Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 5

Substrate

GRASS (JUNCAO)

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JUNCAO (菌草) - Jun(菌) means fungi and Cao(草) means grass in Chinese.

Juncao techniques were invented in China in 1983 by Professor Zhanxi Lin, director of Fujian Agriculture & Forestry University and JUNCAO Research Institute. He used herbaceous plants such as *Musa nana*, *Miscanthus sinensis*, *Dicranopteris dichotoma*, *Miscanthus floridulus* as new cultivation substrates to replace such traditional substrates as sawdust, wheat bran and rice bran. Before Juncao techniques were invented, many species of edible fungi and medicinal fungi, such as shiitake and wood's ear, were mainly cultivated on sawdust or logs. The recent expansion of mushroom production, however, resulted in the over-exploitation of the broad-leaf trees resource and caused a shortage of raw substrate materials. Major mushroom growing countries like Japan and China encountered such problems, which had restricted the further development of large-scale mushroom production. The invention of Juncao techniques has solved the conflict between the increase of fungi production and the protection of ecological environment, and enabled fast and sustainable development of mushroom production.

Definition of Juncao Technology

Juncao is defined as the herbaceous plants that are suitable for cultivating edible and medicinal fungi. Juncao Technology is defined as a series of comprehensive techniques to cultivate edible and medicinal fungi and produce mycelium protein forage with Juncao. The Juncao Industry is defined as the industry utilizing Juncao technology and other relative techniques.

The conversion rate of solar energy into Juncao grasses is 6-8 times higher than that for broad-leaf trees. With Juncao techniques, 1kg of dry grass can be converted into about 1kg of fresh mushrooms. This technology combines ecological, economical and social benefits. Therefore, the Juncao Industry is a new ecological industry that possesses the advantages of a high utilization rate of natural resources and great potential for continual development.

Juncao Species as Substrate Material for Mushrooms

From 1983 to 2003, 37 Juncao species have been selected which are suitable for mushroom cultivation using the three-stage selection method (Table 1).

The test results of the Fujian Agriculture University Central Laboratory show that the nutrients contents of most Juncao species are richer than those of sawdust (Table 2). The contents of protein, nitrogen, fat, phosphorus, potassium, and magnesium in Juncao are higher than those in sawdust of broad-leaf trees. Among them, the protein content of wild *Dicranopteris dichotoma, Neyraudia reynaudiana, Saccharum arundinaceum, Phragmites communis, Miscanthus floridulus* and *Themeda gigantea* is 2-4 times as much as that of sawdust. Their fat, nitrogen, phosphorus, potassium and magnesium contents are respectively 101-216%, 232-353%, 225-685%, 346-908%, and 191-303% of those of sawdust.

Table 1. Juncao species suitable for mushroom cultivation

Pennisetum pupureum Schum.	*Arundo donax* L.
Pennisetum sinense Mez	*Triticum aestivum* L.
Pennisetum alopecuroides Steud.	*Oryza sativa* L.
Phragmites communis Trin.=P. australis (Cav.) Steudel	*Achnatherum splendens* (Trin.) Nevski
Neyraudia reynaudiana (Kunth) Keng ex Hitchcock	*Arundinella hirta* Tanaka
Miscanthus floridulus Warb. Ex K. Schum. & Lauterb.	*Arundinella nepalensis* Trin.
Miscanthus sacchaiflorus Hack.	(=*A. brasiliensis* Raddi)
Miscanthus sinensis Anderss.	*Spartina anglica* C.E.Hubb.
Saccharum arundinaceum Retz.	*Musa nana* Lour.
Saccharum sinense Roxb.	*Setaria anceps* Stapf ex Massey
Saccharum robustum Brandes & Jiesw. ex Grassl	*Eichhornia crassipes* Solms
Themeda gigantea (Cav.) Hack. ex Duthie var. villosa	(=*E. speciosa* Kunth)
Themeda gigantea Hack. ex Duthie var. caudata	Gossypium hirsutum L.
Paspalum wettsteinii Hack.	(=*G.. herbaceum* L.)
Paspalum dilatatum Trin.	*Medicago sativa* L.
(=P. dasypleurum Kunze ex Desv.)	*Helianthus annuus* L.
Vetiveria zizanioides Stapf	*Ferula sinkiangensis* K.M,Shen
Sorghum propinquum (Kunth) Hitchcock	*Dicranopteris dichotoma* (L.)Farw.
Sorghum sudanense Stapf	*Dryopteris ampla* Kuntze
Cymbopogon citrates Stapf	

Table 2. Nutrients contents of Juncao (%)

Nutrients Contents (%)	Protein	Fiber	Fat	N	P	K	Ca	Mg
Sawdust	1.19	84.82	0.93	0.19	0.02	0.11	0.22	0.03
Dicranopteris dicnotoma	3.75	72.10	2.01	0.60	0.09	0.37	0.22	0.08
Neyraudia reynaudiana	4.42	58.80	1.72	0.67	0.14	0.96	/	0.09
Saccharum arundinaceum	2.75	62.50	0.99	/	/	0.76	0.17	0.09
Phragmitas communis	3.19	72.50	0.94	0.51	0.08	0.85	0.14	0.06
Miscanthus floridulus	3.56	55.10	1.44	0.57	0.08	0.90	0.30	0.10
Themeda gigantea	3.85	51.1	1.38	0.61	0.05	0.72	0.19	0.08
Pennisetum purpureum	5.91	68.88	/	/	0.18	0.78	0.40	0.24
Spartina atierniflora	9.90	23.58	2.96	/	/	/	/	/
Sorghum propinquum	4.17	49.47	/	/	0.08	0.46	0.44	0.17

Medicinal and Edible Fungi Species Cultivated with Juncao

45 fungi species have been selected which are suitable to be cultivated with Juncao as the cultivation substrates (Table 3).

Table 3. Mushroom species suitable to be cultivated with Juncao

Agaricus bisporus (J.E.Lang) Pilát
Agaricus blazei Murill
Coprinus comatus (O.F.M&unml;ll.)Gray
Dictyophora rubrovolvata M.Zang, D.G.Ji & X.X Liu
Dictyphora duplicate (Bosc)E. Fisch.
Pleurotus ostreatus (Jacq.) Quél.
Pleurotus sapidus (Schulzer) Sacc.
Pleurotus rhodophyllus Bres.
Pleurotus sajor-caju(Fr.) Singer
Pleurotus citrinopileatus Singers
Pleurotus cystidiosus O.K.Mill.
Pleurotus abalones Y.H.Han, K.M.Chen & S.Cheng
Pleurotus eryngii (D.C.) Gillet
Pleurotus tuber-regium (Rumph.ex Fr.) Singer
(=Lentinus tuber-regium (Fr.)Fr.)
Armillariella mellea (Vahl) P. Karst.
(=Armillaria mellea (Vahl) P.Kumm.)
Armillariella tabescens (Scop.) Singer
(=Armillaria tabescens (Scop.) Emel.)
Lentinus edodes (Berk.) Singer
(=Lentinula edodes (Berk.) Pegler)
Collybia velutipes (Curtis) P.Kumm.
(=Flammulina velutipes (Curtis) Singer)
Tricholoma giganteum Massee
(=Macrocybe gigantea (Massee) Pegler & Lodeg)
Hypsizygus mamoreus (Peck) H.E. Bigelow
Oudemansiella radicata (Relhan) Singer
(=Xerula radicata (Relhan)Fr.)
Stropharia rugosoannulata Farl. ex Murrill
Pholiota nameko (T. Itô)S.Ito & S. Imai
Volvariella volvacea (Bull.) Singer
Agrocybe cylindracea (DC.) Gillet
Pholiota aegerita (V. Brig.) Quél.
(=Agrocybe aegerita (V. Brig.) Singer)
Hericium erinaceus (Bull) Pers.
*Poria cocos (*Schwein.*)* F.A. Wolf
(=Wolfporia extensa (Peck) Ginns)
Ganoderma lucidum (Curtis) P Karst.
Ganoderma sinense J.D.Zao, L.Y.Hsu&X.Q.Zhang
Coriolus versicolor (L.) Quél
(=Trametes versicolor (L.) LIoyd)
Grifola frondosa (Dicks.) Gary
Grifola albicans Imazeki
Auricularia auricula (Hook. f.)Underw.
(=Auricularia auricula-judea (Fr)Schröt)
Auricularia cornea Ehrenb.
Auricularia polytricha (Mont.) Sacc.
Auricularia peltata LIoyd
Auricularia delicate (Fr.) Henn.
Auricularia mesenterica (Dick.) Pers.
Tremella fuciformis Berk.
Tremella aurantia Schwein.
Tremella cinnabarina Bull.

Oyster Mushroom (*Pleurotus ostreatus*) Cultivation with Juncao

Oyster Mushroom (*P. ostreatus*) can utilize a wide range of the available culture materials due to its great adaptability. In addition, it is easy to cultivate with simple technology and has a short growth cycle. A large variety of Juncao and crop stalks are suitable for the culture medium for mushrooms. Under certain suitable conditions, 4-5 weeks are enough from spawning to harvesting. Due to these advantages, *P. ostreatus* will be one of the most important mushroom species and will make a great contribution towards solving the problem of the lack of protein in developing countries.

Nutrition

P. ostreatus is classified as a wood-saprophytic fungus. In nature, it grows on the dead branches of broad-leaf trees, such as poplar, willow, elm, maple, beech and Chinese ilex. In artificial cultivation, either logs or sawdust can be used as the culture substrate. A proper amount of rice bran and sugar can be added in order to promote the mycelia growth and fruit body formation while cultivating with sawdust. In Japan, the amount of rice bran added is up to 36-40% in *P. ostreatus* cultivation with sawdust.

The research done in 1986 discovered that JUNCAO, such as *Neyraudia reynaudiana*, *Miscanthus floridulus*, *Saccharum arundinaceum*, *Themeda gigantea*, *Miscanthus sinensis*, *Spartina anglica*, *Pennisetum purpureum* and *Sorghum propinquum*, are high quality culture materials for *P. ostreatus* cultivation. They can be used as substitutes for sawdust and partially as substitutes for rice bran. Moreover, *P. ostreatus* can also be cultivated with corncob, wheat straw, bagasse, *Musa nana*, *Pistia strationtes*, rice straw and other crop stalks.

Temperature

The appropriate temperature range for spore germination is 24-28℃. Mycelia can grow properly at 7-35℃, while the best range is 20-25℃. The suitable temperature range for fruit body growth is 10-28℃ varying among the different strains. In accordance with the suitable fruiting temperature, strains are classified into psychrophilic (12-15℃), mesophilic (16-22℃) and thermophilic (20-26℃) types. *P. ostreatus* growing in the lower portion of the suitable temperature range generally are of a higher quality.

Humidity

The water content of substrate suitable for mycelial growth is about 65%. Mycelial growth is inhibited if the water content is less than 50%. In bottle cultivation of *P. ostreatus*, mycelia can grow properly under conditions where the air humidity is 65-70%. During the fruiting period, the suitable air relative humidity can be as high as 85-90%. If the relative humidity is less than 85%, the growth of fruit bodies will be slowed down. The mushroom quality will also be negatively affected if the relative humidity is higher than 95%.

Ventilation

P. ostreatus is an aerobic fungus. Its fruitbodies cannot grow normally without fresh air. Although fairly high concentrations of carbon dioxide will not affect the growth of mycelia, such is not the case for that of the fruitbodies. When the concentration of carbon dioxide is higher than 600 ppm, the stipes elongate and cap growth is inhibited. Due to the lack of oxygen, fruitbodies cannot form, or they become malformed.

Illumination

Although mycelia can grow properly even in total darkness, the primordia formation and fruit-body growth require a certain amount of light. Primordia formation is only possible after 12- hour illumination with an intensity of 200 lux. For the proper growth of fruitbodies, the light intensity should be within 50-500 lux. The color of the caps is also related to the light intensity, so insufficient amounts of illumination will lead to pale colored caps.

pH value

Mycelia can grow properly when the pH value is between 4 and 7.5, whereas the most suitable range is pH 5.5-6.0.

Cultivation Steps of Oyster Mushroom with Juncao

Juncao pretreatment

Because of their different biological characters, the harvesting, processing and storage of Juncao is different from that of sawdust. Successfully undertaking the three steps below will help growers realize the full potential of Juncao's nutritional value.

Juncao harvesting

Due to the high nitrogen content of *Dicranopteris dichotoma*, *Neyraudia reynaudiana* and other Juncao, the harvesting season and weather must be carefully chosen. If harvesting takes place during rainy days, drying and processing will become more difficult and this will result in mildew and lower utilization rates of the Juncao. Therefore, harvesting must be arranged in 5-7 days that are sunny. Harvesting time depends on the different species of Juncao and cultivated fungi. For example, *Dicranopteris dichotoma* can be cropped in the whole year, but is best harvested from May to July. *Neyraudia reynaudiana*, *Miscanthus floridulus* and other Juncao of grass family are best cropped in flowering and heading stages. *Neyraudia reynaudiana* used for cultivation of shiitake (*Lentinus edodes*), *Auricularia peltata* and *Auricularia polytricha* should be cropped after heading and aging, whereas those used for cultivation of enokitake (*Flammulina velutipes*), Straw mushroom (*Volvariella volvacea*), *Pholiota nameko* and *Pleurotus sajor-caju* should be cropped just before heading.

Juncao drying

After cropping, grasses must be placed in the sunlight to dry thoroughly, a process that is always affected by the weather. Thus, growers should try to store Juncao before the rainy season. Two storage methods are commonly employed: indoor storage in dry rooms and outdoor haystack storage. For outdoor storage, waterproof coverings are important. For both methods, great care must be taken for fire-prevention. Loose grasses normally occupy large spaces indoors, and they are easily dampened outdoors, so it is necessary to process them into powder immediately after drying. Juncao powder with a small volume is convenient for both storage and long distance transport.

Juncao processing

Processing of Juncao is very different from that of sawdust and rice straw because of the physical structure and nutritional differences. Special Juncao grinders are necessary. The size of the grinder sieve also depends upon the different species of Juncao. For example, a sieve whose holes are of diameter about 2.5mm is used for *Dicranopteris dichotoma* while a sieve with holes of a diameter of 3.0-3.5mm is usually suitable for *Neyraudia reynaudiana*.

Juncao powder storage

Juncao powder must be stored in dry rooms. Otherwise, it will become mildewed or blocked, which will exhaust the nutrients and lower the nutritional value of Juncao.

Substrate formula

Some substrate formulas for *P. ostreatus* cultivation are listed below.

1. *Miscanthus floridulus* 60%, *Sorghum propinquum* 10%, *Pennisetum purpureum* 6%, wheat bran 17%, rice bran 5%, gypsum powder 1.5%, sugar 0.5%.
2. *Miscanthus floridulus* 72%, *Pennisetum purpureum* 5%, wheat bran 15%, rice bran 5%, calcium carbonate 2%, sugar 1%.
3. *Miscanthus floridulus* 49.5%, *Sorghum propinquum* 20%, wheat bran 20%, rice bran 8%, calcium carbonate 2%, sugar 0.5%.
4. *Miscanthus floridulus* 43%, *Neyraudia reynaudiana* 33%, wheat bran 16%, rice bran 6%, calcium carbonate 1.5%, sugar 0.5%.
5. *Pennisetum purpureum* 75%, Phragmites communis 6%, wheat bran 10%, rice bran 6%, calcium carbonate 2%, sugar 1%.

Mixing substrate materials

Growers should weigh all the raw materials according to the substrate formulas, stir the Juncao powder, wheat bran and rice bran evenly, and then pour them into the mixer. They should first add additional nutrients into the water, followed by calcium carbonate or gypsum powder. They should put the mixture into a mixer after it has been stirred thoroughly for 30-40 minutes. Note that the water content of substrate should be 62-65% and the pH value should be 5.5-6.5.

Filling substrate into container

In the case of bottle cultivation, growers should use 850mL plastic bottles and put 500-550g of wet materials in the bottles. In case of bag cultivation, they should use 24 x 44cm plastic bags and put 1.8-2.2kg of wet materials in each bag. Mixed materials should be packed tight in containers as soon as mixing is done. Growers should make sure to clean the surface of bottles or bags.

Sterilization

In the case of high-temperature sterilization, growers need to keep the temperature of the sterilization room at 121℃ for 2 hours while they need to maintain the compost at a temperature of 100℃ for 6 hours for normal-temperature sterilization. Growers need to make sure to record the temperature of both the room and compost at scheduled times. When sterilization is finished, they should lower the temperature slowly. They should open the exhaust valve only after the temperature is lower than 80℃ or the pressure falls to 0.5kg/cm^2.

Inoculation

The temperature of the substrate should be cooled down to 18-25℃ before inoculation. Growers should keep the room temperature between 8 and 15℃ and the humidity less than 60%. Growers need to make sure to inoculate according to the micro-organism and bacteria-free processing rules. They should not use old spawn and should get rid of old spawn on the surface of spawn bottles before inoculation.

Mycelia culture

Bottles and bags should be sterilized by using ultraviolet light and medical liquids before being moved into the culture room. The appropriate room temperature for mycelial growth is 20-24℃ and the appropriate relative humidity is 65-70%. Growers are advised to ventilate with fresh air but no light is required. Growers should observe the temperature variations and mycelia growth situation frequently. It takes about 18-24 days until the substrate is fully colonized.

Primordia formation

In the case of bottle cultivation, growers are advised to scratch the surface of the bottle for primordial induction. To induce fruiting, the water content of substrate should be increased. Growers can add 10-15mL water into each 850mL bottle, pour out the water and place the bottles upside down on the culture shelves. After 20-30 minutes or when no more water drips, they should then turn them right side up again. An alternative way is just to let the compost dry naturally after adding water. An appropriate room temperature is 13-14℃ and an appropriate room moisture is 85-95%. Adequate ventilation is required. Buttons appear in 6-8 days.

Fruiting and cropping

According to the strain type used, appropriate room temperatures vary: 12-15℃ (psychrophilic), 16-22℃ (mesophilic) or 20-26℃ (thermophilic). Growers need to keep room moisture at 90-95%. They should increase the air flow by turning on the exhaust for 30-50 seconds per half hour. A certain amount of scattered light is required. It takes about one week for full fruiting. If the crop is harvested when the caps are nearly open and flat, a high yield will be gained. On the other hand, mushrooms can be preserved for a long period if they are harvested before the cap spreads out.

Culture of second mycelia generation

After harvesting the first flush of mushrooms, growers should keep the room temperature at 20-24℃ and the room moisture at 60-70% in order to induce a second flush. They should make sure to clean the racks of old spawn after cropping and level the surface of the bottles. At this point no light is required, but fresh air is necessary. The second flush should start 8-10 days later. Primordia induction and fruiting are same as with the first flush.

Artificial Planting of Juncao

From the past years' practice, we strongly suggest that mushroom growers who want to adapt Juncao as the culture substrate should plant Juncao too. This is because wild Juncao normally does not grow in fields, a fact that always causes inconvenience and low yields. The yield of planted Juncao can reach 30,000kg per Mu (about 667m^2).

We recommend two methods for Juncao cultivation: artificial reproduction and closing hillsides to facilitate grassing. Wild *Dicranopteris dichotoma* has a wide distribution and always grows in tracks. So growers can get high yield by just closing hills to facilitate grassing, an action that makes transplanting unnecessary. If possible, growers should apply fertilizer before the rainy days in spring, as this will which result in higher yields.