



7

CULTIVATION OF OTHER ECONOMICALLY IMPORTANT AND MEDICINAL MUSHROOM

We have seen that there are number of mushrooms that are cultivated in different parts of the world. We have discussed about the mushrooms that can be cultivated on a substrate after composting or on substrate as such but after its pasteurization at 60-65°C. There are number of other edible mushrooms like shiitake, king oyster, etc and medicinal mushrooms like *Ganoderma*, *Cordyceps*, etc that are gaining popularity. These mushrooms can be grown on saw dust or other materials but need autoclaving of substrate, that is, sterilization at temperature above 120°C for an hour or so. In this lesson we will discuss the cultivation techniques of some of these mushrooms.



OBJECTIVES

After reading this lesson you will be able to:

- understand that substrate preparation for some mushrooms needs autoclaving;
- use of different types of materials for growing different mushrooms;
- demonstrate the cultivation of shiitake, king oyster, *Ganoderma* and *Cordyceps*.

7.1 CULTIVATION OF SHIITAKE

Shiitake (*Lentinula edodes*) is at present the number one mushroom in terms of production in the world. It is mainly cultivated in People's Republic of China, Japan, Taiwan, S. Korea and United State of America and it has unique taste and flavour. It is also known for the medicinal properties.



Notes

Cultivation of Other Economically Important and Medicinal Mushroom

Cultivation of shiitake was initially done on wood logs and in some parts it is still cultivated on wood logs. Mushroom production on wood logs required long time and with passage of time wood log availability started declining. A method of cultivation on saw dust of broad leaved trees was devised where the bags become like logs after spawn run. This is referred as Synthetic Log Cultivation. This method is practiced in Taiwan, Mainland of China, Singapore, Japan, Thailand, etc. As specific type of saw dust of broad leaved trees is required and it is at times not easily available, efforts have been made to grow it on alternate materials like corn cobs, wheat straw, etc. Only selected strains can be grown on such substrates. Normally it grows at low temperature below 20°C.

In synthetic log cultivation mainly wheat grain spawn is used. Sawdust spawn and wood plug spawn are used in wood log cultivation. Grain spawn is prepared by method described in earlier chapter on spawn production. Saw dust spawn is prepared using formula like Saw dust 78%, Sucrose 1%, Wheat bran 20%, Calcium carbonate 1% and Water content 65%. We here describe the method of substrate preparation, spawning and cropping of shiitake mushroom.

7.1.1 Substrate Preparation

The commercial cultivation can be carried out on sawdust of broad leaf trees mainly tuni, mango, safeda, oak, maple and poplar. Most of the formulations include about four parts saw dust and one part wheat or rice bran. We add small amount of CaCO_3 (1.5%), gypsum (0.5%), Sugar (1%), citric acid (0.2%), etc. to the mixture of sawdust and bran. In literature there are different types of formulations. Some strains do well only on particular formulation. Some of the formulations in use are as under:

1.
 - Saw dust 80 kg
 - Wheat bran 20 kg
 - Cane sugar 1.3%
 - CaCO_3 1.5%
 - Citric acid 0.2%
 - CaSO_4 0.5%
2.
 - Corn cobs 40 kg
 - Saw dust 10 kg
 - Wheat bran 12.5 kg
 - Cane sugar 1 kg
 - Pectin 15 g
 - Urea 20 g
3.
 - Rice straw 50%
 - Wheat straw 20%
 - Wheat bran 19 kg
 - Saw dust 20%
 - CaCO_3 1 kg
4.
 - Sugar cane bagasse 50 kg
 - Rice bran 12.5 kg
 - Gypsum 1.5 kg
 - Potassium Sulphate 15 g
 - Urea 15 g
 - Magnesium Sulphate 10 g



Adjust the water content to 60-65% and pH to 5.5-6.0 using gypsum and lime. Soluble ingredients (citric acid, sugar, sulphates, etc.) are usually dissolved first in water before mixing; saw dust has to be soaked at least for one day and rice straw for three hours. All the ingredients are thoroughly mixed.

7.1.2 Bag Filling and Autoclaving

We fill 1.5 to 2 kg of prepared substrate in the heat resistant polypropylene bags just after mixing and wetting the substrate (Otherwise fermentation and contamination may start). Top of the bag is pushed through plastic ring and plugged with non-absorbent cotton. The bags are given cylindrical shape and autoclaved without delay to avoid fermentation in the bags. Sterilization is carried out in an autoclave at 22 psi for 1½-2 hours.

7.1.3 Spawning and Spawn Run

We do the spawning under aseptic conditions after cooling down the substrate to room temperature. Cotton plug is removed; grain spawn is introduced @ 3% and again plugged. We place the bags in cropping rooms where these are generally incubated in a 4 h / 20 h light/ dark cycles at 23-25°C. Spawn run may take 60-80 days or more depending upon the strain. During this period it goes through mycelial growth, mycelial coat, mycelial bump formation and browning stage (Fig. 7.1). Bumps are clumps of mycelium, commonly formed on the surface of most strains after 9-10 weeks. These bumps can turn into mushroom primordia at a later stage but most of them abort. Fluctuating temperatures and high CO₂ promotes bump formation. This is followed by development of Pigmentation. At this stage some aeration should be provided when the bumps have formed for this phase to occur. After longer spawn runs (more than 60 days) the surface of the colonized substrate may begin to turn brown (Fig.7.1), some exudates may be there during spawn running. We remove the polypropylene when bags have partially (half or one third) turned brown. The coat will gradually become hard. While outside of the substrate should be hard, the inside should be softer and moist.



Bags are filled with substrate and autoclaved



Spawning is done before laminar flow



Notes



Complete spawn run



Mycelial bump formation starts



Browning and coat hardening starts



Shiitake mushroom crop

Fig. 7.1: Different stages of cultivation of shiitake mushroom

7.1.4 Fruiting

Fruiting is induced when we provide suitable temperature, high RH, good ventilation and cold water/ shock treatment. A schedule of various parameters is as given below (Table 7.1):

Table 7.1: Various cropping parameter

Stages/Activity	Days	Temperature °C	Light intensity (Lux)	Humidity (%)
Incubation	50-120	20-30	500-1000	65-70
Induction	2-4	10-20	500-1000	85-95
Fruiting	7-14	12-18	500-1000	60-80
Rest	7-21	20-30	None	65-70
Induction	2-4	10-20	500-1000	85-95

Harvest the shiitake mushroom by holding the stalk of the mushroom and break it from the substrate. Don't tear them from the surface. Harvest the mushrooms at an early stage. Don't water the scars left after harvesting for 3-4 days. Two



strains DMR-Shiitake 38 (suitable for cultivation on saw dust) and DMR Shiitake 388 (suitable for cultivation on wheat straw) have been released by ICAR-Directorate of Mushroom Research Solan.



INTEXT QUESTIONS 7.1

State True or False

- (i) Shiitake is mainly cultivated in China, Japan, S. Korea and Taiwan.
- (ii) Normally it grows at low temperature below 20°C.
- (iii) It can be cultivated on any type of saw dust.
- (iv) Substrate must be autoclaved and spawning may be done under fully sterile conditions.
- (v) Cold shock treatment is required for induction of fruiting.

7.2 CULTIVATION OF KING OYSTER

King oyster has gained popularity in in the last 2-3 decades in China, Japan, Europe and other parts of the world. For example cultivation of king oyster (*Pleurotus eryngii*) using bottle was introduced in Japan in 1993 when its production was merely 1910 tonne and it increased to 40475 tonnes by 2016 (Fig.7.2).

Production of king oyster in Japan (1993-2016)

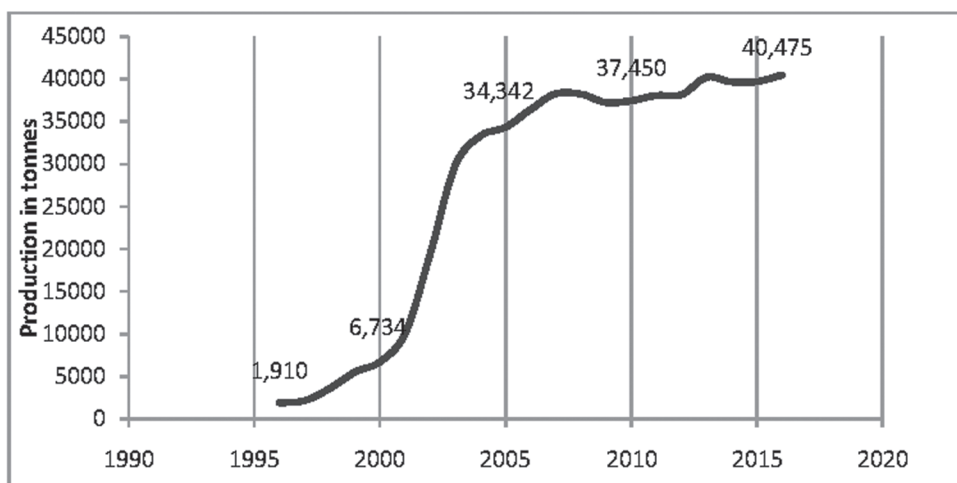


Fig. 7.2: Changes in production of *P. eryngii* in Japan in last 25 years



Notes

The substrate has 65% moisture and pH of about 6.5 after sterilisation. We fill about 1 to 1.5 kg wet substrate in each bag made of polypropylene (PP) or in pp bottles, autoclave and spawn these. Thereafter we keep bags for spawn run at 23-27°C and after complete spawn run we lower the temperature to 14-16°C. Light and aeration is needed for normal fruiting (Fig 7.3).



Bags are filled, autoclaved, cooled and spawned



Fully colonized bags



Bags are opened after complete spawn run



Development of fruitbody

Fig. 7.3: Different steps in the cultivation of king oyster



INTEXT QUESTIONS 7.2

State True or False

- (i) King oyster is replacing *Pleurotus oesteratus* in China and Japan.
- (ii) In China it is mainly cultivated on cottonseed hull + saw dust supplemented with wheat bran and lime.
- (iii) In India, we do not use liquid spawn for cultivation of king oyster mushroom.
- (iv) Temperature required for spawn run and fruiting is 25 and 16°C respectively.
- (v) It can also be cultivated on wheat or rice straw.



7.3 CULTIVATION OF MEDICINAL MUSHROOM- *GANODERMA LUCIDUM*

Mushrooms are not only edible, but are also known for their medicinal properties. Some mushrooms are consumed only because of their medicinal uses and are grouped as medicinal mushrooms. Of the 15000 known species, about 300 are medicinal mushrooms. Oriental countries like China, Japan and Korea have been pioneer in traditional knowledge about medicinal properties of mushrooms. Many traditionally used mushrooms belonging to genera, *Auricularia*, *Flammulina*, *Ganoderma*, *Grifola*, *Lentinula*, *Trametes (Coriolus)*, *Schizophyllum*, *Cordyceps* and *Tremella* have been demonstrated to possess significant medicinal properties. *Ganoderma lucidum*, commonly called Reishi mushroom, is the most popular medicinal mushroom in China and has been used for a wide range of health benefits ranging from preventive measures and maintenance of health to regulation and treatment of chronic as well as acute ailments. Reishi is used as medicine and not as food because it is bitter, corky and hard. Its market value is basically as herbal medicine and dietary supplement.

Reishi mushroom can be grown seasonally in the low cost growing rooms preferably polyhouses and also in the environmentally controlled cropping rooms. As the mushroom is intended to be used exclusively as medicine, it has to be grown organically.



Fig. 7.4: Pinheads and fruit bodies of *Ganoderma* with whitish top

Reishi mushroom is cultivated on the sawdust of the broad-leaved trees like mango, poplar, coconut, sheesham, etc. To sawdust, obtained from the sawmills, we add 20% wheat bran and wet it to a level of 65% moisture. One per cent of calcium sulphate (gypsum) and One per cent of calcium carbonate (chalk powder) are added per kg sawdust to get a pH of 5.5. The mixed substrate (700 g dry wt;



Notes

2.1 kg wet wt) is filled in polypropylene bags the mouth of which is then plugged with cotton after putting a plastic ring like grain spawn pack of mushrooms in polybags.

The bags are then sterilized in autoclave at 22 p.s.i. for 2 hours. After cooling, the substrate is spawned with wheat grain or saw dust spawn @ 3% on the dry weight basis under sterile conditions. Spawn-run (incubation) is done at 28-35°C in the closed rooms (high carbon dioxide) and darkness. After the complete spawn run (bags white all over), which takes about 25 days, polythene top is cut at the level of the substrate totally exposing the top side and proper conditions for fruiting or pinning (temp. 28°C, 1500 ppm CO₂, 800 lux light, 95% RH) are provided.



Fig. 7.5: Mature crop of Ganoderma

Once the pins have grown up enough to form the cap as indicated by the flattening of the whitish top of the pinhead, relative humidity is reduced to 80% and more fresh air is introduced to achieve around 1000 ppm CO₂. Once the cap is fully formed as indicated by yellowing of the cap margin (that is otherwise white), temperature is lowered to 25°C and RH (Relative humidity) is further reduced to 60% for cap thickening, reddening and maturation of the fruit-bodies.

Full maturity is indicated when the cap is fully reddish brown and spores are shed on the top of the cap. Harvesting is done by the tight plucking, holding the root with one hand and pulling up with another; scissors and knives can also be used but no residual bud may be left after harvesting. One cycle of the growing takes 10-15 days. After harvesting the first flush, conditions for pinning are again switched on (i.e. 28°C, 95% RH, 1500 ppm CO₂, 800 lux light) for starting and completing the second flush. Depending upon the conditions, 2-3 flushes appear and a total 25% B.E. can be achieved. One crop cycle takes about four months. Conditions required at various stages are summed up below:



Notes

Stage	Activity/Conditions
Ingredients	Saw dust (1 kg) + Wheat bran (0.2 kg) + CaCO ₃ (10g) + CaSO ₄ (10g)
Substrate preparation	Wetting (65%), bag filling (2.1 kg), autoclaving at 22 p.s.i. for 2 hr
Spawning	Saw dust or wheat grain spawn @ 3% on dry weight basis
Spawn run	28-32°C, high CO ₂ , dark
Pinning	28-32°C, RH 95%, 1500 ppm CO ₂ , light > 800 lux
Cap formation and growth	28-32°C, RH 80%, 1000 ppm CO ₂
Cap maturation	25°C, RH 60%
Harvesting	Fully reddish brown cap, tight plucking

Some workers consider *Ganoderma* a mild pathogen of broad leaved trees and many countries like Australia, New Zealand do not allow its direct entry. Hence it is important that due care is taken during cultivation to check that the spores do not get distributed freely and the substrate after cultivation is properly cooked out to prevent accumulation of inoculum in the fields or forest areas. The used substrate may be dried and burnt as fuel.

Harvested mushrooms are washed with water and dried at low temperature (<50°C) in the cabinet driers, preferably at 35°C in the dehumidifying cabinet drier. Freeze drying is, however, the best option. *Reishi* mushroom has very high dry matter (45% i.e. 450 g dry from 1 kg fresh).



INTEXT QUESTIONS 7.3

Answer the following questions

- (i) Approximately how many species of medicinal mushrooms are currently known.
- (ii) Which is the most popular medicinal mushroom in China?
- (iii) *Ganoderma* is grown on the sawdust of the broad-leaved trees
(True or False).



Notes

- (iv) Humidity is reduced from 95% to 60% over days as we move from pinning to cap maturation (True or False).
- (v) Some workers consider *Ganoderma* a mild pathogen of broad leaved trees and many countries like Australia, New Zealand do not allow its direct entry (True or False).

7.4 CULTIVATION OF *CORDYCEPS*

7.4.1. *Cordyceps sinensis*

Cordyceps is a genus of Ascomycetes fungi that includes about 400 species. Most of these grow on insects. *C. sinensis* is the most important species due to its medicinal properties and it is found in higher reaches of Himalaya (Nepal, Bhutan and Tibet as well as the bordering areas of India and China) at a height of 3000 to 5500 meter. The medicinal properties are attributed to Cordycepin (Fig. 7.6) and some polysaccharides. It is regularly collected from high reaches. When snow starts to melt in May to July, you can see many collectors going in search of this mushroom. Commonly it is called Kira ghas (in India) or Yartsa gunbu/ Yarsagumba in Tibet). In India it is found in Pithoragarh region of Utrakhand. Collectors pluck mushroom before it can mature and scatter its spores (Fig. 7.7) which is resulting in destruction of this Himalayan goldmine.

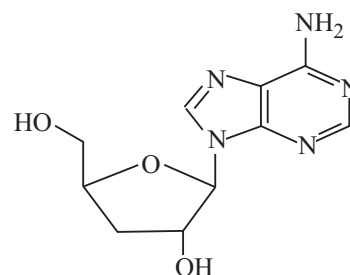


Fig. 7.6: Cordycepin (3'-deoxyadenosine), is a derivative of the nucleoside adenosine

Now we know that *C. sinensis* parasitizes ghost moth larvae. This fungus can grow on many artificial substrates. Also, ghost moth larvae have been successfully reared. Its cultivation was achieved in China in 2013. Large scale artificial cultivation has been recently achieved and 10 ton of this mushroom was cultivated in China in 2016.

It can be cultured in liquid medium containing 1.25% glucose, 1.25% sucrose, 0.02% peptone, 0.0625% yeast powder, 0.025% KH_2PO_4 , 0.0125% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0025% Vit B₁ and natural pH at 24 °C. Based on DNA analysis, it was found to differ from other *Cordyceps* species and has now been renamed as *Ophiocordyceps sinensis*. In the next section we will describe the cultivation of another species namely *Cordyceps militaris*.



***Cordyceps*
(Kira-ghas)**

Fig. 7.7: Collection of *Cordyceps sinensis* in higher reaches

7.4.2 *Cordyceps militaris*

Lab and Culture preparation

C. militaris is cultivated under lab conditions where facilities for control of temperature, humidity and filtered fresh air are available. Cultivation is done in Jars kept on racks just like we keep bags or bottles in a spawn lab. It is cultivated at 16-24°C and requires 800-1000 lux light for its fruiting. During cultivation relative humidity needed is 70-80%.

Potato Dextrose Agar (PDA) medium is used for maintaining and multiplication of the culture. To make PDA, take about 250 g potato, peel these and dice into small pieces. Put 200 g diced potatoes in one litre distilled water and boil for 15-20 minutes or till potatoes become semi-soft. Sieve it and to clear solution add 20 g dextrose and 14 g agar. Boil it, pour in test tubes, autoclave and prepare slants. Pure culture of this mushroom can be procured from outside or made from fruit-body using tissue culture method described in chapter of spawn (clean fruit body, cut into two parts and from upper part in the middle take small tissue and put it in test tube). Cultures are kept in dark for 8-10 days for spread of mycelium. Mycelium will be of pure white in colour due to its growth in dark. When kept in



Notes

light it turns orange in 4-5 days. We can also multiply the culture in Petri plates for its use for preparation of liquid culture.

Liquid Spawn

To begin with, mycelium is cultured on a liquid medium. For preparation of liquid spawn liquid medium is prepared using glucose 30 g, peptone 10 g, yeast extract 5 g, KH_2PO_4 1 g, MgSO_4 0.5 g per litre of distilled water. The medium is poured in flasks, autoclaved, cooled and culture from the test tubes is transferred to sterilized liquid medium and kept on shaker for 4-5 days for preparation of liquid spawn. It may be possible to make liquid spawn on Potato broth, that is same medium as used for multiplying cultures but without adding agar-agar.

Substrate Preparation

First step in preparation of the substrate is the preparation of nutrient solution. For this we add the following in 1000 ml distilled water.

Glucose	20 g
Peptone	5 g
Yeast extract	3 g
KH_2PO_4	2 g
MgSO_4	0.5 g
Tri Ammonium Citrate (TCA)	1 g
Vit B ₁	50 mg
Vit B ₁₂	10 mg
Multivitamin	10 mg

There are other formulations for preparation of nutrient medium wherein the quantity of above ingredients may vary and potato extract, silkworm larvae powder or herbal eggs may be added along with other ingredients like glucose, peptone, MgSO_4 , yeast extract, Vit B₁, etc. Vit B₁ is an important ingredient in all combinations (when eggs are added then after mixing in blender, the foam from the top can be removed).

Second step is the supplementation of brown rice with above nutrient medium. Brown rice is soaked in water for 30 minutes, cleaned and washed thoroughly. Thereafter, it is kept on a sieve or cotton cloth and allowed to dry for 30 minutes (various workers have used substrates other than rice also for the cultivation of this mushroom).



Take 20-25 g rice per jar, add about 40 ml nutrient solution to it and cover the jar with autoclavable cap or polypropylene bag and autoclave at 15 psi for 40-50 minutes. A small hole is made in the cap and it is plugged with cotton. This is sufficient for aeration required during growth. We cool the jars and add 5-10 ml liquid spawn. Whole operation is done in Laminar Flow cabinet to ensure that there is no contamination. Jars are moved left to to right to spread the liquid medium over the solid medium.

Cultivation

After inoculation with liquid spawn the jars are kept in dark for 8-10 days at 20-22°C, RH 65-70%. Jars can be covered with dark polythene or cloth to create dark conditions. Mycelium spreads in the medium and because of its growth in dark, it is of white colour. After complete colonization of the substrate, the jars are kept in light for a week when the colour of mycelium turns orange. Thereafter we provide 1000 lux light for 12 hours daily. It may take 7 days for yellowing, another 2 weeks for bubble and then pinheads formation. When distinct pinheads are formed we can reduce the light to 8-12 hours and it may take another 5-6 week till mushrooms attain harvestable size of 4-6 cm. It can take up to 72 days after we expose the colonize substrate and the total time required for one crop from inoculation to harvest can be up to 3 months. Fresh air may be needed at regular intervals to avoid high carbon dioxide build up in the incubation room. When head of mushroom turns club shaped, it is ready for harvest (Fig.7.8). The mushrooms are plucked out of jars and dried. It can be sold as such or used for making different types of nutraceuticals/ supplements. Cordycepin is an important ingredient and cultural conditions affect the production of cordycepin.



Fig. 7.8: Crop of *C. militaris*



Notes



INTEXT QUESTIONS 7.4

State True or False

- (i) *Cordyceps* is a genus of Ascomycetes fungi that includes about 400 species.
- (ii) Colour of mycelium of *Cordyceps militaris* is white when cultured in dark.
- (iii) Total time taken for one crop of *C. militaris* is up to three months.
- (iv) Cordycdpin, the compound to which medicinal properties are attributed, is much higher in *C. militaris* than in *C. Sinensis*.
- (v) Cultural conditions affect the production of cordycepin.



WHAT YOU HAVE LEARNT

Let us recapitulate the important points we have learnt in this lesson:

- Edible mushrooms like shiitake, king oyster, etc and medicinal mushrooms like *Ganoderma*, *Cordyceps* can be grown on saw dust or other materials but need autoclaving of the substrate.
- The commercial cultivation of shiitake can be carried out on sawdust of broad leave trees mainly tuni, mango, safeda, oak, maple and poplar.
- Spawn run may take 60-80 days. During the period it goes through mycelial growth, mycelial coat, mycelial bump and browning stage.
- Shiitake grows at temperature below 20 °C and needs cold shock treatment by dipping in cold water for induction of fruiting in every flush.
- King oyster has gained popularity in China and Japan and is replacing *Pleurotus ostreatus* in these countries.
- In China it is cultivated on cottonseed hull + saw dust supplemented with wheat bran and lime. Temperature required for fruiting is around 16°C.
- *Ganoderma* is an important medicinal mushroom and can be grown on the sawdust of the broad-leaved trees (mango, poplar, coconut, sheesham).
- High humidity is required for pinhead formation, but it is gradually reduced for cap maturation.
- *Cordyceps sinensis* is collected from nature in higher reaches of Himalaya at a height of 3000 to 5500 meter.

Cultivation of Other Economically Important and Medicinal Mushroom

- *C. militaris* can be cultivated in lab and one crop cycle can take up to three months.
- Cordycdpin, the compound to which medicinal properties are attributed, is much higher in *C. sinensis* than in *C. Militaris*.



Notes



TERMINAL EXERCISE

1. What are the possible reasons for lack of popularity of these mushrooms in India?
2. What shock treatment is given to block of shiitake to induce fruiting?
3. Which of the following materials cannot be used as substrate for cultivation of shiitake?
 - (a) Wheat/rice straw
 - (b) Sugarcane bagasse
 - (c) Saw dust of narrow leaved trees
 - (d) Saw dust of broad leaved trees
 - (e) Corn cobs
4. What type of substrate can be used for the cultivation of king oyster and what temperature is required for its fruiting?
5. Describe the changes required to be made from pinning to cap maturation in *Ganoderma*.
6. What are common names of *Cordyceps sinensis* and in which region of India it is collected?



ANSWERS TO INTEXT QUESTIONS

7.1

- (i) True (ii) True (iii) False (iv) True (v) True

7.2

- (i) True (ii) True (iii) True (iv) True (v) True



Notes

Cultivation of Other Economically Important and Medicinal Mushroom

7.3

- (i) 300 (ii) Ganoderma (iii) True (iv) True (v) True

7.4

- (i) True (ii) True (iii) True (iv) False (v) True

SUGGESTED ACTIVITY

In China species like *Cordyceps sinensis*, *Morchella* spp have been reported to be cultivated. Try to collect information about their cultivation.

Key Learning Outcomes

- Prepare substrate and manage economically important and medicinal mushroom crops viz. *Ganoderma*, *Cordyceps* mushrooms.
- Pick, grade and pack the harvested economically important and medicinal mushroom.