CHAPTER ONE

INTRODUCTION

The word Mushroom is derived from the French word “Mousseson” (Muceron) from mousse. Moss means fast growing (Ramsbottom, 1954). Mushrooms are generally termed as the edible or poisonous gill bearing fleshy agarics. Generally agarics or fleshy species of other group of fungi bearing cap and gills on the underside producing spores are recognized as mushroom (Philips, 1981). The term "Mushroom" applies only to the “Agarics” which are commercially cultivated. The general form of an agric fruiting body is umbrella shaped with central stipe supporting a cap or pilus with numerous radially arranged gill or lamellae on the lower side of a cap. (Webster, 1970)

Mushroom is defined as “macro fungus with distinctive fruiting body which can be either epigeous or hypogenous”. The macro fungi in the sense that they have large fruiting body can be easily seen with naked eyes and can easily be picked up by hands. (Chang and Miles,1993). In a narrow sense, the word mushroom also refers only to fruit body.

Mushroom are saprophytic growing on dead organic matters of vegetative origin, they can utilize almost all agriculture wastes as substrates (Adejoye et.al. 2006). Mushroom are fungi which can produce the complex substances on which they grow for the absorption of their nutrition.

Mushrooms are heterotrophic because of lack of chlorophyll so that they can’t prepare their food themselves and depend upon others for their nutrition. Mushrooms are higher fungi belonging to the class Basidiomycota and Ascomycota.

They reproduce by spores which under proper condition germinate in to hyphen. Their hyphen (collectively mycelia) absorb nutrient from substrate, substrate then colonies, gives pin head and grow to fruit body under proper growing condition. Fruit bodies produce spore under the gills for next generation.

Mushrooms and toadstools are terms applied rather loosely to the fruit bodies of fleshy gill fungi and are commonly used to denote edible and poisonous species. Respectively, they

form a small part of the enormous range of organism called fungi. Their essential characteristic is the lack of green pigment and this puts the fungi in a separate kingdom from other plants (Oderinde et.al, 1985)

Even though mushrooms were used by ancient Greeks and Roman people for their food (Knowing or unknowing their nature and importance) grown naturally but their cultivation process was not known exactly at that time. The first intentional cultivation of mushroom was practiced in china during the sixth century followed by the cultivation of *Auricularia auricula* on wood logs (Chang and Miles, 1993) but the first systematic cultivation of mushroom was made in France during 1650. A French Gardner Jean De La Quin Tinye in (1700 A.D) first introduced the method of cultivation of temperate mushroom (*Agarics bisporus*). This method was spread from France to England and then to America. In his method, mushroom spawn grows in manure, however; the culture of mushroom using bottle spawn and in sterilized manure was investigated many years later in 1894. During the progressive study of its cultivation till now, one of the important milestone in mushroom cultivation is the method of cultivation of mushroom in green house which was initially practiced in Sweden around 1754 (Singh, 2007).

Miller defined mushrooms as the tern applied to both edible and poisonous species of agarics as the gilled mushroom. Similarly Purukayastha and Chandra (1985) pointed out that agarics or fleshy species of other group of fungi are recognized as " Mushrooms'' which may be edible, inedible, poisonous or non- poisonous. According to dictionary of fungi, mushroom was defined as any agarics like or agarics as from Agaricaceae having edible value.

Rinaldi and Tyndalo (1972) defined mushrooms as the structure that are commonly known mushroom which are nothing else but the fruiting bodies of those organism that the mycologists call higher fungi or macromycetes (large fungi) even though the dimensions of the caps of some mushroom might be only few millimeters across.

Cultivation of edible mushrooms with agricultural residues, such as rice and wheat straw is a value added process to convert these materials, which are otherwise considered to be

wastes, into human food. It represents one of most efficient biological ways by which these residues can be recycled (Madan et al, 1987).

The cultivation of Pleurotus is a profitable agro-business. *It* is considered as an agricultural enterprise that challenges the combined skills of both industrial and agricultural technology.

The division of plant pathology (National Agriculture and Research Centre) was first to introduce mushroom cultivation in Nepal. At that time, Nepalese scientist developed the growing technology of white buttom mushroom and extended to farmers in 1977. It utilized the synthetic media of paddy straw which was harvested twice a year in Kathmandu. Few farmers grew mushroom before the introduction of that technology but the number of buttom mushroom cultivation has been increased years after that (Manandhar, 2004).

Commercially *agarics* and *Pleurotus ostreatus* are very much cultivated in Nepal. Among others, the few cultivated species in Nepal are *Pleurotus sajar- caju* and shitake (*Lentinus edodes*). Species of *Auricularia* has also been recommended for cultivation in Nepal.

Ganoderma lucidum, a medicinally very important mushroom, is being experimented in the division of plant pathology although it has been a cultivated experiment by some growers in Nepal. No large farming of this species has been done so far except one or more private farm. (Singh, 2007)

Though Nepal has very good agro climatic growing condition for mushroom cultivation, the number of farmers involved commercially in mushroom cultivation is not satisfactory. Mushroom cultivation in Nepal has spread up to 25 districts up to now and is spreading rapidly in many parts of Nepal. Because of the popularity of mushroom, the mushroom production in Nepal has reached 700 metric tons in a year (Singh, 2007).

Practically, in Nepal, there are different names given to mushrooms in different language such as chyaun in Nepali, Bammhukan in Newari, shymo or shyamu in tamang, shamu in Sherpa, chyabo in Gurung, Mugan in magar, pat in Limbu, Chhani in Tharu and Kakurmutta in Hindi (Adhikari, 2000).

1.1 Oyster mushroom

Pleurotus is the scientific name for the oyster mushroom. The word *Pleurotus* has its origin from Latin *Pleurotus* (sideways) which refers to the sideways growth at the stem with respect to the cap. It is commonly known as "Kanne chayu'' in Nepal.

In nature, oyster mushrooms appear in cluster on dead trees from late fall to spring and are distributed all over the world (Lee Jiyul, 1993) especially in subtropical zone and temperate zone and subtropical forest and temperate forest. *Pleurotus* species can be grown on various agricultural waste material within temperature rays of 20°C to 30°C. *Pleurotus sajar- caju* can tolerate temperature up to 30°C although it fruits faster and produces larger mushrooms at 25°C. *Pleurotus ostreatus* is the so- called low temperature *Pleurotus* fruiting mostly at 12°C-20°C (Pathak et.al, 2000).

Pleurotus species are saprotrophs that act as a primary decomposes of wood especially deciduous tree and beech trees in particular. Visually, the basidiocarp or fruit bodies of an Oyster Mushroom have three distinct parts. (1) A fleshy shell or spatula shaped cap (pileups) (2) a short or long lateral or central stalk called stipe (3) long ridge and furrows underneath the pileus called gills or lamellae. The gills stretch from the edge of the cap down to stalk and bear the spores. The spores are smooth, cylindrical. The spores are heterothallic and germinate easily on any kind of mycological media and within 48- 96 hr whitish thread like colonies develop.

The mycelium of *Pleurotus species* is pure white in color. Basidiospore on germination form primary mycelium, fusion between two compatible primary mycelium develops in to secondary mycelium which has clamp connection and it is fertile. The fruit bodies of the mushroom are distinctly shell fan or spatula shaped with different shades or white, cream, grey, yellow, pink or light brown depending upon the species. The color of the sporophores is extremely variable character influenced by the temperature light intensity and nutrients of the substrate (Suman and Sharma, 2005).

Up to now around 70 species of *Pleurotus* have been recognized and out of these there are about 25 species cultivated commercially all around the world which are given as *P.*

ostreatus, P. Florida , P. flabellatus. P. sajar-caju. P. sapidus. P. cystidious . P. eryngii. P. Fossulatus, P. opuntiae , P. cornucopiae, P. yuccae, P. platypus , P. djamore , P. Australis,, P. populinus, P. levis, P. columbinus (Suman and Sharma, 2005).

1.2 *Pleurotus ostreatus*

Pleurotus ostreatus is the second most cultivated edible mushroom worldwide after *Agarics bisporus*. Due to its economic and ecological value and medicinal properties,*Pleurotus ostreatus* is active lignin degrade in the forest which can degrade a large variety of lignocelluloses substrate and other waste which are produced primarily through the activities of agricultural, forest and food processing industries in a short time in comparison to other edible mushroom.

It was first cultivated in Germany as a subsistence measure during the Great War and is now grown commercially around the world for food. Latin planarity (sideways) refers to the sideways growth of the stem with respect to the cap while Latin *Ostreatus* refer to the shape or the cap which resembles the bivalve of the same name.

The cap measurement may range from 5-15 cm diameter while the stipe is 0.5 cm long. The pileus is white to grey or dull brown in color, surface smooth, margin irregular and curved (Purukayastha and Chandra, 1976).

1.3 Nutritional Value of Mushroom;

Nowadays, Mushroom has become a part of every continental dish because of its nutritional value. It contains large number of nutrients, proteins, carbohydrates, fat, vitamin, mineral, fiber etc. that is why mushroom may be considered as pool of nutrient. It is also known as vegetable meat. Fats occur in mushroom in minor amount especially compared with protein and carbohydrate and the fatty fraction consists predominantly of unsaturated fatty acid such as linoleic acid, which are the perfect food for maintaining a healthy heart and cardiovascular system.

Mushrooms are rich in protein. The protein content in a fresh mushroom is about 3% (Tiwari, 2007). The protein content is intermediate between that of animal's protein and

vegetable protein (Kurtzman, 1979) almost equal to that of core milk more than either potato or cabbage. The protein content in mushroom is less than the protein content of meat, fish, eggs and cheese but they are twice as such as vegetable and with exception of peas and other legumes and 4 times and 12 times than those of orange and apples respectively. They have high percentage of all essential amino acid. They are also low in cholesterol, carbohydrate. It is considered as ideal food for diabetic patient due to absence of starch. It is also useful for perplex willing to decrease the body weight.

They are good source of vitamin such as Thiamin (B_1), Riboflavin (B_2), Nicotinic acid and Pathogenic acid. They also contain Vitamin C (Ascorbic acid) and vitamin K. Vitamin A.D appears to be present only in very small amounts. They are also good source of minerals. They are exceptionally such in calcium and phosphorus than many fresh fruit and vegetables (Suman and Sharma, 2005).

Due to presence of large number of nutrition on a mushroom, it may be useful food to solve malnutrition problem (Manandhar, 2004). Mushroom too have excellent flavors table and no doubt chunk of nutrient so the Greeks & Proman described them as “foods for the god”.

The consumption of edible and medicinal mushrooms by humans is an age-old practice. Higher fungi are abundant sources of a wide range of useful natural products and new products with interesting biological activities. The discovery of bio-active compounds, including anti-tumor substances, has stirred a growing interest in such mushrooms from industry, the media and the scientific community (Pokhrel et. al, 2006).

1.4 Medicinal value of mushroom:-

Mushroom has been valued through the world as both food and medicine for thousands of years. They may be used as antiviral, anticancer, anti hypertensive, anti cholesterol, antitumourous etc. They are useful against diabetes, ulcer and lung diseases (Quimio, 1978).

They have very low calories and contain 80-90% of water and at the same time they have low sodium carbohydrate and fat content and high fiber content so they are useful for us aiming for weight loss.

The presence of fiber makes them important nutrient for brain. Mushrooms are excellent source of potassium which helps lower blood pressure and decrease the risk of heart attack so mushrooms are recommended to people suffering from hypertension. Mushroom also helps to fight against cancer. It has also been found that red mushroom improves blood circulation and cell quality, it allows internal organ to work properly strong than weak constitute and increase the immune system. They also help to maintain healthy metabolism. Mushrooms exactly help to stop migraine, headache and is also beneficial for people suffering from mental illness like obsessive and compulsive disorder (Geuders, 1974).

1.5 Significance of mushroom cultivation

Mushroom cultivation not only reduces the environment impact of the water used as substrates but also provides an economically acceptable alternative for the production of food of superior taste and quality as well as high valued added metabolites such as enzymes (Philippoussis and Zervakis, 2001).

Mushroom mycelia (vegetative growth) are important in ecosystem because they are able to biodegrade the substratum and therefore the wastes of agricultural production. *Pleurotus* is primarily found in decomposes of wood and vegetable residues (Zadrazil and Kurtzman,1981).

Mushroom cultivation not only helps to balance protein shortage especially in developing countries like Nepal but also helps to decrease pollution by degrading or recycling the organic waste material, solid waste which sense to be matters of headache to government especially in context of Nepal.

Mushroom cultivation like other cultivation doesn't need large area so this method is useful to farmers having even small land or even in their small place of home (baranda) which will help then to uplift their lifestyle.

The substrate used after harvesting may be used as fertilizers and soil conditions for growth of plants. Additionally fermented residues could be used as animal feed after mushroom cultivation.

The cost for mushroom cultivation is less than other cultivation or cash crops – also the period of cultivation is less. It requires little space, inexpensive and easily available raw material and has quick return, so the mushroom cultivation may play significant role for the development of farmers.

1.6 OBJECTIVE, JUSTIFICATION AND LIMITATION

1.6.1 Objectives of the study

1.6.2 General objective

The main objective of the study was to examine alternative substrate for the cultivation of *Pleurotus ostreatus*.

1.6.3 Specific Objective

The specific objectives are as follows:-

(1) to identify the mycelial growth, yield and biological efficiency of *Pleurotus ostreatus;*

(2) to cultivate the *Pleurotus ostreatus* in different alternative substrates;

(3) to conserve environment by recycling agricultural and industrial wastes.

1.7 Justification of the study.

Due to the scarcity and lack of rice straw because of its frequent use, this study focuses on alternative ways to grow mushroom on other substrates. Due to the property of mushroom to grow on waste material and convert or biodegrade them into highly nutritional food, it is considered as a natural recycler which helps in reducing environment pollution to a great extent.

The raw material used for mushroom cultivation are available very cheaply and in every farmers doors which are supposed to be suitable to cultivate in Nepalese environment being fast maturing crops. It can be a good source of income to farmers if they are made conscious about its cultivation process and its importance or significance.

The people living in the rural and urban areas have deficiency of protein causing malnutrition. The mushroom can fulfill this requirement by providing adequate supply of calories. Mushrooms are also useful for people suffering from different diseases.

1.8 Limitation of the study:-

The limitation of the research work are listed as follows,

(i) Only three substrate viz.; corn cob, paper wastes and grass wastes were used in this experiment.

(ii) In this experiment only cultivation of mushroom was carried out but nutritive and toxicity test were not done.

(iii) Only one species of mushroom was selected in this experiment.

CHAPTER TWO

LITERATURE REVIEW

Oyster mushroom can be grown on various agricultural residue such as corn cob and leaves, cotton waste, sugarcane baggage and leaves, grasses, rice husk and water hyacinth leaves as good substrates (Singh, 2007).

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

Khan et.al. (1989) studied the yield performance of four strains of oyster viz; *Pleurotus ostreatus* (strain 467) *Pleurotus florida* strain (3526*) Pleurotus sajar-caju* and *Pleurotus ostreatus* on paddy straw during winter. The temperature of growing room varied between 16^0C and 24^0C during the course of the experiment. The results showed that *P. ostreatus* (strain 467) was the most productive strain followed by *P. florida* (strain NRRL 3526) *P. sajar*-caju and *P. ostreatus*.

Cho et. al (1981) reported that a mixture of cotton seed husk and sawdust was a great substrate for the growth of *Pleurotus* sajar-*caju* in Australia. Supplementation of the substrate with wheat bran resulted in a significant increase in the yield of mushroom. The result indicated that light was inhibitory to mycelial growth. However, exposure to light after formation of pin heads was important to avoid abnormalities in fruiting bodies.

In the scope of study, waste paper has been used as main substrate where as waste of husk rice is co-substrate, results indicated that waste of rice increased important cultivation parameters of *Pleurotus ostreatus* mushroom's such as mycelia development and mushroom yield. Mixture based on waste paper and husk rice gave more yield than only waste paper. The best mycelia development and yield was obtained mixtures of waste paper with husk rice in the ratio 75 and 26.

Das & Mukherjee (2007) studied *Pleurotus ostreatus* using dry weed plants like *Leonotis species Sida acuta, Parthenium argentatum, Ageratum conyzoides, Cassia sophera, Tephrosia purpurea & Lantana camera, Leonotis species* was found to be the best substrate in fruit body production of *Pleurotus ostreatus* when it was mixed with rice straw (1:1 wet wt/wet wt.) for mushroom initiation. The fruiting time for *Pleurotus ostreatus* was also less on *Leonotis species* than on any other weed substrate tested in this investigation. *T. purpurea* was the last suited weed for oyster mushroom cultivation. The main problem of oyster mushroom cultivation on weed substrates was found to be low yield in the second flush that could be overcome by blending weed plants with rice straw.

According to a study of plant pathology division (1996), 500 grams of moist rice straw (approximately 167gms dry) was packed into heat resistance polypropylene bags of 12" × 6" size for the cultivation of *L. sajar*-caju. A piece of PVC (polyvinyl 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. *sajar*-caju spawn at the rate of 10 gram/ 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 was harvestable four days later. Three flushes were harvested at the interval of 15-20 days. On an average 40% of the dry substrate was converted into fresh mushroom by weight.

Donini et.al. (2009) did the research to evaluate the cultivation of the strains of *P. ostreatus* in elephant grass substrate supplemented with different consisted in the use of elephant grass substrate supplemented with soy, wheat, rice or corn bran in concentration of 0%, 10% or 20% poured in flasks that were inoculated with spawns of BF24, DF33 and HF19 strains or *P.ostreatus* and incubated at room temperature 20°C-28°C after the complete colonization of the substrate. The flasks were transferred to a fructification chamber with temperature between 20°C- 26°C and average damp of 75%- 90%. The BF24 strain was found to be the most productive one in relation to the others and the supplementation & the

elephant grass with wheat bran in concentration or 10 and 20% favors higher productiveness and BE for the BF24, DF33 and Hs 19 strains or P. ostreatus.

Jwanny et.al. (1995) studied the technical feasibility of using agricultural waste (mango wastes) as a substrates for the cultivation of *Pleurotus ostreatus* NRRL-0366. when comparing the B.E. of mushroom production, the highest yield or fruiting bodies was obtained using a mixture or dead waste and rice straw at a rite (1:1) (11.96%) followed by a mixture (3:1) (11.16%). The lowest one was the mixture 2:1(9.19%) funning *P. ostreatus* NRRL- 0366 can also be cultivated on mango waste supplemented with rice straw at a different ratio. The best one was the 1:1 mixture 10.18% where as the lowest was a mixture. 3:1 (6.4%)

Yildiz et. al. (2002) studied that yield values, diameters & numbers of fruit bodies obtained from the cultivation of *P. ostreatus* mushroom were determined and the effects of different substrate combinations on productivity were investigated. Wastes of some lignocellulogic material as leaves as hazelnut (L.H), leaves of Tilia (LT) and leaves of European Aspen (LEA), Wheat straw (WS), sawdust (S), waste paper (WP) were used for producing *P. ostreatus*. The best main material & best substrate combination of mushroom productivity were WS and WS+ WP (50%+50%) respectively. Mixture which involves WP generally produced higher yield values. Mixtures which contained bran (26%) increased the risk or contamination. The lowest yield and smallest fruit body diameter values.

El- Kattan and Salama (1995) carried out the study to evaluate legume waste and gypsum as possible additives to rice straw for cultivation of the oyster mushroom *P. ostreatus* and *P. florida*. Both yield and quality of oyster mushroom have been improved by supplementing rice straw with legume waste. The highest beneficial effect on yield was achieved when rice straw was supplemented with 50% legume waste. The B.E. was 121.3 and 116.2 for *P. ostreatus* and *P. florida* respectively. Lower mushroom yield were obtained from the two species when substrate composed of either lower or higher percentage of legume waste had been used. The highest mushroom yield was obtained with substrate composed of equal amounts of rice straw and legume waste with 5% gypsum. These yield were however, lower

than these obtained without gypsum addition. Clearly, the gypsum addition adversely inhibited the favorable effect observed with legume waste on mushroom yield.

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).

Mishra (2002) suggested that holes in polythene bag should be of 1 inch diameter at a distance of 4 inch, so that there will be six holes on 12 inch × 18 inch sized bags. Similarly, he suggested for the use of fifty gram 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 band and keeping of ready bags in dark at 20°C- 25°C were also suggested.

CHAPTER THREE

MATERIALS AND METHODS

The field study was conducted in August-September in Balambu area and more information is obtained from different areas of farmers. The agricultural wastage such as paper waste, grass waste was collected from Kritipur and corn cob was collected from Kanchanpur district of far western part of Nepal.

3.1 Mushroom spawns (seeds):-

The pure spawn of *Pleurotus ostreatus* was obtained from national agriculture research council (NARC) Khumaltar, Nepal for experiment.

3.2 Preparation of substrate:-

The preparation of substrate from the raw materials was based on dry weight of each component before mixing; corn cob, paper wastage and grass wastage were the substrate used in this research and the supplements with 10% chicken manure and rice bran.

At first corn cob and grass wastage are chopped into small pieces. Each of these substrate were collected from Kirtipur and Kanchanpur and individually mixed with chicken manure, rice bran, in the proportion of 9:1. The substrate without supplement was considered as control. Each of these treatments along with control was replicated five times during the process. The paper waste was collected from the University compound, which were thrown during the student union elections.

Those mixtures were than soaked in tap water for about three hours to provide the moisture. The excess water was drained off by using palm method. The moisture was about 65%. One kg wet weight of prepared substrate was placed into polypropylene bags of dimension 20cm × 15cm then, autoclaved at 121°C and 15 lbs pressure for one hour and was allowed to cool overnight.

3.3 Spawning:-

The spawn were kept on these bags uniformly approximate (10 gm) by placing them in laminar air flow to prevent them from external contaminations. Then these bags were packed in properly with hands so as to exclude any air inside it and the mouth of the bags were closed with cotton plug and tied by rubber band.

3.4 Incubation and Fruiting:-

Those bags were than kept in dark room with same places between them in a room temperature of 20°C – 25°C. In four or five weeks, the substrates appear white due to the growth of the mycelium. After the colonization of mycelium the plastic bags were cross slashed to allow the mushroom to grow out. These bags were watered (sprayed) twice a day to maintain humidity or the moisture, their mycelia than developed pin head which grew to fruit body.

3.5 Harvesting and Yield:-

Mushrooms were harvested when they were matured. Harvesting was done by hands holding the steps at the base and twisting lightly. Fresh yield was recorded by weighting. The number of mushroom production was counted of each packet.

3.6 Data recorded:-

The data were recorded and analyzed in different aspects. The length of the mycelium growth was measured from the mouth of the bag toward bottom side in a weekly basis using ruler until it fully covered the bag. The time taken for the full colonization and appearance of pinhead in different substrates was recorded. The days taken by pinhead to form the mature fruiting bodies in different substrates were also recorded. The data were recorded for the first and second crops. The weight of crops in different substrates and the number of *Pleurotus ostreatus* in first and second harvest in different substrates were also noted. The mushrooms are counted and weighed and fresh yield was determined.

3.7 Size of mushroom

It was calculated by using the following formula:-

Size of mushroom = total weight of fresh mushroom harvested／total number of mushroom harvested

3.8 Biological efficiency:

The mushroom yield for each trial was compared and the biological efficiency was calculated as indicated below (Royce, 1985).

The formula used for the calculation of biological efficiency was,

B.E. (%) = (fresh weight of mushroom／dry weight of substrate) × 100%

CHAPTER FOUR

RESULTS

4.1 MYCELIAL DEVELOPMENT PERIOD

In the present study, corn cob supplemented with rice bran showed faster rate of mycelium growth (4.75 cm/ week) followed by chicken manure supplement and control in the first week (Table 1.1). Corn cob supplemented with rice bran showed mycelial growth (8.62cm/week) followed by chicken manure supplement and control in second week and similar result was found in third and fourth week, respectively.

Paper waste supplemented with rice bran showed higher mycelial growth (4.27 cm/ week) during the spawn run in first week followed by chicken manure supplement (3.64 cm/ week) and control (3.38cm/week) in the first week. Paper waste supplemented with rice bran showed mycelial growth (8.34cm/week) followed by chicken manure supplement (8.05cm/week) and control (8.0 cm/week) in the second week and similar result was found in third and fourth week, respectively.

Grass wastes supplemented with rice bran (4.15cm/week) showed higher mycelial growth followed by chicken manure supplement (4.04 cm/week) and control (3.96 cm/ week) in the case of first week. It showed slower rate of mycelial growth in comparison to all other treatments (Table 1.1). The rice bran showed best supplement for mycelial growth in all substrates.

The colonization period, primordial formation period and first harvest day were significantly different in each substrate with supplements. The fastest colonization period (29.0 days), primordial formation period (34.0 days) and first harvest period (54.0 days) was found in corn cob supplement with rice bran whereas the slowest colonization period (43.25 days), primordial formation period (47.5 days) and first harvest period (66.75 days) was found in grass wastes alone (control) among all the treatments (Table 1.2).

4.1.1 Comparison of weekly mycelial growth of *Pleurotus ostreatus* on different substrates

Substrate	Supplement	First week (cm)	Second Week (cm)	Third Week (cm)	Fourth Week (cm)
Corn cob	**Control**	4.12±0.35 (n=5)	8.32±0.10 (n=5)	12.6±0.65 (n=5)	16.4±0.22 (n=5)
	Rice bran	4.75±0.5 (n=4)	8.62±1.11 (n=4)	16.0±1.42 (n=4)	16.50±1.49 (n=4)
	Chicken manure	4.27±0.510 (n=5)	8.57±1.07 (n=5)	12.85±2.05 (n=5)	16.25±2.94 (n=5)
Paper waste	**Control**	3.38±0.31 (n=5)	8.00±1.20 (n=5)	12.37±2.17 (n=5)	16.0±0.45 (n=5)
	Rice bran	4.27±0.33 (n=5)	8.34±0.29 (n=5)	12.54±0.32 (n=5)	16.0±0.63 (n=5)
	Chicken manure	3.64±0.50 (n=5)	8.05±0.69 (n=5)	12.54±0.23 (n=5)	16.0±0.45 (n=5)
Grass waste	**Control**	3.96±0.63 (n=4)	7.14±0.77 (n=4)	11.06±1.28 (n=4)	15.93±1.25 (n=4)
	Rice bran	4.15±0.19 (n=4)	7.80±0.54 (n=4)	11.89±1.30 (n=4)	15.34±1.09 (n=4)
	Chicken manure	4.04±0.48 (n=4)	7.72±0.48 (n=4)	11.31±1.58 (n=4)	15.71±2.18 (n=4)

Note: Mean ±SD, n= number of replicates **Table 1**

4.1.2 Comparison of colonization period, primordial formation and first harvest days of *Pleurotus ostreatus* on different substrates

Substrate	Supplement	Colonization period(days)	Primordial formation (days)	First harvest (day)
Corn cob	**Control**	34.0±0 (n=5)	40.0±1.0 (n=5)	61.4±0.54 (n=5)
	Rice bran	29.0±0 (n=4)	34.0±0.5 (n=4)	54.0±0 (n=4)
	Chicken manure	36.2±0.45 (n=5)	42.0±0.70 (n=5)	62.6±1.34 (n=5)
Paper waste	**Control**	34.50±0 (n=5)	38.4±0.45 (n=5)	56.50±0 (n=5)
	Rice bran	33.0±0 (n=5)	37.2±0.45 (n=5)	57.0±0 (n=5)
	Chicken manure	34.0±0 (n=5)	36.8±0.45 (n=5)	56.0±0 (n=5)
Grass waste	**Control**	43.25±0.5 (n=4)	47.5±0.57 (n=4)	66.75±0.96 (n=4)
	Rice bran	42.0±0 (n=4)	46.0±0.82 (n=4)	65.5±0.58 (n=4)
	Chicken manure	43.0±0.57 (n=4)	47.0±1.64 (n=4)	65.5±1.92 (n=4)

Note: Mean± SD, n= number of replicates **Table 2**

4.2 NUMBER OF FRUITING BODIES AND SIZE OF MUSHROOM

The maximum number of fruiting bodies of *Pleurotus ostreatus* was observed in all substrates supplemented with rice bran (Table 1.3) but the substrate, corn cob supplemented with rice bran showed higher number(71.5) of fruiting bodies followed by chicken manure supplement (61.2) and control (59.4).

The total number of fruiting bodies in paper waste supplemented with rice bran showed (60.50) followed by chicken manure supplement which showed (60.20) and control (60.0). Similarly, grass waste supplement with rice bran was (35.0) number of fruiting bodies followed by chicken manure supplement (27.25) and control (26.75).

The number of fruiting bodies decreases in second flush compared to first flush among all the treatment. Rice bran played good supplement to increase the number of fruiting bodies in case of all the substrates.

The highest size of *Pleurotus ostreatus* was found in grass waste with control (10.31) whereas lowest size of *Pleurotus ostreatus* was found in paper waste with chicken manure supplement (6.08) among all the treatments (Table 1.4).

The size of *Pleurotus ostreatus* was directly affected by the number of fruiting bodies. As the number of fruiting bodies was increased, the size of *Pleurotus ostreatus* in the paper waste supplemented with chicken manure decreased. Similar results were found in grass waste and corn cob when supplemented with rice bran. (Table1.4)

4.2.1 Numbers of fruiting bodies in the first and second flush of *Pleurotus ostreatus* on different substrates

Substrate	Supplement	Number of fruiting bodies in first flush	Number of fruiting bodies in second flush	Total number of fruiting bodies
Corn cob	**Control**	33.4±2.70 (n=5)	26.0±2.64 (n=5)	59.4±4.27 (n=5)
	Rice bran	40.25±1.29 (n=4)	31.25±0.95 (n=4)	71.5±1.73 (n=4)
	Chicken manure	34.8±2.28 (n=5)	26.4±1.68 (n=5)	61.2±3.63 (n=5)
Paper waste	**Control**	34.0±1.64 (n=5)	26.0±1.45 (n=5)	60.0±2.97 (n=5)
	Rice bran	35.6±4.28 (n=5)	26.9±2.07 (n=5)	62.50±5.04 (n=5)
	Chicken manure	34.6±1.51 (n=5)	25.6±1.14 (n=5)	60.20±2.0 (n=5)
Grass waste	**Control**	16.0±1.63 (n=4)	10.75 ±2.22 (n=4)	26.75±2.22 (n=4)
	Rice bran	21.0±2.59 (n=4)	14.0±2.44 (n=4)	35.0±4.96 (n=4)
	Chicken manure	17.75±1.70 (n=4)	9.5±1.0 (n=4)	27.25±2.64 (n=4)

Note: Mean±SD, n= number of replicates **Table 3**

4.2.2 Size of *Pleurotus ostreatus* on different substrates (mean ±SD)

Substrates/Supplements	Size of Pleurotus ostreatus		
	Rice bran	Chicken manure	Control
Corn cob	8.05±0.23	8.91±0.43	8.15±0.70
Paper waste	8.23±0.83	6.08±0.16	6.35±0.17
Grass waste	10.09±0.56	10.13±0.61	10.31±0.60

Table 4

4.3 YIELD OF FRUITING BODIES

In the first flush, the maximum yield of *Pleurotus ostreatus* was produced in corn cob with rice bran supplements(352.5gm) followed by chicken manure(339gm) and control(316gm). The yield of *Pleurotus ostreatus* in the paper waste with rice bran supplement was (289gm) followed by chicken manure (243gm) and control (234gm). Similarly, the yield of mushroom on grass waste supplemented with rice bran (197.5gm) followed by chicken manure supplement (190gm) and control (186.25gm).

In the second flush, the yield of *Pleurotus ostreatus* in corn cob supplemented with rice bran was (250gm) followed by chicken manure supplement (260gm) and control (190gm). Similarly, the yield of *Pleurotus ostreatus* was obtained from the paper waste with rice bran supplement (190.7gm) followed by chicken manure (177gm) and control (150gm). Similarly, the yield of Pleurotus *ostreatus* was obtained from grass waste with rice bran supplement was (161.25gm) followed by chicken manure supplement (113.75gm) and control (101.25gm).

The yield of cumulative flushes(total yield) resulted higher in corn cob with rice bran supplement (602.5gm) followed by corn cob with chicken manure supplement (545gm) and corn cob in control (506gm) among all the treatments (Table 1.6).

Corn cob supplemented with rice bran showed highest biological efficiency (109.5%) whereas grass wastes in control showed lowest biological efficiency (52.26) among all the treatments (Table1.5).

4.3.1 Biological efficiency of *Pleurotus ostreatus* on different substrates

Substrates/Supplements	Biological efficiency of *Pleurotus ostreatus (%)*		
	Rice bran	**chicken manure**	**control**
Corn cob	109.5±4.23	99.08±3.40	91.99±2.52
Paper waste	88.36±2.68	76.35±3.46	69.81±3.36
Grass waste	65.22±6.22	55.22±4.15	52.26±1.17

Note: Mean±SD

Table 5

4.3.2 Yield of first and second harvest of *Pleurotus ostreatus* on different substrates

Substrate	Supplement	First yield(gm)	Second yield(gm)	Total yield(gm)
Corn cob	**Control**	316±18.16 (n=5)	190±7.90 (n=5)	506±13.87 (n=5)
	Rice bran	352.5±11.9 (n=4)	250±15.81 (n=4)	602.5±23.27 (n=4)
	Chicken manure	339±16.73 (n=5)	206±5.47 (n=5)	545±18.70 (n=5)
Paper waste	**Control**	234±8.94 (n=5)	150±12.74 (n=5)	385±18.50 (n=5)
	Rice bran	289±7.41 (n=5)	190.7±10.36 (n=5)	486±14.74 (n=5)
	Chicken manure	243±7.58 (n=5)	177±12.54 (n=5)	430±19.03 (n=5)
Grass waste	**Control**	186.25±9.46 (n=4)	101.25±8.54 (n=4)	287.5±6.45 (n=4)
	Rice bran	197.5±14.43 (n=4)	161.25±20.96 (n=4)	358.75±34.24 (n=4)
	Chicken manure	190±14.71 (n=4)	113.75±9.46 (n=4)	303.75±22.86 (n=4)

Note: Mean± SD, n= number of replicates. **Table 6**

CHAPTER FIVE

DISCUSSION

The capacity of *Pleurotus species* to grow on agricultural wastes due to their ligninolytic enzymes and other adaptive enzymes that is generally necessary for completion of fungal life cycle (Martinez et. al, 1994; Jenning and Lysek, 1999). Growing oyster mushroom is becoming more popular throughout the world because of their abilities to grow at a wide range of temperature utilizing various lignocelluloses (Khan and Garcha, 1984; Pidgeon and Anderson; 1981, Mueller and Gawley, 1983).

The formation of fruiting bodies is different stage in the life cycle, the generative stage, from mycelial colonization of the substrate, the vegetative stage and there are physical, chemical and genetic differences between the two stages (Zadrazil, 1978; Stamets and Chilton, 1983; Danai et. al, 1998). The spawn running, pin head formation and fruiting bodies formation are three important phases in the cultivation of mushroom requires proper humidity and temperature, temperature requires 25°C for spawn running and 17°C-20°C for fructification which showed good result (Shah et. al, 2004).

Wheat straw is the principle substrate for oyster mushroom growing although adequate production can be achieved through use of wheat straw with the addition of supplements that substantially increase the yield per unit weight. (Zadrazil and Grabb;, 1983) in this study we used paper waste, grass waste and corn cob as a substrate supplemented with rice bran and chicken manure. Addition of supplements with substrates significantly increased the spawn running, pin head formation, fruit body formation and mushroom yield.

5.1 Effect of spawn running

In the present study, the corn cob showed fastest mycelial growth, rapid primordial formation and first harvest day with addition of rice bran as supplement. This fastest growth may be due to the high nutritional value in combination of both substrates as corn cob and supplement as rice bran. The result followed by paper wastes with rice bran and grass wastes with rice bran. This result is supported by the result of Philippoussis et al, 2006. According to Philippoussis et al (2006), we found that the *Lentinula edodes* mushroom

cultivation in different substrates with different supplements. Among them fastest mycelial growth, colonization day, fruit body formation, fruit yield and biological efficiency was found in corn cob substrates. This result may be due to the higher levels of water soluble sugars, particularly hemicelluloses which could have high growth phase, prior to the breakdown of lignin and cellulose. In return, the highly colonized substrate had high mycelial 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 increase the mycelial growth rate and the degradation of polysaccharide compound is associated with the fruiting stage (Bano et. al, 1993).

Zhang., et. al, 2002, studied the *Pleurotus sajar-caju* mushroom cultivation in the two substrates, rice straw and wheat straw in the two form of grinding and chopping and he observed that ground rice straw yield higher mushroom growth rate and yield than the chopped straw, this is because it ruptured the cell wall of the straw to a greater degree, potentially making the nutrients in the straw more accessible for mushroom growth. Similar result were obtained in our study because the ground forms of corn cob substrate yield fast mycelial growth, primordial formation, fruiting bodies formation and highest yield of mushroom.

In the present study, the composition of corn cob substrate with rice bran showed fastest mycelial development as compared with the composition of corn cob substrate with chicken manure supplement. This is due to the presence of cellulose, hemi cellulose and lingo-cellulosic substance present in rice bran but low mycelial growth in corn cob substrate with chicken manure as supplement was found. This is due to the high nitrogen content in chicken manure, nitrogen inhibit the growth of mycelial development. Similar trend of result was found in the other substrate and the supplements used in this study.

5.2 Fruiting bodies formation

In the present result, the fruiting bodies appeared 3-5 weeks after primordial formation and took 34-46 days after inoculation of spawn. These findings are conformity with Tan (1981) who reported that *Pleurotus ostreatus* and other species on cotton waste took 2-3 weeks for

fruit body formation after spawn running. Similarly, these results agree with Quimio (1978) who reported that fruiting bodies appear 3-4 weeks after inoculation of spawn.

Baysal et al, 2003, studied spawn running, pin head and fruit body formation and mushroom yield of oyster mushroom on waste paper substrate with peat, chicken manure and rice bran. The fastest spawn running, pin head formation, fruit body formation and highest yield was found 20% rice bran but more peat and chicken manure has negative effect on growth, which is similar to our results. In this experiment, the number of fruiting bodies was higher in case of all substrates supplemented with rice bran but the size of mushroom decreases. This is probably due to consumption of high nutrients, increase in number of primordial and lack of space for growth.

5.3 Yield of mushroom

Corn cobs were best and cheap alternative substrate for the cultivation of oyster mushroom with the supplement rice bran followed by paper waste and grass waste among all the treatments. This may be due to the higher degradation of various constituents of corn cob substrates by *Pleurotus ostreatus.*

In the present study, the yield of *Pleurotus ostreatus* was found 506 gm/kg in corn cob substrate in control where as it was resulted 602.5 gm/kg 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 corn cob substrate. Das and Mukherji (2006) analyzed that organic supplements such as rice bran mixed with *Leonotis species* as substrate which increases not only growth parameter but also increases the yield of mushroom of *Pleurotus ostreatus*. He found that the best yield in addition of rice bran as supplement with *Leonotis species* as substrate yield 1390gm/kg of substrate whereas without supplement, it gives 1024 gm/kg substrate. Similar observation has also been made by several other researches like (Baysal et al, 2003) found that paper waste as a substrate mixed with rice husk as supplement increases mushroom yield as compared to sole waste paper substrate. The highest yield was obtained by waste paper as substrate mixed with 20% rice bran. Thus, supplements change the decomposition rate and also the sequences of decomposition of substrate component which helps to increase the yield of mushroom.

In this study, the grass waste showed lowest in yield of *Pleurotus ostreatus* among all the treatments. This may be due to fewer breakdown of cellulosic and lignin of substrate. In our result, the biological efficiency of *Pleurotus ostreatus* on grass wastes supplemented with rice bran was highest (65.22%) followed by chicken manure (55.22%) and control (52.26%). Similarly, Basak and Chanda et al (1996) reported that the biological efficiency of *Pleurotus Sajarkaju* in rice straw was 53.8%. Therefore, the yield of mushroom depends on genetic properties of fungal species, substrate quality and culture condition. Substrate quality includes moisture content, ph value and lingo-cellulosic activity of the substrate and mycelium.

CHAPTER SIX

CONCLUSIONS AND RECCOMENDATIONS

Conclusion

According to the results obtained in the present research, it can be concluded that the corn cob supplemented with rice bran showed the superior mycelial growth among all the treatments as well as control. Therefore, rice bran can be used as best appropriate supplement for the cultivation of oyster mushroom. Corn cob and paper waste supplemented with rice bran could be alternate substrate for the cultivation of Pleurotus ostreatus.

Recommendations:

Since, mushrooms are very rich source of protein and hence could be more effective in decreasing protein deficiency as well as malnutrition problem. So farmers should be provided the technical information regarding the mushroom cultivation.

Further study should be done with different concentration and the mixture of corn cob, paper waste and grass wastes. Due to the ability of mushroom to degrade organic waste material and grow on them, mushroom cultivation can even be done in every house which could be more effective in decreasing the pollution, fulfill the malnutrition and livelihood promotion for the income generation in the rural areas.

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PHOTO PLATES

1.  2. 

***Photo 1 & 2: Spawn running**

3.  4 . 

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***Photo 3: full colonization**

5. 

6. 

7. 

8. 

9. 

10. 

***Photo 5, 6,7,8,9 & 10: primordial formation**

11. 
12. 

13. 

***Photo 11,12 & 13: Fruiting bodies**

14. 

***Photo 14**: **Fresh mushroom**