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Biology and Growth Characteristics of Edible Mushroom: Agaricus compestris, Agaricus bisporous, Coprinus comatus

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Abstract: *Agarics, commonly known as gill- fungi, mushroom or toadstools produce conspicuous basidiocarps. Agarics may be edible, poisonous or unpalatable. Edible agarics are commonly known as mushrooms and poisonous ones the "toad stools". They are mostly saprophytic, growing commonly in lawns, pasture and gardens. In the present study the biology and growth characteristics of three edible mushrooms viz. Agaricus bisporus, A. compestris and Coprinus comatus was studied. Among different carbon sources sucrose, fructose, maltose, lactose and glucose favoured maximum mycelia growth rate. Soybean meal, bran and yeast extract were found to be the best nitrogen sources which favoured maximum growth rate of mycelium. The three inorganic salts KH2PO4, MgSO4 and KNO3 promoted high mycelia growth rate and vigority in all the three edible mushrooms. The vitamins and natural components promoted high mycelia growth rate. The optimum temperature and pH for maximum growth rate were found to be 30^{0}C and 8.0 respectively all the three edible mushrooms*

Keywords: *Mycelial growth, Agarics, Carbon sources, Nitrogen sources, Growth factors, Vitamins*

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I. Introduction

Agarics, commonly known as gill- fungi, mushroom or toadstools produce conspicuous basidiocarps, the spore- bearing fruiting body of Basidiomycota. Agarics may be edible, poisonous or unpalatable. Edible agarics are commonly known as mushrooms and poisonous ones the "toad stools". They are mostly saprophytic, growing commonly in lawns, pasture and gardens. *Agaricus compestris, Agaricus bisporous, Coprinus comatus, Lepiota* sp, *Marasmius* sp, *Trocholoma* sp. are edible mushrooms. Poisonous agarics include *Amanita muscaria, A. phalloides, A. verna* etc.

A mushroom develops from a nodule, or pinhead, less than two millimeters in diameter, called a primordium which is typically found on or near the surface of the substrate. It is formed within the mycelium. The primordium enlarges into a rounded structure of interwoven hyphae roughly resembling an egg, called a "button". The button has cottony roll of mycelium, the universal veil that surrounds the developing fruiting body. As the egg expands the universal veil ruptures and may remain as a cup or volva at the base of the stalk, or as warts or volval patches on the cap. Many mushrooms lack a universal veil and therefore they do not have either a volva or volval patches. A second layer of tissue called partial veil often covers the blade like gills that bear spores. As the cap expands, the veil breaks and remnant of the partial veil may remain as a ring or annulus around the middle of the stalk or as fragments hanging from the margin of the cap. The ring may be skirt like as in Amanita, collar like as in *Lepiota*, or mrely the faint remnants of a Cortina, which is typical off genus *Cortinarius*. Mushroom lacking partial veils do not form an annulus. The stalk (stip) may be central and support the cap in the middle, or it may be off-central and or lateral as in *Pleurotus* and Panus. Mushroom in the genera *Agaricus*, *Amanita*, *Lepoita* and *Pluteus*, have free gills that do not extend to the top of the stalk as in *Omphalotus* and *Pleurotus*. A hymaenium is a layer of microscopic spre-bearing cells that cover the surface of gills. In Mushroom and other agarics, usually four basidiospores develop on the tips of sterigmata, which extend from club-shaped basidia.

Many species of mushroom seemingly overnight, growing or expanding rapidly. This phenomenon is called mushrooming. All species of mushroom take several days to form primordial mushroom fruiting bodies, though they do not expend rapidly by the absorption of fluids. The cultivated and field mushrooms initially form a minute fruiting body, referred to as the pin stage. They then slightly expanded (buttons). Once the buttons are formed, the mushrooms rapidly pull in water from its mycelium and expend by inflating preformed cells.

Mushrooms are a low calorie food usually eaten cooked or raw and as garnish to a meal. Dietary mushrooms are good source of B vitamins, such as riboflavin, niacin and pantothenic acid, and the essential minerals, viz., Selenium, Copper and Potassium. Fat, carbohydrate and calorie are low, with absence of vitamin

'C' and Sodium [1] (Wikipedia, 2012). When exposed to ultraviolet light, natural ergosterol in mushrooms produces vitamin D_2 [2] (Koyyalamudi *et al*., 2009). This process has been exploited for the functional food retail market.

Edible mushrooms, popularly known as the meat of the vegetable world [3] (Haas and James, 2009), are used extensively in cooking. Most mushrooms have been commercially grown on mushroom farms. *Agaricus bisporus* is the most popular mushrooms and is considered safe to eat because it is controlled in controlled, sterilized environments. There are several verities of *A.bisporus* grown commercially, viz., whites, crimini, Portobello, shiitake, maitake or hen-of-the-woods, oyster and enoki. In recent years, increasing affluence in developing countries has led to a considerable growth in interest in mushrooms cultivation, which is now seen as a potentially important economic activity for small farmers.

Some species of mushrooms are poisonous. *A. bisporus* contains carcinogens called hydrazines, the most abundant of which is agaritine. However, the carcinogens are destroyed by moderate heat when cooking [4] (Siegar, 2010). There is no single trait by which all toxic mushrooms can be identified. Additionally, even edible mushrooms may produce allergic reaction in susceptible individual, from a mild asthmatic response to severe anaphylactic shock [5] (Ammirati *et al*., 1985).

China is the world largest edible mushroom producer [6] (All business. Com, 2010). The country produce about half of all cultivated mushroom, and around 2.7kg of mushrooms are consumed per person per year by over a billion people.

Many mushroom species produce secondary metabolites that can be toxic, mind-altering, antibiotic, antiviral, or bioluminescent. Several species can cause severe and unpleasant symptoms. In addition, due to the ability of mushrooms to absorb heavy metals, including radioactive, European mushrooms may include toxicity from the 1986 Chernobyl disaster and continue to be studied [7, 8, 9, 10, 11] (Belarus, 2008; Seref Turhan *et al*., 2007; Eila Kostiainen and Jarko Ylipieti, 2008; Howley, 2010, FOR, 2008).

Mushrooms with psychoactive properties have played a role in native medicine tradition all around the world. They have been used as mental and physical healing. Shamon or curandera (priest-healer) is one such traditional mushroom [12] (Hudler, 2000). Psilocybin mushrooms, commonly known as 'magic' mushrooms or shroones possess psychedelic properties and have reported as facilitating profound and life changing insights often describes as mystical experiences [13, 14) (Griffiths *et al*., 2006, 2008). Psilocybin in psychedelic mushrooms (e.g. *Psilocybe cubensis*), is being studied for its ability to help people suffering from psychological disorders such as Obsessive- compulsive disorder. Minute amounts have been reported to stop cluster and migraine headache. The extracts for medicinal mushrooms contain polysaccharides, glycoprotein and proteoglycans, which modulate immune system responses and inhibit cardiovascular, anti-viral, anti bacterial, ant- parasitic, anti-inflammatory, and anti-diabetic properties [15, 16] (Smith *et al*., 2012; Borchers *et al*., 2008).

For millennia, mushrooms have been valued as edible and medical provisions for humankind. With the popularization of mushroom farming and/or industrialization, mushroom production worldwide continues to increase. It is estimated that more than 10 million metric tons of edible and medicinal mushrooms were produced a few years ago in various countries [17] (Royse, 2005). Mushroom production can convert the huge lignocellulosic waste material into a wide diversity of products (edible or medicinal food, feed and fertilizers), protecting and regeneration the environment. In addition, the mushroom production can generate equitable economic growth that has already had an impact at national and regional levels. This impact is suspected to continue increasing and expanding in the future, because more than 70% of agriculture and forest materials are non productive and have been wasted in the processing. The mushroom conversion has been named the non-green revolution [18, 19] (Chang, 1999; Chang, 2005). However, the mushroom science is a relatively new applied science and the mushroom industry is still small compared to many plant crops, so the investment is limited. As a consequence, scientific research on mushrooms generally lags behind that of plant and animal [20] (Sonnenberg, 2005).

All commercial strains and most wild isolates of Mushrooms have a so-called secondary homothallic life cycle. Most basidia produce two spores and the four post meiotic nuclei are distributed to these spores in such a way that each spore receives two non-sister nuclei [21] (Evans, 1959). Since these nuclei have opposite mating type, each spore will produce fertile heterokaryotic mycelium upon germination. Only a small portion of the basidia (1.3%) produce 4 spores each receiving one post-meiotic nucleus and will form infertile homokaryotic mycelium. In breeding programs, these homokaryons are selected from different strains and used to make hybrids and produce a next generation.

Callac and Kerrigan have described a tetrasporic variety of *A. bisporus* from the Sonoran desert of California [22] (Callac *et al*. 1993). Most of the basidia of this variety produce 4 spores that are haploid, and mating between compatible homokaryons leads to fertile heterokaryotic mycelia. This variety displays mainly a heterothallic life cycle. At last, a rare tetrasporic variety of *A. bisporus* has been found recently that has a true homothallic life cycle [23] (Callac *et al*. 2003). Each spore has one haploid nucleus and can produce fruit

bodies. All three varieties found are completely interfertile. The different varieties are designated as *A. bisporus* var. *bisporus* (secondary homothallic), *A. bisporus* var. *eurotetrasporus* (true homothallic) and *A. bisporus* var. *burnetti* (heterothallic).

The heterothallic trait (mainly 4-spored basidia with homokaryotic spores) is dominant in crosses between 2-spored and 4-spored varieties. The MAT gene and the main determinant for the basidial spore number (bsn) are located on chromosome I [24]. (Imbernon *et al*. 1996). Genetic maps of this chromosome of the secondarily homothallic and heterothallic varieties show a high degree of synteny [25] (Callac *et al*. 1997), indicating the close relationship between these varieties.

All commercial lines of the button mushroom and most wild isolates have a secondarily homothallic life cycle. Only a low percentage of the spores are homokaryotic (haploid). These spores originate from rare 4-spored basidia. Genetic analysis has shown that recombination occurs [26, 27] (Summerbell *et al.* 1989; Kerrigan *et al.* 1993) but at a low frequency. Sonnenberg *et al*., (2008) [28] observed low recombination frequencies, i.e. 1 to 2 recombination events per individual per generation. There is, however, a normal independent segregation of homologue chromosomes. Analysis of marker segregation in heterokaryotic single spore isolates (derived from 2-spored basidia) shows that many loci are heterothallelic. This indicates that most spores receive a pair of non-sister nuclei. The typical life cycle is thus directed to the preservation of heterozygosity in its offspring and might prevent accumulation of deleterious alleles. Contrary to the '*bisporus*' variety, the '*burnettii*' variety has predominantly 4 spores per basidium and there are indications that this variety shows a higher recombination frequency. This reproductive system thus seems to be directed to out breeding. In hybrids between bisporic and tetrasporic varieties, maps are extended due to higher recombination frequencies [29, 25] (Sonnenberg *et al.* 1996; Callac *et al*. 1997). Callac *et al*. (1997) [25] found in 52 single spore isolates from a *bisporic* variety no recombination between markers on chromosome I. In a cross between *bisporic* and *burnettii* varieties, 23% of the offspring were found to be recombinant for markers on chromosome I.

The increased recombination frequency in hybrids between the bisporic and tetrasporic varieties compared to the bisporic variety indicates that the tetrasporic variety has a higher recombination frequency than the bisporic variety. However, no data on marker segregation from tetrasporic offspring are available.

Mazhieka *et al*. (2006) [30] have studied the meiotic process in the heterothallic and secondarily homothallic varieties of *A. bisporus*. They have used a previously developed method of microspreading of basidial nuclei to study meiotic prophase I [30] (Mazheika *et al*. 2006). The EM observations show a large variation in morphologies in chromosome pairing in bisporic and tetrasporic varieties. Clear, vague or absence of axial elements (AE) and synaptonemal complexes (SC) are observed.

A phenomenon that is relevant to mushroom breeding is chromosome length polymorphism (CLP). The size of fungal genomes allows separation of intact chromosomes by pulse field gel electrophoresis [31] (Mills and McClusky, 1990). This technique has shown that strains within a species have a considerable variation in chromosome length [32] (Zolan, 1995). This has been observed also in mushroom producing species [33, 34, 35, 36] (Kerrigan *et al*. 1993; Sonnenberg *et al*., 1996; Larraya *et al*., 1999; Kim *et al*. 2000). The origin of the length differences is not known and it is assumed that homologues of strains which show CLP have more than one heterologous region. A source for the generation of CLP in sexual reproducing fungi is meiosis.

The biological characteristics and growth performance of three edible mushrooms viz., *Agaricus bisporus, Agaricus compestris* and *Coprinus comatus* have not been studied so far and hence the present investigation has been undertaken.

II. Materials and Methods

The work was conducted on three species of edible mushroom viz., *Agaricus compestris, A.bisporus* and *Coprinus comatus*, collected from local mushroom farms (Patna). These mushrooms were cultured separately in malt yeast extract agar medium. The spawn was prepared and planted in well prepared culture media in the laboratory.

Culture media preparation: Five different cuilture media were prepared for laboratory cultivation these edible mushrooms. The composition of these media was as follows:

Medium A: yeast extract 2 g, KH2PO4 1 g, MgSO4 0.5 g, agar 20 g, distilled water 1000 mL; Medium B: glucose 20 g, KH2PO4 1 g, MgSO4 0.5 g, agar 20 g, distilled water 1000 mL; Medium C: glucose 20 g, yeast extract 2 g, VB1100 mg, agar 20 g, distilled water 1000 mL; Medium D: glucose 20 g, yeast extract 2 g, agar 20 g, MgSO4 0.5 g, KH2PO4 1 g, distilled water 1000 mL; Medium E: potato 200 g, sugar 20 g, soybean meal 2 g, KH2PO4 0.5 g, agar 30 g, distilled water 1000 mL.

Effect of Carbon nitrogen sources: The PDA (Potato Dextrose Agar) medium was used for the activated experiment. This medium consisted of potato 200 g, glucose 20 g, agar 20 g, pH neutral (7.0) per liter of distilled water. When mycelia had grown all over Petri dishes, it was punched into 5 mm homogeneous

pieces at the periphery of colonies by the puncher. These pieces of mycelia were used for the next experiment. In order to study the effect of carbon sources on growth 2% of different carbon sources viz., glucose, sugar, maltose, starch, lactose, CMC–Na, fructose was added individually to the basal medium A. A medium containing no carbon source was used as a control. These media were autoclaved at 121°C for 30 min. Mycelium was inoculated into the test medium under aseptic operation after autoclaving. Culture dishes were inoculated and incubated at 25°C in a BOD incubator. All experiments were performed in sextuple. The diameter of colonies was measured every 24 hours and the mycelia growth was observed until the mycelium completely covered the Petri dishes [37] (Shim S M, 2005). In order to study the effect of nitrogen source on growth performance of these mushrooms 2% addition level of yeast extract, peptone, beef extract, carbamide, KNO3, bran, soybean meal was added individually to the basal medium B. No added medium served as a control.

Effect of inorganic salt and growth factor on growth performance: The effect of inorganic salts on growth performance of these three mushrooms the medium B was used. Three different concentrations viz., 0.5%, 1% and 1.5% were set for the three inorganic salts (KH_2PO_4, $MgSO_4$, KNO_3).Similarly the effect of growth factors like Vitamin C, Vitamin B1 and Vitamin E, natural components (potato, bean sprout, malt extract, hay, mushroom, corn) on growth performance was evaluated. Apart from out natural components, 0.01 g/L addition level was added individually to the basal medium C. About 20 g/L of the natural components was extracted with distilled water and was individually added to the basal medium. Unsupplemented basal medium served as control [38] (LU T *et al*., (2017).

Orthogonal test: Medium D was used as the basal medium for orthogonal test. All the above four factors viz., carbon source, nitrogen source, inorganic salt, growth factor) were respectively selected best three levels to carried out the four factors of exercise at three levels orthogonal design as suggested by Lu T and Bau Tolgor (2013) [39].

Single factor test of temperature and pH: On the basis of optimal growth of mycelium cultures were obtained by orthogonal test. To investigate the temperature favorable for the mycelial growth of *A. bisporus, A. compestris* and *Coprinus comatus* the culture of each was incubated for 10 days at 5 different temperature viz., 15^0C, 20^0C, 25^0C, 30^0C and 35^0C in a BOD incubator. Medium E was used as the basal medium. To screen the effect of pH on the growth of *A. bisporus, A. compestris* and *C. comatus*, the basal medium E was adjusted to the range of pH 5.0, 6.0, 7.0, 8.0 and 8.5 with 1 M NaOH or HCl and was incubated for 10 days at 25°C [40] (Rizal L M, 2015).

III. Results

Biology and Taxonomy of *Agaricus compestris, A. bisporus* and *Coprinus comatus*

***Agaricus* (Figure-1, 2 and 3)**

Terrestrial saprophytic fungus usually found on decaying leaves, logs, manure pastures, woodland litter and meadows during the rainy season.

Systematic position: Kingdom: Mycota; Division: Eumycota; Sub-division: Basidiomycotina; Class: Hymenomycota; Sub-class: Holobasidiomycetiae; Order: Agaricales; Family: Agaricaceae; Genus: *Agaricus*; Species: *A. compestris* (L.) *A. bisporus* J. E. Lange

Somatic body consisted of a stalked fruiting body with a fleshy cap at its top (pileus); gills radially arranged on its underside with chocolate-brown spores. Vegetative structure consisted of a mass of thread-like hyphae (mycelia), found underground; mycelia of two types, the primary and secondary; primary mecelium hyaline, monokaryotic and septate, ephemeral; Secondary mycelium dikaryotic, dolipore septate, perennial and branched; mycelia hyphae entangled together, forming root-like white hyphal cords known as rhizomorphs; Rhizomorphs developed into fruiting bodies, basidiocarps commonly known as mushrooms. The dikaryotic mycelium growing away from the centre in the form of a ring, forming a circular colony in the soil also known as fairy rings or fairy circles.

Sporocarp or basidiocarp, the only upper ground part of the fungus consisted of a stem or stalk, known as stipe, which holds a cap or pileus at the top. The underside of the pileus consisted of numerous radially arranged, plate-like gills, which contained spore-bearing basidia. The developing gills were protected by a partial veil which later formed a ring of tissue on the stipe, the annulus.

Agaricus compestris (meadow mushroom) was distinguished by their white cap, stocky stature, non-staining surfaces and flesh, pink-then-brown gills. Their spores were 6.5-8.5µm long.

Agaricus bisporus was distinguished by their long stem with tapering base; flesh white throughout, not changing colour when sliced; spores dark brown, 6.5-8.5 X 4.5-5.5µm, ellipsoid; smooth; thick-walled; 4-sterigmata.

Figure-1: Basidiocarps of *Agaricus bisporus*

Figure-2: Basidiocarps (Fruiting bodies) of *Agaricus compestris*

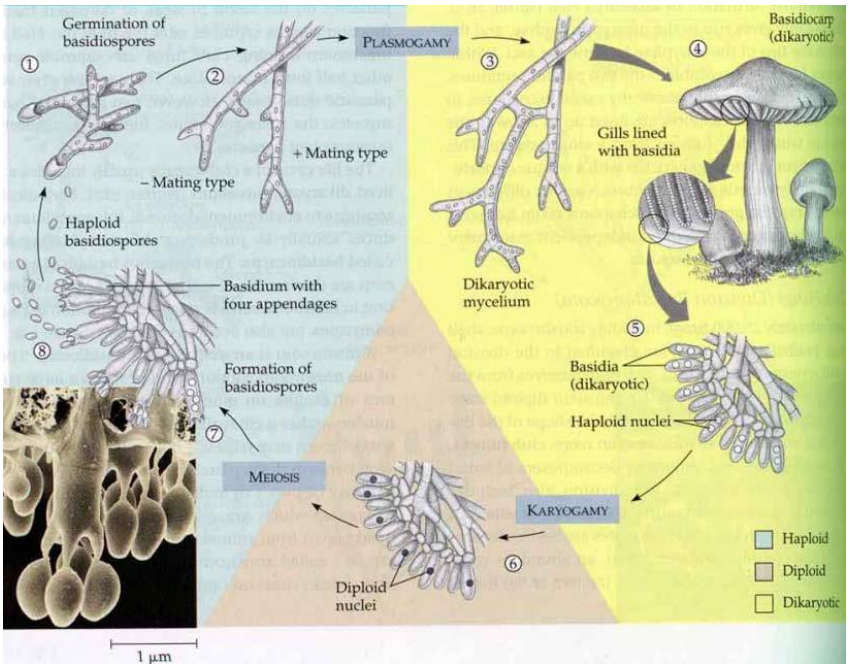

Figure-3: Diagramatic representation of life cycle of *Agaricus*

***Coprinus comatus* (Figure-4, 5 and 6)**

Coprinus comatus (the shaggy ink cap, lawyer's wig, or shaggy mane) terrestrial, saprophytic,usually growing on lawns, along gravel roads and waste areas. The young fruiting bodies first appeared as white cylinders emerging from the ground, then the bell-shaped caps opened out. The caps were white, and covered with scales distinguishing its origin of the common names. The gills beneath the cap were white, then pink, then turned black and secreted a black liquid filled with spores and hence the name "ink cap".

Taxonomic position

Kingdom: Mycota; Division: Eumycota; Sub-division: Basidiomycotina; Class: Hymenomycota; Sub-class: Holobasidiomycetiae; Order: Agaricales; Family: Agaricaceae; Genus: *Coprinus*; Species: *C. comatus* O.F.Müll

The stipe measured 10–40 centimetres high by 1–2.5 centimetres diameter; lacked pleurocystidia; Spores black-brown,Spores 9-13 x 7-9 µm; elliptical; smooth; with a central to slightly eccentric pore. Basidia 4-spored; surrounded by brachybasidia.

Figure-4: Basidiocarps (Fruiting bodies of *Coprinus comatus*)

Figure-5: Basidia and brachybasidia of *C. comatus*

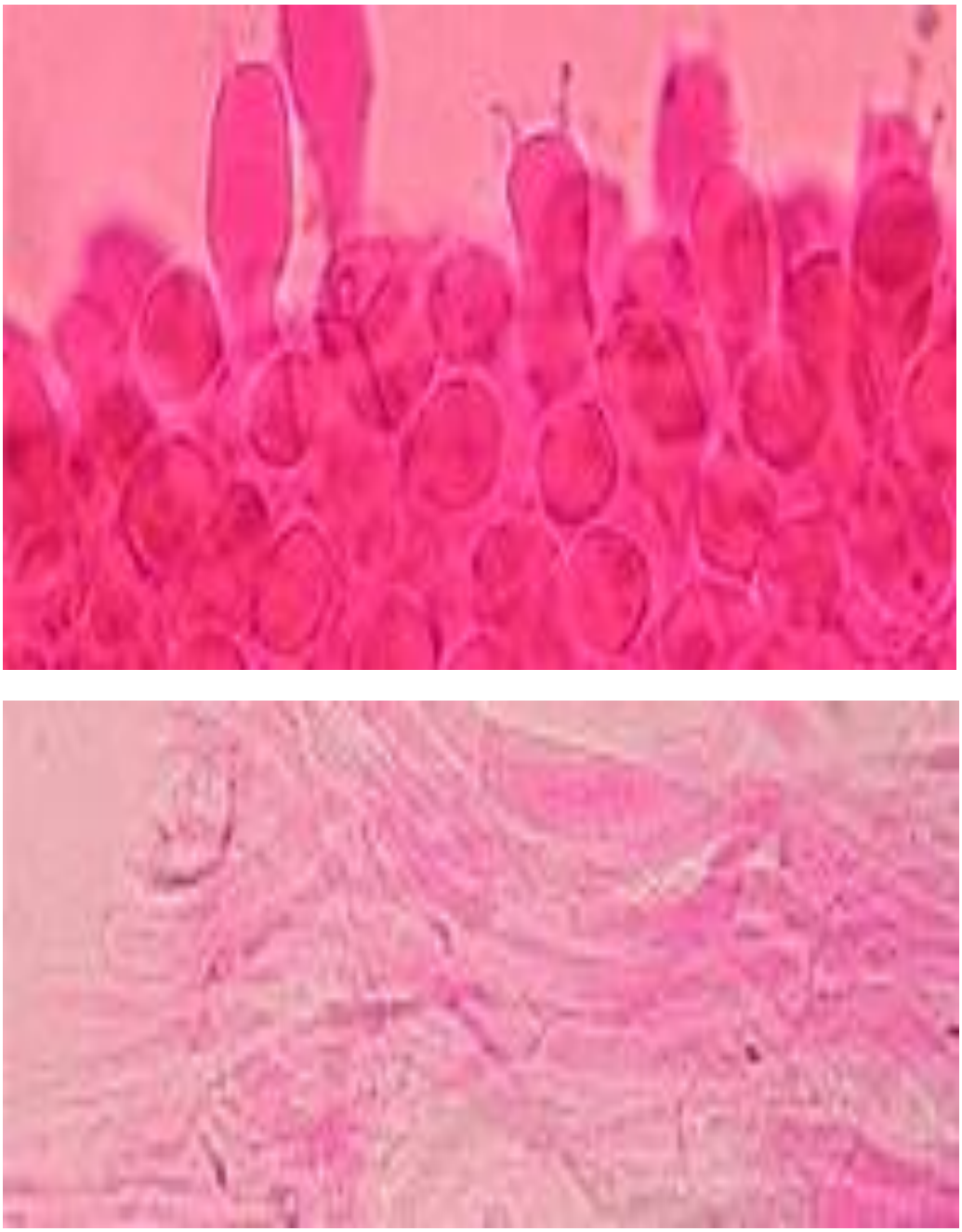

Figure-6: Compact mycelia of *C. comatus*

The results related to the in vitro growth pattern of *Agaricus bisporus*, *A. compestris* and *Coprinus comatus* have been presented in Table-1 to 6 and Figure-7- 14.

Table-1: Effect of different carbon sources on mycelia growth of three edible mushrooms (Mycelial growth in mm/d of average value of six replicates ±SE)

Carbon sources	*A. bisporus*		*A. compestris*		*C. comatus*	
	Mycelial growth rate in mm/d	Vigority	Mycelial growth rate in mm/d	Vigority	Mycelial growth rate in mm/d	Vigority
Sucrose	5.25±0.45	+++	5.35±0.35	+++	4.85±0.26	+++
Fructose	5.35±0.26	+++	5.45±0.31	+++	4.65±0.32	+++
Maltose	5.25±0.21	+++	5.35±0.45	+++	4.87±0.17	+++
Lactose	5.17±0.19	+++	5.15±0.18	+++	4.25±0.21	+++
Glucose	5.21±0.21	+++	5.30±0.21	+++	4.35±0.24	+++
CMC-cellulose	3.25±0.26	++	3.65±0.24	++	3.75±0.23	++
Starch	2.85±0.24	+	2.75±0.21	+	2.67±0.24	+

Table-2: Effect of different nitrogen sources on mycelia growth of three edible mushrooms (Mycelial growth in mm/d of average value of six replicates ±SE)

Nitrogen sources	*A. bisporus*		*A. compestris*		*C. comatus*	
	Mycelial growth rate in mm/d	Vigority	Mycelial growth rate in mm/d	Vigority	Mycelial growth rate in mm/d	Vigority
Soybean neal	5.35±0.35	+++	5.75±0.25	+++	4.95±0.24	+++
Bran	4.45±0.24	+++	5.25±0.21	+++	4.75±0.32	+++
Yeast extract	5.85±0.11	+++	5.65±0.25	+++	4.81±0.15	+++
KNO_3	2.65±0.15	++	2.75±0.16	++	4.97±0.23	+++
Peptone	1.45±0.23	+	1.55±0.25	+	1.65±0.14	+++
Beef extract	2.75±0.24	++	1.65±0.14	+	1.95±0.13	++
Carbamide	0.18±0.05	+	0.17±0.05	+	0.15±0.07	+

Table-3: Effect of inorganic salts on growth performance of three edible mushrooms (Mycelial growth in mm/d of average value of six replicates ±SE)

Salt	Concentration (%)	*A. bisporus*		*A. compestris*		*C. comatus*	
		Growth in mm/d	Vigor	Growth in mm/d	Vigor	Growth in mm/d	Vigor
KH_2PO_4	0.5	4.85±0.21	+++	4.83±0.22	+++	4.35±0.31	+++
	1.0	4.95±0.01	+++	4.97±0.23	+++	4.45±0.41	+++
	1.5	5.15±0.23	+++	5.21±0.27	+++	4.75±0.31	+++
$MgSO_4$	0.5	4.35±0.11	+++	4.26±0.31	+++	4.25±0.27	+++
	1.0	4.25±0.16	+++	4.67±0.41	+++	4.75±0.28	+++
	1.5	4.95±0.25	+++	5.15±0.25	+++	4.95±0.15	+++
KNO_3	0.5	3.17±0.26	+++	3.65±0.27	+++	3.55±0.17	+++
	1.0	3.15±0.20	+++	4.16±0.21	+++	3.75±0.19	+++
	1.5	4.25±0.17	+++	5.27±0.20	+++	4.21±0.23	+++

Table-4: Effect of growth factors and natural products on growth performance of three edible mushrooms (Mycelial growth in mm/d of average value of six replicates ±SE)

Growth factors and natural components	*A. bisporus*		*A. compestris*		*C. comatus*	
	Growth in mm/d	Vigor	Growth in mm/d	Vigor	Growth in mm/d	Vigor
Vitamin C	4.18±0.24	+++	4.25±0.31	+++	3.98±0.18	+++
Vitamin B1	4.15±0.21	+++	4.27±0.11	+++	3.95±0.29	+++
Vitamin E	4.25±0.23	+++	4.31±0.26	+++	4.15±0.27	+++
Potato	4.45±0.17	+++	4.48±0.31	+++	4.17±0.31	+++
Bean sprout	4.55±0.22	+++	4.65±0.20	+++	4.25±0.41	+++
Malt extract	4.16±0.23	+++	4.21±0.28	+++	4.31±0.24	+++
Hay	4.12±0.18	+++	4.18±0.32	+++	4.17±0.23	+++
Mushroom	4.65±0.25	+++	4.75±0.24	+++	4.47±0.14	+++
Corn	4.35±0.31	+++	4.45±0.21	+++	4.35±0.16	+++

Table-5: Effect of different temperature on growth performance of three edible mushrooms (Mycelial growth in mm/d of average value of six replicates ±SE)

Temperature ^{0}C	*A. bisporus*		*A. compestris*		*C. comatus*	
	Growth in mm/d	Vigor	Growth in mm/d	Vigor	Growth in mm/d	Vigor
15	1.75±0.22	+	1.85±0.21	+	1.65±0.17	+
20	4.15±0.11	+++	4.35±0.16	+++	3.75±0.19	++
25	4.75±0.13	+++	4.95±0.16	+++	3.85±0.17	++
30	5.25±0.17	+++	5.37±0.32	+++	4.77±0.32	+++
35	3.71±0.12	++	3.65±0.21	++	3.55±0.21	++

Table-6: Effect of different pH on growth performance of three edible mushrooms (Mycelial growth in mm/d of average value of six replicates ±SE)

pH	A. bisporus		A. compestris		C. comatus	
	Growth in mm/d	Vigor	Growth in mm/d	Vigor	Growth in mm/d	Vigor
5.0	2.26±0.24	++	2.25±0.31	++	2.12±0.18	++
6.0	3.15±0.21	++	3.17±0.11	++	3.21±0.29	++
7.0	4.21±0.23	+++	4.35±0.26	+++	4.37±0.27	+++
8.0	5.35±0.17	+++	5.57±0.31	+++	4.85±0.31	+++
8.5	5.27±0.22	+++	4.75±0.20	+++	4.35±0.41	+++

Degree of vigority is represented by + sign
+++ = high mycelia growth
++ = intermediate growth
+ = poor mycelia growth

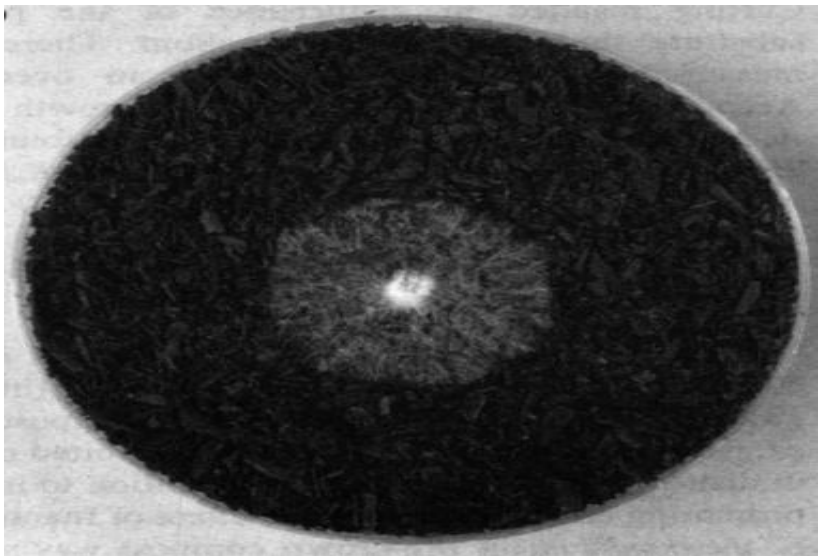

Figure-7

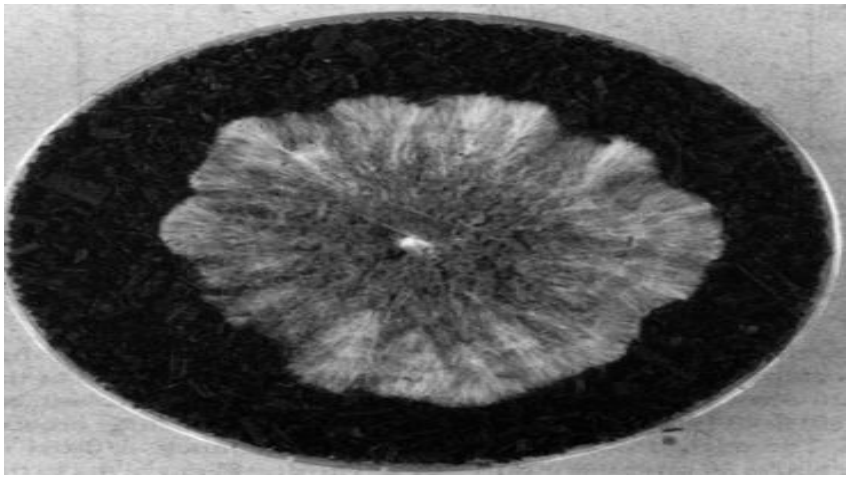

Figure-8
Figure: 7 and 8: Mycelial growth pattern of *Agaricus bisporus*

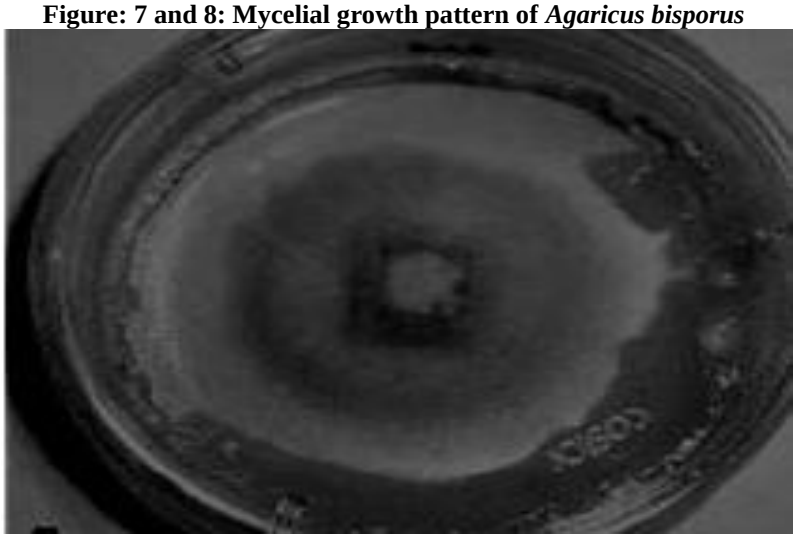

Figure-9

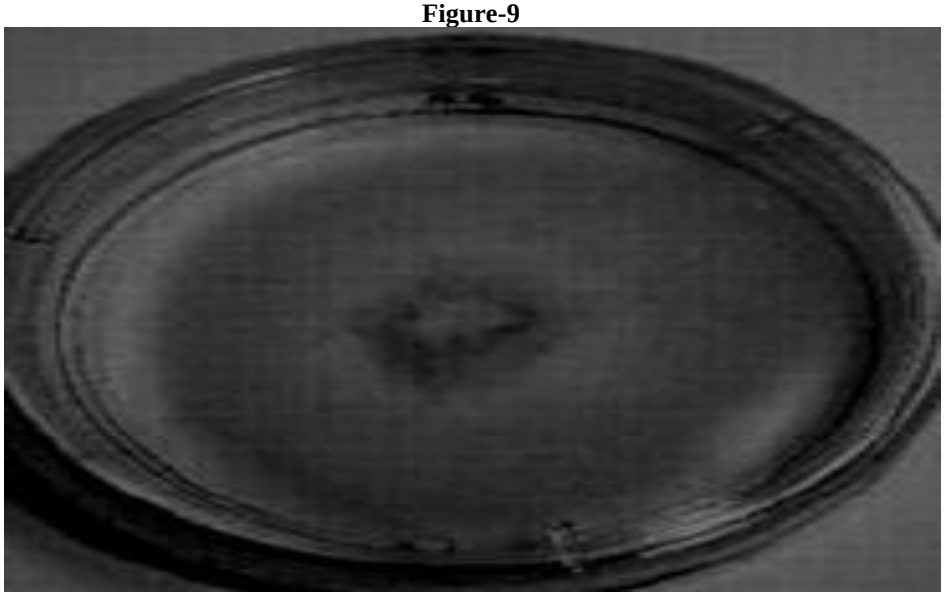

Figure-10

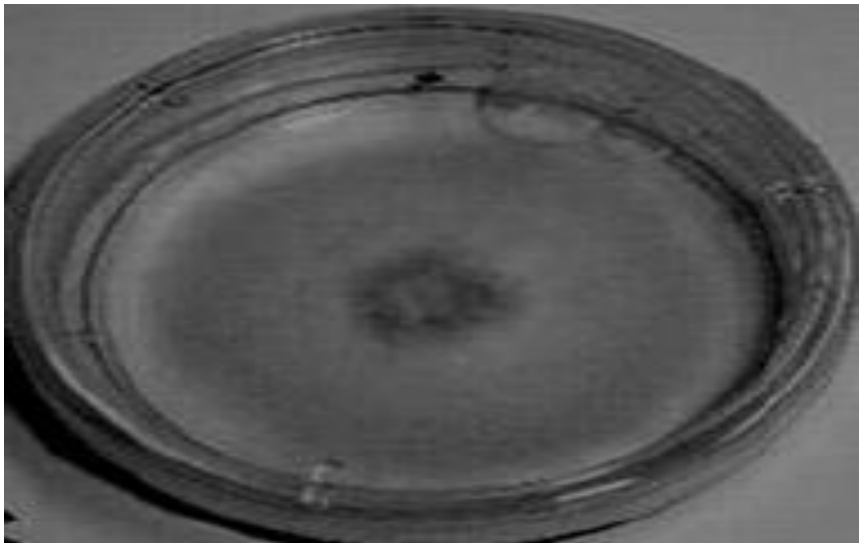

Figure-11

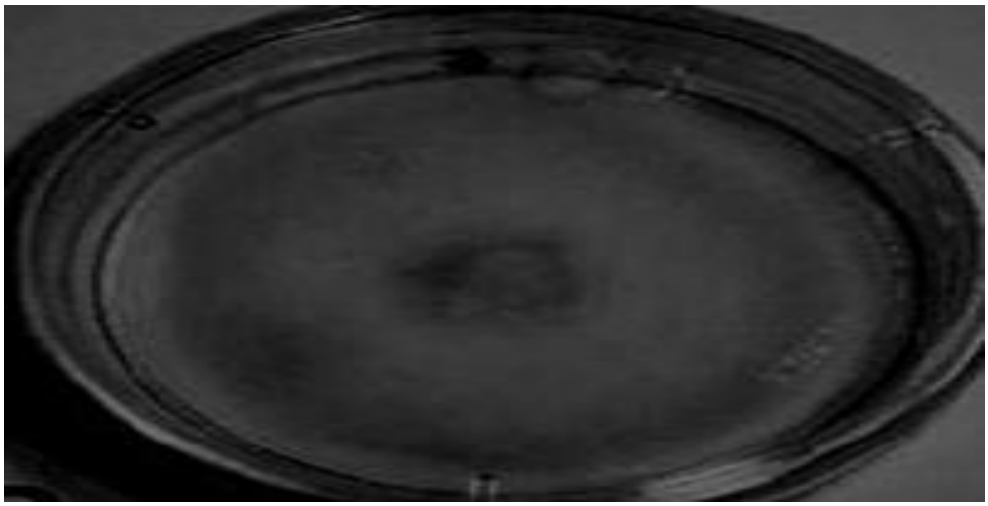

Figure-12

Figure: 9-12: Mycelial growth pattern of *Agaricus compestris*

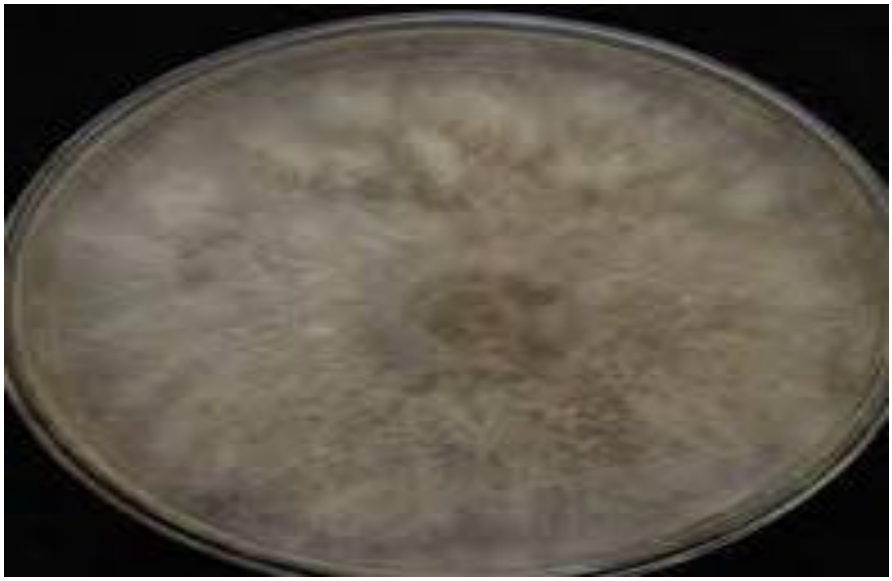

Figure-13

Figure-14

Figure: 13 and 14: Mycelial groath pattern of *Coprinus comatus*

Effect of carbon sources on mycelia growth: The effect of seven different carbon sources on the growth of mycelium in three edible mushroom has been presented in Table-1. From the results it is evident that sucrose, fructose, maltose, lactose and glucose favoured maximum mycelia growth rate in the range of 5.15 mm/d to 5.45 mm/d in *Agaricus bisporus* and *A. compestris*, and in the range of 4.25 mm/d to 4.87 mm/d in *Coprinus comatus*. In comparision to *Agaricus bisporus* and *A. compestris* the mycelia growth rate of *Coprinus comatus* was less. The mycelial vigourity was high in all these soluble sugars in all the three edible mushrooms. Among insoluble carbohydrates the CMC-cellulose favoured moderate growth rate of mycelia, 3.25 mm/d to 3.65 mm/d in *A. bisporus* and *A. compestris*, and 3.75 mm/d in *Coprinus comatus*. The growth rate of mycelia was less in media supplemented with starch (2.75 mm/d in *A. compestris*, 2.85 mm/d in *A. bisporus* and 2.67 mm/d in *C. comatus* (Table-1; Figure-7 to 14). The mycelia vigourity was intermediate in CMC-cellulose but poor in media supplemented with starch.

Effect of Nitrogen sources on Mycelial growth: The effect of seven different nitrogen sources on the growth rate of mycelium in three edible mushroom has been presented in Table-2. From the results it is clear that Soybean meal, bran and yeast extract favoured maximum growth rate of mycelium in all the three edible mushrooms. The mycelia growth rate was moderate in media supplemented with KNO_3 and Beef extract, but poor in presence of peptone and carbamide. In *Agaricus bisporus* the growth rate of mycelium was maximum in yeast extract (5.85 mm/d) followed by Soybean meal (5.35 mm/d) and Bran (4.45 mm/d). In *A. compestris*

Soybean meal promoted maximum growth rate (5.75 mm/d) followed by Yeast extract (5.65 mm/d) and Bran (5.25 mm/d). *Coprinus comatus* exhibited a more or less similar trend of mycelia growth rate in the range of 4.75 mm/d to 4.95 mm/d. The mycelial vigority was high in media supplemented with Soybean meal, Bran and Yeast extract (3+) in *A. bisporus* and *C. comatus* but moderate in *A. compestris* (2+) with Soybeal meal. KNO_3 and Beef extract favoured moderate mycelia growth in *A. bisporus* but poor growth rate in *A. compestris* and C. comatus (+). Peptone and carbamide promoted very poor mycelia growth rate in all the three edible mushrooms (Table-2).

Effect of inorganic salts: The effect of three different concentration of inorganic salts viz. KH_2PO_4, $MgSO_4$ and KNO_3 on mycelia growth rate of *Agaricus bisporus , A. compestris* and *Coprinus comatus* was investigated (Table-3). From the results it is evident that all the three inorganic salts promoted high mycelia growth rate and vigority in all the three edible mushrooms. The optimum mycelial growth rate of all the three edible mushrooms was achieved at 1.5 % KH_2PO_4, $MgSO_4$ and KNO_3. However, growth rate was slightly less in media supplemented with KNO_3 in comparison with other two salts (Table-3).

Effect of growth factors and natural components: The effect of three vitamins viz. Vitamin C, Vitamin B1 and Vitamin E and six natural components on the growth rate of three edible mushrooms was studied (Table-4). The results revealed that all the vitamins and natural components promoted high mycelia growth rate in *A. bisporus, A. compestris* and *C. comatus* (Table-4). The mycelia growth vigour was also high in all the three mushrooms with these vitamins and natural components.

Effect of temperature on mycelia growth rate: The growth rate of mycelium was poor at 15^0C (1.75 mm/d in *A. bisporus*; 1.85 mm/d in *A. compestris*; 1.65 mm/d in *C. comatus*). The growth rate increased with increasing incubation temperature. The optimum temperature for maximum growth rate was 30^0C in all the three edible mushrooms. The growth rate declined to 3.75 mm/d in *A. bisporus*; 3.65 mm/d in *A. compestris* and 3.55 mm/din *C. comatus* at 35^0C (Table-5).

Effect of pH on mycelia growth rate: The growth rate of mycelium was poor (2.12 mm/d – 2.26 mm/d) at pH 5.0 in all the three edible mushrooms. The optimum pH was found to be 8.0 at which the growth rate of mycelium was high in all the three edible mushrooms (Table-6).

IV. Discussion

Edible mushrooms possess the ability to utilize a wide spectrum of nutrients as sources of energy. Being nutritional heterotrophs, they obtain their carbon requirements from organic carbon sources, including various carbohydrate and non-carbohydrate compounds. With an effective battery of enzymes, they are able to utilize a wide array of such substances with high degree of efficiency.

Fungi exhibit carbon heterotrophy and obtain their carbon-requirement from various organic sources. In the present investigation effect of seven organic carbon sources on the growth rate of *Agaricus bisporus, A. compestris* and *Coprinus comatus* was studied. It was found that the disaccharide sugars viz., sucrose, maltose, lactose and monosaccharide sugar glucose and fructose favoured highest growth rate in comparison to polysaccharides like CMC-cellulose and starch. It can, therefore, be concluded that disaccharides and monosaccharides are more easily utilizable sources of carbon that the polysaccharides. The present findings gain support from the work of Xiao Yu ZHANG *et al*., (2018) [43] who have studied the effect of different carbon sources, on the growth rate of a mushroom *Auricularia villosula* and observed a more or less similar results.

Like carbon sources, nitrogen is also used both for functional as well as structural purposes by fungi. The form of nitrogen has a profound effect on metabolism of fungi. In the present investigation the effect of different organic and inorganic nitrogen sources on the growth rate of *A. bisporus, A. compestris* and *Coprinus comatus* was studied. It was observed that the soybean meal, bran and yeast extract promoted highest mycelia growth rate of these edible mushrooms than inorganic nitrogen (KNO_3). The peptone and beef extract were found to promote poor mycelia growth. In presence of carbamide the mycelia growth rate was very poor. It can, therefore, be concluded that nitrate-nitrogen (KNO_3), peptone, beef extract and carbamide less assimilable by these three edible mushrooms.

In the present investigation it was observed that the growth factors viz., Vitamin C, Vitamin B1 and Vitamin E, and natural components like potato, bean sprout, malt extract, hay, mushroom and corn promoted luxuriant mycelia growth of *Agaricus bisporus, A. compestris* and *C. comatus*. A temperature of 30^0C and pH in the range of 8.0-8.0 was found optimal for luxuriant growth of these three edible mushrooms. The present findings gain support from the work of Xiao Yu ZHANG *et al*., (2018) [41] who have studied the effect of different carbon sources, nitrogen sources, vitamins growth factors, temperature and pH on the growth rate of a mushroom *Auricularia villosula* and observed a more or less similar results. Gizaw (2010) [42] studied the

cultivation yield performance of Pholitota nameko on different agro-industrial wastes. Lu *et al*., (2017) [38] have studied the physiological behavior of wild edible mushroom Leucocalocybe mongolica and found a more or less similar pattern. Zhu *et al*., (2011) [43] have studied the effect of inorganic salts and growth factors on the mycelia growth of Morchella esculenta and found a more or less similar results. Wang Jing (2013) [44] investigated the morphological development and domestication of *Auricularia polytricha*. Wang *et al*., (2014) [45] have studied the cultivation characteristics of four wild species of *Auricularia*. Shim *et al*., (2005) [37] have studied the cultural conditions for the mycelia growth of *Macrolepiota procera* and found a more or less similar pattern of in vitro growth characteristics like those of *A. bisporus, A. compestris* and *Coprinus comatus*.

V. Conclusion

It can be concluded that *Agaricus bisporus*, *Agaricus compestris* and *Coprinus comatus* exhibited luxuriant mycelia growth in solid media supplemented with monosaccharide and disaccharides as carbon sources, organic nitrogen sources like Soybean meal, bran and yeast extract, and growth factors (Vitamin C, Vitamin B1 Vitamin E) and natural components of plant origin at temperature 30^0C and pH 8.0.

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