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# Optimization of culture conditions for vegetative growth of an indigenous strain of *Lentinus sajor-caju* (Fr.) Fr.

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## ABSTRACT

**The impact of media composition, temperature, pH, incubation period and light and dark conditions on the vegetative growth of local strain of *Lentinus sajor-caju* (Fr.) Fr., an edible lignicolous mushroom, is presented. The mushroom mycelium gave best mycelia growth on Malt Yeast Extract Agar solid medium and Yeast Glucose liquid medium. Optimum temperature of 33!, incubation period of 11 days, pH range from 4.0-5.0 and dark condition favoured the best mycelia growth.**

**Keywords:** Lignicolous, vegetative growth, incubation period, solid media, liquid media

The genus *Lentinus* Fr. is an agaricoid mushroom which belongs to Phylum *Basidiomycota*, Class *Agaricomycetes*, Order *Polyporales* and Family *Polyporaceae* (Pegler, 1983; Singer, 1986). The species of *Lentinus* are wood decaying (Hibbett *et al.*, 1993) and are reported to grow in nature on variety of special substrates (Morais *et al*., 2000, Philippousis *et al*., 2001). Till date the world over as many as 546 records of this genus are recognized in the MycoBank (MycoBank 2020). In India the genus *Lentinus* is represented by 20 valid species (Sharma and Atri, 2015). From economic point of view a number of species of this genus are used for personal consumption due to their esculent and supposedly therapeutic properties. Local strain of *L. sajor-caju* (Fr.) Fr., on which the present investigation has been carried out, is one such taxon having nutritionally and nutraceutically important constituents because of which it can be considered in the category of functional foods (Joly and Perreau 1977, Corner 1981, Chin 1981, De Leon *et al*., 2012, Singdevsachan *et al*., 2013, Reneses *et al*., 2016). *L. sajor-caju* is reported to be one of the most common mushrooms of palaeotropical forests with its distribution extending from Equatorial and Southern Africa to South East Asia and North West Australia (Pegler 1983). In nature the fungus produces large sized sporophores having infundibuliform pileus with inrolled margins and annulate stipe. From India edibility of this mushroom has been reported by number of investigators including Purkayastha and Chandra (1985), Verma *et al.* (1995) and Acharya *et al.* (2017). Some of the investigators including Gulati *et al.* (2011), Singdevsachan *et al.* (2013), Sharma and Atri (2014), Reneses *et al.* (2016) and Acharya *et al.* (2017) evaluated *L. sajor-caju* for its nutritional components including carbohydrates, proteins, dietary fibres, minerals, vitamins and documented it as a healthy food which has ability to lessen the difficulties of hunger and malnutrition. The sporophores of this mushroom contain all the essential as well as non-essential amino acids required for human beings (Sharma *et al.* 2012, Afiukwa *et al.*, 2015). In view of the culinary significance and the presence of nutraceutically important constituents in

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*L. sajor- caju*, the impact of variable cultural and physiochemical parameters with particular reference to the requirement of optimum temperature, favourable pH, light and dark conditions and incubation period for the vegetative growth has been investigated and the results of the study undertaken on these lines are presented in this manuscript.

## MATERIALS & METHODS

### The material

The culture of *L. sajor-caju* was raised by pure tissue culture method (Yadav, 2005) from the sporophore of *L. sajor-caju* collected from the dead part of the stem of *Bauhinia variegata* growing at Renuka Lake Nahan (Sirmour), Himachal Pradesh and used for the present study. The culture was maintained by subsequent sub-culturing on Malt Extract Agar (MEA) solid medium at 28±1°C temperature and deposited in CSIR-Institute of Microbial Type Culture Collection, Institute of Microbial Technology (IMTECH) Chandigarh under MTCC accession number 10945.

### Chemicals

Chemicals used during the present investigation are from standard sources like HiMedia and Sigma-Aldrich.

### Sterilization of glassware and media

The glassware was rinsed thoroughly with normal tap water and then immersed in Sulphuric Acid–dichromate solution for overnight (Tuite, 1969). Later this glassware was washed using Teepol solution, rinsed in normal tap water and finally with distilled water. Glassware to be used was sterilized by dry heat sterilization (hot air oven at 170 °C for 1 hour) followed by moist heat sterilization (autoclave at 15 lbs, 121 °C for 30-60 minutes). Media and other solutions to be used were also sterilized by autoclaving.

### Inoculum production and inoculation

For inoculation, the fungal culture maintained on Malt Extract Agar (MEA) solid medium was used. Complete inoculation procedure was performed aseptically under Laminar air flow. For all the experiments, as a source of inoculums, uniform small media discs bearing 0.9 mg to 1 mg of mycelia load were implanted in the centre of the flasks or Petri plates containing solidified medium while 1mL of mycelia homogenate was used as inoculum for inoculation in the flasks containing the liquid medium.

### Media evaluation for the vegetative growth

For the maximum and best vegetative growth of *L. sajor-caju* both solid as well as liquid media were evaluated. To investigate the suitable medium for vegetative growth the experiment was performed using 14 different solid media, namely Malt Yeast Extract Agar (MYEA), Yeast Extract Agar (YEA), Malt Extract Agar (MEA), Glucose Peptone Yeast Extract Agar (GPYEA), Glucose Peptone Agar (GPA), Potato Dextrose Agar (PDA), Wheat Grain Extract Agar (WGEA), Sabouraud Dextrose Agar (SDA), Milk Powder Agar (MPA), Gram Grain Extract Agar (GGEA), Elliott Agar (EA), Glucose Asparagine Agar (GAA), Czapek Solution Agar (CSA), Glucose Yeast Agar (GYA) and 14 different liquid media, namely Yeast Glucose Medium (YGM), Potato Dextrose Broth (PDB), Malt Broth (MB), Glucose Peptone Medium (GPM), Glucose Asparagine Medium (GAM), Richard Solution (RS), Rye Seed Medium (RSM), Maltose Peptone Broth (MPB), Dimmick Medium (DM), Asthana and Hawker Medium (AHM), Bilai Medium (BM), Czapek Solution (CS), Peptone Water (PW) and Trace Salt Solution (TSS). All the media were prepared as per the standard composition (Tuite 1969, Kirk *et al.* 2008). For this purpose 25 mL of respective media was dispensed in 100 mL sterilized Erlenmeyer flasks and three replicates were prepared for each. All the inoculated flasks were incubated at

28±1°C temperature as the specimen was collected at the prevailing temperature of 28°C.

**Effect of temperature on the vegetative growth**

To determine the optimum temperature for mycelia growth of *L. sajor-caju* the best evaluated Malt Yeast Extract Agar solid medium and liquid Yeast Glucose Medium were used as the basal media. Various temperatures, viz. 17°C, 21°C, 25°C, 29°C, 33°C and 37°C were selected to conduct the experiment. For setting up the experiment uniformly 25 mL medium was dispensed into each 100 mL Erlenmeyer flasks. Three replicates were prepared for each selected temperature.

**Incubation period for the vegetative growth**

For the determination of incubation period for the mycelia growth of *L. sajor-caju*, the experiment was conducted using the basal liquid Yeast Glucose Medium. For the experimental set up, 30 mL medium was poured in the 100 mL sterilized Erlenmeyer flasks and three replicates were prepared. The inoculated flasks were incubated at 33±1°C optimum temperature for 16 days. The mycelium was harvested on daily basis to measure the change in growth with time.

**Effect of different pH levels on the vegetative growth**

To test the favourable pH for the vegetative growth of *L. sajor-caju*, Malt Yeast Extract Agar solid medium and liquid Yeast Glucose Medium were taken as the basal media. The different pH levels, i.e. 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 were selected. The pH of the basal media was stabilized by using the citrate and phosphate buffers and need based adjustment of pH was done using 1N HCl and 1N NaOH. To set the experiment 25 mL of the medium was poured in sterilized Erlenmeyer flasks and three replicates of each pH level were prepared. The inoculated flasks were incubated at 33±1°C optimum temperature.

**Effect of light and dark conditions on the vegetative growth**

For screening the influence of illumination and dark conditions on the vegetative growth of *L. sajor-caju*, the experiment was performed using basal Malt Yeast Extract Agar solid medium as well as liquid Yeast Glucose Medium. For this purpose 20 mL Malt Yeast Extract Agar medium was aliquoted in the sterilized Petri dishes and 25 mL Yeast Glucose Medium was dispensed in 100 mL Erlenmeyer flasks. Triplicates were prepared for illumination and dark conditions. For the lighted condition, the inoculated Petri dishes were incubated in the incubator with artificial light of 70 Lux while for the dark condition, the inoculated Petri dishes were wrapped with black paper and incubated in the incubator. The inoculated flasks were incubated at 33±1°C optimum temperature.

**Observation criteria for results**

The incubated Erlenmeyer flasks containing solid media were observed daily until full ramification and diameter of mycelia growth was measured in centimetre scale to estimate the vegetative growth. Colony morphology like shape form, margin edge and elevation of colony were also recorded (Gunasekaran 1995). The inoculated Erlenmeyer flasks containing liquid media were incubated for 15 days. After 15 days, the mycelia mat was collected, thoroughly washed and kept in small pre-weighed Petri plates. These Petri plates with mycelia mat were kept in an oven maintained at 65 °C. The mycelia growth was recorded by measuring the dry weight of the mycelium after the completion of the experiment using OHAS Adventure TM digital weighing machine. The results obtained during the present study were also analysed statistically. The results are presented here.

## RESULTS

### Pure culture

The mycelia mat of *L. sajor-caju* in pure culture is very thick and dense and grows in concentric rings with filamentous, radiating margins. Mycelium is white in colour in the initial stages till 3-4 days and turns yellowish brown with maturity (Fig. 1).

**Fig. 1.** Five days old pure culture of *L. sajor-caju*

### Evaluation of solid media for vegetative growth

Out of the 14 solid media evaluated, the mushroom mycelium gave the maximum vegetative growth on Malt Yeast Extract Agar (MYEA) medium with radial extension of 6.3 cm and 1.82 cm growth on an average daily basis. Very thick and dense mycelia mat was observed on Malt Yeast Extract Agar medium. The vegetative growth (6.3 cm) was also comparable

**Table 1:** Colony diameter, growth rate and mycelia characteristics of *L. sajor-caju* on solid media

| Medium | Colony Diameter (cm) ± S. D. | Growth Rate (cm/day) | Colony Characteristics |
|---|---|---|---|
| Malt Yeast Extract Agar (MYEA) | 6.3 ± 0 | 1.82 | Mycelia mat very thick, dense, white, filamentous, colony round with radiating margins, turns yellowish brown on 4$^{th}$ day of incubation |
| Yeast Extract Agar (YEA) | 6.3 ± 0 | 1.60 | Mycelia mat thick, white, filamentous, colony colour turns brown after 6$^{th}$ day of incubation |
| Malt Extract Agar (MEA) | 6.3 ± 0 | 1.60 | Mycelia mat white and comparatively less thick than that on Malt Yeast Extract Agar and Yeast Extract Agar, turns brown after 6$^{th}$ day of incubation |
| Glucose Peptone Yeast Extract Agar (GPYEA) | 5.4 ± 0.2 | 1.33 | Mycelia colony show concentric growth and form cottony white mat |
| Glucose Peptone Agar (GPA) | 5.03 ± 0.05 | 1.26 | Fine, thin filaments of mycelium formed |
| Potato Dextrose Agar (PDA) | 4.7 ± 0.1 | 1.15 | Thin mycelia filaments growing in concentric rings |
| Wheat Grain Extract Agar (WGEA) | 4.53 ± 0.15 | 1.11 | Colony with extremely fine filaments, transparent in colour |
| Sabouraud Dextrose Agar (SDA) | 4.27 ± 0.05 | 1.01 | Thick filaments growing in concentric rings, mycelium colour becomes light brown on 4$^{th}$ day of incubation |
| Milk Powder Agar (MPA) | 4.23 ± 0.2 | 1.13 | Extremely fine transparent mycelia filaments formed |
| Gram Grain Extract Agar (GGEA) | 4.17 ± 0.05 | 1.04 | Round colony formed with radiating margins and thin filaments |
| Elliott Agar (EA) | 3.8 ± 0.10 | 0.86 | Fine filaments in round colony with radiating margins |
| Glucose Asparagine Agar (GAA) | 3.7 ± 0.17 | 0.92 | Extremely thin, transparent, scattered mycelia filaments formed |
| Czapek Solution Agar (CSA) | 2.43 ± 0.15 | 0.61 | Very poor mycelia growth |
| Glucose Yeast Agar (GYA) | 1.97 ± 0.15 | 0.30 | Very thin mycelia mat formed |

with the growth on Yeast Extract Agar and Malt Extract Agar media, however the rate of growth was faster on Malt Yeast Extract Agar medium (1.82 cm/day) in comparison to growth on Yeast Extract Agar medium (1.60 cm/day) and Malt Extract Agar medium (1.60 cm/day media). As compared the mycelia growth was minimum on Czapek Solution Agar medium (2.43 cm) and Glucose Yeast Agar medium (1.97 cm), respectively. Hence Malt Yeast Extract Agar medium was evaluated as the best solid medium out of all the solid media used for evaluation during the recent study. The results are summarized in Table 1. Statistical comparison of growth on different solid media using t values showed that differences between most of the 91 media combinations were highly significant (0.01 level of significance). The differences were significant (i.e. 0.05 level) in 3 combinations (WGEA vs SDA, WGEA vs MPA, SDA vs GGEA) and non-significant in 7 combinations (no difference in MYEA vs YEA, MYEA vs MEA, and YEA vs MEA; non-significant difference in PDA vs WGEA, SDA vs MEA, MPA vs GGEA and EA vs GAA).

**Evaluation of liquid media for vegetative growth**

During the present investigation, 9.56 mg/mL vegetative growth was recorded in Yeast Glucose Medium, which was maximum amongst the evaluated media. The next best mycelia growth (7.03 mg/mL) was obtained in Potato Dextrose Broth followed by Malt Broth (5.24 mg/mL). Least vegetative growth was recorded in Dimmick Medium (1.11 mg/mL) and Asthana and Hawker Medium (1.01 mg/mL). No vegetative growth was there in Bilai Medium, Czapek Solution, Peptone Water and Trace Salt Solution media. Since Yeast Glucose Medium supported the maximum vegetative growth and hence this medium was selected as basal liquid medium. Results have been summarized in Table 2. Statistical comparison of growth on different liquid media using t values showed that differences between most of the 91 media combinations were highly significant. The differences were just significant in 2 combinations (RS vs MPB, MPB vs AHM) and non-significant only in 3 combinations (RS vs RSM, MPB vs DM, DM vs AHM).

**Table 2:** Effect of liquid media on the mycelia growth of *L. sajor-caju*

| Medium | Mycelium Dry Weight (mg/mL) ± S.D. |
|---|---|
| Yeast Glucose Medium (YGM) | 9.56 ± 0.17 |
| Potato Dextrose Broth (PDB) | 7.03 ± 0.30 |
| Malt Broth (MB) | 5.24 ± 0.35 |
| Glucose Peptone Medium (GPM) | 4.13 ± 0.05 |
| Glucose Asparagine Medium (GAM) | 2.46 ± 0.12 |
| Richard Solution (RS) | 1.83 ± 0.30 |
| Rye Seed Medium (RSM) | 1.75 ± 0.05 |
| Maltose Peptone Broth (MPB) | 1.29 ± 0.18 |
| Dimmick Medium (DM) | 1.11 ± 0.12 |
| Asthana Hawker Medium (AHM) | 1.01 ± 0.08 |

No growth on Bilai Medium (BM), Czapek Solution (CS), Peptone Water (PW) and Trace Salt Solution (TSS)

**Evaluation of suitable temperature for vegetative growth**

The results obtained during the present study revealed that at 33°C temperature the maximum radial mycelia extension (6.3 cm) with 1.77 cm growth on an average daily basis was recorded on Malt Yeast Extract Agar solid medium while maximum vegetative growth (9.93 mg/mL) was there on dry weight basis in Yeast Glucose liquid medium. The mycelia mat was quite dense and thick at 33°C temperature. In comparison next best vegetative growth was recorded at 37 °C. The vegetative growth was minimal at 17°C. At 37°C temperature, although the colony diameter was comparable but the mycelia dry weight obtained was on the lower side in comparison to mycelia dry weight obtained at 33°C. Since the colony characteristics, density of the mycelia mat and growth of mycelium was better at 33°C, hence 33°C temperature was evaluated as the best temperature for the vegetative growth of mycelium of *L. sajor*

**Table 3:** Effect of temperature on mycelia growth of *L. sajor-caju*

| Temperature (°C) | Colony Diameter (cm) on Malt Yeast Extract Agar Medium ± S.D. | Growth Rate (cm/day) | Mycelium Dry Weight in Yeast Glucose Medium (mg/mL) ± S.D. |
|---|---|---|---|
| 17 | 1.73 ± 0.15 | 0.43 | 5.17 ± 0.24 |
| 21 | 2.37 ± 0.06 | 0.59 | 5.80 ± 0.33 |
| 25 | 4.57 ± 0.21 | 1.14 | 6.73 ± 0.14 |
| 29 | 5.0 ± 0.10 | 1.24 | 8.23 ± 0.33 |
| 33 | 6.3 ± 0 | 1.77 | 9.93 ± 0.33 |
| 37 | 6.3 ± 0 | 1.60 | 8.60 ± 0.30 |

*caju* on the solid as well as liquid media. The effect of various temperatures on the vegetative growth of *L. sajor-caju* has been summarized in Table 3). The differences in growth at different temperatures were highly significant (0.01 level) on both liquid and solid media as indicated by t values.

## Incubation period for the vegetative growth using yeast glucose medium

On the basis of the results achieved, during the initial days of incubation there was very slow growth of mycelium, which increased rapidly after 5th day of incubation and achieved maximum level (10.94 mg/mL) on 11th day of incubation. Thereafter the vegetative growth remained static on 12th (10.63 mg/mL) and 13th (10.13 mg/mL) days of incubation, after which the growth gradually declined with increasing days of incubation. The mycelia growth during incubation period is presented in Table 4. As per the results obtained, the maximum mycelia growth was achieved on 11th day of incubation.

**Table 4:** Effect of incubation days on mycelia growth of *L. sajor-caju* in Yeast Glucose Medium

| Incubation Days | Mycelium Dry Weight (mg/mL) ± S.D. |
|---|---|
| 1 | 0 |
| 2 | 0.15 ± 0.05 |
| 3 | 0.44 ± 0.05 |
| 4 | 1.36 ± 0.04 |
| 5 | 2.70 ± 0.06 |
| 6 | 4.10 ± 0.05 |
| 7 | 5.90 ± 0.07 |
| 8 | 7.06 ± 0.07 |
| 9 | 8.01 ± 0.15 |
| 10 | 9.12 ± 0.09 |
| 11 | 10.94 ± 0.38 |
| 12 | 10.63 ± 0.03 |
| 13 | 10.13 ± 0.03 |
| 14 | 9.83 ± 0.03 |
| 15 | 9.14 ± 0.15 |
| 16 | 8.77 ± 0.08 |

## Effect of different pH levels on the vegetative growth

The results of the experiment revealed that maximum mycelia growth was there at pH 4.5 on Malt Yeast Extract Agar solid medium (6.4 cm) with 1.29 cm growth on an average daily basis as well as in Yeast Glucose liquid medium (8.37 mg/mL). Very thick and dense mycelia mat was observed at pH 4.5. It was followed by growth at pH 4.0 in solid as well as liquid media. At the pH 7.5, 8.0, 8.5 and 9.0 negligible or no growth occurred at all. The results of the present investigation revealed that acidic pH (3.0-6.0) significantly enhanced the mycelia growth of *L. sajor-caju* whereas mycelia growth was poor in alkaline pH (Table 5). The differences in growth at different pH levels were highly significant on both solid and liquid media as indicated by t-values.

**Table 5**: Effect of different pH levels on mycelia growth of *L. sajor-caju*

| pH | Colony Diameter (cm) on Malt Yeast Extract Agar Medium ± S.D. | Growth Rate (cm/day) | Mycelium Dry Weight in Yeast Glucose Medium (mg/mL) ± S.D. |
|---|---|---|---|
| 3.0 | 4.33 ± 0.06 | 0.88 | 3.79 ± 0.29 |
| 3.5 | 5.10 ± 0.30 | 1.01 | 5.60 ± 0.11 |
| 4.0 | 5.66 ± 0.15 | 1.15 | 7.12 ± 0.10 |
| 4.5 | 6.4 ± 0 | 1.29 | 8.37 ± 0.34 |
| 5.0 | 3.50 ± 0.10 | 0.62 | 6.85 ± 0.12 |
| 5.5 | 2.80 ± 0.10 | 0.58 | 5.88 ± 0.02 |
| 6.0 | 2.46 ± 0.06 | 0.55 | 5.14 ± 0.09 |
| 6.5 | 2.06 ± 0.05 | 0.31 | 4.11 ± 0.06 |
| 7.0 | 1.03 ± 0.05 | 0.25 | 3.35 ± 0.09 |
| 7.5 | 0 | 0 | 2.41 ± 0.07 |
| 8.0 | 0 | 0 | 0.21 ± 0.04 |
| 8.5 | 0 | 0 | 0 |
| 9.0 | 0 | 0 | 0 |

### Effect of light and dark conditions on the vegetative growth

The results of the present investigation revealed that the maximum vegetative growth of *L. sajor-caju* mycelium was there under dark condition both on Malt Yeast Extract Agar solid medium (with maximum radial mycelia extension of 9.0 cm) and Yeast Glucose liquid medium (with maximum radial mycelia extension of 7.02 mg/mL) in comparison to growth under light condition. The mycelia density, colony thickness and the rate of growth was faster under dark condition as compared to the light condition. The results obtained are shown in Table 6. As per the results of the experiment, dark condition favours the maximum mycelia growth.

## DISCUSSION

The growth and development of mushrooms is influenced greatly by various factors such as substrate composition, composition of culturing media, temperature, pH, luminance, aeration and humidity (Chang and Miles 2004, Stott and Mohammed 2004). For determining best media supporting excellent vegetative growth of *L. sajor-caju*, both solid as well as liquid media were evaluated. The results of the present investigation revealed that the maximum radial extension of secondary mycelium with thick, dense mycelia mat was obtained on Malt Yeast Extract Agar with 1.82 cm/day growth on an average daily basis. Kalaw *et al.* (2016) documented that Potato Dextrose Agar showed best vegetative growth with

**Table 6:** Effect of light and dark conditions on the mycelia growth of *L. sajor-caju*

| Condition | Colony Diameter (cm) on Malt Yeast Extract Agar Medium ± S.D. | Growth Rate (cm/day) | Mycelium Dry Weight in Yeast Glucose Medium (mg/mL) ± S.D. |
|---|---|---|---|
| Dark | 9.00 ± 0 | 1.80 | 7.02 ± 0.53 |
| Light (70 Lux) | 5.13 ± 0.12 | 1.04 | 5.37 ± 0.21 |

thick mycelia density and mycelia extension of 49.70 mm, 51.13 mm and 53.49 mm in *Lentinus tigrinus* strain A and B and *L. sajor-caju*, respectively. *L. sajor-caju* did not grow well on Potato Dextrose Agar medium during the present study which is at variance from the observations of Kalaw *et al.* (2016). The mycelia growth of *L. connatus* on Yeast Extract Agar medium as reported by Atri *et al.* (2011) is also comparable to the growth of *L. sajor-caju* mycelium on Malt Extract Agar and Yeast Extract Agar media. While working with *L. sajor-caju* De Leon *et al.* (2017) reported Coconut Water Gelatin (94.01 mm) as the best solid medium which supported very thick mycelium density. The results documented by De Leon *et al.* (2017) are altogether at variance with the presently obtained results which may be because of the use of indigenous nutrient sources in the culture media. Shahtahmasebi *et al.* (2017) evaluated Malt Extract Glucose Agar (7.45 mm) followed by Malt Extract Agar (6.62 mm) solid media as best media for culturing of *L. tigrinus.*

During the present investigation out of 14 liquid media evaluated, Yeast Glucose Medium showed highest mycelia growth (9.56 mg/mL). Dulay *et al.* (2015) used broths of different indigenous nutrient resources for culturing *L. tigrinus* and *L. sajor-caju* and reported Rice Bran decoction as the best liquid medium for the efficient, highest mycelia growth of *L. tigrinus* (11.53 g) and *L. sajor-caju* (9.75 g). The growth of *L. sajor caju* reported by Dulay *et al.* (2015) on Rice Bran decoction is almost comparable to the growth of this species achieved on Yeast Glucose Medium. Presently *L. sajor-caju* did not grow well on Glucose Peptone Medium, however Atri *et al.* (2011) while working with *L. connatus* documented the maximum vegetative growth on Glucose Peptone (71.25 mg). Shahtahmasebi *et al.* (2017) evaluated Malt Extract Glucose (1.62 g) liquid medium as best medium to culture *L. tigrinus*. It is apparent from the above comparison that every species has its own specific media requirement as for vegetative growth is concerned.

While investigating the temperature requirements using Malt Yeast Extract Agar solid medium and Yeast Glucose liquid medium, 33°C temperature was evaluated to support maximum vegetative growth of *L. sajor-caju*. As compared Atri *et al.* (2007) while working with *L. squarrosulus* documented 30±2°C as the optimum temperature for the vegetative growth on both solid as well as liquid media which is on a slightly lower side in comparison to the temperature requirement for the presently evaluated *L. sajor-caju*. In case of *L. strigosus,* Vargas-Isla and Ishikawa (2008) evaluated higher temperature (35 C) requirement for the maximum vegetative growth. Dulay *et al.* (2012) evaluated 32°C temperature requirements for the culturing of *L. tigrinus*, which is similar to the temperature requirement of *L. sajor-caju* studied presently. As is the case in other species of *Lentinus*, Klomklung *et al.* (2014) observed 30°C as the optimum temperature for the mycelia growth of *L. connatus* and *L. roseus*. In line with the present investigation Kalaw *et al.* (2016) also recorded 32°C as the optimum temperature for maximum vegetative growth of *L. sajor-caju* and *L. tigrinus*. Similarly Tantratian *et al.* (2019) elucidated 30°C as optimum temperature while working with *L. squarrosulus*. These observations are in conformity with the results of the present study on *L. sajor-caju*. From these observations it becomes quite apparent that in consonance with other *Lentinus* species, *L. sajor caju* also require relatively higher temperature of 33°C for vegetative growth.

While investigating the growth pattern of *L. sajor caju* during 16 days period of experiment for determining the incubation period, a definite sigmoid pattern of growth was observed. On the basis of results achieved, the growth reached to the maximum level (10.94 mg/mL) on 11$^{th}$ day of incubation. At initial stages of growth in *Lentinus* spp. lipid synthesis takes place but later the lipid content decreases rapidly, which may be the reason behind the reduced mycelial biomass after 11th days of mycelial growth. This phenomenon is also reported by Song *et al.*

(1989) in *Lentinus edodes*. Similar pattern in vegetative growth has been documented in different species of *Lentinus* by different investigators but every species required different incubation period for vegetative growth. The incubation period of 6 days was observed by Vargas-Isla and Ishikawa (2008) for highest mycelia growth of *L. strigosus*. As compared for maximum vegetative growth of *L. squarrosulus,* De Leon *et al.* (2013) reported incubation period of 8 days. Klomklung *et al.* (2014) recorded 1 week incubation period for maximum vegetative growth of *L. connatus* and 8 days for *L. roseus*. For maximum vegetative growth of *L. tigrinus,* Shahtahmasebi *et al.* (2017) documented incubation period of 8 days. Similarly Tantratian *et al*. (2019) reported the requirement of incubation period of 25 days while working with *L. squarrosulus*. The range of incubation period reported by various investigators in different *Lentinus* species extended from 6 days in *L. strigosus* (Vargas-Isla and Ishikawa 2008) to 25 days in *L. squarrosulus* (Tantratian *et al.*, 2019) and the incubation period required for maximum growth by *L. sajor-caju* is found to be within this range..

During the present investigation, 6.4 cm maximum mycelium radial extension and 8.37 mg/mL highest mycelia growth was recorded at pH 4.5. In case of *L. squarrosulus,* Atri *et al*. (2007) documented maximum mycelium extension (5.83 cm) and mycelia dry weight (15 mg/mL) at pH 4.0. These observations are in conformity with the present investigation where pH 4.5 has been evaluated as the most suitable pH level for the vegetative growth of *L. sajor-caju*. Klomklung *et al*. (2014) while working with *L. connatus* and *L. roseus* documented acidic pH of 5.5 for maximum mycelia extension of 12.76 mm and 8.43 mm, respectively. Kalaw *et al.* (2016) recorded pH 6.0 for maximum mycelia growth of *L. sajor-caju* (44.50 mm). During the evaluation of effect of various pH levels on the vegetative growth of *L. sajor-caju*, De Leon *et al.* (2017) observed maximum colony diameter (97.31 mm) with thick mycelia mat at acidic pH of 5.0. The present study revealed that acidic pH (3.0 - 6.0) is favourable for the vegetative growth of *L. sajor-caju* as compared to the basic and neutral pH which is in conformity with the recommendations made by Atri *et al.* (2007), Klomklung *et al.* (2014) and Kalaw *et al.* (2016) while working with different species of *Lentinus* including *L. sajor-caju.*

Many investigators including Chang and Hayes (1978), Leatham and Stahmann (1987), Kitamoto *et al.* (1999), Sakamoto *et al.* (2004), Vargas-Isla and Ishikawa (2008), Dulay *et al.* (2012), Kalaw *et al.* (2016) investigated the effect of illumination on the development of basidiomycetous fungi and reported it as an important factor for the luxuriant mycelia growth as well as for sporophore production. The results of the present study revealed that in case of both solid and liquid media the maximum mycelia growth was observed when incubated under dark condition. Similar to the present study Atri *et al.* (2007) documented darkness as the favourable condition for high mycelia growth in case of *L. squarrosulus.* On the same line Vargas-Isla and Ishikawa (2008) while working with *L. strigosus* also reported maximum mycelia biomass production (397.04 mg) in the absence of light. For *L. tigrinus*, Dulay *et al.* (2012) revealed that secondary mycelium grew best under dark condition. Kalaw *et al.* (2016) documented dark condition for maximum vegetative growth of *L. tigrinus* and *L. sajor-caju*. These observations are in conformity with the presently obtained results while investigating the impact of light and darkness on the vegetative growth of *L. sajor caju*. The present results clearly suggest dark condition as most favourable for best secondary mycelia growth which is in conformity to the similar observations documented by Atri *et al.* (2007), Dulay *et al.* (2012) and Kalaw *et al.* (2016) while working with different *Lentinus* species.

## CONCLUSION

The results of the present study revealed that Malt Yeast Extract Agar solid medium and Yeast Glucose liquid medium are the best media which support the maximum vegetative growth of *L. sajor-caju* with

thick and dense mycelia density when cultured at 33°C maintaining acidic pH of the medium under dark conditions when incubated for 11 days. The present study is quite helpful in understanding the cultural requirements of *L. sajor-caju* for enhancing the vegetative growth of this mushroom.

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