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Effect of Sterilization of Substrate by Hot Water Treatment on Prevalence of Contaminants and Yield Attributes of Oyster Mushroom (*Pleurotus ostreatus*)

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Abstract: An experiment was conducted to evaluate the influence of hot water treatment of rice straw on yield, yield attributes and contamination of oyster mushroom (*Pleurotus ostreatus),* rice straw subjected to hot-water treatment at 60^0C, 80^0C and 100^0C temperature. Each temperature was maintained for 1hr, 2 hour and 3hour, which were compared with untreated (without hot water treatment). There were significant effects of substrate pretreatment methods on contamination, yield attributes and biological efficiency. Different dominant contaminants were found *such as, Trichoderma* harzianum, *Coprinus spp., Apergillus niger* and *penicillium* sp. were obtained during the incubation and cultivation period of the spawn. The contamination of rice straw was higher at non-treated spawn packets and from 60^0C treated packets. Better performance, such as BE was 57.44%, the economic yield (280g), the highest average diameter of pileus (5.0 cm) and the highest average length of stipe (3.70 cm) were obtained from the treatment of 80^0C for 3 hours.
Keywords: Oyster mushroom, *Trichoderma* harzianum, *Coprinus spp., Apergillus niger , penicillium* sp

INTRODUCTION

Mushrooms are becoming increasingly important and common in human diets, due to their nutritional [1,2] and medicinal characteristics [3]. *Pleurotus* mushrooms, commonly known as oyster mushrooms, grow in the wild in tropical, subtropical and temperate regions and are easily artificially cultivated.

Among all mushrooms, *Pleurotus ostreatus* is very popular species of oyster mushroom in Bangladesh because it can be cultivated artificially for suitable weather and climatic condition. Mushroom substrates are contaminated by various kinds of mycoflora. Most of them act as competitor moulds thereby spawn run is adversely affected either by competition for food material or through production of toxic substances [4]. Several causes were reported for mushroom substrate contamination [5]. Sterilization of substrates is much more appropriate method for effective and smooth cultivation of mushroom to remove the existence of a number of microorganisms [6]. The common weed molds associated with edible mushrooms can be controlled by several treatments of substrates of mushrooms. Substrates for commercial production of *Pleurotus ostreatus* must be pasteurized in order to minimize the contamination. Among the substrate treatments, hot water treatment is very common method in Bangladesh but the farmers have lack of appropriate knowledge of sterilization of substrates. Sterilization of substrates is not an easy job for the cultivation of mushroom and the right sterilization time and temperature depend on the possible pathogens in a given substrate material [7]. The substrates for cultivating edible mushrooms e.g. *Pleurotus ostreatus,* has been reported to require varying degrees of pre-treatment in order to promote growth of the mushroom mycelium to the exclusion of other microorganisms [8]. The purpose of this research is to estimate the growth of pathogen on lignocellulosic substrates after hot water treatments with different temperature and time durations to know which of them are more effective to avoid contamination in Bangladesh and to determine the influence on yield attributes of oyster mushroom during mushroom production.

MATERIALS AND METHODS

The research was conducted in mushroom culture house (MCH) and in the laboratory of Sher-e-Bangla Agricultural University during March-June, 2013. Rice straw was used as Substrate and *Pleurotus ostreatus* species and strain NAMDEC oyster mushroom-4 was used in this experiment. Mother

culture was collected from Mushroom Development Institute (NAMDEC), Savar.

Preparation of substrates

Dry rice straw was chopped to 4-5 cm length and then the straw was soaked in water for 16 hours and drained out water. Conidia of microorganism collected from contaminated packets were massively produced in chickpea grain in a conical flask (Figure-1). After colonization of mycelium, a suspension of conidia in distilled water was prepared under laminar flow where conidia were released by shaking. Then, water was filtered using cheesecloth and dropped into an Erlenmeyer flask. Concentration of conidia was adjusted to $47x10^7$ conidia/100mL using a Haemacytometer and one (1) Liter of water suspension of conidia of microorganisms were inoculated with 10 kg chopped straw.

Fig-1: **Microorganism grown on chickpea**

Hot water treatment of rice straw

Microorganism inoculated substrates were treated maintaining at 60^0C, 80^0C and 100^0C temperature. Each temperature was maintained for 1hourr, 2 hours and 3 hours. Ten (10) different sterilization practices were used as treatments. Therefore the straw was taken off from hot water and left on a perforated sieve for removing the excess water for few hours.

Preparation of spawn packets

Then $CaCO_3$ were added with rice straw @ 1% on dry weight basis. The measured materials were taken in a plastic bowl and mixed thoroughly by hand and moisture was increased by adding water up to 65%. Hot water treated straw were filled into 9x12 inch polypropylene bag at 500 g/packet with 50 g mother spawn into three layers in each bag. The filled polypropylene bags were prepared by using plastic neck and plugged their mouth by inserting absorbent cotton and covered with brown paper.

Incubation of spawn packets

The spawn packets were incubated in a dark room at 20-22°C temperature until colonization of mushroom mycelium. After completion of the mycelium colonization the spawn packets were transferred to the culture house.

Cultivation of spawn

The spawn packets were opened by cutting "D" shape on the shoulder and removed the plastic sheet which opened surface of substrate was scraped slightly with a tea spoon for removing the thin whitish mycelial layer. Then the spawn packets were soaked in water for 15 minutes and invested to remove excess water for another 15 minutes. The packets of each type were placed separately on the floor of culture room and covered with newspaper. The moisture of the culture room was maintained 80-90% relative humidity and 20-25^0C respectively. The light around 200-300 lux and ventilation of culture house was maintained uniformly.

Harvesting of mushroom

The matured fruiting body was identified by curial margin of the cap [9]. Mushrooms were harvested by twisting to uproot from the base. The yield was collected up to 5^{th} flush during the harvesting period.

Isolation and purification of competitor moulds

Competitor moulds fungi were collected from the contaminated packets in sterilized Petri dishes with the help of a sterile forceps and thereafter transferred into PDA plates under *in vitro* conditions. Inoculated PDA plates were incubated at 25 ^{0}C ± 2 ^{0}C for 3 to 4 days. A single colony was isolated from the PDA plate and again transferred to PDA plates for obtaining the pure culture. All the pure cultures were kept in refrigerator at 4 ^{0}C for preservation.

Data collection and statistical analysis

The spawn packets were investigated daily and the data on different parameters were recorded a regular basis on percentages (%) of contaminated spawn packets, detection of pathogen from contaminated packets and diseased fruiting bodies, number of primodia, number of effective fruiting bodies per spawn packet, fresh weight of individual fruiting body(g), biological yield (g) in different flushes, economic yield (g), dry weight of individual fruiting body (g), diameter and length of pileus of fruiting body. The bio efficiency (BE) of mushroom was calculated by using the following formula as: % Bio-efficiency (BE) = Fresh weight of mushroom (g) / Dry weight of substrate (g) X 100 [10].

The experiment was laid out following completely randomized design (CRD) with two factors and five replications. The recorded data in this experiment was analyzed using MSTAT-C computer program and means were separated using LSD test at appropriate level of significance, where effect showed significant difference at 5% level of probability.

RESULTS AND DISCUSSION

Contaminants of oyster mushroom (*Pleurotus ostreatus)*

The most dominant contaminants during the cultivation period of the spawn were found *Trichoderma* spp, *Coprinus spp.*, *Apergillus* spp and *penicillium* spp. (Plate-1).

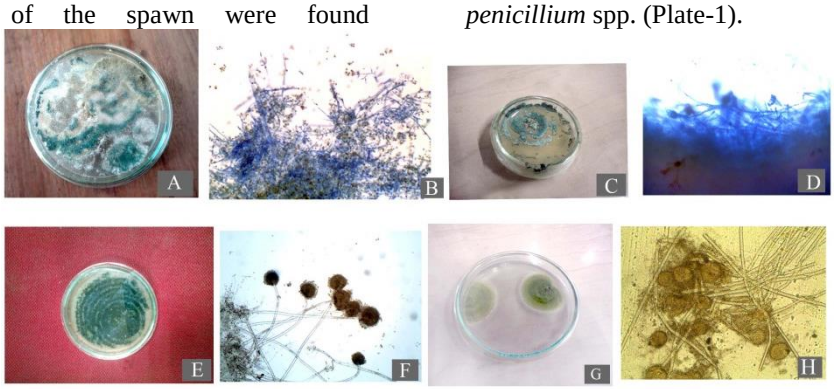

Plate-1.A: **Pure culture of *Trichoderma* isolated from contaminated packets, B- Pathogenic structure of *Trichoderma hazianum* produced in culture, C- Pure culture of *Penicillium sp,* D- Microscopic Structure of *Penicillium,* E- Pure culture of Aspergillus *niger,* F- Pathogenic structure of *Aspergillus niger,* G-Pure culture of *Aspergillus flavus,* H- Microscopic Structure of *Aspergillus flavus***

Maximum contaminants were detected from hot water treatment at 60^0C spawn and untreated packets, whereas minimum prevalence was recorded from hot water (at 80^0C for 3 hours) treated packets. Initially *Trichoderma* produced a dense white mycelium, which was difficult to distinguish from the mushroom mycelium. *Trichoderma* was often mistaken for *Penicillium* or *Aspergillus* molds (and vice versa), being that all three looked very similar on the surface of spawn packets and are not easy to identify apart without the microscopic analysis, especially before sporulation. However, the mycelial mat gradually turned green in color due to heavy sporulation, which is a characteristic symptom of green mold disease. *Trichoderma* spp. produce whitish mycelia indistinguishable from those of the mushrooms during spawn run, therefore it is difficult to recognize the infection at this stage [10, 11]. *Trichoderma* species are present at the initial phase of substrate preparation, then they disappear with the pasteurization, but they can be found again in the substrate after spawning (inoculation with *Pleurotus*), during spawn run (incubation phase) and the harvesting cycles [13, 14]. The contamination of the hot water pasteurized substrate may have occurred probably due to inadequate temperature and time used during pasteurization, since the literature is quite variable with reference to these characteristics. Ten weed mycoflora namely *Aspergillus spp*, *Penicillium* spp., *Rhizopus stolonifer* and *Trichoderma harzianum* were found to be associated with the substrate of oyster mushroom [14]. Green mould competes with the mushroom for space, nutrients as well as causing chemical alteration of the substrate, which hinders mushroom development [10].

Coprinus spp

During the fructification stage, the fruiting bodies of different *Coprinus* species were immerged from spawn packet of untreated or at 60^0C for 2-3 hours treated packets (plate-2). The caps were different shapes. Some are bluish black bell shaped and the stipe is slender, long and grown in clusters through the cutting portion. The contaminated packets were isolated and destroyed quickly to protect the healthy packets.

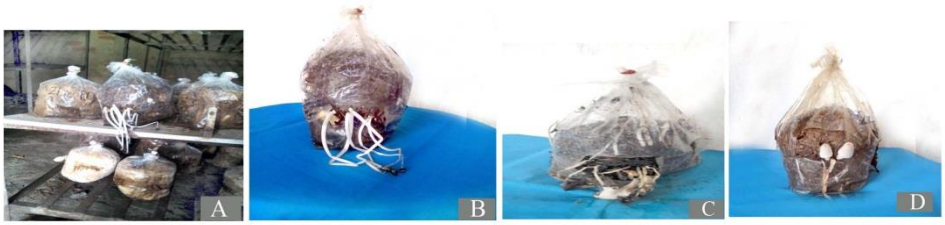

Plate-2. A: **Contamination with *Coprinus* spp, B & C- *Coprinus lagopus, D-Coprinus micacious***

Coprinus micacious

The microorganism species that competed with *Pleurotus* sp. after pasteurization with hot water (80°C for 2 h) were the fungi *Penicillium* sp. and *Trichoderma* sp., probably due to the partial breakdown of cellulose and hemicelluloses, thus making them available to competitors [15]. The contamination of the hot water pasteurized substrate may have occurred probably due to inadequate temperature and time used during pasteurization, since the literature is quite variable with reference to these characteristics. Substrate sterilization is not ideal since both beneficial and harmful organisms in the substrate are killed [16], while the substrates were recommended to sterilize for 24 h at 70°C [17]. *Coprinus lagopus*, a common weed fungus for mushrooms was found to occur in a mushroom industry for spawn production of *Pleurotus ostreatus* in Bangladesh. He also stated that proper attention and

care to be paid for appropriate pasteurization and sterilization of the substrate used for developing spawn [18].

Effect of hot water treatment of rice straw on biological efficiency, economic yield and dimension of fruiting bodies of oyster mushroom (*Pleurotus ostreatus)*

Table-1: Effect of temperature on economic yield, biological efficiency and dimension of fruiting bodies

Treatment	Biological Efficiency (%)	Economic yield (g)	Diameter of pelius (cm)	Stipe length (cm)
Te_1	16.83 c	78.97 c	3.17 c	2.28 c
Te_2	46.75 a	227.10 a	4.48 a	3.02 a
Te_3	22.50 b	109.80 b	3.63 b	2.54 b
Te_4	0.40 d	1.667 d	0.67 d	0.36 d
$LSD_{(0.05)}$	1.715	7.007	0.181	0.178

Te_1=Hot water treatment at 60^0C, Te_2 =Hot water treatment at 80^0C, Te_3 =Hot water treatment at 100^0C, Te_4= untreated

Effect of temperature of hot water treatment of rice straw on biological efficiency, economic yield and dimension of fruiting bodies of oyster mushroom (*Pleurotus ostreatus)*

In the present study, considering the hot water treatment temperature the maximum biological efficiency, economic yield, diameter of pileus and stipe length was recorded from 80^0C temperature treated packets, which was 46.75%, 227.10g, 4.48cm and 3.02cm respectively. The minimum biological efficiency (0.40%), economic yield (1.667 g), diameter of pelius (0.67cm) and stipe length (0.36cm) was observed untreated packets (Table-1).

Table-2: Effect of time of hot water treatment on economic yield, biological efficiency and dimension of fruiting bodies of oyster mushroom

Treatments	Biological Efficiency	Economic Yield	Diameter of pileus (cm)	Stipe length (cm)
Ti_1	19.14 b	91.80 b	3.01 b	1.84 b
Ti_2	22.24 a	107.90 a	3.26 a	2.49 a
Ti_3	23.49 a	113.40 a	2.69 c	1.81 b
$LSD_{(0.05)}$	1.485	6.068	0.157	0.154

Ti_1 =Hot water treatment for 1 hour, Ti_2 =Hot water treatment for 2 hours, Ti_3 =Hot water treatment for 3 hours

Effect of duration of time of hot water treatment of rice straw on biological efficiency, economic yield and dimension of fruiting bodies of oyster mushroom (*Pleurotus ostreatus)*

The biological efficiency and economic yield ranged from 19.14% to 23.49% and 91g to 113.40g in different duration of time, respectively which was statistically different to each other. In the present study, the highest economic yield and biological efficiency was obtained from 3 hours treated packets while the lowest was recorded from untreated packets (Table-2). The highest diameter of pileus (3.26cm) and length of stipe (2.69cm) was found in the treatment for two hours. The highest length of stipe (2.49cm) was recorded from two hours treated packets. The lowest stipe length was observed at 1 hour and 3 hours treated packets where there was no significant variation.

Table-3: **Effect of hot water treatment of rice straw with interaction of different temperature and time duration on economic yield, biological efficiency and dimension of fruiting bodies**

Treatments	Biological Efficiency	Economic yield(g)	Diameter of pelius (cm)	Stipe length (cm)
T_1 (Te_1 x Ti_1)	18.56 g	86.20 g	2.86 ef	1.84 f
T_2 (Te_1x Ti_2)	9.840 i	47.21 i	3.90 bc	2.94 c
T_3 (Te_1 x Ti_3)	22.10 f	103.50 f	2.74 f	2.06 e
T_4 (Te_2X Ti_1)	32.56 c	156.80 c	3.40 d	2.10 e
T_5(Te_2X Ti_2)	50.24 b	244.20 b	5.04 a	3.70 a
T_6 (Te_2x Ti_3)	57.44 a	280.20 a	5.00 a	3.26 b
T_7 (Te_3x Ti_1)	24.24 e	119.20 e	3.76 c	2.36 d
T_8 (Te_3x Ti_2)	28.86 d	140.30 d	4.10 b	3.32 b
T_9 (Te_3x Ti_3)	14.40 h	69.99 h	3.02 e	1.94 ef
T_{10} (Te_4x T_{i1})	1.20 j	5.00 j	2.00 g	1.08 g
T_{11} (Te_4 x T_{i2})	0.00 j	0.00 j	0.00 h	0.00 h
T_{12} (Te_4 x T_{i3})	0.00 j	0.00 j	0.00 h	0.00 h

$LSD_{(0.05)}$	1.917	7.834	0.203	0.199

In a column the figures having a common letter(s) do not differ significantly.

Note: T_1= Hot water treatment at 60^0C for 1hr, T_2= Hot water treatment at 60^0C for 2 hours, T_3= Hot water treatment at 60^0C for 3 hours, T_4= Hot water treatment at 80^0C for 1 hr, T_5= Hot water treatment at 80^0C for 2 hours, T_6= Hot water treatment at 80^0C for 3 hours, T_7= Hot water treatment at 100^0C for 1hr, T_8= Hot water treatment at 100^0C for 2 hours, T_9=Hot water treatment at 100^0C for 3 hours, T_{10}=control, T_{11} and T_{12}=untreated

Economic yield and biological efficiency

In the present study, depending on interaction of time and temperature, the biological efficiency and economic yield varied from 57.44% to 1.20% and 280 to 5g in different treatments respectively which was statistically different to each other. The highest economic yield and biological efficiency was obtained from 80^0C for 3 hours treated packets and the lowest was recorded from untreated packets (Table-3). Biological efficiency (BE) 60.22% and 23.78% was found from sugarcane bagasse pasteurized packets at 60^0C temperature for 3 hours and 2 hours, respectively [19]. BE was recorded 75.83% from wheat straw immersion in 80^0C for 90 minutes hot water [20].

Diameter of pileus and length of stipe

The stipe length and pileus diameter development in oyster mushroom were significantly ($P<0.05$) effected by substrate pretreatment as shown in Table- 3. The highest average diameter of pileus (5.0 cm) and the average length of stipe (3.70 cm) were observed from 80^0C for 3 hours treated. The lowest average diameter of pileus (5.50 cm) and length of stipe (1.08 cm) was found from untreated packets. The mean stipe length was 4.53cm and the pelius diameter was 4.37 cm in sugarcane bagasse pasteurized at 80^0C for 3 hours [19].

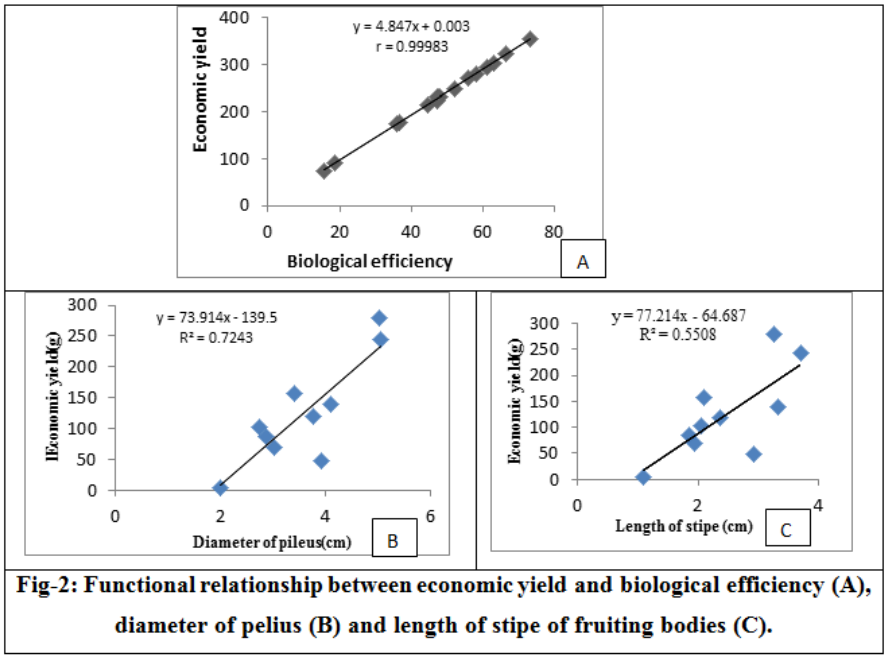

Fig-2: Functional relationship between economic yield and biological efficiency (A), diameter of pelius (B) and length of stipe of fruiting bodies (C).

Relationship of biological efficiency, diameter of pelius and stipe length with economic yield

The economic yield of mushroom was strongly correlated positively with BE. The value of correlation (r=0.99983) was strong and linear. The relationship could be expressed by the regression equation by y=4.8477y+0.003. The relationship between economic yield & the diameter of pileus of fruiting bodies negatively correlated (R^2=0.550), the equation is y=77.21x-64.68 and with stipe length of fruiting bodies negatively correlated (R^2=0.724), the equation is y=73.19x-139.5.

Table-4: Effect of temperature on yield attributes of oyster mushroom

Treatment	Number of Primordia	Number of effective fruiting bodies	Average weight of single fruiting body (g)
Te_1	50.07 c	18.07 c	5.27 b
Te_2	98.73 a	34.53 a	6.90 a
Te_3	56.67 b	20.93 b	5.29 b
Te_4	4.33 d	0.733 d	0.91 c
$LSD_{(0.05)}$	**3.9**	**1.517**	**0.357**

Te_1=Hot water treatment at 60^0C, Te_2 =Hot water treatment at 80^0C, Te_3 =Hot water treatment at 100^0C, Te_4= untreated

Effect of temperature of hot water treatment of rice straw on yield attributes of oyster mushroom (*Pleurotus ostreatus)*

Primordia initiation numbers were significantly different among the four treatments. Higher number of primordia (98.73) were initiated at 80^oC treated rice straw followed by 100^0C (56.67) treated rice straw and 60^0C (50.07) treated rice straw (Table 4). The least number of primordia (4.33) were initiated on untreated rice straw when it was not given any hot water treatment. The number of effective fruiting bodies in different treatments differed significantly (Table 4) depending on hot water treatment temperature. The highest numbers of effective fruiting bodies (34.53) were found in when rice straw was treated at 80^0C for mushroom cultivation. The lowest numbers of effective fruiting bodies (0.733) was found when rice straw was untreated. Considering the temperature of hot water treatments, the highest average weight of fruiting body (6.90g) was recorded from 80^oC treated spawn packets, on the other hand, the lowest (0.91 g) from untreated packets (Table 4). The result of the present study corroborates with the study of previous researcher, that the highest number of primordia of oyster mushroom was found in sterilized paddy straw at first flush whereas the lowest was obtained with saw dust [21]. Effect of time of hot water treatment of rice straw on yield attributes of oyster mushroom (*Pleurotus ostreatus).*

Table-5: Effect of time on yield attributes of oyster mushroom

Treatments	Number of Primordia	Number of effective fruiting bodies	Average weight of fruiting body (g)
Ti_1	48.25 c	15.65 c	5.29 a
Ti_2	52.75 b	19.00 b	4.63 b
Ti_3	56.35 a	21.05 a	3.86 c
$LSD_{(0.05)}$	**3.377**	**1.314**	**0.31**

Ti_1 =Hot water treatment for 1 hour, Ti_2 =Hot water treatment for 2 hours, Ti_3 =Hot water treatment for 3 hours

The number of primordia was found with significant variation in all treatments. The highest number of primordia (56.35) was found from 3 hours hot water treated packets and the lowest (48.25) from 1 hour hot water treated packets (Table 5). The similar trends were also observed in case of number of effective fruiting bodies. The highest number of fruiting bodies (21.05) of oyster mushroom was found from 3 hours treated packets and the lowest (15.65) was recorded from 1 hour treated packets (Table 5). The number of fruiting bodies was found to vary significantly among all treatments. The highest (5.29 g) average weight of fruiting body was obtained from 1 hour treated packets, whereas the lowest (3.86 g) from 3 hours hot water treated packets (Table 5). These results were found statistically different in different time duration. The highest number of fruiting bodieswere obtained in non-sterilized paddy straw through polypropylene bag system [22] and the highest fruiting bodies were found in paddy straw also with same system [23]. The number of fruiting bodies (32.70) were recorded from wheat straw immersed in hot water (80^0C) for 90 min [20].

Table-6: **Combined effect of different temperature and duration of time of hot water treatment of rice straw on several yield attributes of oyster mushroom (*Pleurotus ostreatus)***

Treatments	Number of Premordia	Number of effective fruiting bodies	Average weight of fruiting body (g)
T_1 (Te_1 x Ti_1)	61.60 c	18.80 f	4.93 e
T_2 (Te_1xTi_2)	36.20 e	7.00 g	7.02 b
T_3 (Te_1 xTi_3)	52.40 d	28.40 c	3.87 f
T_4 (Te_2XTi_1)	64.20 c	22.20 e	7.30 b
T_5(Te_2XTi_2)	110.6 b	44.40 a	5.65 d
T_6(Te_2xTi_3)	121.40 a	37.00 b	7.76 a
T_7 (Te_3xTi_1)	54.20 d	19.40 f	6.20 c
T_8(Te_3xTi_2)	64.20 c	24.60 d	5.86 cd
T_9 (Te_3xTi_3)	51.60 d	18.80 f	3.82 f
T_{10}(Te_4x T_{i1})	13.00 f	2.20 h	2.72 g
T_{11}(Te_4 x T_{i2})	0.00 g	0.00 i	0.00 h
T_{12}(Te_4 xT_{i3})	0.00 g	0.00 i	0.00 h
$LSD_{(0.05)}$	4.36	1.697	0.4

Note: T_1= Hot water treatment at 60^0C for 1hr, T_2= Hot water treatment at 60^0C for 2 hours, T_3= Hot water treatment at 60^0C for 3 hours, T_4= Hot water treatment at 80^0C for 1 hr, T_5= Hot water treatment at 80^0C for 2 hours, T_6= Hot water treatment at 80^0C for 3 hours, T_7= Hot water treatment at 100^0C for 1hr, T_8= Hot water treatment at 100^0C for 2 hours, T_9=Hot water treatment at 100^0C for 3

hours, T_{10}=control, T_{11} and T_{12}=untreated. Effect of hot water treatment of rice straw with interaction of different temperature and time duration on yield attributes

Number of premordia

The lowest average number of primordia /packet was observed from the treatment T_0 (13.00) and the highest average number of primordia /packet was found in the treatment T_6 (121.40) followed by T_5 (110.40), T_8 (64.20), T_1 (61.60) and T_4 (64.20) were obtained identical number of premordia (Table 6). Pattern of number of premordia, number of effecting fruiting body, weight of fruiting bodies with economic yield of different treatments of oyster mushroom *(Pleurotus ostreatus)* was showed in figure-3. The result of the present study was more or less similar with this study. The number of primordia and the average yield of oyster mushroom significantly varied with the substrates used [24].

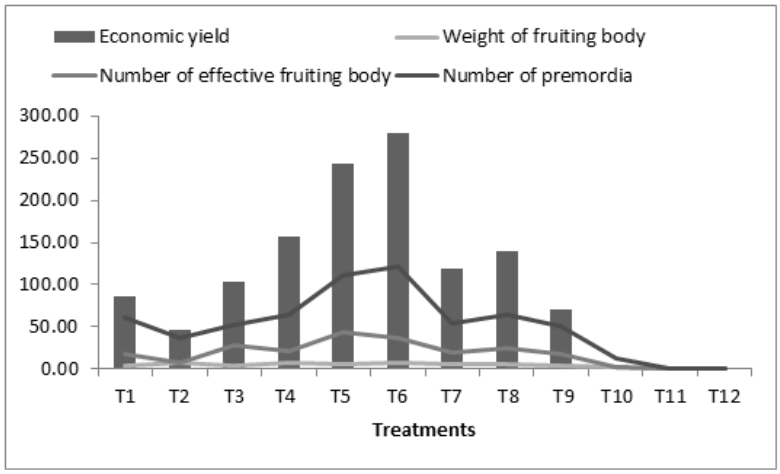

Fig-3: Pattern of number of premordia, number of effecting fruiting body, weight of fruiting bodies and economic yield of different treatment of oyster mushroom *(Pleurotus ostreatus)*

Number of effective fruiting bodies and average weight of fruiting body (g)

The number of effective fruiting bodies in different treatments differed significantly and ranged from 44.40 to 2.20. The highest average number of fruiting body/packet was observed in the treatment T_5 (44.40) and the lowest average number of fruiting body/packet was in the treatment control (2.20) (Table 4). The highest average weightof fruiting bodies were found 7.76g from 80^0C for 3 hours treated rice straw packets. The lowest average weight of fruiting bodies was found 2.72g from untreated rice straw packets. The numbers of effective fruiting bodies ranged from 97.25 -27.75 from different amount rice straw was used for the cultivation of *Pleurotus salmoneo-stramineus*. This result differed with the findings of present study, this variation may occur due to the amount of rice straw and other strain .

CONCLUSION

Performance of biological efficiency, economic yield and other yield attributes of oyster mushroom at 80°C were better compared to treatment at 60°C or 100^0C, on the other hand hot water treatment for 3 hours also gave better result and the prevalence of contaminants were low. It can therefore be concluded that hot water treatment of rice straw in drum at 80°C for 3 hours would be more applicable and feasible technique of substrate pre-treatment that can be adopted to produce a good yield of oyster mushroom in most rural areas in Bangladesh.

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