Research Paper
*Impact Factor - **2.262***
Peer Reviewed Journal

IJMDRR
E- ISSN –2395-1885
ISSN -2395-1877

STUDIES ON GROWTH CONDITIONS OF WILD EDIBLE MUSHROOM *CLAVULINA RUGOSA* (COKER, 1923) COMER, CHOSEN FROM NORTH WESTERN HIMALAYAS

Dr. Renu Rana
Associate Professor, Department of Botany, RKMV Shimla.

Abstract
Clavulina rugosa., commonly known as the white coral fungus or the crested coral fungus,[1] is a white- or light-colored edible coral mushroom. Fruit bodies, which are generally white- to cream-colored, can be up to 8 centimetres tall, and 2–4 cm broad. The coral "arms" are sparingly branched (3–4 times), 2–4 mm wide, smooth, and sometimes wrinkled longitudinally. The tips are cristate, having small pointed projections, and will often darken with age or in dry weather. The fruit bodies have no distinctive odor, and a mild taste. Main 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 Clavulina rugosa was investigated under laboratory conditions. The aim of the Clavulina rugosa 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.

Key Uses: Coral Fungus, Sparingly Branched, Cristate,Distinctive, Projections Clavulina Rugosa.

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 mushroom into pure culture and to study its nutritional requirements. The present study was undertaken on cultural characteristics of edible mushroom i.e. *Clavulina rugosa*.

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

Clavulina rugosa 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 5^0C.

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± 5^0C 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^{o}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 *Clavulina rugosa* on different solid media

Ten solid media (Tuite, 1969) were tried for the circular growth **of** *Clavulina rugosa* (Plate-II). The circular growth in petriplates was recorded after ten days of incubation at an ambient temperature (25^{o}C ± .5°C). The mean colony diameter of mycelium (± standard deviation) in different solid media is numerically and graphically presented in Table 1and Fig1, respectively.

On analyzing through one-way ANOVA with Tukey's multiple comparison test, it was observed that the differences of colony diameter means between Potato Dextrose Agar and Yeastal Potato Dextrose Agar medium was least significant (HSD: 0.00; F-value: 27434.180; P ≤ 0.05). Whereas the comparison of means observed between Glucose Yeast Agar and Malt Agar; Wheat, Grain Extract and Maize Grain Extract was non-significant (HSD: 0.000; F-value: 27434.180; P ≤ 0.1). Rest of the solid media pairs revealed a very significant difference of comparison means (HSD: 0.000; F-value; 27434.180; P ≤ 0.001) (Table1.1).

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

Hence, Yeastal Potato Dextrose Agar medium was used as basal solid medium for further studies on *Clavulina rugosa*.

Growth of mycelium of *Clavulina rugosa* in different liquid media

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

On analysing through one-way ANOVA with Tukey's multiple comparison test it was observed that the difference of mycelial weight (mg) means between Glucose-Asparagine and Czapek's solution was non-significant (HSD:0.00; F-value:1068.164; P ≤ 0.1). Whereas, rest of liquid media pairs revealed very significant difference (HSD: 0.00; F-value: 1068.164; P ≤ 0.001) (Table 2.1).

Results of five liquid media tested for the growth revealed maximum mycelial growth in Glucose-Asparagine, whereas Asthana and Hawker's solution permitted minimum mycelial growth. The mean mycelial weight in Glucose-Asparagine was significantly more than all other liquid media tested.

Effect of Temperature

To study the effect of different temperature values on growth of mycelium of *Clavulina rugosa*, pure culture of *Clavulina rugosa* was inoculated in flasks containing basal medium. These flasks were incubated in at a temperature range of 5°C to 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.

On analysis through one-way ANOVA with Tukey's multiple comparison test to compare the means of mycelial weight it was realized that growth between 5°C and 40°C was non-significant (HSD: 0.00; F-value: 10664.599; $P \leq 0.1$). The remaining pairs of temperature showed very significant difference in the means of mycelial weight of *Clavulina rugosa* (HSD: 0.00; F-value: 10664.599; $P \leq 0.001$) (Table 3.1).

It was concluded from the results that temperature values 25°C and 15°C permitted maximum and minimum mycelial growth of *Clavulina rugosa*, respectively. The growth ceased completely at 5°C and 40°C. The mean mycelial weight at 25°C was significantly more than at all other temperature values studied.

Hence, 25°C was considered as the optimum temperature for culturing *Clavulina rugosa* for further studies.

Effect of Hydrogen ion concentration (pH)

To analyze the effect of different pH values on the mycelium growth of *Clavulina rugosa*, the pH of the basal medium in flasks was adjusted in the range of 3.5-8.5 accordingly, with the help of pH meter and inoculated and incubated at a temperature 25 ± .2°C. The mean mycelial weight (mg) (± standard deviation) at different pH values is numerically and graphically presented in Table 4and Fig. 4, respectively.

Analysis through one-way ANOVA with Tukey's multiple comparison test to compare the mean difference of mycelial weight revealed that mycelial growth of *Clavulina rugosa* was non-significant between pH pairs 5.0 and 7.0; 4.5 and 7.5; 4.0 and 7.5; 4.0 and 8.0 and 7.5; 4.0 and 8.0 and 7.5 and 8.0 (HSD: 0.00; F-value; 6533.468; $P \leq 0.1$). The remaining pairs of pH values were found to be having very significant difference (HSD: 0.00; F-value: 6533.468; $P \leq 0.001$) (Table 4.1).

Results concluded that maximum mycelial growth of *Clavulina rugosa* was noticed at pH 6.0.

Effect of light and darkness

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

Student's t-test, revealed that weight of mycelium of *Clavulina rugosa* in dark was statistically very significant than light conditions (t-value: - 99.557; $P \leq 0.001$) (Table 5.1).

Table 1: Colony diameter of *Clavulina rugosa* 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)	8.80 ± .100
3.	Pridham Yeast Malt Dextrose (PYMD)	8.22 ± .104
4.	Glucose Yeast Agar (GYA)	7.78 ± .076
5.	Malt Agar (MA)	7.82 ± .029
6.	Wheat Grain Extract (WGE)	6.87 ± .058
7.	Maize Grain Extract (MGE)	7.02 ± .029
8.	Horse Gram Extract (HGE)	3.77 ± .058
9.	Pea Extract (PE)	2.95 ± .050
10.	Czapek's Dox (CD)	2.23 ± .058

*** Incubation period of 10 days**

Table 2: Weight of mycelium (mg) of *Clavulina rugosa* in different liquid media

Sr. No.	Name of the medium	Weight of mycelium (mg) (mean ± SD)
1.	Glucose-Asparagine	118.15 ± .254
2.	Czapek's solution	97.95 ± .586
3.	Dimmick's solution	95.59 ± .525
4.	Richard's solution	64.43 ± 2.968
5.	Asthana and Hawker's solution	50.92 ± .951

*** 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 *Clavulina rugosa* on different solid growth media

Sr. No.			1	2	3	4	5	6	7	8	9	10	HSD	F-value
	Growth Media		PDA	YPDA	PYMD	GYA	MA	WGE	MGE	HGE	PE	CD		
		Mean (cm)	8.600	8.800	8.217	7.783	7.817	6.867	7.017	3.767	2.950	2.233	0.00	27434.180
1.	PDA	8.600	0.00	-.200*	.383***	.817***	0.783***	1.733***	1.583***	4.833***	5.650***	6.367***		
2.	YPDA	8.800		0.00	.583***	1.017***	.983***	1.933***	1.783***	5.033***	5.850***	6.567***		
3.	PYMD	8.217			0.00	.433***	.400***	1.350***	1.200***	4.450***	5.267***	5.983***		
4.	GYA	7.783				0.00	.034 NS	.917***	.767***	4.017***	4.833***	5.550***		
5.	MA	7.817					0.00	.950***	.800***	4.050***	4.867***	5.583***		
6.	WGE	6.867						0.00	-.150 NS	3.100***	3.917***	4.633***		
7.	MGE	7.017							0.00	3.250***	4.067***	4.783***		
8.	HE	3.767								0.00	.817***	1.533***		
9.	PE	2.950									0.00	.717***		
10.	CD	2.233										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 *Clavulina rugosa* in different liquid media

Sr. No.			1	2	3	4	5	HSD	F-value
	Liquid Growth Media		Glucose Asparagine	Czapek's Solution	Dimmick's Solution	Richard's Solution	Asthana and Hawker's Solution		
		Mean (mg)	118.15	97.95	95.59	64.43	50.92	0.00	1068.164
1	Glucose-Asparagine	118.15	0.00	20.19***	22.55***	53.72***	67.22***		
2	Czapek's Solution	97.95		0.00	2.36 NS	33.52***	47.03***		
3	Dimmick's Solution	95.59			0.00	31.17***	44.67***		
4	Richard's Solution	64.43				0.00	13.50***		
5	Asthana and Hawker's Solution	50.92					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 *Clavulina rugosa* at different temperatures

Sr. No.	Temperature(in ^{0}C)	Weight of mycelium (mg) (mean ± SD)
1.	5	0.00 ± .000
2.	10	15.82 ± .718
3.	15	29.96 ± .705
4.	20	65.08 ± .141
5.	25	119.34 ± .390
6.	30	54.99 ± .857
7.	35	33.27 ± 1.280
8.	40	0.00 ± .000

*** Incubation period of 10 days**

Table 4: Weight of mycelium (mg) of *Clavulina rugosa* at different pH values

Sr. No.	pH	Weight of mycelium (mg) (mean ± SD)
1.	3.5	36.32 ± .470
2.	4.0	39.17 ± .763
3.	4.5	41.09 ± .984
4.	5.0	60.29 ± .275
5.	5.5	89.69 ± .505
6.	6.0	116.50 ± .350
7.	6.5	78.33 ± .577
8.	7.0	59.47 ± .475
9.	7.5	39.60 ± .529
10.	8.0	38.64 ± .768
11.	8.5	29.41 ± .343

*** Incubation period of 10 days**

Table 3.1: Differences between the means and One-Way ANOVA with Tukey's Multiple Comparison Test for weight (mg) of mycelium of *Clavulina rugosa* at different temperatures

Sr. No.			1	2	3	4	5	6	7	8	HSD	F-value
	Temperature		5	10	15	20	25	30	35	40		
		Mean (mg)	.00	15.82	29.96	65.08	119.34	54.99	33.27	0.00	0.00	10664.599
1	5	.00	0.00	-15.82***	-29.96***	-65.08***	-119.34***	-54.99***	-33.27***	0.00 NS		
2	10	15.82		0.00	-14.14***	-49.26***	-103.52***	-39.18***	-17.45***	15.82***		
3	15	29.96			0.00	-35.12***	-89.38***	-25.04***	-3.31***	29.96***		
4	20	65.08				0.00	-54.26***	10.08***	31.81***	65.08***		
5	25	119.34					0.00	64.35***	86.07***	119.34***		
6	30	54.99						0.00	21.73***	54.99***		
7	35	33.27							0.00	33.27***		
8	40	0.00								0.00		

Table 4.1: Differences between the means and One-Way ANOVA with Tukey's Multiple Comparison Test for the weight of mycelium of *Clavulina rugosa* at different pH values

Sr. No.			1	2	3	4	5	6	7	8	9	10	11	HSD	F-value
pH			3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5		
		Mean (mg)	36.32	39.17	41.09	60.29	89.69	116.50	78.33	59.41	39.60	38.64	29.51	.000	6533.468
1.	3.5	36.32	0.00	-2.85***	-4.77***	-23.27***	-53.37***	-80.18***	-42.01***	-23.15***	-3.28***	-2.32***	6.81***		
2.	4.0	39.17		0.00	.019***	-21.13***	-50.52***	-77.33***	-39.17***	-20.30***	-.43 NS	.53 NS	9.65***		

3.	**4.5**	41.09			0.00	-19.2***	-48.59***	-75.41***	-37.24***	-18.37***	1.49 NS	2.45***	11.58***	
4.	**5.0**	60.29				0.00	-29.39***	-56.21***	-18.04***	.83 NS	20.69***	21.65***	30.78***	
5.	**5.5**	89.69					0.00	-26.81***	11.35***	30.22***	50.09***	51.05***	60.17***	
6.	**6.0**	116.50						0.00	38.17***	57.03***	76.90***	77.86***	86.99***	
7.	**6.5**	78.33							0.00	18.87***	38.73***	39.69***	48.82***	
8.	**7.0**	59.47								0.00	19.87***	20.83***	29.95***	
9.	**7.5**	39.60									0.00	.96***	10.09***	
10.	**8.0**	38.64										0.00	9.13***	
11.	**8.5**	29.51											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 *Clavulina rugosa* in best liquid medium (Glucose- asparagine) in light and darkness

Sr. No.	Treatments	Weight of mycelium (mg) (mean ± S.D)
1.	Light	77.189 ± .206
2.	Dark	95.210 ± .240

*** Incubation period of 10 days**

Table 5.1The significance of differences between the Means as determined by Student's t-test for mycelium weight of *Clavulina rugosa* in light and dark conditions

Sr. No.	Treatments	Weight of mycelium (mg) (mean ± SD)	t-value
1.	Light	77.187 ± .206	-99.551
2.	Dark	95.200 ± .240	

*** Incubation period of 10 days**
***** P ≤ 0.001;** P ≤ 0.01; * P ≤ 0.05; NS: Non-Significant Difference at P ≤ 0.10**

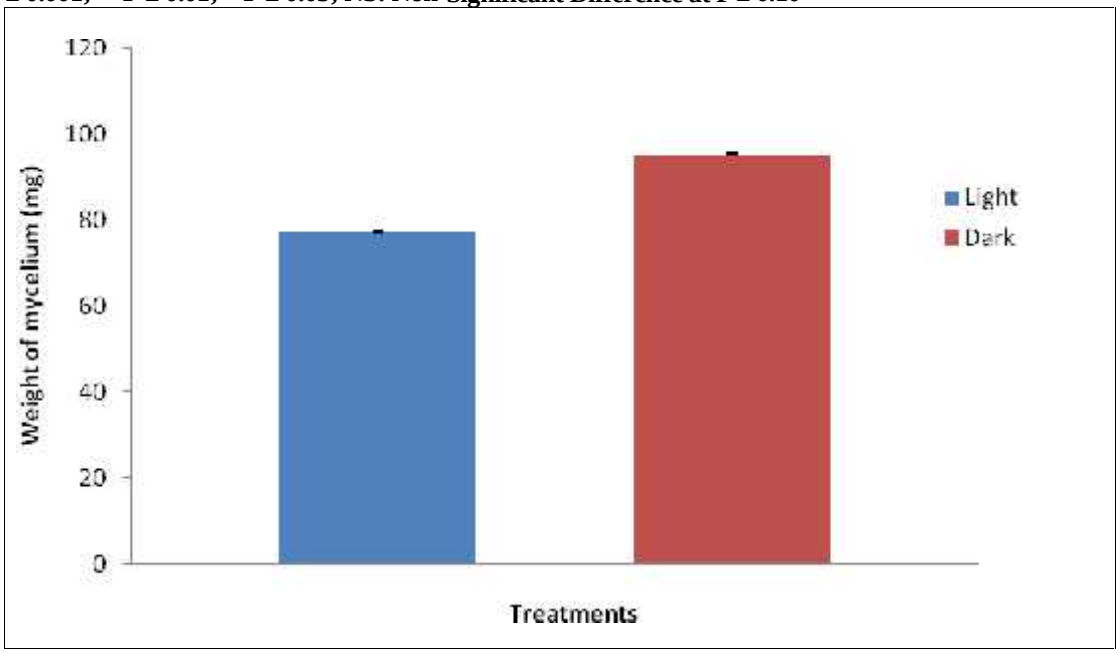

Fig. 5:Weight of mycelium (mg) of *Clavulina rugosa* under light and darkness.

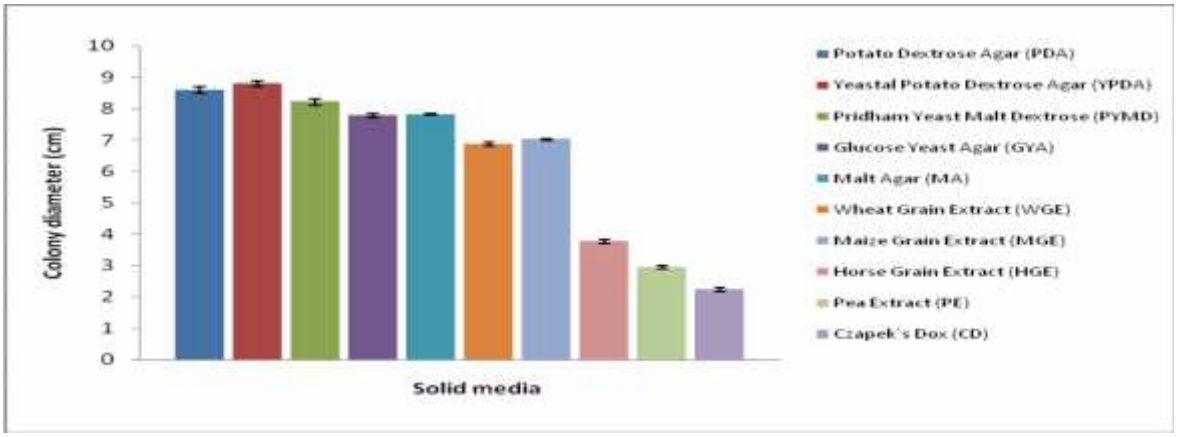

Fig.1 Colony diameter (cm) of *Clavulina rugosa on* different solid media.

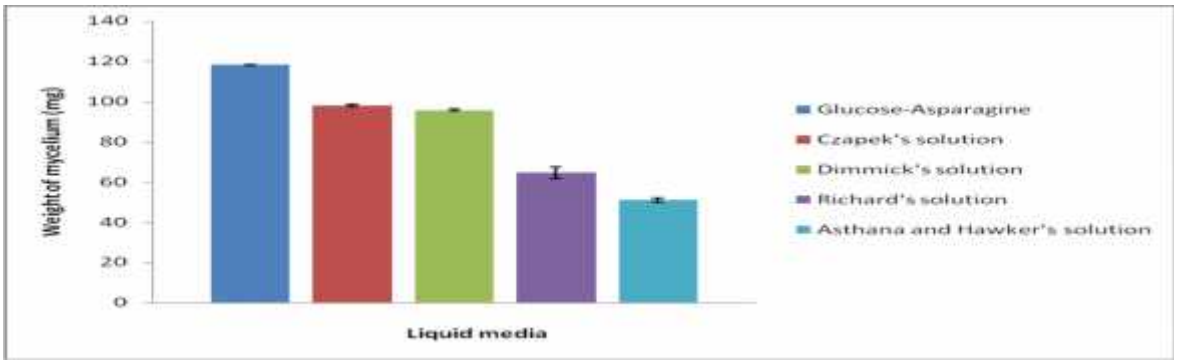

Fig. 2 Weight of mycelium (mg) of *Clavulina rugosa* in different liquid media.

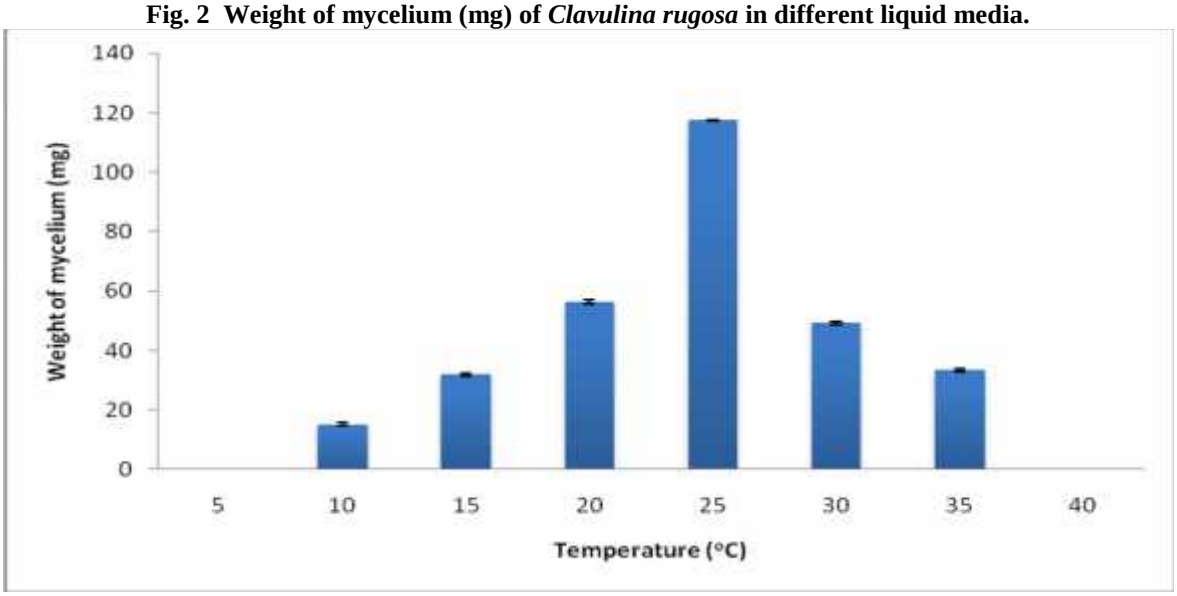

Fig.3: Weight of mycelium (mg) of *Clavulina rugosa* at different temperatures

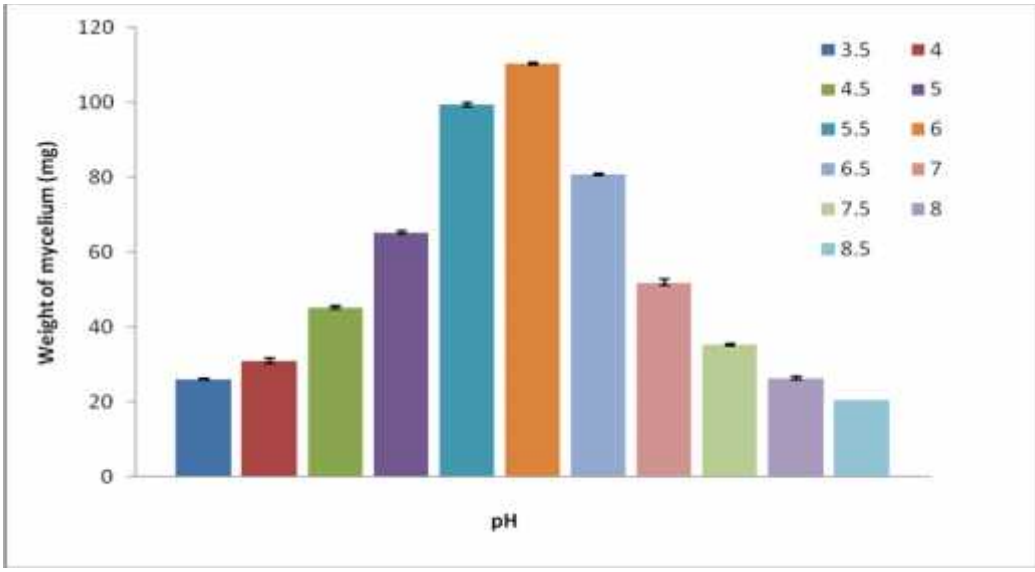

Fig.4: Weight of mycelium (mg) of *Clavulina rugosa* at different pH values

Results

Results derived from the ten solid media tried for the growth of *Clavulina rugosa*, 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 *Clavulina rugosa* 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 *Clavulina rugosa* 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 *Clavulina rugosa* 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 *Clavulina rugosa* 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 *Clavulina rugosa* were investigated with the cultures raised from their basidiocarps.

The literature has references showing evidence of best growth of mushrooms mycelium on Yeastal Potato Dextrose Agar (YPDA) and Potato Dextrose Agar (PDA). 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 ai.,* (1987) also recorded the YPDA as the best medium for culturing *Coprinus lagopus.*

Among five liquid media tested for determining their comparative suitability for vegetative growth of *Clavulina rugosa* investigated, 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*^{O}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^{o}C)were investigated on hyphal growth of *Pleurotus flabellatus* . The temperature for hyphal growth of *Pleurotus flabellatus* varied from 20^{o}C to 31^{o}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^{o}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 their growth of *Clavulina rugosa* the mycelium was grown in the best suited liquid medium at different levels of pH. It was recorded that maximum growth of majority of the wild edible mushrooms studied 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 *Clavulina rugosa* revealed pH 6.0 as suitable for mycelial growth.

It is evident from the results that there is decrease in mycelial growth of *Clavulina rugosa*, 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 *Clavulina rugosa* 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 *Clavulina rugosa*..However, the highest growth rate of these fungi was recorded in Yeastal Potato Dextrose Agar medium followed by potato dextrose agar; Czapek's Dox medium supported least growth of *Clavulina rugosa.* 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 *Clavulina rugosa* 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 *Clavulina rugosa* was recorded at pH 6.0.(Table 4 and Fig.4). Growth of mycelium of *Clavulina rugosa* was better in dark than under light conditions (Table 5 and Fig.5).

The study on *Clavulina rugosa* 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.

References

1. Ali, M.B., Islam, M.N., Mian, I.H. and Rahman, M.M. 2004. Influence of Physical factors on mycelial growth of oyster mushroom. *Journal of Subtropical Agricultural Research and Development.* **2**: 86-90.
2. Chandra, A. and Purkayastha, R.P. 1977. Physiological studies on Indian edible mushrooms. *Trans. Brit. Mycol. Soc.* **69**: 63-70.
3. Chaturvedi, A. 1987. Cultural and Cultivation studies on a *Pleurotus* sp. from Himachal Pradesh. M.Phil. Dissertation, H.P. University, Shimla.
4. Gupta, Y. 1990. Nutritional requirements of *Morchella* species. *Indian. J. Mycol. Pl. Pathol.* **20**: 98 (Abstr.).
5. HongTao, S. ChunYan, H., LuZhang, W. BaiSong, Z., ShuKai, S. and RenE, Y. 2005. Effects of different environmental factors on the growth of *Pleurotus nebrodensis* mycelium. *Journal of Fungal Research.* **3**: 1-22.
6. Jandaik, C.L. and Kapoor, J.N. 1975a. Cultural studies on some edible fungi. *Ind. J. Mush*. **1**: 22-26.
7. Jandaik, C.L. and Kapoor, J.N. 1975b. Nutritive value of mushroom *Pleurotus sajor-caju. Mush. J.* **36**: 408-410.
8. Jandaik, C.L. and Kapoor, J.N. 1976a. Amino acid composition of *Pleurotus sajor-caju* (Fr.) Singer. *Mush.* J. **41**: 145-156.
9. Jandaik, C.L. and Kapoor, J.N. 1976b. Amino acid composition of the protein of *Podaxis pisitillaris* (L. ex. Pers.) Morse an edible mushroom *Ind. J. Mush* **2**: 33-37.
10. Jandaik, C.L. and Thianga, S. 1981. Studies on cultivation and food value of *Macrolepiota procera. Mush Sci.* **11**: 725-733.
11. Jandiak, C.L. Bhandari, A.R., Arora, C.L. and Rangad, C.O. 1978. Chemical composition of some edible fungi. *Mush Sci.* **X**: 685-688.
12. Krieger, L.C.C. 1967.The mushroom Handbook. Dover Publication, New York.
13. Kucharska, R. 2010. Research on cultivation of wild mushrooms in Poland: Cauliflower mushroom, beefsteak fungus and St. George's mushroom. *Badania nad technologia upgrawy grzybow dziko rosnacych w polsce: Szamacika galezistego ozorka debowego I majowki, woisennej.* pp. 10.
14. Kumar, A. 1987. Studies of some mushroom families in North Western Himalayas. Ph.D. Thesis, H.P. University, Shimla.
15. Lakhanpal, T.N., Sharma, R. and Singh, L. 1988. Systematic and ectomycorrhizal relationships of some mushrooms in North-west Himalayas. Proceedings of the first Asian conference on mycorrhizae. January 29-31: 66-69.
16. LiRong, H., LiLi, H., YangJie, Y., GuanYan, M. and HongWei, S. 2004. Effect of different cultivation conditions on hyphal growth of *Pleurotus flabellatus*. *Edible Fungi of China*. **23**: 29-30.
17. Mehta, K.B. 1985. Studies on physiology and cultivation of *Pleurotus sapidus.* Ph.D. Thesis, Himachal Pradesh Krishivishva Vidyalaya SNS nagar Solan.
18. Mehta, K.B. and Bhandal, M.A. 1988. Mycelial growth variation of six *Pleurotus* species at different temperatures. *Ind. J. Mush.* **14**: 64-65.
19. Mukherjee, R. and Nandi, B. 2000. Effect of pH on submerged mycelial production by *Pleurotus* spp. in a medium with lignocellulosic biomass. *Journal of Mycopathological Research.* **38**: 65-69.
20. Nair, G. and Devi, B. 1986-1987. Physiological studies on *Coprinus lagopus. Ind. J. Mush.* **XII-XIII**: 31-36.
21. Pegler D.N. and Gibson, I.A.S. 1972. *Armillaria mellea*. IMI-Description of Fungi and Bacteria. **33**: Sheet 321.
22. QinnGhe, C., XiaoYu, Y., TianGwi, N., Cheng, J. and QuiGang. M. 2004. The screening of cultural condition and properties of xylanase by white-rot fungus *Pleurotus ostreatus. Process Biochemistry.* **39**: 1561-1566.
23. Rangad, C.O. and Jandaik, C.L. 1977. Cultural studies on some *Pleurotus* species. *Ind. J. Mush.* **3**: 13-17.
24. Rangad, C.O., and Jandaik, C.L. 1982. Ecological and nutritional requirements of some edible fungi. *Ind. J. Mush.* **8**: 29-40.
25. Shad, O.S. 1989. Biological studies on *Morchella* species (Morels) of Himachal Himalayas. Ph.D. Thesis, H.P. University, Shimla.

26. Shad, O.S. 1989. Biological studies on *Morchella* species (Morels) of Himachal Himalayas. Ph.D. Thesis, H.P. University, Shimla.
27. Singh, L. and Lakhanpal, T.N. 1988. Physiological studies on mycelial growth of *Octaviania densa* (Rodw.) Cunn. *Ind. J. Mush.* **14**: 48-50.
28. Singh, N.S. and Rajarathnam, S. 1977. *Pleurotus eous* (Berk.) Sacc. A new cultivated mushroom. *Current Science.* **46**: 617-618.
29. Song, Z., XiaMei, H. and HaiYan, X. 2004. Studies on growth media and conditions of liquid culture for *Morchella conica. Journal of Fungal Research.* **2**: 11-15.
30. Tuite, J. 1969. Plant Pathological methods: fungi and bacteria. Burgess Publishing Company Minn. USA.
31. Yadav, A. and Yadav, K. 2012. Physiological study of two ectomycorrhizal fungi isolated from Kumaun Himalaya. *Indian Forester.* **138**: 17-21.