

The Productivity of Genotypes of *Hericium erinaceus* (Bull.: Fr.) Pers. on Wheat Straw and Poplar Sawdust Based Growing Media

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Received: 5.11.2020 | Revised: 3.12.2020 | Accepted: 9.12.2020

ABSTRACT

The genotypes of *Hericium erinaceus* He-101, He-102, He-104, He-105, He-107, He-108, He-109, and growing media made of wheat straw+20% wheat bran (GM1) and poplar sawdust+20% wheat bran (GM2) were studied for spawn run, harvestings, numbers, yield, biological efficiency, cropping period, and average fruit body weight. The spawn run of He-108 was completed within a minimum of 24 and 26 days on GM 1&2, respectively, however, early harvesting of He-101 was recorded within 42 (GM1) and 43 days (GM2) after spawning. He-104 resulted highest 205.7 g yield /1.5 kg GM 1 (31.65% BE) with 11 highest average numbers of mushroom /1.5 kg GM1 and 19.22 g highest average fruit body weight with maximum 5 harvestings and 94 days of total cropping period. The performance of GM1 was found superior to GM 2. The GM1 led to the shortest duration of spawn run (27 days), the highest number of harvestings (3), the highest number of fruits (7/bag), the highest yield (115 g/bag), and the highest BE (17.66%) on paired 't' test. While the short-duration crop of 66 days was recorded in GM2 with an incomplete spawn run.

Keyword: *Hericium erinaceus*, Wheat straw, Poplar sawdust, Genotype, Yield, BE.

INTRODUCTION

Hericium erinaceus (Bull.: Fr.) Pers. is an edible and medicinal mushroom and has been used for centuries in traditional Chinese medicine and Japanese cuisine. It is a member of toothed fungi and ecologically behaves like white-rot fungi of several hardwood trees such as Beech, Oak, Poplar, and Pine trees.

Morphologically it has a long spiny hymenophore that looks like a white snowball (Conde et al., 1972) with no distinct cap and stipe. The hot water extract of mushroom is considered a healthy drink and strengthens immunity and neurological health (Kolotushkina et al., 2003).

Cite this article: Sholyavei, Sh., & Mishra, S. K. (2020). The Productivity of Genotypes of *Hericium erinaceus* (Bull.: Fr.) Pers. on wheat Straw and Poplar Sawdust Based Growing Media, *Ind. J. Pure App. Biosci.* 8(6), 396-405. doi: <http://dx.doi.org/10.18782/2582-2845.8454>

The fruit body is composed of numerous bioactive compounds like phytosterol; β -sitosterols, ergosterol (Rahman et al., 2014), polysaccharides; β -D glucans (Moldavan et al., 2007), phenol analogous; hercenons, and benzyl alcohol derivatives hericenones (Mizuno, 1999; & Ma et al., 2010), diterpenoid derivatives; erinacine (Lee et al., 2014; & Ma et al., 2010). The only recent breakthrough of its first cultivation in India was in Directorate Mushroom Research (DMR), Solan, Himachal Pradesh. The present research work on *Hericium* was initiated in Uttarakhand State, India after receiving seven genotypes from DMR, Solan. They were tested in the natural climate of winter season in the Tarai region of Uttarakhand State on the growing media made of wheat straw and poplar sawdust for the spawn run, harvesting, cropping period, number, yield biological efficiency, and average fruit body weight. Though, *H. erinaceus* was cultivated earlier on various substrates, such as wheat straw, paddy straw, sawdust, cottonseed hulls, sugarcane bagasse, corncobs, supplemented with wheat bran (Oei, 1996). But during the present investigation wheat straw and poplar sawdust are selected as they are available in plenty in the Tarai region of Uttarakhand State.

MATERIALS AND METHODS

Genotypes

The pure culture of seven genotypes of *Hericium erinaceus* (*He-101*, *He-102*, *He-104*, *He-105*, *He-107*, *He-108*, and *He-109*) was obtained from the DMR, Solan, and maintained on Potato Dextrose Agar (PDA) medium to produce master spawn and subsequent commercial spawn.

Master and commercial spawn were prepared from boiled unbroken wheat grains with which 2% CaSO_4 and 0.5% CaCO_3 were thoroughly mixed (Gupta & Sharma, 1994). CaSO_4 and CaCO_3 mixed wheat grains were filled into thermostatic glass bottles of 250 ml capacity @100g/ bottle. The bottles were plugged with non-absorbent cotton and a piece

of butter paper. Ready bottles were autoclaved at 20 lbs for 2 hours. The autoclaved bottles were allowed to cool in an aseptic place followed by inoculation with a pure culture of all 7 genotypes of *Hericium erinaceus*. The ready bottles were incubated at 25°C for about 25 days. The same steps were adopted for the production of commercial spawn using autoclavable polypropylene bags of 1 kg capacity instead of glass bottles. The prepared bags were inoculated from master spawn. The inoculated bags of commercial spawn were incubated at 25°C and get ready for spawning within 25 days after inoculation.

Growing medium

The growing medium 1 (GM1) and 2 (GM 2) were prepared from fresh wheat straw and poplar sawdust, respectively with which wheat bran was added for supporting firm mycelial growth of *Hericium erinaceus*. In preparation of growing media 1, 70 kg of fresh dry wheat straw was soaked in a water tank overnight followed by sun-drying on a pre-disinfested cemented platform for 4 hrs. Moist wheat straw was thoroughly mixed with 14 kg wheat bran and packed in autoclavable polypropylene bags @ 1.5 kg/ bag followed by plugging them with thermostatic polypropylene ring, non-absorbent cotton, and butter paper. Ready bags were autoclaved at 20 lbs for 2 hours. The next day, the autoclaved bags were inoculated with the commercial spawn @ 90 g /bag. Inoculated bags were incubated at 25°C for spawn run.

Growing media 2 was made of 117 kg dry poplar sawdust in which 23 kg wheat bran was added. Total 140 kg dry mixture was getting moist with 70 liters of fresh tap water. The moist mixture was heaped overnight to have uniform moisture. Very next day, the process of bagging, autoclaving, inoculation and incubation was done similar to those of GM1. The inoculated bags of GM1 and GM2 were tagged with date of inoculation, rate of spawning, name of genotype, number of bags, and number of replication. The details of GM1 and GM2 with their treatments and replications are mentioned in table 1.

Table 1: Calculations of GM1 and GM2

Activity	Wheat straw	Sawdust
Quantity of dry substrate (A)	70 kg	117 kg
Quantity of dry wheat bran (B)	14 kg	23 kg
Total dry matter C (A+B)	84 kg	140 kg
Addition of water in the dry matter C (D)	126 liter	70 liter
Total weight of wet substrate E (C+D)	210 kg	210 kg
Weight of one bag	1.5 kg	1.5 kg
Number of genotypes	7	7
Number of replications	5	5
Number of bags per replication	4	4
Total number of bags	7X 5X4 = 140	7X 5X4 = 140
Spawning rate with commercial spawn (6% on a wet weight basis of GM)	90 g	90 g

After completion of the spawn run, polythene bags were removed completely from the logs of GM1 but polypropylene bags of GM2 were folded upside down to half of the substrate level and punched holes at the bottom. The process of cultivation of *Hericium erinaceus* was started in October. In this month the temperature reached down to about 25°C which is an ideal temperature for spawn run.

As temperature constantly decreased, it would have been favorable for the development of fruiting of *Hericium erinaceus* and found most favorable from November to December (15-20°C). The observations on the yield were recorded at the three ranges of temperatures above 20°C, 20-15°C, and 15-12°C. The crop room was kept in dark. A regular spray of water was provided in the morning to maintain 90- 95% RH during the cropping. Usually, 4-5 days were taken for primordia formation but sometimes it extended 7 days. After 8-10 days of primordia formation, harvesting was done. The crop was maintained until the mushrooms stopped coming out of the bags. At most 4 harvestings were obtained. Data of yield (g) and the

number of fruits per bag were recorded for all the genotypes grown on both the media.

Physical properties of GM 1 and 2

Wheat straw and sawdust were characterized by their particle size, bulk density, particle density, and porosity. The particle size of wheat straw was measured by Vernier Caliper. However, the particle size of sawdust was measured under a microscope with the help of the ocular and stage micrometer at 40X. The bulk and particle density of both the substrates were calculated using the method as suggested by Blake and Hartge, 1986. In which two cylindrical beakers of 1000ml capacity were filled up with wheat straw and sawdust separately to the half of the beaker with the gentle press at each step of filling. The respective cylindrical beakers are dried in a hot air oven at 100°C for 6 hours and weight. The dry substrates were water up to 800 ml in each cylinder and heat gently to remove entrapped air inside the media then make the volume up to 1000 ml and weight. The bulk density, particle density, and porosity were calculated by the following formula and concerned data are mentioned in table 2.

$$\text{Bulk density g/cm}^3 \text{ (BD)} = \frac{\text{Dry weight of medium}}{\text{volume of medium}}$$

$$\text{Particle density g/cm}^3 \text{ (PD)} = \frac{\text{Dry weight of medium}}{\text{Volume of dry medium}}$$

$$\text{Porosity \% (P)} = \frac{\text{Particle density} - \text{Bulk density}}{\text{Particle density}} \times 100$$

Table 2: Physical properties of growing medium 1 and 2

Parameter	Observation	
	GM1	GM2
Particle size	15-25 mm	0.8-1.2 mm
Radius of the beaker (R)	5 cm	5cm
Height of beaker up to 500 ml mark (H)	6.5cm	6.5cm
Weight of empty 1000 ml beaker (W ₁)	250g	250g
Weight of beaker with dried medium (W ₂)	288.43g	336.98g
Weight of only dried medium (W ₃)= W ₂ -W ₁	38.43g	86.98g
Weight of beaker with dry medium and water added up to 1000 ml mark (W ₄)	1222.92g	1179.67g
Weight of beaker with 1000 ml water (W ₅)	1225g	1225g
Volume of medium filled up to 500 ml (V ₁) = $\pi \times R^2 \times H$	$3.14 \times (5)^2 \times 6.5 = 510.25\text{cm}^3$	
Volume of dry medium (V ₂)= (W ₅ -W ₁) - (W ₄ -W ₂)	40.51cm ³	132.31cm ³
Bulk density (BD) = W ₃ /V ₁	0.075g/cm ³	0.170g/cm ³
Particle density (PD) = W ₃ /V ₂	0.949g/cm ³	0.657g/cm ³
Porosity (P) (%)	92.48 %	74.12 %

Statistical analysis

The experimental data were recorded on the duration of spawn run, a total number of harvestings, cropping period (days), number, yield (g), biological efficiency (% BE), and average fruit body weight (g), of the genotypes of *Hericium erinaceus*. The respective data were statistically analyzed using Web-Based Agricultural Statistics Software Package (WASP 2.0) at www.ccari.res.in/wasp2.0/index.php. One factorial RBD was used in all crop room experiments Standard deviation (SD) and critical difference (CD) were also calculated at a 5% level of significance. A paired T-test was used to compare the GM1 and GM2 by using the means of their respective treatments.

RESULTS AND DISCUSSION

Spawn run

The mycelium of genotype *He-107*, *He-108*, *He-109*, and *He-108* of *Hericium erinaceus* was spread fast and colonized entire growing medium 1 (wheat straw + 20% wheat bran of dry weight of wheat straw) and growing medium 2 (sawdust + 20% wheat bran of dry weight of sawdust) within 24-25 and 26 days, respectively (Table 3 and photo 1). The genotype *He-104* was grown very slow on GM 1 and 2 both to be colonized within 29 and 35 days after inoculation. Growing medium made of sunflower seed hull 8 parts and wheat bran 2 parts was also colonized by *Hericium*

erinaceus within 25 days (Figlas et al., 2007). Hassan (2007) observed that the growth of mycelium of *Hericium erinaceus* was slow on the oak sawdust and wheat straw-based growing medium and colonized them completely within 40 and 38 days at a 2% spawning rate (wet weight basis of growing medium). However, in the present study, the 6% spawning rate (on a wet weight basis of growing medium) was successfully reduced the time of spawn run in both the growing medium. As per the paired 't' test the growing medium 1 and growing medium 2 has a statistically significant difference for the duration of spawn run at a 5% level of significance (table 7).

Harvesting and cropping period

In the growing medium 1 and 2, the first harvest of *He-101* and *104* was done at 42, 43 days, and 43, 45days after spawning, respectively (table 4). However, the remaining genotypes were differed statistically and taken a comparatively long duration for the first harvest ranged from 51-55 days in GM1 and 47-57 days in GM2. Hassan (2007) took 1st harvest of *Hericium erinaceus* at 14 and 20 days after completion of spawn run in wheat straw and sawdust based growing medium, respectively.

The harvesting frequency of mushroom fruits of all genotypes of *Hericium erinaceus* was studied under natural conditions. It was invariably found that the first harvest of

Hericium erinaceus crop of all genotypes was late due to higher temperature recorded around 25°C in the crop room at the beginning of November, and then in the coming months, the temperature was reduced gradually by 12°C. As the temperature was down by 20-15°C, the proceeding harvestings have been achieved comparatively in less time. After each harvesting generally 4-5 days were taken for new primordia formation. Re-lowering of temperature extends the time of new primordia formation by 7 days for 3rd and 4th flush, and the next 8-10 days were taken for their development to attain the right stage of the harvesting. The fruits of genotype *He-104* and *105* harvested a maximum of 4 times at different intervals from GM1 within a maximum cropping period of 94 and 91 days, respectively. However, in GM 2 average number harvestings were limited by 1-2 only among all genotypes within 63-71 days of average cropping period (table 5).

Number and yield of mushroom fruits

In wheat straw-based growing medium 1, the genotype *He-104*, and *He-107* produced maximum 10 numbers of fresh mushroom. However, these genotypes were differed in their yields and BE. Maximum 205.70 g (31.65% BE) yield of *He-104* was recorded/1.5 kg wet substrate with highest 19.22 g average fruit body weight. This genotype also gave uniform fruiting in all the bags, with bigger fruit size. The yields and BE and average fruitbody weight of *He-107* and *He-109* were statistically *at par* but inferior to the genotype *He-104* (table 6). The remaining genotypes *He-105*, *He-101*, *He-108*, and *He-102* were found poor in the yield, BE and average fruit body weight. Hassan (2007) observed the highest 140g (39.4% BE) - 157 g (43.5% BE) of *Hericiium erinaceus* /kg wheat straw. Figlas et al. (2007) obtained 37.2% BE in sunflower seed hull with 20% wheat bran. Even all the genotypes of present research grown on the same climatic conditions showed different BE ranged from 5.14% to 31.65%. It might be due to their different inherent quality characters. Kirchhoff (1996) also found 14 heterokaryotic strains of *Hericiium erinaceus* showed a large variation in the yield and quality

of the sporophores ranging from 5%-33% on the wet weight of the substrate.

Sawdust based growing medium 2 resulted in fairly fewer numbers, yield, BE, and average fruit body weight among genotypes (Table 6), nevertheless, a maximum of 3 fruits of genotype *He-101* were harvested from 1.5 kg wet substrate and highest 42.22 g (5.63% BE) yield and 15.81 g average fruit body weight of genotype *He-108* were recorded from 1.5 kg wet substrate. In contrast to the above results, Hassan (2007) recorded the highest yield and highest BE of *Hericiium erinaceus* in the sawdust based growing media. Ehlers and Schnitzler (2000) had grown six strains of *Hericiium erinaceus* on fine beech sawdust with 20% wheat bran and obtained a 254.3g yield of fresh mushroom/kg substrate. Ko et al. (2005) obtained 31±2% BE of *Hericiium erinaceus* in the oak sawdust substrate with 20% rice bran. Similarly, Eisenhut and Fritz (1995) recorded a 29.3% average BE of *Hericiium erinaceus* in the substrate of sawdust. Siwulski et al. (2009) obtained BE of three *H. erinaceus* strains cultivated on beech sawdust substrates ranging from 20.1- 28.4%.

The comparative effect of growing medium 1 and 2 was also studied. It was found that GM1 was superseded GM2 for spawn run, harvestings, cropping period, number of fruits, yield, and their BE. However, the cropping period was long (83 days) with GM1 than that of 66 days cropping of GM2 (table 7). Further, both the GMs were statistically *at par* with their respective duration of the first harvest and average fruit body weight. It was observed that mycelia of all tested genotypes couldn't penetrate deep in the GM2 bags during spawn run and restricted superficially. The fine sawdust of GM2 might be also made the logs compact and impermeable of air in comparison to the wheat straw of GM1. Consequently, the sawdust logs resulted in poor numbers of mushrooms and their yield. The bags of GM2 were rarely formed fruit after the first harvest. According to Figlas et al. 2007 higher mycelial growth rate of *Hericiium erinaceus* (3.3 mm/day) was observed with large sunflower seed hull size (12x 4 mm) with the irrespective

presence of wheat bran in comparison to a powder of 40 mesh particles of the same substrate. Curvetto et al. (2002) stated that the faster mycelial growth rate of *Pleurotus ostreatus* strains in linear growth test did not

always lead to higher biological efficiency. Hassan, 2007 has seen the longest spawn run time with the highest yield and the highest BE in the growing medium of sawdust than the growing medium of wheat straw.

Table 3: Spawn run of genotypes of *Hericium erinaceus*

Genotype	Spawn run in days	
	Growing medium 1	Growing medium 2
<i>He</i> -101	28 ^{bc}	31 ^c
<i>He</i> -102	28 ^{bc}	35 ^e
<i>He</i> -104	29 ^c	35 ^e
<i>He</i> -105	27 ^b	33 ^d
<i>He</i> -107	25 ^a	28 ^b
<i>He</i>-108	24^a	26^a
<i>He</i> -109	25 ^a	29 ^b
CD at 5%	1.04	1.15

Superscripts of mean values represent significant differences.

Table 4: First harvest of *Hericium erinaceus* after spawning

Genotype	Duration for the first harvest after spawning (days)	
	Growing medium 1	Growing medium 2
<i>He</i>-101	42^a	43^a
<i>He</i> -102	45 ^a	47 ^b
<i>He</i> -104	43 ^a	45 ^{ab}
<i>He</i> -105	52 ^{bc}	55 ^{cd}
<i>He</i> -107	55 ^c	57 ^d
<i>He</i> -108	52 ^{bc}	53 ^c
<i>He</i> -109	51 ^b	54 ^{cd}
CD at 5%	3.57	3.28

Superscripts of mean values represent significant differences.

Table 5: Harvesting frequency and cropping period of genotypes of *H. erinaceus*

Genotype	Average number of harvesting (Nos.)		Average cropping period (days)	
	GM 1	GM 2	GM 1	GM 2
<i>He</i> -101	3 ^{ab}	2	86 ^{ab}	63
<i>He</i> -102	2 ^b	1	55 ^c	63
<i>He</i> -104	4 ^a	1	94 ^a	71
<i>He</i> -105	4 ^a	2	91 ^a	63
<i>He</i> -107	3 ^{ab}	1	87 ^{ab}	71
<i>He</i> -108	2 ^b	2	76 ^b	71
<i>He</i> -109	3 ^{ab}	1	91 ^a	63
CD at 5%	1	NS	12	NS

Superscripts of mean values represent significant differences.

Table 6: Number of fruits and yield of genotypes of *H. erinaceus*

Genotype	Growing medium 1			Growing medium 2		
	Total number of fruits per bag of 1.5 kg	Total yield (g) per bag of 1.5 kg	Average wt. of fruit body (g)	Total number of fruits per bag of 1.5 kg	Total yield (g) per bag of 1.5 kg	Average Wt. of fruit body (g)
<i>He</i> -101	5.40 ^c	81.40 ^d (12.52)	15.07	3.17 ^a	27.31 ^b (3.61)	8.62
<i>He</i> -102	3.55 ^d	33.40 ^e (5.14)	9.41	1.33 ^{cd}	6.33 ^c (0.84)	4.76
<i>He</i> -104	10.70 ^a	205.70 ^a (31.65)	19.22	1 ^d	21 ^b (2.8)	21
<i>He</i> -105	8.35 ^b	115.60 ^c (17.78)	13.84	1.67 ^c	21.66 ^b (2.89)	12.97
<i>He</i> -107	10.10 ^a	147.10 ^b (22.63)	14.56	2.33 ^b	24.33 ^b (3.24)	10.44
<i>He</i> -108	4.62 ^{cd}	80.10 ^d (12.32)	17.34	2.67 ^b	42.22 ^a (5.63)	15.81
<i>He</i> -109	8.10 ^b	140.50 ^{bc} (21.62)	17.35	1.67 ^c	26 ^b (3.47)	15.57
CD at 5%	1.344	28.29 (4.35)	-	0.66	6.72 (0.89)	-

Superscripts of mean values represent significant differences.

The values in parenthesis represent BE (%)

Table 7: Comparative effect of growing medium 1 and 2 on quantitative characters of *Hericum erinaceus* using the paired T-test

Quantitative character	Growing medium	Mean of samples	T-statistics > T-table (0.05)	Significance
Spawn run	GM1	27 days	2.93 > 2.17	S
	GM2	31 days		
First harvest	GM1	49 days	0.70 < 2.17	NS
	GM2	51 days		
Number of harvestings	GM1	3 nos	4.26 > 2.17	S
	GM2	1 nos		
Cropping period	GM1	83 days	3.05 > 2.17	S
	GM2	66 days		
Number of fruits	GM1	7 nos	4.86 > 2.17	S
	GM2	2 nos		
Yield	GM1	115 g	4.20 > 2.17	S
	GM2	24 g		
Average fruit body weight	GM1	15.25 g	1.06 < 2.17	NS
	GM2	12.73 g		
BE	GM1	17.66%	4.36 > 2.17	S
	GM2	3.21%		

NS = Not significant; S = Significant





























Strain	Spawn run		Fruit body	
	Growing medium 1	Growing medium 2	Growing medium 1	Growing medium 2
<i>He-101</i>				
<i>He-102</i>				
<i>He-104</i>				
<i>He-105</i>				
<i>He-107</i>				
<i>He-108</i>				
<i>He-109</i>				

Photo 1: Variability in spawn run and sporophore type on Growing Medium 1 & 2

CONCLUSION

The particle size, bulk density, particle density, and porosity of the wheat straw and sawdust of GM1 and GM2 had a remarkable effect on the biological parameters of *He-101*, *He-102*, *He-104*, *He-105*, *He-107*, *He-108*, and *He-109*. The wheat straw of GM1 with 15-25 mm particle size, 0.075g/cm³ bulk density, 0.949 g/cm³ particle density, and 0.92048% porosity was superseded to GM2 of sawdust having 0.8-1.2 mm particle size, 0.170g/cm³ bulk density, 0.657 g/cm³ particle density, and 74.12% porosity for the spawn run, harvesting, cropping period, fruit numbers, yield, and biological efficiency. One more difference was observed between GM1 and GM2. The bags of GM2 of incomplete spawn run were fruited, however, the bags of GM1 were fruited after completion of spawn run and hence GM2 produced fairly fewer numbers, yield, BE, and average fruitbody weight of mushroom in the long period of cultivation. Overall the performance of wheat straw-based GM1 was superior to the sawdust-based GM2. Further, refinement of the growing medium is required for the higher production of the *Hericium ernicious* in a short period.

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