REVIEW OF LITERATURE
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Traditionally, fungi have been considered as plants but basic scientific studies have revealed specific characteristics that differentiate them from plants. Most fungal cells are surrounded by a rigid cell wall similar to that bounding the cubical plant cells while studies on fungal cell chemistry have shown that numerous fungi contain chitin, a substance associated with the hard exoskeleton of insects. Besides, a number of fungal cells store glycogen, a typical polysaccharide found in animal cells. The situation is further complicated by the occurrence of some groups of fungi that form flagellated mobile zoospores and creeping amoeboid cells both characteristics of animals, but these structures are subsequently transformed into fruiting bodies typical of many fungi. Under modern classification, fungi have therefore been placed in a group distinct from plants and animals and assigned to the kingdom
Mycetaceae, distinct from the traditional kingdom plantae (Alexopolous and Mims, 1979).

Most of the cultivated edible mushrooms belong to the order Agaricales of the class Basidiomycetes which is characterized by the formation of gills beneath their umbrella-shaped caps known as pilei. If a section of gill is examined under the microscope, spores are observed on their surface. The spores start to fall as the cap fully expands indicating the maturity of the Mushroom. The spores are so minute that they float on air and are carried by the wind. Eventually, they fall to the ground, usually with rains and if the environmental conditions (temperature and moisture) are favourable, the spores germinate to give rise to a mass of mycelium. This is the stage of the vegetative phase of Fungus that, given an unrestricted amount of nutrients and favourable growth conditions, is capable of unlimited growth. The mycelium developed from the germinating spore is called the primary mycelium and is usually uninucleate. This stage is short lived because mycelia from different spores tend to ramify and fuse to from the secondary mycelium, which continues to grow vegetatively. These mycelia are separate and any fragment of it is capable of forming new colonies because each cell contains all the necessary organelles for independent growth.
The fusion of two haploid nuclei, called Karyogamy, results in the formation of a diploid nucleus in the basidium. Soon after, this diploid nucleus undergoes meiosis (or reduction division) and produces four haploid nuclei. The four haploid nuclei eventually migrate outside the basidial cell, through projections called sterigmata, into four basidiospores. These spores continue to develop until they are forcefully liberated from the basidia and propelled into free space. After only a few hours of spore production and release, the mushrooms are in their last hour of life. Bacteria, other fungi and insects may infest the mushroom, reducing the once majestic mushrooms into a soggy mass of tissues that melts into the ground from which it sprung (Stamets and Chilton, 1983).

Mushrooms and other fungi do not have chlorophyll and hence can not manufacture food directly from carbon dioxide and water. They must be provided with food in the form of simple molecules, which are required for their growth and development. Through the mycelium, fungi absorb food from the substrate on which they grow. The mycelium produces enzymes and digest complex carbohydrates, lipids and protein which are then easily absorbed by the hyphae. The hyphae are usually microscopic and can penetrate the substrate. During this stage, called the spawn
stage, mushroom growth takes place, and energy is stored until it is time to form fruiting bodies or to start reproduction.

In the life cycle of mushroom, the fruiting stage is the formation of the visible mushroom, formed from an aggregation of hyphae or mycelium. The mushrooms start as small pin head or primordia that rapidly enlarges into the button stage. The mushroom then differentiates into an umbrella like structure and ultimately forms gills underneath. At the edge of the gills special cells are formed where two nuclei, originating from different mycelial cells, eventually fuse doubling their chromosome number. These cells are called basidia and are the focal point in the reproductive phase of the mushroom.

Mushrooms have a habit of appearing suddenly in nature. This property explains the many superstitions associated with them. The most dominant stage of their life cycle takes place below the ground within the substrate on which they grow throughout the year, awaiting a favourable season for fruiting. This fruiting season is usually during cool and moist weather. Recent research developments have, however, enabled mushrooms to grow artificially under controlled or semi-controlled conditions.
Some mushrooms are interestingly edible owing to their exotic taste, however there are many mushrooms which are deadly poisonous. Unfortunately, no general guidelines are available for distinguishing edible mushrooms from the poisonous ones. A non-specialist can determine the edibility or toxicity of a given species only by carrying out an accurate identification by consulting experts in the subject or illustrated relevant literature. Moreover, identification of a mushroom at the generic level may not be adequate because within a given genus eg. Lepiota, some species are edible while other species are highly poisonous. Several species of Amanita are reported to be extremely poisonous but obvious symptoms do not appear until 8-12 hrs after consumption.

Amatoxin, the poisonous compound present in non-palatable mushrooms is not destroyed by boiling or processing. Some less poisonous mushrooms produce only nausea or gastric upsets within 60 minutes of ingestion, the absorption of the poison into the blood can be prevented by the induced vomiting in time.

The hallucinogenic mushrooms produce some toxic compounds that affect the nervous system. These compounds produce symptoms like visions, unusual sensations, the results may be fatal. Antigens such
as atropine, thiocytic acid and dextrose can prevent irreparable damage to liver and kidneys. Some suggestions have been made to avoid the mushroom poisoning which should be strictly followed prior to its consumption. Uncooked mushrooms should not be eaten until it is absolutely made sure that it is edible. Eating wild button mushroom should be avoided because it may not be possible to distinguish between edible and poisonous at button stage. Consumption of over-mature, insect-infested, diseased, wilting mushrooms or those that ooze out a white milky juice when cut, should be avoided. When eating a species of mushroom, even though it is known to be edible, only a small amount should be taken. Mushrooms partially eaten by animals or insects may not be necessarily fit for human consumption. Conclusively, mushrooms must be thrown away in case of any doubt.

For successful identification of mushrooms, a comprehensive knowledge on the structure of fungi, their habitats and way of living is essentially required. To identify a given mushroom it is necessary to examine the fruiting bodies with utmost care. A fresh fruiting body is more suitable for identification than staled or dried one. A good reference material usually a book with colour pictures of different known mushrooms may be a basic requirement. A key is usually provided in most
reference texts to simplify the mushroom identification. While using the reference, one should have the knowledge of specific characteristics of mushrooms to be identified, which include i) size, colour and consistency of the cap and the stalk, ii) mode of attachment of gills to the stalk, iii) spore colour in mass and iv) chemical tests or reactions.

The colour of the gills is a good indication of the spore colour, however experienced mycologists rely on 'spore print' to the real colour of the spores.

The mode of gill attachment to the stem indicates the genus of the mushroom hence it should be carefully noted. To determine the mode of attachment, the mushroom is cut longitudinally through the cap, exposing the point of attachment of gills to the stem.

The environment in which the mushroom is picked should also be noted. It is important to note whether the mushroom grows directly on the ground, on decaying wood, on a living tree trunk or on compost. One should not overlook the species of trees on which the mushrooms are found growing or the type of grasses or moss present in the area where the mushrooms are collected from.
There is no single reference book in which all mushrooms are illustrated or described. In most cases, mushroom species in publications are grouped by region or locality, for example, North American mushrooms, mushrooms of the western hemisphere, mushrooms of Japan, etc. while certain mushrooms are easy to identify, many are not. In fact, there is a great number of look-alikes. To avoid any unpleasant experiences, specially when identifying for the purpose of determining edibility, experts should always be consulted.

GROWTH IN COMPOST

Straatsma et al. (1991) studied growth of Agaricus bisporus mycelium on sterile compost. The mycelium on sterile compost extended at a linear rate of 4 mm/day. Mehta and Dhar (1919) recorded mycelial growth rate of multisporic cultures after 7 days of incubation. The average downward mycelial growth varied between 2.0 to 4.3 cm. Analysis of the radial growth in selected pedigrees of Agaricus bisporus was done by Stockton and Horgen (1993). Radial growth was analyzed in a number of mushroom strains (field collected, commercial and intraspecific hybrids) at temperatures ranging from 14-28°C. The intraspecific hybrids gave greater radial growth measurements than both the heterokaryons from which the parental homokaryons were isolated and the homokaryons
themselves. Singh (1994) studied the growth rate of different strains of *A. bisporus* (P-1, P-2, MS-39, NCS-5, NCS-15, NCS-12 and S-11) on compost upto 17 days from inoculation and found that the maximum and minimum growth rate per day was recorded for the strains NCS-12 and P-2 as 4.62 and 2.83 mm, respectively.

**GROWTH IN CASING SOIL**

Before initiation of fruiting the growth of fungus forms mycelial pad or stroma in casing soil. The growth rate of *Agaricus bisporus* in casing soil was recorded to be 5 mm/day by Rainey et al. (1986). Singh (1994) studied the growth rate of different strains of *Agaricus bisporus* (P-1, P-2, MS-39, NCS-5, NCS-15, NCS-12 and S-11) on the casing soil upto 17 days from inoculation and reported that the maximum and minimum growth rate (mm per day) occurred in the strain P-1 (4.03 mm) and S-11 (1.76 mm), respectively.

**MYCELIAL GROWTH ON LIQUID MEDIA**

Singh (1994) conducted an experiment to determine the amount of mycelial growth produced by different strains of *Agaricus bisporus* (P-1, P-2, MS-39, NCS-12, NCS-5, NCS-15 and S-11) on modified malt extract liquid medium on the 25th day of inoculation.
The mycelial weight obtained from the strain NCS-12 (99.00 mg) was significantly higher than other strains on this medium and the minimum mycelial weight was produced by the strain P-1. Some other workers have also determined linear growth rate and cultural characteristics in strains of *Agaricus bisporus*. Dudka (1978) presented information on the above parameters after 9 and 54 days, for the strains 45, 125, 200, Czech 11, Vir 1 and B on five nutrient media. Lemke (1978) compared growth rate of some *Agaricus bitorquis* strains with some *Agaricus bisporus* strains on two different agar media. *Agaricus bitorquis* growth rate was not influenced by the choice of medium while *A. bisporus* strains grew faster on wheat kernel agar than on malt agar. Straatsma (1993) used laboratory media for measuring growth parameters of *Agaricus bisporus* mycelial extension on compost agar and directly by biomass measurements in liquid compost extract medium. Measurement of *A. bisporus* biomass in liquid medium gave a value of 0.9 µ/day.

**Food and nutritional values of mushrooms**

Mushroom is a good source of food constituents viz; protein, carbohydrate, minerals and vitamins. The nutritional value of common edible mushrooms has been summarized as under:
Protein

Protein is one of the most important nutrients of commonly consumed food. Interest in the food value of mushroom has focused primarily on their high protein content. Reported protein values varied considerably between species of mushrooms. There may also be variations within lines or strains of some species, depending on tissues type, stage of development, substrate, cultural conditions, or even in accuracy in the methods of analysis used. In most cases the protein values are calculated as total nitrogen based on Kjeldahl analysis x 6.25. A value of 4.85, which was taken into account the quantity of non-protein nitrogen content, is also some times used. A number of investigators have worked out protein content in various species of mushrooms. The protein contents ranged from 21.32 to 43.02% in Volvariella volvacea (Chang, 1964; Lee and Chang, 1975; Garcha, 1976; Li and Chang, 1982), 19.4-37.1% in Agaricus bisporus (Weaver et al. 1977); 10.5 to 27.4% in Pleurotus ostreatus (Bano and Rajanathnum, 1982; Khanna and Garcha, 1984); 37.19% in Pleurotus florida (Khanna and Garcha, 1984); 17.5% in Lentinula edodes (Bano and Rajarathnum, 1982), 21.9% in Flammulina velutipes (Sawada, 1965) and 8.1% in Auricularia auricular-judae (Adriano and Cruz, 1933).
As mentioned above, a wide variation in the protein content could be found in different mushroom species which may be ascribed to the intrinsic compositional variability in the mushroom (Crisan and Sonds, 1978). Although, mushrooms are still inferior to standard protein sources like meat, fish, eggs and cheese, their protein content is twice as high as that of most vegetables with the exceptions of peas and other legumes (Manning, 1985).

Carbohydrates

In addition to water, carbohydrate constitutes the main component of mushrooms. A range of 3 to 28% (on a fresh weight basis) carbohydrates in various species of mushrooms has been reported by Crisan and Sonds (1978). This consist of a large range of compound such as pentose, hexose, disaccharides, amino sugars, sugar alcohols and sugar acids as reported for *Agaricus bisporus*. Polymeric carbohydrates include glycogen which serves as an energy storage compound comparable to starch in higher plants. Tomar (1965) reported a carbohydrate content ranging between 3.84 and 4.88% of the fresh weight in eight strains of *A. bisporus*. For *V. volvacea*, Li and Chang (1982) reported a carbohydrate content fluctuating between 40 and 50% of the dry weight of the mushroom. This level increases from the button to the egg then to the elongation stage, but
drops abruptly at the mature stage. Bano and Rajarathnum (1982) reported a range of 46.6 to 81.1% for carbohydrates in the different dried species of *Pleurotus*. This compares 60% for *A. bisporus* and 67.5% for *L. edodes*.

**Minerals**

Like most vegetables, mushrooms are good sources of minerals. *Volvariella volvacea* is reported to be rich in potassium, sodium and phosphorus and, together with calcium and magnesium, these constitute 56-70% of the total ash content (Li and Chang, 1982). While potassium, calcium and magnesium remain the same during the maturation of sporophores, the sodium and phosphorus levels decrease as the mushroom matures. Potassium and phosphorus are also the main constituents of the ash of *Pleurotus* (Bano and Rajarathnam, 1982). Iron is present in appreciable amounts. Calcium is not significant in *Lentinula edodes*. *Agaricus bisporus* is reported to contain considerable amounts of potassium, phosphorus, copper and iron, but does not contain appreciable quantities of calcium (Manning, 1985). Phosphorus and iron, predominating on the gills and the surface layer of the pileus respectively, can supply a good proportion of the recommended daily dietary needs.

Canned and dried mushrooms are reported to contain a higher mineral content than fresh mushrooms.
(Crisan and Sands, 1988) although the increased amounts of sodium and potassium in canned mushrooms are attributed to the mineral composition of the brine used in canning.

**Vitamins**

Mushrooms are also good sources of B vitamins. Thiamine (vitamin B₁), riboflavin (vitamin B₂), nicotinic acid and pantothenic acid are abundantly found in mushrooms. These vitamins are stable and not affected during heat processing or canning of mushrooms. Vegetables are reportedly poor sources of vitamins B₁₂, the deficiency of which may lead to anemia. Hayes and Hand (1981) have shown that as little as 3 grams of mushroom may provide the recommended daily intake of vitamin B₁₂. Mushrooms also contain vitamin C (ascorbic acid) and vitamin K (Manning, 1985). However, vitamins A, D and E are reported to be present only in very low quantities (Anderson and Fellers, 1942). Li and Chang (1982) have given an account of vitamin contents in some edible mushrooms. In Agaricus bisporus, the thiamine, riboflavin and niacin contents were reported to be 1.1, 5.0 and 55.7 mg/100g air dried weight respectively (Altamura et al. 1987) while in V. volvacea these contents were 0.32, 1.63 and 47.55 mg/100g air dried weight respectively (Cheng, 1979).
Mushrooms: The Genetics and Breeding

Cultivated edible mushrooms mostly belong to the order Agaricales of the class Basidiomycetes. The sexual reproduction in edible mushrooms takes place by somatogamy or spermatization, the characteristics of the class. Basically, sexual reproduction involves fusion of the cytoplasm of two mating individuals (plasmogamy), nuclear fusion (karyogamy) and reduction of chromosomes from diploid to haploid (meiosis) which results in the formation of four haploid spores. In mushrooms, the spores resulting from sexual reproduction are called basidiospores which may be self-fertile or self-sterile.

The phenomenon of self fertility (or homothallism) is very common in fungi as a whole but, only about 10% of the species are self-fertile in Basidiomycetes compared with 90% self-sterile or heterothallic ones. In self-fertile homothallic species, a spore can germinate and form fruiting bodies by itself. Homothallism may be either primary or secondary. In primary homothallism, the homokaryotic mycelium, which proceeds through heterokaryosis and produces the fruiting bodies or completes its sexual stage, comes from a single meiotic uni-nucleate basiospores, this is the case in Volvariella. Secondary homothallism, exemplified by Agaricus bisporus is exhibited by fertile
homokaryotic mycelium resulting from a basidiospore carrying at least two meiotic nuclei of different mating types. In heterothallic species, the uninucleate spores germinate into homokaryotic mycelium that may cross or fuse with another compatible mycelium or spore before the sexual cycle is completed. Here the mating system may be either bipolar, i.e. governed by one factor A (unifactorial), or tetrapolar, i.e. governed by two factors A & B (bifactorial).

It is essential to know the sexual life cycle of the mushrooms before one can go ahead with breeding studies or line improvement in edible mushrooms.

The following are the known sexual patterns in some of the cultivated edible mushrooms. Primary homothallism takes place in Volvariella volvaca while secondary homothallism occurs in Agaricus bisporus. In Agaricus bitorquis unifactorial heterothallism has been reported whereas Lentinula edodes, Pleurotus ostreatus and Auricularia polytricha are characterized by bifactorial heterothallism.

Improved lines of several edible cultivated mushrooms have been developed either by selection or through hybridization (or cross breeding).
Selection can be made from multispore, monospore or tissue cultures. In most cases, selection is made before attempting to cross or hybridize the mycelium. Selection from monosporous cultures is done only on homothallic species such as Volvariella volvacea and Agaricus bisporus; selection from multispores or tissue cultures is done on heterothallic species. Genetic improvement is also achieved by selection after hybridization.

In hybridization or cross breeding, two monospores cultures are mated and the resulting hybrids are grown and selected for better qualities. For heterothallic species, only hybrids resulting from compatible breeding stocks are selected. Some specific techniques used in line improvement of mushrooms are described as under:

i) Monospore or single spore selection

Compared with multispore cultures, monospore cultures that are fertile may offer a better prospect obtaining new lines because they are variable in properties such as rate of growth, shape of fruiting bodies and productivity. This variability makes it possible to develop new lines of Agaricus bisporus by selection. Valuable hereditary factors exhibit their full potential in monospore rather than in multispore culture.
To prepare monospore cultures, a mushroom fruit with the cap still closed but with the veil underneath becoming very thin, as in *Agaricus*, is cleaned and laid flat on a filter paper lining a Petri-dish. This is then covered with a beaker, the Petri-dish and beaker are previously sterilized at 150°-200°C for 2 hrs. The veil will open and discharge the spores, after one or two days on the filter paper. The beaker then is removed and a sterilized lid is placed on the dish. The spore print is stored in the refrigerator for future use. The filter paper with the spore print is aseptically cut into strips and one small strip placed in 10 ml of sterile distilled water. For every spore loads, a dilution series may be made. A suspension (0.1 ml) of the spore is then plated in a 2 cm wide band on a nutrient medium in Petri-dish. To stimulate germination of *Agaricus*, grains of *Agaricus* spawn are placed at opposite ends of the agar surface. Isolated spores will start to germinate 5-7 day later and can be observed by examining the Petri-dish under the microscope. After marking with a felt-tipped pen, the germinating spores are transferred with a portion of the agar to a new medium in a test tube or Petri-dish.

After 2 or 3 weeks, the first selection can be made using the type of mycelium produced on the agar
slant surface as a guide. Mycelia that are slow growing and are of the oppressed type, as well as those that are fluffy, should be discarded because they are likely to produce low yields. The strand types should be further selected and tested for yield and quality of fruiting body by preparing spawn from each selected piece of mycelium. Fruiting trials are conducted first in small trays and later in larger trays in replications.

ii) Selection from multispore cultures

A spore suspension collected as described above, is mixed with warm and liquefied agar medium and allowed for solidification. Fused mycelia grow on the surface of agar within 3-5 days. A piece of the mycelia plug is transferred to fresh agar medium and the first selection is made after one week.

iii) Simple mixing

In this technique two fertile strains are obtained through monospore or multispore culture. They are inoculated close to each other on an agar plate and allowed to grow together. The mycelium along the line of juncture is inoculated in spawn bottles to prepare spawn for further selection.

iv) Cross breeding
Cross breeding is used to obtain fertile cultures from homokaryons of heterothallic species like *Agaricus bitorquis, Lentinula edodes* and *Pleurotus* spp. Two homokaryons or monospore cultures, obtained as previously described, are planted on the surface of an agar medium and allowed to grow in dual culture. If these homokaryons are compatible, their hyphae will fuse at the junctions giving rise to heterokaryon. A piece of these heterokaryotic mycelia is then transferred to fresh agar medium and a different type of mycelial growth results. The heterokaryotic condition is revealed by the resulting thick, fluffy and stringy mycelium compared with the thin and slow growing mycelium of the homospore culture (or the homokaryons). Clamp connections along the mycelium as observed under the microscope indicate that the dikaryotization or heterokaryolism has occurred within the mycelium. The resulting hybrids are then grown up to fruiting bodies and new lines improved in terms of yield, early maturity and quality of fruiting bodies, are selected.

The studies of Indian *Agaricus* in the Eighteenth Century and early parts of 19th Century were done mainly in Europe. Montangne (1942, 1946) was the first person to report members of *Agaricales* from India. The most notable contributions were made by M.J. Berkeley and P. Hennings among the European
workers. A total of 164 species from 39 genera have been described in between 1850 and 1882 (Berkeley, 1850, 1851 a, b, c, d, 1852 a, b, 1854 a, b, c, d, 1867, 1882). At the beginning of 19th century, Hennings (1900, 1901) described another 68 species from 32 genera. Apart from these pioneering studies, Leveille (1846), Cooke (1881, 1888, 1890, 1891), Currey (1874), Masse (1898, 1899, 1901, 1906, 1907, 1912), Woodrow (1903), Mc Rae (1910), Sydow and Butler (1911), Theisen (1911) and Graham (1915) have also made significant contributions towards the study of Indian Agaricales. Some of the USA workers reported 8 genera and 18 species (Lloyd, 1898-1925, 1904-1919; Murrill, 1924). The work of Berkeley, Masse and other European mycologists was mainly based on study of dried specimens sent to them from India, and hence may be quite inadequate with some exceptions. Information drawn from studies of fresh materials is, therefore, quiet necessary for reliable determination of many of these species.

It appears from the survey of literature on taxonomic studies of Indian fungi that the members of Agaricales are neglected group. After this period of intensive work by European and American workers, there was a significant decrease in taxonomic studies on Indian Agaricales till 1960. A total number of 70 species have been described by various Indian authors
between 1900 and 1960. After 1960, there was again an increase in taxonomic studies on Agaricales and a total number of 147 species were reported between 1960 and 1979. Even though the first record of an Indian Agaricus was made more than 130 years ago by Montagne (1842), not much headway had been made in the systematic study of the members of the large group which contains about 3250 species in 220 genera. A survey of literature on Indian Agarics reveals that only 457 species in 76 genera have been reported from South India comprising the four states viz. Tamilnadu, Kerala, Karnataka and Andhra Pradesh.

With the growing awareness about nutritional and culinary value of mushrooms among public, politician as well as bureaucrats, higher emphasis is being given on their cultivation, besides utilizing naturally growing wild mushrooms. India as a whole is bestowed with tropical, subtropical and temperate climatic conditions having good scope for production of different edible fungi.

Some of the most important commercially produced and utilized mushrooms in different states of the country are briefly described as under:
Volvariella volvacea (Bull. Ex. Fr) Sing.

It is commonly known as the straw mushroom. Its cultivation must have begun in China almost three hundred years ago but it was only around 1932-1935 that the mushroom was introduced into the Philippines, Malaysia and other south-east Asian countries (Chang, 1982). Since then its cultivation has spread over other countries where suitable conditions favourable for its growth persist.

Several other species of Volvariella have also been reportedly cultivated for consumption as food. V. bombacina (Schaeellff. Ex. Fr) Sing and V. diplasia (Berk. & Br.) Sing have been cultivated in India. The validity of V. diplasia is, however, doubtful since it differs from V. volvacea in that the former is white in colour whereas the later is blackish. V. bombacina on the other hand also differs from V. volvacea in terms of habitat as well as colour and then also raises doubts regarding its validity until more information on physiological aspects to substantiate the compatibility is available (Kurtzman and Chand-Ho, 1982).

Volvariella mushrooms are best harvested at the button stage (egg shaped fruiting bodies) when the valva or the universal veil is not broken. The yields depend upon the substrate used. Generally, rice straw
alone gives a lower yield than in combination with cotton waste.

**Pleurotus spp.**

Pleurotus, being one of the choice edible mushrooms, can be cultivated in tropics. It has gained importance in the last decade and is now being cultivated in many countries in the subtropical and temperate zones. In Europe, it is known as the Oyster mushroom (*P. ostreatus*) while in China it is called the Abalone mushroom (*P. abalones*). Several other species, now available for cultivation, include *P. sajar-caju, P. florida, P. eryngii, P. sapidus, P. columbianus, P. cornucopiae* and *P. flabellatus*.

Falck (1971) for the first time described the cultivation of Pleurotus on tree stumps and logs. Later, Etter (1929) reported the production of fruiting bodies of *P. ostreatus* in culture. Block et al. (1958, 1959) gave an extensive account on the requirements of the mushroom for sawdust cultivation. It took about 10 more years to commercialize the production of this mushroom in the USA and Europe (Kalberer and Vogel, 1974; Zadrazil, 1978; Kurtzman, 1979).

The earliest record for Pleurotus cultivation in India appears to be that of Bano and Srivastava
(1962). Jandick (1974) first introduced the now popular tropical species, *P. sajar-caju*. Quimio (1978) first reported the fruiting of *P. flaballatus*, a locally collected pink isolate of *Pleurotus* from the Philippines. Therefore, a small *Pleurotus* industry (*P. cystidiosus* and *P. sajar-caju*) developed in the Philippines which was supplementary to the straw (*Volvariella*) and button (*Agaricus*) mushroom industries. *Pleurotus* were introduced into Thailand at an earlier date (FAO, 1983). As in the Philippines, however, it took several years before they became popular. In Taiwan, the first reported commercial cultivation of *P. cystidiosus* was by Jong and Peng (1975). Roxon and Jong (1977) were the first to introduce the Indian species (*P. sajar-caju*) to the USA.

*Agaricus bitorquis*

It is believed that *Agaricus bitorquis* may have been cultivated intermittently for many decades (San Antonio, 1984; Singer, 1961). According to Singer (1961) this species has been referred in the literature under many synonyms such as *Agaricus bitorquis* (Quil) Sacc; *Psalliota bitorquis* Quil), *Agaricus rodmanii* Peck, *Agaricus compestris* var. edules Vitt etc.
Agaricus bitorquis, sometimes referred to as the field mushroom, is closely related to Agaricus bisporus (Singer, 1961; Hintz et al., 1988). The first scientific experiments on the cultivation of this species were conducted in the late 1960's (Cailleux, 1969; Hasselbach and Mutters, 1971; Poppe, 1972). Breeding work was begun shortly afterwords (Fritsche, 1978) and has continued to the present. A. bitorquis can be grown on composts suitable for the production of A. bisporus. Some advantages of A. bitorquis include viral resistance, longer shelf-life, resistance to bruising and a higher optimum growth temperature. Both spawn run temperatures and production temperatures are about 5°C higher than those required by A. bisporus. The tendency of development of pests at higher temperature is, however a disadvantage. The time to first flushes and period between flushes for same lines of A. bitorquis may also take longer than those for A. bisporus.

Lentinula edodes

Lentinula edodes (Berk.) Pegler, commonly known as 'Shiang-gu' in china and "Shiitake" in Japan was possibly first cultivated in China during AD 1000 and 1100 (Chang and Mites, 1987). Some authors believe that the cultivation techniques developed in China were latter introduced into Japan, the major producer of L. edodes (Ito 1978; Royse et al. 1985). Chang and
Mites (1987) have given a more detailed account of the historical record of *L. edodes*.

*Lentinula*, being a widely distributed genus of subtropical and tropical zones contains at least 5 species viz; *L. edodes* *L. lateritia*, *L. boryhana*, *L. guarapiensis* and *L. novaezelandacae* (Pegler, 1983). This genus was long thought to be a later synonym of the much larger genus *Lentinus* Fr. However, based on studies of the hyphal structure, Pegler (1975) found it to be distinct from *Lentinus edodes* (Berk.) Singer. The taxonomic confusion with *Lentinula edodes* is evident by about 15 name changes it has undergone since it was first described as *Agaricus edodes* in 1877 (Pegler, 1975).

**Tremella fuciformis**

*Tremella fuciformis* Berk, commonly known as 'Silver ear' mushroom or white gelly fungus, has been used as a special luxury food item for many decades in China. Its early cultivation was similar to that used for *Lentinula edodes*. Logs were placed in the vicinity of logs on which the fruiting bodies of *Tremella* were present and inoculation simply occurred by chance dissemination of spores from the fruiting bodies. Later, the inoculation of pure culture spawn, which was composed partly of wood or of saw-dust and rice bran was introduced and subsequently adopted by
commercial producers (Chen and Hou, 1978). Large scale cultivation on a synthetic substrate, using a mixed culture inoculum, was started in 1980’s in Fujian Province in China (Huang, 1982).

A pure culture of *Tremella fuciformis* can be used for the cultivation of white gelly fungus on sawdust, but a mixed culture technique, developed in China, gives better yields. The mixed culture spawn consists of the mushroom *Tremella fuciformis* and the ascomycete *Hypoxylon archeri* which forms a feather like mycelium. The later serves as the 'biological factor' or as the 'friend of the mycelium' which helps the *Tremella* in the digestion of the wood and provides some residual nutrition.

The nitrogen deficiency in mushroom substrate is reported to be fulfilled by adding several nitrogen rich supplements like chicken manure, brewer’s grains, urea and cotton seed cake. However, no attention has been paid to supplement the equally important elements like sulphur and phosphorus which take part in catalyzing several biochemical activities. The phosphorus deficiency in the growing medium limits the availability of phosphorus and also stops the assimilation and utilization of nitrogen by the mushroom mycelium and thereby adversely affects its productivity. The use of biofertilizers in
mushroom cultivation is, therefore, gaining importance for raising a quality crop (Ahlawat and Verma, 2002).

The post-mushroom substrate or 'spent' obtained after the crop harvest, creates various environmental problems including ground water contamination and nuisance. In the past, considerable efforts have been made towards profitable utilization of this post-mushroom substrate and mitigation of environmental pollution. It has been found to be a good nutrient source and hence used as an organic fertilizer in agriculture, for purification of contaminated soil, water and air and also in the reclamation and improvement of soil health (Ahlawat and Sagar, 2001).

The mushroom productivity is dependant on the availability of quality spawn, free from any contamination. It has been estimated that about 15-20% of the commercial spawn is lost due to contamination by several fungi, bacteria or actinomycetes every year. The substrate (grains) used for spawn production is the main source of contamination (Bahl, 1989; Moorthy and Mohanan, 1996; Suman, 1993). Decontamination of substrate is, therefore, one of the most important activities in mushroom production. It can be accomplished in several ways ie. sterilization by autoclaving
(Lozana, 1990; Quimio, et al. 1990), steam and hot water pasteurization (Stamets, 1993; Vedder, 1991), dry heating (Kalberer, 1989) and chemical treatments (Stamets, 1993, Chitale and Sing, 1994; Earanna, 1994,). Ahlawat et al. (1971) reported that addition of antibiotics after autoclaving the substrate was found effective in reducing the contamination. Among the antibiotics ampicillin, streptocycline, streptomycin and tetracycline were found, more effective at 50 μg g⁻¹ of spawn substrate. Autoclaving and steam sterilization may be expensive due to the high cost of equipments. A low cost method using a metallic rotary 180 L drum with interrupted revolution every 15 min under continuous heating was demonstrated by Curvetto et al. (1997) to decontaminate a bulk substrate consisting mainly of sunflower seed hulls, in the cultivation of Pleurotus sajar-caju. This requires comparatively shorter time, produces effective decontamination and also allows a thorough mixing of the spawn into the mass substrate.

Effects of some phyto-extracts of some commonly available plants were determined on the mycelial growth of Volvariella volvacea and its common competitor mould, Sclerotium rolfsii by Pani and Parta (1997). Among the phyto-extracts used, they found leaf extract of tamarind (Tamarindus indica) most promising followed by seed extract of soapnut
(Sapindus trifoliata) and root extract of drum stick (M. oleifera) in suppressing the growth of S. rolfsii vis-à-vis V. volvacea in laboratory as well as field conditions. Tamarind leaf extract caused maximum inhibition of S. rolfsii and least interference with the mycelial growth of the edible fungus.

While studying the effect of diflubenzuron, a chitin synthesis inhibitor, on the mycelial growth and yield of Agaricus bisporus, Arora et al. (1997) observed that the dosage of the chemical upto 50 ppm showed a considerable increase in the mycelial growth and yield whereas, an increase in dosage from 50-200 ppm caused a gradual decrease in colony diameter as well as yield of A. bisporus. Diflubenzuron was used in mushroom crop for the first time in 1975 in England to control strains of Lycoriella auripilla (Wina) resistant to organophosphorus insecticides (Binns, 1975) but later on the treatment of the substrate or casing layer with this chemical emerged as standard control measure for the mushroom flies throughout the world (Kalberer, 1978; White, 1981).

MORPHOLOGY

Atkinson (1961) described different species of Agaricus on the basis of morphological characteristics. Smith and Singh (1973) gave a detailed outline to study the fungal fruiting bodies.
Mehta and Dhar (1991) studied vegetative and sporophore characters of 15 different multispore cultures namely, MS 1 to 15 of Agaricus bisporus and they found an average pileus diameter of 3.43 cm, pileus thickness 0.98 cm, stipe length 3.3 cm, stipe diameter 1.49 cm and gill cavity 3.55 cm. Thakur and Dhar (1993) studied morphology of Agaricus strains and reported the average pileus diameter of 3.78 cm, pileus thickness 1.08 cm, stipe length 2.79 cm, stipe diameter 1.64 cm and gill cavity 4.05 cm. Singh (1994) working with the strains P-1, P-2, NCS-5, NCS-12, NCS-15, MS-39 and S-11 as check reported that the pileus diameter varied from 3.12 (NCS-15) to 1.14 cm (MS-39); the length of the stipe from 2.60 (P-1) to 0.67 cm (MS-39); the diameter of the stipe from 2.60 (NCS-12) to 1.50 cm (P-1). The gill cavity of pileus did not vary significantly among the strains.

**GROWTH IN COMPOST**

Straatsma et al. (1991) studied growth of Agaricus bisporus mycelium on sterile compost. The mycelium on sterile compost extended at a linear rate of 4 mm/day. Mehta and Dhar (1991) recorded mycelial growth rate of multispore cultures after 7 days of incubation. The average downward mycelial growth in selected pedigrees of Agaricus bisporus was done by Stockton and Horgen (1993). Radial growth was analyzed in a number of mushroom strains (field
collected, commercial and intraspecific hybrids) at temperatures ranging from 14-28°C. The intraspecific hybrids gave greater radial growth measurements than both the heterokaryons from which the parental homokaryons were isolated and the homokaryons themselves. Singh (1994) studied the growth rate of different strains of *Agaricus bisporus* (P-1, P-2, MS-39, NCS-5, NCS-15, NCS-12 and S-11) on compost upto 17 days from inoculation and found that the maximum and minimum growth rate per day was recorded for the strains NCS-12 and P-2 as 4.62 and 2.83 mm, respectively.

**GROWTH IN CASING SOIL**

Before initiation of fruiting, the fungus groups to form mycelial pad or stroma in casing soil. The growth rate of *Agaricus bisporus* in casing soil was recorded to be 5 mm/day by Rainey et al. (1986). Singh (1994) studied the growth rate of different strains of *A. bisporus* (P-1, P-2, MS-39, NCS-5, NCS-15, NCS-12 and S-11) on the casing soil upto 17 days from inoculation and reported that the maximum and minimum growth rate (mm) per day occurred in the strains P-1 (4.03 mm) and S-11 (1.76 mm) respectively.

Singh (1994) conducted an experiment to determine the amount of mycelial growth produced by
different strains of Agaricus bisporus (P-1, P-2, MS-39, NCS-12, NCS-5, NCS-15 and S-11) on modified malt extract liquid medium on the 25th days of inoculation. The mycelial weight obtained from the strain NCS-12 (99.00 mg) was significantly higher than other strains on this medium and the minimum mycelial weight was produced by the strain P-1. Some other workers have also determined linear growth rate and cultural characteristics of strains of Agaricus bisporus. Dudka (1978) presented information on the above parameters after 9 and 54 days, for the strains 45, 125, 200, Czech 11, Vir 1 and B on five nutrient media. Lemke (1978) compared growth rate of some Agaricus bitorquis strains with some Agaricus bisporus strains on two different agar media. Agaricus bitorquis growth rate was not influenced by the choice of medium while A. bisporus strains grew faster on wheat kernel agar than on malt agar. Straatsma (1993) used laboratory media for measuring growth parameters of Agaricus bisporus mycelium. Mycelial extension on compost agar and directly by biomass measurements in liquid compost extract medium. Measurement of A. bisporus biomass in liquid medium gave a value of 0.9 μ/day.

POST HARVEST PHYSIOLOGY OF MUSHROOMS

The harvested sporophore of the cultivated mushroom continues its post harvest development,
including both maturation and senescence changes, similar to those if allowed to remain growing in the bed (Beelman, 1987). Beelman (1988) studied the factors influencing post harvest quality and shelf life of fresh mushrooms. He discussed microbial deterioration of Agaricus bisporus structures during storage.

Respiration and transpiration

The cultivated mushroom contains mannitol which is the source of respiratory substrate (Hammond and Nicholas, 1975). Respiration rate of mushrooms increases with an increase in the storage temperature and O₂ concentration (Anantheshwarn and Ghosh, 1997). San Antonio and Hegg (1964) reported that transpiration from the surface of mushroom is due to the lack of epidermal structure of mushrooms. It causes loss of weight and freshness, and accelerates senescence.

Post harvest deterioration

Mushrooms contain large quantities of water and, therefore, suffer considerable weight loss during transpiration and storage. Thus, in turn, causes serious economic losses, due to reductions in weight and quality (Grey, 1970). After harvesting, fresh mushroom enters a deterioration phase. Cap opening,
stem elongation, changes in colour (browning), texture and taste, loss of water as a result of transpiration and respiration, additional loss from surface cracks and bruises during growth and handling are the usual symptoms of senescence (Salunkhe et al. 1991). Gormley (1975) categorized quality according to the whiteness (L values), and suggested that a L value less than 80 is considered unacceptable by the wholesalers-

<table>
<thead>
<tr>
<th>Category</th>
<th>Hunter L values</th>
<th>Panel description of whiteness</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>&gt; 93</td>
<td>Excellent</td>
</tr>
<tr>
<td>2</td>
<td>90-93</td>
<td>Very good</td>
</tr>
<tr>
<td>3</td>
<td>86-89</td>
<td>Good</td>
</tr>
<tr>
<td>4</td>
<td>80-85</td>
<td>Fair</td>
</tr>
<tr>
<td>5</td>
<td>69-79</td>
<td>Poor</td>
</tr>
<tr>
<td>6</td>
<td>&lt; 69</td>
<td>Very poor</td>
</tr>
</tbody>
</table>

During the post harvest deterioration, the mushrooms soften and toughen (Beelman, 1987). The pileus of the mushroom rapidly looses its stiffness during storage (Mc Garry and Burton, 1994). During post harvest development, dry matter is exported from stipe to pileus (Hammond and Nicholas, 1976). When stipe is trimmed prior to storage, pileus opening and mushroom senescence are delayed (Ajlouni et al. 1992). Rama et al. (1995) studied the changes of the
surface texture of mushrooms during post harvest storage. The energy required to compress the side, decreased by 55 percent compared to 26 percent for the top of the mushroom, after two days of storage. Guthrie (1984) described a 7 point scale for determining the maturity index and considered a maturity index greater than 4 as unacceptable.

The initial bacterial population at harvest is an important factor in the deterioration of fresh mushroom during storage (Beelman, 1987). The microorganisms of concern are: Pseudomonas tolasii, Clostridium botulinum and Staphylococcus aureus. Pseudomonas tolasii is associated with browning of the mushroom tissues.

**Discolouration, phenolics and browning enzymes**

The browning process in Agaricus bisporus results from an initial enzyme catalyzed oxidation of endogenous phenols into coloured quinines (Nicholas et al. 1994). The latter spontaneously undergo an oxidative polymerization yielding different melanins, the ultra structure of which has been studied by Hegnauer et al. (1995). Two explanations for the increased tissue browning after harvest (Smith et al. 1993) are (a) that the tyrosinase becomes activated and (b) access between tyrosinase and its phenolic substrates is increased. The biochemical composition
of *A. bisporus* including its phenolic constituent has been reviewed by Forest (1990). Among the several phenols, the main direct precursors of melanogenic guanines appeared to be L-tyrosine, L-Dopa, GHB and GDHB. Their amount inside the sporophore showed large variations, depending on the strain, the tissue and the development stage (Rast et al. 1979 and Forest and Arpin, 1991). The level of sporophore phenols may be the main factor involved in the ability to brown (Burton, 1988; Burton et al. 1993). Rai and Saxena (1989) reported the biochemical changes during post harvest storage of button mushroom (*Agaricus bisporus*). The biochemical changes include decreased activity of polyphenol oxidase which catalyses the browning.

**Protease(s) and deterioration**

Burton et al. (1993) have found a marked increase of protease activity during storage which would bring about the activation of latent tyrosinase, as well as possible lysis of membranes allowing a better access of substrates to the enzyme. The increase in protease activity during storage has also been reported (Murr and Morris, 1975; Rajaratnam et al. 1982). This enzyme may be involved in fungal tissue autolysis and changes in sporophore texture (Villanueva, 1966). A serine protease has
been characterized from senescent sporophores of A. bisporus (Burton et al. 1993).

**Longevity of mushrooms at different storage temperatures**

Sethi and Anand (1978) reported that mushroom can not be stored for more than 24 hours at room temperature or one to two weeks at refrigerated temperature. Saxena and Rai (1988) reported that mushrooms can be stored in non-perforated polythene bags for 4 days at 5°C without much deterioration, and the washing of mushrooms in 0.5 percent potassium metabisulphite improves the whiteness, which eventually deteriorates slowly during storage. Maini et al. (1987) reported that treating mushrooms at lower concentration of potassium metabisulphite was found suitable for short term storage (24 hours). Henze (1989) reported that the optimum temperature for storage upto a week is 0-1°C at <90 percent RH. Uniecka (1986) studied the influence of the storage conditions on the shelf life of Agaricus bisporus strains Somycel 9, 92, 53, 653, 11 and Hauser A 8.8. The best temperature for storage was 0-1°C. At this temperature mushrooms could be stored for 7-9 days.
PROTEIN PROFILING

Almost all analytical electrophoresis of proteins is carried out in polyacrylamide gels under conditions that ensure dissociation of the proteins into their individual polypeptide subunits and that minimize aggregation. Most commonly, the strongly anionic detergent SDS is used in combination with a reducing agent and heat to dissociate the proteins before they are loaded on the gel. The denatured polypeptides bind SDS becomes negatively charged. Because the amount of SDS bound is almost always proportional to the molecular weight of the polypeptide and is independent of its sequence, SDS-polypeptide complexes migrate through polyacrylamide gel in accordance with the size of the polypeptide. At saturation, approximately 1.4 g of detergent is bound per gram of polypeptide chain(s) (Sambrook, Fritsch, Maniatis, 1989; Bunker and Rueckert, 1969; Weber and Osborne, 1969). Reynolds and Tanford (1970) gave the gross conformation of protein-sodium dodecyl sulphate complexes.

The discontinuous buffer system of SDS-PAGE used is based on Laemmli (1970). The discontinuous buffer system was originally devised by Ornstein (1964) and Davis (1964). Laemmli (1970) by using an improved method of gel electrophoresis, discovered many unknown proteins in bacteriophage T. He demonstrated
that many phage proteins can be separated with improved method of disk-electrophoresis in SDS. This system combines the high resolution power of disk-electrophoresis with the capability of SDS to breakdown proteins into their individual polypeptide chains. The proteins are also separated according to their molecular weight. Lawrence and Shepherd (1980) examined the protein subunits of 98 wheat cultivars using a discontinuous gel-electrophoresis system. Beitz and Wall (1972) reported that the glutenin aggregates can be broken down into their component subunits by treatment with an agent which breaks disulfide bonds, such as 2-mercaptoethanol and an agent which disrupts hydrophobic interactions and hydrogen bonds, such as the anionic detergent SDS. According to Lawrence and Shepherd (1980) following these treatments the component subunits can be separated by SDS-PAGE. In discontinuous buffer system the buffer in the reservoirs is of a different pH and ionic strength from the buffer used of cost the gel. The sample and the stacking gel contain Tris-Cl (pH 6.8), the upper and lower buffer reservoirs contain Tris-glycine (pH 8.3), and the resolving gel contains Tris-Cl (pH 8.8) (Sambroock, Fritsch, Maniatis, 1989). The discontinuous pH, two-gel systems, first concentrate the proteins in the sample into a narrow starting zone, give better resolution of protein bands (Lawrence and Shepherd, 1980).
Six strains of Agaricus bisporus (Namely, 11, 17, 53, 67, 76 and 85) were compared for their protein content, contents of non-protein nitrogenous compounds and for their protein heterogeneity with respect to solubility behaviour and electrophoretic pattern (Kumar et al. 1991). Paranjpe and Chen (1979) analyzed quantitatively and qualitatively soluble protein form various tissues of the different morphogenetic stages of Agaricus bisporus. The protein profiles of the caps, stalks, pins or primordial and the mycelium were different when studied by polyacrylamide-gel electrophoresis. In order to investigate the nature of the factor which induces a horizontally growing mycelium of A. bisporus to from a varietal or erect basidiocarp they studied the soluble proteins and the phenol-oxidizing enzymes during different stages of the developing mushroom. The isozyme banding pattern of peroxidase and cytochrome oxidase were also studied by polyacrylamide-gel electrophoresis. A comparison of the tissue extracts from the different growth stages revealed that the pins contained the highest amounts of soluble proteins. In the mature basidiocarps, the caps contained the highest amounts of proteins amongst different tissues. The difference in the nature of the proteins was reflected in the banding patterns as seen by acrylamide gel electrophoresis. According to Purkayastha and Nayak (1981) gel
electrophoretic analysis of soluble proteins of fruit bodies of Calocybe indica at different developmental stages reveals that 20, 18 and 16 protein bands are present in button (B), stipe with well differentiated pileus (SWP) and stipe with tiny pileus (SP) respectively. Mature fruit bodies (MF) also show 18 protein bands. Six to seven unique bands usually occur in B, SP and MF but only 3 have been detected in SWP. Certain bands which appear in one stage disappear in subsequent stages, while others remain unaltered. The presence of common protein bands indicates the presence of common biochemical activities in different stages of development. The unique bands found in a particular stage of fruit body could be responsible for different types of tissue formation or expansion or both. Chang and Chan (1973) studied the different protein patterns at different stages of development of a basidiocarp of Volvariella volvaeca.

Perry, Matcham, Wood and Thurston (1993) described the structure of laccase protein and some features of its synthesis by the commercial mushroom Agaricus bisporus. SDS-PAGE analysis of extractcellular laccase protein (secreted by A. bisporus into the medium during mycelial growth), purified from compost extract, showed a predominant
band of 65 KDa molecular mass, together with lesser amounts of smaller polypeptides.

**YIELD**

Evaluation of *Agaricus bisporus* strains under seasonal growing conditions was done by Dhar and Kapoor (1990). Strains S-11, 310, UMX-15, TM-7, L-20 and M-2 were evaluated under tropical winter conditions in Delhi (long method of composting) and under temperature summer conditions in Kashmir (short method of composting). Higher yield were obtained on short method compost compared with the long method compost. Strains S-11, 310, 791, TM-79 and M-2 performed well in all trials. The highest yield under Delhi conditions (22.4 kg/q compost) was obtained with strain S-310 and under Kashmir conditions (17.9 kg/q compost) with strain TM-79. Jin (1990) studied the yield performance of fluffy and oppressed type mycelium of the same strain of *Agaricus bisporus* and reported that fluffy type tended to give high yield whereas, the oppressed type showed good quality. Mehta and Dhar (1991) evaluated 9 strains of *A. bisporus* for yield performance. The strains NCS-14, NCS-5, NCS-11, NCS-6 and NCS-15 were at par in terms of yield and yielded significantly higher than S-11, MS-39, P-2 and NCS-12. Singh (1990; 1991) conducted the yield evaluation trials of different strains of *A. bisporus*, and reported that strains varied in
their yield performance between 6.96 to 10.12 kg/q compost. Chadha (1992) gave an account of different strains of *A. bisporus*. He reported that strains S-11 were introduced in sixties while strains RRL 89, S-22 and S-649 were introduced during 1965-83. The strains S-11 and S-310 were good yielder, while TM-7 and L-20 was moderate yielder. Phutela and Garcha (1995) performed strain evaluation for higher yield in *Agaricus bisporus*. P-1 was considered the best, yielding 8.86 kg mushroom per quintal compost under natural conditions on long method compost. MS-39 performed equally well giving 7.38 kg mushroom per q compost. Rourke (1995) discussed the factors affecting yield variation. He reported that a large proportion of yield variation is due to growing unit, while only a little variation is due to spawn culture. Singh (1994) reported that the strains P-1 and NCS-5 were the best performers in terms of yield in comparison to the strains P-2, MS-39, NCS-15, NCS-12 and S-11 (Cantelo, 1984). Some reports on the successful use of this chemical for the control of sciarid fly infesting *Agaricus bisporus* are also available (Baba, 1990; Arora, 1992).