EFFECT OF GROWTH REGULATORS ON YIELD OF MILKY MUSHROOM (*Calocybe indica*)

by

Mr. Jadhav Amar Namdev
(Reg. No. 16/257)

DIVISION OF PLANT PATHOLOGY AND AGRICULTURAL MICROBIOLOGY

COLLEGE OF AGRICULTURE, KOLHAPUR

MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI-413722, DIST-AHMEDNAGAR
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A Thesis submitted to the

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In partial fulfilment of the requirements for the degree

of

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2018

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part
there of has not been submitted
by me or other person to any
other University or Institute
for a Degree or
Diploma

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Date:

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Maharashtra State (INDIA)

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The assistance and help received during the course of this investigation have been duly acknowledged.

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Place:

Date: **(A. N. Jadhav)**

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LIST OF ABBREVIATIONS AND SYMBOLS

%	:	Percentage
/	:	Per
@	:	At the rate of
+	:	Plus
=	:	Equal
BE	:	Biological Efficiency
°C	:	Degree Celsius
C.D.	:	Critical Difference
Cm	:	Centimeter
etc.	:	et cetra (and so on)
et al	:	And others
Fig.	:	Figure
FYM	:	Farm Yard Manure
g	:	Gram(s)
GA	:	Gibberellic Acid
hr.	:	Hour
IAA	:	Indole- 3- Acetic Acid
IBA	:	Indole- 3- Butyric Acid
i.e.	:	That is (id est.)
Kg	:	Kilogram
M	:	Meter
MEA	:	Malt Extract Agar
mg	:	Milligram
ml	:	Milliliter
No.	:	Number
PDA	:	Potato Dextrose Agar
ppm	:	parts-per-million
q	:	quintal
RH	:	Relative Humidity
SE	:	Standard Error
viz	:	Namely
wt	:	weight
X	:	Multiplication
YPDA	:	Yeast Potato Dextrose Agar

ABSTRACT

EFFECT OF GROWTH REGULATORS ON YIELD OF MILKY MUSHROOM (*Calocybe indica*)

by

Mr. Jadhav Amar Namdev

A candidate for the degree

of

MASTER OF SCIENCE (AGRICULTURE)
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AGRICULTURAL MICROBIOLOGY

Research Guide	: Dr. C. T. Kumbhar
Division	: Plant Pathology and Agricultural Microbiology
Major Field	: Agricultural Microbiology

In the present investigation attempts were made to study the effect of various growth regulators and to find out most effective dosage of the growth regulator for enhancing the yield of milky mushroom (*Calocybe indica*). The investigation was undertaken during March-April, 2018 at the Plant Pathology Section of College of Agriculture, Kolhapur.

Results of the investigation overtly indicated that the growth regulators tried at different concentrations in the present investigation, aiming at promoting growth and sporophore yield of *Calocybe indica*, exhibited significantly varied response to days required for pinhead formation and first harvest, period between flushes, diameter of pileus, length of stipe, number of sporophores, yield of sporophores and biological efficiency. However, amongst the various growth regulators used in the present investigation, spraying of gibberellic acid (GA) 20 ppm significantly demonstrated its efficacy over other treatments in reducing days required for pinhead formation (30.00 days after spawning), first harvest (35.66 days after spawning) and period between flushes(7 days). Furthermore, this treatment was also found most effective amongst all the treatments in augmenting diameter of pileus (15.40 cm), length of stipe (19.94 cm), number of sporophores (22.00), total yield of sporophores (850.12 g) and biological efficiency (85.01%). Protein content of sporophores remained unaffected due to use of different growth regulators.

From the present investigation it is concluded that spraying of gibberellic acid at 20 ppm concentration on beds, at the time of casing, is highly beneficial for obtaining sporophores with large diameter, longer stipes and exuberant yield of sporophores of milky mushroom (*Calocybe indica*).

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1. INTRODUCTION

India is the seventh largest and second most densely populated country in the world and has crossed 100 crore population in the year 2000. The major problem today, is of malnutrition and under nutrition in the developing countries, like India. To meet the needs of the ever increasing population, agricultural scientists and nutritionists are looking for the technologies by which more quality food can be grown with less land resources. Incidentally, mushrooms can be used as a 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 proteins may not be available and are expensive (Pandey, 2002). Consequently, mushrooms have been recognized as an alternating source of good quality food, as they are capable of producing the highest quantity of protein per unit area and time. India produces about 600 million tones of agricultural byproducts, which can profitably be utilized for the cultivation of mushrooms. Currently, we are using 0.04% of these residues for producing around 1.2 lakh tones of mushrooms (Prakasam, 2012). Huge quantities of lignocellulosics and other organic waste residues are generated annually through the activities of agricultural forest and food processing industries. Most of the mushrooms possess enzyme complexes that enable them to attack and degrade these industrial and agricultural byproducts thereby resulting in a high valued food protein suitable for direct consumption.

Mushrooms are large reproductive structures of edible fungi belonging to either *Ascomycotina* or *Basidiomycotina*. The mushrooms comprise a large heterogenous group having various shapes, size, colour, appearance and edibility. Of more than 2000 edible species, about 70 genera having 300 species are reported from India. However, only a few have been brought under cultivation on commercial scale. About 80 mushrooms have been grown experimentally, 20 are cultivated commercially and 4-6 are species produced on industrial scale throughout the world (Chang and Miles, 1992). Production of edible mushrooms has been industrially developed in more than 80 countries, which has increased rapidly during the last 20 years to 3.5 million tones as per FAO (Singh *et al*., 2011).

Mushrooms are consumed for their deliciousness and nutritive value. Scientific studies on mushrooms during the last six to seven decades have revealed that, apart from being a component of forest eco-system, they are nutritionally and medicinally important flora on the earth. Mushrooms are excellent as a food; as they provide a full protein diet containing twenty one amino acids besides vitamins and minerals. Being easily digestible (70-90%), mushroom protein is considered superior to vegetable proteins. They are as well liked for their delicious flavour, low calorific value and high protein contents, vitamins of B group and minerals. Mushrooms contain 20–40% proteins on a dry weight basis and no cholesterol, and are almost fat free (Walde *et al*., 2006). They are good source of non-starchy carbohydrates, dietary fiber, proteins, minerals and vitamins (Kulshreshtha *et al*., 2009). Several mushrooms have been screened for their bio-active properties and many compounds such as polysaccharides, mainly β-D glucan, heteroglycans, hexose correlated compounds, pachymanans, proteioglycans; terpenoides such as ganoderic acid, ganoderol, ganodermic acid and, compounds like germanium and ergosterol have been isolated and commercialized (Hobbs, 1996; Wani *et al*., 2010).

Mushroom farming today is being practiced in more than 100 countries and the production is increasing at an annual rate of 6-7%. In some developed countries of Europe and America, mushroom farming has attained the status of a high-tech industry with extensive mechanization and automation. China leads in world mushroom production by cultivating more than 20 different types of mushrooms on commercial scale (Prakasam, 2012). USA is the second largest producer of mushrooms, sharing 16 % of the world production (Prakasam, 2012; Singh *et al*., 2011).

Mushroom production in India started in the 70s, but with the development of technologies for environmental control and understanding of the cropping systems, mushroom production shot up from mere 5000 tonnes in 1970 to over 1,20,000 tonnes in 2011 and out of which milky mushroom contributes 920 tonnes (Prakasam, 2012). India contributes only 1% in the world mushroom production (Singh and Shwet Kamal, 2012). Agro-climatic diversity in different states and regions of the country, abundance of agricultural residues and availability of cheap labors provide the best opportunity for Indian growers to take up the cultivation of edible as well as medicinal mushrooms as a profitable enterprise.

Integration of mushroom cultivation in the existing farming systems will not only be the supplementary source of income to the farmers but also will ensure proper recycling of agricultural residues. Improvement in dietary values, bioremediation and diversification of agriculture are the other advantages of mushroom cultivation. There is vast scope for establishing mushroom cultivation as a highly remunerative enterprise with innovative ideas in packing, preparation of value added mushroom comestibles such as biscuits, *papads*, pickles, soup powder etc. and prudent marketing strategies.

Milky mushroom (*Calocybe indica* Purkay & Chandra), commonly known as summer mushroom or *doodh chhataa, is* a member of Lyophyllaceae family. It was reported for the first time by Purkayastha and Chandra from west Bengal in 1974. Like oyster mushroom, it can also be cultivated on a wide variety of substrates. Nutritionally, it is rich in carbohydrates (49%), proteins (22%), ash (13%), fiber(13%) and various minerals like Ca, Fe, Zn, Mg, Mn and Se (Nuhu *et al*., 2008; Pushpa and Purushothama, 2010). Apart from this, it has antioxidant and antitumor properties (Selvi *et al.,* 2011). The biological efficiency of this mushroom is much greater than that of oyster mushroom (Senthilnambi *et al.,* 2011). Milky mushroom is basically a summer mushroom, requiring temperature within a range of 30-35^{0}C and relative humidity of 85-90% for spawn run and fruit body development (Senthilnambi *et al.,* 2011). Obviously, its cultivation stands to be the best option for summer season mushroom cultivation. Fruit body of this mushroom is very attractive, comparatively bigger and fleshy than other cultivated mushrooms. Milky mushroom having shelf life period of 5-7 days at room temperature level compared to other species cultivated, making it more valuable and easy to process of handling, transport and storage level. Subsequently, this mushroom can bring very much interest to mushroom cultivators (Maynard, 1970). But all these attributes have so far failed to popularize its cultivation among the mushroom growers. As a result, till today its cultivation is confined to very few parts in the country (Anonymous, 2011). Therefore, there is need to introduce the cultivation technology of this mushroom into new agro-climatic zones of the country, as the biological efficiency of this mushroom is much greater than that of oyster mushroom (Navathe *et al*., 2014).

Refinement in cultivation technology of mushroom is a continuous process. The problems encountered in the cultivation, ultimately resulting in low biological efficiency, are overcome by use of novel ideas and techniques by the researchers working in the field of mushroom science all over the world. The biological efficiency of cultivated mushrooms depends on many factors such as mushroom species, substrate, availability of required micronutrients and hormonal balance maintained by these factors. Many researchers have worked on the performance of a single mushroom species under a given set of conditions. *Pleurotus sajor-caju* and *P. florida* are the most commonly experimented oyster mushrooms. Nonetheless, the research work on milky mushrooms in India is mainly confined to use of various locally available, cheap agro-waste material or enrichment of such substrates with protein rich organic supplements. The effect of micronutrients and growth regulators on biological efficiency of this mushroom has so far remained neglected. Hence, with an aim in view to augment the yield of milky mushroom, it was thought worthwhile to conduct an experiment on use of growth regulators in the cultivation of milky mushroom. The present investigation, therefore, was proposed in this direction with the following prime objectives:

1. To study the effect of various growth regulators on yield of milky mushroom
2. Standardization of dose of growth regulators for milky mushroom production

2. REVIEW OF LITERATURE

Research work on milky mushrooms (*Calocybe* indica) in India is mainly curbed to use of various locally available cheap agro-waste material or fortification of such substrates with protein rich organic supplements. The effect of micronutrients and growth regulators on biological efficiency of this mushroom has so far remained neglected. Hence, with an aim in view to augment the yield of milky mushroom, the present investigation was conducted to study the effect of growth regulators on sporophore yield of milky mushroom. The literature reviewed from all the possible sources on use of chemicals such as growth regulators on biological efficiency of milky mushroom has been presented in this chapter.

2.1 Effect of Growth Regulators on Growth of Mushroom

Shukla (1995) reported that spraying of Indole butyric acid (IBA) @ 5 and 10 ppm enhanced the mycelial growth of *L. edodes*.

Bhardwaj (2004) studied the effect of growth regulators on growth of mycelium and sporocarps of milky mushroom (*Calocybe indica*) and found that the mean mycelial weight was significantly similar at 10 and 20 ppm concentrations of Gibberellic acid (GA_3), Indole -3- Acetic Acid (IAA) and Indole -3- Butyric Acid (IBA). He recorded maximum mean mycelial weight of 191.3 mg with GA_3 at 10, 20 and 30 ppm concentrations. This was followed by 183.9 mg in IBA, 170.8 mg in IAA and 164.0 mg in control. Further he reported that the mean mycelial weight with GA_3 and IBA were statistically similar but differed significantly from that of IAA and control, both of which had statistically similar mycelial weights. He further found that gibberellic acid at 10 ppm was the most promising mycelial growth promoter, as the highest mycelial weight of 217.1 mg was recorded in this treatment. This was followed by 20 ppm of IBA that recorded significantly lower mycelial weight of 202.0 mg. Mycelial weights in all other treatments were significantly low. In addition, he found that the mycelial weight in the flasks having 30 ppm of IBA and, 20 and 30 ppm of IAA was significantly similar to that recorded with control. In regard to growth of sporocarps, he found that among all the test growth regulators, only GA_3 produced longer stipe, with mean stipe length of 11.68 cm. Mean stipe length in the bags receiving other regulators like IAA and IBA was statistically similar to untreated control. He further reported that the concentration of 10 ppm GA_3 enhanced the stipe length the most, thereby indicated it as the optimum dose. Other growth regulators did not have any effect on stipe length.

Mukhopadhyay *et al.* (2005) conducted a study to assess the effect of plant growth hormones on biomass production of *Pleurotus sajor-caju*. They grew this fungus under submerged condition in deproteinized whey supplemented with diammonium hydrogen phosphate and plant growth hormones *viz.*, Indole-3-acetic acid (IAA), gibberellic acid (GA_3) and kinetin (KIN). Based on the experimental data, they reported that the hormones at different concentrations increased the biomass production of *P. sajor-caju* by 15–26%.

Dey *et al.* (2007) conducted an experiment to study the effect of IAA and NAA on mycelial colony proliferation of milky mushroom and varietal performance of different mushroom varieties on to different media. Based on the data, they found that MEA medium supplemented with 5 mg L^{-1} IAA had the highest mycelial growth (8.20 cm) and it was lowest (6.50 cm) with 20 mg L^{-1} at 21 DAI (days after inoculation); NAA at 10 mg L^{-1} had the best mycelial growth (8.10 cm) and poor (6.90 cm) with 5 mg L^{-1} at 21 DAI. When they studied the combined effect of the growth regulators, 5 mg L^{-1} IAA + 10 mg L^{-1} NAA had the maximum mycelial growth (8.80 cm), while the lowest (6.80 cm) was with 20 mg L^{-1} IAA + 5 mg L^{-1} NAA at 21 DAI.

Guo *et al.* (2009) conducted an investigation to appraise the effects of phytohormones including Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) on mycelial growth of medicinal mushroom *Phellinus linteus*. In the investigation they found that, under the optimal IAA, IBA and NAA concentration of 1.0, 1.5 and 5.0 mg/l, the maximal mycelial diameter reached to 8.6 ± 0.4, 7.3 ± 2.6 and 9.0 ± 1.0 mm, respectively. Moreover, they reported that the mycelial biomass and exopolysaccharide (EPS) production with addition of 5.0 mg/l NAA in a shake flask were 6.24 ± 0.18 g/l at 168 h and 0.86 ±0.01g/l at 192 h, which were enhanced by 15.98 and 56.36% in comparison to control, respectively. Results of their experimentation explicitly indicated that NAA at the proper concentration was beneficial in stimulating mycelial growth and EPS biosynthesis, whereas it could not alter the molecular structure of EPS.

Dey *et al.* (2011) conducted an experimentation to find out the effect of two plant growth regulators, gibberellic acid (GA) and naphthalene acetic acid (NAA) at 20, 50, 100 and 200 ppm concentration on growth, yield and yield attributes of button mushroom. In this experiment, they found that the least number of days (13.6) were required from casing to primordial initiation in the treatment of 100 ppm GA_3.

Pani (2011) conducted *in vitro* and *in vivo* studies to evaluate the effect of growth regulators on milky mushroom (*Calocybe indica*). In this investigation, in the submerged culture, he tested Indole Acetic Acid (IAA), Indole Butyric Acid (IBA), Naphthalene Acetic Acid (NAA), Kinetin, 2, 4-D and Maleic Hydrazide (MH) at 10, 25 and 50 ppm. He found that all but MH increased the biomass production. Furthermore, he found that GA_3 was most effective in inducing significantly higher mycelia yield at all doses; however, the maximum dry biomass (851.4 mg) was obtained at 50 ppm. It was followed by Kinetin (675.1mg, 10 ppm). Further he reported that the higher dose (50 ppm) of NAA was toxic, while lower concentrations (10, 25 ppm) were significantly stimulating. Unlike GA_3, he reported that the increase in concentration of Kinetin decreased the rate of vegetative multiplication. IAA also sustained appreciable dry weights (390.8- 432.2 mg). In case of IBA, he found that the increase in mycelia harvest over control was statistically insignificant.

Uddin *et al.* (2012) conducted an experiment to investigate the mycelial colony proliferation of different mushroom species *viz*., oyster (*Pleurotus florida*), milky (*Calocybe indica*) and button (*Agaricus bisporus*) in different media (PDA, YPDA and MEA) and different combinations and concentrations (0, 1, 5, 10 and 20 ppm) of different growth regulators (IAA and NAA). In this investigation, they observed the best mycelial growth of 5.50 cm at 21 days after inoculation (DAI) with 10 ppm IAA, 4.03 cm at 18 DAI with 5 ppm NAA and 5.38 cm at 21 DAI with control. Furthermore, in case of combined effect of IAA and NAA, they found the best mycelial colony proliferation (6.72 cm) with 10 ppm IAA + 5 ppm NAA at 21 DAI. From the investigation, they concluded that malt extract supplemented with 10 ppm IAA+ 5 ppm NAA was the best for mycelial colony proliferation of button mushroom.

Atri *et al*. (2013) experimented to study the effect of five growth regulators (GA_3, IAA, IBA, NAA and Kinetin) at various levels on mycelial growth of *Lentinus squarrosulus*. In this investigation they found that the gibberellic acid at 5 ppm level had maximum mycelial growth of the mushroom fungus.

Pal *et al*. (2013) evaluated five different growth regulators *viz*., Naphthaline acetic acid (NAA), Gibberellic acid, Cytokinin, 2, 4-Dichlorophenoxy acetic acid (2, 4-D) and Indole butyric acid (IBA) at 2 ppm concentration for their effect on mycelial growth of *Pleurotus eous*. In this experiment they recorded the highest mycelial growth of *Pleurotus eous* in gibberellic acid incorporated medium. However, they found that it was the least in 2, 4-D incorporated medium. Moreover they found that the different growth regulators as well influenced the period required for spawn run of *P. eous*. During July 2007, they observed significantly earlier spawn run in gibberellic acid (10.33 days) followed by cytokinin (12.33 days); whereas it was significantly delayed in 2, 4-D (15.67 days) followed by control (14.00 days), NAA (14.00 days) and IBA (13.00 days). Furthermore in October, they found that the spawn run was also quicker in gibberellic acid (11.33 days), whereas it was slower in 2, 4-D (17.33 days), followed by cytokinin (13.67 days), NAA (13.33 days), IBA (13.33 days) and control (13.33 days). The mean of two months data of this experiment explicitly indicated that gibberellic acid incorporated substrate required considerably less (10.83 days) period for spawn run of *P. eous*. In other growth regulators spawn run period varied from 13.16-16.5 days.

Atri and Guleria (2013) conducted an experiment with an objective to investigate the effect of biochemical sources *viz.*, vitamins, phytohormones and trace elements on the vegetative growth of *Lentinus cladopus* Lév. In this experiment they raised the culture of *L. cladopus* in malt broth liquid medium for investigating the role of vitamins (Ascorbic Acid, Biotin, Nicotinic Acid, Thiamine), phytohormones (Gibberellic Acid, Indole-3-Acetic Acid, Indole-3-Butyric Acid, Kinetin) and trace elements (Iron, Boron, Manganese, Molybdenum, Zinc). Results of their experiment explicitly substantiated that Thiamine at 10 ppm, Indole-3-Butyric Acid at 15 ppm and Iron at 1 ppm supported the maximum mycelial growth of *L. cladopus*. Thus, from the investigation they concluded that the vegetative growth of *L. cladopus* can be enhanced by adding Thiamine, Indole-3-Butyric Acid and Iron in basal medium.

Chauhan (2013) reported that the highest mycelial growth (131 mg) of *P. djamor* occurred by the addition of 25 ppm gibberellic acid followed by 50 ppm kinetin (123.5 mg) and again 10 ppm gibberellic acid (120.6 mg). Further they reported that all the growth regulators increased the vegetative growth of the fungus in comparison to control (90 mg). NAA at 10 ppm and 25 ppm concentrations had very little positive effect on growth of the fungus.

Mohapatra and Behera (2013) evaluated eight plant growth regulators for their possible role in yield improvement of *Volvariella volvacea*. In this experiment, they reported that the days required for appearance of pinhead and number of fruiting bodies were 9 and 38.67, respectively. Additionally, they found that the period taken for pinhead emergence was lowest (8 d) in 2 % glucose treated beds whereas, it was highest (10.67 d) in control beds. This exploratory investigation indicated that IAA @ 200 ppm stimulated early pinning (9 d) in *V. volvacea*.

Nasr and Mahdipour (2013) conducted a study mainly aimed to examine the effect of natural and synthetic growth regulators (IAA, NAA) and different media (YPDA, MEA, and PDA) on the growth of mycelium of the two mushrooms *viz., Agaricus bisporus* and *Pleurotus florida*. They examined the treatments in different media and under various densities of growth hormones (0, 1, 5, 10, 20 ppm). Based on results of the study, they reported the maximum mycelium growth (6.8cm) of *A. bisporus* at IAA 10 ppm and NAA 5 ppm and that of *P. florida* at IAA 5 ppm and NAA 0 ppm.

Harith (2014) reported that addition of growth hormones, consisting of β-indole acetic acid (IAA) combined with 6-Benzylaminopurine (BAP) at a concentration of 0.5 mg/L each, enhanced mycelial growth rate of *F. velutipes* at 10.53 mm/day compared to non-supplemented malt extract agar (MEA) medium (7.83 mm/day).

Krishnamoorthy and Balan (2015) appraised growth regulators like indole acetic acid, indole-3-butryic acid, gibberellic acid and kinetin for their effect on sporophore size of milky white mushroom. Results of their experiment clearly indicated that GA_3 at 40 ppm and kinetin increased the pileus diameter slightly higher, but none of the growth regulators had a significant influence on the stipe length.

Atri and Kaur (2016) testified from the experimentation that gibberellic acid at 5 ppm and boron at 5 ppm concentration supported the maximum vegetative growth of *Pleurotus sapidus*. The present findings helped to understand the biochemical requirements of *P. sapidus* for enhancing the vegetative growth of this mushroom in the basal medium.

Joshi (2016) studied the effect of growth regulators, micronutrients and chemical fertilizers on biological efficiency of four oyster mushrooms *Pleurotua sajor-caju*, *P. citrinopileatus*, *P. florida* and *H. ulmarius*. She reported no growth of *Pleurotus sajor-caju* at all the three concentrations (500, 1000, 1500 ppm) of NAA. Further she reported that at 500 ppm concentration of IAA, 0.9 cm growth was observed but it was less than the control (2.0 cm). GA_3 had positive results at all the three concentrations (500, 1000, 1500 ppm). She observed maximum growth (3.2 cm) at 1500 ppm concentration of GA_3, which was followed by 3.0 cm at remaining two concentrations. Cycocil had mycelial growth but it was less than growth in control. On the basis of these results she concluded that GA_3 had positive effect on the mycelial growth of *P. sajor-caju*.

Ramachela and Sihlangu (2016) investigated the effects of various hormonally treated substrates on mycelial development, pinning and biomass of *Pleurotus ostreatus*. For the said experimentation they used 3×4 experimental layout i.e. three substrates: *Urochloa panicoides*, *Zea mays* and *Datura stramonium* and four hormonal treatments: cytokinins, auxins, gibberellins and control. In this experiment, they observed significant differences in mycelial development, pinning, fruit caps and biomass. Hormones used in the experiment had significant effect on cap size and stipe length. They reported cap sizes as: auxins: 13.42 cm, cytokinins: 9.9 cm and gibberellins: 7.13 cm and; stipe lengths as: 6.93, 8.83 and 11.03 cm, respectively.

2.2 Effect of Growth Regulators on Yield and Yield Contributing Parameters of Mushroom

Halbert and Schisler (1986) tested 9 potential growth regulators (GR's) to study their ability to affect yield (kg/m^2) and/or average size (g/mushroom) of sporophores of *Agaricus bisporus*. The GR's used in this study were flurprimidol, cycocel, succinic acid dimethylhydrazide, ancymidol, gibberellic acid, 6-benzyladenine, α-napthalene acetic acid, caffeine and theophylline. They applied 2 ml of GR solution or water control to the casing surface, on the day that primordial were first visible. Results of this investigation indicated that some of the compounds at some concentrations effected yield and size. These results suggest potential use of GR's to produce larger fruiting bodies without a reduction in yield.

Shukla (1995) reported that spraying of Indole butyric acid @ 5 and 10 ppm induced maximum number of sporophores in *L. edodes*.

Eswaran and Ramabadran (2000) conducted studies on physiological, cultural and postharvest aspects of oyster mushroom *Pleurotus eous.* They applied 3 plant hormones such as gibberellic acid (GA), Indole-3-acetic acid (IAA) and 6-benzyl amino purine (BAP) at 1, 10, 100 and 200 ppm with atomizer on surface of the mycelial mat as soon as sporophore initials appeared on beds. Results of their experimentation showed that GA at 100 ppm was the best in improving the yield of *P. eous*.

Ramanujam (2003) reported that application of different concentrations of plant hormones increased the number, weight of sporophores and biological efficiency of *Pleurotus* spp. Further he reported the increased number, weight of sporophores and biological efficiency when GA_3 (100 ppm) was applied. He speculated that the stimulatory action of GA might be due to increase in cell division and cell elongation. The results revealed that the sporophore yield increased significantly in all the treatments, when compared to control. He reported the highest sporophore yield of 342.66 g with the application of GA @ 100 ppm.

Jansi (2010) evaluated the effect of gibberellic acid and glutamic acid on yield of *Pleurotus florida* by using paddy straw substrate. He sprayed the growth regulators at 500 ppm concentration on mushroom beds after mycelial run. From the data obtained, they reported maximum yield of the mushroom with the spray of glutamic acid.

Rostum (2010) conducted different experiments on *Pleurotus ostreatus.* In the first experiment he used Gibberellin (GA_3) in the beginning of the pinhead stage, at the concentrations of 0 ppm or 250 ppm or 500 ppm or 1000 ppm GA_3. In the second experiment he used Benzyl Adenine (BA) in the same stage and in the same concentrations. From results of the experiment he reported that the weight of fruiting body, total yield and biological efficiency of the mushroom increased with increase in the concentration of GA_3. By using 500 ppm of GA_3, he reported increase in fruiting body weight to 12.5g, total sporophore yield to 910.8 g/kg of substrate and the biological efficiency to 91%. Further they found that by using 500 ppm BA he found increased fruiting body weight to 11.7 g, total yield to 621.6 g/kg of substrate and the biological efficiency to 62 %.

Dey *et al.* (2011) conducted an experimentation to find out the effect of two plant growth regulators, gibberellic acid (GA) and naphthalene acetic acid (NAA) at 20, 50, 100 and 200 ppm concentration on growth, yield and yield attributes of button mushroom. In this investigation, they obtained the higher number of fruiting bodies in NAA 50 ppm (70.6), GA_3 200 ppm (72.2) and NAA 100 ppm (67.8); while the highest yield was obtained in NAA 100 ppm (348.62 g/3kg of compost) followed by GA_3 50 ppm (336.52g/3kg of compost), NAA 50 ppm (336.09g/3kg of compost) and GA_3 100 ppm (313.55g/3kg of compost).

Pani (2011) conducted *in vitro* and *in vivo* studies to evaluate the effect of growth regulators on milky mushroom (*Calocybe indica*). In this investigation, in the submerged culture, he tested Indole Acetic Acid (IAA), Indole Butyric Acid (IBA), Naphthalene Acetic Acid (NAA), Kinetin, 2, 4-D and Maleic Hydrazide (MH) at 10, 25 and 50 ppm. His field trials unveiled that spraying 50 ppm GA_3 on the emerging primordia increased the number as well as size of mushrooms and produced significantly higher yield (82.2% biological efficiency) compared to control (67.8% biological efficiency). Further they reported that the increase in sporophore yields, though was not statistically significant than control, was also recorded with 10 ppm Kinetin (76.0 % biological efficiency), 25 ppm IAA (73.1% biological efficiency) and 25 ppm NAA (71.6% biological efficiency).

Mohapatra and Behera (2013) evaluated eight plant growth regulators for their possible role in yield improvement of *Volvariella volvacea*. In this experiment, they obtained the highest yield of 920 g/bed from the application of Indole Acetic Acid (IAA) @ 200 ppm. However, they reported that this yield level was at par with the yield level realized by the treatment that received urea at 2.5 % concentration (883.33 g/bed). Further they reported that with the exception of micronutrient mixture application at 0.25 % and gibberellic acid at 50 ppm, all other treatments were either equal or more than the yield realized from the control (water spray) treatment (753.33 g/bed). This exploratory investigation indicated that IAA @ 200 ppm stimulated higher yield (13.14 %) in *V. volvacea* with an yield increase of 22.12 % over control.

Pal *et al*. (2013) evaluated five different growth regulators *viz*., Naphthaline acetic acid (NAA), Gibberellic acid, Cytokinin, 2, 4-Dichlorophenoxy acetic acid (2, 4-D) and Indole butyric acid (IBA) at 2 ppm concentration for their effect on yield of *Pleurotus eous*. In this experiment they recorded the highest yield of *Pleurotus eous* in gibberellic acid incorporated medium. However, they found that it was the least in 2, 4-D incorporated medium.

Sarker and Chowdhury (2013) evaluated the effect of gibberellic acid (GA_3) on growth and yield performance of oyster mushroom. They sprayed GA_3 with eleven doses *viz.*, 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm at the primordia initiation stage to evaluate its effect on growth and yield performance of the mushroom. In this experiment, they observed that at 10 ppm level GA_3 gave the highest economic yield and dry weight. They obtained increased fresh economic yields to about 30% and 34% and, 80% and 115% increased dry weight with the application of GA_3 at the first and second harvests, respectively in comparison to control. Moreover, GA_3 had a positive effect on number of effective fruiting body, stalk length, pileus diameter, biological yield, economic yield and dry economic yield. Results of this experimentation implied that GA_3 at 10 ppm/packet would be the best possible concentration for production of oyster mushroom.

Krishnamoorthy and Balan (2015) appraised growth regulators like indole acetic acid, indole-3-butryic acid, gibberellic acid and kinetin for their effect on yield of milky white mushroom. Results of their experiment clearly indicated that GA_3 at 40 ppm increased the yield of sporophores. Further they reported that the average weight of individual mushrooms was high when GA_3 and kinetin were sprayed.

Joshi (2016) studied the effect of growth regulators, micronutrients and chemical fertilizers on biological efficiency of four oyster mushrooms *Pleurotus sajor caju*, *P. citrinopileatus*, *P. florida* and *H. ulmarius*. She reported the highest yield (401.10 g/500 g dry weight of substrate) along with biological efficiency (80.22%) using GA_3 @ 1500 ppm treatment.

Kaur (2016) conducted an experiment aimed to increase the biomass production and yield potential of *Calocybe indica* using three growth regulators namely Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) and gibberellic acid (GA_3). In the present investigation, he reported maximum yield with GA when sprayed at pinhead formation (64.3 kg/q) and at all stages (71.1 kg/q); with IBA at 1^{st} flush (75.9 kg/q) and at all stages (70.9 kg/q); with IAA at 1^{st} flush (58.2 kg/q) and at all stages (62.1 kg/q). Further he reported no significant variation in the yield potential of milky mushroom with supplementation of growth regulators in substrate at the time of spawning.

2.3 Effect of Growth Regulators on Protein Content of Mushroom

Paul *et al*. (1994) reported that IAA, GA_3 and Kinetin at an optimum concentration increased the biomass production of food yeast *Kluyveromyces fragilis* grown in deproteinized whey, but they did not observe the effect of hormones on protein content of biomass.

Mukhopadhyay *et al.* (2005) while experimenting to assess the effect of plant growth hormones on biomass production of *Pleurotus sajor-caju* found that the increase in protein content of the mycelia was relatively small (3–5%) over the control. Further they observed maximum enhancement with Indole-3-acetic acid. They could harvest 28% more protein from 1l of whey containing 2mg/l of indole-3-aceticacid.

3. MATERIALS AND METHODS

Aiming at to study the effect of various growth regulators and standardization of dosage of the growth regulators for augmenting the yield of milky mushroom (*Calocybe indica*), the present investigation was undertaken during March-April, 2018 at the Plant Pathology Section of College of Agriculture, Kolhapur.

3.1 Materials

3.1.1 Spawn Production

3.1.1.1 Pure culture of *Calocybe indica*

Pure culture of *Calocybe indica* was obtained from the Mycologist, All India Coordinated Mushroom Improvement Project, College of Agriculture, Pune.

3.1.1.2 Mass multiplication of *Calocybe indica* culture

Whisky glass bottles of 180 ml capacity were used for making potato dextrose agar slants for mass multiplication of *Calocybe indica* culture.

3.1.1.3 Production of mother spawn and planting spawn

Polypropylene bags (size, 20 cm x 30 cm; gauge, 175), calcium carbonate, glucose saline glass bottles of 500 ml capacity, non-absorbent cotton etc. were used for spawn production. Wheat grain was used as a base material for production of spawn.

3.1.2 Bed Preparation

High density polythene bags of 45 cm x 55 cm size (100 gauge) were used for bed preparation. Well dried, chopped wheat straw was used for cultivation of *Calocybe indica*. It was procured from the Agronomy Farm of College of Agriculture, Kolhapur.

3.1.3 Disinfection of Mushroom House

Formaldehyde [37-41% w/v of E Merck (India) Ltd.] at 2 per cent concentration was used for fumigation of mushroom cultivation rooms. In addition, bavistin and copper sulphate were also used at higher concentrations of 10 per cent for elimination of fungal flora in mushroom house. Dichlorovos (DDVP) was used at the concentration of 0.2% for killing insect pests in mushroom house.

3.1.4 Casing Material

Casing material used in the study consisted of mixture of farm yard manure (FYM) and garden soil in 1:1 proportion. The casing material was subjected to intermittent sterilization at 121^0C for 1 h, for 3 consecutive days in an autoclave.

3.1.5 Laboratory Nutrient Media

Potato dextrose agar (PDA) was used for maintenance of *Calocybe indica* culture.

3.1.6 Fungicides and Chemicals

Various fungicides and chemicals *viz.*, bavistin, ethanol 70 per cent, copper sulphate, formaldehyde, calcium carbonate etc. needed in the investigation were used wherever necessary. All the inorganic chemicals used in the experiment were of analytical grade. Growth regulators (GA, IAA and IBA) used were of LR grade.

3.1.7 Chemicals Used for Protein Estimation

H_2SO_4, $CuSO_4.5H_2O$, K_2SO_4, NaOH, methyl red/bromocresol green indicator solution and boric acid Solution.

3.1.8 Glasswares

The Borosil brand glasswares *viz.*, Petriplates, beakers, conical flasks, measuring cylinders, test tubes etc. were used wherever necessary.

3.1.9 Equipments

The laboratory equipments *viz.*, laminar air flow, hot air oven, autoclave, B.O.D. incubator, refrigerator, electronic balance etc. were used wherever necessary.

3.1.10 Miscellaneous Material

Inoculating needle, chop cutter, metallic pan, knives, plastic drum (200 l capacity), labels, spirit lamp etc. were used for the investigation.

3.2 Methods

3.2.1 Maintenance of Pure Culture of *Calocybe indica*

Pure culture or so called master culture of *Calocybe indica* was was maintained on PDA medium by monthly serial transfers to fresh medium and incubated at 28 $\pm$ 1^0C for 7 days. Subcultures full of mycelium after 7 days incubation at 28 $\pm$ 1^0C were stored in refrigerator at 4 $\pm$ 1^0C till further use.

3.2.2 Mass Multiplication of *Calocybe indica* Culture

Potato dextrose agar slants were prepared in whisky glass bottles of 180 ml capacity and *Calocybe indica* culture was inoculated aseptically under laminar flow. The inoculated slants were kept for incubation at 28 $\pm$ 1^0C till the complete growth of fungal mycelium on the agar slants.

3.2.3 Spawn Production

The method recommended by Garcha and Kalra (1979) was followed for spawn production of *Calocybe indica*.

3.2.3.1 Spawn substrate preparation

Only whole wheat grains were selected, weighed and cleaned by washing 2-3 times with tap water and put in a metallic vessel. The water was filled in the vessel to the level so that grains were just immersed in it. The grains were cooked in boiling water to the extent that starch did not come out of the grains. After cooking, the grains were spread on a wide wire gauze mesh for the excess water to trickle down. These were kept overnight in airy place. Next morning 4% calcium carbonate and 2 % gypsum, calculated on the weight of these boiled grains, were mixed. Wide-mouthed glucose saline glass bottles were used for the preparation of mother spawn whereas, heat resistant polypropylene bags were used for preparation of planting spawn. The boiled grains were filled into these glass bottles and polypropylene bags (size, 20 cm x 30 cm; gauge, 175) upto ¾ capacities and plugged with non-absorbent cotton. Bottles and polypropylene bags were sterilized in autoclave at 20 lbs/sq. inch for one hour. After sterilization, bottles and bags were cooled and shaken well. These bottles and bags were transferred to a sterile (UV-sterilized) laminar flow chamber.

3.2.3.2 Preparation of mother spawn

Glucose saline glass bottles were used for preparation of mother spawn for reasons of convenience. The autoclaved bottles containing sterilized wheat grains were inoculated aseptically by transferring small mycelial bits from the 7 day old culture of *Calocybe indica* that was multiplied on whisky bottle slants. The bottles were gently shaken to disperse the inoculum for facilitating multiple contacts of the grains with the inoculum. The bottles were then incubated at $26 \pm 1^{0}C$ in an incubator for 15 days.

3.2.3.3 Preparation of planting spawn

The autoclaved polypropylene bags containing sterilized wheat grains were inoculated under laminar flow with the mother spawn. Four to six spatula fulls were poured into each polypropylene bag containing 500 g wet sterilized wheat grains, under aseptic conditions. The bags were replugged with cotton in laminar flow and the contents were shaken gently to disperse the inoculum uniformly in the substrate. These bags were then incubated in incubator at $28 \pm 1^{0}C$ for 7-10 days. The spawn was ready for use after full impregnation of the spawn substrate with the mycelium.

3.2.4 Preparation of Cultivation Substrate

The cultivation substrate was prepared by hot water treatment as described by Singh and Dwivedi (1991). Freshly harvested well dried wheat straw was chopped in to 5-6 cm bits. It was then soaked in fresh water for 12 hours. The excess water was allowed to drain off and it was then pasteurized by treating it with hot water. The hot water treatment involved filling a metallic pan with chopped straw and water and heating the contents of the pan by means of wood and coal fire until the temperature of water and substrate reached 80^0C. This temperature was maintained for 90 minutes after which the straw was removed and the excess water drained away. The substrate was transferred to spawning room and cooled on a flat, clean surface.

3.2.5 Disinfection of Mushroom House

The rooms meant for spawning, spawn running and cropping were cleaned and disinfected by spraying 0.2 per cent carbendazim and 0.2 per cent dichlorovos (DDVP) with a knapsack sprayer. After spraying, the rooms were kept airtight for 24 h and then opened for fresh air circulation.

3.3 Effect of Growth Regulators on Yield of Milky Mushroom

Various growth regulators at different dosages were appraised in the present investigation in order to find out the most effective growth regulator and its dosage for augmenting the sporocarp yield of *Calocybe indica.*

3.3.1 Experimental Details

3.3.1.1 Treatments

The treatments consisted of spraying the mushroom beds with aqueous solutions of growth regulators, after full spawn running. The treatments were:

T_1 Gibberellic Acid (GA) 5 ppm
T_2 Gibberellic Acid (GA) 10 ppm
T_3 Gibberellic Acid (GA) 15 ppm
T_4 Gibberellic Acid (GA) 20 ppm
T_5 Indole -3-Acetic Acid (IAA) 5 ppm
T_6 Indole -3-Acetic Acid (IAA) 10 ppm
T_7 Indole -3-Acetic Acid (IAA) 15 ppm
T_8 Indole -3-Acetic Acid (IAA) 20 ppm
T_9 Indole- 3- Butyric Acid (IBA) 5 ppm
T_{10} Indole -3- Butyric Acid (IBA) 10 ppm
T_{11} Indole -3- Butyric Acid (IBA) 15 ppm

T_{12} Indole -3- Butyric Acid (IBA) 20 ppm

T_{13} Control

3.3.1.2 Replications: 3

3.3.1.3 Design

Data were tested for their significance by employing completely randomized design.

3.3.2 Preparation of Mushroom Bed

The spawning was done in pre-fumigated room (48 h with 2% formaldehyde). Pasteurized cultivation substrate was layer spawned in high density polythene bags of size 45 cm x 55 cm (100 gauge) by using freshly prepared pure wheat grain spawn at 2 per cent on wet weight basis. These bags had 25-30 pinhole perforation all over for ventilation of substrate. Few pinholes were also made at bottom of the bag for drainage. Each bag contained 3.5 kg wetted straw (one kg dry straw). The mouth of bag was closed with a rubber band.

3.3.3 Crop Management

Mushroom beds thus prepared were then stacked on iron shelves in spawn run room. Complete darkness and 30± 2^0C temperature was maintained in this room till completion of mycelial run. Beds with full white mycelial growth were opened and transferred to the cropping room on a cemented floor. These beds were cut into two equal halves. In order to study the effect of growth regulators on the yield of sporophores, required quantity of the chemicals were dissolved in 1 L distilled sterile water to prepare growth regulator solutions. These solutions were sprayed on the fully colonized substrate as per the treatments. After spraying of the growth regulators topmost layer of the substrate in each bed was covered with 2 cm thick layer of sterilized casing material (garden soil + farm yard manure, 1: 1 v/v). Casing layer was kept moist by giving gentle sprays of water as and when required. In the cropping room, 30 ± 2^0C and 85-90 per cent relative humidity was maintained. The humidity in the cropping room was maintained with the help of mist blowers which were run for 5-10 minutes, 4-5 times in a day. Beds were exposed to diffused light throughout the cropping period. Second spray of the growth regulator solutions was given on the same beds after the first and second harvests. Three replications were maintained for each treatment (Plate 3.1).

3.3.4 Harvesting

Watering was withheld a day before harvesting. Harvesting of sporophores was done before spore shedding. Fresh weight of mushroom was recorded immediately after harvesting.

3.3.5 Observations

1. Days required for spawn run
2. Days required for pinhead formation
3. Days required for first harvest
4. Period between flushes
5. Number of sporophores
6. Breadth of pileus
7. Length of stipe
8. Yield of sporophores per picking
9. Total yield
10. Biological efficiency
11. Protein content

Biological efficiency was calculated by the following formula.

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

3.3.6 Protein Analysis

Protein content in the sporophores was analyzed by Micro Kjeldahl's Digestion method.

3.3.7 Statistical Analysis

The data obtained from the trial were statistically analyzed in Completely Randomized Design as per the procedure described by Panse and Sukhatme (1985).

4. RESULTS AND DISCUSSION

Aiming at to study the effect of various growth regulators and standardization of effective dosage of the growth regulators for augmenting the yield of milky mushroom (*Calocybe indica*), the present investigation was undertaken during March-April, 2018 at the Plant Pathology Section of College of Agriculture, Kolhapur. Results obtained in the present investigation are presented in this chapter.

4.1 Days Required for Complete Spawn Run

Since the growth regulators, aimed to study their effect on growth and yield of milky mushroom (*Calocybe indica*), were sprayed on the mushroom beds after completion of spawn running, the data pertaining to days required for completion of spawn run did not show significant differences amongst the various treatments of growth regulators. Days required for completion of spawn run ranged from 14.33 to 16.33 days in all beds (Table 1).

4.2 Days Required for Pinhead Formation

Data depicted in Table 4.1 and Fig.4.1 overtly indicated that the growth regulators tried at different concentrations in the present investigation, aiming at promoting growth and sporophore yield of *Calocybe indica*, exhibited significantly varied response to days required for pinhead formation.

Amongst the various growth regulators used in the present investigation, spraying of gibberellic acid (GA) 20 ppm significantly demonstrated its efficacy over other treatments by considerably reducing the period required for pinhead formation. Primordia in this treatment appeared on 30.00 days after spawning. This treatment, thus, indubitably induced pinning earlier than rest of the treatments, which took 31.33 to 36.33 days to induce pinning, the latest being in control. Period required for pinhead formation with this treatment, thus, was explicitly found to be curtailed by 6.33 days in comparison to untreated control. Spraying of indole-3-acetic acid (IAA) 20 ppm and indole-3-butyric acid (IBA) 20 ppm were the next best treatments, which induced pinning in 31.33 and 31.00 days, respectively. However, days required for pinning with these treatments differed non-significantly, which clearly substantiates that spraying of indole-3-acetic acid 20 ppm and indole-3-butyric acid 20 ppm had similitude effect in hastening the pinning. These treatments were followed by the treatment of gibberellic acid 15 ppm. This treatment took 32.22 days for inducing the pinning.

To summarize, the results clearly indicated the efficacy of gibberellic acid at 20 ppm over other treatments in curtailing the days required for pinhead formation, which is exemplified from the early pinning with this treatment.

Table 4.1. Effect of growth regulators on days required for spawn run, pinhead formation, first harvest and period between flushes in milky mushroom (*Calocybe indica*)

Treatment	Number of days required for spawn run	Number of days required for pinhead formation	Number of days required for first harvest	Period between flushes (number of days)
T_1 Gibberellic Acid (GA) 5 ppm	15.00	34.67	42.00	9.33
T_2 Gibberellic Acid (GA) 10 ppm	14.67	33.33	40.00	9.00
T_3 Gibberellic Acid (GA) 15 ppm	15.33	32.33	38.67	8.33
T_4 Gibberellic Acid (GA) 20 ppm	15.33	30.00	35.67	7.00
T_5 Indole -3-Acetic Acid (IAA) 5 ppm	16.00	35.00	42.33	9.33
T_6 Indole -3-Acetic Acid (IAA) 10 ppm	16.33	35.33	42.67	9.33
T_7 Indole -3-Acetic Acid (IAA) 15 ppm	14.67	33.33	40.33	8.67
T_8 Indole -3-Acetic Acid (IAA) 20 ppm	14.33	31.33	37.00	8.00
T_9 Indole- 3- Butyric Acid (IBA) 5 ppm	15.33	34.67	42.33	9.33
T_{10} Indole -3- Butyric Acid (IBA) 10 ppm	14.67	35.33	43.00	9.33
T_{11} Indole -3- Butyric Acid (IBA) 15 ppm	15.00	33.67	40.67	8.67
T_{12} Indole -3- Butyric Acid (IBA) 20 ppm	14.33	31.00	37.33	8.00
T_{13} Control	16.00	36.33	44.33	9.67
S.E. m±	0.83	0.29	0.42	0.32
CD at 5%	NS	0.86	1.24	0.94

Pal *et al.* (2013) reported that gibberellic acid incorporated substrate required considerably less (10.83 days) period for spawn run of *P. eous*. Moreover, earlier researchers as well have reported the role of growth regulators in enhancing mycelial growth of array of edible mushrooms (Shukla, 1995; Bhardwaj, 2004; Mukhopadhyay *et al.*, 2005; Dey *et al.*, 2007; Guo *et al.*, 2009; Pani, 2011; Uddin *et al.*, 2012; Atri *et al*., 2013; Pal *et al*., 2013; Atri and Guleria, 2013). Thus, the effect of growth regulator on mycelial growth, as reported by the foregoing researchers, might have reflected in earlier pinning in the present investigation. Mohapatra and Behera (2013) obtained early pinning in 9 days in *Volvariella volvacea* with IAA @ 200 ppm. Moreover, Dey *et al.* (2011) found that the least number of days (13.6) were required from casing to primordial initiation in the treatment of 100 ppm GA_3 in button mushroom. Thus, results of the present investigation are in agreement with results of these researchers.

4.3 Days Required for First Harvest

Data summarized in Table 4.1 and Fig.4.2 pertaining to days required for first harvest of sporophores of milky mushroom (*Calocybe indica*) clearly imply that the growth regulators, tried in the present investigation, at different concentrations had influential effect in curtailing days required for first harvest of sporophores. The sporophores with various treatments matured in 35.66 to 44.33 days.

Various growth regulators, tried at different concentrations, varied considerably in their potentiality to hasten maturity of sporophores. Amongst the various growth regulators, spraying of gibberellic acid (GA) 20 ppm supported significantly earliest maturity of sporophores, which demonstrated its efficacy over other treatments in reducing days required for first harvest of sporophores. Sporophores in this treatment became ready for harvest on 35.66 days after spawning. In contrast, sporophores in rest of the treatments became ready for harvest in 37.00 to 44.33 days, the latest being in untreated control. Period required for first harvest of sporophores with this treatment, thus, was curtailed by 8.67 days in comparison to untreated control beds. Spraying of indole-3-acetic acid (IAA) 20 ppm and indole-3-butyric acid (IBA) 20 ppm were the next best treatments, in which sporophores became ready for harvest in 37.00 and 37.33 days, respectively. However, days required for first harvest of sporophores with these treatments differed non-significantly, which implies that spraying of indole-3-acetic acid 20 ppm and indole-3-butyric acid 20 ppm had identical effect in hastening maturity of sporophores. These treatments were followed by the treatment of gibberellic acid 15 ppm. Sporophores in this treatment became ready for harvest in 38.66 days.

To summarize the results, the treatment of spraying of gibberellic acid (GA) 20 ppm justified its outweigh performance, in comparison to rest of the treatments, in hastening maturity of sporophores.

Pal *et al.* (2013) reported that gibberellic acid incorporated substrate required considerably less (10.83 days) period for spawn run of *P. eous*. Similarly, Mohapatra and Behera (2013) obtained early pinning in 9 days in *Volvariella volvacea* with IAA @ 200 ppm. Dey *et al.* (2011) as well found that the least number of days (13.6) were required from casing to primordial initiation in the treatment of 100 ppm GA_3 in button mushroom. Likewise, early formation of primordia in the present investigation might have led to correspondingly earlier maturity of sporophores in the mushroom beds those received spray of various growth regulators.

4.4 Period between Flushes

Data pertaining to period between flushes of sporophores of milky mushroom (*Calocybe indica*) suggested that the growth regulators, tried in the present investigation at different concentrations, had significant effect on period between flushes of sporophores (Table 4.1 and Fig. 4.3).

Growth regulators, tried at different concentrations in the present investigation, showed considerable variation in period between flushes of sporophores. This period between flushes with various treatments ranged between 7.00 and 9.66 days. However, amongst the various treatments of growth regulator, spraying of gibberellic acid (GA) 20 ppm was found most effective in reducing the period between flushes of sporophores. Least number of days to the tune of 7.00 between flushes of sporophores was recorded with this treatment. Period between sporophore flushes with this treatment was, thus, found to be abridged by 2.66 days, in comparison to control. On the contrary, period between flushes of sporophores in rest of the treatments ranged between 8.00 and 9.66 days, the maximum being in untreated control. Spraying of indole-3-acetic acid 20 ppm, indole-3-butyric acid 20 ppm, gibberellic acid 15 ppm, indole-3-acetic acid 15 ppm and indole-3-butyric acid 15 ppm were the next best treatments in reducing period between flushes. However, period between flushes of sporophores with these treatments differed non-significantly, which implies that these treatments had identical effect in this regard.

To summarize the results, the treatment of spraying of gibberellic acid (GA) 20 ppm was most effective in curtailing period between flushes of sporophores.

4.5 Diameter of Pileus

Data presented in Table 4.2 and Fig. 4.4 pertaining to diameter of pileus of sporophores of milky mushroom (*Calocybe indica*) overtly substantiated that the growth regulators, tried in the present investigation, at different concentrations significantly influenced the size of sporophores.

In the present investigation, pileus showed significantly wide variation with regard to diameter due to various treatments of growth regulators. Diameter of pileus due to various treatments of growth regulators ranged between 9.32 cm and 15.40 cm. However, amongst the various growth regulators experimented, spraying of gibberellic acid (GA) 20 ppm significantly enhanced size of pileus. Diameter of pileus with this treatment was increased remarkably and it was to the tune of 15.40 cm. In contrast, diameter of pileus in rest of the treatments ranged between 9.32 cm and 14.48 cm, the least being in untreated control. Diameter of pileus with this treatment, thus, was improved by 6.08 cm, in comparison to those of sporophores obtained in untreated control beds. Spraying of indole-3-acetic acid (IAA) 20 ppm and indole-3-butyric acid (IBA) 20 ppm were the next best treatments, in which pileus of sporophores measured 14.48 cm and 14.21 cm in diameter, respectively. However, diameter of pileus of sporophores recorded with these treatments did not differ significantly, which explicitly substantiated that spraying of indole-3-acetic acid 20 ppm and indole-3-butyric acid 20 ppm had similitude effect in improving pileus size. These treatments were followed by the treatment of gibberellic acid 15 ppm, which recorded pileus diameter of 13.29 cm.

To summarize the results, the treatment of spraying of gibberellic acid (GA) 20 ppm testified outweigh efficacy, in comparison to rest of the treatments, in increasing size of pileus.

Many earlier researchers have reported varied response of growth regulators to size of sporophores. Krishnamoorthy and Balan (2015) reported that GA_3 at 40 ppm and kinetin increased the pileus diameter of milky mushroom slightly higher. Sarker and Chowdhury (2013) observed that GA_3 had a positive effect on pileus diameter of oyster mushroom. Ramachela and Sihlangu (2016) observed that the hormones used in the experiment had significant effect on cap size. In the present investigation, Gibberellic acid at 20 ppm increased the pileus diameter considerably. Thus, results of the present investigation are in conformity with results of these researchers.

Table 4.2. Size of sporophores of milky mushroom (*Calocybe indica*) as influenced by use of various growth regulators

Growth regulator	Diameter of pileus (cm)	Length of stipe (cm)
T_1 Gibberellic Acid (GA) 5 ppm	10.62	14.94
T_2 Gibberellic Acid (GA) 10 ppm	12.36	16.44
T_3 Gibberellic Acid (GA) 15 ppm	13.29	17.65
T_4 Gibberellic Acid (GA) 20 ppm	15.40	19.94
T_5 Indole -3-Acetic Acid (IAA) 5 ppm	10.71	15.10
T_6 Indole -3-Acetic Acid (IAA) 10 ppm	10.96	14.82
T_7 Indole -3-Acetic Acid (IAA) 15 ppm	11.92	16.39
T_8 Indole -3-Acetic Acid (IAA) 20 ppm	14.48	18.72
T_9 Indole- 3- Butyric Acid (IBA) 5 ppm	10.32	14.70
T_{10} Indole -3- Butyric Acid (IBA) 10 ppm	10.44	15.20
T_{11} Indole -3- Butyric Acid (IBA) 15 ppm	12.10	16.21
T_{12} Indole -3- Butyric Acid (IBA) 20 ppm	14.21	18.90
T_{13} Control	9.32	13.66
S.E. m±	0.24	0.23
CD at 5%	0.69	0.67

4.6 Length of Stipe

Data presented in Table 4.2 and Fig. 4.5 pertaining to stipe length of sporophores of milky mushroom (*Calocybe indica*) clearly validated that the growth regulators, tried in the present investigation, at different concentrations had significantly profound effect on size of sporophores.

In the present investigation, stipe of sporophore showed significantly wide variation with regard to length, due to various treatments of growth regulators. Stipe length due to various treatments of growth regulators ranged between 13.66 cm and 19.94 cm. However, amongst the various growth regulators experimented, spraying of gibberellic acid (GA) 20 ppm yielded sporophores with longest stipes, having mean stipe length to the tune of 19.94 cm. In contrast, stipe length in rest of the treatments ranged between 13.66 cm and 18.90 cm, the least being in untreated control. Stipe length with this treatment was increased remarkably by 6.28 cm, in comparison to that recorded in sporophores harvested from untreated control beds. Spraying of indole-3-acetic acid (IAA) 20 ppm and indole-3-butyric acid (IBA) 20 ppm were the next best treatments, in which stipe of sporophores measured 18.90 cm and 18.72 cm in length, respectively. However, length of stipe of sporophores recorded with these treatments did not differ significantly from each other, which explicitly substantiated that spraying of indole-3-acetic acid 20 ppm and indole-3-butyric acid 20 ppm had similitude effect in improving stipe length. These treatments were followed by the treatment of gibberellic acid 15 ppm, which recorded stipe length of 17.65 cm.

To summarize the results, the treatment of spraying of gibberellic acid (GA) 20 ppm testified outweigh efficacy, in comparison to rest of the treatments, in increasing length of stipe.

Earlier researchers have reported varied response of growth regulators to size of sporophores. Results of the present investigation are partly in agreement with Krishnamoorthy and Balan (2015) who reported that GA_3 at 40 ppm and kinetin increased the pileus diameter slightly higher, but none of the growth regulators had a significant influence on the stipe length of milky mushroom. Sarker and Chowdhury (2013) observed that GA_3 had a positive effect on stalk length of oyster mushroom. Bhardwaj (2004) found that GA_3 produced longer stipe, with mean stipe length of 11.68 cm. He further reported that GA_3 at concentration of 10 ppm enhanced the stipe length the most, thereby indicated it as the optimum dose. Ramachela and Sihlangu (2016) observed that the hormones used in the experiment had significant effect on stipe length. Thus, results of the present investigation are in conformity with the later.

4.7 Number of Sporophores

Data presented in Table 4.3 and Fig. 4.6 pertaining to number of sporophores of milky mushroom (*Calocybe indica*), revealed significantly varied response of different growth regulators at different concentrations.

Table 4.3. Number of sporophores and yield of milky mushroom (*Calocybe indica*) per harvest as influenced by various growth regulators

Treatment		Number of sporophores obtained in all harvests	Yield of sporophores (g)		
			First harvest	Second harvest	Third harvest
T_1	Gibberellic Acid (GA) 5 ppm	14.00	449.57	115.60	77.07
T_2	Gibberellic Acid (GA) 10 ppm	15.67	495.82	127.50	84.99
T_3	Gibberellic Acid (GA) 15 ppm	18.00	528.79	135.97	90.65
T_4	Gibberellic Acid (GA) 20 ppm	22.00	595.08	153.02	102.02
T_5	Indole -3-Acetic Acid (IAA) 5 ppm	14.33	454.17	116.79	77.85
T_6	Indole -3-Acetic Acid (IAA) 10 ppm	13.67	462.03	118.81	79.20
T_7	Indole -3-Acetic Acid (IAA) 15 ppm	16.67	500.00	128.57	85.71
T_8	Indole -3-Acetic Acid (IAA) 20 ppm	20.33	566.05	145.57	97.03
T_9	Indole- 3- Butyric Acid (IBA) 5 ppm	13. 33	459.34	118.11	78.74
T_{10}	Indole -3- Butyric Acid (IBA) 10 ppm	14.00	456.90	117.49	78.32
T_{11}	Indole -3- Butyric Acid (IBA) 15 ppm	16.33	491.69	126.43	84.28
T_{12}	Indole -3- Butyric Acid (IBA) 20 ppm	19.67	557.77	143.43	95.62
T_{13}	Control	12.00	421.33	108.34	72.23
S.E. m±		0.41	5.35	1.38	0.92
CD at 5%		1.21	15.65	4.03	2.68

Growth regulators assessed at different concentrations showed considerably wide-ranging variation in number of sporophores of *Calocybe indica*. Total number of sporophores, obtained from all the flushes, with various treatments of growth regulators ranged between 12.00 and 22.00. However, amongst all the treatments, spraying of gibberellic acid (GA) 20 ppm yielded significantly highest number of sporophores to the tune of 22.00. This treatment, thus, was found most effective than rest of the treatments and yielded 83.33% more sporophores in comparison to that obtained in untreated control beds. Spraying of indole-3-acetic acid (IAA) 20 ppm and indole-3-butyric acid (IBA) 20 ppm was the next best set of performers, which yielded 20.33 and 19.67 sporophores, respectively. The number of sporophores obtained with these two treatments differed non-significantly, indicating parity in their effectiveness. The treatment of spraying gibberellic acid at 15 ppm was the next treatment in performance, which yielded 18.00

sporophores. On the contrary, number of sporophores in rest of the treatments ranged between 12.00 and 16.67, the least being in untreated control.

To abridge the results, spraying of gibberellic acid (GA) 20 ppm was the most effective in augmenting number of sporophores of *Calocybe indica*.

Researchers earlier have documented response of growth regulators to number of fruiting bodies in variety of edible mushrooms. Shukla (1995) reported that spraying of Indole butyric acid @ 5 and 10 ppm induced maximum number of sporophores in *L. edodes*. Ramanujam (2000) reported that application of different concentrations of plant hormones increased the number of sporophores in *Pleurotus* spp. Further he reported increased number of sporophores when GA_3 (100 ppm) was applied. He speculated that the stimulatory action of GA might be due to increase in cell division and cell elongation. Mohapatra and Behera (2013) reported that the number of fruiting bodies of *Vovariella volvaceae* were 38.67. Dey *et al.* (2011) obtained the higher number of fruiting bodies of button mushroom in NAA 50 ppm (70.6), GA_3 200 ppm (72.2) and NAA 100 ppm (67.8). Pani (2011) documented that spraying 50 ppm GA_3 on the emerging primordia increased the number of mushrooms in *Calocybe indica*. Sarker and Chowdhury (2013) observed that GA_3 had a positive effect on number of effective fruiting body of oyster mushroom. Results of the present investigation are in agreement with results of these studies.

4.8 Yield of Sporophores per Harvest

Data presented in Table 4.3 and Fig. 4.7 in regard to yield of sporophores of milky mushroom (*Calocybe indica*), revealed significantly varied response of different growth regulators at different concentrations in all the three harvests.

All the treatments of growth regulators testified exuberant yield of sporophores per harvest, in comparison to that obtained in untreated control. Growth regulators assessed at different concentrations showed considerably wide-ranging variation in yield of sporophores of *Calocybe indica*. However, amongst all the treatments, spraying of gibberellic acid (GA) 20 ppm yielded significantly highest yield of sporophores in all the three harvests, which was to the tune of 595.08 g, 153.02 g and 102.02 g, in first, second and third harvests, respectively. This treatment, thus, was found most effective than rest of the treatments, which yielded 41.23%, 41.24% and 41.24% increase in yield over untreated control in first, second and third harvests, respectively. Spraying of indole-3-acetic acid (IAA) 20 ppm and indole-3-butyric acid (IBA) 20 ppm were the next best treatments in augmenting sporophore yield. These treatments yielded 566.05 g and 557.77 g yield of sporophores in first harvest; 145.57 g and 143.43 g in second harvest and; 97.03 g and 95.62 g in third harvest. However, sporophore yield obtained with these two treatments did not differ statistically from each other in all the three harvests, which explicitly indicated that these two treatments had parity in their effectiveness in augmenting sporophore yield of *Calocybe indica*. The treatment of spraying gibberellic acid at 15 ppm was the next treatment in performance. Least sporophore yield was obtained from untreated control beds.

To abridge the results, spraying of gibberellic acid (GA) 20 ppm was the most effective in augmenting sporophore yield of *Calocybe indica*.

4.9 Total Yield of Sporophores per 1000 g Dry Weight of Substrate

Data presented in Table 4.4 and Fig. 4.8 (Plate 4.1, 4.2 and 4.3) pertaining to total yield of sporophores of milky mushroom (*Calocybe indica*) per 1000 g dry weight of substrate, revealed significantly varied response of different growth regulators at different concentrations tried.

Table 4.4. Total yield and biological efficiency of milky mushroom (*Calocybe indica*) as influenced by various growth regulators

Growth regulator		Total sporophore yield (g)	Biological efficiency (%)
T_1	Gibberellic Acid (GA) 5 ppm	642.25	64.22
T_2	Gibberellic Acid (GA) 10 ppm	708.32	70.83
T_3	Gibberellic Acid (GA) 15 ppm	755.41	75.54
T_4	Gibberellic Acid (GA) 20 ppm	850.12	85.01
T_5	Indole -3-Acetic Acid (IAA) 5 ppm	648.81	64.88
T_6	Indole -3-Acetic Acid (IAA) 10 ppm	660.05	66.01
T_7	Indole -3-Acetic Acid (IAA) 15 ppm	714.28	71.43
T_8	Indole -3-Acetic Acid (IAA) 20 ppm	808.64	80.87
T_9	Indole- 3- Butyric Acid (IBA) 5 ppm	656.20	65.62
T_{10}	Indole -3- Butyric Acid (IBA) 10 ppm	652.71	65.27
T_{11}	Indole -3- Butyric Acid (IBA) 15 ppm	702.41	70.24
T_{12}	Indole -3- Butyric Acid (IBA) 20 ppm	796.81	79.68
T_{13}	Control	601.90	60.19
S.E. m±		7.65	0.76
CD at 5%		22.36	2.23

All the treatments of growth regulators exemplified substantial increase in total yield of sporophores per 1000 g dry weight of the substrate, in comparison to that obtained in untreated control. Growth regulators evaluated at different concentrations showed noticeably comprehensive variation in total yield of sporophores of *Calocybe indica*. However, amongst all the treatments, spraying of gibberellic acid (GA) 20 ppm yielded significantly highest yield of sporophores to the tune of 850.12 g. Spraying of gibberellic acid (GA) @ 20 ppm, thus, was found most effective than rest of the treatments, which yielded 41.23% increase in yield over untreated control. Spraying of indole-3-acetic acid (IAA) 20 ppm and indole-3-butyric acid (IBA) 20 ppm were the next best treatments in augmenting total yield of sporophores. These treatments yielded 808.64 g and 796.81 g yield of sporophores, respectively from all the three harvests. However, total sporophore yield obtained with these two treatments did not differ statistically from each other, which explicitly indicated that these two treatments had parity in their effectiveness in augmenting sporophore yield of *Calocybe indica*. The treatment of spraying gibberellic acid at 15 ppm was the next treatment in performance. Least sporophore yield was obtained from untreated control beds.

To abridge the results, spraying of gibberellic acid (GA) 20 ppm was the most effective in augmenting total sporophore yield of *Calocybe indica*.

4.10 Biological Efficiency

Data presented in Table 4.4 and Fig. 4.9 revealed significant variation in biological efficiency of milky mushroom (*Calocybe indica*) due to various treatments of growth regulators tried at different concentrations.

In the present investigation, growth regulators evaluated at different concentrations showed significant variation in biological efficiency of *Calocybe indica*, which ranged between 60.19 and 85.01%. However, amongst all the treatments, spraying of gibberellic acid (GA) 20 ppm revealed significantly highest biological efficiency to the tune of 85.01%. Gibberellic acid (GA) @ 20 ppm, thus, was found to be most effective than rest of the treatments in enhancing biological efficiency of *Calocybe indica*. Spraying of indole-3-acetic acid (IAA) 20 ppm and indole-3-butyric acid (IBA) 20 ppm were the next best treatments in augmenting biological efficiency. These treatments displayed biological efficiency to the tune of 80.87% and 79.68%, respectively. However, biological efficiency recorded with these two treatments did not differ statistically from each other, which explicitly indicated that these two treatments had parity in their effectiveness in augmenting biological efficiency. The treatment of spraying gibberellic acid at 15 ppm was the next treatment in performance. Least biological efficiency was obtained in untreated control treatment.

To abridge the results, spraying of gibberellic acid (GA) 20 ppm was the most effective in enhancing biological efficiency of *Calocybe indica*.

Many scientists have documented yield increase due to use of array of growth regulators. Halbert and Schisler (1987) found potential use of GR's to produce larger fruiting bodies without a reduction in yield of *Agaricus bisporus*. Shukla (1995) reported that spraying of Indole butyric acid @ 5 and 10 ppm induced maximum number of sporophores in *L. edodes*. Eswaran and Ramabadran (2000) found that GA at 100 ppm was the best in improving the yield of *P. eous*. Ramanujam (2000) found increased number, weight of sporophores and biological efficiency of *Pleurotus* spp. when GA_3 (100 ppm) was applied. Rostum (2010) found that the weight of fruiting body, total yield and biological efficiency of the *Pleurotus ostreatus* were increased with increase in the concentration of GA_3. Pani (2011) unveiled that spraying 50 ppm GA_3 on the emerging primordia increased the number as well as size of mushrooms and produced significantly higher yield of *Calocybe indica*. Pal *et al*. (2013) recorded the highest yield of *Pleurotus eous* in gibberellic acid incorporated medium. Sarker and Chowdhury (2013) documented that at 10 ppm level GA_3 had a positive effect on biological yield, economic yield and dry economic yield of oyster mushroom. Mohapatra and Behera (2013) found that IAA @ 200 ppm stimulated higher yield in *V. volvacea* with an yield increase of 22.12 % over control. Krishnamoorthy and Balan (2015) found increased the yield of milky mushroom with GA_3 at 40 ppm. Kaur (2016) reported maximum yield of *Calocybe indica* with GA when sprayed at pinhead formation and at all stages. Results of the present investigations are in general agreement with the results of foregoing scientists.

4.11 Protein Content

Table 4.5. Protein content of sporophore of milky mushroom (% on dry weight basis) as influenced by different growth regulators

Growth regulator	Protein content (% on dry weight basis)
T_1 Gibberellic Acid (GA) 5 ppm	27.25
T_2 Gibberellic Acid (GA) 10 ppm	28.01
T_3 Gibberellic Acid (GA) 15 ppm	29.21
T_4 Gibberellic Acid (GA) 20 ppm	27.86
T_5 Indole -3-Acetic Acid (IAA) 5 ppm	27.45
T_6 Indole -3-Acetic Acid (IAA) 10 ppm	28.82
T_7 Indole -3-Acetic Acid (IAA) 15 ppm	29.64
T_8 Indole -3-Acetic Acid (IAA) 20 ppm	28.64
T_9 Indole- 3- Butyric Acid (IBA) 5 ppm	27.14
T_{10} Indole -3- Butyric Acid (IBA) 10 ppm	28.02
T_{11} Indole -3- Butyric Acid (IBA) 15 ppm	29.58
T_{12} Indole -3- Butyric Acid (IBA) 20 ppm	28.51
T_{13} Control	27.14
S.E. m±	0.80
CD at 5%	NS

Data pertaining to protein content (per cent on dry weight basis) in sporophore of milky mushroom, depicted in Table 4.5, revealed non-significant differences among various treatments of growth regulators tried at different concentrations. The results, thus, clearly indicated that the protein content in sporophore of milky mushroom was not influenced by different treatments of growth regulators.

5. SUMMARY AND CONCLUSION

In the present investigation attempts were made to study the effect of various growth regulators and to find out most effective dosage of the growth regulator for enhancing the yield of milky mushroom (*Calocybe indica*). The investigation was undertaken during March-April, 2018 at the Plant Pathology Section of College of Agriculture, Kolhapur. Results obtained in the present investigation are summarized in this chapter.

5.1 Summary

Results of the investigation overtly indicated that the growth regulators tried at different concentrations in the present investigation, aiming at promoting growth and sporophore yield of *Calocybe indica,* exhibited significantly varied response to days required for pinhead formation and first harvest, period between flushes, diameter of pileus, length of stipe, number of sporophores, yield of sporophores and biological efficiency. However, amongst the various growth regulators used in the present investigation, spraying of gibberellic acid (GA) 20 ppm significantly demonstrated its efficacy over other treatments in reducing days required for pinhead formation (30.00 days after spawning), first harvest (35.66 days after spawning) and period between flushes(7 days). Furthermore, this treatment was also found most effective amongst all the treatments in augmenting diameter of pileus (15.40 cm), length of stipe (19.94 cm), number of sporophores (22.00), total yield of sporophores (850.12 g) and biological efficiency (85.01%). Protein content of sporophores remained unaffected due to use of different growth regulators.

5.2 Conclusion

From the present investigation it is concluded that spraying of gibberellic acid at 20 ppm concentration on beds, at the time of casing, is highly beneficial for obtaining sporophores with large diameter, longer stipes and exuberant yield of sporophores of milky mushroom (*Calocybe indica*).

6. LITERATURE CITED

Anonymous, 2011. DMR Annual Report, Directorate of Mushroom Research, Indian Council of Agricultural Research, Chambaghat, Solan-173- 213 (HP), pp.84.

Atri, N. S. and Guleria, L. 2013. Evaluation of vitamins, phytohormones and trace element requirements of *Lentinus cladopus* Lev. International Journal of Pharmacy and Pharmaceutical Sciences, 5: Suppl. 4.

Atri, N. S. and Kaur, B. 2016. Effect of growth regulators and trace elements on the vegetative growth of *Pleurotus sapidus* quél. Int J Pharm Pharm Sci., 8(11): 283-287.

Atri, N. S., Kumari, B., Singh, R. and Upadhyay, R. C. 2013. Effect of vitamins and growth regulators on vegetative growth of *Lentinus squarrosulus.* Mycosphere Online Edition, 1080-1090.

Bhardwaj, G. 2004. Studies on *Calocybe indica* (P. & C.) – The milky mushroom. Ph.D. (Agri.) thesis submitted to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan-173230 (H.P.), India.

Chang, S. T. and Miles, P. G. 1992. Mushrooms biology –a new discipline. Mycologist, 6**:** 4-5.

Chauhan, P. 2013. Studies on cultivation of *Pleurotus djamor* (Rumph.) Boedijn. M.Sc. (Agri.) thesis submitted to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan-173230 (H.P.), India.

Dey, B. C., Hossain, M. M., Akanda, A. M. Kamruzzaman, M., Zakaria, M., Choudhary, M. B. K. and Sarker, N. C. 2011. Effect of different plant growth regulators on the yield and yield attributes of white button mushroom. Bangladesh J. Mushroom, 5(1): 35-41.

Dey, R. C., Nasiruddin, K. M. and Munsur, M. A. Z. Al. 2007.Effect of different hormones, media and variety on mycelia growth of mushroom. J. Bangladesh Agril Univ., 5(2): 181-187.

Eswaran, A. and Ramabadran, R. 2000. Studies on some physiological, cultural and postharvest aspects of oyster mushroom, *Pleurotus eous*(Berk.) sacc. Tropical Agricultural Research,12:360-374.

Garcha, H.S. and Kalra, K.L. 1979. Spawn production and its viability. Indian J. Microbiol. 19(4): 214-216.

Guo, X., Zou, X. and Sun, M. 2009. Effects of phytohormones on mycelial growth and exopolysaccharide biosynthesis of medicinal mushroom *Pellinus linteus.* Bioprocess Biosyst Eng., 32: 701–707.

Halbert, C. R. and Schisler, L. C. 1986. Effects of growth regulator compounds on yield and size of the commercial mushroom *Agaricus bisporus.* Proc. Intl. Sym. Scientific and Technical Aspects of Cultivating Edible Fungi. The Penna. State Univ., University Park, PA, USA, July, 1986, pp. 79-90.

Harith, N. B. 2014.Cultivation of *Flammulina velutipes* (Golden needle mushroom/ Enokitake) on various agroresidues. M.Sc. thesis submitted to University of Malaya, Kaulalumpur.

Hobbs, C. 1996. Medicinal mushrooms: an exploration of tradition, healing and culture. Botanica Press, Santa Cruz, CA.

Jansi, J. J. 2010. Studies on the effect of glutamic acid gibberellic acid on the yield and protein content of oyster mushroom *Pleurotus florida* using paddy straw as a substratum. Journal of Basic and Applied Biology, 4 (3):217-220.

Joshi, S. S. 2016. Comparative studies on biological efficiency of oyster mushrooms. M.Sc. (Agri.) thesis submitted to Dr.Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.).

Kaur, R. 2016. Effect of growth regulators on biomass production and yield potential of milky mushroom *Calocybe indica* (P & C). M.Sc. (Agri.) thesis submitted to Punjab Agricultural University, Ludhiana, Punjab.

Krishnamoorthy, A. S. and Balan, V. 2015. A comprehensive review of tropical milky white mushroom (*Calocybe indica* P & C). Mycobiology, 43 (3): 184-194.

Kulshreshtha, M., Singh, A. and Vipul, D. 2009.Effect of drying condition on mushroom quality. Journal of Engineering Science and Technology, 4(1): 90-98.

Maynard, A. J. (Ed) 1970. Methods in Food Analysis, Academic Press New York, p. 176.

Mohapatra, K. B. and Behera, B. 2013. Influence of plant growth regulators on biological efficiency of straw mushroom, *Volvariella volvacea*. Mushroom Research, 21(1):59-61.

Mukhopadhyay, R., Chatterjee, S., Chatterjee, B. P. and Guha, A. K. 2005. Enhancement of biomass production of edible mushroom *Pleurotus sajor-caju* by plant growth hormones. Process Biochem, 40(3):1241-44.

Nasr, N. and Mahdipour, F. 2013. The effect of different growth regulators and media on the mycelial growth of two mushrooms *Agaricus bisporus* and *Pleurotus florida*. Int J Agri Crop Sci., 6(8): 478-84.

Navathe, S., Borkar, P. G. and Kadam, J. J. 2014. Cultivation of *Calocybe indica* (P& C) in Konkan Region of Maharashtra, India.World Journal of Agricultural Research, 2(4): 187-191.

Nuhu, A., Rahul, A., Khan, A., Ara, I., Mi, S. J., Min, L. W. and Tae, L. S. 2008.Nutritional analysis of cultivated mushrooms in Bangladesh – *Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus florida*, and *Calocybe indica*. Mycobiolog, .36(4):228-232.

Pal, D. P., Mishra, S.P., Shukla, C. S. and Verma, L.R. 2013.Effect of growth regulators on mycelia growth and yield of *Pleurotus eous.* International Journal of Agricultural Sciences, 10(1):151-153.

Pandey, M. and Tewari, R. P. 2002. Milky mushroom grown on paddy straw. The Hindu. 93,24.

Pani, B. K. 2011. Influence of some growth regulators on biomass production and sporophore yield of milky mushroom (*Calocybe indica*). Asian J. Exp. Biol. Sci., 2(2): 328-331.

Panse, V. G. and Sukhatme, P. V. 1985. Statistical Methods for Agriculture workers. ICAR Publ. New Delhi. 3rd Edn. pp. 347.

Paul, D., Guha, A. K. and Chatterjee, B. P. 1994. Effect of plant growth hormones on *Kluyveromyces fragilis* grown on deproteinized whey. Biochem Arch,10**:** 277-83.

Prakasam, V. 2012. Current scenario of mushroom research in India. Indian Phytopath., 65(1):1-11.

Purkayastha, R. P. and Chandra, A. 1974. New species of edible mushroom from India. Transactions of British Mycological Society, 62: 415-418.

Pushpa, H. and Purushothama, K. B. 2010. Nutritional analysis of wild and cultivated edible medicinal mushrooms.World Journal of Dairy and Food Sciences, 5(2).

Ramachela, K. and Sihlangu, S. M. 2016. Effects of various hormonal treated plant substrates on development and yield of *Pleurotus ostreatus.* Cogent Food & Agriculture, 2: 1276510.

Ramanujam, K. 2003. Technology Development and training for production and preservation of oyster mushroom. Final Report of ICAR Ad-hoc Scheme 8 (42)/95 Hort. II.

Rostum, A. N. 2010. Use of some growth regulators to improve yield, storage life, and medicinal properties of oyster mushroom. The Iraqi Journal of Agricultural Sciences, 41(6):48-60.

Sarker, R. R. and Chowdhury, A. K. M. S. H. 2013. Effect of different doses of GA_3 application at primordia initiation stage on the growth and yield of oyster mushroom. J. Bangladesh Agril. Univ., 11(1): 5–10.

Selvi, S., Umadevi, P., Murugan, S. and Giftson Senapathy, J. 2011. Anticancer potential evoked by *Pleurotus florida* and *Calocybe indica* using T_{24} urinary bladder cancer cell line. African Journal of Biotechnology, 10 (37):7279-7285.

Senthilnambi, D., Eswaran, A. and Balabaskar, P. 2011. Cultivation of *Calocybe indica* (P & C) during different months and influence of temperature and relative humidity on the yield of summer mushroom. African Journal of Agricultural Research, 6 (3):771-773.

Shukla, A.N. 1995. Effect of hormones on the production of Shiitake.*Lentinus edodes* (Bark.) Sing. Mushroom Research, 4: 39-42.

Singh, M and Kamal, S. 2012. Agricultural Today (Edited by Khan, M.J.) p. 83-87

Singh, M., Vijay, B., Kamal, S. and Wakchaure, G. C. 2011. Mushrooms-Cultivation,marketing and consumption. DMR, Solan. pp. 266.

Singh, R.P. and Dwivedi, R.R. 1991. Standardization of substrate for production of *Pleurotus sajor-caju* (Fr.) Sing. Indian Mushrooms. In Proc. National Symposium on Mushrooms, Thiruvanathapuram. pp. 102-103.

Uddin, M. J., Nasiruddin, K. M., Haque, M. E., Biswas, A. K. and Islam, M. S. 2012. Influence of different media, variety and growth regulator on mycelia colony proliferation of mushroom. J. Environ. Sci. & Natural Resources, 5(1): 223 – 227.

Walde, S. G., Velu, V., Jyothirmayi, T., and Math, R. G. 2006. Effect of pretreatments and drying methods on dehydration of mushroom. Journal of Food Engineering, 74: 108-115.

Wani, B. A., Bodha, R. H. and Wani, A. H. 2010. Nutritional and medicinal importance of mushrooms. Journal of Medicinal Plants Res, 4(24):2598-2604.

7. VITA

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MASTER OF SCIENCE (AGRICULTURE)

AGRICULTURAL MICROBIOLOGY

2018

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