Studies on seasonal cultivation of *Ganoderma lucidum* (Leyss. ex Fr.) Karst.

By

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CERTIFICATE – I

This is to certify that this thesis entitled: "**Studies on seasonal cultivation of *Ganoderma lucidum* (Leyss. ex Fr.) Karst."** submitted for the degree of Master of Science in the subject of **Plant Pathology** to the **Chaudhary Charan Singh Haryana Agricultural University, Hisar** is a bonafide research work carried out by **Mr. Jagdeep Singh**, **Admn. No. 2012A73M** under my supervision and no part of the thesis has been submitted by him for any other degree.

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

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CERTIFICATE – II

This is to certify that this thesis entitled: "**Studies on seasonal cultivation of *Ganoderma lucidum* (Leyss. ex Fr.) Karst."** submitted by **Mr. Jagdeep Singh, Admn. No. 2012A73M** to the **Chaudhary Charan Singh Haryana Agricultural University, Hisar** in partial fulfillment of the requirements for the degree of **Master of Science** in the subject of **Plant Pathology** has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an **External Examiner.**

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MAJOR ADVISOR **EXTERNAL EXAMINER**

HEAD OF THE DEPARTMENT

DEAN, POST-GRADUATE STUDIES

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Place: Hisar
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ABBREVIATIONS

˚C	degree Centigrade (Celsius)
et al.	*et alia* = and others
BE	biologocal efficiency
Gm	gram (s)
hrs.	Hours
i.e.	id est = that is
Kg	Kilogram
Ml	millilitre (s)
min.	minute (s)
Mm	millimeter (s)
Ph	negative logarithm of hydrogen ion concentration
M	meter (s)
psi	per square inch
viz.	Namely
%	per cent
≥	greater than or equal
@	at the rate of
Fig. (s)	figure (s)
w/w	weight/weight
CO_2	carbon di oxide

CHAPTER – I

INTRODUCTION

Mushrooms are the fruiting bodies of macrofungi. These occur seasonally all over the world in various habitats, varying from sandy plains to thick forests or green meadows to roadside pathways. The most fascinating concept of mushroom science is the production of highly nutritious fruit bodies of excellent taste from waste. As such, evaluation of locally available agro and forestry residues is the first essential steps towards standardization of cultivation of any mushroom. Mushroom cultivation has become one of the most profitable agribusiness that could produce good quality food products from different substrates and help to dispose them in an eco-friendly manner (Bano *et al.*, 1993).

Out of more than 14,000 species of mushroom in the world, around 700 have been known for medicinal properties. At present there are at least 270 species of mushrooms that are known to have various therapeutic properties (Ying *et al.,* 1987). Red mushroom (*Ganoderma lucidum*) is a medicinal mushroom. In India, it is also called by different names like "Jarh Phorh" while in Haryana, popularly called "Satpatra" and "Hirdo". This fungus also causes root rot diseases in various forest tree species (Venkataraman, 1936). Commonly known as 'lacquered dark' with glossy exterior it is being widely used in the countries of pacific region as an herbal medicine for the treatment of various diseases (Stavinoha *et al*., 1991). It has been widely used in the folk medicine of China and Japan for 4,000 years especially in the treatment of various ailments (Kabir, 1987).

Hung and Nhi (2012) conducted a study to evaluate the nutritive value, total phenolic compounds and antioxidant activity of five popular Vietnamese mushrooms *viz.*, *Pleurotus ostreatus, Volvariella volvacea, Lentinula edodes, Auricularia polytricha* and *Ganoderma lucidum*. Protein, lipid, ash and total carbohydrate content of *G. lucidum* was 13.3, 3.0, 1.4 and 82.3 per cent, respectively. Furthermore, total bound phenolic content of *G. lucidum* was also higher thus having more antioxidant capacity. Boh *et al.* (2007) observed that polysaccharides, proteins (peptidoglycans) and triterpenes are three major physiologically active constituents of this mushroom.

In India ethno-medicinal value of *G. lucidum* was first reported by Harsh *et al.* (1993). Modern research has revealed its active ingredients to be very good source of triterpene and ganodermic acid, having molecular structure similar to that of steroid hormones. It also contains polysccharides, ergosterol, unsaturated fatty acid, vitamins and minerals. Polysaccharides present in this fungus exhibit immunomodulatory and anti- tumour effect (Zhu and Lin, 2005; Zhu and Lin, 2006). Many polysaccharide-bound proteins produced by basidiomycetes fungi have been classified as anti-tumour chemicals by the US

National Cancer Institute (Jong and Donovick, 1989). Recent reports are in agreement with the ancient literature where it has been mentioned to increase the memory and preventing forgetfulness in old age besides having promising antiviral activity against HIV also (Kim *et al*., 1994). Ganodermic acid isolated from *G. lucidum* has anti-coagulating effect and lowers cholesterol level considerably (Morigawa *et al*., 1986). The basidiocarp of *G. lucidum* is a popular remedy to treat various human ailments such as chronic bronchitis, hepatitis, hypertension, arthritis, gastritis, tumour growth, diabetes, immunological disorder etc. (Ling Zhu *et al*., 2007). Since ancient times this mushroom was known for its use in Chinese and Japanese traditional medicines and it is presumed that its artificial cultivation might have started first in these countries and then spread to Korea, Malaysia and Thailand subsequently (Rai, 2003).

Current world production of *G. lucidum* is around 6000 tonnes, half of which comes from China (Verma and Prasad, 2010). World trade in this mushroom is in the range of 1.5 billion US$, while it is about Rs.120 crores per annum in India (Geetha *et al.*, 2012). *G. lucidum* is probably the first medicinal mushroom to gain importance in India. An attempt has been made in India to study the potential of medicinal mushrooms as an additional crop towards diversification. Selection of a mushroom species for cultivation depends on the appropriate availability of raw materials required for the species and the suitability of environmental conditions. Most of the mushrooms are being cultivated on agro-residues like sawdust/wood chips/wheat straw/paddy straw. In India, these raw materials are available in plenty and the country produces about 600 million tones of crop residues per year (Tewari and Ahlawat, 2007).

Indian conditions are quite congenial for cultivation of medicinal mushrooms and this may fetch more returns in the market (Thakur, 2005). The cultivation of *G. lucidum* follows a typical fruiting behaviour and in the beginning its stem is red and cap is yellow with white margin and the growth continues with colour of the pileus turning brownish red. Margin of the pileus stop growing after attaining the size of 4-7 cm but red colouration continues and mature fruit body shows brownish red colouration with no white margin (Rai, 2003). The growth, spore release and spore germination in *G. lucidum* occur over a wide range of conditions. This versatility of fungus may explain the ubiquitous occurrence through a wide range of climates from various states in India. Keeping in view, the scanty information available on the cultivation of this mushroom in India, present work was undertaken with following objectives:

1. Mycelial growth optimization of *Ganoderma lucidum.*
2. Cultivation of *Ganoderma lucidum* using different agro-residues

CHAPTER–II

REVIEW OF LITERATURE

Ganoderma lucidum (Leyss. ex Fr.) Karst. is a basidiomycetous fungus that grows on decaying logs and tree stumps. It is believed to be the oldest mushroom used in medicine and one of the most respected medicinal mushrooms even today. It is also known as Lingzhi in Chinese; Reishi, Mannentake, or Sachitake in Japanese; and Youngzi in Korean. *G. lucidum* belongs to the family Polyporaceae of the order Aphylophorales and contains triterpenoids, polysaccharides, aminoacids, fungal immunomodulatory proteins and steroids. Several triterpenes e.g. ganoderans A, B and C isolated from *G. lucidum* fruit bodies have been shown to have a strong hypoglycemic effect (Chang and Miles, 2004). All parts of this mushroom namely spore, mycelium and basidiocarp are used for health purpose and for pharmaceutical uses. The medicinal mushrooms are commonly prepared either as hot water extract, concentrate or in powdered form (Smith *et al.*, 2002). The availability of wild *Ganoderma* spp. is inadequate and unsafe to consume, thus its cultivation is essential to meet the demand. In this connection, the cultivation have been attempted and described during the last twenty years.

The literature on various aspects of *G. lucidum* is reviewed under the following heads:

2.1 OCCURRENCE AND DISTRIBUTION

Ganoderma spp. is a diverse fungus which occurs in its natural habitat throughout the world and about 250 species have been described worldwide (Moncalvo and Ryvarden,

1997). *G. lucidum* grows near stumps of oak and other broad leaved tree species in summer, autumn and monsoon in the wild and is distributed in tropical and warm temperate areas of India. The fruit bodies of *G. lucidum* occur in different colours such as Chinese red, bright yellow and white. Initial fruit body of *G. lucidum* is white which turns into yellow shades and finally into varnished, reddish to reddish brown shade, quite beautiful and distinctive. The red variety of *G. lucidum* is most commonly used for nutraceutical purposes and commercially cultivated in China, Taiwan, Japan, Korea and North America and has gained wide popularity in other parts of the world (Doshi and Sharma, 1997). *G. lucidum* is an annual and in wild it does not grow more than once a year like some polypores, its fruiting body is quite tough and can last for month. It has been added to the American Herbal Pharmacopoeia and Therapeutic Compendium (Perumal, 2009).

2.2 TAXONOMY

Ganoderma is morphologically most complex genus of the polypores. The prevailing disagreement in the classification of the Polyporaceae results from the multiplicity of criteria upon which the several systems of classification are based. The morphological characteristics of the basidiocarp were used as main basis for distinguishing genera and species in the sub-family Ganodermoideae (Donk, 1933). Donk (1964) raised the sub-family Ganodermoideae to the rank of family Ganodermataceae including *Ganoderma* and *Amauroderma* as two distinct genera. *Ganoderma* comprises species that have only derm and are distributed throughout the tropics and temperate zones (Furtado, 1965**).** The genus *Ganoderma* was established by Karsten (1881) with *G. lucidum* as the only species. Patouillard (1889) used basidiospore morphology for separating groups of ganodermoid polypores and divided *Ganoderma* into two sections; *Ganoderma* with ovate basidiospores and *Amauroderma* with globose to subglobose basidiospores. Taxonomically, *Ganoderma* belongs to the phylum: Basidiomycota; order: Aphyllophorales and family: Ganodermataceae (Alexopoulous *et al.*, 1996). In all, 219 species within the family have been assigned to the genus *Ganoderma* of which *G. lucidum* (W. Curt.: Fr.) P. Karsten is the type species (Moncalvo, 2000).

2.3 NUTRITIONAL STATUS

Hung and Nhi (2012) reported protein, lipid, ash and total carbohydrate content of *G. lucidum* 13.3, 3.0, 1.4 and 82.3 per cent, respectively and the total bound phenolic content of *G. lucidum* was also high which is responsible for its high antioxidant activity. Polysaccharides, proteins (peptidoglycans) and triterpenes are the three major physiologically active constituents in *G. lucidum* (Boh *et al.,* 2007; Zhou *et al*., 2007). Rajamannar *et al.* (2000) analyzed the proteins to differentiate *Ganoderma* isolates and found that all isolates shared common proteins. Shamaki *et al.* (2012) reported polysaccharides, proteins, amino acids, vitamins, steroids, lipids and minerals in the fruit body of *G. lucidum.*

2.4 MORPHOLOGICAL CHARACTERIZATION

Different characteristics such as shape and colour (red, black, blue/green, white, yellow and purple) of the fruit body, host specificity and geographical origin are used to identify individual members of the *Ganoderma* species. Basidiocarp of this genus has a shiny surface associated with the presence of thick walled pilocystidia embedded in an extracellular melanin matrix (Moncalvo, 2000). Fruit bodies of *G. lucidum* are stipitate, dimidiate or reniform and rarely suborbicular, thick, corky and yellowish in margin and then turn brownish in mature part with shining on the surface. The margin is usually thin or truncate often slightly incurved. The shape and size of basidiospores and cuticle cells have been considered as two most important characters in the genus *Ganoderma*. Nuss (1982) suggested that *G. lucidum* may produce two types of basidiospores. The type produced early in the season is said to germinate only after the insect ingestion and probably is dispersed in this manner. The other type of basidiospore is the one produced throughout the rest of the growing season. This type is widely air-dispersed and germinates readily on agar and malt medium without special treatment.

2.4.1 ESTABLISHMENT OF PURE CULTURE

Venkataraman (1936) reported that *G. lucidum* grew well on malt agar medium and the same has been reported to support good growth of *G. lucidum* by earlier workers also (Adaskaveg and Gilbertson, 1987; Biley *et al*., 2000; Lomberh *et al*., 2002). Potato dextrose agar has been also found to be good medium but it took slightly more time (Booth, 1971; Biley *et al.,* 2000). Sharma and Thakur (2010) reported that radial growth of *G. lucidum* was higher in malt extract agar added with linseed extract medium.

2.5 PHYSIOLOGICAL CHARACTERISTICS

To determine the optimal conditions and specific requirements for mycelial growth and development of this mushroom, physiological studies are necessary**.**

2.5.1 MOISTURE

Berovic and Habijanic (2000) reported that for *G. lucidum* cultivation and polysaccharides production, a moisture content of 70 per cent in the solid substrate was critical while, moisture fractions higher than 70 per cent promoted good growth and polysaccharides production and *vice-versa*. Rai (2003) reported from India that 65 per cent moisture in the substrate was optimum for the mycelial growth and for the successful cultivation. On the other hand, Veena and Pandey (2006) reported 65 per cent substrates moisture was optimum for mycelial growth and cultivation of *G. lucidum.*

2.5.2 TEMPERATURE

Temperature is one of the most important factor for the mycelial growth, fruiting and quality of mushroom. The genus *Ganoderma* has most divergent temperature requirements known in any genus of the cultivated mushrooms. The effect of environmental conditions on

the mycelial growth of *G. lucidum* was investigated in shake flask cultures and it grew well in the temperature range of 30-35˚C (Liau *et.al*., 1998). Adaskaveg and Gilbertson (1986) reported that it has an optimum temperature range of 30-34˚C with the maximum growth temperature of 37˚C and exhibited the highest growth rate of 7-8 mm/day. *G. lucidum* is a mesophilic fungus with a temperature range of 0-50˚C and an optimum between 15˚C and 40˚C as observed by Griffin (1994). Rai (2003) found that 35˚C is optimum for its mycelial growth. On the other hand, temperature range of 28-30˚C has been reported as optimum for the vegetative growth of *G. lucidum* by Dadwal and Jamaluddin (2004). Whereas, Veena and Pandey (2006) reported that 30±1˚C was optimum for the mycelial growth and cultivation of *G. lucidum*. Song *et al*. (2007) observed that *G. lucidum* had maximum mycelial growth rate at 25-35˚C while, Negi *et al*. (2008) reported 32±1˚C most suitable for the optimum yield of the fruiting bodies of *G. lucidum* under the high humid conditions (85-90% RH). On the contrary, Iqbal *et al.* (1997) reported that its growth was maximum at 30˚C after 12 days of inoculation; however it reduced drastically below 25˚C and above 35˚C. Bajwa *et al.* (2005) while, working with influence of different media and temperature recorded that a temperature of 25˚C and potato dextrose agar being the best followed by Malt extract media at the same temperature. The cultivation of *G. lucidum* in green house was done at temperature of 24-28˚C with low light intensity and a yield of about one ton was obtained from an area of 420 m² (Boawei *et al*., 1998).

2.5.3 pH

The hydrogen-ion concentration (pH) of media/substrates influenced the growth and development of mushroom. It was found that pH definitely affected mycelial growth. A culture system having constant pH at 3 and 6 exhibited improved mycelial growth of *G. lucidum* (Min *et al*., 1999). Mishra and Singh (2010) revealed that local isolates of *G. lucidum* from Uttarakhand preferred acidic pH (5.0-6.0) for their growth. Iqbal *et al*. (1997) reported that the growth of fungus was maximum at pH 5.5-6.5 whereas, a pH of 4.8 has been reported as optimum for the mycelial growth of *G. lucidum* (Rai, 2003). Negi *et al*. (2008) observed that pH 5.5-6.5 was optimum for fruiting body yield of *G. lucidum*.

2.6 SPAWN PRODUCTION

Though different substrates have been reported by various workers, wheat grains are most widely used for the spawn production of *G. lucidum*. The grains should be fresh, unbroken, insecticide or fungicide untreated and insect undamaged. Munjal (1973) prepared wheat grains spawn adopting similar method as for button mushroom spawn production. Stamets and Yao (2000) reported rye grains as the basal medium for spawn making of *G. lucidum*. The use of wheat grains spawn @ 2 per cent dry weight of the substrate was best for optimizing the yield of *G. lucidum* (Rai, 2003). On the other hand, Mishra and Singh (2006) reported maximum yield from wheat grains spawn applied @ 5 per cent on dry weight basis.

Veena and Pandey (2010) used sorghum grains as the basal medium for spawn production and the rate of spawn varied from 6-8 per cent on wet weight basis of the substrate. The use of bajra grains as a basal medium for spawn production has also been reported by Sharma and Thakur (2010).

2.7 CULTIVATION TECHNOLOGY

Artificial culture and cultivation of *G. lucidum* was initially attempted by Henmi (1937). Successful cultivation of this mushroom on sawdust/wheat straw + rice/wheat bran had been attempted in India (Mishra and Singh, 2006; Rai, 2003; Veena and Pandey, 2004) in which mycelium aggregated to form pinheads of about 0.5 to 0.8 cm diameter, white in colour and pinheads appeared after 5-8 days after opening of the bags and continued to appear for 3-4 days. Approximately, 30-40 per cent of pinheads abort and rest grow to form long red stems with white tips. Triratana and Chaiprasert (1991) cultivated *G. lucidum* in sawdust bags. The substrate was a mixture of pararubber (*Hevea brassiliensis*) sawdust and rice bran five per cent and packed in polypropylene bags of 300 gm each. The bags were incubated at 27-32˚C after spawn run and were exposed to 80 per cent relative humidity with natural indoor daylight of about 150 lux. The average yield from the first harvest varied between 6.7-16.9 gm/bag. Similarly, cultivating *G. lucidum* on sawdust from different trees including *Hevea brassiliensis, Dipterocarpus alatus, Pentacme suavis* and *Tectona grandis* and mixed with the supplements such as rice bran 5.0-7.5 per cent was attempted having relative humidity 80 per cent and 150-200 lux light. The sawdust of *H. brassiliensis* supplemented with rice bran (7.5%) gave the highest biological efficiency. Chiu *et al.* (2000) artificially cultivated *G. lucidum* on sawdust compost supplemented with wheat bran @ 10 per cent in an environmental chamber with ≥80 per cent relative humidity at 28˚C under 16 hrs light and 8 hrs dark photoperiod. Cultivation of *G. lucidum* on poplar sawdust fortified with 20 per cent wheat bran resulted in 10-15 per cent biological efficiency with overall average of 12 per cent (Rai, 2003). Similarly, Dadwal and Jamaluddin (2004) cultivated *G. lucidum* on logs and wooden chips of *Delonix regia* at 28-30˚C and 70-90 per cent relative humidity. The results indicated that wooden chips showed better growth and fruit body production however, the wooden logs did not show the spread of mycelium as fast as in wooden chips in polybags, resulting into meagre fruiting bodies production.

2.7.1 SUBSTRATES

Commercial cultivation of *G. lucidum* has been reported with a variety of substrates. Sawdust was found to be a best substrate for cultivation of *G. lucidum* (Triratana and Chaiprasert, 1991). Mayzumi *et al.* (1997) reported cultivation on Japanese oak, apricot and keyaki logs. Cha and Yoo (1997) cultivated *Ganoderma* on sawdust and oak logs. Siwulski and Sobieralski (2001) observed that mycelium of strain CS 95 and strain LZ 1 grew faster through the birch sawdust substrate. Rai (2003) reported cultivation of *G. lucidum* on wheat

straw, bagasse and 20 per cent sawdust. Dadwal and Jamaluddin (2004) observed better mycelial growth and production of fruit bodies on chips and logs of *Delonix regia* and similar results were also reported by Tiwari *et al*. (2004). Veena and Pandey (2006) evaluated different substrates for the cultivation of *G. lucidum* and reported sawdust and wood chips as the best substrate. Singh *et al.* (2007) reported maximum biological efficiency on wheat straw and sawdust (3:1) substrate. On the other hand, Song *et al*. (2007) reported whey permeate as a best substrate for the cultivation of *G. lucidum.* Veena and Pandey (2010) evaluated a wide range of substrates including agricultural residues such as wheat straw, rice bran and wheat bran. Apart from conventional substrates, some unique substrates were also evaluated like coffee stem sawdust, spent coffee ground, winery wastes and vine wastes (Jaramillo *et al*., 2010). Similarly, Jandaik *et al*. (2010) investigated the effects of four forestry byproducts (sawdust of oak, mango, khair and tuni) and three agricultural residues (paddy straw, wheat straw and soybean residues) on growth characteristics (spawn run and primordial formation) and yield of *G. lucidum*. Among substrates agriculture residues supported better yield and higher biological efficiency of *G. lucidum* compared to forestry byproducts.

2.7.2 SUPPLEMENTS

Higher yield of *G. lucidum* was reported on rice bran, ground corn and ground sorghum (Triratana and Chaiprasert, 1991). Chen (1999) reported rice bran or wheat bran as an essential ingredient for the cultivation of *G. lucidum.* Veena and Pandey (2006) evaluated different substrates for the cultivation of *G. lucidum* and reported sawdust and wood chips as the best substrate followed by its combination with other substrates *viz*., rice bran, wheat bran and finger millet. Mishra and Singh (2006) have reported maximum biological efficiency of *G. lucidum* on wheat straw supplemented with 5 per cent rice bran. Sawdust of *Alnus nepalensis* mixed with rice bran (10%) was found to be a good substrate (Negi *et al*., 2008). Sharma and Thakur (2010) reported that mycelial colonization was very fast when sugarcane bagasse and sunflower straw were supplemented with 15 per cent wheat bran. Peksen and Yakupoglu (2009) reported tea waste as new supplement for substrate mixture in cultivation of *G. lucidum*. Veena and Pandey (2011) reported the enormous potential of paddy straw for the cultivation of *G. lucidum* in combination with sawdust and rice bran. Jandaik *et al.* (2010) investigated the effects of four forestry byproducts and three agricultural residues along with four supplements (wheat bran, rice bran, corn flour and gram powder) on growth characteristics, spawn run, primordial formation and yield of *G. lucidum*. The highest yield (82.5 gm) and biological efficiency (27.5%) were recorded from paddy straw supplemented with wheat bran, which invariably resulted in significantly higher yield compared to the unsupplemented check or other supplements used in this study. Erkel (2009) reported the effects of various kinds of sawdust and bran on the yield of *G. lucidum*. Three kinds of sawdust (poplar, oak and beech) and bran (wheat, rice and corn) were used as a substrate

media in *G. lucidum* cultivation. The highest yield and biological efficiency were obtained from oak sawdust compared to the other sawdusts and also from wheat bran compared to the other brans. The yield and biological efficiency of rice bran at whole combinations were lower than other treatments while, substrates containing wheat bran gave the highest yield. So, Erkel obtained the highest yield with oak sawdust and wheat bran compared to other dusts and brans. Azizi *et al*. (2012) studied the effect of sawdust, malt extract and wheat bran on mycelial growth of *G. lucidum*. Three kinds of sawdust (beech, poplar and hornbeam) as basal medium were mixed with two levels of wheat bran (5% and 10% w/w) and malt extract (2.5% and 5% w/w) as medium supplement for production of *G. lucidum*. The results showed highest mycelial growth rate (10.6 mm/day) in a combination of beech sawdust with 2.5 per cent malt extract and 10 per cent wheat bran.

2.8 BIOLOGICAL EFFICIENCY

The biological efficiency is influenced by a number of factors like temperature, pH, relative humidity, photoperiod, substrates, supplements etc. Different workers obtained varying yield of *G. lucidum* and this may be due to the influence of above factors. Triratana and Chaiprasert (1991) obtained 12-17 per cent biological efficiency using some strains of *G. lucidum* on sawdust whereas, Rai (2003) reported biological efficiency between 10-15 per cent and similarly Mishra and Singh (2006) obtained 15-17 per cent biological efficiency. On the other hand, Negi *et al.* (2008) obtained 32 per cent biological efficiency by using strains OE-52 of *G. lucidum.* Jaramillo *et al.* (2010) reported the average biological efficiency of 24.2 per cent in two flushes. Similarly, Veena and Pandey (2011) observed maximum biological efficiency of 29.90 per cent with the combination of sawdust: paddy straw: rice bran (22:5:67.5:10). Further, Azizi *et al.* (2012) showed that various kinds of sawdust affects fruiting body yield, biological efficiency and mycelial growth rate significantly. The highest fruiting body yield and biological efficiency (102.58 gm/kg and 12.89%, respectively) were found using hornbeam sawdust. The beech sawdust promotes the mycelial growth rate more than other sawdust and the best combinations for high yield (142.44 gm/kg) and biological efficiency (18.68%) were obtained in a combination of poplar sawdust with 5 per cent malt extract and 10 per cent wheat bran.

CHAPTER – III

MATERIAL AND METHODS

The present investigation entitled "Studies on seasonal cultivation of *Ganoderma lucidum"* was carried out in the Mushroom Technology Laboratory, Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, during 2012-2014. The materials and methods employed during the course of investigation have been described under the following heads:

3.1 MATERIALS

3.1.1 Glassware and equipments

Glasswares used for present study was of borosilicate make, sterilized plastic plates, 250 ml empty milk bottles, polypropylene bags and polythene bags, these were used for physiological studies, spawn preparation and cultivation of *G. lucidum*.

3.1.2 Chemicals

The standard analytical grade chemicals were used for the present study.

3.1.3 Culture

The culture of *G. lucidum* was obtained from DMR, Solan (H.P.).

3.2 METHODS

3.2.1 Sterilization of equipments

Glassware was sterilized at 180˚C for 2 hours in hot air oven and media was sterilized in an autoclave at 15 psi pressure (121.6˚C) for 20 minutes.

3.2.2 Maintenance of the culture

The Potato Dextrose Agar (PDA) medium slants were inoculated with mushroom mycelium under aseptic conditions and incubated at 30±1˚C. Culture of *G. lucidum* was maintained on potato dextrose agar medium at 4±1˚C.

3.2.3 Preparation of standard inoculums

Potato dextrose agar (PDA) medium was used as basal medium for the preparation of inoculum. A mycelial disc of five mm was cut from seven days old culture of *G. lucidum* with the help of sterilized cork borer under aseptic conditions and incubated. The cultures thus obtained were used as inoculum for the different experiments in the present investigation.

3.2.4 Physiological studies

The studies on different physiological parameters of the fungus *G. lucidum* were conducted under *in vitro* conditions. The mushroom (*G. lucidum*) was cultured on different substrates namely wheat straw, saw dust and wheat straw + saw dust (1:1) as a medium to carry out the experiments of different substrate moisture regimes, select the best substrate and its moisture content for further experimentation.

3.2.4.1 Effect of different substrate and moisture regimes on mycelial growth

The experiment was carried out by measuring the mycelial growth of *G. lucidum* using wheat straw, saw dust and wheat straw + saw dust (1:1) as substrate having different moisture regimes 60±2 per cent, 70±2 per cent, 80±2 per cent and 90±2 per cent. The different moisture content of substrates were adjusted by Gravimetric method and after adjusting the desired moisture content, substrates were sterilized in an autoclave. Five gram of each sterilized substrate was kept in nine cm diameter sterilized Petri plates having required moisture content and each treatment had five replications and the experiment was completely randomized design (CRD). The uniform five mm mycelial bits of *G. lucidum* was placed at the centre of Petri plates containing different substrate having different moisture content under aseptic conditions and were incubated. The observations for radial mycelial growth were recorded at an interval of 72 hrs. It was observed that wheat straw + saw dust (1:1) at 70±2 per cent moisture content supported the maximum mycelial growth of *G. lucidum*. Thus the above said substrate with 70±2 per cent moisture content selected for further experimentation.

3.2.4.2 Effect of different temperature regimes on mycelial growth

The required amount of substrate with 70±2 per cent moisture content was selected and sterilized in an autoclave. After sterilization five gram substrate was kept in sterilized Petri plates and five replications of each treatment with CRD were maintained. These plates were inoculated under aseptic conditions with five mm mycelial discs of actively growing culture of *G. lucidum.* The plates were incubated at different temperatures *viz.,* 25±1˚C, 30±1˚C, 35±1˚C and 40±1˚C. The observations for mycelial growth were recorded at an interval of 24 hrs.

3.2.4.3 Effect of different pH on mycelial growth

The required amount of substrate having 70±2 per cent moisture content was taken and the different pH *i.e.* 4.5, 5.0, 5.5 and 6.0 were adjusted by using standard normal solution of NaOH and HCl and sterilized. Each Petri plates had five gram substrate and each treatment replicated five times in a CRD. These plates were inoculated under aseptic conditions with five mm mycelial discs of actively growing culture and incubated. The observations for mycelial growth were recorded at an interval of 24 hrs.

3.2.5 Effect of substrate supplementation on mycelial growth of *G. lucidum*

3.2.5.1 Effect of different doses of wheat bran as a supplement

Different doses (10, 20 and 30%) of wheat bran were added as supplement (wet weight) to the substrate for selection of optimum dosage of supplement application and unsupplemented substrate served as control. Thereafter, it was filled in large size test tubes (150 ml), plugged and autoclaved. The autoclaved tubes were inoculated with uniform sized mycelial discs of *G. lucidum* and incubated at 30±1˚C. The experiment was conducted in a CRD with five replications for each treatment. The observations were recorded for the time taken to colonize the substrate.

3.2.5.2 Evaluation of different substrates supplemented with wheat bran

The best supplement dose (wheat bran @ 20%) was selected in the previous experiment and used furthur to evaluate the effect of different substrates fortified with supplement on mycelial growth of *G. lucidum.* The different substrates were supplemented with 20 per cent wheat bran. The experiment had six combinations of substrates and supplement namely wheat straw, saw dust, wheat straw + wheat bran, saw dust + wheat bran, wheat straw + saw dust and wheat straw + saw dust + wheat bran. These were filled in large sized test tubes (150 ml) with three replications for each treatment and autoclaved. Sterilized tubes were inoculated with uniform sized mycelial discs of *G. lucidum* and incubated at 30±1˚C. The experiment was conducted in CRD and observations were recorded for the time taken to colonize the substrate.

3.2.6 Evaluation of suitable substrates for spawn production

The different substrates evaluated for the spawn production were wheat grains, bajra grains and sorghum grains and saw dust. Healthy grains were cleaned, washed and boiled for 20 min in 1.5 times water till the grains became tender. The grains were allowed to remain in water for another 10 min without further heating. The excess water was drained and grains were spread on a clean plastic sheet for surface drying and allowed to cool. Saw dust was soaked overnight in water and excess water was drained and spread for surface drying. The substrates were mixed with three per cent calcium sulphate and one per cent calcium carbonate on wet weight basis to obtain the desired pH and to avoid the stickiness of substrates. Thereafter, these were filled in clean 250 ml glass bottles, plugged with non-absorbent cotton and sterilized at 22 psi for 2 hrs. After cooling, the bottles were shaken to restore transparency of glass and were inoculated with uniform sized culture mycelial discs of *G. lucidum.* The bottles were incubated at 30±1˚C and the experiment was CRD with five replications for each treatment. The observations were recorded for linear growth of mycelium at 72 hrs interval until colonization of the substrate.

3.2.7 Cultivation of *G. lucidum*

Among the different evaluated agro-residues in above experiments, the best substrate (wheat straw + saw dust) with and without supplement (wheat bran @ 20%) and wheat grains based spawn were selected for cultivation of *G. lucidum*. The mixture of wheat straw + saw dust supplemented with 20 per cent wheat bran was taken up for the cultivation of this mushroom and substrate without wheat bran served as control. Cultivation of *G. lucidum* was carried out in two seasons during August-October, 2013 and February-April, 2014 under natural conditions. The cultivation of *G. lucidum* involved following steps:

3.2.7.1 Substrates preparation

The substrates wheat straw and saw dust were mixed in equal proportion and soaked in the water for 10 hrs. After draining excess water, these were spread on clean flour and amended with three per cent calcium sulphate and one per cent calcium carbonate. The mixed substrate was filled in polypropylene bags (500 gm/bag) and plugged with non-absorbent

cotton after putting a plastic ring in the neck. Thereafter, bags were sterilized at 22 psi for 2 hrs. On the other hand, substrate with supplementation were prepared after soaking of substrates (wheat straw and saw dust) and draining excess water to which wheat bran was added @ 20 per cent. Thereafter, the same procedure was followed as described above.

3.2.7.2 Spawning

The sterilized bags containing substrate/substrate + supplement were spawned aseptically with wheat grains based spawn @ 3 per cent (dry weight) and put in mushroom growing house.

3.2.7.3 Cropping

During spawn run, the mushroom house had nearly dark condition with a temperature range of 25-35˚C. The relative humidity (≥ 90%) was maintained by regularly spraying water in the mushroom house and the windows and doors of mushroom house were opened for minimum time to have high CO_2 concentration. After spawn run, fresh air was introduced by opening the windows/doors regularly to reduce the CO_2 concentration in mushroom house and lights were switched on to provide about 500 lux brightness, coupled with regular spraying of water to have high humidity. These conditions were maintained throughout the growing period except that during cap thickening and maturity, humidity was lowered (approximate 70%) by reducing the number of sprays in the mushroom house. Upon complete colonization the bags were cut with blade exposing the upper side.

3.2.7.4 Harvesting

The mature fruit bodies (with well differentiated pileus, gills and stipes) were picked up by giving gentle twist without disturbing the smaller ones and the harvesting index was that fruit bodies were harvested when the margin of the pileus stopped growing after attaining the size of 4-7 cm and mature fruit bodies were brownish red with no white margin.

3.2.7.5 Observation

The observations were recorded for time taken for spawn run, time taken for pin head formation, time taken for first harvest, time taken for last harvest, number of flushes, number of fruit bodies per bag, weight of fruit bodies (gm/bag) and disease incidence. The biological efficiency (fresh weight) was calculated and the data was analyzed statistically.

$$\text{BE (\%)} = \frac{\text{Yield of fruit body}}{\text{Weight of substrate}} \times 100$$

3.2.7.6 Experimental design

The experimental design was a completely randomized design with five replications for each treatment, except otherwise stated.

CHAPTER – IV

EXPERIMENTAL RESULTS

Ganoderma lucidum is a high temperature medicinal mushroom. The present investigation were undertaken to standardize the seasonal cultivation of this mushroom. So, an attempt was made with the aim to find out the suitability of locally available agro-residues for its cultivation under natural conditions. To achieve the above objectives studies concerning mycelial growth optimization and seasonal cultivation of *G. lucidum* using different agro-residues were undertaken. In the present work observation on various aspects like effect of moisture content, temperature, pH of the substrates, type of substrate and supplement for growth and development of *G. lucidum* and the substrate for its spawn production were recorded.

The results of present investigation are described in the subsequent paragraphs under following heads:

1. PHYSIOLOGICAL STUDIES
2. EFFECT OF SUBSTRATE SUPPLEMENTATION ON MYCELIAL GROWTH OF *G. LUCIDUM*
3. EVALUATION OF SUITABLE SUBSTRATES FOR SPAWN PRODUCTION
4. CULTIVATION OF *G. LUCIDUM*

4.1 Physiological studies

Prior to domestication of the fungus it is important to know the various physiological requirements for its vegetative growth. Hence *in vitro* physiological studies on *G. lucidum* were undertaken and results are presented under following heads:

4.1.1 Effect of different substrate and moisture regimes on mycelial growth

The observations regarding effect of substrate moisture content on radial mycelial growth of the mushroom on different substrates was measured and the results are presented in Table 1. The results indicated that, different moisture content of the substrates showed the different mycelial growth. In the present investigation four different substrate moisture content *viz*., 60±2 per cent, 70±2 per cent, 80±2 per cent and 90±2 per cent were maintained in different substrates and radial mycelial growth was recorded at an interval of 3, 6, 9 and 12 days.

It is evident from the results that, in case wheat straw as substrate no growth was recorded at any of the moisture content upto three days of incubation. On the other hand, where saw dust was used as substrate the mycelial growth was observed at moisture level of 70±2 per cent and 80±2 per cent. The fungus did not grow when moisture was below 70±2

per cent and more than 80±2 per cent. When both the substrates (wheat straw + saw dust) were mixed in equal proportions radial mycelial growth was 5.9 mm at 70±2 per cent moisture followed by 4.7 mm (80±2%), 3.1 mm (60±2%) and 2.7 mm (90±2%) moisture levels after three days of incubation.

Table 1: Effect of different substrates and its moisture content on radial growth of *G. lucidum*

Sr. No.	Moisture content (%)	Radial growth* (mm) after days											
		Wheat Straw				Saw Dust				Wheat Straw + Saw Dust (1:1)			
		3	6	9	12	3	6	9	12	3	6	9	12
1.	60	0	4.2	11.6	20.8	0.0	5.2	16.1	30.3	3.1	6.5	29.9	38.7
2.	70	0	7.6	15.9	29.8	2.4	10.8	23.2	38.6	5.9	13.2	35.0	45.0
3.	80	0	6.7	15.2	24.6	1.7	8.6	21.4	36.4	4.7	10.4	31.3	41.3
4.	90	0	2.8	9.1	16.6	0.0	3.6	11.4	22.2	2.7	5.6	15.7	30.9
5.	CD (p=0.05)	-	1.1	1.0	1.2	0.6	0.8	1.0	0.9	0.9	1.2	1.1	1.2

*Average of five replications
Note: The moisture content was maintained at ±2 per cent of the desired level.

Whereas, after 12 days of incubation maximum radial growth recorded was 45 mm at 70±2 per cent moisture followed by 41.3 mm (80±2%), 38.7 mm (60±2%) and 30.9 mm (90±2%) moisture. From the data it is evident that the substrates supported good mycelial growth of *G. lucidum* upto 12 days of incubation and among the two substrates and their combination maximum growth of *G. lucidum* were in case of wheat straw + saw dust (45 mm) followed saw dust (38.6 mm) and wheat straw (29.8 mm) at 70±2 per cent moisture content.

On the other hand, minimum growth (30.9 mm) was recorded on wheat straw + saw dust followed by saw dust (22.2 mm) and wheat straw (16.6 mm) alone at 90±2 per cent moisture content of the substrates. Thus best growth of *G. lucidum* was measured when wheat straw + saw dust was used as substrate having 70±2 per cent moisture content as compared to the other treatments.

4.1.2 Effect of different temperature regimes on mycelial growth

The observation regarding influence of different temperature regimes on mycelial growth were recorded under *in vitro* conditions by culturing the fungus in Petri plates having wheat straw + saw dust as substrate. The radial growth was recorded for each treatment and data thus obtained after analyses are presented in Table 2.

The perusal of the results indicated that *G. lucidum* can grow at all the temperature evaluated in this study. However, the maximum growth was achieved with the 30±1˚C (45 mm) followed by 35±1˚C (42.2 mm), 25±1˚C (31.1 mm) and 40±1˚C (23.8 mm) after 12

days of incubation. Thus a temperature of 30±1˚C was found to be the most suitable for mycelial growth of this mushroom which showed maximum radial growth of 45 mm after 12 days of incubation and growth was significantly higher than at other temperature taken in this experiment.

Table 2: Effect of different temperature on radial growth of *G. lucidum*

Sr. No.	Temperature (˚C)	Radial growth* (mm) after days									
		3	4	5	6	7	8	9	10	11	12
1.	25	0.0	1.5	4.1	6.8	11.2	15.8	20.2	24.5	27.1	31.1
2.	30	2.7	4.9	8.4	16.7	21.2	25.3	31.0	37.1	42.9	45.0
3.	35	1.5	3.6	6.7	14.2	18.3	22.2	28.2	33.5	38.6	42.2
4.	40	0.0	1.3	3.2	5.4	8.4	11.2	15.1	16.7	20.1	23.8
5.	CD (p=0.05)	0.4	0.5	0.8	0.7	0.7	1.2	0.9	1.0	0.8	0.7

*Average of five replications
Note: The temperature was maintained at ±1˚C for the different treatments.

4.1.3 Effect of different pH on mycelial growth

To find out the suitable pH for the growth of *G. lucidum*, the substrate pH was adjusted to different pH levels *viz.*, 4.5, 5.0, 5.5, 6.0. The results presented in Table 3 indicates that *G. lucidum* grow at pH range of 4.5 to 6.0. However, the maximum growth was achieved when pH of the substrate was 5.5 (45 mm) followed by 5.0 (42.7 mm), 6.0 (41.3 mm) and 4.5 (24.8 mm). So, the pH of 5.5 was found to be the most suitable for mycelial growth of *G. lucidum* which showed maximum radial growth of 45 mm after 12 days of incubation. This radial growth was significantly more than the radial growth at all other pH treatments. All the observations of radial growth statistically differed from each other.

Table 3: Effect of different pH on radial growth of *G. lucidum*

Sr. No.	pH	Radial growth* (mm) after days									
		3	4	5	6	7	8	9	10	11	12
1.	4.5	1.2	2.9	5.6	7.7	9.0	10.9	12.1	16.6	21.3	24.8
2.	5.0	2.0	4.0	9.2	15.5	20.3	24.2	28.2	32.3	38.4	42.7
3.	5.5	2.8	5.2	10.7	17.6	22.1	26.5	31.4	36.8	42.8	45.0
4.	6.0	2.1	3.6	8.6	13.3	18.8	22.6	25.5	31.2	36.3	41.3
5.	CD (p=0.05)	0.5	0.6	1.0	1.0	0.9	1.1	1.0	0.9	0.9	1.0

*Average of five replications

Table 4: Effect of different moisture, temperature and pH on mycelial growth of *G. lucidum*.

Sr. No.	Moisture content (%)	Radial growth* (mm) after 12 days				
		Growth	Temperature (˚C)	Growth	pH	Growth
1.	60	39	25	31	4.5	25
2.	70	45	30	45	5.0	43
3.	80	41	35	42	5.5	45
4.	90	31	40	24	6.0	41

*Average of five replications
Note: The moisture content was maintained at ±2 per cent of the desired level and temperature was maintained at ±1˚C for the different treatments.

Thus, from the above results it was concluded that a mixture of wheat straw + saw dust substrate in equal proportion having 70±2 per cent moisture with a 5.5 pH upon incubation at 30±1˚C upto for 12 days supported maximum radial mycelial growth (Table 4).

4.2 Effect of substrate supplementation on mycelial growth of *G. lucidum*

4.2.1 Effect of different doses of wheat bran as a supplement

To find the optimum rate of supplementation (wheat bran) on mycelial growth of this mushroom, wheat bran @ 10, 20 and 30 per cent was added to the substrate as supplement and unsupplemented substrate (wheat straw + saw dust) served as control. The observations were recorded (Table 5) for linear growth of *G. lucidum*. The results upon analysis indicated that growth of *G. lucidum* was observed at all the supplement doses used in the present study. However, maximum growth was supported when wheat bran was added @ 20 per cent (8.8 days) followed by 30 per cent (10.6 days) and 10 per cent (12.4 days). Thus supplementation with wheat bran @ 20 per cent was most suitable for growth of *G. lucidum* as substrate which colonized in 8.8 days.

Table 5: Effect of supplement doses on linear growth of *G. lucidum*

Sr. No.	Substrate + Supplement rate (%)	Complete* colonization (days)
1.	Wheat straw + saw dust (1:1) (Control)	13.6
2.	Wheat straw + saw dust (1:1)+ wheat bran @ 10	12.4
3.	Wheat straw + saw dust (1:1)+ wheat bran @ 20	8.8
4.	Wheat straw + saw dust (1:1)+ wheat bran @ 30	10.6
5.	CD (p=0.05)	1.4

*Average of five replication

4.2.2 Evaluation of different substrates supplemented with wheat bran

To know the most suitable and economical substrate for mycelial growth of *G. lucidum* the substrates and supplement were screened for the best mycelial growth. The

observations were recorded for time taken to completely colonize the substrates and data concerning, growth initiation (days) and complete colonization (days) of the substrates. A perusal of data placed in Table 6 revealed that, the substrates supplemented with 20 per cent wheat bran exhibited maximum growth than unsupplemented substrate (Plate 1). The earliest growth (2 days) of *G. lucidum* was observed when wheat straw + saw dust supplemented with 20 per cent wheat bran. Similarly mycelial growth was initiated (2.3 days) when wheat straw or saw dust alone supplemented with wheat bran. On the other hand, in case of wheat straw + saw dust and saw dust without any supplement more time was taken for growth initiation (2.7 days) and the slowest growth was recorded when wheat straw was alone (4.3 days). Similar trend was observed regarding complete colonization of the substrate. The minimum time was observed (8.3 days) in case of wheat straw + saw dust supplemented with wheat bran followed by wheat straw + wheat bran, saw dust + wheat bran, wheat straw + saw dust, saw dust and wheat straw after 10.7, 11.7, 13.3, 13.7 and 15.7 days, respectively.

Table 6: Effect of substrate and supplement on linear growth of *G. lucidum*

Sr. No.	Substrates and Supplement	*Growth in days	
		Initiation	Completion
1.	Wheat straw	4.3	15.7
2.	Saw dust	2.7	13.7
3.	Wheat straw + wheat bran (20%)	2.3	10.7
4.	Saw dust + wheat bran (20%)	2.3	11.7
5.	Wheat straw + Saw dust (1:1)	2.7	13.3
6.	Wheat straw + Saw dust + wheat bran (20%)	2.0	8.3
7.	CD (p=0.05)	0.9	1.3

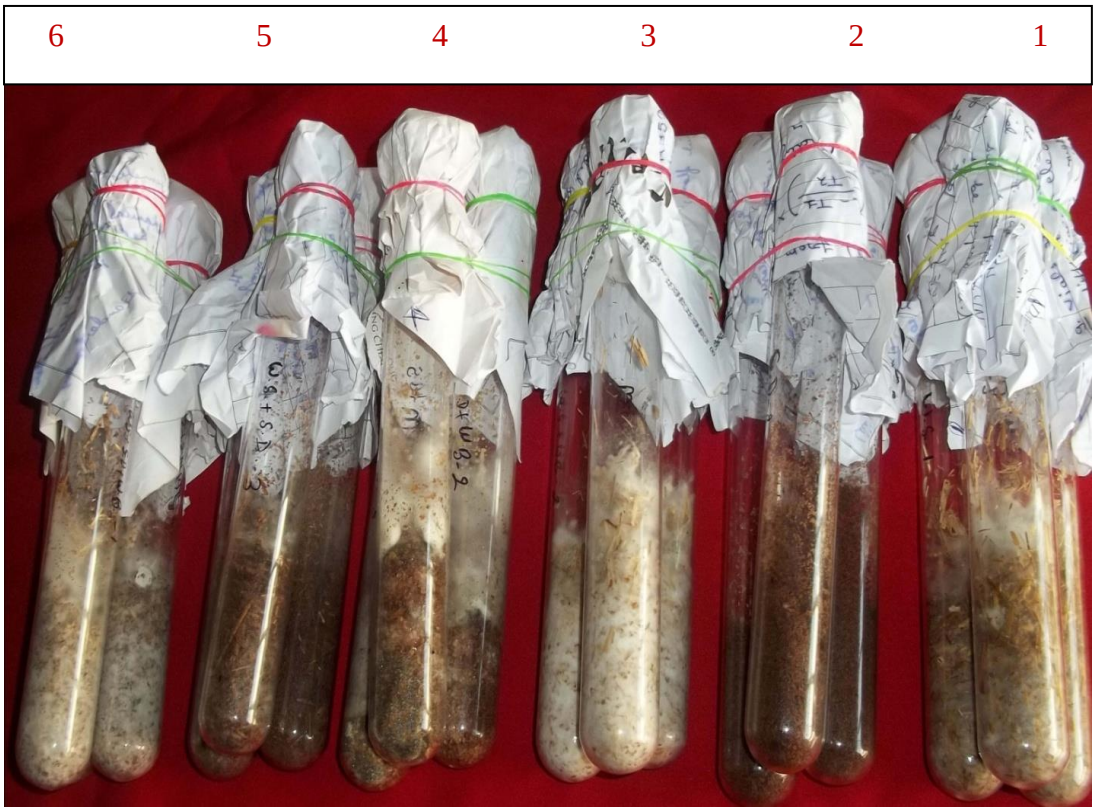
*Average of three replications

4.3 Evaluation of suitable substrates for spawn production

The four substrates selected for spawn production were wheat grains, bajra grains, sorghum grains and saw dust. The growth of mycelium was measured at 72 hrs interval until complete colonization of the substrate. An evident from Table 7 that, among the four evaluated substrates, fastest mycelial spread was observed in wheat grains where complete impregnation of mycelium was recorded at 12 days (Plate 2) followed by sorghum grains, bajra grains and saw dust which showed complete mycelial spread at 14, 16 and 19 days, respectively. The highest mycelial growth 67.8 mm was reported in wheat grains followed by sorghum grains (60.8 mm), bajra grains (51.4 mm) and saw dust (47.0 mm).

Plate: 1

Effect of different substrate and supplement on linear growth of *G. lucidum*



1. Wheat straw, 2. Saw dust, 3. Wheat straw + wheat bran, 4. Saw dust + wheat bran,
5. Wheat straw + saw dust, 6. Wheat straw + saw dust + wheat bran.

Plate: 2

Linear growth of *Ganoderma lucidum* on different spawn substrates

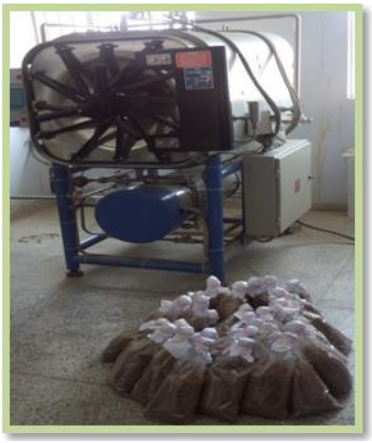
(a) Saw dust (b) Bajra grains (c) Wheat grains (d) Sorghum grains

Wheat grains based spawn

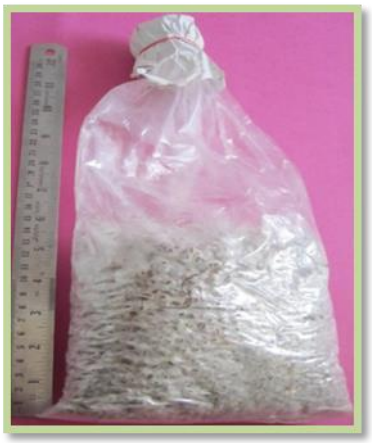
Plate: 3

Cultivation steps for *Ganoderma lucidum*

A. Substrate preparation



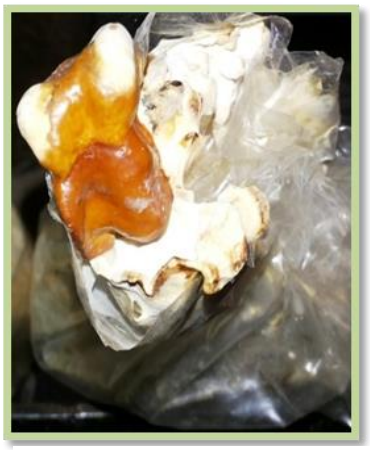
B. Bags filling & autoclaving



C. Wheat grains based spawn

D. Spawn run

E. Pinhead formation



F. Fruit-body formation

Table 7: Effect of different spawn substrates on linear growth of *G. lucidum.*

Sr. No.	Substrates	*Growth (mm) after days				Complete* Colonization (Days)
		3	6	9	12	
1.	Wheat grains	19.0	32.6	51.0	67.8	12.0
2.	Bajra grains	8.6	21.8	34.8	51.4	16.8
3.	Sorghum grains	12.0	25.6	43.4	60.8	14.8
4.	Saw Dust	6.6	17.6	30.6	47.0	19.4
5.	CD (p=0.05)	1.9	2.7	2.3	2.5	1.0

*Average of five replications

4.4 Cultivation of *G. lucidum*

The different parameters for *Ganoderma lucidum* growth were evaluated in the above experiments to find the optimum temperature, substrate pH, substrate type, substrate moisture, supplementation and substrate for spawn production. Thereafter, the cultivation of *G. lucidum* was carried out during August-October, 2013 and February-April, 2014 under natural conditions. The substrates wheat straw and saw dust were mixed in equal proportion and after soaking in water overnight the excess water was drained and amended with three per cent calcium sulphate and one per cent calcium carbonate. Thereafter, wheat bran was added and filled in polypropylene bags, plugged and autoclaved. Another set had substrate mixture only without any supplementation. After sterilization and cooling, the bags were inoculated with wheat grains based spawn under aseptic conditions. The bags were placed in the mushroom house under natural conditions whereas; humidity and CO_2 concentration were maintained as per requirement by spraying of water and opening and closing of doors and windows of mushroom house. The observations were recorded for yield and yield parameters and the results are depicted in Table 8, 9 and plate 3.

Table 8 shows that, time for completion of spawn run was less (13.00 days) in supplemented substrate than unsupplemented substrate (15.80 days). The time taken for pinhead formation, first harvest and last harvest was 14.60, 25.00 and 25.00 days respectively being maximum in wheat straw + saw dust and minimum 11.40, 22.00 and 22.60 days respectively when substrate was supplemented with 20 per cent wheat bran. Regarding the number and weight of fruit body per bag supplemented substrate had significantly more fruit body than the unsupplemented and the maximum number of fruit body being (8.40) and fruit body weight (143.80 gm) was also higher in supplemented substrate. Significant difference was recorded in the biological efficiency of *G. lucidum* in both the treatments and it was higher (28.76%) in supplemented substrate than unsupplemented (20.84%) during 2013.

The second year data revealed that (Table 9), time for completion of spawn run was also less (15.40 days) in supplemented substrate than (17.80 days) in substrate without supplement.

Table 8: Effect of substrates and supplement on growth parameters of *G. lucidum* during 2013

Sr. No.	Substrates* & Supplement**	DFSR[1]	DFPF[2]	DFFH[3]	DFLH[4]	NOF[5]	NOFB[6]	WOFB[7] (gm/bag)	BE[8] (%)	MDI[9] (%)
1.	Substrate	15.80	14.60	25.00	25.00	2.00	6.00	104.20	20.84	12.00
2.	Substrate + supplement	13.00	11.40	22.00	22.60	2.00	8.40	143.80	28.76	8.00
3.	t-value	4.22	4.44	3.00	2.75	-	4.71	19.37	19.37	0.58
4.	Sig. (p=0.05)	0.00	0.00	0.02	0.03	-	0.00	0.00	0.00	0.58

* Wheat straw + saw dust
** Wheat bran @ 20 per cent
1: Days for spawn run, 2: Days for pinhead formation, 3: Days for first harvest, 4: Days for last harvest, 5: Number of flushes,
6: Number of fruit body per bag, 7: Weight of fruit body per bag, 8: Biological efficiency, 9: Mould (green) disease incidence

Table 9: Effect of substrates and supplement on growth parameters of *G. lucidum* during 2014

Sr. No.	Substrates* & Supplement**	DFSR[1]	DFPF[2]	DFFH[3]	DFLH[4]	NOF[5]	NOFB[6]	WOFB[7] (gm/bag)	BE[8] (%)	MDI[9] (%)
1.	Substrate	17.80	15.40	23.00	19.20	2.00	5.40	97.60	19.52	16.00
2.	Substrate + supplement	15.40	13.60	21.60	18.20	2.00	7.00	131.40	26.28	12.00
3.	t-value	3.39	1.99	2.75	1.44	-	2.67	15.56	15.56	0.53
4.	Sig. (p=0.05)	0.01	0.08	0.03	0.19	-	0.03	0.00	0.00	0.61

* Wheat straw + saw dust
** Wheat bran @ 20 per cent
1: Days for spawn run, 2: Days for pinhead formation, 3: Days for first harvest, 4: Days for last harvest, 5: Number of flushes,
6: Number of fruit body per bag, 7: Weight of fruit body per bag, 8: Biological efficiency, 9: Mould (green) disease incidence

The time taken for pinhead formation, first harvest and last harvest was 15.40, 23.00 and 19.20 days respectively being maximum in wheat straw + saw dust and minimum 13.60, 21.60 and 18.20 days respectively in supplemented substrate. The maximum number of fruit body being (7.00) and fruit body weight (131.40 gm) per bag was also higher in supplemented substrate than unsupplemented. The biological efficiency of *G. lucidum* was higher (26.28%) in supplemented substrate as compare to unsupplemented substrate (19.52%) during 2014.

It could be observed from the two year data of 2013 and 2014 that, supplemented substrate gave significantly higher biological efficiency than unsupplemented substrate. The crop duration was three months in both the years and only two flushes were obtained during 2013 and 2014 cropping period. The incidence of competitor mould was more during 2014 as compare to 2013. Thus it was also observed that, biological efficiency was higher (28.76%) during 2013 than (26.28%) during 2014.

CHAPTER –V

DISCUSSION

Among the various cultivated mushrooms, *Ganoderma lucidum* is known to have excellent medicinal attributes but the existing knowledge of its biological nature and cultivation technique is scanty and fragmentary. The present investigation was undertaken to domesticate *G. lucidum* commercially so that quality mushroom could be supplied to the end user (pharmaceutical industry) and it can significantly contribute towards the mushroom market in India. In the present study, research relating to mycelial growth optimization and cultivation technique by using different agro-residues was conducted. The outcome of these studies would undoubtedly enrich the existing knowledge and assist in successful cultivation and commercialization of this medicinal mushroom as well as may help in crop diversification.

In the course of studying the influence of different substrates and its moisture content, temperature and pH on mycelial growth of *G. lucidum*, it was revealed that all these parameters had significant influence on the growth of this mushroom.

Moisture content of substrate is considered to be an important and effective tool for the mycelial growth optimization. The moisture of substrate is used by the fungus for nutrients uptake for its growth and development. In the present study, different moisture levels of substrates supported good mycelial growth of *G. lucidum.* Maximum growth was obtained at a moisture content of 70±2 per cent followed by 80±2 per cent, 60±2 per cent and 90±2 per cent in all the substrates *viz.*, wheat straw, saw dust and wheat straw + saw dust (1:1). The results are in accordance with the studies of Berovic and Habijanic (2000) wherein they reported 70 per cent moisture content of substrate optimum for *G. lucidum* growth. On the other hand, some other workers observed that 65 per cent moisture level in the substrate was optimum for mycelial growth and for successful cultivation of *G. lucidum* (Rai, 2003; Veena and Pandey, 2006). Amongst three different substrates evaluated for mycelial growth, wheat straw + saw dust (1:1) supported significantly higher growth followed by saw dust and wheat straw alone at 70±2 per cent moisture content and minimum at 90±2 per cent substrate moisture. Singh *et al*. (2007) also observed wheat straw and saw dust to be the suitable substrates for its cultivation. On the other hand, Rai (2003) achieved successful cultivation of *G. lucidum* when wheat straw, bagasse and saw dust and their combinations were used as substrates. Veena and Pandey (2010) also observed the suitability of agricultural residues such as wheat straw, rice bran and wheat bran as substrates for growth and development of *G. lucidum*.

Temperature has been reported to influence the growth and development of fungi to a great extent. Among different genera of cultivated mushroom, genus *Ganoderma* has most

divergent temperature requirements. In the present studies mycelial growth of *G. lucidum* was optimum at 30±1˚C followed by 35±1˚C, 25±1˚C and 40±1˚C. Similar results have been reported by Veena and Pandey (2006); as well as Liau *et al.* (1998) who observed optimum mycelial growth of *G. lucidum* at a temperature range of 30-35˚C. On the other hand, Adaskaveg and Gilbertson (1986) observed temperature range of 30-34˚C to be optimum with the maximum growth temperature of 37˚C. Griffin (1994) reported that *G. lucidum* is a mesophilic fungus as it can grow above 0˚C and below 50˚C with an optimum range between 15˚C and 40˚C. Song *et al.* (2007) observed that *G. lucidum* had maximum mycelial growth rate at 25-35˚C while, Negi *et al*. (2008) reported 32±1˚C was most suitable for the optimum yield of the fruiting bodies of *G. lucidum*. Similarly, temperature range of 28-30˚C has been reported as optimum for the vegetative growth of *G. lucidum* by Dadwal and Jamaluddin (2004). On the contrary Iqbal *et al.* (1997) reported that, its growth was maximum at 30˚C after 12 days of inoculation, however it reduced drastically below 25˚C and above 35˚C. Hence, the present studies are in agreement with the work done by earlier workers.

The hydrogen-ion concentration (pH) of media/substrates also plays an important role on the growth and development of mushroom. In the present investigation the maximum mycelial growth of *G. lucidum* were recorded at pH 5.5 and optimum range was between 5 and 6 while, at pH 4.5 the growth was highly retarded. The studies carried out by earlier workers (Iqbal *et al*., 1997; Negi *et al*., 2008; Mishra and Singh, 2010) are in agreement with the present work. On the contrary, a culture system having constant pH at 3 and 6 exhibited improved mycelial growth of *G. lucidum* (Min *et al*., 1999). While, Rai (2003) reported that pH 4.8 was optimum for the mycelial growth of *G. lucidum.*

Organic or inorganic substances added to the main substrate for the direct utilization by the mushroom constitutes supplements. Types of supplements differ as per the nutritional requirement of the mushroom and supplementation has positive role in mycelial growth, development and also on the quality of mushroom. In the present studies wheat bran was used as supplement.

Wheat bran supplement when added in different doses (10, 20 and 30%) to the substrate (wheat straw + saw dust), earliest colonization occurred *i.e.* 8.8 days when wheat bran supplement was @ 20 per cent; this was followed by 10.6 days (30%) and 12.4 days (10%). The present study supports the earlier work done by Chen (1999) who reported wheat bran to be an essential ingredient for the cultivation of *G. lucidum.* On the other hand, Sharma and Thakur (2010) observed that mycelial colonization was very fast when sugarcane bagasse and sunflower straw were supplemented with 15 per cent wheat bran. Similarly, Azizi *et al*. (2012) studied the effect of sawdust, malt extract and wheat bran on mycelial growth of *G. lucidum*, proved that highest mycelial growth rate *i.e.* 10.6 mm/day in a combination of beech sawdust with 2.5 per cent malt extract and 10 per cent wheat bran. Work of Gurung *et al*.

(2012) also pointed out that, most medicinal mushrooms were successfully cultivated when 20 per cent wheat bran was added to the basic substrate saw dust.

Studies carried out to find the suitable and economical substrate-supplementation for *G. lucidum* growth; revealed that substrate supplemented with 20 per cent wheat bran exhibited higher growth than unsupplemented ones. The earliest (2 days) growth was observed when combination of wheat straw + saw dust substrate supplemented with 20 per cent wheat bran. The time taken for substrate colonization was minimum (8.3 days) in case of wheat straw + saw dust substrate supplemented with wheat bran followed by 10.7, 11.7, 13.3, 13.7 and 15.7 days in wheat straw + wheat bran, saw dust + wheat bran, wheat straw + saw dust without supplementation, saw dust alone and wheat straw respectively. The present findings also supported the work of Jandaik *et al.* (2010), who investigated four forestry byproducts and three agricultural residues along with four supplements (wheat bran, rice bran, corn flour and gram powder) for growth characteristics, spawn run, primordial formation and yield of *G. lucidum*. The highest yield (82.5 gm) and biological efficiency (27.5%) were recorded from paddy straw supplemented with wheat bran, which invariably resulted in significantly higher yield compared to the unsupplemented check or other supplements used in this study. Similarly, Veena and Pandey (2011) also reported the enormous potential of cereal straw for the cultivation of *G. lucidum* in combination with sawdust and rice bran. On the other hand, Erkel (2009) while studying the effect of various kinds of sawdust and bran on the yield of *G. lucidum* and obtained the highest yield and biological efficiency from oak sawdust and wheat bran combination. Veena and Pandey (2011) also obtained maximum yield with the combination of sawdust: paddy straw: rice bran. Thus present studies confirms that wheat straw + saw dust (1:1) supplemented with 20 per cent wheat bran is ideal for *G. lucidum* growth.

Spawn, the planting material for mushroom is an important component of mushroom cultivation, as it influence the yield and quality. An attempt was made to find the suitable substrate for spawn production by taking three types of grains *i.e.* wheat, sorghum and bajra and saw dust were used as substrates for mycelial growth of *G. lucidum.* The study revealed that wheat grains based material was an ideal substrate for spawn production as it supported faster and highest vegetative growth followed by sorghum grains, bajra grains and least growth being in case of saw dust. These findings are in agreement with the work of other workers as well (Rai, 2003; Mishra and Singh, 2006). On the contrary, Veena and Pandey (2010) reported sorghum grains as the basal medium for *G. lucidum* spawn production. While, Sharma and Thakur (2010) stated that bajra grains as basal medium for its spawn production.

The mushroom was cultivated by using wheat straw + saw dust in equal proportion as substrate and this was supplemented with 20 per cent wheat bran. *G. lucidum* was grown as

per method described under the head materials and method (3.2.7) during August-October, 2013 and February-April, 2014. The results also pointed out that supplementation with wheat bran increased the biological efficiency from 20.84 to 28.76 per cent during 2013 and from 19.52 to 26.28 per cent during 2014, indicating that addition of supplement increased the yield. The variation in biological efficiency during 2013 and 2014 may be attributed to the higher incidence of competitor mould during 2014 owing to mushroom house conditions prevalent during cultivation period. This is in agreement with the work of Negi *et al.* (2008), where 32 per cent biological efficiency of *G. lucidum* was obtained. Similarly, Jaramillo *et al.* (2010) also obtained two flushes of *G. lucidum* with a biological efficiency of 24.20 per cent. The present investigation also in agreement with the work of Veena and Pandey (2011) where they obtained a biological efficiency of 29.90 per cent with a combination of cereal straw and saw dust supplemented with bran.

It can be inferred from the above discussion that, a mixture of wheat straw + saw dust in equal proportion supplemented with 20 per cent wheat bran having 70±2 per cent moisture with 5.5 pH and incubated at 30±1˚C supported dense mycelial growth and upon spawning with wheat grains based spawn gave two flushes of *G. lucidum* with a biological efficiency of 28.76 per cent.

CHAPTER –VI

SUMMARY AND CONCLUSION

The present study embodies "Studies on seasonal cultivation of *Ganoderma lucidum*". The research explorations presented in the preceeding pages were conducted on various aspects of mycelial growth optimization and cultivation technology of *G. lucidum* by using different agro-residues. The summaries and conclusions of present investigation are as under:

Among the evaluated substrates having different moisture content maximum mycelial growth was obtained at a moisture level of 70±2 per cent in substrate wheat straw + saw dust (1:1), followed by 80±2 per cent, 60±2 per cent and 90±2 per cent moisture in all the substrates *viz.*, wheat straw + saw dust (1:1), saw dust and wheat straw alone.

Though *G. lucidum* can grow at a wide range of temperature, maximum mycelial growth was obtained at 30±1˚C followed by 35±1˚C, 25±1˚C and 40±1˚C.

Among the different pH levels of evaluated substrate, maximum mycelial growth of *G. lucidum* was recorded at a pH of 5.5 and range was between 5 and 6 while, at pH 4.5 the mycelial growth was highly retarded.

A mixture of wheat straw + saw dust (1:1) having 70±2 per cent moisture, with 5.5 pH and upon incubation at 30±1˚C upto 12 days supported maximum mycelial growth of *G. lucidum*.

Wheat bran supplement when added in different doses to the substrate wheat straw + saw dust (1:1), the earliest colonization was reported (8.8 days) when a dose of 20 per cent and variation from this supplement dose reduced the growth.

Time taken for *G. lucidum* growth initiation and complete colonization was minimum when a combination of wheat straw + saw dust (1:1) supplemented with 20 per cent wheat bran as compared to other substrate-supplement combination and irrespective of supplement.

Wheat grains based material was an ideal substrate for spawn preparation as it supported faster and highest vegetative growth of *G. lucidum* followed by sorghum grains, bajra grains and saw dust.

The supplemented substrate gave higher biological efficiency from 20.8 to 28.7 per cent in 2013 and 19.5 to 26.2 per cent in 2014 as compared to unsupplemented one with cropping duration of three months in both the cultivation seasons of *G. lucidum*.

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ABSTRACT

Title of thesis	:	**Studies on seasonal cultivation of *Ganoderma lucidum* (Leyss. ex Fr.) Karst.**
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Title of degree	:	**Master of Science**
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Keywords: *Ganoderma lucidum*, mycelial growth, substrate, supplement, biological efficiency.

Ganoderma lucidum (Fr.) Karst (Polyporaceae) is an important medicinal mushroom. Amongst three substrates, different moisture content, temperature regimes and pH levels evaluated for mycelial growth of this mushroom. Wheat straw + saw dust (1:1) with 70±2 per cent moisture content was ideal. The optimum temperature and pH for its mycelial growth was 30±1˚C and 5.5, respectively though the mushroom can grow in the temperature range of 30-35˚C and pH 5-6. Among three different doses of wheat bran evaluated as supplement, the dosage of 20 per cent was the best when wheat straw + saw dust (1:1) was used as substrate. Out of four base materials used for spawn production, wheat grains were most suitable. Studies carried on cultivation of *G. lucidum* during 2013 and 2014 on wheat straw + saw dust substrate supplement with wheat bran, amended with (3%) calcium sulphate and (1%) calcium carbonate, filled in polypropylene bags, sterilized, inoculated with (3%) wheat grains based spawn and placed in mushroom house in dark at 30±1˚C with high CO_2 and ≥85 per cent relative humidity, was optimum in terms of spawn run, pinhead formation, no. of fruit body, yield and biological efficiency. Two flushes were obtained in three months crop cycle and maximum biological efficiency of 28.7 per cent was achieved in supplemented substrate during 2013 cropping season.

MAJOR ADVISOR **SIGNATURE OF THE STUDENT**

HEAD OF THE DEPARTMENT

CURRICULUM VITAE

a)	Name of the Student	:	Jagdeep Singh
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c)	Place of Birth	:	Hisar (Haryana)
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j)	Academic qualifications :		

Degree	University/ Board	Year of passing	Per centage/ OGPA	Subjects
Matric	HBSE	2006	8.20/10	Science, English, Social Science, Mathematics, Sanskrit, Hindi
Intermediate	HBSE	2008	7.20/10	English, Biology, Chemistry, Physics, Hindi
B.Sc. (Hons.) Agriculture	CCSHAU, Hisar	2012	6.90/10	All Agricultural Subjects
M. Sc. Agriculture	CCSHAU, Hisar	2014	8.15/10	Plant Pathology

k) Co-curricular activities, certificate and training courses:

- Six month experience of RAWE.
- Merit certificate on first prize in cricket in the 'Inter College Games &Sports 2010 - 11', at CCS HAU, Hisar.
- Merit certificate of Nation Service Scheme at CCS HAU, Hisar.
- Certificate on basic and advanced computer training at CCS HAU, Hisar.
- Certificate on 'Training related to Production, Processing, Marketing of Product and Accounts' at HSDC Sirsa.
- Training certificate on 'Commercial Agriculture' at CCS HAU, Hisar.
- Training course on 'Bee-keeping' at CCS HAU, Hisar.
- Training course on 'Mushroom Production Technology' at CCS HAU, Hisar.
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- Participation in a seminar organized by "Indian Soiety of Mycology and Plant Pathology" at PDKV Akola, Maharashtra.

I, hereby, declare that all the information given in the resume is true to the best of my knowledge.

Dated:

Place: **(Jagdeep Singh)**

UNDERTAKING OF THE COPYRIGHT

I, **Jagdeep Singh,** Admn. No. **2012A73M** undertakes that I give copy right to the CCS HAU, Hisar of my project report entitled **"Studies on seasonal cultivation of *Ganoderma lucidum* (Leyss. ex Fr.) Karst."**

I also undertake that, patent, if any, arising out of the research work conducted during the programme shall be filed by me only with due permission of the competent authority of CCS HAU, Hisar.

Signature of student