KAVAKA 60 Special Issue: 84-92 (November 2024) DOI: 10.36460/Kavaka/60/sp/2024/84-92

Effects of Physicochemical Factors on Vegetative Growth of *Lentinus cladopus* - A Wild Edible Mushroom

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(Submitted on August 31, 2024; Accepted on September 22, 2024)

ABSTRACT

***Lentinus cladopus* Lév is an edible lignicolous mushroom which was collected from the wild and subsequently cultured with a view to investigate the effect of variable temperatures, incubation period, pH and different carbon and nitrogen sources for maximum mycelia growth. During the investigations undertaken optimum temperature of 30±1 °C, incubation period of 10 days and pH ranging between 4.0-5.0. Dextrose amongst the carbon sources and Glycine amongst the nitrogen sources were found to support maximum vegetative growth.**

Keywords: *Lentinus cladopus*, Incubation period, Vegetative growth, pH, Carbon sources, Nitrogen sources.

INTRODUCTION

The genus *Lentinus* Fr. is a member of Class - Agaricomycetes, Order - Polyporales and Family - Polyporaceae (Kirk *et al.*, 2008). It is characterized by lignicolous, xeromorphic, and tough to leathery fruit bodies having lamellae with serrated margins. In the context, the hyphal system is dimitic with either skeletal or binding hyphae associated with generative hyphae (Pegler, 1977; Singer, 1986). Its species are widely distributed especially in subtropical regions with as many as 684 records in the world over (MycoBank, 2024). As compared from India only 20 species are documented so far (Sharma and Atri, 2015). Many of its species including *L. connatus*, *L. cladopus*, *L. cochleatus*, *L. crinitus*, *L. glabratus*, *L. lepidus*, *L. kauffmanii*, *L. polychrous*, *L. sajor-caju*, *L. squarrosulus*, *L. torulosus* and *L. tuberregium* are reported to possess variable degree of edibility (Pegler, 1983).

L. cladopus Lév, on which the present investigations have been undertaken, is reported from in India, Thailand and Philippines (Natarajan, 1978; Karunarathna *et al.*, 2011; De Leon *et al.*, 2013; Sharma and Atri, 2015). The mushroom grow in caespitose clusters and is characterized by membranous, whitish, smooth basidiocarps having convex to sub-infundibuliform pileus and cylindrical, concolorous stipe (Pegler 1977; 1983). It is an edible mushroom and a potential source of nutritional, nutraceutical food components (Gulati *et al.*, 2011; Sharma and Atri, 2014). This mushroom is collected by the local communities and reported to be edible in different parts of India (Natarajan and Raman, 1981; Verma *et al.*, 1995; Gulati *et al.*, 2011; Tanti *et al.*, 2011; Sharma and Atri, 2014). The prominent tribes of Nagaland state of India are reported to collect *L. cladopus* and use it for personal consumption (Choudhary *et al.*, 2015). Natarajan and Raman (1980) reported the *in vitro* production of fertile fruit bodies of *Lentinus cladopus* in liquid culture. Different defined, undefined and natural media were used. Among the various natural media such as beetroot extract, carrot extract, 5% malt extract, sawdust-carrot extract and tomato extract, sawdust-carrot extract was the best in producing normal fruitbodies of *Lentinus cladopus* in 23 d in still culture. While working on this mushroom, Atri and Lata (2013) discussed various parameters including media requirements, substrates, etc. for its culturing and cultivation. At the same time, Atri and Guleria (2013) also documented the effect of trace elements, and different concentrations of growth regulators, and vitamins on the vegetative growth of *L. cladopus*. In the present study, the effect of different temperatures, incubation period, pH, carbon and nitrogen sources on the mycelial growth of *L. cladopus* are presented.

MATERIAL AND METHODS

The material

Lentinus cladopus fructifications were gathered from the rotten portion of live stem bole of *Albizia chinensis* (Osbeck) Merrill from Palampur (Himachal Pradesh). Pure culture of the mushroom was raised by using soft tissue from the confluence point of sporophore with the stipe following the methodology given by Yadav (2005). The culture was purified and maintained by repeated subculturing on Potato Dextrose Agar (PDA) at 28±1ºC in Mycology laboratory, Department of Botany, Punjabi University Patiala, Punjab, India. Pure culture was deposited in CSIR-IMTECH (Institute of Microbial Technology Chandigarh) India under Microbial Type Culture Collection (MTCC) accession number 10948.

Chemicals used

The chemicals used were agar, amino acids (Glycine, L-Proline, DL-Serine, DL- Methionine, DL-nor-Leucine, L-Hydroxyproline, DL-β-Phenylalanine, DL-Aspartic Acid, L-Ornithine monohydrochloride, L-Cystine), carbon sources (arabinose, galactose, mannose, ribose, starch, xylose, rhamnose, melibiose, glucuronic acid, citric acid), dextrose, potassium dichromate, malt extract, maltose, peptone extract, sulphuric acid and yeast extract They chemicals were of Merck and Sigma grade. Double distilled water was used for the experiments.

Sterilization

The glassware were rinsed thoroughly with tap water and then immersed in sulphuric acid - dichromate solution (potassium dichromate - 100 g + concentrated sulphuric acid - 600 mL + water - 400 mL) for 24 h (Tuite, 1969). Glassware were washed by dipped in sulphuric acid - dichromate solution, then by using soap solution Teepol, followed by rinsing with tap water and then with the distilled water. Sterilization of glassware was done by dry heat sterilization in hot air oven at 170ºC for 60 min followed by moist heat sterilization in an autoclave at 15 lbs pressure and 121ºC temperature for 1 h. Media and other solutions to be used were also subjected for sterilization in the autoclave. The heat sensitive chemicals such as amino acids, growth regulators and vitamins were sterilized using millipore filters (0.22 μm pore size). Different inoculating aids such as inoculation needles, forceps, cork borer, etc., were dipped in ethyl alcohol and then sterilized over flame of gas burner to red hot.

Inoculum production and inoculation

The fungal culture maintained on Potato Dextrose Agar (PDA) solid medium was used for inoculation. Every time, 5 d old mycelia were used to carry out the various experiments. Inoculation was done aseptically under laminar air flow. Uniform small medium discs with mycelia load of 0.9 mg - 1.0 mg were implanted in the centre of the flasks or Petri plates containing solid medium and 1.0 mL of homogenized culture was used as the inoculum for inoculation in the flasks containing liquid media.

Effect of temperature on the mycelial growth

Experiment to determine the favourable temperature for maximum mycelial growth of *L. cladopus* was conducted on the best evaluated Malt Extract Agar solid medium and Malt Broth liquid medium. For the experiment, 5 different temperatures i.e., 20ºC, 25ºC, 30ºC, 35ºC and 40ºC were considered. For each temperature, 3 replicates were kept by pouring 20 mL of medium in 100 mL sterilized conical flasks. Later on, the inoculated flasks were incubated at 30±1ºC for 10 d.

Incubation period for the mycelial growth

To investigate the incubation period for the vegetative growth of *L. cladopus*, experiment was performed on Malt Broth (MB) basal liquid medium. For this purpose, 20 mL of medium was dispensed in the 100 mL sterilized conical flasks and 3 replicates were prepared for harvesting the mycelia on daily basis. Then, the inoculated flasks were incubated at 30±1ºC for 16 d and the mycelial mats were harvested on daily basis for observations.

Effect of different hydrogen ion concentration (pH) on the mycelial growth

Experiment for the determination of suitable hydrogen ion concentration (pH) for best mycelial growth of *L. cladopus* was performed on the Malt Extract Agar solid medium as well as Malt Broth liquid medium. Different pH levels of 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 were considered and the media were adjusted by using 1 N NaOH and 1 N HCl. Stabilization of pH of the media was done by using citrate and phosphate buffers. Three replicates of each pH level were prepared by pouring 20 mL of the medium in the

100 mL sterilized flasks and inoculated flasks were incubated at 30±1ºC for 10 d.

Effect of carbon sources on the mycelial growth

To observe the effect of different carbon sources on the vegetative growth of *L. cladopus*, 10 carbon sources, namely arabinose, galactose, mannose, ribose, starch, xylose, rhamnose, melibiose, glucuronic acid and citric acid were selected. Dextrose, 15 g/L (carbon source) in the Malt Broth (MB) basal medium was replaced with the selected carbon sources on the basis of their molecular formulae and molecular weight. Polysaccharides selected as carbon source were added at a rate of 5 g/L in the medium (Gaur and Agnihotri, 1979). Two controls were kept, one without adding any carbon source and second with dextrose as a carbon source in the medium. Three replicates of each carbon sources and controls were prepared by pouring 20 mL of medium in 100 mL sterilized conical flasks. Later on, the inoculated flasks were incubated at 30±1ºC for 10 d.

Effect of nitrogen sources on the mycelial growth

To study the effect of nitrogen sources on the mycelial growth of *L. cladopus*, 10 different nitrogen sources, namely Glycine, L-Proline, DL-Serine, DL- Methionine, DL-nor-Leucine, L-Hydroxyproline, DL-β-Phenylalanine, DL-Aspartic Acid, L-Ornithine monohydrochloride and L-Cystine were selected. Peptone, 1 g/L (nitrogen source) in the basal medium was replaced by selected nitrogen sources on the basis of their molecular formulae and molecular weight. Two controls were kept, one without adding any nitrogen source and second with peptone as a nitrogen source in the medium. Three replicates of each nitrogen sources and controls were prepared by transferring 20 mL of medium in 100 mL sterilized conical flasks. Later on, the inoculated flasks were incubated at 30±1ºC for 10 d.

Observation criteria

The inoculated and incubated conical flasks containing solid media were observed on daily basis and the diameter of mycelial colony was recorded in centimeter scale to determine the mycelia growth. Elevation of colony, margin edge and shape of colony were also recorded during growth (Gunasekaran, 1995). Inoculated conical flasks of liquid media were kept for 16 d incubation period. Mycelial mats collected after 16 d were then thoroughly washed and kept in an oven maintained at 65ºC in small pre-weighed Petri plates. Dry weight of mycelial mat was measured after the completion of the experiment using OHAS Adventure TM digital weighing machine. The results were subjected to statistical analysis.

RESULTS

Effect of temperature for mycelial growth

In the experiment conducted, the maximum radial mycelial extension (4.52 cm) on an average daily basis was recorded on Malt Extract Agar solid medium at 30ºC. In the liquid Malt Broth medium, maximum mycelial growth (17.26 mg/mL) on dry weight basis was recorded at 30ºC. At the end of the experiment, white coloured mycelial mat was formed which was quite dense and thick. In comparison, next best mycelial growth was recorded at 25ºC, and the minimum vegetative growth was there at 40ºC. The colony characteristics, density of the mycelial mat and growth of mycelia was best at 30ºC both in the solid as well as liquid media. Hence, 30ºC was evaluated as the optimum temperature for the mycelial growth of *L. cladopus* on both solid as well as liquid media. The effect of various temperatures on the mycelial growth of *L. cladopus* is presented in **Table 1**.

Table 1: Effect of temperature on mycelial growth of *L. cladopus* in solid and liquid media.

Temperature (˚C)	Colony diameter (cm) on Malt Extract Agar medium ± S.D.	Mycelium dry weight in Malt Broth (mg/mL) ± S.D.
20	3.42 ± 0.34	14.83 ±0.54
25	4.34 ± 0.17	15.22 ±0.54
30	4.52 ± 0.10	17.26 ±1.27
35	1.15 ± 0.10	0.48 ±0.25
40	0.75 ± 0	0.13 ± 0.04

Effect of incubation period on mycelial growth in Malt Broth

During the initial days of incubation, there was very slow growth of mycelium in the medium, which increased rapidly after 4th d of incubation and achieved maximum level (12.01 mg/mL) on 10th d of incubation. Thereafter, the mycelial growth remained static on 11th d (11.20 mg/mL) and 12th d (11.06 mg/mL), thereafter growth gradually declined with the passage of time. Thus, 10 d of incubation gave best result. The mycelial growth during different incubation period with standard deviation is presented in **Table 2.**

Table 2: Mycelial growth of *L. cladopus* during incubation period in liquid medium.

Days	Mycelial dry weight in Malt Broth (mg/mL) ± S.D.
1	0
2	2.40 ± 0.35
3	3.21 ± 0.51
4	4.98 ± 1.81
5	5.79 ± 0
6	7.37 ± 0.64
7	8.11 ± 0
8	9.41 ± 1.00
9	10.70 ± 0.16
10	12.01 ± 0.07
11	11.20 ± 0.14
12	11.06 ± 0.77
13	10.99 ± 0.24
14	10.82 ± 1.18
15	9.67 ± 0.26
16	9.40 ± 2.65

Effect of different pH levels on mycelial growth

Maximum mycelial growth was observed at pH 4.5 both on Malt Extract Agar solid medium (4.13 cm) as well as in Malt Broth liquid medium (6.18 mg/mL). At acidic pH 4.5, very thick and dense mycelial mat was observed. It was followed pH 4.0 in solid as well as liquid medium. Negligible or no growth occurred at pH 8.0, 8.5 and 9.0. The results of the present investigation revealed that acidic pH (4.0, 4.5, and 5.0) significantly enhanced the mycelial growth of *L. cladopus* whereas mycelial growth was poor at alkaline pH (**Table 3**).

Table 3: Effect of different pH levels on mycelial growth of *L. cladopus* on solid and liquid medium.

pH	Colony diameter (cm) on Malt Extract Agar medium ± S.D.	Mycelial dry weight (mg/mL) in Malt Broth ± S.D.
3.0	2.33 ± 0.20	1.75 ± 0.52
3.5	2.93 ± 1.24	3.75 ± 1.24
4.0	4.00 ± 0.13	5.41 ± 0.79
4.5	4.13 ± 0.10	6.18 ± 0.31
5.0	3.33 ± 0.57	3.83 ± 0.23
5.5	2.10 ± 0.23	1.74 ± 0.86
6.0	1.88 ± 0.05	0.75 ± 0.07
6.5	1.64 ± 0.98	0.65 ± 0.17
7.0	1.08 ± 0.22	0.66 ± 0.32

pH	Colony diameter (cm) on Malt Extract Agar medium ± S.D.	Mycelial dry weight (mg/mL) in Malt Broth ± S.D.
7.5	0.76 ± 0.07	0.75 ± 0.00
8.0	0 ± 0	0.58 ± 0.17
8.5	0 ± 0	0.34 ± 0.00
9.0	0 ± 0	0.13 ± 0.14

Evaluation of different carbon sources on mycelial growth

Out of the 10 carbon sources and 2 controls used, the maximum mycelial growth was obtained in the control with dextrose as carbon source (10.28 mg/mL) in the Malt Broth (MB). This was followed by arabinose (8.11 mg/mL), galactose (7.53 mg/mL), mannose (7.15 mg/mL), ribose (6.35 mg/mL) and starch (6.08 mg/mL). Least mycelial growth was recorded in dextrose deficient control (3.23 mg/mL). In the medium with glucuronic acid and citric acid as carbon source, no vegetative growth was recorded. Arabinose supported the maximum vegetative growth next to dextrose as a carbon source (**Table 4**). Hence Arabinose can serve as a good substitute for the replacement of dextrose in the basal medium of as a carbon source.

Table 4: Effect of different carbon sources on mycelial growth of *L. cladopus.*

Carbon source	Mycelium dry weight in Malt Broth (mg/mL) ± S.D.
Control (with Dextrose as carbon source)	10.28 ± 2.44
Arabinose	8.11 ± 0.86
Galactose	7.53 ± 0.45
Mannose	7.15 ± 1.10
Ribose	6.35 ± 0.72
Starch	6.08 ± 0.59
Xylose	6.02 ± 0.42
Rhamnose	5.53 ± 0.46
Melibiose	4.68 ± 0.83
Control (without carbon source)	3.23 ± 0.41
Glucuronic acid	0 ± 0
Citric Acid	0 ± 0

Evaluation of different nitrogen sources on mycelial growth

Out of the 10 nitrogen sources and 2 controls used, the maximum mycelial growth was achieved in the medium containing Glycine (8.98 mg/mL) followed by control with Peptone as a nitrogen source (8.85 mg/mL), L-Proline (8.34 mg/mL) and DL-Serine (8.14 mg/mL) in the Malt Broth medium. Least mycelial growth was recorded in the medium containing L-Cystine (2.74 mg/mL). Glycine in the basal medium gave maximum vegetative growth, hence it proved as the best nitrogen source than Peptone. Thus, as a Nitrogen source glycine can be an excellent replacement for peptone in the basal medium. The mycelial dry weight with ± standard deviation (S.D.) is presented in **Table 5**.

Table 5: Effect of different nitrogen sources on the vegetative growth of *L. cladopus.*

Nitrogen sources	Mycelial dry weight in Malt Broth (mg/mL) ± S.D.
Glycine	8.98 ± 1.15
Control (with Peptone as nitrogen source)	8.85 ± 1.20
L-Proline	8.34 ± 0.50
DL-Serine	8.14 ± 1.06

Nitrogen sources	Mycelial dry weight in Malt Broth (mg/mL) ± S.D.
DL- Methionine	7.81 ± 1.16
DL-nor-Leucine	7.50 ± 0.52
L-Hydroxyproline	7.20 ± 1.07
DL-β-Phenylalanine	7.00 ± 0.83
Control (without nitrogen source)	6.83 ± 0.44
DL- Aspartic acid	6.79 ± 0.86
L-Ornithine monohydrochloride	6.26 ± 0.99
L-Cystine	2.74 ± 0.33

DISCUSSION

Various factors such as composition of culturing medium, temperature, pH, dark and light conditions, relative humidity, aeration and composition of substrates influence the mycelial growth and domestication of fungi (Chang and Miles, 2004; Scott and Mohammed, 2004). Amongst the physical parameters, temperature is found to be an important environmental factor which is essential for the mycelial growth. The maximum mycelial growth in Malt Extract Agar (4.52 cm) and Malt Broth (17.26 mg/mL) was recorded at 30±1ºC. Manjunathan and Kaviyarasan (2011) documented the maximum mycelial extension (96.12 mm) of *L. tuberregium* at 25ºC whereas the minimum growth was documented at 40ºC. Dulay *et al.* (2021) recorded 30ºC as the favourable temperature supporting best mycelial growth while working on 14 isolates of *Lentinus*. Kalaw *et al.* (2021) reported 30 ˚C optimum temperature for the vegetative growth of seven different strains of *Lentinus.* Lata and Atri (2022) documented 33ºC as the optimum temperature to culture *L. sajor-caju* under laboratory conditions. In the case of *L. tigrinus*, Kalaw *et al*. (2023) reported 30ºC temperature quite suitable for fast mycelial growth.

To measure the increase or decrease in the mycelial growth in *L. cladopus*, the mycelial mat was harvested on daily basis for 16 d. The maximum mycelial growth of 12.01 mg/mL was recorded on the 10th d of incubation, but De Leon *et al.* (2013) reported maximum growth of this species on 8th d of incubation. Klomklung *et al*. (2014) documented 7 d incubation period for *L. connatus* and 8 d for *L. roseus*. Shahtahmasebi *et al.* (2017) reported incubation period of 8 d for maximum mycelial growth of *L. tigrinus*, while Lata and Atri (2022) reported 10 d for *L. sajor-caju.*

One of the important parameter for mushroom culturing and cultivation is hydrogen ion concentration. The present results showed that acidic pH favoured the vegetative growth of *L. cladopus* and there was a sharp decline in vegetative growth in basic pH. The maximum vegetative growth of *L. cladopus* in Malt Extract Agar (4.13 cm) and Malt Broth (6.18 mg/mL) was recorded at pH 4.5. Manjunathan and Kaviyarasan (2011) recorded pH 6.5 for the maximum growth of *L. tuberregium* and Atri *et al*. (2007) recorded pH 4.0 for mycelial growth of *L. squarrosulus.* Dulay et al. (2021) have found pH 5.0 – 7.0 as favourable to support the maximum mycelial growth of 14 different isolates of *Lentinus.* Acidic pH 5.0 was found as the optimum for culturing 7 different species of *Lentinus* by Kalaw *et al.* (2021). While working on *L. sajor-caju*, Lata and Atri (2022) documented acidic pH 4.5 for the maximum mycelial extension (6.4 cm) on Malt Yeast Extract agar and maximum mycelial yield (8.37 mg/mL) in Yeast Glucose medium. In the case of *L. tigrinus*, Kalaw *et al.* (2023) reported pH 5.0 – 7.0 for the fastest mycelium growth.

Amongst the nutritional parameters, mushrooms require carbon and nitrogen for structural and functional purposes besides growth regulators, trace elements and vitamins in small amount for their nutrition (Atri and Guleria, 2013). In the present study, arabinose as a carbon source in the basal medium was found to support the maximum mycelial growth (8.11 mg/mL) of *L. cladopus* followed by dextrose. Gbolagade *et al*. (2006) documented fructose, followed by glucose as the most suitable carbon source for the vegetative growth of *L. subnudus*. For *L. squarrosulus*, Atri *et al.* (2009) have reported maximum mycelial growth in the medium containing dextrose as carbon source followed by fructose, whereas Anike *et al.* (2015) found starch supported the maximum mycelial yield (4.31 g/L) amongst 8 carbon sources

tested. Das *et al.* (2015) observed the maximum mycelial dry weight of *L. squarrosulus* in fructose (17.45 g/L) followed by maltose (17.12 g/L). Manjunathan and Kaviyarasan (2011) found that among 10 carbon sources, glucose supported the maximum mycelial yield of *L. tuberregium* While working with *L. swartzii*, *L. strigosus* and *L. tigrinus*, Dulay *et al.* (2020) demonstrated that mycelial growth is favourably supported by starch, fructose and sucrose, respectively. Observation of the current research revealed that the mycelial growth of *L. cladopus* was maximum in the basal medium containing dextrose as carbon source. *L. cladopus* and other species of *Lentinus* grow well in the medium that contains hexose sugars as the carbon source. Observations of the present study are mostly in agreement with the conclusion drawn by Gbolagade *et al*. (2006), Atri *et al.* (2009), Manjunathan and Kaviyarasan (2011) and Das *et al.* (2015) who worked with other species of *Lentinus.*

Amongst the nitrogen sources evaluated during the present investigation, Malt Broth liquid medium with peptone as nitrogen source gave high yield of mycelia growth of *L. cladopus.* In the basal medium, when peptone was substituted by glycine as nitrogen source maximum vegetative growth (8.98 mg/mL) was observed. Hence, glycine proved to be the best nitrogen source even better than peptone. Gbolagade *et al.* (2006) reported maximum mycelial dry weight of *L. subnudus* with L-glutamic acid as nitrogen source. Atri *et al*. (2009) evaluated DL-Leucine as the best nitrogen source for high mycelial yield (14.30 mg/mL) of *L. squarrosulus* followed by glycine (14.05 mg/mL). Amongst 10 different nitrogen sources evaluated for the mycelial growth of *L. tuberregium* by Manjunathan and Kaviyarasan (2011), yeast extract (68.23 mg/30 cm^3) followed by beef extract (64.56 mg/30 cm^3) supported the maximum mycelial growth. While working with 3 different strains of *L. squarrosulus*, Anike *et al.* (2015) investigated effect of 8 nitrogen sources on mycelial growth and demonstrated that yeast extract, which is a organic nitrogen source, showed the maximum mycelial yield of 5.24 g/L , 5.61 g/L and 6.12 g/L in strains 340, 339 and 218, respectively. In the case of *L. squarrosulus*, Das *et al.* (2015) reported substantial vegetative growth (9.77 mg/mL) with the amendment of yeast extract followed by peptone (5.68 mg/mL). Dulay *et al.* (2020) investigated the effect of different nitrogen sources on mycelial growth of *L. swartzii*, *L. strigosus* and *L. tigrinus* and found ammonium chloride, yeast extract, malt extract supported the high mycelial yield. The results obtained during the present investigation showed that amendment with amino acid glycine in the basal medium, followed by peptone in Malt Broth basal medium supported maximum mycelia growth of *L. cladopus.* The results of the present study are in line with the observations of Gbolagade *et al.* (2006) documented while working with *L. subnudus*, Manjunathan and Kaviyarasan (2011) with *L. tuberregium*.

CONCLUSION

The present work clearly established that the optimal temperature, acidic pH, incubation period and biochemical nutrients such as carbon, nitrogen are quite important for the luxuriant mycelial growth of *Lentinus cladopus*. The study is important to understand the role of some major factors for culturing *L. cladopus* to increase its mycelial yield. As is evident from the previous study, *L. cladopus* being edible mushroom is rich in the nutritional and nutraceutical constituents which are quite beneficial for human health. In view of this, there is a substantial scope for its domestication and large-scale production for use in the welfare of the society and to make it available for consumption as food.

ACKNOWLEDGEMENTS

Dr. Lata, the senior author extends her gratitude to Head, Department of Botany, Punjabi University Patiala, Punjab, India to provide the laboratory facilities for executing the present investigation. Financial aid in the form of SAP grant from UGC and FIST grant from DST, Government of India to the Department of Botany at Punjabi University, Patiala during the tenure of the present work is gratefully acknowledged. Dr. Gaurav Bhakri is grateful to Dean and Head, Department of Chemistry and Biochemistry, Sharda School of Basic Sciences and Research, Sharda University, Greater Noida, Uttar Pradesh, India for support and encouragement during manuscript drafting.

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