CHAPTER ONE

INTRODUCTION

1.1 Background

Mushrooms belong to a group of living organisms which are known as fungi. They are the members of higher fungi belonging to the class Basidiomycetes and some are Ascomycetes. The fruiting bodies of Basidiomycetes are known as basidiocarps and the fruiting bodies of Ascomycetes are known as ascocarps (Chang & Hayes, 1978).Mushroom is very unique by gical systems in lacking chlorophyll and therefore, it does not require sun r ability to degrade the inedible plant materials and biosynthesis of edib is very high in compare to other organisms (Gogoi, 2006).

Mushrooms depend on organic decays or living plants for their nutrition, and have ability to degrade cellulose, hemicelluloses, lignin and in turn produce edible fruiting bodies, which have characteristic aroma and flavors (Pandey & Ghosh, 1996). Mushrooms are also good sources of proteins, vitamins and minerals. Protein content in mushroom varies from 19-40 %. Most of the essential amino acids are available in mushroom (Kurtzman, 1975). The new source of food protein should be economic and acceptable in taste, odor and other characters to needy local population (Milner, 1991). Mushrooms fall in this category, which not only have good nutritional value but also have high productivity per unit area and time. Thus, mushroom can be taken as a good means to combat malnutrition problem in developing countries (Rai, 1990).

Mushrooms have become a part of human diet since long. In a country where conventional proteins of both, animal and plant origins are not available in required quantities to a large section of population, any attempt to supplement diet with alternative sources of protein would be a welcoming venture (Shukla,1991).

Fungi play many vital roles in forest and ecosystems depend upon fungi's ability to decompose organic plant matter soon after it is rendered available. Mushroom also

helps in decomposing plant and bioconverting directly into palatable protein. Thus, it serves as the natural ecological agent to recycle and recovery of the plant material (Kaul & Dhar, 2007).

Mushrooms are capable of agro-waste degradation which are useless by-products and can be recycled to produce additive food in form of mushroom for human consumption. Thus, the environmental pollution can be reduced by disposal of agricultural wastes. Further, used compost from mushroom growing may also be recycled for use as animal feeds, soil conditioning and fertilizer (Suman & Sharma, 2005).

In spite of their toxicity, the mushrooms are used for various purposes. They are used for remedies of different diseases, for decoration, for commercial trade etc. *Morchella* spp and *Ophiocordyceps sinensis* found in high altitude of Nepal and used as trade both in national and international markets due to their medicinal value (Adikari, 2008a, 2008b; Devkota, 2008). Mushrooms are the source of extra ordinary power and have medicinal properties like anticancerous, anticholesteral, antitumorous. They are useful against diabetes, ulcer and lunge diseases (Quimio, 1990). The mushrooms like *Ganoderma lucidium*, *Coriolus hirsutus* and *Pycnoporus cinnabarinus* are used for heal cut and wounds (Adhikari, 2000)

Mushroom cultivation is one of the efficient ways by which residues can be recycled. It may also offer economic incentives for agribusiness to examine these residues as valuable resources and develop new enterprises to use them to produce nutritious mushroom products. Therefore, the mushroom cultivation may become one of the most profitable agri-business that could produce food products from different substrates and help dispose of them in an environmentally friendly manner. The cultivation of edible mushrooms offers one of the most feasible and economic method for the bioconversion of agro-lignocellulosic wastes (Bano *et al.,* 1993; Cohen *et al.,* 2002).

Mushroom cultivation does not require too much land fertilizer and other costly inputs. Substrates for its cultivation constitute a major part of waste product which is easily available in village. Because of cheap availability particularly, the farmers can reduce their poverty. Economic value of mushroom is also very high. It has a

significantly higher rate of economic return (Fanadzo *et al*, 2010).Thus, at present, it has a great potentiality for the enhancement of economic status of people in the countries like Nepal.

In Nepal, mushroom cultivation has tremendous prospect although, at present only some wild types of mushrooms are eaten by rural folk (Adikari, 2000; Manandhar, 2005).Modern technology has made possible to grow mushrooms under control and semi-control conditions. The simple, economical and commercial methodology for cultivation of some known edible mushrooms has been evolved in Nepal (Khadge, 2006).

Lentinus sajor-caju (Rumph.: Fr.) Fr. is generally referred to as oyster mushroom and popularly known as 'kanye chyau' in Nepal. Its synonyms is *Pleurotus sajor- caju* (Fr.) Singer (Adikari, 2000). It is mostly cultivated by farmer in Nepal and sold in market which is grown in paddy straw. And so often, it is known as paddy straw mushroom.

Based on climatic and cultural requirements, three species of mushrooms are recommended for cultivation in Nepal. They are : button mushroom (*Agaricus bisporus)*, which was introduced during the year 1979-80, oyster mushroom (*Lentinus sajor-caju)* introduced to cultivate during the year 1983-84, and paddy straw mushroom (*Volvariella volvacea*), introduced during the year 1982 (PPD, 1992-1999). The cultivation of *L sajor-caju* is done during winter season in Terai region (22-26 ^{o}C) and use as summer crop in the hills of Nepal (25-30 ^{o}C). Oyster mushroom cannot be grown in Terai during the summer season due to high temperature value i.e 30 -40 ^{o}C (Manandhar, 2004).

Oyster mushroom is largely grown on paddy and wheat straw which is becoming more costly substrate because of high demand. Therefore, the present investigation was carried out to search out different agricultural waste for the successful cultivation of oyster mushroom. Though paddy straw is considered as the best substrate in terms of yield, it has become necessary to find cheap and alternate substrates due to the higher cost of paddy substrates and it's non –availability in certain areas.

1.2 Justification

Due to the following importance, the oyster mushroom is selected in the present study.

- Public demand for edible mushroom has been mounting day by day in the last few years due to the supply by mushroom growers at a reasonable price.
- It helps in the poverty alleviation as well as malnutrition which are common in the rural region of Nepal.
- It is highly profitable crop and different agricultural wastes can be used for its cultivation. Low cost lignocellulosic materials can be used for the extra income generation.
- Present cultivation practices are mainly focused only on rice straw. Therefore, the identification of low cost substrates is to be established in the present fact context which is need for the sustainable mushroom production.

1.3 Objectives

- To identify the low cost substrates and supplements for oyster mushroom cultivation.
- To determine the productivity of certain agricultural waste products used as substrates.

1.4 Limitation

- Only few agricultural wastes were used as substrates in this experiment.
- The nutritive and toxicity test of mushroom were not done.
- During the thesis work, there was problem of load shedding so only one species was selected for cultivation.

CHAPTER TWO
LITERATURE REVIEW

2.1 Morphology of *Lentinus sajor – caju*

Mushroom is a general term applied to the fruiting body of fleshy fungi. Most of the mushrooms belong to the class Hymenomycetes and subdivision Basidiomycotina. They are characterized by a hymenenal layer, which bears basidiospores (Shukla, 1991).

Lentinus sajor-caju (Rumph.: Fr.) Fr. is a gill fungus. Sporophores (basidiocarp) of this fungus are solitary or in group which occur on decaying plants of *Euphorbia royleana*. Pileus is 5-14 cm in diameter, sometimes lobed and folded at maturity having a white to grey or dull brown in colour; surface smooth, margin irregular and curved. Gills (lamellae) are distinctly formed, decurrent and remain white at fresh and become yellow when dry. Stipe 1-3 × 0.1-0.2 cm, white, rigid and solid, flesh semi-coriaceous when fresh, rigid on drying. Basidia 35-55 × 5-12 μ, clavate. Hymenophoral trama irregular. Basidiospores 6.5 - 7.5 × 2.7-3.3 μ, cylindrical, smooth, non-amyloid, spore mass white (Purkayastha & Chandra, 1976).

Distribution of *L. sajor-caju* was found in Japan, China, North America, India and Nepal (Adikari, 2000)

2.2 Historical review of mushroom cultivation

Mushroom has become part of human diet since time immemorial. Its importance is mentioned in ancient Hindu book VEDA (Rigveda, Yajurveda, Samveda and Athrvaveda) nearly 3893091 years back (Adhikari, 1981-82).

In Europe, mushroom cultivation without any systematic processes was done even during the Roman times around 900 AD. However, the practice cultivation of European forms of mushroom in a systematic manner was originated in France during the 17th century (Singh, 2007).

First attempt of cultivation of button mushroom, began in vicinity of Paris around 1650, which, later spread to whole Europe (Shukla, 1991).

2.3 Factors affecting oyster mushroom production

2.3.1 Temperature and relative humidity

Zandrazil (1978) indicated that optimum temperature for fruiting body formation of *Pleurotus sajar-caju* was 23 °C, however, fructification could take place at 30°C too. Similarly, Quimio (1990) reported that *P.sajor-caju* might tolerate temperature of 28-30°C.Shukla (1991) stated that temperature and relative humidity of the rooms after opening of the bags should be 20-25°C and 65-75 % or more, respectively. Khader (1993) mentioned that oyster mushroom could grow at moderate temperatures ranging from 22 to 28°C. Pandey & Ghosh (1996) reported that oyster mushroom can be grown when room temperature is between 20 – 30°C and relative humidity 70 %.

Singh (2007) reported that the most suitable temperature is 20 to 22°C for buton mushroom cultivation (*Agaricus*) and 26 to 28°C for *Pleurotus* spp. Although, different species exhibited different temperature preferences, much variation has not been note in their requirements for pH and relative humidity, as most of the species tested, preferred 5.6 as the optimum pH and 70-90 % as the optimum relative humidity (Paroda & Chadha, 1996).

Mishra (2002) mentioned temperature and relative humidity requirements for different types of mushrooms as noted below (Table 1)

Table No. 1: Temperature and relatives humidity requirements for different types of mushrooms

Mushroom species	Mycelial development		Mushroom production		Relative humidity (%)
	Optimum temperature (°C)	Temperature range (°C)	Optimum temperature (°C)	Temperature range (°C)	
Agaricus bisporus	20 – 25	1-32	14/16-16/18	10-20	80-90
Pleurotus sajar-caju	20 - 26	18-30	25	20-28	80-85
Pleurotus florida	25	16-27	25	25-28	80-85
Pleurotus ostreatus	20-25	5-25	10-15	10-15	80-85
Volvariella volvacea	25-30	20-40	25-30	30-40	80-85
Lentinus edodes	20	5-35	15-20	8-20	80-85
Pleurotus colombinus	12-20	12-20	10-20	10-15	70-75

2.3.2 Room condition

For inducing fructification, ventilation has been found to play a key role. Well-ventilated room was required during fruiting of oyster mushroom (Shukla, 1991), while less light intensity was found more effective to induce bunching of the fruiting bodies (Paroda & Chadha, 1996).

Ventilation is responsible for the maintenance of congenial environmental conditions and also for the removal of toxic gas by the introduction of adequate fresh air. Good aeration is essential for a healthy crop, as ventilation is one of the important factors governing mushroom production (Tiwari, 2007)

2.3.3 Substrates and ingredients

Oyster mushroom can be grown on various agricultural residues such as corn cob and leaves, cotton waste, sugarcane bagasse and leaves, grasses rice hulls and water hyacinth leaves as good substrates (Quimio, 1990; Singh, 2007).

Three different kinds of biomass, namely *Populus deltoides*, *Eupatorium adenophorum* and sericulture waste were used individually for the cultivation of *Pleurotus sajor -caju* alone and mixed with paddy straw (Patrabansh & Madan, 1997)

Pani *et al*. (1998) reported that the highest yields were obtained with 50 % *Brassica* and 50 % rice straw for cultivation of *Pleurotus sajor-caju* on *Brassica* crop residues like rape and mustard in India.

Plant Pathology Division (1991) reported that *P. sajor-caju* could be cultivated in the mixture of barley straw and paddy straw in an equal proportion for higher yields.

Anyakorah & Olatunji (2001) cultivated oyster mushroom on different agro industrial wastes and reported that *P. sajor- caju* grew on all cellulosic wastes but cotton waste had the highest yield. Mishra (2002) indicated that *Pleurotus* spp. could be grown on paddy straw, maize stalk, wheat straw, millet straw, maize husk, sawdust etc. Oyster mushroom can be cultivated on unfermented substrates. Therefore, composting is not necessary.

2.4 Cultivation

2.4.1 Spawn preparation

Aneja (1996) suggest that grain filled bottles should be autoclaved for two consecutive days at 121°C for 30 minutes. He also suggested that incubation of inoculated bottles at 25 – 25 °C in darkness for 3 weeks, and shaking of containers at intervals for an even distribution of mycelium is required.

Mane *et al.* (2007) reported that wheat grains were boiled for 15-20 mins and mixed with 4 % $CaCO_3$ and incubated at (27±2 %) °C for mycelial growth for 12-15 days until the mycelium fully covered the grains.

2.4.2 Methods of substrate preparation

Oyster mushroom (*Lentinus sajor-caju*) production by using various sized plastic bags was also studied (Plant Pathology Division, 1992). Pieces of paddy straw were put into the plastic bags of 12"x 6" size in 3-4 alternate layers of spawn (2% by weight) and straw. As against conventional method, the plastic bags were removed by cutting with a sharp knife and the substrates kept in a room at 26-28°C and 80% relative humidity. Average yield of fresh mushroom per sample was 41% of the dry matter as against 30% in the perforated, bigger sized (22"×14") bags.

According to a study of Plant Pathology Division (1996), 500 g of moist rice straw (approximately 167 g dry) was packed into heat resistant polypropylene bags of 12" × 6" size for the cultivation of *L sajor-caju*. A piece of PVC (Polyvenyl chloride) pipe of 1.5" ×1.5" size was inserted into its mouth and plugged with cotton. Bags were autoclaved at 121°C for one hour and inoculated with *L sajor-caju* spawn at the rate of 10 g per bag after cooling. Incubation was done at 25°C for four weeks. Plastic cover was removed and the substrates transferred into culture room, where relative humidity was adjusted to 80-85%. Complete impregnation of mycelium was observed after incubation for 21-25 days. Four days after removal of plastic bags, young mushroom primordial came out from the surface, which were harvestable 4 days later. Three flushes were harvested at the intervals of 15-20 days. On an average, 40% of the dry substrate was converted into fresh mushroom by weight.

Cultivation technique of oyster mushroom (*L sajor-caju*) on fermented compost was also studied (Plant Pathology Division, 1998). Ingredients used were paddy straw, urea, Diammonium Phosphate (DAP) and agriculture lime. Treatments comprised of autoclaved and not autoclaved, chopped (2-5 cm long) paddy straw soaked in tap water for 12 hours. Excess water was drained off for four hours and then mixed with urea (596 g), DAP (392 g) and agriculture lime (1000 g) thoroughly. The mixture was stacked in a heap of 1 ×1 ×1 cubic meter size under shade. Three days after stacking, agriculture lime was mixed and another heap prepared. After 6 days, the compost was divided into two parts. First part was packed into polypropylene bags of 18” × 12” size at the rate of 2 kg compost per bag. These bags were autoclaved at 121°C for one hour. After cooling under laminar flow, the bags were inoculated with spawn of *Lentinus sajor-caju*. All bags were incubated at an ambient temperature (25-27 °C) under dark for three weeks. Then, the bags were kept in a cultivation room. The second part of compost was not autoclaved, but simply inoculated and packed. Mean BE from autoclaved treatment was 48% and from not autoclaved treatment 43.07%. Thus, the biological efficiency of oyster mushroom varied from 43-48% on moist compost. It indicated that oyster mushroom could be cultivated on compost also.

Study of cultivation of oyster mushroom (*L. sajor-caju*) in a substrate (paddy straw) mixed with different ingredients was also performed by Plant Pathology Division (1999). The five treatments were 90% paddy straw and 10% chicken manure, 90% paddy straw and 10% rice bran, 95% paddy straw and 5% urea, 90% paddy straw and 10% sawdust and 100% paddy straw, which yielded 966 g, 425 g, 0 g, 1229 g and 550 g respectively, in a bag size of 30 × 45 cm, which were filled with 3 kg of moist substrate and other ingredients with five replications. All bags were autoclaved 121°C for one hour. Each bag was inoculated aseptically with 100 g of spawn of *L.sajor-caju* and incubated at 25-27°C in dark for 3 weeks. Water was sprayed on the bag just to keep it moist after full colonization. The first flush of mushroom was harvested 10 days after removal of polythene bags. Yield of the mushroom was highest in the treatment 90% paddy straw and 10% chicken manure. Addition of urea was detrimental to the growth of oyster mushroom.

Paddy straw with different ingredients, such as sawdust, rice bran, maize powder, mustard meal and chickpea flour were used to find out the effect on *Pleurotus*

ostreatus. The Biological efficiency obtained were 5.6 % on rice bran, 10.6 % on maize powder and 7.9 % on mustard meal (Plant Pathology Division, 2000).Similarly, the cultivation of red mushroom (*Ganoderma lucidium*) was done on the wooden based ingredient such as sawdust with rice bran supplementation. (Plant Pathology Division, 2008)

The use of composted or uncomposted substrates varies according to personal preference .Uncomposted rice straw may be used but it should first be chopped to length of 4-6 cm. The straw is the soaked overnight in clean water, then drained the following morning before adding the supplements (20 % rice bran). A special squeezer mixer may be used to facilitate draining and mixing before bagging (Quimio, 1990).

2.4.3 Spawning and incubation

One bottle of spawn (about 250 g) was needed for 5 kg of dry paddy straw for inoculation (Shrestha *et al*., 1999). They also recommend that holes of 1 cm size on the 12” ×18” sized plastic bags should be at the distance of 10 cm. After mixing the substrates with spawn and ingredients, it should be filled and compressed slightly to make the straw compact (Singh, 1995).

Mishra (2002) suggested that, holes in polythene bag should be of 1” diameter at a distance of 4”, so that there will be 6 holes on 12”×18” sized bags. Similarly, he suggested for the use of 50 g of spawn in this sized bag. Light pressure after spawning, sprinkling with some amount of spawn at the top of the bags, tying of polythene bags with rubber bands and keeping of ready bags in dark at 20-25 °C were also suggested.

2.5 Growth and yield of oyster mushroom

2.5.1 Mycelium development and primordial formation

Three weeks after inoculation, white mycelia were seen inside the polythene bag (Shrestha *et al*., 1999). When the mycelia spread on the surface of the substrate, the polythene bags were removed and the compost placed on a shelf or a platform made up of bricks, bamboo or wooden block (Singh, 1995).Water should be sprinkled 1-2

times per day or as required on compost and floor to maintain the required temperature (20 – 25°C) and humidity (80 – 90 %). After 3-4 days of plastic removal, small primordia had appeared on the compost (Mishra, 2002).

2.5.2 Harvesting

After three to four days initiation of primordia, mushrooms become ready for harvest. Whether mushrooms are ready or not for picking can be judged from their cap. When the mushrooms mature, the edges of caps fold upward and inward and become thinner. It is the right time for picking (Shukla, 1991). He also mentioned that for hand picking, the stripes at the base are hold with the fingers and gently pulled out the mushrooms. Harvesting with knife, leaves a stub on compost, which later rots and enhances contamination. However, Shrestha *et al*. (1999) and Mishra (2002) suggested to harvest by sharp knife at the base rather than by hand at the intervals of 10 – 15 days.

2.6 Diseases and pests

Oyster mushrooms suffer from various fungal, bacterial and viral diseases. A number of competitor moulds, such as *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Tricoderma* spp., *Mucor* spp., *Rhizopus* spp. are recorded (Paroda & Chaddha, 1996).

The main sources of infection during cultivation of mushrooms are air, water, human beings and rodents. Besides these, substrates and spawn are also responsible for infection. Generally, several types of insects can attack mushrooms. In Nepal, the main mushroom pests are sciarids (fungal gnats), phorids (hump-backed), cecids (midges), tarsonemid mites, pepper mites, predatory mites , eelworms and other fungal diseases (Tiwari, 2007).

2.7 Mushrooms and their research status in Nepal

Mushroom survey and research was begun in Nepal by 1854, and till now, about 700 species of mushrooms have been identified. Among them, 10% species are found in tropical, 35% in subtropical, 65% in temperate, 16% in alpine and 4% in Himali zones of the country (Adhikari, 2000).

In 1984, mushroom development programme was introduced in Plant Pathology Division which establish laboratory for spawn production. In 1992, CAT was established in private sector for spawn production. This unit is mainly concentrated till now on research of two types of mushrooms, namely button mushroom (*Agaricus bisporus*) and oyster mushroom (*Lentinus sajor-caju*). At the beginning, some works were also done on *Lentinus edodes* and *Volvariella volvacea* (Manandhar, 2004)

The experimental cultivation mushroom in Nepal started since 1972 with the works of Singh & Nisha (1973). They reported the successful cultivation of Nepalese *Pleurotus* sp. mushroom using different substrates such as *Qurecus*, *Pinus* and *Euphorbia*.

In National Herbarium and Plant Laboratories under the Department of Plant Resources a Godawary, the experimental cultivation of *Lentinula edodes* was carried on in 1991(Adhikari & Manandhar, 1993).The experimental cultivation of *Lentinula edodes* also stared in the promises of Khumaltar.

Annonymous (NARSC, 1989) incorporate the news on mushroom improvement, list of 6 species of mushrooms gathered from Kathmandu valley and the comparative studies on cultivation of *Lentinus sajor-caju*, Agarious bisporous and *Volvariella volvacea* on paddy and other substrates.

Adhikari (2000) reported the nutrient components found in the wild edible mushrooms of Nepal. Pandey & Bhudathoki (2007) analyzed the protein content in 35 species of wild and two species of cultivated mushrooms (*Agaricus bisporous* & *Pleurotus sajar caju*) gathered from various places through Bradford's method. The amount of protein in *L. sajor caju* was found 0.64mg/ml.Rana & Giri (2007) reported the nutritional value of 11 wild edible mushrooms collected from Sagarmatha National Park.

In Nepal, the commercial cultivation was induced in 1978, using the spawn of *Agaricus bisporus* by farmers. After 2001, they started to produce the spawn and fruiting bodies of mushrooms (*Agaricus bisporus*, *Pleurotus ostreatus*, *Lentinula edodes*, *Lentinus sajor -caju* and *Ganoderma lucidium*) at commercial scale (Adikari, 2010).Several species were found cultivated in the Kathmandu valley by different growers(Singh, 2007). They are Kanye- *Pleurotus eryngii*, *P. ostreatus*, *Lentinus sajar-caju*, Gobre- *Agaricus bisporus*; Mrige(Shitake)-*Lentinus edodes*; Parale-

Volvariella volvacea; Dadhu chyau- *Ganoderma lucidium*; and others- *Pholiota nameko*, *Flammulina velutipes* and *Grifola frondasa*.

Ganoderma lucidium, a medicinally very important mushroom is being experimented in the Division of Plant Pathology, NARC. Although it has been cultivated experimentally by some growers in Nepal, no longer has farming of this specis been done so far. One or more primate farms have started producing spawn of this mushroom (Singh, 2007).

Mushroom growers buy seeds either from the Plant Pathology Division, NARC or from the private seed production centers. There are several private sector spawn production centers in Kathmandu. It can be assumed that the production of mushrooms in Nepal has reached 700 metric tones now (Singh, 2007).
Plant Pathology Division (1995-2008) produces spawn of button and oyster mushroom in limited quality. Nowadays, the production decrease due to number of private sectors for spawn productions (Figure 1).

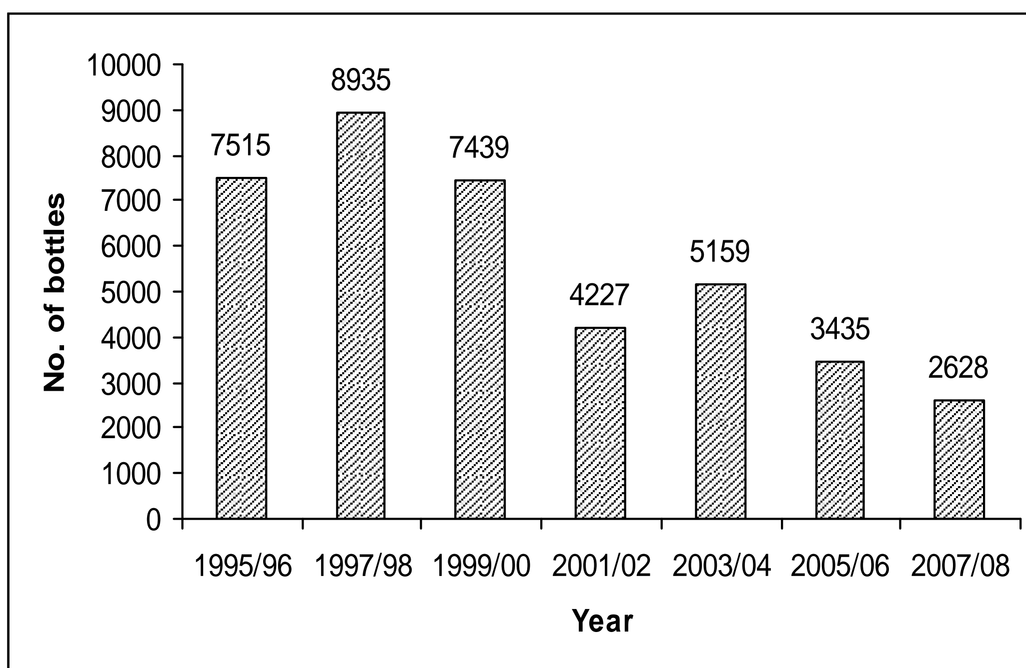


Figure No. 1: Distribution of mushroom seed (spawn) through Plant Pathology Division, Khumaltar

CHAPTER THREE
MATERIALS AND METHODS

3.1 Experimental site

The experiment was conducted in the laboratory of Central Department of Botany, Tribhuwan University in Plant Pathology Unit, Kirtipur, Kathmandu. The agricultural residue such as pea wastes and maize stalks were collected form Kirtipur and banana leaves were collected from tropical belt (Siraha district) of eastern part of Nepal.

3.2 Mushroom spawn

The pure strain (seed spawn) of *Lentinus sajo-caju* was obtained from National Agriculture Research Council (NARC), Nepal for experiment.

3.3 Treatments

The different agricultural wastes, namely pea waste, maize stalk and banana leaves were taken as substrates. Rice bran and chicken manure were used as supplements for the cultivation of mushroom. Each of six treatments (with supplements) was replicated five times and remaining three treatments (control) was replicate thrice times. The treatments were as below:

Treatment No.	Composition (dry weight basis)
T1	100 % Pea waste (Control)
T2	90 % Pea waste + 10 % Rice bran
T3	90 % Pea waste + 10 % Chicken manure
T4	100 % Maize stalk (Control)
T5	90 % Maize stalk + 10 % Rice bran
T6	90 % Maize stalk + 10% Chicken manure
T7	100 % Banana leaves (Control)
T8	90 % Banana leaves +10 % Rice bran
T9	90 % Banana leaves + 10 % Chicken manure

3.4 Disinfection of room

The laboratory room was sprayed with 70 % ethyl alcohol for disinfection before starting the experiment. The room was cleaned and well ventilated.

3.4 Substrate preparation

3.4.1 Chopping and soaking of substrates

The substrates were chopped in 2-3 cm size pieces. They were washed and soaked in water (Photo-2, 10, 18) for 24 hours to moisten them thoroughly and were kept on the steep floor so as to remove the excessive moisture from the substrates to get 60-65 % moisture level. After that, 10 % rice bran and 10 % chicken manure was mixed in each substrate respectively as supplementary activator substances (Photo-27). The moisture percentage was calculated by drying 100g wet substrate in an oven at 70°C until constant weight, with three replicates per sample. The substrates were filled in the polypropylene bag of 25 cm | 15 cm in size at the rate of 400 gm per bag on dry weight basis (1 kg wet weight) and their mouths were plugged by sterilized cotton with the help of rubber band (Photo-26).

3.4.2 Sterilization

The polythene bags with substrates were autoclaved at 121°C at 15-20 lbs pressure for an hour in the laboratory of CDB, Kirtipur. After that, the bags were allowed to cool for some period before spawning.

3.4.3 Spawning (Inoculation)

After cooling the polythene bags, they were inoculated with the spawn *Lentinus sajar caju* at the rate of 2 % per bag according to the dry weight of substrates. The spawn were kept in each packet with well manner, compressed properly with hand, and mouth of the bag tied with a rubber band so as to minimize air space. In this way, packets for all the treatments were prepared. All the activities were carried out aseptically in clean room to avoid contamination of the substrates and spawn by using ethanol. The bags were than inoculated for spawn running under complete darkness at controlled temperature of 25°C.

3.4.4 Incubation

The bags were kept into a disinfected dark room until the mycelium has fully penetrated to the bottom of the substrate. In 3 or 4 weeks, the substrate appears white due to the growth of the mycelium.

3.4.5 Bag removal

Some primordia were formed after completing mycelial growth (Photo-13, 21). Mouth of polypropylene bags were opened and the bags cut with a knife vertically from an upper point downward and removed carefully, when whitish mycelial growth had spread to both, lower and upper sides from the inoculated zone.

3.4.6 Watering

The substrate packets were watered lightly immediately after removal of the plastic bags. Three times watering a day was done on the substrates and later 2 times during first flush, one time during second flush.

3.5 Harvesting and yield

Mushrooms were harvested when they were mature. Harvesting was done by hand holding the stipes at the base and twisting lightly. Fresh yield was recorded by weighing. The number of mushroom production was counted of each packet.

Biological efficiency (BE): It was calculated by using the following formula.

$$\text{BE (\%) X} \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \mid 100$$

Size of mushroom: It was calculated by using the following formula.

$$\text{Size of mushroom X} \frac{\text{Total wt. of fresh mushroom harvested}}{\text{Tolal no. of mushroom harvested}}$$

3.6 Moisture percentage

The moisture content of mushroom was found out by weighing fresh mushroom and kept in incubator at temperature 70° C for 24 hrs (Photoplate-31, 32). It was expressed in percent and calculated by the formula -

$$\text{Moisture content (\%)} \; X \frac{\text{Weight of fresh mushroom - Weight of dry sample}}{\text{Weight of fresh sample}} \; | 100$$

3.7 Data collection

3.7.1 Mycelial growth increment

Mycelia growth was measured in centimeters as the length of the mycelium spreading form the mouth of the bag toward the bottom side at a weekly interval using the ruler until it has fully cover whole bag (Photo-25).

3.7.2 Colonization period

The number of days to mature in each treatment was observer and recorded (Photo-5, 12, 20).

3.7.3 Pin head days

The number of days to produce pin heads after each treatment was observed and recorded (Photo-13).

3.7.4 Number of mushroom harvested

The number of mushroom production in each substrate were collected and counted. After that, weight of fruit bodies was taken with the help of electronic balance in each fruiting bags.

3.7.5 Weight of harvested mushroom

The weight of the mushroom harvested from the each fruiting bags was recorded. After that, dry weight of fruiting body was also recorded.

3.8 Statistical analysis

The data were analyzed by using SPSS computer programme (Version 12.0). Analysis of variance (ANOVA) and the Duncan's multiples range test was used to determine significant differences between the means of yields of mushroom.

CHAPTER FOUR
RESULT

4.1 Mycelia development period

In the present study, pea wastes supplemented with rice bran showed higher mycelial growth (6.04 cm/week) followed by chicken manure supplement and control in first week. (Table 2).There was no significant difference in mycelia growth between pea waste supplemented with chicken manure and rice bran in first week. Pea wastes supplemented with rice bran showed mycelia growth (12.33 cm/week) followed by chicken manure supplement and control in second week and similar result was found in third week. There was significant difference ($p<0.05$) in mycelium growth between pea waste supplemented with chicken manure and rice bran during second and third week. (Table 2)

Maize stalks supplemented with rice bran showed faster rate of mycelium growth (6.46 cm/week) during the spawn run in first week. There was significant differences ($p<0.05$) in mycelium growth between maize stalks supplemented with rice bran and chicken manure in first, second and third week respectively (Table 2)

Banana leaves supplemented with rice bran (4.86 cm /week) showed higher mycelium growth followed by chicken manure supplement (4.22 cm/week) and control (3.84 cm/week) in case of first week. It also showed slower rate of mycelium growth in comparison to all other treatments. There was significant differences ($p<0.05$) in mycelium growth between banana leaves supplemented with rice bran and chicken manure in first, second week and third week respectively (Table 2).

The colonization period, primordial formation and first harvest day were significantly different ($p<0.05$) in each substrates with supplements (Table 3). The fastest colonization period (22.80 days), primordial formation period (29.00 days) and first harvest period (32.80 days) was found in maize stalk supplemented with rice bran whereas the slowest colonization period (35.00 days), primordial formation period (41.80 days) and first harvest period (44.40 days) was found in banana leaves alone (control), among all the treatments (Table 3).

Table No. 2: Comparison of weekly mycelial growth of *Lentinus sajar caju* on different substrates (Mean ± SD, n = number of replicates)

Substrates	Supplements	First week (cm)	Second week (cm)	Third week (cm)
Pea wastes	Control	4.70 ± 0.40 [b] (n = 3)	10.50 ± 0.79 [b] (n = 3)	16.50 ± 0.26 [b] (n = 3)
	Rice bran	6.04 ± 0.28 [a] (n = 5)	12.22 ± 0.61 [a] (n = 5)	17.44 ± 0.43 [a] (n = 5)
	Chicken manure	5.54 ± 0.32 [a] (n = 5)	11.14 ± 0.62 [b] (n = 5)	16.74 ± 0.46 [b] (n = 5)
Maize stalks	Control	5.73 ± 0.40 [b] (n = 3)	11.60 ± 0.89 [b] (n = 3)	17.27 ± 0.21 [b] (n = 3)
	Rice bran	6.46 ± 0.32 [a] (n = 5)	12.68 ± 0.50 [a] (n = 5)	18.24 ± 0.64 [a] (n = 5)
	Chicken manure	5.66 ± 0.39 [b] (n = 3)	10.92 ± 0.69 [b] (n = 3)	17.32 ± 0.37 [b] (n = 3)
Banana leaves	Control	3.84 ± 0.35 [b] (n = 5)	7.72 ± 0.69 [b] (n = 5)	11.28 ± 1.07 [b] (n = 5)
	Rice bran	4.86 ± 0.30 [a] (n = 5)	9.42 ± 0.49 [a] (n = 5)	13.86 ± 0.90 [a] (n = 5)
	Chicken manure	4.22 ± 0.38 [b] (n = 3)	8.42 ± 0.70 [b] (n = 3)	12.38 ± 1.20 [b] (n = 3)

Different letters along the column indicate significant differences of the mean ($p = 0.05$) according to the Duncan Multiple Range Test.

Table No. 3: Comparison of colonization period, primordial formation and first harvest days of *Lentinus sajar-caju* on different substrates (Mean ± SD, n = number of replicates)

Substrates	Supplements	Colonization period (Days)	Primordial formation (Days)	First harvest (Days)
Pea wastes	Control	29.33 ± 1.15 [a] (n = 3)	36.00 ± 1.00 [a] (n = 3)	38.67 ± 0.58 [a] (n = 3)
	Rice bran	23.20 ± 1.30 [c] (n = 5)	30.60 ± 1.14 [b] (n = 5)	34.20 ± 0.84 [b] (n = 5)
	Chicken manure	27.40 ± 1.14 [b] (n = 5)	35.20 ± 1.30 [a] (n = 5)	38.80 ± 1.48 [a] (n = 5)
Maize stalks	Control	24.33 ± 0.58 [a] (n = 3)	31.33 ± 0.58 [a] (n = 3)	35.00 ± 1.00 [a] (n = 3)
	Rice bran	22.80 ± 1.10 [b] (n = 5)	29.00 ± 1.58 [b] (n = 5)	32.80 ± 1.79 [b] (n = 5)
	Chicken manure	25.20 ± 0.84 [a] (n = 3)	31.80 ± 0.84 [a] (n = 3)	35.20 ± 0.84 [b] (n = 3)
Banana leaves	Control	35.00 ± 1.00 [a] (n = 5)	41.80 ± 3.49 [b] (n = 5)	44.40 ± 3.29 [a] (n = 5)
	Rice bran	28.20 ± 0.84 [c] (n = 5)	36.00 ± 1.22 [a] (n = 5)	39.60 ± 1.14 [b] (n = 5)
	Chicken manure	33.20 ± 1.30 [b] (n = 3)	40.40 ± 2.07 [b] (n = 3)	43.20 ± 2.17 [a] (n = 3)

Different letters along the column indicate significant differences of the mean ($p = 0.05$) according to the Duncan Multiple Range Test.

4.2 Number of fruiting bodies and size of mushroom

The maximum number of fruiting bodies of *Lentinus sajor-caju* was observed in all substrates supplemented with chicken manure (Table 4).But the substrate, maize stalk supplemented by chicken manure showed highest number (61.80) of fruiting bodies followed by rice bran supplement (48.00) and control (44.67).

The total number of fruiting bodies in pea waste supplemented with chicken manure showed (52.60) followed by rice bran supplement (44.20) and control (40.67). Similarly, banana leaves supplemented with chicken manure was (26.60) number of fruiting bodies followed by rice bran supplement (24.40) and control (19.40). There was significantly difference ($p<0.05$) in total number of fruiting bodies between maize stalk supplemented with rice bran and chicken manure respectively. Similar result was found in pea wastes also whereas there was no significantly different in the number of fruit bodies between banana leaves supplemented with rice bran and chicken manure respectively (Table 4).

The number of fruiting bodies decreases in second flush compare to first flush among all the treatments. Chicken manure played good supplement to increase the number of fruiting bodies in case of all the substrates.

The highest size of *L. sajor-caju* was found in maize stalk with rice bran supplement (7.28) whereas lowest size of *L. sajor-caju* was found in pea waste with chicken manure supplementation (4.40) among all the treatments (Table 5).

The size of *L. sajor-caju* was directly affected by the number of fruiting bodies. As the number of fruiting bodies was increased, the size of *L. sajor-caju* in pea waste supplemented with chicken manure decreased. Similar results were found in banana leaves and maize stalk when supplemented with chicken manure (Table 5).

Table No. 4: Number of fruiting bodies in first and second flush of *Lentinus sajar-caju* on different substrates

Substrates	Supplements	No. of fruiting bodies in First Flush	No. of fruiting bodies in Second Flush	Total no. of Fruiting bodies
Pea wastes	Control	26.00 ± 2.00 [b] (n = 3)	14.67 ± 1.15 [b] (n = 3)	40.67 ± 3.06 [b] (n = 3)
	Rice bran	27.60 ± 1.82 [b] (n = 5)	16.60 ± 1.14 [b] (n = 5)	44.20 ± 2.59 [b] (n = 5)
	Chicken manure	32.40 ± 3.85 [a] (n = 5)	20.20 ± 3.19 [a] (n = 5)	52.60 ± 6.62 [a] (n = 5)
Maize stalks	Control	28.00 ± 1.00 [b] (n = 3)	16.67 ± 0.58 [b] (n = 3)	44.67 ± 1.15 [b] (n = 3)
	Rice bran	30.00 ± 3.39 [b] (n = 5)	18.00 ± 1.73 [b] (n = 5)	48.00 ± 4.53 [b] (n = 5)
	Chicken manure	37.00 ± 1.58 [a] (n = 5)	24.80 ± 2.28 [a] (n = 5)	61.80 ± 3.03 [a] (n = 5)
Banana leaves	Control	13.20 ± 2.28 [b] (n = 5)	6.20 ± 1.30 [c] (n = 5)	19.40 ± 3.51 [b] (n = 5)
	Rice bran	16.40 ± 1.14 [a] (n = 5)	8.00 ± 0.71 [b] (n = 5)	24.40 ± 1.52 [a] (n = 5)
	Chicken manure	16.60 ± 1.14 [a] (n = 5)	10.00 ± 1.58 [a] (n = 5)	26.60 ± 2.30 [a] (n = 5)

Different letters along the column indicate significant differences of the mean ($p = 0.05$) according to the Duncan Multiple Range Test.

Table No. 5: Size of *Lentinus sajar-caju* on different substrates (Mean ± SD)

Substrates / supplements	Size of *Lentinus sajar-caju*		
	Control	**Rice bran**	**Chicken manure**
Pea wastes	5.51 ± 0.28	6.78 ± 0.24	4.52 ± 0.71
Maize stalks	6.29 ± 0.76	7.28 ± 0.37	4.40 ± 0.29
Banana leaves	5.01 ± 1.07	6.32 ± 0.56	4.93 ± 0.35

4.3 Yield of fruiting bodies

In the first flush, the maximum yield of *Lentinus sajor-caju* was produced in maize stalk with rice bran supplementation (213.17 g) followed by control (158.96 *g)* and chicken manure supplement (156.50 g).

Yield of *L. sajor-caju* in pea waste with rice bran supplement was (185.23 g) followed by chicken manure supplement (128.51 g) and control (137.51 g). Similarly, the yield of mushroom on banana leaves supplemented with rice bran was (102.36 g) followed by chicken manure supplement (84.50 g) and control (70.92 g).There was significant difference (p>0.05) in yields of *L. sajor-caju* obtained from all three substrates when supplemented with rice bran and chicken manure respectively (Table 6).

In the second flush, the yield of *L. sajor-caju* in pea waste supplemented with rice bran was (114.30 g) followed by chicken manure supplement (105.79 g) and control (86.72 g) .Similarly, the yield of *L. sajor-caju* was obtained from the maize stalk with rice bran supplement was (134.96 g) followed by control (121.70 g) and chicken manure supplement (115.08 g). Similarly, the yield of *L. sajor-caju* was obtained from the banana leaves with rice bran supplement was (51.10 g) followed by chicken manure supplement (23.98 g) and control (45.78 g). There were no significant difference between the yields obtained from all three substrates when supplemented with rice bran and chicken manure respectively in second flush (Table 6).

The yield of cumulative flushes (total yield) resulted higher yield in maize stalk with rice bran supplement (348.13 g) followed by pea waste with rice bran supplement (299.53g) and maize stalk in control (280.66g) among all the treatments (Table 6).

Maize stalk supplemented with rice bran showed highest B.E. (87.08 %) whereas banana leaves in control showed lowest B.E. (23.72 %) among all the treatments (Table 7).

Table No. 6: First and second flush of *Lentinus sajor caju* on different substrates

Substrates	Supplements	First Yield (g)	Second Yields (g)	Total Yields (g)
Pea wastes	Control	137.51 ± 12.61 [b] (n = 3)	86.72 ± 13.07 [b] (n = 3)	224.23 ± 23.36 [b] (n = 3)
	Rice bran	185.23 ± 11.49 [a] (n = 5)	114.30 ± 10.28 [a] (n = 5)	299.53 ± 13.12 [a] (n = 5)
	Chicken manure	128.51 ± 10.59 [b] (n = 5)	105.79 ± 7.22 [a] (n = 5)	234.30 ± 16.49 [b] (n = 5)
Maize stalks	Control	158.96 ± 18.56 [b] (n = 3)	121.70 ± 14.84 [b] (n = 3)	280.66 ± 33.06 [b] (n = 3)
	Rice bran	213.17 ± 12.68 [a] (n = 5)	134.96 ± 5.39 [a] (n = 5)	348.13 ± 15.73 [a] (n = 5)
	Chicken manure	156.50 ± 6.97 [b] (n = 5)	115.08 ± 4.61 [b] (n = 5)	271.57 ± 6.58 [b] (n = 5)
Banana leaves	Control	70.92 ± 13.19 [c] (n = 5)	23.98 ± 5.19 [b] (n = 5)	94.90 ± 13.81 [c] (n = 5)
	Rice bran	102.36 ± 4.74 [a] (n = 5)	51.10 ± 6.06 [a] (n = 5)	153.46 ± 4.35 [a] (n = 5)
	Chicken Manure	84.50 ± 7.93 [b] (n = 5)	46.78 ± 8.64 [a] (n = 5)	131.28 ± 16.00 [b] (n = 5)

Different letters along the column indicate significant differences of the mean ($p = 0.05$) according to the Duncan Multiple Range Test.

Table No. 7: Biological efficiency of *Lentinus sajar-caju* on different substrates (Mean ± SD)

Substrates / supplements	Biological efficiency of *Lentinus sajar-caju*		
	Control	**Rice bran**	**Chicken manure**
Pea wastes	56.06 ± 5.84	74.88 ± 3.27	58.57 ± 4.12
Maize stalks	70.16 ± 8.26	87.03 ± 3.93	67.89 ± 1.64
Banana leaves	23.72 ± 3.45	38.37 ± 1.08	32.82 ± 4.00

All the three substrates were significantly different ($p<0.001$) between the yield obtained from the first and second harvest (Table 8). Banana substrate showed highly significant difference in first harvest (85.92 g) than the second harvest (40.66 g) but pea substrate showed lower significant difference between the first harvest (152.40 g) and second harvest (104.67 g) respectively. However, among all three substrates, highest yield was obtained in first harvest than the second harvest during the cultivation of *Lentinus sajor-caju* (Table 8).

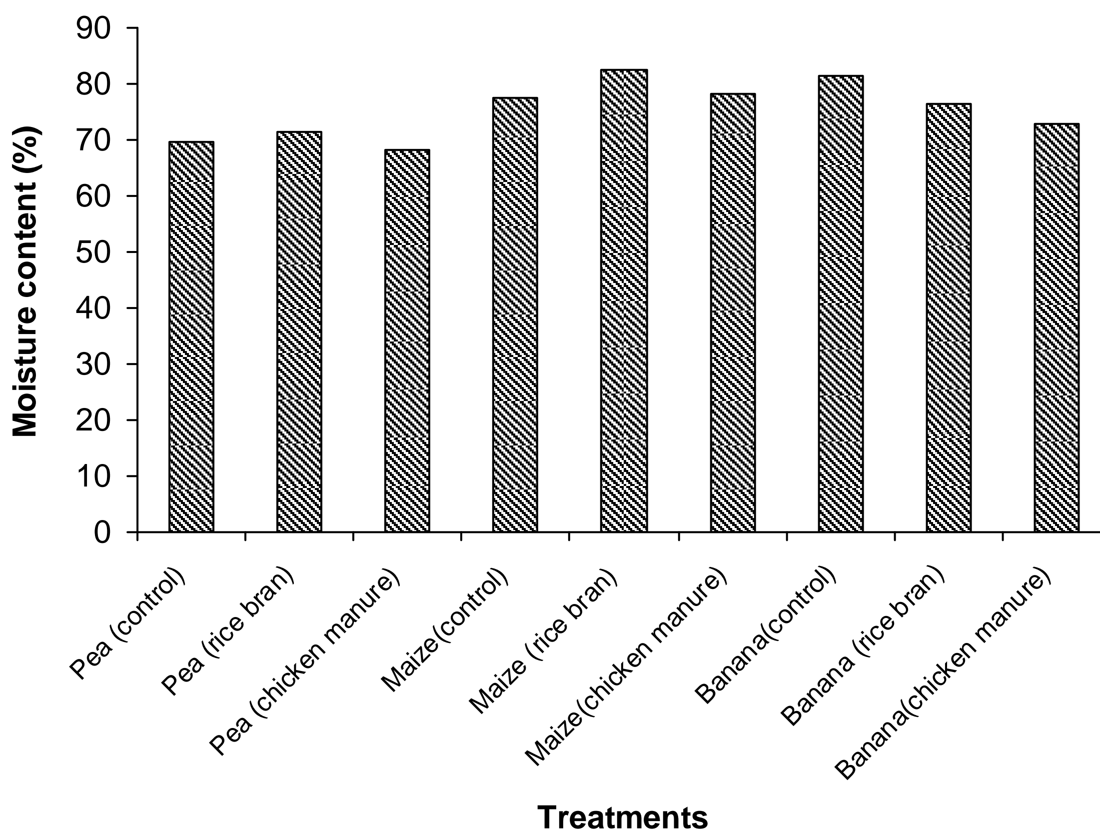
Table No. 8: Comparison between yield of first and second harvest in different substrates (Mean ± SD, n = number of replicates)

Substrates	First Harvest (g)	Second Harvest (g)	t - test
Pea wastes	152.40 ± 29.15 (n = 13)	104.67 ± 14.17 (n = 13)	t = 5.310 (p < 0.001)
Maize stalks	178.86 ± 30.41 (n =1 3)	124.25 ± 11.74 (n = 13)	t = 6.039 (p < 0.001)
Banana leaves	85.92 ± 15.86 (n = 15)	40.66 ± 13.82 (n = 15)	t = 8.338 (p < 0.001)

4.4 Moisture content (%)

The moisture percentage of fruiting bodies was highest in maize stalk supplemented with rice bran, followed by banana leaves in control and maize substrate supplemented with chicken manure respectively among all the treatments. However, the lowest moisture percentage of fruiting bodies was obtained in pea waste in control.

Figure No. 2: Moisture content (%) of fruiting bodies after harvest in different treatments



CHAPTER FIVE
DISCUSSION

5.1 Effect of mycelium growth

In the present study, the maize stalk showed fastest mycelial growth, rapid primordia initiation and fast harvest day, with addition of rice bran as a supplement. This might be due to high quality nutritional value in combination of both substrate as maize stalk and supplement as rice bran. According to Oei (2003), substrate having high quality lignin and cellulose contents takes a longer time to start pinning compared to the substrates with low content nutritional value. This is because the mycelia remains vegetative for a longer period hence the vigorous growth and late pinning and poor nutritional substrates exhibited low mycelia densities making them prone to contamination especially by the green mold. Thus, it can be concluded that fast mycelial growth rate is necessary for fast mycelia covering of substrate.

In return, the highly colonized substrates had high mycelia densities. The result from this experiment showed that yield of the mushroom was directly related to the spread of the mycelium into the substrate. Adding growth limiting mineral and nutrient can increased the mycelial growth rate and the degradation of Polysaccharide compounds is associated with the fruiting stage (Bano *et al*., 1993).

The paper residues supplemented with rice bran was described by Baysal *et al* (2003). These authors obtained an increase in the biological efficiency with the increasing concentration (10 and 20 %) of rice bran during the production of *Pleurotus ostreatus*. This fact can be related to our research where the addition of supplement with different substrates resulted in an increase in productivity and biological efficiency, except for the maize stalk which had a decrease on these variables as the addition of chicken manure as a supplement.

The major components of lignocellulosic wastes used for mushroom cultivation are cellulose, hemicellulose and lignin. Growth and fruiting bodies are dependent on the ability of the particular mushroom to attack these components as nutrient sources. Thus, different substrates showed the different results, however supplements share a little sufficient in mycelial growth and yield of mushroom.

5.2 Fruiting bodies formation

In the present result, the fruiting bodies appeared 4-6 weeks after primordial formation and took 32-44 days later after inoculations of spawn. These findings are conformity with Quimio (1999) who reported that formation of fruiting bodies was 3-4 weeks after inoculation of spawn. Similarly, Tan (1981) reported that *P. ostreatus* and other species on cotton waste took 2-3 weeks for fruit body formation after spawn running. This result is consistent with our results.

According to Bayal *et al* (2003) , the fastest mycelial growth(15.8 days), pin head formation (21.4 days) and fruit body formation (25.6 days) were analyzed with the substrate (waste paper) composted of 20 % rice husk in weight.

In our experiments, the number of fruiting bodies was higher in case of all substrates supplemented with chicken manure but the size of mushroom decreases .This may be probably due to consumption of high nutrients, increase in number of primordial and lack of space for growth.

5.3 Yield of mushroom

Maize stalk were best and cheap alternative substrate for the cultivation of oyster mushroom (*Lentinus sajor-caju*) with the supplements rice bran followed by pea wastes and banana leaves among all the treatments. This may be due to higher degradation of various constituents of maize substrate by *L. sajor caju*.

In the present study, the yield of *L. sajor caju* was found 280.66 g/kg substrate in maize substrate in control where as it was reported 348.13 g/kg substrate with the addition of rice bran as a supplement in the same substrate. It might be due to fast rate of degradation of cellulosic and lignin by the addition of rice bran in the maize substrate. Mane *et al* (2007) analyzed that organic supplements such as groundnut oilseed cake, gram powder and rice bran not only affected growth parameters but also increased yields of *L. sajor caju.* He found that the best yield in addition of rice bran supplement with cotton stalks + pigeon pea stalks and leaves + wheat straw yielded 756.88 g/kg substrate, whereas without supplement it gave 703.22 g/kg. Similar observations have also been made by several other researchers (Bano *et al*., 1993;

Plant Pathology Division, 2000). Thus, supplements change the decomposition rate and also the sequences of decomposition of substrates components.

In this study, the banana leaves showed very low in yield of *L. sajor-caju* among all the treatments. This may be due to fewer breaks down of cellulosic and lignin substrate. In our result, the biological efficiency of *L. sajor-caju* on banana leaves supplemented with rice bran was highest (38.37 %) followed by chicken manure supplement (32.82 %) and control (23.72 %). Similarly, Obodai (2003) reported that biological efficiency of *Pleurotus ostreatus* in banana leaves was 37.15%. Therefore, the yield of mushroom depends on genetic properties of fungal spp (subspecies, strain), substrate quality and culture conditions. Substrate quality includes moisture contents, lime concentration, resultant pH value and lignocellulosic activity of the substrate and mycelium.

Lentinus sajor-caju and *Pleurotus ostreatus* were grown in banana tree straw, supplemented with rice bran (5%) by Bonatti *et al* (2004).He resulted that *L. sajor-caju* presented higher biological efficiency (7.51%) than *P. ostreatus* (6.34%).This result is not consistent with our result.

5.4 Moisture content (%) in fruiting bodies

In the present study, moisture percentage of fruiting bodies of rice bran supplemented with rice bran was found 82.48 %. Bonatti (2004) resulted that the moisture of *Pleurotus ostreatus* was higher when cultivated in rice straw (88.08 %) than in banana straw (83.17 %).Adikari (2000) analyzed that moisture content in fruiting bodies of wild edible mushroom, *Pleurotus cornucopie* was found 89.35 %.

Patrabansh & Madan reported that moisture content in the fruiting bodies of *Lentinus sajor-caju* on different substrate such as *Populus deltoides*, *P. deltoides* + paddy straw(1:1), *P. deltoides* + paddy straw (1:2), *Eupatorium adenophorum*, *Eupatorium adenophorum* + paddy straw (1:1) , *Eupatorium adenophorum* + paddy straw (1:2), sericulture waste, sericulture waste + paddy straw(1:1), sericulture waste + paddy straw(1:2) were 90.75 %, 89.69 %, 91 %, 89.48 %, 90.42 %, 90.99%, 90.20 %, 90.30 %, 90.86 %,90.99 % respectively. This value was high than our studied. This may be due to effect of different substrates with different composition.

CHAPTER SIX
CONCLUSION & RECOMMENDATIONS

6.1 Conclusion

- According to the results obtained in the present research it can be concluded that the maize stalk supplemented with rice bran showed the superior mycelial growth among the all treatments as well as control. Therefore, rice bran can be used as best appropriate supplement for cultivation of oyster mushroom.

- The supplementation of pea waste with chicken manure in the concentration of 10 % favour higher number of fruiting bodies of *Lentinus sajor-caju.*

- Maize stalk and pea waste supplemented with rice bran could be alternative substrates for cultivation of *L. sajar caju.*

6.2 Recommendations

- The technical information should be share to farmers for growing and spawn production of best quality, and in order to control the price of mushrooms in the market place.

- Further study should be done with different concentration and mixtures of banana leaves, maize stalk and pea wastes.

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PLATE – I
Experiment on banana leaves

Photo1: Chopped pieces

Photo 2: Soaked in water

Photo 3: Spawn running

Photo 4: Spawn running

Photo 5: Full colonization

Photo 6: Primordia formation

Photo 7: Fruiting bodies

Photo 8: Fruiting bodies

PLATE – II
Experiment on maize stalks

Photo 9: Maize stalks

Photo 10: Soaked in water

Photo 11: Spawn running

Photo 12: Full colonization

Photo 13: Primordia formation

Photo 14: Fruiting bodies

Photo 15: Fruiting bodies

Photo 16: Fruiting bodies

PLATE – III
Experiment on pea wastes

Photo17: Chopped leaves

Photo18: Soaked in water

Photo 19: Spawn running

Photo 20: Full colonization

Photo 21: Primordia formation

Photo 22: Fruiting bodies

Photo 23: Fruiting bodies

Photo 24: Fruiting bodies

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Head of

PLATE – IV

Photo 25: Measurement of mycelia growth

Photo 26: Spawn running in control

Photo 27: Substrate with supplement

Photo 28: Fruiting bodies

Photo 29: Fruiting bodies

Photo 30: Fruiting bodies

Photo 31: Fresh mushroom

Photo 32: Dried mushroom