Part I Shiitake

Chapter 4

Shiitake Bag Cultivation

COFFEE RESIDUES

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Enzymes in the shiitake metabolism allow the fungi to degrade and use cellulose, hemicelluloses and various quantities of lignin for their nutrition. Among agricultural by-products available for use in shiitake cultivation, coffee residues are one of the most potentially importance substrates because million tons of coffee residues are produced every year in more than 50 countries. However, due to the presence of substance unfavorable for mushroom growing including caffeine, tannins and polyphenols, these residues have not been utilized beneficially and their disposal is a serious concern for the processing units.

Coffee Residues as an Alternative Substrate

There are several different coffee residues, including coffee husks, coffee pulp and spent grounds. Coffee husk is produced in the dry process of the separation of the coffee berries while coffee pulp is obtained by the wet process of extracting coffee from the berries. Spent coffee grounds are produced during the processing of raw coffee powder to prepare instant coffee. The nutrition and harmful substances of main coffee residues are shown in the Table 1.

Table 1. Nutrition and harmful substances in coffee residues (%)

Component	Coffee husk	Coffee pulp	Coffee spent ground
Protein	9.2-11.3	8.5-12.1	10.3-12.2
Lipids	2.0-2.3	1.5-2.0	15.2-17.9
Cellulose	13.2-27.6	15.1-20.3	13.2-18.4
Ash	3.3-4.1	5.5-6.8	4.5-6.3
Extract not-nitrogen	57.8-66.1	45.5-54.3	41.0-49.8
Tannins	4.5-5.4	1.8-2.4	1.2-1.5
Caffeine	0.8-1.1	0.5-0.7	0.02-0.08

Note : The value varies according to the coffee species and processes.

Unfortunately, the coffee husk and pulp have large amounts of caffeine and tannins, and the harmful substances, especially caffeine, have a negative effect on mushroom growth and inhibit the growth of mushroom mycelium. While coffee residues are highly nutritious, most do require treatment before used as substrate for shiitake cultivation and the costs of pre-treating coffee husk and pulp can hinder their wide usage. Spent coffee grounds are the exception and do not reqire a caffeine removal processing stage (Thielke, 1989).

Figure 1. Coffee residues **A**: Coffee husk **B**: Coffee pulp **C**: Coffee spent ground (Photo courtesy of Carmenza L. Jaramillo)

Pre-treatment of Coffee Residues

The pretreatment of coffee residues, especially coffee husk and pulp, aims at detoxification and removal of the most caffeine possible. Among the several possible methods for caffeine removal, the most frequently chosen are filtration with boiling water, degradation by fermenting microorganisms, and bioremediation using with *Pleurotus* spp.

The boiling water method

The coffee husks or pulp are boiled in water for 15 minutes, and then the water is drained off. The residues can then be used for the shiitake cultivation. This method is efficient, but it does generate a large amount of water with harmful substances. It is thought that some potentially nutritious components are lost through boiling, but no specific research has been done on this specific possibility.

The use of fermentating microorganisms

There are many microorganisms, such as *Rhizopus arrizus*, *Phanerochaete* and *Aspergillus* spp., which can partially degrade the caffeine and tannins, achieving reductions of 65-92% for caffeine and 45-65% for tannins (Brand *et al*., 2000, 2001 and 2002; Pandey *et al*., 2000). One week of fermentation after inoculation can suffice for this process.

Bioremediation with Pleurotus spp.

The *Pleurotus* mushroom has a strong ability to degrade or absorb some harmful substances. It has been reported that *Pleurotus* can be used to absorb caffeine from substrate materials because this fungus can accumulate caffeine in the fruiting bodies and thereby diminish the caffeine content in the coffee residue substrate. According to the research (Fan *et al*., 2000a), the fruiting body of *Pleurotus* can contain 0.157% caffeine while the raw coffee has 1% caffeine. The accumulated caffeine in the fruiting body is generally acceptable because most consumers are accustomed to drinking coffee. The harvested oyster mushrooms could perhaps be marketed as a new product with a stimulating effect and the coffee residues in the spent *Pleurotus* substrate could then be utilized for shiitake production. Certainly some addition of supplements would be required before the second use, as the oyster mushrooms would also consume some of the basic nutrition in the substrate.

Shiitake Bag Cultivation with Coffee Residue

Formula

Treated coffee residues are good substrates for shiitake cultivation, but they make loose bags when used alone. For this reason coffee residues are usually supplemented with some additional cellulosic substrates such as sawdust and rice or wheat bran in order to create a desirable texture for filling shiitake bags. The formula used is shown in Table 2.

Filling the bag

Polyethylene or polypropylene plastic bags 25-35cm long and 15-20cm wide are used. All materials are weighed and prepared according to the formula, then mixed evenly with clean water at a ratio of 1:1.2. The water content is then adjusted to 55%, a level suitable for filling the bags. The mixture is packed into plastic bags easily, neither tightly nor loosely. Generally, the bags should contain half a kilogram of dried substrate. Once filled, they are tied with string or covered with cotton and a neck ring.

Table 2. Formulae of shiitake bag cultivation using coffee residues

Formula	Main substance	Nitrogen source	Supplementation
1	Coffee husk 50%, Sawdust 29%	Wheat or rice bran 20%	Gypsum 1%
2	Coffee pulp 50%, Sawdust 28%	Wheat or rice bran 20%	Gypsum 1%, Calcium superphosphate 1%
3	Coffee husk 50%, Coffee spent ground 29%	Wheat or rice bran 20%	Gypsum 1%
4	Coffee pulp 40%, Coffee spent ground 39%	Wheat or rice bran 20%	Gypsum 1%

Note: The formula can be adjusted according to local resources.

Sterilization

After filling, the bags are transferred to a special steaming room and sterilized at 97℃ for 8~10 hours, or put in an autoclave for 2 hours at 1.1kg/cm². If the bags are sterilized in an autoclave, the strings should be tied loosely or the bag should be perforated and covered with a filter. On the second day, when the temperature has dropped to 60℃, the bags are taken out and put into an inoculation room that has been cleaned in advance and prepared for cooling during the inoculation period.

Spawning

Spawn is generally bought from reliable suppliers, and inoculation is conducted under a flush lamina desk or in an inoculation box equipped with a UV light. Inoculation can be performed when the bags have cooled to the point that the temperature at the bag surface is less than 25℃. The bags can be inoculated either at the sealed side or the other, according to preferences of each farm.

Management at mycelial growth stage

During the spawn run, attention should be paid to maintaining a constant temperature around the bags. Substrate bag piles should be turned after one week in order to even the temperature for each tier of bags. When the white mycelia cover the whole surface of the bags (Fig. 2), the bags should be carried into the fruiting house.

Figure 2. Coffee residue substrate bag fully colonized by shiitake mycelia

Figure 3. Shiitake fruiting bodies from coffee residues (Photo courtesy of Gerardo Mata)

Management at fructification stage

Growers are advised to move the fully colonized bags to growing house and arrange them there for fructification. The growing house should be covered by plastic and shade netting that allows some natural light to come through. When the color of substrate surface turns from light brown to dark brown and yellow water droplets appear, it is the time for primordial induction. About one week later, the primordia will start to form. After the fruiting bodies grow to 2cm in diameter, they can be watered directly overhead. There will be 4 or 5 flushes in total (Fig. 3). If the substrate is immersed in water for 1 or 2 days, in some cases another flush or two are possible.

In experiments the biological efficiency of this production can reach 90% (Fan *et al*., 2000b), but farmers can usually harvest 0.3 to 0.5kg of fresh shiitake mushrooms from 1kg of dried substrate.

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