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RESEARCH ARTICLE

STUDIES ON GROWTH CONDITIONS OF WILD EDIBLE MUSHROOM *HELVELLA CRISPA* FRIES SELECTED FROM NORTH WEST HIMALAYAN REGION

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ABSTRACT

Helvella crispa is an ancient term for an aromatic herb. The specific epithet crispa comes from Latin and means curled or wrinkled - a reference to the contorted cap or saddle of this woodland fungus. The saddle-shaped cap may have two or three major undulations and many minor curled contortions. The upper surface is smooth and cream or occasionally pinkish or pale ochre; the underside is pale ochre and slightly downy. The upward tapering stem is white and ornately furrowed or fluted; it is hollow and has thin, elastic flesh. 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 *Helvella crispa* was investigated under laboratory conditions. The aim of the *Helvella crispa* 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.

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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. *Helvella crispa.* 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

Helvella crispa 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-

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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 40^0C, 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 *Helvella crispa* on different solid media

Ten solid media were tested for the circular growth (Plate-II) the circular growth in petriplates was recorded after 10 days of incubation at ambient temp (25°C ± .5°C). The mean colony diameter of mycelium (± standard deviation) in different solid media is numerically and graphically presented in Table 1 and Fig.1, respectively. The analysis through one-way ANOVA with Tukey's multiple comparison test revealed that the difference of colony diameter means of mycelium between maize Grain Extract and Horse Gram Extract was significant (HSD-0.000; F-value-8873.028; P≤ 0.01). The difference between the Colony diameter means of Potato Dextrose Agar medium Pridham Yeast Malt Dextrose medium; wheat Grain Extract-Pea Extract medium was non significant (HSD:0.000; F-value-8873.028; $p \leq 0.10$). While, the comparison of means in rest of solid media pairs revealed very significant differences (HSD: 0.000; F-value 8873.028; $p \leq 0.001$) (Table 1). It can be concluded from the results of study on ten solid media that Yeastal Potato Dextrose Agar showed maximum colony diameter whereas, Maize Grain Extract showed minimum colony diameter. The mean colony diameter of Yeastal Potato Dextrose Agar was significantly more than all other media tested. Thus, yeastal potato Dextrose Agar medium was selected as best solid medium for the mycelial growth of *Helvella crispa*. Hence, it was used as basal solid medium in subsequent studies.

Table 1. Colony diameter of *Helvella crispa* on different solid media

Sr. No.	Name of the medium	Colony diameter (cm) (mean ± SD)
1.	Potato Dextrose Agar (PDA)	8.48 ± .076
2.	Yeastal Potato Dextrose Agar (YPDA)	8.87 ± .104
3.	Pridham Yeast Malt Dextrose (PYMD)	8.38 ± .076
4.	Glucose Yeast Agar (GYA)	5.82 ± .104
5.	Malt Agar (MA)	5.22 ± .028
6.	Wheat Grain Extract (WGE)	3.75 ± .087
7.	Maize Grain Extract (MGE)	2.52 ± .028
8.	Horse Gram Extract (HGE)	2.83 ± .057
9.	Pea Extract (PE)	3.88 ± .076
10.	Czapek's Dox (CD)	5.20 ± .259

* Incubation period of 10 days

Table 2. Weight of mycelium (mg) of *Helvella crispa* in different liquid growth media

Sr. No.	Name of the medium	Weight of mycelium (mg) (mean ± SD)
1.	Glucose-Asparagine	116.61 ± .940
2.	Czapek's solution	98.99 ± .305
3.	Dimmick's solution	92.78 ± .599
4.	Richard's solution	68.17 ± 1.444
5.	Asthana and Hawker's solution	65.62 ± .555

* Incubation period of 10 days

Growth of mycelium of *Helvella crispa* in different liquid media

Five liquid media were tried for the growth of mycelium in terms of weight (mg) of *Helvella crispa*. The weight of mycelium was measured after 10 days of incubation at 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 (mg) means between Richard's Solution Asthana and Hawker's Solution showed significant difference (HSD:0.00; F-value 1601.458; $P \leq 0.05$). No liquid medium pair revealed non-significnat diference between means of mycelial weight. While, rest of liquid media pairs revealed very significant difference (HSD: 0.00; F-value 1601.458; $P \leq 0.001$) (Table 2). It can be concluded from the results of study on five liquid media tried that Glucose-Asparagine showed maximum growth. While, Asthana and hawker's Solution allowed minimum mycelial growth. The mean mycelial weight in Dimmick's solution was significantly more than all other liquid media tested. Therefore, Glucose-Asparagine was selected as best liquid medium for growing the cultures of *Helvella crispa* and Glucose-Asparagine was used as basal medium in subsequent studies.

Table 1. Differences between the means and One-Way ANOVA with Tukey's Multiple Comparison Test for colony diameter (cm) of mycelium of *Helvella crispa* on different solid growth media

Sr. No.	Growth Media		1	2	3	4	5	6	7	8	9	10	HSD	F-value
			PDA	YPDA	PYMD	GYA	MA	WGE	MGE	HGE	PE	CD		
		Mean (cm)	8.483	8.867	8.383	5.817	5.217	3.750	2.517	2.833	3.883	5.200	.000	8873.028
1.	PDA	8.483	0.00	383***	.100 NS	2.667***	3.267***	4.733***	5.967***	5.650***	4.600***	3.283***		
2.	YPDA	8.867		0.00	.483***	3.050***	3.050***	5.117***	6.350***	6.033***	4.983***	3.667***		
3.	PYMD	8.383			0.00***	2.567***	3.167***	4.633***	5.867***	5.550***	4.500***	3.183***		
4.	GYA	5.817				0.000	.600	2.067***	3.300***	2.983***	1.933***	.617***		
5.	MA	5.217					0.00	1.467***	2.700***	2.383***	1.333***	.017 NS		
6.	WGE	3.750						0.00	1.233***	.917***	-1.33 NS	-1.450***		
7.	MGE	2.517							0.00	.317**	-1.367***	-2.683***		
8.	HE	2.833								0.00	-1.050***	-2.367***		
9.	PE	3.883									0.00	1.317***		
10.	CD	5.200										0.00		

Table 2. Differences between the means and One-Way ANOVA with Tukey's Multiple Comparison Test for weight (mg) of mycelium of *Helvella crispa* in different liquid growth media

Sr. No.	Liquid Growth Media		1	2	3	4	5	HSD	F-value
			Glucose Asparagine	Czapek's Solution	Dimmick's Solution	Richard's Solution	Asthana and Hawker's Solution		
		Mean (mg)	111.61	98.99	92.78	68.17	65.62	0.00	1601.458
1	Glucose- Asparagine	111.61	0.00	12.61***	18.83***	43.44***	45.98***		
2	Czapek's Solution	98.99		0.00	6.22***	30.83***	33.37***		
3	Dimmick's Solution	92.78			0.00	24.61***	27.15 ***		
4	Richard's Solution	68.17				0.00	2.54**		
5	Asthana and Hawker's Solution	65.62					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.

Effect of temperature

To record the effect of different temperatures on the mycelial growth, pure culture of *Helvella crispa* was inoculated in the flasks containing basal liquid medium i.e. Glucose-Asparagine. The flasks were incubated at a temperature range of 5-40^{o}C in separate incubators. The mean mycelial weight (± standard deviation) at different temperatures is numerically and graphically presented in Table 3 and Fig.3, respectively. One-way ANOVA with Tukey's multiple comparison test was applied to compare the weight of mycelium and it was found that growth was not significant between 5^{o}C and 40^{o}C (HSD: 0.000; F-value: 20322; P ≤ .1). While, the remaining pairs of temperatures showed very significant difference in the means of mycelial weight (mg) (HSD: 0.000; F-value: 20322.380; P ≤ 0.001) (Table 3). It was concluded from the results that maximum and minimum mycelial growth was recorded at 25^{o}C and 10^{o}C, respectively. The mycelial growth ceased completely at 5^{o}C and 40^{o}C. The mean mycelial growth at 25^{o}C was significantly more than all other temperatures studied. Thus, 25^{o}C was considered as the optimum temperature for growing *Helvella crispa* in the cultures for further studies.

Effect of Hydrogen ion concentration (pH)

To study the effect of different pH values on the mycelial growth, pure culture of *Helvella crispa* was inoculated in the basal liquid medium (Glucose-Asparagine). The pH of the liquid medium in flasks was adjusted in the range of 3.5-8.5 accordingly with the help of pH meter and incubated at a temperature (25 ± .2^{o}C) in an incubator. The mean mycelial weight (± standard deviation) at different pH values is numerically and graphically presented in Table 4 and Fig 4, respectively. One-way ANOVA with Tukey's multiple comparison test was applied to compare the difference of mycelial weight means and it was observed that the difference between the means of mycelial weights (mg) between pH 3.5-8.0 was non-significnat (HSD:0.00; F-value-13965.664; P ≤ 0.10) while, at the rest of the pH pairs very significant difference in the means of mycelial weight (mg) (HSD:0.00; F-value:13965.664; P ≤ 0.001) (Table 4) was observed. It was concluded from the results that maximum and minimum mycelial growth was recorded at pH 6.0 and pH 8.5, respectively. The mean mycelial weight (mg) at pH 6.0 was significantly more than at all other pH values studied. Thus, pH 6.0 was considered as optimum pH value for growing *Helvella crispa* in the cultures for further studies.

Table 3.Weight of mycelium (mg) of *Helvella crispa at* different temperatures

Sr. No.	Temperature(in^{0}C)	Weight of mycelium (mg) (mean ± SD)
1.	5	.00 ± 0.000
2.	10	13.00 ± .953
3.	15	28.52 ± .501
4.	20	59.99 ± .268
5.	25	113.70 ± .608
6.	30	75.88 ± .498
7.	35	35.45 ± .193
9.	40	0.00 ± .000

* Incubation period of 10 days

Table 4. Weight of mycelium (mg) of *Helvella crispa* at different pH values

Sr. No.	pH	Weight of mycelium (mg) (mean ± SD)
1.	3.5	26.03 ± .058
2.	4.0	31.00 ± .793
3.	4.5	45.23 ± .404
4.	5.0	65.21 ± .386
5.	5.5	99.44 ± .504
6.	6.0	110.38 ± .304
7.	6.5	80.73 ± .390
8.	7.0	51.87 ± .783
9.	7.5	35.26 ± .226
10.	8.0	26.40 ± .400
11.	8.5	20.44 ± .041

* 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 *Helvella crispa* at different temperatures

Sr. No.	Temperature		1	2	3	4	5	6	7	8	HSD	F-value
			5	10	15	20	25	30	35	40		
		Mean (mg)	.00	13.00	28.52	59.99	113.70	75.88	35.45	O.00	.000	20322.380
1	5	.00	-0.00	-13.00***	-28.52***	-59.99***	-113.70***	-75.88***	35.35***	0.00 ^{NS}		
2	10	13.00		0.00	-15.52***	-46.99***	-100.70***	-62.88***	-24.45***	13.00***		
3	15	28.52			0.00	-31.47***	-85.18***	-47.36***	-6.93***	28.52***		
4	20	59.99				0.00	-53.71***	-15.89***	24.53***	59.00***		
5	25	113.70					0.00	37.82***	78.25***	113.70***		
6	30	75.88						0.00	40.42***	75.87***		
7	35	35.45							0.00	35.45***		
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 *Helvella crispa* 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)	26.03	31.00	45.23	65.21	99.44	110.38	80.73	51.87	35.26	26.40	20.44	.000	13965.664
1.	3.5	26.03	.00	-4.97***	-19.20***	-39.18***	-73.41***	-84.35***	-54.69***	-25.83***	-9.23***	.36 ^{NS}	5.59***		
2.	4.0	31.00		0.00	-14.23***	-34.21***	-68.44***	-79.33***	-49.73***	-20.87***	-4.26***	4.60***	10.56***		
3.	4.5	45.23			0.00	-19.98***	-54.21***	-65.15***	-35.49***	-6.63***	.36^{NS}	18.84***	24.79***		
4.	5.0	65.21				0.00	-34.23***	-45.17***	-15.51***	13.35***	29.95***	38.82***	44.78***		
5.	5.5	99.44					0.00	-10.94***	18.71***	47.57***	64.18***	73.04***	79.00***		
6.	6.0	110.38						0.00	29.66***	58.52***	75.12***	83.99***	89.95***		
7.	6.5	80.73							0.00	28.86***	45.47***	54.33***	60.29***		
8.	7.0	51.87								0.00	16.61***	25.47***	31.43***		
9.	7.5	35.26									0.00	8.86***	14.82***		
10.	8.0	26.40										0.00	5.96***		
11.	8.5	20.44											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.

Effect of light and darkness

To study the effect of light and darkness on the growth of *Helvella crispa*, the basal liquid medium (Glucose-Asparagine) adjusted at pH 6.0, was inoculated and incubated at 25° ± .2°C in 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. It is clear from the results that the growth of mycelium was better in dark (110.393 ± .526 mg) than light (92.157 ± .372 mg) (Table 5.1). On application of Student's-test it was found that weight of mycelium in dark was statistically more significant than light conditions (t-value -49.058; P ≤ 0.001). Thus, the results proved that maximum mycelial growth was supported by darkness.

Table 5. Weight of mycelium (mg) of *Helvella crispa* in best liquid medium (Glucose- asparagine) in light and darkness

Sr. No.	Treatments	Weight of mycelium (mg) (mean ± SD)
1.	Light	92.157 ± .372
2.	Dark	110.393 ± .526

* Incubation period of 10 days

Table 5.1. The significance of differences between the means as determined by Student's t-test for mycelium weight of *Helvella crispa* in light and dark conditions

Sr. No.	Treatments	Weight of mycelium (mg) (mean ± SD)	t-value
1.	Light	92.157 ± .372	-49.058
2.	Dark	110.393 ± .526	

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

RESULTS

Results derived from the ten solid media tried for the growth of *Helvella crispa*, 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 *Helvella crispa* 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 ≤ 0.001) (Table 2.1). Maximum and minimum growth of *Helvella crispa* 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 *Helvella crispa* 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 *Helvella crispa* 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).

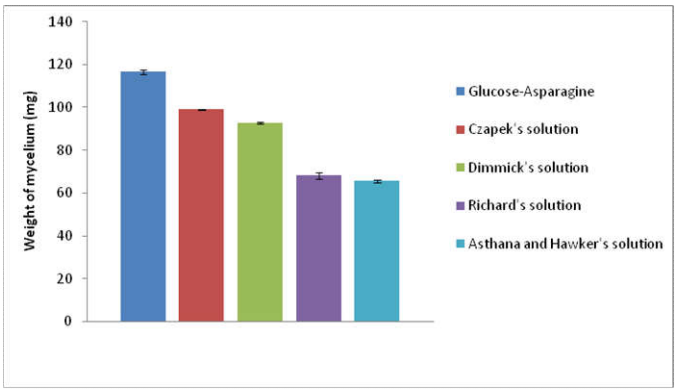

Fig. 1. Colony diameter (cm) of Helvella crispa on different solid media

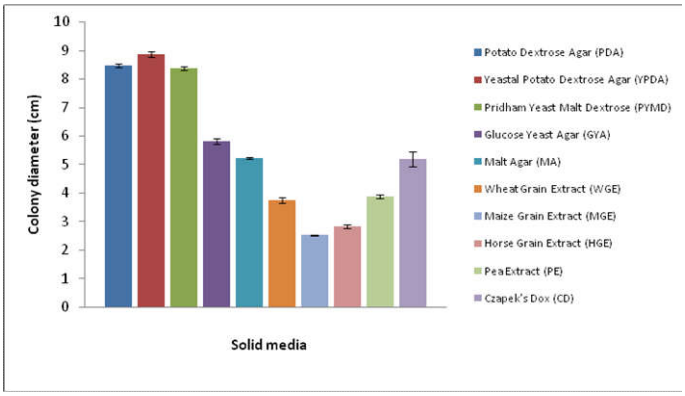

Fig. 2. Weight of mycelium (mg) of Helvella crispa in different liquid media

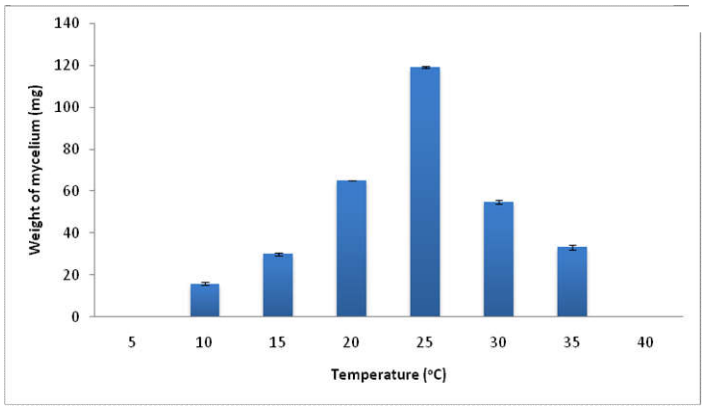

Fig. 3. Weight of mycelium (mg) of Helvella crispa at different temperatures

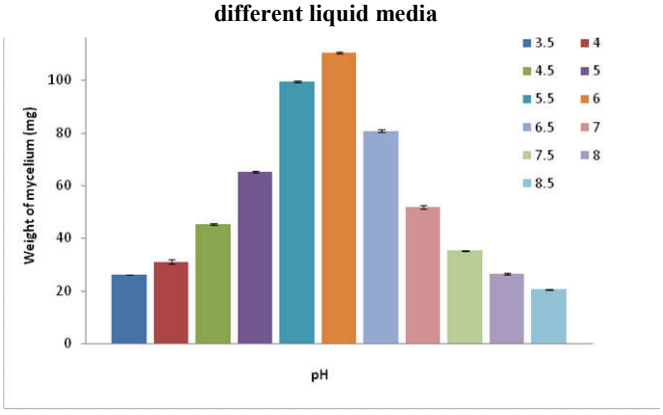

Fig. 4. Weight of mycelium (mg) of Helvella crispa at different pH values

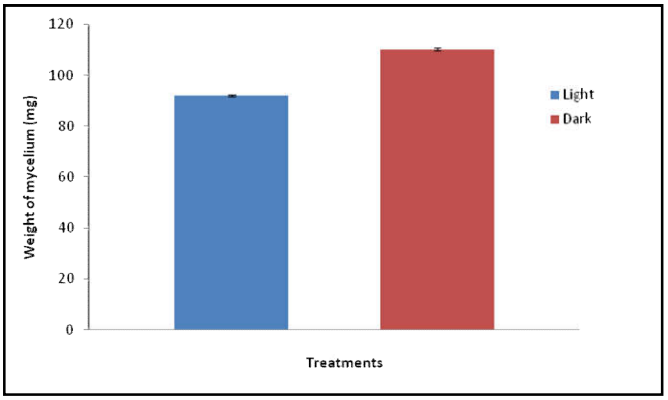

Fig. 4. Weight of mycelium (mg) of Helvella crispa at different pH values

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 *Helvella crispa* were investigated with the cultures raised from their basidiocarps. The literature has references showing evidence of best growth of *Helvella crispa* 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.*

For determining the comparative suitability for vegetative growth of *Helvella crispa,* five liquid media were 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*^{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 *Helvella crispa* the mycelium was grown in the best suited liquid medium at different levels of pH. It was recorded that maximum growth 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 *Helvella crispa*, 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 *Helvella crispa* 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 *Helvella crispa.* However the highest growth rate of these fungi was recorded in Yeastal Potato Dextrose Agar medium; Czapek's Dox medium supported least growth of *Helvella crispa.* 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 *Helvella crispa* 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 *Helvella crispa* was recorded at pH 6.0 (Table 4 and Fig.4).

Growth of mycelium of *Helvella crispa* was better in dark than under light conditions (Table 5 and Fig.5). The study on *Helvella crispa* 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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