

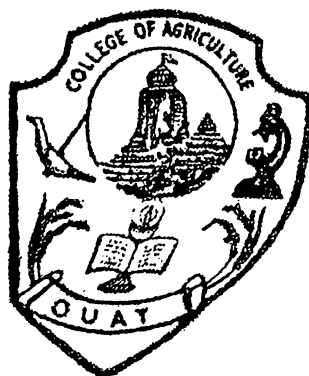
# **IMPROVEMENT OF BIOEFFICIENCY OF PADDY STRAW MUSHROOM**

**A  
THESIS SUBMITTED TO THE  
ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY,  
BHUBANESWAR  
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS  
FOR THE DEGREE OF**

**MASTER OF SCIENCE IN AGRICULTURE  
(PLANT PATHOLOGY)**

**BY**

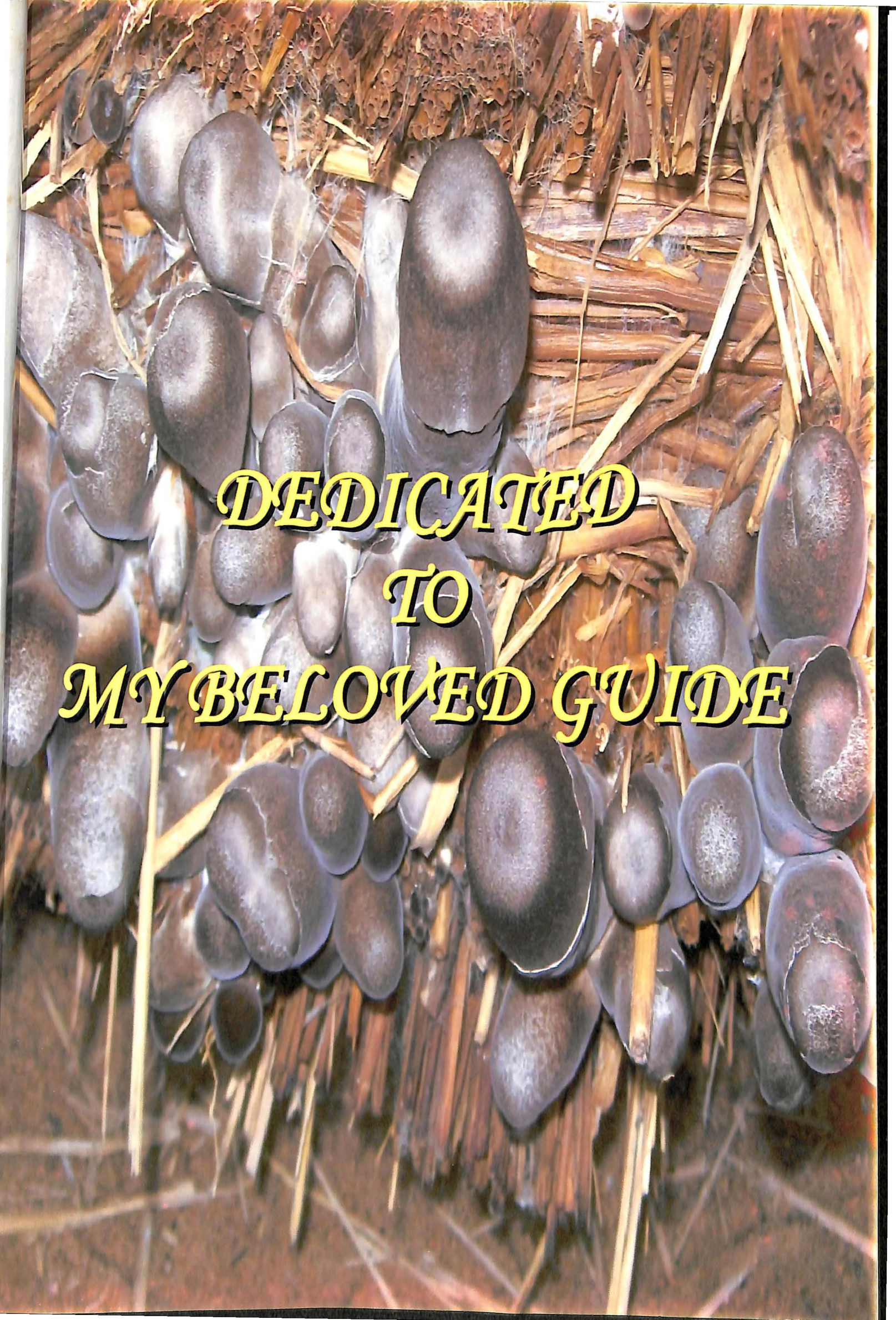
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A photograph of a bird's nest filled with dark, oval-shaped eggs, with the text "DEDICATED TO MY BELOVED GUIDE" overlaid in yellow. The nest is made of dry, brown twigs and sticks, and the eggs are arranged in a somewhat circular pattern. The text is centered and written in a bold, yellow, serif font with a black outline.

**DEDICATED  
TO  
MY BELOVED GUIDE**



ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY  
DEPARTMENT OF PLANT PATHOLOGY  
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**CERTIFICATE- I**


*This is to certify that the thesis entitled “IMPROVEMENT OF BIOEFFICIENCY OF PADDY STRAW MUSHROOM” submitted in partial fulfillment of the requirement for the award of the degree of MASTER OF SCIENCE (AGRICULTURE) in the discipline of (PLANT PATHOLOGY) to the Orissa University of Agriculture and Technology, Bhubaneswar in an authentic record of bonafide research work carried out by SURENDRA DELKI under my guidance and supervision.*

*This research work in original and no part of this thesis has been submitted for any other degree or diploma. The assistance received during the course of investigation has been acknowledged by him.*

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## CERTIFICATE-II

This is to certify that the thesis entitled "**IMPROVEMENT OF BIOEFFICIENCY OF PADDY STRAW MUSHROOM**" submitted by **SURENDRA DELKI** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in the discipline of **PLANT PATHOLOGY** has been approved by the Student's Advisory Committee after an oral examination on the same in collaboration with the External Examiner.

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## ABSTRACT

Paddy straw mushroom cultivation has been commercialized in the state of Orissa since 1992. However, at present the level of productivity is low (10 % B.E.). There is scope for yield improvement through using productive strains and effective substrate management. During the present course of investigation, attempts have been made to explore the effect of substrate management on the productivity of *Volvariella volvacea*.

Among the different inorganic salts and their combinations supplemented to cereal grain substrate, calcium carbonate @ 3.0% supported the highest mean mycelial growth of 108.8mm at 10 days after inoculation.

Pre-soaking of substrate for six hours registered significantly highest yield of 1028.75 g/bed with a corresponding biological efficiency of 14.69 % among the six soaking periods evaluated.

Substrate soaking in 2.0% calcium carbonate solution resulted in early appearance of pinheads (8.00 d), highest number of fruiting bodies / bed (54.50) and yield (968.75 g) with the biological efficiency of 13.83% among the four concentrations evaluated.

Eight plant growth regulators were tried for their possible role in promoting mushroom productivity. The highest yield of 920.00 g/bed was realized from the application of Indole acetic acid @ 200 ppm concentration in comparison to control (753.33 g/bed).

The semi-composed paddy straw substrate pasteurized either through physical or chemical agents proved its inferiority in terms of yield and biological efficiency (7.27 and 7.40 % B.E. respectively) in comparison to the conventional method of cultivation (11.31 % B.E.).

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# *Chapter I*



## **INTRODUCTION**



# INTRODUCTION

---

Mushrooms have been used and prized as a delicacy for more than two thousand years. The Greek philosopher, Theophrastus (372-287B.C.), wrote that mushrooms gathered from farm lands, fields and meadows were valued as food. During the centuries that followed and through the Middle Ages, the Greeks and Romans, most especially, considered mushrooms as a special food. The association of fungi with thunderstorms was common in mythology, and it was formerly believe that mushrooms were formed by lightening and thunderstorms.

Many years before the development of mushrooms production in the United States and Europe, the Chinese had long been growing mushrooms but of different species. Literature references indicate (Chang and Miles,1987) that *Auricularia auricula* was first cultivated in China in A.D.600 while *Flammulina velutipes* was grown around A.D. 800-900. Cultivation of other mushrooms such as *Lentinula edodes*, *Volvariella volvacea*, and *Tremella fuciformis* was first recorded in China in the year 1000, 1700, and 1800 respectively. The European button mushroom (*Agaricus bisporus*) was cultivated in France around 1600A.D. on a composted substrate.

The rapid development and growth of the mushroom industry from a primitive cave culture into one using more highly technical and controlled methods was stimulated in the 1960s. With the establishment of laboratories for research on mushroom growing not only in United States and Europe but also in Japan, Taiwan and Korea, improved technologies on the use of mushroom growing houses and use of

pure culture spawn resulted in the rapid and increased production of mushrooms worldwide. Mushrooms are now produced in some 80 countries around the world (Flegg, et al. 1985). However, China is the largest producer and consumer of mushrooms in the world (Wakchaure, 2011).

Mushroom is considered to be a complete, health food and suitable for all age groups. Mushrooms are rich in protein, dietary fibre, vitamins and minerals. The digestible carbohydrate profile of mushroom includes starches, pentoses, hexoses, disaccharides, amino sugars, sugar alcohols and sugar acids. The crude fibre composition of the mushroom consists of partially digestible polysaccharides and chitin. Edible mushrooms commonly have insignificant lipid level with higher proportion of polyunsaturated fatty acids. All these results in low calorific yield from mushroom foods. Mushrooms do not have cholesterol. Instead, they have ergosterol that acts as a precursor for Vitamin D synthesis in human body. The protein content of edible mushrooms is usually high, but varies greatly. The crude protein content of mushrooms varied from 12-35% depending upon the species. The free amino acids composition differs widely but in general they are rich in threonine and valine but deficient in sulphur containing amino acids (methionine and cysteine).

Mushrooms produce a wide range of enzymes that can degrade complex agricultural, industrial and forest wastes like cereal straws, coconut and coffee waste, millet straws, saw dust, sugarcane bagasse, cotton waste etc. which are available in huge quantities in our state that can be utilized for production of protein rich food. Wheat and paddy straw have become surplus possibly due to green revolution and

breakthrough in rice production. Mushroom cultivation offers great scope for its proper utilization. Thus cultivation of edible mushroom could be an economically viable proposition for the bio-conversion of lingo-cellulosic wastes. Though a variety of substrates have been used for cultivation, the most popular substrate is still paddy straw in rice producing countries and cotton waste in industrialized area (Chang, 1978).

Modern classification has placed the fungi in a group distinct from plants and animals and has assigned them to the Kingdom Myceteae, distinct from the traditional Kingdom Plantae (Alexopolous and Mims, 1979). Most of the cultivated edible mushrooms belong to the order Agaricales of the class Hymenomyces belonging to the sub-division Basidiomycotina. This order is composed of fungi forming fleshy, usually umbrella like fruit bodies. The term mushroom refers to this fruit body, which is otherwise called as the basidiocarp.

Species of mushroom have particular preference to temperature, relative humidity and substrate. Generally spring in the temperate regions and rainy season in the tropical region are the best season of the year to go for mushroom hunting.

India is gifted with distinct season namely summer, rainy and winter. Accordingly, mushroom can be selected and cultivated round the year. Straw mushroom can be selected and cultivated round the year. Straw mushroom (*Volvariella spp.*) can be grown in summer and rainy season in the temperature range of 25 – 40°C. Oyster mushroom (*Pleurotus spp.*) can be grown in winter season when

temperature is about 20 – 30°C and the white button mushroom (*Agricus bisporus*) can be grown in winter season in the temperature range of 15 to 22° C

The paddy straw mushroom (*Volvariella volvacea*) is cultivated from West Africa to India and the far East (Chang and Miles, 1987). Large scale production has been extended to Taiwan, Malaysia and South Korea (Dal caire, 1987). However it is the most popular mushroom of Thailand, China, Japan, Korea, Philippines, Indonesia, Taiwan, India. The cultivated species of *Volvariella* is recognized by various names such as Chinese mushroom, Paddy straw mushroom, Nanhua mushroom and warm or tropical mushroom.

Thomas *et al.* (1943) first cultivated the paddy straw mushroom at the College of Agriculture, Coimbatore. Thereafter, many improvement and modification in the growing method were worked out at several research centres in India (Asthana, 1947; Rath, 1961; Ramakrishnan *et al.*, 1980 and Mohapatra *et al.*, 2011). Being as tropical mushroom, it is highly suitable for northern plains as well as costal and plateau areas of South India.

Three species of *volvariella* are grown in India namely, *V. volvacea*, *V. diplasia*, *V. esculenta*. The climatic condition in the plains of India are suitable for commercial cultivation of paddy straw mushroom. The growing season starts in March and continues upto end of October which provide ideal temperature for appearance of fruit bodies. Vegetative growth of *volvariella* occur profusely at 32-

34°C. An important feature of *Volvariella* is its fast growth; only 14-15 days are required from spawning to harvest.

However, poor crop management during any phase of production of *Volvariella* will decrease the yield substantially. Straw mushroom has got the ability to use cellulosic materials more effectively than other cultivated mushroom.

The primordium of the mushroom can be formed 8-10 days after spawning under favourable environmental condition and on a suitable growth medium. *Volvariella* has been considered to be one of the easiest mushroom to cultivate, but even under appropriate condition, the biological efficiency is lower than that of most other cultivated mushroom. A variety of waste materials has been used for cultivation of paddy straw mushroom such as paddy straw, banana leaf, saw dust, cotton waste, water hyacinth and oil palm pericarp waste. (Ahlawat and Kumar, 2005)

Paddy straw mushroom has been popular vegetarian diet of the people of Orissa since long. People in the rural areas were in the habit of collecting this mushroom grown naturally on straw piles during the rainy season. But it could not find a regular place in the diet due to its non availability in other seasons. Now the scenario has changed altogether and it is cultivated 8-10 months a year. Besides, method for off-season cultivation of paddy straw mushroom in the coastal ecosystem of the state are in the offing.

The conditions in the most of the agro-climatic situations of Orissa are suitable for taking up cultivation of a number of edible mushrooms. Diverse agro-waste for mushroom cultivation are also available in abundance. Paddy straw, the main input of mushroom cultivation is plentifully available at cheaper rates. Besides, cultivation is comparatively easier, as it involves minimum investment, labour and space. Mushroom cultivation has been commercialized in the state since 1992 with the establishment of Centre of Tropical Mushroom Research and Training in the Department of Plant Pathology, Orissa University of Agriculture and Technology, involving thousands of growers in both the coastal as well as the interior districts round the year.

Besides the availability of paddy straw in abundance, other agricultural wastes such as straw of jowar, bajra and ragi, maize stalks and cobs, paddy husk, coir pith, jute stick and wastes of agro-based industries like cotton, jute waste, paper pulp, saw dust etc. are available.

In India, Orissa is the leading state in terms of straw mushroom production. About 2835 tonnes of mushroom is produced every year in the state generating a turnover of over 20 crores of rupees. It is cultivated under thatched roof (indoor cultivation) or as an intercrop in the coconut plantations (outdoor cultivation) in the coastal ecosystem.

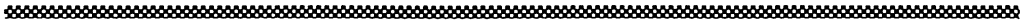
Farmers utilize paddy straw for cultivation of straw mushroom as it is plentifully available at a cheaper rate throughout the state. In most cases, cultivation is done on

non-pasteurized substrate with incorporation of pulse powder or wheat bran. However, the biological efficiency varies from 10-15%, which is low. Further, paddy straw is prone to mushroom contaminants. But there is a scope for yield increase by using the productive strains of the species and by effective substrate management in terms of appropriate straw processing before raising beds along with cultivation in controlled condition using semi-composted substrate.

In this context, studies have been planned on improvement of spawn quality through enrichment of spawn substrate with inorganic salts, substrate management through appropriate period of soaking in calcium carbonate solution, application of growth regulators and cultivation in controlled condition with semi-composted substrate for obtaining higher productivity.



# *Chapter II*



## **REVIEW OF LITERATURE**



# REVIEW OF LITERATURE

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Paddy straw mushroom is an edible mushroom of the tropics and sub-tropics. It was first cultivated in China as early as in 1822. Around 1932-35, the straw mushroom was introduced into Philippines, Malaysia, and other South- East Asian countries by overseas Chinese. In India this mushroom was first cultivated in early 1940's. In India, 19 edible species of *Volvariella* have been recorded but cultivation methods have been devised for three of them only viz: *V. esculenta* (mass) Sing., *V. diplasia* (Berk and Br.) Sing. and *V. volvacea* (Bull.ex Fr) Sing. A review on available literatures has been exercised particularly in the area of research included in this thesis in relation to cultivation procedure of *V. volvacea*.

## 2.1 Straw Mushroom Production : Present Scenario

Paddy straw mushroom is the 6<sup>th</sup> largest cultivated mushroom in the world with an annual production of 180800 metric tonnes contributing to 6.1% of the total mushroom production (Chang and Miles, 2004).

It has been in cultivation in the coastal states like Orissa, Andhra Pradesh, Tamil Nadu, Kerala and West Bengal (Ahlawat and Tewari, 2007).

**Mohapatra et al.** (2010) indicated that under conventional method of farming, the yield is quite unstable and low with an average bioefficacy of 10%. This was attributed to the use of poor quality spawn and inappropriate after care of beds and management of biotic and abiotic disorders.

**Singh (1911)** opined that straw mushroom cultivation has been restricted to Orissa. This variety has low yield and poor keeping quality. However, pasteurized paddy straw substrate supplemented with cotton seed hulls could give better productivity.

At present, total production of paddy straw mushroom in Orissa is about 2835 tonnes per annum, emanating from over 3500 production units out of which 95% are seasonal ones (Anon, 2011).

## **2.2 Spawn Production**

### **2.2.1 Effect of substrate on spawn quality**

**Yong (1978)** recommended the use of leafmeal of Ipil ipil (*Leucaena leucocephala*) as a good substrate for mushroom spawn production.

**Purkayastha et al, (1980)** reported that maximum production of fruit bodies would be obtained from spawn based on wheat grain, about 65% increased in yield was obtained with grain spawn as compared to paddy straw spawn. Rice husk and rice bran would also be considered for spawn preparation. Spawn prepared from cotton seed waste failed to produce, any fruiting bodies.

**Bisht and Harsh (1984)** indicated that waste tea leaves could be used with boil wheat grain for spawn production of *Volvariella volvacea*.

**Mascarenhas and Inamdar (1991)** recommended the use of hay spawn for better productivity and quicker mycelial run. Spawn prepared with waste tea leaves

showed poor performance in terms of vegetative growth as well as production of fruiting bodies.

**Chang and Miles (1994)** used the rice straw *Azolla (Azolla pinnata)* Ramie (*Bachmeria nivea*) leaves and Ipil ipil (*Leucaena leucocephala*) leaves as substrate of spawn which gave good results.

**Upadhyay et al. (2004)** indicated that the cereal grain like wheat, maize, bajra, jawar carry a greater reserve of food material per grain to sustain the inoculum of mushroom mycelium.

**Mohapatra et al, (2010)** recorded highest sporophore yield (1103.33g/bed) raised from paddy grain spawn with bioefficiency of 14.95%.

### **2.2.2 Supplementation of Spawn Substrate**

**Chang (1978)** observed that performance of spawn based on paddy straw without meal was equal to the spawn prepared on rice straw or wheat straw or sugarcane bagasse added with wheat or gram powder.

**Tewari (1985)** reported that spawn prepared on paddy straw pieces mixed with 4% and 3% gypsum exhibited dense mycelial growth and brick red chlamydospores and on sorghum mixed with 4% and 6% gypsum produced similar yields. He explored the preparation of spawn on sterilized chopped paddy straw and sorghum with varying amount of calcium carbonate (0 to 10%) and gypsum (0 to

6%). Production of spawn on rice straw was best when 4 to 5% gypsum was added while sorghum with 4 to 6% calcium carbonate gave good results.

**Mohammed and Shah** (1989) experimented tea leafs as substrates of spawn with supplementation of rice bran (0, 5, 10 and 20%). It was revealed that the rate of growth of mycelium increased to a maximum of 10cm with 10% bran. But it declined to control level or below as the proportion of bran increased to 20%.

**Mascarenhas and Inamdar** (1991) reported that Bengal gram (10%) in spawn gave the maximum mycelial growth as well as highest yield when compared to 1% bengal gram and rice bran.

**Upadhyay et al.** (2004) reported that cereal grains mixed with gypsum and chalk powder could raise the pH of grains to around 7 to 7.5, which was congenial for growth of *Volvariella volvacea*.

### **2.3 Evaluation of Substrate for Mushroom Production**

A variety of waste materials has been used for cultivation of paddy straw mushroom which include : paddy straw (Chang,1965), water hyacinth (Chang and Mok, 1971), oil palm bunch (Naidu, 1971), oil palm pericarp waste (Graham and Yong, 1974; Yong and Graham, 1973), banana leaves and saw dust (Chua and Ho, 1973), cotton waste (Chang, 1974, Hu *et al.*, 1973 and Yau and Chang, 1972), sugarcane bagasse (Hu *et al.*, 1973, 1976).

**Chang (1974)** outlined the cultivation of *Volvariella volvacea* on cotton waste compost. Fermented cotton waste mixed with 4% rice and wheat bran and 4 to 6% limestone gave higher production.

**Pal and Roy (1982)** studied the agronomic management practices for successful cultivation of *Volvariella volvacea* in the plain of West Bengal and indicated that leaves of banana, corn and sugarcane bagasse were equally suitable with that of paddy straw.

**Khan et al. (2002)** evaluated eight different agricultural wastes and found 50% sugarcane bagasse and 50% cotton waste as the highest yielder in paddy straw mushroom (*Volvariella volvacea* strain P.K.101).

**Obodai et al. (2003)** indicated that the biological efficiencies of two strains of *Volvariella volvacea*, VVI and VVO were highest on banana leaves (72 and 43 percent, respectively) among three lignocellulosic wastes evaluated.

**Kaur et al. (2004)** reported that rice straw beds supplemented with rice bran gave the maximum yield of 21.80kg for MTCC 957strain and 27.1kg for VVI strain per 100kg substrate out of eight supplements evaluated. Cotton waste and its compost gave the lowest yield when used as a substrate.

**Pramod et al.** (2004) studied the production of fruiting bodies by *V. volvacea* on various substrates and observed that beds with oil palm bunch waste produced greatest number of sporocarps (24) with the highest biological efficiency.

**Belewu and Belewu** (2005) observed that the biological efficiency of *V. volvacea* on banana leaf substrate was 15.21%. Full colonization of substrate was recorded in 15 days. The studies revealed potential of banana leaves as a good substrate for the cultivation of paddy straw mushroom and the spent substrate as a viable ingredient in ruminant feed.

**Mahbuba et al.** (2008) reported that the highest biological efficiency of straw mushroom (20.34%) was observed on rice straw, while 8.62%, 8.44%, 5.07%, 2.77% and 1.37% B.E. were obtained from rice straw + cotton waste, cotton waste, rice straw + sugarcane bagasse and sugarcane bagasse respectively.

**Ukoima et al.** (2009) cultivated straw mushroom on various farm wastes and reported that the highest yield of 345g was recorded from palm fibre, followed by 231g from rice husk and 146g from saw dust. Palm fibre was considered the most suitable farm waste for growing *V. volvacea*.

**Onuoha et al.** (2009) screened agro waste materials like paddy straw, oil palm fibre, saw dust and a mixture of oil palm fibre and saw dust and found that growth and production of fruit bodies on oil palm fibre was similar to that of paddy straw. The production of fruit bodies on the mixture of oil palm fibre and saw dust was scanty.

Saw dust alone as a substrate produced few fruit bodies that were comparatively small in size.

## **2.4 Substrate Management for Mushroom Production**

**Yee and Yung** (1980) reported that cultivation of *Volvariella volvacea* was favoured by alkaline pH of the compost which suppressed the growth of weed fungi including *Coprinus* sp., *Aspergillus* sp., that produced metabolites antagonistic to rice straw mushroom.

**Bhavani Devi** (1982) reported that the optimum period of soaking the straw before layout of the bed was found to be 3 to 15 hours. However, the maximum yield was obtained from bed prepared with straw pre-soaked for 6 hours with a moisture content of 73-75%.

**Thakur and Yadav** (2006) opined that fresh yield of straw mushroom in 18 hours of wetting was considerably higher and varied from 1.79 to 11% with an average of 5.71% as against partial composting in which the yield varied from 3.31 to 6% with an average yield of 4.8%.

**Ahlawat and Tewari** (2007) reported that immersing of bundles in clean water with 2% calcium carbonate for 12-18 hours in a cemented water tank could improve the bioefficiency of straw mushroom substantially.

**Behera et al**, (2010) observed that soaking of straw in 2% calcium carbonate powder for 6 hours gave the highest biological efficiency of 10.17% along with lowest incidence of competitor moulds out of ten different treatments evaluated.

## **2.5 Growth regulators for yield enhancement**

**Despande and Tamhane** (1982) observed that the yield of straw mushroom was increased 17.3% by spraying with a 2% glucose solution. Spraying with 200 ppm IAA and NAA increased the yield by 10.6 and 8%, respectively.

**Kundu** (2003) reported that vitamins viz; thiamine, biotin, pyridoxine and inositol were more or less equal in improving growth and protein production of the test fungi at different levels.

**Ahlawat** (2011) found stimulatory effect of IBA on mycelial growth of *Agaricus bisporus* under *In vitro* conditions. The spray stimulated early pinning as well as higher yield of mushroom. The results are equally well both in *A. bisporus* and *A. bitorquis*.

## **2.6 Indoor Cultivation**

An indoor cultivation technology was introduced in the early 1970s enabling cultivation all the year round and varied types of agricultural wastes can also be used. The introduction of cotton waste in place of paddy straw in Hong kong increased and stabilized the yield considerably (Chang, 1979).

**Quimio *et al*, (1990)** indicated that the indoor cultivation technology is now-a-days practised in Thailand, Indonesia, Vietnam, Singapore and Malaysia. Biological efficiency of about 25-50% can be obtained by using cotton waste. The combination of rice straw and cotton waste also gave good yield of about 21.8 - 27%.

**Quimio (1993)** refined the indoor method of paddy straw mushroom cultivation for obtaining higher yields as compared to conventional method of cultivation.

**Ahlawat and Tewari (2007)** observed that cotton waste under indoor method of cultivation gives a higher and more stable yield (30 – 40%) along with early fructification and harvesting.



# *Chapter III*



# **MATERIALS & METHODS**



# MATERIALS AND METHODS

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The present investigations were undertaken on the collection, purification and multiplication of the test fungus, improvement of spawn quality through enrichment of spawn substrate with inorganic salts, management of substrate through soaking in lime water and through application of growth regulators, standardisation of compost preparation and indoor farming of paddy straw mushroom to evaluate the biological efficiency.

The materials used and methods followed in the present study are as follows :

## **Test Fungus**

The present study was limited to only *Volvariella volvacea*. The test fungus was procured from the Centre of Tropical Mushroom Research and Training, Department of Plant Pathology, Orissa University of Agriculture and Technology, Bhubaneswar.

## **Maintenance of Culture**

The pure culture of the fungus was maintained on Potato Dextrose Agar (PDA) slants throughout the period of investigation. Sub-culturing of the fungus was done at an interval of 15 days and stored at  $28\pm 1^{\circ}\text{C}$ . Seven days old pure mycelial cultures of the test fungus was used in various studies.

## **Cleaning and Sterilization of Glasswares**

Borosil glasswares such as culture tubes, petridishes, conical flasks etc. were used throughout the period of investigation. Standard procedures for cleaning and sterilization of glasswares (Riker and Riker, 1936) were adopted. All the glasswares were cleaned in dilute solution of potassium dichromate and sulphuric acid (60g potassium dichromate per litre distilled water, 60ml of concentrated sulphuric acid added slowly to it) followed by thorough washing with tap water and subsequent rinsing with distilled water before use. Petridishes, culture tubes etc. wrapped with paper were sterilized in hot air oven at 160°C for 2 hours.

## **Sterilization by Flame**

The scalpels, inoculating needles, glass rods, nichrome wire loops etc. were sterilized by dipping them in 70% Ethanol followed by flaming over spirit lamp.

## **Inoculation and incubation**

Strict aseptic conditions were maintained in the inoculation chamber at the time of inoculation. Agar blocks of approximately 1cm X 1cm size containing the mushroom inoculum were taken out of the culture starts with the help of inoculating needle and inoculated in flat bottles containing Potato Dextrose Agar media or in spawn bottles were incubated at room temperature unless otherwise specified.

## **Preparation of Potato Dextrose Agar (PDA)**

Potato Dextrose Agar, the most commonly used medium for growing paddy straw mushroom was prepared as follows :

Two hundred grams of peeled and sliced potato was boiled in 500ml of distilled water till the tissues were softened. Then the extract was filtered through a clean muslin cloth. Twenty gram agar was boiled in 500ml of distilled water taken in enamel mug till it melted completely. Both the solutions were mixed subsequently. Twenty gram of Dextrose was added and the volume was made upto 1000ml.

Before sterilization, aliquots of 10ml of the medium were taken in culture tubes of 40ml capacity for preparation of agar slants. Media to be poured into petridishes were taken in Erlenmeyer conical flasks. The culture tubes and the conical flasks were plugged with non-absorbent cotton and autoclaved at 15 p.s.i. for 20 minutes. In order to suppress bacterial contamination streptomycin sulphate @ 500mg per 1000ml was added to the medium before autoclaving. These were subsequently stored in a refrigerator at 5-8°C for further use.

### **Preparation of Potato Dextrose broth (PD)**

Potato Dextrose, the most commonly used liquid medium for growing paddy straw mushroom fungus was prepared as follows :

200 gram of peeled and sliced potatoes was boiled in 500ml of distilled water till the tissues were softened. Then the extract was filtered through a clean muslin cloth. Twenty gram of Dextrose was added and the volume was made upto 1000ml. Streptomycin sulphate @ 500mg per 1000ml was added for the suppression of bacterial contamination. One hundred ml of broth was poured into each of the conical

flasks and plugged with non-absorbent cotton and autoclaved at 15 p.s.i. for 20 minutes. These were subsequently stored in a refrigerator at 5-8°C for further use.

## **Spawn Preparation**

Spawn is a living ramified mycelium multiplied under highly hygienic conditions and used as material for cultivation of mushroom under controlled climatic conditions throughout the year.

Wheat grains were used as the base material for multiplying the mycelium in the spawn bottles. The bold, healthy and pesticide free grains were chosen for substrate purpose.

The procedure of spawn preparation was as follows :

1. The wheat grains were washed several times to remove the suspended particles or foreign materials.
2. Other grains were boiled in a container with water till they softened. Care was taken that in no case starch oozed out of the grains.
3. The boiled grains were spread in a thin layer over a polythene sheet under shade for draining out excess water from grain surface to avoid greasiness.
4. Calcium carbonate powder @2% of the dry substrate was thoroughly mixed with the grains for maintenance of proper pH (6.5 – 7.0) in the spawn substrate.
5. Then the grains were filled upto  $\frac{3}{4}$ <sup>th</sup> of the wide mouthed empty saline bottles (450ml capacity).

6. The bottles were plugged with non-absorbent cotton and sterilized in an autoclave at 22 p.s.i. for 2 hours.
7. After sterilization the bottles were allowed to cool overnight and then transferred to the inoculation chamber.
8. The inoculation chamber was sterilized by spraying 40% formaldehyde solution. The sterilized bottles were inoculated under laminar flow kept in an inoculation chamber. The inoculating needle was properly sterilized by flame and transfer of the culture was made close to the flame of the spirit lamp.
9. Sterilized bottles were inoculated with agar blocks (1cm X 1cm) containing mushroom culture of one week old. The inoculated bottles were incubated at  $28 \pm 1^{\circ}\text{C}$  for 10-15 days.
10. The mycelium covered the bottles in about 10 days and that time the spawn was ready for cultivating mushroom in large scale.

### **Precautions made**

1. The inoculation was done under aseptic condition.
2. In case there was green or black mould development in the spawn bottles, the entire contents of the bottle was destroyed.
3. One month old spawn was used in the field trials.

## **Cultivation of *Volvariella***

### **Materials used in bed preparation**

Hand threshed tall indica variety of rice was mostly used for preparation of bed. The straw bundles were stored in shade to avoid wetting. Paddy straw was made into bundles of around 15cm diameter. Approximately, 2' length of straw bundles was taken by cutting both the ends with chaff cutter for field experiments. The resulting bundle weighed 500g approximately.

### **The farm house**

All the experiments were conducted in the farm house of Centre of Tropical Mushroom Research and Training, Department of Plant Pathology, Orissa University of Agriculture and Technology, Bhubaneswar.

The structure of farm house of CTMRT was as follows :

Length	-	50'
Breadth	-	20'
Roof	-	Asbestos
Floor	-	Cemented
Windows	-	Wider, covered with fine wire net

### **Light and Air**

To regulate the light and air in the farm house of CTMRT the wider windows were covered with gunny bags which were opened in the East. West direction so that light in the morning and evening percolated through the open windows.

## **Humidity**

To maintain humidity, the gunny bag screens were sprayed with water during cultivation. Moreover, aerial spraying of water was done and floor was also kept moist regularly while watering the substrate.

## **Temperature**

As the straw mushroom required little higher temperature, the experiments were conducted from the month of March to October.

## **Construction of bed platform**

The platforms were made-up of bamboo stricks in square size of 2'X2' on bamboo pillars or bricks raised upto 6" height from floor in order to avoid soil-borne contamination with competitor moulds and insect, pest, infestation. Alternatively, the mushroom beds were prepared on bricks which were also raised 6" from floor. In order to conduct the experiments in scheduled time, racks were made two tiers, so that more number of beds could be accommodated under the same shade.

## **Preparation of mushroom bed**

Twenty bundles of paddy straw weighing about 10kg dry weight were used in each bed 2'x2'x1'9" (length x breadth x height). Paddy straw bundles of 2' length and 15cm diameter were used. The un-crumpled straw devoid of leafy materials was preferred. The tied bundles were soaked in clean and cold water for 6 hours. The straw was sterilized chemically with 125ml of Formalin and 7.5g Bavistin in 100 litres of water (Vijay and Sohi, 1987). The wet bundles were removed from water and

kept inclined for 4 to 6 hours to drain off excess water. Then the uneven ends of bundles were cut by chaff cutter.

Three soaked straw bundles with their butt ends on one side were placed lengthwise very close to each other on the bamboo frame. Then another set of three bundles were placed over them in similar manner but with butt ends on the opposite side. This layer was the first layer where straw bundles were placed in East-West direction. Similarly in second layer, the first three bundles were placed in opposite North-South direction i.e. butt ends right angles to the butt ends of first layer and then another three bundles were placed also in North-South direction with butt ends in the opposite side in order to complete the second layer. Similarly the third layer was prepared over the second layer in East-West direction i.e. similar to the first layer with their butt ends opposite to the second layer. The fourth or top most layer was prepared over the third layer but in a similar way to the second layer setting the butt ends opposite to third layer. The thickness of the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> layers was 6" each, except the fourth or the top most layer which was 3" in thickness. Thus in each layer, six bundles of straw were used whereas two bundles were used in the fourth layer.

Finally, after making the paddy straw bed, it was pressed gently to remove the air gaps in the bed to create appropriate compactness for the mycelial run. All the four sides were trimmed to give the bed a square size shape. Thus each bed was having length 24", breadth 24" and height 21" size.

### **Spawning and additive supplementation**

Three hundred gram spawn was used in each bed of the experiments which was 3% of the dry weight of the substrate. The spawn bottles were broken and the

spawn cakes were split into number of spawn bits of thumb size i.e. 3.0cm each. After the first layer was prepared, spawn bits of thumb size were put on the four sides of the layer at 3" apart leaving 3" from edge. No spawning was done in the centre. After spawning, wheat bran powder was spread over the spawn bits to facilitate the mycelial growth. In a similar way spawning was done in the second layer. But in case of third layer, the bed was spawned on the entire surface. So 50% of the total spawn was spread in the third layer and rest 50% was distributed equally on the first and the second layer. Two hundred gram of wheat bran (2.0%) was spread over the spawn bits after spawning. Like spawning, 50% of the wheat bran powder was supplemented in the third layer, rest 50% was distributed equally on the first and second layer.

### **Maintenance of bed**

The paddy straw mushroom beds were covered with transparent polythene sheets in order to maintain optimum temperature and humidity and also to protect the beds from rain, wind and external contaminants.

The accumulation of moisture in form of water droplets underside the polythene was prevented. The polythene sheet was covered for 8-10 days till budding. Watering was done regularly, after removal of polythene sheet from the substrate keeping in view the climatic conditions.

Watering was avoided at the pin head stage of mushroom in order to prevent abortion and rotting of pin heads. Watering was done with the help of hand compression sprayer (12 lit capacity)

## **Fruiting of straw mushroom**

Within 8 – 10 days, pin heads were developed and the polythene cover was removed. Within 10 to 14 days from spawning fruits of first flush were harvested and then polythene was covered. Then the second harvest from the second flush was recovered after 7 to 10 days.

### **3.1 Effect of inorganic salts and their combinations on spawn quality**

The experiment was conducted in order to ascertain the effect of two inorganic salts and their combinations as supplements to boiled cereal grains in improving growth rate *Volvariella volvacea*.

The clean and boiled wheat grains were dried under shade for draining out excess water as per standard protocol. Two inorganic salts are : calcium carbonate and calcium sulphate singly and in various combinations were thoroughly mixed with the grains for maintenance of pH in the medium. Eight treatments were designed for the purpose as follows:

1. Control (boiled wheat only)
2. Boiled wheat + 2% calcium carbonate
3. Boiled wheat + 3% calcium carbonate
4. Boiled wheat + 2% calcium sulphate
5. Boiled wheat + 3% calcium sulphate
6. Boiled wheat + 1% calcium carbonate + 1% calcium sulphate
7. Boiled wheat + 1.5% calcium carbonate + 1.5% calcium sulphate
8. Boiled wheat + 0.5% calcium carbonate + 2.0% calcium sulphate

These calculations were on dry weight basis of grains used for spawn preparation. In treatments receiving the combination of two salts, first calcium sulphate and calcium carbonates were mixed separately and then the mixture of both was thoroughly mixed with the grains. These mixing was done on smooth and clean surface with gloves in hands to avoid contamination. The grains were filled up in bottles, plugged and sterilized at 22p.s.i for two hours. The sterilized and cooled bottles were inoculated with agar blocks containing the mushroom culture under laminar flow and incubated at  $28 \pm 1^\circ\text{C}$  for 10 days. Five replications were maintained for each treatment in Randomized Block Design.

Observation on downward mycelial growth rate at 72 hours interval was recorded upto 10 days at incubation. Reduction in spawn quality if any was also observed and recorded.

### **3.2 Effect of substrate soaking period on mushroom productivity**

The trial on substrate soaking period was conducted to find out the appropriate period of soaking of the paddy straw substrate for realizing better productivity.

Good quality paddy straw bundles were collected, made to 1.5' length by trimming both the ends and soaked in clean and cold water for 2, 4, 6, 8, 10 and 12 hours. Bundles were taken out, excess water drained-off to 65-70% substrate moisture and beds were raised as per standard procedure. The experiment was laid out in Randomized Block Design with four replications in each treatment. Transparent polythene sheets were covered on the beds and appropriate after care was taken. After the emergence of pin head, the polythene cover was removed, watering done as and

when required and the fruits of first flush were harvested followed by providing the polythene cover once again. Then the second harvest from second flush was recorded.

Observations on days taken for emergence of pinhead, number of fruiting bodies, weight of fruiting bodies and biological efficiency were recorded.

$$\text{Percent Biological Efficiency (BE)} = \frac{\text{Fresh weight of mushroom} \times 100}{\text{Dry weight of substrate}}$$

### **3.3 Pre-soaking of substrate in different concentrations of lime water and its effect on mushroom productivity**

The experiment was designed to find out the appropriate concentration of calcium carbonate in water for soaking of straw substrate before raising beds so as to obtain better yields.

Good quality paddy straw bundles were collected, made to 1.5' length by trimming both the ends and soaked in lime water at 0.5, 1.0, 1.5 and 2.0% concentrations for 6 hours. One control treatment (water without calcium carbonate) was maintained for comparison. Bundles were taken out, excess water drained-off to 65-70% substrate moisture and beds were raised as per standard procedures. The experiment was laid out in Randomized Block Design with four replications in each treatment. Transparent polythene sheets were covered on the beds and appropriate aftercare was taken. After the emergence of pin heads, the polythene cover was removed, watering done as and when required and the fruits of first flush were harvested followed by providing the polythene cover once again. Then the second

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harvest from the second flush was recovered. Observations on days taken for emergence of pin head, number of fruiting bodies, weight of fruiting bodies and biological efficiency were recorded.

### **3.4 Effect of application of growth regulators on mushroom productivity**

An exploratory investigation was undertaken to evaluate some plant hormones, micronutrient mixtures, carbon and nitrogenous compounds for their possible role in promoting the yield of paddy straw mushroom.

The experiment was designed in Randomized Block Design with treatments as follows :

**Table 1: Growth regulators with their concentration and dosage.**

<b>Sl. No.</b>	<b>Treatment</b>	<b>Concentration</b>	<b>Dosage</b>
1.	Glucose	2%	20g/litre water
2.	Urea	2.5%	25g/litre water
3.	Multiplex micronutrient mixture	0.25%	2.5ml/litre water
4.	Multiple samras (amino acid mixture)	0.2%	2.0ml/litre water
5.	Multiplex mushroom spray	0.2%	2.0ml/litre water
6.	Bayer planofix (NAA)	200 ppm	1.0ml/4.5litre water
7.	Hi media IAA	200 ppm	1.0g/4.5 litre water
8.	Hi media GA	50 ppm	50mg/litre water
9.	Control (Water spray)	--	--

Three replications were maintained for each treatment. A control with water spray was maintained for the sake of comparison.

Straw mushroom beds were raised after processing of good quality straw as per protocol. Transparent polythene sheets were covered on the beds and appropriate aftercare was taken. After the emergence of pinheads, the polythene cover was removed. The chemicals were sprayed as per the recommended doses on to the mushroom primordia gently through a hand compression sprayer. Beds were watered as and when required. The fruits of first flush were harvested and the beds were covered once again for recovery of the second flush. Observations on days taken for emergence of pinhead, number of fruiting bodies, weight of fruiting bodies and biological efficiency were recorded.

### **3.5 Evaluation of biological efficiency of straw mushroom in semi-composted substrate**

The study was undertaken to ascertain the role of semi-composted substrate in promoting the biological efficiency of straw mushroom in indoor conditions.

The experiment was designed as per standard protocol (Quimio, 1993). The straw was made to small pieces (2") by chaff cutter. A pile was made (1.5m high X 1.5m wide) by addition of sufficient quantity of water mixed with 1% calcium carbonate and left to ferment in the open, first turning was given after 2 days and 5% wheat bran was mixed along with water. Again the pile was formed and left for fermentation for the next 2 days after which second turning was given and left for 2 days for fermentation. The substrate was then steam pasteurized at 10 p.s.i. for 2 hours in autoclave and allowed to cool naturally. Another lot of semi-composted

substrate was pasteurized with 1.5 litres of formalin and 50 g Bavistin, Carbendazin 50WP per 3 quintals of base material for comparing the productivity with the steam pasteurized substrate. Besides, control beds were raised following conventional method of cultivation. Each treatment was replicated seven times.

The compost was spread on shelves with a thickness of 10-15 cm and spawned with fresh spawn at 2% of the dry weight of the compost. The pieces of broken spawn were inserted in compost at a depth of 2 – 2.5cm at a distance of 12-15cm. Spawn was covered with the displaced compost and bed with thin plastic sheet.

The cultivation was done at ambient temperature (28-30°C) during spawn running for the next 5-6 days. Water and light were not provided during spawn running period, however, little ventilation was given.

The plastic sheets were removed after spawn run was complete with little sprinkling of water over the beds. Light and intermittent fresh air were also provided for initiation and development of primordia.

Observation on number of fruit bodies, weight of fruit bodies and biological efficiency were recorded per 100kg of compost on dry weight basis and compared with the control (Non-composted substrate).

### **3.6 Statistical analysis**

Mean values of each character were worked out by dividing the total with corresponding number of observations. The standard Error (S.E.) and Critical Difference (C.D.) were calculated by using formula (Gomez and Gomez, 1983).

Standard Error of Mean  $[SE(m)] = \sqrt{EMS/r}$

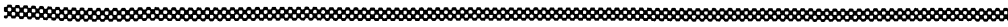
Critical Difference (C.D.) =  $\sqrt{EMS/r} \times 1.414 \times t$  value at error

Degree of freedom at 5% level of significance where, r = number of replications.

EMS = Error Mean Square.



# *Chapter IV*



## **EXPERIMENTAL RESULTS**



## EXPERIMENTAL RESULTS

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### 4.1 Effect of inorganic salts and their combinations on spawn quality

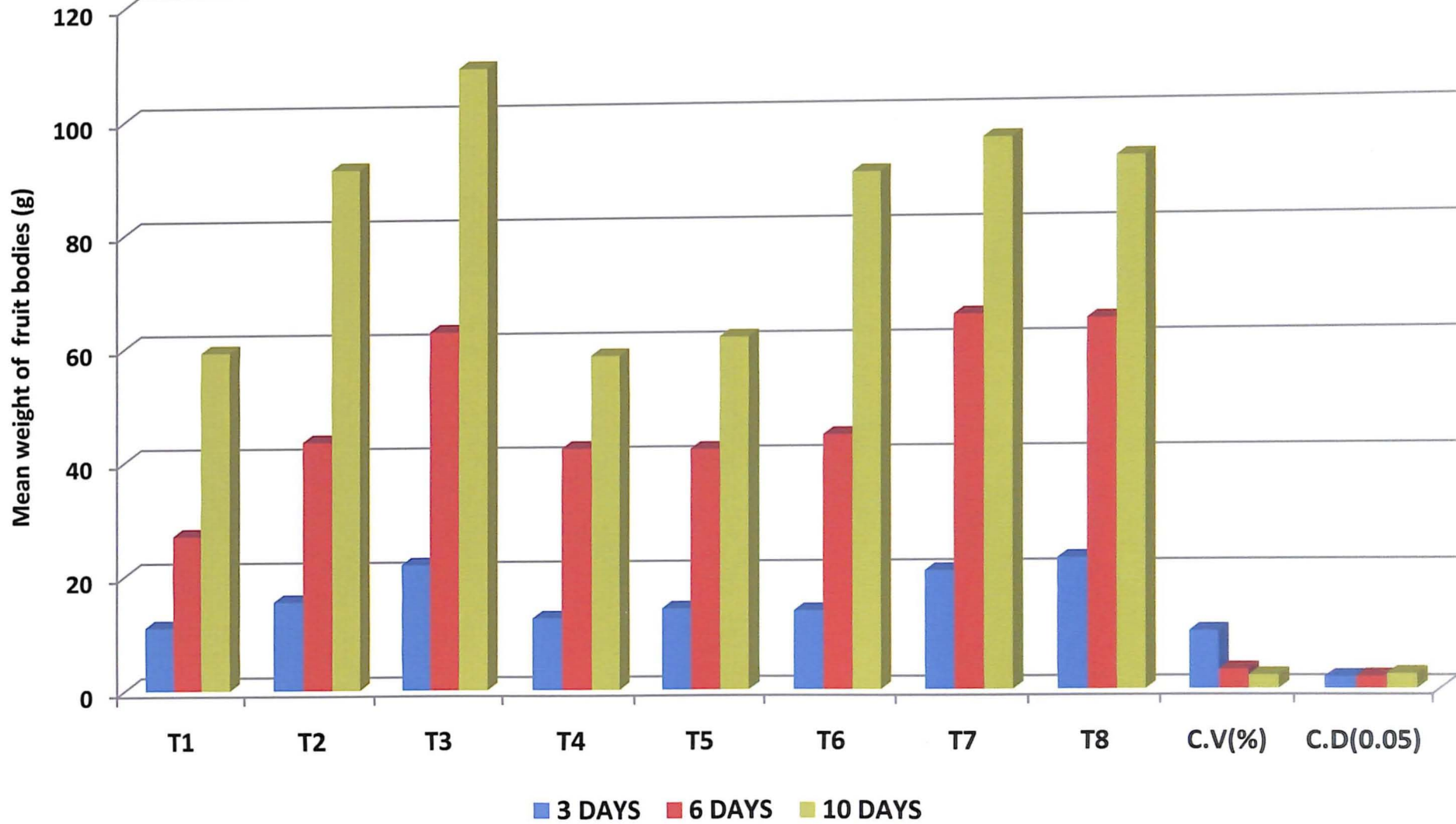
In order to evaluate the potential of few inorganic salts and /or their combinations as supplements to improve the spawn productivity, two salts viz. calcium carbonate and calcium sulphate along with their combinations at different dosages were supplemented with boiled wheat grain before sterilization. Mycelial growth rate of *Volvariella volvacea* was recorded treatmentwise at three days interval up to the end of the incubation period (10 days). The mean data on growth rate of mycelium on three days are presented in Table 2.

The analysed data revealed that out of the eight treatments tried, boiled wheat supplemented with 3% calcium carbonate exhibited highest mean mycelial growth of 108.8mm followed by boiled wheat supplemented with calcium carbonate and calcium sulphate @ 1.5% each (95.8mm) at 10 days of experimentation. The mycelia growth was in the range of 58.2-108.8mm in the experiment. Further it was observed that calcium carbonate was superior to calcium sulphate as a supplement to wheat grain in respect of promoting spawn quality and reducing the incubation period. Boiled wheat along with 0.5% calcium carbonate and 2.0% calcium sulphate, as recommended at the national level as standard was found to be inferior in comparison to boiled wheat along with 3% calcium carbonate. Therefore, calcium carbonate alone @3% or combination of calcium carbonate and calcium sulphate @1.5% each was superior over other treatments in the investigation (Fig 1).

**Table-2: Effect of inorganic salts on spawn quality**

Treatment	Mycelial growth rate on wheat grain substrate(mm)		
	Days after spawn inoculation		
	3	6	10
T1-Boiled wheat	11	27.0	59.2
T2-Boiled wheat + 2% calcium carbonate	15.4	43.4	91.2
T3-Boiled wheat + 3% calcium carbonate	21.8	62.6	108.8
T4-Boiled wheat + 2% calcium sulphate	12.4	42.0	58.2
T5- Boiled wheat + 3% calcium sulphate	14.0	41.8	61.4
T6- Boiled wheat +1% calcium carbonate + 1% calcium sulphate	13.6	44.2	90.0
T7-Boiled wheat + 1.5% calcium carbonate + 1.5% calcium sulphate	20.4	65.0	95.8
T8- Boiled wheat + 0.5% calcium carbonate +2.0% calcium sulphate	22.6	64.2	92.4
C.V(%)	9.93	3.28	2.30
C.D(0.05)	2.00	2.07	2.52

**Fig. 1 : Effect of inorganic salts on spawn quality**



## **4.2 Effect of substrate soaking period on mushroom productivity**

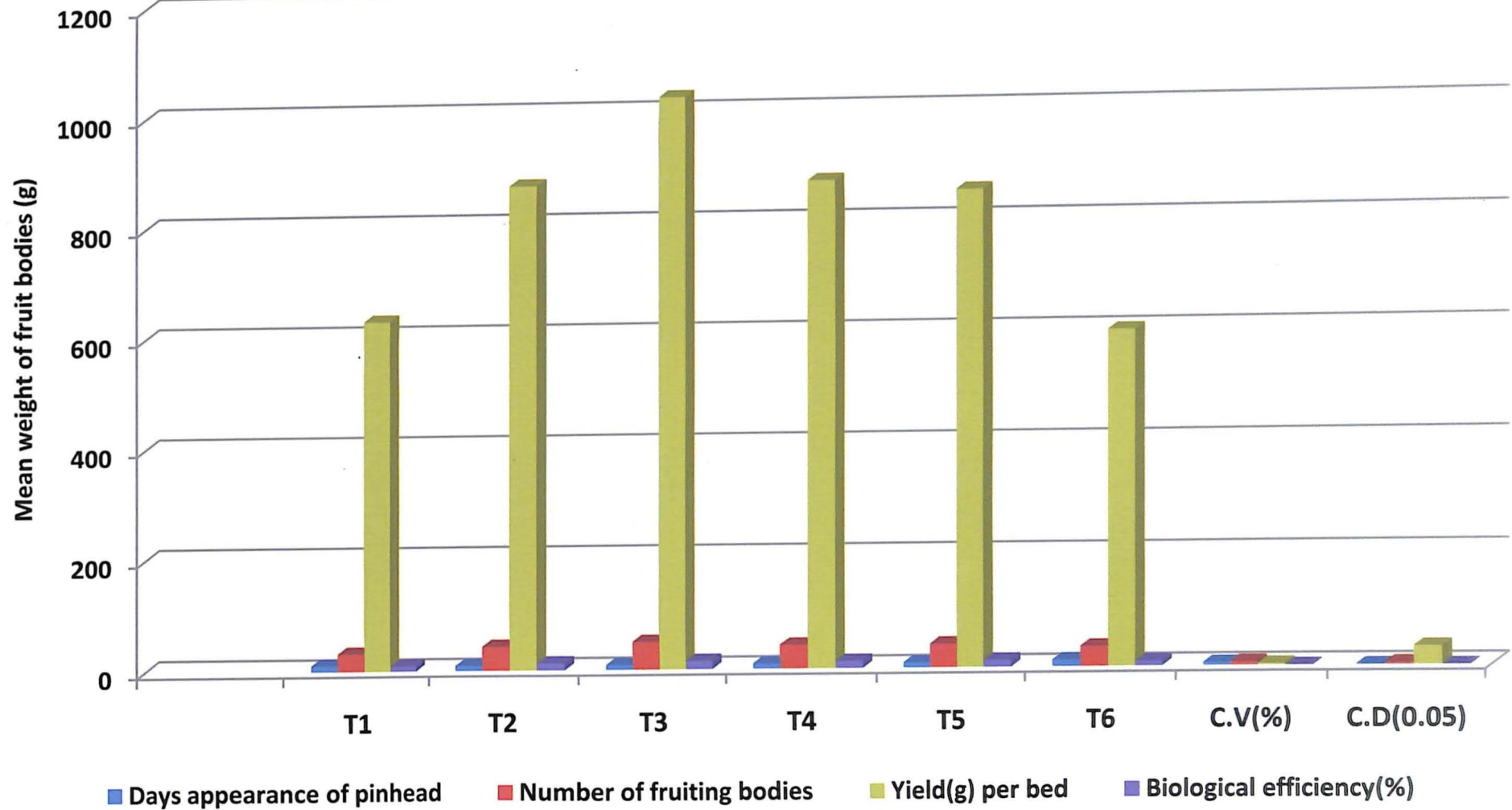
Presoaking of the substrate in clean and cold water appropriately before raising mushroom beds is required to obtain good mycelial run as well as better fruiting. To ascertain the optimum period of soaking, the experiment was designed with six treatments with varied periods. The mean data on days to appearance of pinheads, number of fruiting bodies and yield /bed (g) along with biological efficiency of individual treatments are presented in Table 3.

The data indicated that pre-soaking of substrate for six hours registered significantly the highest yield of 1028.75g per bed with a corresponding biological efficiency of 14.69%. The same treatment produced maximum fruiting bodies per bed (49.25) and the days to appearance of pinheads was numerically lowest (8.50d). Further, pre-soaking for a period of four hours yielded 872.50g of fruiting bodies which was statistically at par with pre-soaking for 8 hours (875.00g) and pre-soaking for 10 hours (855.00g) with the realized biological efficiencies of 12.46, 12.50 and 12.21% respectively. It was also found that soaking for 2 hours and 12 hours were inferior in yield performance having yielded 630.00g and 600.00g per bed respectively. The yield level in the trail varied in between 600.00-1028.75g per bed (Fig 2 and Plates 1-4).

**Table-3 Effect of substrate soaking period on mushroom productivity**

<b>Treatment</b>	<b>Days appearance of pinhead</b>	<b>Number of fruiting bodies</b>	<b>Yield(g) per bed</b>	<b>Biological efficiency (%)</b>
T1-Soaking for 2 hours	10.50	31.25	630.00	9.00
T2- Soaking for 4 hours	8.75	42.75	872.50	12.46
T3-Soaking for 6 hours	8.50	49.25	1028.75	14.69
T4-Soaking for 8 hours	9.00	42.00	875.00	12.50
T5-Soaking for 10 hours	8.75	41.50	855.00	12.21
T6-Soaking for 12 hours	11.75	35.50	600.00	8.57
C.V(%)	4.78	6.08	2.68	---
C.D(0.05)	0.69	3.70	32.72	---

**Fig. 2 : Effect of substrate soaking period on mushroom productivity**





*Plate 1 : Soaking of substrate for 2 hours*



*Plate 2 : Soaking of substrate for 4 hours*



*Plate 3 : Soaking of substrate for 6 hours*



*Plate 4 : Soaking of substrate for 8 hours*

### **4.3 Pre-soaking of substrate in different concentrations of lime water and its effect on mushroom productivity**

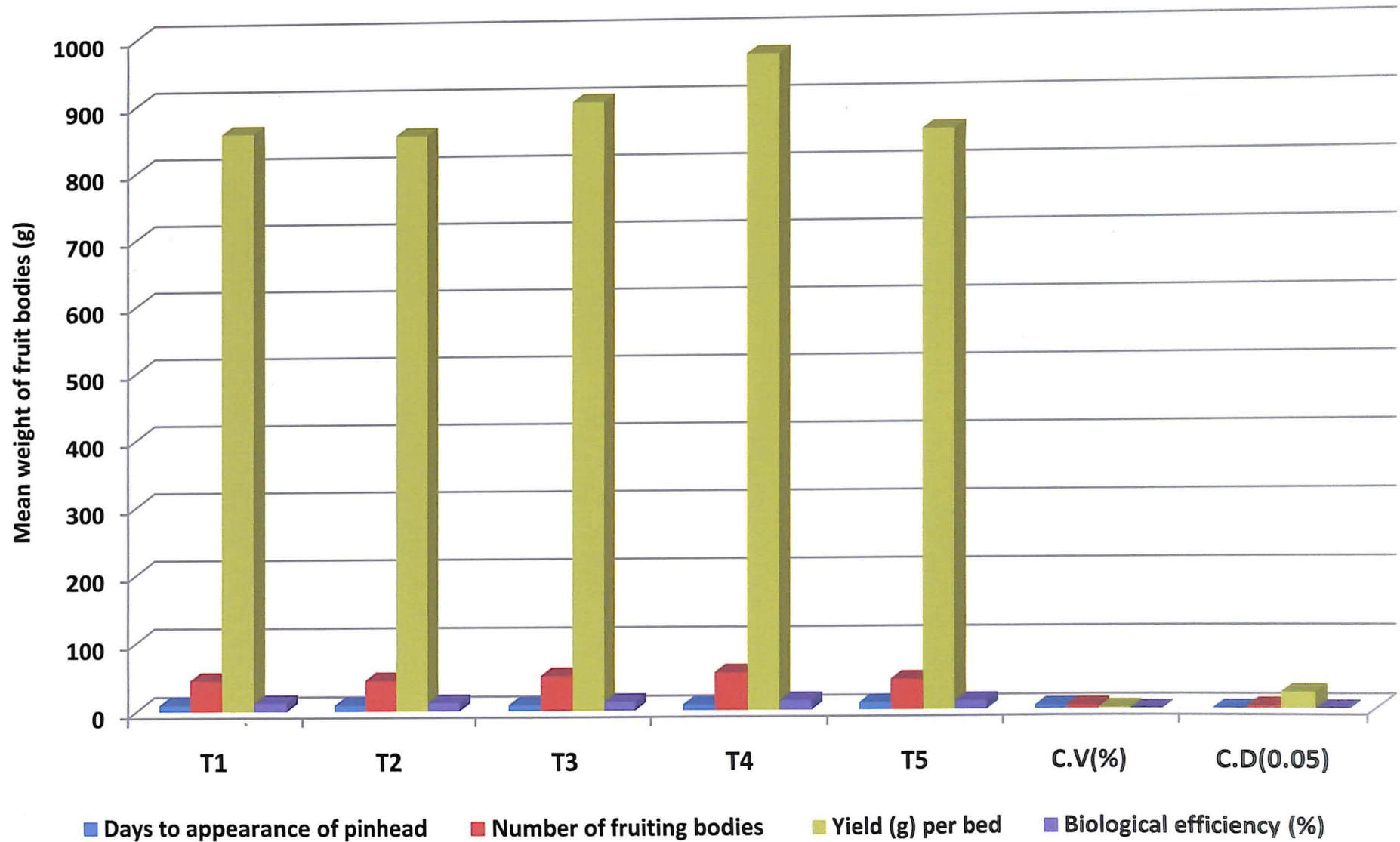
Substrate soaking in calcium carbonate amended water at suitable concentration is necessary to reduce the acidity of the medium which suppresses the competitor moulds and in turn improves yield appreciably. Calcium carbonate at four dosages was supplemented with water along with control(no supplementation) to evaluate their yield potential. The mean data on days to appearance of pinhead, number of fruiting bodies and yield (g) per bed is presented in Table 4.

The data indicated the superiority of the treatment that received 2.0% lime water soaking with lowest period for appearance of pinheads (8.00d), highest number of fruiting bodies per beds (54.50) and yield (968.75g) with the corresponding biological efficiency of 13.83% which was followed by pre-soaking in 1.5% calcium carbonate having given a yield of 900.00g (12.85% B.E.) lime water at 0.5-1.0% concentration found inferior in terms of days to appearance of pinhead, number of fruiting bodies and yield of sporophores. The yield realized from the non-supplemented water was low (855.00g/bed) which was statistically at par with the water amended with 0.5-1.0% calcium carbonate (857.50 and 852.50g/bed respectively) (Fig 3 and Plates 5-6).

**Table-4 Effect of pre-soaking of substrate in lime water on mushroom productivity**

<b>Treatment</b>	<b>Days to appearance of pinhead</b>	<b>Number of fruiting bodies</b>	<b>Yield (g) per bed</b>	<b>Biological efficiency (%)</b>
T1-0.5% lime water	9.25	44.75	857.50	12.25
T2-1.0%lime water	8.75	44.50	852.50	12.17
T3-1.5% lime water	8.50	50.50	900.00	12.85
T4-2.0% lime water	8.00	54.50	968.75	13.83
T5- Control (plain water)	10.25	43.75	855.00	12.21
C.V(%)	4.89	5.00	1.72	-
C.D(0.05)	0.67	3.66	23.47	-

**Fig. 3 : Effect of pre-soaking of substrate in lime water on mushroom productivity**





*Plate 5 : Pre-soaking of substrate in 2.0% lime water*



*Plate 6 : Pre-soaking of substrate in plain water.*

#### **4.4 Effect of plant growth regulators on mushroom productivity**

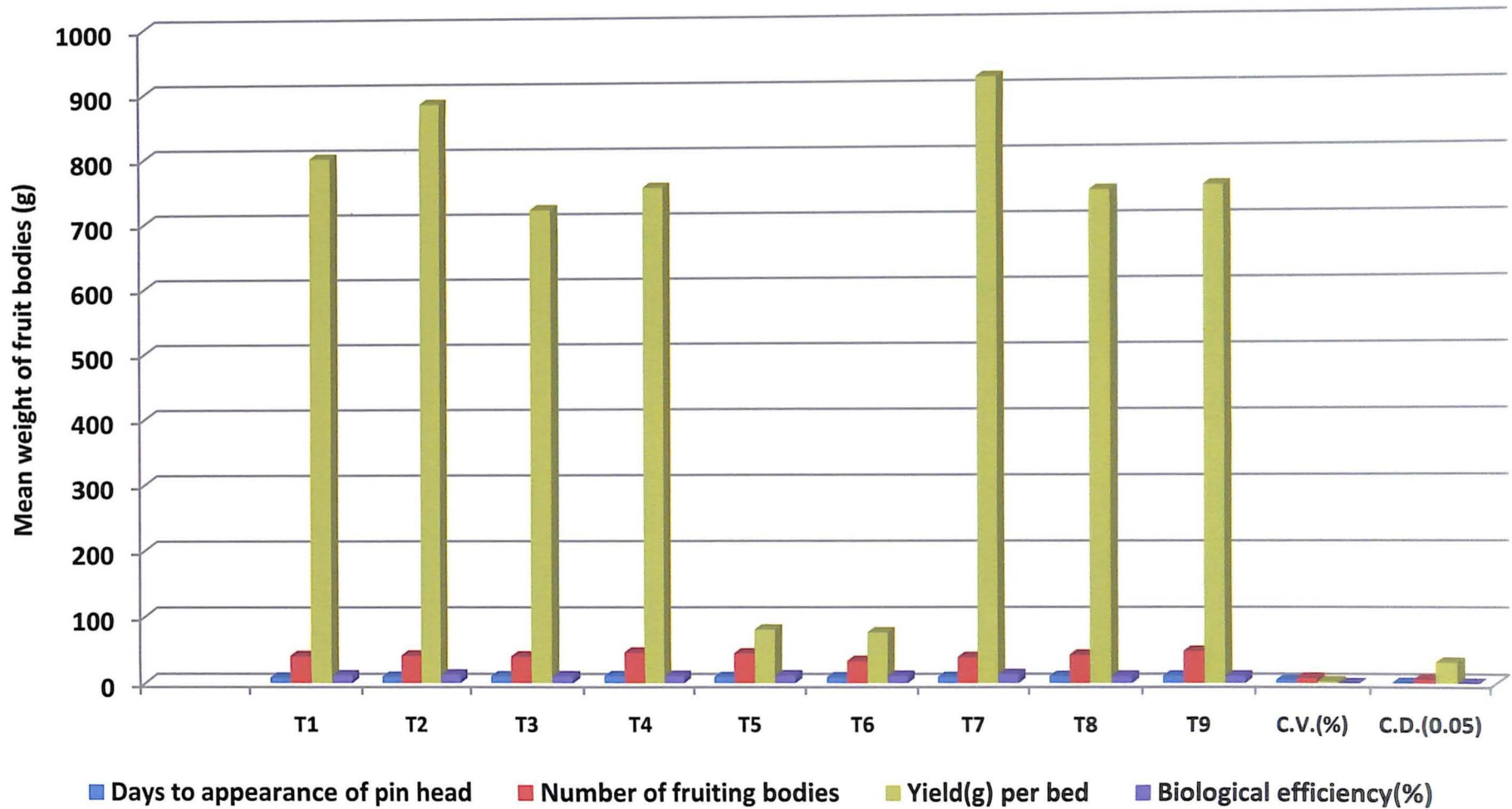
In an attempt to evaluate eight plant growth regulators for their role in yield improvement, an experiment was designed and data were recorded on days to appearance of pinhead, number of fruiting bodies and yield(g) /bed and presented in Table 5.

The analyzed data of the influence of eight treatments on mushroom yield and Yield attributing parameters indicated that the highest yield of 920.00g /bed was recorded from the application of Indole acetic acid (IAA) @ 200ppm with the days to appearance of pinhead and number of fruiting bodies at 9.00d and 38.67% respectively. However, this yield level was at par with the yield level realized by the treatment that received Urea at 2.5% concentration (883.33g/bed). It was observed that, with the exceptions of micro-nutrient mixture application at 0.25% and GA at 50ppm, all other treatments were either equal or more than the yield realized from the control (water spray) treatment (753.33g/bed). Further, the days taken for pinhead emergence was lowest (8.00d) in glucose (2%) treated beds, whereas, it was found highest (10.67d) in control beds. The investigation was exploratory in nature and needed repetition for ascertaining the role of growth regulators on mushroom (Fig 4 and Plates 7-15).

**Table-5 Effect of plant growth regulators on mushroom productivity**

<b>Treatment</b>	<b>Days to appearance of pin head</b>	<b>Number of fruiting bodies</b>	<b>Yield(g) per bed</b>	<b>Biological efficiency(%)</b>
T <sub>1</sub> -Glucose 2%	8.00	40.33	800.00	11.42
T <sub>2</sub> -Urea 2.5%	9.33	41.00	883.33	12.61
T <sub>3</sub> -Multiplex micro nutrient mixture 0.25%	10.00	39.67	720.00	10.28
T <sub>4</sub> - Multiplex samras (amino acid mixture) 0.2%	10.33	45.67	753.33	10.76
T <sub>5</sub> - Multiplex mushroom spray 0.2%	9.00	44.33	803.33	11.47
T <sub>6</sub> -Bayer planofix(NAA) 200ppm	8.67	33.00	760.00	10.85
T <sub>7</sub> -Hi Media IAA 200ppm	9.00	38.67	920.00	13.14
T <sub>8</sub> - Hi Media GA 50ppm	10.33	42.00	746.67	10.66
T <sub>9</sub> -Control (water spray)	10.67	47.67	753.33	10.76
C.V.(%)	4.42	7.77	2.57	----
C.D.(0.05)	0.73	5.57	39.79	----

**Fig. 4 : Effect of plant growth regulators on mushroom productivity**





*Plate 7 : Bed treated with 2% glucose.*



*Plate 8 : Bed treated with 2.5% urea.*



*Plate 9 : Bed treated with 0.2% multiplex micronutrient mixture.*



*Plate 10 : Bed treated with 0.2% multiplex samras (amino acid mixture).*



*Plate 11 : Bed treated with 0.2% multiplex mushroom spray.*



*Plate 12 : Bed treated with 200 ppm Bayer planofix (NAA).*



*Plate 13 : Bed treated with 200 ppm Hi media IAA*



*Plate 14 : Bed treated with 50 ppm Hi media GA*



*Plate 15 : Bed treated with Control (Water spray)*

#### **4.5 Effect of semi-composted substrate on mushroom productivity in indoor condition**

An exploratory investigation was undertaken to ascertain the role of semi-composted substrate in indoor condition in improving the yield of paddy straw mushroom, *V.volvacea*. Two partially composted substrates pasteurized physically and chemically were compared with conventional method of cultivation in terms of mushroom yield and biological efficiency. The mean data recorded on days to pinhead emergence, number of fruiting bodies and yield (Kg) per 100Kg dry substrates are presented in Table 6.

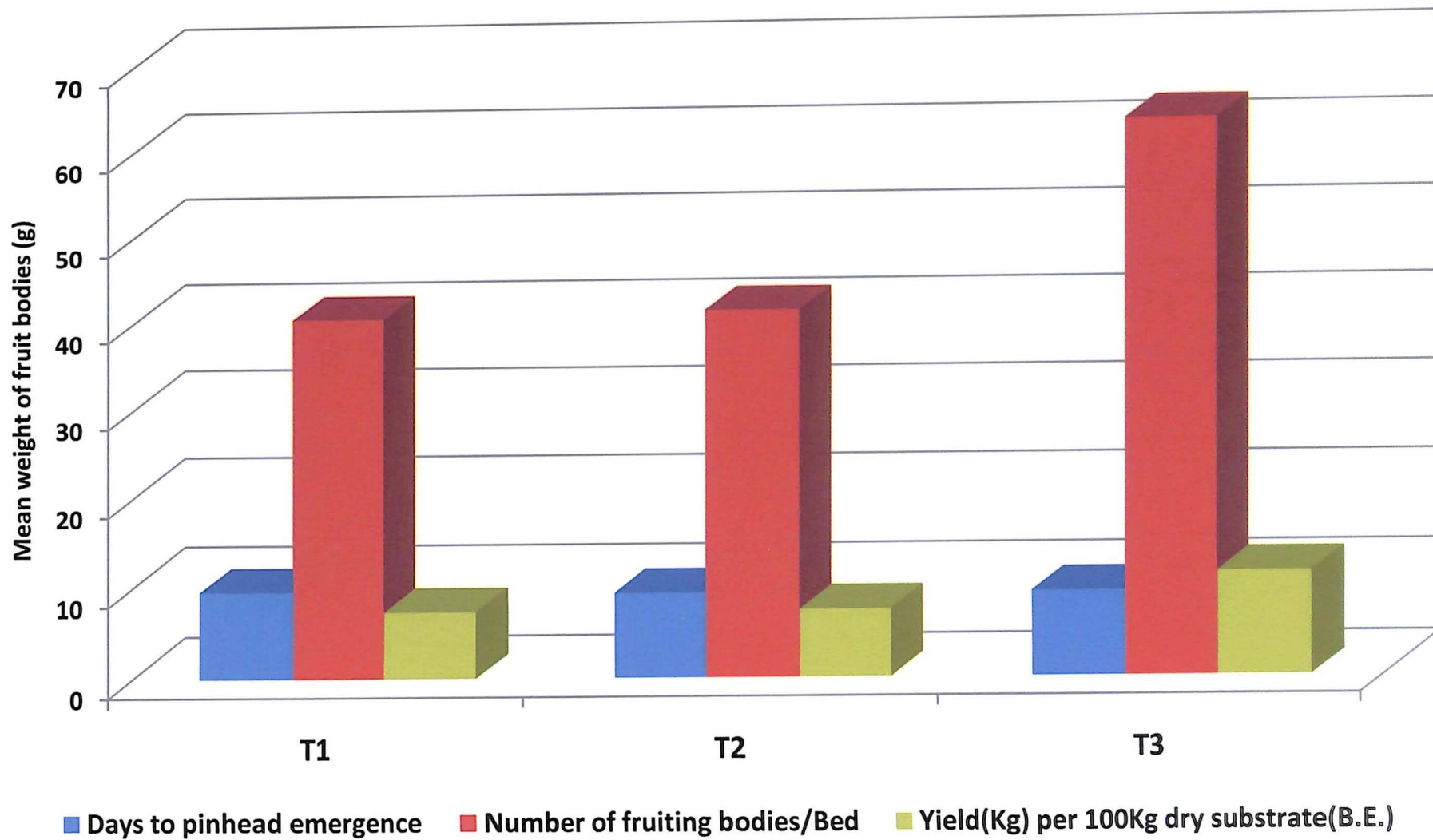
The data revealed that conventional method of cultivation could realize better yields (11.31Kg/100Kg dry substrate) over the cultivation done on partially composted substrate employing both the pasteurization methods. The steam pasteurized and the chemical pasteurized partially- composted substrates could yield 7.27Kg and 7.40Kg mushrooms per 100Kg dry substrate with less fruiting bodies as compared to conventional method. However, the days taken for pinhead emergence was almost equals in all the three treatments. The sub-standard yields realized from the partially-composted substrate needed confirmation through repetition of the trial (Fig 5 and Plate-16-17).

**Table-6 Effect of semi-composted substrate on mushroom yield**

<b>Treatment</b>	<b>Days to pinhead emergence</b>	<b>Number of fruiting bodies/bed</b>	<b>Yield(Kg) per 100Kg dry substrate(B.E.)</b>
T1-Semi-composted steam pasteurized	9.57	40.42	7.27
T2-Semi-composted chemical pasteurized	9.28	41.14	7.40
T3-Conventional method	9.28	62.85	11.31

**\*Mean of seven replications**

**Fig. 5 : Effect of semi-composted substrate on mushroom yield.**





*Plate 16 : Mushroom cultivation in composted substrate*



*Plate 17 : Mushroom cultivation in non-composted substrate*



# *Chapter V*



## **DISCUSSION**



## DISCUSSION

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Paddy straw mushroom (*Volvariella volvacea*) also known as Chinese mushroom, ranks sixth among the cultivated mushrooms of the world. It is a fast growing mushroom and under favourable growing conditions total crop cycle is completed within 3 weeks time. This mushroom can use wide range of cellulosic materials with a C:N ratio of 40-60, quite high in comparison to other cultivated mushrooms. The fast growing nature, easy cultivation technology and great acceptability at the consumers' level make this mushroom unique among the cultivated edible mushrooms. Presently this mushroom is more popular in the coastal states like Orissa, Andhra Pradesh, Tamil Nadu, Kerala and West Bengal. In fact, this has become economic and nutritional mainstay of the predominantly agrarian population of the coastal states.

Higher rate of conversion of the lingo cellulosic agro-wastes into protein rich fruit bodies largely depends on spawn quality. The acidity of the spawn substrate has to be neutralized or made fairly alkaline to promote the growth of *V. volvacea* and thereby to improved spawn quality. Different concentrations of Gypsum (calcium sulphate) and Chalk powder (Calcium carbonate) are mixed with cereal grains before sterilization for pH improvement and reduction in moisture content. The study on the effect of inorganic salts and their combinations on spawn quality included 8 treatments including the boiled wheat without supplementation. Data recorded on mycelial growth of *V. volvacea* at 3, 6 and 10 days after inoculation revealed that boiled wheat supplemented with 3% calcium carbonate exhibited highest mean mycelial growth of 108.8mm followed by boiled wheat supplemented with calcium

carbonate and calcium sulphate at 1.5% each (95.8mm) at 10 days of inoculation. It was observed that treatment of calcium sulphate both at 2 and 3% concentration were inferior in terms of mycelial growth rate resulting in lengthening of the spawn incubation period. Upadhyay *et al.* (2004) observed that boiled wheat supplement with 0.5% calcium carbonate and 2.0% calcium sulphate had good mycelial run. However, the findings of the present investigation ascertained that calcium carbonate alone at 2 - 3% concentration or combination of both at 1.5% concentration each could lead to better quality spawn.

Substrate soaking period has a tremendous bearing on mushroom productivity, longer period of soaking facilitates decomposition of straw resulting in decreased C:N ratio which is unfavourable for *V.volvacea*. The experiment on substrate soaking period indicated that soaking for a period of six hours significantly highest yield of 1028.75g/bed with a biological efficiency of 14.69%. Lengthy soaking periods upto 12 hours reduced to the biological efficiency of the substrate. This finding was in agreement with the findings at Bhavani Devi (1982) and Mohapatra *et al.* (2010). Belewu and Belewu (2005) reported that pre-soaking of substrate even for a period of 4 hours could give better yields in paddy straw mushroom. Ahlawat and Tewari (2007) were of the view that soaking straw for a period of 12 -14 hours was necessary to get good yields under conventional method of cultivation. However, this study ascertained that 4 – 8 hours of soaking was optimum to obtain a good crop of straw mushroom in outdoor farming. Quality of straw pre-soaked for longer periods would lead to deterioration of yield and quality of mushrooms.

Soaking of substrate in water amended with calcium carbonate has been in practice to reduce the acidity of the medium for promotion of mycelial growth of *V. volvacea*, to suppress the acid loving competitor moulds and in turn to improve productivity. Acidity of paddy straw medium has been a great obstacle in yield improvement of this low yielding mushroom. Hence, the investigation was designed to find out the appropriate dosage of calcium carbonate to be amended with water before soaking substrate. The results showed a substantial yield increase of straw mushroom in lime water treated substrate as compared to control. Substrate pre-soaking in 2% lime water register significantly highest sporophore yield (968.75g/bed) with a biological efficiency of 13.83% among all the treatments which agreed to the findings of Ahlawat and Tewari (2007) and Ahlawat (2011). The lime water treated beds also had low counts of *Coprinus*, the main competitor of straw mushroom.

Sizeable number of works have been done by research workers across the country and outside on the effect of plant growth regulators on straw mushroom productivity. However, nothing concrete has emerged out of the investigations till date. To ascertain this fact, a trial was designed with 9 growth regulators including the control during the growing season of *V. volvacea*. Organic carbon and Nitrogen sources, micronutrients and amino acid mixtures and few plant hormones were evaluated for the purpose. The study revealed that among the growth regulators tried highest yield (920.00g/bed) along with biological efficiency (13.14%) was obtained from Indole acetic acid 200ppm treatment having a modest mycelia run period of 9 days. However, this treatment was statistically at par with the treatment that received 2.5% Urea application having yield of 883.33g/bed and biological efficiency of

12.6%. It was also observed that application of micronutrient mixture @0.25% and Gibberallic acid @ 50ppm (10.28% and 10.66% B.E. respectively) did not exhibit any yield advantage over control (10.76% B.E.). Reports are available on use of these hormones in horticultural crops either to improve the yield or their quality. Mushrooms are different from higher plant because of the nature of growing medium and other aspects, but still reports are available on use of hormones in mushroom cultivation. Deshpandey and Tamhane (1982) reported that the yield of straw mushroom was increased by 17.3%, 10.6% and 8.0% by spraying mushroom bed with 2% glucose solution, 200ppm IAA and 200ppm NAA respectively. Ahlawat (2011) indicated that IBA @0.1% (w/v) stimulated early pinning as well as higher yield in *Agaricus bisporus*. However, the findings of the above study needs repetition in order to ascertain the role of plant growth regulators on mushroom productivity and to recommend the results therefrom to the growers.

Cultivation of paddy straw mushroom following traditional method has been less rewarding. However, indoor cultivation utilizing cotton waste substrate alone or in combination with paddy straw has become semi-industrialized in some of the South-East Asian countries with higher and more stable yield (30-40% B.E.). The role of semi-composted paddy straw substrate alone in promoting the straw mushroom productivity has been experimented by many workers (Quimio, 1993; Thakur and Yadav, 2007; Ahlawat and Tewari, 2007; Ahlawat, 2011). In this context, the exploratory investigation was planned to evaluate the semi-composted and pasteurized paddy straw substrate for its efficiency in promoting mushroom yield in comparison to non-composted substrate. Results were, however, quite discouraging. It was observed that the semi-composted steam pasteurized and the chemical pasteurized substrate were

poor yielders (7.27 and 7.40% B.E. respectively) in comparison to non-composted substrate (11.31% B.E.). Ahlawat and Tewari (2007) were of the view that the semi-composted paddy straw substrate could yield over 25% biological efficiency in indoor conditions. However, the present investigation was not in agreement with the above finding. The investigation being exploratory in nature, needs further repetition before going for any recommendations to the commercial growers.



# *Chapter VI*



## **SUMMARY & CONCLUSION**



## SUMMARY AND CONCLUSION

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In Orissa, paddy straw mushroom cultivation is by and large, an outdoor enterprise. Cultivation is done in the interspaces of coconut plantations in the coastal ecosystem with traditional bed method. Farmer use paddy straw as the substrate which is abundantly available in the state. In most cases cultivation is done on non-pasteurized substrate with incorporation of pulse powder or wheat bran as the supplement. Under uncontrolled situation, the cultivation is subjected to several biotic and abiotic stress limiting the productivity up to 10 percent, which is low. However, there is scope for yield improvement by using high yielding strains and appropriate substrate management. During the present course of investigation, a series of experiments were conducted to find out the role of substrate management on the productivity of *Volvariella volvacea*.

The investigation on the effect of inorganic salts and their combinations on spawn quality revealed the superiority of spawn substrate supplemented with 3 percent calcium carbonate on a dry weight basis in terms of highest mean mycelia growth (108.8mm) exhibited by the fungus at 10 days of experimentation.

Pre-soaking of substrate for six hours in water was superior to pre-soaking for lengthy periods in terms of realization of yield (1028.75g/bed) and biological efficiency (14.69 %). The same treatment was also associated with lowest period for emergence of mushroom primordia (8.50d) and production of highest number of fruit bodies (49.25). The conventional method of 12 hours of pre-soaking was inferior in terms of yield (600.00g/bed) and biological efficiency (8.57 %).

Substrate soaking in different concentration of lime water indicated that soaking in 2 percent lime water for 6hours produced highest yields (968.759g/bed) with as biological efficiency of 13.83 %. This treatment also resulted in earlier appearance of pinheads (8.00d) and production of highest number of fruiting bodies (54.50). The yield realized from substrate soaking in non-supplemented water was low (855.00g/bed).

Eight plant growth regulators were evaluated for their role in yield improvement of *V. volacea* over control (water spray). Application of Indole Acetic Acid (IAA) at 200ppm was found superior among the treatments in giving the highest yield (920.00g/bed) with 13.14percent biological efficiency. However this yield level was at par with the yield realised from the application of Urea @2.5% concentration (883.33g/bed) with 12.61percent biological efficiency. Application of micronutrient mixture at 0.25percent and gibberlic acid @50ppm had no role on yield improvement.

Conventional method of cultivation of non-composted substrate proved better in terms of yield (11.31kg/100 kg dry substrate) in comparison to semi-composted stem pasteurized (7.27 kg/ 100kg dry substrate) and chemically pasteurized substrate (7.40kg/ 100kg dry substrate).The low yields realized from the partially composted substrates need confirmation through repetition.



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