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OPTIMIZATION OF GROWTH CONDITIONS FOR LARGE SCALE PRODUCTION OF MYCELIUM FROM DIFFERENT *MORCHELLA* SPP. OF HIMALAYAN REGION

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Abstract

Morchella spp. is commonly known as morels. This mushroom is delicious and highly priced treasure of Himachal Pradesh. In Himachal Pradesh, it is commonly known as "Guchhi". This mushroom occurs in wild and not cultivated till date. The present study is done to determine the optimum conditions for mycelial growth of *Morchella* species. Mycelial cultures of *Morchella* species namely *M. crassipses M. angusticeps, M.semilibera, M. conica* and *M. esculenta* were taken for investigation. Total five parameters were taken under observation i.e. pH, temperature, C:N ratio, different media and effect of light in solid as well as broth media. The pH taken for observation ranges from pH 5 to pH 9 that is acidic to alkaline. Study revealed that good rate of mycelium growth observed in alkaline pH in comparison to acidic pH. Incubation temperature taken was 5ºC, 15ºC, 20ºC, 25ºC and 30ºC. Best temperature recorded was 25 ºC for the growth of mycelia. Different media used were 2% of Malt extract Agar (MEA), Yeast malt agar (YMA), Sabouraud's Dextrose Agar medium (SDA), Glucose peptone agar medium (GPA) and (PDA) Potato Dextrose Agar. Maximum radial growth and biomass production was observed in PDA and MEA followed by average growth in other media used for mycelium production. Carbon and Nitrogen (C:N) ratio was taken to analyse the requirement of Carbon and Nitrogen for accelerating mycelial growth of different *Morchella* species. Results of this study revealed that four species showed maximum yield at 20:2 except *M. conica* which showed maximum biomass production at 10:2. Parameter of light revealed that maximum growth was recorded in the presence of artificial light as compared to growth observed in dark for all five species of *Morchella*.

Key words: *Morchella,* pH, Temperature, Different media, Biomass, Carbon and Nitrogen Ratio.

Introduction

Mushrooms are well acknowledged for their economical, nutritional and medicinal values. These are used as a source of functional food as well as traditional remedy for curing many diseases. A mushroom found in wild contains mineral nutrients which are helpful in regulating metabolism of human body. They are delicious and antioxidant rich food hence popular in all age groups. *Morchella* which is commonly known as "Guchhi" found in Himalayan range in the early spring and monsoon season. In present study mycelial cultures of five species are taken to analyze the effect of fermentation factors like temperature, different media, pH, Carbon Nitrogen ratio and artificial light for determining best suited range for luxuriant mycelial growth. Gbolagade *et al*., (2006) suggested that environmental factors like pH, temperature, source, substrate type are responsible for determining the nutritional composition of any mushroom. Temperature play key role in growth and metabolites production as different studies suggested that metabolic reactions are temperature dependent variables. Mushrooms have different range of temperature for their development and this might be due to their habitats. Each mushroom grows on their optimal temperature which may vary from acidic to neutral for development of mycelium and formation of basidiocarp (Kalmis *et al*., 2008). Source and substrate are also important for the growth of mushroom fruiting bodies. Zanetti and Ranal, (1997) suggested that if source of carbon and nitrogen is not in balanced ratio that may decrease productivity of mushroom mycelia and fruiting bodies. Ratio of carbon and nitrogen starchy sources must be present in a balanced amount to enhance mycelium growth (Ryu *et al*., 2015). Aim of the study is to know

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the suitable conditions for enhanced production of the mycelia on large scale.

Material and Methods

Pure mycelial cultures of *Morchella* spp. were analyzed for the optimization of growth conditions on different parameters like temperature, different media, pH, Carbon Nitrogen ratio and light for determining best suited range for luxuriant mycelial growth. The pure cultures were maintained on MEA (Malt extract agar) for further studies.

Effect of temperature

8 mm disc of mycelium was inoculated in the sterilized Petri dishes containing 2% MEA which was incubated at different temperatures ranging from 5, 15, 20, 25 and 30°C. The diameter of the mycelium radial growth was measured in an interval of 2 days for 8 days in agar media during incubation. Malt extract broth (MEB) was prepared in 250 ml Erlenmeyer flask and inoculated aseptically with 8 mm disc of the fresh mycelial culture. Three replicates were maintained and incubated for 14 days at different temperature to get maximum growth. After 14 days mycelial mat was took by filtering through filter paper (Whatman No.1) dried in lyophiliser and finely crushed into dried powder.

Effect of pH

Mycelial cultures growth was observed at different pH i.e. 5, 6, 7, 8 and 9 on 2% MEA medium in Petri plates and liquid media in flasks. An adjustment to pH was done by 1M NaOH and HCl (N/10) (Amin *et al.*, 2008).

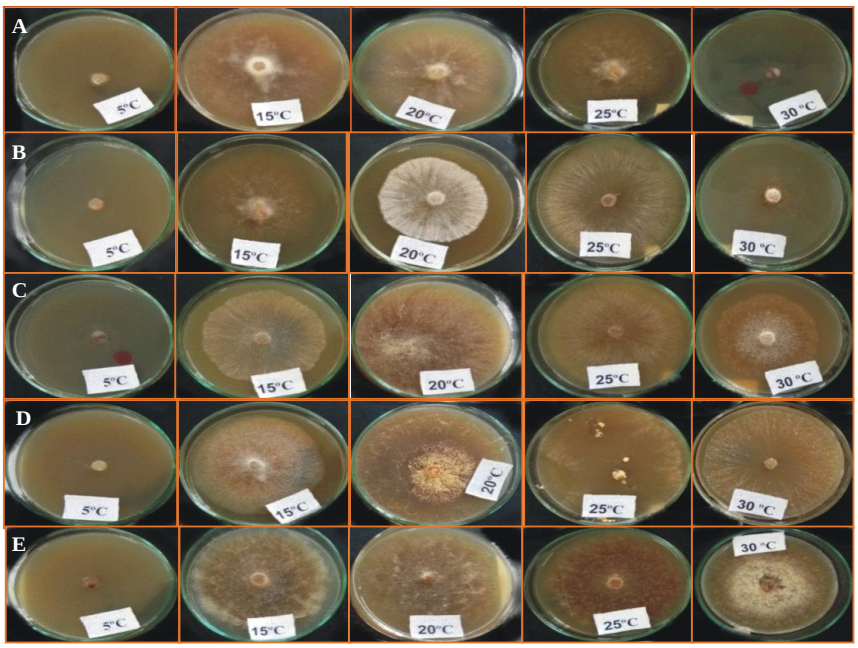

Fig. 1: Effect of different temperature on radial growth of *M. crassipses* (A); *M. semilibera* (B); *M. angusticeps* (C); *M. conica* (D) and *M. esculenta* (E).

Effect of Carbon (C), Nitrogen (N) sources ratio

Source is essential requirement for the growth of mycelia so carbon source was glucose and nitrogen source was ammonium tartrate. The medium agar and broth for growth were used consist of 2 g of ammonium tartrate, 20 g of glucose, 1g of KH_2PO_4, 0.2g of NaH_2PO_4, 5g of $MgSO_4.7H_2O$, 1.46g of 2, 2-dimethylsuccinate, 100 µg of thiamine HCl, 100 µg of $CaCl_2$, 100 µg of $FeSO_4$, 20 µg of $CuSO_4$, 10 µg of $MnSO_4$ per liter of medium for liquid culture and 20g of agar added for petri plates. The pH was kept up ±7 before autoclaving at 15 psi and 120°C for 20 min. Thiamine HCl was included in the medium after autoclaving. 6 treatments prepared to create different ratio of C:N were 20:2, 20:1, 20:0.5, 10:2, 5:2 and 5:0.5. Bits of 8 days old agar (6 mm diameter) were inoculated by active mycelia cultured before in 2% MEA (Malt extract agar) and MEB (Malt extract broth). Each treatment was processed in triplicates at optimum temperature under aseptic and controlled conditions.

Effect of different media

2% of Malt extract, Glucose peptone, Yeast malt, Sabouraud's dextrose and potato dextrose agar media were taken to analyse the effect of different media on the radial growth and biomass production of mycelia. Media and petri dishes (20 mm) were autoclaved at 121°C for 20 minutes. The mycelium discs (8 mm) of each *Morchella* spp. were placed in petri dishes containing each culture medium under aseptic conditions and incubated at 25°C temperature. The mycelium radial diameter was measured in mm every 2 days for 7 days and for biomass production all above described media were taken as broth and incubated for 14 days at optimum temperature (Nguyen and Ranamukhaarachchi, 2020)

Effect of light

The radial growth of mycelia and biomass production was observed in artificial light (500 lux) and for dark conditions plates and flasks were covered with aluminum foil. The mycelium discs (6 mm) placed in petri plates and flasks were inoculated with five discs in 100 ml of broth. Further these were incubated for radial growth of mycelia and biomass production at optimum temperature in 2% MEA for 7 and 14 days respectively (Priya and Geetha, 2016).

Table 1: Effect of different temperature on radial growth and biomass production.

S. No.	Mushrooms	5°C	15°C	20°C	25°C	30°C
Radial growth at different temperature (mm/day)						
1	*M. crassipses*	0±0	4.9±0.2^{b}	4.6±0.6^{b}	4.1±0.6^{b}	6±0.2^{a}
2	*M. angusticeps*	1.6±0.4^{d}	3.2±0.4^{c}	4.1±0.2^{b}	5.2±0.4^{a}	1.7±0.2^{d}
3	*M. semilibera*	1.3±0.6^{c}	4±0.4^{b}	4.6±0.2^{ab}	5±0.2^{a}	4±0^{b}
4	*M.conica*	0.2±0.4^{d}	3.6±0.4^{c}	4.5±0.2^{b}	9.6±0.4^{a}	4±0.4^{b}
5	*M.esculenta*	0±0	3.4±0.4^{c}	4.2±0.2^{bc}	11.6±0.4^{a}	4.9±0.4^{b}
Biomass yield at different temperature (mg/ml)						
1	*M. crassipses*	0±0	0.28±0.04^{d}	0.59±0.02^{b}	1.19±0.04^{a}	0.53±0.02^{c}
2	*M. angusticeps*	0.19±0.05^{e}	0.33±0.05^{d}	0.86±0.01^{b}	2.28±0.01^{a}	0.59±0.60^{c}
3	*M. semilibera*	0.03±0.05^{e}	0.37±0.07^{d}	1.18±0.03^{b}	2.08±0.09^{a}	0.68±0.07^{c}
4	*M.conica*	0.09±0.06^{d}	0.98±0.02^{b}	1.19±0.01^{a}	1.18±0.09^{a}	0.85±0.03^{c}
5	*M.esculenta*	0±0	0.60±0.01^{c}	0.68±0.05^{c}	2.09±0.09^{a}	0.83±0.01^{b}

In each row different letters means significant difference at P<0.05.
Values are Mean ±SD (n=3)

Statistical Analysis

Statistical analysis was done by one way annova of variance (ANNOVA) followed by Tukey's HSD test using SAV v.9.1.3 program. Differences at p*<0.05 were considered to be significant. Experiments were carried out in triplicates.

Results

Effect of different temperature

In case of *M. crassipses* luxuriant growth was observed at temperature 30°C(6±0.2 mm) followed by 15°C(4.9±0.2 mm) shown in fig. 1A and maximum biomass yield was recorded at temperature 25°C (1.19 ±0.04 mg/ml). In case of *M. angusticeps* maximum radial growth was recorded at temperature 25°C (5.2±0.4 mm) followed by 20°C(4.1±0.2 mm) shown in fig. 1B and maximum biomass production was also observed at 25°C (1.19±0.04 mg/ml) but other temperatures showed either average growth or least growth. *M. semilibera* showed a non-significant difference at the range 15-30°C with maximum growth at temperature 25°C(5±0.2 mm) shown in fig. 1C and maximum biomass yield was recorded at temperature 25°C(2.08±0.9 mg/ml). Best temperature for radial growth of mycelium was observed at 25°C(11.6±0.4 mm) shown in fig. 1E followed by other temperatures but showing poor mycelial growth for *M. esculenta* and maximum biomass production was also observed at 25°C (2.09±0.9 mg/ml). *M. conica* showed best radial growth at 25°C(9.6±0.4 mm) and least growth observed at temperature 5°C(2.9±0.4 mm) shown in fig.1D and maximum biomass yield was also observed at 25°C (1.18±0.01 mg/ml). As the temperature increases 15°C onwards the mycelium culture changes from white to brown and also observed sclerotia formation at temperature 25°C. All results are shown in table 1.

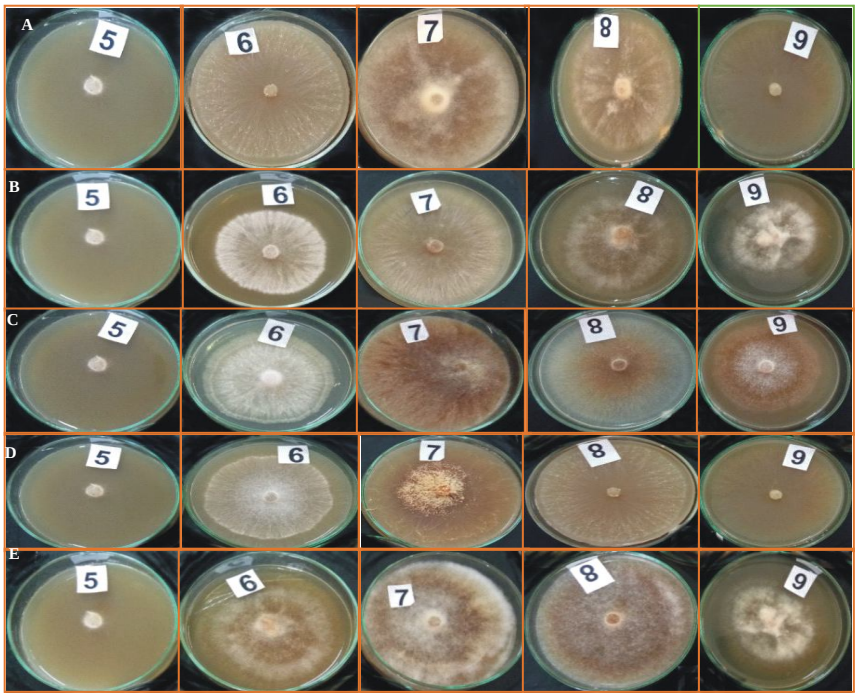

Fig. 2: Effect of different pH on radial growth of *M. crassipses* (A); *M. semilibera* (B); *M. angusticeps* (C); *M. conica* (D) and *M. esculenta* (E).

Effect of different pH

All species of *Morchella* showed variation in radial growth and biomass production shown in table 2 at different pH. The *M. crassipses* showed maximum radial growth of (8.3±0.14 mm/day) at pH 8 and least radial growth was recorded (4.5±0.2 mm/day) at pH 6 whereas biomass yield was observed maximum at pH 7 (2.31±0.01 mg/ml) shown in fig. 2A. The *M. angusticeps* showed maximum radial growth of (9.6±0.2 mm/day) at pH 8 and least radial growth was recorded (2.5±0.23 mm/day) at pH 9 shown in fig. 2B whereas biomass yield was observed maximum at pH 7 (2.25±0.01 mg/ml). *M. semilibera* exhibited maximum radial growth (9.6±0.2 mm/day), at pH 8 and average growth was observed at pH 6 and pH 7 shown in fig. 2C whereas biomass yield was observed maximum

Table 2: Effect of different pH on radial growth and biomass production.

S. No.	Mushrooms	pH5	pH6	pH7	pH8	pH9
Radial growth at different pH (mm/day)						
1	*M. crassipses*	0±0	4.5±0.2^{c}	6.3±0.17^{b}	8.3±0.14^{a}	4.6±0.23^{c}
2	*M. angusticeps*	0±0	3.3±0.23^{c}	6.7±0.11^{b}	9.6±0.2^{a}	2.5±0.23^{c}
3	*M. semilibera*	0±0	3.7±0.23^{b}	7.6±0.11^{a}	9.6±0.2^{a}	3.8±0.23^{b}
4	*M.conica*	0±0	3.8±0.23^{c}	7.4±0.2^{b}	10±0.15^{a}	3.8±0.23^{c}
5	*M.esculenta*	0±0	3.1±0.23^{b}	8.4±0.2^{a}	10.7±0.1^{a}	2.26±0.23^{b}
Biomass yield at different pH (mg/ml)						
1	*M. crassipses*	0±0	1.19±0.01^{b}	2.31±0.02^{a}	1.12±0.05^{c}	1.24±0.01^{b}
2	*M. angusticeps*	0±0	0.89±0.02^{b}	2.25±0.01^{a}	0.92±0.02^{b}	0.72±0.08^{c}
3	*M. semilibera*	0±0	1.15±0.13^{b}	2.28±0.02^{a}	1.11±0.07^{b}	1.05±0.11^{b}
4	*M.conica*	0±0	1.08±0.08^{b}	2.32±0.04^{a}	1.21±0.01^{b}	1.17±0.15^{b}
5	*M.esculenta*	0±0	0.85±0.05^{c}	2.34±0.07^{a}	1.32±0.04^{b}	0.67±0.05^{d}

In each row different letters means significant difference at P<0.05.
Values are Mean ±SD (n=3)

(2.32±0.04 mg/ml) at pH 7. The *M. conica* showed high range of radial growth of mycelium (10.0±0.15 mm/day) and minimum growth (3.8±0.23 mm/day) was recorded at pH 6 shown in fig. 2D while biomass yield was recorded maximum (2.32±0.04 mg/ml) at pH 7. The *M. esculenta* showed highest radial growth (10.7±0.1 mm/day) at pH 8 and lowest radial growth (2.26±0.23 mm/day) was analysed at pH 9 shown in fig. 2E whereas biomass yield was observed maximum (2.34±0.07 mg/ml) at pH 7. There was no growth of mycelia at pH 5. The mycelium of all *Morchella* spp. shown color change from white to brown as the pH turns acidic to alkaline with the formation of compact mass called sclerotia.

Effect of Carbon and nitrogen ratio

In *M. crassipses* there was effect on radial growth because of variation in C:N ratio. Least radial growth was observed in 10:2 (0.7±0.2 mm/day) and 5:0.5 (0.43±0.6 mm/day) with non-significant difference whereas maximum radial growth was observed at C:N ratio 20:2 (5.26±0.05 mm/day) shown in fig. 3A and biomass yield was observed maximum at 20:2 (1.31±0.01 mg/ml). In *M. angusticeps* maximum radial growth was observed in 20:1 (4.2±0.43 mm/day) followed by 20:2 with non-significant differences and least growth was observed at 5:0.5 (0.93±0.04 mm/day) shown in fig. 3B and maximum biomass yield was recorded at 20:2 (1.05±0.1 mg/ml) in liquid media. The *M. semilibera* showed maximum radial growth at 20:2 (4.93±0.15 mm/day) followed by 10:2 (4.23±0.23 mm/day) with non - significant difference and average growth was observed in other C: N ratio shown in fig. 3C while in broth C:N was 20:2 (1.23±0.03 mg/ml) and poor mycelial growth was observed at 5:0.5 (0.22±0.08 mg/ml). In case of *M. conica* maximum radial growth was observed in 10:2 (9.16±0.23 mm/day) and least growth was observed at 5:2 (1.26±0.05 mm/day) shown in fig. 3D whereas biomass yield was maximum at 10:2 (2.31±0.2 mg/ml) followed by 20:2 (2.28±0.6 mg/ml) and 20:1 (2.228±0.05 mg/ml) with non-significant difference. The *M. esculenta* showed maximum radial growth at 20:2 (10.23±0.15 mm/day) shown in fig. 3E and biomass growth of mycelia was also observed maximum at 20:2 (3.48±0.04 mg/ml). All values were given in table 3.

Effect of different media

The *M. crassipses* showed luxuriant radial growth of mycelia (6.93±0.83 mm/day) on Potato dextrose agar plates followed radial growth on (3.6±0.4 mm/day) on Malt extract agar plates shown in fig. 4A whereas

Table 3: Effect of carbon and nitrogen ratio on radial growth and biomass production.

S. No.		20:2	20:1	20:0.5	10:2	5:2	5:0.5
Radial growth at different Carbon and Nitrogen ratio (mm/day)							
1	*M. crassipses*	5.26±0.05^{a}	3.26±0.05^{a}	2.53±0.05^{c}	0.7±0.2^{c}	2±0.26^{b}	0.43±0.06^{c}
2	*M. angusticeps*	4.2±0.43^{ab}	4.3±0.46^{a}	3.06±0.1^{b}	3±0.2^{bc}	2.43±0.1^{c}	0.93±0.04^{d}
3	*M. semilibera*	4.93±0.15^{a}	2.6±0.17^{b}	1.26±0.23^{a}	4.23±0.23^{a}	1.83±0.24^{a}	0.86±0.37^{b}
4	*M.conica*	9.16±0.23^{b}	9.13±0.20^{b}	8.8±0.1^{b}	9.23±0.11^{a}	1.26±0.05^{c}	0±0
5	*M. esculenta*	10.23±0.15^{a}	9.93±0.15^{a}	4.36±0.23^{bc}	1.53±0.05^{cd}	0±0	0.46±0.05^{b}
Biomass yield at Carbon and Nitrogen ratio (mg/ml)							
1	*M. crassipses*	1.31±0.01^{a}	0.18±0.01^{b}	0.633±0.6^{c}	0.18±0.05^{e}	0.49±0.05^{d}	0±0
2	*M. angusticeps*	1.05±0.10^{a}	1.05±0.11^{a}	0.77±0.04^{b}	0.75±0.05^{b}	0.61±0.02^{b}	0.24±0.06^{c}
3	*M. semilibera*	1.23±0.03^{a}	0.65±0.05^{c}	0.38±0.05^{d}	1.06±0.06^{b}	0.46±0.07^{d}	0.22±0.08^{e}
4	*M.conica*	2.28±0.06^{ab}	2.28±0.05^{ab}	2.20±0.019^{b}	2.31±0.02^{a}	0.32±0.06^{c}	0±0
5	*M. esculenta*	3.48±0.04^{a}	2.48±0.04^{b}	1.09±0.05^{c}	0.38±0.04^{d}	0±0	0.12±0.01^{e}

In each row different letters means significant difference at P<0.05. Values are Mean ±SD (n=3)

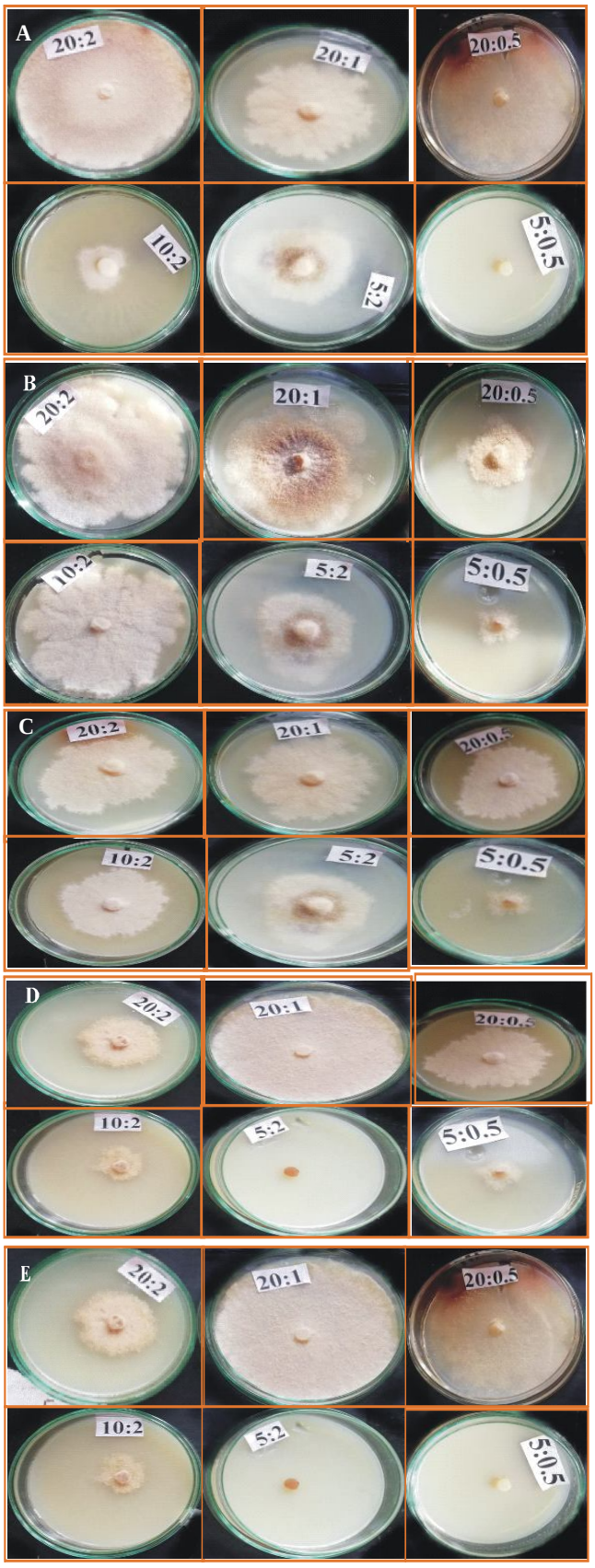

Fig. 3: Effect of carbon and nitrogen sources on radial growth of *M. crassipses* (A); *M. semilibera* (B); *M. angusticeps* (C); *M. conica* (D) and *M. esculenta* (E).

biomass yield was recorded maximum in Malt extract broth (2.12±0.02 mg/ml). In *M. angusticeps* radial growth was observed maximum on Potato dextrose agar plates (9.06±1.28 mm/day) and poor growth was observed in rest of the media used for study shown in fig. 4B while maximum biomass yield was recorded in Malt extract broth (2.28±0.04 mg/ml). The *M. semilibera* showed best radial growth (9.06±1.28) on PDA plates and least growth was observed on GPA (2.26±0.61 mm/day) shown in fig. 4C whereas maximum biomass production (2.50±0.14 mg/ml) was recorded in Malt extract broth. The *M. conica* exhibited maximum radial growth (2.86±0.11 mm/day) on PDA plates shown in fig. 4D while maximum biomass production was analysed in Malt extract broth (2.86 ±0.11 mg/ml). The *M. esculenta* showed maximum radial growth (13.3±0.6 mm/day) observed in Potato dextrose media shown in fig. 4E and maximum biomass yield was observed in Malt extract broth (3.27±0.01 mg/ml). All results have been given in table 4.

Effect of light

In all five species of *Morchella* we observed that radial growth shown in fig. 5 and biomass production shown table 5 showed significant difference in the presence and absence of light. Each species grew fairly well in the presence of artificial light (500 lux) whereas growth was arrested in darkness. *M. esculenta* showed maximum radial growth of (10.8±0.4 mm/day) and biomass production was recorded (3.31±0.04 mg/ml) in artificial light.

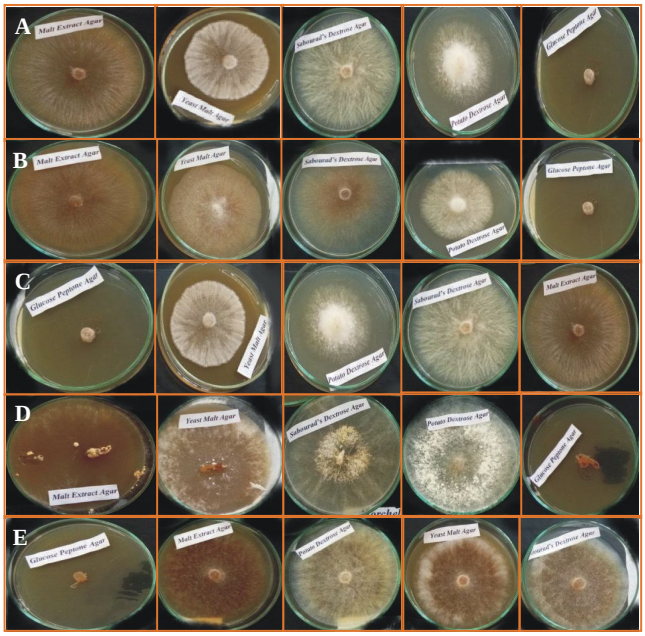

Fig. 4: Effect of different media on radial growth of *M. crassipses* (A); *M. semilibera* (B); *M. angusticeps* (C); *M. conica* (D) and *M. esculenta* (E).

Table 4: Radial growth and biomass yield in different media.

S. No.	Mushrooms	MEA	YMA	SDA	PDA	GPA
Radial growth at different media (mm/day)						
1	*M.crassipses*	3.6±0.4^{bc}	3.6±1.05^{bc}	3.73±1.06^{b}	6.93±0.83^{a}	1.6±0.4^{c}
2	*M.angusticeps*	5.6±0.4^{b}	1.86±0.49^{c}	2.8±0.4^{c}	9.06±0.83^{a}	1.46±0.83^{c}
3	*M.semilibera*	6±0.4^{b}	2.8±0.8^{c}	6.4±0.4^{b}	9.06±1.28^{a}	2.26±0.61^{c}
4	*M.conica*	8.53±0.83^{a}	5.6±0.4^{d}	6±0.4^{c}	2.05±0.21^{b}	2±0.8^{c}
5	*M.esculenta*	10±0.4^{b}	6.6±0.46^{c}	5.4±0.4^{c}	13.3±0.6^{a}	1.6±0.8^{d}
Biomass yield at different media (mg/ml)						
1	*M.crassipses*	2.12±0.02^{a}	0.94±0.07^{bc}	0.91±0.06^{c}	1.06±0.03^{ab}	0.61±0.04^{c}
2	*M.angusticeps*	2.28±0.04^{a}	0.87±0.02^{d}	0.76±0.02^{c}	1.63±0.06^{b}	0.55±0.03^{d}
3	*M.semilibera*	2.50±0.14^{a}	0.97±0.65^{abc}	0.86±0.02^{bc}	1.68±0.04^{ab}	0.55±0.01^{c}
4	*M.conica*	2.86±0.11^{a}	0.34±0.07^{d}	0.86±0.01^{b}	2.75±0.01^{ab}	0.67±0.02^{d}
5	*M.esculenta*	3.27±0.01^{a}	1.27±0.07^{c}	1.36±0.03^{c}	2.21±0.04^{b}	0.64±0.06^{d}

In each row, different letters mean a significant difference at P<0.05.
Values are Mean ±SD (n=3)

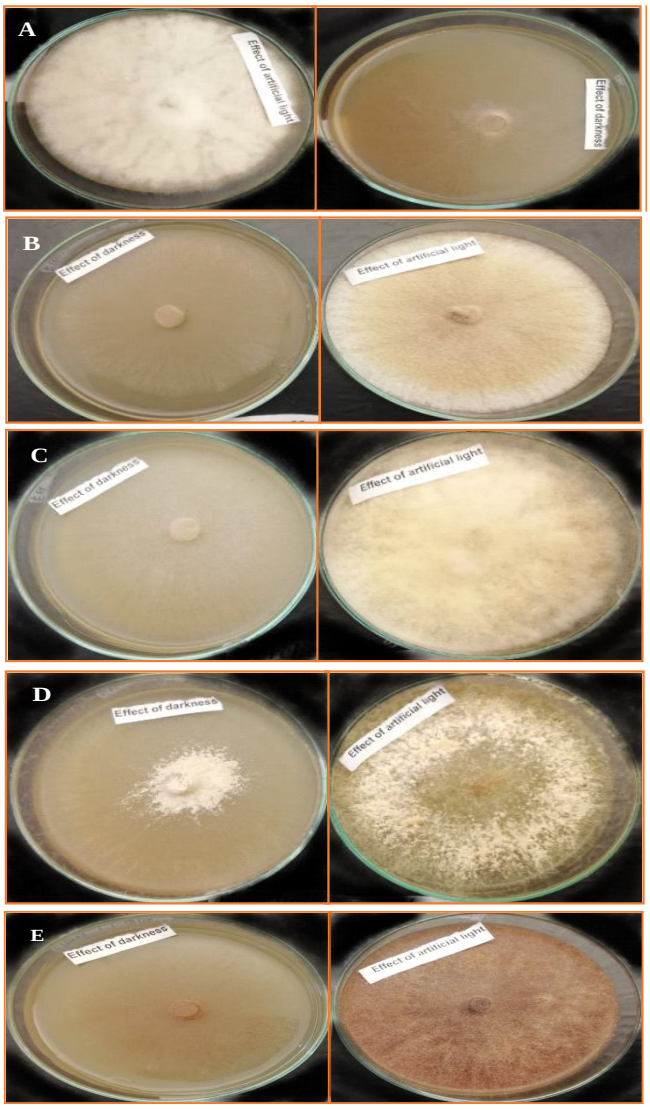

Fig. 5: Effect of light on *M. Crassipses* (A); *M. semilibera* (B); *M. angusticeps* (C); *M. conica* (D) and *M. esculenta*1 (E).

Discussion

This study revealed that *Morchella* species exhibited luxuriant growth in Malt extract media followed by Potato Dextrose media at pH 7-9 and temperature ranging from 15-25ºC in the presence of light. Our results of effect of pH are in accordance with the study done by (Karunarathna *et al*., 2014; Yamanka, 2003; Shih *et al*., 2007; Chang *et al*., 2006) for different mushrooms. These studies reported that pH, range 5-7 is best for growth of mycelia. In previous reported study done by (Jo *et al*., 2010; Xavier *et al*., 2007; Jayasinghe *et al*., 2008) suggested that mushrooms taken for analysis showed maximum growth of mycelia at temperature range of 25-30ºC. These results are in favor of our findings for *Morchella* species which grows in moderate temperature range of 15-25ºC. In case of carbon nitrogen ratio it could be deduced that all species of *Morchella* require high carbon and high nitrogen except *M. conica* for their mycelial growth. Growth media is equally important factor in enhancing growth of mycelium. In present study MEA and PDA media found to be best culture media support the growth of mycelia. Similar study was done by (Singh and Verma, 2000) for the different strains of *M. esculenta* and results suggested that Malt extract media is best suitable media for the growth of mycelia. (Shrestha *et al*., 2006) done a study for analyzing growth of *Cordyceps militaris* mycelia in the presence of light and suggested that light is critical single factor which contributes most in the dense mycelial growth as compare to dark. But in this study we found

Table 5: Effect of light on radial growth and biomass production.

S.No.	Mushrooms	Effect of light	Effect of darkness
Effect of light on radial growth (mm/day)			
1	*M. crassipses*	8.63±0.15^{a}	2.8±0.4^{b}
2	*M.angusticeps*	6.6±0.43^{a}	1.46±0.15^{b}
3	*M. semilibera*	7.76± .05^{a}	2.13±0.05^{b}
4	*M.conica*	8.46±0.30^{a}	5.2±0.4^{b}
5	*M.esculenta*	10.8±0..4^{a}	7.46±0.13^{b}
Effect of light on biomass yield (mg/ml)			
1	*M. crassipses*	0.87±0.18^{a}	0.46±0.05^{b}
2	*M.angusticeps*	0.74±0.05^{a}	0.60±0.04^{b}
3	*M.semilibera*	0.86±0.03^{a}	0.75±0.05^{b}
4	*M.conica*	2.42±0.07^{a}	1.07±0.15^{b}
5	*M.esculenta*	3.31±0.04^{a}	1.78±0.09^{b}

In each row, different letters mean a significant difference at P<0.05. Values are Mean ±SD (n=3)

maximum growth in the presence of artificial light as compare to dark.

Conclusion

Mushrooms are present in different habitats so having different requirements for their growth. Substrate and other physiological parameters are very essential requirements for the growth of any living organisms like bacteria, fungi and plants. Growing media, pH, temperature and source of nutrients like carbon and nitrogen are very important stimulants for the better growth of mycelia. Overall results revealed that *Morchella* species grows best in Malt extract media followed by Potato Dextrose media at pH 7-9 and temperature ranging from 15-25ºC in the presence of artificial light. Presence of various bioactive compounds in mycelial cultures makes mushrooms rich in antioxidants and medicinal properties. As Morels cannot grow artificially in lab conditions so we can use its mycelium in the form of functional food. To get more biomass we need to standardize these few factors like temperature pH, light effect, nutritional requirement and carbon source. Our study can help researchers to produce mycelial biomass on industrial scale.

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