Preface

The Action Research Institute, in keeping track with high escalation of growth of mushroom culture in India, has conducted a number of action researches on available technology of mushroom spawn production with a view to simplify the techniques so that any person living in rural or urban area and having literacy or no literacy may make the best use of this technology with an ease and a command. The Institute has also made attempts to improvise a number of low-cost gadgets so that a person with very small and limited means may not find any problem in setting up and running a mushroom spawn production unit at the household level to meet his requirements and the requirements of others. In consonance with this development, the technology schedule that is to be followed up and the investment schedule which is to be taken care of by the mushroom spawn producers have very systematically been presented in this MANUAL to facilitate smooth functioning of small size mushroom spawn production units at the growers level.

This Mushroom Spawn Production Manual for Small Size Primary Unit has been developed for incorporation in the Kit which is specially designed for those who intend to produce mushroom inoculum from liquid culture to media without opening the mouth of either container of culture and media. This eliminates the risk for contamination to a considerable extent, and thereby reduces the cost, increases the availability and escalates the prospect of promotion of mushroom very high.

The intending mushroom spawn producer is expected to learn the techniques of producing mushroom grain spawns in packets by ways of transferring mushroom inoculum from liquid culture to grain medium without opening the mouth of either container of culture and medium.

Technology Schedule

The intending mushroom spawn producer is to learn and practise all production functions relating to -

I. Processing Grain Medium

II. Processing Liquid Medium

III. Sterilizing Processed Media

IV. Inoculating Sterilized Media

V. Incubating Inoculated Media

1. Processing Grain Medium

A. Collect wheat grains of good quality and remove dust, waste materials as well as broken and immatured grains.

B. Take a measure of 1150 g of clean wheat for preparing grain spawns in 8 polypropylene packets.

C. Wash the measured wheat grains thoroughly, if possible in running water, and soak it in clean water for an hour. Change water frequently to prevent fermentation in case of longer period of soaking.

D. Boil clean water (@ 2 liters of water per kg of wheat) in a container, aluminium cauldron (dekchi), and add to it water-soaked grains. Put off the fuel-line as soon as boiling starts.

E. Allow a gap for a few minutes to see that grains have become tender but not splitted.

F. Drain excess water by pouring the contents in a bamboo basket, or perforated plastic basket or on a sieve made up with iron-wire-net having mesh smaller than wheat grain.

G. Unload the contents after one hour and spread the boiled wheat grains on a clean cloth or polythene sheet.

H. Allow the boiled grains to dry up overnight.

I. Mix lime with the grains (@ 20 g per kg of dry wheat in

preparing spawns in polypropylene packets).

J. Add, if there is an apprehension for a bacterial attack, any bacteriostatic compound like chloramphenicol capsule (e.g. enteromycetin capsule 250 mg : @ 1 capsule per kg of dry wheat).

K. In preparing grain spawns in polypropylene (pp) packets -

i) Take a pp packet (20 cm by 12 cm) and fill in it 200g of prepared grains.

ii) Put the package in another pp packet.

iii) Roll a piece of non-absorbent cotton in a cylindrical form with a length of 5 cm and a circumference of 8 cm.

iv) Use the rolled cotton to plug the mouth of the double-packet container.

v) Place the cotton-plug inside at one corner of the mouth and bring the rest with small foldings on the top of the plug.

vi) Tie a knot with cotton thread at 2 cm down the edge of the double packet.

II. Processing Liquid Medium

A. Select some old but solid potatoes of good quality.

B. Take a measure of 125g of potato and wash them thoroughly in fresh water.

C. Peel off each potato and cut it into 1.5 cm cubes.

D. Wash the cubes lightly in fresh water.

E. Boil 4 litres of water and allow it to cool down.

F. Take out 2 litres of clean water by way of siphoning and leave the rest with sediments at the bottom of the container.

G. Add the potato-cubes in an aluminium container with I litre of clean water and boil the contents until the potato-cubes become soft enough but do not split.

H. Take out potato-cubes and allow the liquid to cool down.

I. Add clean water to make up the volume of the liquid to 1 litre

and filter the liquid through a thin layer of absorbent cotton.

J. Add 20g of dextrose to the liquid and stir till dextrose gets thoroughly dissolved.

K. Take in a small container a little amount of the liquid and add to it the contents of 1 capsule of enteromycetin. Stir the liquid till the contents get thoroughly dissolved. Add the mixture to potato-dextrose medium and shake them to mix up thoroughly.

L. Wash 7 bottles (used saline bottles) and add in each bottle a few broken glass—pieces. Pour 60 ml of the liquid in each of 7 bottles.

M. Plug the mouth of each bottle by placing a thin layer of non-absorbent cotton between the rubber-cock and the glass-mouth of the bottle. Place a thin layer of cotton-pad on the top of the rubber-cock. Cover the mouth by a piece of thick paper and tie a knot over the paper at the neck of the bottle.

III. Sterilizing Processed Media

A. Sterilize the bottles or the packets containing grain medium at a pressure of 15 pounds per square inch (i.e., at a temperature of 121°C) for one hour a day in two consecutive days, or continuously for 2 hours on the same day. Sterilize the bottles containing liquid medium at a pressure of 15 pounds per square inch (i.e., at a temperature of 121°C) once for half an hour.

Carry on the following operations to do the sterilization of the media —

i) Take adomestisize pressure-cooker and check before use that sufficient water (about 1.5 litres) at the bottom of the cooker is present beneath the perforated lid and to effect it make use of an additional support for resting the perforated lid on the surface of water. To make this effective, use 3 pieces of galvanized pipe or similar such devices, each having a height of 4 cm.

ii) Place the media containers (bottles or packets) inside the cooker and on the perforated lid, keeping them at perpendicular to the base of the cooker.

iii) Place on the top a piece of old newspaper as a cover. Clean the lid and close it firmly, keeping the air-vent open.

iv) Put the pressure-cooker on heat and wait for the steam emitting through the air-vent. Close, after few minutes, the air-vent by pressing the automatic pressure valve on it.

v) Wait for the first whistle to come out and count 30 minutes to put off the fuel-line in case of sterilization of liquid medium and 60 minutes in case of sterilization of grain medium.

vi) Allow the inside pressure to go down by itself.

vii) Finally remove the automatic pressure valve with care and uncover the lid of the cooker.

viii) Bring out the sterilized media while hot and allow them to cool down at room temperature.

IV. Inoculating Sterilized Media

A. Collect good contamination-free liquid culture prepared in a bottle plugged with a rubber-cock, following the method as developed by Action Research Institute.

B. Shake the culture-bottle to effect a to-and-fro movement of glass piece inside which will break the mycelial mat into finer fragments, producing a uniform distribution of mycelial suspensions in the liquid.

C. Sterilize the syringe and the needle well before inoculation.

d. Close the door and window of the room or the chamber within the room, earmarked for purpose of inoculation, and spray a mild disinfectant containing thymol to drop any floating spore from the air inside the room.

E. Leave the room or he chamber for purpose of inoculation and close the door behind.

F. Enter into the room or the chamber for purpose of inoculation and close the door behind.

G. Wash the top of the working table, aluminium tray etc. with

lysol and clean syringe, needle and your hands thoroughly with cotton soaked in alcohol or rectified spirit.

H. Light a spirit-lamp and keep it close to working hands to avail a micro-organism free zone and to flame the needle from time to time.

I. Wipe the neck of the bottle of the liquid culture and the exposed surface of the rubber-cock with a piece of cotton soaked in alcohol or rectified spirit.

J. Flame the needle, and the while flaming draw sufficient air inside the syringe.

K. Wipe the needle with a piece of cotton soaked in alcohol or rectified spirit.

L. Insert the needle through the smaller hole in the rubber-cock, invert the bottle and push the air inside.

M. Draw the liquid culture in the syringe and turn the bottom of the bottle down to rest on the working table.

N. Withdraw the needle of the syringe and keep the rubber cock-covered with a piece of cotton soaked in alcohol or rectified spirit.

O. Carry on the following operations for inoculation of grain medium in packets.

   i) Take a packet of grain medium and wipe a place anywhere on the margin at the bottom of the packet with a piece of cotton soaked in alcohol or rectified spirit.

   ii) Insert the needle through the space on the margin thus cleaned and push 1 ml of liquid culture on the grains inside the packet.

   iii) Withdraw the needle and perform a line-sealing just above the needle-puncture with the help of an electric sealer or a candle-flame.

   iv) Wipe the needle with a piece of cotton soaked in alcohol or rectified spirit and repeat the process to inoculate the next packet.

   v) Make complete use of the liquid culture drawn in the syringe and wash the syringe thoroughly with alcohol or rectified spirit. Flame the needle to redness, and while

flaming draw sufficient air inside the syringe. Push the air inside the bottle containing liquid culture and draw some amount of liquid culture in the syringe for further use.

vi) Repeat the process for inoculation of the next batch of packets and continue with the operations till all grain packets are inoculated.

P. Carry on the following operations for inoculation of liquid medium in bottles —

i) Take a bottle containing sterilized liquid medium and clean the neck of the bottle as well as the exposed surface of the rubber-cock with a piece of cotton soaked in alcohol or rectified spirit.

ii) Insert the needle of the syringe through the smaller hole in the rubber-cock, push 5 ml of liquid culture inside, take the needle of the syringe out and shake the bottle lightly.

iii) Wipe the needle with a piece of cotton soaked in alcohol or rectified spirit and repeat the process to inoculate the next bottle of liquid medium.

iv) Make complete use of the liquid culture drawn in the syringe and wash the syringe thoroughly with alcohol or rectified spirit. Flame the needle to redness, and while flaming draw sufficient air inside the syringe. Push the air inside the bottle containing liquid culture and draw some amount of liquid culture in the syringe for further use.

v) Repeat the process for inoculation of the next batch of bottles of liquid medium and continue with the operations till bottles, each containing 60 ml of liquid medium, are inoculated.

V. Incubating Inoculated Media

A. Incubate the inoculated media in a dark room at room temperature.

B. Agitate the contents to effect uniform and rapid growth of mycelia by shaking the inoculated grain bottle and pressing

the inoculated grain packet lightly on the second and fourth day and by shaking lightly the bottle containing inoculated liquid medium on the third and sixth day of inoculation.

C. Allow a period of two to three weeks for incubation to facilitate mycelial growth. Check every day every packet of bottle for any expression of contamination during growth. Discard the packet or bottle as a contaminated one whenever you find a coloured patch or even a trace of it inside the bottle or packet.

D. Use fully grown and contamination-free grain spawn or liquid culture after three weeks from the day of inoculation, i.e. at the end of the incubation period that marks completion of mycelial growth in the medium.

Investment Schedule

In carrying on the production functions as spelt out in the technology schedule, the intending mushroom spawn producer will be required to make use of a few tools and accessories as well as some raw materials. Some of these tools and accessories may be available from within the household. But it is difficult for a commoner to procure certain materials from local market. Many small-scale operators have reported procurement of such articles of very low grade and at a very high cost. These problems have led the Action Research Institute to design and develop a number of Mushroom Spawn Production Kits to suit the variable needs of mushroom spawn producers. To start with, an intending mushroom spawn producer may procure one Small-size Primary Spawn Production Kit which costs Rs. 500.00 and contains the following articles:

1. Mushroom Liquid Culture 1 Small Bottle
2. Polypropylene Packets (20X12 cm) 400 pcs
3. Non-absorbent Cotton 1 pkt (400g)
4. Cotton Thread 1 pkt
5. Calcium Carbonate 1 pkt (1 kg)
6. Dextrose 1 phial (20g)
7. Enteromycetin Capsule 1 pc
8. Rectified Spirit 500 ml in 5 Bottles
9. Lysol 100 ml in 1 Bottle
10. Formaldehyde 100 ml in 1 Bottle
11. Electric Sealer 1 pc
12. Spirit Lamp 1 pc
13. Match box 1 pc
14. Hand Sprayer 1 pc

15. PVC Tube 1 m
16. Syringe (30 ml) with Needle (18 size) 1 set
17. Napkin 1 pc
18. Blade 1 pc
19. Manual for the Kit 1 copy.

This kit contains liquid of different sorts in 8 small bottles fitted with rubber-cocks. The empty bottles as available in the household may be used to store the contents of 7 bottles, other than the liquid culture bottle. The seven bottles thus available may be used for preparation of liquid culture. The 9 items (2 to 10 which will be exhausted after preparation of 400 spawn packets and 14 bottles of liquid culture may be procured from market or from Action Research Institute.

In addition to the materials supplied in the Kit, the mushroom spawn producer will be required to collect the following articles from within the household:

1. Pressure Cooker (domestic size) 1 pc
2. Kerosene Stove/Hot Plate/Heater/Gas Oven 1 pc
3. Aluminium Cauldron (dekchi) with Lid 1 set
4. Strainer with Handle 1 pc
5. Small Tools and Accessories
6. Wheat (or any cereal)
7. Potato.

A mushroom spawn producer operating at the household level will be required to make an investment of Rs 600.00 (Rs 500.00 to buy a Kit and Rs. 100.00 as working capital), and after taking into account all coasts relating to purchase of raw materials, depreciation of non-recurring items, labour, management, capital investment, contamination loss and storage loss he will be able to sell out one packet of mushrooms spawn (200g) to a dealer at Rs 3.50, and the dealer may sell it to a mushroom grower at Rs 4.50. It will ensure him with a steady supply of spawn for running with his schedule of mushrooms production and to help others by way of making available the most important input for cultivation of mushroom.