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Evaluation of shiitake mushroom (*Lentinula edodes*) strains on different substrates

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Abstract
Shiitake is an important specialty mushroom grown throughout the world. It has a large brown colored umbrella shaped cap with white gills and stalks. It grows throughout the year on the decaying wood of broad leaved trees. It is popular because of its high nutritional and medicinal properties. It is known to have limited genetic diversity and therefore, a limited number of strains for exploitation at commercial level. The selection of a substrate which is cheap and abundantly available requires further studies. Therefore, four promising strains obtained from Directorate of Mushroom Research (DMR), Solan (H.P.) were evaluated at All India Coordinated Research Project (Mushroom) centre, Hisar (Haryana) for their growth characters on wheat straw and saw dust substrates in 2017-18.

Wheat straw and saw dust wet substrates were supplemented with wheat bran at 20% and $CaCO_3$ at 1% and filled in polypropylene bags, autoclaved, cooled, spawned, incubated and treated in chilled water for fruiting. The experiment was laid out in RBD design and four replications. The observations were taken on spawn run (days), first harvest (days), number of fruiting bodies per bag, yield (g) per bag, total yield (kg/100 kg dry substrate) and average fruit body weight (g).

In wheat substrate the duration of spawn run and first harvest was significantly as low as 53.8, 87.5 days, respectively in strain LE17-01 as compared to other strains. The number of fruiting bodies was significantly more at 16.2 in strain LE17-03. The average fruit body weight was significantly as high as 10.9 g in LE17-04 as compared to other strains. The total yield was significantly highest at 14.9 kg /100 kg dry wheat substrate in LE17-01 as compared to other strains. In saw dust substrate, the duration of spawn run was significantly as low as 61.5 days in LE17-01 whereas, duration of first harvest was significantly lowest at 97.0 days in strain LE17-03. The number of fruiting bodies at 17.4 was observed significantly more in strain LE17-04 as compared to other strains. The average fruit body weight was significantly as high as 10.7 g in strain LE17-01 as compared to other strains. The total yield was significantly highest at 17.5 kg /100 kg dry saw dust substrate in LE17-01 as compared to other strains.

Keywords: *Lentinula edodus*, mushroom, saw dust, shiitake, strain, substrate, wheat straw

Introduction
The global consumption of edible mushrooms is doubled in 15 years and has reached near 40 MT per year (Royse *et al*., 2017) [15]. Among these, shiitake mushroom is an important edible mushroom popular throughout the world for its nutritional and medicinal properties and occupies first place in production among the cultivated edible mushrooms (Royse and Sánchez 2017) [16]. It is mainly produced in China and Japan where China is the largest exporter and consumer. It is a large mushroom with brown colored umbrella shaped cap with white gills and stalks. It grows on logs of fallen trees under natural conditions but now it has become possible to cultivate under controlled conditions on various agro-wastes throughout the year.

It is a good source of nutrients and is being used as a medicinal tonic for over two thousand years. It has been found to contain 90% water, 7% carbohydrates, 2% protein, less than 1% fat, vitamins B and minerals. It has also been found to contain Vitamin D when exposed or dried under sunlight or artificial light (Ko *et al*., 2008; Cardwell *et al*., 2018) [16, 3]. It has many bioactive compounds, secondary metabolites useful in pharmaceutical and food industries (Jiang *et al*., 2010; Yildiz *et al*., 2015; Duran-Rivera *et al*., 2020) [11, 23, 8] and recognized as a functional food (Zhu *et al*., 2015) [24]. It has also been found to possess the bioactive molecules to promote immunity (Xiaoshuang *et al*., 2015) [21]. The nature of bioactive compounds, chemical composition, flavor, smell of mushroom fruits has been found to be varied based on substrates used for its cultivation (Smith *et al*., 2002; Gaitan-Hernandez *et al*., 2017; Gaitan-Hernandez 2020) [19, 10, 9]. It has anticancer, antiviral, immunomodulatory, antioxidant, antibacterial, antifungal, antidiabetic, anti-atherosclerotic characteristics (Kang *et al*., 2012; Rahman *et al*. 2018; Afiati *et al*., 2019) [12, 14, 1].

The worldwide production of its spent substrate is 12.5 MT per year (Wei *et al*., 2020) [20] and it may also be exploited for production of fermentable sugars through enzymatic saccharification (Xiong *et al*. 2019; Chen *et al*., 2020; Chen *et al*., 2021; Chen *et al*. 2022) [22, 4, 6, 5]. There are few reports on genetic diversity in this mushroom. Significant differences in biological efficiencies and mushroom size(s) were observed among twenty four genotypes of shiitake mushroom cultivated on saw dust substrate (Diehle and Royse 1986) [7]. The strain DMRO-388s recorded the highest bio-efficiency at 85.63% on saw dust and the strain DMRO-327 with 53.02% on wheat straw whereas, the mycelial colonization was rapid on saw dust, while the sporophore formation was found earlier on wheat substrate. They also observed that saw dust gave higher yield over the wheat straw but the fruiting was earliest on wheat straw (Annepu *et al.*, 2019) [2]. The highest biological efficiency at 145.11% was found in strain IE-256 on sorghum stubbles and the highest production rate at 1.69% in strain IE-245 whereas, the highest yield was observed at 41.96% in strain IE-256 on sorghum stubbles (Gaitan-Hernandez *et al*., 2020) [9]. However, it is known to have a low genetic diversity (Sharma *et al*., 2018; Duran-Rivera *et al*. 2020) [18, 8] and therefore, a selection of a promising strain within the available genetic resources and selection of a strain specific substrate for their cultivation is required to be studied.

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Materials and Methods

Study area: The present study was carried out during 2017-18 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 four strains LE17-01, LE17-02, LE17-03, LE17-04 during 2017-18 for their evaluation on wheat straw and saw dust substrates of broad leaved trees 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 25ºC 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 of broad leaved trees were soaked overnight separately in freshwater. Then these substrates were dried in shade to have moisture of 55%. Then the substrates were supplemented with wheat bran @ 20% and calcium carbonate ($CaCO_3$) at 1.0% 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 126º C for 2h. The autoclaved bags were allowed to cool and spawned asceptically at 5% on wet weight basis.

Cultivation technology

These bags were kept in incubation room on racks at 24 °C for spawn run. After complete spawn run, it was kept as such till the substrate turned brown. Then the substrates were removed from polypropylene bags and dipped the blocks in chilled water at 4-5 °C for about 10 minutes. After chilled water treatment blocks were kept at 18-20 °C in incubation room for fruiting. After fruit harvest, the substrate blocks were given a rest of 16 days and then chilled water treatment was repeated to get second fruit harvest.

Statistical design and replications

There were four replications per treatment or strain and 6 bags per replication. The experiment was randomized as per RBD design.

Observations recorded

The observations were taken on spawn run (days), time taken for first harvest (days), number of fruiting bodies, total yield per bag, yield (kg/100 kg dry substrate) 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

Growth characteristics of *Lentinula edodes* strains cultivated on wheat straw substrate

In wheat straw, the spawn run was significantly at the earliest in 53.8 days in strain LE17-01 followed by 58.5 days in LE17-02 and was significantly as much as 63.5 days in strain LE17-04. The first harvesting was done at 87.5 days in strain LE17-04 and it was significantly at minimum as compared to other strains, it was followed by 89.0 days in LE17-02 strain. The strain LE17-04 took as many as 92.5 days in first harvest and it differed significantly as compared to other strains. The number of fruiting bodies was significantly maximum at 16.2 per bag in strain LE17-03 followed by 15.2 in strain LE17-01 and significantly minimum at 13.0 in strain LE17-04. The average yield was significantly maximum at 148.8g per bag in strain LE17-01 followed by 141.3 g/bag in strain LE17-04 and significantly minimum at 126.3g per bag in strain LE17-02. The average fruit body weight was significantly maximum at 10.9 g in strain LE17-04 followed by 9.9 g in strain LE17-01 and significantly minimum at 8.0 g in strain LE17-03. The total yield was found to be highest at 14.9 kg/100kg dry substrate in LE17-01 strain followed by 14.1 kg/100 kg dry substrate in LE17-04 strain and however, both were statistical at par in yield. The yield was found to be lowest at 12.6 kg/100 kg dry substrate in LE17-02 strain (Table 1, Fig. 1).

Table 1: Growth characteristics of *Lentinula edodes* strains on wheat straw substrate

Strain	Spawn run (days)	Time taken for first harvest (days)	Number of fruiting bodies	Total yield/bag	Yield (kg/100 kg dry substrate)	Average fruit body weight (g)
LE17-01	53.8	87.5	15.2	148.8	14.9	09.9
LE17-02	58.5	89.0	14.3	126.3	12.6	08.9
LE17-03	60.5	92.5	16.2	127.5	12.8	08.0
LE17-04	63.5	91.8	13.0	141.3	14.1	10.9
CD at 5%	1.02	1.84	0.6	9.39	0.94	0.44

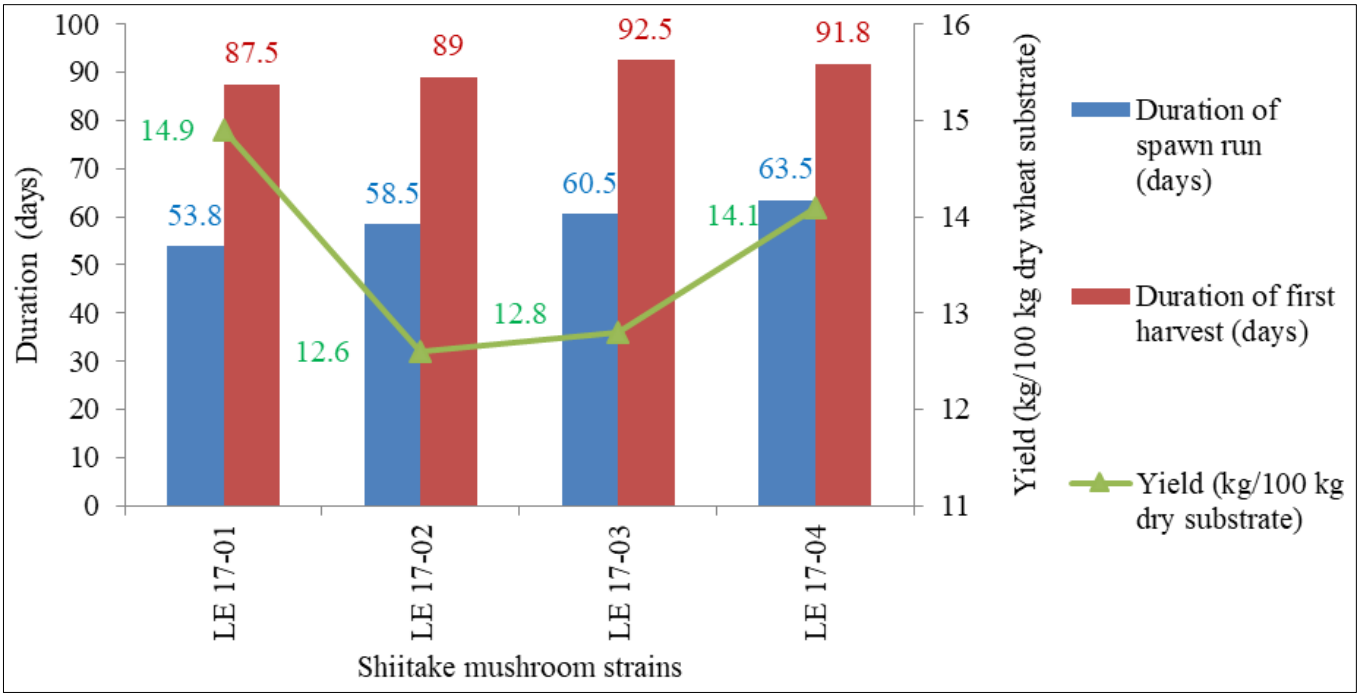

Fig 1: Growth characteristics of *Lentinula edodes* strains on wheat straw substrate

Growth characteristics of *Lentinula edodes* strains cultivated on saw dust substrate

In saw dust substrate, the spawn run was significantly as early as 61.5 days in strain LE17-01 followed by 61.8 days in LE17-03 and it was significantly as late as 65.8 days in strain LE17-04. The first harvesting was done at 97.0 days in strain LE17-03 and it was significantly at minimum as compared to other strains followed by 99.8 days in LE17-02 strain. The strain LE17-01 took significantly as many as 100.8 days in first harvesting and differed significantly as compared to other strains. The number of fruiting bodies were significantly maximum at 17.4 per bag in strain LE17-04 followed by 16.3 in strain LE17-01 and significantly minimum at 15.1 in strain LE17-03. The average yield was significantly maximum at 175 g per bag in strain LE17-01 followed by 172.5 g/bag in strain LE17-04 and significantly minimum at 155 g per bag in strain LE17-03. The average fruit body weight was significantly highest at 10.7 g in strain LE17-01 followed by 10.2 g in strain LE17-03 and significantly lowest at 9.7 g in strain LE17-02. The total yield was highest at 17.5 kg/100kg dry substrate in LE17-01 strain followed by 17.3 kg/100 kg dry substrate in LE17-04 strain and however, both were statistical at par in yield. The yield was found to be lowest at 15.5 kg/100 kg dry substrate in LE17-02 strain (Table 2, Fig. 2).

Table 2: Growth characteristics of *Lentinula edodes* strains on saw dust substrate

Strain	Spawn run (days)	Time taken for first harvest (days)	Number of fruiting bodies	Total yield/bag	Yield (kg/100 kg dry substrate)	Average fruit body weight (g)
LE17-01	61.5	100.8	16.3	175	17.5	10.7
LE17-02	63.3	99.8	15.9	155	15.5	09.7
LE17-03	61.8	97.0	15.1	155	15.5	10.2
LE17-04	65.8	99.0	17.4	172.5	17.3	09.9
CD at 5%	2.45	NS	0.45	9.46	0.95	0.36

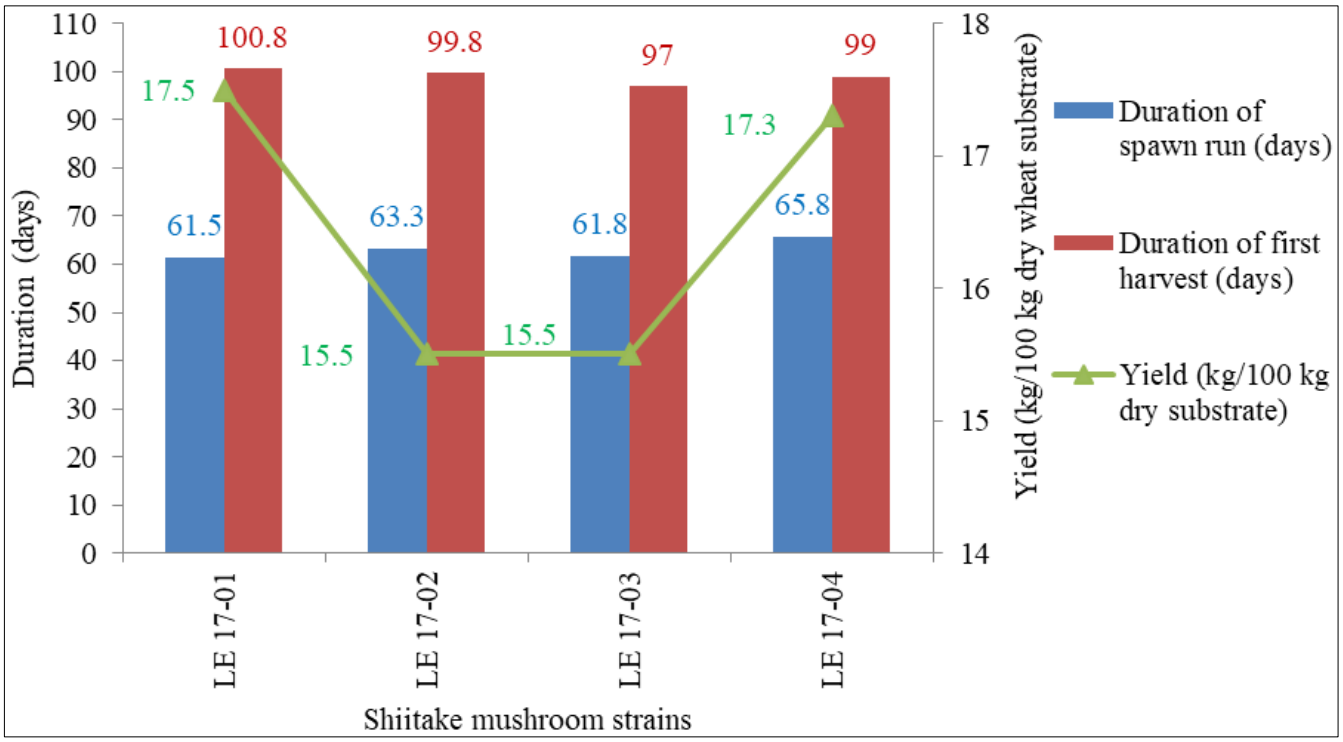

Fig 2: Growth characteristics of *Lentinula edodes* strains on saw dust substrate

Discussion

The strain LE17-01 had fastest spawn run in 53.8 days with highest yield at 14.9 kg/100 kg dry substrate whereas, strain LE17-02 gave lowest yield at 12.6 kg/100 kg dry substrate in wheat substrate. In saw dust substrate, strain LE17-01 had earliest spawn run in 61.5 days, highest fruit body weight at 10.7 g and maximum yield at 17.5 kg/100 kg dry substrate whereas, strain LE17-02 had lowest fruit body weight at 9.7 g and gave lowest yield at 15.5 kg/100 kg dry substrate in saw dust substrate. The duration of spawn run and first harvesting was found to be at minimum in wheat substrate in all four strains as compared to their respective durations in saw dust substrate, whereas, number of fruiting bodies, yield per bag (g), yield (kg/100 kg substrate) and fruit body weight (g) were at maximum in all four strains cultivated in saw dust substrate as compared to their respective character in wheat straw substrate. The strain HE 17-01 performed better as compared to other strains in comparing spawn run (days), yield per bag (g) and yield (kg/100 kg substrate) in both substrates. The strain HE 17-02 was poor in yield as compared to other strains in both substrates.

All the strains were found to be different in growth characteristics on both substrates. It may be due to the inherent genetic variability in strains. Among the substrates, saw dust substrate gave higher yield but wheat straw was found to be helpful in reducing time of spawn run and first harvesting. In a similar study, significant differences in biological efficiencies and mushroom size(s) were observed among twenty four genotypes of shiitake mushroom cultivated on saw dust substrate (Diehle and Royse 1986) [7]. The effect of different genotypes, substrates and their interactions on yield and yield attributing factors has also been found in a study. According to their study, strain DMRO-388s recorded the highest bio-efficiency at 85.63% on saw dust and the strain DMRO-327 with 53.02% on wheat straw. The mycelial colonization was rapid on saw dust, while the sporophore formation was found earlier on wheat substrate. They also observed that breakdown of phenolic compounds, lignin and hemicelluloses in wheat straw and saw dust varied in different strain. The saw dust substrate gave higher yield over the wheat straw but the fruiting was earliest on wheat straw (Annepu *et al.*, 2019) [2]. Therefore, there is possibility of variations in production of enzymes in different strains to degrade phenolic compounds, lignin, hemicelluloses etc. and hence, resulted in variations in growth characteristics. The saw dust was also found to be a better substrate as compared to wheat straw for all strains in terms of yield. There was no prevalence of relationship between incubation period and yield; spawn run time and fruit body weight in the present study but in an earlier study biological efficiency was found to be correlated with incubation period, and mushroom size with spawn runs days (Royse 1985) [17].

In an earlier study, the biological efficiency, production rate, fibre content, phenolic compound content had also been found to be affected by strain and substrate type. The highest biological efficiency and yield were noticed in strain IE-256 whereas the highest production rate was observed in strain IE-245 on sorghum stubble. Similarly, fiber and phenolic compound contents of the carpophores were also found to be affected by strain and substrate (Gaitan-Hernandez *et al.*, 2020) [9]. These studies also corroborated with our findings that growth characters like spawn run and first harvest time, number of fruiting bodies, yield and fruit body weight were found to be affected by strain and substrate type.

Conclusion

Among the four shiitake mushroom strains it is recommended that strain LE17-01 is better in terms of spawn run time of 61.5 days with highest fruit body weight at 10.7 g and maximum yield at 17.5 kg/100 kg dry substrate whereas strain LE17-02 had lowest fruit body weight at 9.7 g and minimum yield at 15.5 kg/100 kg dry substrate in saw dust substrate. The growth characters of mushroom were found to be influenced by strain and substrate type.

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References

1. Afiati F, Firza SF, Kusmiati, Aliya LS. The effectiveness β-glucan of Shiitake mushrooms and saccharomyces cerevisiae as antidiabetic and antioxidant in mice sprague dawley induced alloxan. International Conference on Biology and Applied Science (ICOBAS), AIP Conference Proceedings 2120, 070006-1–070006-8, Published by AIP Publishing. 2019, 978-0-7354-1860-8/$30.00.
2. Annepu SK, Sharma VP, Barh A, Kumar S, Shirur M, Kamal S. Effects of genotype and growing substrate on bio-efficiency of gourmet and medicinal mushroom (*Lentinula edodes* (Berk.) Pegler. Bangladesh Journal of Botany. 2018;48(1):129-138.
3. Cardwell G, Bornman JF, James AP, Black LJ. A review of mushrooms as a potential source of dietary vitamin D. Nutrients. 2018;10(10):1498.
4. Chen F, Martín C, Finell M, Xiong S. Enabling efficient bioconversion of birch biomass by Lentinula edodes: regulatory roles of nitrogen and bark additions on mushroom production and cellulose saccharification. Biomass Conversion and Biorefinery, 2020, https:// doi.org/10.1007/s13399-020-00794-y.
5. Chen F, Martín C, Lestander TA, Grimm A, Xiong S. Shiitake cultivation as biological preprocessing of lignocellulosic feedstocks – Substrate changes in crystallinity, syringyl/guaiacyl lignin and degradation-derived by-products. Bioresource Technology. 2022;344:126256.
6. Chen F, Xiong S, Gandla ML, Stagge S, Martín C. Spent mushroom substrates for ethanol production – Effect of chemical and structural factors on enzymatic saccharification and ethanolic fermentation of *Lentinula edodes*-pretreated hardwood. Bioresource Technology, 2021, https://doi.org/10.1016/j.biortech.2021.126381 (In press).
7. Diehle AD, Royse DJ. Shiitake cultivation on sawdust: evaluation of selected genotypes for biological efficiency and mushroom size. Mycologia. 1986;78(6):929-933.
8. Duran-Rivera B, Rojas-Rodas F, Silva-Lopez W, Gomez-Suarez C, Castro-Restrepo D. Molecular identification of Shiitake [*Lentinula edodes* Berk (Pegler)] and production of secondary metabolites with biotechnological potential. Bionatura. 2020;5(3):1183-88.
9. Gaitan-Hernandez R, Aquino-Bolanos EN, Maricela H, Salmones D. Yield and phenolic content of shiitake mushrooms cultivated on alternative substrates. Emirates Journal of Food and Agriculture. 2020;32(3):188-197.
10. Gaitan-Hernandez R, Barradas Zavaleta MA, Aquino-Bolanos EN. Productivity, physicochemical changes, and antioxidant activity of Shiitake culinary-medicinal mushroom *Lentinus edodes* (Agaricomycetes) cultivated on lignocellulosic residues. International Journal of Medicinal Mushrooms. 2017;19(11):1041-1052.
11. Jiang T, Jahangir MM, Jiang Z, Lu X. Influence of UV-C treatment on antioxidant capacity, antioxidant enzyme activity and texture of postharvest shiitake (*Lentinus edodes*) mushrooms during storage. Postharvest Biology and Technology. 2010;56(3):209-21.
12. Kang MY, Rico CW, Lee SC. *In vitro* antioxidative and antimutagenic activities of oak mushroom (*Lentinula edodes*) and king oyster mushroom (*Pleurotus eryngii*) by products. Food Science and Biotechnology. 2012;21:167-73.
13. Ko JA, Lee BH, Lee JS, Park HJ. Effect of UV-B exposure on the concentration of vitamin D2 in sliced shiitake mushroom (*Lentinus edodes*) and white button mushroom (*Agaricus bisporus*). Journal of Agricultural and Food Chemistry. 2008;50(10):3671-3674.
14. Rahman MA, Abdullah N, Aminudin, N. *Lentinula edodes* (shiitake mushroom): An assessment of *in vitro* anti-atherosclerotic bio-functionality. Saudi Journal of Biological Sciences. 2018;25:1515-1523.
15. Royse DJ, Baars JJP, Tan Q. Current Overview of Mushroom Production in the World. In: Edible and Medicinal Mushrooms: Technology and Applications. Edn 2, Wiley Blackwell, New York, 2017.
16. Royse DJ, Sanchez JE. Productión mundial de setas *Pleurotus* spp. con énfasis en países iberoamericanos. In: J. E. Sánchez and J. E. Royse (Eds.), La Biología, el Cultivo y las Propiedades Nutricionales y Medicinales de las Setas *Pleurotus* spp. ECOSUR, Tapachula, 2017, 17-25.
17. Royse DJ. Effect of spawn run time and substrate nutrition on yield and size of the Shiitake mushroom. Mycologia. 1985;77(5):756-762.
18. Sharma VP, Annepu SK, Barh A, Shirur M, Kamal S. Genetic divergence and cluster analysis in Shiitake genotypes based on yield related traits with commercial breeding significance to shorten the production period. International Journal of Vegetable Science. 2018, https://doi.org/10.1080/19315260.2018.1433264.
19. Smith JE, Rowan NJ, Sullivan R. Medicinal mushrooms: A rapidly developing area of biotechnology for cancer therapy and other bioactivities. Biotechnology Letters. 2002;24:1839-1845.
20. Wei M, Xiong S, Chen F, Geladi P, Eilertsen L, Myronycheva O, *et al*. Energy smart hot-air pasteurisation as effective as energy intense autoclaving for fungal preprocessing of lignocellulose feedstock for bioethanol fuel production. Renewable Energy. 2020;155:237-247.
21. Xiaoshuang D, Stanilka JM, Rowe CA, Esteves EA, Nieves C, Spaiser SJ, *et al.* Consuming *Lentinula edodes* (Shiitake) mushrooms daily improves human immunity: A randomized dietary intervention in healthy young adults. Journal of the American College of Nutrition. 2015;34(6):478-487.
22. Xiong SJ, Martin C, Eilertsen L, Wei MG, Myronycheva O, Larsson SH, Lestander TA, *et al*. Energy-efficient substrate pasteurisation for combined production of shiitake mushroom (*Lentinula edodes*) and bioethanol. Bioresource Technology. 2019;274:65-72.
23. Yildiz O, Can Z, Laghari A, Sahin H, Malkoc M. Wild edible mushrooms as natural source of phenolics and antioxidants. Journal of Food Biochemistry. 2015;3:148-154.
24. Zhu F, Du B, Bian Z, Xu B. β-Glucans from edible and medicinal mushrooms: Characteristics physicochemical and biological activities. Journal of Food Composition and Analysis. 2015;41:165-173.