High Yield Cultivation Techniques for Year-round Production of *Lentinula edodes* in a Solar Greenhouse

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Abstract: Cultivation of *Lentinula edodes* (shiitake mushroom) in the solar greenhouse can provide the appropriate environment for its growth and development, implement the year-round production, improve the yield and quality of shiitake mushrooms, and increase the biological transformation rate to over 150%. Adopting this model technique can prolong the production time and increase the number of harvests. In addition, this model technique has many other advantages such as prolonging the time that the mushroom can be supplied to the market, the convenient management and the higher work efficiency. Therefore, it is worth promoting and applying such techniques.

Key words: Shiitake mushroom, solar greenhouse, year-round production, high yield cultivation

1 Introduction

The shiitake mushroom has high value in terms of nutrition and medication, and it is one of the most popular edible fungi cultivated widely in China. In the past ten years, the cultivation of shiitake mushroom has been developing very fast. The cultivation regions are gradually moving towards the northern parts of China. The scale of cultivation and the annual yield have been greatly enhanced. These facts make great contributions to the regulation of the structure of the agricultural industry, enrichment of the markets' supply and promotion of the farmers' revenues.[1, 2] However, in the north of China, the shiitake mushroom is mainly cultivated in the fall season but not very extensively during springtime. Thus, the harvest times are concentrated from late November to May of the following year, which results in a situation where there are difficulties in selling the shiitake mushrooms during the in-season. Furthermore, no mushrooms are available during the off-season, and the cultivation houses cannot be fully used.[3] Therefore, based on the previous researches by others[4, 5], we studied the year-round cultivation of the shiitake mushroom in the solar greenhouse from 2001 to 2004. Our results demonstrated that, compared to the normal greenhouses, the solar greenhouse can provide a more suitable environment for the growth and development of shiitake mushrooms, which can increase the biological transformation rate to over 150% and the ratio of investment to production to 1:5. The following summaries of the year-round cultivation technique are references for the people who live in the northern regions and wish to cultivate shiitake mushrooms.

2 Seasonal Arrangement and Production Cycle

The city of Linfen is located in the south of Shanxi Province and has the climatic characteristics of both a warm temperate zone and a continental climate that include obvious differences between the four seasons: i.e. cold winters and hot summers, a dry spring and damp fall, and heterogeneous precipitation which dramatically concentrates in July, August and September. There is a great difference in temperature between seasons, and during the day and night. The highest temperature in summer is 38°C and the lowest temperature in winter is -15°C, which averages out at 13.6°C over the year. The optimum temperature for the growth of shiitake myce

lium is 22-27°C. The optimal temperature for primordial differentiation is 8-21°C depending on the strains. The temperature range for the growth of the fruit body is 5-25°C. We can make use of the solar greenhouse to grow mycelia and to fruit (which are carried out in different temperature greenhouses). In winter, the lowest temperature in the solar greenhouse can remain above 5°C (without heating), which benefits the fruiting of low to mid-temperature strains. In the summer, the highest temperature is only around 25°C (covering with grass curtains during daytime), which offers the possibility to fruit in the hot summer.

The shiitake mushroom must be cultivated twice a year, in both spring and fall, to achieve the year-round production in the solar greenhouse. The production processes for both spring and fall cultivation include the preparation of material, packing, sterilization, inoculation, growth of mycelium, turning of colour, through summer (not for fall cultivation), fruiting and harvesting and so on, in which the key point is the determination of the proper time for inoculation. Fall cultivation should be started after the high temperature period of the summer; otherwise it easily leads to mycelium burning. Fruiting should be finished before the end of summer to prolong the fruiting time and increase the yield. According to the temperature changes in the solar greenhouse all the year round, the optimal inoculation period should be from the end of August to September. The growth period of mycelium is from September to November. The turning of colour is almost at and end in early November. The period of fruiting is from mid-November to early August of the next year.

The spring inoculation time is determined on the basis of the achievement of the turning of colour in mycelium bags before the local upcoming high temperature summer, so that the mycelium in the bags can sustain the high temperature in the summer. Therefore, the optimal inoculation period of spring cultivation should be from late January to the end of February. After the inoculation, mycelia are grown in the solar greenhouse from February to June. By late May, the top of the solar greenhouse should be covered with grass curtains, which can decrease the temperature inside to prevent burning of the mycelium. At the end of June, the turning of colour is almost completed. From early July to the end of August, the mycelium bags should be shaded in the solar greenhouse. The time from inoculation to the end of summer should be less than 180-200 days or it will affect the yield. The fruiting time is from September to March of the next year. The year-round cultivation arrangements in the solar greenhouse are presented in Table 1.

Table 1. Year-round cultivation arrangements in the solar greenhouse

Cultivation season	Strain	Inoculation time	Growth of mycelia	Through summer	Fruiting time
Spring cultivation	939, 135, 9015, 241-4	Late January-end of February	February - June	July-August	September-March of next year
Fall cultivation	Nos.6 and 7 GuHuang	End of August - September	September - November		Mid-November - following August

3 Cultivation Management Procedures

3.1 Cultivation strain

Picking the right strains is the basis of success to achieve year-round production of the shiitake mushroom. The better strains always have several common characteristics. Firstly the mycelium grows quickly and more strongly than mycelium of contaminating fungi. Secondly, the fruiting is regular and the interval between the harvests is shorter. Thirdly, the density of fruiting is moderate, solitary or fasciculate. Fourthly, fruit body shape is better with a thick cap and short stem, suitable size, good quality and high yield. According to the climatic characteristics LinFen City and the optimal conditions for the shiitake mushroom, and after the comparing experiments

on the different shiitake strains, we believe it is better to choose the 939, 135, 9015 and 241-4 strains (with growth times of 120-150 days) for spring cultivation. After summer, fruiting starts at the beginning of September and the quality of the mushrooms is excellent. For the fall cultivation, it is better to select strains such as No.6 and No.7 GuHuang (brought in from Fujian). Their nutritional growth time is 50-55 days. Fruiting starts in mid-November after the turning of colour.

3.2 Substrates

The main substrate materials for shiitake cultivation are sawdust from broadleaf trees and bran. The sawdust must be fresh, free of rainwater, free of mildew and the right size. Leftovers from timber processing factories will suffice, but hardwood is better than softwood. The bran must be fresh and not mildewed. Based on our experiments, two good formulae are:

Formula 1: sawdust 83-85%, bran 15-17%, gypsum 2%, corn (crushed) 2%, lime 0.2%, KH_2PO_4 0.2%, $MgSO_4$ 0.4-0.5% and Duo-Jun-Ling 0.2 %-0.3%.

Formula 2: sawdust 40%, wheat straw powder 40%, bran 20%, gypsum 2%, corn (crushed) 2%, lime 0.2%, KH_2PO_4 0.2%, $MgSO_4$ 0.4-0.5 % and Duo-Jun-Ling 0.2-0.3%.

The ratio of materials to water for both formulae is 1:1.1-1.5.

After mixing the materials, the mixture is packed into heat resistant plastic bags (24 × 55 cm). There are two ways to pack the bag. One is by hand and the other is by machine. The bags should not be packed too tight, with 2 kg of the dry mixture per bag. After packing the bags, it is required to immediately sterilize the mixture using pots at normal pressure. The bags should be placed in the pot in the form of a strip and the door of the pot should be sealed airtight. The process of mixing the materials to placing the bags in the pot should be completed within six hours. The longer it takes, the easier for the mixture to ferment, which adversely affects the quality. The sterilization time varies depending upon the amount of the mixture in the pot. If the pot contains 2500-3000 kg mixture, then 16-18 hrs are needed for sterilization at 100°C and normal air pressure. For spring cultivation, the sterilization time can be reduced to decrease the ripeness of the material in order to meet the longer time required for the growth of mycelium.

3.3 Inoculation and growth of mycelium

The spawn is inoculated into the sterilized mixture in an inoculation box[6]. One person on one side of the box punctures the holes (three holes a row and three rows around each bag) and immediately hands the bag to the other person who is sitting on the other side of the box. The second person inserts the spawn into the holes. After finishing the inoculation, the inoculated bags are placed in a new plastic bag. One week after inoculation, when the mycelia grow and extend to about 2-4 cm, the outside plastic bag can be removed. There are two spawn strains that can be used. One spawn strain is made from sawdust and the other from branches. One bottle of the branch spawn strain can be inoculated into more than ten bags and it is easy to inoculate and has a high mycelium growth rate. It also guarantees the quality of mycelium, curtails the period of culture and increases the yield.

The inoculated bags should be stacked in the form of a square in the greenhouse and never be stacked higher than six layers (eight layers for spring cultivation). One week later, the outside bags can be removed in a sunny, non-windy day.

Puncturing some holes in the bags will increase the supply of oxygen, speed up the growth of mycelia and

promote the diffusion of heat to prevent the temperature inside from rising too high. For spring cultivation, since the temperature is relatively lower in the early period and the growth of mycelia is slower and longer, not many holes should be made during this period. Generally speaking, 80-100 holes should suffice and should be pricked three times. When the diameter of the colonies around the inoculation holes reaches 8-10 cm, 5-6 holes should be made around each inoculation hole. These air exchange holes should be about 1 cm away from the edge of the colonies and 1 cm deep. When the mycelia are connected within the bags, more holes (5-8) about 1.5 cm deep and 1.0 cm away from the edge of the mycelia circle should be made. For a third time, when the bag is completely colonized by mycelia, 20-30 holes, 1.5 cm deep, should be made evenly around the bag. For bags inoculated with the branch spawn strain, when the diameter of the colonies reaches 10 cm, the branches should be plugged out. This will increase the supply of oxygen and promote the growth of mycelia. Later, some air exchange holes should also be made. For fall cultivation, after inoculation, since the temperature is still higher and the growth of mycelium is faster, more holes should be made; 120-150 holes should be made 5-6 times.
The number and depth of holes, and the time of puncturing will vary depending upon both the condition of the mycelium growing inside the bags, and the temperature and humidity of the greenhouse. When making the holes, the following must be taken into account:
(i) it is not wise to make holes on high-temperature days; otherwise it is easy for the mycelia to burn and the edge of the holes to become mildewed.
(ii) the holes should be made gradually.
(iii) where the bag has tumour-shape protrusions, or has contaminated spots, or the colony is not in the right place, holes should not be made.
During the growth of mycelium, two more parameters should be monitored. Firstly, the relative humidity in the culture room must be kept around 70%. Secondly, the air in the room should be kept fresh so that the growth of mycelium is faster. Without the removal of the outside bag, the mycelia in the bags can grow to form tumour-like protrusions on the surface in 30-40 days; 45-55 days after inoculation, the bags will be full of tumour-like protrusions, which means that the mycelia are physiologically mature and will change colour very soon. However, the tumour-like protrusions cannot be too concentrated. If there are no tumour-like protrusions after 30-45 days, it means that either the temperature was too high, or that oxygen was scarce during that period, which will affect the fruiting. Growth of mycelium for spring cultivations is relatively longer compared to fall cultivations.

3.4 Turning of colour

The nutritional transformation reaches the peak level stage 45-55 days after fall inoculations. The turning of colour starts from the 45th day and ends on the 55th day. For spring inoculations, the turning of colour is completed within 55-65 days. During the whole cultivation process, including the turning of colour and fruiting, the bags are not removed in order to maintain the humidity. The turning of colour usually takes place in the mycelium growth greenhouse. The proper conditions for the turning of colour include maintaining the temperature between 18-24°C and the air humidity at about 75%, requiring some dispersed light, and having good ventilation. High temperatures can result in the secretion of yellow water, so a hole on the bottom of bag must be made to release the water to prevent rotting. The speed of turning of colour will be slowed if the temperature is either above 28°C or below 12°C. Too much humidity will lead to uncontrolled mycelium growth, while dryness will cause the bags to lose water and make the turning of colour difficult. For spring cultivation, the turning of colour should be finished before summer begins to prevent the mycelia from being contaminated with other fungi. For fall cultivations, after the turning of colour, bags are ready for promoting budding and fruiting.
If the turning of colour is going well, the insides of the bags should have a brown colour and they should be shiny, fruit normally and have good quality and high yield. On the other hand, if the turning of colour is not good, they will fruit either early or late, and the quantity and the quality of shiitake mushrooms will be lower.

3.5 Management of spring inoculated bags through summer

After spring inoculation, correct management of the inoculated bags through the high temperature in summer (July-August) becomes very important. The temperature for mycelium growth ranges between 5-32°C and the optimal temperature is 22-27°C. The mycelium will stop growing when the temperature reaches 33°C. If the mycelium is left at a temperature of 33°C or higher for a long time, it will die. Since our local temperature is quite high in summer, the main task in summer is to reduce the greenhouse temperature by every means to ensure the mycelia go through summer safely. There are several ways to manage this situation: 1) arrange the bags in a triangle or in the form of square with no more than five layers, and do not keep stacks too close, or place one layer of bags on each shelf to allow good ventilation; 2) during a long period of hot days, increase the covers on top of the greenhouse and close windows and doors to reduce heat radiation; 3) on cloudy or rainy days, close windows and doors and keep the inside dry to prevent contamination with other fungi; 4) increase ventilation and decrease temperature by opening doors and windows in the evening and closing them during the daytime, and keep the inside temperature of the bags under 30°C; if some of the bags are contaminated, they must be removed immediately to prevent further contamination; 5) try not to move the bags around; if some bags need to be moved, do it on a hot early afternoon; stimuli from moving bags can promote budding and result in early ripeness, which makes it harder to manage; also, moving them too many times may cause too much turning of colour and the skin of mycelium to become too thick. Both will affect fruiting.

3.6 Fruit body development

After the physiological ripeness of mycelia in fall cultivations, or spring cultivations through summer, the bags need to be moved into the greenhouses in which the shiitake mushrooms will be produced and finally harvested. Whether it is managed scientifically or not has a great influence upon the yield and quality of the mushrooms, so we should manage well the following aspects:

3.6.1 Budding

There are three aspects in the management of budding, that is, promoting budding, protecting buds and dispersing buds.
Promoting budding is to promote the primordia to differentiate into baby mushrooms. Since the bags lose much water through summer, we should inject water into the bags to restore the water content in the bags to about 55%. In order to promote budding, we should do our best to provide the conditions which are beneficial to the growth and differentiation of primordia: 1) keep the temperature in the greenhouse to between 10-18°C and the difference in temperature from day to night to be about 8-10°C; the stimulation of low temperature and temperature difference will make bud formation easy; 2) maintain the relative humidity in the greenhouse at about 85% and the plastic membrane should be sealed to avoid too much humidity if it rains continuously for a long time; 3) keep the quantity of water in the culture material at 55-60%; 4) keep good ventilation in the greenhouse to make sure there is sufficient oxygen supply and use some indirect lightings. However, if the above conditions are provided but the buds still do not show up, we should use the method of shaking. Shaking is also an effective method for promoting budding.
Protecting buds means we use blades to cut out the plastic membrane around the buds to prevent them from being squeezed after they are formed. Then, the baby mushrooms can be outside of the membrane to get ample sunlight and fresh air for their growth. This helps to maintain the temperature and the humidity, increase the oxygen supply and promote fruiting.
If the mushroom buds are too concentrated, we should disperse the buds. We should cut off the small and the dense buds and retain the big and the scattered ones, which can promote the mushroom body to grow.

3.6.2 Fruiting

Generally, we can harvest six to seven times in spring cultivations, and eight to nine times in fall cultivations. When the temperature is correct, we can harvest three times a month. However, due to the lower temperature in winter, it is only once a month. Based on the seasons of fruiting, we can divide the shiitake mushrooms into four types: fall mushrooms, winter mushrooms, spring mushrooms and summer mushrooms. The climatic conditions in greenhouses vary in different seasons and different periods, so we must take different measures to achieve the goal of producing high quality mushrooms.

Management of the fall mushrooms Mushrooms harvested from September to November are fall mushrooms. The mycelia in the over-summer bags have stored large quantity of nutrients after four to five month's growth. With the temperature gradually becoming lower in September, corresponding measures should be taken to manage fruiting after the buds are formed. This will depend on the weather conditions, the temperature and humidity in the greenhouse, as well as the sunlight conditions.

Management of the winter mushrooms The period from December to February is the time of winter mushroom production. It is relatively colder in the winter in the northern regions of China. Although the sunlight can increase the temperature in the solar greenhouse to 27℃ in the daytime, the temperature in the greenhouse can only be maintained around 5℃ at night. Thus, the important part of management in this stage is to raise the temperature of the greenhouse. There are some effective methods for adjusting the temperature of the greenhouse such as thickening the covers, reducing the times of ventilation and the duration of ventilation (ventilation in the higher temperature noon is better). If the air humidity in the greenhouse is not enough, it can be adjusted by spraying water. The temperature in the greenhouse should be kept at 10-20℃ and the humidity 80-90%.

Management of spring mushrooms With the gradual increasing temperature, spring (March- June) becomes the main stage of mushroom production for the fall cultivation. The characteristics of this stage include a long period of correct temperature for fruiting, shorter intervals between harvests and more harvests. Therefore, the management should be determined according to the temperature and humidity in the greenhouse, and sunlight illumination. Generally speaking, water after each harvest.

Management of summer mushrooms For summer (July-August) fruiting, there are several points that should be taken into account. Firstly, because water evaporates quickly due to the high temperature, water should be sprayed frequently, 3-5 times in the daytime (especially at noon) and 1-2 times at night. At the same time, we should inject water into the bags, which not only provides ample water for fruiting and decreases the temperature inside the bags, but also reduces the diseases caused by too much humidity. Secondly, the top of the solar greenhouse must be covered with grass curtains so that fruiting is not affected by direct sunlight. At the same time, it reduces the temperature inside the greenhouse. The temperature inside the greenhouse should be kept to no more than 26℃, which not only stimulates budding but also decreases the rate of fruiting to improve the quality of the shiitake mushrooms. Thirdly, in order to reduce the humidity and increase the supply of oxygen, ventilation should be increased and should be done in the morning or evening when the temperature is right. Finally, since the temperature is usually very high in summer, the bottom three layers can produce more mushrooms than the top three layers (little or none). Thus, we should switch the bags in the top layers with the bottom layers after each harvest in order to increase the overall yield.

4 Conclusions

In comparison with normal greenhouses, the solar greenhouse can provide the appropriate environment for the

growth and development of shiitake mushrooms, achieve year-round production, and increase the quantity and quality of shiitake mushrooms. In addition, cultivating the shiitake mushrooms in the solar greenhouse can prolong the production period, shorten the interval between harvests and produce excellent mushrooms. This technique can supply the market all-year round and is easy to manage. It also can increase work efficiency. It is calculated that about 20 tons of substrate materials are required for one production cycle inside a 480 m^2 solar greenhouse, and the total cost of supplies and labour fee is ¥14,000. About 30 tons of fresh shiitake mushrooms are produced every production cycle and the net profit is between ¥50,000-¥70,000.

Making use of the solar greenhouse to produce shiitake mushrooms can enhance the utilization ratio of the production facilities and the greenhouse as well as increase the production efficiency of the greenhouse. This is a new mode of cultivation that can achieve great economic benefits in a relatively short period of time and benefit the shiitake mushrooms' industrial and professional production. Therefore, it is worth spreading and applying, and will greatly help to develop shiitake mushroom's industrialization in the north regions of China.

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