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Evaluation of non-conventional lignocellulosic residues for Shiitake mushroom (*Lentinula edodes* (Berk.) Pegler) cultivation

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Abstract

Non-conventional substrates were evaluated to raise Shiitake mushroom including cereal, legume and oilseed wastes wherein cereal straw performed better than other residues. Mycelial run (42.00 days) and mycelial coat hardening (48.00 days) was fastest on rice straw followed by wheat straw while maximum time was taken by sawdust substrate. The other colonization parameters like mycelial bump formation (MBF), browning, formation of pinheads and production of mature fruit bodies was significantly higher on cereal residues. Among legumes, soybean straw was colonized in 38.67 days but pinhead formation and mature fruits took 95.33 and 101.67 days, respectively. Morphological parameters like number of fruit body, weight of fruit body, pileus diameter, stalk length and stalk diameter were significantly better on cereal residues. However, pileus diameter, stalk length and stalk diameter of fruit bodies was higher on sawdust substrate (control) while biological efficiency (25.36 per cent) was more on cereal substrates.

Keywords: Biological efficiency, colonization stages, *Lentinula edodes*, non-conventional substrates

Shiitake mushroom, *Lentinula edodes* (Berk.) Pegler, cultivation has become more and more popular in recent years. Several anti-tumor effects have been documented for the bioactive component lentinan, which is isolated from the fruit bodies of shiitake mushrooms. It has been proven to have therapeutic implications in several cancer therapies (Bisen *et al.* 2010). Nations with friendly climates and plentiful wood supply, such as China, Taiwan, Japan, Korea, etc., growing shiitake on logs made of natural wood is a long-standing custom. But the production of shiitake mushrooms has been revolutionized by intense cultivation utilizing wood shavings or finely powdered sawdust (SD) from broad-leaved trees. To grow shiitake mushrooms, sawdust and wood shavings are combined to create synthetic wood logs. In terms of production efficiency and time, this artificial log cultivation approach outperforms the natural log method (Shen *et al.* 2004). According to Sharma *et al.* (2017), commercial shiitake mushroom growing in India is still in its infancy and requires significant support to grow as a business.

The traditional method, which mostly employs sawdust from logs and hardwoods, is not only expensive but also has a significant application as mulch in the paper sector. In place of natural logs, synthetic log cultivation is significantly quicker and more useful. Specifically, sawdust-based synthetic log cultivation has increased production and consumption of shiitake mushrooms (Elisashvili *et al.* 2015; Tarushi and Sud 2022). Consequently, research is focused on using different agro-residues as production substrates for shiitake growth. The possibility of growing shiitake on straw-based substrates was investigated by Mata *et al.* (2001), Philippoussis *et al.* (2003) and Gaitan-Hernandez *et al.* (2006). Further, information on the comparative productivity of superior strains on wheat straw (WS) and sawdust (SD) is needed to promote shiitake cultivation on non-conventional agro-residues like wheat straw (WS). It is thought that the substrate may have undergone various physical and chemical changes during the different phases of fungal growth, which could have led to the formation of mushrooms (Gaitan-Hernandez *et al.* 2011). Consequently, many agricultural waste products are also used in the shiitake mushroom business as replacement substrates. By using locally easily accessible agro-residues, cultivation expenses can be reduced and the environmental harm that results from burning various agro-residues in the field after harvest is minimized (Chittaragi and Kumar 2021). Considering this, the present study is designed to use a range of cereal substrate (wheat, riceand maize straw), leguminous waste (soybean, lentil and pea straw) and oilseed substrates (linseed and mustard) to evaluate the shiitake mushrooms' bio-efficiency on different substrates in light of their potential uses in medicine and commerce.

Materials and Methods

All the experiments were conducted at Centre for Mushroom Research and Training unit, Department of Plant Pathology, CSKHPKV, Palampur.

Maintenance of Pure Culture

The shiitake strain DMRO 327 obtained from the CMRT Unit, Department of Plant Pathology, CSKHPKV, Palampur is used in present investigation. The pure culture is maintained by regular sub culturing on PDA slants and kept at 20±2ºC in the BOD incubator.

Preparation of spawn

Spawn was prepared on sawdust substrate using standard procedure (Ashrafuzzaman *et al.* 2009; Tarushi *et al.* 2020). For this method, the poplar sawdust was used. Calcium carbonate was applied at 0.1 % of the mixture. The moisture level of the mixture was maintained at 65 % with tap water. Bottles were filled with 200g. The bottles were sterilized in an autoclave for 1 h at 121°C under 1.5 kg/cm^2 pressures and allowed to cooled down for 24 h and transferred to a clean bench. For inoculation process, the sterilized glass rod was used to make a hole in the center of the bottle and shiitake mushroom mycelium was placed aseptically in the opening of the spawn bottles. Inoculated bottles were incubated at 22±2°C. The growth of the shiitake mycelium was observed at 10 days interval.

Substrates preparation

The cereal (wheat, rice and maize), legume (soybean, pea and lentil), oilseed (mustard and linseed) straw substrates were cut in 3-5 cm pieces; this straw was soaked in fresh water overnight so as to have a final moisture content of 65-70 %. The calcium carbonate was added @ 1 % to all bags to maintain the pH of the substrates. The wet substrate was mixed well manually and then filled in polypropylene bags (40 × 25 cm) @ 500g dry substrate per bag and compressed with hand to make it compact. After putting a plastic ring and cotton plug, the bags were sterilized by autoclaving at 20 psi for 90-120 minutes. The bags were allowed to cool down for 24hrs. Later, the bags were inoculated aseptically with sawdust spawn @ 2-3 % on wet weight basis and kept in an incubation room at 20-23°C temperature. Poplar sawdust substrate was used as control following similar procedure. The data on different parameters recorded periodically.

Data collection

The parameters such as time taken for completion of mycelial run, mycelial coat formation, mycelial bump formation, browning, pinning and fruiting was noted at different intervals. The number of fruit bodies per bag and average weight of fruit bodies were also calculated. The biological yield, economic yield, biological efficiency and dry yield were deduced by using the formula (Chang and Miles 2004).

Biological Yield (BY)

Biological yield in g/1000 g packet was recorded by weighing the whole cluster of fruit bodies without removing the lower hard portion.

i. **Economic Yield (EY)**

Economic yield in g/1000 g packet was recorded by weighing all the fruiting bodies in a packet after removing the lower hard portion.

ii. **Biological Efficiency**

Biological efficiency in each harvest and total harvests was calculated by using the formula:

$$\text{B.E.}(\%) = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

iii. **Dry yield:**

Mushroom sample was taken in a paper envelop and weighed. The mushroom was then oven dried at 60^{0}C for 24 hours and weighed again. The weight of blank envelope was subtracted from the weight of fresh mushroom plus weight of envelope. The dry yield was computed using the following formula:

$$\text{Dry Yield (g/kg packet} = \frac{\text{EY X Oven dry weight of sample (g)}}{\text{Fresh weight of sample}}$$

Fructification

To obtain a homogenous production it is necessary to undertake a thermal shock, which consists in subjecting the substrate to a change in temperature namely lowering the temperature of the substrate to 4 - 10ºC. For this purpose, the cultural blocks were dipped in ice-chilled water for 15-30 min and then transferred to the fructification room. To maintain the humidity of the bags, water was supplied at regular basis.

Statistical analysis

The statistical analysis of the collected data was done by using OPSTAT software.

Results and discussion

Effect of agro-waste substrates on colonization stages of *Lentinulaedodes*

Among all cereal substrates tested, rice straw took least number of days for completion of mycelial run (42.00 days) and mycelial coat hardening phase (48.00 days) followed by wheat straw and these were statistically on par with each other (Table 1). However, wheat straw took lesser number of days for the mycelial bump formation (69.67 days), browning (79.33 days), pinning (89.67 days) and mature fruit body production (94.67 days) followed by rice straw and these were statistically on par with each other. In case of leguminous substrates, soybean substrate took minimum number of days for completion of mycelial run (38.67 days), mycelial coat hardening phase (48.67 days), mycelial bump formation stage (74.96 days), browning (85.67 days), pinning (95.33days) and mature fruit body production (101.67 days) followed by lentil substrate and these were statistically different from each other. Oilseed substrates were also used for the cultivation of mushroom and it was observed that mustard substrate took lesser number of days for completion of mycelial run (48.03 days), mycelial coat hardening phase (58.67 days), mycelial bump formation (82.12 days), browning (95.33 days), pinning (106.87 days) and mature fruit production (110.57 days) followed by linseed substrate which were statistically on par with each other. All incubation stages and mature fruit body production was in lesser number of days when compared with control on poplar sawdust substrate which took 120.33 days to produce mature fruit bodies. Among all the tested substrates, wheat straw gave best results (Figure 1), completing all the stages of incubation and production of fruit bodies in lesser number of days as compared to other substrates and control on sawdust.

These results are in accordance with the findings of Chittaragi and Kumar (2021) who used wheat straw and pearl millet straw as a substrate for cultivation of shiitake mushroom and reported that the pearl millet has fastest mycelial run (38 days) as compared to sawdust (44 days).Puri *et al.* (2011) cultivated two distinct strains on various sawdust and agricultural wastes separately and in combination. The combination of wheat straw and poplar sawdust (1 kg) demonstrated the shortest colonization time (55 days). Ramkumar *et al.* (2010) reported that ill-filled paddy with additive $CaCO_3$ improved all the growth stages as compare to other substrates (sorghum grain, silver oak sawdust, sugarcane trash and paddy straw) and

Table 1. Colonization stages of *Lentinula edodes* on different agro-waste substrates

Substrates*	Mycelial run (days)	Mycelial coat hardening (days)	Mycelial bump formation (days)	Browning (days)	Pinning (days)	Baby shiitake formation (days)	Mature fruit body production (days)
Wheat	42.67	48.33	69.67	79.33	89.67	92.33	94.67
Rice	42.00	48.00	71.09	82.79	91.67	96.00	98.33
Corn	47.33	56.33	80.03	93.67	105.33	107.67	109.67
Soybean	38.67	48.67	74.96	85.67	95.33	99.33	101.67
Lentil	52.01	58.04	80.33	89.67	98.85	104.23	106.68
Linseed	48.87	61.17	85.67	97.67	106.87	108.67	110.57
Mustard	48.03	58.67	82.12	95.33	107.43	110.33	112.41
Control	54.33	64.00	83.33	102.33	112.33	117.33	120.33
CD	1.76	2.86	3.28	3.52	4.14	4.43	3.83

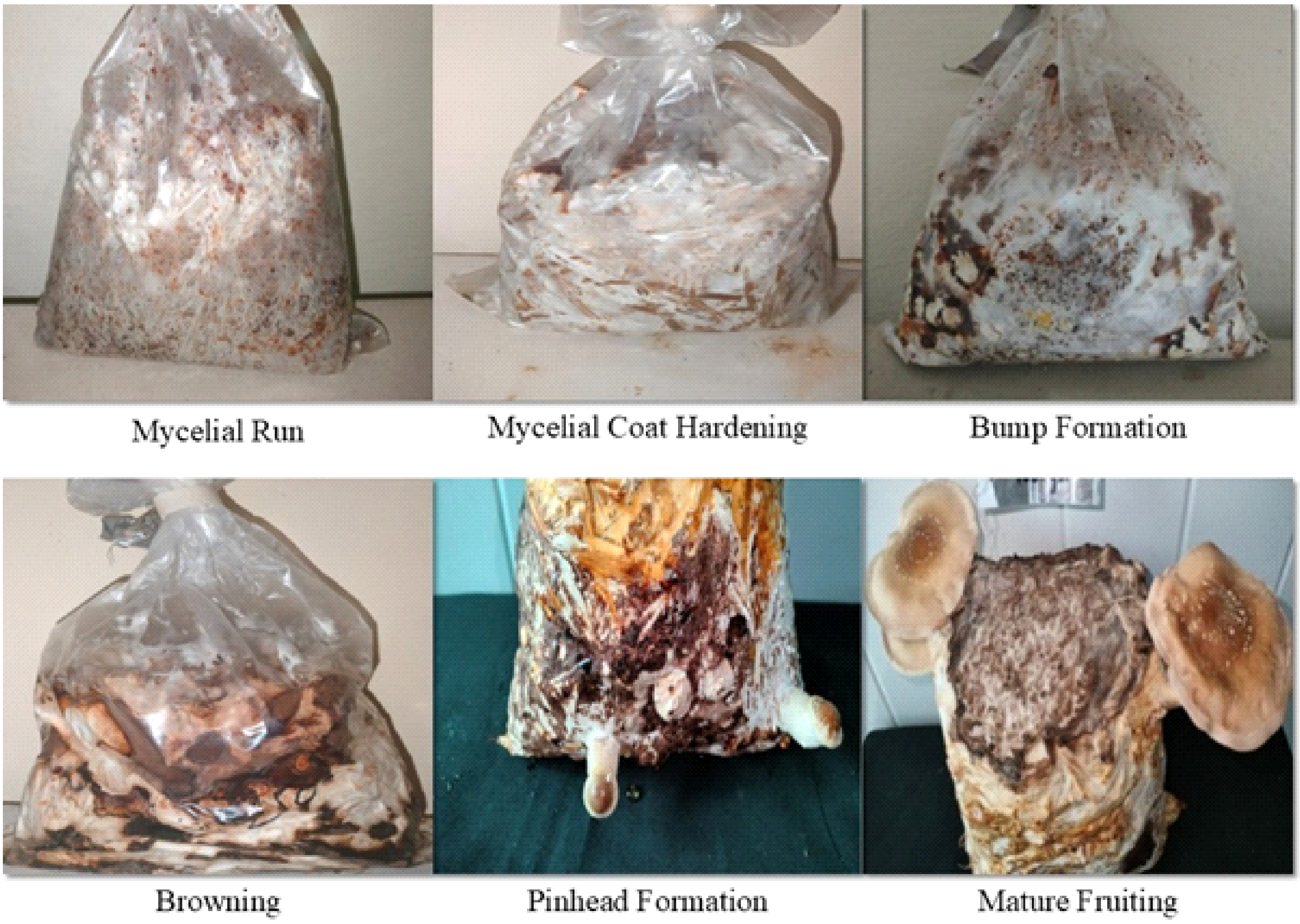

Fig. 1 Different colonization stages of *Lentinulaedodes* on wheat straw

additives (corn flour and gypsum).

Effect of agro-waste substrates on the morphological characters of *Lentinula edodes*

The data represented in the Table 2 indicate the morphological characters of fruit bodies on different agro-waste substrates. Among all the substrates tested, maximum number of fruit bodies were recorded on wheat straw (10.34) which was comparable to control (10.78) minimum on lentil (5.71) and no fruiting was observed on pea substrate. In cereal substrates, the maximum mean number of fruiting bodies were observed on wheat straw (10.34) followed by rice straw

Table 2. Basidiocarp parameters of *Lentinula edodes* on different agro-waste substrates

Substrates	Fruit bodies (no.)	Weight of fruit bodies (g)	Pileus Diameter (cm)	Stalk length (cm)	Stalk width (cm)
Wheat	10.34	23.21	7.26	5.18	1.27
Rice	9.02	20.70	6.76	5.77	1.40
Corn	7.337	17.73	6.31	4.60	1.29
Soybean	7.36	18.23	6.66	4.48	1.22
Lentil	5.71	19.70	6.40	4.40	1.29
Linseed	5.27	16.28	6.34	3.80	1.42
Mustard	5.14	14.50	5.83	4.37	1.39
Control	10.78	22.50	8.10	7.20	1.42
CD	0.36	0.71	0.91	1.00	0.27

(9.02) whereas, in leguminous substrates, the maximum mean number of fruiting bodies were recorded on soybean substrate (7.36) followed by lentil substrate (5.71). Similarly, in oilseed substrates, maximum number of fruiting bodies was observed on linseed substrate (5.27) followed by mustard substrate (5.14). The maximum weight of fruit bodies was also observed on wheat substrate (23.21 g) followed control (22.50 g) which were statistically similar to each other and minimum on mustard (14.50 g). Cereal substrates tested revealed that the maximum fruit weight was recorded on wheat substrate (23.21 g) and rice substrate (20.70 g) and corncob substrate (17.73 g) respectively. Whereas, in leguminous substrates, the maximum weight of fruit body were recorded on lentil substrate (19.70 g) followed by soybean substrate (18.23 g). On the other hand, on oilseed substrates maximum fruit weight were observed on linseed substrate (16.28 g) followed by mustard substrate (14.50 g). Other parameters like pileus diameter, stalk length and width were maximum in sawdust substrate. However, amongst cereals wheat straw, legumes soybean and oilseeds linseed straw gave better results when compared to their counter parts.

Our results corroborated with Desisa *et al.* (2023) who investigatedthe *Lentinula edodes* (Pegler) production on seven different substrates: 100% sugarcane bagasse (S1), 80% sugarcane bagasse, 20% cow dung (S2), horse manure (S3), chicken manure (S4), cottonseed hulls (S5), sugarcane ûlter cake (S6), and sugarcane trash (S7) and found that the cap diameter of S3 (13.33 cm) and S2 (12.33 cm) were signiûcantly larger and the S1, S5, and S7 were signiûcantly smaller. Chittaragi and Kumar (2021) used wheat straw and pearl millet straw as a substrate for cultivation of shiitake mushroom concluded that the wheat straw has highest number of fruiting bodies per bag (13.5/bag). Similarly, Hoa *et al.* (2015) found that in *Pleurotus ostreatus* (PO) and *Pleurotus cystidiosus* (PC) the variations in the substrates' total carbon and total nitrogen content, which explain the variation in the C:N ratio, have a significant impact on the growth of the mycelium and the formation and development of the fruiting bodies in mushrooms. According to Philippouss is *et al.* (2003), substrate mixes with reduced C/N encouraged early sporophore induction. In an experiment they found that wheat straw promote the quality and early fructification of mushrooms.

Effect of different agro waste substrates on the various yield parameters of *Lentinula edodes*

The data presented in Table 3 and Figure 2 depict the yield parameters of *L. edodes* on different non-conventional lignocelluloses wastes, the mean highest biological yield (127.07 g), economic yield (116.40 g), dry yield (13.13 g) and biological efficiency (25.41%) was observed on sawdust substrate but these values were statistically on par with wheat straw followed by rice straw. However, minimum biological yield (70.61 g), economic yield (62.12 g), dry yield (4.42 g) and biological efficiency (14.12%) were observed in mustard substrate. In case of leguminous substrates, the highest mean biological yield (87.25 g), economic yield (81.49 g), dry yield (7.25 g) and biological efficiency (17.32%) were recorded on soybean substrate followed by lentil substrate. Among oilseed substrates, the maximum biological yield (75.53g), economic yield (68.01g), dry yield (5.39g) and biological efficiency (15.11%) were observed on

Table 3.Yield parameters of *Lentinula edodes* on agro-waste substrates

Substrates	Biological yield (g/kg)	Economic yield (g/kg)	Dry yield (g/kg)	Biological efficiency (%)
Wheat	126.80	115.42	14.78	25.36
Rice	118.81	109.40	11.08	23.76
Corn	85.29	78.26	6.91	16.99
Soybean	87.25	81.49	7.25	17.32
Lentil	85.00	75.31	7.01	16.87
Linseed	75.53	68.01	5.39	15.11
Mustard	70.61	62.41	4.42	14.12
Control	127.07	116.40	13.13	25.41
CD	1.21	3.75	0.45	0.24

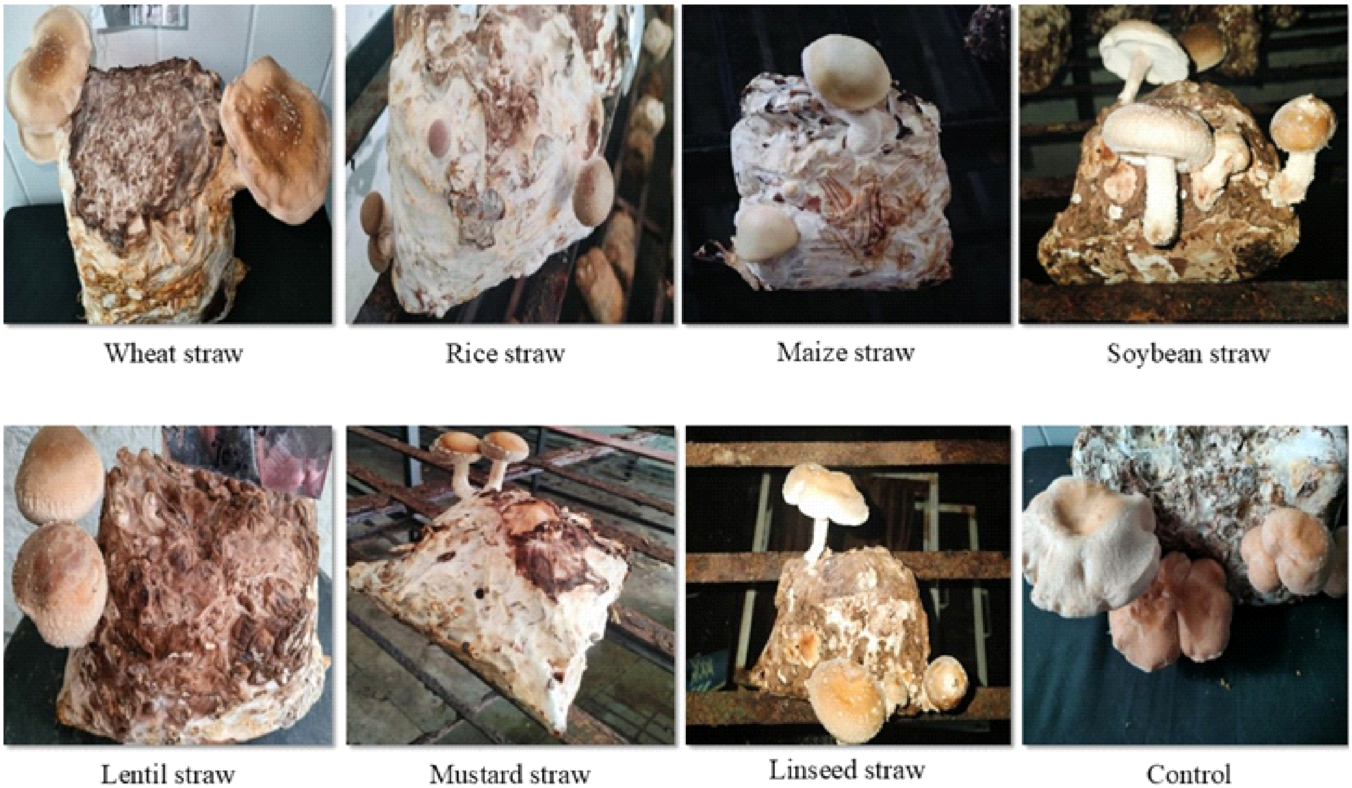

Fig. 2 Biological yield of *Lentinulaedodes* among cereal, legume and oilseed substrates

linseed followed by linseed substrate.

The results are in conformity with the findings of Puri *et al.* (2011) who also reported that the highest yield of 80.4g/500g of dry substrate in the Ll strain and the corresponding highest biological efficiency of 45.9 per cent were recorded for wheat straw substrate, while the highest yield of 61.1g/500g of dry substrate and the corresponding highest biological efficiency of 40.7 per cent on wheat straw substrate for the L2 strain were compared with sawdust, which had biological efficiencies ranging from 3.8 to 24.2 per cent for all the sawdust substrates. The fact that the mushroom fungus frequently breaks down wheat straw indicates that the *L. edodes'* hydrolytic and oxidative enzymes broke down the cellulosic and hemi-cellulosic components of the straw's cell wall, aiding in the colonization process. Puri and Kumar (2012) who evaluated cultivation of *L. edodes* on various agro wastes *viz*., wheat straw, pulse waste, banana leaves cotton waste, sugarcane bagasse and corn stalk and found that the wheat straw show highest yield and biological efficiency and maximum crude fiber content and ash was found in fruit bodies grown on cotton waste. Similarly, Philippoussis *et al.* (2003) studied the impact of various lignocelluloses substrates on shiitake mushroom crop performance, including oak-wood sawdust (OS), wheat straw (WS), and maize cobs (CC) and discovered that wheat straw (WS) increased earliness and crop productivity (BE 54.17%) by presenting shorter cropping times and homogeneous yield distribution throughout flushes, while WS and CC produced the highest yield and heavier basidiomata during the first two flushes. Morais *et al.* (2000) used four strains of *Lentinusedodes* (Berk.) Sing. (L. e. 1-3, L. e. am, L. e. 16-3, and L. e. 6-6) and four lignocelluloses substrates (rice straw, Chestnut sawdust, barley and Pinus sawdust) packed in 500 and 1000g polypropylene bags and discovered that the highest biological efficiency was found in L. e. am strain (59.5%) with block size of 500 g and lowest was found in L.e. 16-3 strain (18.9%) with block size of 1000g.

Conclusion

The study concluded that non-conventional substrates (lignocellulosic substrate) straw of cereal, legume and oilseed can be used for the cultivation of Shiitake mushroom. These substrates colonized the straw at the faster pace as compared to conventional substrate (sawdust). The morphological and yield parameters were comparable to control. Non-conventional substrates can be a good alternate to conventional sawdust substrate as it is difficult to get broad-leaved trees sawdust easily and has a significant application as mulch in the paper industries. The fruit body characters like compactness, texture and quality were also similar and comparable to sawdust substrate.

Conflict of interest: Authors declare no competing interest.

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