Cultivation of *Pleurotus* spp in China

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Abstract: *Pleurotus* mushrooms are very popular in China and more than ten species of *Pleurotus* are currently cultivated on an industrial scale, including *P. ostreatus P. cornucopiae* and *P. citrinopileatus*. In 1986, the combined yield of all *Pleurotus* spp was only 108,000 tonnes but output in 2003 had increased to 2,488,000 tonnes. In recent years, new species such as *P. erygyii* and *P. nebrodensis* have gained prominence and annual production of these species has expanded rapidly. In 2001, annual yields of *P. eryngii* and *P. nebrodensis* were 21,022 tonnes and 7,343 tonnes, respectively. In 2003, these figures had increased to 114,107 tonnes and 52, 223 tonnes respectively, representing increases of 443% and 611%, respectively in just two years. The main reasons for this rapid increase in mushroom production are: (i) Chinese farmers are able to use as growth substrates many local by-products, e.g., cereal straw, cotton seed hulls, etc, which are usually considered as waste materials, and (ii) innovative and improved cultivation methods adapted to local climates and conditions. In this paper, several methods for cultivating *P. eryngii* and *P. nebrodensis* are described.

Key words: Cultivation, mushrooms, *Pleurotus* spp, *Pleurotus erygii, Pleurotus nebrodensis*

1 Introduction

China is now the leading producer and consumer of edible mushrooms. In 2003, production of edible mushrooms in China was estimated to be 10.38 million tonnes, accounting for over 80% of mushroom production worldwide. *Pleurotus* mushrooms are very popular in China and more than ten species of *Pleurotus* are currently cultivated on an industrial scale, including *P. ostreatus P. cornucopiae* and *P. citrinopileatus*. In 1986, the combined yield of all *Pleurotus* spp was only 108,000 tonnes. However, by 2003, production of *Pleurotus* mushrooms had increased to 2,488,000 tonnes (Table 1). In terms of output, *Pleurotus* spp ranked second only to *Lentinula edodes* among all the different edible mushrooms cultivated in China before 2000 and, since 2001, it has held the top position.

Table 1. Yields of *Pleurotus* spp in China (Tonnes)

Year	*Pleurotus* spp	*P. eryngii*	*P. nebrodensis*
1986	108,000		
1997	760,000		
1998	1,020,000		
1999	1,538,000		
2000	1,723,000		
2001	2,594,000	21,022	7,343
2002	2,647,000	72,366	34,325
2003	2,488,000	114,107	52,223

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respectively, representing increases of 443% and 611%, respectively in just two years. The main reasons for this rapid increase in mushroom production are: (i) Chinese farmers are able to use as growth substrates many local by-products, e.g., cereal straw, cotton seed hulls, which are usually considered as waste materials, and (ii) innovative and improved cultivation methods adapted to local climates and conditions. In this paper, several methods for cultivating *P. eryngii* and *P. nebrodensis* are described.

2 Cultivation Characteristics

2.1 Nutrition

P. eryngii requires high nitrogen levels, and a carbon:nitrogen ratio of 60:1 is recommended. Under laboratory conditions, glucose and soybean powder represent suitable sources of carbon and nitrogen, respectively. The mycelium will grow faster when wort or peptone is added to the medium.[1]
A carbon:nitrogen ratio of 40-25:1 is recommended for *P. nebrodensis*. Mycelia growth is also enhanced by the addition of wort, yeast extract or peptone to the medium.[2]

2.2 Temperature

Guo Meiying[3] reported that *P. eryngii* grew at temperatures between 6-32°C, with an optimum temperature for mycelial growth of 24°C. The most suitable temperature range for pinning was between 12-15°C, and from 10 to 18°C for fruiting body formation.
Mycelial growth of *P. nebrodensis* was optimal between 24-26°C. The most suitable temperature range for pinning and fruiting in this species was 15-20°C, with only 18-22 days required to harvesting. When the temperature was maintained at 8°C±1°C, 25 days were required before commercial harvesting. No fruit body formation occurred when temperatures were maintained below 5°C, whereas temperatures above 23°C caused fruiting body abnormalities and increased the likelihood of bacterial infection. temperature Fluctuations in excess of 15°C killed the fruiting body.

2.3 Moisture

In the case of *P. eryngii*, the water content of the substrate and the relative humidity should be maintained between 65-70% and 75-90%, respectively to realize optimum yields. Watering directly on to the fruiting body should be avoided in order to reduce the chances of rot.[4]
For *P. nebrodensis*, the water content of the substrate and the relative humidity should be maintained between 60-70% and 70-85%, respectively.[5]

2.4 Aeration

P. eryngii prefers high levels of aeration, especially during the fruiting stages, otherwise the fruit body will grow slowly and abnormalities will occur.[6]
Mycelial growth of *P. nebrodensis* is stimulated by high concentrations of CO_2. Cai Yanshan[7] reported that CO_2 concentrations in the substrate during the incubation stage varied from 300 to 220,000 ppm. However, the primordia need fresh air and the CO_2 concentration should controlled at levels between 30-1,000 ppm. During fruiting body development, the CO_2 concentration should be maintained at 1,500 ppm.

2.5 Light

The mycelium of *P. eryngii* is reported to grow well in dark conditions but diffused light of between 200-1000 lux intensity is required for fruiting body development.[8]
Light was also found to be unnecessary for mycelial growth of *P. nebrodensis* but light intensities between 500-1000 lux promoted fruiting body development.

3 Growth Substrates

3.1 *P. eryngii*

P. eryngii will grow on sawdust, cotton seed hulls, maize stalks and bagasse. Better harvests result when bran, corn flour, cotton seed hulls or soybean stalks are added to the basic substrate. Several substrate formulations are widely used in difference areas of China according to local conditions and climate. The following are representative examples:
(I) sawdust, 36%; cotton seed hulls, 36%; bran, 20%; powdered soybean stalks, 6%; gypsum, 1%; calcium superphosphate, 1%.
(II) Sawdust, 30%; cottonseed hulls, 25%; bran, 15%, powdered soybean stalks, 5%; gypsum, 1%; calcium superphosphate, 1%, maize core, 18%; maize powder, 5%.
(III) Sawdust, 22%; cotton seed hulls, 22%; bran, 20%; powdered soybean stalks, 29%, gypsum, 1%, calcium superphosphate, 1%, maize powder, 5%.
(IV) Sawdust, 73%; bran, 25%; gypsum, 1%; calcium superphosphate, 1%.
Li Rongcun[9] reported that the addition of barley lees to these substrates increased yields by 18-32%.

3.2 *P. nebrodensis*

Chen Wenliang[10] reported biological efficiencies of 94% when a substrate formulation consisting of 80% cottonseed hulls, 18% bran, 1% cane sugar, and 1% gypsum was used for the cultivation of *P. nebrodensis*. A popular formulation now used in China consists of 67% cottonseed hulls, 30% sawdust, 1% lime and 2% gypsum. In the main maize producing areas, a common substrate formulation is 80% maize core, 13% bran, 5% rapeseed cake, 1% lime and 1% gypsum.[11] In Henan Province, farmers prefer to use pure cottonseed hulls to produce *P. nebrodensis*.

4 Cultivation Technologies

4.1 *P. eryngii*

Currently, three cultivation methods are used to grow *P. eryngii* in China: bag, casing and bottle.

4.1.1 Plastic bag cultivation method

Polypropylene bags (17 x 33 x 0.005 cm) that can be sterilized under high pressure conditions, or polythene bags (15 x 55 x 0.005 cm) that can be sterilized under conditions of normal pressure, each containing 500 g and 800-1,000 g of dry substrate, respectively are used to grow *P. eryngii*. In the case of the longer bags, 4-5 inoculation holes are required.
After inoculation, the plastic bags are placed in dark incubation rooms maintained at 25°C, and mycelial colonization of the substrate is normally complete within 30-40 days. Selecting the most suitable time to open bag

is very important, and a period of 10-20 days should elapse before moving the bags to the growing rooms. Usually, if sawdust or cotton seed hulls are the main substrates, 15-20 days after completion of mycelial growth is required, whereas 10-15 days are needed if crop stalks are the main substrates. Weather conditions are another key factor: the temperature should be maintained between 10-18°C, and a relative humidity of 85-90% is most suitable for promoting fruit body formation. Growing rooms should be aerated for 20-30 mins two-to-three times everyday. Under such conditions, primordia will be formed within 8-15 days.
Once the primordia appear, the room temperature should be adjusted to between 8-20°C. Temperatures above 22°C will cause the bud to die. In cases where the bags are housed in plastic sheds, aeration and watering will normally be carried out at noon. At the bud stage, the relative humidity should be adjusted to 90%, and subsequently reduced to 85% when the cap of fruit body has reached 2-3cm in size. When the temperature is high and the relative humidity below 80%, watering is necessary. However, water should not be applied directly to the fruiting body otherwise etiolation will occur and the risk of bacterial infection will be increased. Therefore, before watering, the fruiting bodies are normally covered with paper or plastic film. After the first harvest, the bag should be either affused or soaked, and the whole procedure repeated.

4.1.2 Casing cultivation method

Six to ten days after complete colonization of the bagged substrate, the plastic bag is removed completely and the contents of each bag placed on a bed approximately 3-4cm apart. The bags are then covered with casing soil, containing 16-20% water, to a thickness level of 3-4cm. The casing soil must not be allowed to become too dry or too wet. The cultivation rooms should be aerated for about 30 minutes every morning and evening. All other conditions are the same as those described for the Plastic Bag Cultivation Method. The primordia will appear within 10-20 days. Some growers will revert to this method of cultivation after they have collected a second harvest of fruiting bodies using the standard Plastic Bag Cultivation Method.

4.1.3 Bottle cultivation method

Normally, this method is adopted only by mushroom companies operating modern factories. According to Gao Junhui,[12] after inoculation, bottles are incubated in a dark room. For the first 20 days, the temperature should be maintained at 18°C, after which it is increased to 23°C for a further 15 days. During the incubation stage, the relative humidity should be kept between 60-80%, and the CO_2 concentration below 3,000 ppm. After scratching (removal of the top 1-2 cm of substrate), bottles are transferred to cultivation rooms where they are kept for 7-10 days at 14-15°C, under conditions of 80-90% relative humidity, a CO_2 concentration below 2,000 ppm, and a light intensity of between 50-200 lux. Initially, the bottle is inverted but, once the fruit body appears, the bottle is stood upright and the conditions in the cultivation rooms readjusted to a temperature of between 16-18°C, 75-90% relative humidity, a CO_2 concentration below 3000 ppm and a light intensity of 50-500 lux. Cultures take 10-13 days to reach maturity, and normally only a single flush is harvested.

4.2 *P. nebrodensis*

4.2.1 Plastic bag cultivation method

Different growers will select different substrate formulations to grow *P. nebrodensis* but all adopt the plastic bag cultivation method using sterile 17 x 33 x 0.005 cm plastic bags. After inoculation, the bags are transferred to dark rooms for incubation. Yu Zhongben[13] divided this stage into five steps:
Step 1: One to seven days after inoculation.
The spawn germinates during the first 24 hr, and grows into the substrate within 48hr. Three days later, the

room temperature will increase and some cooling down will be necessary. The temperature should be maintained between 25-28°C. During this period, the mycelium can extend 2-3cm.

Step 2: Days 8-15.

The bags need to be moved for the purpose of aeration and precautions should be taken to reduce the risk of contamination. The temperature should be maintained between 25-30°C, and the relative humidity below 65%. During this period, the mycelium will extend 5-8cm.

Step 3: Days 15-22.

During this period, the mycelium will normally colonize half of the bag. The incubation room must be kept clean and dry with the relative humidity maintained at about 60% and the temperature between 26-28°C.

Step 4: Days 23-30.

During this time the mycelium will extend throughout at least 70% of the bag and, in some cases, completely. The temperature should be kept below 25°C; if temperatures are allowed to rise to 28-32°C, extensive development of a mycelial hull will occur that can reduce the yield. The light intensity should be controlled at 50 lux or a mycelia hull will again be formed.

Step 5: Days 31-90.

Temperatures of between 20-30°C are good for the growth of *P. nebrodensis* at this stage. Some liquid will form in the bag due to fungal metabolism and should be removed from the bags on a weekly basis in order to avoid rotting.

Different temperature regimes are required at different stages of development. To induce fruiting in *P. nebrodensis*, fluctuations in temperature of 10-15°C over a period of 10-15 days are required. The plastic bag is then opened and the old mycelia hull at the top removed. The temperature should be maintained between 14-17°C. Watering should be carried out five days later, and the relative humidity and light intensity maintained at 85-90% and 600 lux, respectively. Normally 15 days are required for the fruit body to appear. When the bud first appears, the temperature should be between 8-12°C and the relative humidity about 85%. When the fruit body reaches 5 cm in size, the temperature should be raised to 12-15°C, the light intensity adjusted to 800-1000 lux and increased aeration provided. Normally, 10-15 days are required for the fruit body to grow up. Usually, only one fruiting body is produced for each bag.

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