INVESTIGATION ON THE EDIBLE SPECIES OF *Coprinus* AND STANDARDISATION OF TECHNIQUES FOR ITS LARGE SCALE ARTIFICIAL CULTIVATION

BY

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THESIS

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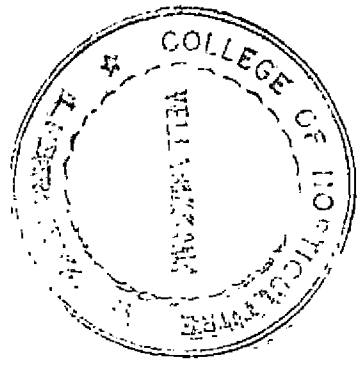

DECLARATION

I hereby declare that this thesis entitled "Investigation on the edible species of Coprinus and standardisation of techniques for its large scale artificial cultivation" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

(GEETHA D)

College of Agriculture,
Vellayani,
December, 1982.

CERTIFICATE

Certified that this thesis entitled "Investigation on the edible species of Coprinus and standardisation of techniques for its large scale artificial cultivation" is a record of research work done independently by Smt.Geetha,D under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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(GEETHA D)

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INTRODUCTION

INTRODUCTION

Mushrooms are a heterogenous group of fleshy fungi and their cultivation has developed into a large scale industry since it provides an excellent source of nutritive and delicious food. These are rich in proteins, vitamins and minerals, while poor in fat and carbohydrates. Their protein is having a better digestibility than other protein foods. They are liked for their better flavour, taste and high food value. Mushrooms are also known to possess medicinal properties

The mushrooms of the genus Coprinus are commonly known as ink caps and are characterised by black spore deposits and conversion of cap and gills into black inky fluid. They vary in size and grow on dung, recently manured ground, humus and wood. Most of the larger ones are edible in immature stages. They must be picked before they mature and used almost at once. These mushrooms have excellent flavour and fine texture, but for culinary purpose, they are not as popular as other mushrooms because of the dirty colour of the spores and smaller size of fruit bodies.

Species of Coprinus are suited for the cultivation under the tropical conditions utilising various farm and industrial waste products. They are found to occur on cowdung manure, paddy straw and guinea grass stumps and among these Coprinus lagopus (Fr.) Fr. is found to be the best growing one on paddy straw beds. Very limited studies have been carried out so far on the morphology, nutrition and suitability of the large scale cultivation of any species of Coprinus. Taking into consideration of these points the present study was undertaken on the following aspects.

1. Collection, identification and isolation into pure culture of different species of Coprinus locally available.

2. A critical study of the natural substrate on which native Coprinus occur in the local conditions.

3. Comparative studies on the nutritive value of different edible species of Coprinus.

4. Physiological studies to standardise the nutritional requirements of edible species of Coprinus.

5. Standardisation of techniques for spawn

production using various raw materials like straw, grasses, grains, etc.

6. Standardisation of techniques for artificial cultivation of edible species of Coprinus using various raw materials like straw, compost, salvinia, banana dried leaves and pseudostem, sesamum waste, vegetable waste, etc.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The first reference to Coprini in botanical literature is found in the statement by Theophrastus, Circa 300 B.C., that the fungi which grow on dung have no bad smell. Persoon (1801), in his Synopsis fungorum, gathered together all the lamellated fungi exhibiting deliquescence into a section of the genus Agaricus and called this section Coprinus because of the fact that many of the species come up on the dung of herbivorous animal. Fries (1821) retained this arrangement, so that for several decades such a fungus as Coprinus comatus was known to Mycologists as Agaricus comatus. However, Fries, with the publication of his Epicrisis Systematis Mycologici, (1836-1838), raised Persoon's section Coprinus to generic rank, a status which has been rightly maintained by all Modern Systematists. Ih thus happens that all the fungi having fruit bodies of Inaequi-hymeniiferous or Coprinus type are included in the single genus Coprinus.

Singer (1975) enumerated the characters of the genus Coprinus with the type species being C.comatus as follows. "Pileus usually conical or campanulate in youth, more rarely initially subglobose, then expanding in many species especially in the small ones; structure of

the epicutis and velar layers varied; margin frequently deeply plicate-furrowed along the back of the lamellae which generally have parallel sides and mostly disappear in age by autodeliquescence starting from the edge upwards, free or sinuate, or adnexed or adnate; hymenium consisting of isolated basidia arranged rather regularly among sterile cells-pseudo paraphyses; characteristic large cystidia very frequently present on the sides of the lamellae; cheilocystidia proper not differentiated in most species, but the edges of the lamellae often heteromorphous because of the presence of large, loosely attached spherocysts; spore print black or fuscous; spores under the microscope blackish and opaque, more rarely fuscous and opaque or transparent, but always very deeply coloured by a pigment, with a germ pore, smooth, more rarely warty, echinuate, reticulate or angular; basidia normal but rarely clavate, usually cylindric or even narrowed in the middle, (1) - 2 - (3) - 4 - spored, hymenophoral trama regular; stipe central and more or less straight; veil present or absent and if present often indistinctly double, often condensed into an annulus or with an annulus in the lower part of the stipe and the veil then resembling a volva, usually also apparent on the pileus, rarely with a true, well developed cup shaped volva at the base; context

usually white or whitish, flesh very thin and fragile to almost absent in the tiniest species and clamp connections more often present. They are seen on dung or on soil, sand, peat, on various fabrics, on living Basidiomycetes around living tree trunks, on dead wood, etc., also on buildings, often forming small sclerotia. The development of carpophore is mostly or always hemiangiocarpous. The auto deliquescence of the lamellae is obvious where true coprini grow under optimal conditions. This, combined with the peculiar type of hymenophore and hymenium should make it easy to distinguish Coprinus".

Bilgrami et al. (1975) listed out the following 21 species of Coprinus as recorded for India upto 1977. Berkely (1856) reported the occurrence of C.comatus Fr. and C.hookeri Berk. on grassy places and C.vellereus Berk. on deadwood and soil, Ellis and Everhart (1897) reported the occurrence of C.stellaris Quel. on dung of Zebra. Hennings (1901) observed C.spraguei Berk. & Curt. on botanical gardens. Bose (1920) reported C.fimbriatus Berk.Br. on dung. Rea (1922) reported several species of Coprinus occurring on various substrates viz., C.cinereus (Schaeff.) Cooke and C.filiformis Berk. & Br. on dung of Nilghai; C.ephemerus (Bull.) Fr. on rabbit dung;

C.gibbsii Massee. & Crossl., C.hendersonii (Pers.) Berk. and C.stellaris Quel. on dung of Zebra; C.papillatus (Batsch.) Fr. and C.nycthemerus Fr. on cowdung. Hole (1927) reported C.ephemereus (Bull.) Fr. on rabbit dung. Butler and Bisby (1931) reported the occurrence of C.niveus (Pers.) Fr. on dungs and heaps of rotten straw. Ginai (1936) reported the occurrence of C.nycthemerus Fr. on cowdung; C.cinereus (Schaeff) Cooke. and C.filiformis Bork. & Br. on dung of Nilghai. Banerjee (1947) observed C.comatus Fr. on lawns, refuse dumps, especially on ashes and C.micaceus (Bull.) Fr. around trees, stumps and fence posts. Saksena and Mehrotra (1953) recorded the occurrence of C.atramentarius (Bull. ex Fr.) Fr. Lange and Smith (1953) reported C.disseminatus (Pers.ex Fr.) S.F. Gray on and around stumps and C.stellatus Butler on dung. Ghosh et al. (1967) reported C.disseminatus (Pers. ex Fr.) S.F. Gray and C.comatus Fr. on soil and dung. Saxena et al. (1969) reported the occurrence of C.comatus Fr. on dung of kangaroo, deer and Nilghai.

Certain species of Coprinus were found to occur on seeds on conifers (Munjal & Sharma, 1975). Purkayastha Chandra (1976) reported the occurrence of sporophores of C.atramentarius (Bull. ex Fr.) Fr. growing scattered or

in dense clusters on gardens, waste places, richly manured soil and around tree stumps. They also reported C.comatus (Mull. ex Fr.) S.F. Gray sporophores growing singly, scattered or in clumps on grassy land in lawns, gardens, fields, roadsides and refuse dumps and C.micaceus (Bull. ex Fr.) Fr. sporophores growing usually in dense clumps or scattered on ground, base of living trees, around stumps and rarely on logs. Bhavani Devi (1982) reported the occurrence of C.lagopus (Fr.) Fr. as a weed fungus in the paddy straw beds of Volvariella volvacea (Bull.ex Fr.)Sing.

II. Isolation

Emmons (1954) isolated C.micaceous from sputum of diseased animal. Buller (1958) obtained pure culture of C.lagopus from horse dung balls. Kurtzman (1978) isolated C.fimetarius from straw. North (1980) reported a method for isolating C.cinereus mycelia from manure heaps and described that it grew well on a simple minimal medium, pH 6.8 containing one amino acid asparagine and one vitamin thiamine. Seal (1981) reported the isolation of C.cinereus (Schaeff ex Fr.) S.F. Gray Sensu Konr from amended straw at pH 8.3. Results indicated an initial preference of C.cinereus for hemicellulose break down followed by cellulose attack. C.cinereus under alkaline condition was able to

convert straw with a low ruminant digestibility to a product much enhanced and at least equivalent to good quality hay in its nutritional requirements.

III. Developmental Morphology and spore characteristics

The spores of the Coprini were discovered in 1729 by the Florentine botanist Micheli, who observed them on the gills with the microscope. Muller (1780) described C.comatus in the Flora Danica. He gave some excellent life-size illustrations showing the fruit bodies in various stages of development, including the deliquescence of the gills and the production of inky drops from the revolute pileus margin and also a sketch of a surface view of the hymenium as seen with the microscope. Bulliard (1791) gave a good illustration of the cystidia on the gills of a species of Coprinus. Link (1809) published illustrations of the hymenium of a Coprini in which he represented the basidia as thecae, each theca containing four rows of spores. The first illustration of a cross section of the hymenium of a Coprinus showing basidia and paraphyses correctly drawn were published by Corda (1837). Massee (1896) gave a revision of the genus Coprinus in which he enumerated 169 species and discussed their general morphology, distribution, habitat and their classification.

Buller (1958) studied the developmental steges and spore liberation of C.lagopus in detail. He reported that the stipe of the fruit body elongated rapidly before spore discharge and just as the stipe was ceased to elongate, pileus expanded and flattened, cheilocystidia were separated from one another and the pileus melted to a watery stump. After spore discharge, the stipe was collapsed and the whole fruit body was fallen to the ground. Haard and Kramer (1970) reported that basidiocarps of some species of Coprinus matured in the later afternoon or evening of each day and began to discharge spores. Spore numbers peaked about midnight, then rapidly decreased, as autolysis of basidiocarps occurred. Watling (1971) observed that the spores of most coprophilous and lignicolous fungi germinated immediately when plated on agar, but some did not germinate due to the presence of self inhibitors produced by a high concentration of spores. Heintz and Niederpruem (1971) studied the ultrastructure of quiescent and germinated basidiospores and oidia of C.lagopus and reported that C.lagopus has two haploid spore stages, sexually produced basidiospores and asexually formed oidia. Both were capable of germinating to reform the vegetative thallus. They differed in morphology, size and pigmentation, but

followed similar developmental pathways during germination. Bret (1977) described the role of cap and mycelium on the stipe elongation of C.congregatus Bull. ex Fr. The cap is essential for complete stipe elongation in developing fruit bodies. Stipe elongation also depends on the presence of vegetative mycelium at the base of fruit body. The dry weight of stipe increased during the entire period of elongation. The dependence of stipe on cap and vegetative mycelium at each stage of fruit body development diminished progressively as the fruit body approached maturity. Chapman and Barankovich (1979) studied the germination of basidiospores and oidia of C.domesticus and reported that the basidiospores germinated over a wide temperature range (optimum 28 to 35°C) and the optimum pH range was 5.5 to 7.1. Similar germination percentages occurred for basidiospores and oidia in all agar media except those containing K_2HPO_4-$MgSO_4$. $7\ H_2O$ - microelements $(NH4)_2SO_4$ and plain agar. Basidiospores had a high germination per cent than oidia in all liquid media except biotin-thiamine. Maximum germination for both occurred on agar with cornmeal and malt-extract-liquid medium.

IV. Physiology

Farlow (1881) reported the growth of Coprinus on

the surface of water contained in a glass jar. Koske and Leathers (1969) reported that Keyworth's broth supported good vegetative growth of Coprinus mycelium in shake culture, but did not yield carpophores. Watling (1971) studied the collection techniques of Homobasidiomycetes and reported that coprophilous fungi can be conveniently collected simply by taking some of the substrate, air drying carefully and incubating in a damp chamber. The fruit bodies developed normally. He also reported that the fruit bodies of Coprinus were formed normally in culture. When the stipe of Coprinus species was used for culturing, the fruit bodies appeared directly on the stipe tissue, while other species fruited directly from the pad of hyphae produced from a spore or spore group. In certain strains of C.pellucidus, fructification was obtained, when bacteria were also present in the culture. Cheng-Ho and Yee (1977) studied the physiology of Volvariella volvacea and C.cinereus and reported that both preferred a neutral environment whereas C.cinereus preferred an acidic one. Both could use a number of carbohydrates and could decompose hemicellulose and cellulose. C.cinereus was more efficient in utilizing hemicellulose with sodium nitrate as nitrogen source. Kurtzman (1978) reported that

a temperature of 35-40°C and relative humidity of 90 per cent were best for the growth of C.fimetarius. North (1980) reported that C.cinereus grew well at pH 6.8, in a simple minimal medium, containing one amino acid asparagine and one vitamin thiamine. Seal (1981) reported the growth of C.cinereus in alkaline condition.

V. Cultivation

Kurtzman (1978) described the cultivation of C.fimetarius in large scale and reported that straws of various species of plants have been used successfully as a substrate along with a nitrogen supplement like calcium nitrate was most successful. Paper was used to replace upto 50 per cent straw. He obtained good yield in Pakistan in five days using paddy straw, paper and a nitrogen source calcium nitrate. Kurtzman (1978) also reported that, with long straw and small inoculum, the yield of fresh mushroom was 16 per cent of dry weight of the straw, while with larger inocula, chopped straw and calcium nitrate yields were larger and less time was required for production. The fresh weight of mushroom was about 60 per cent of dry weight of straw, when the culture was well handled.

Kurtzman (1978) used sorghum grain spawn for his work. He reported that a temperature of 20 to 30°C and a relative

humidity of 90 per cent were good for the growth of C.fimetarius. About 48 hours were necessary for spawn run through the substrate and the buds appeared in two days after the initials were seen on the bed.

VI. Nutritive value

Kurtzman (1978) reported that C.fimetarius contained about 6.4 per cent nitrogen on a dry weight basis, which was equal to 40 per cent crude protein. This figure was too high for true protein, but these mushrooms probably contained nearly 30 per cent protein on dry weight basis. Shobha and Punekar (1981) reported the nutritive value of four mushrooms viz., Agaricus bisporus, Armillaria sp., Coprinus sp. and Pluteus sp. The mushrooms were analysed for their proximate constituents, minerals and vitamins. The four mushrooms were good sources of proteins, phosphorus, iron and vitamins, but poor sources of fat, carbohydrates, calcium and vitamin C. The four species of mushrooms compared favourably with food stuffs like beef meal, yeast and soybean.

VII. Edibility

Child (1952) reported the inability of Coprini to sentisize man to ethyl alcohol. C.atramentarius, C.comatus and C.micaceus were prepared in a number of ways and were

given to a subject who was known to react to alcohol sentisizing drugs. The Coprini produced no untoward effects and did not sensitize the subject to alcohol. It was suggested that the reports alleging that Coprini sentize to alcohol might be explained by the accidental inclusion of Panaeolus in the cooking vessel. The edibility of C.atramentarius was reported by Atkinson (1961), Krieger (1967) and Kaul (1971) also. Atkinson (1961) reported the edibility of C.comatus (Mull.ex Fr.)S.F.Gray and C.micaceus (Bull.ex Fr.) Fr. Christensen (1966) reported the edibility of C.atramentarius, C.comatus, C.micaceus and C.sterquilinus. Kaul and Kachroo (1970) reported that European authors have classed C.comatus Fr. as one of the best among edible fungi and the villagers of Jammu and Kashmir collected its closed button stage for consumption. C.micaceus (Bull.) Fr. also offered a good meal to the villagers. Ghosh et al. (1974) reported the edibility of C.comatus (Muller ex Fr.) S.F.Gray. Quimio and Suayan (1976) reported that, although the species of Coprinus have excellent flavour and fine texture, for culinary purpose they are not as popular as the others because of the dirty colour of spores.

VIII. Preservation

Most members of the genus Coprinus are ephemeral and so without refrigeration, it will not remain fresh for more than eight hours. They deliquesce and turn to black inky mass when get old. Kurtzman (1978) reported that C.fimetarius could be preserved by refrigeration, drying and canning and this species remained good for more than four days with mechanical refrigeration. Kurtzman (1978) described that, soon after harvest, mushrooms should be cooled, blanched or dried at 60 to 80°C. Blanching in boiling water for one minute preserved the flavour and destroyed the autolytic enzymes responsible for ephemeral characteristics. The blanched mushrooms were used for canning and they remained for an indefinite period.

MATERIALS AND METHODS

MATERIALS AND METHODS

I. Collection, identification and preservation of mushroom

A survey was conducted in and around the campus of College of Agriculture, Vellayani during May-June 1982 and the commonly occurring species of *Coprinus* viz., *C.lagopus* (Fr.) Fr., *C.disseminatus* (Pers.ex Fr.)S.F.Gray and *C.ephemerus* (Bull.ex Fr.) Fr. were collected. The morphological characters of all collected mushrooms were studied in detail. It was found that *C.lagopus*(Fr.)Fr. was the most commonly occurring species. The same was observed as a regular weed fungus in beds of *Volvariella volvacea* (Bull.ex Fr.) Sing.

Twenty five sporocarps of various stages of development of this mushroom were collected from mushroom beds of *V.volvacea* and their morphological characters were studied in detail following the data sheet developed by Bhavani Devi (1982) (Appendix I). The identity of mushrooms was confirmed by Dr.D.N.Pegler, Royal Botanic Gardens, England. This mushroom was used for all further studies.

The morphological terms used in the present study are those according to Singer (1975). All the colours in

the macroscopic descriptions were according to 'Dictionary of Colour' (Maerz and Paul, 1950) and sited under results by appropriate plate number as given in the Dictionary. The specimens were dried in a Sigg Dorrex Dehydrator at 70°C and preserved in the laboratory in sealed polythene covers. Specimens were also preserved by wet method using FAA solution. Collections were deposited in the Herbarium of the College of Agriculture, Vellayani, Trivandrum.

Melzer's reaction tests (Melzer, 1934) were carried out on the surface, context of the pileus and stipe and spores. Approximately one square cm fresh tissue from the pileus and stipe of mushroom button was dissected out with a clean razor blade and placed in the depression of porcelain spot plate. A drop or two of the Melzer's reagent was applied on the tissues and allowed to stand for a minimum of 15 minutes. The reactions indicated by a colour change were observed.

Reaction tests were also carried out using 3 per cent aqueous potassium hydroxide, hydrochloric acid (11 M) and concentrated sulphuric acid. Composition of all the reagents, media and chemicals used are given in Appendix-II.

Spore prints were taken on a white sheet of paper. Sporophores were collected from bed just before maturity. and the stipe was cut off beneath the pileus. The pileus was then placed with its gills down on a white sheet of paper and covered with a bell jar and kept undisturbed for 5 to 10 minutes to get a clear spore print. Subsequently permanent spore prints were made by the same method on a white sheet of paper coated with gum arabic.

II. Isolation and purification of C.lagopus (Fr.) Fr.

The isolate of C.lagopus was obtained from paddy straw beds of V.volvacea, raised at the Department of Plant Pathology, in which it appeared as a weed mushroom. For the isolation, tissues of the mushroom were cut from the pileus as well as stipe and surface sterilized with 95 per cent ethyl alcohol for one minute. These bits were inoculated on petri dishes containing 15 ml of potato dextrose agar media and incubated at room temperature (29 ± 2°C). When the maximum growth was obtained, after 6-7 days, it was aseptically transferred to potato dextrose agar slants. The isolate was then purified by the hyphal tip method and maintained on potato dextrose agar slants by subculturing periodically.

III. Spore germination

Germination of basidiospores was studied by germinating spores in slides as well as by the hanging drop method. Spore suspension was prepared by shaking a 10 cm disc of mature pileus with 15 ml of sterile water in a test tube. The time taken to start spore germination and also the average measurement of germ tubes of 10 spores were taken at an interval of two hours, by observing through the High Power objective (45 X) of compound microscope. The drawings were made by means of camera lucida.

The per cent of germination at different temperature viz., 20, 25, 30, 35 and 40°C after one hour of incubation was also noted.

IV. Developmental morphology

Studies were conducted to observe the different stages of development of the fungus after spawning till maturity. The time taken for deliquescence of the pileus was also recorded. The developmental stages of the fungus is divided into five stages viz., pinhead stage, tiny button stage, button stage, elongation stage and mature stage following Chang and Yau (1971) who adopted the same for V.volvacea.

V. Nutritional studies

1. Growth of C.lagopus in different media

In order to find out the best medium for the growth of C.lagopus the different media viz., potato dextrose agar, oat meal agar, Czapek's agar and Richard's medium were used.

The composition of the media were given in Appendix-II.

Growth on solid media

The different solid media were prepared and autoclaved at 1.02 kg/cm^2 for 15 to 20 minutes. An aliquot of 15 ml of each medium was plated on sterilized petri dishes and inoculated in the centre with a 5 mm disc of the fungus, cut out from an actively growing seven day old culture and incubated at room temperature (29 $\pm$ 2°C). The colony diameter was taken at an interval of 24 hours for seven days. Four replications were maintained in each treatment.

Growth on liquid media

The liquid media were prepared and 50 ml of each medium was transferred to 250 ml conical flasks and autoclaved at 1.02 kg/cm^2. The media were then inoculated by 5 mm culture disc of the fungus cut out from an actively growing culture and incubated at room temperature (29 $\pm$ 2°C). After ten days of incubation, the mycelial mat was filtered

through a Whatman No.1 filter paper and kept in an oven at 70°C. The dry weights were taken until a constant weight was obtained. Four replications were maintained in each case.

2. Effect of different temperature on the growth of fungus

In order to assess the best temperature for the maximum growth of the fungus, potato dextrose broth was prepared as described above. The medium was inoculated by 5 mm culture disc of the fungus from an actively growing seven day old culture and incubated at different temperature viz., 20, 25, 30, 35, 40 and 45°C. After ten days of inoculation, the mycelial mat was filtered, dried at 70°C and dry weights were taken till two consecutive weights were equal. Three replications were maintained for each treatment.

3. Effect of pH on growth of the fungus

Potato dextrose broth was prepared and initial pH was adjusted to 5, 6, 7, 8, 9, 10, 11 and 12 by adding 0.1 N Hydrochloric acid or 0.1 N Sodium hydroxide as the case may be and the pH was adjusted by means of a Digital pH metre (ELICO Private Ltd.). Fifty ml of each medium was taken in 250 ml Erlenmeyer conical flask and autoclaved at 1.02 kg/cm^2. The medium was inoculated by 5 mm culture disc of the fungus from an actively growing

seven day old culture and incubated at room temperature (29 ± 2°C) for 10 days. The mycelial mat was filtered, dried at 70°C and dry weights were taken till constant weights were obtained. Three replications were kept in each case.

4. Effect of light and darkness on the mycelial growth of C.lagopus

Fifteen ml of potato dextrose agar media was plated on sterilised petri dishes and 5 mm culture disc of the fungus from an actively growing seven day old culture was placed at the centre. One set of dishes were placed under ordinary light condition and the other set of dishes were wrapped with black paper and incubated in complete darkness. Seven replications were kept in each case and the colony diameter was taken at an interval of 24 hours for six days.

5. Effect of different carbon sources on the growth of C.lagopus

Czapek's broth was used as the basal medium and various mono and disaccharides viz., dextrose, lactose and maltose were substituted for sucrose in the basal medium so as to give the same per cent of carbon in each case. Samples without the addition of any sugar was taken as control.

Fifty ml of each medium was taken in 250 ml conical flasks and autoclaved at 1.02 kg/cm^2. The medium was then inoculated

by a 5 mm culture disc of fungus, cut out from an actively growing seven day old culture and incubated at room temperature (29 ± 2°C). After 10 days, the mycelial mat was filtered and dry weights were taken, after drying at 70°C, till constant weights were obtained. Four replications were maintained in each case.

6. Effect of different sources of nitrogen on the growth of C.lagopus

Organic as well as inorganic forms of nitrogen viz., asparagine, sodium nitrite and ammonium chloride were substituted for sodium nitrate in Czapek's medium, so as to give the same per cent of nitrogen in each case. As in the previous experiment, 50 ml medium was taken in each flask, sterilized and were inoculated with 5 mm culture disc of the fungus. The flasks were incubated at room temperature (29 ± 2°C) for 10 days. The mycelial mat was filtered through a Whatman No.1 filter paper and dry weights were taken after drying in an oven at 70°C till two consecutive weights were equal. Flasks without any nitrogen source were taken as control. Four replications were kept in each case.

VI. (a) Effect of different spawn substrates on the mycelial growth and sporocarp production of C.lagopus

Paddy straw, dried salvinia, red gram, horse gram,

bengal gram, green gram and wheat grains were used as substrates for spawn production and their comparative efficiency assessed.

Preparation of spawn on straw and salvinia

Colourless empty bottles of 750 ml capacity were filled with paddy straw bits (2.5 cm in length) leaving a space of 7-8 cm at the top, and were soaked by immersing in water for about 12-15 hours. The bottles were taken out and kept upside down for 3-4 hours to drain the excess water from the straw. Five gram of coarsely powdered red gram was added to each bottle and plugged with cotton. The bottles were autoclaved at 1.02 kg/cm^2 for 1 to 2 hours per day consecutively for two days.

Salvinia was collected and sundried for one week. Dried material was chopped and bottles were filled as described above.

Preparation of grain spawn

Four hundred gram of grain was boiled for 3 to 5 minutes with equal volume of water. The excess water present was drained off and 15 to 20 g of calcium carbonate were mixed thoroughly. Clean colourless bottles of 750 ml capacity were filled with the above grain, leaving 7 to 8 cm

space at the top. The bottles were sterilized as above and allowed to cool down. Mycelial bits from seven day old actively growing pure culture of the fungus were inoculated aseptically and incubated at room temperature (29 ± 2°C). Three replications were maintained in each case and visual observations on the mycelial growth of fungus were recorded after 15 days and graded as follows:

+++	Good mycelial growth
++	Moderate mycelial growth
+	Very poor mycelial growth

The spawns prepared on each of the substrates were used to spawn paddy straw beds. Twenty day old spawn was used to lay out conventional standard beds and the yield of sporocarps were recorded.

(b) Effect of different temperature on the mycelial growth of fungus on spawn bottles

Spawn bottles were prepared using the substrates, as described above and were inoculated with the mycelial bits of fungus and maintained at different temperatures, viz., 25, 30, 35 and 40°C for 20 days. After 20 days of incubation, the mycelial growth of the fungus was noted and visual observations were recorded and graded as follows:

+++++ Very good mycelial growth

++++ Good ,, ,,

+++ Moderate ,, ,,

++ Scanty ,, ,,

\+ Very scanty ,, ,,

\- No growth

(c) Effect of different organic amendments on the yield of sporocarps of C.lagopus

To find out the influence of different organic amendments on the yield of C.lagopus, the following amendments were used, viz., powdered green gram, bengal gram, horse gram, red gram, wheat flour and cow dung. Conventional rectangular straw beds were laid out with 4 kg paddy straw. 150 g of each amendment were sprinkled uniformly before and after placing the spawn. Beds without the addition of any of these amendments were maintained as control. The yield of fresh mushroom was recorded for each bed. Three replications were maintained for each treatment.

VII. (a) Influence of different types of straw bed for the maximum production of sporocarps of C.lagopus

1. Rectangular beds with paddy straw twists

Rectangular beds with straw twists were laid, following the method of Jeyarajan and Ramaiah (1974) and Bhavani Devi (1982). Four kilogram of straw were made into

twists of 3-4 m long and 7-8 cm in diameter. These twists were made into small bundles and kept immersed in water for 12-15 hours. The pre-soaked twists were placed on a raised wooden plateform (70 x 50 cm) in a zig-zag manner after draining the excess moisture. Second twist was placed over the first row in the opposite direction which form the first layer of bed. One bottle spawn was divided into small bits of 3-5 cm in size in the case of paddy straw spawn and 4-5 g in the case of grain spawn and placed 10 cm apart along the periphery of the bed. A total quantity of 150 g coarsely powdered red gram was sprinkled uniformly over the spawn at each bed. The same procedure was followed for placing the remaining twists and they were spawned and sprinkled with red gram powder, successively making altogether of 3 layers. The entire bed was compacted by pressing and was completely covered with transparent polythene sheet. The yield of fresh mushroom was recorded in each case.

2. Rectangular beds with chopped straw

Rectangular beds were made out of 4 kg of straw chopped into small bits of 20-30 cm in length. The paddy straw was kept immersed in water for about 15 hours. Wooden boxes of dimension 50 x 35 x 30 cm with both sides opened were filled with pre-soaked chopped straw.

The straw was spread from the base in 5 cm thickness. Spawn bits were placed on the periphery. Red gram powder was sprinkled before and after spawning. Successive layers of straw were made and spawned as before, till the height of bed reached the height of the box. The bed was made compact by pressing from the top and the box was removed. The whole bed was covered with polythene sheet. Three replications were kept and the yield of mushroom was recorded.

(b) Effect of different substrates on the yield of C.lagorus

Four different locally available raw materials, viz., paddy straw, waste paper, dried salvinia and banana pseudostem were used in the layout of the mushroom beds.

1. Beds on paddy straw

Conventional straw beds were laid using twists of paddy straw weighing 3 kg.

2. Beds on salvinia

Three kilogram of Salvinia was collected, sundried for one week and used as such for the preparation of beds following the method described earlier in the case of chopped straw.

3. Beds using a combination of chopped straw and paper

Mushroom beds were laid, following the method of Kurtzman (1978). 2.25 kg of dry chopped straw and 0.75 kg of paper bits were soaked in 10.5 litres boiling water containing 200 g of calcium nitrate. Then the whole material was placed on a tray and cooled. After about 15 hours of cooling, beds were laid on wooden boxes of size 50 x 35 x 30 cm with both sides opened. One bottle spawn and 150 g red gram powder were used to spawn one bed and the fresh weight of mushroom was recorded.

4. Beds on banana pseudostem

Fresh banana pseudostems were taken and vertical cuts were made at different places. Spawning was done at the cut places. Red gram powder was placed before and after spawning. The pseudostem was covered with polythene sheet. One bottle spawn and 150 g red gram powder were used. Three replications were kept and the fresh weight of mushroom was recorded.

VIII. Preservation of mushroom

1. Refrigeration:- 50 g of mushroom buttons were collected and stored in refrigerator at 10-15°C. Samples were kept in open and closed polythene bags. Visual observations were taken after 24, 48 and 72 hours respectively.

2. Dehydration:- 100 g of fresh mushroom buttons were collected and dried in a Sigg Dorrex dehydrator at 70°C. The dried buttons were packed in polythene bags or stored in airtight containers.

3. 250 gram of mushroom buttons were taken and dried in a Sigg Dorrex dehydrator at 70°C. This was powdered and stored in polythene bags.

4. Preservation in brine:- Brine of 1 to 7 per cent concentrations were prepared by dissolving sodium chloride in sterile water. An aliquot of 150 ml each of the solution was transferred to clear conical flasks of 250 ml capacity and autoclaved at 1.02 kg/cm^2. Fresh fruit bodies in button stage were collected, washed in tap water and dipped in boiling water for 1-2 minutes. Five buttons each were transferred aseptically to each flask containing the brine and were incubated at room temperature. Three replications were maintained for each treatment.

The microflora of the above preserved mushrooms was estimated following the serial dilution technique, at weekly interval for a period of 6 weeks during storage period.

A 10^{-7} dilution of brine was prepared and the bacterial, actinomycetes and fungal population from the

preserved samples were estimated employing nutrient agar, Martin's Rose Bengal agar and Kuster's agar.

IX. Chemical Analysis

1. Analysis of moisture

Twenty gram of fresh mushroom buttons were taken in a flat bottomed dish and dried in a Sigg Borrox Dehydrator at 70°C, till a constant weight was obtained. The loss in weight was taken and the percentage of moisture was determined.

$$\text{Percentage of moisture} = \frac{\text{loss in weight of mushroom} \times 100}{20}$$

2. Analysis of Fat

The fat was analysed by Soxhlet method (Lees, 1975). 5 g of dried powdered mushroom was taken in a filter extraction thimble, the end of which was plugged with fat free cotton wool. The thimble along with the contents was placed in the central syphon portion of soxhlet apparatus. A previously weighed 250 ml flask containing 40 ml each of analytical grade petroleum ether and diethyl ether was connected to the soxhlet syphon and condenser. The sample was extracted under reflux, on a water bath for 5-6 hours. The mixed ether was distilled off and the flask

with the contents was dried in an oven at 105°C for about 3 hours. The flask with the contents was cooled in a dessicator and weight was taken. The drying and weighing were continued till two consecutive weights were equal. The percentage of extractable fat was calculated by

$$\text{Percentage of fat} = \frac{(\text{weight of flask with fat} - \text{weight of flask without fat}) \times 100}{5}$$

3. Analysis of crude fibre

Two gram of fresh mushroom button was boiled in a 500 ml conical flask with 200 ml of 1.25 per cent H_2SO_4 for about 30 minutes. It was then filtered through a muslin cloth and washed 3 times with 50 ml boiling water. The residue was then boiled with 200 ml of 1.25 per cent NaOH for about 30 minutes and filtered through muslin cloth. It was washed thoroughly with 25 ml of boiling 1.25 per cent H_2SO_4 and then with 50 ml boiling water. A final washing with 25 ml absolute alcohol was also done and the residue was dried at 110°C in an oven for about 6 hours. It was then cooled in a dessicator and weighed to constant weight and expressed in percentage.

$$\text{Percentage of crude fibre} = \frac{\text{Dry weight of fibre} \times 100}{2}$$

4. Analysis of protein

The percentage of nitrogen in the sample was determined by microkjeldahl method and the crude protein was determined by multiplying the percentage of nitrogen by 6.25.

0.2 g of dried powdered mushroom was digested with 15 ml of conc. H_2SO_4 and one g of digestion mixture (a mixture of K_2SO_4, $CuSO_4$ and selinium powder in the ratio 10:1:0.1) for about 1/2 - 1 hour till the solution became clear. The digested material was made upto 100 ml. Ten ml of digested material was transferred to the distillation apparatus. A 100 ml conical flask with 5 ml of 4 per cent boric acid solution and 2-4 drop of methyl red-methylene blue mixed indicator was placed at the condenser tip. 8-10 ml of $NaOH-Na_2S_2O_3$ solution was then added to the distillation flask. About 15 ml distillate was collected and it was titrated against 0.00904 N HCl. The end point was indicated by the appearance of a violet colour. A blank experiment with all the ingredients except the sample was also done. The percentage of nitrogen in the sample was calculated by

$$\text{Percentage of Nitrogen} = \frac{(\text{Titre value} - \text{blank value}) \times 0.00904 \times 100 \times 100 \times 0.014}{0.2 \times 10}$$

5. Analysis of total carbohydrates

The method described by Aminoff et al. (1970) was followed to determine the total carbohydrates. One g of oven dried powdered mushroom was digested with 0.00901 N HCl for about one hour. The digested material was titrated against a boiling mixture of 10 ml each of alkaline potassium ferrocyanide and sodium hydroxide, using methylene blue as indicator. The end point of the titration was indicated by the appearance of a light yellow colour. A blank experiment was also done using glucose, instead of the sample.

$$\text{Percentage of carbohydrates} = \frac{\text{Blank value} \times 0.00901 \times 100 \times 100 \times 0.95}{\text{value of the sample} \times 1}$$

0.95 = conversion factor

6. Analysis of aminoacids

The amino acids in the mushroom sample was analysed by descending paper chromatography, using the solvent n-Butanol-Acetic acid-water mixture (4:1:5 $V/v_{/v}$). The amino acid extract of the mushroom sample was prepared in alcohol and was spotted, along with the standard amino acids on a Whatman No.1 filter paper. The side of the filter paper on which spotting was done, was dipped in the trough

containing the solvent and placed in the chromatographic chamber. The solvent was allowed to run, for about 12 hours. The paper was taken out, the solvent front marked and the paper was dried at 60°C for 20 minutes. The chromatogram was sprayed with 0.25 per cent ninhydrin in acetone. The number of amino acids in the mushroom sample was noted and were identified by comparing with the standard amino acids.

X. Edibility trial

Edibility of C.lagopus were studied on four Red Eyed White rabbits of weights 2 kg each. Of these, three rabbits were fed with dried powdered mushroom along with the normal diet of 750 g grass and 70 g wheat flour and one was maintained as control. They were fed with 5 g mushroom powder for the first seven days and the quantity was increased by 2.5 g per week, upto 15 g for a total period of 5 weeks. After 35 days of feeding, the weights of rabbits were taken. Visual observations on the external appearance of rabbits were also recorded daily.

XI. Biomass production by C.lagopus, V.volvacea and P.sapidus

About 50 ml of potato dextrose broth was taken in 250 ml Erlenmeyer flasks and autoclaved at 1.02 kg/cm^2. The media were inoculated by 5 mm culture disc of fungus from an actively growing seven day old culture of

C.lagopus, V.volvacea and Pleurotus sapidus and were incubated on a Rotary shaker at 200 x 260 strokes per minute. The cultures were exposed to diffused sunlight daily for half an hour and again placed on shaker. After five days of shaking, the mycelial pellets were filtered through a Whatman No.1 filter paper and dried at 70°C in Sigg Dorrex dehydrator and dry weights were recorded. After shaking for five days, the flasks were allowed to remain stationary and further changes were observed.

RESULTS

RESULTS

I. Collection, Identification and Preservation of mushroom

A survey was conducted in and around the campus of College of Agriculture, Vellayani, during May-June 1982 and the commonly occurring species of Coprinus viz., C.lagopus (Fr.) Fr., C.disseminatus (Pers. ex Fr.) S.F.Gray and C.ephemerus (Bull. ex Fr.) Fr. were collected. The morphological and microscopical characters of the mushrooms collected were recorded on the data sheet prepared by Bhavani Devi (1982). The macro and micro characters were enumerated as follows.

1. Coprinus lagopus (Fr.) Fr.

Pileus:- 5 to 30 mm diameter in button stage and 30 to 80 mm in mature stage; cylindric oval at first, later campanulate with a somewhat pointed apex; white in colour at first, then grey and later turns black; surface covered with dense white scales at first and then scales break into patches and fall down leaving the cap shiny. Pileus was splitted radially before autodigestion.

Scales:- 1 to 1.5 mm long, broadest where they were attached to the pileus flesh and the free end was projected into the air, soft, white and more or less conical and clear in young fruit bodies.

Gills:- 7 to 8 gills per mm, 2 to 8 mm wide, length varied with pileus size, white at first and later turns black.

Stipe:- Length 1 to 5 cm before and 10 to 15 cm after elongation, 5-7 mm in diameter, white hollow and slightly hairy, a tap root like base which penetrate the surface was also present and it tapered upwards.

Spores:- Black, elongated oval with a germ pore, 8.25 - 12.5 x 5.5 - 7 µm (Fig.1.a)

Basidia:- Long and short basidia present, tetraspored
Long basidia - 30 x 8.5 µm
Short basidia - 22.5 x 7.5 µm

Cystidia:- Pleurocystidia and cheilocystidia present.

Pleurocystidia:- Elongated oval in shape, rounded at the apex and bulged in the middle and contracted into a stalk at the base, 20 - 30.45 x 10 - 14.75 µm (Fig.1.b).

Cheilocystidia:- Elongated club shaped, 60 - 64.45 x 32.5 - 36.80 µm.

Veil, volva and ring:- Absent

Spore print:- Black (Plate 1)

Collected as a weed fungus on paddy straw beds of V.volvacea, from cowdung manure and guinea grass stumps.

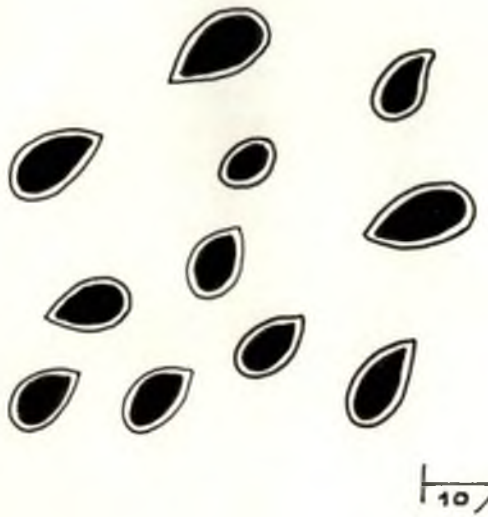


FIG: 1 a. BASIDIOSPORES

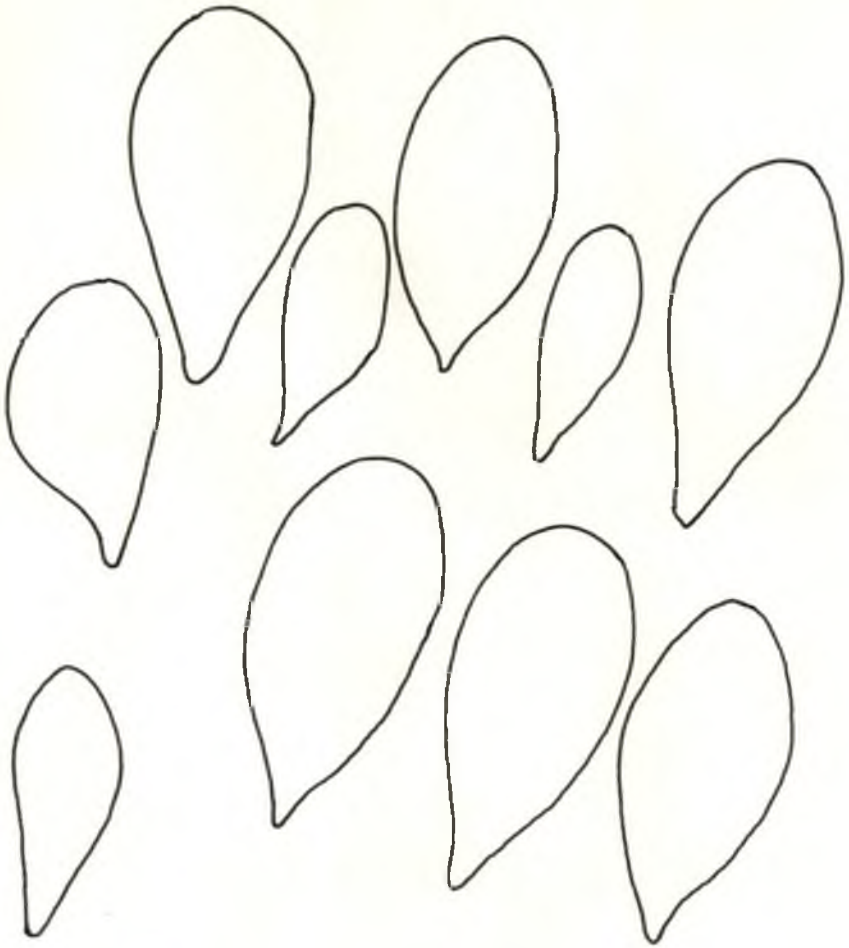
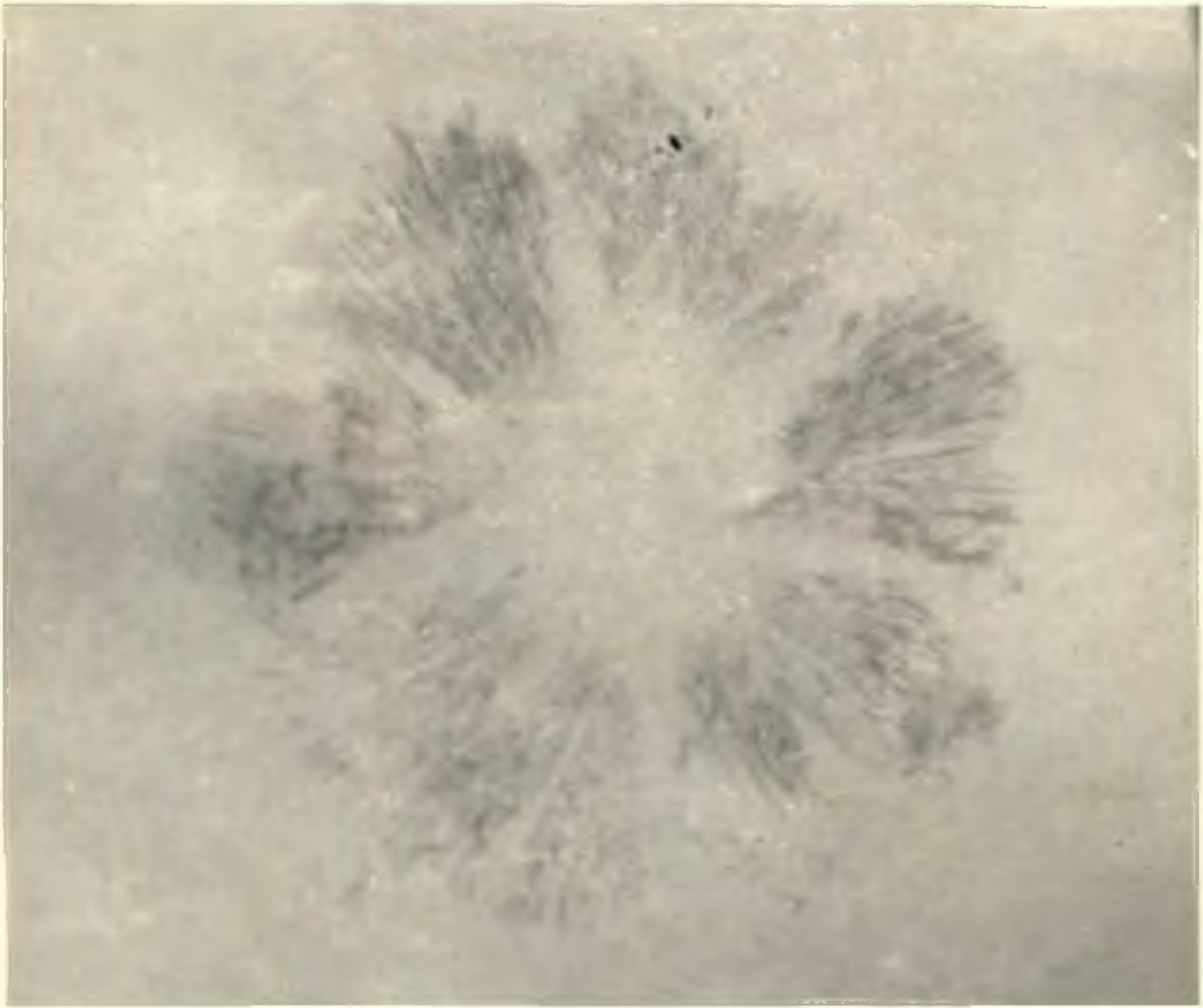


FIG: 1 b. PLEUROCYSTIDIA



2. C.disseminatus (Pers.ex Fr.) S.F.Gray

Pileus thin, membraneous, furrowed, fragile, ovate, 10 to 15 mm in diameter, later campanulate, whitish or pale buff to grey, powdery, except the disc which remains yellowish. Flesh one mm thick in centre, becoming very thin at the margin. Gills thin, two mm wide, wedge shaped and adnate, at first greyish white and then black at maturity. Stipe 2.5 to 4 cm long, whitish, thin, fragile, hollow, very slightly tapering upwards. Basidia tetraspored, tetramorphic, longer ones much protruding and narrow. Pleurocystidia absent, cheilocystidia large and obtuse. Spores 7-9 x 3.5 - 5.5 µm, smooth, flattened, rather broadly fusiform with a distinct germpore.

Collected as a weed furngus on paddy straw beds of V.volvacea.

3. C.ephemerus (Bull. ex Fr.) Fr.

Pileus 1 to 1.5 cm in diameter, very thin, at first elongated oval, later campanulate, flat at maturity, striate or delicately furrowed when young with narrow prominent fold. When old, tan to reddish brown in centre and paler towards the margin. Pileus margin wavy.

Gills narrow, linear, at first white, then brownish later black, slightly liquifying. Stipe 3 to 4 cm long, 1 to 1.5 mm in diameter, thin, white, tan towards the lower portion, hollow and fragile. Spores ovate to obovate, 16 to 17 x 9 to 10 µm. Spore print - Black. Collected from rabbit dung.

The identity of all the above described species were confirmed by comparing their characters with the already reported ones (Bhavani Devi, 1982) and that of C.lagopus further confirmed by Dr.D.N.Pegler.

The specimens were preserved by drying at 70°C in Sigg Dorrex Dehydrator and also preserved in FAA solution, by wet method and deposited in the Herbarium unit, Department of Plant Pathology, College of Agriculture, Vellayani, Trivandrum.

Melzer's reaction tests were carried out on the surface, context of the pileus and stipe and spores of C.lagopus, C.ephemerus and C.disseminatus. The surface, context of the pileus and stipe were found to be pseudoamyloid, while the basidia and basidiospores were inamyloid. The surface, context of the pileus and stipe were remained unchangeable on treatment with 3 per cent potassium hydroxide, while they attained a bluish violet

colour on treatment with 11 M hydrochloric acid and reddish brown, with concentrated sulphuric acid. The spores were found to be black coloured, on treatment with the above reagents.

II. Isolation and purification of mushroom

C.lacopus appeared as a weed mushroom on paddy straw beds of V.volvacea, at the Department of Plant Pathology, was isolated and purified by hyphal tip method and maintained on potato dextrose agar slants by subculturing periodically.

Spore germination

Germination of spores in slides and hanging drop were studied and germination of 15 basidiospores were observed. After 1 to 2 hours of incubation, small protuberances of size 8.75 x 3.5 µm were observed on spores. These protuberances changed into germ tubes of size 14-28 x 3.5 µm after 4 to 5 hours of incubation. After 7-8.5 hours of incubation, the germ tubes attained a size of 30 - 35 x 3.5 µm (Fig.2). Table-1 indicated that the germination per cent was more at 30°C followed by 25 and 35°C. The germination per cent was low at 20°C and 40°C. No germination was observed at 15°C and 45°C.

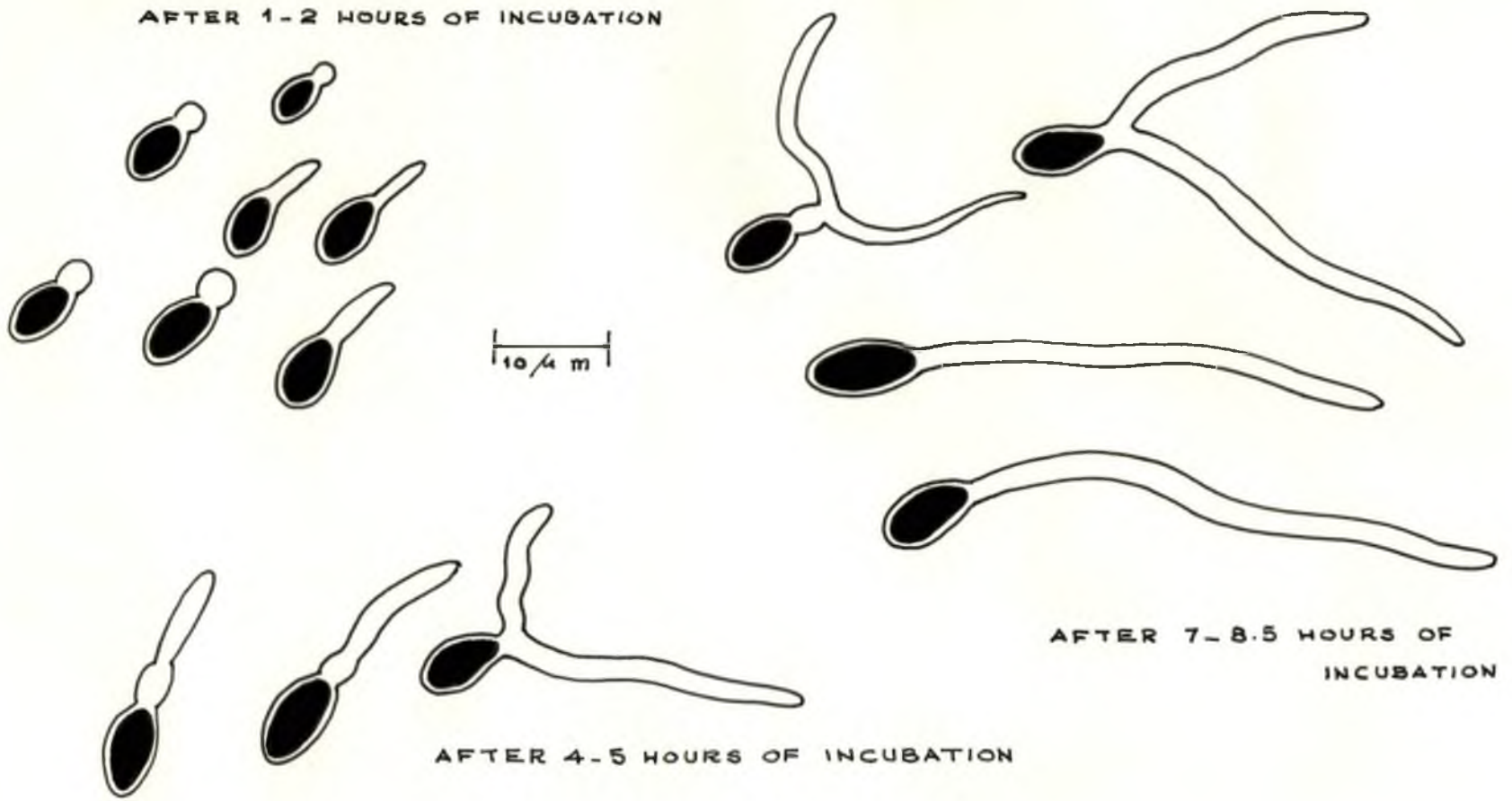


FIG: 2. GERMINATION OF BASIDIOSPORES

Table-1

Germination of basidiospores at different temperatures after 1 hour of incubation

Temperature (°C)	Germination per cent	
	Hanging drop technique	Spore germination in slides
15	-	-
20	12.50	11.40
25	22.95	20.50
30	30.45	25.50
35	20.00	18.75
40	2.40	-
45	-	-

IV. Developmental morphology

Studies were conducted to observe the different stages of development of mushroom from spawning till maturity. The developmental stages of mushroom is divided into 5 stages viz., pinhead stage, tiny button stage, button stage, elongation stage and mature stage (Plate 2) and the details are presented in the Table-2. Mushrooms of pinhead stage of size 4 mm began to appear on the bed, after 72 hours (3 days) of spawning. The pinhead stage remained as such for two more days. After 120 hours (5 days) of spawning, the pinheads attained a size of 5-6 mm. The vertical section showed that the pileus and stipe were not well differentiated. After 144 hours (6 days) of spawning, the pinheads attained the tiny button stage with a stipe length of 2-2.5 cm and pileus diameter of 1.5 - 3 cm. The vertical section revealed differentiation of stipe and pileus. After 150 hours of spawning, the buttons attained a stipe length of 4-5 cm and pileus diameter of 2.5-4 cm. The colour of the buttons changed from white to ashy grey during afternoon hours. After 158 hours of spawning, the buttons reached the elongation stage, with a stipe length of 10-15 cm. During the mature stage (160 hours after spawning)

Tiny button

Button

Table-2

Comparative morphology of different stages of development of C.lagopus

Stages of development	Duration after spawning		Morphological characters	Measurement of different structures (cm)		
	in days	in hours		Whole basidiocarp length.	Pileus diameter	Stipe length
1. Pin head	3	72	Appears as a hyphal knot, Pileus and stipe not differentiated, white.	0.4	-	-
2. Tiny button	6	144	Pileus and stipe differentiated, slightly bigger than pin head	2.5-3	1.5-3	2-2.5
3. Button	6½	150	Cylindric-oval at first and later companulate with a somewhat pointed apex, white and surface covered with dense white scales.	5-6	2.5-4	4-5
4. Elongation	6½	158	The colour of the button changed from white to ashy grey and the stipe elongates	12-16	2.5-4	10-15
5. Mature	6¾-7	160-163	The Pileus was expanded and splitted radially	12-16	2.5-4	10-15

the pileus was expanded and splitted radially. Auto-digestion of the pileus started after 162-163 hours, from the periphery of the gills towards the centre and the gills liquified into a black inky fluid. The expansion of the pileus, following autodigestion was observed during late nights. After the completion of the autodigestion, the stipe collapsed and the whole fruit body was fallen to the ground.

V. Nutritional studies

1. Growth of C.lagopus in different media

a) Growth on solid media (Table-3)

The data revealed that the mycelial growth of the fungus was maximum on potato dextrose agar, followed by oat meal agar. Richard's medium and Czapek's medium favoured least growth of the fungus. The radial growth of the fungus reached the maximum on the 5th day itself on potato dextrose agar, followed by 6th day on oat meal agar. 4 to 6 sporocarps were observed on potato dextrose agar and oat meal agar media, after 3 to 4 days of inoculation. The mycelial growth was very thin in Czapek's medium and 2 to 3 thin sporocarps appeared after 5 to 6 days of inoculation (Fig. 3).

Table-3

Growth of C.lagopus in different solid media

Sl. no.	Media	Colony diameter in cm (average of 4 replications)						
		after 24 hrs.	after 48 hrs.	after 72 hrs.	after 96 hrs.	after 120 hrs.	after 144 hrs.	after 168 hrs.
1.	Potato dextrose agar	2.40	3.97	5.63	7.92	8.63	9.00	9.00
2.	Oat meal agar	1.45	3.22	4.35	5.57	6.47	7.93	9.00
3.	Czapek's medium	1.85	5.13	7.45	9.00	9.00	9.00	9.00
4.	Richard's medium	1.93	2.37	2.87	3.55	4.57	4.70	5.07

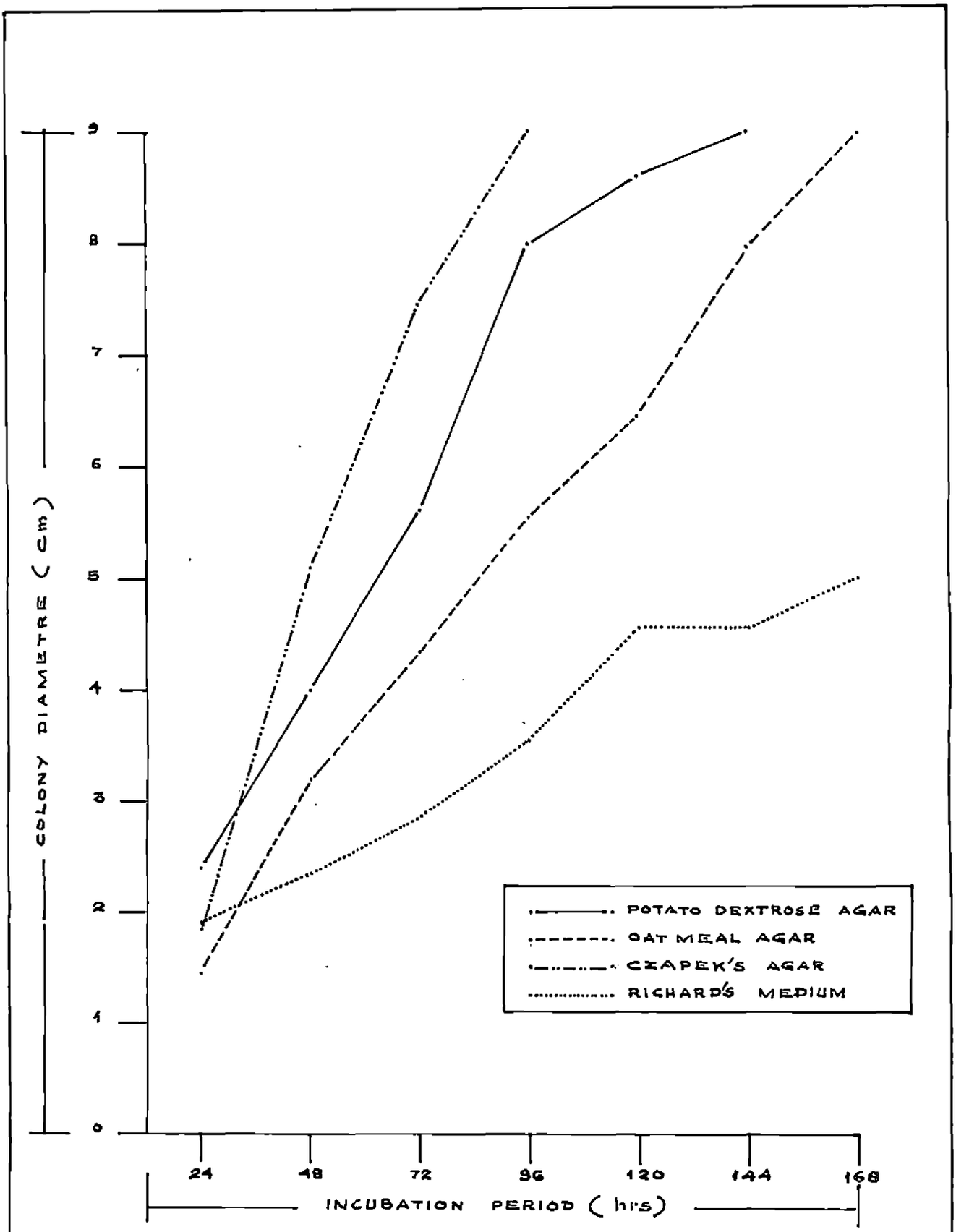


FIG:3. RADIAL GROWTH OF *Coprinus lagopus* (Fr.) Fr. IN DIFFERENT SOLID MEDIA

b) Growth on liquid media (Table-4)

Experiments on the growth of C.lagopus on liquid media revealed the presence of maximum dry weight of mycelium in potato dextrose media (1800 mg) followed by oat meal medium (1235 mg). There was significant difference between these two treatments. Richard's medium and Czapek's medium were the least favoured media for the development of mycelium of fungus. 5 to 8 large sporocarps were observed after 4 to 5 days of inoculation in potato dextrose medium and oat meal medium, while 2 to 3 small sporocarps were appeared in Czapek's and Richard's medium (Fig. 4 and Appendix III).

2. Effect of different temperature on the growth of C.lagopus (Table 5 & Fig.5)

From the table, it is clear that the growth of the fungus was best at 35°C (1076.67 mg) followed by a temperature of 30°C and 40°C respectively (976.67 mg and 568.33 mg). The growth was poor at 25°C and 45°C (495 mg and 383.33 mg). There was no growth at temperature less than 25°C and more than 45°C. There was significant difference between all the treatments.

3. Effect of pH on fungus growth (Table-6)

Studies on the influence of initial pH of media on the growth of C.lagopus showed that the organism can

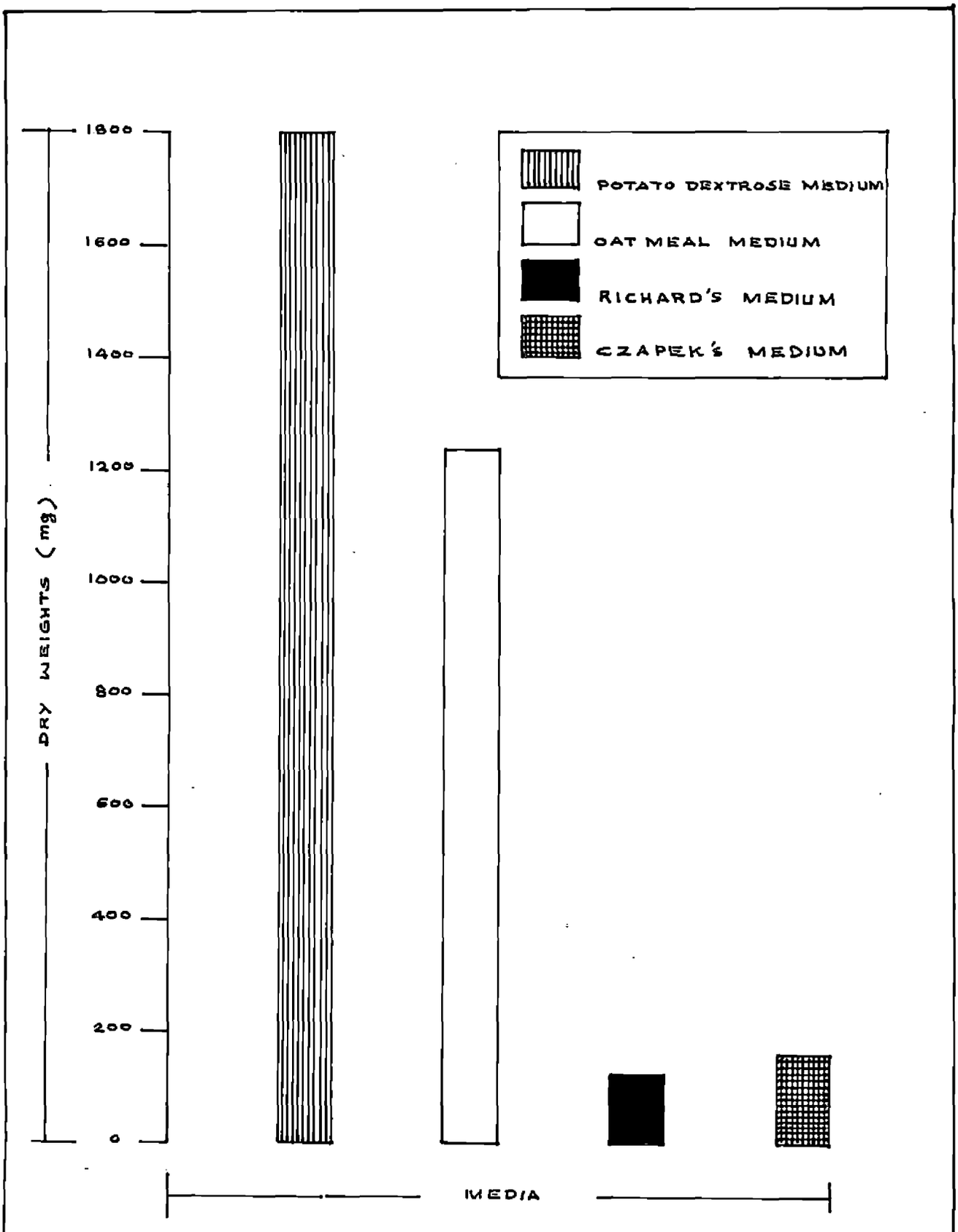


FIG: 4. GROWTH OF *Coprinus lagopus* (Fr.) Fr. IN DIFFERENT LIQUID MEDIA

Table-5

Effect of different temperature on the growth of *C.lagopus* in potato dextrose broth

Sl. no.	Temperature (°C)	Dry weight of mycelium (mg) (average of 4 replications)
1.	25	495.00
2.	30	976.67
3.	35	1076.67
4.	40	568.33
5.	45	383.33

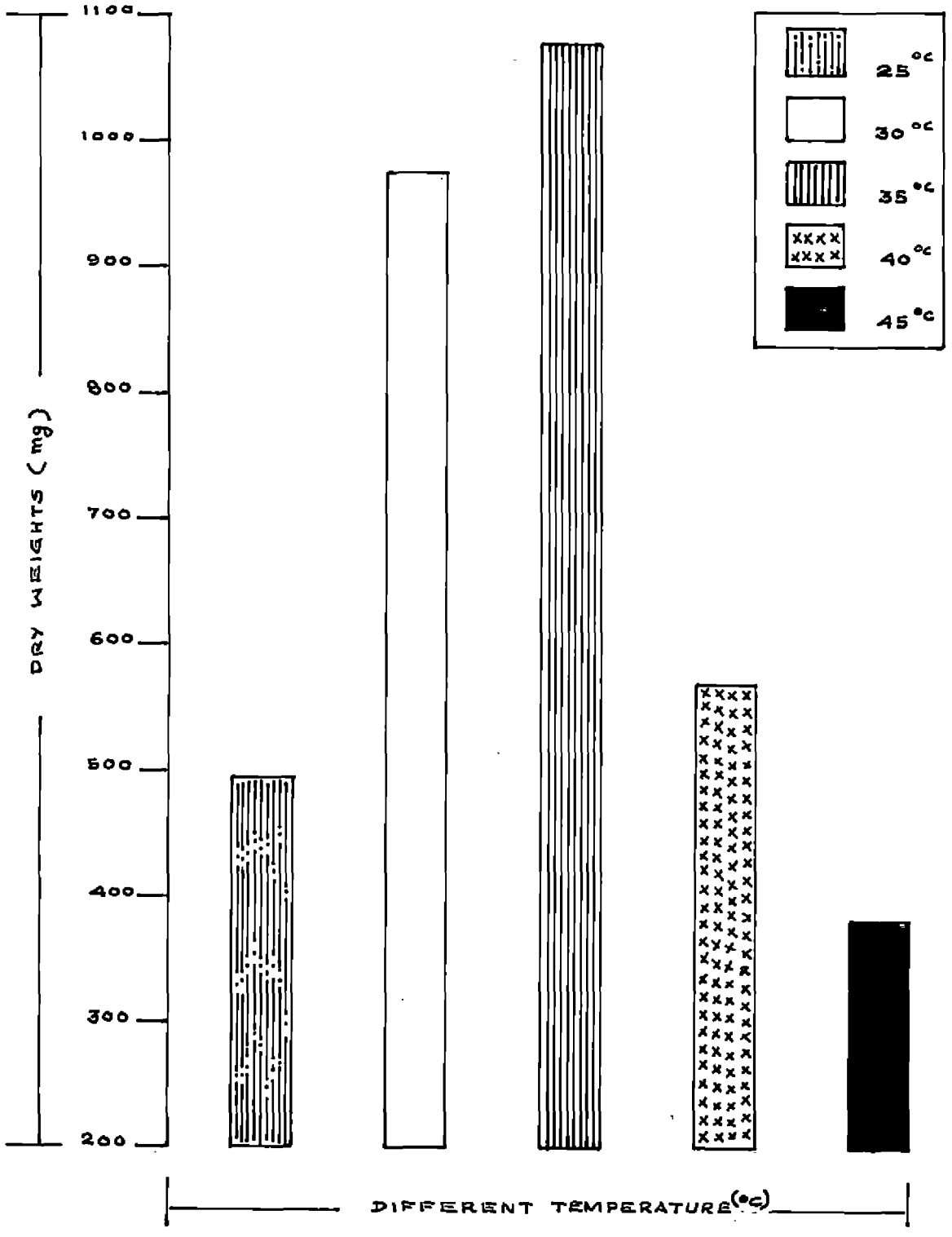


FIG: 8. EFFECT OF DIFFERENT TEMPERATURE ON THE GROWTH OF *Coprinus Lagopus* (Fr.) Fr.

Table-6

Effect of pH on the growth of C.lagopus

Sl. no.	Initial pH of medium.	Dry weight of mycelium (mg) (average of 3 replications)
1.	4	253.33
2.	5	1975.00
3.	6	1893.00
4.	7	1083.00
5.	8	867.00
6.	9	856.67
7.	10	771.67
8.	11	631.67
9.	12	353.33

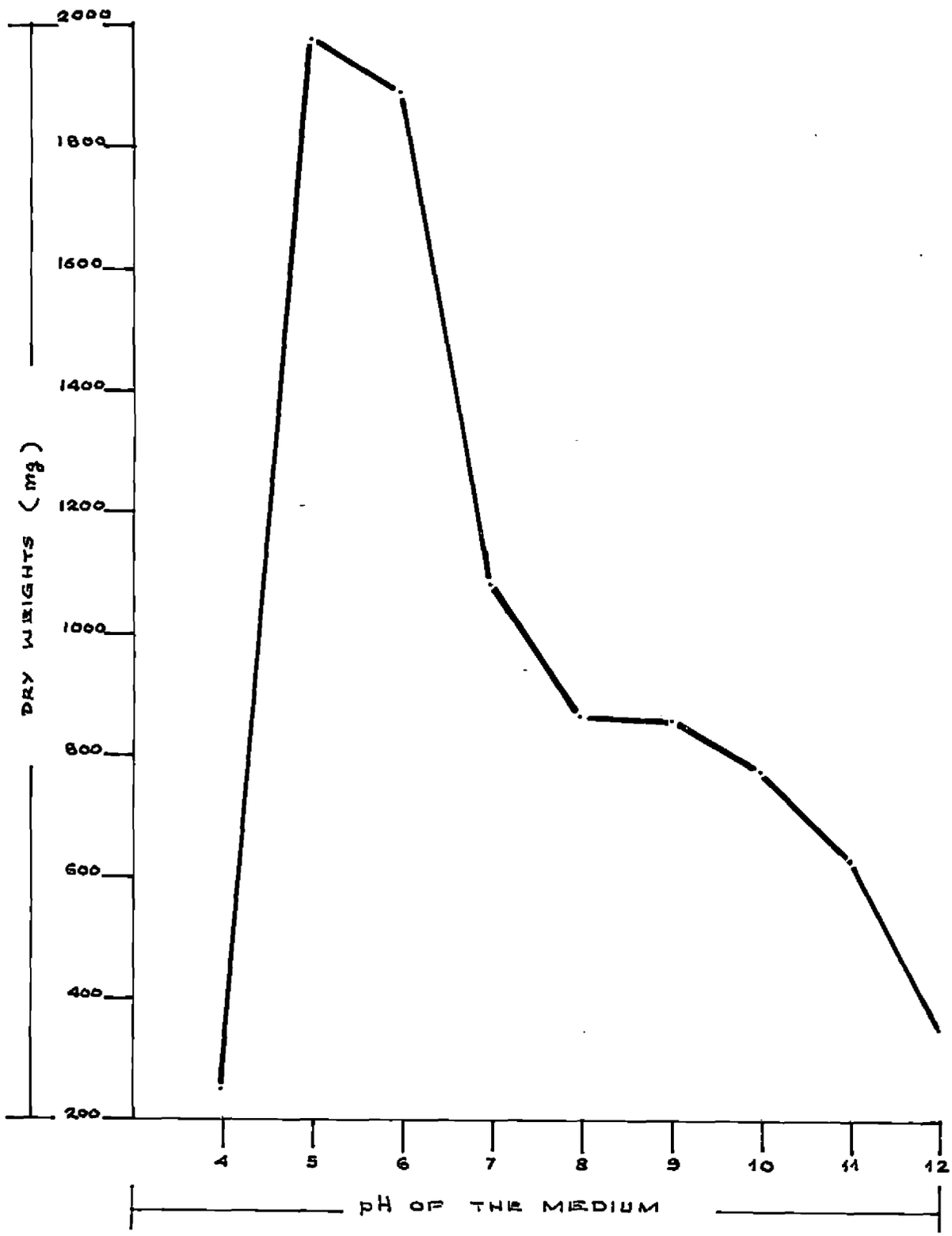


FIG: 6. EFFECT OF pH ON THE GROWTH OF *Coprinus lagopus* (Fr.) Fr.

Table-2

Comparative morphology of different stages of development of C.lagopus

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3. Button	6¼	150	Cylindric-oval at first and later campanulate with a somewhat pointed apex, white and surface covered with dense white scales.	5-6	2.5-4	4-5
4. Elongation	6½	158	The colour of the button changed from white to ashy grey and the stipe elongates	12-16	2.5-4	10-15
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Table-3

Growth of C.lagopus in different solid media

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3.	Czapek's medium	1.85	5.13	7.45	9.00	9.00	9.00	9.00
4.	Richard's medium	1.93	2.37	2.87	3.55	4.57	4.70	5.07

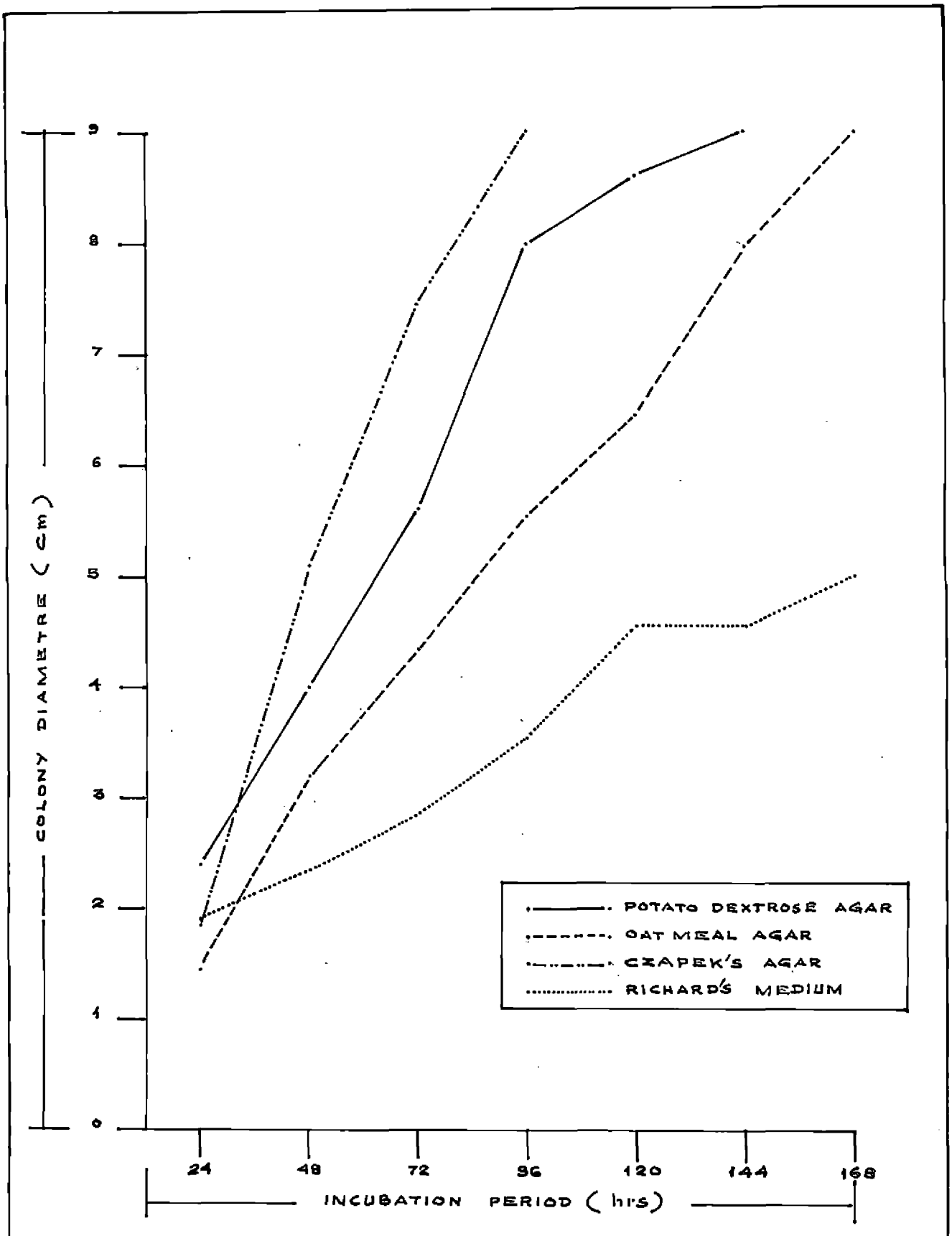


FIG:3. RADIAL GROWTH OF *Coprinus lagopus* (Fr.) Fr. IN DIFFERENT SOLID MEDIA

Table-4

Growth of C.lagopus in different liquid media

Sl. no.	Media	Dry weight of mycelium (mg) (average of 3 replications)
1.	Potato dextrose medium	1800
2.	Oat meal medium	1235
3.	Richard's medium	125
4.	Czapek's medium	160

CD at 0.05 level = 133.25

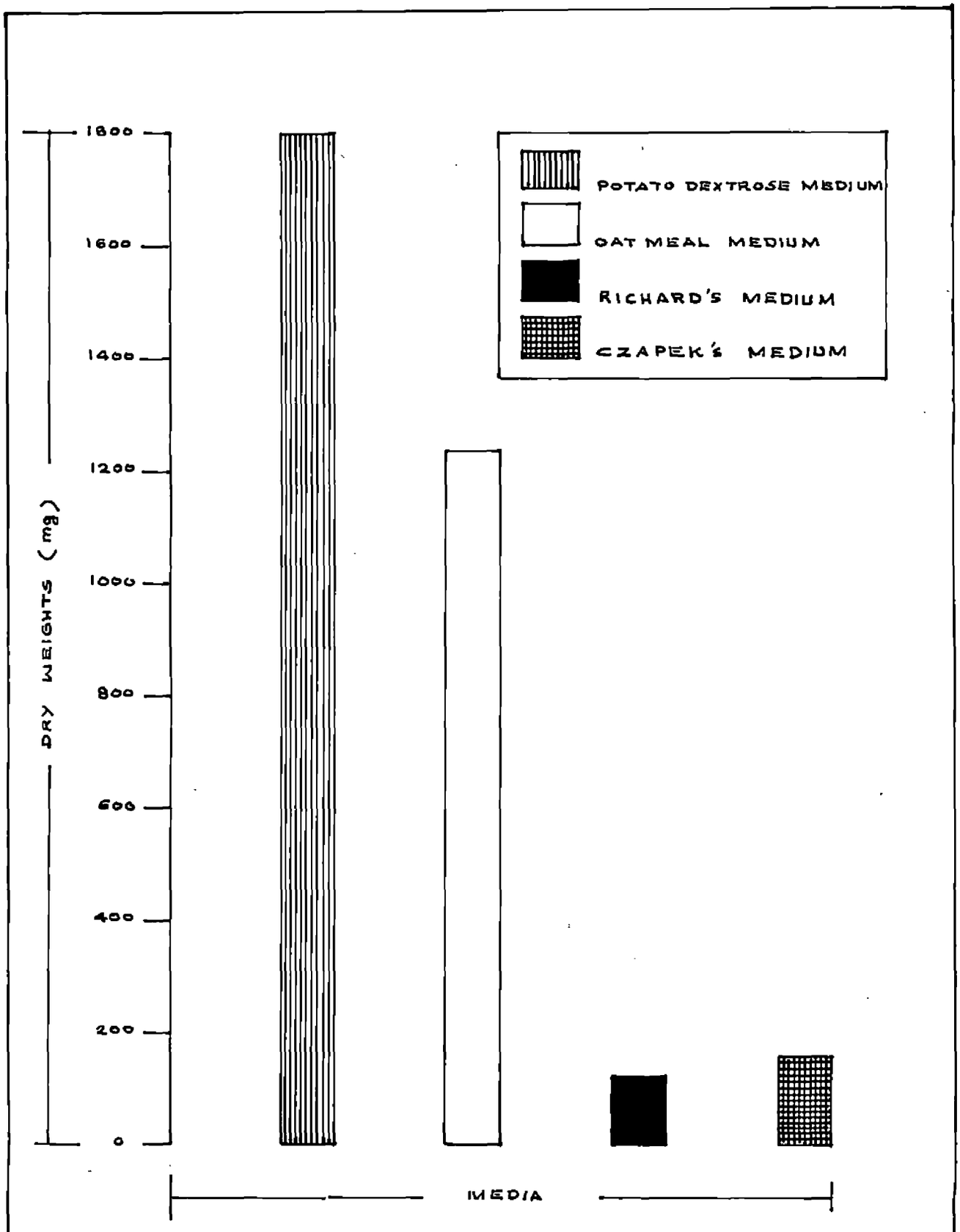


FIG: 4. GROWTH OF *Coprinus lagopus* (Fr.) Fr. IN DIFFERENT LIQUID MEDIA

Plate-3. Growth of C.lagoriis at pH 5

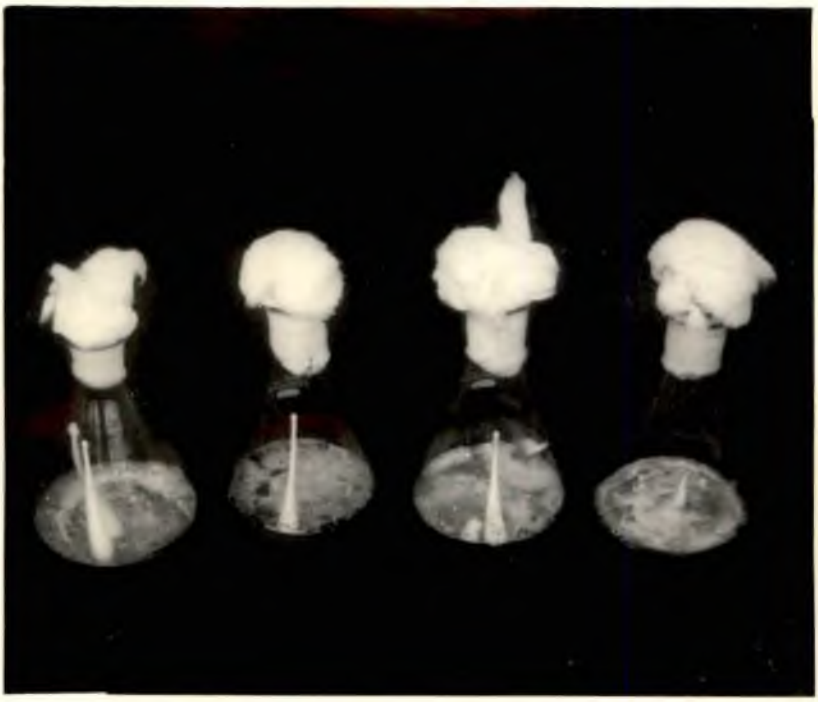
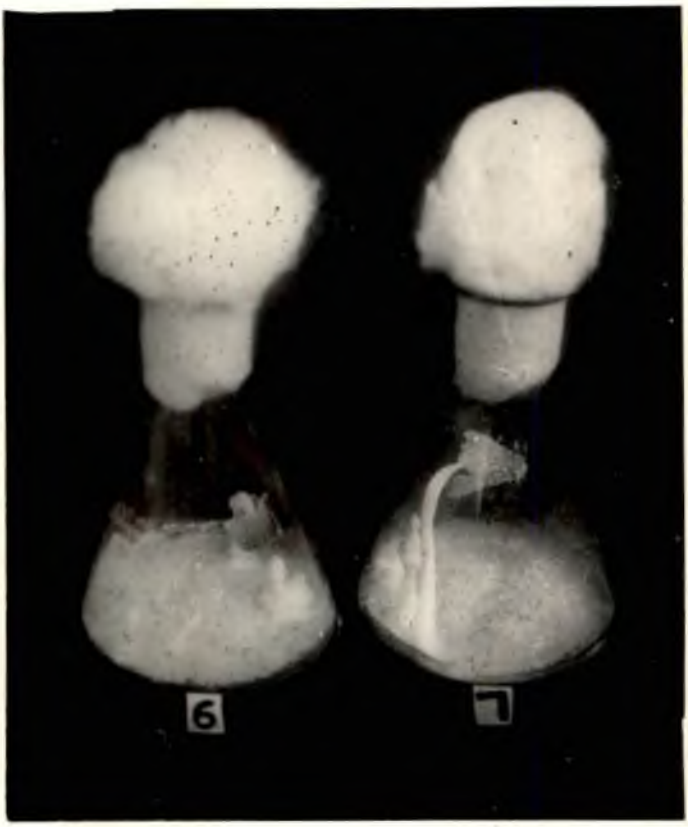


Plate-4 Growth of C.lagoriis at pH 6 & 7



grow on a wide range of pH from acidic to alkaline. The fungus attained maximum growth at pH 5 (1975 mg). As the pH was increased above 6, there was a gradual decrease in dry weight. The growth of the fungus was poor at pH 4 and 12 respectively (253.33 and 353.33 mg.) (Fig.6 and Plate 3 and 4).

4. Effect of light and darkness on the mycelial growth of C.lagopus (Table-7)

The data indicate that maximum radial growth (9 cm) was observed in petri dishes incubated under ordinary light condition, while the radial growth reached only 5 cm in petri dishes incubated under darkness. Sporocarps were observed on petri plates incubated under light condition. They were absent in petri plates incubated under darkness (Fig.7).

5. Effect of different sources of carbon on the growth of fungus

Table-8 on the dry weight of mycelium reveal that maltose was the best source of carbon for the growth of C.lagopus (268.25 mg), followed by dextrose and sucrose respectively (186.50 mg and 160 mg). The growth was poor when lactose was used as carbon source (140 mg). Significant differences were observed between all the treatments.

Table-7

Effect of light and darkness on the mycelial growth of C.lagopus

Sl. no.	Time interval	Colony diameter in cm (average of 3 replications)	
		Darkness	Light
1.	After 24 hours	1.52	2.40
2.	,, 48 ,,	2.53	3.97
3.	,, 72 ,,	3.35	5.63
4.	,, 96 ,,	5.22	7.92
5.	,, 120 ,,	5.32	8.63
6.	,, 144 ,,	5.32	9.00

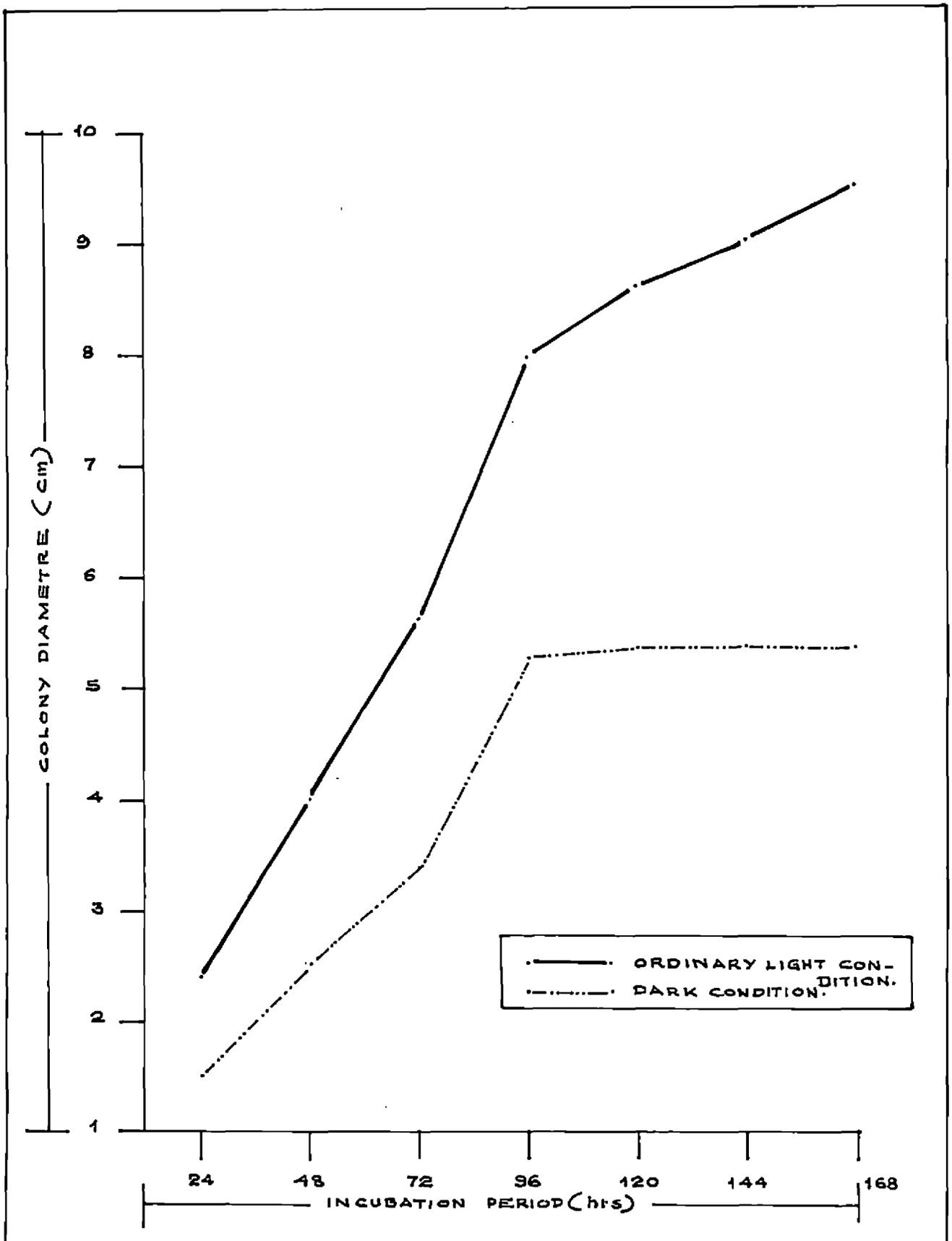


FIG: 7. EFFECT OF LIGHT AND DARKNESS ON THE MYCELIAL GROWTH OF *Coprinus lagopus* (Fr.) Fr.

Table-8

Effect of different sources of carbon on the growth of C.lagopus

Sl. No.	Carbon source	Dry wt. of mycelium (mg) (average of 4 replications)
1.	Sucrose	160.00
2.	Lactose	140.00
3.	Maltose	268.33
4.	Dextrose	186.67
5.	Control	25.00

CD at 0.05 level = 16.68

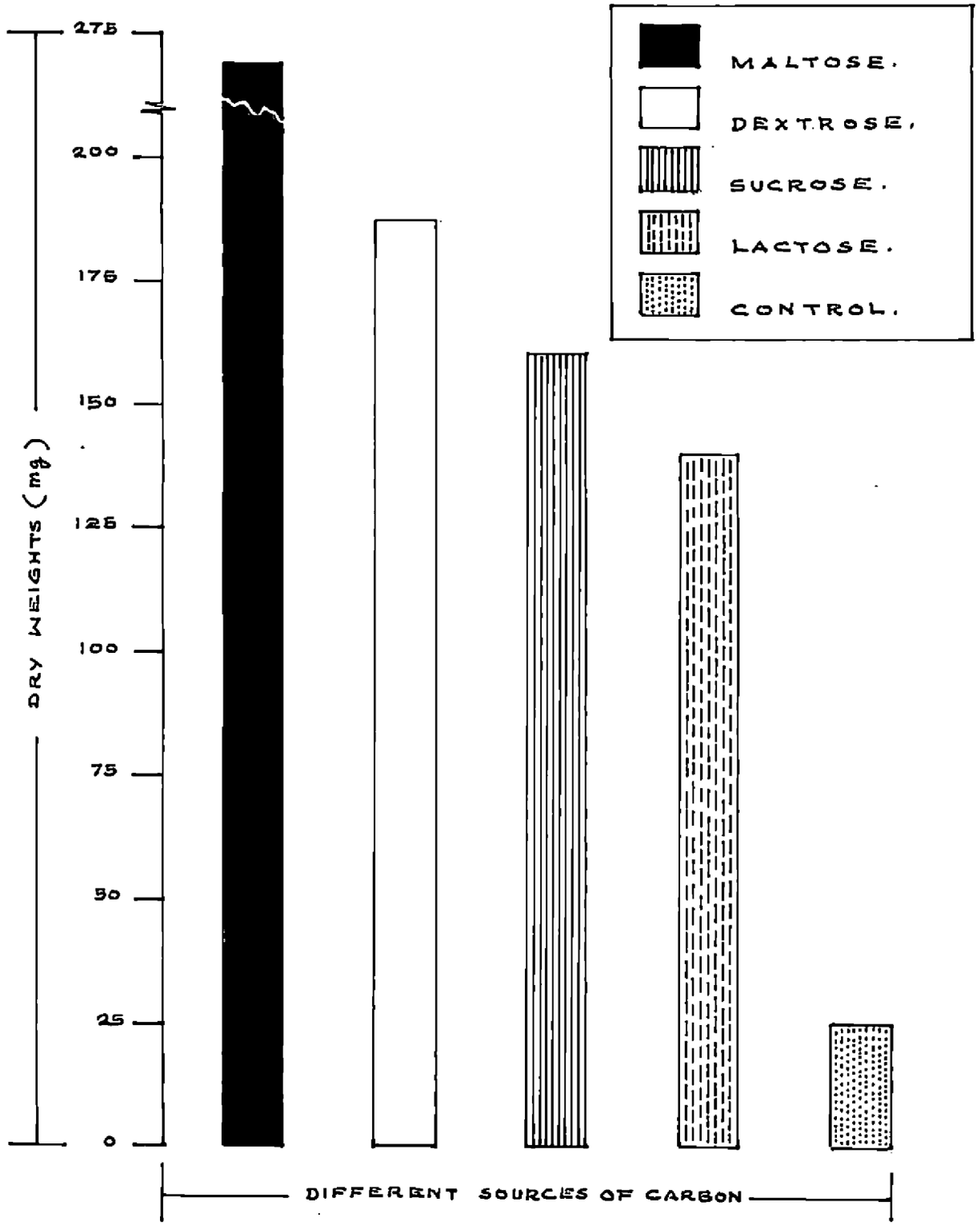


FIG: 8. EFFECT OF DIFFERENT SOURCES OF CARBON ON THE GROWTH OF *Coprinus lagopus* (Fr.) Fr.

The colour of the mycelial growth was pure white when maltose was used as carbon source and greyish white when dextrose and sucrose were used. 6 to 8 small sporocarps were observed in all the media except in the medium with lactose as carbon source. The mycelial growth was very sparse and greyish white in control flasks (Fig.8 and Appendix-IV).

6. Effect of different sources of nitrogen on the growth of C.legopus (Table-9)

The data reveal that sodium nitrate was the best source of nitrogen for the growth of fungus (160 mg) followed by sodium nitrite (131.50 mg). Ammonium chloride and asparagene produced significantly lesser growth. The growth was very poor in control flasks. The mycelial growth was greyish white in all the flasks (Fig. 9 and Appendix-V).

VI. Effect of different spawn substrates on the mycelial growth and sporocarp production of C.legopus (Table-10 and Plate 5)

The data reveale that green gram, red gram, horse gram, bengal gram and wheat grain supported best mycelial growth on 18th day. A steady increase of the mycelial growth was noticed from 3rd day onwards, reaching

Table-9

Effect of different sources of nitrogen on the growth of C.lagopus

Sl. no.	Nitrogen source	Dry wt. of mycelium (mg) (average of 4 replications)
1.	Sodium nitrate	160.00
2.	Sodium nitrite	131.50
3.	Ammonium chloride	86.50
4.	Asparagine	76.00
5.	Control	54.75

CD at 0.05 level = 14.38

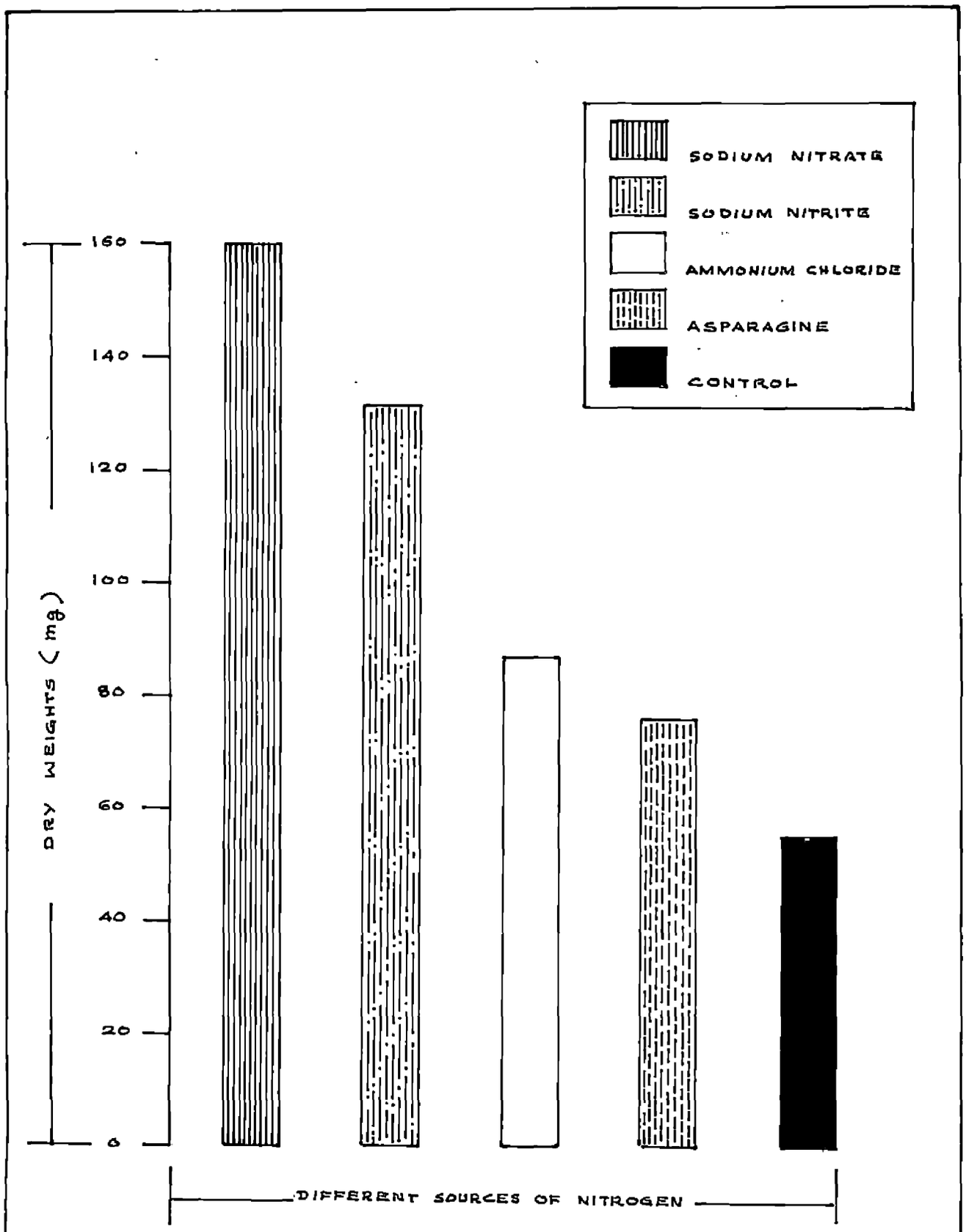


FIG: 9. EFFECT OF DIFFERENT SOURCES OF NITROGEN ON THE GROWTH OF *Coprinus lagopus* (Fr.) Fr.

Table-10

Effect of different spawn substrates on the mycelial growth of C.lagopus

Sl. no.	Substrate	Mycelial growth
1.	Red gram	+++
2.	Horse gram	+++
3.	Bengal gram	+++
4.	Wheat grain	+++
5.	Green gram	+++
6.	Paddy straw	++
7.	Salvinia	+

+++ Good mycelial growth
++ Moderate mycelial growth
+ Poor mycelial growth

Plate-5 Growth of *C. lagopus* in spawn bottles

the maximum on 18th day. Spawn prepared on paddy straw showed only moderate growth and 25-30 days were taken for complete growth. Very poor growth was noticed in the case of salvinia.

a) Yield of fresh sporocarps of C.lagopus from beds laid out with spawn prepared from different substrates (Table-11)

Table-11 reveal that maximum yield of mushroom was recorded from the beds spawned with green gram spawn (704 g) followed by bengal gram and red gram spawn which were on par (673 and 670.67 g). The yield was significantly low in beds laid out with horse gram and wheat spawn (510.33 g and 531.33 g). The yield was very poor in paddy straw spawn (339 g). No yield was recorded from beds spawned with salvinia spawn (Fig.10 and Appendix-VI).

b) Effect of different temperature on the mycelial growth of C.lagopus on spawn bottles (Table-12)

Visual observations on the growth rate of C.lagopus in spawn bottles containing different substrates incubated at different temperatures are given in Table-12. Results showed that red gram, horse gram, bengal gram, green gram and wheat grains supported very good mycelial growth at 30°C and 35°C and scanty growth at 25°C and 40°C. Paddy straw supported moderate growth of C.lagopus at

Table-11

Yield of fresh sporocarps of C.lagonus from beds laid out with spawn prepared from different substrates on straw beds using 4 kg paddy straw

Sl. no.	Spawn substrates	Yield of mushroom in (g) (average of 3 replications)
1.	Red gram	670.67
2.	Horse gram	510.33
3.	Bengal gram	673.00
4.	Green gram	704.00
5.	Wheat	531.33
6.	Paddy straw	339.00
7.	Salvinia	--

CD at 0.05 level of significance = 30.95

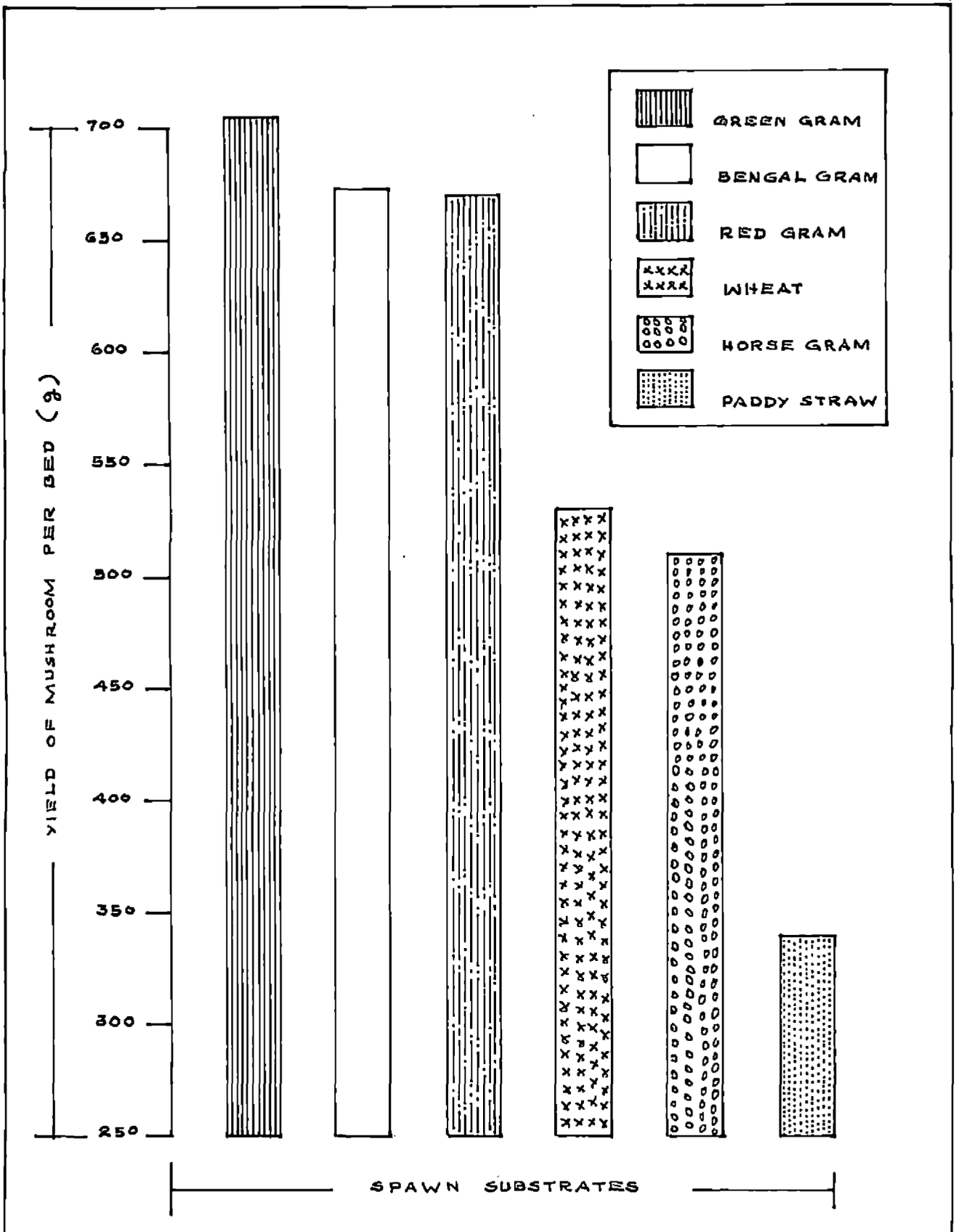


FIG: 10. EFFECT OF DIFFERENT SPAWN SUBSTRATES ON THE YIELD OF FRESH SPOROCARPS OF *Coprinus lagopus* (Fr.) Fr.

Table-12

Effect of different temperature on the mycelial growth of C.lagopus on spawn bottles

Sl. no.	Substrate	Mycelial growth on 20th day of incubation at different temp. (°C)			
		25	30	35	40
1.	Red gram	++	++++	+++++	++
2.	Horse gram	++	++++	+++++	++
3.	Bengal gram	++	++++	+++++	++
4.	Wheat grain	++	++++	+++++	++
5.	Green gram	++	++++	+++++	++
6.	Paddy straw	+	+++	+++	+
7.	Salvinia	-	+	+	-

+++++ Very good mycelial growth
++++ Good " "
+++ Moderate " "
++ Poor " "
+ Very poor " "
- No growth

30°C and 35°C and very scanty growth at 25°C and 40°C. Very scanty mycelial growth was observed in salvinia at all the temperatures tested.

c) Effect of different organic amendments on the yield of sporocarp of C.lagopus (Table-13)

To find out the influence of different organic amendments on the yield of C.lagopus, experiments were laid out with different organic amendments and the data obtained are given in Table-13. The maximum yield was obtained from beds amended with green gram powder (663 g) followed by bengal gram (651.33 g), red gram (606.67 g), wheat flour (483.33 g) and horse gram (450.67 g). The yield was significantly higher in all the treatments over the control except with cowdung slurry (Fig. 11 and Appendix-VII).

VII. (a) Influence of different types of straw bed for the maximum production of sporocarps of C.lagopus

In order to study the influence of different types of straw bed for the maximum production of sporocarps of C.lagopus rectangular beds were laid using 4 kg of paddy straw as twists and chopped straw. The results revealed that a significant increase in yield (704 g) was obtained from rectangular beds laid out with paddy straw twists.

Table-13

Effect of different organic amendments on the yield of sporocarp of C.legerus

Sl. no.	Amendments used	Yield of sporocarps (g) (average of 3 replications)
1.	Wheat flour	483.33
2.	Green gram	663.00
3.	Bengal gram	651.33
4.	Horse gram	450.67
5.	Red gram	606.67
6.	Cowdung slurry	285.00
7.	Control	260.00

CD at 0.05 level = 14.38

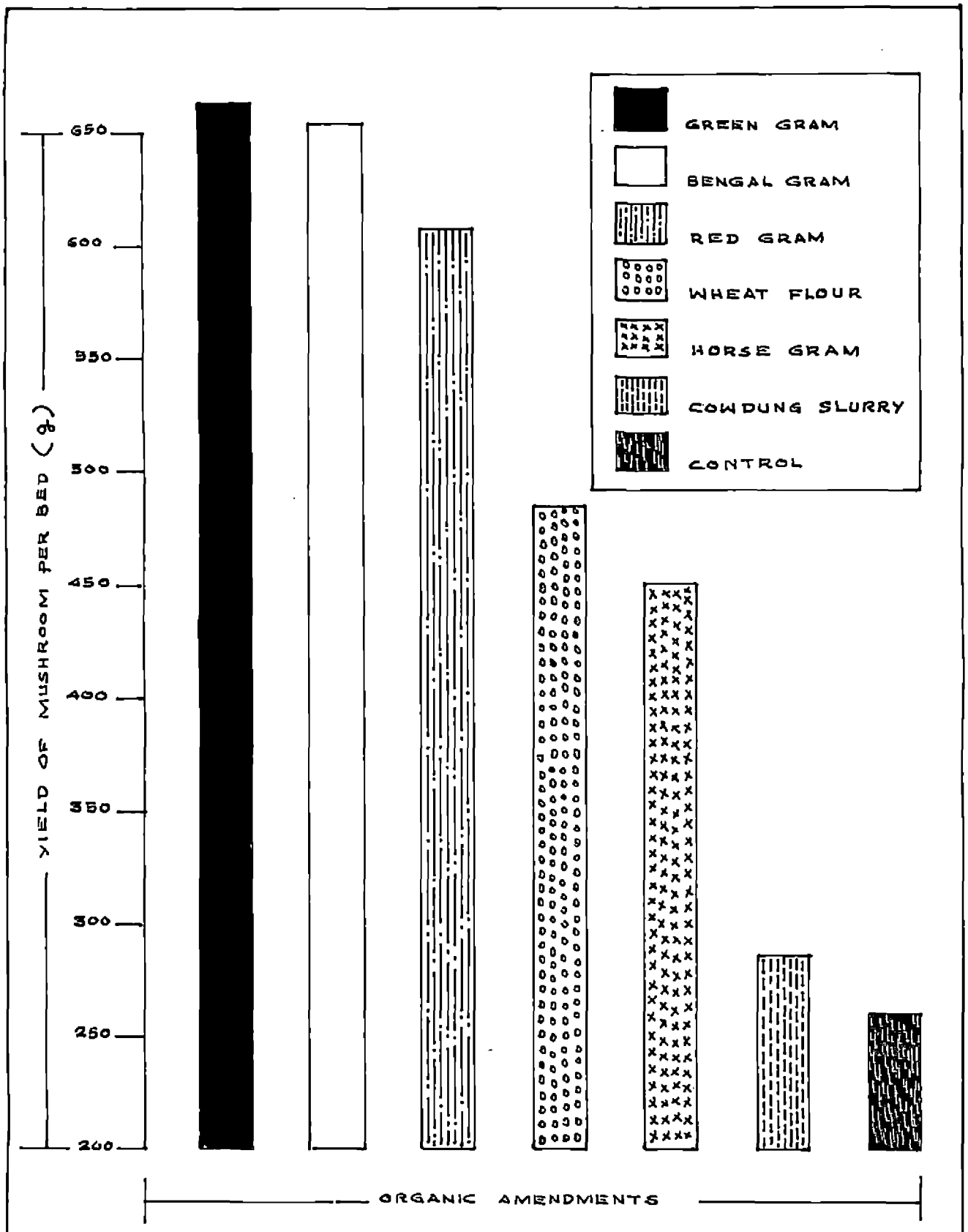


FIG: 11. EFFECT OF DIFFERENT ORGANIC AMENDMENTS ON THE YIELD OF SPOROCARPS OF *Coprinus lagopus* (Fr.) Fr.

The yield was very poor (378 g) from beds laid out with chopped straw.

(b) Effect of different substrates on the yield of C.lagopus

Table-14 showed that maximum yield was obtained (525 g) from conventional paddy straw beds (Plate 6,9and 8). Beds prepared with a mixture of chopped straw and paper at a ratio of 2.25:0.75 yielded only 508.33 g. From a single banana pseudostem 120 g was obtained (Plate 7), while no yield was obtained from beds laid out with salvinia.

VIII. Preservation

1. Refrigeration:- Visual observations of fresh sporocarps of C.lagopus kept under refrigeration revealed that the samples which were kept in open polythene bags remained fresh after 48 hours of storage and they started shrinking after 72 hours of storage. Visual observations of the samples kept in closed polythene bags showed that, after 24 hours of preservation, the buttons started deteriorating with the accumulation of moisture in plastic bags and they liquified producing a bad smell.

2. Dehydration:- Properly dehydrated mushrooms could be preserved effectively by keeping them in polythene bags (Plate 10). Visual observation of the dehydrated mushrooms

Table-14

Effect of different substrates on the yield of C.lagopus

Sl. no.	Substrates	Fresh wt. of mushroom (g) (average of 4 replications)
1.	Paddy straw twists	525.00 (substrate-3 kg)
2.	Chopped straw and paper	508.33 (,,)
3.	Salvinia	—
4.	Banana pseudostem	120.00 (one complete pseudostem)

Plate-6 Sporocarps of C. lagopus on beds of paddy straw twists.

Plate-7 Sporocarps of C. lagopus on banana pseudostem

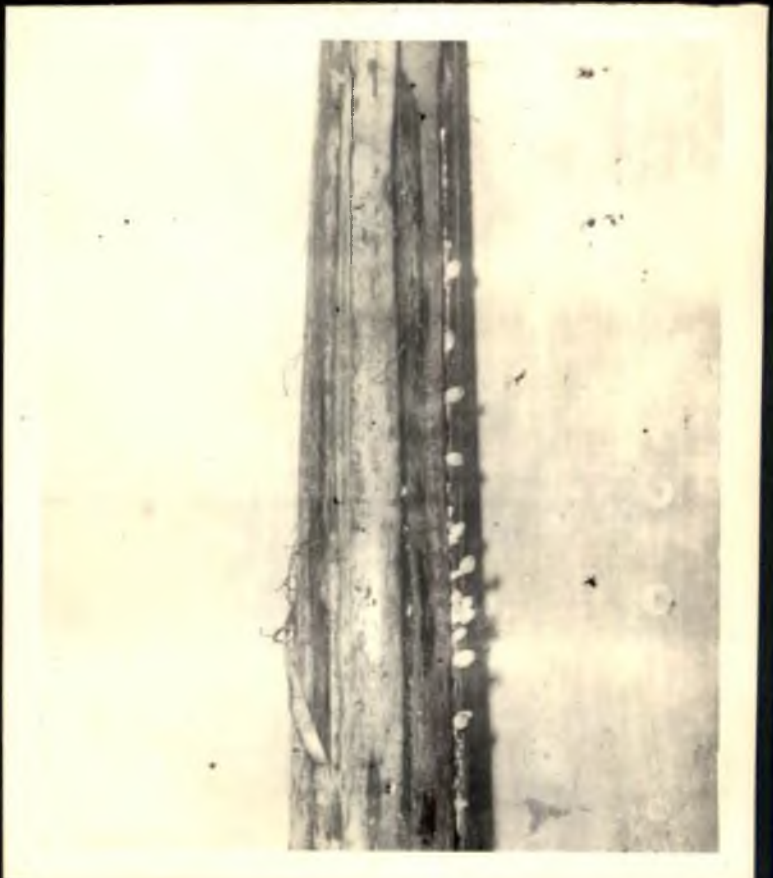
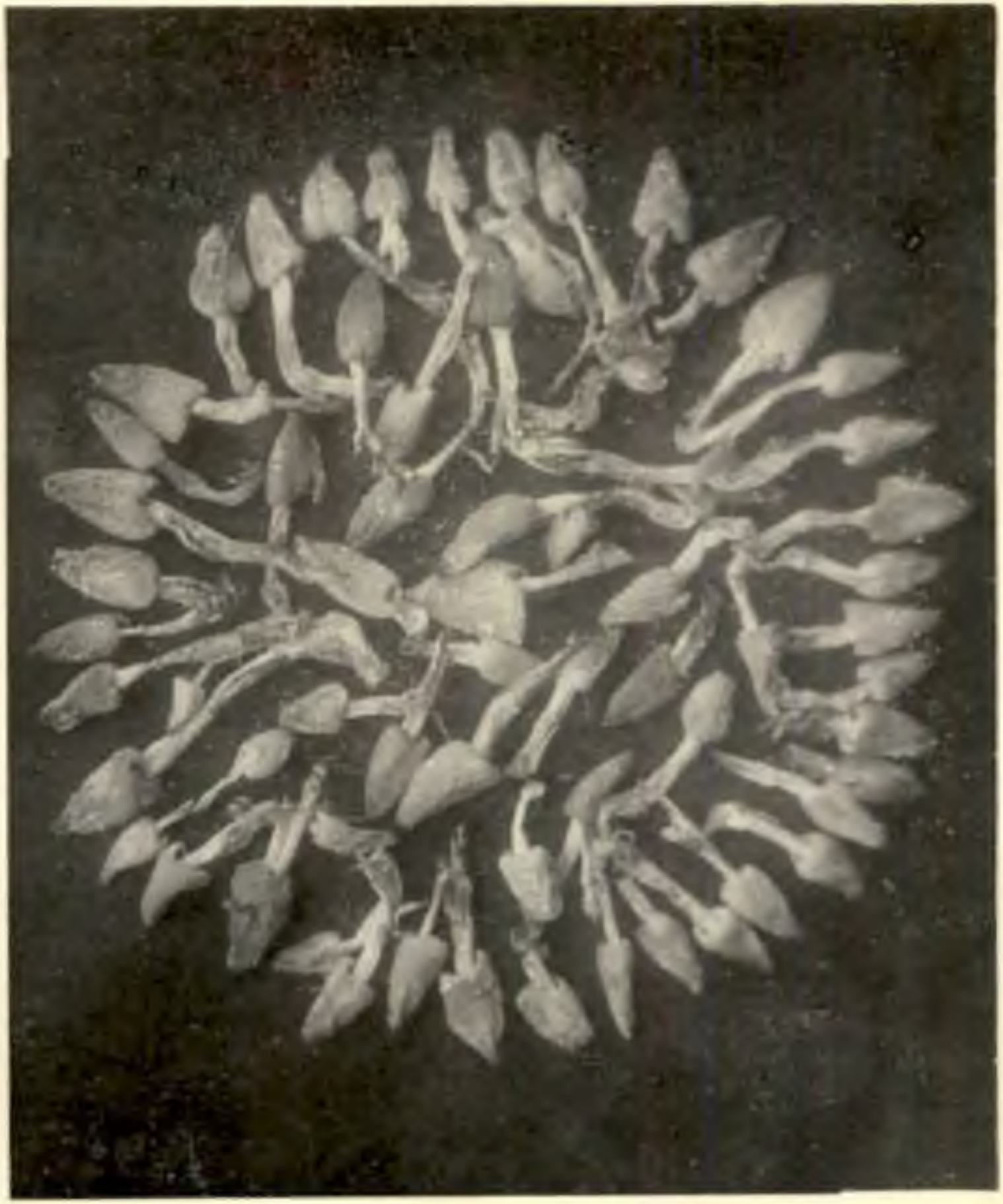


Plate-8 Yield of fresh sporocarps of *C.lagopus* from beds of paddy straw twists

Plate-9 A bunch of sporocarps of *C.lagopus*



kept in polythene bags and in air tight containers revealed that the samples were free from the attack of microorganisms, while the samples kept open were found to be infected by common species of Aspergillus, Penicillium and Bacteria.

3. Preservation in brine

Fresh mushrooms at button stages were harvested, cleaned and preserved in different concentrations of brine (1 to 7 per cent). The duration of storage was 6 weeks. Visual observation of the preserved mushroom at different concentrations of brine revealed that the mushrooms retained more or less the same original colour of the materials preserved. Table-15 showing the microbial assay of preserved mushrooms conducted at weekly interval showed that it was free from any bacterial growth in those preserved on 5, 6 and 7 per cent brine upto 4 weeks. The population of bacteria and fungi are more in 1 to 4 per cent brine. The results indicated a gradual reduction in bacterial population as the concentration of brine increased. Actinomycetes was absent in all the treatments throughout the experimental period.

IX. Nutritive value of C.lagopus

The nutritive value of the sporophore of C.lagopus in button stage was assessed and the data are given in

Table-15

Total microbial flora of preserved mushroom in different concentrations of brine

(microbial population expressed in ml x 10^{x})

Brine (conc.)	Incubation period in weeks																	
	1			2			3			4			5			6		
	F	B	A	F	B	A	F	B	A	F	B	A	F	B	A	F	B	A
1%	-	-	-	-	-	-	6	13	-	7	18	-	8	28	-	8	28	-
2%	-	-	-	-	-	-	5	12.5	-	5	13.5	-	6	25	-	6.5	26.33	-
3%	-	-	-	-	-	-	8.5	10.33	-	4	14.33	-	4	24.5	-	5.5	23.33	-
4%	-	-	-	-	-	-	-	8.33	-	3	12.33	-	3	22.5	-	-	20	-
5%	-	-	-	-	-	-	-	-	-	-	-	-	7	18	-	12	20	-
6%	-	-	-	-	-	-	-	-	-	-	-	-	6	16.5	-	8	13.3	-
7%	-	-	-	-	-	-	-	-	-	-	-	-	6	12.33	-	6	9.5	-

F = Fungi B = Bacteria A = Actinomycetes

Table-16

Per cent composition of protein, crude fibre, carbohydrate, fat and moisture content of C.lagopus

Sl. no.	Contents	Per cent
1.	Moisture	91.250
2.	Carbohydrate	5.090
3.	Protein	1.529
4.	Crude fibre	0.625
5.	Fat	0.576

Table-16. The data indicated that moisture formed the major constituent of mushroom (91.25 per cent), followed by carbohydrate (5.095 per cent) and protein (1.529 per cent). The crude fibre content was 0.625 per cent and fat content was 0.576 per cent.

Qualitative analysis of aminoacid composition of C.lagopus

The qualitative analysis of amino acid composition of sporocarps indicated the presence of 14 amino acids, out of which ten were identified viz., leucine, proline, ornithine, serine, valine, tryptophan, glycine, arginine, tyrosine and phenylalanine.

X. Edibility trials

Edibility trials of C.lagopus (Fr.) Fr. on Red Eyed White rabbits revealed that there was no significant difference in the weights of rabbits which were fed with dried mushroom powder as against the control. No change was observed in the external appearance of rabbits given with dried mushroom powder to that of control rabbit.

XI. Biomass production by C.lagopus, V.volvacea and Pleurotus sapidus in submerged culture

The studies indicated that C.lagopus was very good in the production of mycelial pellets (Plate 11).

The maximum dry weight of 2.5 g per 50 ml potato dextrose broth was obtained in the case of C.lagopus while the dry weights were poor in V.volvacea and P.sapidus (1.5 g and 1.85 g respectively). The sporocarp primordia were observed in flasks kept stationary after five days of shaking in the case of C.lagopus and P.sapidus, while no primordia were observed in the case of V.volvacea.

DISCUSSION

DISCUSSION

A survey conducted in and around the College of Agriculture, Vellayani Campus revealed that the commonly occurring species of Coprinus were Coprinus lagopus (Fr.)Fr., C.disseminatus (Fers. ex Fr.) S.F.Gray and C.ephemerus (Bull.ex Fr.) Fr. Among the three species, the most commonly occurring one was C.lagopus. The culture of C.lagopus used for the present study was isolated from paddy straw beds of Volvariella volvacea (Bull.ex Fr.)Sing. raised at the Department of Plant Pathology. The mushroom was tentatively identified as C.lagopus (Fr.) Fr. by comparing its characters which was confirmed by Dr.D.N.Pegler of Royal Botanic Gardens, England and the characters of the commonly occurring C.lagopus collected from paddy straw, guinea grass stumps and cowdung manure, from Vellayani were found to be in identical with that of C.lagopus appeared on paddy straw. The identity of C.disseminatus and C.ephemerus were confirmed by comparing its characters with the already reported ones (Bhaneni Dei, 1982). Only very few information is available regarding the nutritional and cultivation aspects of C.lagopus. Buller (1958) studied the morphology of C.lagopus. Kurtzman (1978) has conducted studies regarding the cultivation of C.fimetarius. No study has been done so

far regarding the nutritional and cultivation aspects of C.lagopus in India and this is the first work regarding its cultivation.

The macro and microscopic characters of the mushrooms were studied in detail. The diameter of the pileus of C.lagopus varied from 5 to 30 mm in the button stage to 30 to 80 mm in the mature stage. They appeared as cylindric oval at first and later campanulate with a pointed apex, white in colour at first, then grey and finally turned to black. The pileus is fleshy in C.lagopus while it is thin, membraneous and 10 to 15 mm in diameter in the case of C.disseminatus and C.ephemerus. It is ovate at first and later campanulate, whitish or pale buff to grey, powdery except the disc which remains yellowish in the case of C.disseminatus while the pileus is oval, later campanulate and flat at maturity and when old tan to reddish brown in centre and pales towards the margin in C.ephemerus.

The gills of C.lagopus are 2 to 8 mm wide, length varied with pileus size, white at first and later turns black, while the gills are very thin, 2 mm wide, wedge shaped and adnate in C.disseminatus and narrow and linear in C.ephemerus.

The stipe length varied from 1 to 5 cm before and 10 to 15 cm after elongation, 5 to 7 mm in diameter, hollow, white and hairy in C.lagopus, while the stipe was only 2.5 to 4 cm long, thin, white, hollow and fragile in C.ephemerus and C.disseminatus.

The basidiophores of C.lagopus and C.ephemerus were more or less the same in dimension and shape. They were black, elongated oval with a germ pore and 8.25 to 12.5 x 5.5 to 7 µm in dimension in C.lagopus and were ovate to obovate and 16 to 17 x 9-10 µm in C.ephemerus. The spores were smaller (7 to 9 x 3.5 to 5.5 µm) smooth, flattened and rather broadly fusiform with a distinct germ pore in the case of C.disseminatus.

Both pleurocystidia and cheilocystidia were present in C.lagopus. Pleurocystidia, elongated and oval in shape, rounded at the apex and bulged in the middle and contracted into a stalk at the base and has a dimension of 20 to 30.45 x 10 to 14.75 µm. Cheilocystidia, elongated and club shaped and 60 to 64.45 x 32.5 to 36.80 µm in dimension. In C.disseminatus pleurocystidia were absent and large cheilocystidia were present.

The above details agree with those reported by Buller (1958) and Bhavani Devi (1982).

Studies conducted to observe the different stages of development of mushroom from spawning till maturity indicated that the different stages of development can be divided into 5 stages viz., pin head, tiny button, button, elongation and mature stage, following Chang and Yau (1971) who adopted the same for V.volvacea. Mushrooms on pinhead stages of size 4 mm began to appear on bed after 72 hours of spawning and they attained tiny button stage after 144 hours. There was differentiation of stipe and pileus in tiny buttons. After 158 hours, they attained elongation stage and after 160 hours, the mature stage in which the pileus was expanded and splitted radially. The expanded pileus remained as such for one or two hours. Then the autodigestion takes place from the periphery of the gills towards the centre and the gills liquify to a black inky fluid. The expansion and autodigestion of the pileus were observed during late nights. These studies indicated that C.lagopus has got a nocturnal pattern of spore dispersal.

In order to get a spore print the fully expanded pileus was immediately cut and kept so as to avoid the autodigestion. Experiments showed that only 5 to 10 minutes is sufficient to get a detailed spore print, while

The stipe length varied from 1 to 5 cm before and 10 to 15 cm after elongation, 5 to 7 mm in diameter, hollow, white and hairy in C.lagopus, while the stipe was only 2.5 to 4 cm long, thin, white, hollow and fragile in C.ephemerus and C.disseminatus.

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The above details agree with those reported by Buller (1958) and Bhavani Devi (1982).

Melzer's reaction tests were carried out and the surface, context of the pileus and stipe were found to be pseudoamyloid, while the basidia and basidiospores were inamyloid.

The fungus C.lagopus was isolated from paddy straw beds of V.volvacea, purified by hyphal tip method and maintained on potato dextrose agar slants by sub-culturing periodically. Kurtzman (1978) isolated C.fimetarius from straw.

The per cent of germination of basidiospores noted at different temperature after one hour of incubation revealed that the optimum temperature range for basidiospore germination was 25 to 30°C. The germination per cent was very low at 20°C and 40°C. The percentage germination was maximum at 30°C (30.45 and 25.50). No germination was observed at temperatures below 20°C and above 40°C. This indicates that in our climatic conditions, the basidiospores of C.lagopus germinate freely at room temperature. Chapman and Barankovich (1979) reported that the germination of basidiospores of C.domesticus occur over a wide temperature range and the optimum was 28 to 35°C.

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In order to get a spore print the fully expanded pileus was immediately cut and kept so as to avoid the autodigestion. Experiments showed that only 5 to 10 minutes is sufficient to get a detailed spore print, while

in other species especially in V.volvacea the usual time taken for getting a clear spore print is 1 to 2 hours (Bhavani Devi, 1982). In nature the fully expanded stage of C.lagopus is rarely seen because of its quick autodigestion. Buller (1958) studied the mechanism of autodigestion and spore liberation. Tracy (1955) and Iten and Matile (1970) reported the presence of autolytic enzyme chitinase in the deliquescing caps of Coprinus especially in C.lagopus. Bush (1974) observed the presence of β (1-3-)-gluconase in the deliquescing caps of Coprinus.

In vitro studies on the nutritional aspects of the fungus indicated that maximum mycelial growth and dry weights were obtained on potato dextrose medium and oat meal agar (1800 mg and 1235 mg). Czapek's medium and Richard's medium favoured least growth of the fungus. Five to eight sporocarps were observed after 4 to 5 days of inoculation in potato dextrose and oat meal medium while two to three small sporocarps appeared in Czapek's and Richard's medium.

Studies on the effect of different temperature on fungus growth revealed that the growth of the fungus was best at 35°C (1076.67 mg) followed by 30°C and

40°C respectively (976.67 mg and 568.33 mg). The growth was very poor at 25°C and 45°C (495 and 383.33 mg) and no growth was observed at temperatures below 25°C and above 45°C. This is in agreement with the work of Kurtzman (1978) who reported that C.fimetarius can be grown in a wider temperature range from less than 20°C and upto 42°C, the preferred range being 35 to 40°C and Chakraborty and Rahman (1979) who reported that the optimum temperature for the growth of the normal colonies of C.lagopus was 37°C and North (1980) who reported that an incubation temperature of 37°C is used for the growth of vegetative mycelia of C.cinereus, as this temperature is probably high enough to discourage the growth of non-coprophilous fungi.

Studies on the influence of initial pH of media on the growth of C.lagopus showed that the organism can grow on a wide range of pH from acidic to alkaline. The fungus attained maximum growth at pH 5 (1975 mg) and as the pH was increased above 6, there was a gradual decrease in growth and poor growth was observed at very low and high pH, viz., 4 and 12 (253.33 and 353.33 mg). Seal (1981) reported the growth of C.cinereus in an alkaline environment and he has isolated the fungus from

amended straw at pH 8.3. Chang-Ho and Yee (1977) reported that C.cinereus preferred an acidic pH. North (1980) also reported that C.cinereus grew well on a simple minimal medium of pH 6.8.

Studies on the effect of light on the mycelial growth of C.lagopus indicated that best growth and sporocarp production were observed at ordinary light condition while the beds kept in darkness supported poor mycelial growth and no sporocarp production. Buller (1931) observed an inhibitory effect of day light on basidiocarp formation of C.sterquilinus. He reported that the development of basidiocarp rudiments less than 1 mm long were inhibited by light, but as these became larger than 3 mm, light was no longer inhibitory. Buller (1958) reported that as compared with the lighted fruit bodies the darkened ones differed in having stipes which were relatively longer and which tapered towards the base and having narrower gills and pointed and narrow pileus. Light inhibits the development of the tapering pseudorhizal stipe.

In vitro studies on the best source of carbon for the growth of fungus indicated that maltose was the best source of carbon (268.25 mg) followed by dextrose and

sucrose respectively.(186.50 and 160 mg). The growth was poor (140 mg) with lactose as carbon source. Chang-Ho and Yee (1977) reported that the fungus C.cinereus could make use of all carbohydrates like xylose, mannose, cellobiose and soluble starch with the exception of lignin.

Among the organic and inorganic sources of nitrogen tested, sodium nitrate was the best for the mycelial growth, the dry weight being 160 mg, followed by sodium nitrite (131.50 mg). Ammonium chloride and asparagine produced significantly lesser growth. Chang-Ho and Yee (1977) reported that C.cinereus was more efficient with sodium nitrate as nitrogen source.

In order to standardise the cultivation techniques of C.lagopus under Kerala condition, the following trials have been carried out. Studies on the suitability of different spawn substrates on the mycelial growth and sporocarp production of C.lagopus revealed that green gram, red gram, horse gram, bengal gram and wheat grains favoured good mycelial growth and sporocarp production and among these green gram spawn yielded maximum sporocarps (704.09 g) followed by bengal gram and red gram spawn (673 and 670.67 g). The yield was low in horse gram and wheat. The yield was very poor in paddy straw spawn (339 g) comparing with the above grain spawn.

Kurtzman (1978) reported that C.fimetarius grows faster on sorghum grain.

The studies on the effect of different temperature on the mycelial growth of C.lagopus in spawn bottles showed that a temperature of 30 to 35°C is best for the mycelial growth in spawn bottles. Though the fungus attained some growth in liquid media at 25 and 45°C, very poor growth was observed in spawn bottles and the growth was not proceeded even after 20 days.

Studies on the suitability of organic amendments for sporocarp production indicated that maximum yield (663 g) was obtained when green gram was used followed by red gram, bengal gram, wheat flour and horse gram. The yield was significantly poor with cowdung slurry (285 g). No study has been conducted in this aspect with respect to C.lagopus.

Studies on the influence of different types of straw bed for the maximum production of sporocarp of C.lagopus revealed that significant increase in yield (704 g) was obtained from rectangular beds with twists of paddy straw, weighing 4 kg. The yield was poor (378 g) from beds laid out with chopped straw.

Studies on the effect of different substrates on the yield of C.lagopus revealed that maximum yield was obtained (524 g) from paddy straw beds of 3 kg, followed by 508.33 g using a mixture of chopped straw and paper at 3:1 ratio. From a single banana pseudostem, 120 g was obtained, while no yield was obtained from beds laid out with salvinia. These studies indicated that beds using twists of paddy straw is best for the cultivation of C.lagopus. Kurtzman (1978) reported the large scale cultivation of C.fimetarius in a mixture of chopped straw and paper 3:1 and 200 g calcium nitrate. He also reported that straws of various species have been used successfully as a substrate for its cultivation.

Studies regarding the preservation of mushroom indicated that these mushrooms can be preserved in refrigerated condition upto 3 days and after that they start shrinking. It can also be preserved by drying at 70°C and also by powdering after drying. Preservation in various concentrations of brine indicated that the microbial contamination was low at 5, 6 and 7 per cent concentration, while it was more at lower concentration. Kurtzman (1978) reported that immediately after harvesting, C.fimetarius must be cooled, blanched or dried at 60 to 80°C. If they are cooled quickly, Coprinus will be useful

for more than 3 days. Blanching Coprinus in boiling water for one minute will preserve the flavour and destroy the autolytic enzymes responsible for the ephemeral characteristics and once blanched the mushrooms are ready for canning or other preservation which will keep them for an indefinite period.

Studies on the nutritive value of C.lagopus revealed that the major constituent is moisture (91.25 per cent) followed by 5.095 per cent carbohydrates, 1.529 per cent protein, 0.625 per cent crude fibre and 0.576 per cent fat. These mushrooms are poor in protein compared to other mushrooms, but the carbohydrate content is high. Crisan and Sands (1978) reported that C.comatus (fresh) contain 92.2 per cent moisture, 2.54 per cent protein, 0.33 per cent fat, 5.88 per cent carbohydrate and 0.75 per cent fibre and C.atramentarius (fresh) contain 92.3 per cent moisture, 2.09 per cent protein, 0.57 per cent fat, 5.33 per cent carbohydrate and C.atramentarius (mature) contain 0.93 per cent fibre.

Comparative studies on the submerged culture production of mycelial biomass of C.lagopus, V.volvacea and Pleurotus sapidus revealed that C.lagopus was very good in the production of mycelial pellets and maximum dry

weight (2.5 g) was got, while the dry weights were poor in V.volvacea and P.sapidus (1.5 g and 1.85 g respectively). The sporocarp primordia were observed in flasks kept stationary after 5 days of shaking in the case of C.lagopus and P.sapidus while no primordia were observed in the case of V.volvacea. Kurtzman (1978) observed the formation of fruit bodies of P.sapidus in flasks kept stationary after shaking for about 30 days.

Edibility trials of C.lagopus on Red Eyed White rabbits indicated that there was no significant difference in the weights of rabbits which were fed with dried mushroom powder as against the control. No change was observed in the external appearance of rabbits given with dried mushroom powder to that of control rabbit. With these results, C.lagopus can be regarded as edible mushroom. C.fimetarius was reported to be edible by Kurtzman (1978), C.atramentarius, C.comatus and C.micaceous were reported to be edible by Purkayastha and Chandra (1976).

SUMMARY

SUMMARY

A survey conducted in and around College of Agriculture, Vellayani Campus indicated that Coprinus lagopus (Fr.) Fr., Coprinus disseminatus (Pers.ex Fr.) S.F.Gray and Coprinus ephemerus (Bull.ex Fr.) Fr. are the commonly occurring species of Coprinus and among these C.lagopus was the most common one. The macro and microscopic characters of the mushrooms were studied in detail.

The isolate of the fungus C.lagopus was obtained from paddy straw beds of Volvariella volvacea raised at the Department of Plant Pathology, College of Agriculture, Vellayani. The fungus was purified by hyphal tip method and maintained in potato dextrose agar slants by subculturing periodically. The trials in germination of basidiospores showed that small germ tubes appeared on spores after one to two hours of incubation. The per cent of germination noted at different temperature after one hour of incubation revealed that the optimum temperature range for basidiospore germination is 25 to 35°C.

Studies conducted to observe the different stages of development of mushroom from spawning till

maturity revealed that the different stages of development can be divided into five stages viz., pin head, tiny button, button, elongation and mature stage. Mushrooms of pin head stages of size 4 mm began to appear on bed after 72 hours of spawning and they attained tiny button stage after 144 hours in which there is differentiation of stipe and pileus. After 158 hours, they attained elongation stage and after 160 hours, the mature stage, in which the pileus was expanded and splitted radially. Again after one or two hours, autodigestion takesplace from the periphery of the gills towards the centre and the gills liquify to a black inky fluid. The expansion and autodigestion of the pileus were observed during late night. The fully expanded pileus was immediately cut and kept on a white sheet of paper and spore print was taken. Five to ten minutes was necessary to get a clear spore print. In nature, the fully expanded stage of C.lagopus is rarely seen because of its quick autodigestion.

In vitro studies on the nutritional aspects of the fungus indicated that maximum mycelial growth and dry weights were obtained on potato dextrose medium and

oat meal medium. Five to eight sporocarps were observed in the media four to five days after inoculation. Studies on the effect of different temperature on fungus growth revealed that a temperature of 30 to 35°C is optimum for the growth of C.lagopus and no growth was observed at temperatures below 25°C and above 45°C. Studies on the influence of initial pH of media on the growth of C.lagopus showed that the organism can grow on a wide range of pH from acidic to alkaline. The growth was maximum at pH 5 and as the pH was increased above 6, there was a gradual decrease in dry weight.

Studies on the effect of light on the mycelial growth of C.lagopus indicated that best growth and sporocarp production were observed at ordinary light condition, while the petri plates kept in complete darkness supported poor mycelial growth and no sporocarp production. In vitro studies on the best source of carbon and nitrogen for fungus growth indicated that maltose was the best source of carbon and sodium nitrate was the best source of nitrogen.

The techniques for artificial cultivation of C.lagopus were standardised. Studies on the suitability of different spawn substrates on the mycelial growth and

sporocarp production revealed that green gram, red gram, horse gram, bengal gram and wheat grains favoured good mycelial growth and sporocarp production. A temperature of 30 to 35°C was found to be the best for the mycelial growth in spawn bottles. The yield of sporocarp in mushroom beds was increased by the addition of organic amendments and the maximum yield was obtained when green gram was used, followed by red gram, bengal gram, wheat flour and horse gram. Studies on the influence of different types of straw bed for the maximum production of sporocarps of C.lagopus revealed that significant increase in yield was obtained from rectangular beds with twists of paddy straw. Maximum yield of sporocarps of C.lagopus was obtained from paddy straw beds and also from beds with a mixture of chopped straw and paper at the ratio of 3:1 amended with calcium nitrate.

Studies on the preservation of the sporocarps indicated that these mushrooms can be preserved in refrigerated condition upto three days. These can also be preserved by drying in a dehydrator at 70°C and also by powdering after drying. Preservation in various concentrations of brine indicated that the microbial contamination is low at five, six and seven per cent

concentrations, while it was more at lower concentrations.

Studies on the nutritive value of C.lagopus revealed that the major constituent is moisture (91.25 per cent) followed by 5.095 per cent carbohydrates, 1.529 per cent protein, 0.625 per cent crude fibre and 0.576 per cent fat. The qualitative analysis of amino acid composition of sporocarps indicated the presence of 14 amino acids, out of which ten were identified viz., leucine, proline, ornithine, serine, valine, tryptophan, glycine, arginine, tyrosine and phenylalanine. Comparative studies on the mycelial biomass production by submerged culture, revealed that C.lagopus was very much suitable for the production of mycelial pellets than V.volvacea and P.sapidus. Sporocarp primordia were observed in flasks kept stationary after five days of shaking in the case of C.lagopus and P.sapidus while no primordia were observed in the case of V.volvacea. Edibility trials of C.lagopus on Red Eyed White Rabbits indicated that it can be considered as edible since no visible disorders were observed on rabbits fed with dried and powdered sporocarps of C.lagopus.

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* Originals not seen

APPENDICES

Appendix-I

DATA SHEET USED FOR THE MORPHOLOGICAL DESCRIPTIONS OF MUSHROOM

Name:

Locality:
Soil type:
Habitat:
Vegetation:
Substratum:

Habit:

Collected by:

Name:

Date:

MORPHOLOGY

I. PILEUS

Shape:
Diameter:
Texture:
Colour:
Surface:
Margin:

II. VEIL

III. VOLVA

IV. ANNULUS

V. GILLS

Colour:
Texture:
Number per cm:

Cystidia:
Type:
Shape (Fig.1-b)

VI. STIPE

Shape:
Colour:
Size:
Surface:
Base:

VII. SMELL

VIII. SPORE

Shape(Fig.1.a):
Colour:
Size:

IX. SPORE PRINT

Colour

X. OTHER CHARACTERS

Appendix-II

COMPOSITION OF THE REAGENTS, CHEMICALS AND MEDIA USED FOR THE STUDY

Reagents and Chemicals

1. Melzer's reagents (Melzer, 1934)

Potassium iodide	-	1.5	g
Iodine	-	0.5	g
Water	-	20	ml
Chloral hydrate	-	22	g

2. Potassium hydroxide - 3 per cent

3. Hydrochloric acid - 11 M

4. Concentrated sulphuric acid

Media

1. Potato dextrose agar

Peeled potato	-	250	g
Glucose	-	20	g
Agar	-	15	g
Distilled water	-	1000	ml
pH	-	6 to 6.5	

2. Czapek's agar

Sucrose	-	30	g
Sodium nitrate	-	2	g
Dipotassium phosphate	-	1	g
Magnesium sulphate	-	0.5	g
Potassium chloride	-	0.5	g

Ferrous sulphate	-	0.01 g
Agar	-	15 g
Distilled water	-	1000 ml

3. Richard's medium

Potassium nitrate	-	10 g
Potassium dihydrogen phosphate	-	5 g
Magnesium sulphate	-	2.5 g
Ferric chloride	-	0.02 g
Sucrose	-	50 g
Agar	-	15 g
Distilled water	-	1000 ml
pH	-	6.6 to 7.2

4. Oat meal agar

Oats	-	100 g
Agar	-	15 g
Distilled water	-	1000 ml
pH	-	6 to 6.5

5. Nutrient agar

Peptone	-	10 g
Beef extract	-	5 g
Distilled water	-	1000 ml
Agar	-	20 g

6. Martin's Rose Bengal Streptomycin Agar

Dextrose	-	10 g
Peptone	-	5 g
Potassium dihydrogen phosphate	-	1 g
Magnesium sulphate	-	0.5 g
Rose Bengal	-	(1 part in 30,000 parts of the medium)
Agar	-	20 g
Streptomycin	-	30 g
Distilled water	-	1000 ml

7. Kuster's Agar

Glycerol	-	10 g
Casein	-	3.3 g
Sodium chloride	-	2 g
Dipotassium hydrogen Phosphate	-	2 g
Magnesium sulphate	-	0.05 g
Calcium carbonate	-	0.02 g
Iron sulphate	-	0.01 g
Agar	-	15 g
Distilled water	-	1000 ml

Appendix-III

EFFECT OF DIFFERENT LIQUID MEDIA ON THE GROWTH OF C.LAGOPUS

(Analysis of variance Table)

Source	S.S.	df	Mean sum of squares	F calculated
Total	8293150	15		
Treatment	8203400	3	2734466.6	365.61*
Error	89750	12	7479.166	

* Significant at 0.05 level

CD = 133.25

Mean of treatments and ranking

T_1	T_2	$\overline{T_3}$	T_4
1800	1235	125	160

Appendix-V

EFFECT OF DIFFERENT SOURCES OF NITROGEN ON THE GROWTH OF C.LAGOPUS

(Analysis of variance Table)

Source	S.S.	df	M.S.S.	F calculated
Total	31108.95	19		
Treatment	29740.20	4	7435.05	81.48*
Error	1368.75	15	91.25	

* Significant at 0.05 level

CD = 14.38

Mean of treatments and ranking

T_1	T_2	T_3	T_4	T_5
160	131.5	86.5	75	54.75

Appendix-VI

EFFECT OF DIFFERENT SPAWN SUBSTRATES ON THE YIELD OF FRESH SPOROCARPS OF C.LAGOPUS

(Analysis of variance Table)

Source	S.S.	df	M.S.S.	F calculated
Total	294908.2	17		
Treatment	291276.2	5	58255.24	192.48*
Error	3632	12	302.66	

* Significant at 0.05 level

CD = 30.95

Mean of treatments and ranking

T_4	T_3	T_1	T_5	T_2	T_6
704	673	670.67	531.66	510.33	339

Appendix-III

EFFECT OF DIFFERENT LIQUID MEDIA ON THE GROWTH OF C.LAGOPUS

(Analysis of variance Table)

Source	S.S.	df	Mean sum of squares	F calculated
Total	8293150	15		
Treatment	8203400	3	2734466.6	365.61*
Error	89750	12	7479.166	

* Significant at 0.05 level

CD = 133.25

Mean of treatments and ranking

T_1	T_2	T_3	T_4
1800	1235	125	160

Appendix-IV

EFFECT OF DIFFERENT SOURCES OF CARBON ON THE GROWTH OF C.LAGOPUS

(Analysis of variance Table)

Source	S.S.	df	M.S.S.	F.calculated
Total	125686.95	19		
Treatment	123853.20	4	30963.3	253.27*
Error	1833.75	15	122.25	

*Significant at 0.05 level

CD = 16.68

Mean of treatments and ranking

T_3	T_4	T_1	T_2	T_5
268.25	186.5	160	140	25

Appendix-VII

EFFECT OF DIFFERENT ORGANIC AMENDMENTS ON THE YIELD OF SPOROCARPS OF *C. LAGOPUS*

(Analysis of variance Table)

Source	S.S.	df	M.S.S.	F calculated
Total	508070	20		
Treatment	498015.3	6	83002.55	115.57*
Error	10054.7	14	718.19	

* Significant at 0.05 level

CD = 46.93

Mean of treatments and ranking

T_2	T_3	T_5	T_1	T_4	T_6	T_7
663	651.33	606.67	483.33	450.66	285	260

GLOSSARY

Adnate	- gills attached to the stipe with their entire width
Agaric	- any gill fungi
Amyloid	- colour reaction with Melzer's reagent - black or slightly greyish if amyloid, brown to purplish brown when pseudo-amyloid, yellowish if inamyloid (negative)
Annulus	- a ring like partial veil, or part of it round the stipe after expansion of pileus
Basidium	- the spore mother cell of basidiomycetes, bearing spores on short sterigmata
Buttons	- young unexpanded cap
Campanulate	- bell shaped
Cheilocystidium	- cystidium in the edge of a gill
Clavate	- club like
Context	- the hyphal mass between the superior surfece and subhymenium or trama or basidiocarps
Cystidia	- sterile, unicellular light coloured large cells in the hymenium of basidiomycetes
Disc	- central portion of the upper surface of a mushroom

Echinulate	- covered with minute spines
Fuscous	- smoky or dull brown
Hymenium	- a fertile layer that bears either basidia and basidiospores or asci and ascospores mixed with paraphyses etc.
Hymenophore	- the portion of the carpophore which bears the hymenium
Lamellate	- having gills
Obovoid	- inversely ovate
Paraphyses	- sterile structures look like threads or filaments occurring in hymenium
Pileus	- that portion of carpophore which resembles umbrella like cap
Pleurocystidium	- cystidium on the sides of the gill
Plicate	- folded into pleats
Pseudorhiza	- a root like extension of the stipe
Sinuate	- gills notched at the stipe
Spherocyst	- a spherical cell present in the trama
Sporophore	- a fruit body of a mushroom
Stipe	- stem or stalk of fungal fruit bodies
Striate	- marked with tiny streaks
Subhymenium	- the layer of interwoven hyphae between the hymenium and trama giving rise to basidia

Trama	- the tissue lying between two hymenial layer, usually consisting of densely packed or loosely interwoven hyphae.
Veil	- usually membraneous structure or sometimes spider web like. It envelops the part or the entire carpophore
Volva	- the cap like basal remains of the universal veil after expansion of the fruit body
Wart	- scales in the surface of the pileus

INVESTIGATION ON THE EDIBLE SPECIES OF *Coprinus* AND STANDARDISATION OF TECHNIQUES FOR ITS LARGE SCALE ARTIFICIAL CULTIVATION

BY

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ABSTRACT

A survey conducted in and around the College of Agriculture, Vellayani Campus revealed that the commonly occurring species of Coprinus were Coprinus lagopus (Fr.) Fr., C.disseminatus (Pers. ex Fr.) S.F. Gray and C.ephemerus (Bull.ex Fr.) Fr. and among these, the most commonly occurring species was C.lagopus. The culture of C.lagopus used for the present study was isolated from paddy straw beds of Volvariella volvacea raised at the Department of Plant Pathology and maintained on potato dextrose agar slants by subculturing periodically.

The different stages of development of mushroom from spawning till maturity can be divided into 5 stages viz., pinhead, tiny button, button, elongation and mature stage. For the full development and auto-digestion of the sporophore of C.lagopus it took 161-162 hours from the time of spawning. In nature the fully expanded stage of C.lagopus is rarely seen because of its quick autodigestion.

Potato dextrose and oatmeal medium were the best media for the growth of C.lagopus and a temperature of 30 to 35°C was suitable. The fungus could grow on a wide range of pH from acidic to alkaline and the growth

was maximum at pH 5. Light was essential for the mycelial growth and sporocarp production. Maltose and sodium nitrate were the best sources of carbon and nitrogen respectively for the mycelial growth.

Green gram, red gram, horse gram, bengal gram and wheat grains favoured good mycelial growth and sporocarp production in spawn bottles and a temperature of 30 to 35°C was found to be best for the mycelial growth in spawn bottles. Green gram powder was the best organic amendment for sporocarp production.

Rectangular beds with paddy straw twists was the best type of straw bed for the cultivation of C.lagopus. Paddy straw twists and a mixture of chopped straw and paper at 3:1 ratio and 200 g calcium nitrate were found to be the best substrates for its cultivation.

These mushrooms could be preserved in refrigerated condition upto 3 days. They could also be preserved in brine and by drying and powdering. The major constituent of C.lagopus is moisture (91.25 per cent) followed by 5.095 per cent carbohydrate, 1.529 per cent protein, 0.625 per cent crude fibre and 0.576 per cent fat. It contained 14 aminoacids

out of which ten were identified viz., leucine, proline, ornithine, serine, valine, tryptophan, glycine, arginine, tyrosine and phenylalenine. C.lagopus was good in the production of mycelial pellets by submerged culture. Edibility trials of C.lagopus on Red Eyed White Rabbits indicated that it can be considered as edible since no external disorders were observed on rabbits fed with dried and powdered sporocarps of C.lagopus.