Weather-based analysis of shiitake yield and scientific intervention of shiitake cultivation in North East India.

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I hereby declare that the work embodied in this thesis entitled studies on studies on the weather effect of shiitake yield and standardization of traditional cultivation in Nort East India" is research work done by me under the supervision and guidance of **Dr. M. MATHIYAZHAGAN Associate professor of Botany, Silapathar Science College, Silapathar.** I further declare that this work has not been submitted earlier in full or in parts to any other University for the award of any other degree, Diploma , Associateship, Fellowship or other similar titles.

Hitesh Doley.

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LIST OF ABBREVIATIONS AND SYMBOLS

sp, spp	**: Species (singular and plural)**
Viz	**: Namely**
et al.	**: and other co workers**
Sl. No.	**: Serial number**
SEd	**: Standard error deviation**
CD	**: Critical difference**
CV	**: Coefficient of variation**
cm	**: Centimetre**
Fig	**: Figure (s)**
0 c	**: Degree Celsius**
kg	**: Kilogram (s)**
ha	**: Hector (s)**
mg	**: Milligram (s)**
gm	**: Gram (s)**
ml	**: Millilitre**
mm	**: Millimetre**
MSL	**: Mean Sea Level**
No	**: Numbers**
Max.	**: Maximum**
Min.	**: Minimum**
Temp.	**: Temperature**

CHAPTER-I

INTRODUCTION

Edible fungi belong to the Basidiomycota, which are macroscopic. Mushroom fungi are multicellular saprophytic fungi that are found in the rain forests. These edible fungi are hypogeal and found in soil, rainforest, wood, decomposed plant waste, etc. Shiitake mushrooms (*Lentiola edodes*) are found in the altitude range of 300–800 MSL. The Lentinula genus was proposed by Earle (1909). "Shii" means the hardwood log known as *Castanopsis cuspidata*, and "take" means the mushroom. Shiitake mushrooms can produce lignin and cellulolytic enzymes that are capable of degrading wood (Jong, 1989). It is commonly known as the golden oak mushroom, oak wood mushroom, and black forest mushroom. This method of mushroom cultivation is eco-friendly to nature and provides sustainable sources of income. Shiitake mushrooms are favoured for growing on lignocellulosic substrates rich in lignin because they secrete an enzyme called ligno cellulolytic enzyme (Leatham, 1986). Shiitake mushrooms provide a source of protein, vitamins, and minerals (Khan and Kausar, 1981). Shiitake mushrooms are used in traditional Chinese medicine for purposes such as immunomodulatory, antibacterial, antiparasitic, antiviral, hypocholesterolemic, and hepatoprotective (Hobbs, 2000). In India, the largely cultivated mushroom is *Agaricus,* followed by shiitake (Boa 2004). The Shiitake mushroom is one of the world's six most popular edible mushrooms, accounting for 17% of total production (Chang and Miles, 2004; Milnd Chang, 1997). For the cultivation of shiitake mushrooms, these logs are continuously exposed to natural weather conditions. The traditional method of cultivating the shiitake mushroom is largely affected by the weather. They used to collect the fruiting body of shiitake mushrooms seasonally from December to April. In Arunachal Pradesh, these shiitake mushrooms are naturally found in *Meliosma simplicifolia*. They collect the fallen *Meliosma simplicifolia* logs and keep them in their yard in the open field. For the cultivation of shiitake mushrooms, these logs are continuously exposed to natural weather conditions and are the traditional way of cultivating the shiitake mushroom. They will be the source of income for the farmers during the above period. The yield is sometimes marginal to abundance. We have aimed to study the effect of weather parameters on yield.

1. Survey and exploitation of edible shiitake mushroom occurrences and cultivation by the tribal people of Arunachal Pradesh
2. Standardization of cultivation methodology of shiitake mushrooms on native wood logs to increase the yield

CHAPTER-II

REVIEW OF LITERATURE

Lentinus edodes (Berk.) Singer, a white rot wood decay fungus, inhabits the wood of many hardwood tree species in Asia, especially those belonging to the family Fagaceae, e.g., oaks , Castanopsis (shii), chestnuts, hornbeam, beach, and also grows on Pasania (Lithocarpus) in Japan (Chang and Hayes, 1978). It ranks second among the cultivated mushrooms in the world from the standpoint of total production (Chang and Miles, 1987).

Lentinus edodes is known as "Shiitake" in Japan and "Shiang-gu" or "Hoang-mo" in China. The Japanese name is a combination of "shii" and "take". The word "shii" refers to the shii tree, one of the many tree species that it grows on in nature, and "take" is a Japanese word for mushroom (Chang and Hayes, 1978). Because Japan is the world's leading producer of shiitake mushrooms, the mushroom is known as "shiitake" or "Japanese mushroom." However, a search through the historical accounts reveals that it was perhaps in China that the edibility of the mushroom was first recognised and its initial cultivation technology developed.

The earliest records pertaining to this mushroom in Chinese have been investigated by Chang and Miles (1987).

They have attempted to put the stages of development of cultivation technology of Lentinus into historical perspective, and in doing so, they acknowledge the useful account of Zang (1981), in which he described the story of Wu San Kwung, who is known by both legend and history as the originator of Lentinus mushroom cultivation. The author has freely drawn upon their account of the early cultivation of Lentinus in China.

From the account, it emerges that Wu San Kwung lived during the Sung Dynasty (960–1127) and greatly hunted for wild mushrooms for food in the forests of the high mountains. His keen observation of the manner of growth of Lentinus in the wild enabled him to suggest, devise, and improve the initial cultivation technology of this mushroom. The farmers in his country were poor, and his efforts helped them improve their financial position. In recognition of this contribution, he is worshipped in temples erected in his honour, and a festival is held every year from July 16–19 (on the Chinese calendar) to thank Wu San Kwung for his concern for the welfare of the people.

In Wang Cheng's 1313, in the "Book of Agriculture," he wrote the section on the cultivation of fungi. He gathered information from tell-tales on mushroom cultivation. His book contains a brief but accurate description of the sequential steps involved in Lentinus cultivation.

The body of evidence gathered by Chang and Miles (1987) suggests that the cultivation of Lentinus was introduced into Japan from China. They cite Singer (1961), whose account of Lentinus cultivation is frequently referred to, and point out that Shiitake presented by the natives of Kyushu to the emperor Chuai in Japan in the year 199 A.D. is more likely to have been collected from the wild than cultivated. However, Ito (1978) considers the shiitake presented to the emperor to be the product of some primitive form of cultivation. But he also

states the probable Shiitake cultivation initiation in China 800 years ago, from where it was first introduced into Japan by Chinese farmers. Singer also suggests that a primitive form of Shiitake cultivation was introduced into Japan by Chinese farmers, and toward the end of the 17th or beginning of the 18th century, semicultivation techniques had been developed by the Japanese. However, Nakamura (1983) does not accept that shiitake cultivation was introduced into Japan from China. But it is no way to denigrate the Japanese to agree with the concept that they received a foreign idea and accepted it (Ito, 1978; Singer, 1961). Whatever account is accepted, the Japanese have over time evolved such technology for shiitake cultivation that they lead the world in its cultivation and production. Lentinus edodes occurs naturally on broad-leaved tree species throughout Asia. It is reported from China, Korea, Thailand, Burma, Nepal, North Borneo, the Philippines, Japan, Papua, and New Guinea (Mori et al., 1976). Shiitake cultivation on wood logs is the oldest method, but gradual improvements have led to its cultivation on sawdust. There are other methods that have been developed recently, but using logs or sawdust are the two substrates that are used most commonly.

1. CULTIVATION ON WOOD LOGS

For cultivation of the Shiitake mushroom on natural wood logs, approximately 84 hardwood tree species have been used (Ito and Imai, 1925; San Antonio, 1981; Leatham, 1982; Farr, 1983; Kuo and Kuo, 1983; Harris, 1986; Singer and Harris, 1987; Cotter et al., 1985 ;Cotter and Flynn, 1989). But the most commonly used species are Quercus serrata and Q. acutissima (Ito, 1978). Traditional Shiitake cultivation on wood logs was first compiled and described by Singer (1961) and has been recently redescribed in detail by Ito (1978). The details given are reviewed as follows:

1.1 Preparation of the Inoculum

The inoculum consists of pure culture spawn, impregnated in sawdust or of small wedge-shaped pieces of wood called "Tanegoma". A mixture of sawdust and 2% rice bran is used as the medium of the spawn. It is autoclaved and inoculated with mycelium of L. edodes and incubated at 24–28 C. In 'Tanegoma' spawn, inoculum is grown on autoclaved wedge-shaped, round wedge-shaped, or rod-shaped pieces of wood of various species of oak. Rod-shaped pieces are usually about 1.5 cm in diameter and 2.0 cm in length. The latter method is considered better for inoculation efficiency.

1.2 Preparation of Bed Logs

Wood is used as a substrate for shiitake cultivation. Instead of beds, the logs of certain woods are used for mushroom growing, and these logs are called "bed logs." Trees for cultivation are felled in autumn from coppices. The time of cutting is important and crucial for proper cultivation. If a cut is made at the wrong time, the bark strips off easily and contamination with weed fungi takes place before the shiitake mycelium has had a chance to become established. Another important item to be considered is the sugar content of the wood, which in oak, begins to increase considerably when about one-third of the leaves have turned red in autumn and continues to increase until just before budding in spring. Consequently, the mycelium finds a high percentage of easily available carbohydrates in wood and develops rapidly once it has

been inoculated on such wood. After trees are felled, the trunks remain in the forest. Just before inoculation, they are cut into logs measuring 1–1.5 m in length and 5–15 cm in diameter. The thicker branches are also used.

1.3 Inoculation

If solid pieces of inoculum are used, these are drilled or pounded into the log holes. In the case of sawdust spawn, the incisions or holes are more or less the same size as those used with wood chip spawn. The holes and incisions are painted with hot wax in order to prevent evaporation. The number of holes or incisions is calculated to correspond to about 1 hole per 1,000 sq cm.

1.4 Laying

After inoculation, the 'bed logs' are placed in a position favorable for the development of the mycelium. This is done on a site specially selected for the "laying operational for the development of the mycelium. This is done on-site specially selected for "laying operation." The site is called a "laying yard. This operation is as important as having good logs and a perfect inoculum.

The laying yard should never be excessively moist because of the danger of contamination of the logs with other more hygrophilous competing wood destroyers. The laying yard is often located in the evergreen grove, which is well ventilated, i.e., accessible to winds, out of reach of ground fogs in the low valleys, and, if necessary, drained by small ditches. If the "laying" takes place during the rainy season, very little watering will be necessary, but during the very dry season, the logs should be watered lightly. The optimum temperature for the development of the mycelium of Shiitake under these circumstances is between 24–28°C. On the 'laying yard', the 'bed logs' are put in an obliquely upright position at a small angle with the surface of the earth, with a single log laid crosswise to increase aeration.

1.5 Raising

After the mycelium has developed thoroughly through the wood, which may happen after one year or usually a year and a half after inoculation, depending on the kind of wood, spawn, weather, laying yard conditions, and Shiitake strain, the logs are transferred to another place, called the "raising yard". This transfer is meant to be an adaptation to conditions optimal for the fruit bodies, in contrast to the conditions optimal for the development of the vegetative phase of the fungus, the mycelium. For fruiting, the desired temperature is between 12 and 20°C. This is the reason that cropping takes place in early spring or late autumn. At the same time, the fruiting of Shiitake requires considerably more moisture than mere vegetative growth. The raised yard must be better shaded and a high relative humidity must be maintained, i.e., 90%. The logs are placed more upright in the raising yard. Ordinarily, the transfer of the logs will take place during the winter, so they should be ready for cropping in early spring.

1.6 Cropping

Shiitake fruit bodies grow in the spring and fall. Once the logs begin to produce, they will continue to do so for 3-6 years in a particular season. The producing logs are kept wet by water. There is no other care necessary except daily harvesting. The fruit bodies, at the picking time, should be young, just at the time when they begin to sporulate, with a strong convex pileus and traces of the veil visible. Optimum yield from natural logs has been reported to be as high as 9–35% over a 6-year harvesting period (San Antonio, 1981).

REVIEW OF SUGGESTIVE PARAMETERS

Among the cultivated mushrooms, the cultivated white mushroom, Agaricus bisporus, is well known to the European people and the Shiitake (the forest mushroom, Lentinus edodes), to the Oriental people, and both these enjoy a good reputation internationally as food (ItO/1981). The popularity of Shiitake has now spread to the west as well, where it is known as the "Black Forest Mushroom." Shiitake has been cultivated in many Asian countries since very early times, but unfortunately, it has not been possible to cultivate it under Indian conditions so far. That is why the present studies were taken up. Before giving details of the investigations undertaken herein, a review of literature, especially on aspects considered in the present study, is given in the following pages.

PHYSICAL ELEMENTS 3

Before cultivation of any fungus is taken up, it is essential to bring the fungus into culture and study its basic requirements as well as the physical parameters, because the conditions vary from one place to another and even from strain to strain.

3.1. Consistent Media Growth

The evaluation of a fungus for its initial vegetative growth is done on solid media. The best-suited medium is then used for studying the effects of other physiological parameters. For the mycelial growth of Lentinus edodes, potato dextrose yeast extract agar has been reported as the best medium (Jodon and Royse, 1979). The best mycelial growth of L. edodes has been recorded on potato dextrose agar, malt extract agar, and yeast extract agar medium (Campbell and Slee, 1987). Song et al. (1987) studied the growth of the mycelium of L. edodes on various solid media, and the maximum mycelial growth was recorded on Macaya-Lizano agar medium. Hunt et al. (1988) also reported the best mycelial growth on modified Macaya-Lizano agar medium. In general, the culture of L. edodes in the laboratory is reported to be maintained on potato dextrose agar, malt agar, oat meal agar, and complete agar medium (Chang and Miles, 1989). Potato dextrose yeast extract agar medium and potato dextrose agar medium have also been widely recommended for maintaining cultures of L. edodes (Jodon and Royse, 1979; Royse, 1985; Toyamasu and Zennyozi, 1981; Badham, 1988; Tan and Chang, 1989a). A nutritionally weak medium is preferred for maintenance of the culture of L. edodes as it lowers the metabolic rate of the organisms and this prolongs the period between transfers.

Growth in Liquid Media Liquid media

They have been used by a number of investigators to establish cultures to study the nutritional requirements of the fungi. Some have investigated the possibility of growing mushroom mycelium in a wide range of liquid media as a potential source of fungal biomass for use as food (Block et al. £!•/ 1959). Static culture techniques were employed in nutritional investigations by Treshchow (1944), while Ivanovich (1965) used liquid cultures for cytological investigations. Ishikawa (1967) reported the best mycelial growth of L. edodes on malt extract medium. Song et al. (1987) reported the best mycelial growth of L. edodes in molasses in twelve different media tested and minimum growth in Czapek-Dox medium. Raaska (1990) used yeast malt extract medium enriched with 0.26% and 1.0% fine alder sawdust and 1.45% of CaSO, respectively. The yeast malt extract medium consisted of 2% malt and 0.1% yeast extract was used as a control. The best mycelial growth was obtained in yeast malt extract, supplemented with CaSO.

Temperature Influence

Incubation temperatures are important in determining the rate of growth of a fungus (Haye S, 1978). The best mycelial growth of L. edodes has been reported at 25°C. At lower temperatures, such as 45 °C in a liquid culture, mycelia die quickly (Tokimoto and Komatsu, 1978).Toyamasu and Zennyozi (1981) also observed 25°C as the optimal temperature for the mycelial growth of L. edodes. Han et al. (1981) studied the effect of temperature on pure culture isolates (CH-7, CH-14, and CH-25) of L. edodes and reported 26 C as the optimal temperature for both the isolates. Good growth was recorded at 28°C for CH-25 and at 20–26°C for CH-14. The optimal temperature range for mycelial growth varied with the isolates of L. edodes, and it might be correlated with the optimal temperature for fructification. L.J. edodes and Campbell and Slee (1987) reported 22 C as the optimal temperature for the vegetative growth of L.J. edodes. Hunt et al. (1988) recorded 25 C as the best temperature for the mycelial growth of L. edodes. Song et al. (1987) reported 20–25°C as the best temperature range for the best mycelial growth of L. edodes. Jong (1989) reported that the mycelium of L. edodes could grow within a range of 5°C to 32°C with best growth at 25°C and that constant temperature was better for mycelial growth than fluctuating temperature. Song et al. (1990) reported maximum mycelial growth of L. edodes at 25 C. Raaska (1990) also reported 25°C as the best temperature for maximum mycelial growth of L. edodes. Therefore, it is clear that L. edodes prefers a temperature of around 25 °C for producing good mycelial growth.

3.4 Hydrogen-Ion Concentration (pH) Effects

A fungus will grow maximally over a certain range of pH values in a medium and will fail to grow at high and low extremes. The mechanism of pH action differs at different concentrations of hydrogen ions (Cochrane, 1958). Hiroe and Kamiyoshi (1937) studied the effect of pH on the vegetative growth of L. edodes and reported pH 5.0 to 6.0 as the optimum pH for the best mycelium growth. However, Ishikawa (1967) reported 3.5 pH as the best for maximum mycelial growth of L. edodes. Song et al. (1987) reported the best mycelial growth of L. edodes at pH 4.3 and 4.8. Song et al. (1990) further reported the maximum mycelial growth of L. edodes at 4.5 pH. Raaska (1990) observed 3.2 to 4.8 as the optimum pH range for best mycelial growth of L. edodes. Therefore, it is clear that the optimum pH range for best mycelial growth of L. edodes is around 3.5 to 6.0.

PHYSIOLOGICAL PARAMETERS 3.5

The Effect of Carbon Sources As in other heterotrophic organisms, carbon compounds serve two essential functions in the metabolism of fungi. They supply, in the first place, the carbon needed for the synthesis of the compounds which go to make up the living cell-proteins, nucleic acids, cell wall materials, reserve foods, and so on; in a typical fungus, about 50 percent of the dry weight is carbon. Second, the sole source of appreciable amounts of energy is the oxidation of carbon compounds, which may account for half or more of the carbon supplied to a culture (Cochrane, 1958). Studies on carbon nutrition have emphasised the utilisation of carbohydrates. The carbohydrate content may consist of a large variety of compounds. Soluble sugars have been recorded to be better carbon sources than starch and wood flour (Block et al., 1959). Different carbon sources have been tried by a number of workers to study the mycelial growth of L. edodes. A large number of carbon sources, including monosaccharides, oligosaccharides, and polysaccharides, have been reported to be satisfactory for the vegetative growth of L. edodes. They sustain good growth of the fungus at a concentration of 3–5% in the liquid culture (Yoshida et al., 1965; Ishikawa, 1967). Sugimori et al. (1971) reported good mycelial growth of L. edodes with ethanol and glycerin as noncarbohydrate substances. Leatham (1985) considered D-glucose as the most efficient carbon source for the vegetative growth of L. edodes. Tan and Chang (1989b) obtained the best mycelial growth of L. edodes with 3% sucrose. Song et al. (1987) reported the best mycelial growth of L. edodes with glucose among 26 different carbon sources tried. Although comparable growth occurred with fructose and trehalose, no mycelial growth was reported with aesculin, arabinose/mannose, or Dxylose. Song et al. (1990) recorded the best mycelial growth of L. edodes with glucose.

3.6 Nitrogen Sources' Influence

There is no optimum amount of nitrogen for a culture; the demand depends, in the first instance, on the carbon supply, but in principle at least, any factor may change the apparent optimum concentration of the nitrogen source. In general, however, inorganic or organic nitrogen is taken up rapidly during the growth phase (Cochrane, 1958). Ishikawa (1967) studied the effect of nitrogen sources on the vegetative growth of L. edodes and reported the best mycelial growth with peptone, certain 1-amino acids, urea, and various ammonium salts, whereas nitrates and nitrites were not useful for the growth. Han et al. (1981) reported the best mycelial growth with ammonium chloride, ammonium nitrate, and acetamide, while no growth was observed with sodium nitrite and potassium nitrite among ten different nitrogen sources studied. Leatham (1985) reported L-glutamic acid as the only significant nitrogen source for the best mycelial growth of L. edodes. Kawamura and Goto (1980) also reported L-glutamic acid is the best nitrogen source for the vegetative growth of L. edodes. Song et al. (1987) recorded the best mycelium growth with ammonium chloride and relatively low potassium nitrate. Good growth was observed with ammonium nitrate, while no growth was reported with ammonium acetate or sodium nitrite. Among the amino acids used, aspartic acid is reported to be the best, but relatively low growth has been observed with asparagine. The Effect of Vitamins or Growth Regulators Organic compounds that are required in minute amounts for growth may be called vitamins or growth factors. These two categories are distinguished on the basis of their concentrations. Vitamins are usually required in micromolar concentrations or less and function as co-enzymes, whereas other growth factors are usually required at higher

concentrations, up to 100 pm, and function as structural elements (Griffin, 1981). The vitamin requirement for the vegetative growth of L. edodes has been studied by different workers. Hiroe and Ikuda (1960) and Ishikawa (1967) reported thiamine as the only essential vitamin for the mycelial growth of L. edodes. Leatham (1983) recorded the best mycelial growth with a mixture of vitamins and less growth with thiamine. Han et al. (1981) recorded the maximum mycelial growth with a 5 ppm concentration of indole acetic acid and 10 ppm of gibberellic acid. Tan and Chang (1989b) studied the effects of growth regulators (indole acetic acid, kinetin, and gibberellic acid) at concentrations of 5, 10, 50, 100, and 300 ppm w/v. At all concentrations, gibberellic acid showed good mycelial growth. Indole acetic acid showed poor growth, while kinetin had an inhibitory effect on the mycelial growth of L. edodes. Tan and Chang (1989a) studied the effect of indole acetic acid on different strains of L. edodes at concentrations of 100 and 300 ppm and recorded better growth in comparison to control.

3.7. Effect of Trace Elements

Fungi have relatively large requirements for phosphorus, potassium, sulfur, and magnesium and much smaller but definite requirements for at least five micronutrients (trace elements and minor elements). The known essential micronutrients are iron, zinc, copper, manganese, and molybdenum. Manganese, iron and zinc at 2 mg/litre have been reported to promote mycelial growth of L. edodes in addition to magnesium, sulfur, potassium, and phosphorus. The mixture of copper, cobalt, and molybdenum accelerated the growth under suitable concentrations (Ishikawa, 1967). (1987) reported that an increase in the concentration of potassium phosphate in the medium from 0.5-2.0 (10 M) resulted in an increase in the yield of mycelium. However, further increases in phosphate ions decreased the mycelial growth . Raaska (1990) recorded the best mycelial growth in cultures supplemented with calcium sulphate.

4. SPAWN PRODUCTION

Mushroom spawn consists of a medium impregnated with mushroom mycelium. It is produced by taking a pure strain of mycelium from a culture tube and multiplying it. To ensure high quality spawn, sterile conditions are required throughout the spawning process (Przybylowicz and Donoghue, 1990). Previously, fermented manure or tobacco stems were used as a substrate. As spawn substrates, a variety of materials, both alone and in various combinations, can now be used, including rice straw cuttings, sorghum, rye, and wheat grains, cotton wastes, sawdust, and used tea leaves. The materials used for spawn making are relative rather than absolute. They could differ from place to place and country to country (Chang and Quimio, 1982). For Shiitake cultivation, originally no artificial inoculum was used. Later on,the use of spore suspension came into vogue. Then started the use of pure culture spawn on sawdust and plugs (wood plug spawn), followed by the liquid spawn, grain spawn, comb spawn, etc., all of recent origin. These methods of spawn preparation are described below. In the original method, the inoculum consisted of logs bearing Shiitake mycelium. Such logs were dragged to a suitable site of new logs, which would be in the process injected by the Shiitake mycelium through "natural" spore dissemination from the developing fruiting bodies of the old logs (Singer and Harris, 1987). Ito (1978) described the earlier techniques used to cultivate Shiitake as called "Hodagi". In this technique, small hatchet notches were cut on the trunks, which would serve as the receiving sites for wind-borne spores. Some of these spores would germinate to produce

mycelium, which would spread into the wood and develop into fruit bodies. But this method was dependent on natural elements and no regular production of shiitake could be expected from this method. Furthermore, the grower would have to wait patiently for the shiitake production to begin. To ensure the supply of inoculum, they started the use of spore papers in the form of spore prints into the incisions in the logs (Singer and Harris, 1987). An improvement to this type of spore inoculum was the suspension method, introduced about 75 years ago (Ito, 1978). The spores were harvested either from basidiocarps in nature or from those under cultivation. The spore suspension was placed in holes chopped in the logs. This method, though more reliable than the one described above, was still crude and uncertain. The reliability increased with the use of pure culture spawn (Quimio et al., 1990). The pure culture spawn is reported to be produced by inoculating a bottle of sterilised horse manure with tissue cultures from a quality mushroom or spores germinated under sterile conditions. Production of bottle spawn has led to the development of various types of pure culture spawn which differ principally in the preparation of the spawn substrates and the ingredients, e.g., manure spawn, grain spawn, and perlite spawn (Chang and Miles, 1989). Singer and Harris (1987) described that the Shiitake spawn method is similar to the method used in the case of other mushrooms, but the medium used for the spawn is logically one adapted to the biology of the Shiitake mycelium. In other words, the substratum must consist at least partly of wood. The mycelium of L. edodes grows well on sawdust mixed with rice-bran (4:1). The sawdust substrate was autoclaved and inoculated with tissue or spores and incubated at a temperature of between 24 and 28 C. This substrate had higher nutrient and moisture levels and was usually inoculated into larger holes in the logs. Therefore, the mycelium began colonising wood more rapidly (Przybylowicz and Donoghue, 1990). This made it possible to select and propagate Shiitake mycelium with improved vigour and high yield. Singer (1961) reported that in the 1920's K. Kitayama was the first person to develop the pure culture sawdust spawn of Shiitake. A disadvantage of this method is that it has a higher surface-to-volume ratio because of its particulate nature. This makes it more susceptible to drying out during the spawn run. Therefore, sawdust-filled inoculations must be sealed (Shinkosha, 1981). An alternative kind of spawn used very frequently consisted of autoclaved pieces of wood in different shapes. Mori (1987) reported that Dr. Kisaku Mori was the first person to develop this method. In 1935, the late Dr. Mori started the Mushroom Research Institute in Kiryu, Japan, and after many attempts, he was able to invent pure culture wood chip spawn in 1942. The mycelium was transferred from a petridish containing the original culture or any medium (sawdust, rice bran, malt extract, etc.) onto the wood plugs, which were subsequently overgrown by the white mycelium of the fungus after incubation at 24-28 °C. Often, one end of the plugs was tapered to facilitate insertion into the log. Sawdust spawn is said to have several advantages, including the fact that it is easier to use than sawdust spawn and that it resists drying better due to its less exposed surface (Shinkosha, 1981). Under moist conditions, sealing plug inoculation holes to prevent water loss is not needed. This considerably decreases the time and labour needed for inoculation (Przybylowicz and Donoghue, 1990).

Itavaara (1989) prepared the liquid spawn for commercial cultivation of shiitake on a sawdust substrate. The spawn was prepared by inoculating the yeast malt extract medium with the mycelium of shiitake. The medium was prepared by using 2% malt extract and 0.1% yeast extract, and spawn was ready in five days in comparison to weeks in other techniques. Saito (1976) stated that liquid spawn, consisting of mycelium suspended in a liquid slurry, has been injected into logs, but the results obtained were poor. Further, in the liquid spawn of Shiitake, the homogenization effect has been studied by Raaska (1989) and reported that strong and even

quite short homogenization times of the mycelium prevented the spawn growth. Strong homogenisation of the mycelium produced many short mycelium pieces whose ability to regenerate after homogenisation was poor. The Shiitake spawn was grown in a stationary liquid medium. Before the inoculation to the sawdust medium, the mycelium was ruptured by homogenisation. Itavaara (1989) reported that liquid spawn is a good adaptation to the sawdust medium. The spawn should be homogeneous without pellets because inoculations are made by injections. In addition, large amounts of the spawn should be produced with minimum labour. Another form of spawn used is grain spawn for the production of shiitake on sawdust substrate. Hu and Lin (1972) reported a general grain spawn used for different cultivated mushrooms. This grain spawn consisted of a known quantity of healthy wheat grains, which were washed several times and soaked in water for 18–24 hours. Then the grains are boiled for about 15 minutes, air dried, and mixed with 2% gypsum (to prevent the grains from sticking together) and 4% calcium carbonate (to adjust the pH). These are put in 1/2 litre milk bottles, plugged and sterilised twice at 20 lbs of pressure for 30 minutes. The contents of the bottles are agitated 2-3 times during the incubation period for the uniform and rapid distribution of mycelia in grains. They noticed that grain spawned several advantages: it was easy to produce and handle; it was more effective and vigorous. For the cultivation of Shiitake, Tan and Chang (1989a) prepared grain spawn as follows: Then, 200 g of wheat grains, 2 g of CaCO, and 150 ml of tap water were mixed, sterilized, and inoculated with mycelia of shiitake. Royse's (1985) grain spawn had the following ingredients: rye grain, 80 g; sawdust, 6 g; CaCO, 1 g; and water, 150 ml. Diehle and Royse (1986) prepared grain spawn by taking 6 g sawdust; 115 g pre-cooked millet; 1 g brewer's yeast; and 1.5 g rice bran. Another type of spawn used for Shiitake production on logs is the "comb spawn," consisting of thin slices of colonised wood which are inserted into sawcuts in the logs (Fujimoto, 1987). Lelley (1989) reported the use of fireboard instead of wood wafers for the cultivation of shiitake. It is clear that with the passage of time in the commercial cultivation of Shiitake, the most commonly used spawns are sawdust or grains and solid spawn. From among the different spawn types, it has been reported that wood plug spawn is superior for log cultivation, while sawdust spawn, liquid spawn, and grain spawn are best for sawdust cultivation (Campbell and Slee, 1987).

5. CULTIVATION

Lentinus grows in nature on dead hardwood logs in a warm, moist climate. This combination of warm temperatures and plentiful moisture prevents excessive drying of the wood and promotes rapid fungal growth (Singer, 1961).

For the cultivation of L. edodes, different hardwood tree species have been used by workers in the bed log production of shiitake (Ito and Imai, 1925; San Antonio, 1981; Leatham, 1982; Farr, 1983; Kuo and Kuo, 1983; Harris, 1986; Singer and Harris, 1987). Nutalaya and Putaragetvit (1981) reported the best bed log production of L. edodes. However, the best production of shiitake has been reported from small-diameter oak wood logs, however (Singer, 1961; Chang and Hayes, 1978; Leatham, 1982; Cotter et al., 1985; Cotter and Flynn, 1986).

Peng (1989) studied the production of shiitake on a number of tree species and reported that the tree species of Liquidamber formosona, Castanopsis hystrix, and Elaeocarpus sylvestris

were excellent, while other species such as Mallust peniculatus, Sapium discolor, and Acacia manqium were also found ideal for growing shiitake. Cotter and Flynn (1986) stated that the production of shiitake on white and chestnut oak (Quercus alba and Q. prinus) was excellent, but poor in pink oak (Q. palustris). Mori (1987) reported that 2-Serrata and Q. acutissima were the best-suited tree species for the production of shiitake.

Shiitake cultivation on log wood encountered many problems, and an alternative method of cultivation on sawdust has now become more widely accepted in different parts of the world due to the easy cultivation of Shiitake on it. In sawdust, the wood structure is disrupted and more surface area is exposed. This gives the mycelium better access to the wood, so the fungus can grow and degrade the sawdust more rapidly (Przybylowicz and Donoghue, 1990). The sawdust enriched with rice bran has been used by different workers to cultivate Shiitake (Ando, 1974; Han et al., 1981; Ishikawa, 1967; Song, 1983; Royse, 1985; Diehle and Royse, 1986; Ho, 1989). Shiitake was cultivated on a sawdust mixture of hardwood tree species, enriched with 10% rice bran and 0.2% CaCO. A sawdust compost of hardwood tree species, preferably Quercus, Acer and Betula, was supplemented with a sugar source and a nitrogen vitamin-mineral source was also added (Han et al., 1981).

Sawdust, wheat bran, and grains were used in a ratio of 8:1:1 as the substrate to cultivate Shiitake (Royse, 1985). Diehle and Royse (1986) cultivated Shiitake on a sawdust substrate consisting of sawdust (maple-60%, birch-40%) mixed with rice bran and millet. Triratana and Raksatul (1984) cultivated Shiitake on pararubber tree sawdust supplemented with rice bran-5%, corn starch-0.4%, magnesium sulfate-0.1%, and calcium sulfate-1.0%. The same substrate was used by Triratana and Tantikanjana (1989) to study the effects of some environmental factors on the morphology and yield of L. edodes. Tan and Chang (1989a) studied the effects of 17 formulations of sawdust mixed with different nutrients, i.e.,

used tea leaves, wheat bran, wheat flour, CaCO, CaSO, MgSO, sucrose, indole acetic acid, tannic acid, and stannous chloride and reported a double yield with the addition of 10% wheat flour to a basal sawdust medium. Kalberer (1989) cultivated Shiitake on a heat-treated substrate of sawdust mixed with rice bran and calcium carbonate.

Chang and Miles (1989) reported that shiitake was cultivated on agricultural by-products or residues such as bagasse, sugar beet residue, cotton seed hulls, peanut hulls, and corncobs. Wang et al. (1989) reported that shiitake has been cultivated on the bark wastes of different tree species. The bark consisted of nanmu (Taiwan phoeba and/or common machilus), Taiwan acacia, white popinae, and others, and adequate quantities of chicken manure were added to provide 1.5% N.

Ando (1974) reported the fruiting of L. edodes on liquid synthetic medium at a minimum of 57 days after inoculation. Leatham (1983) reported the fructification of L. edodes on defined liquid medium supplemented with oat flour within 40 days after inoculation. Chang and Miles (1990) reported the cultivation of L. edodes on a straw and woodchip mixture supplemented with feather meal. Benomyl was also added. Denison and Donoghue (1992) reported high quality mushroom production on alder-based media, which was used as an alternative to oak sawdust.

Ito (1978) reported that cultivation on a supplemented sawdust substrate provides a more rapid and controlled method of cultivation of shiitake than is presently possible with sectioned logs.

From amongst the different materials used for the cultivation of Shiitake, it becomes evident that wood logs and sawdust are the two main substrates which have been commercially used for Shiitake cultivation in different parts of the world.

6. EFFECT OF ENVIRONMENTAL FACTORS ON FRUITING

6.1 Temperature

The fruiting body production in edible fungi alongwith other factors depends on certain optimum/ which may be the same as for vegetative growth or different. In general, optimum temperature for fructification production in most edible fungi is lower than optimum temperature for vegetative growth (Quimio et al., 1990). Fruiting in Lentinus edodes may be induced in two ways: by lowering the temperature, increasing humidity within and outside the substrate and by increase in light. The low temperature range determined for fruiting body development in L. edodes was between 3-12 C which may differ depending upon the stock or strain used (Nukuzimu et a_l., 1959; Nagai et al., 1962; Ando et al., 1969). Campbell and Slee (1987) observed 20 C, 10-15 C and 10°C as the optimum temperatures in three different strains, respectively. Ito (1978) reported 12-20 C as the optimal temperature for the fruiting of L edodes, whereas. Miller and Jong (1987) reported 10°C as the optimum temperature for fruiting body production. Han et al. (1981) observed 16°C as the best temperature for fruiting body production in Shiitake. Royse (1985) obtained good crop of Shiitake at 18°C. Kalberer (1989) reported 17-19°C as the best temperature range for fruiting body formation in L. edodes. It is clear, therefore, that L. edodes prefers a temperature range between 10-20 C for best fruiting body production.

6.2 Light

Light regulates reproductive development in a wide range of fungi. Many fungi are apparently uninfluenced in reproduction by light in the visible range; i.e., they do equally well in darkness, continuous light or alternate darkness and light. Depending on the fungus and developmental stage, light may stimulate or inhibit development (Barnett and Lilly, 1950). The effective wavelengths are generally in their near ultraviolet, blue, red or far-red spectral region (Tan, 1977). Ishikawa (1967) designed an experiment to determine the effect of light exposure during spawn run and fruit body initiation of L. edodes. The cultures were exposed to continuous light at four light intensities (50, 100, 500, 1000 lux) for five exposure periods (0, 10, 30, 60, 80 days). He found an apparent interaction between light intensity and exposure period. The culture exposed to continuous light of 50 lux for 80 days produced 2.8 times more mushrooms by those exposed for 80 days to 1000 lux. Komatsu (1963) reported that light irradiation played a role in the process of fruit body development and especially for gill and spore formation. And under various filtered light conditions, using coloured cellophane papers fruit bodies developed abnormally and sporulation diminished. Ando (1974) reported that depending on the growth medium, blue and/or pink wavelengths were necessary for fruiting. Leatham and Stahmann (1987) studied the effect of red and blue light and stated that red light with 22-340 lux gave highest yield of L. edodes. Hunt et al. (1988) stated that for sporophore production light was provided at 450 lux, abnormal sporophores were produced at low intensity, i.e., approximately 22 lux. Kalberer (1989) reported that best fruiting flush was obtained by providing low light intensity (between 22-150 lux). Ishikawa (1967) reported that light is necessary for fruiting of L. edodes/ though it inhibits vegetative growth. Therefore, it can be concluded that L. edodes prefers a light range between 22-150 lux for the production of fruiting bodies.

6.3 Relative Humidity

Some fungi have specific substrate moisture optima that may depend, to some extent, on the type of substrate used. Cotter et _al. (1985) and Cotter and Flynn (1986) reported that the fruitings of L. edodes were induced by soaking wood logs in cool water for 2-3 days. The logs were then stacked in an area of high humidity, i.e., 90% to get good crop. Royse (1985) reported

95-98% humidity best for the production of Shiitake mushroom on sawdust. Jong (1989) reported 80% as the best moisture percentage for fructification of Shiitake. Hunt et al^. (1988) reported 90%, as the best moisture percentage while Chang and Miles (1989) and Kalberer (1989) stated that the moisture percentage at 85-90% and 95-99%/ respectively was best for the fructification of L. edodes. Przybylowicz and Donoghue (1990) reported that 55-68% moisture content in sawdust culture and 35-65% log moisture content is optimum for the primordial formation of L. cdodos. From the above, it can be concluded that moisture percentage range between 80-99% favours the best fructification production in L. edodes.