STUDIES ON MILKY MUSHROOM (*Calocybe indica* P&C) PRODUCTION IN CHHATTISGARH

M.Sc. (Ag.) Thesis

By

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COLLEGE OF AGRICULTURE
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M.Sc. (Ag.) Thesis

Submitted to the

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By

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CERTIFICATE – I

This is to certify that the thesis entitled **"Studies on milky mushroom (*Calocybe indica* P&C) production in Chhattisgarh"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafide research work carried out by **Anurag Kerketta** under my guidance and supervision. The subject of the thesis has been approved by Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma (Certificate awarded etc.) or has been published/ published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

Date: 21-07-2016

H. K. Singh 21/07/2016
(Major Advisor and Chairman)

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Member: Dr. D. Khokhar — 21.07.16

Member: Dr. (Smt.) G. Chandrakar — 21/7/2016

Member: Dr. A.S. Kotasthane — 21/7/2016

CERTIFICATE – II

This is to certify that the thesis entitled **"Studies on milky mushroom (*Calocybe indica* P&C) production in Chhattisgarh"** submitted by **Anurag Kerketta** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur, (C.G.) in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** in the **Department of Plant Pathology** has been approved by the External Examiner and Student's Advisory Committee after oral examination.

Date: 27.8.2016

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Approved/Not approved _____

Director of Instructions _____

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Abbreviation	Description
%	Per cent
@	At the rate of
S. No.	Serial Number
p.s.i.	Per square inch
lbs	Pound
BE	Biological Efficiency
°C	Degree Celsius
cm	Centimeter
g	Gram
CD	Critical Difference
SEm	Standard error of mean
CG	Chhattisgarh
et al.	And others
Fig.	Figure
i.e.	That is
viz.,	namely
Kg	Kilo gram
No.	Number
mm	Millimeter

LIST OF ABBREVIATIONS

Abbreviation	Description
IGKV	Indira Gandhi Krishi Vishwavidyalaya
TNAU	Tamil Nadu Agricultural University
ACRP	All India Co-ordinated Research Project
CRD	Completely Randomized Design
AICMIP	All India Co-ordinated Mushroom Improvement Project
DMR	Directorate of Mushroom Research
CG	Chhattisgarh
CAGR	Cumulative Annual Growth Rate
GPS	Global Positioning System

THESIS ABSTRACT

a) Title of Thesis	: "Studies on Milky Mushroom (*Calocybe indica* P&C.) Production in Chhattisgarh."
b) Full Name of the Student	: Anurag Kerketta
c) Major Subject	: Plant Pathology
d) Name and Address of the Major Advisor	: Shri H.K. Singh (Scientist) Department of Plant Pathology, College of Agriculture, I. G. A. U. Raipur (C. G.).
e) Degree to be awarded	: Master of Science in Plant Pathology

Signature of the Student

Signature of Major Advisor 21/07/2016

Date: 21-07-2016

Signature Head of Department

ABSTRACT

Present study entitled "**Studies on Milky Mushroom (*Calocybe indica* P&C.) Production in Chhattisgarh.**" was carried out in the Mushroom Research Laboratory, Department of Plant Pathology, College of Agriculture, IGKV, Raipur.

Cultural characteristics and radial growth of different strain of *Calocybe indica* was studied on different media and temperature to know the suitable medium and temperature for its growth and development. Among the tested media and temperatures, the maximum radial growth observed in strain CI-522 on potato dextrose agar medium and both strain CI-522 and strain CI-524 at temperature 30°C was found to be superior for radial growth with cottony white mycelial growth and entire margin. Spawn development of different strain of *Calocybe*

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indica on different cereal grain was also studied. Among cereal grains, sorghum grains took significantly less time for spawn development with strain CI-524 followed by strain CI-4. The minimum time required for spawn run and primordial initiation in both strain CI-522 and strain CI-524 with wheat grains raised spawn and different grain raised spawn also in wheat straw. Higher yield and biological efficiency (%) was recorded in strain CI-524 in sorghum grains raised spawn followed by wheat grains raised spawn. In strain comparison for yield and yield attribute, strain CI-524 was recorded as superior strain followed by strain CI-4, whereas strain CI-1was found as lowest performer. The combination of compost + vermicompost found to be best as casing materials and in combination of wheat + Lathyrus found to be suitable straw substrates for cultivation of *C. indica.*

शोध ग्रन्थ सारांश

शोध ग्रन्थ का शीर्षक	–	"छत्तीसगढ़ में सफेद दुधिया मशरूम (कैलोसाइब इंडिका पी और सी.) के उत्पादन का अध्ययन".
छात्र का पूर्ण नाम	–	अनुराग केरकेट्टा
मुख्य विषय	–	पादप रोग विज्ञान
मुख्य सलाहकार का नाम और पता	–	श्री एच. के. सिंह (वैज्ञानिक) पादप रोग विज्ञान विभाग, कृषि महाविद्यालय रायपुर
प्रदान की जाने वाली उपाधि	–	एम.एस.सी. (कृषि) पादप रोग विज्ञान

Harsingh 21/07/2016
मुख्य सलाहकार के हस्ताक्षर

Anurag
छात्र के हस्ताक्षर

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विभागाध्यक्ष के हस्ताक्षर

सारांश

वर्तमान अध्ययन जिसका शीर्षक "छत्तीसगढ़ में सफेद दुधिया मशरूम (कैलोसाइब इंडिका पी और सी) के उत्पादन का अध्ययन" है, मशरूम अनुसंधान प्रयोगशाला, पौध रोग विज्ञान विभाग, कृषि महाविद्यालय रायपुर, इं.गां.कृ.वि.वि. में सम्पन्न हुआ।

कैलोसाइब इंडिका के विभिन्न प्रभेद के वृध्दि एवं विकास के लिए उपयुक्त माध्यम और तापमान को जानने के लिए उसका (विभिन्न प्रभेदो का) विभिन्न माध्यम और तापमान पर अध्ययन किया गया । परीक्षण किये गये माध्यमो एवं तापमानो में प्रभेद सी आई.– 522 का कॉटनी सफेद कवकजाल एवं नियमित किनारा के साथ अधिकतम अर्ध्दव्यास (दीप्तिमान) वृध्दि पोटैटो डेक्स्ट्रोज अगार माध्यम पर देखा गया। एवं दोनो प्रभेद सी.आई.–522 और सी.आई.–524 के अधिकतम अर्ध्दव्यास/दीप्तिमान वृध्दि के लिए 30 डिग्री सेल्सियस तापमान उपयुक्त पाया गया। कैलोसाइब इंडिका के विभिन्न प्रभेद का विभिन्न धान्य दानो पर बीज विकास का अध्ययन भी किया गया। सभी धान्य दानो में से बाजरा के दानों में प्रभेद सी.आई..–524 का बीज विकास के लिए सार्थकपूर्ण सबसे कम समय लगा। उसके बाद प्रभेद सी.आई.–4 में देखा गया। गेहूँ के भूसे में, गेहूँ दानों में तैयार किये गये दोनो प्रभेद सी.आई.–522 और सी.आई.–524 के बीज (स्पान) में कवकजाल विकास/बीज विकास एवं पिनहेड विकास के लिए सबसे कम समय लगा और विभिन्न दानो में तैयार किये गये बीज में भी। सबसे ज्यादा उपज और जैव क्षमता/बायोलोजिकल दक्षता (%) प्रभेद सी. आई..–524 में बाजरा दानो में तैयार किये गये बीज में पाया गया उसके बाद गेहूँ दानो में तैयार किये बीज में पाया गया। कैलोसाइब इंडिका की ,खेती के लिये कम्पोस्ट + वर्मी कम्पोस्ट का मिश्रण केसिंग पदार्थ के रूप में सबसे अच्छा पाया गया, और गेहूँ + तिवड़ा (भूसा) का मिश्रण उपयुक्त पाया गया।

CHAPTER-I
INTRODUCTION

Fungi are cultivated worldwide for the production of edible mushrooms. It belongs to either division Basidiomycotina or Ascomycotina and may be epigeal or hypogeal. The vegetative parts of mushroom mainly consist of thread like long thin mycelium and form fruiting bodies under suitable conditions. The total number of edible and medicinal fungi is over 2,300 species. Cultivated mushrooms have become popular with over 200 genera of useful macro fungi in the world. The most common ones that are produced and consumed are button mushrooms (*Agaricus bisporus*), shiitake mushrooms (*Lentinula edodes*), and oyster mushrooms (*Pleurotus* spp.) and accounted for nearly 76% of the global mushroom market size in 2013. Today, commercially grown species are button and oyster mushrooms, followed by paddy straw, milky mushroom, etc. In India, most of the cultivated mushrooms are white button *(A. bisporus),* oyster *(Pleurotus* spp.), paddy straw *(Volvariella* spp.) and white milky *(Calocybe indica).*

Mushrooms are liked for their delicious flavour, low calorific value and high protein contents, vitamins of B group and minerals. It contains 20–40% proteins on a dry weight basis and no cholesterol, and is almost fat free (Walde *et al*., 2006). Mushrooms are a good source of non-starchy carbohydrates, dietary fiber, protein, mineral and vitamins (Kulshreshtha *et al*., 2009). The carbohydrates in the mushrooms are at a level of 4.5 to 5.0 percent but are in the form of glycogen, chitin and hemicellulose instead of starch. The fat contain is as low as 0.3 % but is rich in linoleic acid, an essential fatty acid. In view of increasing demand of high quality food with an increasing world population, mushrooms will be an important source of proteins that can replace meat for a major part.

Mushrooms are spongy, fleshy, porous, and fruity parts of a fungus, and are mostly grouped as vegetables. Due to increased recognition of its medicinal and nutritional values, coupled with the realization of the income generating potential of fungi through trade, the demand for mushrooms has been on the rise. The edibility of mushrooms depends on the absence of poisonous content and its

desirable taste and aroma. Being a rich source of nutrition and being fat, cholesterol, and gluten free, and very low in sodium content, mushrooms are gaining popularity among health conscious consumers. The global market for mushrooms was valued at $29,427.92 million in 2013. This market is projected to grow at a cumulative annual growth rate (CAGR) of 9.5% from 2014 to reach $50,034.12 million by 2019. Europe dominated the market in 2013, and is projected to be the fastest growing market for mushrooms between 2014 and 2019, followed by the Asia Pacific region (Market report/Mushroom report). Mushroom production in India started in the 70s but with the development of the technologies for environmental controls and understanding of the cropping systems, mushroom production shot up from mere 5000 tonnes in 1990 to about 1,20,000 tonnes at present and out of which milky mushroom contributes 900 tonnes. India contributes only 1% in world mushroom production (Manjit Singh and Shwet Kamal, 2009).

Among the cultivated mushroom, the milky mushroom *(Calocybe indica* P&C*)* is a potentially new species to the world mushroom growers. It is a robust, fleshy, milky white, umbrella like mushroom, which resembles button mushroom. The species is suitable for hot humid climate and can be cultivated indoor in high temperature and high humidity areas. It grows well at a temperature range of 25-35°C and relative humidity more than 80%. Milky mushroom can be cultivated throughout the year in the entire plains of India. The cultivation technology is very simple, involves less cost and no special compost is needed for the cultivation. The cultivation process resembles that of oyster mushroom but for the additional process of casing. The mushroom can be harvested from 24-28 days after spawning and the total crop cycle is only 45-50 days. Most importantly, the milky mushroom has an extended shelf life of 3-5 days compared to other cultivated species, making it more amenable to handling, transportation and storage. So, there is a growing interest among the farmers towards milky mushroom.

Calocybe indica is a tropical edible mushroom of Indian origin and can be cultivated indoor in high temperature and humidity areas (Purkayastha & Chandra, 1974). *C. indica* commonly known as the milky mushroom was commercialized as a new variety *C. indica*, var. APK2 from the Tamil Nadu Agricultural University

(TNAU), Coimbatore, India and can be cultivated throughout the summer season (Krishnamoorthy *et al.*, 1998). DMR Milky-334 is another variety of *C. indica* that has been released by Directorate of Mushroom Research Solan in 2013 for its commercial cultivation (DMR Annual Report 2013). Another similar or related species is *Macrocybe* and a variety named DMR Macrocybe-01 has also been released for commercial cultivation. Milky mushroom is well appreciated due to its large-sized milky white sporophores, simple production technology and low capital investment. Commercial cultivation of this species is still in its infancy in India. It is suitable for hot humid climate and can be cultivated almost throughout the year in India except few places (Pani, 2012). It contains 32.3% protein on dry weight basis and possesses 41% crude fibers, different types of vitamins, minerals. Due to their high content of vitamin, protein and minerals, mushrooms are considered as "Poor man's Proteins". Mushrooms can be used for the food to solve the malnutrition problem. Mushrooms have good nutritional value particularly as a source of protein that can enrich human diets, especially in some developing countries where animal protein may not be available and are expensive (Pandey, 2004).

C. indica can be cultivated in all types of lignocellulosic agricultural residues such as paddy, wheat, barley, maize, groundnut haulms, grasses, cotton and leaf fall of trees and sugarcane leaves banana stem and different grass. Presently all the commercial growers are utilizing paddy straw and wheat straw for *C. indica* cultivation. India is generating about 700 million MT of agricultural waste besides fruit and vegetable residue, coir dust, husk, dried leaves, pruning, coffee husk, tea waste, etc. A large amount of the agricultural wastes are burnt or left in the field for composting and incorporation in the soil for fertility. In this process a large amount of potent source of organic carbon and nutrients are lost, which otherwise could be recycled back to the field.

The first report of wild occurrence of *Calocybe indica* Purkayastha & Chandra, commonly called "Dhuth chatta" (means "milky white mushroom" originated from India). *Calocybe indica* an edible mushroom, native to India was first described by Purkayastha and Chandra (1947) (Purkayastha, 1984-85; Pandey and Tewari, 1993). Even though attempts were made to grow *C. indica*

(Purkayastha and Nayak, 1981), Pandey and Tewari, (1993) only limited success was achieved in increasing the bio-efficiency and productivity of this mushroom. For several decades, people from West Bengal (Eastern Indian State) have collected these mushrooms and sold in local markets. In nature, milky white mushrooms are seen grown on humus rich soil in agricultural fields or along the roadside in tropical and subtropical parts of India, especially in the plains of Tamil Nadu (South Indian State) and in Rajasthan (located in the western edge of India). These mushrooms grow every year between the months of May and August, which normally coincides with sufficient showers after a prolonged dry spell. *C. indica* is mainly a grassland species, saprophytic (organisms which obtain nutrients from dead organic matter) in nature and sometimes ectomycorrhizal (symbiotic relationship with root of some plants) with *Cocos nucifera*, *Borassus flabellifer*, *Tamarindus indicus*, and *Peltophorum ferruginum*. Detailed studies on preferential physiological and cultural requirements for growing *C. indica* were reported. Since this mushrooms is morphologically similar to *Agaricus bisporus* (button mushroom), it has been quite popular in southern Indian states and slowly getting popular in other countries (China, Malaysia, and Singapore). Methods have been developed to cultivate this mushroom on commercial scale since late nineties. Chhattisgarh has been bestowed with huge diversity of *Calocybe indica* and Mushroom Research Laboratory, IGKV Raipur has a huge collection of C. indica from different areas of CG (AICMIP Annual Report 2015-16). However a systematic study of this species is still lacking in context to this particular state or Central India *per se.*

Keeping the above fact in mind and the variations in growth and yield of *C. indica* strains collected from different growing areas, the current study entitled "**Studies on milky mushroom (*Calocybe indica* P&C) production in Chhattisgarh**" was undertaken to assess the effect of various parameters related to milky mushroom *(C. indica)* collected from CG. The objectives of the study were as follows:

1. To study the cultural characteristics of milky mushroom (*Calocybe indica*).

2. To study the development of spawn of milky mushroom (*Calocybe indica*) on different grain substrates.

3. To evaluate different strains of Milky mushroom (*Calocybe indica*) for yield and yield attributing characters.

CHAPTER-II
REVIEW OF LITERATURE

This chapter deals with the brief review of the research work done earlier related to production technology of *C. indica* and various parameters affecting its mycelial growth and yield. The literature pertaining to proposed studies thoroughly reviewed. The work done on other species of mushrooms was also reviewed wherever necessary.

2.1 To study the cultural characteristics of *C. indica* on different media and at different temperature

2.1.1 Effect of different media on radial growth of *C. indica*

Singh *et al.,* (2009) reported that *Calocybe indica* grew well on all the tested media but maximum linear growth (7 cm) was recorded on wheat extract agar medium followed by PDA and paddy straw decoction agar medium.

Kumar *et al.,* (2011) studied on five strains (*i.e*., CI-3, CI-4, CI-5, CI-6, and CI-10.) of milky mushroom. They investigated that the maximum radial growth was found in malt extract agar medium followed by potato dextrose agar medium and minimum in sugarcane bagasses extract agar medium on 8 days of observation. The mycelia growth of each strain varied significantly in all the media tested. In malt extract agar media, maximum radial growth of mycelium (9 cm) was observed in CI-6. The other strains showed significant variation with each other ranging from 3.2 to 8.9 cm. they also studied on temperature requirement of *Calocybe indica* and reported that among the tested temperatures, all strains showed maximum mycelial growth at 28°C followed by 32°C and minimum at 20°C on 8 d of incubation. The mycelia growth of each strain varied significantly at all the temperature tested. At 28°C temp. Strain CI-6 showed maximum radial growth (9 cm). However other strains showed variation to each other giving diametric growth from 8-9 cm. at 32°C, max avg. mycelial growth 8.4 cm was recorded in strain CI-4 which was superior to other strains. The least growth in strains was recorded at 20°C in CI-3 (3.1 cm) and CI-10 (3.3 cm).

Krishnamoorthy *et al.,* (2015) reported that the majority of the time required for maximum mycelia growth in culture media like potato dextrose agar

or malt extract agar was 8 to 10 days. The pH requirement was reported to have a wide optimal range, between 5.5 and 8.5. The optimum temperature for mycelia growth and mushroom production was reported to be around 30-35°C. At temperatures below 25°C or above 38°C the growth of *C. indica* was not supported. As far as the mycelia production in liquid broth was concerned, potato dextrose broth yielded the maximum of 0.22 g in 100 ml of broth, while malt extract broth produced 0.19 g per 100 ml in 8 days at pH 6 when incubated at 30°C.

2.1.2 Effect of temperature on radial growth of *C. indica*

Varshney (2007) reported temperature requirement from 25-35°C for mycelial growth of *Calocybe indica.* All strains of *Calocybe indica* showed maximum mycelial growth at 28°C followed by 32°C and minimum at 20°C. At 28°C temperature on 8th day's strain CI-6 was at par showing maximum diametric growth of mycelium (9.0 cm) in observations.

Shukla *et al.,* (2013) studied on effect of temperature on mycelia growth of the strains of milky mushroom (*Calocybe indica)* viz. CI- 4, CI- 6, CI- 7, C1- 8, Cl- 9 & Cl- 10. They reported that the most suitable temperature for fast and full mycelial impregnation and growth was 30°C.

Shukla *et al.,* (2014) reported mycelial growth of six strains (CI-5, CI-6, CI-7, CI-8, CI-9, CI-10) of *Calocybe indica.* All strains showed maximum mycelial growth at 35°C at 4^{th}, 6^{th}, and 8^{th} day. However, on 2nd day some of the strains like CI-5 and CI-6 showed maximum mycelial growth at 25°C while that of other strains were at 30°C.

Singh *et al.,* (2015) studied on five strains (i.e. APK-2, CI-6, CI-8, CI-9, CI-10) of *Calocybe indica* and incubated at five different temperature *viz.*, 21, 24, 27, 30, and 33°C. The results revealed that all strains showed maximum mycelial growth at 30°C followed by 27°C and minimum at 21°C on 3rd, 5th, 7th and 9th day's observations. However, the mycelial growth of each strain varied significantly at all the temperature tested. At 30°C temperature on 9th day's strain APK-2 showed maximum radial growth (full growth) of mycelium (9.0 cm).

2.2 Evaluation of different grain substrates in development of spawn and yeild

Prasuna (2002) studied different grain substrates for spawn production of *C. indica* and reported early spawn development on wheat grain followed by jowar grains.

Senthilnambi *et al.,* (2011) conducted an experiment to find the suitability of different grains as spawn substrates and their effect on yield parameters of *C. indica.* The results revealed the supremacy of sorghum grains as the most suitable substrate for early spawn run, which took only 13.7 days for hundred percent mycelial growths. The yield and number of buttons harvested were found maximum in the sorghum grain spawn followed by ragi grain spawn. The maize grain substrates took 19 days for complete spawn run and recorded low yield when compared to other spawn substrates. The days for pin head formation and first harvest of the crop were earlier in case of sorghum grain spawn followed by ragi spawn.

Krishnamoorthy A. S. and Venkatesh Balan (2015) stated that sorghum or wheat grains were found to be the best substrates for spawn production of *C. indica.*

Sofi *et al.,* (2014) investigated on effect of different grains and alternative substrates on Oyster mushroom (*Pleurotus ostreatus)* production. They used various cereals *viz.*, wheat grains (*Triticum aestivum,* Barley (*Hordium vulgare),* Maize (*Zea mays),* Millets *(Setaria italika)* as grain substrates for planting spawn. The maximum and minimum growth rates were seen in corn and millet substrate, respectively.

2.3 Strain evaluation and effect of environment on yield of *C.indica*

Kaur *et al.,* (2011) studied on nine strains of *Calocybe indica* (Ci-1, Ci-2, Ci-3, Ci-4, Ci-5, Ci-6, Ci-7, Ci-9 and APK-2). Ci-1 to Ci-7, Ci-9 and APK-2 were grown on wheat straw following the standard adapted cultivation technology. Observations on days of spawn run, number of fruit bodies, yield and average weight of fruit body were recorded along with stipe length and pileus diameter of the random sample of each strain. Spawn run period ranged from 31 to 40 days. The number of fruit bodies harvested was maximum for Ci-7 (1890) followed by Ci-4 and lowest in Ci-9 (890). The biological efficiency, estimated from the

harvested yield (kg q-1 dry straw), was maximum in strain Ci-3 (81.28%). Five strains Ci-4, Ci-5, Ci-6, Ci-7 and APK-II gave yields at par with each other whereas Ci-1, Ci-2 and Ci-9 showed low biological efficiency (47.82-51.28%). Average weight of fruit body ranged from 38 to 53 g with maximum in Ci-3, Ci-6 and Ci-9 strains and least in Ci-7 strain. The pileus diameter of strains ranged between 6.2 and 9.4 cm and stipe length between 10 and 14.2 cm. considering the yield potential of *C. indica* strains, only one strain Ci-3 had maximum biological efficiency. Similar results for *C. indica* strain Ci-3 under natural climatic condition of Punjab have been reported by Mangat (2005) and Kaur (2010).

R. Elaiya Raja and P. Ganesh (2012) studied and found the most favourable and unfavourable month for the cultivation of *Calocybe indica* and the influence of temperature and relative humidity on mushroom yield. Significant variation in the yield and other parameters existed when *Calocybe indica* was grown during different periods of study the year of 2011 to 2012. In summer months (March to July), a general increase in mushroom yield was observed but variation of temperature during other months did not effect the yield . The maximum yield was recorded in the month of May and June 2012 . However, the yields obtained in the months of May to November 2012 were found to be on par with that of the above observation. Mushroom attained harvesting maturity almost one day earlier during monsoon months. In addition, during this period, the average weight of individual mushroom was found to be comparatively high was noticed during summer monsoon.

Kumar *et al.,* (2015) found that the minimum time was observed during June-August 2008-09 for spawn run (19 and 18 days), while for pin head formation (12 and 11 days) and first harvesting (20.0 and 19.7 days) were noticed during July-September for strain CI-4 and CI-6, respectively. Maximum numbers of pinhead initiation were recorded during April-June. However maximum number of fruiting bodies were harvested during September-November in CI-4 and CI-6 strains. However, maximum yield per kg substrate and average weight per fruit body were recorded during July-September from strain CI-4 and CI-6, respectively.

2.4 Effect of age and quantity of spawn on milky mushroom production

Pani (2011) investigated the effect of age of spawn and it's quantity on sporophore production of *Calocybe indica.* Quickest substrate colonization (15 days) and primordial initiation (30 days) as well as highest number (6) and weight of sporophores (70.5 % BE) was recorded in 21 days old spawn. The average pileus diameter and stipe length were 14.6 and 16.7 cm, respectively. A single sporophore weighed about 117.5 g. Mushroom yield decreased with increase in spawn age. A spawn dose of 200 g/ kg of dry substrate was found optimum.

2.5 Evaluation of straw substrates and casing material on biological efficiency of *C. indica*

Amine *et al.,* (2010) investigated effect of different substrates and casing materials on the growth and yield of *Calocybe indica.* The number of effective fruiting bodies, the biological economic yield & the biological efficiency were statistically similar all of the casing material used. The maximum biological efficiency was found in the cow dung & loamy soil (3cm thick) was the best casing material and the rice straw was the best substrate for the commercial cultivation of *Calocybe indica.*

Pani (2012) investigated that the effect of depth and time of casing on sporophore production of *Calocybe indica*. Results indicate that 2 cm thick casing layer was ideal for quickest primordial initiation, higher sporophore number and maximum yield. There was no significant difference in these attributes between 1 and 2 cm casing depths. Thickness beyond 2 cm gradually decreased the mushroom yield and delayed the fruiting. Casing at the time of spawning was best in terms of quickest fructification (28 days), higher sporophore number (6) and maximum yield (74.6 % BE). There were no significant variations in yield attributes when casing was done just after substrate colonization. Delay in casing (18-25 days) resulted in gradual reduction in the number and weight of sporophores. It was concluded that a casing depth of 2 cm at the time of spawning should be allowed to obtain higher production of *C. indica*.

Subramanian *et al.*, (2015) determine the effect of casing layer thickness in the growth and yield of *Calocybe indica,* an indigenous tropical edible mushroom. The pure culture was grown in potato dextrose agar medium and maintained. The

spawn of *Calocybe indica* was prepared from the pure culture using white sorghum seeds as substrate. The spawn was inoculated in the paddy straw substrate filled bags and maintained in the mushroom cultivation chamber for the spawn running and growth of mushrooms. After the complete mycelium spreading in the bags, the casing process were done with varying thickness of casing layer viz. 1.5, 2.5 and 3.5 cm. The bags were monitored for the growth of the mushrooms and yield in each of the bags were recorded. The bags with casing layer thickness of 2.5 cm recorded the maximum yield and bioefficiency.

Pani (2011) conducted a study to evaluate straw of ten popular paddy varieties of Orissa for sporophore production of the milky mushroom, *Calocybe indica*. The variety CR-1014 recorded the highest mushroom yield (70.5 % BE) followed by Kanchan (69.9 % BE), Jagabandhu (69.6 % BE), Lalat (68.5 % BE), Swarna (66.1 % BE), Pratikshya (63.3 % BE), CR-1018 (61.2 % BE), CR-1009 (55.3 % BE), IR-64 (53.2 % BE) and IR-36 (53.1 % BE).

Navathe *et al.,* (2014) conducted an experiment and paddy straw was encased separately with four different casing materials *viz.*, vermicompost, sand + soil, spent biogas slurry, sand +soil+ spent biogas slurry to assess their effect on the biological efficiency of *C. indica.* It was revealed that among the four treatments maximum biological efficiency was obtained on paddy straw encased with the combination of sand + soil + spent dried biogas slurry (180.32%). This was followed by paddy straw encased with vermicompost (176.8%), spent dried biogas slurry (130%) and sand + soil (79.94%), respectively. Though the biological efficiency was numerically higher on paddy straw encased with combination of sand + soil + spent dried biogas slurry than paddy straw encased with vermicompost, yield on both the treatments was statistically at par. These two treatments were statistically significant with the remaining two treatments. It is evident from these results that the combination of sand + soil + spent dried biogas slurry as well as vermicompost are ideal casing materials for getting maximum biological efficiency of *C. indica* on paddy straw substrate.

Rawal *et al.,* (2014), tested three different combinations of substrates *viz*., wheat straw + paddy straw (1:1), wheat straw + paddy straw (1:2), wheat straw + paddy straw (2:1) along with wheat straw and paddy straw alone for yield

performance. Two year pooled data showed that substrates vary for period taken to spawn run, pin head formation and 1st harvest. The wheat straw alone substrate significantly took minimum days for spawn run; pinhead formation as well as 1st harvest followed by wheat straw + paddy straw (2:1), and wheat straw + paddy straw (1:1). In terms of average production, wheat straw alone substrate was found significantly superior than others by giving the maximum yield followed by wheat straw + paddy straw 2:1 ratio. The significant lower yield was obtained from paddy straw alone substrate. However significantly maximum number of fruit bodies were obtained from the substrate wheat straw + paddy straw (2:1), followed by wheat straw alone. The average fruit body weight was found maximum in case of wheat straw + paddy straw (2:1), followed by wheat straw alone (91.05g), however minimum weight was obtained in wheat straw + paddy straw 1:1 ratio. *Calocybe indica* strain APK-2 recorded maximum average biological efficiency and average mushroom size in wheat straw alone substrate followed by wheat straw + paddy straw (2:1) combination.

Vijaykumar *et al.,* (2014) conducted an experiment to find out the efficacy of different substrates such as paddy straw, wheat straw, soybean straw, coconut coir pith, cotton wastes and sugarcane bagasses for cultivation of milky mushroom. Among the six different substrates, wheat straw substrates was superior which recorded minimum days for spawn run, pinhead formation and for first harvest with highest no. of fruiting bodies, highest diameter, maximum yield and highest biological efficiency. Paddy straw was the next best superior substrate for cultivation of milky mushroom.

Kumar *et al.,* (2015) reported the effect of different agricultural wastes alone or in combination on yield of two strains (CI-6 and CI-4) of *C. indica.* Minimum time was recorded for spawn run in wheat straw (WS) substrates alone , while days for pinhead formation and days for first harvesting was observed in the combination of WS+PS (paddy straw) substrates with the ration of 2:1 in strain CI-4 and CI-6, respectively. Maximum yield per kg dry substrates was harvested in case of CI-4 with WS+BS (Brassica straw) (1:2) and CI-6 with WS+BS (2:1) combination. However, maximum average weight per fruit body was recorded in WS+BS (1:1) combination from strain CI-4 and CI-6.

2.6 Influence of different supplements on the commercial cultivation of *C. indica*

Alam *et al.,* (2010) investigated the most suitable supplements and their levels for the commercial cultivation of milky white mushroom. Rice bran, maize powder, and wheat bran with their different levels were used as supplements to evaluate the yield and yield contributing characteristics of *C. indica*. Primordia initiation was observed between 13.5 and 19.3 days. The results indicated that the 30% maize powder supplement was effective for producing viable fruiting bodies. The maximum diameters of the pileus and stalk were observed with 30% maize powder. The highest biological and economic yield and biological efficiency were also obtained with 30% maize powder as a supplement. The results indicate that increasing the supplement level resulted in less biological efficiency, and that 30% maize powder was the best supplement level for rice straw substrate to cultivate milky white mushrooms.

Krishnamoorthy *et al.,* (2015) reported that the addition of different supplements with the substrates influenced the spawn run, days for pinning, number of pinhead initiation, flushing pattern and overall mushroom yield.

CHAPTER-III
MATERIALS AND METHODS

The materials and methods were used during the present investigation are as follows:

3.1 Experimental site

The research experiments were conducted in Mushroom Research Laboratory, Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur (C.G.).

3.2 Washing and Sterilization

During the experimental work, BOROSIL make glasswares were used. The glasswares prior to use were washed with water and detergent powder followed by rinsing with tap water. The glasswares were dried and sterilized in oven at 180°C for 2 hours. The inoculation needle, knife, scissors, blades and cork borer were sterilized by dipping them in 95% alcohol followed by heating over flame.

3.3 Collection of *C. indica* from different geographical locations of C.G., their maintenance and source of other materials

The pure cultures of five strains of *C. indica* were prepared from fresh fruiting bodies, collected from different geographical locations of Chhattisgarh. The cultures were further multiplied on PDA (potato dextrose agar) medium and maintained at 30°C. The pure cultures were also stored at 4°C for further work. The other materials *i.e.* straw substrates [wheat (*Triticum aestivum*) straw, mustard (*Brassica compestris*) straw, pigeon pea (*Cajanus cajan*) straw, Lathyrus (*Lathyrus sativa*) straw and maize (*Zea mays*) straw] empty glucose bottles, polypropylene bags, cereal grains [maize *(Zea mays),* sorghum *(Sorghum bicolor),* bajra *(Pennisetum typhoids),* ragi *(Panicum miliaceaum)* and wheat *(Triticum aestivum)*], chemicals *viz.*, streptomycin, alcohol, calcium carbonate, calcium sulphate, bavistin and formalin and other materials were procured from Department of Plant Pathology, IGKV, Raipur.

3.4 Statistical analysis

Completely randomized design (CRD) and factorial with CRD was used for statistical analysis. All the statistical analysis was performed by using online software OPSTAT and WASP analysis in the Department of Statistics and Social Science. The critical difference (C.D.) was calculated at 5% levels.

3.5 Preparation of media

During the present investigation the following media were used.

Table 3.1 Different types of media, their ingredients and composition

Media/Contents		Quantity
i) Potato dextrose agar (PDA) medium		
Potato (Peeled and sliced)	-	200 g
Dextrose	-	20 g
Agar	-	20 g
Distilled water	-	1000 ml
ii) Potato sucrose agar (PSA) medium		
Potato (Peeled and sliced)	-	200 g
Sucrose	-	20 g
Agar-Agar	-	20 g
Distilled water	-	1000ml
iii) Wheat extract agar medium		
Wheat grains	–	200 g
Dextrose	-	20 g
Agar-agar	-	20 g
Distilled water	-	1000 ml
iv) Malt extract agar medium		
Malt extract	-	20 g
Agar-agar	-	20 g
Distilled water	-	1000 ml
v) Asthana & Hawker's medium		
Sucrose	-	5 g
Potassium dihydrogen phosphate	-	1.75 g

Magnesium sulphate	-	0.75 g
Potassium nitrate	-	3.5 g
Agar- agar	-	20 g
Distilled water	-	1000 ml

The prepared media were sterilized in autoclave at 15 lbs psi for 30 minutes and kept in refrigerator for further studies.

3.6 Meteorological data

The meteorological data on daily temperature (maximum, minimum and average) and relative humidity (morning and evening) of mushroom growing house were recorded and used during present study.

3.7 General methods

3.7.1 Preparation of mother spawn

Mother spawn was prepared from pure cultures of five strains (CI-1, CI-4, CI-522, CI-524 and CI-530) of *C. indica.* Wheat, maize, sorghum, bajra and ragi grains were used as substrate for preparation of mother spawn. Clean healthy and bold grains were taken but broken and undersized grains were discarded then these were thoroughly washed and dipped in water for 10-12 hours. The soaked grains were cooked for 10-15 minutes till they become soft without rupturing of epidermis. Thereafter, the excess water was drained and grains were spread on muslin cloth so that excess moisture can be eliminated. In cooled grains, Gypsum and Calcium carbonate (2% w/w) were mixed thoroughly. These prepared grains were filled in conical flask (250 ml) and plugged with non absorbent cotton. Each flask contains 100g grains and sterilized at 20 lbs psi for 2 hours. After cooling, inoculation was done with active growing mycelium of five strains of *C. indica* then incubated at 30°C till the mycelium covered the entire grain surface. The spawn was prepared by this ways called as mother spawn and prepared in sufficient quantity required for the proposed research work. For all kinds of studies mother spawn was multiplied on wheat grains in similar way as described for mother spawn.

3.7.2 Preparation of planting spawn

It was prepared in poly propylene bag (6" × 11" -150 gauges) wheat grains were processed and filled in bag in similar way as mother spawn. Each bag contained 250g wheat grains and thereafter these bags were sterilized. After cooling bags were aseptically inoculated with 10-15 gm of mother spawn in laminar air flow and incubated at 30°C. The inoculated bags were incubated and frequently examined for any types of contamination and those exhibiting contaminations were immediately discarded and those showing white, silky, uniform, strand mycelial growth covering all the grains were used for experimentation.

3.7.3 Preparation of mushroom bag

The wheat straw (chopped) was used as substrate for growing of *C. indica*. The substrate was dipped in water (which has already mixed with 75ppm Carbendazim and 500ppm formaldehyde) for 14 hours as per the method described by Vijay and Sohi (1987). Another method was used for treatment of straw in which overnight ten kilograms of wheat straw was soaked along with mixed 500g Calcium Carbonate ($CaCo_3$). Thereafter, excess water was drained off the next morning and straw was spread over on sloppy, cemented floor till the moisture content of straw remained 65-70 per cent. Before spawning, formaldehyde was sprinkled on floor, and thereafter the spawn was mixed in substrate through layering method @ 4 per cent on wet weight basis in all experiments. The spawned substrate was filled in poly propylene bags (18" × 27" -150 gauges) and mouth of the spawned bag was tied with nylon rope. The spawned bags were kept in mushroom growing room, where appropriate temperature (25-35°C) and relative humidity (80-90 per cent) were maintained by frequently sprinkling of water on walls and floor. After complete colonization of substrate by mushroom mycelium (spawn run), casing were done.

3.7.4 Casing

To promote sporophore production in milky mushroom, garden soil + FYM (1:1) was added as surface layer to the fully colonized straw bed. This casing layer was usually 2.0 to 5.0 cm deep and usually applied approximately after 2 weeks of

spawning. Vermicompost alone and in combination with garden soil + FYM (1:1) were also used as casing material

3.7.5 Picking of the mushroom

Picking was done by slight twisting and pulling of sporophores. Three flushes were harvested from the same bed at an interval of 10-15 days.

3.7.6 Weighing of sporophore

The freshly harvested sporophore was immediately weighed with the help of single pan balance with a sensitivity of 1g.

3.7.7 Yield of mushroom

The cumulative yield in each replication was recorded by summing up the fresh weight at the number of picking, which was represented as weight (g) per unit of dry straw substrate.

3.7.8 Biological efficiency

The yield was expressed in biological efficiency and calculated using the formula (Chang *et al.,* 1981).

$$\text{Biological efficiency \%} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

3.8 Effect of media on radial growth

Five different media *i.e.* Potato dextrose agar, potato sucrose agar, wheat extract, malt extract and Asthana and Hawker's, medium were used to know the effect of media on mycelial growth of 5 strains of *C. indica.* 20 ml of each medium was poured in sterilized Petridishes. At the time of pouring a little amount of streptomycin was added in each medium to inhibit the bacterial growth. After solidification of medium the plates were centrally inoculated with a 7 days old culture of respective strain of *C. indica.* The inoculated plates were incubated at, 30°C and 4 replications were maintained for each treatment. The observations were

recorded for radial growth in till the mycelial growth in any treatment reached at the periphery of the plates.

3.9 Effect of temperature on radial growth

The plates containing 20 ml medium were inoculated with actively growing pure culture of each strains of *C. indica.* These inoculated plates were kept at different temperature *i.e.*, 22°C, 25°C, 30°C, and 33°C for incubation. The observations were recorded for radial growth. Four replications were kept in each treatment.

3.10 Effect of different grains on spawn development

Five different grains were used to know the effect of spawn development of five strains of *C. indica.* The flask containing 200g was inoculated with actively growing pure culture of each strains of *C. indica.* These inoculated flasks were kept at 30 ±1°C temperature for incubation. The observations were recorded for 100% mycelial growth on grains surface. Four replications were kept in each treatment.

3.11 Effect of different methods of straw treatment on yield of *C. indica*

Wheat straw was treated by two methods one was chemical method and another was $CaCO_3$ and used to production of different strain of *C. indica* and the observation was recorded for spawn run, pin head initiation, pileus diameter, stipe length, stipe diameter and yield with BE %.

3.12 Effect of re-casing on yield of *C. indica*

The yield obtained from the bags. After three harvesting, on the bags re-casing was done and observation was recorded for pin head initiation and yield of *C. indica.*

3.13 Effect of different casing materials on yield of *C. indica*

Various types of casing materials viz., compost {garden soil + FYM (1:1)}, vermicompost and combination of compost + vermicompost (1:1) were used to casing the bag and observation was noticed for spawn run, pin head initiation and yield of *C. indica.*

3.14 Evaluation of different straw substrates for yield of *C. indica*

Different types of straw substrates [wheat, wheat + Lathyrus, wheat + Mustard, wheat + arhar and wheat + maize (1:1)] were used to know suitable substrates for cultivation of *C. indica.* The observation was recorded for spawn run pin head initiation and yield of *C. indica.*

3.16 Spore print

The pileus/cap of a fresh sporophore from each strain was removed with the help of a scalpel and the entire pileus was kept on a transparency sheet as well as black sheet and covered with a bowl which was covered with a moist blotting paper from inside.

CHAPTER- IV
RESULT AND DISCUSSION

4.1 Cultural characteristics of *C. indica* strains collected from different geographical areas of Chhattisgarh on different media and at different temperature

4.1.1 Effect of different media on radial growth of *C. indica* strains

An extensive survey was conducted across CG. and five different strains of *C. indica* were collected from different geographical areas and the GPS details of the collected *C. indica* strains given below (Table 4.1 and Plat 4.0). Under *in vitro* condition, influence of five media *viz.* Potato dextrose agar, potato sucrose agar, wheat extract, malt extract and Asthana and Hawker's, media were studied to see their effect on radial growth of five strains (CI-1, CI-4, CI-522, CI-524 and CI-530) of *C. indica* and results are presented in Table- 4.2.

There was significant difference in radial growth of different strains of *C. indica* on different media under the study. On potato dextrose agar (PDA) the significantly highest (80.62 mm) radial growth showed by strain CI-522 followed by strain CI-4 (80.12 mm), strain CI-530 (74.62 mm) and strain CI-524 (66.12 mm) while, the lowest (62.50 mm) radial growth was recorded in strain CI-1.

For radial growth of different strains of *C. indica* on potato sucrose agar (PSA) medium, the significantly maximum (80.12 mm) radial growth was noticed in strain CI- 4, followed by strain CI-522 (78.75 mm), strain CI-530 (76.00 mm) and strain CI-524 (69.75 mm) whereas, the minimum (60.00 mm) radial growth was showed by strain CI-1.

The significantly higher (56.25 mm) radial growth of different strains on wheat extract agar (WE) agar medium was recorded in strain CI-522 than other strains followed by strain CI-4 (55.75 mm), strain CI-530 (49.50 mm), strain CI-1 (47.25 mm) respectively. It was least in strain CI-524 (38.37 mm).

On malt extract agar (ME) medium the significantly maximum (64.62 mm) radial growth was recorded in strain CI-524 followed by strain CI-522 (55.62 mm), strain CI-4 (50.37 mm) and strain CI-530 (46.37 mm) whereas, the significantly minimum (*43*.75 mm) radial growth was found in strain CI-1.

On Asthana & Hawker's medium, the significantly highest (57.00 mm) radial growth was found in strain CI-4 followed by strain CI-524 (54.75 mm), strain CI-522 (52.50 mm) and strain CI-530 (59.24 mm) whereas, the minimum (46.02 mm) radial growth was found in strain CI-1.

Table 4.1 Collection of different *C. indica* strains from different geographical area of Chhattisgarh and their GPS detail .

Sl. No.	**Local Accession No & GPS Position**	**Name of fungi**	**Habitat/ Substrates**	**Place**	**Date of collection**	**Edibility**	**Designation**
1	CI-1 **N 21^0 15.714' E 81^0 34.765' Altitude 1094ft**	*Calocybe indica** Milky mushroom	Beneath Peepal Tree Rhizospere region	Raipur Dharampura	13.08.2015	Edible	DMRO-747
2	CI-4 **N 20^{0}13.412' E 81^{02}2.738' Altitude 1240ft**	*Calocybe indica** Milky mushroom	Beneath Peepal Tree Rhizospere region	Kanker	09.08.2015	Edible	DMRO-748
3	CI-522 **N 20^0 43.388' E 81^0 59.612' Altitude 1215ft**	*Calocybe indica** Milky mushroom	Beneath Peepal Tree Rhizospere region	Gariyaband Bhamni Forest	11.08.2015	Edible	DMRO-749
4	CI-524 **N 21^0 12.945' E 82^0 12.828' Altitude 1036ft**	*Calocybe indica** Milky mushroom	Beneath Karanj Tree Rhizospere region	Sirpur forest	27.08.2015	Edible	DMRO-750
5	CI-530 **N 21^0 16.717' E 81^0 34.660' Altitude 1096ft**	*Calocybe indica** Milky mushroom	In between branches of Peepal tree	Raipur, Professor Colony	14.08.2015	Edible	DMRO-751

The radial growth of different strains of *C. indica* differed significantly with respect to different media. The strain CI-1 showed maximum (62.50 mm) radial growth on PDA. It was significantly higher than other medium and the lowest (43.75 mm) radial growth of strain CI-1 was recorded in ME followed by A&H medium (46.62 mm), WE (47.25 mm) and PSA (60.00 mm) respectively.

The strain CI-4 was showed significantly maximum (80.22 mm) radial growth on both PDA and PSA followed by A&H medium (57.00 mm) and WE medium (55.75 mm) whereas, the minimum (50.37) radial growth was noticed on ME.

The significantly highest (80.62 mm) radial growth of strain CI-522 was recorded on PDA followed by PSA medium (78.75 mm), WE medium (56.25 mm) and ME medium (55.62 mm) and it was significantly least (52.50 mm) on A&H.

The strain CI-524 was showed significantly maximum (69.75 mm) growth on PSA medium followed by PDA medium (66.12 mm), ME medium (64.62 mm) and A&H medium (54.75 mm) while, the minimum radial growth was found on WE medium.

The significantly maximum (76.00 mm) radial growth of strain CI-530 was recorded on PSA followed by the PDA (74.62 mm), A&H (49.75 mm) and WE (49.50 mm) and it was showed minimum (46.37 mm) on ME.

On an average, the radial growth of different strains of *C. indica* differed significantly. It was maximum (64.74 mm) in CI-522 and next were CI-4 (64.67 mm), CI-530 (59.24 mm), CI-524 (58.72 mm) and CI-1 (52.02 mm).

Among the tested media the highest (72.92 mm) radial growth of *C. indica* was noticed in potato sucrose agar medium and it was significantly superior to other medium. The lowest (49.42 mm) radial growth was found in wheat extract medium followed by potato dextrose agar (72.79 mm), malt extract (52.14 mm) and Asthana & Hawker's medium (52.12 mm) (Plate-4.2).

The present findings are close to the results obtained by Singh *et al.,* (2009) reported that *C. indica* grew well on all the tested media but maximum linear growth (7 cm) was recorded on wheat extract agar medium followed by PDA. Krishnamoorthy *et al.,* (2015) reported the majority of the time required for more radial growth in culture media like PDA or malt extract medium is 8 to 10 days.

Table 4.2 Effect of different media on radial growth of different strains of *C. indica*

MEDIA	Radial Growth of Strains (mm)*					AVERAGE	Colony characteristics of strains of *C. indica*
	CI-1	CI-4	CI-522	CI-524	CI-530		
PDA	62.50	80.12	80.62	66.12	74.62	72.79	All strains showed cottony white, dense mycelial growth with entire margin.
PSA	60.00	80.12	78.75	69.75	76.00	72.92	Cottony white growth with entire margin but the strain CI-4 and CI-530 showed concentric ring.
WE	47.25	55.75	56.25	38.37	49.50	49.42	All strains showed cottony white, dense mycelial growth with entire margin.
ME	43.75	50.37	55.62	64.62	46.37	52.14	All strains showed cottony white, dense mycelial growth with entire margin.
A&H	46.62	57.00	52.5	54.75	49.75	52.12	Cottony white with entire margin. Thin and less mycelial growth was recorded in contrast to all tested media.
AVERAGE	52.02	64.67	64.74	58.72	59.24		

	SEm±	CD (5%)	
Strains	0.69	1.96	
Media	0.69	1.96	
Strains × Media	1.56	4.40	

(*) - Average of four replications.

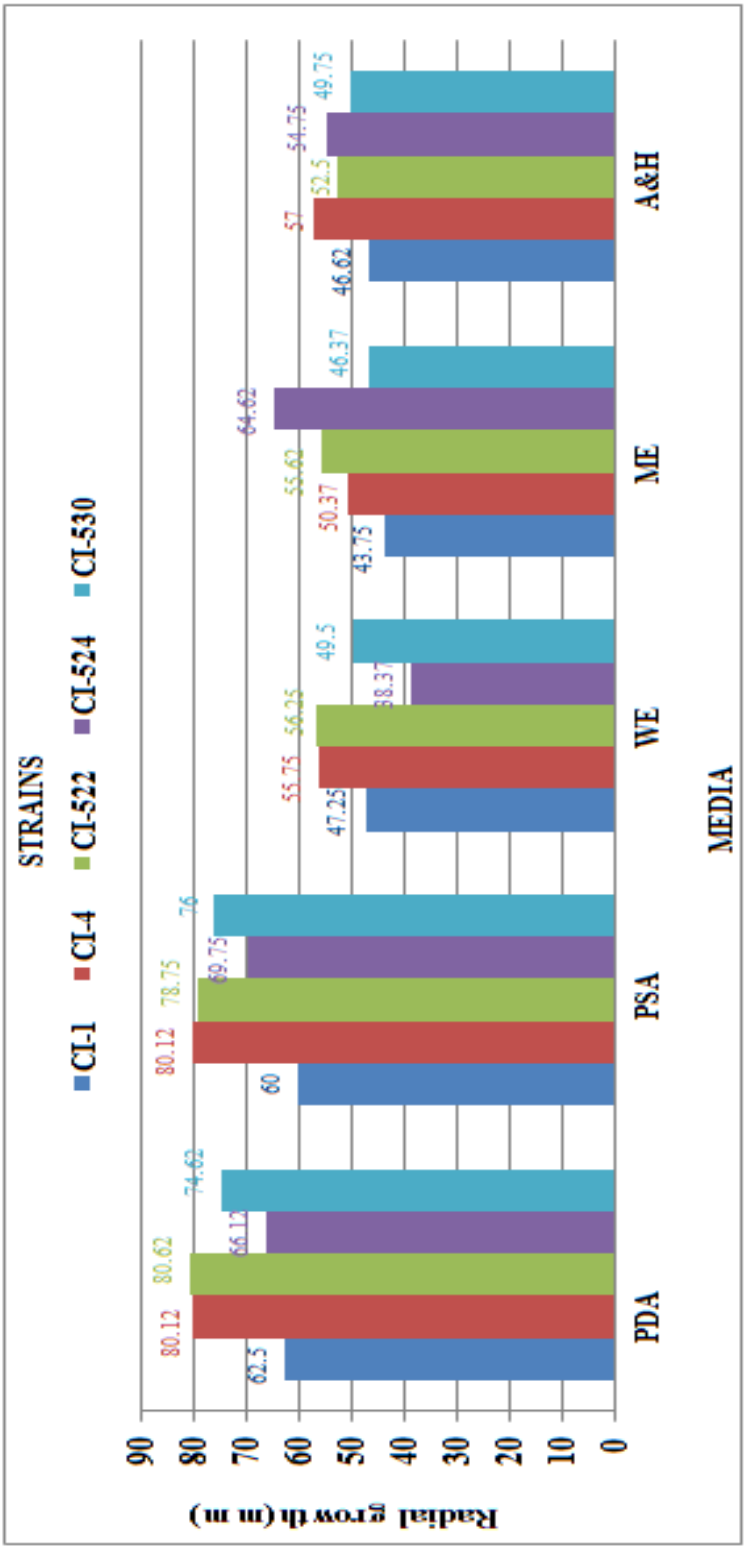


Fig. 4.1- Effect of different media on radial growth of different strain of *Calocybe indica.*

Plate 4.0- Diversity of milky mushroom in Chhattisgarh

Plate 4.1b- Effect of different media on radial growth of different strains of *Calocybe indica.*

4.1.2 Effect of different temperatures on radial growth of *C. indica*

Effect of different temperatures on radial growth of different strain of *C. indica* was studied and the results are depicted in Table 4.2, (Plate-4.3).

There was significant difference in radial growth of different strains of *C. indica* at different temperatures under study. At 22°C temperature the significantly highest (35.75 mm) radial growth was showed by strain CI-4 followed by strain CI-530 (35.50 mm) and two strains CI-522 and CI-524 (33.12 mm) while, strain CI-1 showed minimum (29.27 mm).

The radial growth of different strains of *C. indica* at 25C° temperature differed significantly, the significantly highest (56.87 mm) radial growth was noticed in two strains CI- 4 and CI-522, followed by strain CI-530 (55.37 mm) and strain CI-1 (51.50 mm) whereas, the minimum (43.75 mm) radial growth was showed by strain CI-524.

The significantly maximum (80.62 mm) radial growth was recorded in two strain CI-522 and CI-524 at 30°C followed by strain CI-4 (80.12 mm) and strain CI-530 (74.62 mm) whereas, the strain CI-1 showed minimum (62.25 mm) radial growth.

At 33°C temperature, the significantly highest (58.50 mm) radial growth was found in strain CI-4 followed by two strains CI-522 & CI-524 (58.00 mm) and strain CI-1 (53.62 mm) whereas, the minimum (50.75 mm) radial growth was found in strain CI-530.

The radial growth of different strains of *C. indica* differed significantly with respect to different temperatures. The strain CI-1 showed maximum (62.25 mm) radial growth at 30°C. It was significantly higher than other temperatures and the lowest (29.37 mm) radial growth of strain CI-1 was recorded at 22°C followed by 25°C (46.62 mm) and 33°C (60.00 mm) respectively.

Strain CI-4 showed significantly maximum (80.12 mm) radial growth at 30°C followed by 33°C (58.50 mm) and 25°C (56.87 mm) whereas, the minimum (37.75) radial growth was noticed at 22°C. The significantly highest (80.62 mm) radial growth of strain CI-522 was recorded at 30°C followed by 33°C (58.00 mm)

and 25°C (56.87 mm) while, it was significantly least (33.12 mm) at 22°C temperature.

The strain CI-524 showed significantly maximum (80.62 mm) growth at 30°C followed by 33°C (58.00 mm) and 25°C (64.62 mm) while, the minimum (33.12 mm) radial growth was found at 22°C temperature.

The significantly maximum (74.62 mm) radial growth of strain CI-530 was recorded at 30°C followed 25°C (55.37 mm) and 33°C (50.75 mm) whereas, it was minimum (34.50 mm) at 22°C temperature.

There was significant difference on an average radial growth of *C. indica* at different temperatures. The highest (75.64 mm) radial growth of *C. indica* was noticed at 30°C and it was significantly superior to other temperature. The lowest (33.17 mm) radial growth was found at 22°C followed by 33°C (44.17 mm) and 25°C (41.49 mm).

There was considerable difference in average radial growth of different strain of *C. indica.* The growth was significantly higher (57.81 mm) in CI-4, whereas, it was lower (49.18 mm) with CI-1, followed by CI-530 (53.81 mm), CI-524 (53.87 mm) and CI-522 (57.15 mm). It was observed that the growth of different strains was much influenced at different temperature (22-33°C). (Fig.-4.2)

Varshney (2007) reported temperature requirement from 25-35°C for mycelial growth of *Calocybe indica.* All strains of *Calocybe indica* showed maximum mycelial growth at 28°C followed by 32°C and minimum at 20°C. At 28°C temperature on 8th day's strain CI-6 was at par showing maximum diametric growth of mycelium (9.0 cm) in observations. Shukla *et al.,* (2013) studied on effect of temperature on mycelia growth of the strains of milky mushroom (*Calocybe indica)* viz. CI- 4, CI- 6, CI- 7, C1- 8, Cl- 9 & Cl- 10. They reported that the most suitable temperature for fast and full mycelial impregnation and growth was 30°C.

Table 4.3 Effect of different temperature on radial growth of different strains of *C. indica*

TEMPERATURE	Radial Growth of Strains (mm)*					AVERAGE	Colony characteristics of strains of *C. indica*
	CI-1	CI-4	CI-522	CI-524	CI-530		
22°C	29.37	35.75	33.12	33.12	34.5	33.17	In all strain absolute white mycelial growth with entire margin.
25°C	51.50	56.87	56.87	43.75	55.37	41.49	In all strain absolute white mycelial growth with entire margin.
30°C	62.25	80.12	80.62	80.62	74.62	75.64	In all strain absolute white mycelial growth with entire margin.
33°C	53.62	58.50	58.00	58.00	50.75	44.17	Cottony white mycelial growth with entire margin but difference in strain CI-524, in which concentric ring was observed.
AVERAGE	49.18	57.81	57.15	53.87	53.81		
			SEm±		**CD (5%)**		
Strains			0.618		1.752		
Temperature			0.553		1.567		
Strains× Temperature			1.236		3.505		

(*) - Average of four replications.

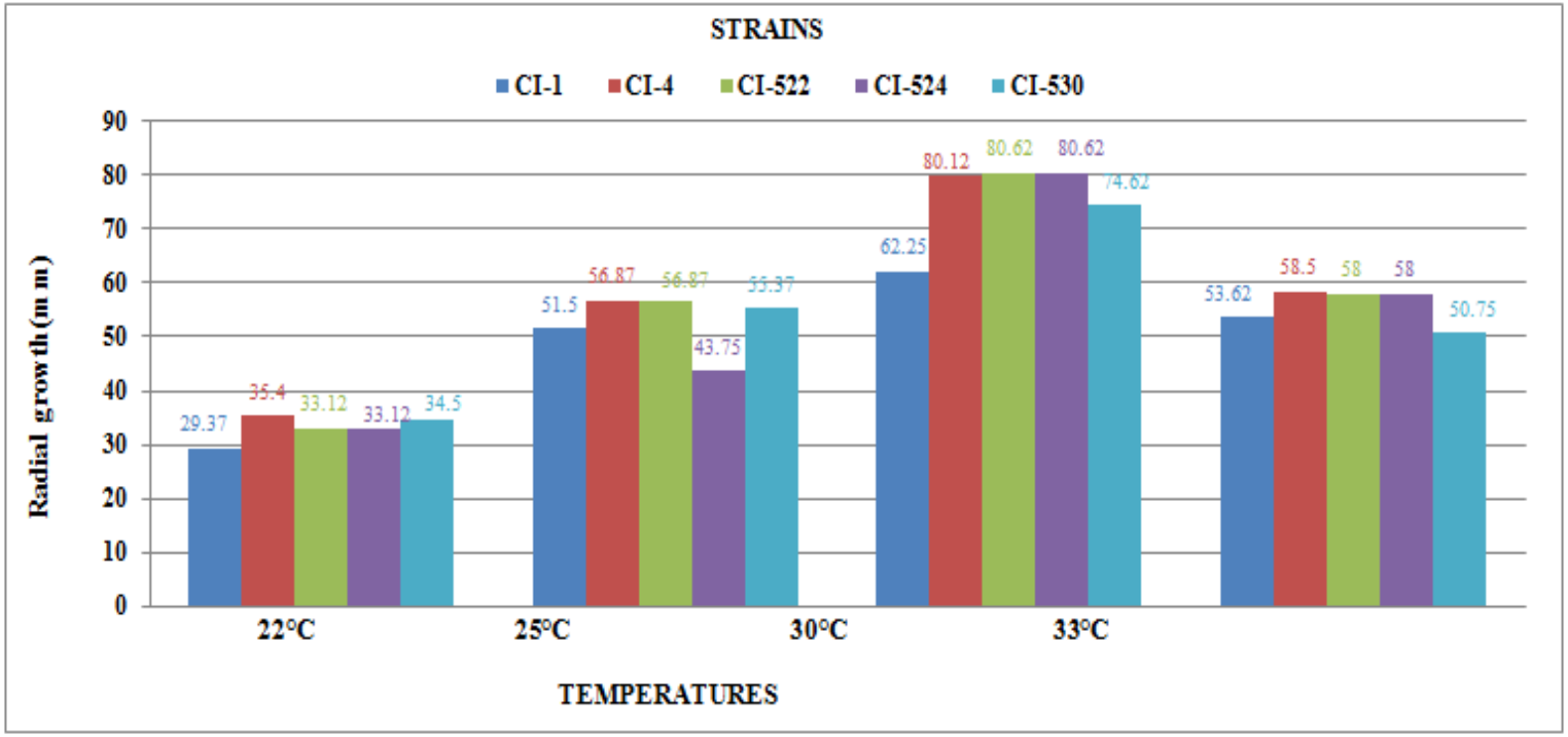


Fig. 4.2- Effect of different temperatures on radial growth of different strain of *Calocybe indica*.

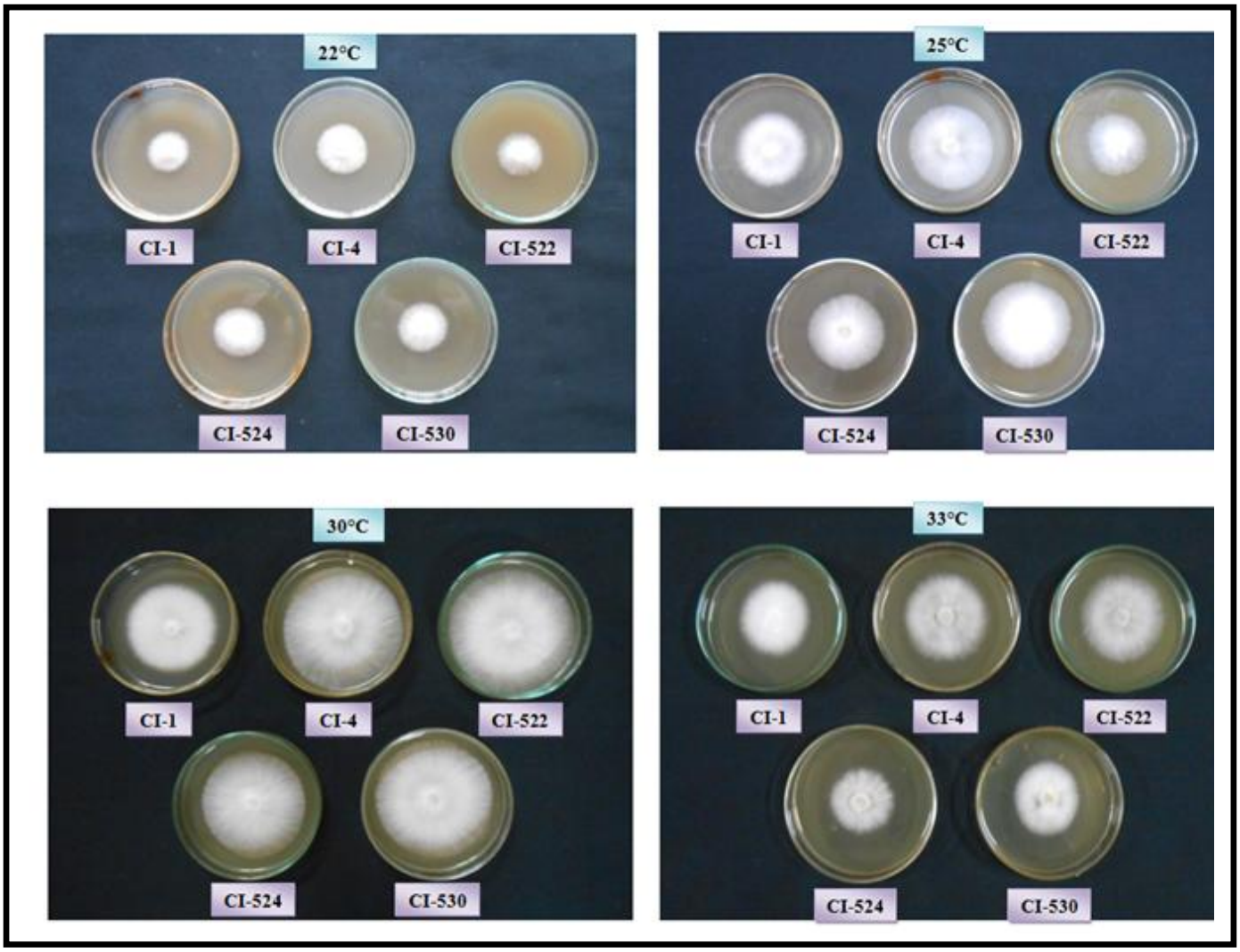


Plate 4.2- Effect of different temperature on radial growth of different strain of *Calocybe indica.*

4.2 Evaluation of different grain substrates for spawn development

Evaluation of different grain substrates were studied for spawn development of *C. indica* and subsequently data is presented in table 4.4 (Fig 4.3 and Plate 4.3). There was significant difference in spawn development of different strains of *C. indica* on different grains.

The numbers of days for spawn development of different strains of *C. indica* on different grains was differed significantly under study. On wheat grains, the significantly minimum (14.00 days) time required for spawn development took by strain CI-522 followed by two strains CI-4 and CI-530 (14.25 days), strain CI-554 (14.50 days) and it was delayed by strain CI-524 (14.75 days).

For spawn development of different strains of *C. indica* on maize grains, the significantly less (16.00 days) was noticed in strain CI-530, followed by two strain CI-1 and CI-4 (16.25 days) and two strain CI-522 and CI-524 which took more (17.25 days) numbers of days for spawn development.

On sorghum grains, the significantly minimum (12.00 days) time was recorded in strain CI-524 than other strains and next were strain CI-4 (12.25 days), strain CI-1 (13.50 days) and strain CI-522 (14.25 days). However, it was delayed (16.00 days) in strain CI-530.

The number of days required for complete spawn development on bajra grains, the significantly minimum (17.25 days) time was recorded in two strains CI-1 and CI-4 followed by the rest three strains CI-522, CI-524 and CI-530.

On ragi grains, the significantly quicker (13.25 days) time took for spawn development by strain CI-1 than other strains. However, delayed (16.00 days) duration for spawn development was found in strain CI-522 followed by strain CI-4 (15.75 days), strain CI-524 (14.25 days) and strain CI-1 (13.25 days).

The number of days required for spawn development of different strains of *C. indica* differed significantly with respect to different grains. The strain CI-1 took minimum (13.25 days) time on ragi grains followed by sorghum grains (13.50 days), wheat grains (14.50 days) and maize grains (16.25 days) whereas, it was delayed (17.25 days) on bajra grains.

The strain CI-4 took significantly minimum (12.25 days) time for spawn development followed by wheat grains (14.25 days), ragi grains (15.75 days) and

maize grains (16.255 days), whereas, the maximum (17.25 days) time was noticed on bajra grains.

The significantly less (14.00 days) time by strain CI-522 was recorded for spawn development on wheat grains followed by sorghum grains (14.25 days), ragi grains (16.00 days) and maize grains (17.25 days) while, it was significantly more (18.25 days) on bajra grains.

The strain CI-524 took significantly minimum (12.00 days) number of days for spawn development on sorghum grains followed by ragi grains (14.25 days), wheat grains (14.75 days) and maize grains (17.25 days) whereas, the maximum (18.25 days) time was recorded on bajra grains.

The significantly less (14.00 days) time by strain CI-530 was recorded on ragi grains followed by wheat grains (14.25 days) and maize and bajra grains(16.00 days) whereas, the significantly more (18.25 days) required on bajra grains.

On an average there was significant difference noticed in spawn development of *C. indica* on different grains. The number of days required for spawn development by *C. indica* differed significantly. On different grains, by *C. indica*, the time required for complete spawn development was significantly less in sorghum (13.6 days) followed by wheat grain (14.35 days), ragi (14.65 days) and maize (16.65 days) whereas, significantly more time was required on bajra (17.85 days).

The time required for spawn development of different strains of *C. indica* differed significantly. It was minimum (14.95 days) in CI-1 and next were CI-4 (15.15 days), CI-522 (15.95 days), CI-524 (15.3 days) and CI-530 (15.7 days).

The present findings are very close to the results obtained by Prasuna (2002) studied different grain substrates for spawn production of *C. indica* and reported early spawn development on wheat grain followed by jowar grains. Krishnamoorthy, A. S. and Venkatesh, Balan (2015) stated that sorghum or wheat grains were found to be the best substrates for spawn production of *C. indica.*

Table 4.4 Evaluation of different grain substrates for spawn development of *C. indica*

GRAINS	Complete spawn development by strains (days)*					AVERAGE	Mycelial characteristics
	CI-1	CI-4	CI-522	CI-524	CI-530		
Wheat	14.50	14.25	14.00	14.75	14.25	14.35	White mycelial growth, all grains were covered. Grains were tightly held with each other by all strain.
Maize	16.25	16.25	17.25	17.25	16.00	16.65	Contrast to other tested grains, grains were loosely held with each other.
Sorghum	13.50	12.25	14.25	12.00	16.00	13.60	White mycelial growth, all grains were covered. Grains were tightly held with each other by all strain.
Bajra	17.25	17.25	18.25	18.25	18.25	17.85	White mycelial growth, all grains were covered by all strain. Grains were tightly held with each other
Ragi	13.25	15.75	16.00	14.25	14.00	14.65	In CI-1 scattered mycelial growth was observed. Grains were tightly held with each other.
AVERAGE	14.95	15.15	15.95	15.30	15.70		
		SEm±		**CD (5%)**			
Strains		0.099		0.280			
Grains		0.099		0.280			
Strains × Grains		0.222		0.626			

(*) - Average of four replications.

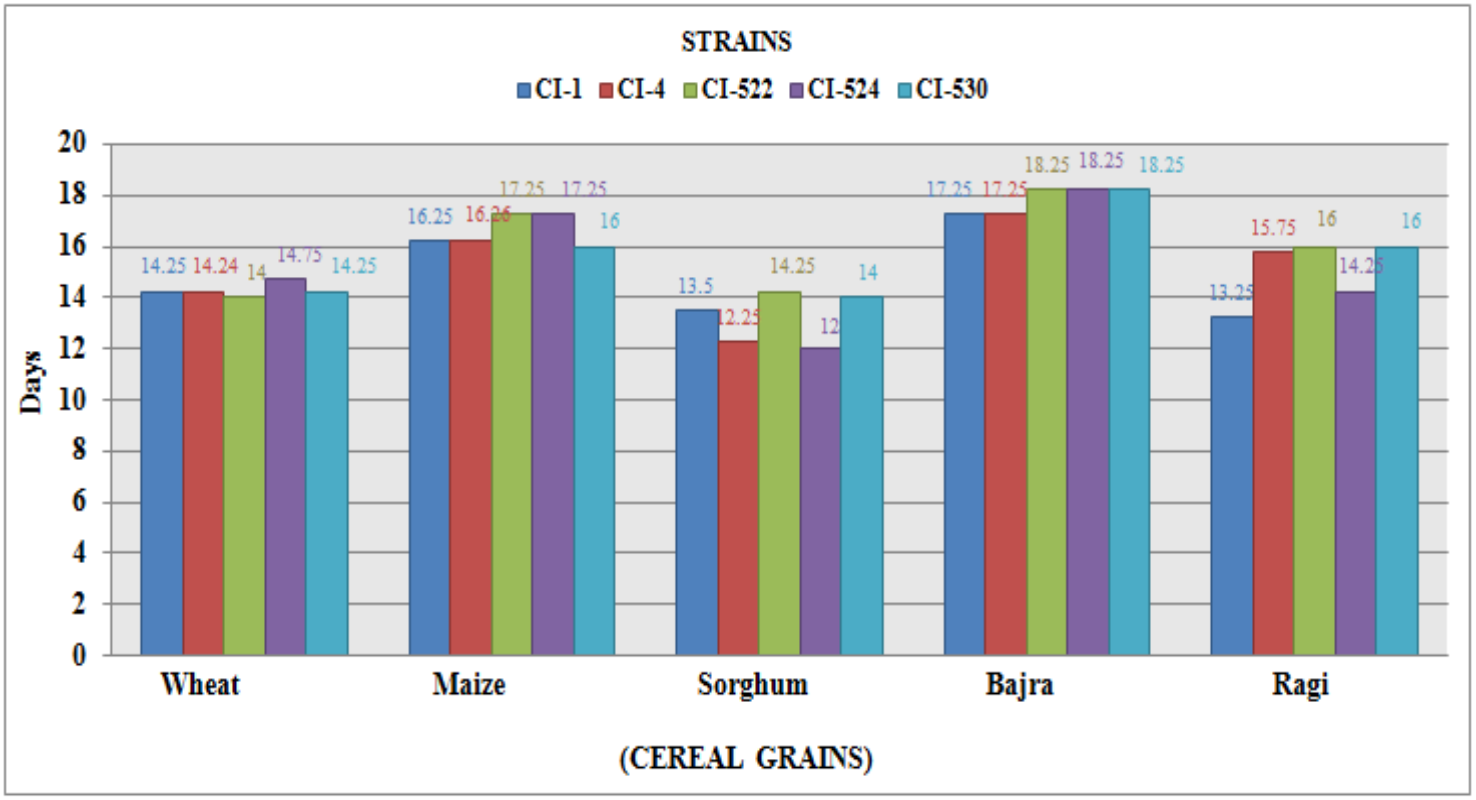


Fig. 4.3- Evaluation of different cereal grains for spawn development of *Calocybe indica*.

Plate 4.3- Evaluation of spawn development of different strains of *Calocybe indica* on different grain substrates.

4.3 Studies on yield attributes of different strain of *C. indica* on wheat substrate

4.3.1 Yield performance of different strain of *C. indica* on wheat grain raised spawn in wheat straw substrate treated by chemical

Five strains of *C. indica* were grew in wheat straw substrate treated by chemical method and studied for spawn run, pin head initiation, weight of sporophores, stalk length, stalk diameter, pileus diameter and yield. The data is presented in Table 4.5.

The period for spawn run in five strains of *C. indica* differed significantly. Spawn run was faster in both strains CI-522 and CI-524 (12 days) followed by strain CI-530 (13 days), and CI-4 (14 days) while it was slower (15 days) in strain CI-1. Pin head initiation of *C. indica* also differed significant with the strains. Pin head initiation was quicker in strain CI-524 and CI-522 (7 days) whereas it was delayed in strain CI-1 (9 days).

Pileus diameter, stalk length and of different strain of *C. indica* differed significantly with each other. Pileus diameter was significantly more in strain CI-524 (11.26 cm) followed by strain CI-4 (8.68 cm), strain CI-1 (5.51 cm) and strain CI-530 (4.90 cm) while it was less (3.63 cm) in strain CI-522 of *C. indica.* The significantly highest stalk length was recorded in strain CI-524 (19.6 cm) followed by strain CI-530 (14.8 cm), CI-4 (12.6 cm) and CI-1 (10.2 cm). Whereas, lowest (9.9 cm) stalk length was recorded in strain CI-522 of *C. indica.* The stalk diameter different strain of *C. indica* did not show any significant difference with each other. It was varied from 2.96-4.10 cm. The average weight of *C. indica* also did exhibit significant difference with each other strains. The average weight of sporophores was significantly maximum (82 g) in strain CI-524 followed by strain CI-4 (75g), strain CI-522 (75g) and strain CI-530 (57 g) whereas it was minimum (54 g) in strain CI-1.

The yield of five strains of *C. indica* differed significantly with each other. The yield of *C. indica* was significantly maximum in strain CI-524 (696.66g) with BE (69.66%) followed by strain CI-530 (485g) with BE (48.5%), strain CI-4 (318g) with BE (31.83%) and strain CI-1 (273.33g) with BE (27.33%) while it was minimum in strain CI-522 (226.67g) with BE (22.66%) (Fig. 4.4).

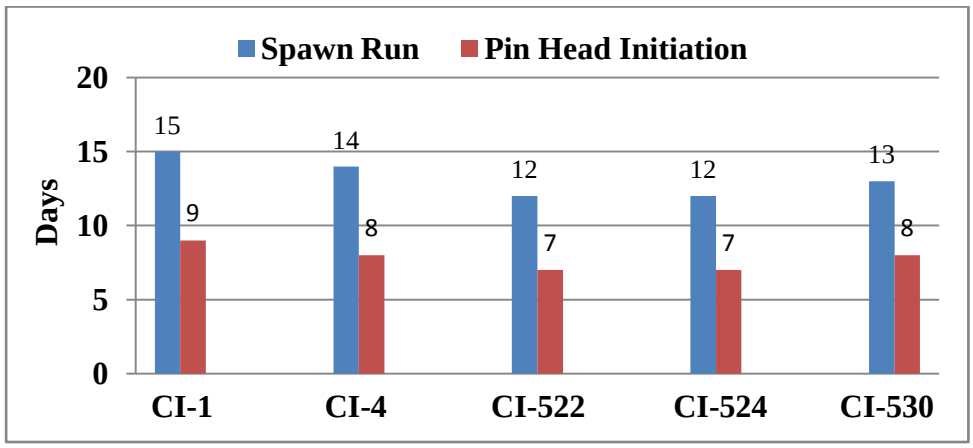
Table 4.5 Yield performance of different strains of *C. indica* on wheat grain raised spawn in wheat straw substrates treated by Chemical

Yield g/ kg dry wheat substrate									
S No	**Strains**	**Spawn Run (Days)***	**Pin head Initiation (Days)***	**Pileus Diameter (cm)****	**Stipe Length (cm)****	**Stipe Diameter (cm)****	**Average Weight (g)****	**Yield (g)***	**BE (%)**
1.	**CI-1**	15	9	5.51	10.2	2.96	54	273.33	27.33
2.	**CI-4**	14	8	8.68	12.6	4.00	75	318.33	31.83
3.	**CI-522**	12	7	3.63	9.9	3.58	64	226.67	22.66
4.	**CI-524**	12	7	11.26	19.6	4.10	82	696.67	69.66
5.	**CI-530**	13	8	4.90	14.8	3.50	57	485.00	48.50
	SEm±	0.33	0.33	1.47	1.51	0.34	5.95	20.38	
	CD (5%)	1.24	1.05	4.34	4.45	NS	17.57	64.23	

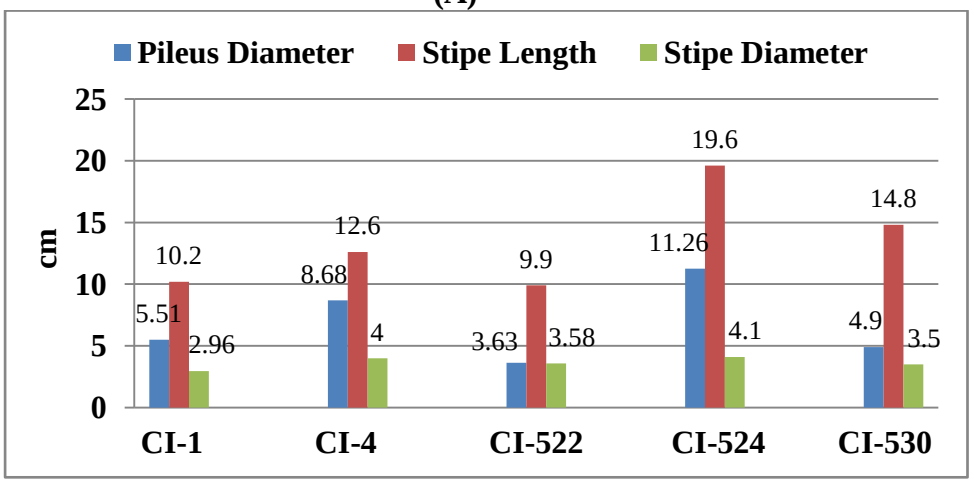
(*) – Average of three replication.

(**) – Average of five sporophore.

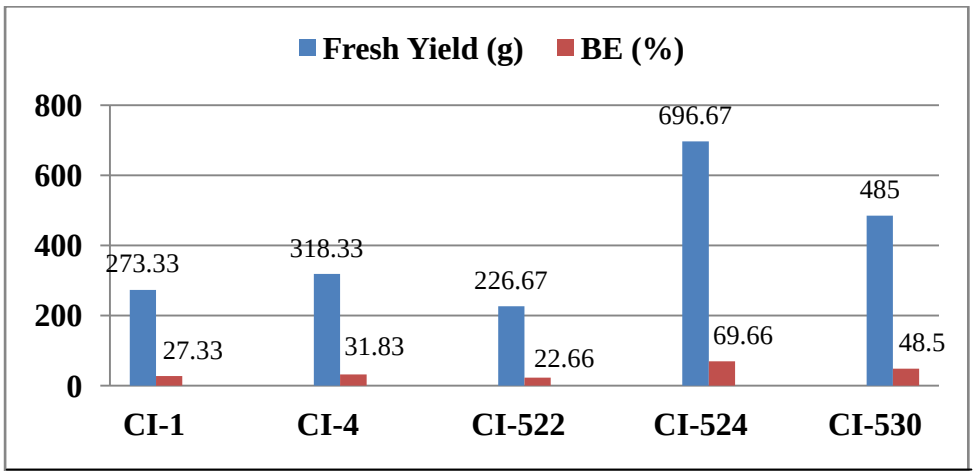
(NS) – Non significant.



(A)



(B)



(C)

Fig. 4.4- (A) Spawn run and pin head initiation (B) Pileus diameter, stipe length and stipe diameter (C) Fresh yield and biological efficiency of different strain of *Calocybe indica* in wheat grain raised spawn in wheat straw treated by chemical.

Spawning

Spawn run

Casing

Case run

Pin head

Fruiting bodies

Fig. 4.4a- Different stage in production of *Calocybe indica*.

CI-1

CI-4

CI-522

CI-524

CI-530

Plate 4.4- Fruiting bodies of different strains of *Calocybe indica.*

4.3.2 Yield performance of different strains of *C. indica* on wheat grain raised spawn in wheat straw substrate treated by $CaCO_3$

Five strains of *C. indica* were grew in wheat straw substrate treated by chemical method and studied for spawn run, pin head initiation, weight of sporophores, stalk length, stalk diameter, pileus diameter and yield. The data is presented in Table 4.6.

The period for spawn run in five strains of *C. indica* did not show any significant difference with each other. It varied from 13-14 days. Pin head initiation of *C. indica* differed significantly with the strains. Pin head initiation was quicker in strain CI-524 followed by CI-530 (8 days), CI-522 (10 days) and strain CI-4 (12 days) whereas it was delayed in strain CI-1 (14 days).

Pileus diameter of different strain of *C. indica* differed significantly with each other. Pileus diameter was significantly more in strain CI-524 (8.99 cm) followed by strain CI-530 (6.60 cm), strain CI-4 (5.67 cm) and strain CI-522 (3.40 cm) while it was less (3.25 cm) in strain CI-1 of *C. indica.* The stalk length and stalk diameter did not show any significant difference in strain of *C. indica.* The stalk length was varied from 8.70-12.30 cm and stalk diameter was varied from 2.72-3.64 cm. The average weight of *C. indica* also did exhibit significant difference with each other strains. The average weight of sporophores was significantly maximum (73.00g) in strain CI-524 followed by strain CI-4 (66.00g), strain CI-530 (62.00g) and strain CI-522 (55.80g) whereas it was minimum (50g) in strain CI-1.

The yield of five strains of *C. indica* differed significantly with each other. The yield of *C. indica* was significantly maximum in strain CI-524 (590g) with BE (59%) followed by strain CI-530 (406.67g) with BE (40.66%), strain CI-4 (320g) with BE (32%) and strain CI-522 (265g) with BE (26.5%) while it was minimum in strain CI-1 (150g) with BE (15%) (Fig. 4.5).

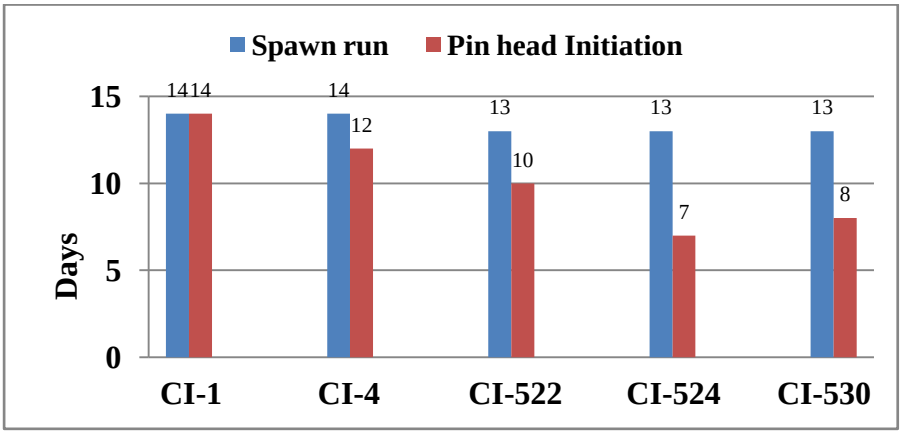
Table 4.6 Yield performance of different strains of *C. indica* on wheat grain raised spawn in wheat straw substrates treated by $CaCO_3$

Yield g/ kg dry wheat substrate									
S No	**Strains**	**Spawn Run (Days)***	**Pin head Initiation (Days)***	**Pileus Diameter (cm)****	**Stipe Length (cm)****	**Stipe Diameter (cm)****	**Average Weight (g)****	**Yield (g)***	**BE (%)**
1.	**CI-1**	14	14	3.25	10.80	2.72	50.00	150.00	15.00
2.	**CI-4**	14	12	5.67	12.00	3.12	66.00	320.00	32.00
3.	**CI-522**	13	10	3.40	8.70	3.46	55.80	265.00	26.50
4.	**CI-524**	13	7	8.99	12.00	3.64	73.00	590.00	59.00
5.	**CI-530**	13	8	6.60	12.30	3.60	62.00	406.67	40.66
	SEm±	0.44	0.29	1.19	1.52	0.29	4.12	14.16	
	CD (5%)	NS	0.93	3.52	NS	NS	12.17	44.62	

(*) – Average of three replication.

(**) – Average of five sporophore.

(NS) – Non significant.



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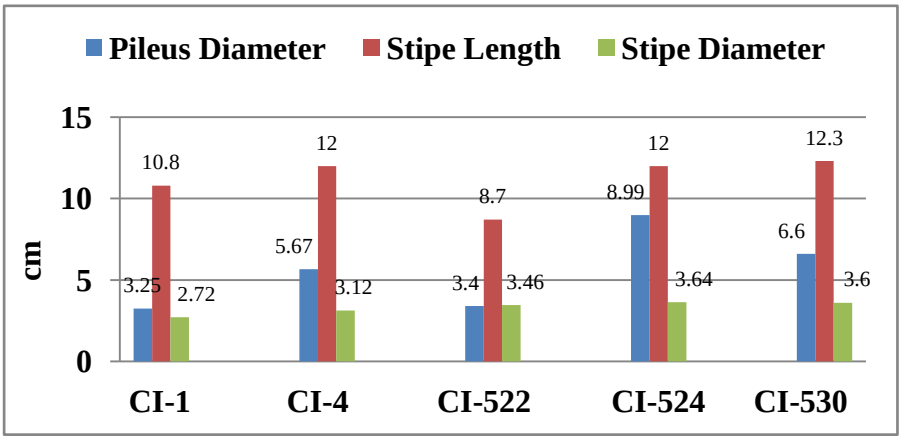

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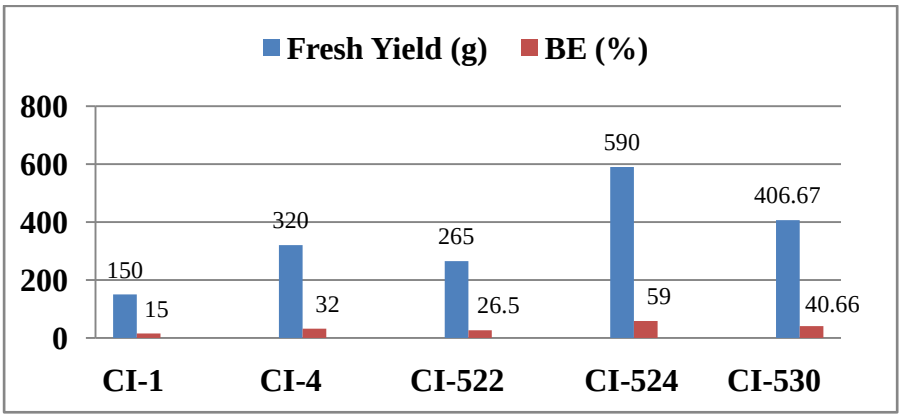


(C)

Fig. 4.5- (A) Spawn run and pin head initiation (B) Pileus diameter, stipe length and stipe diameter (C) Fresh yield and biological efficiency of different strain of *Calocybe indica* in wheat grain raised spawn in wheat straw treated by $CaCO_3$.

Fig. 4.5- Cultivation of *Calocybe indica* in growing room.

4.3.3 Yield performance of different strains of *C. indica* in wheat grain raised spawn after re-casing in wheat straw substrate

C. indica were studied for pin head initiation, pileus diameter, stalk length, stalk diameter, average weight and yield performance after re-casing. The result depicted in Table 4.7.

Time taken for pin head initiation differed significantly with each other strains. Pinhead initiation was quicker in strain CI-524 and CI-530 (7 days) whereas it was delayed in strain CI-522 (9 days). There was no pin head in strain CI-1.

Pileus diameter, stalk length and stalk diameter also differed significantly with different strains. The pileus diameter was significantly maximum (10.40 cm) in strain CI-524, followed by CI-4 (8.14 cm) and CI-522 (6.68 cm) whereas it was minimum (4.46 cm) in strain CI-530. The stalk length was significantly more (16.1 cm) in strain CI-524 and it was minimum (9.14 cm) in strain CI-522. Stalk diameter also differed significantly. The maximum (4.54 cm) stalk diameter was recorded in strain CI-524, followed by CI-4 (4.00 cm) and CI-522 (3.96) while the minimum (3.78 cm) stalk diameter was recorded in strain CI-530. The average weight of sporophore did not show any significant difference with strains, it varied from 54.38-70.36g.

The fresh yield of different strain of *C. indica* after re-casing also differed significantly with each other. The maximum (255g) fresh yield with BE (25%) was obtained in strain CI-524, followed by CI-530 (198.33g) with BE (19.83%) and CI-4 (193.33g) with BE (19.33%) whereas the minimum fresh yield was recorded in strain CI-522 (168.33g) with BE (16.83%).

4.3.4 Yield performance of strain CI-524 of *C. indica* on different grains raised spawn on wheat straw substrate

Different grains like wheat, maize, sorghum, bajra and ragi were used to raise the spawn of *C. indica* (CI-524) and their influence on spawn run, pin head formation, stalk length, stalk diameter, pileus diameter and yield were studied. The data are presented in table 4.8.

Table 4.7 Yield performance of different strains of *C. indica* in wheat grain raised spawn after re-casing in wheat straw substrate

Yield (g)/kg dry wheat substrate								
S. No.	**Strain**	**Pinhead Initiation (Days)***	**Pileus Diameter (cm)****	**Stipe Length (cm)****	**Stipe Diameter (cm)****	**Average Weight (g)****	**Yield (g)***	**BE (%)**
1	**CI-1*****	-	-	-	-	-	-	-
2	**CI-4**	8	8.14	11.0	4.00	70.36	198.33	19.83
3	**CI-522**	9	6.68	9.14	3.96	54.38	168.33	16.83
4	**CI-524**	7	10.40	16.1	4.54	73.00	255.00	25.50
5	**CI-530**	7	4.46	14.42	3.78	66.00	193.33	19.33
	SEm±	0.33	0.93	0.99	0.22	5.80	6.45	
	CD (5%)	1.08	2.80	2.98	NS	NS	21.05	

(*) – Average of three replication.

(**) – Average of five sporophore.

(***)- No pin head initiation.

(NS) – Non significant.

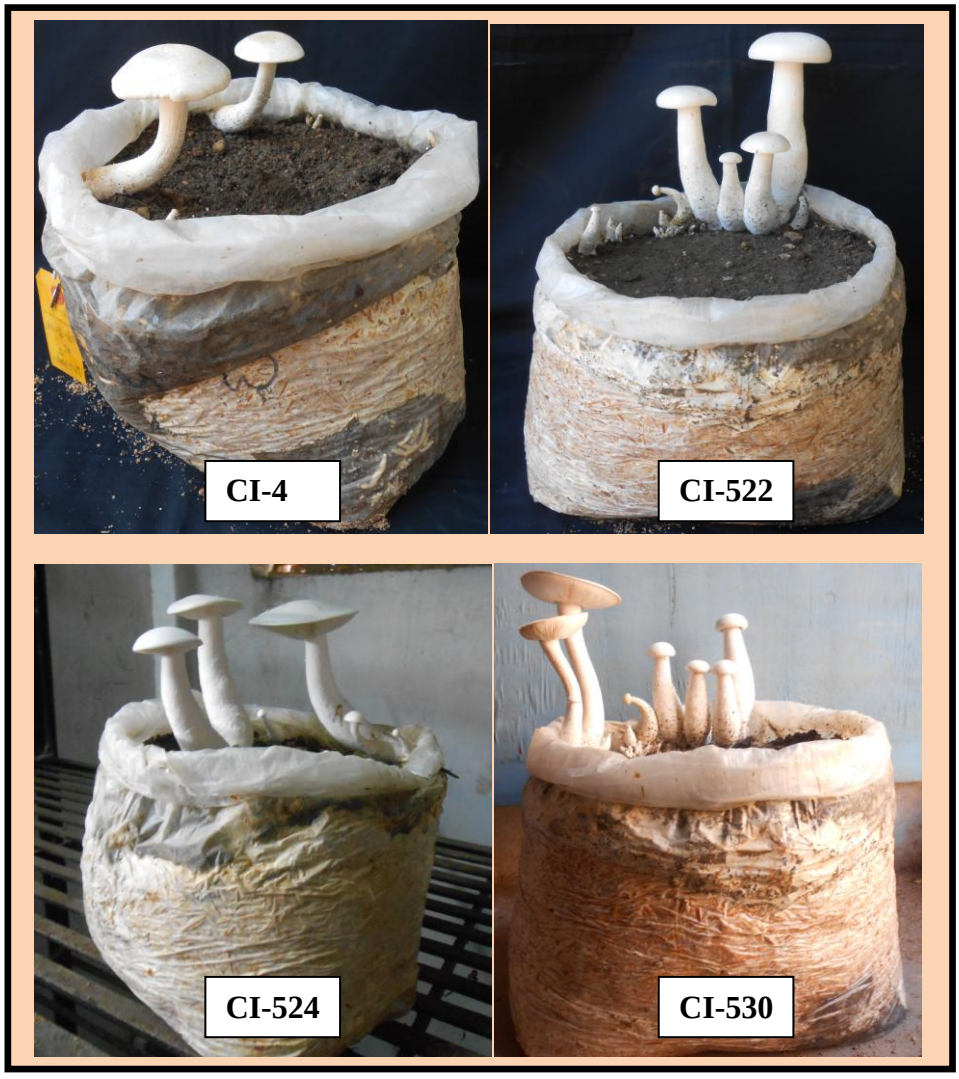


Plate 4.6- Evaluation of different strain of *Calocybe indica* for fruiting bodies in wheat grains raised spawn after re-casing in wheat straw.

The number of days required for spawn run by strain CI-524 differed significantly with respect to different grains. Spawn prepared on sorghum and bajra grains required significantly less time (11 days) for spawn run whereas, took significantly more time by maize (13 days), wheat and ragi grain based spawn (12 days) for spawn run by strain CI-524 of *C. indica*. Similarly pin head initiation was quite earlier (6 days) in sorghum, bajra and ragi grain followed by wheat grain (7 days). However, pin head initiation took significantly more time when spawn was prepared using maize (8 days).

The other parameters like stalk length of strain CI-524 differed significantly. The stipe length of strain CI-524 was highest in the fruit body produced from bed inoculated with wheat grain based spawn (17.80 cm) followed by sorghum (17.60 cm), ragi (12.00 cm) and maize (11.50 cm). However, the minimum length was showed by bajra (11.40 cm) grain. The average weight of sporophores, pileus diameter and stalk diameter did not show any significant difference with the type of grains used. However, the average weight of sporophores varied from 54-85g, pileus diameter from 5.66-10.36 cm, stalk diameter from 3.16-4.44 cm.

The fresh yield of strain CI-524 differed significantly with type of grains used. The highest yield recorded on sorghum grains (718.33g) with BE (71.83%) followed by wheat grains (685g) with BE (68.50%) and minimum yield was recorded in ragi (283.00g) with BE (28%) followed by bajra grains (310g) with BE (31 %) and maize grains (486.67g) with BE (48.66%) (Fig.4.6).

Senthilnambi *et al.,* (2011) found the supremacy of sorghum grains as the most suitable substrate for early spawn run, which took only 13.7 days for hundred percent mycelial growths. The yield and number of buttons harvested were found maximum in the sorghum grain spawn followed by ragi grain spawn. The maize grain substrates took 19 days for complete spawn run and recorded low yield when compared to other spawn substrates. The days for pin head formation and first harvest of the crop were earlier in case of sorghum grain spawn followed by ragi spawn.

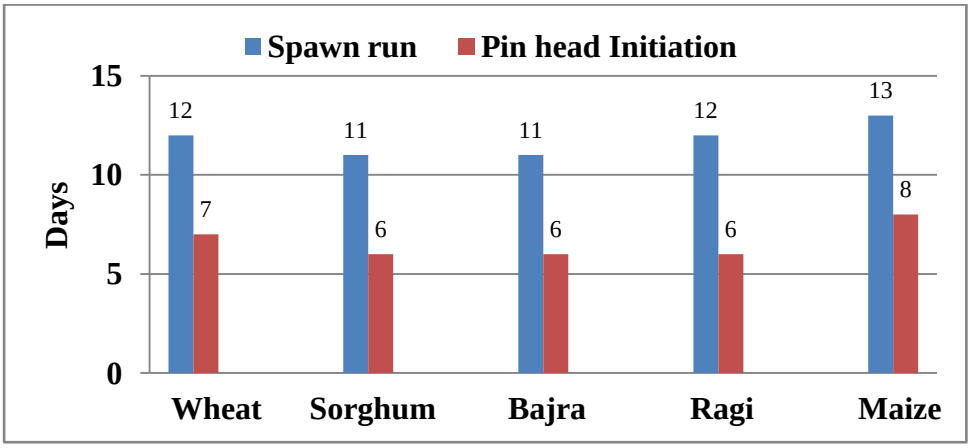
Table 4.8 Yield performance of strain CI-524 on different grains substrates raised spawn in wheat straw substrate

Yield g/ kg dry wheat substrates									
S No	**Grains**	**Spawn Run (Days)***	**Pin head Initiation (Days)***	**Pileus Diameter (cm)****	**Stipe Length (cm)****	**Stipe Diameter (cm)****	**Average Weight (g)****	**Yield (g)***	**BE (%)**
1.	**WHEAT**	12	7	10.36	17.80	4.44	85	685.00	68.50
2.	**SORGHUM**	11	6	9.24	17.60	3.24	66	718.33	71.83
3.	**BAJRA**	11	6	5.68	11.40	3.34	62	310.00	31.00
4.	**RAGI**	12	6	6.93	12.00	3.50	60	283.00	28.30
5.	**MAIZE**	13	8	5.66	11.50	3.16	54	486.67	48.66
	SEm±	0.29	0.29	1.50	1.50	0.31	8.78	13.22	
	CD (5%)	0.93	0.93	NS	4.44	NS	NS	41.68	

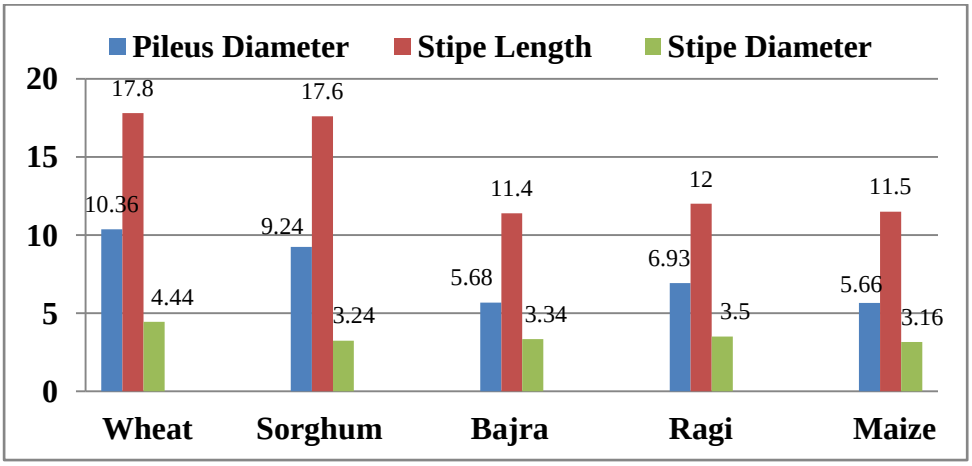
(*) – Average of three replication

(**) – Average of five sporophore

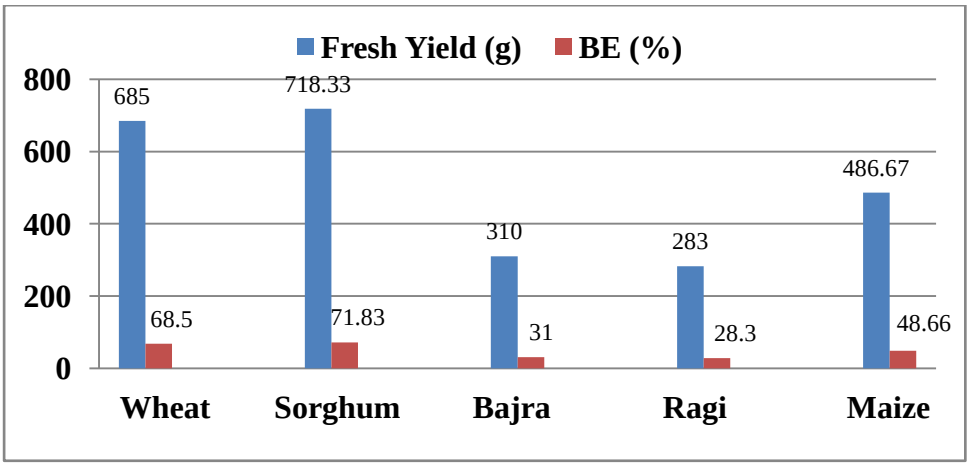
(NS) – Non significant



(A)



(B)



(C)

Fig. 4.6- (A) Spawn run and pin head initiation (B) Pileus diameter, stipe length and stipe diameter (C) Fresh yield and biological efficiency of strain CI-524 of *Calocybe indica* in different grains raised spawn in wheat straw substrate.

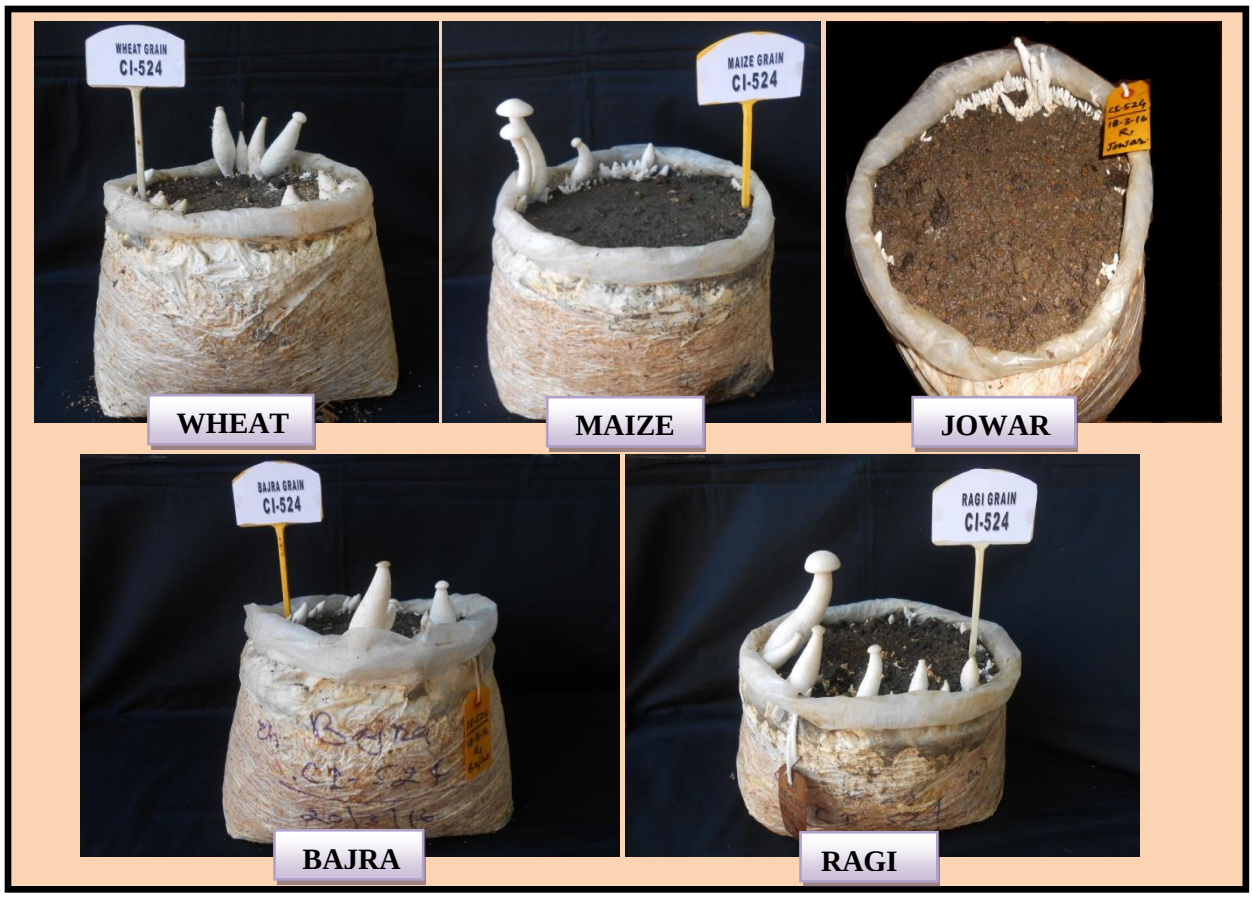


Plate 4.7- Evaluation of strain CI-524 of *Calocybe indica* for pin head on different grains raised spawn on wheat straw.

Plate 4.8- Evaluation of strain CI-524 of *Calocybe indica* for fruiting bodies on different grains raised spawn on wheat straw.

4.4 Studies on effect of different casing materials and straw substrates on yield of strain CI-524 of *C. indica*

Various casing materials were studied on spawn run, pinhead initiation and yield of strain CI-524 of *C. indica.* The results obtained are given in Table 4.9.

Different casing materials did not show any significant difference with each other in spawn run and pin head initiation. It varied from 11-12 days for spawn run. The pinhead initiation was from 10-11 days.

The fresh yield of strain CI-524 of *C. indica* differed significantly with different casing materials. Compost + Vermicompost gave significantly higher yield (813.33g) with BE (81.33%) followed by vermicompost (748.33g) with BE (74.83%) and compost [Garden soil + FYM (1:1)] gave minimum yield (690g) with BE (69.0%).

4.9 Effect of different casing materials on yield of strain CI-524 of *C. indica*

Yield (g)/ kg dry wheat substrate					
S. No.	**Treatment**	**Spawn Run (Days)***	**Pin head Initiation (Days)***	**Yield (g)***	**BE (%)**
1	**Compost** [Garden soil + FYM (1:1)]	12	11	690.00	69.00
2	**Vermicompost**	12	11	748.33	74.83
3	**Compost + Vermicompost (1:1)**	11	10	813.33	81.33
	SEm±	0.43	1.13	19.41	
	CD (5%)	NS	NS	67.17	

(*)- Average of three replication,

NS- Non significant.

To find out the suitable substrate for growth and yield of *C. indica*, wheat and four substrates combined with wheat straw were evaluated. The results are presented in Table 4.10.

Table 4.10 Influence of different straw substrates on yield of strain CI-524 of *C. indica*

Yield (g)/ kg dry straw substrate					
S. No.	**Treatment**	**Spawn Run (Days)***	**Pin head Initiation (Days)***	**Yield (g)***	**BE (%)**
1	**Wheat**	12	7	696.67	69.66
2	**Wheat + Lathyrus (1:1)**	13	11	765.00	76.50
3	**Wheat + Mustard (1:1)**	13	10	390.00	39.00
4	**Wheat + Arhar (1:1)**	12	11	405.00	40.50
5	**Wheat + Maize (1:1)**	13	12	435.00	43.50
	SEm±	0.21	0.69	13.10	
	CD (5%)	0.66	2.20	41.29	

(*)- Average of three replication.

NS- Non significant.

The time required for spawn run by strain *C. indica* varied significantly with respect to different substrate used. Spawn run was earlier in alone wheat and wheat + Arhar (12 days) followed by three combination wheat + lathyrus, wheat + mustard and wheat + maize (13 days).

The time taken for pin head initiation differed significantly with respect to different substrates. Pin head initiation was quicker in alone wheat straw (7 days) followed by Wheat + mustard (10 days), two combination Wheat + Lathyrus and

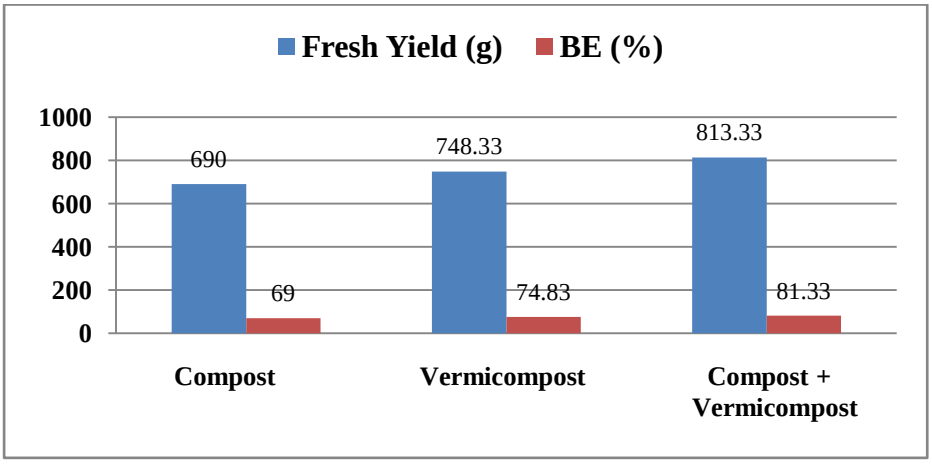


Fig. 4.7- Effect of different casing materials on yield and biological efficiency of strain CI-524 of *Calocybe indica.*

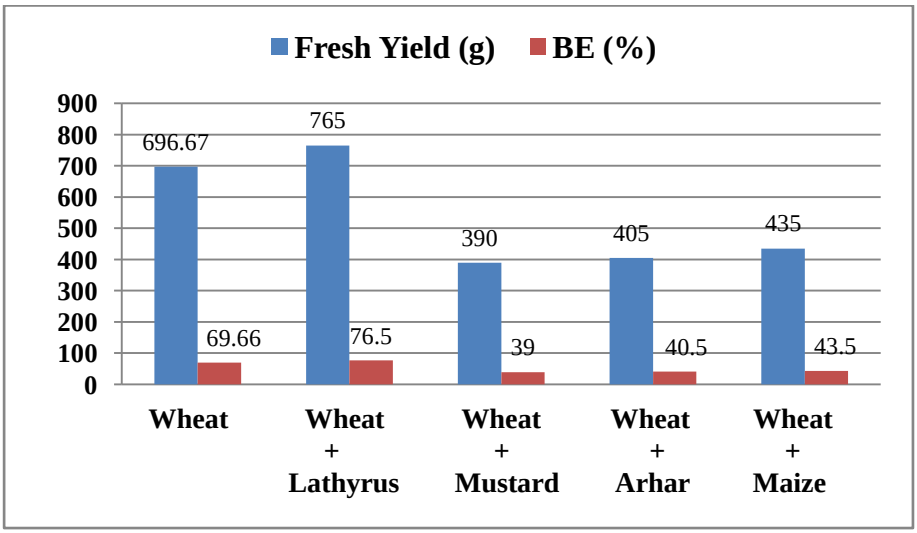


Fig. 4.8- Evaluation of different straw substrates for yield and biological efficiency of strain CI-524 of *Calocybe indica*.

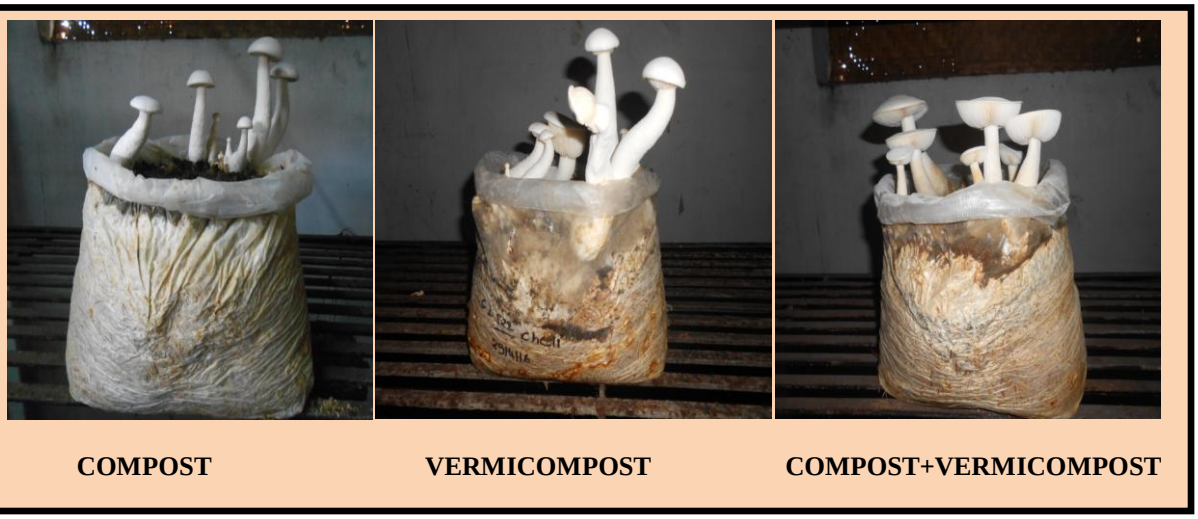


Fig. 4.9- Effect of different casing materials for fruiting bodies of strain CI-524 in wheat straw substrate.

Fig. 4.10- Effect of different combination of straw substrates for fruiting bodies of strain CI-524 in wheat straw substrate.

Wheat + Arhar (11 days). However, spawn run period was significantly delayed in combination wheat + maize (12 days).

The fresh yield of *C. indica* also differed significantly with respect to different substrates. The combination of wheat + Lathyrus straw gave significantly maximum yield (765g) with BE (76.5%) followed by alone wheat straw (696.67g) with BE (69.66%), wheat + maize (435g) with BE (43.50%) and the combination of wheat + Arhar straw (405g) with BE (40.50%) whereas the minimum fresh yield (390g) with BE (39%) was obtained from the combination of wheat + Sarso straw.

4.5 Spore print

Spore print was studied and result revealed that flat pileus kept over-night for fine and deeply texture print. The print can be used as taxonomic descriptor. Sporeprint gives information about the colour of the basidioospores, attachment of the stipe to the pileus, spacing between gills, diameter of the pileus as well as stipe and many other taxonomic parameters.

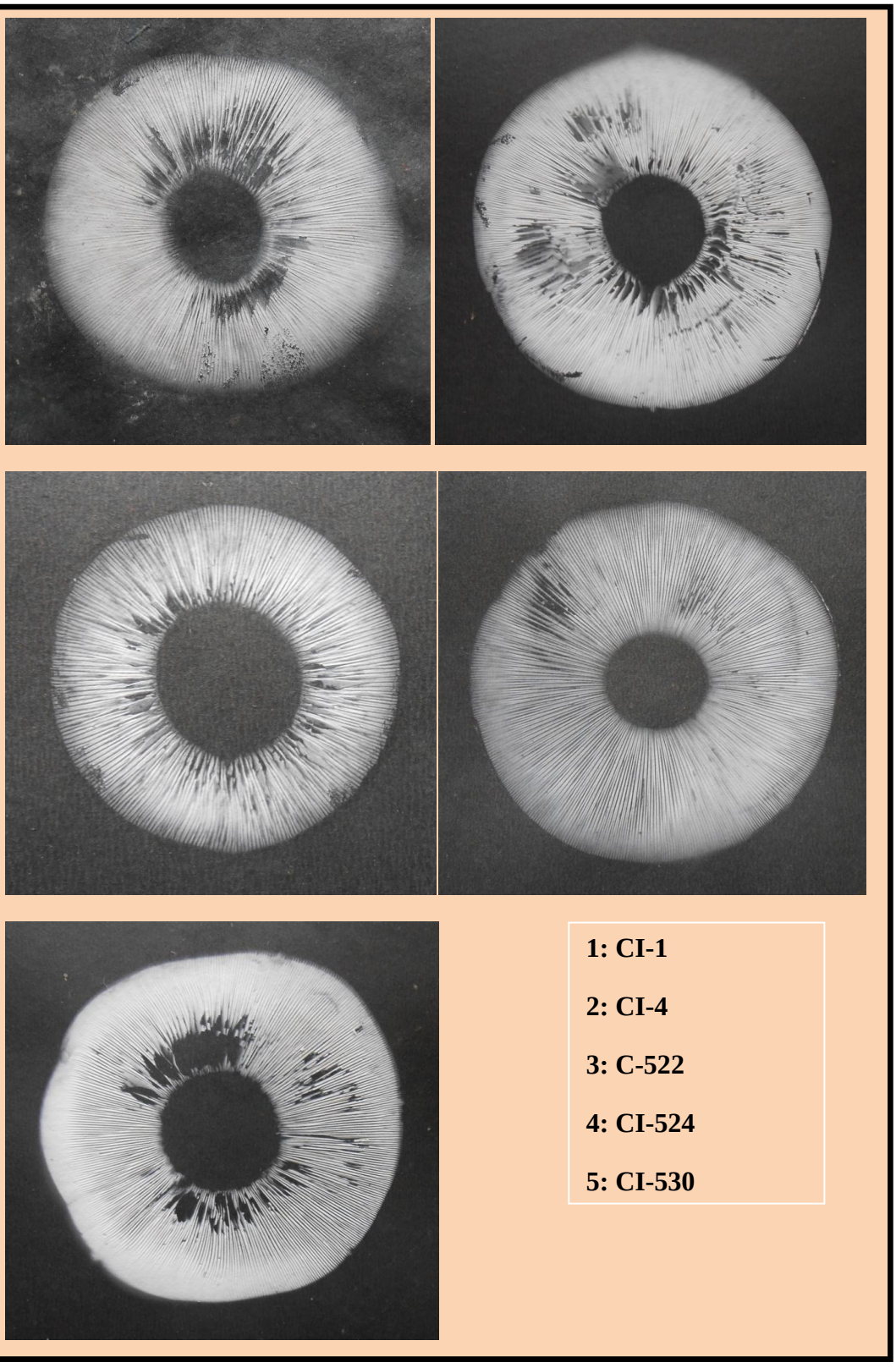


Plate 4.11 a- Spore prints of different strains of *Calocybe indica.*

CONC

The present investigation entitled "Studies on milky mushroom (*Calocybe indica* P&C) production in Chhattisgarh" was undertaken in the Department of

Plant Pathology, IGKV, Raipur. The salient findings obtained, discussed and summarized are as:

1. Potato sucrose agar medium was excellent for mycelial growth of *C. indica.*
2. The highest mycelial growth of *C. indica* was recorded at 30°C temperature.
3. Maximum radial growth was recorded in strain CI-522, CI-4, CI-524, CI-530 and CI-1 respectively but strain CI-524 was higher yielder contrast to other tested strains.
4. Sorghum grains were found to be superior for spawn development and yield performance followed by wheat grains..
5. Performance of *C. indica* was better in strain CI-524 followed by strain CI-530, strain CI-4 and strain CI-522 while strain CI-1 was poor in yield performance.
6. The Combination of compost + vermicompost was found to be best as casing material.
7. The combination of wheat + lathyrus was found to be suitable as straw substrate for production of *C. indica*.

Suggestion for future work:

1. Characterization of various strains of *C. indica* at morphological, molecular level should be undertaken.
2. Enhancement of yield through breeding programs.
3. Commercialization of the production technology of *C. indica.*
4. To develop an economical, feasible and sustainable production technology of *C. indica* using most commonly available substrates for its production; and a suitable bio-product for molds management.

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APPENDIX-A

Meteorological data of growing house

Month- March

Date	Temperature (°C)		Relative Humidity (%)	
	Minimum	Maximum	Morning	Evening
18-03-2016	24.4	33	72	65
19-03-2016	24	33	72	65
20-03-2016	26.1	33	72	65
21-03-2016	24	33	65	50
22-03-2016	26	33	65	52
23-03-2016	26	33	60	52
24-03-2016	26	33	62	65
25-03-2016	26.5	33	60	65
26-03-2016	27.3	33	62	62
27-03-2016	27.3	33	62	62
28-03-2016	27.3	33	82	82
29-03-2016	27.7	33	89	74
30-03-2016	28.4	33	84	75
31-03-2016	28.4	33	84	68

Month- April

Date	Temperature (°C)		Relative Humidity (%)	
	Minimum	Maximum	Morning	Evening
01-04-2016	28.2	33	87	76
02-04-2016	28.2	33	87	76
03-04-2016	28.2	33	85	76
04-04-2016	28.2	33	82	75
04-04-2016	28.3	33	81	75
06-04-2016	29.5	33	70	73
07-04-2016	28.5	33	73	75

08-04-2016	28.8	33	80	75
09-04-2016	29.2	33	72	65
10-04-2016	29.5	33.4	69	65
11-04-2016	29.5	33.6	68	65
12-04-2016	29.5	33.6	65	65
13-04-2016	29.6	30.6	65	65
14-04-2016	29.5	31.6	65	65
15-04-2016	30.2	35	67	65
16-04-2016	24.4	33	68	65
17-04-2016	24	33	68	65
18-04-2016	26.1	33	67	65
19-04-2016	24	33	68	65
20-04-2016	26	33	65	65
21-04-2016	26	33	64	65
22-04-2016	26	33	63	71
23-04-2016	26.5	33	62	64
24-04-2016	27.3	33	65	62
25-04-2016	27.3	33	58	65
26-04-2016	27.3	33	57	65
27-04-2016	27.7	33	56	65
28-04-2016	28.4	33	53	60
29-04-2016	28.4	33	59	60
30-04-2016	28.2	33	62	60

Month- May

Date	**Temperature (°C)**		**Relative Humidity (%)**	
	Minimum	**Maximum**	**Morning**	**Evening**
01-05-2016	28.2	33	56	56
02-05-2016	28.2	33	56	55
03-05-2016	28.2	33	56	55
04-05-2016	28.3	33	51	55

05-05-2016	29.5	33	52	55
06-05-2016	28.5	33	51	55
07-05-2016	28.8	33	58	56
08-05-2016	29.2	33	57	56
09-05-2016	29.5	33.4	57	56
10-05-2016	29.5	33.6	58	56
11-05-2016	29.5	33.6	51	56
12-05-2016	29.6	30.6	52	56
13-05-2016	29.5	31.6	52	57
14-05-2016	30.2	35	48	57
15-05-2016	31.2	35	48	58
16-05-2016	31.2	35	61	58
17-05-2016	32.2	36.5	59	58
18-05-2016	33	35	59	57
19-05-2016	33	35.5	59	61
20-05-2016	32.5	35.6	62	62
21-05-2016	33	35.6	65	62
22-05-2016	33	35.6	65	62
23-05-2016	32.3	36	65	62
24-05-2016	32.3	36	62	62
25-05-2016	33	35.5	61	62
26-05-2016	32	36	60	62
27-05-2016	29	36	76	60
28-05-2016	29	35	76	60
29-05-2016	29.5	35	75	60
30-05-2016	29.6	35	76	61
31-05-2016	30.2	35	75	61

Month- June

Date	Temperature (°C)		Relative Humidity (%)	
	Minimum	Maximum	Morning	Evening
01-06-2016	29	35	77	72
02-06-2016	28.5	35.5	77	72
03-06-2016	33	36	71	72
04-06-2016	33	35	70	68
05-06-2016	33	35	72	68
06-06-2016	31.2	35	76	65
07-06-2016	31.1	35.5	75	60
08-06-2016	30	36.5	76	65
09-06-2016	30	35.5	77	62
10-06-2016	30	35.5	76	62
11-06-2016	30	35.5	76	60
12-06-2016	30.1	35.5	72	60
13-06-2016	30	35	75	60

APPENDIX-B

ANOVA Table 1. Effect of different media on radial growth of different strain of *C. indica*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated
Strains (A)	4	2,208.865	552.216	56.730
Media (B)	4	11,315.715	2,828.929	290.618
Interaction (A X B)	16	2,195.785	137.237	14.098
Error	75	730.063	9.734	
Total	**99**	**16,450.428**		

Two Way Mean Table

	B1	B2	B3	B4	B5	Mean A
A1	62.250	60.000	47.250	43.750	46.625	**51.975**
A2	80.125	80.125	55.750	50.375	57.000	**64.675**
A3	80.625	78.750	56.250	55.625	52.250	**64.700**
A4	66.125	69.750	38.375	64.625	54.750	**58.725**
A5	74.625	76.000	49.500	46.375	49.750	**59.250**
Mean B	**72.750**	**72.925**	**49.425**	**52.150**	**52.075**	

TABLE OF SEM, SED AND C.D.

Factors	C.D.	SE(d)	SE(m)
Factor (A)	1.969	0.987	0.698
Factor (B)	1.969	0.987	0.698
Factor (A X B)	4.404	2.206	1.560

ANOVA Table 2. Effect of different temperatures on radial growth of different strain *C. indica*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated
Strains (A)	4	752.450	188.113	30.791
Temperatures (B)	3	18,125.509	6,041.836	988.945
Interaction (A X B)	12	1,021.850	85.154	13.938
Error	60	366.563	6.109	
Total	**69**	**16,450.428**		

Two Way Mean Table

	B1	B2	B3	B4	Mean A
A1	29.375	51.500	62.250	53.625	**49.188**
A2	35.750	56.875	80.125	58.500	**57.813**
A3	33.125	56.875	80.625	58.000	**57.156**
A4	33.125	43.750	80.625	58.000	**53.875**
A5	34.500	55.375	74.625	50.750	**53.813**
Mean B	**33.175**	**52.875**	**75.650**	**55.775**	

TABLE OF SEM, SED AND C.D.

Factors	C.D.	SE(d)	SE(m)
Factor (A)	1.752	0.874	0.618
Factor (B)	1.567	0.782	0.553
Factor (A X B)	3.505	1.748	1.236

ANOVA Table 3. Evaluation of different grain substrates for on radial spawn development of different strain of *C. indica*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated
Strains (A)	4	13.340	3.335	16.958
Grains (B)	4	233.340	58.335	296.619
Interaction (A X B)	16	76.760	4.798	24.394
Error	75	14.750	0.197	
Total	**99**	**338.190**		

Two Way Mean Table

	B1	B2	B3	B4	B5	Mean A
A1	14.500	16.250	13.250	17.250	13.500	**14.950**
A2	15.750	14.250	16.250	17.250	12.250	**15.150**
A3	17.250	14.000	16.000	18.250	14.250	**15.950**
A4	14.750	14.250	17.250	18.250	12.000	**15.300**
A5	16.000	14.250	16.000	18.250	14.000	**15.700**
Mean B	**15.650**	**14.600**	**15.750**	**17.850**	**13.200**	

TABLE OF SEM, SED AND C.D.

Factors	C.D.	SE(d)	SE(m)
Factor (A)	0.280	0.140	0.099
Factor (B)	0.280	0.140	0.099
Factor (A X B)	0.626	0.314	0.222

ANOVA Table 4. Spawn run of different strain of *C. indica* on wheat grains raised spawn in wheat straw treated by chemical method.

Source of Variation	DF	SS	MS	F-Cal	F-Table
Treatment	4	23.06667	5.766667	12.35714	3.47805
Error	10	4.666667	0.466667		
Total	**14**				

TABLE OF SEM, SED, CV, T-value AND C.D.

Factors	SE(m)	SE(d)	CV(%)	T-value	C.D.
Spawn run	0.394405	0.557773	5.047759	2.228139	1.242796

ANOVA Table 5. Primordial initiation of different strain of *C. indica* on wheat grains raised spawn in wheat straw treated by chemical method.

Source of Variation	DF	SS	MS	F-Cal	F-Table
Treatment	4	8.6666667	2.166667	6.5	3.47805
Error	10	3.333333	0.333333		
Total	**14**				

TABLE OF SEM, SED, CV, T-value AND C.D.

Factors	SE(m)	SE(d)	CV(%)	T-value	C.D.
Primordial initiation	0.333333	0.471405	7.216878	2.228139	1.05035

ANOVA Table 6. Pileus diameter of different strain of *C. indica* on wheat grains raised spawn in wheat straw

Source of Variation	DF	SS	MS	F-Cal	F-Table
Treatment	4	193.7446	48.43615	4.469104	2.866081
Error	20	216.76	10.838		
Total	**24**				

TABLE OF SEM, SED, CV, T-value AND C.D.

Factors	SE(m)	SE(d)	CV(%)	T-value	C.D.
Pileus diameter	1.472277	2.082114	48.4419	2.085963	4.343214

ANOVA Table 7. Stalk length of different strain of *C. indica* on wheat grains raised spawn in wheat straw

Source of Variation	DF	SS	MS	F-Cal	F-Table
Treatment	4	317.64	79.41	6.959684	2.866081
Error	20	228.2	11.41		
Total	**24**				

TABLE OF SEM, SED, CV, T-value AND C.D.

Factors	SE(m)	SE(d)	CV(%)	T-value	C.D.
Stalk length	1.510629	2.136352	25.17041	2.085963	4.456352

ANOVA Table 8. Stalk diameter of different strain of *C. indica* on wheat grains raised spawn in wheat straw

Source of Variation	DF	SS	MS	F-Cal	F-Table
Treatment	4	4.1304	1.0326	1.762116	2.866081
Error	20	11.72	0.586		
Total	**24**				

TABLE OF SEM, SED, CV, T-value AND C.D.

Factors	SE(m)	SE(d)	CV(%)	T-value	C.D.
Stalk diameter	0.342345	0.484149	21.09996	2.085963	1.009917

ANOVA Table 9. Average weight of sporophore of different strain of *C. indica* on wheat grains raised spawn in wheat straw

Source of Variation	DF	SS	MS	F-Cal	F-Table
Treatment	4	2826	706.5	3.980282	2.866081
Error	20	3550	177.5		
Total	**24**				

TABLE OF SEM, SED, CV, T-value AND C.D.

Factors	SE(m)	SE(d)	CV(%)	T-value	C.D.
Average weight	5.958188	8.42615	20.06463	2.085963	17.57664

ANOVA Table 10. Yield/bag of different strain of *C. indica* on wheat grains raised spawn in wheat straw

Source of Variation	DF	SS	MS	F-Cal	F-Table
Treatment	4	443983.3	110995.8	89.03409	3.47805
Error	10	12466.67	1246.667		
Total	**14**				

TABLE OF SEM, SED, CV, T-value AND C.D.

Factors	SE(m)	SE(d)	CV(%)	T-value	C.D.
Yield/bag	20.38518	28.829	8.827042	2.228139	64.23501

ANOVA Table 11. Spawn run of strain CI-524 of *C. indica* on different grains raised spawn in wheat straw treated by $CaCO_3$

Source of Variation	DF	SS	MS	F-Cal	F-Table
Treatment	4	4.266667	1.066667	4	3.47805
Error	10	2.666667	0.266667		
Total	**14**				

TABLE OF SEM, SED, CV, T-value AND C.D.

Factors	SE(m)	SE(d)	CV(%)	T-value	C.D.
Spawn run	0.298142	0.421637	4.327356	2.228139	0.939466

ANOVA Table 12. Primordial initiation of strain CI-524 of *C. indica* on different grains raised spawn in wheat straw

Source of Variation	DF	SS	MS	F-Cal	F-Table
Treatment	4	6.266667	1.566667	5.875	3.47805
Error	10	2.666667	0.266667		
Total	**14**				

TABLE OF SEM, SED, CV, T-value AND C.D.

Factors	SE(m)	SE(d)	CV(%)	T-value	C.D.
Primordial initiation	0.298142	0.421637	8.419529	2.228139	0.939466

ANOVA Table 13. Pileus diameter of different strain of *C. indica* on wheat grains raised spawn in wheat straw

Source of Variation	DF	SS	MS	F-Cal	F-Table
Treatment	4	91.00074	22.755018	1.996808	2.866081
Error	10	227.8655	11.39327		
Total	**14**				

TABLE OF SEM, SED, CV, T-value AND C.D.

Factors	SE(m)	SE(d)	CV(%)	T-value	C.D.
Pileus diameter	1.509521	2.134786	44.56317	2.085963	4.453085

ANOVA Table 14. Stalk length of strain CI-524 of *C. indica* on different grains raised spawn in wheat straw

Source of Variation	DF	SS	MS	F-Cal	F-Table
Treatment	4	221.96	55.49	4.884683	2.866081
Error	10	227.2	11.36		
Total	**14**				

TABLE OF SEM, SED, CV, T-value AND C.D.

Factors	SE(m)	SE(d)	CV(%)	T-value	C.D.
Stalk length	1.507315	2.131666	23.97198	2.085963	4.446577

ANOVA Table 15. Stalk circumference of strain CI-524 of *C. indica* on wheat grains raised spawn in wheat straw

Source of Variation	DF	SS	MS	F-Cal	F-Table
Treatment	4	5.4296	1.3574	2.71263	2.866081
Error	20	10.008	0.5004		
Total	**24**				

TABLE OF SEM, SED, CV, T-value AND C.D.

Factors	SE(m)	SE(d)	CV(%)	T-value	C.D.
Stalk diameter	0.316354	0.447392	20.00536	2.085963	0.933244

ANOVA Table 16. Average weight of sporophore of strain CI-524 of *C. indica* on different grains raised spawn in wheat straw

Source of Variation	DF	SS	MS	F-Cal	F-Table
Treatment	4	2776	694	1.797927	2.866081
Error	20	7720	386		
Total	**24**				

TABLE OF SEM, SED, CV, T-value AND C.D.

Factors	SE(m)	SE(d)	CV(%)	T-value	C.D.
Average weight	8.786353	12.42578	30.04111	2.085963	25.91972

ANOVA Table 17. Yield/bag of strain CI-524 of *C. indica* on different grains raised spawn in wheat straw

Source of Variation	DF	SS	MS	F-Cal	F-Table
Treatment	4	495183.3	123795.8	235.8016	3.47805
Error	10	5250	525		
Total	**14**				

TABLE OF SEM, SED, CV, T-value AND C.D.

Factors	SE(m)	SE(d)	CV(%)	T-value	C.D.
Yield/bag	13.22876	18.70829	4.613331	2.228139	41.6846

RESUME

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