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Lentinus squarrosulus (mont.): successful domestication and regional adaptability in Orlu, Imo State, eastern Nigeria

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Abstract

Lentinus squarrosulus is a wild edible mushroom utilized globally not only for its nutritional values but also for its medicinal and mycoremediation potentials. Domestication of this mushroom will make available mother culture and spawn for research and propagation, and ensure year-round availability for economic and sustainable development. Tissue culture was prepared and actively growing mycelia were inoculated onto grain spawn. Cultivation trials were conducted using sawdust from various wood species, including *Treculia africana* (African breadfruit), *Mangifera indica* (Mango), *Dacryodes edulis* (African pear), and mixed sawdust from various wood species. Substrates were composted, sterilized, inoculated with the spawn of *L. squarrosulus* and incubated. Growth was monitored, recorded and fruiting bodies harvested. Results of domestication revealed that mother culture was produced in 5 - 7 days spawned in 14 days and was available for research, and cultivation. *L. squarrosulus* mycelia colonized all the substrates used to varied degrees with the mycelial running time ranging from 30.4 days to 34.8 days. Mycelia running time on *T. africana* differed significantly from *D. edulis.* Fruiting bodies were successfully harvested from 38 to 68 days with the maximum number of fruiting bodies (40 ± 9.47) and highest yield of 89.03 ± 29.41 g obtained from *T. africana* in three flushes. This was followed by *M. indica* (35, 54.27 ± 14.64 g). *Dacryodes edulis* sawdust recorded the lowest yield (23, 32.31 ± 11.34 g). *M. indica* sawdust had the broadest pileus diameter (6.45 ± 1.97 cm) and longest stipe (2.83 ± 0.49 cm). In conclusion, *L. squarrosulus* has the potential to be domesticated in Orlu, Imo State, with *T. africana* sawdust as a suitable substrate for cultivation.

Keywords – Cultivation – Edible – mushroom – Sawdust – Tissue culture – Yield

Introduction

Mushroom production in Africa is very low and contributes little or nothing to the world's total yearly production (Onyango et al. 2011). *Agaricus* sp., *Pleurotus* sp., *Lentinula* sp., and *Ganoderma* sp. are among the cultivars of mushrooms that dominate the market. In Nigeria, research and production of mushrooms are still emerging and the species mostly studied are those of the genus *Pleurotus* (Nurudeen et al. 2014). Although lignocellulosic wastes on which mushrooms are cultivated abound, the major difficulty in growing mushrooms in Nigeria is that growers use exotic strains from imported mother culture which are expensive (Musieba et al. 2012). These imported mother cultures lack regional adaptability, produce low yields, and are vulnerable to pests and diseases, according to Otieno et al. (2015). Therefore, cultivation of *L. squarrosulus*,

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an indigenous wild edible mushroom that grows at elevated temperatures is a better option since it will grow faster than these exotic strains imported from temperate regions.

Inadequate identification, research and documentation have made the use of these mushrooms to be restricted. Because of this, the public is therefore ignorant of the importance of mushrooms. In Nigeria, the alluring potential of mushrooms is not fully harnessed to solve industrial and economic growth due to a lack of research (Okhuoya et al. 2010). Mushrooms are hand-picked from farms, forests, and decaying woods; some are picked at the start of the rainy season, and others at its end. As a result, they are seasonal and inaccessible all year round for mushroom enthusiasts. Currently, there is a threat of mushroom extinction because of the degradation of their natural habitats as a result of the growing human population and practices like climate change, bush burning, urbanization, deforestation, and firewood gathering (Okigbo & Nwatu 2015, Nteziryayo et al 2022). Hunting for mushrooms in the woods requires extra effort, putting mushroom hunters at risk of being attacked by wild creatures (Osarenkhoe et al. 2014). In addition, mushroom hunting increases the possibility that one may pick and consume poisonous species, which can result in anything from mild to severe gastrointestinal illnesses to fatalities. Nigeria produces a lot of lignocellulose wastes, which overwhelms the nation's disposal infrastructure and causes environmental problems (Okhuoya et al. 2010).

The necessity of domesticating *Lentinus squarrosulus* and cultivating them commercially is highlighted by the aforementioned problems. It is anticipated that commercial mushroom cultivation and domestication would provide a solution to these issues. A lot of nations have started cultivating mushrooms for commercial purposes. Researchers have focused their efforts on domesticating wild mushrooms to make them available all year round, prevent the consumption of dangerous species, and lessen the risk associated with mushroom hunting (Musieba et al. 2012). Mushroom cultivation is one of the most viable, affordable, cost-effective, and biotechnological approaches for lignocellulosic waste bioconversion (Hussien et al. 2016). The method can also reduce air pollution caused by lignocellulosic waste dumping and burning (Singh et al. 2020). Mushroom production is an effective method of converting agricultural waste into useful protein (Garcia-Delgado et al. 2015) and has enormous potential for income and jobs (Thakur 2020).

Lentinus squarrosulus is a tropical white rot fungus that is a member of the Polyporaceae family. This naturally occurring edible fungus grows on decaying tree trunk logs, old stumps, and exposed or buried tree roots (De Leon et al. 2013). They thrive on a wide range of substrates and inhabitants. Nigeria has documented cases of *Lentinus squarrosulus* cultivation on sawdust, leaves, forest tree bark, and palm press fiber (Adesina et al. 2011, Osibe & Chiejina 2015, Obodai et al. 2014). *Lentinus squarrosulus* also grows on chicken manure, rice straw, cassava peels, and andropogon straw among other substrates. It grows swiftly, has a limited life span, degrades quickly, and is easily shredded by rain (Roy & Krishnappa 2018). *Lentinus squarrosulus* has not been widely cultivated in Nigeria due to complications and difficulty in spawn preparation and hasn't been cultivated extensively to yield fruiting bodies, according to Omar et al. (2011).

Some researchers have, however, been successful in cultivating and domesticating them in East Africa (Hermawa 2021). *Lentinus sajor-caju* is the first species of *Lentinus* known to exist as domesticated strains; other species include *L. strigosus, L. tigrinus, L. squarrosulus*, and most recently, *L. swartzii* (Dulay et al. 2017). There has been no report of domestication of *Lentinus squarrosulus* in the eastern part of Nigeria especially in Imo state, the only reports available were on cultivation trials. Therefore, domestication of *L. squarrosulus* is necessary in eastern part of Nigeria. This study aimed to domesticate the wild edible mushroom *L. squarrosulus,* exploit its regional adaptability for economic and sustainable development, make available mother culture (germplasm) and spawn for research and propagation, and ensure year-round availability as an alternative protein source while utilizing the readily available saw dust posing nuisance in the environment in Orlu, Imo State.

Materials & Methods

Sample collection

Fresh *Lentinus squarrosulus* fruiting bodies were hand-picked from decaying logs of wood from various farms in Okporo, Amaifeke and Ihioma in Orlu Local Government Area, Imo State, wrapped in sterile Ziploc bags, and transported to the lab following Hussien et al. (2014). Their morphology was studied by a mycologist and was tentatively identified by comparing them to the available original taxonomic literature as stated in mycological treaties (Karunarathna et al. 2011).

Collection of substrates

Three different wood types: *Mangifera indica* (Mango), *Treculia africana* (African breadfruit), and *Dacryodes edulis* (African pear) were procured from the Orlu Timber Market, located on the New Orlu - Owerri Express Road in Orlu, Imo State and sawed into sawdust. Mixed sawdust (sawdust from various wood species) was also collected from the timber market. They were sun-dried individually for seven days to prevent deterioration.

Tissue culture

The tissue culture of *Lentinus squarrosulus* was isolated using the method described by Tibuhwa (2012) with slight modification. Fresh fruiting bodies of *L. squarrosulus* were cleaned with 70% ethanol on the outer surface to get rid of any surface contaminants and other microfungi. A tiny sample of inner tissue from the pileus/stipe was excised, inoculated onto malt extract agar and cultivated for seven days at 27 ± 2^{o}C. The pure culture was obtained by subculturing onto malt extract agar and storing it at 4^{o}C on a malt agar slant.

Spawn preparation and inoculation

To prepare spawn in sorghum grains, the method of Singh et al. (2017) was employed. Two kilograms of *Sorghum bicolor* grains were purchased, cleaned under running water, and soaked overnight to allow for optimal water absorption. The next day, the grains were drained, parboiled for 15 minutes, and drained until the dripping stopped. Ten percent rice bran was used as a nitrogen source, and 1% calcium carbonate ($CaCO_3$) was added to correct the pH. The mixture was poured into spawn bottles, sealed, and autoclaved at 121^{0}C (15 psi) for 30 minutes before cooling for 24 hours. *L. squarrosulus* culture was inoculated into each sterile spawn bottle so that the mycelia side of the plug was in direct contact with the spawn. The bottles were incubated at 27 ± 2^{o}C.

Bulk substrate preparation

Mature spawn was used for fruiting body production on sawdust of three different wood types and mixed sawdust. The method of Carrasco et al. (2018) was adopted with modifications to prepare bulk substrates. Ten percent rice bran was added to each of the substrates as an additional nitrogen source. The pH was adjusted to reduce greasiness by adding 2% calcium carbonate ($CaCO_3$). All of the components were properly mixed with the substrates and covered with a polythene sheet to prevent drying and left to compost.

Bagging and inoculation

After being composted, the substrates were packaged in transparent polypropylene bags that can withstand heat, each containing 1 kg of composted substrate in five replicates, tied, and pasteurized at 1000^{o}C for three hours. The substrates were cooled, and then mature spawn was introduced and left to incubate at room temperature.

Cropping and harvesting of fruiting Bodies

Once the substrates had been colonized by mycelia, the bags were taken to the cropping room, cut open at both ends, and irrigated to initiate fructification. The humidity was maintained twice daily by sprinkling the floor and the bags with clean water. The formation and growth of the

mushrooms were monitored every day. Observations and documentation of total mycelia running, pin head appearance and fruiting body development in various substrates were made. When they reached maturity, fruiting bodies were collected. Data was gathered on the size (pileus diameter, stipe lengths), weight and yield of fruiting bodies.

Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the data collected. With Statistical Analysis Software (SAS), version 9.2, mean significant differences ($p < 0.05$) between treatments were compared using the Least Significant Difference (LSD) test.

Results

Tissue culture and spawn production

Tissue culture production is the first step in the domestication of mushrooms. Tissue culture of *L. squarrosulus* was successfully obtained on malt extract agar (Fig. 7a) and preserved on slants at 4^{o}C. Mycelia of *L. squarrosulus* ramified the grain spawn successfully (Fig. 7b).

Growth and fruiting body production of *Lentinus squarrosulus* on different substrates

This is the second phase of the domestication trial. *Lentinus squarrosulus* was cultivated on the four different wood species in eight replicates, and the result of the best five replicates was selected. The results on the overall time taken for full colonization (mycelia running) of the substrates, pin head initiation/emergence, and maturity of fruiting bodies of *L. squarrosulus* cultivated on sawdust of four different wood species are illustrated below. This study showed that *L. squarrosulus* can be grown commercially on sawdust of different wood species. The four substrates used in the study supported the growth and fructifications of *L. squarrosulus* but in varied degrees according to substrate type.

Colonization time /mycelial running time of domesticated *L. sqaurrosulus* on the different substrates

Treculia africana (breadfruit) sawdust recorded the fastest/ shortest colonization time (30 ± 1.95 days) followed by *Mangifera indica* (mango) sawdust (33 ± 4.02 days) and mixed sawdust (sawdust from different wood species) (33 ± 3.56 days). *Dacryodes edulis* (African pear) recorded the slowest/longest colonization time (35 ± 2.17days).

Pinhead emergence/initiation of domesticated *L. squarrosulus* on different substrates

Significant variations were seen in the mycelia mass stimulation for pin head (primodia) appearance in the various substrates following mycelia running. The appearance of pin heads occurred in the same sequence as in the period of time it took for full ramification of substrates (colonization time/ mycelial running). It took relatively longest time in *Dacryodes edulis* sawdust (39 ± 2.17 days) in the first flush.

Maturation time of domesticated *Lentinus squarrosulus* on different substrates

Among the three wood species, *T. africana* is the ideal substrate for early maturation of *L. squarrosulus* (38 ± 1.64 days). This was followed by *M. indica* (39 ± 3.30 days). Fruiting bodies of *L. squarrosolus* harvested from *D. edulis* sawdust had the longest maturation duration (42 ± 1.79 days). Harvest was in three flushes.

Yield attributes of domesticated *Lentinus squarrosulus* on different substrates

The yield component results for *Lentinus squarrosulus* are shown below. The weight of the fruiting bodies, size, and quantity are all considered its yield. For yield attributes of *L. squarrosulus*, *T. africana* sawdust yielded the highest number of mature fruiting bodies (40 ± 9.47), followed by *M. indica* (35 ± 8.55), then *D. edulis* (23 ± 4.40) and finally mixed sawdust (21

± 4.38) in three flushes (Fig. 4). Fruiting bodies with the highest weight in gram in harvest of three flushes were harvested from *T. africana* (89.03 ± 29.41 g). *D. edulis* recorded the least weight (32.31 ± 11.34 g). The parameters for the measurement of size of fruiting bodies are pileus diameter and stalk length. *M. indica* sawdust produced fruiting bodies with the broadest pileus diameter (6.45 ± 1.97 cm) and longest stipe (2.83 ± 0.49 cm) while *D. edulis* sawdust produced fruiting bodies with smallest pileus diameter (3.49 ± 0.99 cm) and shortest stipe (2.07 ± 0.40 cm) of *L. squarrosulus*, mean of three flushes (Fig. 6).

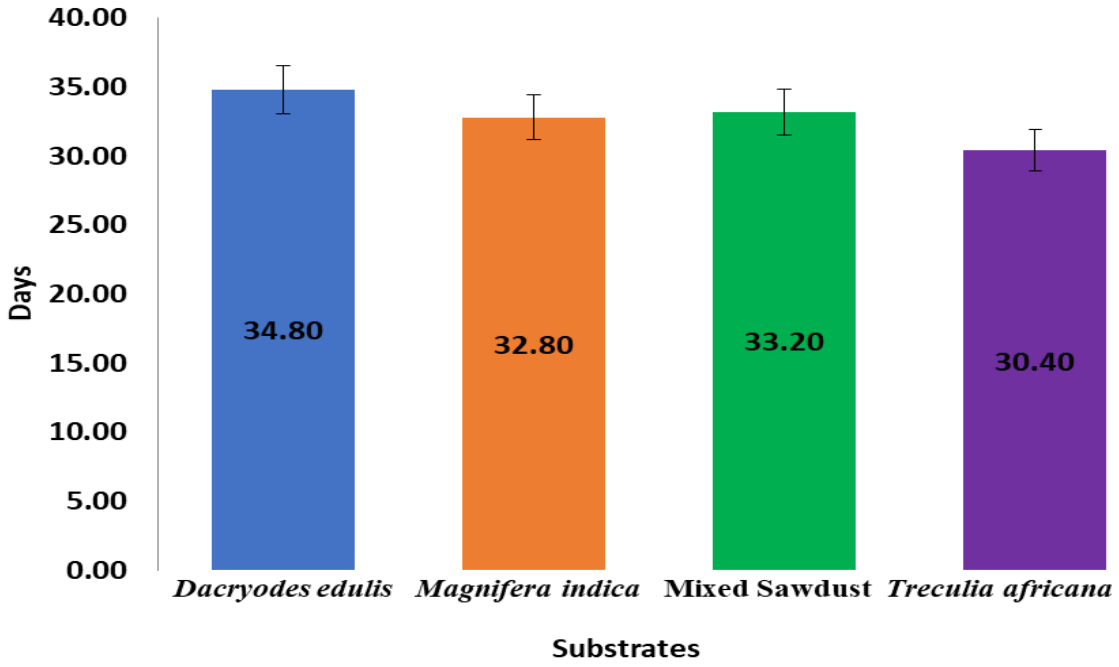

Fig. 1 – Colonization time/mycelial running time of domesticated *L. sqaurrosulus* on the different substrates.

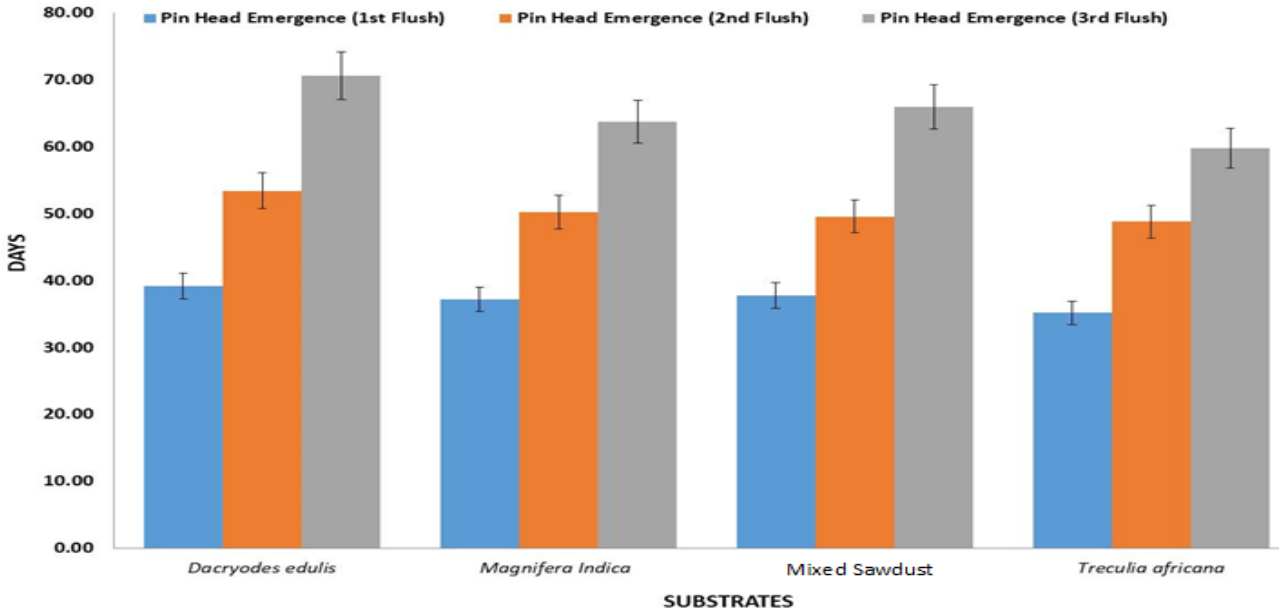

Fig. 2 – Pin head emergence/initiation of domesticated *L. squarrosulus* on different substrates.

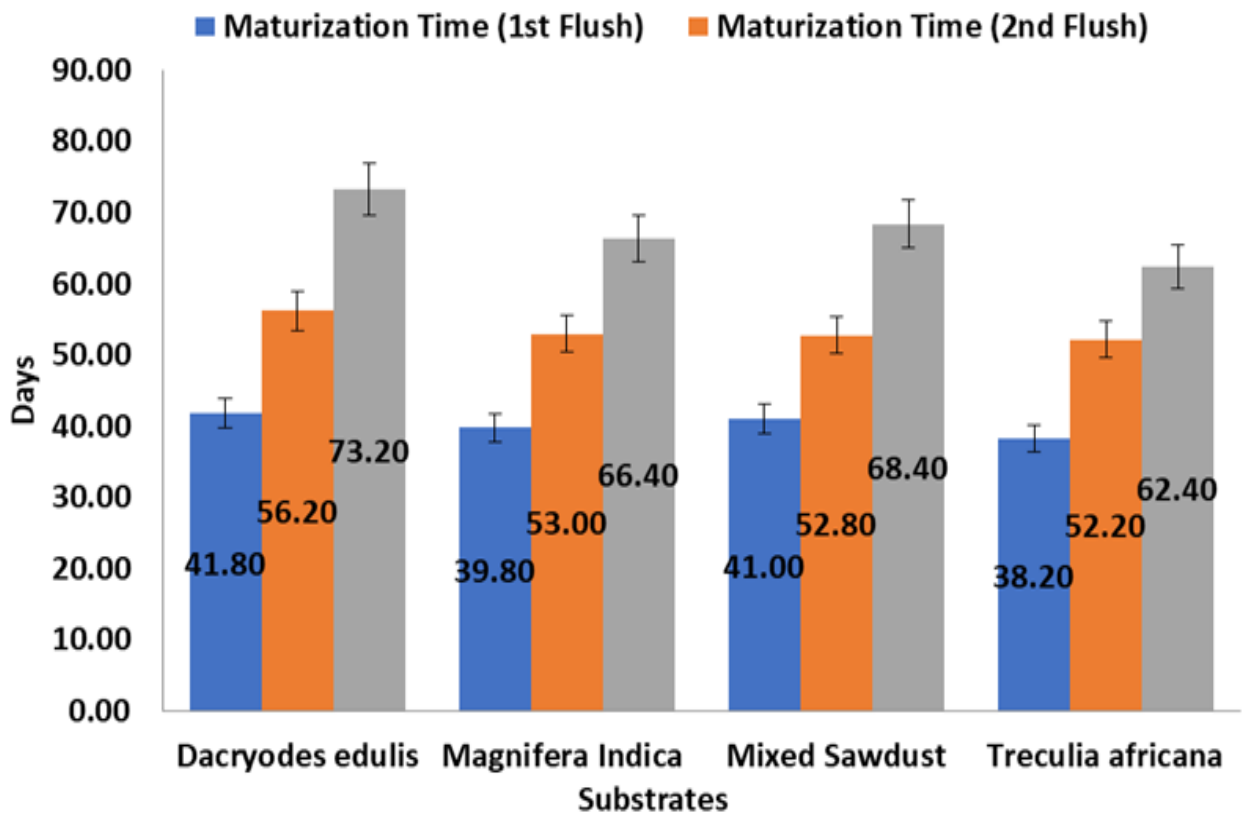

Fig. 3 – Maturation time of domesticated *Lentinus squarrosulus* on different substrates.

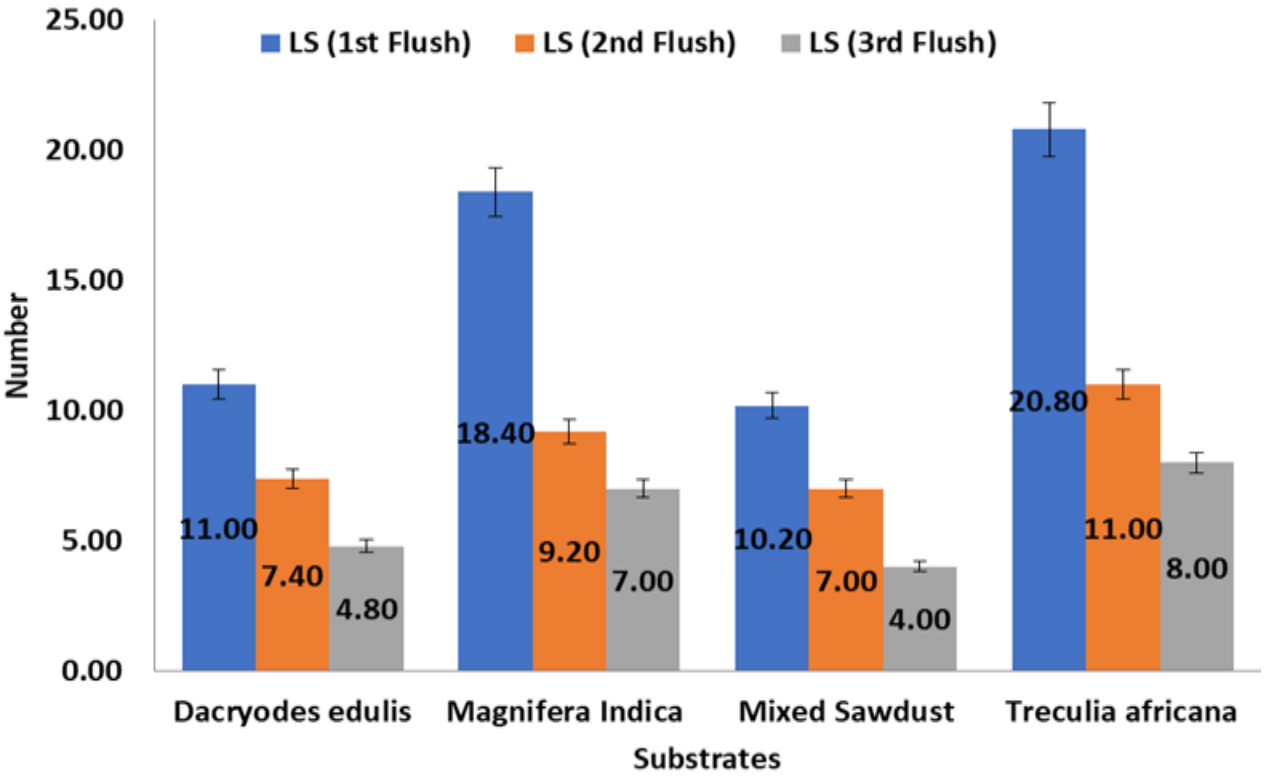

Fig. 4 – Yield (number) of fruiting bodies of domesticated *Lentinus squarrosulus* on different substrates.

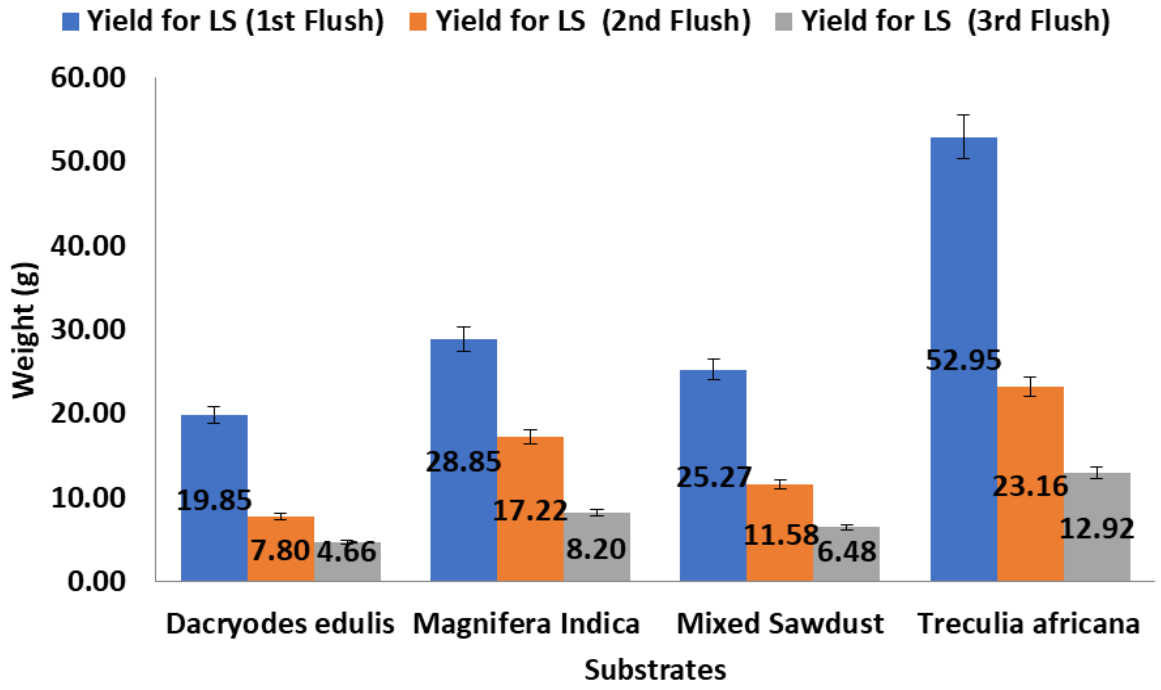

Fig. 5 – Yield (weight) of fruiting bodies of domesticated *Lentinus squarrosulus* on different substrates.

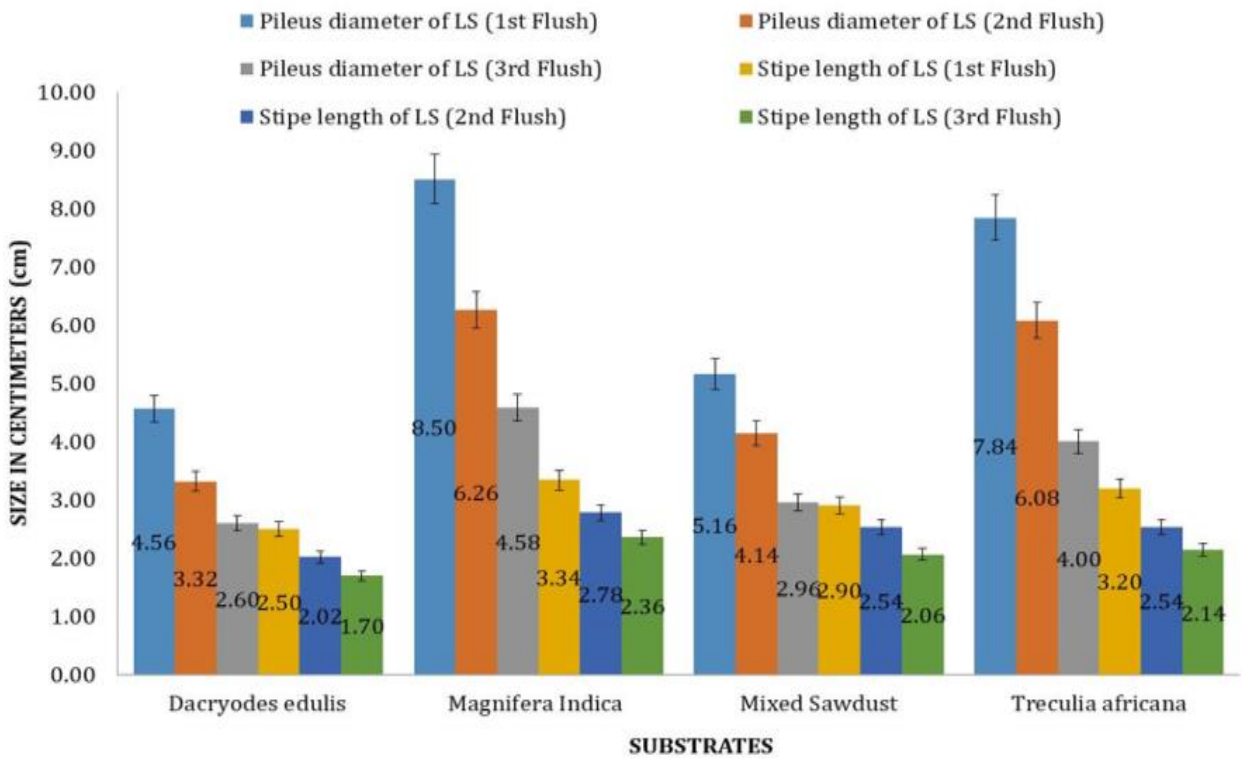

Fig. 6 – Yield (size of pileus diameter and stipe/stalk length) of domesticated *L. squarrosulus* on different substrates.

Fig. 7 – a Tissue culture of *Lentinus squarrosulus*. b Spawn of *Lentinus squarrosulus*. c Mycelium growth on substrate bags. d Fruiting bodies of *Lentinus squarrosulus* in its natural habitat. e – f Mature fruiting bodies of *Lentinus squarrosulus* produced in the domestication study.

According to the above results, all the substrate types supported the growth and fructification of the mushroom under study. However, *Treculia africana* sawdust was superior to all other treatment saw dust in having the shortest cropping cycle and producing the highest yield. The fruiting bodies were of good appearance. These features and their potential values in commercial mushroom production will be discussed.

Discussion

Lentinus squarrosulus is not widely cultivated commercially in Nigeria due to the complications and difficulties in tissue germplasm isolation and spawn production. The results obtained in this study is reporting for the first time the successful domestication of a wild edible mushroom, *L. squarrosulus* in eastern Nigeria. Production of tissue culture is the first step in the domestication of mushrooms. The tissue culture of *Lentinus squarrosulus* was successfully obtained on malt extract agar in this investigation from fresh and juvenile fruiting body of. *L. squarrosulus. Lentinus squarrosulus* mycelia developed on malt extract agar, an artificial medium in 5–7 days. Nteziryayo et al. (2024) recorded an incubation time of 6 days for germplasm isolation of *L. squarrosulus*. De Leon et al. (2017) also reported an incubation period of 6 days for tissue culture isolation of *Lentinus squarrosulus* These reports are in line with this study. The capacity to generate mother culture for use in cultivation is one of the most crucial features of domesticating mushrooms. *Lentinus squarrosulus* mother cultures were prolific on grain spawn (sorghum), and the grains were ramified in 14 days. Consequently, the mother culture (germplasm) of *L. squarrosulus* is available for research and commercial cultivation. Nteziryayo et al. (2024) reported an earlier incubation time for *L. squarrosulus* spawn production on sorghum grains (12 days). Dibaluka et al. (2010) reported an incubation duration of 18 – 21 days for *L. squarrosulus* on maize grains. The spawn was employed in cultivation trials to ascertain the substrates that would support growth and fruiting bodies production. This is the second phase of domestication and it was successful. Similar results were obtained by Adedokun & Okomadu (2017), Nteziryayo et al. (2024).

Mycelial running is the spread of fungus hyphae across the substrate and its colonization, or the growth of mycelia into the substrate. Mycelial running is a sign of how quickly the mycelia use the substrate (Buah et al. 2010, Masarirambi et al. 2001). The domesticated *L. squarrosulus* colonized the four different substrates employed in the study effectively and showed different mycelia running time (colonization time) (Fig 1). Colonization period ranged from 30 days to 35 days and was fastest in *Treculia africana* sawdust and least in *Dacryodes edulis*. Our research aligns with that of Dibaluka et al. (2010). They noted a 28–30 days colonization time. However, some studies contrast this study. It took the *L. squarrosulus mycelia* 16 days to fully ramify the substrate in research reported by De Leon et al. (2017). According to Li (2021), *Lentinus* species took between 18.0 and 25.6 days to colonize substrates made of rice straw and sawdust. For *Lentinus edodes,* Alemu (2015) obtained mycelia running 25 days. These findings recorded shorter cropping cycles when compared to the present study.

Time taken for pin heads to emerge after mycelia running differed in each of the substrates. Pin heads appearance ranged from 32 days - 71 days (Fig. 2) and first appeared on *Treculia africana* sawdust 32 days after inoculation. The time taken for pin head appearance on *T. africana* is not significantly different from *M. indica* and mixed sawdust but is significantly different from *Dacryodes edulis*. There is no significant difference in pin head appearance in *M. indica, D. edulis* and mixed sawdust. This study compares favorably with several other researchers who recorded lengthy pin head emergence. They include findings of Ediriweera et al. (2015) which reported that the pin head initiation period for *L. squarrosulus* in rubber sawdust was 50 to 60 days, as well as Miriyagalla et al. (2022) who observed that the pin head appearance period was 50 to 84 days. However, early pin head emergence was noted by several researchers which do not correspond with the present study. On *Brachystegia nigerica* sawdust, Okhuoya et al. (2005) got early pin heads of *L. squarrosulus* in 20.60 days. Kalaw et al. (2021) observed pin head emergence duration of 21.6 to 33.5 days following inoculation.

On various substrates, fruiting bodies of *L. squarrosulus* took a variety of times to mature. It took the shortest time (38 days) to harvest mature fruiting bodies from *T. africana* sawdust, while on *D. edulis* sawdust it took the longest time (42 days) (Fig. 3). In all the substrates, the maturation duration for *L. squarrosulus* varied significantly. According to Osibe & Chiejina (2015), *L. squarrosulus* harvested from the palm press fiber supplemented with 18% wheat bran matured in 34.33 to 56 days. This is in agreement with the results of this study. On *Spondias mombin* and *Citrus sinensis* logs, Adesina et al. (2011) observed a maturation time of 28 days following inoculation and is in contrast with the findings of the present study.

In terms of yield, *Treculia africana* sawdust generated the highest number of mature fruiting bodies of *L. squarrosulus* (40 ± 9.47) when compared to *Mangifera indica* (35 ± 8.55), *Dacryodes edulis* (23 ± 4.40) and mixed sawdust (21 ± 4.38) (Fig. 4). The quantity of fruiting bodies of *L. squarrosulus* obtained from *T. africana* and *M. indica* sawdust did not significantly change but they differed significantly from those produced from *D. edulis* and mixed sawdust. Numbers of fruiting bodies produced from *D. edulis* did not differ from those of mixed sawdust. On sawdust treated with 15% and 20% rice hull, fruiting bodies of *L. squarrosulus* ranging from 9.33 ± 1.35 to 18.33 ± 6.62 were produced (De Leon et al. 2017). This contradicts the quantity of fruiting bodies of *L. squarrosulus* obtained in this study.

T. africana sawdust produced the maximum yield of *L. squarrosulus* (89.03 ± 29.41 g) and *D. edulis* sawdust produced the lowest yield (32.31 ± 11.34 g) (Fig. 5). More yields were obtained in the first flush than subsequent flushes. Yield of *L. squarrosulus* on *T. africana* differed significantly from all the substrates used in this study. The other three substrates did not differ significantly from one another. This suggests that *Treculia africana* demonstrated to be the appropriated substrate for commercial cultivation of *L. squarrosulus*. De Leon et al. (2017) reported *L. squarrosulus* yield ranging from 43.33 g to 90.00 g, which corresponds with the results of our investigation. Osibe & Chiejina (2015), on the other hand, obtained a yield of *L. squarrosulus* that ranged from 44.14 g to 206.50 g which is in contrast with the findings in this study.

In this investigation, yield did not match with the rate of colonization or primordial emergence. This is consistent with studies conducted by (Liang et al. 2005) and (Oghenekaro et al 2020) which showed that the time of primordial initiation, harvesting intervals, mushroom size, and yield may not correlate to the fastest mycelial growth. The nutrients in the substrates became less available after each flush; a lower yield was obtained in the most recent flush compared to the initial flush. Several additional research findings showed that the quantity of fruiting bodies or yield decreased from one flush to the next (Adesina et al. 2011), (Osibe & Chiejina 2015), (Hussien et al 2016), (De Leon et al. 2017) and (Nteziryayo et al. 2019). These are in line with this study. Hussien et al. (2016) suggested that temperature, humidity, and substrate type are all potential causes of low yield.

The size of a mushroom is an important factor in determining its market value; broad pilei on fruiting bodies may make them more appealing to buyers. Pileus diameter is a good indicator of mushroom size (Dibaluka et al. 2010). The longest stalk (stipe) length of 3.37 ± 0.59 cm and the widest pileus diameter of 8.50 ± 1.34 cm were produced by *L. squarrosulus* on *Mangifera indica* sawdust, while the smallest pileus diameter of 4.56 ± 0.60 cm and the shortest stipe length of 2.50 ± 0.46 cm were obtained from *Dacryodes edulis* sawdust in the first flush (Fig. 6). Comparing treatment means, the pileus diameter and stipe length of *L. squarrosulus* harvested from *Mangifera indica* and *Treculia africana* sawdust did not significantly differ from one another, but they did differ from the other two substrates used. Adesina et al. (2011) measured the cap diameters of *L. squarrosulus* on *Gmelina* sawdust treated with wheat bran and found that the biggest cap diameter measured 7. 11±1.50 cm and the lowest were 4.36±0.60 cm, as well as the longest stipe length 5.11 ± 1.52 cm and the smallest stipe was 2.32 ± 0.33 cm. In a substrate enriched with 5% rice hull, largest pileus diameter (7.24 ± 0.73 cm), and the stipe length (4.30 ± 5.09 cm) of *L. squarrosulus* were obtained (Dulay et al. 2012). A diameter of 7.19 ± 0.73 cm was also measured for the greatest pileus in a substrate enriched with 20% rice hull. These results disagree with the results in this study.

Differences in the structure and chemical content of substrates may be responsible for variations in colonization times, pin head emergence, and fructification of sporocaps. According to Mata et al. (2005), the temperature of the incubation environment, light, and humidity all have impact on how quickly mushrooms spawn. Temperature, pH, the presence of inhibitory substances, sterilizing procedures, and cultivating methods can all have an impact on growth of mushrooms (Akinyele et al. 2012). The variances were caused by ambient factors, the quantity of substrates utilized, the size of the polypropylene bags, and the chemicals added to the substrates (Ediriweera et al. 2015).

This present domestication trial demonstrated *L. squarrosulus'*s potential for domestication through germplasm isolation, spawn production, substrate colonization and the subsequent development of fruiting bodies. *L. squarrosulus* was successfully domesticated in Orlu, Imo State, eastern Nigeria because it was harvested from a farm with a climatic condition similar to the area where it was grown. They could adapt to the environmental/climatic condition. Utilizable elements found in the sawdust used in cultivation aided in the growth and development of the fruiting bodies of *L. squarrosulus*. Simple observations revealed that the domesticated *L. squarrosulus* fruiting bodies were similar to the wild counterparts. Successful domestication and commercial cultivation of *L. squarrosulus* have been described by a number of authors in the past (Adesina et al. 2011, De Leon et al. 2017, Nteziryayo et al. 2019).

In conclusion, to determine the cultivability of the wild edible fungus, *Lentinus squarrosulus*, a domestication study was conducted. According to the study, *L. squarrosulus* may be grown in Orlu, Imo State, Nigeria, on sawdust from a variety of wood species. *Treculia africana* sawdust was shown to be the most effective substrate for the commercial production of *L. squarrosulus*, with respect to its effects on mycelial running time, pin head emergence, maturation of fruiting bodies, and yield. There is availability of mother cultures produced from tissue culture of *L. squarrosulus* for research and propagation purposes. Domestication of *L.squarrosulus* will ensure year round availability for mushroom lovers and large-scale commercial production of

L. squarrosulus in Nigeria can help to improve income, bridge protein malnutrition gap and serve as employment opportunities in both rural and urban regions. In view of the findings from the research, we hereby recommend that mushroom farming should be incorporated into agricultural system in Nigeria since the yield of *L. squarrosulus* obtained in this study is satisfactory in a first domestication trial. Sawdust is relatively abundant at no cost and therefore should be utilized in mushroom farming as an alternative to incineration with concomitant air pollution in order to ensure safe and healthy environment.

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