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Centre for Industrial Entomology Development (CIED)  
Hariharbhawan, Lalitpur, Nepal**

**Project Name: “Feasibility and Preparatory Study on Establishment of  
Mushroom Resource Centre in Khopasi, Kavrepalanchowk”**



**Volume-I  
Main Report**



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## Abbreviations

BOQ	Bills of Quantities
CIED	Centre for Industrial Entomology Development
DOA	Department of Agriculture
DFA	Dog Food Agar
DMR	Directorate of Mushroom Research
DUDBC	Department of Urban Development and Building Construction
FAO	Food and Agricultural Organization of the United Nation
FGD	Focus group discussions
GDP	Gross Domestic Product
GoN	Government of Nepal
HEPA	High efficiency particulate air
HORDI	Horticultural Crop Research and Development Institute
HVAC	Heating, Ventilation, and Air-conditioning
ICAR	Indian Council of Agriculture Research
IEF	Institute of Edible Fungi
IS	Indian Standard
KIIs	Key informant's interviews
MC	Moisture content
ML	Milliliter
MoALD	Ministry of Agriculture and Livestock Development
MoF	Ministry of Finance
MSP	Minimum Selling Price
MT	Metric Tons
NAGRC	National Agriculture Genetic Resource Center
NARC	Nepal Agricultural Research Council
NBC	Nepal National Building Code
NMPS	Nepal Mushroom Producer Association
NMRC	National Mushroom Resource Center
NPC	National Planning Commission
NPPC	National Plant Pathology Center
NS	Nepal Standard
OMYA	Oatmeal Malt Yeast Enriched Agar
PDA	Potato Dextrose Agar
PDYA	Potato Dextrose Yeast Agar
PEM	Protein Energy Malnutrition
RCC	Reinforced Cement Concrete
RH	Relative humidity
RKVY	Rashtriya Krishi Vikas Yojana
SMS	Spent Mushroom Substrate
SQCC	Seed Quality Control Center
SWOT	Strength Weakness Opportunity and Threat
TOR	Term of References
W/V	Weight/Volume
W/W	Weight/Weight

## Executive Summary

The world mushroom production has increased at a rapid rate since the late 1990s. The production has increased more than 10-fold during the last four decades. As per the FAO statistics, the most notable increases occurred in China, Japan, USA, the Netherlands and India. China has long been the world's largest mushroom producer country. China's present share in the total world mushroom production is more than 70% which was only 5.7 % in 1978 and has maintained an annual growth rate of 10% for the last three decades. Mushroom is their sixth economically important crop in terms of country's revenue generation. The second largest mushroom producing country is Japan followed by the United States of America, the Netherlands, India, Poland, Spain, Canada and the UK.

China is the main producer of cultivated edible mushrooms with about 967 species, which is considered as almost 50% of the cultivated edible mushroom species worldwide. Two hundred and forty species could be commonly found in the market, and more than 60 species were commercially cultivated across China.

In Nepal, there is great potential for year-round farming of a wide variety of mushrooms due to congenial agro-climatic condition and availability of wide variety of raw substrates (agricultural & forestry wastes). Even today only a handful of species are grown commercially. Mainly white button (*Agaricus bisporus*) and Oyster (*Pleurotus* spp.) mushrooms are available in the local market to common consumers. Shitake (*Lentinus edodes*) and Milky mushroom (*Calocybe indica*) are other popular mushrooms in Nepal which have been farming on a commercial scale (Raut, 2019). Mushroom industry in Nepal is dominated by small-scale producers. According to the statistics by the Nepal mushroom producer's association, there are around 3000 rural households engaged in mushroom production and related activities.

Mushroom is a rich source of protein, vitamins and folic acid also good source of iron. It is highly suitable for heart disease and diabetes patients its cultivation is practiced worldwide for more than 200 years. In Nepal, in recent years its commercial cultivation has started. It is cultivated in all the districts. Mushroom farming can be done by any one i.e. retired person and house wife also as space requirement is low.

The production follows the upward trend. In 2011/12, the total output of mushroom was only 1530 Mt. which has reached 13,241 tonnes in 2020/21. It indicates that the mushroom industry is gradually taking root in Nepal but the pace is rather slow. With the change in the food habit and the recognition of its nutritional and medicinal values domestic market is also increasing rapidly. Current domestic production is insufficient to meet the local demand. To meet the demands Nepal has been importing a huge amount of mushrooms and its products from other countries annually.

Considering the importance of mushroom in the world market, the Centre for Industrial Entomology Development (CIED) under the Department of Agriculture (DOA) has taken initiative to establish mushroom resource center in Khopasi, Kavrepalanchowk with the objective of conservation and maintenance of native and exotic germplasm as well as production and supply of quality pure culture of mushroom to the commercial mushroom growers.

Use of both desk review and consultation with stakeholders were done. Desk review was targeted to review the past and existing mushroom subsector development policies and program of Nepal and India. Besides the policy documents research and development vision and progress made in India to support the mushroom subsector were reviewed for the importance of dedicated mushroom resource center in Nepal.

For the field study key stakeholders at policy level, research institutions, private mushroom laboratories/farms and mushroom growers' association were selected for key informant's interviews (KIIs) and focus group discussions (FGDs). Checklists of a set of open-ended questionnaires for each stakeholder were prepared to complete the KIIs and FGDs.

A critical analysis of relevant issues and review of the existing policies, status of mushroom cultivation, medicinal and edible value and market dynamics in mushrooms, SWOT analysis of mushroom industry in Nepal, opportunities and challenges of Nepalese mushrooms sub-sector is analyzed and on that basis policy and programme intervention for sustainable development mushroom subsector programs that benefits mushroom growers, consumers and needs of mushroom resource center has been recommended.

The Government of Nepal (GoN) does not have mushrooms production, processing and marketing as well as standard and stable subsidy policy. Moreover, the government has never declared the Minimum Selling Price (MSP) of mushroom. The country is not self-sufficient in mushroom requirement and a large quantity of mushroom has been imported from India to meet the demand of large groups of consumers. The Government didn't paid attention to control the quality of mushrooms' pure culture and spawn as a consequence Nepalese mushroom growers vulnerable to losses.

A variety of culture media can be used to grow vegetative mycelium before it is inoculated on a suitable substrate to grow mushrooms. These culture media are also used as substrate for isolation, multiplication, maintenance and preservation of mushroom cultures. For the convenience of the practical uses a few compositions of different media are (1) Potato Dextrose Agar (PDA) ingredients: 200g diced potato, 20 g agar powder, 20g dextrose or ordinary white sugar, 1 liter water used for isolation of mushrooms (2) Water agar (WA): water 1000 ml, agar 20 grams is used for isolation and culture (3) Media for mushroom mycelium are malt extract, yeast agar; potato dextrose, yeast agar; oatmeal, malt, yeast enriched agar; dog food agar and cornmeal yeast glucose agar.

Mushroom spawn can be prepared on any kind of cereal grains like wheat, maize, pearl millet and sorghum etc. The large grains carry a greater reserve of food material per grain to sustain the inoculums of mushroom mycelium until it is established and feeding on the compost, so they may be more effective in poor compost or adverse conditions, Whereas, the small grains provide more points of inoculums per gram of spawn, so if all the grains of both types grow equally well, the small ones will penetrate the compost sooner.

Although cereal grains are substrate for making spawn of any mushroom variety but due to prohibitive cost a variety of agricultural waste like corn cobs, wooden sticks, rice straw, saw dust and tea leaves etc. have also been used. Sawdust of red wood tree species is preferred for making spawn. Wheat bran ranging from 10-20 percent is mixed with sawdust to increase moisture percentage. Paddy straw can be cut into small pieces of 2-3 cm and soaked overnight before using it as spawn substrate.

Liquid nitrogen is a colorless, odorless, and tasteless gas with a boiling point of  $-196^{\circ}\text{C}$  and a freezing point of  $-210^{\circ}\text{C}$ . It is generated on a large scale in air separation plants. In cryopreservation the storage of microorganisms at ultra low temperatures ( $-196^{\circ}\text{C}$  in liquid nitrogen) is regarded as the best method of culture preservation. At  $-196^{\circ}\text{C}$  dormancy is induced, during which organism does not undergo any change either phenotypically or genotypically, provided adequate care is taken during freezing and thawing. This method can be applied to both sporulating and non-sporulating cultures.

Mother cultures should be kept in a test tube in a deep freezer ( $-85^{\circ}\text{C}$ ), where we cannot get continuous power supply, preservation by the liquid paraffin method is more practical because the method does not require electricity. Do not preserve mother cultures for a long term more than 3 years. Do not repeat sub culturing mother cultures incubated at room temperature, because mutation of mother cultures possibly happens during mycelial growth.

Mushroom technicians and cultivators commonly isolate mushroom strains using three methods: tissue isolation, spore isolation, and substrate mycelium isolation. Tissue culture technique is used to bring the edible mushroom to pure culture so that the mushroom fungus can further be used to prepare spawn, which is an essential material for mushroom cultivation. This nucleus culture is grown on Potato Dextrose Agar medium in test tubes. A small tissue from a well-grown mushroom is aseptically transferred to agar medium in a test tube in a culture room. The test tubes are incubated under room temperature for 10 days for full white growth of fungal culture. This is further used for preparation of Mother spawn.

There are various methods on maintenance and conservation of mushrooms culture and good culture collection center adopts more than one method to preserve them. When a new genus or a species is discovered and described, it is generally deposited in an established germplasm bank. This ensures availability of organism for use in future.

After pure mycelia cultures are obtained a wide variety of methods are available for the conservation of mushroom cultures suitable for a particular need e.g., preservation for a relatively shorter period, or a long period. The choice of preservation method depends upon many factors but the availability of necessary equipments and funds is commonly determining in such decision.

The most common contaminants encountered during spawn preparation are species of *Aspergillus*, *Penicillium*, *Trichoderma*, *Cladosporium*, *Chaetomium*, *Alternaria*, *Mucor*, *Rhizopus*, *Fusarium* and *Drechslera*. Grains are the main source of contamination. The fungal contamination can be usually recognized with the typical colors of their mycelium, spores and conidia. If the contaminants are allowed to grow, they may spoil a large number of spawn bags. If such contaminated bags are not timely removed and disinfected it may become a perennial source of contamination. Selection of good quality grains, proper autoclaving and strict hygiene in spawn lab can reduce the contamination to a great extent.

There should be no greenish or blackish spot in the spawn. Such spots indicated contamination. There should be no slimy liquid in the spawn which indicates bacterial contamination. Old spawn should not be used because its vigor might have decreased.

Grain spawn is produced by growing and expanding a mycelium culture on grain. This process begins by hydrating grains overnight before sterilizing in a pressure cooker. The sterilized grains are inoculated with a live mycelium culture. This grain spawn can take anywhere between 10 days to 3 weeks before fully colonized and ready for use.

Based on climatic and cultural requirements, six species of mushrooms are recommended for cultivation in Nepal. They are: button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus sps.*), paddy straw mushroom (*Volvariella volvacea*), Shiitake (*Lentinus lentinula*) milky mushroom (*Calocybe indica*) and Red mushroom *Ganoderma lucidum*. The cultivation of oyster mushroom is done during winter season in Terai region (22-26° C) and use as summer crop in the hills of Nepal (25-30° C). Oyster mushroom cannot be grown in terai during the summer season due to high temperature (30 -40° C). Milky mushroom requires 30-35°C and RH 80-90% is maintained for entire cropping cycle. Favorable temperature for Shiitake mushroom cultivation at 22-26°C, humidity of 80-85%, diffused light and well ventilated conditions, The cultivation technology of red medicinal mushroom *Ganoderma lucidum* requires an optimum temperature of 30-32°C, humidity of 80-85%.

Cultural control, including sanitation, composting and pasteurization is the basis for successful mushroom culture. Cultural practices that can reduce pest fly populations include exclusion, sanitation (washing and sanitizing), shortening crop cycles and post-harvest steam cleaning are the management practices of mushroom flies. Use of clean compost, pasteurization or sterilization of casing soil, good peak heating and fumigation of mushroom house and use of carbendazim/benomyl/ chlorothalonil/TBZ or prochloraz manganese fungicide for the effective management of wet bubble. Yellow mold is found growing in between the compost layer or at the bottom layer. Extract of *Cannabis sativa* is very effective in reducing the growth of yellow mould pathogens without affecting the growth of *A. bisporus* when added in malt extract agar medium @ 5% and use of pasteurized compost, sterilized casing soil may be a good alternative along with addition of P<sub>2</sub>O<sub>5</sub> (0.5%) in compost to prevent crop losses due to yellow mould syndrome.

Besides sun drying, mushrooms can be dried in cabinet dryers at a drying temperature of 55-60°C which gives dehydrated final product of lower moisture content with longer shelf life and better quality.

Mushrooms can be canned whole, sliced and stems and pieces as per demand. The canning process involves cleaning, blanching (5-6 minutes at 95-100°C), filling into can with brine solution (2% salt with 0.1% citric acid or 100 ppm ascorbic acid), and sterilization by heat (118°C), cooling, labelling and packaging.

For preparing mushroom murabba, fresh button mushrooms are graded, washed, pricked and blanched in 0.05% KMS solution for 10 min. Blanched mushroom are then dipped in 50 °Brix sugar solution and refrigerated overnight. Next day mushroom are strained out of sugar solution and to the solution we add 0.1% citric acid and sufficient sugar and heat to attain strength of 60 °Brix. Mushrooms are then again dipped into it and kept overnight. This process is repeated to raise the concentration of syrup to 70 °Brix and mushrooms are dipped into it for 1 week to prepare preserve. The preserve is then drained out of sugar syrup and filled in a container with freshly prepared sugar syrup of 68 °Brix. The containers are then sealed airtight and stored.

The process for making candy is practically the same as that employed for mushroom preserve, with the difference that the produce is impregnated with a higher concentration of sugar (75° Brix) and is also partially dried under shade to attain the chewable consistency. The mushroom candy can be stored up to 8 months with excellent acceptability.

For preparing mushroom chips, freshly harvested button mushrooms are washed, sliced (2 mm) and blanched in 2% brine solution. The mushrooms are dipped overnight in a solution of 0.1% of citric acid + 1.5% of NaCl + 0.3% of red chilli powder. After draining off the solution, the mushrooms are subjected to drying in cabinet dryer at 60°C for 8 hr. Then it is fried in the refined oil and good quality chips are prepared. After mixing the spices, the chips are packed in polypropylene packets and sealed.

Both button or oyster mushroom can be used to prepare delicious and nutritious mushroom biscuits using ingredients viz., refined wheat flour (maida) & mushroom powder ( in 80:20 or 90:10 ratio), sugar (30%), ghee (bakery fats) (45%), baking powder (0.6%), ammonium bicarbonate (0.3%), salt (0.6%), milk powder (1.5%) and vanilla essence (0.02%). For making biscuits all the dry ingredients are finely ground and sieved. Then fat and sugar are mixed well for 5-7 minutes using Dough kneeder. These ingredients are then added to dough kneeder with other dry ingredients for dry mixing. Thereafter, water is added to make dough cohesive and homogenous and mixing is continued till fully done. Then dough is kept for 10 minutes covered with wet cloth. Thin sheets of dough (1.25 cm thick) are made and cut into different shapes of biscuits using different steel dies. These raw cut biscuits are then baked in hot oven (at 180°C) for 20 minutes and after cooling biscuits are ready for packaging.

For preparing mushroom soup powder, dried oyster or button mushrooms are finely ground in a pulverizer to pass through 0.5 mm sieve. This mushroom powder (20g) is then mixed with milk powder (25g), corn flour (40g), salt (8g), sugar (3g), black pepper (2g) and oregano (2g). This soup mix has to be mixed with 6 times quantity of water for the preparation of good quality mushroom soup with characteristic aroma and taste.

The production of spent mushroom substrate (SMS) after crop harvest is a matter of concern because it creates various environmental problems including ground water contamination and nuisance if not handled properly. SMS obtained from various sources vary in its physical and chemical properties. Treatments like rapid salt leaching and re-composting by aerobic or anaerobic methods for one to two years make SMS more suitable for growing flowers, vegetables, fruit, saplings, ornamental shrubs and other horticulture plants of economic importance.

NARC and private sectors are the source of mushroom seed. Quality Spawn Production is a great challenge in case of private laboratories and Nepal's mushroom industry is the lack of quality assurance in the seed (spawn) supply system so that true to type and quality seeds (spawn) could be ensured. Many devastating cases have been reported by producers due to contaminated spawn that in turn leading to large financial losses. Spawn contamination caused by poor production infrastructure, improper handling, poor inventory management, and inaccurate measurement are the serious issues of mushroom seed (spawn) industry of Nepal. It is imperative that lack of regulatory institutions and the seed (spawn) standards in the mushroom subsector in the country are the major reasons for

such consequences. Country lacks the institutional arrangement dedicated to the mushroom subsector development. Similarly, poor human resource development is another weak area in this sector. Though the country is endowed with lots of landraces of mushrooms, country is still to explore the potential native mushroom species that have commercial value. Maintenance of the promising germplasms and production of its pure culture is the far-reaching task of the public institution at this juncture. With the advancement of the mushroom subsector, producers should also be updated with the latest technologies; however, there is dearth of reliable institution to provide such service. Similarly, producer farmers can rarely find the places where they can have easy access to the exposure of the proven technology where they can build their confidence witnessing the result of the demonstration of mushroom production and post-harvest technologies. In the present situation there is no any provision and system and authority of registration of the germplasm, landraces and varieties of mushrooms. Similarly, lack of quality control and lack of technical back up on spawn production are major

To meet the challenges of mushroom industry in Nepal, a dedicated mushroom resource center is urgently needed to provide pure cultures for the production of mushroom spawn and also to provide facilities for the conservation of exotic and locally available edible and medicinal mushroom species.

The proposed mushroom resource center is located within the boundary of Sericulture Farm at Khopasi, Kavrepalanchowk district, facing North-South, 28.2 km east of Kathmandu and 9.1 km south from Banepa Bazar. The climate in Khopasi is warm and temperate. In winter, there is much less rainfall than in summer. In Khopasi, the average maximum and minimum annual temperature is 22.24 °C and 11.28°C, respectively. Precipitation here is about 126 inch per year and a sunshine hour was 5.55 hours and facing of land is such that in daylight sunlight utilization efficiency is enough. Average wind speed of the site is 4.38 km/hr. The large and open area with river on the east and community forest on the west of Khopasi farmland makes it well ventilated and atmosphere is suitable with proper air circulation. Even during hot and summer the temperature of the site remains normal. Very few residential buildings exist nearby resulting in minimal human activities which is less probable to increase in coming 15 to 20 years making the area safe for the construction of the Mushroom Laboratory. It has clay silt with trace of sand soil with steep topography of the land, drainage problem does not exist which prevents the probability of contamination of different pathogens and insect pests on mushrooms. Abundant, clean and non-contaminated underground water supply system makes more sustainability of the center. Contamination of culture/spawn due to livestock can be prevented as livestock farming is limited and not so popular. The area is clean without any dumping area around so the landsite is very suitable for agronomical activities. No industries nearby and nature friendly environment with suitable air quality. Good transportation facilities as proper condition BP Highway near Kathmandu can be used. Unlimited Power Supply at Khopasi/Panauti area is well known for Electricity generation.

The mushroom resource center at Khopasi, Kavrepalanchowk will provide pure cultures and also will have adequate facilities for the conservation of exotic and locally available edible and medicinal mushrooms species. The Centre will have a "Farmers Service Unit" to attend to the problems of the growers with regard to mushrooms crop management,

diseases, pests, substrate quality etc. The centre will analyse and test the samples of mushrooms for diseases/pests and substrates/casing brought by mushroom farmers.

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## 1. BACKGROUND

The tomorrow's agriculture will require complete recycling of nutrients, water and agro-wastes. The increasing population, decreasing per capita arable land, urbanization and industrialization, changes in climate, demand for quality and functional foods, etc. will necessitate us to focus on secondary agriculture and novel crops like mushroom. Diversification in any farming system imparts sustainability and mushrooms are one such component. Commercial production of edible mushrooms represents unique exploitation of the microbial technology for the bioconversion of the agricultural, industrial, forestry and horticultural wastes into nutritious food.

Mushrooms are considered to be the highest producer of protein per unit area and time. Water requirement for mushroom cultivation is far less (25 liters per kg fresh mushrooms) (<https://dmrsolan.icar.gov.in/>) than field crops as they are cultivated indoors. This implies that mushroom has the potential of being a major crop in coming years.

Nepal agriculture will continue to be a main strength of Nepali economy. With the variety of agricultural crops grown today, Nepal is in the process of achieving food security. However, our struggle to achieve food and nutritional security is still on. In future, the ever-increasing population, depleting agricultural land, changes in environment, water shortage and need for quality food products at competitive rates are going to be important issues. To meet these challenges and to provide food and nutritional security to our people, it is important to diversify the agricultural activities in areas like horticulture.

Diversification in any farming system imparts sustainability. Mushrooms are one such component that not only impart diversification but also help in addressing the problems of quality food, health and environment related issues. One of the major areas that can contribute towards goal of conservation of natural resources as well as increased productivity is recycling of agro-wastes including agro-industrial waste. Utilizing these wastes for growing mushrooms can enhance income and impart higher level of sustainability.

### 1.1. Health benefit and nutritional value of mushrooms

- Mushrooms are edible fungus. They provide a range of antioxidants and other nutrients that may contribute to heart health and protect the body from cancer, among other benefits.
- Mushrooms contain protein, vitamins, minerals, and antioxidants.
- The antioxidant content in mushrooms may help prevent lung, prostate, breast, and other types of cancer.
- The fiber, potassium, and vitamin C in mushrooms may contribute to cardiovascular health.
- Oyster and shiitake mushrooms are believed to have the most effective beta glucans that helps to regulate blood sugar, reducing the risk of type 2 diabetes.

Mushroom is considered to be a complete, health food and suitable for all age groups, child to aged people. The nutritional value of mushroom is affected by numerous factors such as species, stage of development and environmental conditions. Mushrooms are rich in protein, dietary fiber, vitamins and minerals. The digestible carbohydrate profile of

mushroom includes starches, pentoses, hexoses, disaccharides, amino sugars, sugar alcohols and sugar acids. The total carbohydrate content in mushroom varied from 26-82% on dry weight basis in different mushrooms. The crude fibre composition of the mushroom consists of partially digestible polysaccharides and chitin (<http://agridaksh.iasri.res.in/html>).

Mushrooms do not have cholesterol. The protein content of edible mushrooms is usually high, but varies greatly. The crude protein content of mushrooms varied from 12 – 35% depending upon the species. The free amino acids composition differs widely but in general they are rich in threonine and valine but deficient in Sulphur containing amino acids (ethionine and cysteine) (<http://agridaksh.iasri.res.in/html>). Nutritive values of different mushroom are given in Table 1.

**Table 1: Nutritional compositions in mushrooms**

<b>Nutrient</b>	<b>Common mushroom</b>	<b>Shiitake mushroom</b>	<b>Oyster mushroom</b>
Moisture (g/100 g)	92.45	89.74	89.18
Energy (kcal/100 g)	22	34	33
Protein (g/100 g)	3.09	2.24	3.31
Fat (g/100 g)	0.34	0.49	0.41
Ash (g/100 g)	0.85	0.73	1.01
Carbohydrate (g/100 g)	3.26	6.79	6.09
Dietary fibre (g/100 g)	1	2.5	2.3
Ergosterol (mg/100 g)	56	85	64
Calcium (mg/100 g)	3	2	3
Copper (mg/100 g)	0.32	0.14	0.24
Iron (mg/100 g)	0.5	0.41	1.33
Magnesium (mg/100 g)	9	20	18
Manganese (mg/100 g)	0.05	0.23	0.11
Phosphorus (mg/100 g)	86	112	120
Potassium (mg/100 g)	318	304	420
Selenium (µg/100 g)	9.3	5.7	2.6
Sodium (mg/100 g)	5	9	18
Zinc (mg/100 g)	0.52	1.03	0.77
Thiamin (mg/100 g)	0.081	0.015	0.125
Riboflavin (mg/100 g)	0.4	0.22	0.35
Niacin (mg/100 g)	3.61	3.88	4.96
Pantothenic acid (mg/100 g)	1.5	1.5	1.29
Pyridoxine (mg/100 g)	0.1	0.29	0.11

**Source:** USDA, Food Data Central. 2019. Available from: <https://fdc.nal.usda.gov>

Mushroom has got diverse applications as food articles such as vegetable, soup, food additive, edible powder, water extract, alcoholic extract, tonic capsule, etc (Jayaraman,1992 and Joshi, 2005). Mushrooms contain various nutritional components like carbohydrates, adenosines, terpenoids, hormones, proteins, vitamins, amino acids, fibers, minerals, essential oils, steroids, etc.

## 1.2. History of Mushroom Cultivation

Mushroom farming has a long and fascinating history, dating back to ancient times. The earliest recorded evidence of mushroom cultivation comes from China, where farmers began growing shiitake mushrooms over 1,000 years ago. However, the practice of cultivating mushrooms for food is thought to have started much earlier, as early as 600 AD with the *Auricularia auricula* on wood logs (Neupane, 2068). Initiation of mushroom cultivation was done by the Division of Plant Pathology, Nepal Agricultural Research Council (NARC) in the year 1974. Cultivation of mushroom in Nepal is *Agaricus bisporus* (1977/1978), Oyster mushroom (*Pleurotus* spp.) (1983/84), *Volvariella volvacea* (1981/1982), Shiitake Mushroom (*Leninula edodes*) (1979-1980), Jelly Mushroom (*Auricularia auriculata*) (1998), Red Mushroom (*Ganoderma lucidum*) (2004), King's Oyster Mushroom (*Pleurotus eryngii*) (Pa rajuli, 2014). *Agaricus bisporus* is the most cultivated variety in the world also called as table mushroom.

## 1.3. Global Mushroom Production

The world mushroom production has increased at a rapid rate since the late 1990s. The production has increased more than 10-fold during the last four decades (Figure 1). As per the FAO statistics 2022, the most notable increases occurred in China, Japan, USA, the Netherlands and India. China has long been the world's largest mushroom producer country. China's present share in the total world mushroom production is more than 70% which was only 5.7 % in 1978 and has maintained an annual growth rate of 10% for the last three decades. Mushroom is their sixth economically important crop in terms of country's revenue generation (Zhang et. al., 2014). The second largest mushroom producing country is Japan followed by the United States of America, the Netherland, India, Poland, Spain, Canada and UK (Table 2).

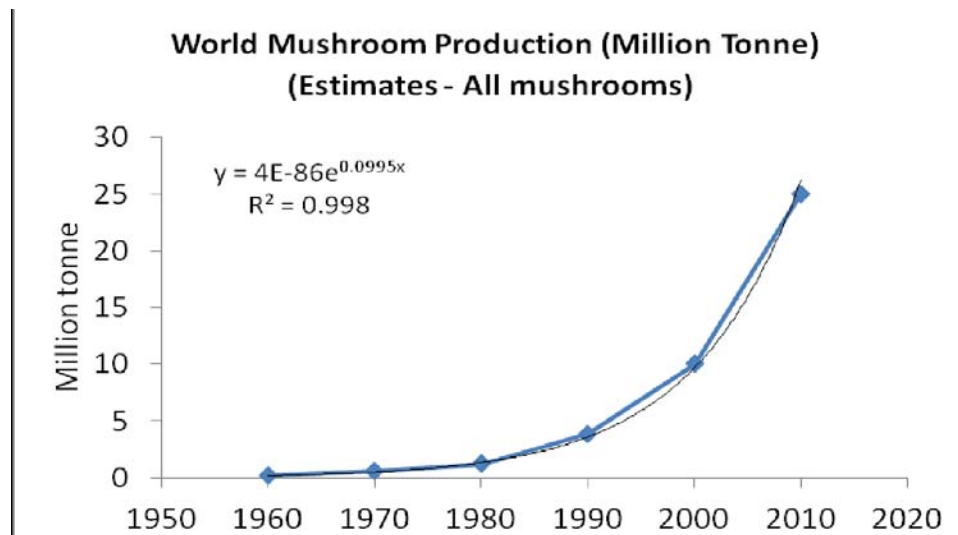


Figure 1: World Mushroom Production (FAO Stat) (in lakh tons)

Most of the mushrooms are wild, yet considerable species are cultivated worldwide. About 38,000 species of mushrooms are known in the world, out of which around 2,000 species

are edible (Chang, 2006). Some of the major cultivated mushroom species are given in Table 2.

**Table 2: Cultivated Species of Mushrooms in the World**

<i>Agaricus bisporus</i>	<i>Ganoderma lucidum</i>	<i>Pholiota adiposa</i>	<i>Pleurotus pulmomarius</i>
<i>Agaricus bitorquis</i>	<i>Grifola frondosa</i>	<i>Pholiota nameko</i>	<i>Pleurotus sajor-caju</i>
<i>Agaricus blazei</i>	<i>Hericium erinaceus</i>	<i>Pleurotus abalonus</i>	<i>Pleurotus salmoneostramineus</i>
<i>Agrocybe cylindracea</i>	<i>Hypsizigus marmoreus</i>	<i>Pleurotus columbinus</i>	<i>Sparassis crispa</i>
<i>Auricularia auricula</i>	<i>Lentinula edodes</i> (Shiitake)	<i>Pleurotus cornucopiae</i>	<i>Stropharia rugosa-annulate</i>
<i>Auricularia polytricha</i>	<i>Lepista nada</i>	<i>Pleurotus eryngii</i>	<i>Tremella fuciformis</i>
<i>Colocybe indica</i>	<i>Lepista sordida</i>	<i>Pleurotus flabellatus</i>	<i>Volvariella volvacea</i>
<i>Coprinus comatus</i>	<i>Lyophyllum decates</i>	<i>Pleurotus nebrodensis</i>	
<i>Dictyophora indusiata</i>	<i>Lyophyllum shimeji</i>	<i>Pleurotus opuntiae</i>	
<i>Flammulina velutipes</i>	<i>Naemarioloma sublaterinium</i>	<i>Pleurotus ostreatus</i>	

Source: Chang, 2006

China is the main producer of cultivated edible mushrooms with about 967 species, which is considered as almost 50% of the cultivable edible mushroom species worldwide. Two hundred and forty species could be commonly found in the market, and more than 60 species were commercially cultivated across China (Li and Xu, 2022).

**Table 3: Top 10 Mushroom Producing Countries in 2020**

Rank	Country	Tons	% of Top 20
1	China	44092400.00	95.26
2	Japan	520080.81	1.12
3	USA	408163.37	0.88
4	The Netherlands	286600.60	0.62
5	India	221564.31	0.48
6	Poland	201612.50	0.44
7	Spain	182994.48	0.40
8	Canada	146154.18	0.32
9	UK	116470.07	0.25
10	Iran	109980.78	0.24

Source: FAO/ 2020 (Rob Cook)

#### 1.4. Scope and Status of Mushroom Production in Nepal

In Nepal, it is estimated that the production of mushrooms has increased about 8.65 times over a decade until 2021 (Table 4). Nepal is blessed with a range of ecosystems that really are ideal for mushroom production. Different mushroom species have been commercially

cultivated in different parts of Nepal. Mushroom farming is going popular day by day and it is a new farming concept of Nepal. Before some year mushroom isn't cultivated in Nepal, people were dependent upon wild mushroom. But now there are many different commercial mushroom farming in Nepal that produces different kinds of mushroom. And people like it day by day but the production is so low that to fulfill the demand of people they have to import it from India. Due to high demand of mushroom among people, there could be high scope of mushroom cultivation in Nepal.

Button mushroom (Gobrechyau) was first introduced in Nepal in 1977 and recommended to farmers for commercial cultivation. Shiitake mushroom, Oyster mushroom, Jelly ear mushroom, Red mushroom (Ratochyau) King's Oyster mushroom, and milky mushroom were introduced in 1979-1980, 1981, 2004, 2012, 2015, respectively.

**Table 4: Status of Mushroom Production in Nepal**

Year	Fresh Mushroom Production (Mt.)	Mushroom Seed Production (Bottle)
2010/11	1530	268,560
2011/12	1,530	268,560
2012/13	1,650	289,624
2013/14	1,900	333,505
2014/15	2,700	425,000
2015/16	9,300	1,488,000
2016/17	10,850	1,545,000
2017/18	10,500	1,540,000
2018/19	11,255	1,588,700
2019/20	12,607	1,599,435
2020/21	13,241	1,600,552

**Source:** Statistical Information on Nepalese Agriculture 2077/78 (2020/21)

In Nepal, there is great potential for year-round farming of a wide variety of mushrooms due to congenial agro-climatic condition and availability of wide variety of raw substrates (agricultural & forestry wastes). Even today only a handful of species are grown commercially. Mainly white button (*Agaricus bisporus*) and Oyster (*Pleurotus* spp.) mushrooms are available in the local market to common consumers. Shitake (*Lentinus edodes*) and Milky mushroom (*Calocybe indica*) are other popular mushrooms in Nepal which have been farming on a commercial scale (Raut, 2019). Mushroom industry in Nepal is dominated by small-scale producers. According to the statistics by the Nepal mushroom producer's association, there are around 3000 rural households engaged in mushroom production and related activities.

The production follows the upward trend. In 2011/12, the total output of mushroom was only 1530 Mt. which has reached 13,241 tonnes in 2020/21 (Table 4). It indicates that the mushroom industry is gradually taking root in Nepal but the pace is rather slow. With the change in the food habit and the recognition of its nutritional and medicinal values domestic market is also increasing rapidly. Current domestic production is insufficient to meet the local demand. To meet the demands Nepal has been importing a huge amount of mushrooms and its products from other countries annually.

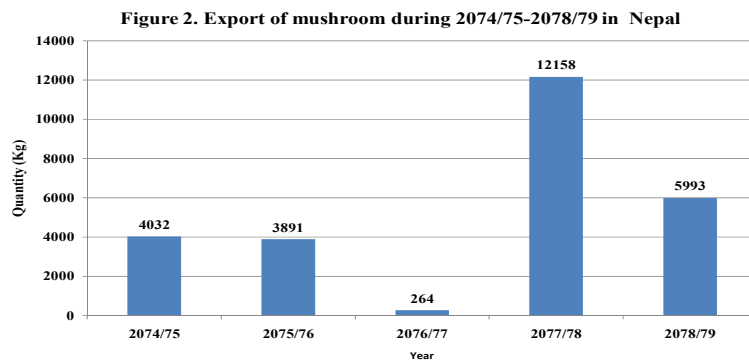
Organized research and production of mushroom in Nepal was started after the establishment of Mushroom Research Unit in Plant Pathology Division-NARC, Khumaltar

in 1974. Research and production program was mainly concerned on seven different mushrooms, namely Button Mushroom (*Agaricus bisporus*), Oyster Mushroom (*Pleurotus* sp.), Shiitake Mushroom (*Lentinula edodes*), Straw Mushroom (*Volvariella volvacea*), Jelly ear Mushroom (*Auricularia auriculata*), Red Mushroom (*Ganoderma lucidum*) and Milky mushroom (*Calocybe indica*). Kings Oyster (*Pleurotus eryngii*)

The mushroom industry is one of the fastest growing sub sectors of agriculture in Nepal. Involvement of private sectors is increasing and support services from the public sectors have been very limited. In Nepal the biggest challenges facing mushroom farmers in the finding a reliable source of mushroom culture and spawn, which are the material used to start the growth of mushrooms. At the time, the private mushrooms farm/laboratories have been importing pure culture from abroad, but it was often unreliable and expensive.

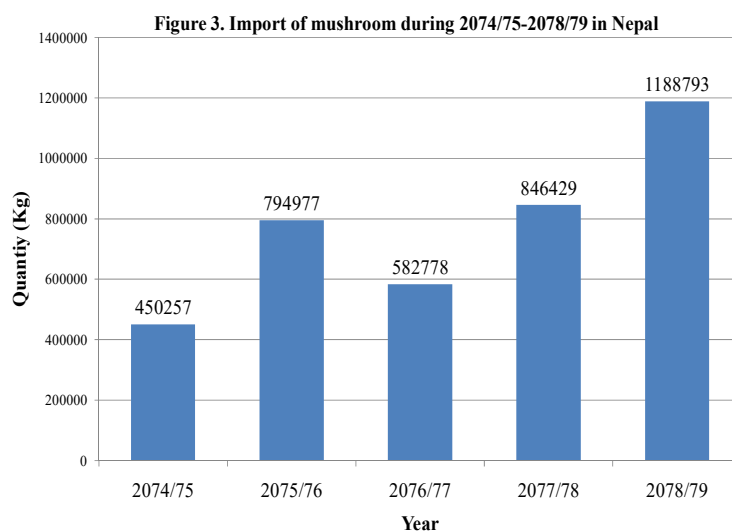
#### 1.4.1. Export and Import of Mushrooms in Nepal

The value of exports of mushrooms, fresh or chilled to Nepal totaled was very nominal as compared to import during the periods of five years from BS 2074/75 to 2078/79. The export of mushrooms, and truffles prepared or preserved otherwise than by vinegar or acetic acid from Nepal totaled 4032 kg in 2074/75 where as in 2078/79 it was 5993 kg (Figure 3).



**Figure 2: Export of Mushroom during 074/75-078/79**

The import of mushrooms to Nepal from other countries over the last five years has seen a significant increase. The value of imports of mushrooms and truffles prepared or preserved otherwise than by vinegar or acetic acid to Nepal total from BS 2074/75 to 2078/79 is presented in Fig 3. The import of mushroom to Nepal in 2078/79 went up by 264% compared to 2074/75. It seems that in future there is high scope of mushroom industry in Nepal.



*Figure 3: Import of Mushroom during 074/75-078/79*

## 1.5 Major challenges and constraints in mushroom subsector in Nepal

### 1.5.1. Major challenges

- Limited scientific research and lack of latest improved technology
- Insufficient investment and inadequate scientific research on mushrooms
- Increasing the price of raw substrate mainly rice straw and wood dust
- Poor quality of spawn
- Poor farm management practices/diseases & pest attack
- Poor harvest management
- Inadequate promotion and marketing activities
- Unstable farm-gate prices and profit margins
- Competition from neighboring countries
- Lack of appropriate mushroom policies and laws
- Government policies are not favorable for small land holders and landless mushroom growers.
- Unavailability of quality spawns throughout the country due to low production or urban oriented production.
- Conservation and maintenance of the promising germplasms and production of its pure culture is the far-reaching task
- Lack of a critical mass of well-trained mushroom technicians and growers to operate mushroom enterprises efficiently and successfully
- Lack of regulatory institutions and quality assurance in the seed (spawn) supply system
- Only exotic cultivars are in the farming system

## **1.5.2. Major constraints**

### **1.5.2.1. Low technological adoption:**

- Low technological options available for interventions:
- Available technologies are costly unless machineries are used
- Adoption of developed technologies are very low
- Technology dissemination: Negligible government involvement in extension not any dedicated organization

### **1.5.2.2. Socioeconomic and policy related constraints**

- No mushroom price fixation
- Perishable commodity- handling and storage facilities limited
- Farmer and consumer awareness
- Specific insurance scheme for mushroom farmers
- Unhealthy marketing

### **1.5.2.3 High cost of production**

- Small-scale and labor-intensive farming
- Higher cost of inputs
- Low productivity
- No mechanization
- Poor technical feedback

There is no technical standard of mushroom seed/spawn production laboratories in Nepal. Weak regulation and no quality assurance and certification system always lead to poor quality of spawn production. So, it needs to managing and generating a sufficient number of well-trained and experienced human resources and creating distinct quality assurance and certification system to standardize the spawn products.

Many spawn producers are not well equipped and trained because of which quality of spawn they produce does not yield effective results. Most of the times the mother culture used by spawn producers are produced by themselves and quality of culture is not reliable. In practice, spawn producers have difficulty to obtain good quality mother culture and source of same is not reliable

Degeneration and mutation of culture, and incidence of bacterial (*Bacillus* spp.) and fungal pathogens (common moulds such as *Trichoderma* spp., *Neurospora* sp.etc.) are some of the problems faced by spawn producers

Quality spawn production is a great challenge in case of private laboratories. No registration, lack of quality control and lack of technical back up on spawn production. Mushrooms resource center is an immediate need for quality control and research.

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## 2. RATIONALE OF THE STUDY

The mushroom industry one of the fastest growing sub sectors of agriculture in Nepal. However, the growth has not been supported by research and effective input supply system. There is very limited scientific research and discourse, insufficient technologies. The increasing growth of mushroom production and consumption in Nepal seems a viable and attractive option that supports poverty reduction aim of government. But while demand for mushrooms continues to grow rapidly and hence the involvement of private sectors is also increasing, support services from the public sectors have been very limited to address the demand of efficient and effective technologies, quality inputs and associated quality regulation.

One of the biggest challenges facing by Nepal's mushroom industry is the lack of quality assurance in the seed (spawn) supply system so that true to type and quality seeds (spawn) could be ensured. Many devastating cases have been reported by producers due to contaminated spawn that in turn leading to large financial losses. Spawn contamination caused by poor production infrastructure, improper handling, poor inventory management, and inaccurate measurement are the serious issues of mushroom seed (spawn) industry of Nepal. It is imperative that lack of regulatory institutions and the seed (spawn) standards in the mushroom sub sector in the country are the major reasons for such consequences. Country lacks the institutional arrangement dedicated to the mushroom sub sector development. Similarly, poor human resource development is another weak area in this sector. Though the country is endowed with lots of landraces of mushrooms, country is still to explore the potential native mushroom species that have commercial value. Maintenance of the promising germplasms and production of its pure culture is the far reaching task of the public institution at this juncture. With the advancement of the mushroom sub sector, producers should also be updated with the latest technologies; however, there is dearth of reliable institution to provide such service. Similarly, producer farmers can rarely find the places where they can have easy access to the exposure of the proven technology where they can build their confidence witnessing the result of the demonstration of mushroom production and post-harvest technologies.

In this context, Government resources allocation based on the growth and outcome of mushroom sub sector is highly justified and establishment of a dedicated institution that stands as the National Mushroom Resource Center (NMRC) would be one of the pivotal steps of government initiatives in this sector. The NMRC has been conceptualized to provide following services:

- a. Collection and preservation of exotic and native strains of mushroom in the country.
- b. Produce true to type certified pure culture and distribute it as per the demand of the country for the research and commercial use.
- c. Provide the advance level training to the technicians, mushroom producers and spawn producers.
- d. Conduct adaptive research in mushroom production and post-harvest technologies and manage the year round demonstration of the advance and protected (controlled conditions) mushroom production technologies to the researcher, technicians and producers.

In this context, CIED has approved the program for an in-depth study for establishment of proposed NMRC. Part of the farm land of the Sericulture Development Center, Khopasi, Kavrepalanchok has been considered as the possible location for the establishment of NMRC. This study would appraise the suitability of the NMRC at that location and prepare the detail plan for the establishment of the NMRC as detailed in this TOR of the study.

### **3. OBJECTIVES**

The overall objective of the assignment is to develop detail plan for the establishment of the NMRC in part of the farm land of the Sericulture Development Center, Khopasi, Kavrepalanchowk District of Nepal and prepare the detail technical and financial plan associated with the establishment and operation of that center.

The specific objectives of the assignment are:

1. To review and draw the lesson learnt from the provision of policy, regulation, institutional arrangement for research and development and working mechanism of government institution associated with mushroom.
2. To review the status of mushroom sub sector in Nepal with due focus on production, technology adoption, seed (spawn) supply, regulation, technology development, extension of the improved technologies, demand and supply of mushroom and its seed (spawn) as well as its forecast.
3. To assess the need of mushroom resource center in Nepal.
4. To assess the topographical, meteorological, socio-economic and technical suitability for the establishment of proposed NMRC in the farm land of the Sericulture Development Center, Khopasi, Kavrepalanchok.
5. To prepare details of mandate, functions and organizational setup of the proposed NMRC with due consideration of federal structure and functional linkages among various institutions.
6. Detailing of the feasible land area including Topographic survey, Soil test and other requirements as per site condition.
7. To prepare the detail master plan including layout, detail structural designs of infrastructures, Detail cost estimates of individual components and in common, Bill of quantities ( BOQ) , specification of the various components/units of proposed NMRC, in the given location.
8. The detail design and cost estimates should include the required HVAC, elcetromechanical and specific requirements to intended mushroom species and purpose.
9. Construction plan of the purposed infrastructures.

## **4. METHODOLOGY**

### **4.1 General**

In order to achieve the aforementioned objective, the use of desk review, field visit and consultation with stakeholders.

### **4.2. Desk Review**

Desk review was targeted to review the past and existing mushroom subsector development including environment requirements, health benefit and nutritional value of mushrooms, demand and supply, organizations and institutions associated with mushroom subsector, mushrooms spawn supply chain, mushroom genetic resource acquisition, conservation, maintenance and utilization, pure culture production, quality control, laboratories and equipment associated with pure culture production and quality control, post-harvest technologies and transfer of mushroom related technologies.

### **4.3. Field Study**

For the field study stakeholders were MoALD, Central Plant Pathology Center-NARC, Genebabank-NARC, Central Biotechnology Center-NARC, Nepal Academy of Science and Technology (NAST), Nepal Mushroom Producer Association (NMPS) and Private Mushroom Laboratories/Farms for the Key Informant Interviews (KIIs) and discussion. The Chief of Agriculture development Division at MoALD the Chief of Central Plant Pathology Center-NARC, Genebabank-NARC and Central Biotechnology Center-NARC; the secretary of Nepal Academy of Science and Technology (NAST); the former and present Chairman of Nepal Mushroom Producer Association (NMPS) and owners (Ares mushroom) of private mushroom laboratories/farms Thankot, Kathmandu were consulted for KIIs and discussion and the collected information from each stakeholder were critically analyzed.

### **4.4 Key Informants Interview (KII)**

Federal Ministry of Agriculture and Livestock Development, National Plant Pathology Research Center-NARC, Nepal Academy of Science and Technology (NAST), Nepal Mushroom Producer Association (NMPS), Private Mushroom Laboratories/Farms and concerned stakeholders etc. were interviewed using key informant interview tool. (Annex1).

### **4.5. Stakeholders workshop**

During the feasibility and preparatory study the inception report was presented by the team leader of the consultant's on BS 2079/11/08 at the meeting hall of CIED Hariharbhawan, Lalitpur in the chairmanship of Mr. Bhoj Raj Shapkota the Director of CIED. Acting DG, NMPS and mushroom and fungi exports were participated. The second stakeholders' workshop was organized at the meeting hall of CIED on BS 2079/12/29 after the preparation of final draft including the suggestions and comments received from inception workshop. The queries, comments and suggestions received from the participants were included in the final report. The final report was presented on BS 2080/3/15 at the meeting hall of CIED Hariharbhawan. The DG of DOA, joint secretary or the chief of the planning, monitoring and foreign aid from MOALD, ADGs from DOA, representative from NARC, SQCC, fungi and mushroom exports and other officials from DOA and CIED were participated in the meeting. The comments and suggestions received from the higher authorities, exports and other participants were included in the final report. The list of the participants in all the three meeting is given in Annex 5.

#### **4.6. Working team composition**

**Team Leader:** The team leader has Master Degree in Plant pathology and more than 30 years work experience in mushroom research and development (gremplasm collection, conservation, characterization and maintenance; pureculture/motherculture production, spawn production; pest and diseases of mushrooms and commercial mushroom production technology of different types of mushrooms). In addition to research work the team leader has expertise on postharvest technology and value addition of mushroom and transfer of mushroom related technologies to the mushroom farmers and entrepreneurs. The team leader bears good knowledge and skills to engagement in policy formulation in close coordination with public and private sector institutions.

#### **Mushroom Expert/Agriculture Expert:**

The mushroom/Agriculture expert has Ph.D. Degree in Agriculture with more than 30 years of professional experience in mushroom sub sector and tissue culture. The agriculture expert has excellent knowledge and experience in the areas of mushroom culture/spawn production and tissue culture. He has also good knowledge and experience on planning, coordination and management of the project.

#### **Structural Engineer:**

The structural engineer bears Masters Degree in structural engineering with more than 10 years' experience and he has good capacity to coordinate with the various team members.

#### **Mechanical Engineer**

The Mechanical Engineering has Master Degree in Mechanical Engineering with minimum 15 years of experience in heating, ventilation, and air conditioning (HVAC).

#### **Electrical engineer**

The Electrical engineer has Master Degree in Electrical Engineering with more than 10 years' experience in related field.

#### **Geomantic Engineer/Surveyor:**

The survey Engineer has minimum Master Degree in Civil Engineering with 5 years of experience in relevant fields.

#### **Event Coordinator and Computer operator:**

In the team the consulting firm hired an event coordinator and computer operator as and when needed and they have bachelor and intermediate degree, respectively with five years' experience in relevant field.

## **5. REVIEW OF MUSHROOM RELATED POLICIES, PROGRAMS AND POLICY INTERVENTIONS**

### **5.1 General Review**

**Mushroom spawn source center:** Many devastating cases have been reported by producers due to contaminated spawn that in turn leading to large financial losses. Spawn contamination caused by poor production infrastructure, improper handling, poor inventory management, and inaccurate measurement are the serious issues of mushroom seed (spawn) industry of Nepal. It is imperative that lack of regulatory institutions and the seed (spawn) standards in the mushroom subsector in the country are the major reasons for such consequences.

**Dedicated institutional arrangement:** Country lacks the institutional arrangement dedicated to the mushroom subsector development. Maintenance of the promising germplasms and production of its pure culture is the far reaching task of the public institution at this juncture. Similarly, producer farmers can rarely find the places where they can have easy access to the exposure of the proven technology where they can build their confidence witnessing the result of the demonstration of mushroom production and post-harvest technologies.

**Introduction of advanced and recent technologies:** Though the country is endowed with lots of landraces of mushrooms, country is still to explore the potential native mushroom species that have commercial value. With the advancement of the mushroom sub sector, producers should also be updated with the latest technologies; however, there is dearth of reliable institution to provide such service. Similarly, poor human resource development is another weak area in this sector.

**Farmers' gate minimum selling price:** The role of the middleman is a serious threat in the field of mushroom farming in Nepal. Middlemen fix the price and they impose a low price. The total profit received by the farmer is low. The market price is very low and the farmer compels to bear the loss. In Nepal there is no any provision of farmers' gate minimum selling price of mushroom.

**Ineffective quarantine service:** Mushroom is coming into the Nepalese market without any administrative barriers from India. It is cheap since there is approximately a 50 percent subsidy to the farmer. The government is not using quarantine service in the border check post for the quality and edible test.

Similar practices in neighboring countries on establishment and operation of mushroom resource center

### **India**

In India the National Centre for Mushroom Research and Training (NCMRT) came into existence at Solan (later renamed as National Research Centre for Mushroom) in 1983. It was upgraded to Directorate of Mushroom Research (DMR) on 2008. It was established with the objectives of undertaking research on all aspects of mushrooms and also to impart training to the trainers and growers. Presently, All India Coordinated Research Project on Mushroom (AICRPM) has its 23 Coordinating and 9 Cooperating centers in 27 States of

the country with the mandate to make survey in the regions, to collect new types of mushrooms, to undertake research and examine the adaptability of mushrooms and their different strains in different agro-climatic zones of the country and to test the developed technology of ICAR-DMR. Currently ICAR-DMR is working on collection, identification, conservation and genetic characterization of mushroom germplasm, development of high yielding varieties, improvements in the production technologies of different edible mushrooms, technology developed for newer specialty mushrooms, integrated pest and disease management, post harvest technologies for various mushrooms and imparting trainings to the trainers, entrepreneurs, growers, unemployed youths, women growers etc. (<https://dmrsolan.icar.gov.in/>)

### **Sri Lanka**

In Sri Lanka, under the Department of Agriculture (DOA) Mushroom Division – HORDI (Horticultural Crop Research and Development Institute) is one of the major research institutes conducting research and training programmes on mushroom cultivation. Their objective is to development of technology for Oyster, Button, Straw and Milky mushroom and promote it as a women's enterprise. The institute also conducts various training programmes targeting to technicians, mushroom growers and entrepreneurs.

(<https://doa.gov.lk/hordi-home/>).

Agriculture Research Station, Telijjawila is a substation of HORDI is responsible for conducting research activities to address the field problems and they also conduct research and training programmes on mushroom cultivation.

Regional Agricultural Research & Development Centre Makandura Sri Lanka is also engages in research and development activities to develop the mushroom cultivation techniques. Ratmalana Mushroom Development and Training Center is another research and training institute who conduct training sessions on cultivation of mushroom for farmers in Sri Lanka (<https://doa.gov.lk/>).

### **China**

**Institute of Agriculture Resource and Resources and regional planning, CAAS:** China is the first largest mushrooms producer of mushroom and third largest exporter in the world in 2021. The National Mushroom Improvement Center was approved by the Ministry of Agriculture and Rural Affairs of China in February 2012, and opened in 2016. It is the only national platform for edible mushroom improvement technology in China. Targeting technical issues in the edible mushroom industry in China, the center combines technological research, development and integration in a systematic way. It conducts the usability evaluation of germplasm resources, innovation in germplasm materials and breeding technology, breeding of new mushroom varieties, preservation and maintenance of mushroom spawn, spawn quality control techniques, and cultivation technologies appropriate for new varieties. In addition, the center strives to be developed into China's comprehensive research and development platform for mushroom germplasm resource efficient use and spawn-related technology, and also to become a national center for high-quality mushroom spawn preservation, maintenance and supply. (<https://iarrp.caas.cn/en/aboutus/structure/scientific/287904.htm>).

### **Institute of Edible Fungi:**

Institute of Edible Fungi (IEF), located in the Yangtze River Delta, was founded in 1960 as a mission-oriented research institute specializing in edible fungi. It was the first institute

in China to provide comprehensive coverage of all research areas applicable to edible fungi and strong technical training in mushroom production.

The institute has a comprehensive and sophisticated scientific research system and a complete range of disciplines. It has five research sections, including laboratory of fungi genetics, activities of the improved and standardized spawn breeding center, laboratory for resource utilization and mushroom cultivation process engineering, laboratory of processing technology and fermentation engineering, and mushroom industry and economics research office.

IEF has close cooperative links with universities and research centers in more than 20 countries and regions. The institute organizes international training workshops on edible & medicinal mushroom production technology on behalf of the Ministry of Science and Technology to help third world countries to cultivate their own edible & medicinal technical experts.

Govt. Subsidies for Mushroom Cultivation: On 2019-10-05 the honorable minister for Agriculture and livestock had approved the certain procedures (karyabidhi) for provincial and local level agriculture program with conditional remarks. Government subsidies for Mushroom Cultivation in Nepal are as follows:

The approved scheme 2023-24 offers financial assistance for Mushroom cultivation via 3 programs as given below:

- Program of public infrastructure and laboratory construction - Subsidy 100% of the total cost.
- Program of community-based infrastructure and laboratory construction- 2 Subsidy up to 85% of the total cost.
- Program of individual Program infrastructure and mushroom laboratory construction - Subsidy up to 50% of the total cost.
- Program of public institution for training and workshop - Subsidy 100% of the total cost.

The above schemes of subsidies will be mainly provision for: 1. Construction and strengthen of Mushroom laboratory capacity and infrastructure. 2. Procurement of machinery and equipment's for mushroom spawn production. 3. Procurement of glassware and chemicals for mushroom spawn production.

In the process of government subsidy policy for mushroom cultivation policy of Government of India also reviewed which are given as follows:

In India, a mushroom cultivation government subsidy was introduced in 2011 under the National Horticulture Board to promote mushroom production and help farmers with their endeavors. This mushroom subsidy scheme provides financial assistance for the farmers to set up their mushroom production plants. The offer is available for individuals and provides a substantial cost as well. Rashtriya Krishi Vikas Yojana (RKVY) scheme 2021-22 offers financial assistance for Mushroom cultivation via 5 programs as follows:

- Program 1 Small Scale Mushroom Production Unit – Subsidy up to 40% of the total cost of Rs. 28,125/unit. However, the max limit is – Rs. 11, 250/unit.

- Program 2 Hi-tech Milky Mushroom Production Units – Subsidy up to 40% of the cost of Rs. 2.50 lakh/unit. However, the max limit is Rs. 1.00 lakh/unit.
- Program 3 Minimal Processing and Value Addition Units of Mushroom – Subsidy up to 40% of the total cost of Rs. 1.00 lakh/unit. However, the maximum cost of Rs. 0.40 lakh/unit.
- Program 4 Small Scale Mushroom Spawn Production Units – Subsidy up to 40% of the entire cost of Rs. 5.00 lakh/unit. However, the maximum cost is up to Rs. 2.00 lakh/unit.
- Program 5 Vermicompost Units for Compost Production – The final total cost of creating compost of dimensions is 30'x8'x2.5' with a cost of Rs. 1.00 lakh. However, this scheme provides subsidies up to 50% of the cost of Rs. 0.50 lakh (*which shall be based on size or even a pro-rata basis*).
- In India, Haryana govt. provides about 40% subsidy to general farmers, while a 90% subsidy to scheduled caste farmers.
- It also provides high-quality pasteurized compost & mushroom spawn at subsidized rates to the farmers.
- Free training is given to Haryanvi farmers under the National Horticulture Mission.

*Source* (<https://www.tractorjunction.com/blog/top-mushroom-producing-states-in-india/>)

Strengthening extension services: Agricultural extension is a system that facilitates access of farmers or their organizations to new knowledge, information and technologies and promotes interaction with research, education, agri-business, and other relevant institutions to assist them in developing their own technical, organizational and management skills and practices. (Christoplos, 2010). Technologies are advancing and the world is being digitalized at a faster rate. Within a few seconds, development and changes happening in one corner of the world can reach the other corner. Countries, people, and markets are affected and influenced by those changes, technologies, and communication and information, no matter where they originate. Nepal, Nepalese, and Nepalese markets are affected and influenced too. Likewise, demands and needs of Nepalese farmers and agri-entrepreneurs are changing and they need improved technologies to improve their efficiency. Extension services must keep examining stakeholder needs and demands and be prepared to advise them accordingly. Agriculture extension service (AES) authority in Nepal should initiate a discussion inviting all agricultural stakeholders, farmers in particular, and assess what worked in the past and what did not work. If some activities did not work, extension professionals must identify why they did not, and what could be and should be done to address the needs and demands of the current and upcoming farmers. AES should train its extension personnel with new competencies. Poor performance of the extension services system is also attributed to the attitude of the extension staff. Extension professionals should be made accountable to their clients, the farmers, the entrepreneurs for their work. Those who are serving effectively should be rewarded. Nepal should bestow high priority to demand-driven, decentralized, pluralistic extension services, and ICTs. Decentralized units have decision making authority and are physically close to their beneficiaries. They plan and implement development programs with their beneficiaries' participation, making them sustainable.

Post-harvest management and product diversification:

Mushroom has very short shelf life, postharvest research and value addition activities has not given due emphasis right from the starting due to no dedicated mushroom research/resource center in the country. Due to highly perishable nature preservation of mushrooms is necessary to minimize the post-harvest losses, for this, improving knowledge and skills in picking, grading and preservation at the appropriate stage, cold storage, refrigerated transportation, suitable processing, appealing packaging and labeling and establish the mushroom value-added chain from farm to ultimate consumers. The processing techniques such as canning, individual quick freezing (I.Q.F.), vacuum freeze drying (VFD), drying, vacuum drying, pickling, steeping preservation, radiation preservation, O<sub>2</sub> & CO<sub>2</sub> analyzer, modified atmosphere packaging machine etc. have been not developed and practiced in Nepal. These practices can be on the basis of their merits per se market demand and end use.

#### Promotional and marketing

Popularizing mushrooms through ICT, including television, radio, advertisements, and posters, as well as mushroom festivals. Participation in the national and international exhibitions and providing financial assistance to small sized mushroom businesses in particular. Ensure year-round supply at a suitable standard rate by time-scheduling crops to achieve a daily relatively uniform output. Promote marketing, processing, and exporting through the private sector.

Farm-gate prices and profit margins are fluctuating

Establishing a support price minimum throughout the year, diversify and develop different mushrooms based on consumer preferences. For more profitable production, enhanced technology is being used to minimize the growing season and crop rotation lengths.

Crop insurance schemes: Agriculture insurance in Nepal has not been successful. Considering the unique nature of Nepalese agriculture and the inequitable socio-economic status of Nepalese farmers, crop insurance has remained a failed attempt in general. Crop insurance in mushrooms has not been practiced in Nepal. Every year one part of Nepal or other food crops are mainly affected by natural calamities and similar conditions might happen in mushroom industry also. Crop insurance protects farmers' losses caused by crop failure. Crop insurance also encourages farmers to adopt modern and innovative technologies that further increase their income.

Coordination and collaboration: Weak coordination and collaboration between federal, provincial and local government for mushroom research and development in Nepal.

## **5.2. SWOT Analysis on Mushroom Industry in Nepal**

A SWOT analysis was done based on the review. There are six strengths that need to be highlighted, with consideration of seven significant opportunities for greater benefit. Whereas, fifteen major weaknesses and four of threats must also be fully addressed.

### **Strength**

- Very conducive agro-climate for year-round farming of a wide variety of mushrooms.
- Prevalence of diverse mycophagous communities.
- Low-cost labor, plentiful cheap supply of a wide variety of raw substrates, building materials, spawn, and other inputs.
- The huge potential of the local market as well as easily accessible vast export market of India.

- Mushroom venture easily adopted even by educated Nepalese youths in comparison to other any agricultural ventures.
- Strategic geo-location.

### **Weakness**

- Lack of a critical mass of well-trained mushroom technicians and growers
- Lack of proper technical advice on mushroom enterprise
- Lack of quality control and certification
- Quality spawn, modernization of cultivation technology, localization of exotic technology, processing facilities
- Lack of well-organized market channels distribution network
- Inadequate scientific research on mushrooms and lack of well-equipped mushroom specific laboratory/resource center
- Lack of appropriate mushroom policies and laws
- Inadequate public awareness
- Insufficient mushroom courses in School and University curriculum
- Poor farm management practices
- Inadequate coordination between public institutions providing services to the rural population
- Insufficient investment
- Poor harvest management and marketing
- Lack of collection and domestication of locally available germplasm suitable for various agro-climatic conditions

### **Opportunity**

- Mushroom can be cultivated in Nepal throughout the year in natural environmental condition and hence year-round production can be taken
- High return can be fetched with low investment and rapid growth of the national and global mushroom market.
- Increasing supply and demand gap in the world trade of mushrooms and the shrinkage of production in countries like Japan, Taiwan, and South Korea due to high labor costs.
- Very suitable and important cottage industry activity in the integrated rural development program can create jobs both in semi-urban and rural areas.
- Need to increase the production and consumption of nutritious horticultural crops to ensure food & nutritional security at the household level to end the hunger and poverty by 2030 (SDG).
- Growing numbers of health-conscious consumers and demand for healthy, quality and organic products.
- Highly potential for the alleviation of Protein Energy Malnutrition (PEM) and also to improve economic standard of the masses.
- Increasing interest in protection and improvement of the environment.

### **Threat**

- Diseases and pests damage on product quality and supply.
- The limited supply of organic pest control products.
- Fierce competition from other neighboring countries.

- Rising input prices (including labor).
- Unstable farm-gate prices and profit margins

## **6. MUSHROOM SPAWN SUPPLY CHAIN**

### **6.1. Present Status of mushroom supply chain in Nepal**

Farmers purchase spawn from spawn producers to cultivate different species of mushrooms. In Nepal, NARC is the only governmental organization that produces spawns and the quantity is very limited. There are many laboratories in private sector that produce spawns in big quantity. Farmers are highly dependent on these private commercial spawn producers.

Spawn producers produce grain spawn by use of their own varieties (strains, stocks). Some producers imported mother culture from India or abroad for production of commercial spawn. However, spawn producers are not well aware about the variety of strain or mother culture used because of which they cannot identify the actual variety of spawn produced by them.

Mushroom yield and biological efficiency are important parameters that need to be understood and optimized by the mushroom producers but biological efficiency is not known to the spawn producers. They are not facilitated or trained to carry out DNA analysis in order to identify variety of spawn produced. As a result, the buyer of these spawn (mushroom growers) use these spawns and cultivate mushrooms but do not know about the species of mushrooms cultivated. The spawn supply chain as a whole is not systematic, scientific and reliable. The supply chain at present is working without proper knowledge among both the producers and buyers as a result in future; small setback can cause greater problems. There are no any separate Mushrooms Resource Centers to facilitate or help these producers and farmers in identification of the species and much research work on mushrooms is pending due to lack of mushroom research centers to carry out the research work.

### **6.2. Identification of Specific Problems in Mushroom Seed Spawn Supply Chain**

In Nepal, commercial spawn producers are not registered as there is no registration system prevalent. Spawn producers are not guided, monitored and inspected by authorized institutions because of that, quality of spawn has been compromised. There is no any information shared on selling such spawns to farmers regarding the name of the producer, date when spawn was prepared, species of spawn, any inspection done on quality and expiry date of spawn. As a result, when mushroom producers suffer from low quality spawn they do not know what went wrong in spawn and where to report their problem. So it is urgent need to register all the mushroom spawn producers so that monitoring of the quality of spawn they produce can be done. Spawn producers need to be monitored and supervised by authorized institution so that the quality controlled of spawn production can be reliable. Also, farmers need to be aware about the spawn quality, species of spawn so that they can cultivate mushrooms effectively.

Spawn producers are not maintaining proper records as to the date of sterilization, incubation temperature maintained and shipping date of spawn of every lot, when any problem arises, they are not able to track the cause of the problem. Same mistakes get repeated frequently and there is no scope for correction of the mistakes in spawn production and such situation affects the supply of quality spawn.

## 7. CONCEPT DEVELOPMENT (NMRC)

### 7.1. Feasibility Analysis of Mushroom Resource Center, Khopasi, Kavrepalanchowk

#### 7.1.1. Site Description

The climate in Khopasi is warm and temperate. In winter, there is much less rainfall than in summer. In Khopasi, the average maximum and minimum annual temperature is 22.24 °C and 11.28°C (Department of Hydrology, 2022), respectively. Precipitation here is about 126 inch per year and a sunshine hour was 5.55 hours and facing of land is such that in daylight sunlight utilization efficiency is enough. Average wind speed of the site was 4.38 km/hr. The large and open area with river on the east and community forest on the west of Khopasi farmland makes it well ventilated and atmosphere is suitable with proper air circulation. Even during hot and summer the temperature of the site remains normal. Very few residential buildings exist nearby resulting in minimal human activities which is less probable to increase in coming 15 to 20 years making the area safe for the construction of the Mushroom Laboratory. It has clay silt with trace of sand soil with steep topography of the land, drainage problem does not exist which prevents the probability of contamination of different pathogens and insect pests on mushrooms. Abundant, clean and non-contaminated underground water supply system makes more sustainability of the center. Contamination of culture/spawn due to livestock can be prevented as livestock farming is limited and not so popular. The area is clean without any dumping area around so the landsite is very suitable for agronomical activities. No industries nearby and nature friendly environment with suitable air quality. Good transportation facilities as proper condition BP Highway near Kathmandu can be used. Unlimited Power Supply as Khopasi/Panauti area is well known for Electricity generation.



*Figure 4: Map of Nepal Showing Kavrepalanchowk District Location*

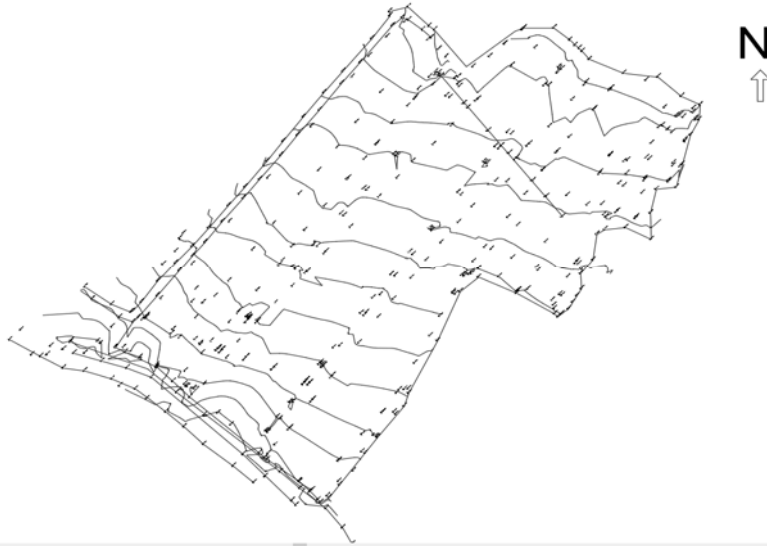


**Figure 5: Kavrepalanchowk District Map**



**Figure 6: Project Area**

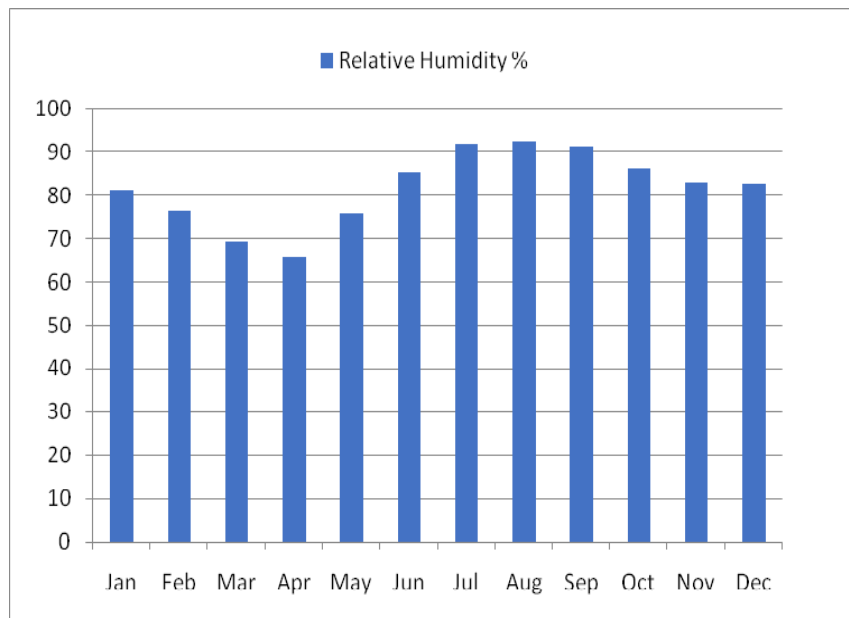
Topographic survey and site visit was done. After topographic survey, it was found that total area of the site is 28551.61sq.m.(56 Ropani-1 Aana-3 Paisa-3.37 Dam).



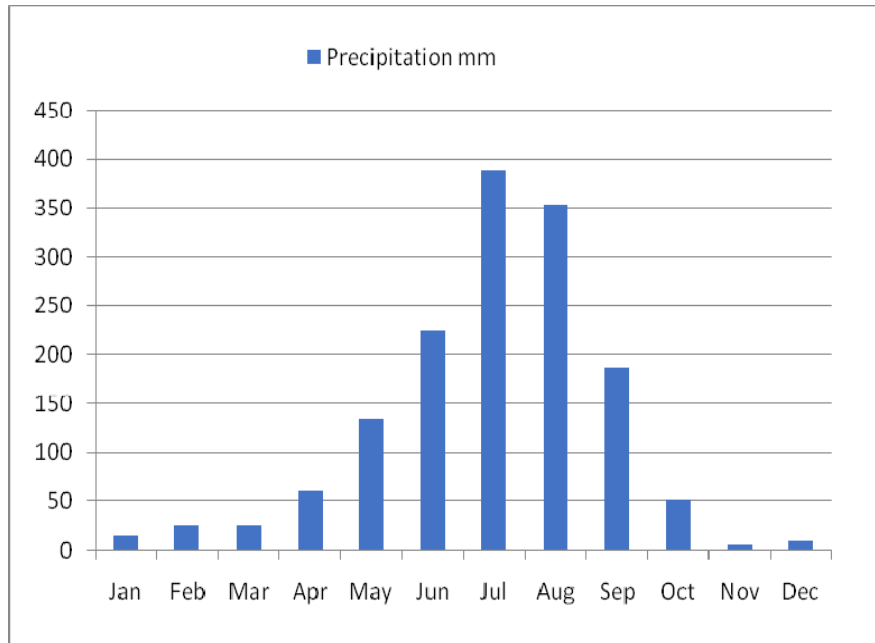
**Figure 7: Topographical Map**

### 7.1.2. Climate

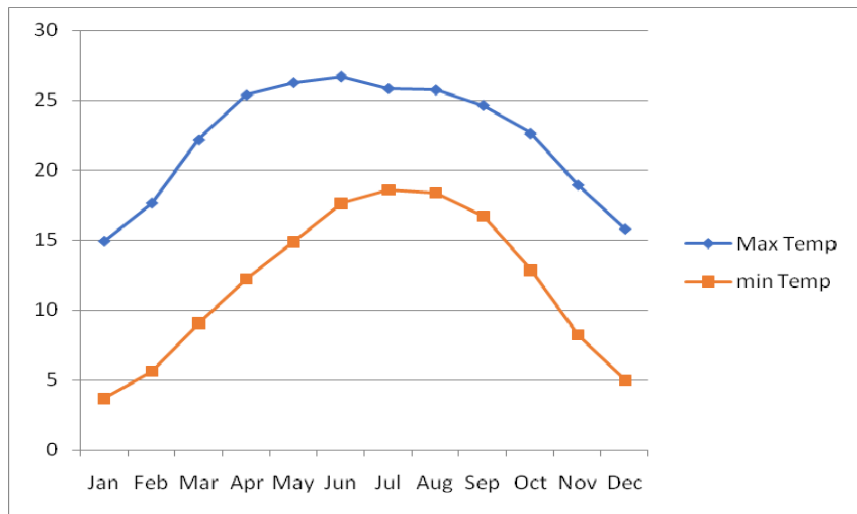
All of the data required was obtained from the department of hydrology and Meteorology. Please refer Annex 5 for detail.



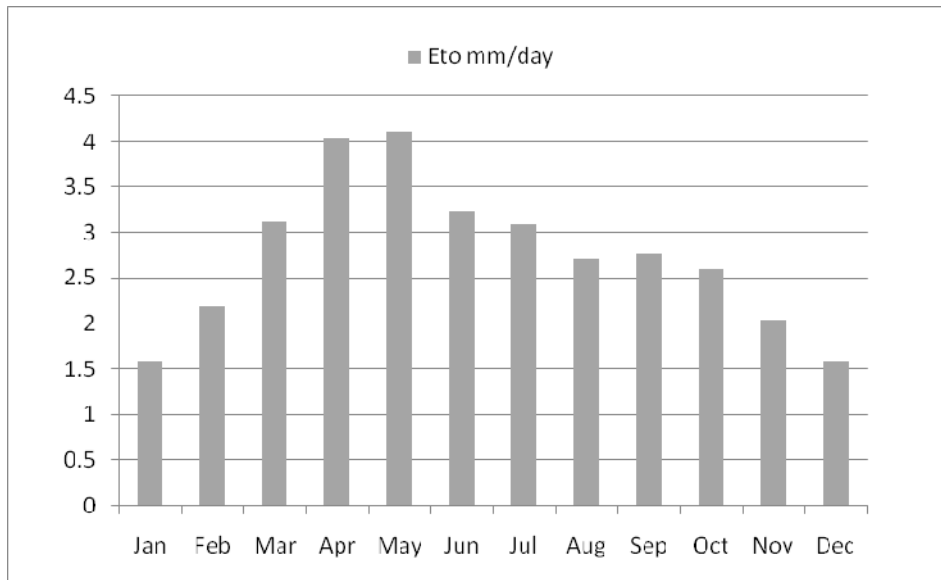
**Figure 8: Relative Humidity %**



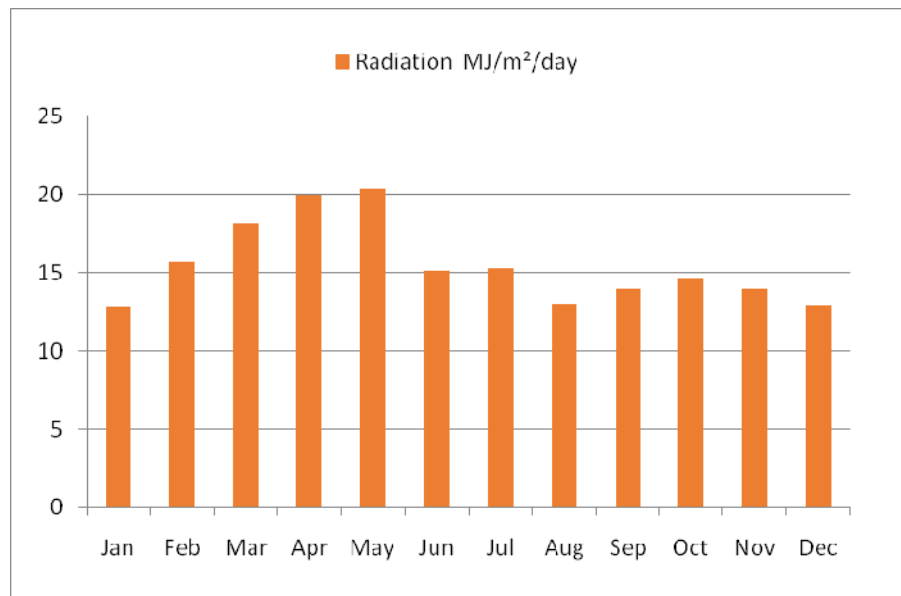
**Figure 9: 24h Accumulated Precipitation**



**Figure 10: Average Maximum and Minimum Temperature in Degree Celsius**



**Figure 11: Evapotranspiration from Crop wat Tool**



**Figure 12: Radiation From Cropwat Tool**

## **8. GOAL, VISION, OBJECTIVES, MANDATE AND WORKING AREAS OF THE MUSHROOM RESOURCE CENTER**

### **8.1. Goal**

The goal of NMRC is to enhance the import substitution and export promotion of mushroom and mushroom related products and increase the employment opportunities in the mushroom subsector through enhanced mushroom seed supply system, utilization of native mushroom species and effective technology dissemination.

### **8.2. Vision**

To enhance the agricultural economy, create credible employment opportunities and increase the livelihood of farmers through promotion of mushroom subsector.

### **8.3. Objectives**

The major objectives of National Mushroom Resource Centre are:

- a. To function as the government institution dedicated to the growth and development of mushroom sub sector.
- b. To regulate and ensure the supply system of quality mushroom spawn within the country.
- c. To address the demand of efficient and effective technologies, quality inputs and associated quality regulation of mushroom sector.
- d. To conduct adaptive research and demonstration activities related to mushrooms.
- e. To build capacity of stakeholders and actors involved in mushroom sub sector.
- f. To explore and preserve the mushroom genetic resources available in the country.
- g. To substitute the import of mushroom pure culture and spawn.
- h. To strengthen the supply chain and value chain of mushroom spawn and mushroom.
- i. To monitor quality of spawn in public and private sectors and enforcing spawn standards and fair price.

### **8.4. Mandate**

- Collection, identification, conservation, utilization and production of edible and medicinal mushroom.
- Apply appropriate mushroom cultivation technology and spawn production and distribution.
- Transfer of technology and capacity building of stakeholders for spawn production.
- The mushroom resource center at Khopasi Kavrepalanchowk will provide pure cultures and also will have adequate facilities for conservation of exotic and native edible and medicinal mushrooms species. The NMRC will have a "Farmers Service Unit" to address the problems of the growers with regard to mushrooms crop management, diseases, pests, substrate quality etc. Testing of samples/substrates/casing the centre will analyses the samples of mushrooms for diseases/pests and substrates/casing brought by mushroom farmers.

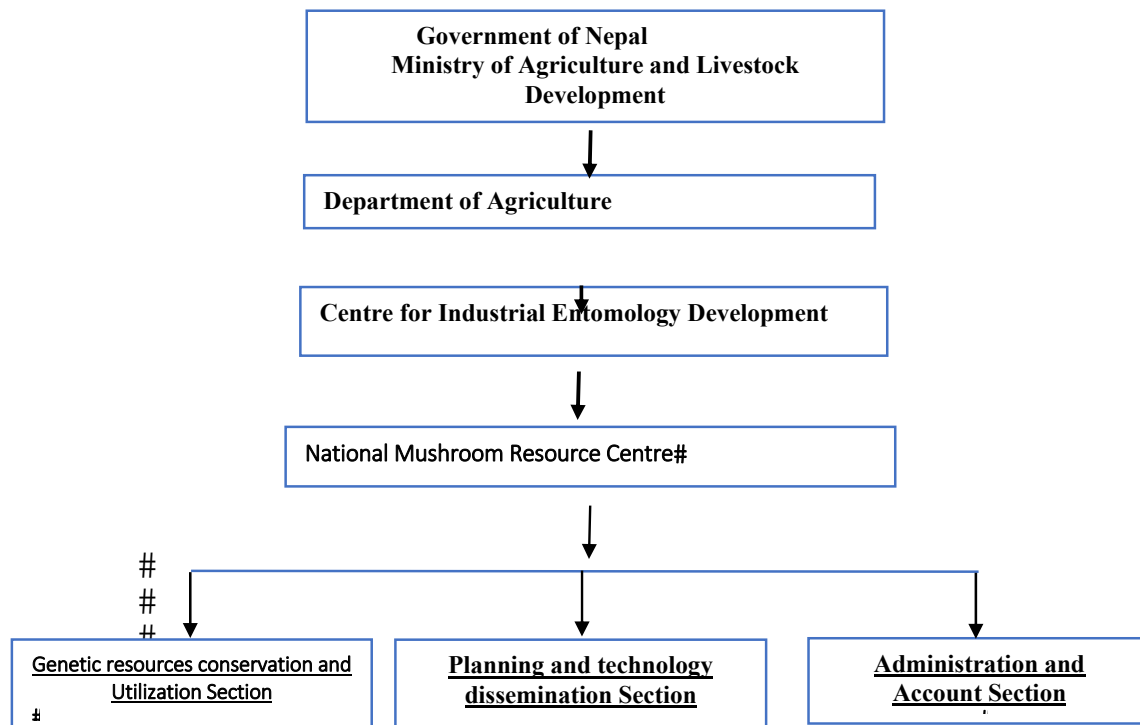
## 8.5. Major activities/Working areas

The NMRC has been conceptualized to provide following services:

- a. Collection, identification, preservation of exotic and native strains of mushroom in the country.
- b. Produce true to type certified pure culture and distribute it as per the demand of the country for research and commercial use.
- c. Provide advanced level training to technicians, mushroom producers, and spawn producers.
- d. Conduct adaptive research in mushroom spawn production, mushroom production, and post-harvest technologies.
- e. Manage year-round demonstration of advanced and protected (controlled conditions) mushroom production technologies to the researcher, technicians, and producers.
- f. Quality regulation and certification of private mushroom spawn producing laboratories through formulation and implementation of legal standards.

## 8.6. Organizational Structure

Under the Centre for Industrial Entomology Development, National Mushroom Resource Centre will function as the major government institution for the development of mushroom sub sector in the country. The proposed institution will have the following organizational structure for its operation:



## 8.7. Major Scope of Proposed Sections

### 8.7.1. Genetic resources conservation and Utilization Section

- Genetic resources acquisition, conservation and maintenance
- Pure culture production and supply

- Adaptive research

### **8.7.2. Planning and technology dissemination Section**

- Technology demonstration
- Post-harvest technology
- Training and Capacity building

### **8.7.3. Administration and Account Section**

- Personnel administration management
- Account administration
- Office logistic management

## **8.8. Working modalities of proposed units of NMRC**

The Mushroom resource center Khopasi, Kavre will function under the umbrella of Department of Agriculture (DOA) and solely work on different aspects of mushrooms. Broadly, the proposed NMRC will comprise the following four units.

### **8.8.1 Germplasm and Conservation Unit**

It will collect native and exotic germplasm/genetic materials of the different species of edible mushrooms from national and international sources and then this unit will conserve and maintain these germplasm/genetic materials for the sustainable and reliable seed (spawn) supply chain within the country.

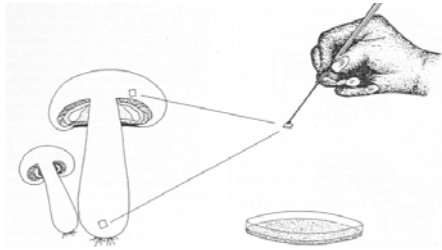
The unit would perform its task as follows:

- Collection, identification, characterization, conservation and utilization of native and exotic mushroom species.
- Pure culture production of native mushrooms.
- Media preparation for isolation of different species of mushroom and preservation of pure culture.
- Production and preservation for mother culture.
- Revival of preserved culture for mass production.
- Mapping of native mushroom.
- Collaborative research with NARC for exotic mushroom culture.

#### **8.8.1.1. Preparation of substrate (Media)**

- Potato dextrose agar (PDA) ingredients: 200g diced potato, 20 g agar powder, 20g dextrose or ordinary white sugar, 1 liter water.
- Wash and weigh the potatoes and cut them into small pieces.
- Boil for about 15 to 20 minutes until they are soft.
- Remove the potatoes.
- Add water to the broth to make exactly 1 liter.
- Add the dextrose and the agar. Be sure to add the right amount of sugar and agar, otherwise the medium will become either too soft or too hard.
- Stir occasionally and heat gently until the agar has melted. The agar should be hot when poured into the test tubes or bottles otherwise it will become lumpy.
- Fill about one fourth of the test tubes.
- Then, seal the tubes or bottles with cotton plugs.

### 8.8.1.2. Isolation of mushroom



**Figure 13: Isolation technique**  
(Source: Upadhaya et. al., 2004)

Aseptic concept :

1. Any article or material used in the processes of isolation must be sterilized.
2. Contamination must be prevented in the processes of isolation and culture.

The ability to produce qualified spawn is the "symbol" of a professional edible fungus industry.

To produce spawn by oneself can reduce the cost. There are two ways to produce spawns:

#### **Tissue isolation:**

This method belongs to asexual reproduction or clone. Theoretically, the spawns got from this method will not have hereditary aberrance, so it is usually used in production.

#### **Spore isolation:**

This method is sexual reproduction. Spore isolation can be divided into two kinds: single spore isolation and multi-spore isolation. Single spore isolation is mainly used to do hereditary breeding research and multi-spore isolation is mainly used in reproduction.

### 8.8.1.3. Sterilization

#### a. Heat sterilization

- i. Dry heat: Oven- 170°C 120 min.
- ii. Moist heat:
  1. Autoclave 121°C 15 lb/in<sup>2</sup> 15~240 min.
  2. Intermittent Sterilization
- iii. Flame sterilization

#### b. Filtration sterilization

#### c. Radiation sterilization

#### d. Sterilization by gases: ethylene oxide,

#### e. Chemical sterilization

### 8.8.1.4. Media

#### 8.8.1.4.1. Media for isolation and culture

For isolation

1. Water agar (WA): water 1000 ml, agar 20 grams

#### 8.8.1.4.2. Media for mushroom mycelium Malt Extract, Yeast Agar (MYA, MYPA)

- 1,000 milliliters (1 liter) water
- 20 grams agar
- 20 grams barley malt sugar

- 2 grams yeast (nutritional)
- 1 gram peptone (optional, soybean derived)

**8.8.1.4.3. Media for mushroom mycelium Potato, Dextrose, Yeast Agar (PDYA, PDYPA)**

1,000 milliliters (1 liter) water

- 300 grams of potato water (i.e.; the broth from boiling potatoes in 2-3 liters of water for one hour)
- 20 grams agar
- 10 grams dextrose
- 2 grams yeast
- 1 gram peptone (optional, soybean derived)

**8.8.1.4.4. Oatmeal, Malt, Yeast Enriched Agar (OMYEA)**

- 1,000 milliliters water (1 liter)
- 80 grams instant oatmeal
- 20 grams agar
- 10 grams malt sugar
- 1 grams yeast

**8.8.1.4.5. Dog Food Agar (DFA)**

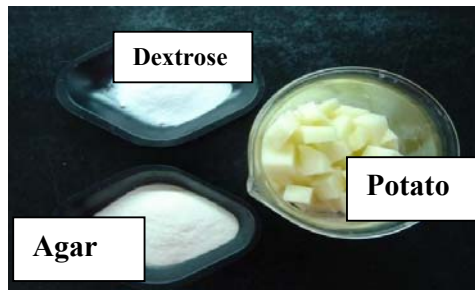
- 1,000 milliliters water (1 liter)
- grams dry dog food
- rams agar

**8.8.1.4.6. Cornmeal, Yeast, Glucose Agar (CYGA)**

- 1,000 milliliters water (1 liter)
- 20 grams agar
- 10 grams cornmeal
- 5 grams malt or glucose
- 1 gram yeast

**8.8.1.4.7. Agar Medium Preparation Potato, Dextrose, Agar (PDA)**

- 1,000 milliliters (1 liter) water
- 200 grams of potato (i.e., the broth from boiling potatoes in 900 of water for 0.5 hour)
- 20 grams agar
- 20 grams dextrose.



*Figure 14: Agar medium preparation*

#### **8.8.1.5. Culture (Strain) Preservation**

- Periodic transfer
- Sterile mineral oil (paraffin oil) overlaying
- Sterile distilled water storage
- Freeze-drying (lyophilization)
- Freezing:
  - a. - 20 ~ - 135°C
  - b. - 196 °C( in liquid nitrogen)

#### **8.8.1.6 LONG-TERM STORAGE OF SEED CULTURE**

Seed culture is the most important element of spawn production. Although there are many ways to store seed culture, the methods for storage in liquid nitrogen (-196°C) or a deep freezer (-80°C) are highly recommended except for *Valariella volvacea* and *Pleurotuscystidiosus*.

It is also common for subcultures of the seed culture to be maintained at 20 to 23°C. In this case, subcultures must be restarted once a year. Household freezers, however, are not suited to long-term storage of seed culture because they do not provide sufficiently low temperatures (-20°C or higher), and the survival rate of seed culture is significantly low. To be on the safe side, the seed culture should be stored using two or more methods simultaneously. When liquid nitrogen is easily available, the method is better than the method by use of a deep freezer.

##### **8.8.1.6.1. Inspection of colonies**

After checking that the colonies on PDA media are growing normally (i.e., presence of sectoring, formation of characteristic circular colonies, absence of flat hyphae, etc.), punch out small fungal disks (6 to 8 mm in diameter) from just inside the colony margin using a sterilized corkborer.

##### **8.8.1.6.2. Storage solution and containers**

For the storage solution, use a solution containing 10% w/v glycerol and 5% w/v trehalose. For cryopreservation, add 1 ml of storage solution to tubes (2 ml) or vials and then sterilize.

#### 8.8.1.6.3. Storage of fungal disks

Place 2 to 5 fungal disks into the sterilized tubes/vials containing storage solution, store the tubes/vials in a refrigerator for 30 minutes to 1 day and night, and then move the tubes/vials to the deep freezer (-80°C).

#### 8.8.1.6.4. Thawing and culturing

Remove the tubes/vials containing seed culture from the deep freezer and perform rapid thawing (30°C for 3 minutes). Remove and transfer the fungal disks to PDA media, being careful not to damage them in a laminar air flow.

#### 8.8.1.6.5. Procedure of Long term storage by Deep Freezer

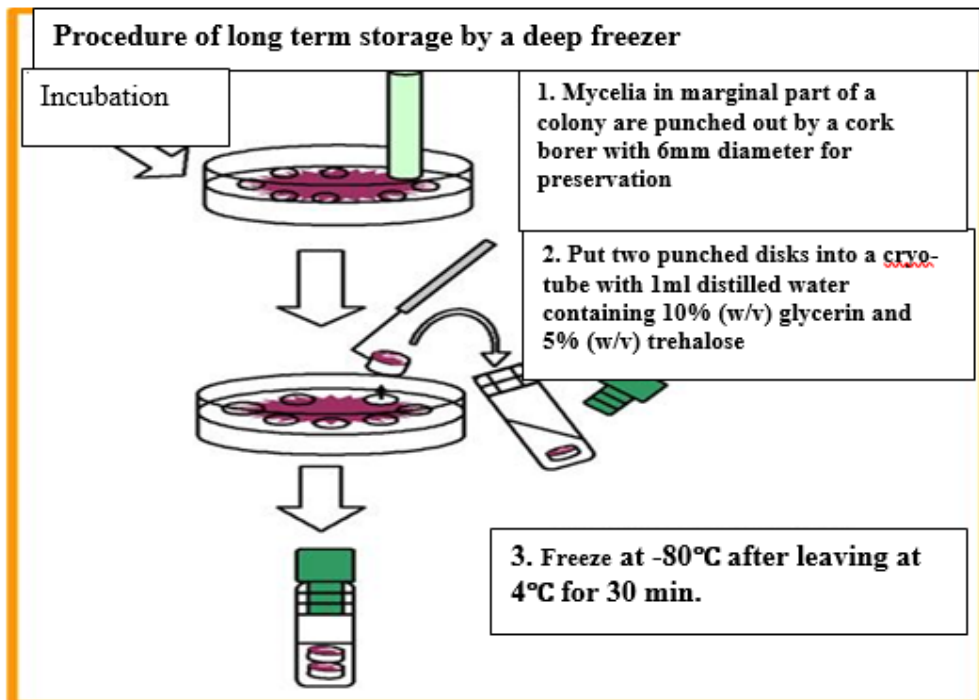
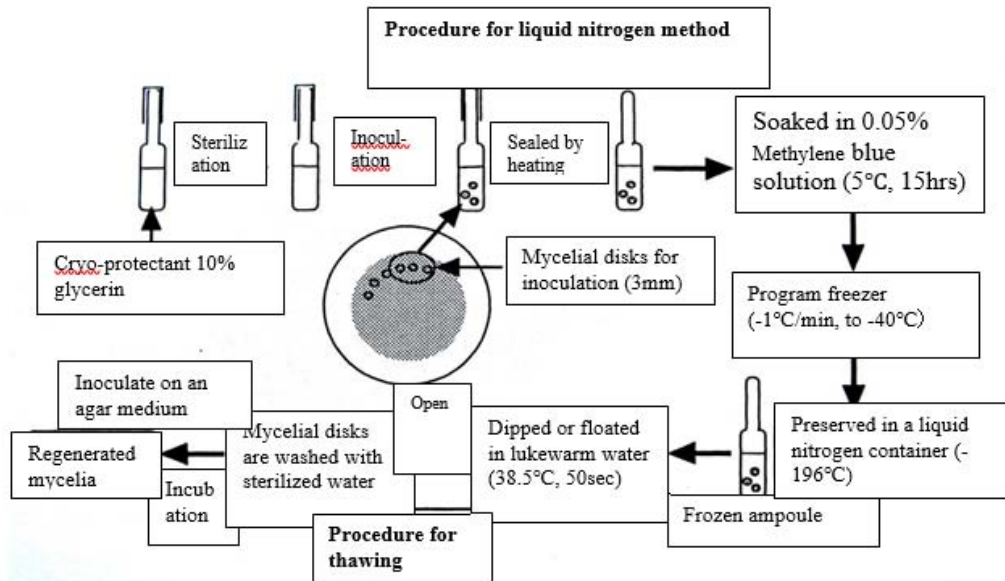


Figure 15: Procedure of long term storage by a deep freeze

## Cryo-Preservation Method-Liquid Nitrogen



**Figure 16: Cryo-preservation method-Liquid nitrogen**  
(Source: Singh et. el.,2001)

### 8.8.1.6.6. Liquid Nitrogen Gas

#### A. Gas phase

1. Temperature: -140°C
2. Type of cryo-vial: Cryo-tube (screw cap type)
3. Advantage: Contamination risk is low as compared to the method for immergence to liquid nitrogen. Workability is higher than the method for immergence to liquid nitrogen.
4. Disadvantage: Temperature is likely to vary when open the lid.

#### B. Liquid phase

1. Temperature: -196°C
2. Type of cryo-vial: Ampule type
3. Advantage: Temperature is uniform inside the container and is kept for a long time even if refilling of liquid nitrogen is delayed.
4. Disadvantage: Workability is slightly low.

#### C. In the case of use of G48/48-6R

1. Gas phase in the container.
2. Liquid nitrogen (LN): 13.1 L
3. Loss of LN: 0.557 L/day
4. Size: Outer diameter 445 mm

- Height 820 mm
  - Top mouth diameter 119 mm
5. The container can accommodate 750 vial tubes (6 canisters).

The apparatus required for liquid nitrogen storage and their tentative costs (June 2023) are give in Table 5.

**Table 5. Apparatus for Liquid Nitrogen Storage**

S.N.	Instrument	Price(N Rs)	Quantity	Total Price(tax included)
1	Storage contained(IX-35)	105,000	2	237,300
2	Container for carry of LN2(30 I)	67,986	2	135,972
3	LN2 withdrawal device	22500	2	45000
4	Refrigerator base caster	18,500	2	37,000
5	Cryo-bials	38	600	22,800
6	Freezing unit for storage			
7	Cane for cryo-bials	1,200	24	28,800
8	LN2 glove	12,500	2	25,000
9	LN2	175	156	27,325
	<b>Total</b>			<b>559,197</b>

The room equipped with ventilation fan for liquid nitrogen can be prepared at NMRC.

Running Cost (Loss of LN) of Liquid Nitrogen			
Container	Evaporation of LN	Cost	
IX-35	0.118 l/day	7,537	NRs. 175/ 1 of LN
IX-47	0.37 l/day	23,634	„

### 8.8.1.7. Incubation

#### 8.8.1.7.1. Long term storage of mother cultures

- (1) Subculture  
Stored in an incubation room (21-23 C) or in a refrigerator
- (2) Paraffin method  
Stored in an incubation room, does not require continuous electricity supply
- (3) Deep freezer method
- (4) Liquid nitrogen method

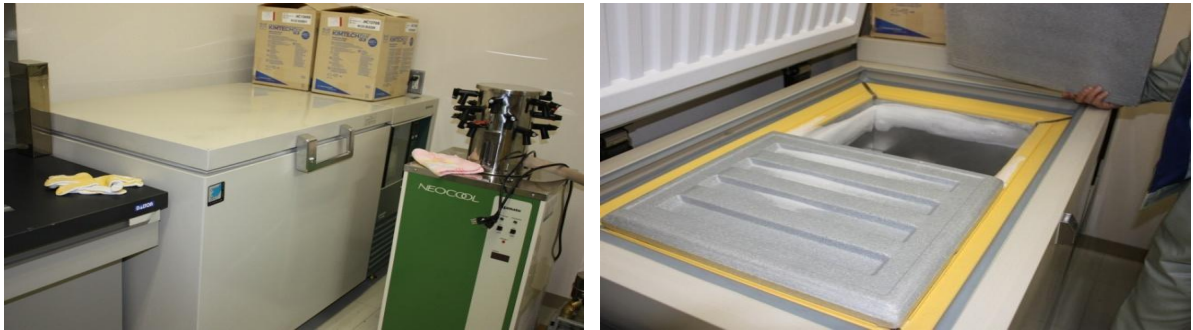
#### 8.8.1.7.2. Liquid Paraffin (Mineral Oil) method

- Storage for 2-3 years can be acceptable, but storage for more than 5 years is not suitable
- This method does not require electricity



*Figure 17: Liquid paraffin (Mineral Oil) method*

### 1-(3) Cryo-preservation method (Storage in a deep freezer)



*Figure 18: Cryo-preservation method (Storage in a deep freezer)*

Cryopreservation is a non-lethal storage of biological material at ultra low temperature. At the temperature of liquid nitrogen (-196 degree) almost all metabolic activities of cells are ceased and the sample can then be preserved in such for extended periods.

#### 8.8.1.7.3. Direct freezing method (Source: Jong et. al., 1984)

##### Procedure

##### A. Materials for preservation

- (1) Mycelia on slant or plate agar
- (2) Mycelia on sawdust based media
- (3) Fruit bodies
- (4) Spawn

##### B. Freezing

- (1) Materials are placed in plastic bags and then sealed tightly
- (2) Then transferred to a deep freezer at -85°C

##### C. Thawing

- (1) Taking out the plastic containers and leave them at a room temperature
- (2) Waite until the materials' temperature goes up to a room temperature
- (3) Wipe drops on the containers with tissue paper
- (4) Regeneration of strains by inoculation of the materials on an appropriate medium

##### Improvements in production of quality spawn

- Preservation of mother cultures
- Making formula of substrate
- Operation of an autoclave
- Cooling after autoclave
- Incubation

### 8.8.1.8. Advantages and Disadvantages of the Long Term Storage Methods

#### 8.8.1.8.1 Subculture

Strains are stored between 21-23°C or ca. 10°C. Easy method but laborious and likely to occur changes in original characters due to multiplication, and some species cannot be stored below 15°C

#### 8.8.1.8.2. Mineral oil (liquid paraffin) method

Strains can be stored at a room temperature but cannot be stored for a long period due to block of air, and no research on the changes in productivity of strains after a long term storage by liquid paraffin method

#### 8.8.1.8.3. Deep freezer method

Practical method but requires continuous supply of electricity. Researches on the changes in productivity of strains are not sufficient

The most reliable method for long term storage of microorganism, and does not requires electricity but running cost is high due to loss of liquid nitrogen by continuous evaporation.

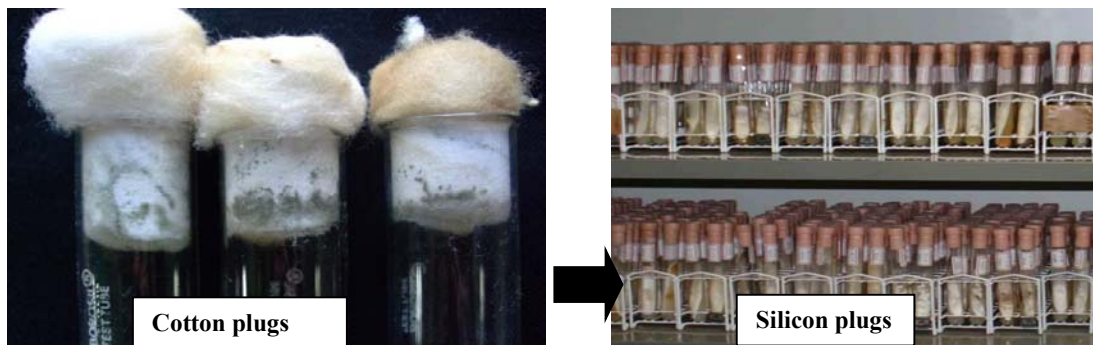


Figure 19: Cotton plugs contaminated in the rainy season

### Culture (strain) Preservation

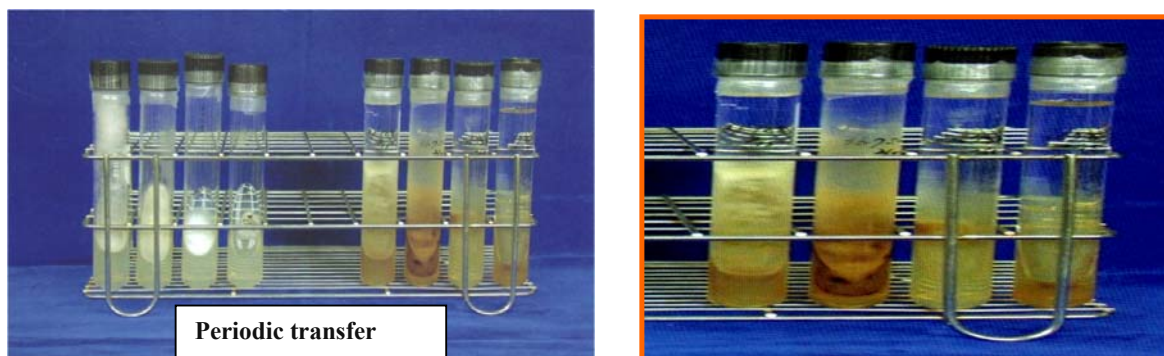


Figure 20: Culture (strain) preservation

### 8.8.2. Pure Culture Production and Supply Unit

This unit would be engaged in the production of certified/quality assured pure culture as per the need of the commercial mushroom seed/spawn producers. This unit would be facilitated with quality test unit to check the quality of pure culture in a regular manner. In addition to these the unit will also responsible for:

- Necessary mass production of laboratory media
  - Preparation of sterilized substrate for research purpose of different species of mushroom
  - Production of mother spawns for commercial and research purpose
  - Prepare the list and the technical specifications of necessary equipment for mushroom pure culture and spawn production.
- The unit will also responsible for working preparation of different types of spawn

### **8.8.2.1. Seed culture preparation for Shiitake mushrooms**

#### **(1) Inoculum inspection and seed culture preparation**

Transfer the stored inoculum onto PDA media, and then incubate at the specified temperature, and check the colonies for any abnormalities in terms of the speed of hyphal growth and colony morphology, etc. If no abnormalities are found, the colonies can be used as an inoculum source for preparing spawn. The spawn can then be used as inoculum for farm-scale production after confirming its ability to form mushrooms by inducing the development of fruiting bodies on part of the spawn. The spawn should be prepared so that the mycelia of the seed culture spread rapidly through the medium and that all areas of the spawn are roughly the same maturity.

#### **(2) Incubation containers**

As containers for preparing spawn, use 850-mL polypropylene bottles (PP bottles) with caps that provide superior filtration ability against fine dust and appropriate breathability. Although urethane or NK caps are often used in practice, these are somewhat inferior in terms of dust protection. In such cases, efforts should be made to ensure that the incubation room for spawn should be clean. In addition, ST caps, which do not have filters, are especially poor in terms of dust filtration ability (although they offer good breathability); thus, the ST caps are never used for spawn production. After the mycelium has spread throughout the medium, the spawn should be allowed to mature for approximately 1 month, after which it can be used as spawn for farm-scale production. Medium preparation and incubation conditions are the same as for farm-scale production.

### **8.8.2.2. Preparation of spawn for farm-scale production**

#### **8.8.2.2.1. Procedure**

1. Prepare medium ingredients 2. Weigh medium materials 3. Homogenize medium using a mixer 4. Add and mix in nutrients 5. Add and mix in water 6. Fill containers with medium and make holes for inoculums 7. Close containers 8. Sterilize 9. Allow to cool 10. Inoculation 11. Incubate and maturation 12. Storage

#### **8.8.2.2.2. Preparation of sawdust medium**

##### **8.8.2.2.2.1. Sawdust spawn**

- Sawdust spawn consists of mushroom mycelium grown on a sawdust/bran mixture similar to the sawdust substrate. Since the mushroom mycelium in the spawn is already adapted to growth on sawdust, it quickly becomes acclimated to the new substrate.

- Sawdust spawn is bound together with mycelium, so it must be broken apart before spawning. This makes it harder to handle than other types of spawn. Spawning rate is usually high, up to 5% (v/v).

#### 8.8.2.2.2.2. Formulas for Creating Sawdust Spawn

- Sawdust 78%
- Rice bran (or wheat bran) 20%
- Sucrose 1%
- Gypsum 1%



*Figure 21: Formulas for creating sawdust spawn*

#### 8.8.2.2.2.3. Sawdust Spawn Preparation

- Preparation the sawdust, rice bran (or wheat bran), gypsum and sucrose.
- Mixed all of the materials, but the gypsum have to mixed with the water before mixing.
- Put into spawn bottles.
- They were sterilization at 121 °C for 2 or 3 hours.

#### 8.8.2.2.2.4. Tree Species and Sawdust Particle Size

Ensure uniform mycelial growth and decomposition of the medium, tree species well suited to decomposition should be selected for preparing sawdust, and the particle size should be relatively small (3 mm or less). Species well suited for sawdust preparation include Nepalese alder (*Alnus nepalensis*), *Castanopsis* spp., and Himalayan hazel (*Corylus ferox*). If the sawdust is dry, water absorbency should be managed by storing the sawdust outdoors for a certain period of time while applying water as needed.

#### 8.8.2.2.2.5. Medium Ingredients

Enough nutrients (rice or wheat bran) should be added to the medium so that the nutrient content is 8 to 10% in wet weight of medium. As rice bran is readily oxidized, it is not suitable for long-term storage and should be used as quickly as possible. Adjust the moisture content to between 59 and 61%. If the initial moisture content is too high, the moisture content after ripening will be 68% or higher. When the spawn with high moisture content is inoculated, the air space in inoculation holes of bed-logs becomes poor and the spawn is prone to shrinkage and dried by vapping of water in the spawn through wax for sealing. After measuring the moisture content of the sawdust and nutrient material using a moisture meter, the amount of material to be added should be calculated using the medium recipe calculation software. The moisture content should be adjusted as precisely as possible.

#### **8.8.2.2.6. Mixing and Packing Medium**

After mixing the sawdust and nutrient material for 15 to 20 minutes, add water and mix for another 30 to 40 minutes. When ambient temperatures are high, mixing for long periods of time promotes bacterial propagation and is not recommended. When a mixer is used, only add medium material to just above the top of the shaft; care must be exercised because if too much material is added, it will not mix uniformly. If the capacity of the mixer is much smaller than the capacity of the sterilizer, mixing will require a long period of time and promote bacterial propagation. Bacterial propagation can change the pH of the medium and, in some cases, lead to the production of antimicrobial substances, resulting in poor mycelial growth after inoculation. The medium must be sterilized immediately after it is prepared. Although a bulk density of 60% (specific weight, 510 g of medium for an 850 ml container) is recommended for general media, for spawn media, given the fineness of the sawdust, a bulk density of 50 to 55% (w/v) is required to ensure sufficient void volume. Medium should be packed into containers in such a way that the medium near the bottom does not become compacted. If the compactness of the medium is not uniform, the degree of spawn maturity will also not be uniform.

#### **8.8.2.2.7. Medium Sterilization**

Indicator species for sterilization include microorganisms capable of growing on the medium used for cultivation, are heat-resistant and grow in the temperature range required for mycelial culture (20 to 27°C). Even if microorganisms are heat-resistant, they are not targeted for sterilization as long as they do not grow on the medium used or at temperatures required for mushroom cultivation.

In general, molds and yeasts have low heat-tolerance and can be killed by heating at 60°C for 5 to 15 minutes. In contrast, bacteria that produce spores have high heat-tolerance. Spore-forming bacteria belong to the genera *Bacillus* and *Clostridium*. *Clostridium* spp., do not grow under aerobic conditions and, thus, are not subject to sterilization in the mushroom farming sector. On the other hand, *Bacillus* spp., which does grow under aerobic conditions, is important sterilization targets in the mushroom farming sector. Of the more than 30 species of bacteria in genus *Bacillus*, those that exhibit high heat-tolerance are referred to as “thermophilic bacteria.” Two representative thermophilic species are *Bacillus stearothermophilus* and *Bacillus coagulans*. Of these two, *B. stearothermophilus* does not grow at temperatures of 28°C or lower. Furthermore, *B. stearothermophilus* and *B. coagulans* do not grow on sawdust/wheat bran medium, even at the suitable temperature (55°C). Meanwhile, mesophilic *Bacillus* spp. grow rapidly on media used for mushroom cultivation and at temperatures required for mycelial growth. From the above, indicator species for sterilization in mushroom cultivation include heat-resistant mesophilic *Bacillus* spp. Examples of sterilization conditions include 8 hours at 99°C (temperature of the medium itself), 30 minutes at 113°C, 5 minutes at 117°C, and 3 minutes or less at 122°C. In cases where the autoclave display temperature (not the temperature of the medium itself) is used, taking into consideration the extra time required for the medium to reach the display temperature (which depends on the autoclave configuration), required sterilization conditions are 30 minutes at 121°C for an 850-mL bottle or 60 minutes at 121°C for 2.5 kg of medium.

#### **8.8.2.3. Relationship Between Absolute Pressure and Temperature**

The relationship between absolute pressure and temperature is shown in Fig. 19. This relationship is not affected by external conditions such as elevation or weather.

Theoretically, absolute pressure is the sum of atmospheric pressure and the pressure inside the autoclave.

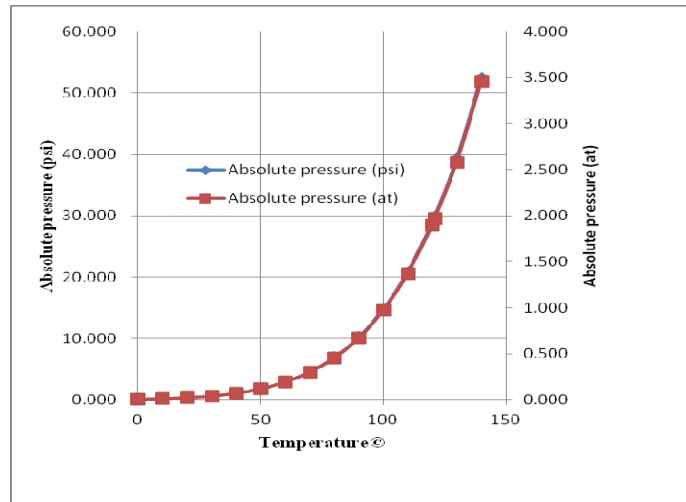


Figure 22: Relationship between absolute pressure and temperature

#### 8.8.2.4. Operation of Autoclaves Used in Experiments

- Do not set up an autoclave in the working room. Do not leave the autoclaved substrate in the working room for cooling.
- Set up the autoclave room near to an inoculation room. After autoclave, immediately bring the autoclaved substrate to the inoculation room and cool it.
- Use of dustless materials for the wall, incubation racks (steel-made one is recommended) and the floor of the inoculation and the inoculation rooms. The inoculation room should not be made at the position facing a road.
- Windows are unnecessary in the inoculation room
- It is most recommended that the inoculation room is equipped with a clean ventilation unit. If the room is big enough, a ventilation fan is unnecessary, but do not work for a long time (more than one hour), because CO<sub>2</sub> concentration more than 2,500 ppm is not Using a temperature data logger set up a system for monitoring the internal temperature of the medium. Place the specified number of bottles or bags in the autoclave and turn on the autoclave. Be careful not to overload the autoclave as this will reduce the autoclave's sterilization efficiency.
- After turning on the autoclave, to replace the air in the chamber as well as in the bottles/bags with steam, leave the hatch slightly open to promote air expelling. Replacement typically requires 1 to 2 hours (depending on the size of the chamber).
- After replacing the air with steam, maintain the chamber at the specified pressure (Table 5). As the absolute pressure is the sum of atmospheric pressure and the pressure measured inside the autoclave, the required chamber pressure will vary with elevation. The pressures required at different elevations are shown in Table 1. It should be noted, however, that these pressures are for when the air in the chamber has been completely replaced by steam. If there is air left in the chamber, a higher pressure is required to reach the target temperature of 121°C. If it is possible to monitor the temperature of the medium itself, the autoclave should be turned off once the temperature reaches 120°C.

**Table 6: Pressure required at different elevations to achieve an autoclave temperature of 121°C.**

	Atm (hpa)	psi (pound force/inch <sup>2</sup> )	at (technical atmosphere, kgf/cm <sup>2</sup> )	Mpa (mega pascal, 10 <sup>6</sup> pa)
Absolute pressure at 121°C	-	29.731	2.090	0.200
Elevation (m) 0	1,013.25	14.689	1.033	0.101
500	950.50	15.949	1.121	0.110
750	919.25	16.402	1.153	0.113
1,000	888.00	16.855	1.185	0.116
1,250	856.75	17.308	1.217	0.119
1,500	825.50	17.761	1.249	0.123
1,750	794.25	18.214	1.281	0.126
2,000	763.00	18.668	1.312	0.129
2,250	731.75	19.121	1.344	0.132
2,500	700.50	19.574	1.376	0.135

- After sterilization is complete, gradually reduce the pressure inside the autoclave. If the pressure is lowered too quickly, the moisture at the surface of the medium is removed, causing the surface layer of medium to dry.
- After the pressure gauge indicates a return to normal pressure (gauge reads 0), quickly remove the medium and allow it to cool in a laminar air flow hood or other clean-air environment.

#### **8.8.2.4.1. Contamination by Return Air**

When the temperature inside the autoclave falls below the boiling point of water, the steam condenses to water, and the volume is reduced by a factor of approximately 1,700. This reduction in volume creates a vacuum condition inside the chamber, causing air to rapidly enter the chamber through the pressure release valve. The return air enters the bags/bottles as well as the chamber. If the return air contains thermophilic spores and the filtering ability of the caps is low, surface contamination of the medium may occur. Many large autoclaves are equipped with a device to filter the return air. However, if no such filter is present, after checking that the pressure inside the autoclave has returned to zero, open the hatch before a vacuum is created within the chamber (i.e., before the steam starts to condense) and move the bottles/bags to a laminar air flow hood (or other clean-air environment). Begin operating the laminar air flow hood (or other air cleaning equipment) beforehand so that the medium in bottles/bags can be cooled in a clean air environment.

#### **8.8.2.5. Inoculation, Incubation, and Storage**

##### **8.8.2.5.1. Inoculation**

After the medium has cooled, move the bags/bottles to the location where inoculation will be performed (hereinafter “inoculation room”) while preventing contact with the outside air. Perform inoculation in a laminar air flow hood. After the temperature of the medium is cooled to a room temperature drop spawn into the inoculation holes and the medium surface. When using the HEPA filter of a laminar air flow hood, the maximum allowable

humidity in the inoculation room is relative humidity (RH) 90%. If the RH exceeds this threshold, the laminar air flow hood will not function properly. As such, ample care must be exercised when selecting an inoculation room. Avoid rooms that remain humid all the time due to evaporation of groundwater through the floor. Also, because mold can grow on wood, avoid the use of wood in spawn production facilities as much as possible and, instead, use aluminum or another metal for room dividers. When spawn production is performed in such regions, it is highly recommended that the inoculation rooms be equipped with a dehumidifier. To ensure that mycelia spread uniformly throughout the bottles, make sure that the spawn falls to the bottom of the inoculation holes before inoculating the medium surface.

#### **8.8.2.5.2. Incubation environment and spawn storage**

The incubation room should be constructed so that it is sealed against contamination by the outside air. In addition, the floor of the incubation room should be insulated to ensure efficient temperature control. The incubation room should be maintained at a temperature that is slightly lower than the optimum temperature for mycelial growth typically 21-23°C. Because microbial contamination occurs more readily when the temperature of the incubation room fluctuates widely, a temperature controller should be used to maintain a constant temperature. Incubation rooms are typically ventilated so that the carbon dioxide (CO<sub>2</sub>) level does not exceed 3,000 ppm. However, if a CO<sub>2</sub> monitor is not available, a timer should be used to ensure that the incubation room is ventilated for 15 minutes every 2 to 4 hours (depending on the number of incubation bags/bottles). The exhaust fan should be equipped with a hood to prevent entry of outside air. Along with the exhaust fan, an air purifier equipped with a HEPA filter should be installed outside the incubation room and configured to allow cooled air to be pumped into the room (Fig. 2). When operating the exhaust fan, the outgoing and incoming air volumes should be checked by measuring the wind speed to ensure that they are balanced. Air should be moved gently through the incubation room to promote air exchange inside the bottles through the caps. Relative humidity inside the incubation room, should be maintained at 60-70%. Because relative humidity can fall below 40% during the dry season, the incubation room should be equipped with an ultrasonic humidifier. Centrifuge-type misters generate large droplets of varying size and do not raise the relative humidity effectively.

Primordia form on the substrate when nearly matured. Because primordia can be formed in extremely low levels of light (0.01 to 0.0001 lx), incubation rooms do not need windows and should be kept as dark as possible. Decomposition of the medium continues for approximately 1 month after the mycelia have spread. After the spawn has matured, it is distributed to mushroom farmers. When distribution is delayed, the spawn should be stored in a cool room (2 to 4°C) to prevent over-ripening. In addition, the spawn should be placed in clean plastic bags or otherwise stored and checked regularly to ensure that it does not dry out. The maximum storage period for spawn is 2 months. Older spawn should be discarded.

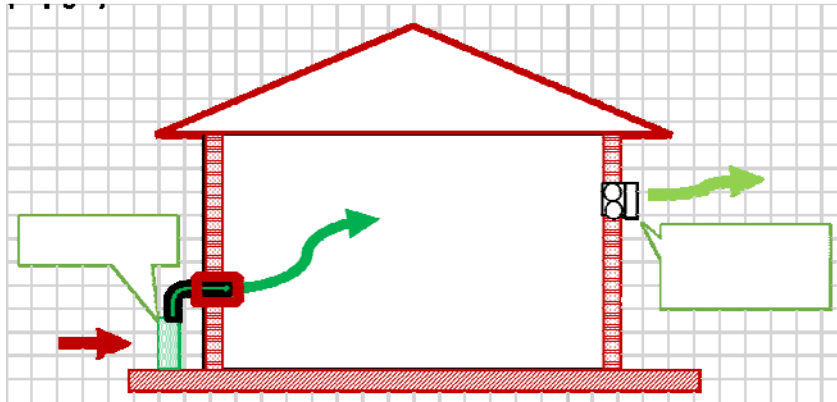


Figure 23: Diagram of a simple air-exchange system

## Microbial contamination: Inspection and countermeasures

### Inspection of the sterilization/cooling processes

Given potential contamination of the medium surface by return air during the cooling process, after cooling, samples of the medium surface (including the surface exposed to the inoculation holes) should be taken using sterile techniques while the sample is in the laminar flow hood. Samples should also be taken from inside the medium (areas that are not in contact with the surface) and transferred onto PDA medium to check for the presence of contaminants. This process is used to check whether contamination has occurred during the sterilization or cooling process.

The nutrient agar with high detection capability is typically used to detect bacteria; however, PDA is also commonly used. Although incubation for bacterial detection is typically performed at 35°C, at this temperature, thermophilic bacteria are also detected. In the mushroom farming sector, incubation is carried out at the optimal temperature for mycelial growth. After incubating the samples for 4 to 5 days at 25-28°C, check for the presence/absence of microbial contamination

### 8.8.2.5.3. Laminar flow hood management

The most important aspects of laminar air flow hood performance are the cleaning function of the HEPA filter (remove 99.97% of particles that are 0.3 µm or larger) and the flow rate of clean air.

- The cleaning function of the HEPA filter is checked using PDA. After running the laminar flow hood for 15 minutes, place 2 or 3 petri dishes containing PDA in the middle of the laminar air flow hood with the lids off for 30 minutes. Incubate the petri dishes in the incubation room for 4 to 5 days. If bacteria are detected when the laminar air flow hood is operated at the maximum allowable humidity (RH90%) or lower, the HEPA filter must be replaced. The cleaning performance of the laminar air flow hood can also be measured using a particle counter. Such particle counters are expensive, but they enable instantaneous checking of cleaning performance. If the laminar air flow hood is operating properly, the particle count for particles 0.3 µm or larger will be zero.
- HEPA filter maintenance requires regular cleaning of the prefilter. When the relative humidity is high, mold grows on dust collected by the prefilter, hindering the inflow of air and increasing the burden on the Sirocco fan.
- Specifications for the output (flow rate) of clean air after the HEPA filter are stipulated in the Japanese Industrial Standards (JIS). For horizontal laminar flow equipment, the flow rate at 10 cm in front of the HEPA filter should be 0.3 to 0.6 m/s. For vertical

flow equipment, the flow rate should be 0.2 to 0.9 m/s. If the flow rate decreases, contaminated air from outside the hood can mix in. As a general rule, the HEPA filter should be replaced when the flow rate falls to half the original flow rate. For horizontal laminar flow equipment, HEPA filters should be replaced when the flow rate falls to 0.2 m/s.

#### 8.8.2.5.4. Inspection and evaluation of spawn

All spawn should be visually inspected for microbial contamination during incubation and before shipping to customers. When doing so, pay attention to the following, as incubation containers that are characterized as follows must be discarded.

- Containers in which the mycelia do not spread completely through the medium within 1 month.
- Containers where the color at the top of the medium and the color at the bottom of the medium differ substantially (maturity not uniform).
- Over-ripe spawns with moisture content of 68% or higher.

#### 8.8.2.6. Manufacturing of Grain Spawn

##### 8.8.2.6.1. Adjustment procedure

1. Clean grains 2. Boil 3. Drain and cool 4. Add additives and mix 5. Fill containers 6. Make cotton plugs 7. Cover plugs with a polypropylene sheet 8. Autoclave 9. Cool 10. Inoculate 11. Incubate



Wash grains with water      boil      Drain & cool      Add calcium carbonate and gypsum



Fill bags      Make cotton plugs      Cover with polypropylene



Fill bottles      Sterilize      Vacuum-sealed bags      Cool in laminar flow hood



Figure 24: Procedure for making grain spawn`

### 8.8.2.6.2. Practical Considerations for Spawn Production

Both wheat and millet are used to make grain spawn, with wheat grains being the most common. In the case of grain spawn, no nitrogen source is added. The procedure for making grain spawn using wheat is described below.

#### 8.8.2.6.2.1. Grain Preparation

Measure out the specified grain amount and wash with water. At this point, discard husks and grains damaged by insects.

#### 8.8.2.6.2.2. Moisture Content Adjustment

Oyster mushroom mycelia grow vigorously at a moisture content of 45-50%, but growth slows at a moisture content of 40% or less. Although mycelia continue to grow vigorously at a moisture content approaching 50%, the grains become too soft, shortening the time that the grains can be used as spawn. Accordingly, grains for farm-scale production should be adjusted to 43-45%. Because the moisture content is determined during the boiling step, this step should be performed consistently. Although moisture content can be measured precisely if a moisture meter is available, if no meter is available, the moisture content can be estimated by calculating the increase in weight after boiling. An increase in grain weight by 1.6 to 1.65 times for fresh grains and by 1.65 to 1.7 times for older grains is optimal for mycelial growth. The equation used to calculate moisture content and sample calculations are presented below.

$$\text{Equation: } b = 100(1+c/a)/((100+c)/a)$$

a = initial grain moisture content before boiling (%)

b = grain moisture content after boiling (%)

c = percent increase in weight before and after boiling (%)

Table 7: Increase in weight and moisture content after boiling of substrate (wheat)

a (Initial MC, %)	c (Increase in weight, %)	b (MC after boiling, %)
6	60	41.3
8	60	42.5
10	60	43.8
12	60	45.0
6	65	43.0
8	65	44.2
10	65	45.5

Note: Normally, grain moisture content is 9 to 13%; however, the initial moisture content of old grains is 8% or lower moisture content (MC).

#### **8.8.2.6.2.3. Draining, Addition of Calcium Carbonate and Calcium Sulfate, and Filling**

After boiling, remove the water on the surface of the grains and allow drying. If grains are not sufficiently dried at this stage, water will pool at the bottoms of bags/bottles during sterilization. After boiling, the grains are removed from the container and spread on a plastic sheet, etc. and left overnight to dry. However, if there are time constraints, an electric fan, etc. can be used. Calcium carbonate and/or calcium sulfate (gypsum) are added to improve the separation of mycelia after inoculation. The amount of calcium carbonate or calcium sulfate added is 0.3 to 0.6% (w/w of medium) and 1.2 to 1.6%, respectively. Since oyster mushrooms are saprophytes that prefer slightly acidic conditions, addition of calcium sulfate (gypsum) by itself is also okay. When making spawn for farm-scale production, used heat-tolerant plastic (polypropylene) bags filled with 200 to 400 g of medium.

#### **8.8.2.6.4. Sterilization of Media**

Special handling is required for sterilization of plastic bags for farm-scale production to ensure proper sterilization. When plastic bags containing the grains are placed randomly in autoclaves, the air expand as the pressure inside the autoclave increases, so that the space between bags disappears and the steam does not flow uniformly. For this reason, the internal temperature of the medium does not increase and complete sterilization does not occur. When placing bags inside an autoclave, form two layers separated by a perforated tray or wire mesh to create a space between layers (to allow movement of steam). Sterilization is carried out in the same manner as for sawdust spawn. The procedure can be facilitated by monitoring the temperature of the medium using a data logger. After sterilization is completed and the pressure gauge reads zero, gradually open the hatch of the autoclave. Move the bags to a laminar air flow hood while they are still under vacuum and allow them to cool in a clean air environment. After sterilization, grain media (bags/bottles) must not be left inside the autoclave to cool. When using bagged media, check the performance of the heat seal of the bags and be sure to use good quality rubber bands that will not be damaged by heat to secure cotton plugs at the tops of the bags. Also, wrap the cotton plugs with a polypropylene sheet to keep them to avoid moistened from the steam and affix the plugs to the bags using a rubber band.

#### **8.8.2.6.5. Criteria for Production of Quality Spawn**

- Do not throw garbage around spawn production facilities, especially spent substrate, contaminated substrate and corn leftovers (*Neurospora* sp. easily propagates).
- Wash your hand with clean water and then wipe with Ethanol (70-80% v/v) before inoculation work.
- Do not do the inoculation work after other jobs.
- Change clothes and shoes before entering an inoculation room (a front room is recommended to be made).
- Do not wear the shawl when enter into the inoculation or incubation room.
- Cleaning the filter of an air-conditioner regularly (record the cleaning day).
- Cleaning the floor after finishing work should be done every day.

**8.8.2.6.6. Mother spawns:** plastic bottles or glass bottles (small bottles of 300~500 mL are recommended).

**Commercial spawn:** plastic bags with cotton plugs, and grains are 200~400 g in weight. Commercial spawn: produce for remote areas, decide the number of spawn of oyster, button mushrooms, and is shiitake included.

**Mother spawns:** decide the number of bottles of mother spawn for distribution to spawn producers and for inoculation to produce commercial spawn.

#### **8.8.2.6.7. The size of shelves for incubation of spawn**

**Materials:** Steel products are better than wooden one to avoid contamination.

Decide the size and the number of stages to clear the number of bottles/bags per one steel shelf for incubation.

The size is 9150 mm (L) ×4600 (W)×1820 (H), and one stage can accommodate 28 bags with 0.7~1.0 kg media. The necessary number of steel shelf varies depending on the types of spawn.

### **Mushroom Spawn Production**

- Spawn is any form of mycelium that can be dispersed and mixed into a substrate.
- It's mushroom mycelium growing on a given substrate such as wheat or sawdust. After the culture has grown throughout the substrate medium, it is called "spawn."
- Spawn can be purchased in a variety of forms, e.g., grain, sawdust, plug and liquid spawn
- The most common forms are grain or sawdust spawns.
- Grain spawn is typically used by commercial cultivators to inoculate sterilized or pasteurized substrates.
- Spawn remains in a healthy state for a very limited period of time, usually for no more than 2 months.
- Even under refrigeration (2-4°C), a noticeable decline in viability occurs.

#### **8.8.2.7.1. Grain Spawn**

Grain is the most popular. Wheat is also utilized.

Advantages: Grain spawn breaks up easily into individual grains which can be evenly distributed through the substrate, thus decreasing the colonization time. Each kernel of grain is full of mycelium, which is protected during inoculation. The spawning rate is generally less than 2% (v/v).

#### **8.8.2.7.2. Liquid Spawn**

- Liquid spawn is slurry of mycelium in a nutrient solution. It can be produced by blending intact mycelium or by culturing in liquid fomenters.
- Advantages of liquid spawn are the large number of inoculum particles put into the sawdust and the ease of inoculation.
- Liquid inoculum is very different from the sawdust substrate into which it is injected. The mycelium must recover from inoculation, and then turn on the enzymes needed to colonize wood. Inoculation success is improved if the mycelium has been adapted to wood extracts prior to inoculation.
- Fortifying the inoculation solution with sugars increases initial growth, but it also increases the risk of contamination. The extra nutrients may act as a reserve for the mycelium, but--unlike grain spawn--this reserve is not already colonized by spawn

mushroom. The purity of cultures used for liquid inoculation must be absolute because a small amount of contamination is spread much farther than with conventional methods. However, under proper conditions, many containers can be inoculated rapidly.

#### 8.8.2.8. Culture (Strain) Preservation

- Periodic transfer
- Sterile mineral oil (paraffin oil) overlaying
- Sterile distilled water storage
- Freeze-drying (lyophilization)
- Freezing:
  - a. - 20 ~ - 135°C
  - b. - 196 °C( in liquid nitrogen)

#### 8.8.3. Adaptive Research Technology Demonstration and Production Unit

This unit will carry out adaptive research and demonstrate the technologies for commercial production, pure culture & spawn production and post-harvest management. In addition, this unit will have production blocks for the different types and species of mushrooms with the necessary facilities to manipulate the production environment (for example temperature, relative humidity, air circulation, light etc.) as per the requirement so that mushroom production could be feasible to the any seasons of the year.

- Research on different parameter of native mushroom
- Carrying out Verification trial of commercial mushroom strain
- Demonstration of new strain of mushroom
- Comparative Study of new strain of mushroom

##### 8.8.3.1. Mushroom Cultivation Technologies

**Table 7: Various types of raw substrate available in Nepal and associated mushroom species.**

Substrate	Mushroom species
Rice Straw	Straw ( <i>Volvariella</i> ), Oyster ( <i>Pleurotus</i> ), Button ( <i>Agaricus</i> )
Wheat straw	Oyster ( <i>Pleurotus</i> ), Button ( <i>Agaricus</i> ), <i>Stropharia</i> , Straw ( <i>Volvariella</i> )
Sawdust	Shiitake ( <i>Lentinus</i> ), Oyster ( <i>Pleurotus</i> ), Ear ( <i>Auricularia</i> ), Reishi ( <i>Ganoderma</i> ), Maitake ( <i>Grifolafrondosa</i> ), Lion's Head or Pom Pom ( <i>Hericium</i> )
Sawdust-straw	Oyster ( <i>Pleurotus</i> ), <i>Stropharia</i>
Cotton waste from textile industry	Oyster ( <i>Pleurotus</i> ), Straw ( <i>Volvariella</i> )
Logs	Nameko ( <i>Pholiota</i> ), Shiitake ( <i>Lentinus</i> ), White jelly ( <i>Tremella</i> )
Crushed bagasse and molasses wastes from sugar industry	Oyster ( <i>Pleurotus</i> )
Water hyacinth/Water lily	Oyster ( <i>Pleurotus</i> ), Straw ( <i>Volvariella</i> )
Banana leaves	Straw ( <i>Volvariella</i> )
Bean straw	Oyster ( <i>Pleurotus</i> )
Sawdust-rice bran	Nameko ( <i>Pholiota</i> ), Ear ( <i>Auricularia</i> ), Shiitake ( <i>Lentinus</i> )

### **8.8.3.1.1. Cultivation of Oyster (Pleurotus species) Mushroom**

This is a tropical and subtropical mushroom. It can be grow on different substrates like paddy straw saw dust, wheat straw etc. This mushroom can be cultivated throughout the year. There are different strain of oyster mushroom such as *Pleurotus sajor-caju*, *Pleurotus florida* and *Pleurotus ostreatus*.

#### **8.8.3.1.1.1. Method of Cutting and Soaking the Straw**

Cultivation of Oyster Mushroom requires clean and dry fresh straw. During summer 'Taichung' variety of straw is recommended. However, cultivation of these mushrooms can be done in rice straw of any variety. First, straw shall be cut into pieces about 2 inches long and chopped straw shall then be soaked in clean water for at least 2 hours in winter season and 15-20 minutes in summer season. Thereafter, the soaked straw shall be kept in shade for 8-12 hours to drain the water out. When squeezing the straw with a first, if the water does not come out and only palm gets wet it should be considered that the moisture of straw is correct.

#### **8.8.3.1.1. 2. Method of Steaming Straw**

A tin or iron drum should be filled with 6-9 inches of water and a wire mesh shall be placed above the surface of water with the help of bricks. The water drenched straw shall be placed on the mesh to the mouth of the drum which later shall be covered with plastic sheet and tied with rope. Earthen pot can be used instead of drum. Then, burn the stove or firewood to steam the straw. In this way after steaming in more than 95 degrees Celsius when hot steam comes out from the mouth of drum, steam continuously for about 3 hours in summer season and 2 hours in winter season. This shall cure the bacterial infections and other organisms shall die. Steaming more hours in summer season is because bacterial infections are more during summer season. After steaming the straw, it is allowed to cold for 12-15 hours. When the temperature is less than 25 degrees Celsius, it can be used for inoculation of spawn (planting spawn).

#### **8.8.3.1.1.3. Quality of Mushroom Spawn**

Mushroom spawn must be of good quality. While selecting spawn, spawn shall be 10-15 days old without any contamination in which mycelium has not spread over top and the growth of mycelium shall be in in one level.

#### **8.8.3.1.1.4. Methods of Inoculation or Planting of Spawn**

When planting spawn, pour the seed from bottle into a clean pot or newspaper using a clean needle. It is very important that both hands are clean while taking out the spawn and planting them. About 10 cm apart, 1 cm holes should be made in plastic bags in which spawn is to be planted. After placing or putting straw up to 2-3 inches in plastic bags, spread 1 teaspoon (15 gram) of spawns on top of the bag in a ring-shaped straw. Then again place 2 inches of straw and spread the spawns ring shaped. Till the plastic bags is filled, plant spawn on the straw layer by layer and after filling the plastic sow some spawns from top and with the help twine the mouth of the bag is covered.

#### **8.8.3.1.1.5. Incubation and Crop Management:**

Place the plastic bags filled with spawn on the dark room with closed windows for 2-3 weeks for incubation. The temperature of room shall be about 20-25 degree Celsius. After

the spread of mycelia all around the plastic bag, the mouth of bag is ready to be opened and sharp knife or blade is used to cut the plastic.

The ball of white straw can be placed on a brick or wooden board or basket or can also be hung using a rope. Sprinkle water 1-2 times a day on these balls so that relative humidity shall be maintained up to 80-90 percent. For this the floor of the room in which mushroom is to be grown shall be made cold using water or the windows of the room shall be covered by wet bags. About 4-5 days after opening the mouth of the plastic bag, the primordia of mushroom will come out like small beads. These lumps become large and turn into mushrooms. After 3-4 days it is time to pick mushrooms. At this time, the ventilation of the room should be good and light is also necessary. If the room is dark, it should be illuminated with a tubelight for 12 hours a day.

#### **8.8.3.1.1.6. Harvesting of Mushroom**

It is very important to cover the nose and mouth with a mask or handkerchief when going to the room to pick the mushrooms. When picking mushrooms, a blade or sharp knife should be used to cut the stems and straw joints so that no part of mushroom shall remain. Otherwise, disease can attack the remaining part and spread in others too. When plucking the mushrooms, don't pull them. The pulling will affect the other mushrooms to grow. Mushrooms can be picked 3-4 times in interval of 10-15 days. After that, there will not be any more production.

#### **8.8.3.1.1.7. Production**

From 1 kg of dried straw, generally 500-700 gm of fresh mushroom can be produced. If the technology is properly adopted, condition is favorable the production can be more.

#### **8.8.3.1.2. Cultivation Technique of White Mushrooms**

The English name of White Mushroom is Milky Mushroom and scientific name is *Calocybe indica*. It can be cultivated in paddy straw, wheat straw etc., It can be cultivated in Terai districts and inner Terai areas such as Chitwan, Dang, plain area (*besi*) and areas of Kathmandu with hot climate in rainy seasons (Baisakh, Jestha, Ashad, Shrawan, Bahdra). The temperature of room or shed should be 20-30 degree Celsius during initiation stage. The temperature of 20-25 degree Celsius is highly recommended. At the time of fruiting, temperature shall be above 30 C up to 37 C and relative humidity shall be 70-80 percent. The pH value of wet straw should be in between 6.5-7.5. The materials necessary for cultivating these mushrooms is similar to that required for oyster mushrooms. The percentage of spawn required for cultivation is 15% i.e., 750 gms of spawns required for 5 kg of straw.

##### **8.8.3.1.2.1. Cultivation Techniques**

The method of cutting, soaking and steaming of straw as well as the process of Inoculation of spawn and maintaining them shall be similar to that of Oyster mushrooms. In order to cultivate white mushrooms, soil is used to cover as a Casing soil.

##### **8.8.3.1.2.2. Casing Soil Preparation**

###### **Soil Filling (Casing)**

- A. Soil treatment should be started 2(two) weeks after planting the spawns to prepare the casing soil for covering the mouth of the bag. Loamy or sticky soil is good for this. When removing the soil from garden, remove about 6 inches of soil from top and the

soil within is taken out, made fine by breaking all the balls. If the 75 kg of soil taken out is too sticky then same can be mixed with 25 kg sand. In 100 kg of this kind of soil, 3 kg of Agricultural Lime (3 percent) and 250 ml of Formalin (2 percent) mixed in 5-liter water shall be prepared and sprayed. Cleaned shovel should be used to mix the heap and should be covered by plastic sheet and treated for 48 hours. After removing the plastic, the smell of formalin should be removed by turning soil twice in a difference of 2-3 days. The soil should have pH of 7.8-8.5. The treated soil can be used after 1 week. For 1 ton of straw, 800-1200 kg of soil is necessary.

- B. Time of casing :After the mycelium has well spread in the plastic bag (20-25 days after planting spawn) treated soil shall be used to cover the mouth of plastic bag and should be 1-1.5 thick to cover the topmost portion of bag.
- C. After covering the mouth of bag with soil, watering shall be done with the help of sprayer such that only the surface of soil gets wet. If too much water is sprayed, the spread of fungus shall be affected.

#### **8.8.3.1.2.3. Room Environment**

After the plastic bag has been covered by casing soil the room temperature shall be maintained above 30, between 37-38 C for 7-10 days. By that time, the mycelium below the surface of soil shall start to spread properly. Fresh air shall enter the room and the relative humidity shall be maintained at 80-90 percent in the room. During summer, floor and walls of room shall be made wet by spraying water on it or jute sack shall be posted on floor and walls and made wet.

#### **8.8.3.1.2.4 Harvesting of Mushroom:**

Same as that of Oyster mushroom.

#### **8.8.3.1.2.5. Production**

5 kg of dried straw shall yield 3-5 kg of mushroom.

#### **8.8.3.1.3. Cultivation Technique of Gobre Chyau (Button Mushroom)**

The scientific name of button mushroom is *Agaricus bisporus*. These mushrooms can be grown artificially. Button mushroom (Gobre ) shall be grown in places where there is circulation of cold air and water. Depending on season it can be cultivated in hills, valley and terai and with the installation of air conditioning equipment to maintain temperature, relative humidity and moisture these mushrooms can be cultivated throughout the year (in 12 months). In General, farmers of Nepal are not financially strong to make such investment because of which after studying the weather it is possible to cultivate mushroom twice in a year in Kathmandu valley.

If you prepare compost and inoculation of spawn in Poush-Magh, mushroom shall grow in Falgun-Baisakh. If you prepare compost and inoculation of spwan in Ashad-Shrawan, mushroom shall grow in Asoj-Mangsir. In similar way, in hilly and terai areas appropriate time to cultivate mushrooms shall be determined by studying local climate.

While cultivating mushrooms, special attention should be given to following factors:

- A. Temperature:

After spawn is inoculated, temperature of room shall be maintained at 20-25 C and same temperature shall be maintained for 10 days after the soil is filled. Mycelium spreads well in this temperature. When the temperature is very low, mycelium cannot spread.

**B. Humidity**

After planting the spawn, the relative humidity shall be maintained at 70-75 percent. The relative humidity shall be at 80-90 percent at the time of pinning of mushrooms. For this during dry weather, water shall be sprayed on the floor to maintain humidity.

**C. Ventilation and Light**

The place or room where mushrooms are to be cultivated shall be well ventilated with good circulation of fresh air inside and air shall not be allowed to move out of the room. Sunlight shall not pass straight in the room. Dim light in the room is more preferable.

**8.8.3.1.3.1. Required Materials Recommended for the Cultivation of Gobre Chyau**

**A. For making fertilizer:**

1. Straw(new):1000 kg
2. Urea: 5 kg
3. Ammonium Sulphate:20 kg
4. Superphosphate (Triple) or D.A.P:10 kg
5. Agricultural lime: 40 kg
6. Mushroom spawn: 20 bottles

**B. Other materials required:**

1. Formalin:1 liter
2. Dithane M-45:125 gm
3. Nuwan:100 ml

For the people interested in growing these mushrooms they are recommended to prepare 250 kg of compost of straw and arrange for other materials accordingly. The following methods shall be followed while cultivating these mushrooms.

**8.8.3.1.3.2. Preparation of Compost**

The straw for preparation of compost shall not be too much wet. Air shall be circulated in the heap of compost properly. The compost shall be well ripened. Too much of ripening of compost shall spoil the compost fully and also if the compost is not ripened properly, there is possibility of overheating of compost bed.

**8.8.3.1.3.3. Appropriate Time to Flip the Compost**

In preparation of compost special attention shall be given at the time of flipping of compost.

- First flip (first turning): On fifth day, compost should be flipped for the first time. While flipping, the dry straw shall be suppressed inside such that inside of the compost shall be kept outside and outer part of compost is suppressed inside.
- Second flip: The compost should be flipped for second time in 10<sup>th</sup> day.
- Third flip: The compost shall be again flipped for third time in 13<sup>th</sup> day.

- Fourth flip: The compost shall be flipped for fourth time in the 16<sup>th</sup> day. From this day, compost shall be mixed with hand and made into a pile. When the compost is flipped this time, Ammonia gas comes out which does not give good odor and causes sore eyes.
- Fifth flip: compost shall be flipped for 5<sup>th</sup> time in 19<sup>th</sup> day. Nothing shall be put in the compost this time.
- Sixth flip: On 22<sup>nd</sup> day, compost shall be flipped for 6<sup>th</sup> time. Proper quantity of pesticides should be sprayed this time for disease and insect's control. For disease control, Dithane M-45 or Dithane Z-78 (2.5 gm per liter) shall be mixed in water and in the same mixture for insects control Nuwan (1 ml per liter) shall be added in mixture. For 1 ton of compost 20 lit of pesticides shall be prepared and sprayed all over the compost.
- Seventh flip: The compost shall be flipped in 25<sup>th</sup> day for 7<sup>th</sup> time. Nothing shall be kept in compost this time.
- Eighth flip: Generally, in summer weather compost shall ripe and be made ready in 28 days.

#### **8.8.3.1.3.4. Preparation of Fed**

After the compost is ready bed shall be prepared in room where mushroom is to be cultivated. The length of compost bed should be 3-4 feet in width and 6-8 inches in height according to the size of the room. If two beds are to be prepared, between two beds 1-1 feet gap shall be maintained. Rack or basket shall be filled with compost for cultivation of mushrooms.

#### **8.8.3.1.3.5. Proper Time to Planting Spawn (Inoculation):**

After 1-2 days of filling the bed with compost the temperature of compost shall be maintained at about 25 C. After the smell of ammonia disappears before planting the spawn a piece of cotton or cloth is made wet with spirit to clean hands, containers in which spawn is to be kept and needle. After this, needle shall be used to mix spawn in the spawn bottle and pour the spawn from the bottle to the clean container. Then, 1-1.5 inches of compost shall be removed from the top of the compost bed and seed shall be sown on same after which same compost shall be used to cover the spawns pressing the compost lightly. It is also recommended that spawn shall be sprinkled on top of the compost.

After planting the spawn the bed shall be treated with 2 percent formalin (in 1 liter water 50 ml formalin) and covered with newspaper. When watering the bed, only newspaper shall be soaked. After planting the spawns, the room temperature shall be maintained at 20-25 C. After 8-10 days white mycelium shall be observed in compost bed and after 15-20 days white mycelium shall spread on all part of compost bed.

#### **8.8.3.1.3.5. Soil Filling (casing)**

Soil preparation shall be similar to that in White mushroom.

- 1) Time to fill the soil: After the spawns have spread well in compost bed and 15-20 days after planting the spawns bed shall be covered with treated soil 1-1.5 thick.

- 2) Method of watering: After covering the compost bed with soil, sprayer is used to water the surface of the casing soil to make it wet.

#### **8.8.3.1.3.6. Room Environment**

After covering the compost bed with casing soil, the room temperature shall be maintained at 20-25 C for 7-10 days. By that time, mycelium start to spread below the surface of casing.

By 11<sup>th</sup> day:

1. The room temperature shall be reduced in between 15 C- 18 C.
2. Fresh air shall be circulated inside the room.
3. Relative humidity in the room shall be between 80-90 percent.

In hot weather, the floor, walls of room shall be made wet by spraying water or wall can be covered by wet jute sack. During winter, the temperature or humidity of room shall be increased through use of heater and boiling water in large pot. Air cooler shall be kept or windows of room shall be opened to keep the room well air circulated. The windows of the room shall be fitted with wire netting having small holes to circulate fresh air inside the room.

#### **8.8.3.1.4. Cultivation Technique of Shiitake (Mrige) Mushroom (*Lentinula edodes*)**

Colour of this mushroom is liver brown, has white dots in body and at a glance looks similar to external color of deer. Different strains of Shiitake mushroom include Mori 290, XR 18 and W4.

##### **8.8.3.1.4.1. Selection of Trees and Log**

These mushrooms cannot be cultivated in all types of trees. For this purpose, walnut trees are considered good. Apart from this, these mushrooms can be cultivated in various deciduous trees of Nepal like Uttis, Katus, Saur, Mauda, Banj, Lakure, Dale Katus. Cultivation can be done on wood of above-mentioned trees that are usually 4-20 years old. The log shall be free from disease, of same size, “**matured bark**” tight and uninjured. The diameter of log shall be 7 to 25 cm (3” to 10”). The tree selected for mushroom cultivation shall be cut from Kartik to Magh (after the leaves start to fall and before the new growth of leaves). Since the sugar content in wood bark is higher during winter wood cut at this time is suitable for shiitake mushroom cultivation. After cutting the tree in order to reduce the amount of moisture in log, the log shall be kept away from sun for 7-15 days. Before planting the mushroom spawn, the humidity of log shall be maintained between 40- 50 percent. If there is less than 20 percent or more than 70 percent of moisture the white fungus shall not spread well. If there is too much moisture harmful insects like *Trichoderma spp.* shall attack the area in which spawns has been cultivated.

##### **8.8.3.1.4.2. Materials Required for Small Scale Cultivation of Shiitake Mushrooms:**

Wooden log, drilling machine to make holes in log, hand drill machine is called Berma, white wax (paraffin wax), fat, sawdust, utensils to melt wax, wooden or bamboo stick (1 ft long), muslin cloth, shiitake mushroom spawn, seed tray, iron needle, plastic sheets (1.5 meters wide), wood measuring tape (1.5 m long), wood cutting axe, wood cutting saw, cotton, spirit are necessary materials required.

##### **8.8.3.1.4.3. Method to Make Holes in Log and Plant the Seed**

By use of drilling bit of 1-1.5 cm, 2.5 cm deep holes are made in wooden log. While making holes in this way leaving 5-5 cm on both top ends of log, in middle holes are made

10 cm apart. The distance from one line to other line shall be 5 cm. When making hole from first line to second line, it should fall in the middle of the first line.

Shiitake mushroom spawn shall be taken out from plastic bag or glass bottle with the help of clean iron needle and placed on clean tray or plastic seat. Hands should be thoroughly cleaned with soap before handling spawns. The spawns should be made into small lumps and the holes of log shall be filled with those lumps. Syringe to plant the spawn and Pressure spawn plugging tools are the equipments found in market.

Three parts of fat, 12 parts of **sallako khoto**, 85 parts of wax shall be melted separately and whole mixture shall be heated in a pot. Wrap up 12 inch long cotton around the wooden or bamboo stick or “**ghochokotuppo**”. A cotton wrapped stick is tightened with thread then dipped in hot steaming wax, fat, “**khoto ko gholma**” and used to fill holes. The solution shall fill the holes in such a way that it does not come above the surface of the wood. The log in which spawns are planted is called bed log.

#### **8.8.3.1.4.4. Management of Bed Log**

The temperature of the place where bed log is kept shall be maintained at 15-22 C. The logs are to be rotated from top to bottom and otherwise frequently. Lay bricks or wood on the bottom so that bed log does not touch the soil. They should be covered with plastic if not kept in store. If covered by plastic sheets, plastic shall be removed 1-2 times in a day so that air circulation is maintained. In hot weather, water should be sprayed in each bed log in alternative or every day.

- After 1-1.5 months the wooden logs shall be turned from upside down and otherwise 3- 6 times. By this time the bed log is 6/7 months old after which they are to be managed differently. Now the logs have to be separated from the group and kept vertically and apart from each other in a store. Bottom of logs shall not touch the soil. Bed logs shall be kept in support of bamboo or wooden stand and 10 cm apart from each other.
- Direct sunlight shall not enter in the room where bed logs are kept and dim light would be appropriate. The temperature of room shall be maintained between 15-20 C. The relative humidity of room shall be 70-80 percent. Also, the moisture content in the room shall be 55-65 percent for which seeing the weather conditions water shall be sprayed in wooden log 1-2 times. If the humidity is less than 32 percent, mushrooms will not grow.
- By this time, the fungus might have well spread in hard wood part of the bed log and if the temperature, light and humidity is well maintained mushroom primordia start to emerge which later grow into the mushroom. It will take around 6-10 days in maintained temperature to reach to the point where mushroom can be harvested.
- After plucking the mushrooms, the bed log has to be soaked in water for 12-24 hours to prevent drying out of bed logs. Again, these bed logs are stored and incubated for 2-3 months at 15 to 20 C temperature and water is sprayed frequently for production of mushrooms.

#### **8.8.3.1.4.5. Harvesting of Mushroom**

While picking mushrooms, they shall be cut either clockwise or counter clockwise or mushroom stem or surface of bed log shall be cut by the knife. No part of mushroom shall remain on the surface.

#### **8.8.3.1.5. Cultivation Technology of Red Mushroom (*Ganoderma Lucidum*)**

This mushroom is used as a multi-purpose medicinal mushroom. It is not used as vegetable like other mushrooms. There are many types of mushrooms in this category. Red mushroom is one of them. Skin of these mushrooms is red and shiny. According to the study so far, cultivation of these mushrooms is considered appropriate in humid places like Kathmandu, Godavari, Nagarkot, Lumle, Kaski from Poush to Magh.

##### **8.8.3.1.5.1. Climate Required for Cultivation**

Cultivation of these mushrooms is considered appropriate in places where temperature of 15 to 25 degree C and humidity of 80-100 percent is maintained. It is considered good if the temperature is 15-16 C especially for 3-4 weeks after planting the spawns. Mushroom disc requires 80-100 percent relative humidity, temperature of 20-25 C, good air circulation and light for growth of these mushrooms. These can be cultivated in wood logs of trees like Uttis, **Khasro**, Katus, wood dust of Uttis and paddy straw.

##### **8.8.3.1.5.2. Selection and Preparation of Disc:**

Its cultivation can generally be done on wood of the abovementioned trees of 4 to 10 years old. Disc shall be made of same size and from matured, tight and uninfected bark of these trees. The diameter of wooden disc is 10-15 cm (4" – 6") and should have wooden frame of corresponding length. The trees selected for mushroom cultivation shall be cut from Kartik to Magh (after leaves of the trees began to fall and before the new growth begins). It is considered appropriate to cut the trees 7-10 days before planting.

After cutting the trees to reduce amount of moisture in logs they shall be kept in cool place for 7-15 days without direct sunlight. Before planting mushroom spawns the moisture content of the mushroom disc should be 45-50 percent. If there is more moisture after planting spawns other harmful fungus like *Trichoderma spp* may attack.

##### **8.8.3.1.5.3. Materials Required for Cultivating Red Mushrooms:**

Wooden disc, Plastic bag 12X14", mushroom spawns, iron needle, plastic sheet 1.5m, wood cutting axe, saw, **sprit**, **sprit lamp** etc.

##### **8.8.3.1.5.4. Method of Steaming Mushroom Disc**

Inside of a tin or iron drum shall be filled with 9 inches of water and a wire mesh shall be placed above the surface of water using bricks. Layers of mushroom discs shall be placed on top of mesh up to mouth of drum. Mouth of drum is then covered by plastic sheet and tied with a rope. Stove or firewood is then burnt to boil the mushroom discs. While boiling at above 96 C when hot steam comes out of mouth of drum steam shall be vaporized for continuous 2-3 hours. This shall kill the infections and other infectious fungus in the

mushroom disc. After the vaporization of mushroom discs, they are cooled down for 15-16 hours and when temperature is below 25 C the disc becomes appropriate for planting the mushroom spawns.

#### **8.8.3.1.5.5. Inoculation of Spawn (method to plant spawns)**

The room shall be cleaned 2 days before planting of the spawns and the formalin solution shall be sprinkled in the room and kept closed. At the time of planting spawns, spawn shall be removed out from the plastic bottle or plastic bag with the help of clean needle and mouth of plastic in which mushroom disc is kept shall be opened. After which, about 50 gms of spawns shall be spread on the top surface of the mushroom disc and mouth of Disc shall be tied with the cotton rope.

#### **8.8.3.1.5.6. Incubation and Mmanagement of Mushroom Disc**

The mushroom disc in which spawns are planted shall be kept in dark room with good air circulation or shed for 2-3 weeks for incubation. The temperature of the room or shed shall be maintained at 15-16 C. If blue/green colored fungus appears in mushroom disc apart from white fungus, it should be removed immediately. If the white fungus in mushroom disc grows into white buds' mouth of mushroom disc shall be ready to be opened. Such mushroom disc then shall be placed in a bed shape in the shed of 4-5 ft height such that discs shall not touch each other. The relative humidity shall be maintained at 90-95% by covering the disc with casing soil lightly and watering the soil 2-3 times from time to time. At this time, ventilation of room shall be good and light is also very important. If the place does not have good light, mushrooms shall not grow into good shape and production will also get affected.

#### **8.8.3.1.5.7. Harvesting of Mushroom**

It is very important to cover the nose and mouth with mask and handkerchief before entering the room where mushrooms are growing or while picking the mushrooms as the spores of the mushrooms are scattered everywhere in the room. While picking the mushroom, a sharp knife shall be used to cut the stem of the mushroom and joint of the mushroom disc that is, no part of the mushroom shall remain. Otherwise, the remaining part can get infected and infections shall spread to other mushrooms also. Mushrooms can be picked for 2-3 times at a gap of 2 months. The mushroom disc used for production can be used next year as well for production. Next year with same mushroom disc and maintained humidity, light, moisture and air circulation production can be done in month of Jestha and Ashad. The production of quantity of mushrooms can be calculated through 45-65% of initial weight of the wood.

### **8.8.3.2. Disease Management Practices in Mushroom Production**

#### **8.8.3.2.1. Management of Mushroom Flies**

- Sticky trap is used for monitoring and management of mushroom flies.
- The trap consists of a 15 W yellow bulb and a polythene sheet of any size coated with mustard oil hanged on the wall. Flies show photo-tactic behavior in the morning and evening hours, all the flies will stick to the polythene sheet.

- This trap is highly popular among the mushroom growers and is widely adopted in almost all the mushroom growing areas of Nepal.
- In addition to the trap, one or two spray application of deltamethrin, malathion or dichlorovos as adulticides on walls and floor is highly effective for fly control.
- Placement of UV fly catcher at the height of 4-5 feet effectively controls mushroom flies.

#### **8.8.3.2.2. Management of Wet Bubble Disease (*Mycogone perniciosa*) in Button Mushroom**

Wet bubble pathogen (*Mycogone perniciosa*) of button mushroom has a world-wide distribution and can cause severe crop losses. Infected fruit bodies, spent mushroom substrate, farm yard compost, substrate material, ground water etc. are the major sources of inoculum. Disease transmits through contaminated irrigation water, air, casing soil, picker's hands, insects (like flies and mites). If the pathogen infects mushroom before the differentiation of stipe and pileus, the sclerodermoid masses are formed. Whereas infection after differentiation results in the production of thickened stipe with deformation of gills. *M. perniciosa* produces small thin-walled phialoconidia on *Verticillium*- like conidiophores and bicellular conidia which are commonly referred to as either aleuriospores or chlamydospores. Use standard crop management practices and be the earliest to jump to any disease control strategies.

1. Wet bubble produced two main symptom types, one if young pin heads are infected they develop monstrous shapes which often do not resemble mushrooms. When infection take place before the differentiation of stipe and pileus the sclerodermoid form resulted, whereas, infection after differentiation resulted in the production of thickened stipe with deformation of the gills.
2. Always do composting on cemented floor
3. Maintain proper moisture in compost and proper pasteurization at 59 °C for 6-8 hrs
4. Proper pasteurization of casing at 65 °C with 65% moisture
5. Treat empty room with 2% formalin
6. Maintain proper hygiene and sanitation in and around mushroom house
7. Use foot dips
8. Harvesting should be done from new rooms to older rooms
9. Use light trap for monitoring and controlling fungal gnats. Drench with 2% formalin before disposing off the bags
10. Maintain 70 °C temperature inside rooms for 8-10 hours
11. Dispose off spent mushroom substrate in pits away from mushroom farm and cover it with layer of soil
  - Ensure the cleanness of machinery and all equipment for spawning and compost filling.
  - Ensure the cleanliness of growing rooms. (Floor, walls, shelves, cloths, racks and other equipments and tools must be thoroughly cleaned and treated with disinfectants)
  - When the work is done, the machinery, equipment and rooms must be cleaned properly
  - Disinfect the machinery, equipment and the corridor following the route of transportation, the nets, cloths and other inventory with 2% formalin solution before starting work

- Maintain the time needed for the contact action of the disinfectant and the processed surface (not less than 20 minutes), then thoroughly ventilate the growing rooms
- During compost filling and spawning, the personnel doing this work isn't allowed to enter a clean corridor or the warehouse, or contact personnel engaged in harvesting mushrooms. The components for the preparation of the casing soil must be stored special places, not allowing them to be mixed
- Keep clean the room where the casing soil is stored along with the area adjacent to it;
- Transport the casing mixture and its components to the growing rooms only in thoroughly washed and cleaned transportation
- After the application of casing layer, immediately remove the remains of the casing mixture from the working corridor and the growing room, then clean the floor machinery and equipment
- During the process, there should not be any work that doesn't have to do with the application of casing layer going on in the working corridor; the passageway must be closed
- Alternatively, a spray of 0.8 percent formalin on to casing surface, immediately after casing, can be effective. However, this concentration can be injurious if used at later stage in crop

#### **8.8.3.2.3. Management of yellow mould disease syndrome**

- Yellow mould syndrome produces three characteristic symptoms in mushroom beds viz., mat formation, confetti and yellow flakes with spore mass inside the compost beds either separately or in combination.
- The causal organisms of yellow mould disease encountered in button mushroom farms are *Myceliophthora lutea*, *Sepedoniumchrysopermum* and *S. maheshwarianum*.
- Although all these yellow mould causing organisms are capable to reduce the mushroom yield, but *M. lutea* is the most devastating fungus causing complete crop failure depending upon the stages of the infection.
- Under seasonal conditions, casing is normally chemically pasteurized and causes the problems of fungicidal residue in the mushrooms. As an alternative, tunnel sterilized casing soil showed drastically reduced fungal counts in comparison to control.
- 0.5% phosphate supplementation in the compost can increase yield upto 98% in comparison to untreated inoculated control and the disease could not establish in any of the treated bags.
- Extract of *Cannabis sativa* is very effective in reducing the growth of yellow mould pathogens without affecting the growth of *A. bisporus* when added in malt extract agar medium @ 5%.
- Use of pasteurised compost, sterilized casing soil may be a good alternative along with addition of P2O5 (0.5%) in compost to prevent crop losses due to yellow mould syndrome.

#### **8.8.3.3. Mushroom Based Value Added Products**

Various value-added products such as mushrooms pickle, jam, sauce, candy, preserve, chips etc. can be prepared from fresh mushrooms whereas from the dried mushroom powder value added products like instant soup mix, bakery products, papad, nuggets etc.

#### **8.8.3.3.1. Mushroom Pickle**

Pickling of mushrooms is an easy home scale process for preservation of mushrooms to a value added product of high market acceptability. For preparing mushroom pickle, mushrooms are washed, sliced and blanched for 5 min in 0.05% KMS solution. The blanched mushrooms are washed in cold water for 2-3 times and the excess water is drained off. Then the mushrooms are subjected to salt curing process, in which 10% sodium chloride is added and kept overnight. The excess water oozed-out of mushroom is removed on the next day and spices & preservatives are mixed to the desired taste and quality of mushroom pickle. To 1 kg mushroom various spices viz. turmeric powder (20 g), black mustard seed powder (35 g), red chilli powder (10 g), cumin seed powder (1.5 g), carom seed (10 g), nigella seed (kalonji)(10 g), fennel seed powder (1.5 g) and mustard oil (200 ml) are added to prepare tasty pickle. Acetic acid and sodium benzoate within the permitted limits are used as preservatives. This pickle can be stored up to one year in the airtight bottles.

#### **8.8.3.3.2. Mushroom Biscuits**

Both button and oyster mushroom can be used to prepare delicious and nutritious mushroom biscuits using ingredients viz., refined wheat flour (*maida*) & mushroom powder ( in 80:20 ratio), sugar (30%), ghee (bakery fats) (45%), baking powder (0.6 %), ammonium bicarbonate (0.3%), salt (0.6 %), milk powder (1.5 %) and vanilla essence (0.02%). For making biscuits all the dry ingredients are finely ground and sieved. Then fat and sugar are mixed well for 5-7 minutes using dough kneeder. These ingredients are then added to dough kneeder with other dry ingredients for dry mixing of 20-25 minutes. Thereafter, water is added to make dough cohesive and homogenous and mixing is continued for 10-15 minutes. Then dough is kept for 10 minutes covered with wet cloth. Thin sheets of dough (1.25 cm thick) are made and cut into different shapes of biscuits using different steel dies. These raw cut biscuits are then baked in hot oven (at 180°C) for 20 minutes and after cooling biscuits are ready for packaging.

#### **8.8.3.3.3. Mushroom Soup Mix**

Mushroom soup mix was developed with 30% oyster mushroom powder, 30% corn flour, 25% milk powder, 8% salt, 3% sugar, 2% black pepper, and 2% oregano. This soup mix has to be boiled for 2 minutes with 14 times quantity of water for the preparation of good quality mushroom soup with characteristic aroma and taste. This mushroom soup mix can be stored for 90 days at ambient temperature and for 180 days at refrigerated temperature without any significant change in sensory, proximate, Vitamin D, antioxidant and microbial quality of soup mix.

#### **8.8.3.3.4. Mushroom Sauce or Ketch-up**

Freshly harvested button mushrooms are washed, sliced and cooked in 50% of water for 20 minutes. Mushroom paste is prepared using a mixer grinder. Then salt (10%), sugar (25%), acetic acid (1.5%), sodium benzoate (0.065%), onion (10%), garlic (0.5%), ginger (3%), cumin (1 %), black pepper (0.1%), red chilli powder (1 %) and arrarote (0.2%) are mixed in the paste and cooked to bring its TSS to 35 °Brix. Then the ketch-up is filled in the sterilized bottles or jars.

#### 8.8.3.3.5 Mushroom Preserves (Murabba)

For preparing mushroom preserve, fresh button mushrooms are graded, washed, pricked and blanched in 0.05% KMS solution for 10 minute. Blanched mushroom is then dipped in 50 °Brix sugar solution and refrigerated overnight. Next day mushroom is strained out of sugar solution and the solution is added with 0.1% citric acid and sufficient sugar to attain strength of 60 °Brix by heating. Mushrooms are then dipped into it and kept overnight. This process is repeated to raise the concentration of syrup to 70 °Brix and mushrooms are dipped into it for 1 week to prepare preserve. The preserve is then drained out of sugar syrup and filled in a container with freshly prepared sugar syrup of 68 °Brix. The containers are then sealed airtight and stored.

#### 8.8.3.3.6. Mushroom Candy

The process for making candy is practically the same as that employed in the case of mushroom preserve, with the difference that the produce is impregnated with a higher concentration of sugar (75°Brix) and is also partially dried under shade to attain the chewable consistency. The mushroom candy can be stored up to 8 months with excellent acceptability.

#### 8.8.3.3.7. Mushroom Chips

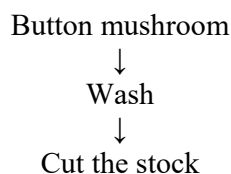
Mushroom chips can be prepared from button or oyster mushroom both. For preparing mushroom chips, freshly harvested mushrooms are washed, sliced (in case of button mushrooms), divided in individual mushrooms from the bunch (in case of oyster mushrooms) and blanched in 2% brine solution. The mushrooms are dipped overnight in a solution of 0.1% of citric acid + 1.5% of NaCl + 0.3% of red chilli powder. After draining off the solution, the mushrooms are subjected to drying in cabinet dryer at 60°C for 8 h. Then it is fried in the refined oil and good quality chips are prepared. After spices mixing, the chips are packed in polypropylene packets and sealed after proper labeling.

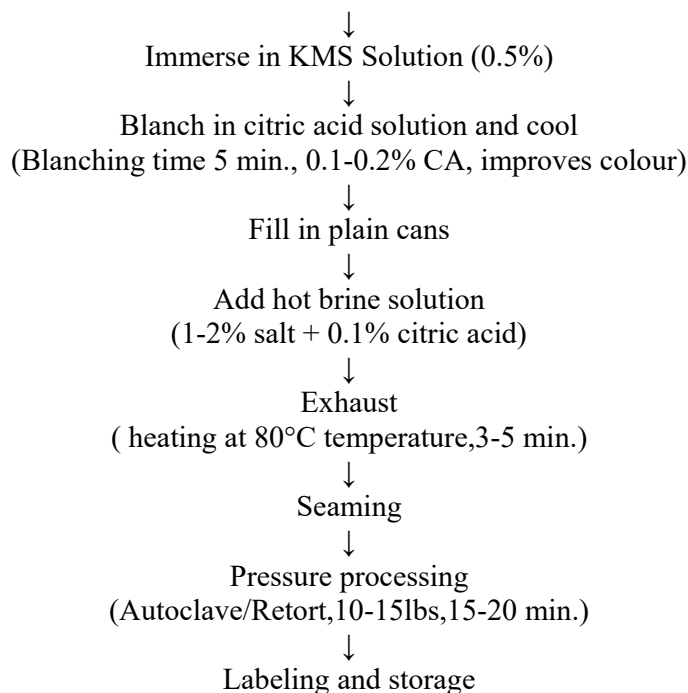
#### 8.8.3.3.8. Mushroom Jam

Development of mushroom jam would aid in preserving mushrooms for a year as a product that is nutritious as well as widely acceptable. For preparation of mushroom jam, washed and blanched mushrooms are ground into a paste. This mushroom paste is then added with sugar (1:1 to paste), pectin (1% of pulp) and citric acid (1% of pulp) and heated with continuous stirring to avoid sticking to pan till it reaches a TSS of 68o Brix. This prepared jam is hot filled in sterilized glass bottles leaving a head space of 0.8 to 1.0 cm. The bottles are then sealed and stored in a cool and dry place.

#### 8.8.3.3.9. Canning of Mushrooms

- Canning is the most common process for preserving mushrooms, particularly *Agaricus* mushroom.
- Canning is divided into six operations; cleaning, blanching, canning, sterilization, cooling, labeling and packing.
- Use of tomato juice is better as canning medium for retaining the mushroom quality than the brine solution





#### 8.8.3.3.10. Mushroom Pickling

- Pickling of mushroom is also a popular method of preserving.
- It is more economically viable way during the surplus periods.
- Sweet chutney from edible mushroom having a shelf life of over a year with better sensory qualities.
- Pickle prepared from paddy straw mushroom was also reported with better quality.

#### Recipe for Mushroom Pickles

- Oil: 10%
- Red chilly: 2.5%
- Mustard seeds: 3%
- Mustard powder: 0.5%
- Methi seeds: 1.6%
- Cumin: 1.5%
- Clove: 0.3%
- Cinnamon: 0.2%
- Pepper: 0.6%

#### 8.8.3.3.11. Mushroom Freezing

1. Individual quick freezing is another popular processing method
2. In this process, raw materials are washed at processing units after receipt from farm, and then the mushrooms are inspected, sliced and graded according to quality.
3. After that, blanched and water cooled mushroom are subjected to tunnel freezing stage. At this stage are cooled in a system having temperature around -40°C and core areas of mushroom pieces acquire a temperature of around -18 C.

4. Subsequently packed in multi-layer poly-bags and stored in a cold storage having temperature – 20°C to -35°C.
5. Vacuum freeze drying (V.F.D) is a further development in mushroom processing. In this process the original shape, quality, colour size, texture, freshness properties of thermal produce are retained.

#### **8.8.3.3.12. Mushroom Drying**

- Drying refers to the removal of water by heat to such a level that the biochemical and microbial activity is checked due to reduced water activity in the produce.
- Pre-treatments: Water blanching of mushroom (*Agaricus bisporus*) for 5 minutes along with 0.5 per cent citric acid, 0.1 per cent KMS and 125 ppm Ethylene diamine tetra acetic acid to improve colour and texture of mushroom slices
- Drying in cabinet-air drier at  $60 \pm 2^\circ \text{C}$  results in high per cent of yield followed by dehumidified drier ( $50 \pm 2^\circ \text{C}$ ).

#### **8.8.3.3.13. Development of Shiitake Mushroom Vegetables Soup Mix**

A mushroom vegetable mixed soup mix was developed using shiitake mushroom powder (20%) along with vegetables mix (containing tomato powder, dried carrot shreds, partially cooked and dried peas, onion powder and garlic powder) (15%), corn flour (27.5%), milk powder (22.5%), salt (9%), sugar (3%), black pepper (2%) and oregano (1%). The developed soup mix was found acceptable based on sensory analysis and contained 2.8 % moisture, 8.62% protein, 71.44% carbohydrate, 4.02% fat, 13.12% ash, 3.47% fiber and 2681.48 IU/g Vitamin D.

#### **8.8.3.3.14. Techniques for Enhancement in Quality and Shelf-Life of Harvested Button Mushroom**

Spray 0.2% solution of  $\text{CaCl}_2$  on mushroom beds starting from pinhead initiation stage up to completion. Wash mushrooms with solution of 0.02% KMS + 100 ppm EDTA. It helps in maintaining superior quality of the stored mushroom. Pack the mushrooms in 100 gauge thick polypropylene bags which help in retaining the quality for a much longer period than packaging in ordinary polythene bags.

#### **8.8.3.3.15. Recycling of Spent Mushroom Substrate (SMS)**

- Fresh SMS contains 1.9:0.4:2.4 (NPK), while 8-16 months old contains 1.9:0.6:1.0 (NPK).
- SMS does not fall in the category of hazardous substances as it does not contain heavy metals.
- SMS obtained from various sources vary in its physical and chemical properties.
- Treatments like rapid salt leaching and re-composting by aerobic or anaerobic methods for one to two years make SMS more suitable for growing flowers, vegetables, fruit, saplings, ornamental shrubs and other horticulture plants of economic importance.
- The use of an-aerobically re-composted spent mushroom substrate as casing material gave superior button mushroom yield with better diseases management.
- SMS of paddy straw, oyster and button mushrooms can be used as feeding material for vermi-composting.

#### 8.8.4. Training and Capacity Building Unit

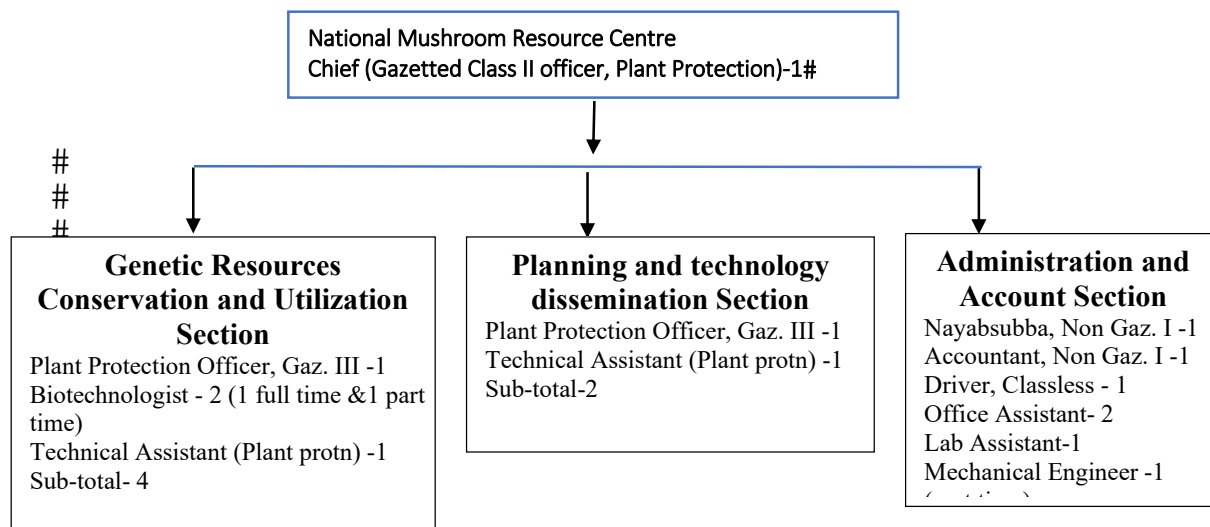
This unit would have advanced and air-conditioned training halls, practical classrooms, dormitories and canteen with necessary facilities and accessories. It will provide training related to various technologies associated with mushrooms for capacity building of technicians, farmers etc. The unit will perform major function as follows.

- Organizing different levels of training programmes on mushroom.
- Participation in national level exhibitions.
- Mentorships: Mentoring provides guidance and knowledge and skills on mushroom production, marketing and value addition.
- Organize workshop and conference for mushroom research and development.
- Develop the training curriculum, training methods and tools for mushroom production skill developments and conduct training courses for mushroom growers, entrepreneurs and technicians.
- Support in evaluation and monitoring of the training and other activities implementation.

#### 8.8.5. Composition of the Staffs for the Proposed NMRC

It is suggested that the proposed institution will have the following staffs for its operation:

S N	Post	Number	Remarks	
		Full time permanent	Full time contract basis	Part time contract
1	Senior Plant Protection Officer, Gazetted Class II officer	1		
2	Plant Protection Officer, Gazetted Class III officer	2		
3	Bio Technologist, (Minimum Bachelor in Biotechnology with proven experience)		1	1
4	Technical Assistant (Plant Protection)	2		
5	Non gazette (Administration)	1		
6	Non gazette (Account)	1		
7	Driver		1	
8	Office Assistants		2	
9	Lab Assistant		1	
11	Electrical Engineer			1
12	Mechanical Engineer			1
Total		7	5	3



## 8.8.6. Terms of Reference for Proposed Technical Staff

### 8.8.6.1. Senior Plant Protection Officer

- Overall leadership, management and strategic direction for the organization.
- To establish relationships and ensure coordination with other line agencies, including research and education institutions, across the three tiers of government.
- Regular assessment of mushroom seed supply system in Nepal and propose the needful programs to address the constraints/problems in the mushroom value chain.
- Provide technical feedback to the CIED and DoA in the formulation of mushroom related policies, plans, strategies and legal instruments.
- Implementation of mushroom related policies, programs and regulations as approved by government.
- Approve the Standard Operating Procedure of the organizations/labs from concerned authorities.
- Strategic planning, policy development, budgeting, resource allocation, and ensuring the effective delivery of services of the institution.
- Ensure the quality of the pure culture supply.
- Adopt the proper internal control system.

### 8.8.6.2. Plant Protection Officer (Genetic Resources Conservation and Utilization Unit)

Plant Protection Officer will perform following duties having due collaboration with the Biotechnologist/s.

- Acquisition of the exotic and domestic mushroom germplasm.
- Conservation of the mushroom germplasms.
- Production and distribution of pure culture and spawn of the promising mushroom species.

- Prepare the follow the Standard Operating Procedure.
- Ensure the quality of the pure culture and spawn by adopting standard quality testing procedures.
- To monitor and regulate various mushroom seed producing laboratories and facilities of the genetic resources conservation.
- To keep records of national data and information related to mushrooms.
- To supervise and guide junior technical staff.
- To establish proper coordination with Biotechnologist.
- To supervise the operation of laboratories for production and preservation of pure culture.
- Serve as the resource person in the training program and other associated activities.
- Assist Chief as per requirement.

#### **8.8.6.3. Plant Protection Officer (Planning and technology dissemination Section)**

- To conduct adaptive research and extension activities related to mushroom.
- To provide technical guidance and services to farmers and other stakeholders
- To keep records of national data and information related to mushrooms.
- To provide training and capacity building of technicians at province and local level as well as seed producers and farmers.
- To supervise and guide junior technical staff.
- To carry out post-harvest related adaptive research.
- To serve as the resource person in the training program and other associated activities.
- Assist Chief as per requirement.

#### **8.8.6.4. Bio Technologist**

- To draft the Standard Operating Procedures (SOP) and its contextual revision.
- To perform tissue culture, spore germination, and spawn production following the approved SOP.
- To supervise and operate the laboratories for the production and preservation of pure culture.
- To preserve (short term and long term) the acquired germplasm of exotic and native species of various mushroom strains and also plan for the backup storage of important species of mushrooms.
- To conduct experiments, analyze data, and publish research findings to contribute to the scientific community's knowledge and advancements in mushroom biotechnology.
- To supervise quality control procedures to ensure the consistency and safety of mushroom pure culture and spawn produced in the laboratories.
- To supervise and operate culture techniques, including media preparation, sterilization, inoculation, and maintenance of mushroom cultures in

laboratory conditions and ensure the supply of pure culture and spawn as per the demand.

- To carry out the quality testing of the pure culture and spawn as per need.
- Serve as the resource person in the training program and other associated activities.
- Assist Chief as per requirement.

#### **8.8.6.5. Technical Assistant /Junior technical assistant**

- To perform research, extension and other activities associated with mushrooms under the supervision of senior staff.
- To coordinate and perform various laboratories operations under the supervision of senior staff.

### **8.9. Functional Linkage With Other Institutions**

NMRC will be in operation under the regular and strategic guidance and supervision of CIED. It will ensure the functional linkages with other institutions as mention below.

#### **8.9.1. Research Institutions**

- NMRC will establish functional linkages with Mushroom Unit of Pathology Division of Nepal Agricultural Research Council (NARC) and will coordinate adaptive research and multi-location trials. NMRC will acquire the innovative technologies and research output from Mushroom Unit of the NARC. Furthermore, NMRC will provide the continuous feedback to the Mushroom Unit to further advance the research work. NMRC will also take part in the Research-Extension-Education platform to share and receive the innovative ideas. Furthermore, a research desk will be ensured for the Scientist/s of the Mushroom Unit of Pathology Division to carry out in-depth collaborative research.
- NMRC will also establish functional linkages with National Agriculture Genetic Resource Centre (Gene Bank). It will manage to exchange mushroom genetic resources for the long term conservation and utilization. Furthermore, it will exchange the outcomes of adaptive research and farmer feedback to further strengthen the research and extension outcomes.

#### **8.9.2. Education Institutions**

- NMRC will have coordination with Plant Pathology Department of the Agriculture and Forest University and Tribhuvan University, Institution of Agriculture and Animal Sciences and periodic exchange of outcomes and research ideas will also be ensured. Further, NMRC will also provide the platform for the graduate and post-graduate student involved in the mushroom research based on the MoU and approval from concerned authorities.

#### **8.9.3. Province and Local Governments**

- NMRC will have coordination with government at Province and Local level for regulation of mushroom seed supply chain, trainings, and capacity building of technicians,

- NMRC will provide the mushroom learning platform for the Province and Local government technicians.

#### 8.9.4. Private Sector and Other Institutions

- NMRC will provide the capacity building training to private mushroom seed producers, mushroom producers periodically. It will guide the entrepreneurs to establish mushroom seed production laboratories and mushroom production business. It will provide the pure culture of the promising species of exotic and native mushrooms as per the demand of private sectors. Further, it will also provide the spawn of the promising species for the farmer trial and demonstration.
- NMRC will facilitate to accomplish the technical requirement of the private mushroom seed production laboratories and will establish close relationship with private entities during the regulation of quality mushroom seed supply.
- Similarly, NMRC will join it hand together with NGOs and INGOs working the field of mushroom development based on MoU and approval from concerned authorities.

#### 8.10. Expected Outcomes of NMRC

After the establishment of National Mushroom Resource Centre in the country, the followings outcomes are expected:

- Rapid growth and development of mushroom subsectors in the nation.
- Self-sufficiency in quality mushroom spawn production and supply.
- Conservation and utilization of exotic and native species of the mushroom.
- Reduction in the import and increase in export of fresh mushroom and its product.
- Commercialization of promising native species of mushrooms and open the avenue for the exports of those species.
- Dissemination of the innovative technologies and outcomes of the adaptive research associated with mushrooms.
- Technological advancement and large-scale production of mushroom in the country.
- Conservation and promotion of native mushroom species found in the country.
- A reliable technical learning platform for the mushroom entrepreneurs across the country.
- NMRC will provide the services as below

S.N.	Type of services	Units	Amount	Remarks
1	Conservation of native species of mushroom germplasm	Number		
2	Conservation of exotic species of mushroom germplasm	Number		
3	Pure culture production			Number of species..... Will be sufficient to produce .....kgs of mushroom spawn.
4	Spawn production			Number of

S.N.	Type of services	Units	Amount	Remarks
				species..... will be sufficient to produce .... kgs of mushroom.
5	Officer level training			
6	Assistant level training			
7	Laboratory training for private sector			
8	Advance level training for farmer			
9	On-site teaching and mentoring to the mushroom entrepreneurs			Through the visit of demonstration facilities

## **9. DEMOGRAPHY AND ETHNIC COMPOSITION**

At the time of the 2011 Nepal census, Panauti Municipality had a population of 47,549. Of these, 68.6% spoke Nepali, 16.4% Tamang, 14.1% Newar, 0.2% Maithili, 0.1% Bhojpuri, 0.1% Hindi, 0.1% Thangmi and 0.1% other languages as their first language.

In terms of ethnicity/caste, 27.7% were Chhetri, 27.2% Hill Brahmin, 18.6% Newar, 17.4% Tamang, 1.5% Sarki, 1.4% Damai Dholi, 1.2% Kami, 1.0% Gharti/Bhujel, 0.8% Sanyasi/Dasnami, 0.7% Magar, 0.7% Pahari, 0.5% Rai, 0.4% Gurung, 0.1% Badi, 0.1% Danuwar, 0.1% Musalman, 0.1% other Terai, 0.1% Thakuri, 0.1% Thami and 0.2% others. In terms of religion, 81.7% were Hindu, 16.1% Buddhist, 1.7% Christian, 0.1% Muslim and 0.3% others. In terms of literacy, 76.3% could read and write, 1.6% could only read and 22.0% could neither read nor write.

## **10. PRIORITIZED PROJECTS**

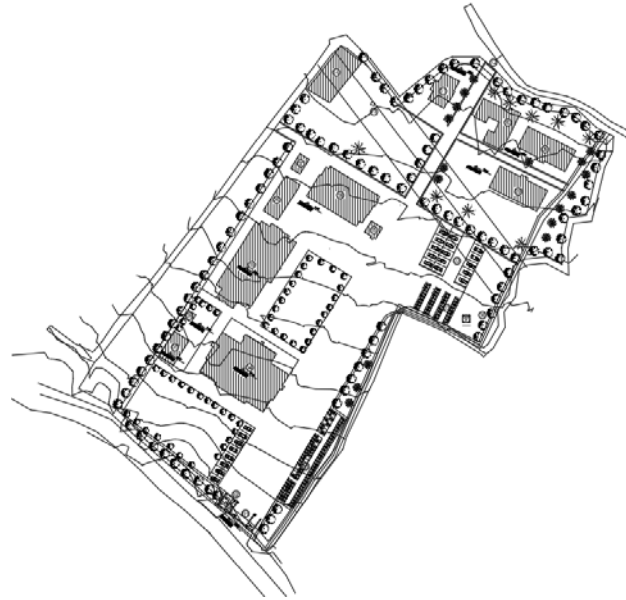
After Visiting the site and looking through its existing conditions, the project was then divided into two categories which specified whether there was new construction to be made or Upgradations of existing structures with further additions of the amenities to facilitate the user.

### **10.1. Planning Concept of Proposed NMRC**

The concept of planning is taken from the site potential of Promoting the concept of NMRC. The Concept was to create the pocket space as an interaction plaza without ruining the existing terrains. The structures are planned considering the existing contour of the site. Requirements for the site were kept as minimum as possible to conserve the existing conditions of the site. The design is basically incorporated so as to give the impression as if the building has emerged from the site. Also, the masterplan is designed considering the local context and the local architecture. Furthermore, it can contribute in the conservation of the complex as well as to make the place lively and convenient. This proposal has also been planned to enhance the build spaces as well as environment of the master plan area.

### **Zoning**

Zoning is followed with the division of the project into Mother Unit as main Laboratory zone, Administrative Zone, Residential Zone and other facilities required for the NMRC. All functions are arranged according to their functional requirement and linkage between them is arranged in the same manner. The space is connected through different interconnecting spaces as open space, pathways, landscape and building itself.



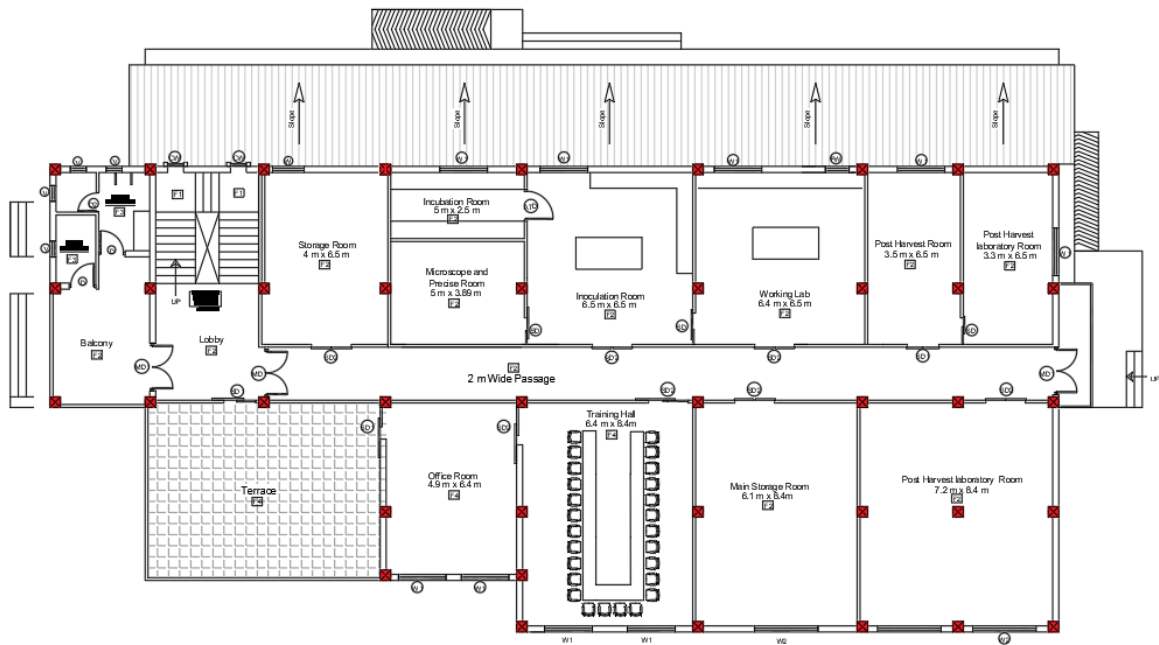
*Figure 25: Masterplan*

A masterplan is the blueprint that shows the future layout of both existing and future proposed infrastructure of the area. Masterplan helps to restrict the haphazard and unplanned establishment. The Draft masterplan of the area was finalized after discussion with the CIED officials and further DPR is prepared based on this masterplan.

Details features included in Masterplan are:

- Boundary of the land and entrance
- Contours of whole area
- Proposed landscaping
- Location of the buildings (Admin Block, Pure Culture Production Lab, Growth Chamber, etc)
- Open Space
- Position of the public utility and Service
- Internal linkage and Parking





**Figure 28: First Floor Plan of Seed Production and Conservation Block**



**Figure 29: 3D View of Seed Production and Conservation Block**

## 10.2.2. Growth Chamber

A growth chamber, also known as a plant growth chamber or plant incubator, is a controlled environment facility designed to provide optimal conditions for mushroom growth, development, and experimentation. It allows researchers, breeders, and horticulturists to manipulate and control environmental factors such as temperature, humidity, light intensity, photoperiod, and carbon dioxide levels to simulate specific growing conditions.

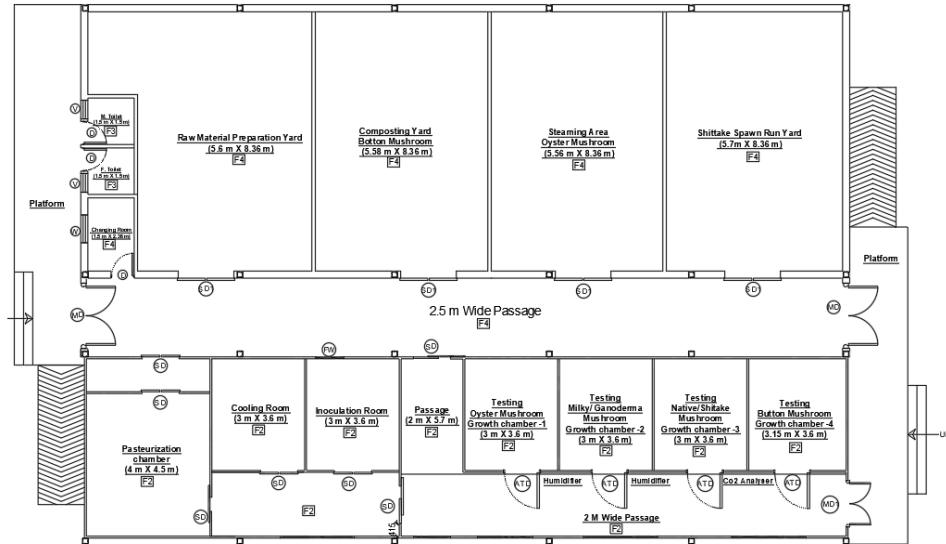


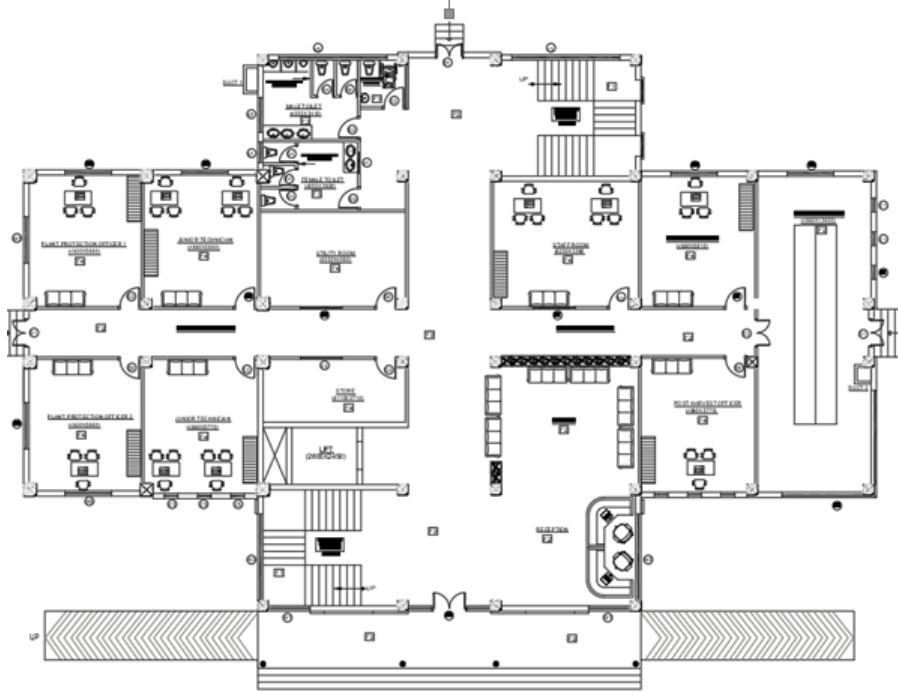
Figure 30: Floor Plan of Growth Chamber`



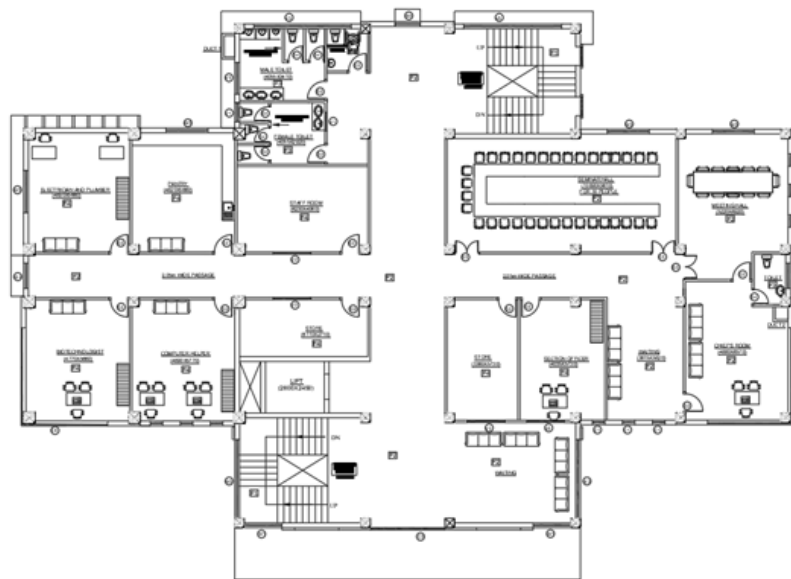
Figure 31: 3D View of Growth Chamber

### 10.2.3. Admin Block

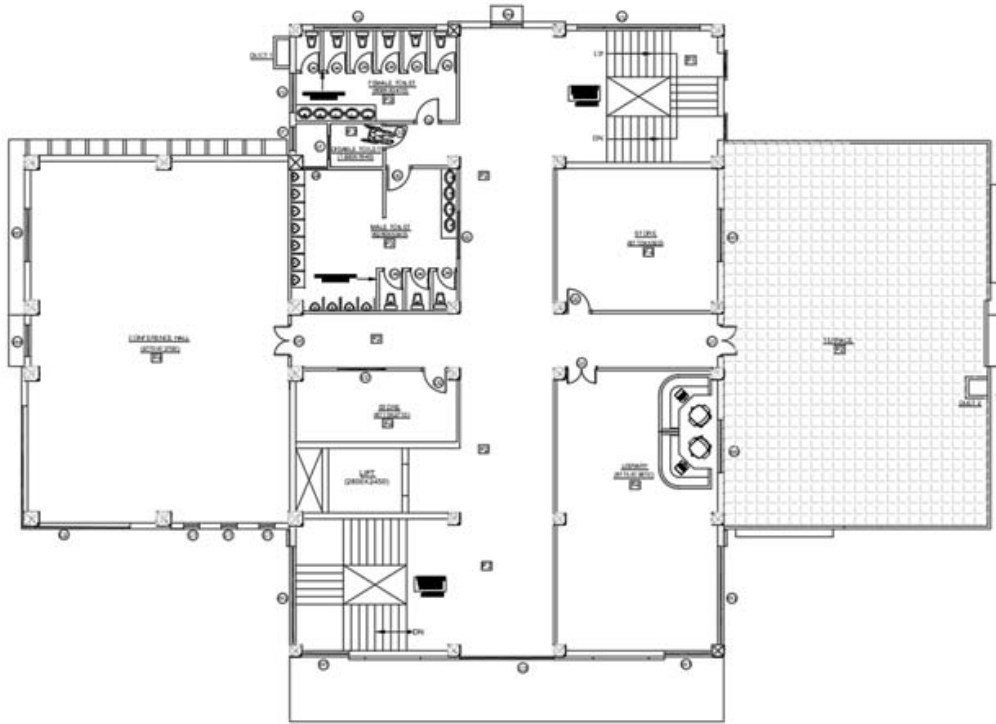
An "admin block" typically refers to a building or a section of a building that houses multiple offices or workplaces. It is designed to accommodate various departments, organizations, or businesses in separate office spaces within the same complex. Office blocks are commonly found in business districts or commercial areas, and they provide a centralized location for professionals to carry out their work.



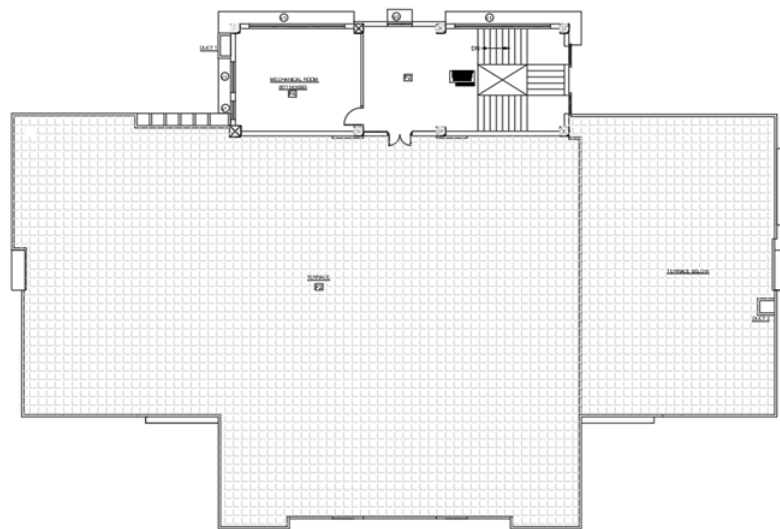
*Figure 32: Ground Floor Plan of Admin Block*



*Figure 33: First Floor Plan of Admin Block*



*Figure 34: Second Floor Plan of Admin Block*



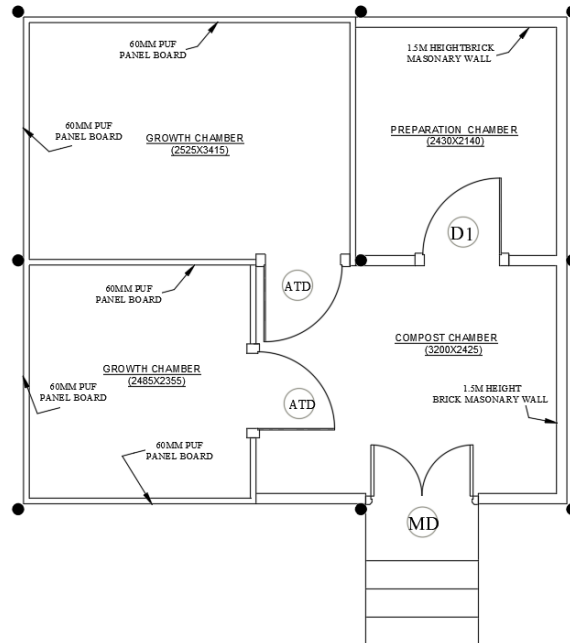
*Figure 35: Top Floor Plan of Admin Block*



*Figure 36: 3D View of Admin Block*

#### 10.2.4. Production Block

This production block has been designed for the sample purpose for the farmers/demonstrators who can be implemented in the field. This design may vary according to site condition.



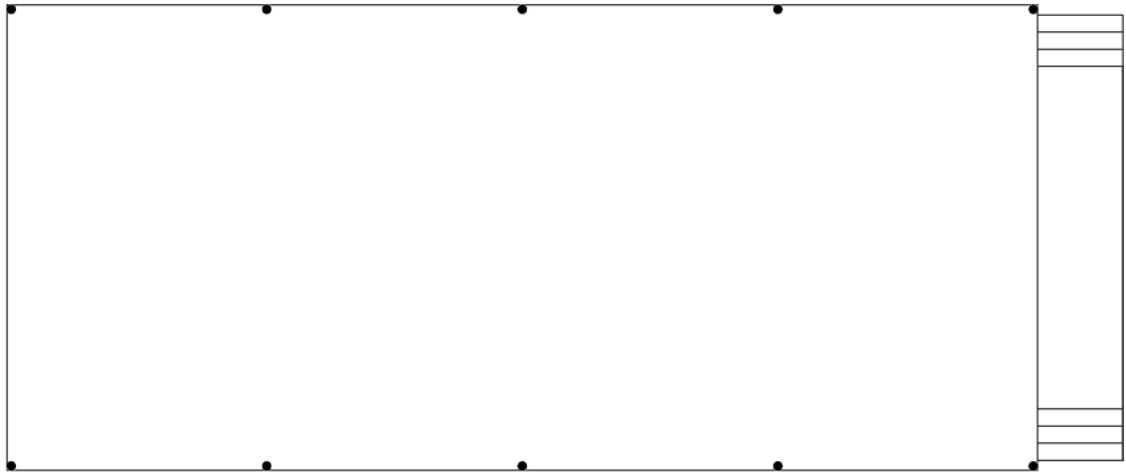
*Figure 37: Plan of Production Block*



*Figure 38: 3d View of Production Block*

#### **10.2.5. Shed**

A shed for hay is a structure specifically designed to store and protects hay bales. It provides a sheltered and dry environment, which helps preserve the quality of the hay and prevents it from being damaged by exposure to moisture or adverse weather conditions.



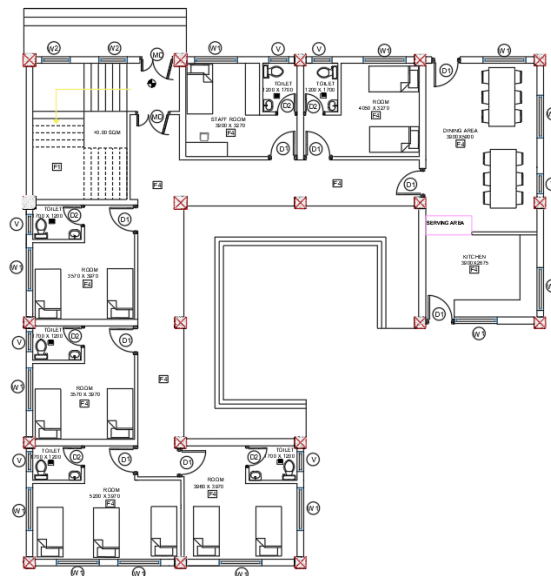
*Figure 39: Floor Plan of Shed*



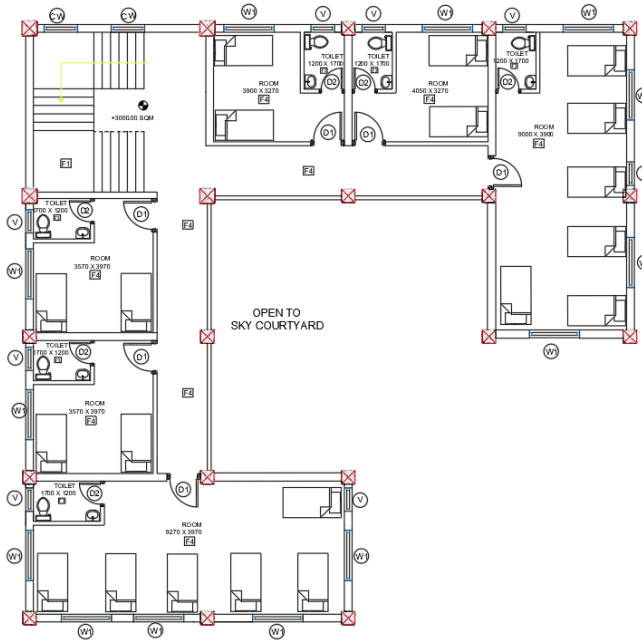
*Figure 40: 3D View of Shed*

### 10.2.6. Hostel

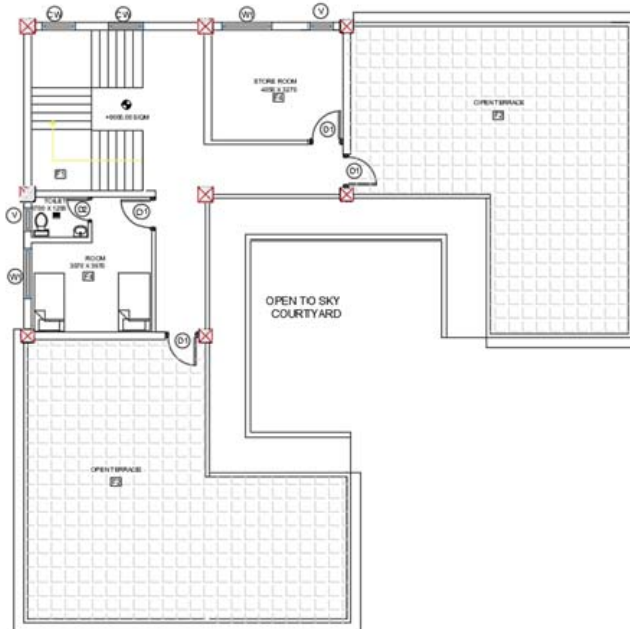
In some cases, research centers or institutes may provide accommodation facilities for visiting researchers, scholars, or students within their premises. These accommodations are often referred to as "research center hostels" or "guest houses." They are specifically designed to cater to the temporary housing needs of individuals involved in research or academic activities.



*Figure 41: Ground Floor Plan of Hostel Block*



**Figure 42: First Floor Plan of Hostel Block**



**Figure 43: Top Floor Plan of Hostel Block**



Figure 44: 3D View of Hostel Block

### 10.2.7. Staff Quarter

Staff quarters in a research center are residential accommodations provided to the staff members employed by the center. These quarters serve as residences for researchers, scientists, support staff, and other employees who work at the research center.

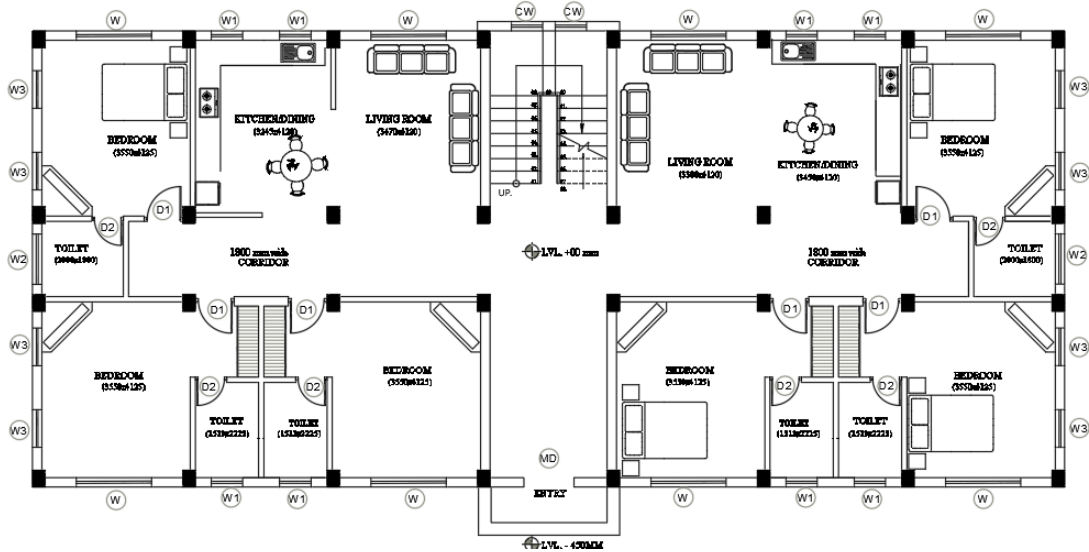


Figure 45: Ground Floor Plan of Staff Quarter

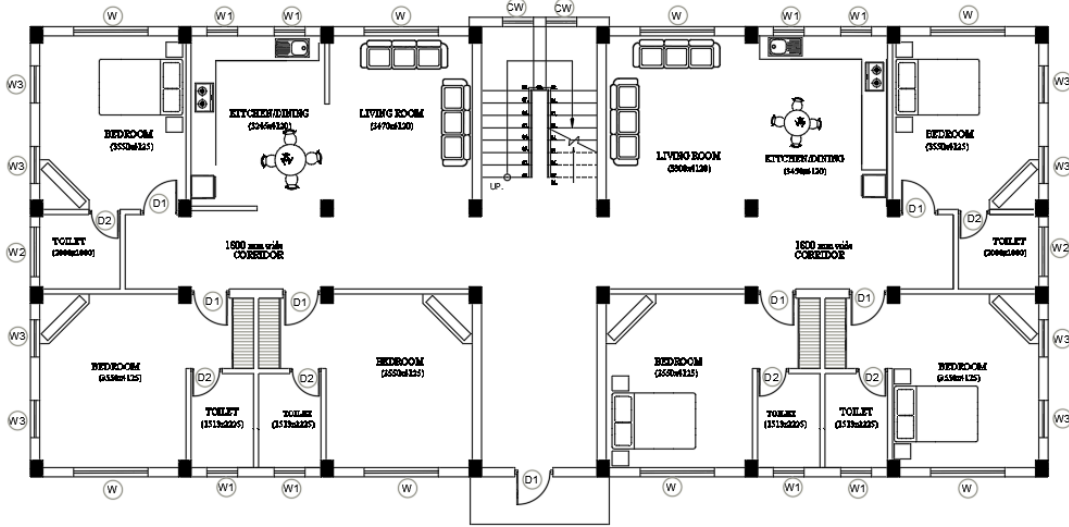


Figure 46: First Floor Plan of Staff Quarter

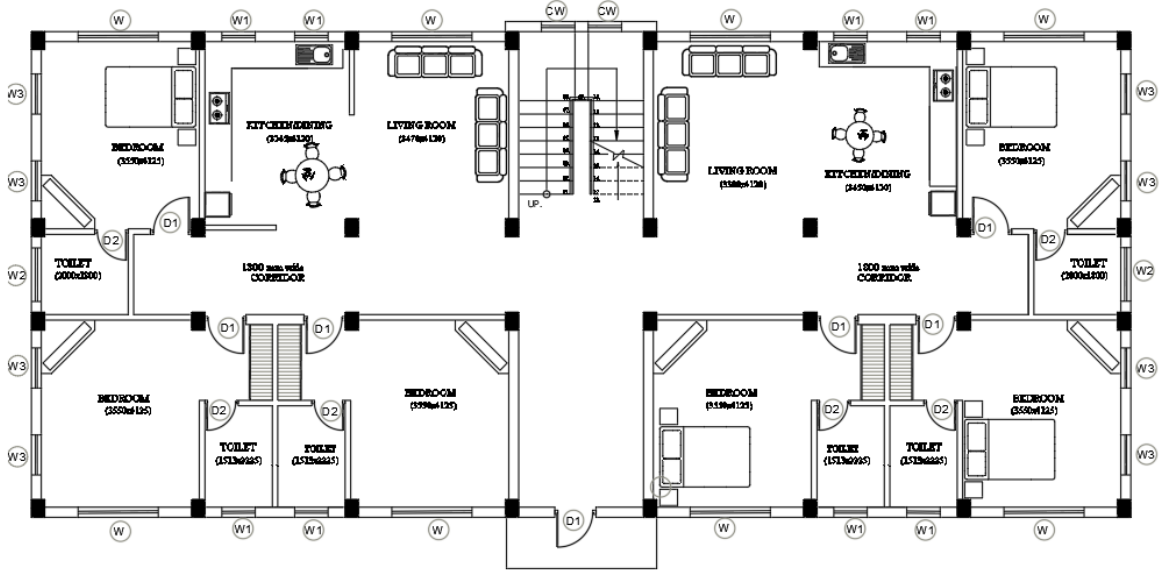


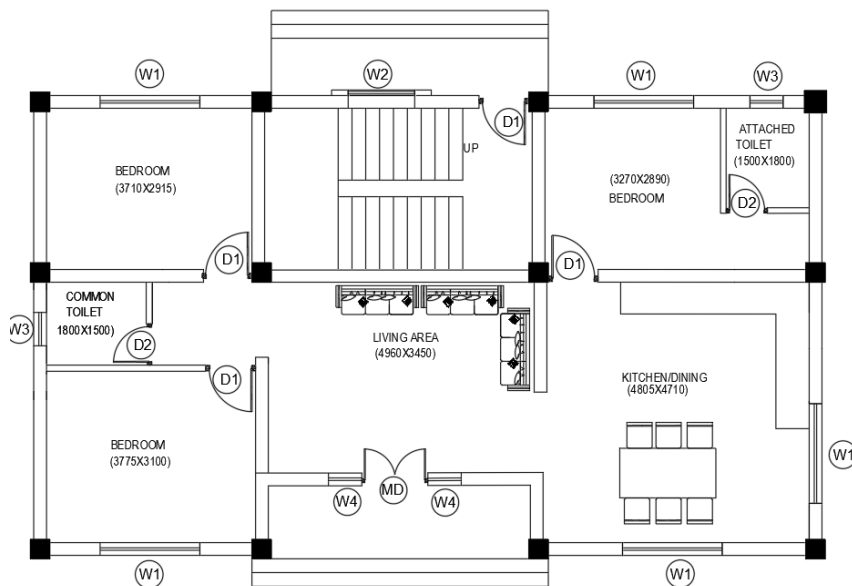
Figure 47: Second Floor Plan of Staff Quarter



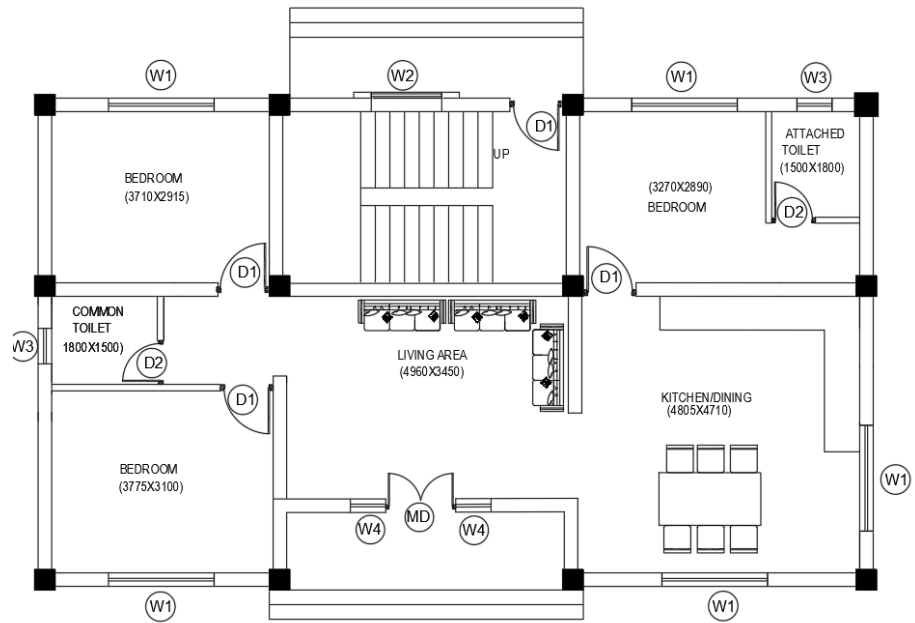
*Figure 48: 3D View of Staff Quarter*

### 10.2.8. Chief's Quarter

In some research centers or institutions, a chief or director's quarter may be provided as a designated residence for the highest-ranking official or administrative leader. This residential accommodation is typically larger and more luxurious compared to staff quarters, reflecting the seniority and responsibilities of the chief or director.



*Figure 49: Ground Floor Plan of Chief's Quarter*



**Figure 50: First Floor Plan of Chief's Quarter**



**Figure 51: 3d view of Chief's Quarter**

### 10.2.9. Toilet

A public toilet, also known as a restroom, bathroom, or lavatory, is a facility that is accessible to the general public for personal hygiene and sanitary needs. Public toilets are typically found in various locations such as parks, shopping malls, airports, train stations, restaurants, and other public spaces.

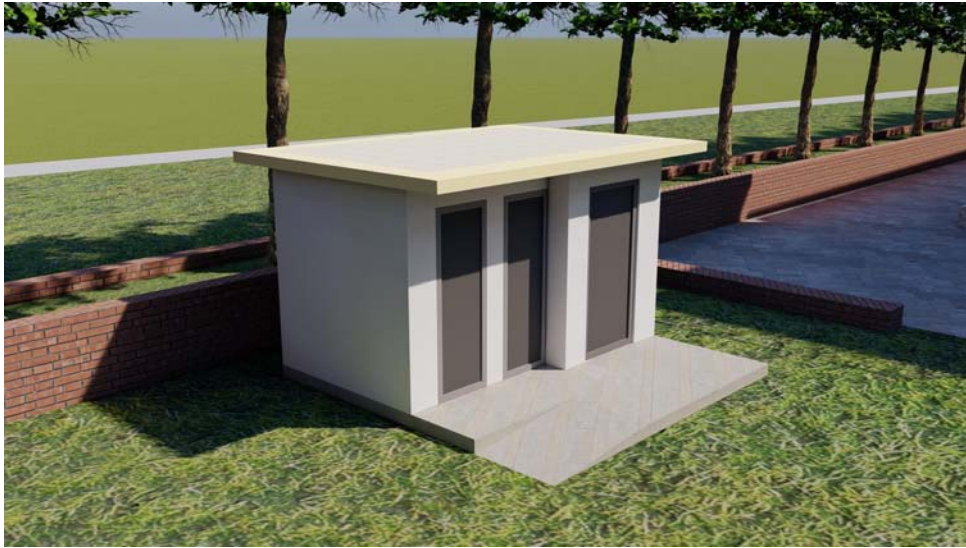


Figure 52: 3d View of Public Toilet

### 10.2.10. Canteen

A canteen in a research center is a designated area or facility where individuals working at the research center can have meals and refreshments. It serves as a communal dining space and provides food services to researchers, scientists, staff members, and visitors within the research center.

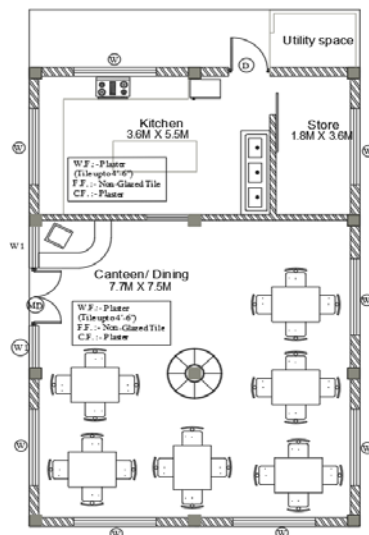


Figure 53: Floor Plan of Canteen



*Figure 54: 3d View of Canteen*

## 11. DETAILED ENGINEERING DESIGN

Based on the scope of work, the consultant had carried out detailed engineering design work of the project. After completion of field work, the consultant had started detailed design works based on the previously prepared conceptual design with incorporation of comments and suggestion received from the client and the other stakeholders of the project. Architectural planning of the whole area have been prepared as master plan and detail of the required structure have been prepared by the Architect. Detailed drawings have been prepared in suitable drawings sheets. After finalization of the architectural design, a structural analysis and design has been done by the structural engineer considering all the loads including earthquake. The detailed structural drawings also have been prepared for the building. Based on the collected information and results of the discussions the consultants designed the building, following the standard codes of practice, norms and guidelines. The relevant codes for the design is Nepal National Building Code (NBC: 1994), Revised NBC: 2020, IS Codes or other relevant codes equivalent to NBC, Municipality Building Bye-Laws, Norms, Rule and Regulation. The space standard prepared by DUDBC/Municipality is followed for the study. As mentioned earlier, the design will, to the extent possible, be guided by Cultural, environmental and climatic factors and reflect state of the art practice in environmental consideration. Following major things are considered while designing of components:

- All detailed design is prepared in accordance with the requirements of structural design and recognized good building practices.
- The architectural design has estimated building work materials which are both attractive and durable. Local availability, of such materials for future maintenance purpose will be a selection criterion. Usage of renewable, indigenous, Low volatile organic compound (VoC) outgassing building materials to the greatest extent possible is preferred.
- Special care is taken to ensure that building details confirm to the intended usage to the space and to the requirements and customs of the users.

For furnishing the working or construction drawings, all plans and sections was fully dimensioned. Information on materials of finishing, renderings is incorporated in the drawing itself. A separate finishing schedule is provided in plan for each room with finished level as per requirement. All elevation is furnished with exterior rendering and finishing schedule. A standard system of cross referencing the drawing is followed so as to facilitate the interpretation of details with respect to their location, all key positions (expansion, contraction and construction joints etc.) and elements (doors, windows, stairs, rails, gutters, etc.) is furnished with construction details in an appropriate scale. In order to avoid 'Minor lapse' or 'blight' detailing and specification is taken note of:

Major factors play a dominant role during the appropriation of building to the site. They are:

- The contour of the site
- The orientation
- The approach road
- The geometry of the site

## **12. QUANTITY AND COST ESTIMATION**

Detailed Quantity estimate has been done based on the Design of the structure. Quantity of all items is estimated separately. The consultant has done cost estimate works based on the GoN norms and district rates and the same has been presented as volume II: Cost estimates. Rate of each items of work is finalized based on the GoN norms and District rate. Transportation cost of the materials is also incorporated in the rate analysis. The rates for various items incorporated in the design have been taken from district rates of same district and the rate of items which are not listed in district rates of the project district have been taken from other relevant districts and transportation rate has been added. For the electrical and sanitary items market rates has also been taken. These are taken based on various similar previous projects performed by the consultant. The Summary of Cost of the Project is presented as below:

Ministry of Agriculture and Livestock Development			
Centre of Industrial Entomology Development (CIED)			
Hariharbhawan, Nepal			
SUMMARY SHEET			
Project name: Feasibility and Preparatory Study on Establishment of Mushroom Resource Centre in Khopasi , Kavrepalanchowk			
Location:Khopasi , Kavrepalanchowk , Nepal			
SN.	DESCRIPTION	TOTAL COST	REMARKS
A	CIVIL WORKS		
1	Gate and Guard House	2,884,854.90	
2	Admin Block	80,506,332.46	
3	Canteen	4,594,831.12	
4	Shed	2,458,744.38	
5	Public Toilet	1,863,342.79	
6	Staff Quarter	66,396,118.32	2 in number
7	Hostel	24,244,618.51	
8	Chief Quarter	12,061,589.44	
9	Secondary Entrance	198,769.11	
10	Production Block	1,534,771.66	
11	Seed Production @ CON Block	54,529,236.65	
12	Growth Chamber	11,322,135.70	
13	Elevated Water Tank	4,288,546.11	
14	Masterplan and Landscaping	8,138,409.51	
15	Mechanical Cost	40,945,488.00	
16	Miscellaneous Cost (Hiring of Instruments, Labour Camp, Social cost, etc.)	3,000,000.00	
	Sub total=	318,967,788.66	
	<b>Provisional Sum</b>		
	Provisional sum for construction of hut, hiring of equipments, social security (2%)	1,594,838.94	
	Insurance	1,594,838.94	
	Lab Test	1,275,871.15	
	As Built Drawing Preparation	425,290.38	
	Total Provisional Sum=	4,890,839.43	
	Total Works with PS=	323,858,628.09	
	<b>Contengency</b>		
	Contengency 4%=	12,954,345.12	
	Physical Contengency 10%=	32,385,862.81	
	Total Without VAT=	369,198,836.02	
	VAT @13%=	47,995,848.68	
	GRAND TOTAL=	417,194,684.70	

### 13. LIMITATIONS

- The total time allocated to complete the assignment was short.
- The concerned KII were not serious about the time factors.

### 14. RECOMMENDATIONS

- Due to industrialization, urbanization and population growth, there will be shrinkage of arable land and availability of natural resources will be a limiting factor. Mushrooms do not compete for arable land and can utilize vertical space; hence this commodity is required to be promoted among the masses. Moreover, the water requirement of this crop is meager in comparison to field crops. Being an indoor crop, the effects of global warming may be lesser on mushroom production as well as productivity. Mushroom industry in the coming years will generate employment opportunities in addition to providing quality food.
- Mushroom cultivation is a labour intensive activity and will provide ample employment opportunity in the coming days.
- In the future, agricultural residues are needed to be recycled /utilized judiciously. Burning of residues or their *in situ* decomposition is not the right strategy as it is creating environmental hazards leading to deterioration of human and soil health. Utilization of agro-residues for mushroom production will not only help to reduce the environmental pollution but will profitably recycle them into quality food, besides improving soil health due to recycling of spent mushroom substrate.
- There is a policy problem with the Nepalese government on minimum selling price and mushroom crop insurance. Indian mushroom is cheaper than Nepalese product since there is a provision of subsidies from the government. Government should ensure minimum support-price for mushrooms and provisions for insurance coverage.
- Strengthen the facilities in the mushroom resource center and research organizations for safe-deposit and retrieval of cultures, using long term preservation techniques like cryopreservation and lyophilization.
- Need to develop of mushroom database depicting available mushroom biodiversity of the country with passport data. Preparation of a data list of endangered mushroom species and their conservation.
- Need of analysis and characterization of mushroom germplasm particularly of edible and medicinal mushrooms using morphological, biochemical and molecular markers and confirmation of accessions for their purity using biotechnological tools.
- Exploitation of mushroom biodiversity is needed for the improvement of commercially available edible mushrooms besides the introduction of new suitable mushrooms from the wild. Similar improvement may be needed in medicinal mushrooms to enhance output of active compounds.
- In oyster mushroom, improvement is required in yield, shelf-life, quality and other nutraceutical properties. It will require further research in the following areas: techniques for rapid single spore isolation and inter-mating for inter and intra-specific hybrids; protoplast fusion between closely related species to incorporate the desirable traits like fruit body size, colour, flavour and texture into the high yielding types; breeding spore deficient/low spore strains/ Lovastatin and vitamin

D rich hybrids with better quality and higher yield and breeding varieties for disease/pest resistance.

- Disseminate and Commercialize mushroom technologies:
- a. **Button mushroom:** Research to improve the seasonal and environmental controlled cultivation of button mushroom for different conditions of Nepal may be required in the near future. Introduction of mechanization and automation, as in the years to come labour will become costlier. Mechanical harvesting with single flushing or reduced flushing strains will be common.
- b. **Oyster mushroom:** Cultivation of oyster mushroom in different parts of the country has tremendous potential, which is yet to be fully harnessed. However, research support to improve the cultivation technology for a sustainable yield of quality produce is very much needed, particularly in the following areas: Yield optimization by methods like improved substrate preparation, supplementation, environment control, etc, to increase its profitability; promoting oyster mushroom by developing ready-to-use kits/ fully spawn run blocks in urban, peri-urban and unexplored areas; use of liquid spawn technology; promotion of mechanized farming with controlled conditions; use of spent straw/substrate for recycling as compost or cattle feed; IPM in oyster mushroom for stable yields
- c. **Paddy straw mushroom:** Potential of this mushroom remains underexploited despite abundant availability of paddy straw and highly suited environmental conditions in Nepal: Efforts are needed to improve the existing technology for consistent and higher yields; integrated disease and pest management strategy for consistent yield; utilization of SMS for white button mushroom casing and vermin composting
- d. Promotion of medicinal mushrooms like *G. lucidum* and *Grifola frondosa* is required considering the national/international market.
- Development of liquid spawn technology for all the cultivated mushrooms: Development of technologies (carriers and containers) to prolong the viability, shelf life and ease in bulk transport of the ready-to-use spawn; private sector to be encouraged for the mass production of quality spawns at local level; development of suitable machineries for quality spawn production for seasonal growing and development of technologies for the hitech spawn production for controlled environment farming; development and enforcement of spawn standards in the country; development of alternative spawn substrate sterilization technique for producing quality spawn to lower the cost of spawn preparation.
- Utilize Spent Mushroom Substrate: SMS has many positive attributes still left for its potential uses. The material has been found to be a good nutrient source for field and horticultural crops because of its nutrient-status. Besides, it has a high cation exchange capacity and has a slow mineralization rates that help in retaining its quality as an organic matter.
- Integrated Pest Management: Epidemiological studies on new competitors and parasitic moulds, bacterial and viral diseases; development of molecular diagnostic tools against major diseases and insect-pests; integrated pest and disease management packages for major mushrooms; investigations on mushroom viruses and development of diagnostics and vaccines against important mushroom viruses; use of botanical pesticides, bio-control-agents and virulent strains of pathogens to control diseases and pests of mushrooms; residual toxicity of chemicals used by the mushroom industry; identification and use of environmentally safe bio-pesticides including chitin synthesis inhibitors, growth regulators, anti-feedants, EPN and *Bacillus thuringiensis* strains to avoid pesticide residues and development

of resistant population/strains; development of quick diagnostic methods for detection of nematodes infestation; use of insect attractants, repellents, chemosterilants, pheromones, kairomones and genetic control and development of biosensors for detection of microbial load.

- **Post-harvest Technology:** Low cost drying technology for the domestic and international market; refinement in modified atmosphere packaging (MAP) and controlled atmosphere packaging (CAP) suiting mushrooms for their increased shelf-life; use of recyclable and biodegradable packing material; substitution of tin cans with alternative materials and reduction in blanching losses during canning; development of low cost freeze-drying and IQF technologies; ready-to-cook recipes, value-addition and product diversification to cover pharmaceutical, cosmetic and fast food industries.
- **Utilize Information Technology:** Development of expert system (ES) for mushroom cultivation, marketing, forecasting and management of insect-pests and diseases; development of data bases of input suppliers, entrepreneurs, farmers, market channels and financial institutions; development of interactive online-website on mushrooms and establishment of teleconferencing facilities; on-line connectivity of Mushroom Library for referencing and dissemination of published information.
- **Transfer of Technology:** As per the proposed organizational hierarchy most of the technology will be developed/evaluated at select R&D establishments. MRC will act primarily as trainers' training centre, where development workers from province, the krishi gyan kendras (AGKs), various palikas including NGOs will be imparted training in the latest technical know-how on mushrooms, who, in turn, will train the prospective growers and entrepreneurs.
- **Human resource development (HRD)** is an important instrument to improve the efficiency and capabilities of scientific as well as technical manpower. There is an urgent urge to train scientists and technical personnel in emerging frontier areas of mushroom research. The MRC plans to give high priority to this aspect and train MRC subject matter specialists in emerging frontier areas of mushroom technology. Exposure of mushroom technicians and scientists to modern techniques and advances in the basic mushroom technologies will be a prime component of HRD. Similarly the technicians will also need to be exposed to the hi-tech part of the production technology in countries where climate controlled mechanized cultivation of mushrooms has attained greater heights. Training in some other related agrionic areas like computer automation and informatics may also be essential.
- **Linkages:** Integrating industry for focused proper utilization of the output; linkages with other national and international institutions/agencies. To develop holistic information covering physiological aspects of mushrooms, biomaterials for mushroom houses, need based softwares, biosafety and biosecurity, mycomolecules, nanoparticles, biofuels, enzymes, etc and Nutrition Lab.
- **Production of mushroom spawn** is important for continuous production of mushrooms. There should be registration system for spawn producers such that commercial spawn can be produced by only after registration. Registered spawn producers shall be supervised, directed and inspected by the authorized organization. The quality of spawn produced by spawn producers should be inspected frequently, if there is any error or drawback identified, organization shall assist spawn producers to correct the error and improve the quality of spawn. The spawn producers shall be made to sell spawn to farmers only after labeling on

spawn and such information tags shall have information about spawn producer's name and place, species, date of preparation, date of inspection and expiry date. The spawn producers should be trained to use appropriate equipment to produce good quality of spawn and make aware about the necessity of proper equipments. Similarly, spawn producers should maintain proper records about date of sterilization, incubation temperature so that in case of any problem it will make ease to track the records, identify the problem and the solution. The government should provide subsidy to the spawn producers for construction of proper laboratory to produce good quality of spawn and also assist to the spawn producers

- MRC can work in future to preserve mother cultures without mutation for long-term and register new varieties developed in private sector to protect breeders' right.
- Government instead of spawn producers shall work on system of long-term storage for varieties so that varieties are preserved in the long run more systematically. Spawn producers can apply for long term storage of the varieties by paying certain charge to MRC and can take in the variety from MRC when they need. This system shall not work unless there is proper interaction between government institutions and spawn producers. So, MRC can work as a medium between government and spawn producers. Also, MRC or government organizations should assist to establish a spawn producers' society for proper communication and grievances of spawn producers can be addressed.
- MRC shall be established to collect commercial strains of *P. ostreatus*, *Agaricus bisporus*, *Lentinula edodes* as well as wild mushrooms as materials for development of hybridized new strains and collect wild mushrooms to reveal mycoflora of Nepal.
- In order to identify the new variety, morphological traits and DNA analysis based on PCR method are required so that mushrooms cultivation of the new species can be differentiated from old varieties according to a standard cultivation method and the techniques for DNA analysis are necessary. MRC shall be established so that it can facilitate DNA analysis.
- MRC shall be established for long-term preservation of pathogens e. g., molds, bacteria and virus without losing their infecting ability on mushrooms so that they can be used for research purpose in future.
- Development of better media for long-term preservation is required. For example: In past strains of *Volvariella volvacea* could not be preserved in low temperature for long term, but recently an update has been made in long-term storage of the fungus through update on revision of media ingredients. So, research works are frequently necessary to be familiar with updates and revisions. MRC shall be established to carry out such research works and be updated.
- Identification of native mushroom was carried out in past by NARC collaborating with Kyushu University, Fukuoka, Japan after which no any major progress is found. MRC shall be established to work on identification of pathogens of mushroom and wild fungi by DNA analysis.
- There is huge potential of mushroom industry in Nepal that could contribute to the national economy within its scope of fresh and processed food demand in the changing socioeconomic contexts. In this focus should be given to context collection, conservation and characterization of native edible and medicinal mushrooms.

- Improving knowledge and skill on the right stage of picking, grading, and preservation, cold storage, refrigerated transportation, proper processing, packaging, labeling etc.
- Establishing the value-added chain of mushroom from farm to the final consumer.
- Organized national and international trade fairs.
- Providing financial support especially for small and medium-sized enterprise.
- Encouraging private sector for marketing, processing, and export.
- Develop and strictly enforce the appropriate mushroom policies and laws.
- Develop specific policies in trading, branding and food standard for mushroom products.
- Monitoring the establishment of spawn units in public and private sectors, and enforcing spawn standards and fair price.
- Involvement of cooperatives and other marketing organizations for providing the required inputs as well as help in viable marketing of mushrooms.
- Technical guidance and financial support to the small scale and export oriented mushroom processing industries.
- Creation of Mushroom Development Board (MDB) like Tea and Coffee Board for promotion of mushroom production, processing and marketing industry in Nepal.
- Guidelines for notification and release of mushroom varieties on the lines for crop standards and variety release.
- In the present context government does not have dedicated mushroom resource center in the field of mushroom technology development and dissemination. Very few Government organizations who are involved in mushroom subsector have acute shortage of financial and trained human resources as well as laboratory facilities. Since the beginning mushroom spawn (seed) producers are suffering due to unavailability of certified true to type pure culture of mushroom species and few private mushroom laboratories/farm have been importing/procuring pure culture of mushroom species from India and abroad. In this scenario it is urgent need to establish a mushroom resource center where technically viable and sustainable for the development of mushroom subsector in the country. So, MRC as an immediate need to promote the mushroom industry in Nepal.
- The location topography and weather conditions of Sericulture Farm in Khopasi, Kavrepalanchowk is quite suitable, because it has facing North-South, the average maximum and minimum annual temperature is 22.24 °C and 11.28°C, respectively. Precipitation is about 126 inch per year and a sunshine hour was 5.55 hours and facing of land is such that in daylight sunlight utilization efficiency is enough. Average wind speed of the site was 4.38 km/hr. The large and open area with river on the east and community forest on the west of Khopasi farmland makes it well ventilated and atmosphere is suitable with proper air circulation. Even during hot and summer the temperature of the site remains normal. Very few residential buildings exists nearby resulting in minimal human activities which is less probable to increase in coming 15 to 20 years making the area safe for the construction of the Mushroom Laboratory. It has clay silt with trace of sand soil with steep topography of the land, drainage problem does not exist which prevents the probability of contamination of different pathogens and insect pests on mushrooms. Abundant, clean and non contaminated underground water supply system makes more sustainability of the center. Contamination of culture/spawn due to livestock can be prevented as livestock farming is limited and not so

popular. The area is clean without any dumping area around so the landsite is very suitable for agronomical activities. No industries nearby and nature friendly environment with suitable air quality. Good transportation facilities as proper condition BP Highway near Kathmandu can be used. Unlimited Power Supply as Khopasi/Panauti area is well known for Electricity generation. So, establishment of mushroom resource center within the boundary of Sericulture Farm in Khopasi, Kavrepalanchowk is quite appropriate.

## 15. CONCLUSION

Nepal is an agriculture dominant country and huge quantities of wide varieties of organic waste are generated from agriculture, forestry, and food processing industries. Mushroom cultivation is an effective bioconversion technology of transforming these wastes into wealth or potentially valuable resources. The production of mushroom has increased by 8.65 times over a period of decade until 2020/21 AD in Nepal. Similarly, mushroom seed output was 268,560 bottles per year in 2010, an increase of almost 6 times over a ten-year period to 1,600,552 bottles per year until 2020/21. Mushroom, a protein-rich wonder food needs more publicity as it deserves. It is then this precious vegetable would solve the problem of protein malnutrition within the country. Mushrooms are an example of a component that not only makes use of vertical space but also aids in the production of high-quality food, as well as environmental sustainability and health. To fulfill the changing needs of food products, it is necessary to promote both mushroom production and consumption. The mushroom industry is gradually taking root in Nepal but the pace is rather slow because of insufficient scientific research and dedicated mushroom resource center. In future, the ever-increasing population, depleting agricultural land, changes in the environment, water shortage and need for quality food products are going to be the vital issues.

There is a need to promote both mushroom production as well as consumption for meeting the changing needs of food items. Thus, the mushroom sector holds huge potentials to contribute significantly to the nation's socio-economic transformation. A dedicated mushroom resource center is urgent needed to provide the pure culture for the production of mushroom spawn and also have to facilities for conservation of the exotic and native and edible and medicinal mushroom species.

NMRC will primarily provide pure cultures to the spawn producers and also to provide facilities for the conservation of exotic and locally available edible and medicinal mushroom species. Similarly, NMRC also perform as a training centre, where R&D workers from various provinces, palikas and others organizations including NGOs will be imparted training in the latest technical know-how on mushrooms, who, in turn, will train the prospective growers and entrepreneurs.

## 16. REFERENCES

- Chang, S. T. 2006. The world mushroom industry: Trends and technological development. *International Journal of Medicinal Mushrooms* 8: 297–314.
- Christoplos, I. 2010. Mobilizing the potential of rural and agricultural extension. Food and Agriculture Organization of the United Nations. <https://bit.ly/2UJqSHd>.
- FAO. 2022. *World Food and Agriculture – Statistical Yearbook 2022*. Rome.
- Li, C and Xu, S. 2022. Edible mushroom industry in China: current state and perspectives. *Applied Microbiology and Biotechnology* 106 (11):1-7.
- GoN. 2020/21. Statistical Information on Nepalese Agriculture. Ministry of Agricultural Development, Agribusiness Promotion and Statistics Division, Kathmandu, Nepal.
- [http://agridaksh.iasri.res.in/html\\_file/mushroom/nutritional\\_properties\\_mush.htm](http://agridaksh.iasri.res.in/html_file/mushroom/nutritional_properties_mush.htm)
- <https://dmrsolan.icar.gov.in/>
- <https://research.wur.nl/en/publications/current-overview-of-mushroom-production-in-the-world>
- <https://rrcultivation.com/blogs/mn/the-history-of-mushroom-farming>
- <https://www.tractorjunction.com/blog/top-mushroom-producing-states-in-india/>
- Jayaraman, J. 1992. Laboratory manual of biochemistry. New Delhi: Willey Eastern LTD.
- Jong, S.C., Levy, A. and Stevenson, R. W. 1984. In Proceedings of the fourth International Conference on culture collection (Eds.Kocur, M and da Silva, vE.), pp.125-136. World Federation for culture collectios, London.
- Joshi, T. R. 2005. Miracle of ganotherapy. Kathmandu: Sajha Prakashan.
- Morris, G.J. 1981. Cryopreservation: An Introduction to cryopreservation in culture collection. Culture center of Algae and Protozoa, Cumbria, UK.
- Morris, G. J., Smith, D. and Coulson, G. E. 1988. A comparative study of the morphology of hyphae during freezing with the viability upon thawing of 20 species of fungi. *Journal of General Microbiology* 134:2897-2906.
- Neupane, S.P 2068. Nepalma Chyau Kheti. Publisher Binita Neupane
- Parajuli, G.P. 2014. Mushroom Research in Nepal: Current Status and Prospects. Nepal Academy of Science and Technology (NAST) and Ministry of Science Technology and Environment. p 19-22.
- Parajuli, G. P, Watanabe K., Khadka B. 2068. Simple Cultivation Technology of Shiitake Mushrooms. JICA/Nepal and Nepal Agriculture Research Council, Plant Pathology Division. Leaflet. p 8.
- Parajuli, G. P, Vaidya M. L, Bastola H, Vaidya G, Tripathi N. 2071. Modern Cultivation Technology of Oyster Mushroom, NARC, Plant Pathology Division, Leaflet. P 8.
- Parajuli G. P, Watanabe K, Bastola H. 2069. Simple Cultivation Technology of Red Mushroom Leaflet, NARC, Plant Pathology Division, P8 .
- Parajuli G. P, Bastola H, Vaidya M. L, Vaidya G, Mahato V. 2071. Simple Cultivation Technology of Button Mushroom, NARC, Plant Pathology Division, Leaflet. P 8.
- Parajuli G. P, Bastola H, Tripathi N, Adhikari B. 2073. Cultivation Technology of Milky Mushroom, NARC, Plant Pathology Division, P 8.
- Raut, J. K. 2019. Current Status, Challenges and Prospects of Mushroom Industry in Nepal. *International Journal of Agricultural Economics*. Vol. 4, No. 4, 2019, pp. 154-160.
- Singh, S. K. Upadhaya, R. C. and Verma, R. N. 2001. Effect of cryoprotectants on preservation of mycelia cultures of edible mushrooms. *Mushroom Research*. 10(2): 67-72.

Upadhaya, R. C., Singh, S. K. and Tiwari, R. P. 2004. Mushroom Spawn Production and Infrastructure Requirements. National Research Center for Mushroom (ICAR). Solan, Himanchal Pradesh. Pp.38.

Zhang Y., Geng W., Shen Y., Wang Y., Dai Y. C. (2014) Edible Mushroom Cultivation for Food Security and Rural Development in China: Bio-Innovation, Technological Dissemination and Marketing. *Sustainability* 6: 2961-2973.

## ANNEXES

### ANNEX 1. Checklist

#### *Checklist for key informant interviews (KIIs)*

##### **A. Ministry of Agricultural and Livestock Development**

- What are the major districts for mushroom production?
- How many mushroom resource centers are in the country?
- What is the present status of mushroom sub-sector in Nepal?
- What are the mushroom development strategies and programs of MoALD?
- What are the initiatives taken by the ministry to introduce the advanced technologies of mushroom?
- What are the initiatives taken by the ministry to conserved edible and non edible mushroom?
- What are the market strategies adopted by MoALD for mushroom?
- Is any change in the policy in recent years?
- How federal ministry working with other stakeholders to strengthen the mushroom industry in Nepal?
- What are the future strategies for mushroom development in the country?
- Besides MOALD, what are the major roles of other ministries to promote mushroom industry?

### **National Plant Pathology Research Center-NARC, Khumaltar**

- How many types of mushrooms are cultivating by NPPC?
- Have you adequate facilities and lab equipments to grow different types of mushroom culture?
- What type of facilities requires to conserving mushroom culture and germplasm?
- What are the opportunities in mushroom sub-sector of Nepal?
- What are the challenges of mushroom research in Nepal?
- What kinds of role have been playing by the NPPC for the development of mushroom sub-sector in Nepal?
- What should be the future direction of NPPC for the development of mushroom sub-sector in Nepal?
- How MOALD and NARC supporting to NPPC for the technology generation of mushroom?
- What are the sources of mushroom germplasm acquisitions by NPPC?
- And how many mushroom germplasms conserved in NPPC?
- How many types of mushroom varieties are released and registered and what is the yield of these mushrooms?
- What shorts of programs are being implemented by NPPC to solve the major issues of mushroom research in Nepal?
- Do you suggest something else for the development of mushroom sub-sector in Nepal?

### **Nepal mushroom producer association (NMPS)**

- What is the status of mushroom production in Nepal?
- How many mushroom entrepreneurs registered in your association and how many are in producing mushroom?
- What are the strategies of NMPS to promote mushroom industry in Nepal?
- Does the government have support to promote mushroom industry through mushroom producer association?
- Are mushroom farmers getting subsidy from Government?
- Are insurance practices effective for mushroom production?
- What the major markets of mushroom in Nepal?
- Does the NMPS have fixed the market price of mushroom?
- What problems do you see for the development of mushroom sub sector in Nepal?
- What are your suggestions for development of mushroom sub-sector in Nepal?
- Are government's existing policies and programs are favorable to promote mushroom industry in Nepal?
- What types of efforts are paying by the NMPS to strengthen the mushroom production and marketing in Nepal?

## ANNEX 2 . List of Apparatus and Lab Equipments

S.N.	Room	Item	Unit
1	Office Room	Computer	4
2		Printer	4
3	Microscope and precise devices room	Electronic balance	3
4		Moisture analyzer	3
5		Microscope	2
6	Laboratory room (line B)	Autoclave (Big size)	1
7		Autoclave (Middle size, new one)	2
8		Autoclave (Middle size)	2
9		Electric cleaner	3
10		Distillation apparatus	3
11		Hot air oven	3
12		pH meter	3
13		Lockers for reagent and glass wares	
14		Experiment desk with water sink	3
15		Cooling room 1	Refrigerator
16	UV light		
17	Air shower		1
18	Inoculation room 1	Laminar flower	3
19		UV light (in the room)	4
20		Deep freezer	3
21		Hot plate round	3
22	Incubation room (5)	Dehumidifier	5
23		Ultrasonic Humidifier 2.4L/hur	5
24		CO <sub>2</sub> analyzer	5
25		Device for measurement of temp. and humidity	5
26	Spawn storage room (2)	Refrigerating machine	2
27		Devices for measurement of temp. and humidity	2
28	Long term Preservation	storage container (1x 35)	2
29		Container for carry of LN2(30L)	2
30		Liquid N2 withdrawel device	2
31		Cryo -bials	600
32		Cane -bials	25
33		Cane for cryo-bials	24
34		Refrigerator base caster	2
35		Glove	2
36		LN2	156
37		Oxygen analyser	1
38	Laboratory Working Room	Lockers for reagent and glass wares	
39		Experiment desk with water sink	
40	Post Harvest	Drier Solar	1
41	Laboratory	Freezer	1

<b>ANNEX 3. List of Different Room/Chamber and Their Features</b>	
Rooms/Chambers	Main Features and Functions
Working room	This room is used for washing, boiling and filling grains with sufficient water supply and drainage, boiling cattle or vessel, this plate form preparation of substrates (media), mixing of substrates and bagging.
Autoclave room	This room is used for sterilization of substrates (media).
Cooling room	This room is used for cooling of substrates after autoclave.
Inoculation room	This room is used for inoculation of desired culture to the substrates, preparation of media for inoculation purpose.
Incubation room	Incubation room shall be environmentally controlled for incubation of inoculated bags at desired temperature.
Cold-rooms, Deep freezer	Storage of spawn after incubation, fully matured spawn bags shall be stored at this room for commercial purpose. This room shall be used for storing fully colonized spawn bags. Long term storage of culture after incubation at deep freezer.
Inoculation room 2	Pouring of media in the laminar flow for aseptic condition. Isolation of native mushroom for tissue culture.
Laboratory autoclave	This room is used for preparation of media and autoclaving of test tubes with media.
Microscope and Precise room	Weighing of chemicals, identifying of native mushrooms with the help of microscope etc.
Changing room	For changing clothes before going to the laboratory.
Office room	This room is mainly used for waiting room of staffs, making notes of daily work plan etc.
Storage room 1	Storage of substrates (media, wheat, saw dust etc.,)
Storage room 2	Storage of chemicals and laboratory equipments
Storage room	Storage of chemicals, media(substrates) for training purpose
Microscope and Precise room	This room is used for handling of microscope for identification of mushrooms culture.
Inoculation chamber	Demonstrate the preparation methods of spawn of mushrooms.
Working lab	Preparation of substrates (media) for spawn production and mushrooms cultivation etc.,
Incubation	Incubate the substrates after inoculation of culture
Post harvest Laboratory	This room is used for value addition work of mushrooms such as drying of mushroom, canning etc.
Raw materials Preparation yard	This room is used for preparation of substrates for cultivation of mushroom.
Composting yard	This site is used for preparation of compost for short term.

Steaming area	This site is used for preparation of substrates for cultivation of oyster mushroom etc.,
Shiitake Mushroom spawn run yard	This area is used for incubation of shiitake inoculated log for incubation purpose and production of shiitake mushroom
Growth Chamber	There are 6 growth chambers. These growth chambers are used for cultivation of different mushrooms for adoptive research purpose. In growth chamber the researchers/producers control humidity, temperature, light, and other factors.
Production of mushroom area	Cultivation of mushroom by using different methods and demonstration of difference in yield quantity through use of different methods.

**ANNEX 4. Room Size of Seed Production and Conservation Block (Ground Floor)**

Room	No. of Room	Room Size
Storage Room	1	3.6 m x 6 m
Working Area	1	7.1 m x 8.6 m
Autoclave Room (Boiler)	1	3 m x 5.5 m
Cooling Room	1	3 m x 5 m
Inoculation Room	2	4 m x 4.1 m
Incubation Room	4	2.52 m x 4 m
Mother Spawn Room	1	2.52 m x 4 m
Changing Room	1	1.8 m x 4 m
Office Room	1	3.5 m x 6 m
Microscope and Precise Room	1	2.8 m x 6 m
Laboratory Autoclave Room	1	5.7 m x 6 m
Buffer Room	1	2 m x 4 m
Cryopreservation Room	1	3 m x 4 m
Cold Room and Deep Freezer	1	3.5 m x 4 m
Machine Room	1	34 m x 3.1 m

## ANNEX 5. Hydrological and Meteorological Data (Source: Department of Hydrology and Meteorology)

Parameter: Relative Humidity  
Station: 1024, Dhulikhel, Kavre

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1993	89.236	85.67	78.04	78.008	84.201	90.663	92.971	93.47	93.394	90.57	91.016	89.138
1994	88.654	86.651	83.914	69.312	79.156	88.408	91.196	92.819	92.289	86.675	86.248	83.811
1995	88.719	87.93	85.188	65.11	73.338	95.261	95.491	94.483	90.446	86.427	87.003	86.87
1996	88.306	85.177	74.996	62.598	73.85	87.884	93.641	93.405	91.78	88.064	86.748	84.437
1997	89.709	84.98	81.29	86.606	82.422	87.201	94.587	95.348	96.758	93.533	95.218	94.726
1998	94.838	86.103	78.479	76.605	87.051	86.386	94.462	96.114	92.973	91.766	89.11	88.477
1999	86.051	79.585	67.393	65.775	82.806	93.586	94.598	94.779	95.163	93.19	90.208	90.066
2000	86.677	77.798	68.575	73.711	84.695	90.888	93.282	95.385	94.21	90.703	89.891	87.488
2001	89.548	82.346	74.946	73.68	89.543	92.263	93.601	93.548	94.616	92.446	93.166	90.633
2002	88.029	89.024	88.058	82.648	85.254	88.76	93.701	92.354	90.633	84.88	84.141	82.846
2003	79.256	77.251	72.067	65.056	65.851	86.213	90.393	91.006	91.91	84.04	83.716	84.088
2004	81.119	73.856	62.585	68.518	74.779	86.216	92.529	91.34	91.45	84.222	78.871	78.714
2005	81.185	69.482	65.725	53.864	65.524	76.155	90.029	93.101	88.214	84.698	81.331	81.42
2006	61.329	74.883	57.279	62.656	82.058	85.98	90.814	91.222	90.383	83.743	81.108	82.904
2007	83.267	85.885	74.475	68.89	72.022	84.988	92.945	92.02	92.883	89.508	84.1	81.567
2008	78.78	70.215	64.643	64.688	75.229	88.751	90.062	91.511	90.161	82.672	80.383	84.408
2009	77.283	67.666	56.311	56.455	76.767	77.903	89.931	93.3	90.331	85.772	75.695	87.163
2010	82.832	75.03	61.47	54.58	64.174	73.055	89.769	92.959	91.97	85.554	83	77.977
2011	77.841	71.992	59.972	60.065	81.656	85.045	91.711	91.683	93.08	85.485	85.65	83.761
2012	70.811	64.881		60.406	59.382	75.098	91.633	91.616	92.256	83.203	77.293	78.3
2013	71.12	72.685	67.127	57.116	68.695	87.425	91.938	91.29	80.308	89.179	77.542	80.304
2014	77.474	68.821	65.462	49.025	65.72	82.031	89.919	92.841	92.053	84.916	83.686	78.488
2015	71.37	74.891	72.232	70.748	64.175	73.311	89.809	92.666	88.226	83.209	83.369	82.585
2016	82.508	66.377	59.779	42.493	73.298	84.818	92.654	89.767	93.633	87.054	77.873	78.116
2017			75.909	68.593	78.229	81.405	87.817	89.416	85.726	78.327	69.055	69.217
2018	70.24	65.205	57.03	68.121	75.417	82.436	89.08	91.467	86.893	75.719	72.22	74.629
2019	72.624	70.566	59.849	74.714	62.003	76.258	87.922	88.054	92.195	84.753	83.575	79.251
2020	80.227	79.507	65.869	68.546	79.085	88.132	90.76	90.525	90.922	79.212	69.928	73.319
2021	69.766	68.393	61.869	53.996	81.198	85.243	91.788	92.474	89.887	89.294	81.908	77.733
2022	81.986	74.556	62.001	66.06	81.869	88.459	89.23	86.951	91.884	81.28	78.452	78.56

Parameter: 24 h accumulated Precipitation  
Station: 1024, Dhulikhel, Kavre

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1993	7.4	3.2	34.9	50.5	165.9	369.9	297	542.8	112.1	36.6	0	0
1994	30.1	26.1	11.3	2.4	162	249.5	433.4	398.1	297.8	0	24	0
1995	6.5	41.4	15.6	5	182.5	442.1	560	397.1	104.6	19.8	58.3	13.2
1996	71.9	11.6	14.2	8.9	67.7	321.6	483.8	398.6	143.3	48	0	0
1997	22.2	8.2	10	92.3	113.6	225.2	438.2	342.7	87.7	10.7	6	99.1
1998	0	27.2	56.9	78.5	152.6	197.2	447.8	625.8	182.8	26.8	13	0
1999	0	0	0	0	149.6	534.6	522.7	398.3	174.4	264.4	0	0
2000	0	4	18.6	70.5	218.6	218.8	373.5	430	172.4	4.2	0	2
2001	2	16	4	21.6	178.3	265.4	384.6	340.6	190.9	60	0	0
2002	30	34.2	80.6	105.4	244.2	194	668.3	569.5	273.8	24	4	0
2003	22.4	84.3	58.3	93.4	50.8	275.6	542.2	377.2	237	13.2	0	24.4
2004	40.2	0	2.2	53.4	137.6	281	414.8	226	233.6	25.8	3.2	0
2005	54.6	11.6	39.8	43.5	57.8	119.8	230.1	506.2	58.8	120.2	0	0
2006	0	0	20.2	118.6	148	224.8	280.4	176.2	187.8	31.8	0	44.5
2007	0	110.6	35.6	116.2	90.4	171.4	286.9	237	289.4	49.2	4	0
2008	6	0	17	37.4	105.3	216	174.1	317.4	239.2	18	0	2
2009	0	0	14.2	18.2	135	134.2	314.2	313	73.7	48.7	0	0
2010									174.2	37.2	0	0
2011	3.5	47.3	16.3	64.2	207.9	267.4	287.3	276.2	257.4	4	26.5	0
2012	9.9	27.2	1.2	98	36.1	110.9	423.2		210.1	0	0	0
2013	6.3	34.2	22.6	53.4	93.4	149.2	293.8	154.7	79.7	122.4	0	0
2014	3	12.5	38.3	12.2	136.3	135.5	332	288.3	213.6	73.2	0	19.4
2015	1.3	25.6	73	65.9	18.2	111.2	368.5	426.7	99.7	29.2	0	0
2016	1.2	10.4	1.3	4.3	138.3	178.7	362.6	132	337.8	91.7	0	0
2017			17.8	126.2	123.2	212.2	299.6	301.7	151.1	14.1	0	0
2018	2.8	3.5	61.6	101.4	166.3	174.2	360.9	400.8	71.9	71.9	0	0
2019	17.2	73.7	13.3	87.6	77.6	115.1	554.4	472.4	274.3	39.7	0	20.5
2020	54.55	21.71	30.1	98	139.1	165.11	339.83	260.3	289.51	6	0	0
2021	0.01	3.24	6.52	42.11	173.42	171.54	496.41	320	135.72	77	0	54.52
2022	0.04	43.02	9.21	83.44	193.8	265.8	293.8	286.1	259.2	138.5	0	0

Parameter: Maximum temperature  
 Station: 1024, Dhulikhel, Kavre

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1993	15.84	19.085	22.355	25.366	27.248	26.933	25.936	25.4	24.913	23.47	20.068	17.858
1994	16.025	17.189	23.319	26.646	28.1	27.346	26.922	26.726	25.226	22.477	18.046	15.032
1995	12.154	14.9	21.87	27.02	29.14	24.873	25.125	25.683	25.47	22.67	18.613	15.619
1996	13.406	16.628	22.242	25.69	28.33	25.531	25.74	25.5	24.457	22.703	19.743	15.661
1997	12.987	15.382	21.929	21.553	26.338	27.063	26.816	26.322	25.1	20.69	18.3	13.834
1998	13.761	16.732	18.967	24	25.703	28.683	25.451	24.822	25.006	23.774	19.45	15.787
1999	15.032	20.575	23.709	29.233	26.629	26.083	25.177	24.812	24.8	21.822	18.816	15.08
2000	14.5	15.982	20.967	25.783	25.516	25.966	26.096	27.306	25.633	23.258	19.05	15.112
2001	14.048	18.464	22.467	26.3	25.612	26.85	26.435	25.854	24.316	22.693	19.5	15.193
2002	14.758	17.821	21.354	23.833	24.483	26.416	25.37	25.564	23.85	22.258	18.75	15.29
2003	13.919	15.924	20.387	25.533	25.887	25.9	25.564	25.874	24.333	22.596	18.683	14.29
2004	13.274	16.896	23.161	24.6	26.016	25.893	24.435	26.032	24.216	21.403	17.116	15.403
2005	13.209	17.5	21.693	25.366	26.645	28.183	25.89	25	25.209	21.758	17.793	15
2006	16.822	19.91	21.838	23.8	24.661	26.216	26.483	26.564	23.933	21.483	18.1	15.112
2007	13.806	14.035	20.064	24.716	26.216	26.39	25.467	26.177	24.866	23.274	19.696	16.58
2008	14.983	17.396	22.241	27.033	27.08	26.733	26.903	25.87	24.733	22.833	19.466	15.854
2009	16.419	19.928	22.338	27.05	25.387	27.216	26.55	25.87	25.133	22.467	18.883	15.568
2010	16.838	17.839	24.693	28.316	28.129	28.8	25.951	25.532	24.15	22.5	19.266	16.112
2011	14.58	17.785	22.564	24.266	25.306	26.083	25.274	26.564	25.083	22.967	17.866	16.145
2012	16.554	18.644		25.776	29.122	28.853	26.054	26.287	25.633	23.316	19.543	17.464
2013	15.916	19.974	24.299	25.94	27.935	26.443	26.187	26.2	18.316	21.467	18.347	15.483
2014	15.364	16.957	21.203	26.23	26.722	27.32	26.729	25.47	24.163	19.716	14.606	11.193
2015	11.08	13.992	16.664	17.94	24.151	24.82	22.258	22.329	22.06	18.474	14.623	10.29
2016	9.996	14.931	18.79	23.733	21.664	23.466	22.345	23.029	21.006	18.625	14.613	12.393
2017			22.312	26.733	26.161	27.73	27.403	26.87	27.65	25.967	23.016	20.654
2018	17.854	21.375	24.567	24.766	25.483	27.4	26.129	22.37				18.348
2019	17.78	18.857	23.467	24.866	28.87	28.666	26.845	26.838	25.233	24.577	22.2	17.661
2020	16.032	18.655	22.951	24.466	25.145	26.223	26.135	27.793	26.253	26.838	21.95	18.822
2021	19.403	21.053	24.612	27.5	24.538	26.716	26.419	26	27.216	25.225	21.133	18.135
2022	16.703	17.857	26.483	27.383	26.193	26.15	27.338	27.887	26.6	24.932	22.5	19.458

Parameter: Minimum temperature  
 Station: 1024, Dhulikhel, Kavre

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1993	3.305	5.492	6.386	10.706	14.751	17.373	18.92	18.851	16.721	12.78	7.586	3.793
1994	2.792	2.877	8.309	10.226	14.374	17.633	18.348	18.096	16.733	11.187	5.86	3.354
1995	2.219	4.828	8.766	12.193	16.926	18.946	18.948	18.887	17.636	13.406	8.81	5.52
1996	4.074	6.244	10.565	11.872	15.122	17.383	19.119	18.283	17.482	13.17	9.223	5.212
1997	3.256	3.775	8.419	10.996	13.625	17.024	19.303	19.07	17.2	10.696	8.2	4.485
1998	3.483	5.892	7.983	12.083	16.177	19.233	19.603	19.345	17.926	15.554	10.486	5.887
1999	3.925	8.303	9.564	14.75	16.032	17.959	19	18.935	18.1	13.919	8.9	6.177
2000	3.887	3.793	7.774	12.383	16.222	18.55	19.032	18.983	17.42	13.29	9.566	4.725
2001	3.854	6.053	8.577	11.889	11.561	9.683	10.516	10.177	9.033	5.112	2.2	4.016
2002	2.774	5.339	8.467	10.833	14.887	17.75	18.529	18.367	16.596	12.37	8.183	4.741
2003	2.983	4.803	7.79	11.616	12.758	16.683	18.241	18.645	17.596	13.387	8.6	4.612
2004	3.554	5.482	10.983	12.966	15.409	17.533	18.441	19.048	17.943	12.225	7.766	5.129
2005	4.048	5.875	9.709	11.816	13.951	17.866	19.077	19.019	18.1	13.106	7.976	4.709
2006	4.519	9.321	8.951	12.116	15.974	17.983	19.464	18.964	17.58	12.661	8.766	5.383
2007	3.838	5.089	8.225	13.066	15.677	18.016	19.041	18.98	17.756	13.935	8.466	4.354
2008	3.677	3.586	8.967	11.633	13.758	17.973	18.877	18.641	17.136	12.65	8.633	6.403
2009	5.064	6.714	8.58	12.883	14.416	17.266	18.75	18.467	16.7	13.287	9.216	5.103
2010	4.112	4.696	10.209	13.016	14.822	17.783	18.709	18.612	17.216	13.403	8.9	3.641
2011	2.354	4.892	7.967	10.666	14.177	16.916	18.112	17.935	16.35	13.387	8.666	4.08
2012	1.993	5.51		11.933	14.58	18.586	19.241	18.612	17.533	11.719	6.453	4.141
2013	2.187	5.432	9.638	12.076	15.819	18.293	18.79	18.248	4.546	11.038	4.757	4.719
2014	4.061	5.446	8.251	11.81	15.17	18.676	19.425	18.68	17.24	12.351	8.866	4.77
2015	4.929	6.039	9.296	11.893	15.019	17.883	17.958	17.435	16.883	12.806	8.923	4.193
2016	3.625	6.351	9.758	13.75	14.854	17.983	18.925	18.538	17.623	14.096	8.1	6
2017			10.593	13.133	14.645	17.793	18.548	18.806	17.833	14.567	9.266	6.806
2018	3.741	7.357	9.848	12.416	14.983	17.966	18.967	18.854	17.5	12.096	8.266	4.874
2019	4.158	6.232	8.287	12.883	14.903	17.666	18.725	18.725	17.65	13.29	10.45	4.693
2020	4.064	5.758	8.661	11.4	14.887	18.133	19.048	19.08	18.433	15	8.533	5.516
2021	5.403	6.642	10.225	12.163	14.851	17.88	18.806	18.596	17.533	15.241	8.666	5.28
2022	4.532	4.428	11.558	14.61	15.564	18.1	18.893	18.725	17.8	13.532	9.3	6.035

**EvapoTranspiration Eto calculation using DHM Data and Cropwat tool of FAO**

Month	Min Temp	Max Temp	Humidity	Wind	Sun	Radiation	Eto
	°C	°C	%	km/day	hours	MJ/m <sup>2</sup> /day	mm/day
Jan	3.67	14.93	81	86	6:59	12.8	1.58
Feb	5.59	17.66	76	104	7:29	15.7	2.18
Mar	9.05	22.19	69	112	7:32	18.1	3.11
Apr	12.19	25.38	66	147	7:32	20	4.04
May	14.86	26.28	76	147	7:00	20.4	4.12
Jun	17.62	26.70	85	130	3:27	15.1	3.24
Jul	18.58	25.85	92	112	2:52	15.3	3.09
Aug	18.39	25.75	92	104	2:44	13	2.72
Sep	16.73	24.64	91	95	4:03	14	2.77
Oct	12.84	22.63	86	86	6:18	14.6	2.6
Nov	8.22	18.96	83	69	7:38	14	2.04
Dec	4.95	15.81	82	69	7:28	12.9	1.59
Average	11.89	22.23	82	105	5:55	15.5	2.8

**ANNEX 6. Capacity of Incubator, Growth Chamber and Production Chamber**

<b>A. Incubator</b>		
Materials	Size	Quantity
Test Tube	10 inches	7800 * 4 bottles
Mother Spawn	800 ml bottle	7800*4 bottles
Spawn Bag	400 gm	1020 packets
<b>B. Growth Chamber and Production Chamber</b>		
Substrate Bag	12 inches	13*4*2=104 packets

**ANNEX 7 List of Participants in the Stakeholder Meeting**

Date: 2079-11-08 (Feb 20, 2023)			
S.N.	Name	Designation	Office
1	Bhoj Raj Sapkota	Chief	Center Industrial Entomology Development
2	Hari Bahadur K.C.	Director General	DOA
3	Dr. Shanta Karki	Deputy Director General	DOA
	Januka Pandit	Deputy Director General	DOA
4	Dr. Mahadev Poudel		DOA
5	Krishna Bhadra Adhikari	Chief	Bagmati Pradesh
6	Sanjib Bimali	KrishiEi. Kri. Yaa	Kri. Pur. Pra. Ke
7	Yam Kumar Shrestha	Chief	Resham Bikash Kendra, Khopasi
8	Suraj Vaidya	Chief	NPPC, Khumaltar-NARC
9	Saroj Adhikari		D.O.A
10	Rajib Raj Bhandari	ba. Bali sa. Aa.	Ke. Kri. Pra.
11	Mina Kandel		Kri. Aa. Bi. Kri. Pra. Bi. Ma.
12	Dr. Bhusan Shrestha,	Fungi Expert	
13	Dr. Buddhi RatnaKhadgi	Mushroom Expert	
14	Bibek Sunar	Ba. Sa. Aa.	Ke. Kri. Pra
15	Santosh Karki	Presdent	Nepal Mushroom Production
16	Sujan Pokhrel	Baa. Sa. Aa.	Bay. Ki. Bi. Ke.
17	Asmita Neupane,	kri. Pra. Aa.	
18	Gopal Parajuli	Team leader	
19	Bishow Prashanna Amatya	Mechanical Engineer	
20	Basista Acharaya	Scientist	NARC
21	Dr.Ram Chandra Adhikari	Agriculture Expert	
22	Jyoti Mani BHattarai,	Structural Engineer	
23	Ajit Shrestha	Structural Engineer	
24	Manjil Bhattarai	Civil Engineer	
25	Sanu Maya Jhakri Magar	Architect	
26	Dr. Prakash Acharaya	Ba. Baa. Bi. Aa	SQCC
27	Sunil Jaisawal	Prabidhisahayak	
28	Sunita Subedi	Accountant	
29	Sujan Bhandari	Na. Su.	
30	Harisharan Budhathoki	Kharidar	
31	Laxmi Gurung		

Date: 2079-12-29 (April 12, 2023)			
S.N.	Name	Designation	Office
1	Bhoj Raj Sapkota	Chief	Center Industrial Entomology Development
2	Hari Bahadur K.C.	Director General	DOA
3	Dr. Shanta Karki	Deputy Director General	DOA
4	Tika Ram Sharma	Chief	Kri. Pu. Bi. Ta. Kri. Ya. Pra. Ke.
5	Dr. Shrimat Shrestha	Director Crop and Horticulture	NARC
6	Krishna Bhadra Adhikari	Chief	Bagmati Pradesh
7	Pharindra Devkota	Senior Plant protection Officer	
8	Yam Kumar Shrestha	Chief	Resham Bikash Kendra, Khopasi
9	Suraj Vaidya	Chief	NPPC, Khumaltar-NARC
10	Saroj Adhikari		D.O.A
11	Mina KandelKri		Aa. Bi. Kri. Pra. Bi. Ma.
12	Dr. Bhusan Shrestha,	Fungi Expert	
13	Dr. Buddhi RatnaKhadgi	Mushroom Expert	
14	Bibek Sunar	Ba. Sa. Aa.	Ke. Kri. Pra
15	Sangita Shrestha	Mushroom Seed Expert	
16	Sujan Pokhrel	Baa. Sa. Aa.	Bay. Ki. Bi. Ke.
17	Asmita Neupane,	kri. Pra. Aa.	
18	Gopal Parajuli	Team leader	
19	Bishow Prashanna Amatya	Mechanical Engineer	
20	Dr.Ram Chandra Adhikari	Agriculture Expert	
21	Jyoti Mani BHattarai,	Structural Engineer	
22	Ajit Shrestha	Structural Engineer	
23	Manjil Bhattarai	Civil Engineer	
24	Sanu Maya Jhakri Magar	Architect	
25	Dr. Prakash Acharaya	Ba. Baa. Bi. Aa	SQCC

Date: 2080-03- (June , 2023)			
S.N.	Name	Designation	Office
1	Bhoj Raj Sapkot	Chief	Center Industrial Entomology Development
2	Hari Bahadur K.C.	Director General	DOA
3	Dr. Shanta Karki	Deputy Director General	DOA
4	Tika Ram Sharma	Chief	Kri. Pu. Bi. Ta. Kri. Ya. Pra. Ke.
5	Suraj Vaidya	Chief	NPPC, Khumaltar-NARC
6	Krishna Bhadra Adhikari	Chief	Bagmati Pradesh
7	Yam Kumar Shrestha	Chief	Resham Bikash Kendra, Khopasi
8	Sunil Singh		DOA
9	Bibek Sunar	Ba. Sa. Aa.	Ke. Kri. Pra
10	Sujan Pokhrel	Baa. Sa. Aa.	Bay. Ki. Bi. Ke.
11	Asmita Neupane,	kri. Pra. Aa.	
12	Sunil Jaisawal		
13	Sunita Subedi		
14	Sujan Bhandari		
15	Harisharan Budhathoki		
16	Dr. Bhusan Shrestha,	Fungi Expert	
17	Gopal Parajuli	Team leader	
18	Bishow Prashanna Amatya	Mechanical Engineer	
19	Dr.Ram Chandra Adhikari	Agriculture Expert	
20	Jyoti Mani BHattarai,	Structural Engineer	
21	Manjil Bhattarai	Civil Engineer	
22	Sanu Maya Jhakri Magar	Architect	

**ANNEX 8. Construction and Implementation Schedule**

**ANNEX 9. Photographs**









