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STUDIES ON GROWTH CONDITIONS OF WILD EDIBLE MUSHROOM *RAMARIA BOTRYTIS* (FR.) RICKEN SELECTED FROM NORTH WEST HIMALAYAN REGION

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ABSTRACT

Ramaria botrytis, is commonly known as the clustered coral, the pink-tipped coral mushroom, or the cauliflower coral. It is an edible species of coral fungus in the family Gomphaceae. Its robust fruit body can grow up to 15 cm in diameter and 20 cm tall, and resembles some marine coral. Its dense branches, which originate from a stout, massive base, are swollen at the tips and divided into several small branchlets. The branches are initially whitish but age to buff or tan, with tips that are pink to reddish. The flesh is thick and white. The fungus is mycorrhizal with broadleaf trees,. Fruit bodies of Ramaria botrytis are edible, and young specimens have a mild, fruity taste. The fungus contains several chemical compounds with in vitro biological activity, and fruit bodies have antimicrobial activity against several species and strains of drug-resistant bacteria that cause disease in human. Major factors essential for growing mycelium in laboratory are nutrients, temperature, pH and light and dark conditions. The impact of these factors on the growth of Ramaria botrytis was investigated under laboratory conditions. The aim of the Ramaria botrytis investigation was to determine optimal conditions for the development of the fungus. The results showed Yeastal Potato Dextrose Agar medium as best solid medium, Glucose-Asparagine as best liquid medium, optimal temperature was 25°C, whereas optimal pH was 6.0 under dark conditions.

KEYWORDS:*coral fungus, clustered, mycorrhizal, massive base, branchlets, Ramaria botrytis.*

INTRODUCTION

Mushrooms have been consumed since ages as a delicacy. Earlier these were collected from natural habitats and cooked either fresh or after drying. With time, cultivation techniques have been worked out for 25-30 edible species out of the 2000 naturally occurring edible mushrooms. Still, there are many more species which are consumed by local inhabitants which are yet to be cultivated.

Before cultivation is taken up of any mushroom it is essential to bring the mush room into pure culture and to study its nutritional requirements. The present study was undertaken on cultural characteristics of edible mushroom i.e. *Ramaria botrytis.*

We are still dependent upon forests for the supply of most of the wild edible mushrooms because they have not been artificially and commercially cultivated till date. The reason being little information about their nutritional requirements and entering of some of the

mushrooms into mycorrhizal association with forest trees..Hence it was considered worthwhile to investigate the nutritional requirements of these wild edible mushroom..The information is recorded on the following parameters: Growth of mycelium on different solid and liquid media; Recording the effect of temperature; pH and light and darkness

MATERIALS AND METHODS

Ramaria botrytis was brought into culture. For raising culture, the fruit bodies of mushroom were wiped gently with sterile cotton moistened with 70% ethanol. Bits of tissues were cut aseptically from the region of rapid cell division and planted in the centre of culture tubes containing sterilized potato-dextrose agar medium..After incubating for 10 days at 22^{0} C± 2^{0} C the actively growing mycelium was transferred to potato-dextrose slants for sub culturing. Throughout the study the culture was maintained on yeastal potato-dextrose agar medium at 5^{0}C.

i) Composition of media

In order to study the effect of different solid and liquid media on growth, ten solid media of the same composition as given by Tuite (1969) were tried. In case of solid media, inoculations were done in petriplates, whereas inoculations were done in 100 ml conical flasks in case of liquid media, 20 ml of the liquid medium in each flask. Three replicates of each medium were taken for the purpose of study.

ii) Sterilization

All glassware was sterilized in an oven at 180± 5^{0}C for 90 minutes. The media were autoclaved at 15 lb pressure per sq. inch (1.0545kg /cm^{2}) for 20 minutes. The inoculation needle and cork borer were initially dipped in ethyl alcohol and then flame sterilized.

iii) Inoculum

Inoculum used during the course of all physiological studies consisted of 5 mm diameter discs cut with the help of pre-sterilized cork borer. Ten days old cultures raised on PDA were used.

iv) Incubation period

Pertiplates containing the basal medium and inoculums were incubated for ten days at 22±2^{0} C in order to raise the culture for further studies.

v) Recording of growth

On solid media, the vegetative growth was recorded by taking the average linear growth of mycelia colony in two directions at right angles, till the petriplates were completely colonized. In the liquid media studies, the mycelia mats were filtered through Whatman No. I filter paper discs of 7.5 cm diameter. These filter papers were previously oven dried at 70 ± 5°C for 3 consecutive days (until constant weight) and weighed, after keeping in moisture free desiccators. After filtration the mycelia mat was again oven dried as above and weighed to record the final dry weight of the same. Throughout the experimentation, three replicates of each treatment were kept and the average was used as a quantitative measure for comparing the growth under different treatments.

vi) Effect of temperature on growth

In this experiment the best solid and liquid medium, out of the 10 tried, were selected for the experiment. The flasks containing basal medium and inoculums were incubated at different temperatures viz. 5, 10, 15, 20, 25, 30, 35 and 40^{0}C, in separate incubators for studying the optimal temperature requirement.

vii) Effect of Hydrogen ion concentration (pH) on growth

In order to study the effect of pH, inoculation was done in different media with pH adjusted at 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5, respectively. The pH of basal medium was adjusted with the help of sodium citrate and sodium phosphate buffers.

viii) Effect of light and darkness on growth

The flasks with best basal liquid medium, with optimum temperature and pH, were given the light and dark treatment. For dark conditions flasks were wrapped with black paper so that no light could enter inside.

OBSERVATIONS

Growth of mycelium of *Ramaria botrytis* on different solid media

Ten solid media (Tuite, 1969) were tested for the growth of *Ramaria botrytis.* The circular growth in petriplates was measured after ten days of incubation at a temperature (25 ± .5°C). The mean colony diameter of mycelium (± standard deviation) in different solid media is numerically and graphically presented in Table 1 Fig. 1, respectively.

Analysis through one-way ANOVA with Tukey's multiple comparison test revealed that the difference of colony diameter means between Wheat Grain Extract and Maize Grain Extract was non-significant (HSD:0.00; F-value: 43354.214; $P \leq 0.1$). Whereas, the comparison of means in rest of the solid media pairs revealed a very significant difference (HSD: 0.00; F-value: 43354.214; $P \leq 0.001$) (Table 1.1).

Results of ten media tried for the growth of Ramaria botrytis indicated that Yeastal Potato Dextrose Agar medium supported its maximum growth while, Czapek's Dox medium permitted minimum mycelium growth. The mean colony diameter of Yeastal Potato Dextrose Agar was significantly more than in all other media tested.

Hence, this medium was used as basal medium for the further studies related to Ramaria botrytis.

Growth of mycelium of *Ramaria botrytis* in different liquid media

Five liquid media (Tuite, 1969) were tried for the growth of Ramaria botrytis. The weight of mycelium was measured after ten days of incubation at an ambient temperature (25 ± .5°C). The mean of mycelial weight (mg) (± standard deviation) in different liquid media is numerically and graphically presented in Table 2 and Fig. 2, respectively.

The one-way ANOVA with Tukey's multiple comparison test revealed that the difference of mycelial weight between Czapek's solution and Dimmick's solution was non-significant (HSD: 0.00; F-value: 10622.770; $P \leq 0.1$). Whereas, difference of mycelium was very significant (HSD: 0.00; F-value: 10622.770; $P \leq 0.001$) (Table 2.1).

Conclusion drawn from the results of five media testedfor the growth of *Ramaria botrytis* that Glucose-Asparagine supported maximum growth, whereas, Asthana and Hawker's solution allowed minimum mycelial growth. The mean mycelial weight in Glucose-Asparagine was statistically very significant than all other liquid media tested.

Therefore, Glucose-Asparagine was selected as best liquid medium for raising cultures of *Ramaria botrytis*. Hence, in subsequent studies this medium was used as basal medium.

Effect of temperature on the growth of mycelium of *Ramaria botrytis*

To study the effect of different temperature on the growth of mycelium of *Ramaria botrytis*, mycelium was inoculated in the flasks containing Glucose-Asparagine as basal medium. These flasks were incubated at a temperature range of 5-40°C in separate incubators. The mean mycelial weight (mg) (± standard deviation) at different temperature values is numerically and graphically presented in Table 3 and Fig.3, respectively.

Analysis through one-way ANOVA with Tukey's multiple comparison test revealed that the mycelial growth at 5°C and 40°C was not significant (HSD: 0.00; F-value: 14463.052; $P \leq 0.1$). The comparison of the means of mycelial weight of remaining pairs of temperature showed very significant difference (HSD: 0.00; F-value: 14463.052; $P \leq 0.001$) (Table 3.1).

It is concluded that the maximum growth of Ramaria botrytis was observed at 25°C and minimum growth was noticed at 10°C. Growth completely ceased at 5°C. Hence, 25°C temperature was considered as the optimum temperature for raising the cultures of *Ramaria botrytis* for further studies.

Effect of Hydrogen ion concentration (pH) on the growth of mycelium of *Ramaria botrytis*

To analyse the effect of different pH values on the mycelial growth of *Ramaria botrytis*, the pH of liquid basal medium i.e. Glucose-Asparagine in flasks was adjusted in the range of 3.5-8.5 accordingly with the help of pH meter, then inoculated and incubated at a temperature (25 ± .2°C) in an incubator. The mean mycelial weight

(mg) (± standard deviation) at different pH values is numerically and graphically presented in Table 4 and Fig.4, respectively.

One-way ANOVA analysis with Tukey's multiple comparison test revealed that the difference between the means of mycelial weight (mg) between pH 4.5, pH 7.0 and pH 4.0, 8.0 was non-significant (HSD: 0.00; F-value: 6533.468; $P \leq 0.1$). While, the difference between means of mycelial weight was significant (HSD: 0.00; F-value: 6533.468; $P \leq 0.01$). The rest of the pH pairs studied, showed very significant difference between means of mycelial weight (HSD: 0.00; F-value: 6533.468; $P \leq 0.001$) (Table4.1).

It is concluded from the results that maximum and minimum mycelial growth of *Ramaria botrytis* was recorded at pH 6.0 and pH 3.5, respectively. The mean mycelial weight (mg) at pH 6.0 was statistically very significant than at other pH values studied.

Thus, pH 6.0 was considered as optimum pH for raising cultures of *Ramaria botrytis* in subsequent studies.

Effects of light and darkness on the growth of mycelium of *Ramaria botrytis*

To study the effect of light and darkness on the growth of *Ramaria botrytis* the flasks containing liquid basal medium Glucose-Asparagine adjusted at pH 6.0 were inoculated and incubated at 25 ± .2°C in light and dark conditions (flasks wrapped in black paper). The mean mycelial weight (mg) (± standard deviation) in light and dark conditions is numerically and graphically presented in Table 5 and Fig.5, respectively.

On applying Student's t-test, it was recorded that weight of mycelium in dark was statistically very significant than under light conditions (t-value: - 157.718; $P \leq 0.001$) (Table5.1

Table 1: Colony diameter of *Ramaria botrytis* on different solid media

Sr. No.	Name of the medium	Colony diameter (cm) (mean ± SD)
1.	Potato Dextrose Agar (PDA)	8.60 ± .100
2.	Yeastal Potato Dextrose Agar (YPDA)	8.92 ± .029
3.	Pridham Yeast Malt Dextrose (PYMD)	8.10 ± .100
4.	Glucose Yeast Agar (GYA)	7.52 ± .029
5.	Malt Agar (MA)	7.85 ± .050
6.	Wheat Grain Extract (WGE)	7.22 ± .029
7.	Maize Grain Extract (MGE)	7.16 ± .058
8.	Horse Gram Extract (HGE)	6.03 ± .058
9.	Pea Extract (PE)	4.50 ± .050
10.	Czapek's Dox (CD)	2.92 ± .029

* Incubation period of 10 days

Table 2.: Weight of mycelium (mg) of *Ramaria botrytis* in different liquid media

Sr. No.	Name of the medium	Weight of mycelium (mg) (mean ± SD)
1.	Glucose-Asparagine	103.63 ± .549
2.	Czapek's solution	88.69 ± .608
3.	Dimmick's solution	88.32 ± .368
4.	Richard's solution	51.74 ± .466
5.	Asthana and Hawker's solution	37.17 ± .294

* Incubation period of 10 days

Table 1.1: Differences between the means and One-Way ANOVA with Tukey's Multiple Comparison Test for colony diameter (cm) of mycelium of *Ramaria botrytis* on different solid growth media

Sr. No.	Growth Media		1 PDA	2 YPDA	3 PYMD	4 GYA	5 MA	6 WGE	7 MGE	8 HGE	9 PE	10 CD	HSD	F-value
		Mean (cm)	8.600	8.917	8.100	7.517	7.850	7.217	7.167	6.033	4.500	2.917	0.00	43354.214
1.	PDA	8.600	0.00	-.317***	.500***	1.083***	.750***	1.383***	1.733***	2.567***	4.100***	5.683***		
2.	YPDA	8.917		0.00	.817***	1.400***	1.067***	1.700***	1.750***	2.883***	4.417***	6.000***		
3.	PYMD	8.100			0.00	.583***	.250***	.883***	.933***	2.067***	3.600***	5.183***		
4.	GYA	7.517				0.00	-.333***	.300***	.350***	1.483***	3.017***	4.600***		
5.	MA	7.850					0.00	.633***	.683***	1.817***	3.350***	4.933***		
6.	WGE	7.217						0.00	.050 NS	1.183***	2.717***	4.300***		
7.	MGE	7.167							0.00	1.133***	2.667***	4.250***		
8.	HE	6.033								0.00	1.533***	3.117***		
9.	PE	4.500									0.00	1.583***		
10.	CD	2.917										0.00		

Table 2.1: Differences between the means and one-way ANOVA with Tukey's Multiple Comparison Test for weight (mg) of mycelium of *Ramaria botrytis* in different liquid media

Sr. No.	Liquid Growth Media		1 Glucose Asparagine	2 Czapek's Solution	3 Dimmick's Solution	4 Richard's Solution	5 Asthana and Hawker's Solution	HSD	F-value
		Mean (mg)	103.63	88.69	88.32	51.74	37.17	0.00	10622.770
1	Glucose- Asparagine	103.63	0.00	14.94***	15.31***	51.89***	66.46***		
2	Czapek's Solution	88.69		0.00	.367 NS	36.95***	51.52***		
3	Dimmcsk's Solution	88.32			0.00	36.58***	51.15***		
4	Richard's Solution	51.74				0.00	14.57***		
5	Asthana and Hawker's Solution	37.17					0.00		

***P ≤0.001;** P ≤0.01; * P ≤0.05; NS: Non-Significant Difference at P ≤0.10;
HSD: Honestly Significant Difference as revealed through Tukey's multiple comparison test.

Table 3: Weight of mycelium (mg) of *Ramaria botrytis* at different temperatures

Sr. No.	Temperature(in ^{0}C)	Weight of mycelium (mg) (mean ± SD)
1.	5	0.00 ± .000
2.	10	15.35 ± .632
3.	15	22.26 ± .528
4.	20	58.11 ± .480
5.	25	104.49 ± .452
6.	30	61.26 ± .647
7.	35	31.89 ± .780
8.	40	0.00 ± .000

* Incubation period of 10 days

Table 4: Weight of mycelium (mg) of *Ramaria botrytis* at different pH values

Sr. No.	pH	Weight of mycelium (mg) (mean ± SD)
1.	3.5	23.56 ± .488
2.	4.0	33.20 ± .242
3.	4.5	55.12 ± .189
4.	5.0	68.23 ± .277
5.	5.5	80.90 ± .283
6.	6.0	109.48 ± .334
7.	6.5	75.25 ± .266
8.	7.0	55.32 ± .401
9.	7.5	40.91 ± .803
10.	8.0	32.36 ± .337
11.	8.5	24.25 ± .332

* Incubation period of 10 days

Table-2.117: Differences between the means and One-Way ANOVA with Tukey's Multiple Comparison Test for weight (mg) of mycelium of *Ramaria botryits* at different temperatures

Sr. No.	Temperature		1 5	2 10	3 15	4 20	5 25	6 30	7 35	8 40	HSD	F-value
		Mean (mg)	0.00	15.35	22.26	58.11	104.49	61.26	31.89	0.00	0.00	14463.052
1	5	.00	0.00	-15.35***	-22.26***	-58.11***	-104.49***	-61.26***	-31.89***	0.00 NS		
2	10	15.35		0.00	-6.91***	-42.76***	-89.14***	-45.91***	-16.54***	15.35***		
3	15	22.26			0.00	-35.85***	-82.23***	-38.99***	-9.6***	22.26***		
4	20	58.11				0.00	-46.38***	-31.56***	26.22***	58.11***		
5	25	104.49					0.00	43.23***	72.59***	104.49***		
6	30	61.26						0.00	29.36***	61.26***		
7	35	31.89							0.00	31.89***		
8	40	0.00								0.00		

Table 2.118: Differences between the means and One-Way ANOVA with Tukey's Multiple Comparison Test for the weight of the mycelium of *Ramaria botrytis* at different pH values

Sr. No.	pH		1 3.5	2 4.0	3 4.5	4 5.0	5 5.5	6 6.0	7 6.5	8 7.0	9 7.5	10 8.0	11 8.5	HSD	F-value
		Mean (mg)	25.56	33.20	55.17	68.23	80.90	109.48	75.25	55.32	40.91	32.36	54.25	.000	6533.468
1.	3.5	23.56	0.00	-9.64***	-31.59***	-44.67***	-57.34***	-85.34***	-51.69***	-31.76***	-17.35***	8.80***	1.21***		
2.	4.0	33.20		0.00	-21.95***	-35.59***	-47.70***	-76.28***	-42.12***	-22.12***	-7.71***	.84 NS	10.85***		
3.	4.5	55.17			0.00	-13.09***	-25.75***	-54.33***	-20.10***	-.17 NS	14.23***	22.79***	32.79***		
4.	5.0	68.23				0.00	-12.67***	-41.25***	-7.01***	12.91***	27.32***	35.87***	45.88***		
5.	5.5	80.90					0.00	-28.58***	5.65***	25.58***	39.99***	48.54***	58.55***		
6.	6.0	109.48						0.00	34.23***	54.16***	68.57***	77.12***	87.13***		
7.	6.5	75.25							0.00	19.93***	34.33***	42.87***	52.89***		
8.	7.0	55.32								0.00	14.41***	29.96***	32.97***		
9.	7.5	40.91									0.00	8.55***	18.56***		
10.	8.0	32.36										0.00	10.01***		
11.	8.5	214.25											0.00		

***P ≤0.001; ** P ≤0.05; * P ≤0.10; NS: Non- Significant Difference at P ≤0.10;
HSD: Honestly Significant Difference as revealed through Tukey's multiple comparison test.

Table 5: Weight of mycelium (mg) of *Ramaria botrytis* in best liquid medium (Glucose- asparagine) in light and darkness

Sr. No.	Treatments	Weight of mycelium(mg) (mean ± S.D)
1.	Light	77.187 ± .185
2.	Dark	95.200 ± .070

* Incubation period of 10 days

Table 5: The significance of differences between the Means as determined by Student's t-test for mycelium weight of *Ramaria botrytis* in light and dark conditions

Sr. No.	Treatments	Weight of mycelium (mg) (mean ± SD)	t-value
1.	Light	77.187 ± .185	-157.718
2.	Dark	95.200 ± .070	

* Incubation period of 10 days

*** P ≤0.001;** P ≤0.01; * P ≤0.05; NS: Non-Significant Difference at P ≤0.10

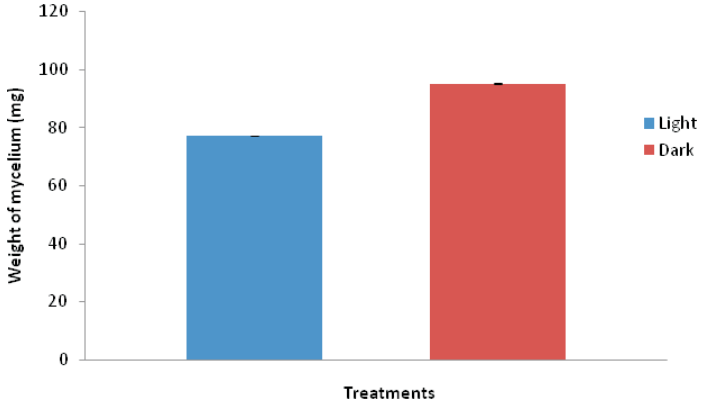

Fig. 2.60: Weight of mycelium (mg) of *Ramaria botrytis* under light and darkness

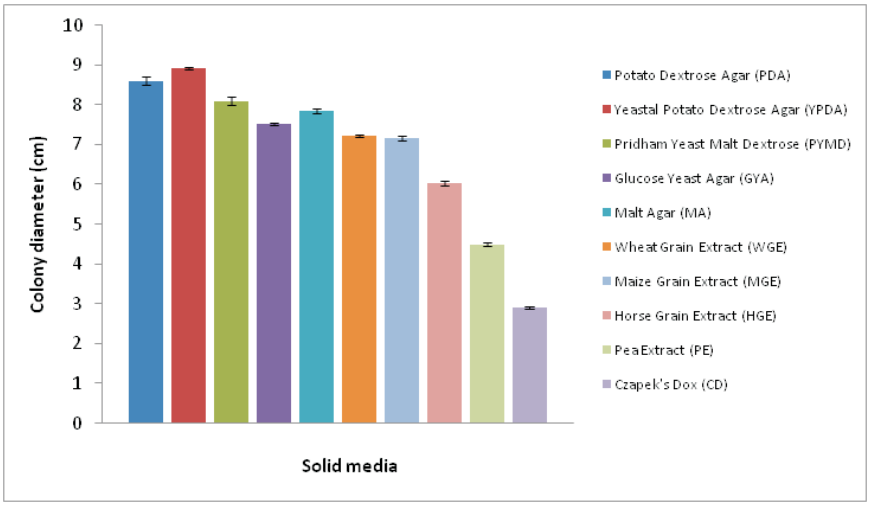

Fig. 1: Colony diameter (cm) of *Ramaria botrytis* on different solid media

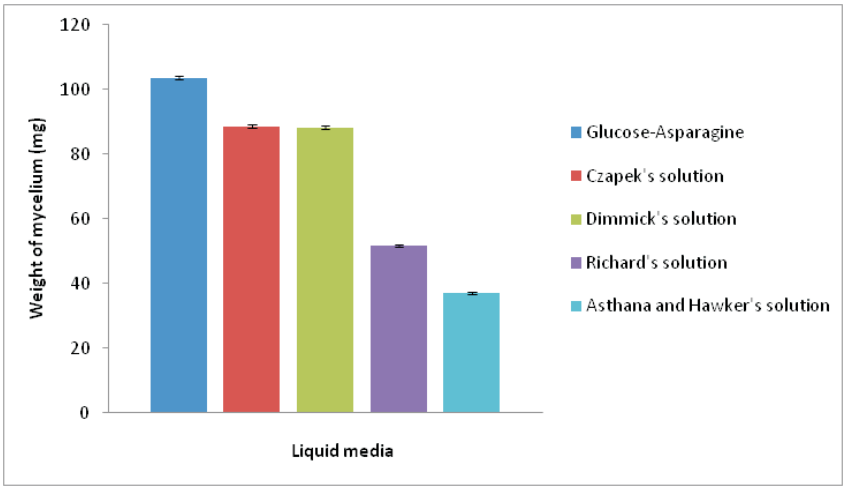

Fig. 2: Weight of mycelium (mg) of *Ramaria botrytis* in different liquid media

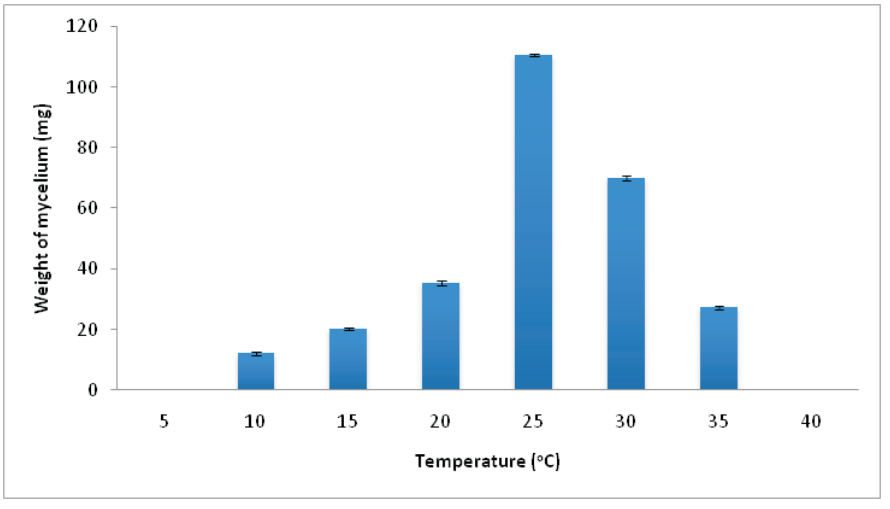

Fig. 3: Weight of mycelium (mg) of *Ramaria botrytis* at different temperatures

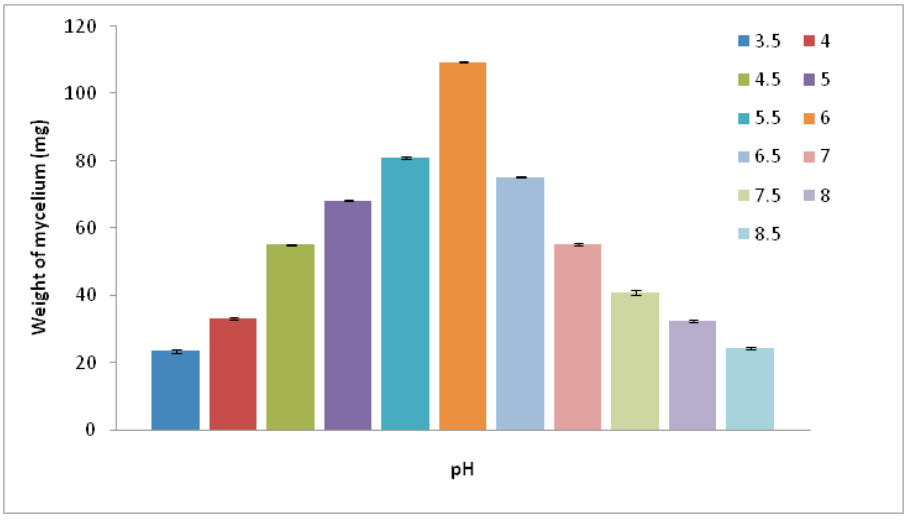

Fig. 4: Weight of mycelium (mg) of *Ramaria botrytis* at different pH values.

RESULTS

Results derived from the ten solid media tried for the growth of *Ramaria botrytis*, clearly indicated that Yeastal Potato Dextrose Agar medium supported maximum growth of mycelium while, Czapek's Dox permitted minimum colony diameter (Table 1). The mean colony diameter of Yeastal Potato Dextrose Agar was significantly more than all other tested solid media (Table 1.1).

Results of five liquid media tried for the growth of *Ramaria botrytis* proved that Glucose-Asparagine showed maximum mycelial weight whereas minimum growth was recorded in Asthana and Hawker's solution (Table 2). Whereas, the comparison of mycelial weight means observed in all the five liquid media pairs was very significant (HSD: 0.00; F-value: 9283.584; $P \leq 0.001$) (Table 2.1).

Maximum and minimum growth of *Ramaria botrytis* occurred at 25°C and 10°C, respectively (Table 3). The growth ceased completely at 5°C and 40°C. The mean mycelial growth was significantly more than at all other temperature values studied (Table 3.1).

Maximum growth of *Ramaria botrytis* was recorded at pH 6.0 and (Table 4). The mean mycelial weight (mg) at pH 6.0 was significantly more than all other pH values studied (Table 4.1).

Regarding growth of mycelium of *Ramaria botrytis* was better in dark than under light conditions (Table 5). Student's t-test, revealed that weight of mycelium in dark was statistically very significant under dark conditions than under light conditions (Table 5.1).

DISCUSSION

A study of detailed growth conditions of an organism is as important as the study of any of its other aspects. In the present study growth conditions regarding (media, temperature, hydrogen ion concentration light and darkness) of *Ramaria botrytis* were investigated with the cultures raised from their basidiocarps.

The literature has references showing evidence of best growth of various mushrooms mycelium on Yeastal Potato Dextrose Agar (YPDA). Good mycelial growth on YPDA has been recorded by Jandaik and Kapoor (1975a) in case of *Pleurotus sajor- caju, Podaxis pistillaris and Phellorina inquinans.* Rangad and Jandiak (1977) also reported YPDA as best medium for growth of different species of *Pleurotus, Agrocybe aegerita Flammulina valutipes and Stropharia rugoso- annulata.* Thianga and Jandaik (1979) also recorded best growth of *M. procera* on YPDA. Chaturvedi (1987) recorded YPDA as best medium for the growth of *P. ostreatus*. Shad (1989) recorded best growth of *M. esculenta, M. conica and M. deliciosa* on PDA. Nair and Devi *et al.*, (1987) also recorded the YPDA as the best medium for culturing Coprinus lagopus.

Among five liquid media tested Glucose-Asparagine supported maximum average mycelial growth. Rangad and Jandaik(1982) also recorded maximum growth of *F.velutipes,Agrocybe aegerita and Stropharia-rugoso-annulata* in Glucose- Asparagine, Mehta (1985) and Chaturvedi (1987) observed Glucose- Asparagine medium to favour maximum vegetative growth of *Pleurotus sapidius and Pleurotus ostreatus*. Singh and Lakhanpal (1988) also recorded maximum growth of *T. himalayansis* in Glucose Asparagine solution. Shad (1989) also found glucose asparagine to support maximum growth of *M. esculanta, M. deliciosa, M. Conica, M. crassipes and M. semilibra.*With regard to the effect of temperatures, it was recorded that all the sixteen mushrooms studied could grow in a wide temperature range of 10- 35°C but failed to grow below 10°C and above 35°C. Rangad and Jandaik (1977) have reported maximum growth of *Agrocybe aegerita and Stropharia rugoso-annulata* at 25°C. Mehta and Bhandal (1988) also recorded growth of *P. ostreatus, P. florida, P. saroj- caju, P. flabellatus, P. sapidus and P. cystidiosus* at 25°C. While, Gupta (1990) recorded 25°C to be the optimum temperature for vegetative growth of *M. esculenta, M. conica, M. crassipes, and M. angusticeps.* The highest radial diameter, mycelia density and dry mycelia weight were recorded at temperature 25°C for *Pleurotus ostreatus* (*Ali et al*., 2004).Effects of temperature (5-34°C)were investigated on hyphal growth of *Pleurotus flabellatus* . The temperature for hyphal growth of *Pleurotus flabellatus* varied from 20°C to 31°C with optimum temperature at 25°C (Li Rong *et al*., 2004). Song *et. al*., (2004) conducted studies on growth conditions of liquid culture for *Morchella conica.* The optimum temperature for *Pleurotus nebrodensis* was 25°C. The studies indicated that the suitable temperature for mycelial growth was 22-28°C although 25°C was optimum (HongTao et. al., 2005). Similarly, Yadav and Yadav (2012) observed 25°C to be the optimum temperature for the growth of

Cantharellus cibarius and Scleroderma bovista.

It is evident from the results that showed maximum growth at 25°C. The growth of mycelium starts decreasing with increase or decrease in optimum temperature. The results are in agreement with the references quoted in the literature.

For recording Optimum pH level for the growth of *Ramaria botrytis* the mycelium was grown in the best suited liquid medium at different levels of pH. Maximum growth of *Ramaria botrytis* occurred at slightly acidic pH i.e. 6.0. This was closely followed by 5.5 and 6.5 in acidic pH range. This finding is in agreement with the optimum pH for *Podaxis pistillaris* which had been recorded to be 6.0 by Jandaik and Kapoor (1975b). Thind and Jandaik (1979) also recorded pH 6.0 as best pH for growth of *Macrolepiota procera*. Rangad and Jandaik (1982) also recorded maximum growth of mycelium at pH 6.0 in *Stropharia- rugoso- annulata*. Nair and Devi (1986-87) also recorded pH 6.0, as optimum pH for the growth of *Calocybe lagopus.* Further, Ali et al., (2004) reported pH 6.0 for the maximum mycelium growth of *pleurotus ostreatus.* During the screening of culture conditions for *P. pulmonarius and P. columbinus*, the best pH was reported 6.0 (QinnGhe *et al*., 2004). Studies of Song *et al*. (2004) on growth conditions of *Ramaria botrytis* revealed pH 6.0 as suitable for mycelial growth.

It is evident from the results that there is decrease in mycelial growth of *Ramaria botrytis*, on either side of optimum pH..In other words, the growth of mycelium increased with decrease in acidity and decreased with increase in basicity upto optimum pH.

Mycelium of *Ramaria botrytis* was found to grow better under dark conditions in comparison to light conditions. Better growth of *S. crispa and T. himalayansis* was also recorded in dark conditions by Sharma (1987) and Lakhanpal *et al*., (1988).

CONCLUSION

All the solid media tested, supported good to moderate growth of this mushroom species. However, the highest growth rate of these fungi was recorded in Yeastal Potato Dextrose Agar medium; Czapek's Dox medium supported least growth of *Ramaria botrytis*. Good growth on YPDA may be ascribed to yeast extract which is known to contain growth enhancing substances like riboflavin. Least growth of this mushroom in remaining extracts may be attributed to the lack of nutrient content required for the growth of fungus used in present investigations.(Table 1 and Fig.1). Out of five liquid media tried Glucose-Asparagine showed maximum mycelial weight (Table 2 and Fig.2). The better growth of fungi in Glucose asparagines may be ascribed to free amino acid asparagine present in the solution.

Maximum and minimum growth of *Ramaria botrytis* occurred at 25°C and 10°C, respectively. The growth ceased completely at 5°C and 40°C (Table 3 and Fig.3). The mean mycelial growth was significantly more than at all other temperature values studied. Maximum growth of *Ramaria botrytis* was recorded at pH 6.0.(Table 4 and Fig.4). Growth of mycelium of *Ramaria botrytis* was better in dark than under light conditions (Table 5 and Fig.5).

The study on *Ramaria botrytis* concludes that it also behaves in the same manner in culture as the other commercially cultivated mushrooms like *Agaricus bisporus,Pleurotus and Volvariella spp.etc.* There is need to develop and standardize the cultivation technology of these wild edible mushrooms for making them commercially cultivable and popular among the common people like other cultivated mushrooms.

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