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Preliminary Studies on the Screening of Substrates for Spawn Production and Cultivation of Indigenous Strain of *Lentinus sajor-caju* (Fr.) Fr.

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ABSTRACT

***Lentinus sajor-caju* (Fr.) Fr. is a lignicolous edible basidiomycetous mushroom. The sporophores of this mushroom are valuable health food with high nutritional and nutraceutical properties. The work presented in this manuscript pertains to the screening of substrate for spawn production and cultivation of indigenous strain of *L. sajor-caju* collected from North West India. In this paper, the vegetative growth of *L. sajor-caju* on seven different substrates and reproductive growth on six different ligno-cellulosic substrates have been evaluated. As in other mushrooms, wheat grains supported the maximum mycelia growth amongst the evaluated substrates. *L. sajor-caju,* when grown on six different ligno-cellulosic substrates, gave maximum biological efficiency (56.06%) on paddy straw substrate on fresh weight basis which was far better in comparison to 44.55% biological efficiency obtained on wheat straw substrate.**

Keywords: Biological efficiency, *Lentinus sajor-caju*, Ligno-cellulosic substrates, Primordia, Spawn, Sporophore

INTRODUCTION

Lentinus sajor-caju is a white wood-rotting basidiomycetous macrofungus which belongs to Family *Polyporaceae* (Class *Agaricomycetes*, Order *Polyporales*). Naturally growing sporophore of this mushroom is large sized having infundibuliform pileus with inrolled margin and annulate stipe. Young sporophores are fleshy and whitish in colour which on maturity become rigid, hard and yellowish brown. Occurrence of this mushroom is reported to extend from Equatorial and Southern Africa to South East Asia and North West Australia (Pegler, 1983).

It is an edible mushroom with acceptable culinary credentials when young and fresh. Number of investigators such as Joly and Perreau (1977) reported it from Vietnam, Corner (1981) from Malaysia, Chin (1981) from Thailand, De Leon *et al*. (2012) from Philippines, Verma *et al*. (1995) from North Eastern Hills regions (India) and Singdevsachan *et al*. (2013) from Odisha (India) have reported its edibility from different parts of the world. It is known by various vernacular names like, "Kuwat-kawayan" and "Ulat" in Philippines, "Kulat-nyelutong" in Thailand and "Vaikol-kaalan" in Tamil Nadu, India. Number of investigators including Gulati *et al*. (2011), Singdevsachan *et al*. (2013), Sharma and Atri (2014), Acharya *et al*. (2017) evaluated various nutritional components like carbohydrates, proteins, dietary fibres, minerals, and vitamins from this mushroom. Sporophores of *L. sajor-caju* contain all essential and non-essential amino acids in substantial amount required for human being (Sharma *et al*., 2012; Afiukwa *et al*., 2015; Lata and Atri, 2017). This mushroom has been reported to serve as a promising source of considerable antioxidant ingredients due to which it can be of utility in pharmaceutical and cosmetic industries (Singdevsachan *et al*., 2013; Sharma and Atri, 2014; Dulay *et al*., 2015; Abdullah *et al*., 2016). *L. sajor-caju* is healthy food source which can be a good option to meet the hunger and malnutrition requirements in the society.

In view of its consumption as food and being a rich source of nutritional, nutraceutical constituents, and availability of meagre work on its domestication, it was decided to domesticate the indigenous strain of *L. sajor-caju* using local ligno-cellulosic substrates.

MATERIAL AND METHODS

The culture used during present investigation is a lab isolate of local strain of *L. sajor-caju* collected from dead bark of *Bauhinia variegata* from Renuka lake, Nahan (Sirmour) in Himachal Pradesh, India during the field survey. The pure culture was raised through tissue culture using the flesh of the sporophore from the point of confluence of stipe with the pileus (Yadav, 2005). It was deposited in MTCC housed at Institute of Microbial Technology, Chandigarh under number MTCC10945. The culture was maintained by subsequent sub-culturing on Malt Extract Agar (MEA) solid medium at 28±1℃. To evaluate different natural substrates for spawn preparation

and sporophore production, the standard methodology given by Quimio *et al*. (1990) was followed.

Substrate evaluation for spawn preparation

As many as seven substrates, namely wheat grains, maize grains, mustard seeds, bajra grains, jowar grains, rice bran, and saw dust were screened for spawn production. All selected substrates were cleaned and washed with tap water to remove dirt, debris, and broken grains. Cleaned substrates were then boiled separately in double distilled water for about 30 minutes. Boiled substrates were sieved and ruptured grains discarded. For adjusting the pH, boiled substrates were mixed with 2% calcium sulphate. Subsequently, 4% calcium carbonate was thoroughly mixed in boiled substrates to prevent stickiness. Each substrate was then filled in test tubes in triplicate and sterilized in an autoclave for 30 minutes at 15 lbs pressure (121 ℃). After autoclaving, all test tubes containing substrates were allowed to cool to ambient temperature. These test tubes were then aseptically inoculated with 2 mycelia disks each bearing 1.5 mg of mycelia load and then incubated at 28±1 ℃. Test tubes were observed for linear mycelia growth using centimetre scale, density and colonization visually on daily basis until full ramification of mycelium took place. Best evaluated substrate was selected for preparation of the mother spawn and subsequent use for inoculation of the substrate.

Evaluation of different ligno-cellulosic substrates for cultivation

For this purpose, locally available ligno-cellulosic natural substrates, namely paddy straw, wheat straw, wooden flakes, sawdust, 1:1 mixture of paddy and wheat straw, and 1:1:1:1 mixture of paddy straw, wheat straw, wooden flakes, and sawdust were used. Procedural steps starting from substrate preparation, substrate spawning, incubation and chilling treatment were followed for cultivation of *L. sajor-caju*.

Substrate preparation

For this purpose, 500 g of each ligno-cellulosic substrate on dry weight basis per bag was used. Chopped substrates were soaked in water for 1-2 days and after every 4-5 hours, water was changed. For disinfection, 15-20 mL of formaldehyde was added to 10 L water used for soaking the substrates. When fully soaked, excess water was decanted off and soaked substrates were given 2-3 washing with fresh water so as to remove the traces of formaldehyde. Subsequently, soaked substrates in triplicate were filled in polypropylene bags and sterilized in autoclave at 121 ℃ (15 lbs pressure) for 1 hour.

Substrate spawning

Autoclaved substrate bags were allowed to cool to ambient temperature. Spawning of substrates was done with mother spawn prepared on wheat grains at the rate of 4% and spawning, substrate bags were uniformly punctured all around for gaseous exchange.

Incubation and chilling treatment

Inoculated bags were incubated at 28±1 ℃ for colonization of the mycelium in the substrate. Polypropylene coverings were removed from the substrate bags when fully colonized. Subsequently, each colonized substrate cylinder was immersed in the ice-cold water for 2-5 minutes for giving chilling (shock) treatment. After shock treatment, fully colonized substrates were transferred to the well-ventilated cropping room in which the temperature was maintained at 28±1 ℃ and relative humidity between 80%-90%. Humidifier was used to maintain humidity.

Harvesting and determination of biological efficiency

Time taken for the emergence of primordia on different substrates was noted. Once a flush of sporophores was harvested, the macroscopic observations and fresh and dry weight of the sporophores were recorded. Calculation of the biological efficiency was done on fresh and dry weight basis. Freshly harvested sporophores were dried till a constant dry weight was obtained at 45 ℃ in the hot air oven. For calculating the biological efficiency on fresh weight basis, dry weight basis and moisture content following formulas were used.

On fresh weight basis Biological efficiency =

$$\frac{\text{Fresh weight of mushrooms}}{\text{Dry weight of substrate}} \times 100$$

On dry weight basis

$$\text{Biological efficiency} = \frac{\text{Dry weight of mushrooms}}{\text{Dry weight of substrate}} \times 100$$

Moisture (%) =

$$\frac{\text{Fresh weight of mushrooms} - \text{Dry weight of mushrooms}}{\text{Fresh weight of mushrooms}} \times 100$$

RESULTS

Evaluation of substrate for spawn production

Out of six substrates used for evaluation, best mycelia ramification was observed on wheat grains (9.90 cm) with 1.29 cm growth on an average daily basis after 8 days of incubation. Mycelium was quite dense and thick and ramification was uniform (**Table 1, Figures 1-7**). In comparison, the mycelia ramification and growth on an average daily basis was not to the extent on maize grains, mustard seeds, bajra grains, jowar grains, rice bran, and saw dust. Amongst all the grains used for evaluation, the growth and ramification of mycelia was found minimum on rice bran (7.63 cm) and sawdust (5.30 cm). Hence, on the basis of results arrived at, out of all the six substrates, wheat grains are recommended as the best substrate for raising mother spawn of *L. sajor-caju*. Mother spawn takes some time to mature and becomes ready for use after 15-20 days of incubation (**Figure 8**).

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Table 1: Linear mycelia growth of *L. sajor-caju* on different substrates used for spawn production

Substrates	Linear Mycelial Growth (cm) ± S.D.	Growth Rate (cm/day)	Number of Days for Full Colonization	Mycelial Characteristics
Wheat Grains	9.90 ± 0	1. 29	8	Very thick and dense mycelial colonization
Maize Grains	9.26 ± 0.42	1.18	9	Thin mycelia colonization
Mustard Seeds	9.00 ± 0.35	1.18	9	Thick and dense mycelia colonization
Bajra Grains	8.93 ± 0.49	1.17	10	Thick dense mycelia colonization
Jowar Grains	7.80 ± 0.75	0.94	9	Thick dense mycelia colonization
Rice Bran	7.63 ± 0.30	0.93	11	Very thin and fine mycelia colonization
Sawdust	5.30 ± 0.36	0.78	Growth stopped after 4 days	Very thin and fine mycelia colonization

Evaluation of ligno-cellulosic substrates for cultivation

Locally available four ligno-cellulosic natural substrates and their mixture (paddy straw, wheat straw, wooden flakes, sawdust, 1:1 mixture of paddy straw and wheat straw and 1:1:1:1 mixture of paddy straw, wheat straw, wooden flakes and sawdust) were used for evaluation, the results of which are discussed in the ongoing account.

Colonization of substrates

Each substrate inoculated with spawn took its own time for complete colonization. Paddy straw substrate was observed with fastest mycelia growth, followed by wheat straw, 1:1:1:1 mixture of paddy straw + wheat straw + wooden flakes + sawdust and 1:1 mixture of paddy straw + wheat straw. Paddy straw substrate was completely ramified with mycelium after 12 days of inoculation (**Figure 9**). While bags of wheat straw substrate took 15 days (**Figure 10**), mixture of four substrates took 23 days (**Figure 11**), mixture of paddy straw and wheat straw took 30 days (**Figure 12**) for complete colonization. In sawdust and wooden flakes alone, the colonization was poor and the mycelium stopped growing after 15 days of inoculation (**Figure 13, 14**).

Sporophore production on different substrates

On paddy straw substrate (**Figures 15 - 17**), fruit body primordia emerged in bunches after the incubation of 29 days and their number varied from 140-240 per 1.5 Kg of substrate on dry weight basis in three bags of 0.5 Kg each. Some primordia got matured into full-fledged fruit bodies within 1-2 days of appearance while others were abortive. An average of 280.31 g/0.5 kg fresh weight of mushrooms were harvested from paddy straw substrate.

On wheat straw substrate (**Figure 18 - 20**), sporophore primordia appeared after 28 days of incubation. In all, 50-165 primordia emerged on the three colonized substrate bags of 0.5 Kg each on dry weight basis. On the same pattern as in case of paddy straw substrate, some primordia matured into sporophores within 1-2 days of appearance while others aborted. From wheat straw substrate, an average of 222.75 g/0.5 kg of fresh mushrooms were harvested..

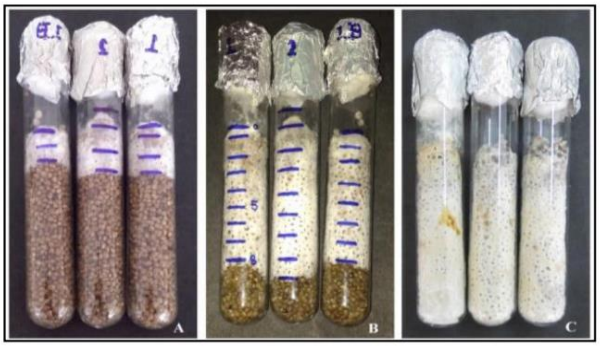

Figure 1. Linear growth on 3rd day (A), 8th day (B) and 9th day (C) on bajra grains

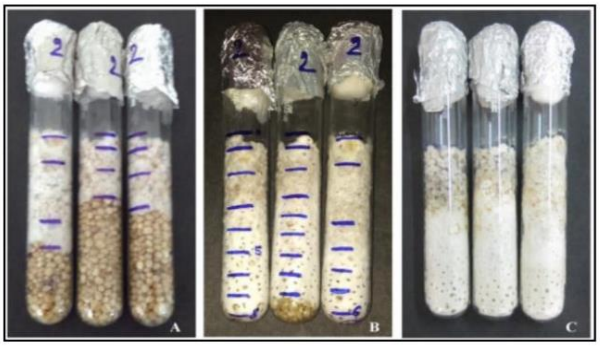

Figure 2. Linear growth on 3rd day (A), 8th day (B) and 9th day (C) on jowar grains

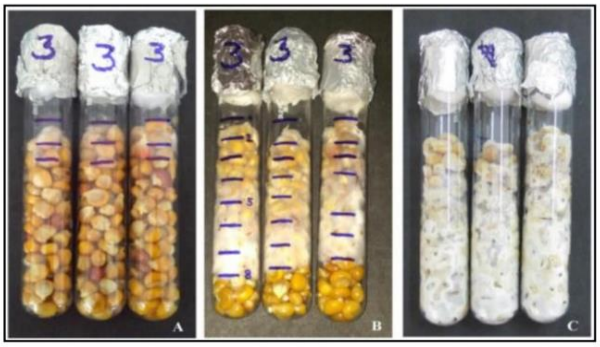

Figure 3. Linear growth on 3rd day (A), 8th day (B) and 9th day (C) on maize grains

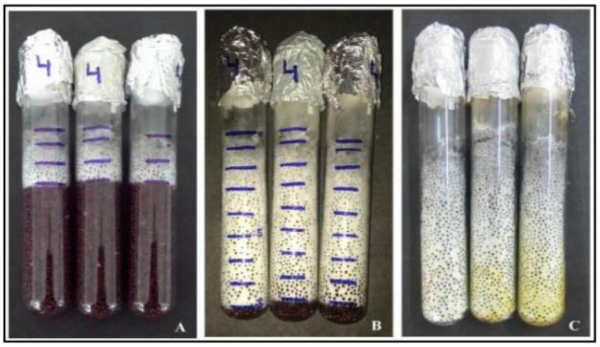

Figure 4. Linear growth on 3rd day (A), 8th day (B) and 9th day (C) on mustard grains

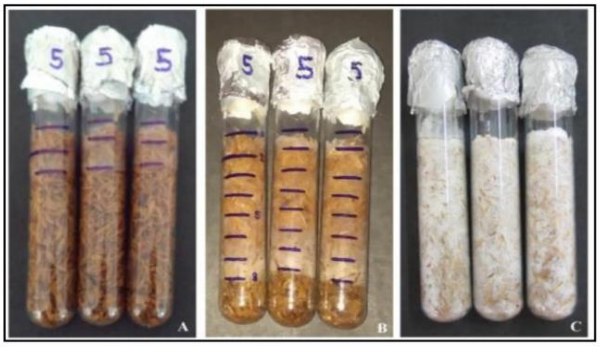

Figure 5. Linear growth on 3rd day (A), 8th day (B) and 9th day (C) on rice bran

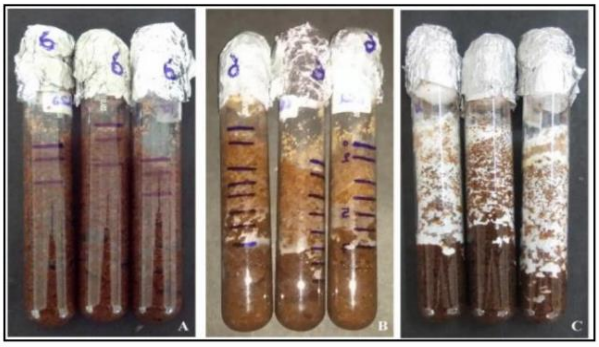

Figure 6. Linear growth on 3rd day (A), 8th day (B) and 9th day (C) on saw dust

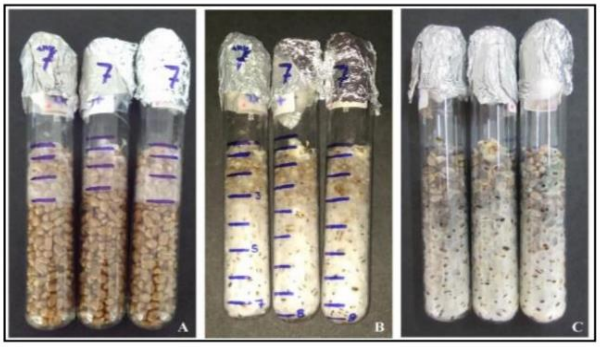

Figure 7. Linear growth on 3rd day (A), 8th day (B) and 9th day (C) on wheat grains

Figure 8. Mother spawn on wheat grains after 15 days

On the mixture of different substrates (paddy straw + wheat straw + wooden flakes + sawdust – 1:1:1:1**)** after 30 days of incubation 60-70 primordia appeared in small bunches (**Figure 21 - 23**), some of which within 1-2 days of appearance matured into full-fledged fruit bodies while others were abortive. On this substrate, on an average 94.65 g/0.5 kg of fresh weight of mushrooms were harvested.

In comparison on wooden flakes, sawdust alone and the substrate mixture containing only two substrates (wheat straw and paddy straw in 1:1 ratio) no fruiting could be obtained in any of the three replicates of each of these despite incubation for 2 months after spawning. This was because in these the mycelia colonization in the substrates was poor and the mycelia growth almost stopped after 15 days of incubation.

Sporophore morphology

The harvested sporophores (**Figures 24, 25**) of *L. sajor-caju* were soft and creamy in colour when young and turned yellowish and coriaceous in consistency with maturity. The size for carpophores varied from 2.0 cm to 12.0 cm in height. Pileus of mushroom was infundibuliform with inrolled margin and its size varied from 3.5-14.0 cm in diameter. The lamellae were linear and crowded on the under surface of the pileus and the stipe was annulate and its size varied between 0.2-6.0 × 0.1-3.0 cm.

Biological efficiency

The biological efficiency and other observations during cultivation of *L. sajor-caju* are summarized in table (**Table 2**). It was observed that on fresh weight basis, *L. sajor-caju* gave maximum yield in the paddy straw substrate followed by wheat straw substrate and 1:1:1:1 mixture of paddy straw, wheat straw, wooden flakes, and sawdust. The mushroom gave 56.06% biological efficiency on fresh weight basis on the paddy straw substrate. By taking into consideration the average dry weight of harvested fruit bodies, the biological efficiency of paddy straw substrate was evaluated at 10.15%. Net moisture percentage has been calculated at 81.89% in the freshly harvested carpophores on paddy straw substrate. On an average, 222.75 g fresh weight of the sporophores were harvested from dry wheat straw substrate giving 44.55% biological efficiency. By taking dry weight (47.30 g) of the harvested mushrooms, 9.46% biological efficiency was obtained. In fresh carpophores, 78.76% net moisture has been calculated. In comparison, only 18.93% biological efficiency was observed in mixture substrate of paddy straw, wheat straw, wooden flakes, and sawdust on the fresh weight basis (94.65 g) while only 2.21% biological efficiency was obtained by taking into account dry weight of the harvested mushrooms with 88.33% net moisture in the carpophores on fresh weight basis.

Table 2: Evaluation of ligno-cellulosic substrates for cultivation of *L. sajor-caju*

Substrate	Net Dry Weight of Substrate/Bag (g)	Number of Primordia	Fresh Weight of Mushrooms/Bag (g)			Average Fresh Weight of Mushrooms (g)	Dry Weight of Mushrooms/Bag (g)			Average Dry Weight of Mushrooms (g)	Net Moisture Content (%)	Biological Efficiency on Fresh Weight Basis (%)	Biological Efficiency on Dry Weight Basis (%)
			Bag 1	Bag 2	Bag 3		Bag 1	Bag 2	Bag 3				
Paddy straw	500	140-240	360.64	230.20	250.10	280.31	60.50	41.00	50.80	50.77	81.89	56.06	10.15
Wheat straw	500	50-165	102.24	241.00	325.00	222.75	36.91	46.00	59.00	47.30	78.76	44.55	9.46
Mixture (1:1:1:1) - Paddy straw + Wheat straw +Wooden flakes + Sawdust	500	60-70	55.00	134.12	94.82	94.65	6.50	14.93	11.72	11.05	88.33	18.93	2.21

Figure 9. Colonized paddy straw substrate

Figure 10. Colonized wheat straw substrate

Figure 11. Colonized mixed substrate - paddy straw + wheat straw + sawdust + wooden flakes (1:1:1:1)

Figure 12. Colonized mixed substrate – paddy straw + wheat straw (1:1)

Figure 13. Colonized sawdust substrate

Figure 14. Colonized wooden flakes substrate

Figure 15. Sporophores on paddy straw substrate bag 1

Figure 16. Sporophores on paddy straw substrate bag 2

Figure 17. Sporophores on paddy straw substrate bag 3

Figure 18. Sporophores on wheat straw substrate bag 1

Figure 19. Sporophores on wheat straw substrate bag 2

Figure 20. Sporophores on wheat straw substrate bag 3

Figure 21. Sporophores on mixed substrate (1:1:1:1) bag 1

Figure 22. Sporophores on mixed substrate (1:1:1:1) bag 2

Figure 23. Sporophores on mixed substrate (1:1:1:1) bag 3

Figure 24. Primordia and young sporophores (with evanescent annulus)

Figure 25. Fully matured sporophores (with evanescent annulus)

DISCUSSION

As for the production of mushroom spawn is concerned from amongst the different substrates used for evaluation including bajra grains, jowar grains, maize grains, mustard seeds, wheat grains, rice bran and sawdust; wheat grains turned out to be the best substrate which supported maximum vegetative growth for producing spawn of *Lentinus sajor-caju*. Similar conclusions were drawn by number of investigators including Singh (2010), Atri *et al*. (2011), Atri and Lata (2013), Das *et al*. (2015), Kupradi *et al*. (2017) and Shahtahmasebi *et al*. (2017) while evaluating different substrates for the production of spawn in case of *L. squarrosulus*, *L. connatus*, *L. cladopus* and *L. tigrinus*. Hussein *et al*. (2016) from Tanzania reported sorghum grains as the other suitable substrate for production of spawn of *L. sajor-caju*. In yet other report, Dulay *et al*. (2021) and Miriyagalla *et al*. (2022) reported rice seeds and corn to be quite suitable for the production of spawn in case of *L. sajor-caju*, *L. squarrosulus* and *L. swartzii*. Also, sorghum grains for spawn production of *L. squarrosulus* (De Leon *et al*., 2017; Mwinyi *et al*., 2022) and rice grains and rice husk for raising spawn of *L. tigrinus* (Dulay *et al*., 2012) are reported to be quite good. From this, it becomes clear that different substrates are being preferred by the investigators in different part of the world. Substrate offering larger surface area, easy availability and its cost are important factors, which needs to be considered while selecting the substrate for evaluation. For production of spawn of *L. sajor-caju*, bajra and jowar grains are equally good. As compared on wheat grains less days were taken by the mushroom mycelium for colonization, better mycelia density around the colonized grains was there besides its easy availability and low cost resulted in the selection of wheat grains for production of spawn.

Locally available ligno-cellulosic natural substrates, namely paddy straw, wheat straw, wooden flakes, sawdust, 1:1 mixture of paddy straw and wheat straw and 1:1:1:1 mixture of paddy straw, wheat straw, wooden flakes and sawdust were screened for the cultivation of *L. sajor-caju*. Mushroom gave maximum biological efficiency (56.06%) on paddy straw substrate on fresh weight basis in comparison to wheat straw substrate (44.55%) and other substrates used.

There are number of species of *Lentinus* which have been cultivated using ligno-cellulosic substrates by different investigators. Some such examples include *L. squarrosulus* (De Leon *et al*., 2013; Das *et al*., 2015; Kupradi *et al*., 2017; Atri *et al*., 2018; Kalaw *et al*. 2021; Miriyagalla *et al*. 2022), *L. connatus* (Atri *et al*., 2011), *L. cladopus* (Atri and Lata, 2013), *L. sajor-caju* (Hussein *et al*., 2016; Kalaw *et al*., 2021; Miriyagalla *et al*., 2022), *L. swartzii* (Dulay *et al*., 2021; Kalaw *et al*., 2021), *L. strigosus* (Kalaw *et al*., 2021) and *L. tigrinus* (Dulay *et al*., 2012; Shahtahmasebi *et al*., 2017). Although, there is lot of work on the cultivation of *L. squarrosulus* in which number of substrates including paddy straw, wheat straw, rice husk, wheat bran, sawdust, wooden logs, cassava bagasse, etc. have been used extensively (De Leon *et al*., 2013, 2017; Kupradi *et al*., 2017; Atri *et al*., 2018; Kalaw *et al*., 2021; Miriyagalla *et al*., 2022). In comparison, very few references of cultivation studies are available on *L. sajor-caju.* Hussein *et al*. (2016) while working on *L. sajor-caju* in Tanzania using mixed substrates of dried banana leaves and wood shred (70% + 30%) reported 52 g/kg of substrate fresh mushrooms giving 18% biological efficiency. As compared, we could achieve much higher (56.06%) biological efficiency on paddy straw substrate, however on 1:1:1:1 mixture of paddy straw, wheat straw, wooden flakes and sawdust the biological efficiency obtained was almost comparable (18.93%) to that achieved by Hussein *et al*. (2016) while cultivating *L. sajor-caju*. Dulay *et al*. (2021) while working with *L. swartzii* found the highest weight of fruiting bodies (37.02 g) and biological efficiency (7.40%) on 7:3 ratio of rice straw and sawdust substrate. Kalaw *et al*. (2021) cultivated *L. sajor-caju*, *L. squarrosulus*, *L. strigosus* and *L. swartzii* and achieved 10.2% biological efficiency in case of *L. sajor-caju*, 7.3% in case of *L. squarrosulus*, 10.5% in case of *L. strigosus* and 6.9% in case of *L. swartzii.* This is substantially on the lower side in comparison to 56.06% biological efficiency achieved when *L. sajor- caju* was cultivated on paddy straw and 44.55% biological efficiency on wheat straw substrate during the present investigation. From Sri Lanka, Miriyagalla *et al*. (2022) could get very small amount (13.98 g) of *L. sajor-caju* sporophores when grown on mango saw dust after incubating the inoculated substrate for as many as 88 days which is much less in comparison to 280.31 g mushroom yield obtained when cultivated using rice straw and 222.75 g fresh weight of mushrooms obtained on wheat straw that too after

incubation of 30-31 days only during the present investigations. Probably this difference in yield and fruiting time seems to be because of the colonization efficiency of the mushroom mycelium of local strain of *L. sajor-caju* and the substrate used for cultivation.

CONCLUSION

The results of the study clearly indicate wheat grains to be the potential substrate for spawn production and paddy straw substrate as the best substrate for the cultivation of *L. sajor-caju.* The results achieved during the present investigation are without use of any supplement and without much improvisation of the conditions for cultivation. By adding supplements and improvising the conditions for cultivation better biological efficiency of the mushroom can be achieved.

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