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Evaluation of *Hypsizygus ulmarius* on different pre-treated substrates

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Abstract
The experiment was conducted in the mushroom farm house of department of plant Pathology, School of Agricultural Sciences and Rural Development, Nagaland University, Medziphema during December 2018 - March 2019 in a Completely Randomized Design (CRD). Disinfection of the substrate is an important step for successful cultivation of mushroom. Here, seven different substrate pre-treatment methods (hot water treatment at 80- 90 ^{o}C, treatment with ginger, garlic and neem extracts at 4% and 6% concentration) were evaluated to study their effect on growth and yield of *Hypsizygus ulmarius.* Fastest spawn run, pin head formation, yield and biological efficiency was recorded from substrate with how water treatment at 80-90 ^{o}C however no growth was recorded on control beds.

Keywords: *Hypsizygus ulmarius*, hot water treatment, ginger, garlic, Neem

Introduction
Hypsizygus ulmarius, is also known as blue oyster mushroom, is a high yielding mushroom which was first introduced in India for commercial production by Indian Institute of Horticultural Research, Bangalore. The mycelium is pure white in colour and when cultivated on suitable substrate, develops into blue coloured pinheads becoming light white upon maturity, the matured mushrooms having three distinct parts- a fleshy cap or pileus with furrows and ridges underneath called gills or lamellae and a central or lateral stalk called stipe. Nutritionally, *H. ulmarius* contains about 23.2% of crude protein, 1.9% starch, 56.1% carbohydrate and 9.1% fibre on dry weight basis (Sethi *et al*., 2012) [7]. The substrate for growing mushroom should be at its best condition for efficient colonization by the mushroom mycelia. Competitors like bacteria and fungi can limit the growth, quality and yield of mushroom (Sharma *et al*., 2018) [8]. Several competitor molds have been reported occurring in the substrates used for the cultivation oyster mushroom when the substrate has not been uniformly or properly sterilized. Hence, in order to remove other competing micro-organisms and improve mushroom mycelial growth and yield, the growing substrate requires some pre-treatment. Hence, sterilization is one of the most crucial step in mushroom substrate preparation.

Materials and Methods
The experiment was conducted at the mushroom farm house of Department of Plant Pathology, School of Agricultural Sciences and Rural Development, Nagaland University during the year 2018-19 following a Complete Randomised Design (CRD) with seven treatments and three replications. The spawns were prepared using wheat and bajra grains, free from disease and insect infestation following the procedure described by Krishnamoorthy (1981) [5].

Hot water treatment
The chopped substrates were soaked in water for 11 hours followed by hot water treatment at 80-90oC for 30 minutes. The treated straws were then taken out, allowing excess water to drain- off while letting it cool.

Plant extracts
Ginger, garlic and neem leaf extracts were prepared at 4% and 6% concentration and then the paddy straws were soaked in solution containing the required per cent of plant extract for 16 - 18 hours (Biswas, 2015) [3].

Cultivation method

Polythene bag method described by Bano and Nagarajan (1976) was followed in the experiment. The pre-treated straws were filled in Polythene bags (45 cm x 30 cm) with holes of 5 mm in diameter at 10cm apart was sterilized and filled with the 670 g of dry substrate and100 g of spawn per bag. The open end of the polythene bag was tied and tagged. The bags were kept on racks in the spawn running room until complete spawn run was observed, then were cut open and transferred to cropping room, where watering was done. Gunny bags soaked with water was used to maintain the desired level of humidity in the cropping room by spreading it on the floor. Harvesting was done when the small primordial converted into a full grown sporophore.

Observations recorded

Spawn run (days)
Pinhead formation (days)
Stipe length (cm)
Pileus diameter (cm)
Yield (g)

Biological efficiency (%)

The percent conversion of dry substrate to fresh mushroom was calculated using the formula given by Chang, 1978

$$B.E = \frac{\text{Fresh weight of the mushroom}}{\text{Dry weight of the straw substrate}} X100$$

Observation on the occurrence of weed fungi and other contaminant

The occurrence of weed fungi and other contaminants was calculated following the scale given by Biswas (2015) [3]

Grade 0: 0% –incidence of contaminants
Grade 1: >0 – 20% coverage by the contaminants
Grade 2: >20 – 40% coverage by the contaminants
Grade 3: >40 – 60% coverage by the contaminants
Grade 4: >60 – 80% coverage by the contaminants
Grade 5: >80 – 100% coverage by the contaminants

$$\text{Percent Contamination Index} = \frac{\text{sum of total scores}}{\text{Maximum rating X total number of observations}} X100$$

Results and Discussion

Effect of different substrate pre-treatments on growth behaviour of *H. ulmarius*

As presented on table 1, T1 (hot water treatment at 80-90 ºC for 30 minutes) was observed to be statistically significant over all the other treatments requiring a minimum time of 15 days for complete spawn run and 18 days for pin head formation over control, which was followed by T7 (6% neem extract) with 20.7 days. The maximum time for complete mycelial run was recorded on T4 (4% garlic extract) with 25 days over control. On control beds it was observed that the results were highly insignificant as there were complete colonization by competitor molds on all the replications. These contaminants compete with the mushroom mycelium for available nutrients, as well as the metabolites produced by these competitors inhibiting the growth of mushroom mycelium (Wang *et al.*, 2016) [11]. Results on pin head formation indicated that T1 was superior over all other treatments requiring minimum time of 18 days for pin head formation, followed by T7 with 22.7 days, while the maximum days required for pinhead formation of 27 days was recorded from T4. The early mycelial run and pin head formation of *H. ulmarius* on hot water treated substrate is similar with the results of Siddhant *et al*. (2014) [9] who stated that *Pleurotus sajor-caju* took the minimum time of 14 days on hot water pasteurized paddy straw for complete mycelial run. The process of sterilization may have reduced the natural temperature sensitive microorganism of the substrate, reducing the competition and improving substrate colonization by mushroom mycelium as it enhances high level conversion of cell wall polysaccharide into sugars, promoting faster mycelial run and better yield. (Saritha and Pandey, 2010) [6]. Khan *et al*. (2016) [4] reported that heat sterilization causes early spread of mycelia and pin head formation which might be due to the killing of microorganisms which would delay the spread of mycelia. The late mycelial run and pin head formation from beds treated with plant extracts may be due to the inhibitory effect of the botanicals against the growth of the mushroom mycelia.

Table 1: Effect of different substrate pre-treatments on growth behaviour of *H. ulmarius*

Treatments	Spawn run (days)	Pin head formation (days)
T1: Hot water treatment at 80-90oC for 30 minutes	15.0 (3.94)	18.0 (4.30)
T2: Soaking in 4% Ginger extract	22.0 (4.74)	24.3 (4.98)
T3: Soaking in 6% Ginger extract	22.3 (4.78)	25.0 (5.05)
T4: Soaking in 4% Garlic extract	25.0 (5.05)	27.0 (5.24)
T5: Soaking in 6% garlic extract	22.7 (4.81)	25.0 (5.05)
T6: Soaking in 4% neem extract	21.7 (4.64)	22.7 (4.81)
T7: Soaking in 6% neem extract	20.7 (4.60)	22.7 (4.81)
T0: Control	0.00 (0.71)	0.00 (0.71)
S.Em±	0.07	0.06
CD (p=0.05)	0.21	0.19

Note: Data in the Table are mean values of three replications and those in parenthesis are square root transformed values

Effect of different substrate pre-treatments on size of *H. ulmarius*

The length of the stipe ranged from 5.4 cm to 6.33 cm (Table 2.). Maximum stipe length was recorded from T4 with 6. 3 cm which was statistically at par with T1 (5.9 cm); T5 (5.9 cm); T2 (5.7 cm) and T6 (5.7 cm) over control. Whereas, minimum stipe length of 5.37 cm was recorded from T7 over control. The largest pileus diameter was recorded from T4 (7.8 cm) which was statistically at par with T5 (7.5 cm) and the minimum pileus diameter was observed from T7 (6.3 cm) over control. The better performance of *H. ulmarius* on hot water treated beds may be due to the softening of the straw during the process of heat treatment enabling the mushroom mycelium to easily digest the nutrients producing better mushroom sizes.

Table 2: Effect of different substrate pre-treatments on size of *H. ulmariu*

Treatments	Size of mushroom	
	Stipe length (cm)	Pileus diameter (cm)
T1: Hot water treatment at 80-90 ^{0}C	5.9	6.7
T2: Soaking in 4% Ginger extract	5.7	6.8
T3: Soaking in 6% Ginger extract	5.4	6.5
T4: Soaking in 4% Garlic extract	6.3	7.8
T5: Soaking in 6% garlic extract	5.9	7.5
T6: Soaking in 4% neem extract	5.7	6.8
T7: Soaking in 6% neem extract	5.4	6.3
T0: Control	0.00	0.00
SEM±	0.30	0.25
CD (p=0.05)	0.91	0.75

Effect of different substrate pre-treatments on yield potential of *H. ulmarius*

The substrate pre-treated by hot water at 80-90 ^{o}C for 30 minutes gave significant response with 655 g/670 g of paddy straw followed by T7 with 465 g and least on T5 with 216.67 g/ 670 g of paddy straw which was statistically at par with T4 with 245 g/670 g of dry paddy straw over control (Table 3.). Highest biological efficiency was recorded on T1 with 97.76% followed by T7 with 69.40% and the least on T5 with 32.33% which was statistically at par with T4 with 36.56%. The result obtained on yield parameters are in agreement with the findings of Atila (2016) [1] who stated that pasteurizing in hot water is one of the best method for the production of oyster mushroom. The better performance of oyster mushroom on heat pasteurized substrate maybe due to the breaking down of lignin-cellulosic bond aiding in softening of substrate during heat pasteurization which helps in easy colonization by the mycelium leading to better yield (Saritha and Pandey, 2010) [6]. Sharma *et al.* (2018) [8] revealed that hot water treated paddy straw substrates produces higher yield of 676 g than chemical treated substrates.

Table 3: Effect of different substrate pre-treatments on yield potential of *H. ulmarius*

Treatments	Yield (gm)	B.E (%)
T1: Hot water treatment at 80-90oC for 30 minutes	655.00	97.76
T2: Soaking in 4% Ginger extract	376.67	56.22
T3: Soaking in 6% Ginger extract	446.67	66.66
T4: Soaking in 4% Garlic extract	245.00	36.56
T5: Soaking in 6% garlic extract	216.67	32.33
T6: Soaking in 4% neem extract	400.00	59.70
T7: Soaking in 6% neem extract	465.00	69.40
T0: Control	0.00	0.00
SEM±	24.11	3.60
CD (p=0.05)	72.28	10.79

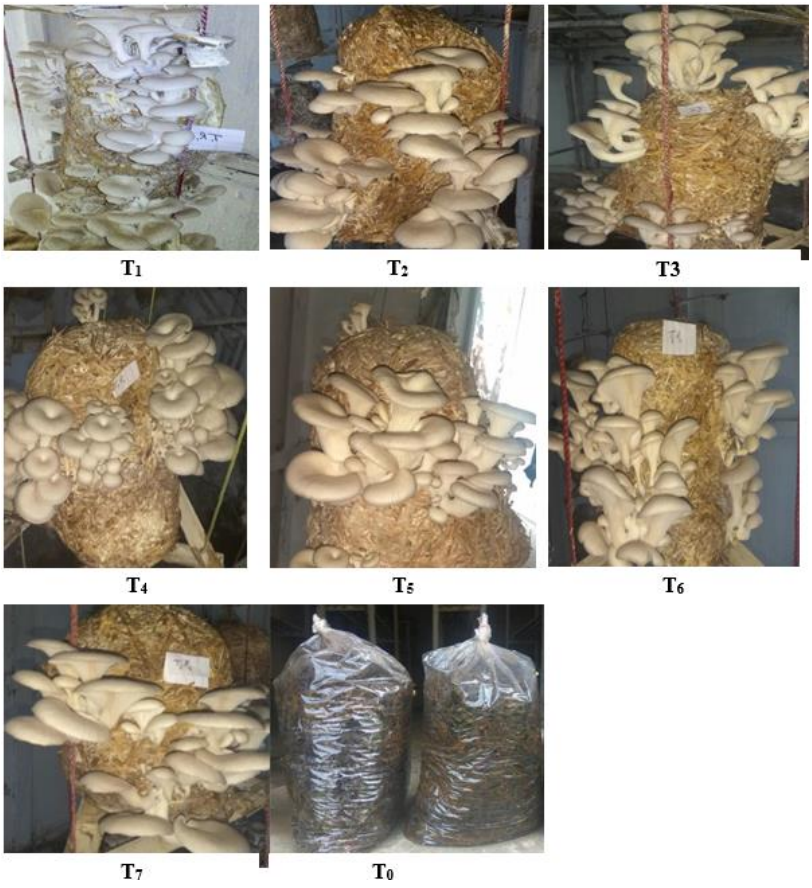

Plate 1: *Hypsizygus ulmarius* on different pre-treated substrate

Table 4: Incidence of competitor molds and weed fungi

Treatments	PCI (%)
T1: Hot water treatment at 80-90 ^{0}C for 30 minutes	0
T2: Soaking in 4% Ginger extract	0
T3: Soaking in 6% Ginger extract	0
T4: Soaking in 4% Garlic extract	33.3
T5: Soaking in 6% garlic extract	33.3
T6: Soaking in 4% neem extract	0
T7 : Soaking in 6% neem extract	0
T0: Control	100

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