-water treatment at 70°C for 20 m was found significantly superior among all the treatments and duration of treatment because the least fungal colonies of 1.9×10^4 and 2.1×10^4 cfu g^{-1} substrate were recorded in this time x treatment interaction in wheat and paddy substrates, respectively (Table 3).

3.6. Impact of substrate treatment methods on economics of Pleurotus florida and P. sajor-caju cultivation

An attempt was made for oyster mushrooms *viz. P. florida* and *P. sajor-caju* cultivation on the substrates treated with different methods and having minimum fungal and bacterial population. The mushroom cultivation was carried out by hanging method in a thatched hut of 30'×60' size containing 1485 bags each having 2.0 kg dry substrate as per method described by Jeet et al., 2022. The economic parameters

like net returns, benefit cost ratio and cost of production of mushroom (₹ kg^{-1}) of *P. florida* and *P. sajor-caju* was determined. It revealed that *P. florida* cultivation in paddy straw substrate treated in hot water at 70°C for 20 m was found to be superior in giving maximum net return at Rs. 161799, benefit cost ratio at 3.03:1 with a minimum cost of production at ₹ 24.7/kg mushroom. It was followed by paddy straw treated chemically with net returns at Rs. 129520, benefit cost ratio at 2.78 with cost of production at ₹ 27.0/kg mushroom. The net returns at ₹ 124174, benefit cost ratio at 2.71:1 with cost of production at ₹ 27.7/kg mushroom were found when substrate of paddy straw heap of 2' height covered by black polythene for 6h was used for *P. florida* cultivation. It revealed that paddy straw treated by different methods was more remunerative as compared to their respective treatments in wheat substrate (Table 4).

Similarly, *P. sajor-caju* cultivation in paddy straw substrate treated in hot water was found to be superior in giving maximum net return at ₹ 113462, benefit cost ratio at 2.43:1 with a cost of production at ₹ 30.9/kg mushroom. It was followed by a net returns at ₹ 93657, benefit cost ratio at 2.29 with cost of production at ₹ 32.8/kg mushroom when paddy straw was treated chemically and used for mushroom cultivation. The net returns at ₹ 84747, benefit cost ratio at 2.17:1 with cost of production at ₹ 34.6/kg mushroom were found when substrate of paddy straw heap of 2' height covered by black polythene for 6h was used for *P. florida* cultivation. The data presented in Table 4 revealed that paddy straw treated by different methods was more remunerative as compared to their respective treatments of wheat substrate (Table 4).

It was very interesting to note that the microbial colonies (bacterial and fungal) were found decreased when substrates were hot-water treated at 70°C for 20 m because of lysis of bacterial and fungal cells but microbial colonies instead of decreasing further increased by increasing the duration of

Table 4: Economics of cultivation of *P. florida* and *P. sajor-caju* on different substrates treatment methods

Treatments	*Pleurotus florida*			*Pleurotus sajor-caju*		
	Net Returns (Rs.)	B:C ratio	Production Cost/kg Mushroom (Rs.)	Net Returns (Rs.)	B:C ratio	Production Cost/kg Mushroom (Rs.)
WS solar heat treated	79327	1.99	37.7	54602	1.68	44.6
WS hot water treated	119180	2.37	31.7	87839	2.01	37.3
PS solar heat treated	124174	2.71	27.7	84747	2.17	34.6
PS hot water treated	161799	3.03	24.7	113462	2.43	30.9
WS chemical treated (Check-1)	91044	2.14	35.1	64693	1.81	41.5
PS chemical treated (Check-2)	129520	2.78	27.0	93657	2.29	38.8

hot-water treatment at 70°C. It may be due to the growth of some thermophillic fungi and bacteria in the substrates, when treated at higher temperatures for longer duration. Since, no literature is available regarding effect of different hot-water and duration of hot-water treatment on microbial population of substrates; therefore, it constitutes the first report. However, Thakur et al. (2001) compared only the mycoflora associated with untreated paddy substrate (1324 cfu g^{-1} of straw) with chemically treated substrate (496 cfu g^{-1}). In an another study, Muhammad et al. (2007) compared different pasteurisation methods like steam pasteurisation, hot-water treatment, and chemical sterilisation of cotton waste substrate and revealed better results only in steam pasteurisation. Similarly, many other researchers have reported the different techniques of substrate pasteurization. Saritha and Pandey (2010) tested various substrates to determine the best pasteurization method for *P. ostreatus* var. *florida* cultivation. They revealed that steam pasteurization at 80°C for 2 h was most effective which gave highest BE at 82.8% followed by hot water treatment at 80°C for 1 h which gave BE at 77.6%. Jongman et al. (2013) reported that *P. ostreatus* × *P. florida* cultivated on steamed maize cobs had significantly higher BE of 69.4% than hot water treated substrates (53.3% BE). Similarly, Ejigu and Kebede (2015) obtained the highest yield of oyster mushroom at 1.58 kg 4 kg^{-1} on hot water treated maize stalk and the lowest at 0.50 kg 4 kg^{-1} of substrate from saw dust. Akhter et al. (2017) revealed that hot water treatment of rice straw at 80°C for 3 h gave better yield of *P. ostreatus* (57.44% BE). Paddy substrate is pasteurized by parboiling method recorded the minimum days (11.2) for spawn run of oyster mushroom. Shreshtha et al. (2021) evaluated that productivity of *P. ostreatus* is high in steam sterilized paddy straw as it took the shortest time for pinhead formation (34.3 days), fruiting body formation (43.6 days), crop duration (89.3 days) and highest BE at 101.38%.

The cultivation of *P. florida* was found to be more

remunerative in hot water treated substrate followed by chemical treated and solar heat treated paddy substrate. It is due to cheap price of paddy straw, low cost involved in its hot water treatment and its better pasteurization. However, the cost involved in solar heat treatment of substrates is negligible as compared to hot water and chemical treatments but in economic terms, it was not found to be as profitable as other methods. Therefore, solar heat treatments of substrates still require more studies; however, it may be exploited as an alternate of chemical treatment of substrates. The cultivation of *P. florida* was found to be more economical on paddy straw hot water treated substrate as compared to *P. sajor-caju* cultivation and it may be due to genetically traits.

Therefore, it can be summarized from the present studies that hot water treatment of different substrates is suitable methods of pasteurization. In previous studies, there is no information on presence of microflora on substrates after pasteurization, but in the present study, fluctuations in bacterial and fungal colonies on both substrates have been estimated under different pasteurization methods. Therefore, in the present scenario, it is a new study with an indication that solar heat treatments of different substrates can be further studied for exploitation in pasteurization of different substrates of oyster mushroom cultivation. The hot-water treatment of wheat and paddy substrates at 70°C for 20 m is the best pasteurization technique.

4. CONCLUSION

Wheat and paddy substrates of 2.0' height covered with black polythene for 6 h in solar heat have lowest bacteria 6.7-7.1×10^9cfu g^{-1} substrate fungi 4.7-4.9×10^4 cfu g^{-1} substrate as compared to highest bacteria 9.2-9.4×10^9 cfu g^{-1} substrate, fungi at 6.9-7.1×10^4 cfu g^{-1} substrate in check. Hot-water treatment at 70°C for 20 min have lowest number of bacteria 3.7-4.3×10^9 cfu g^{-1} substrate and fungi 1.9-2.1×10^4 cfu g^{-1} substrate as compared to highest number of bacteria 8.8-9.0×10^9 cfu g^{-1} substrate, fungi at 7.5-7.9×10^4 cfu g^{-1} substrate in check.

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