The Pharma Innovation Journal 2022; 11(6): 379-384

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(6): 379-384

www.thepharmajournal.com
Received: 19-03-2022
Accepted: 28-05-2022

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Evaluation of *Hericium erinaceus* (Lion's Mane) mushroom strains on different substrates

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Abstract
Lion's mane fruit is white and globe-shaped and popular by its taste, nutritional and medicinal properties. It is grown on agro-wastes under laboratory conditions. Directorate of Mushroom Research (DMR) supplied its nine strains for evaluation on wheat and saw dust substrates. The wet substrates with 60% moisture supplemented with wheat bran were filled in polypropylene bags, autoclaved, cooled, spawned asceptically and kept for incubation. This experiment was laid out in RBD design with five replications. The observations were recorded on time taken for spawn run, first harvest, crop duration and yield.

In wheat substrate, the spawn run days were significantly as low as 29.4 days in HE 18-01, 18-02, 18-08 strains whereas HE 18-03, HE 18-07 completed spawn run in 33.4 days and it was significantly high. The first harvest was obtained in 31.2 days in HE 18-06. The crop duration was significantly as low as 39.0 days in HE 18-06, 18-01 strains. The yield was significantly highest at 18.7 kg/100 kg dry substrate in HE18-08.

In saw dust substrate, the spawn run was significantly as low as 21.2 days in HE 18-09 followed by 23.2 days in HE 18-08 whereas, HE 18-02 completed spawn run in 34.6 days and it was significantly high. The first harvest was obtained in 29.4 days in HE 18-08 and HE 18-09. The crop duration was significantly as low as 38.0-38.3 days in HE 18-09 and HE 18-08 strains whereas, it was significantly as high as 45.4 in HE 18-04. The yield was significantly as high as 23.7 kg/100 kg dry substrate in HE18-08.

Keywords: *Hericium erinaceus*, lion's mane, saw dust, strain, substrate, wheat straw, Yield

Introduction
H. erinaceus belong to family Hericiaceae, class Agaricomycetidae, division Basidiomycota. Its spores are white, elliptic and smooth to slightly rough. The fruiting body is globe-shaped with long, shaggy spines, white colored which turns brown or yellow with age. It is an important edible mushroom widely distributed throughout North America, Europe, China and Japan having good dietetic and pharmacological activities without harmful effects. It is being used in traditional Chinese medicine and people in Asia use it for both culinary and medicinal purposes. It has a mild pleasant taste and can be used for salads, soups, or as a delicious side dish. Friedman (2015) [7] reported that it has high protein of 22 grams per 100 grams of dry mushrooms and also an incredible non-animal source of essential amino acids at 44.25 g amino acid/100 g of crude protein. It can become an integral part of diet for vegans, or those wanting to consume less animal protein. It is also a rich source of carbohydrates, crude fiber, low fats, ash, calcium, thiamin, minerals, vitamins and soluble sugars such as arabitol, glucose, mannitol, inositol and trehalose. It contains physiologically important polysaccharides such as hericenones A-B, erinacines A–I, hericenones C–H, hericirine and polyphenols. These polysaccharides play vital role in regulating and curing various diseases such as blood pressure, cholesterol metabolism, liver problems, cancer, obesity, ulcer and diabetes (Bacha *et al.* 2018) [2]. It has some important components, especially β-glucan polysaccharides, responsible for anti-cancer, immuno-modulating, hypolipidemic, antioxidant and neuro-protective activities along with anti-microbial, anti-hypertensive, anti-diabetic, wound healing properties (Khan *et al.*, 2013) [17]. It is also being used to treat cognitive impairment, Parkinson's disease, and Alzheimer's disease. Its bioactive compounds have been found to promote the expression of neurotrophic factors (Chong, 2019) [5]. It inhibited the growth of *Porphyromonas gingivalis* and may be useful for prevention of periodontal disease (Ichikawa, 2021) [13]. Suleiman (2022) [26] found antioxidant and antiviral activities of the aquatic mushroom extract in a preliminary study and may be used as an essential part of human meals every day to protect against either oxidative stress or viral attack especially,

Covid-19 and other advanced variants such as omicron variant that threaten the human health. It is being used to eat or take in the supplements form.

H. erinaceous is native to North America, Europe and Asia. It is known to be cultivated on wood logs of poplar, oak, alder, aspen, maple, birch, ash, beech, willow, elm etc under natural conditions. It can fruit intermittently for 20 years on the same dead tree and can survive for 40 years (*Slawomir, 2016)* [25]. However, it can be successfully cultivated on different agro-wastes under laboratory conditions. The solid substrates mixed with different supplements like wheat bran, wheat straw, soybean meal, corn meal, vegetables oils or fatty acids, corn cobs, corn meal, cotton chaff, gypsum, sunflower husk, sugarcane, olive pruning residues, rice bran and rice straw are being used for its cultivation under laboratory conditions (Kirchhoff, 1996, Zhang, 2000, Zheng *et al.*, 2002; Kang *et al.*, 2002, Ko *et al.*, 2004; Figlas *et al.* 2007; Siwulski and Sobieralski 2007, Hu *et al.* 2008; Koutrotsios *et al.* 2016, Atila *et al* 2016) [19, 29, 31, 16, 20, 6, 24, 12, 21, 1]. Julian *et al.* (2018) [15] investigated the optimum nutritional requirement for mycelial growth of *H. erinaceous* and observed the highest mycelial growth and density in potato dextrose agar.

Grigansky *et al.* (1999) [9] investigated the physiological characteristics of 14 different strains of *H. erinaceus* on beechwood substrate, agar medium and liquid medium. The strains were found to be very similar in their responses to increasing temperature, water content of the substrate and pH of liquid media. However, there was no growth at 3 and 37 °C, whereas, a temperature of 26°C and a water content of 50-70% in substrate were found suitable for vegetative growth of all strains. Similarly, Thi (2018) [23] reported that it can be cultivated at a temperature of 26 °C and a relative humidity of 85 to 90%.

The cultivation of high quality mushrooms require high yielding strains, minimum crop duration and suitable substrate. There are few studies of the systemic screening of *H. erinaceus* strains. Imtiaj *et al.* (2008) [14] screened different growing conditions of four strains of *H. erinaceus* and observed the highest growth at 25°C excluding one strain which had a similar growth at 20°C temperature. The strains showed highest growth on potato dextrose agar and at a pH of 6. Among the carbon sources, dextrose, fructose, and glucose led to the highest growth, whereas among for the nitrogen sources, alanine and ammonium acetate were found suitable. Among the different substrates, the highest growth of all strains was found on sawdust (Hassan, 2007, Siwulski and Sobieralski, 2007, Atila *et al.*, 2016) [11, 24, 1]. Zongying *et al.* (2015) [32] studied the genetic diversity of 19 commercial strains collected from different localities in China based on random amplified polymorphic DNA (RAPD) and polymerase chain reaction (PCR).

Since, *H. erinaceous* has been found to possess many medicinal properties against many threatening diseases of human beings and is also a rich source of nutrients; therefore, it is a mushroom of high economic value. There are few studies on selection of a high yielding, short crop duration strains and a suitable substrate for its cultivation at commercial level. Therefore, the present study was conducted to identify a high yielding, short crop duration strain for its cultivation on wheat straw and saw dust substrates under laboratory conditions.

Materials and Methods

Study area: The present study was carried out during 2018-19 in Mushroom Technology Laboratory, Department of Plant Pathology, Chaudhary Charan Singh Haryana Agricultural University, Hisar situated at 20^0 10' N latitude,75^0 46' E longitude, altitude 215 m msl in the semi-arid region of North-Western India.

Mushroom strains used in experiment: The DMR, Solan supplied nine strains HE 18-01, HE 18-02, HE 18-03, HE 18-04, HE 18-05, HE 18-06, HE 18-07, HE 18-08 and HE 18-09 during 2018-19 for their evaluation on wheat straw and saw dust substrates at All India Coordinated Research Project (Mushroom) Centre, Hisar (Haryana).

Spawn preparation of mushroom strains: Clean, healthy, and bold wheat grains were used for the preparation of spawn of different strains. The grains were softened by boiling in water for 20 minutes. After cooling, the grains were mixed with $CaCO_3$ and $CaSO_4$ @ 0.5% and 2% (w/w basis), respectively. This prepared substrate was filled in 500 ml glucose/milk bottles and heat-resistant polypropylene bags up to 2/3 volume and plugged with non-absorbent cotton. Then the bottles/bags were autoclaved at 121ºC for 2 hours at 15 lb psi pressure. After sterilization, these bottles/bags were cooled and pure culture was aseptically transferred to them, and further incubated at 25 ºC in BOD incubator for 14 days to allow mycelium to spread on wheat grains. After the complete spread of mycelium on wheat grains it was used for spawning of substrate.

Wheat straw and saw dust substrates preparation: The wheat straw and saw dust substrates were soaked for 12 hours separately in freshwater. Then these substrates were dried in shade to have moisture of 60% and then supplemented with wheat bran @ 20% on dry weight basis. The supplemented wet wheat straw and saw dust substrates were filled separately at 2.0 kg /polypropylene (PP) bag of 45 cm x 30 cm and autoclaved at 121º C for 2h. The autoclaved bags were allowed to cool and spawned asceptically at 3% on wet weight basis.

Cultivation technology: These bags were kept in incubation room on racks at 23-25 °C for spawn run and 18-20ºC for fruiting. There were five replications per treatment or strain and 5 bags per replication. The experiment was randomized as per RBD design. The observations on spawn run (days), time taken for first harvest (days), crop duration (days), yield kg/100 kg dry straw, and average fruit body weight (g) were recorded. A total of two flushes were taken from each bag during the cultivation period. The total mushroom yield of each strain of mushroom was calculated replication-wise by adding the fresh weight of all of the two harvests.

Results

Performance of *H. erinaceous* strains on wheat straw

The data presented in Table 1 revealed that the spawn run was completed in 29.4 days in strain HE 18-01, HE 18-02 and HE 18-08 followed by 29.6 days in HE 18-04 in autoclaved wheat straw. The spawn run took maximum period of 33.4 days in strain HE 18-03, HE 18-07 and it was significantly high as

compared to other strains. The first harvest was obtained in 31.2 days in HE 18-06 followed by 31.4 days in HE 18-05 and both strains were statistically at par but differed significantly as compared to other strains. The crop duration was found to be minimum at 39.0 days in strain HE 18-06 followed by 40.3, 40.5 days in strains HE 18-01, HE 18-05, respectively. A maximum crop duration of 47.9, 47.7 was taken by strain HE 18-04, HE 18-02, respectively and both strains were statistically at par but differed significantly as compared to other strains. The average yield was found to be significantly as high as 18.7 kg/100 kg dry wheat substrate in HE18-08 and it was followed by 18.0 kg/100 kg dry substrate in strain HE 18-01. The yield was as low as 12.7 kg/100 kg dry wheat substrate in strain HE 18-07 followed by 13.1 kg/100 kg dry substrate in strain HE 18-06 and both strains were found to be statistically at par in yield (Table 1, Fig 1).

Table 1: Evaluation of *H. erinaceous* strains on wheat straw substrate

Strain	Spawn run (days)	Time for first harvest (days)	Crop duration (days)	Yield (kg/100kg dry substrate)
HE 18-01	29.4	33.8	40.3	18.0
HE 18-02	29.4	39.6	47.7	16.1
HE 18-03	33.4	35.6	44.8	15.5
HE 18-04	29.6	38.6	47.9	15.1
HE 18-05	29.4	31.4	40.5	16.1
HE 18-06	29.7	31.2	39.0	13.1
HE 18-07	33.4	34.6	42.8	12.7
HE 18-08	29.4	33.6	41.6	18.7
HE 18-09	30.6	34.4	42.6	14.7
CD at 5%	0.728	0.652	0.627	0.615

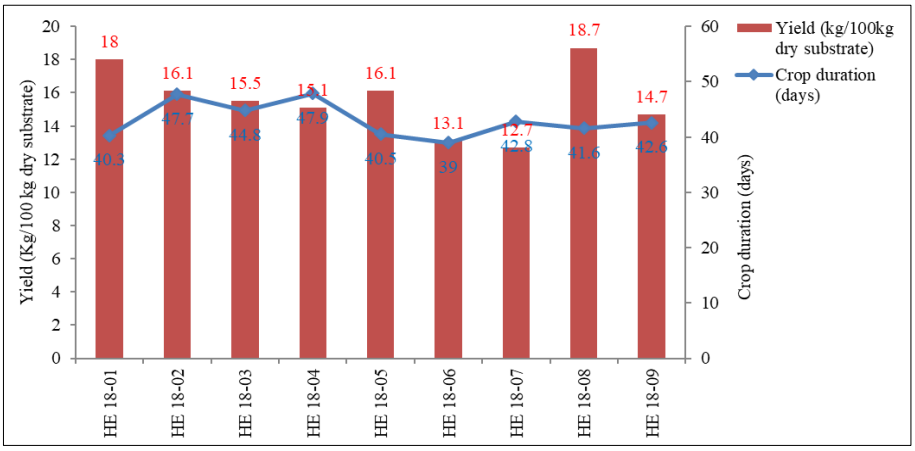

Fig 1: Evaluation of *H. erinaceous* strains on wheat straw

Performance of *H. erinaceous* strains on saw dust

The data presented in Table 2 revealed that the spawn run was completed in 21.2 days in strain HE 18-09 which was significantly minimum as compared to other strains. It was followed by 23.2 days in HE 18-08 in autoclaved saw dust. The spawn run took maximum period of 33.6 days in strain HE 18-04 and it was significantly high as compared to other strains. The first harvest was obtained in 29.4 days in HE 18-08, HE 18-09 followed by 30.6 days in HE 18-01 and these strains differed significantly as compared to other strains. The crop duration was found to be minimum at 38.0, 38.3 days in strain HE 18-09, HE 18-08, respectively and both strains were statistically at par but differed significantly as compared to other strains. It was followed by 40.3, 40.5 days in strains HE 18-01, HE 18-05, respectively. A significantly maximum crop duration of 47.9, 47.7 was taken by strain HE 18-04, HE 18-02, respectively and both strains were statistically at par but differed significantly as compared to other strains. The average yield was found to be significantly as high as 23.7 kg/100 kg dry wheat substrate in HE 18-08 and it was followed by 20.5 kg/100 kg dry substrate in strain HE 18-01. The yield was significantly as low as 12.7 kg/100 kg dry saw dust substrate in strain HE 18-09 followed by 15.1 kg/100 kg dry substrate in strain HE 18-06 (Table 2, Fig 2).

Table 2: Evaluation of *H. erinaceous* strains on saw dust substrate

Strain	Spawn run (days)	Time for first harvest (days)	Crop duration (days)	Yield (kg/100kg dry substrate)
HE 18-01	26.4	30.6	40.2	20.5
HE 18-02	34.6	37.6	44.8	18.5
HE 18-03	32.6	36.6	45.2	19.3
HE 18-04	33.6	36.6	45.4	17.5
HE 18-05	27.4	31.6	40.8	17.2
HE 18-06	27.2	32.6	41.5	15.1
HE 18-07	29.6	32.6	41.4	17.7
HE 18-08	23.2	29.4	38.3	23.7
HE 18-09	21.2	29.4	38.0	12.7
CD at 5%	0.697	0.489	0.497	0.509

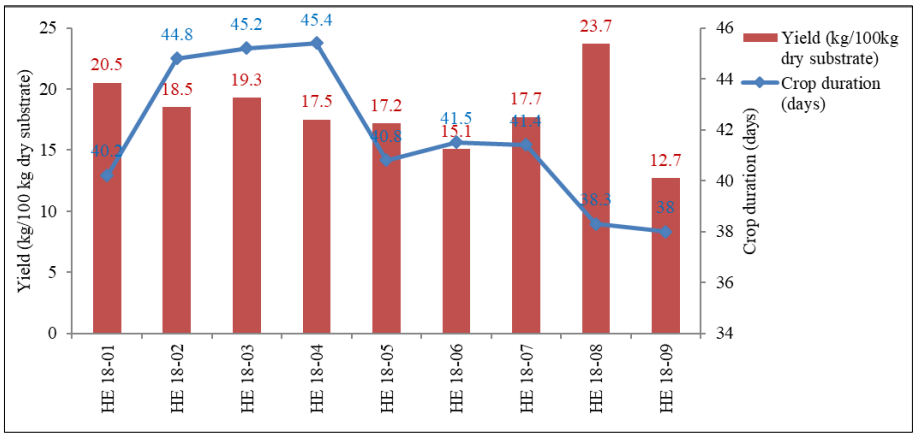

Fig 2: Evaluation of *H. erinaceous* strains on saw dust

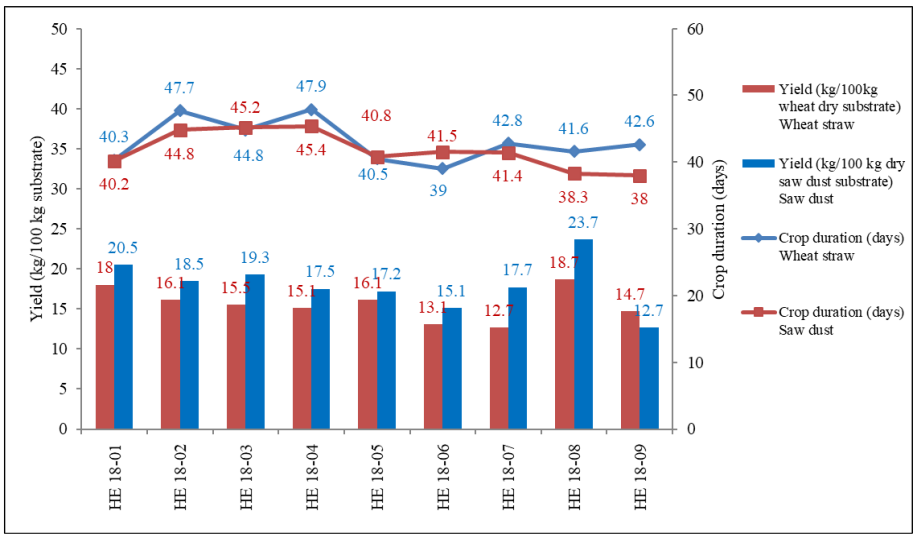

Fig 3: Performance of *H. erinaceous* strains in different substrates with respect to crop duration and yield

High yielding *H. erinaceous* strains

Plate 1: Strain HE 18-08

Plate 2: Strain HE 18-01

The results have also revealed that yield is higher in saw dust substrate supplemented with 20% wheat bran as compared to wheat straw supplemented with wheat bran at 20%. In saw dust substrate, strain HE 18-08 gave highest yield at 23.7 kg/100 kg dry substrate in 38.3 day of crop duration. Similarly, in wheat straw substrate, the same strain gave higher yield at 18.7 kg/100 kg dry substrate in 41.6 days. In all the strains, the yield is higher and crop duration is less in saw dust substrate as compared to wheat straw (Fig 3).

Discussion

The spawn run was as early as 29.4 days in strain HE 18-01, HE 18-02 and HE 18-08 in autoclaved wheat straw. The first harvest was at the earliest in 31.2 days in HE 18-06 followed by 31.4 days in HE 18-05. The crop duration was as short as 39.0 days in strain HE 18-06 and it was as long as 47.9, 47.7 days in HE 18-04, HE 18-02, respectively. The yield was as high as 18.7 kg/100 kg dry wheat substrate in HE18-08 followed by 18.0 kg/100 kg dry substrate in HE 18-01 as compared to lowest yield at 12.7 kg/100 kg dry wheat substrate in HE 18-07.

The spawn run was as early as 21.2 days in strain HE 18-09 followed by 23.2 days in HE 18-08 in autoclaved saw dust. The first harvest was at the earliest in 29.4 days in HE 18-08, HE 18-09 followed by 30.6 days in HE 18-01. The crop duration was as short as 38.0, 38.3 days in strain HE 18-09, HE 18-08, respectively and it was as long as 47.9, 47.7 days

in strains HE 18-04, HE 18-02, respectively. The yield was as high as 23.7 kg/100 kg dry substrate in HE 18-08 followed by 20.5 kg/100 kg dry substrate in strain HE 18-01. It was as low as 12.7 kg/100 kg dry substrate in strain HE 18-09.

The variations in spawn run days, first harvest period, crop duration depend upon the rate of multiplication of strain and it is due to the inherent genetic ability of a strain. The yield varied in different strains and it may also be due to genetic variability of a strain. Kirchhoff (1996) [19] has shown a large variation in yield, quality and colour of the fruit bodies in different strains of *H. erinaceous*. Hassan (2007) [11] reported that the incubation time (37-46 days) and biological efficiency of a mushroom also depend upon strain, suitable substrates and environmental conditions. Bunroj *et al.* (2017) [3] screened six strains of lion's mane in East of Thailand and reported that yield and number of harvestings is determined by strain characteristics. Since, the breeding of elite strains of *H. erinaceus* is hindered by the lack of a genetic and molecular toolbox, therefore, Gong *et al.* (2020) [8] performed resequencing analysis of 127 F1 single-spore isolates and constructed the first high-resolution genetic map of *H. erinaceus* and informed that high-resolution genetic map may be used as reference in future genetic, genomic and breeding studies on *H. erinaceus.*

Various works correlated the mycelia growth in *H. erinaceous* with the activity of different enzymes like β-amylase, protease, cellulose, laccase etc in different substrates and informed that higher the activity of released enzymes resulted in shorter crop duration (Kim *et al.*, 2000; Han, 2003; Hu *et al.*, 2008; Sun *et al.*, 2011; Li, 2014) [18, 10, 12, 27, 22]. Similarly, in the present study, there may be variations in enzymes production by different strains which might have resulted in variations in period of spawn run, first harvest, crop duration and differences in yield of *H. erinaceous* strains.

Recently, many workers have used biotechnological tools for identification of *H. erinaceous* strains because of their bioactive or secondary metabolites importance in medical science research. Chen *et al.* (2017) [4] sequenced the genome to investigate the biosynthesis of bioactive secondary metabolites from *H. erinaceus*. Zeng *et al*. (2018) [28] identifed numerous proteins involved in terpenoid, polyketide and sterol biosynthesis by proteome analysis of *H. erinaceus*. However, they did not predict genes or proteins involved in the biosynthesis of polysaccharides, which are the most important substance in *H. erinaceus*. Zhang *et al.* (2019) [30] predicted the open reading frames (ORFs) and simple sequence repeats (SSRs) from the transcriptome data of the six strains of *H. erinaceous*.

The results have also revealed that yield is higher in saw dust substrate supplemented with 20% wheat bran as compared to wheat straw supplemented with wheat bran at 20%. In saw dust substrate, strain HE 18-08 gave highest yield at 23.7 kg/100 kg dry substrate in 38.3 day of crop duration. Similarly, in wheat straw substrate, the same strain gave higher yield at 18.7 kg/100 kg dry substrate in 41.6 days. In all the strains, the yield is higher and crop duration is less in saw dust substrate as compared to wheat straw. The present findings are in line with the findings of Hassan (2007) [11], Siwulski and Sobieralski (2007) [24] and Atila *et al.* (2016) [1] who obtained the highest yield of *H. erinaceus* when grown on sawdust, whereas, Bunroj (2017) [3] obtained higher yield of *H. erinaceous* strains on rice straw substrates than saw dust. It may be due to better nutritional status of rice straw than rice straw for mushroom cultivation. Therefore, rice straw as well as other crop residues and their mixing with many supplements like wheat bran, rice bran, cotton seed cake, soy cake etc. require further testing.

Conclusion

The strain HE 18-08 gave highest yield at 23.7 kg/100 kg dry saw dust substrate in 38.3 day of crop duration followed by strain HE 18-01 which gave a yield of 20.5 kg/ 100 kg dry saw dust substrate in 40.3 days of crop duration. Similarly, strain HE 18-08 gave higher yield at 18.7 kg/100 kg dry wheat substrate in 41.6 days followed by 18.0 kg/100 kg dry wheat substrate in 40.3 days in strain HE 18-01. Therefore, it is summarized that strain HE 18-08 followed by HE 18-01 are better strains in yield as well as duration of crop is concerned. However, saw dust substrate supplemented with wheat bran at 20% gave higher yield in the range between 12.7-23.7 kg/100 kg dry substrate in short crop duration between 38.0-45.4 days depending upon strain. The wheat straw substrate supplemented with wheat bran at 20% gave yield in the range between 12.7-18.7 kg/100 kg substrate in crop duration between 39.0 to 47.7 days based on strain.

Acknowledgement

The author is thankful to the Directorate of Mushroom Research, Chambaghat, Solan (H.P.) for providing the cultures of different strains of *Hericium erinaceous* for their evaluation at AICRP (Mushroom) centre, Hisar.

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