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Cultural and comparative physiological studies on wild edible and mycorrhizal mushroom species of genus *Boletus* and *Suillus*

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Abstract
The pure cultures of *Boletus edulis* and *Suillus sibiricus* were isolated on Potato Dextrose Agar Medium and Hagem's Agar Medium respectively. Ten different solid media were evaluated and best mycelial growth of *B. edulis* is observed on Modified Melin Norkran's Medium whereas *S. sibiricus* showed best growth on Hagem's Medium. The mycelia grown on best basal medium revealed 20ºC as optimum temperature. The 5.5 is found to be the best pH for the maximum mycelial growth of these two mushrooms. The growth of mycelium is better in darkness and compared to light.

Keywords: culture, edible, mycorrhizal, *Boletus edulis*, *Suillus sibiricus*

Introduction

Boletus edulis and *Suillus sibiricus* are wild edible poroid mushrooms. The Culture characters of many fungi wood-rotting, edible and medicinal mushrooms have been studied by various scientists (Stalpers, 1978; Buchalo, 1988 and Stamets, 2000) [32, 1, 33]. Pure cultures of mushrooms can be derived from the tissue of fruiting bodies, surface sterilized mycorrhizal roots and sclerotia. Members of some genera of mushrooms are often fairly easy to isolate, among these are *Amanita*, *Astraeus*, *Boletus*, *Cortinarius*, *Fascoboletinus*, *Hebeloma*, *Hymenogaster*, *Hysterangium*, *Laccaria*, *Lactarius*, *Leccinum*, *Melanogaster*, *Paxillus*, *Pisolithus*, *Rhizopogon*, *Scleroderma*, *Suillus and Tricholoma* (Molina and Palmer, 1982) [21].

The mushroom's character as a pioneer species, the fact that it is easy to work with in a pure culture medium, as well as its capacity to form mycorrhizae, facilitate the study of their biology (Kropp and Fortin, 1988; Godbout and Fortin, 1992; and Kropp and Mueller, 1999) [18, 12, 19]. For the purpose of mycelial propagation, growth of the fungal strains is affected by many culture conditions, principally pH, temperature and composition of the different media. Within a single species, isolates from different origins can exhibit morphological variations (Castellano and Molina, 1989) [3].

A lot of work was done to describe the various cultural characters of *Psilocybe sensulato* species (Gilmore, 1926; Heim *et al.*, 1963; Dubovoy and Herrera 1968; Watling 1971; Buchalo *et al.*, 2009 and Silva *et al.*, 2016) [11, 15, 7, 37, 2, 30]. The several species of Strophariaceae and *Psilocybe sensulato* were characterized in culture medium for providing relevant taxonomic characters (Walther and Weiβ, 2008) [36]. Singh *et al*. (2000) [31] studied the physico-chemical preferences for best mycelial colonization in some edible mushrooms. Previously cultural studies of different mushrooms like *Gymnopilus* sp. (Fausto-Guerra *et al.*, 2002) [8], *Schizophyllum commune* and *Lentinula edodes* (Reddy *et al.*, 2017) [24] were also carried out in various part of the world.

The present studies is important because the pure culture of these edible fungi can be further utilized for making mushroom spawn for the purpose of mushroom cultivation trials. Both the mushrooms are also ectomycorrhizal, since the pure culture can be used to test *in vitro* mycorrhizal synthesis and production of mass inoculums for the seedling inoculation in the nurseries inoculation programmes.

Materials and methods

Pure Culture Isolation

Pure cultures of *Boletus edulis* and *Suillus sibiricus* were raised from the pileus and stipe portion of the healthy and fresh fruiting bodies. The specimens were first wash with distilled water and then the tissues from pileus and stipe portion were cut with the help of sterilized blade. The bits of tissue (2-3 mm) were taken by sterilized forceps and dipped in 0.1% Mercuric Chloride solution for 5-10 seconds and then washed with sterilized distilled water. Now the tissue was placed on sterilized filter paper to remove the excess moisture. These bits of tissue were then transferred aseptically into Petri plates containing nutrient medium with the help of sterilized forceps. Petri plates were then incubated at ambient temperature for at least 7-9 days and observed regularly for the appearance of culture. The actively growing mycelial colonies were subcultured to obtain pure cultures. Ten solid media have been tried during the present studies. All the media were prepared following Tuite (1969) [35].

Preparation of Inoculum

Inoculum used in this study was obtained from the periphery of actively growing mycelial colonies. Mycelial discs of 5mm diameter were taken out with a presterilized borer under aseptic conditions, to be used as inoculum in different solid media.

Recording of Vegetative Growth in Different Solid Media

Vegetative growth of mycelium in the solid media was measured by taking the diameter of colony in two directions at right angles. Five replicates of each medium were used and average values were taken for comparison of growth in different media. The medium with best vegetative growth was used in further studies i.e. for studying the effect of temperature, pH and light and darkness.

Effect of Temperature

For the study of temperature requirement of the fungus, inoculated Petri plates and flasks were incubated at the following temperatures *viz*. 5, 10, 15, 20, 25, 30, 35 and 40ºC in separate incubators on the best suited solid medium.

Effect of Hydrogen Ion Concentration (pH)

To record the effect of different pH on the growth of this fungus the best solid media was adjusted at different pH levels, *viz*. 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5. The pH was adjusted with the help of N/10 NaOH or N/10 HCl. The pH was checked with the help of digital type Phillips pH meter. The inoculated Petri plates and flasks were incubated at best suited temperature and after that the growth was measured.

Effect of Light and Darkness

Best selected solid medium with optimum pH was inoculated and was given light and dark treatment at optimum temperature. Growth was observed after incubation period.

Statistical Analysis of the Data

The data obtained for mycelial growth under different conditions were from five replicates. All data obtained was statistically analyzed. To find out the significance of difference between the mean values, one way analysis of variance (ANOVA) test and student's t-test was applied. Tukey's multiple compression test was used to determine honestly significant difference (HSD) values for significance among means.

Results

Pure Culture Isolation

Pure culture of *B. edulis* was isolated on Potato Dextrose Agar Medium (PDA). The colour of the colony was white and cottony (Fig. 1. a). The culture of *S. sibiricus* has been isolated on Hagem's Agar Medium. Colony colour changes from white to cream (Fig. 1. c). Mycelia are slow growing. Subculturing of these mushrooms was done on PDA medium.

Fig 1: a) Petriplate containing pure culture of *Boletus edulis* b) Petriplates showing cultural Characteristics of *B. edulis* on different solid media c) Petriplate containing pure culture of *Suillus sibiricus* d) Petriplates showing cultural Characteristics of *S. sibiricus* on different solid media

Cultural Characteristics in Different Solid Media

Ten solid media were evaluated for the linear growth of *B. edulis* and *S. sibiricus*. The mycelial growth of *B. edulis* was slightly more as compared to *S. sibiricus*. Hence the mean colony diameter of mycelium (± standard deviation) in different solid media was recorded after 09 and 10 days of incubation period at ambient temperature (25°C) for *B. edulis* and *S. sibiricus* respectively.

In case of *B. edulis*, the mean colony diameter of mycelium was maximum on Modified Melin Norkran's Medium (8.50±0.16 cm) followed by Potato Dextrose Agar (7.94±0.10 cm), Hagem's Medium (7.60±0.19 cm), Yeastal Potato Dextrose Agar (7.22±0.21 cm), Paridham Yeast Malt Dextrose Medium (7.08±0.17 cm), Malt Yeast Agar Extract (7.00±0.28 cm), Maize Grain Extract (6.24±0.19 cm), Wheat Grain Extract (5.06±0.22 cm) and Martin's Medium

(4.00±0.14 cm). Minimum colony diameter of mycelium was recorded in Dimmick Agar Extract (1.40±0.14 cm) (Fig.1. b and Fig. 2).

The mean colony diameter of mycelium in case of *S. sibiricus* was maximum on Hagem's Medium (7.30±0.42 cm) followed by Modified Melin Norkran's Medium (6.98±0.17 cm), Potato Dextrose Agar (6.52±0.23 cm), Yeastal Potato Dextrose Agar (6.40±0.14 cm), Paridham Yeast Malt Dextrose Medium (6.18±0.21 cm), Dimmick Agar Extract (6.00±0.14 cm), Malt Yeast Agar Extract (5.80±0.14 cm), Wheat Grain Extract (5.46±0.29 cm) and Maize Grain Extract (5.20±0.33 cm). Minimum colony diameter of mycelium was recorded in Martin's Medium (4.86±0.16 cm) (Fig. 1. d and Fig. 2).

It is clear from the results of ten solid media evaluated that the *B. edulis* shows maximum growth in Modified Melin Norkran's Medium whereas Dimmick Agar Extract shows minimum growth of mycelium. The mean colony diameter of mycelium in Modified Melin Norkran's Medium was significantly more than all other solid media evaluated in case of *B. edulis*. Thus, Modified Melin Norkran's Medium was used best solid medium for the mycelial growth of *B. edulis*. Hence it has been now used as basal solid medium in subsequent studies.

In case of S. sibiricus, it is revealed from the results of ten solid media evaluated that maximum growth was recorded in Hagem's Medium whereas Martin's Medium showed minimum growth of mycelium. The mean colony diameter of mycelium in Hagem's Medium was significantly more than all other solid media evaluated except Modified Melin Norkran's Medium. Thus, Hagem's Medium was observed as best solid medium for the mycelial growth of *S. sibiricus*. Hence it has been now used as basal solid medium in subsequent studies.

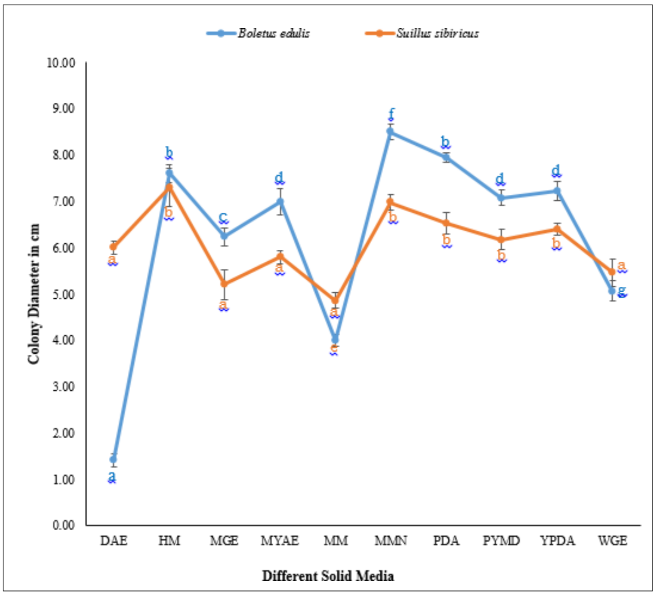

Fig 2: Effect of different solid media on the mycelial growth of *Boletus edulis* and *Suillus sibiricus*. The Mean ± S.D. titled with same letters are not significantly different as revealed through One Way ANOVA with Tukey's Multiple Comparison Test (p≤0.05).

Effect of Temperature

To study the effect of temperature on linear growth of mycelium, the cultures of both mushrooms were inoculated on the basal solid medium. The basal solid media were Modified Melin Norkran's Medium and Hagem's Medium used for *B. edulis* and *S. sibiricus* respectively in petriplates which were incubated at temperature ranging from 5°C to 40°C in different incubators. The mean colony diameter (± standard deviation) at different temperatures is graphically presented in Fig. 3.

In case of *B. edulis*, the mean colony diameter of mycelium at 20°C (8.62±0.25 cm) was maximum and followed by 25°C (7.90±0.14 cm), 15°C (7.10±0.20 cm), 30°C (6.80±0.14 cm) and 35°C (5.66±0.19 cm). Minimum growth was observed at 10°C (5.28±0.21 cm) whereas growth was completely ceased at 5°C and 40°C (Table 10). Whereas the mean colony diameter of mycelium in case of *S. sibiricus* at 20°C (7.52±0.27 cm) was maximum and followed by 25°C (7.04±0.14 cm), 15°C (6.38±0.14 cm), 30°C (5.74±0.17 cm) and 35°C (4.20±0.17 cm). The minimum growth was observed at 10°C (4.06±0.17 cm) whereas growth was completely ceased at 5°C and 40°C (Fig. 3).

It can be concluded from the results that maximum and minimum mycelial growth was recorded at 20°C and 10°C respectively for the both mushrooms. The mycelial growth was completely ceased at 5°C and 40°C. The mean colony diameter of mycelium at 20°C was significantly more than all other temperatures studied. Thus 20°C was considered as the optimum temperature for mycelial growth of both mushrooms. So this temperature was used as in the further studies.

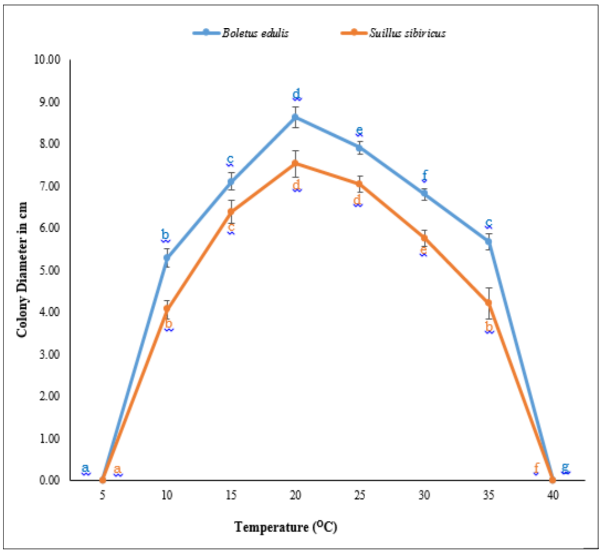

Fig 3: Effect of different temperature on the mycelial growth of *Boletus edulis* and *Suillus sibiricus*. The Mean ± S.D. titled with same letters are not significantly different as revealed through One Way ANOVA with Tukey's Multiple Comparison Test (p≤0.05).

Effect of Hydrogen Ion Concentration (pH)

To record the effect of pH on linear growth of mycelium, the pH of the basal solid medium for both mushrooms were adjusted at different pH levels ranging from 4.0-8.5. For each pH level the fungus was inoculated and incubated at optimum temperature of 20°C in different incubators. The mean colony diameter (± standard deviation) at different pH levels is recorded.

It is clear from the results that the maximum growth of *B. edulis* mycelium was supported at pH 5.5 (8.82±0.34 cm) followed by pH 5.0 (8.14±0.19 cm), pH 6.0 (7.32±0.17 cm), pH 4.5 (7.20±0.23 cm), pH 6.5 (6.84±0.19 cm), pH 7.0 (6.14±0.21 cm) pH 7.5 (5.48±0.17 cm), pH 4.0 (5.18±0.26 cm) and pH 8.0 (4.88±0.17 cm). Minimum growth was recorded in pH 8.5 (4.30±0.14 cm) (Fig. 4).

It is clear from the results that in case of S. sibiricus the maximum growth of mycelium was also supported at pH 5.5 (7.58±0.28 cm) followed by pH 6.0 (7.04±0.27 cm), pH 6.5 (6.60±0.14 cm), pH 5.0 (6.08±0.25 cm), pH 7.0 (5.18±0.33 cm), pH 4.5 (4.72±0.34 cm), pH 7.5 (4.66±0.14 cm), pH 8.0 (4.22±0.26 cm), pH 4.0 (3.40±0.29 cm). The minimum growth was recorded in pH 8.5 (2.96±0.19 cm) (Fig. 4).

It is evident from the results that maximum and minimum colony diameter of mycelium was recorded at pH 5.5 and pH 8.5 respectively for the both mushrooms. The mean colony diameter of mycelium at pH 5.5 was significantly more in most of the pH levels. Thus pH 5.5 was recorded as best for mycelial growth of *B. edulis* and *S. sibiricus*, hence in subsequent experiment to see the effect of light and darkness on the mycelial growth of these mushrooms were carried out at this pH 5.5 only.

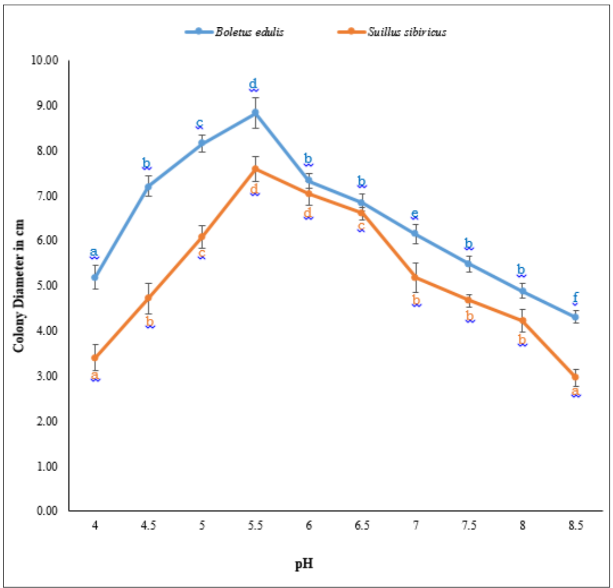

Fig 4: Effect of different pH on the mycelial growth of *Boletus edulis* and *Suillus sibiricus*. The Mean ± S.D. titled with same letters are not significantly different as revealed through One Way ANOVA with Tukey's Multiple Comparison Test (p≤0.05).

Effect of Light and Darkness

To record the effect of light and darkness on the growth of *B. edulis* and *S. sibiricus* mycelium, petriplates containing basal solid medium for both mushrooms adjusted at pH 5.5 were inoculated and incubated at 20°C in light and darkness. The mean colony diameter of mycelium (± standard deviation) at light and darkness conditions is recorded.

In case of B. edulis, the growth of mycelium was better in dark (8.82±0.29 cm) than in light (7.24±0.21 cm) (Fig. 5). It is clear from the results that the growth of mycelium was better in dark (7.60±0.19 cm) than in light (7.10±0.16 cm) in case of *S. sibiricus* (Fig. 5). Thus, it is evident from the results that maximum mycelial growth was supported in darkness as compared to light.

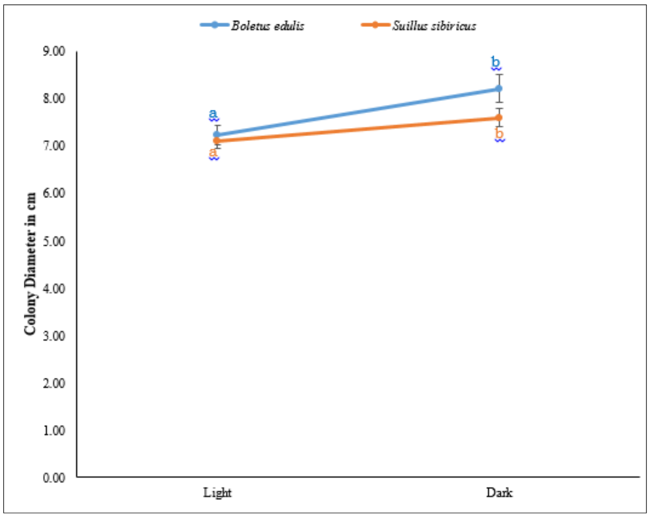

Fig 5: Effect of light and darkness on the mycelial growth of *Boletus edulis* and *Suillus sibiricus*. The Mean ± S.D. titled with same letters are not significantly different as revealed through Student's t-test (p≤0.05).

Discussion

During the present study cultural and physiological studies were carried for *B. edulis* and *S. sibiricus* mushrooms. The similar study was carried out by various researchers, France and Reid (1984) [9] tested *Cenococcum geophilum*, *Pisolithus tinctorius*, *Thelephora terrestris* and *Suillus granulatus* for their ability to grow on different nutrient media. Sharma and Mishra (1988) [29] recorded maximum growth of *Laccaria laccata* on Modified Melin-Norkran's medium. The *Laccaria bicolor* was found to grow at best rate and colony diameter was maximum in Malt Agar Extract (Santiago-Martinez *et al.*, 2003) [26]. The higher average growth in diameter of *Pisolithus tinctorius* strains was recorded in the Potato Dextrose Agar Medium whereas minimum in the Malt Extract Medium (Garcia-Rodriguez *et al.*, 2006) [10]. Sehgal *et al.* (2019 a,b) [27, 28] evaluate different solid media for the best mycelial growth of *Amanita ceciliae* and *Lactarius sanguifluus*. Both mushrooms showed maximum growth on Potato Dextrose Agar Medium.

Chang and Chien (1988) [4] cultured different species of fungi on MMN medium in a temperature ranges from 5°C to 40°C. They concluded that the optimum temperature for most of the fungi ranged from 22°C to 27°C with maximum growth at 25°C whereas in most of the cases the mycelia stopped to below 10°C and above 35°C. Hacskaylo *et al.* (1965) [13] observed 24°C as the optimum temperature for the mycelial growth of *Amanita rubescens* and *Suillus luteus*. Optimal temperature for the mycelial growth lies between 18°C and 27°C for majority of fungi (Harley, 1959) [14], the growth was ceased above 35°C and below 5°C in case of many fungi (Hacskaylo *et al.*, 1965) [13]. In a similar study Hung and Chien (1978) [16] recorded the physiological parameters of two ectomycorrhizal fungi (*P. tinctorius* and *S. bovines*) in five media. Out of five media tested *P. tinctorius* grew well on Modified Melin-Norkran's Medium (MMN) while *S. bovinus* grew equally on all the media tested. Peng and Chien (1988) [22] observed 30°C, 20°C and 25°C as optimum temperatures for the growth of *Boletus* sp., *B. qriseus* and *Suillus grevillei* respectively. Cline *et al.* (1987) [5] showed that optimal growth of *Pisolithus tinctorius* isolates varied between 21°C and 32°C and had reduced growth at 38°C. Sehgal *et al.* (2019 a,b) [27, 28] observed best mycelial growth of *Amanita ceciliae* and *Lactarius sanguifluus* at 20°C and 25°C respectively.

Both the mushroom showed maximum growth at pH 5.5. These results are in consistent with early observations made by Modess (1941) [20] who determined the pH requirements of a large number of Hymenomycetes members. The optima varied between pH 3.5 to pH 5.9 with little growth of many species below pH 2.5 or above pH 6.5. Thapar (1988) [34] reported that the pH requirements varied among different isolates of *Cenococcum graniforme*. *Laccaria laccata* showed maximum colony growth at pH 5.0 while its dry weight was maximum at pH 7.0 (Jha *et al.*, 1990) [17]. These authors also observed better colony diameter of *P. tinctorius* at pH 7.0 and dry weight at pH 6.0. Sanchez *et al.* (2001) [25] observed that the strains of some edible fungi *Lactarius deliciosus*, *Suillus granulatus* and *Suillus luteus* collected from the regions of the Mediterranean showed increased in their biomass production when pH of solid media increased from 4.5 to 5.5 whereas *Tricholoma focale* diminished its biomass with similar increments in pH. However, reported that The *Boletus griseus*, *Suillus bovines*, *Cenococcum graniforme* and *Laccaria laccata* grew profusely at acidic and neutral pH (Peng and Chien, 1988 and Jha *et al.*, 1990) [22, 17]. Sehgal *et al.* (2019 a,b) [27, 28] also observed the best mycelial growth of *Amanita ceciliae* and *Lactarius sanguifluus* at pH 5.0.

The results of the present study are also in agreement with the results of Cochrane (1958) [6] and Hung and Chien (1978) [16] reported the inhibitory effect of light on the growth of *Pisolithus tinctorius* and *Suillus bovines* whereas growth of mycelia was promoted in the darkness. The maximum mycelial growth was observed in case of *Laccaria laccata*, *Amanita muscaria*, *Amanita ceciliae* and *Lactarius sanguifluus* under darkness (Raman and Thiagarajan, 1988 and Sehgal *et al.*, 2019 a,b) [23, 26, 27].

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