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Evaluation of *Pleurotus* species or strains cultivated on wheat substrate under Haryana conditions during 2017-18 to 2020-21

Satish Kumar and Jagdeep Singh

Abstract

The Directorate of Mushroom Research (DMR), Chambaghat, Solan (H.P.) supplied a total of 34 Pleurotus species/strains during different years for their evaluation at All India Coordinated Research Project (Mushroom) at Hisar centre. The wheat straw was hot water treated at 65 °C for 60 minutes and dried till it attains 60 per cent moisture. The polythene bags were filled with 5 kg wet substrate having spawned at 10% of dry substrate. There were five replications per strain and 10 bags per replication. The observations on yield (kg/100 kg dry straw), time taken for first harvest (days) and pileus color were observed. The eleven strains tested during 2017-18 revealed that strain PL 17-11 produced a significantly maximum yield of 63.2 kg/100 kg dry substrate and gave first harvesting in 23.0 days. The ten strains were examined during 2018-19 and strain PL 18-04 produced highest yield of 73.6 kg/100 kg dry substrate and took a time of 25.2 days for first harvest. The strain PL 18-02 took a significantly lowest time of 24.8 days for first harvest. The seven strains evaluated during 2019-20 revealed that strain PL 19-07 produced significantly highest yield of 94.4 kg/100 kg dry straw and first harvesting was completed in 28 days. Similarly, six strains were tested during 2020-21 and strain PL-20-202 produced highest yield of 55.8 kg/100 kg dry straw with 26.2 days of first harvest duration. The four strains PL 17-11, PL 18-04, PL 19-07, PL 20-202 were found to give highest yield at 63.2, 73.6, 94.4 and 55.8 kg/100 kg dry substrate with duration of first harvest from 23-28 days during 2017-18, 2018-19, 2019-20 and 2020-21, respectively. Among these, the strain PL 19-07 was light brown while other three were white in color. It can also be summarized in another way that among 34 strains, 22 strains gave a yield of 50.0 kg or more /100 kg dry substrate and only 12 strains gave yield of less than 50.0 kg/100 kg dry substrate. The duration of first harvest was between 23.0 to 35.5 days and pileus was light brown, brown, dark brown and white in colour.

Keywords: Pleurotus, spawn, species, strains, wheat straw, yield

Introduction

Oyster mushrooms belong to genus *Pleurotus* and the family Pleurotaceae. It is popularly known as 'Dhingri' in India. (Adejoye et al., 2006)^[1]. It is the most popular and widely cultivated edible fungi in the world (Yin *et al.* 2014) ^[24]. It can be grown commercially on a wide range of agricultural wastes with high yield (Banik and Nandi 2004)^[4]. Its world production is about one million tonne and the third-highest among other mushrooms. China is the leading country in its production whereas, in India, its production is about 21000 tonnes annually. The world production of all mushrooms is about 40 million tonnes contributed largely by countries like China, the USA, the Netherlands, Poland, Spain, France, Italy, Ireland, Canada, and the UK, whereas, India produces only 2.59 lakh tonnes of total mushrooms (Anonymous, 2021)^[3]. The Haryana state is also a leading producer of white button mushroom with 21,200 tonnes production during 2020-21 (Anonymous, 2021)^[3]. Oyster mushrooms are currently in high demand for their nutrition, medical benefits and therapeutic attributes (Chang, 1996)^[5]. The fresh mushrooms have an average moisture content of 85-90%, 3% protein, 4% carbohydrates, 0.3-0.4% lipids, and 1% minerals and vitamins. These mushrooms have remarkable medicinal properties (Zhang et al. 2007, Khatun et al. 2015, Nguyen et al. 2016, Golak-Siwulska et al. 2018) ^[26, 9, 17, 7]. It has many medicinal properties such as antimicrobial, antiviral, anti-human immunodeficiency virus (HIV), antineoplastic, antitumor, antimutagenic, antioxidant, hyperglycaemic, hypotensive, antiinflammatory, hepatoprotective, hypocholesterolemic, immunomodulatory, anti-aging (Patel et al., 2012) [18]. Pleurotus florida has been found to possess antioxidant and anticancer properties (Nayana and Janardhanan, 2000; Manpreet et al., 2004)^[16, 12].

The inflammation and platelet aggregation are inhibited by methanol preparations of *P. florida* (Nayana *et al.*, 2004) ^[15]. *Pleurotus sajor-caju* has hypertensive effects through its active ingredients which affect the renin-angiotensin system (Chang, 1996) ^[5].

It has also been shown to have bioremediation characteristics since it is able to degrade contaminants (Rodri'guez *et al.* 2004) ^[21] and biocides (Law *et al.* 2003) ^[10]. It can produce useful enzymes even while degrading waste products (Ina´cio *et al.* 2015) ^[8] as well as being consumed after being part of biofuel production (Chen *et al.* 2020) ^[6].

The widespread malnutrition with the ever-increasing protein gap has further necessitated the search and cultivation of an alternative source of protein. To meet the need for quality food, the mushroom cultivation is now developing as an important industry (Ambili and Nithya, 2014)^[2]. These mushrooms are the easiest, quickest, and cheapest to grow. It has cheap capital expenditures, takes less time to prepare, and uses low-level production equipment, making it more profitable to cultivate than other mushrooms on a commercial scale.

India produces about 600 million tonnes of crop residue every year, with majority being permitted to degrade naturally or burned on the spot which have environmental impact. The Haryana state alone produces around 22 million tonnes of wheat and paddy straw per year. Keeping in view of surplus availability of raw substrate and high scope of its commercialization in future, it becomes essential identifying the promising *Pleurotus* species/strains. Therefore, a total of 34 *Pleurotus* species/strains were evaluated during different years to identify most promising strains under Haryana conditions on wheat straw.

Materials and Methods

The present study was carried out in Mushroom Technology Laboratory, Department of Plant Pathology, Chaudhary Charan Singh Haryana Agricultural University, Hisar during 2017-18 to 2020-21.

Pleurotus species/strains used in experiment: The DMR, Solan supplied 11, 10, 07, 06 *Pleurotus* species/strains during 2017-18, 2018-19, 2019-20 and 2020-21, respectively for their evaluation on wheat substrate at All India Coordinated Research Project (Mushroom) Centre, Hisar (Haryana).

Spawn preparation: 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 121 °C 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 °C 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 substrate preparation: The wheat straw was soaked in freshwater for 12 hours to soften them. After draining the surplus water, the substrate was pasteurised in hot water treatment at 65 °C for 60 minutes and then dried for 4 h till it attains moisture of 60 per cent. The pH of the substrate was adjusted to 7.50 with CaCO₃. The polythene bags of 45 cm x 60 cm were filled with 5 kg wet wheat substrate containing spawn at 10% of dry substrate.

Cultivation technology: These bags were kept in mushroom house on racks at 22-28 °C and 85-90% (R.H.) The relative humidity was maintained in cultivation room by frequent sprays of water on the floor and walls. The light intensity of 800 lux light was provided for 6h on daily basis. There were five replications of each strain and 10 bags/replication and randomized properly as per RBD design. The observations on yield kg/100 kg dry substrae, time taken for first harvest (days) and pileus color of different varieties/strains was recorded during different years i.e. 2017-18 to 2020-21. A total of three flushes were taken from each bag during the cultivation period. The total mushroom yield of each strain/variety of *Pleurotus* was calculated replication-wise by adding the fresh weight of all of the three harvests.

Results

Among the eleven strains evaluated during 2017-18, it was found that a significantly higher yield was obtained from strain PL 17-11 (63.2 kg/100 kg dry substrate) followed by PL 17-08 (59.0 kg) and a minimum yield of 45.2 kg was obtained from PL 17-01 (Table 1). The strain namely PL 17-11 took a minimum of 23.0 days for first harvesting and it was significantly a minimum as compared to strain PL 17-03 which took a significantly maximum time of 35.5 days. The strain PL 17-11 was considered as the best strain in that year which gave maximum yield of 63.2 kg/100 kg dry substrate and took less time of 23 days in first harvest (Table 1, Figure 1, Plate 1). The data presented in Table 1 revealed that among eleven strains, seven strains gave yield between 51.0 to 63.2 kg/100 kg dry substrate and time of first harvest ranged between 23.0 to 32.0 days. The pileus colour also varied among different strains. It was observed as brown, dark brown, light brown and white (Table 1).

Pleurotus species/strain	Yield kg/100kg dry straw	Time taken for first harvest (days)	Pileus colour
PL 17-01	45.2	27.7	Brown
PL 17-02	56.0	26.7	Dark Brown
PL 17-03	48.5	35.5	White
PL 17-04	57.8	27.0	Dark Brown
PL 17-05	51.0	29.3	Light Brown
PL 17-06	51.0	32.0	White
PL 17-07	53.0	28.7	White
PL 17-08	59.0	27.7	White
PL 17-09	48.0	29.7	Brown
PL 17-10	46.3	28.7	Brown
PL-17-11	63.2	23.0	White
CD at 5%	1.62	0.78	-

 Table 1: Evaluation of high yielding varieties/strains of Oyster mushroom (*Pleurotus* spp) during 2017-18

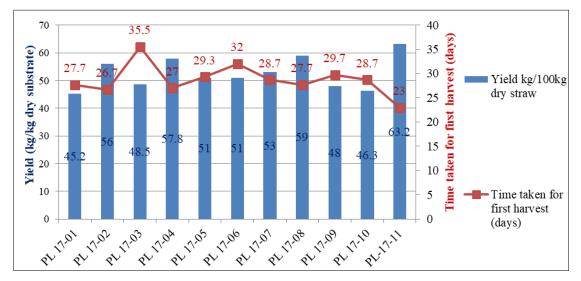


Fig 1: Evaluation of Pleurotus species/strains for yield and time of first harvest during 2017-18

In the year 2018-19, ten strains were evaluated as shown in Table 2. It revealed that a significantly highest yield was obtained from strain PL 18-04 (73.6 kg/100 kg dry substrate) followed by PL 18-07 (68.4 kg) and a significantly lowest yield of 29.6 kg/100 kg dry substrate was obtained from PL 18-06 in that year. The strain PL 18-02 gave the first flush only in 24.8 days followed by 25.2 days in PL 18-06 and PL 18-09 which took significantly a maximum time of 33.4 days for first harvest. The strains PL 18-04, PL 18-07 and PL 18-07

02 were the best yielder giving yield of 73.6 kg, 68.4 kg and 67.6 kg/100 kg dry substrate, respectively and the time of first harvest also remained low within 24.8 to 26.4 days (Table 2, Figure 2, Plate 2). The data presented in Table 2 revealed that among ten strains, three strains gave yield between 67.6 to 73.6 kg/100 kg dry substrate and time of first harvest ranged between 24.8 to 26.4 days. The pileus colour also varied among different strains. It was observed as brown, light brown and white (Table 2).

Table 2: Evaluation of high yielding varieties/strains of Oyster mushroom (*Pleurotus* spp) during 2018-19

Pleurotus species/strain	Yield kg/100kg dry straw	Time taken for first harvest (days)	Pileus colour
PL-18-01	37.4	32.4	Light Brown
PL-18-02	67.6	24.8	Brown
PL-18-03	36.6	31.8	Light brown
PL-18-04	73.6	25.2	White
PL-18-05	40.4	25.4	White
PL-18-06	29.6	33.4	White
PL-18-07	68.4	26.4	White
PL-18-08	46.4	31.6	White
PL-18-09	37.6	33.4	White
PL-18-10	30.6	30.6	White
CD at 5%	0.676	0.686	-

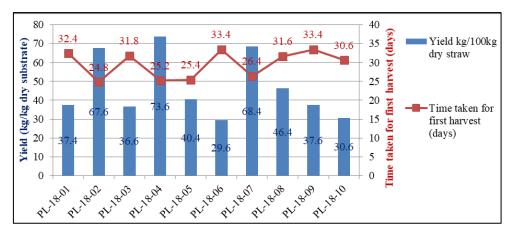


Fig 2: Evaluation of Pleurotus species/strains for yield and time of first harvest during 2018-19

In the year 2019-20, seven strains were cultivated on wheat straw. A significantly higher yield at 94.4 kg/100 kg dry substrate was obtained from strain PL 19-07 followed by 77.2 kg/kg dry substrate in strain PL 19-04 in this year. A

significant lowest yield of 67.0 kg/100 kg dry substrate was obtained from strain PL 19-06. The strain PL 19-01, PL 19-04 gave the first flush only in 26.0 days followed by 26.7 days in PL 19-06 and it was significantly at minimum as compared to

29.3 days taken by strain PL 19-05. The strain PL 19-07 was the highest yielder (94.4 kg/100 kg dry substrate), however the time of first harvest was also significantly low at 28 days as compared to 29.3 days in PL 19-05 (Table 3, Figure 3, Plate 3). The data presented in Table 3 revealed that among

seven strains yield ranged between 67.0 to 94.4 kg/100 kg dry substrate and time of first harvest ranged between 26.0 to 29.3 days. The pileus colour was light brown in strain PL 19-07 and white in rest of strains (Table 3).

Table 3: Evaluation of high yielding varieties/strains of Oyster mushroom (*Pleurotus* spp) during 2019-20

Pleurotus species/strain	Yield kg/100kg dry straw	Time taken for first harvest (Days)	Pileus colour
PL 19-01	73.6	26.0	White
PL 19-02	70.5	27.0	White
PL 19-03	73.4	28.0	White
PL 19-04	77.2	26.0	White
PL 19-05	67.3	29.3	White
PL 19-06	67.0	26.7	White
PL 19-07	94.4	28.0	Light brown
CD at 5%	4.49	0.21	-

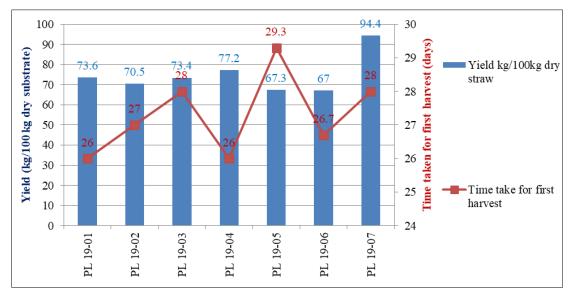


Fig 3: Evaluation of Pleurotus species/strains for yield and time of first harvest during 2019-20

In the year 2020-21, six strains were grown on wheat straw to determine the yield (kg/100 kg dry substrate) and time taken for first harvest. The highest yield was obtained from strain PL 20-202 (55.8 kg/100 kg dry substrate) followed by strain PL 20-205 (54.2 kg) and both the strains were statistically at par while comparing their yield potential in that year. A significantly lowest yield at 42.4 kg/100 kg dry substrate was obtained from strain PL 20-202 and PL 20-201 took a minimum of 26.2 and 26.8 days, respectively

and was statistically at par in time taken for first harvest. The strain PL-20-206 took as high as 29.4 days as compared to other strains. The strain PL 20-202 is the highest yielder (55.8 kg/100 kg dry substrate) and also took the least time at 26.2 days in first harvest (Table 4, Figure 4, Plate 4). The data presented in Table 4 revealed that among six strains, five strains gave yield between 50.0 to 55.8 kg/100 kg dry substrate and time of first harvest ranged between 26.2 to 28.4 days. The pileus colour was white in all strains (Table 4).

Table 4: Evaluation of high yielding varieties/strains of Oyster mushroom (Pleurotus spp) during 2020-21

Pleurotus species/strain	Yield kg/100kg dry straw	Time taken for first harvest (days)	Pileus colour
PL-20-201	52.2	26.8	White
PL-20-202	55.8	26.2	White
PL-20-203	50.0	29.0	White
PL-20-204	51.8	27.8	White
PL-20-205	54.2	28.4	White
PL-20-206	42.4	29.4	White
CD at 5%	5.18	1.21	-



Fig 4: Evaluation of *Pleurotus* species/strains for yield and time of first harvest during 2020-21



Plate 1: (PL 17-11)



Plate 2: (PL 18-04)



Plate 3: (PL 19-07)



Plate 4: (PL 20-202)

Among the 34 *Pleurotus* species/strains tested, there are 22 strains (PL 17-02, PL 17-04, PL 17-05, PL 17-06, PL 17-07, PL 17-08, PL 17-11, PL 18-02, PL 18-04, PL 18-07, PL 19-01, PL 19-02, PL 19-03, PL 19-04, PL 19-05, PL 19-06, PL 19-07, PL 20-201, PL 20-202, PL 20-203, PL 20-204, PL 20-205) which gave a yield of 50.0 kg or more /100 kg dry substrate, and only 12 strains (PL 17-01, PL 17-03, PL 17-09, PL 17-10, PL 18-01, PL 18-03, PL 18-05, PL 18-06, PL 18-08, PL 18-09, PL 18-10 and PL 20-06) which gave yield less than 50.0 kg/100 kg dry substrate.

Discussion

Since, the yield, growth characteristics and pileus color varied among different *Pleurotus* species/strains. The variability in yield and time taken for first harvest (days) of different Pleurotus species/strains was studied among the different Pleurotus species/strains tested during four years. The objective of this study was to identify the Pleurotus species/strains which gave highest yield in short duration under Haryana conditions. During 2017-18, the strain PL 17-11 gave highest yield at 63.2 kg/100 kg dry substrate and also took duration of 23 days in first harvest. Similarly, the strain PL 18-04 was found to give a maximum yield of 73.6 kg/100 kg dry substrate by taking a time of 25.2 days in the first harvest during 2018-19. The strain PL 19-07 was the highest yielder at 94.4 kg/100 kg dry substrate and the time of first harvest was only 28 days during 2019-20. The strain PL 20-202 gave the highest yield at 55.8 kg/100 kg dry substrate and

also took the least time of 26.2 days in first harvest. Therefore, among the 34 Pleurotus species/strains tested at AICRP (Mushroom) Hisar, one highest yielding and taking less number of days for first harvest strain was selected every year from 2017-18 to 2020-21. The strains PL 17-11 (2017-18), PL 18-04 (2018-19), PL 19-07 (2019-20) and PL 20-202 (2020-21) have been found to be promising *Pleurotus* strains during different years of oyster cultivation experiments. Since all these strains were cultivated on wheat straw and gave a yield of 55.8 to 94.4 kg/kg dry substrate. The time of first harvest also ranged between 23.0 to 28.0 days among these four strains. Similarly, Moonmoon et al. (2010) [14] also studied the growth and yield parameters of three strains of P. eryngii by cultivating on saw dust as well as rice straw. The strain Pe-1 on saw dust showed the highest biological yield and efficiency (73.5%) than other strains. Mleczek et al. (2021) ^[13] also recorded the highest biological efficiency (%) for Pleurotus strains on wheat straw. They compared the growth of six different Pleurotus mushroom species and noticed that chemical modification of wheat substrates did not increase yield. Therefore, yield parameter is one of the distinguishing characters to identify different strains. Since the different Pleurotus species/strains were cultivated uniformly on wheat straw pasteurized by hot water treatment without any chemical or non-chemical amendments during all the four years, therefore, there is no contradiction in presenting results on yield and time taken for first harvest. Hence, it is a reliable method to select the best strain based on their yield and time taken for first harvest. However, it is very difficult and time consuming procedure to identify the Pleurotus species/strains based on their morphological, physiological characters and yield potential, but now a days, the sequencing of the genomes is a reliable tool for the identification of various Pleurotus species/strains. Some of the researchers like Riley et al. (2014) [20]; Qu et al. (2016) ^[19]; Wang et al. (2018) ^[23], Zhang et al. (2018) ^[26], Li et al. (2018) ^[11] have made efforts in identification based on genome which is necessary for more quality and comprehensive genetic analyses of these mushrooms. In addition, some Pleurotus species have not yet been sequenced despite of their economic importance. Vieira et al. (2013) [22] analyzed the genetic variability by random amplified polymorphic DNA (RAPD) based on coloring pileus and yield of four commercial strains of Pleurotus ostreatus. The molecular characterization showed two different groups, with 69% similarity between them. Within the group that contained the three samples collected from mushroom growers showed 93% similarities, which showed pileus light gray color and yield statistically different relative to the second group. The second group was only formed by one strain which showed staining of the pileus dark grey and lower yield in the first group. Moreover, Pleurotus species/strains tested in this experiment may also be cultivated under different climatic conditions and various agro-wastes. In spite of selection of strain based on yield potential or duration of crop, the nutritional and medicinal status of a fruiting body of Pleurotus strain is another important aspect which is required to be investigated further.

Conclusion

The *Pleurotus* strains provided by Directorate of Mushroom Research, Solan (H.P.) were evaluated on yearly basis from 2017-18 to 2020-21 by growing on wheat substrate pasteurized in hot water at 65 °C for 60 minutes. Among the

four strains, PL 17-04 strain gave the highest yield at 57.8 kg/100 kg dry substrate and took a minimum of 27 days in first harvest during 2017-18. During 2018-19, among the ten strains, PL 18-04 strain was found to give a maximum yield of 73.6 kg/100 kg dry substrate and also took a time of 25.2 days in the first harvest. Among the seven strains, PL 19-07 was the highest yielder at 94.4 kg/100 kg dry substrate in 28 days of first harvest during 2019-20. During 2020-21, among the six strains, PL 20-202 gave the highest yield at 55.8 kg/100 kg dry substrate and completed first harvest in 26.2 days.

The selection of high yielding strain on yearly basis are PL 17-11, PL 18-04, PL 19-07 and PL 20-202 which had highest biological efficiency at 63.2, 73.6, 94.4 and 55.8 per cent, respectively when cultivated on wheat straw pasteurized by hot water treatment at 65 $^{\circ}$ C for 60 minutes.

Generally, a strain having a yield of 50.0 kg or more/100 kg substrate is considered as a good strain. Therefore, based on these criteria, there are 22 strains which gave a yield of 50.0 kg or more /100 kg dry substrate and only 12 strains gave yield less than 50.0 kg/100 kg dry substrate which may be considered as a poor yielder. The time taken for first harvest ranged between 23.0 to 35.5 days and pileus was light brown, brown, dark brown and white in colour. The *Pleurotus* species/strains tested in this study should also be cultivated under different climatic conditions and on various other agrowastes. Moreover, nutritional and medicinal status of fruiting body of a strain is another important aspect which requires further investigations.

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