Technical Bulletin

Diseases and Competitor Moulds of Mushrooms and their Management

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FOREWORD

The cultivation of Mushrooms is a carefully controlled biological system, however contamination with microorganisms, which are in ways, is inevitable. In India majority of the mushroom holdings are lacking adequate compost preparation, pasteurization and proper environmental control facilities, which lead to the development of various diseases and pests sufficiently to a level to cause considerable yield loss. It is therefore very important for the mushroom growers that they should know the importance of diseases and competitors and should understand the importance of hygiene to grow mushrooms successfully and profitably. I would like to advise the mushroom growers to pay maximum attention to prepare compost/ substrate of optimum quality and maintain highest level of hygiene to avoid these problems. I appreciate the efforts and labour put in by the authors in compiling and editing the bulletin for its use by the mushroom growers and researchers.

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I. INTRODUCTION

Like all other crops, mushrooms are also affected adversely by a large number of biotic and abiotic agents/ factors. Among the biotic agents, fungi, bacteria, viruses, nematodes, insects and mites cause damage to mushrooms directly or indirectly. A number of harmful fungi are encountered in compost and casing soil during the cultivation of white button mushroom. Many of these act as competitor moulds thereby adversely affecting spawn run whereas others attack the fruit bodies at various stages of crop growth producing distinct disease symptoms. At times there is complete crop failure depending upon the stage of infection, quality of compost and environmental conditions. General distribution of various competitor moulds and pathogenic fungi is as follows:

I. Those occurring mainly in compost include: Olive green mould (*Chaetomium olivaceum* and other spp.), Ink caps (*Coprinus* spp.) Green moulds (*Aspergillus* spp. *Penicillium* spp. and *Trichoderma* spp.), Black moulds (*Mucor* spp., *Rhizopus* spp.) and other (*Myriococcum praecox*, *Sporotrichum* sp., *Sepedonium* sp., *Fusarium* spp., *Cephalosporium* spp., *Gliocaldium* spp., and *Papulospora* spp.).

II. Fungi occurring in compost and in casing soil: White plaster mould (*Scopulariopsis fimicola*): Brown plaster mould (*Papulospora byssina*), Lipstick mould (*Sporendonema purpurescens*), False truffle (*Diehliomyces microsporus*) and green moulds.

III. Fungi occurring on and in casing soil and/or on the growing mushrooms: Cinnamon mould (*Peziza ostracoderma*), wet bubble (*Mycogone perniciosa*), Dry bubble (*Verticillium fungicola*), Cobweb (*Cladobotryum dendroides*), Pink mould (*Trichothecium roseum*) and green moulds.

IV. Fungi attacking the fruit bodies only: Fusarial rot (*Fusarium* spp.).

At any phase of growth an undesirable growth or development of certain moulds can occur and can adversely affect the final mushroom yield.

II. FUNGAL DISEASES AND COMPETITOR MOULDS

A. WHITE BUTTON MUSHROOM (*Agaricus bisporus, A.bitorquis*)

a. Diseases

1. DRY BUBBLE

Pathogen : *Verticillium fungicola*

Common Name : *Verticillium* disease, brown spot, fungus spot, dry bubble, La mole.

This is the most common and serious fungal disease of mushroom crop. If it is left uncontrolled, disease can totally destroy a crop in 2-3 weeks (Fletcher *et al*. 1986). *Verticillium fungicola* was major pathogen responsible for considerable yield losses of cultivated mushrooms in Manchuela area provinces of Cuenca and Albacete, Spain (Gela, 1993). In a disease survey of commercial mushroom houses, *V.malthousei* was isolated from 11.3% of mushroom sampled (Foree *et al*. 1974). From India the first report of the heavy incidence of dry bubble disease was from mushroom farms located at Chail and Taradevi (Seth *et al*. 1973). The pathogen has been invariably isolated from the compost and casing samples collected from mushroom farms in Haryana, HP and Punjab (Sharma, 1992). Thapa and Jandaik (1984-85) have recorded the incidence of dry bubble from 25-50% at Solan and Kasauli and upto 15% at Shimla and Chail during 1980-81. Artificial inoculation with the pathogen at the time of spawning and at different loads of inoculum had delayed pinhead formation by 5 days and reduced the number and weight of fruit bodies by 2.26-47.2% and 2.19-38.01%, respectively (Sharma and Vijay, 1993).

Symptomatology : Whitish mycelial growth is initially noticed on the casing soil which has a tendency to turn greyish yellow. If infection takes place in an early stage, typical onion shaped mushrooms are produced. Sometimes they appear as small-undifferentiated masses of tissue upto 2cm in diameter. When affected at later stage, crooked and deformed mushrooms with distorted stipes

and with tilted cap can be seen. When a part of the cap is affected harelip symptom is noticed. Affected mushrooms are greyish in colour. If the infection occurs at later stage, grey mouldy fuzz can be seen on the mushrooms. Sometimes little pustules or lumps appear on the cap. On fully developed sporophores, it produces localized light brown depressed spots. Adjacent spots coalesce and form irregular brown blotches. Diseased caps shrink in blotched area, turn leathery, dry and show cracks. Infected fruit bodies are malformed, onion shaped and become irregular and swollen mass of dry leathry tissue (Sharma, 1994). In *A.bitorquis*, the dark brown blotches caused by *V.fungicola* var *aleophilum* are sometimes covered with a layer of grey coloured mycelium particularly in the centre. In *A.bisporus* it causes minor spotting though in variety Les Miz-60 it causes fruit body deformation. An isolate of *V.psalliote* from *A.bitorquis* causes more confluent brown spots on *A.bitorquis* but could not infect *A.bisporus* (Zaayan and Gams, 1982).

Causal Organism : *Verticillium fungicola*

The fungus produces numerous one celled thin walled, oblong to cylindrical, hyaline conidia, 3.5-15.9 x 1.5 - 5u on lateral or terminal, verticillately branched conidiophores (200-800 x 1.5-5.0 u). Conidiophores are relatively slender and tall. Conidia accumulate in clusters surrounded by sticky mucilage. The fungus abounds in soil.

Epidemiology : *Verticillium* is carried on to the farm by infected casing soil. Spread is carried out by infected equipments, hands and clothing. Phorid and sciarid flies are also known to transmit this disease (Renker and Bloom, 1984). Under laboratory conditions sciarids and phorids were found to transmit 84-100% and 76-100% of *V. fungicola* respectively, into two different media (Kumar & Sharma, 1998). Mites are also known to transmit the disease from infected to healthy mushroom (Fikete, 1967). The fungus is soil borne and spores can survive in the moist soil for one year. It also perpetuates through resting mycelium from dried bulbills and in spent compost. The optimum temperature for disease development is 20°C. The period from infection to symptom expression is 10 days for the distortion symptoms and 3-4 days for cap spotting at 20°C. The pathogen grows best at 24°C. However,

V.fungicola var *aleophilum* and *V.psalliotae* grow best at higher temperature (27°C) (Fletcher *et al*. 1986). High humidity, lack of proper air circulation, delayed picking and temperature above 16°C favour its development and spread (Sohi, 1988). It becomes more common when cropping is extended beyond 61 days. A number of wild growing fleshy fungi also serve as source of inoculum. Air borne dust is also major source of primary infection and may enter houses through exhaust vents. If infection occurs early, it causes more severe malformation of fruit bodies. Holmes (1971) reported that inoculum introduced before 21st day caused low mushroom yield and high disease incidence. However, inoculum introduced after 14 days of casing caused the highest disease incidence. According to Nair and Macaulley (1987) when crops of *A.bisporus* and *A.bitorquis* were infected at casing with *V.fungicola* var *fungicola* and *V.fungicola* var *aleophilum* respectively, a relatively high incidence of disease was observed but disease was less in the crops infected at spawning or after second flush. Reduction of temperature from 20C to 14C and RH from 90% to 80% for 5 days could not reduce the severity of the disease. All the commercial strains are susceptible (Sharma 1994). However, Poppe (1967) in pot trials found brown strain from France most resistant to dry bubble disease.

Management

a) Physical methods : Use of sterilized casing soil, proper disposal of spent compost and proper hygiene and sanitation are essential to avoid primary infection (Sharma, 1994). Wuest and Moore (1972) reported that treating mineral soil with aerated steam at 54.4°C for 15 minutes eliminated *V.malthousi* that had been experimently established for 17 days in axenic soil culture. Further in 1973, Moore and Wuest reported that thirty minute treatment with aerated steam at 60°C and 82°C, hindered spore germination and soil colonization by *V.malthousei* more than similar treatment at 98°C. Heat treatment of infected casing layer at 63°C for one hour completely prevented spore germination (Poppe, 1967).

b) Biological method : According to Trogoff and Ricard (1976) spraying casing soil with $100x10^6$ *Trichoderma* propagules/litre/m^2 controlled *V.malthousei* in

several trials on naturally infected mushroom holdings where dry bubble disease was endemic. Under laboratory conditions, leaf extracts of *Callistemon lanceolatus, Cannabis sativus, Citrus* sp., *Euclyptus* sp., *Dhatura* sp., *Urtica dioica, Solanum khasianum* and *Thooja compacta* caused 27.77%, 13.05%, 16.66%, 22.22%, 5.55%, 6.66%, 22.77% and 27.77% inhibition, respectively of *V.fungicola* (Sharma and Kumar 1998-99) Bhat and Singh (2000) reported 5 bacterial isolates effective against *V.fungicola*.

c) Chemical methods : In laboratory trials *V.malthousei* was controlled by Zineb on a large scale, Bercema - Zineb 80 used at 0.1 - 1.2% controlled the disease when used before and between the flushes (Philipp, 1963). *V.malthousei* was controlled by 3 sprays with Dithane Z-78 at 0.25 or 0.50% or Hexathane at 0.30% given at the time of casing, at pinhead formation and after flushes of crop (Seth *et al*. 1973). Application of chlorothalonil as a drench reduced the incidence of *V.fungicola* tolerant to certain benzimidazole fungicides. However, incorporation of chlorothalonil into the casing layer caused toxicity to crop and depressed the yield (Gandy and Spencer, 1976). However, Zaayen and Rutjens (1978) obtained good control with 2 application of Daconil 2787 (chlorothalonil) at 3g/m^2 without any adverse effect on yield. Treatment should be applied directly after casing and again 2 weeks later. According to Geijn (1977) disease can be controlled by spraying with carbendazim, benomyl or thiophenate methyl at 100, 150 and 200g/100m^2, respectively in 100-150 litres of water immediately after casing. Cased beds can also be treated with 0.5% formalin or 100g carbendazim, 150g benomyl or 200g thiophenate methyl in 100-150 litres of water per m^2 of bed. Zaayen (1979) obtained highest yield with chlorothalonil at 3g/ litre water /m^2 applied directly after casing and again 2 weeks later. Good control of *V.fungicola* was achieved by spraying with prochloraz manganese at 60g/ 100m^2 within 7 days of casing and subsequently at 2 weeks intervals (Fletcher and Hims, 1981). Fungicides triadimefon (1g/m^2), prochloraz (1g/m^2),

Delsene M (carbendazim + maneb) (8g/m^2) and chlorothalonil (2g/m^2) applied after casing increased the yield from 39.9% in untreated controls to 56.7, 56.3, 54.6 and 53.1%, respectively (Gandy and Spancer, 1981). Zaayan and Adrichem (1982), Russell (1984), Eicker (1987) recommended the application prochloraz + manganese complex (Sportak 50WP) at 1.5g a.i/m^2, 9 days after casing. However, only partial control has been achieved by intensive use of prochloraz in Spain (Gela, 1994). Eicker (1984) recommended the application of Tecto as drench (450g a.i thiabendazole/dm^3) at dosages of 1.838g a.i/m^2 after casing and 1.44g a.i/m^2 between each break. Application of Amitrole T at 50g, paraquat at 10g and diuron at 20-40g after 24 hours of inoculation were effective against *V.malthousei* (Popple, 1972). Zaayan and Geijn (1979) suggested new possibilites for control of diseases which advocated application of formaldehyde (2 litre/100 litres of water/100m^3) immediately after casing for effective management of disease. If disease reappears, replace formaldehyde by benlate, bavistin, Topsin M throughout one cultivation cycle. If *V.fungicola* becomes resistant to these fungicides, chlorothalonil (3g /m^2) can be used immediately after casing and again 14 days later or Curamil (pyrazophos) (at 0.5ml/m^2) can be applied after casing and thereafter at weekly intervals. According to Flegg (1968) fumigation with methyl bromide at a CTP of 600 oz/hr/ 1000 cu.ft or more can provide a satisfactory alternative to cook out with live steam.

2. WET BUBBLE

Pathogen : *Mycogone perniciosa*

Common Name : Wet bubble, La mole, white mould, bubble, *Mycogone* disease

Wet bubble in white button mushroom incited by *Mycogone perniciosa* Magn. has been reported as one of the serious diseases from almost all the major mushroom growing countries of the world. Bubbles or mole (*M. perniciosa*), first described from Paris in 1888, is stated to be responsible for the heaviest losses in mushroom beds in France, England and United States (Nielson, 1932). The disease has also

been reported to assume serious proportions in other major mushroom growing countries of the world such as United Kingdom, Netherlands, USA, China, Taiwan, South Africa, Brazil, Hungary, Australia and Poland from time to time. In India, this disease was reported for the first time in 1978 from some mushroom farms in Jammu and Kashmir (Kaul *et al*., 1978). Later, this disease has been reported from the States of Himachal Pradesh, Haryana and Maharashtra (Sharma, 1994, Sharma and Kumar, 2000, Bhatt and Singh, 2000).

Symptomatology : Many workers have described Symptoms of wet bubble at different stages of mushroom development. Smith (1924) recognised two main symptom types, infected sporophores and sclerodermoid masses, which he considered to be the result of infection by *M. perniciosa* at different stages in the development of the sporophores. Thus, when infection took place before the differentiation of stipe and pileus the selerodermoid form resulted, whereas, infection after differetration resulted in the production of thickned stipe with deformation of the gills (Fletcher and Ganney, 1968). Garcha (1978) described the symptoms in the form of white mouldy growth on the mushrooms, leading to their putrifaction (giving foul odour) with a golden brown liquid exudate. Hsu and Han (1981) reported that the infected sporophores may be recognised by two symptoms, one is tumorous form, infected from pinheads, and other is malformation, infected at later stage. Both types of infections may exude water drops on the surface of infected sporophores. These water drops later change into amber colour. Tu and Liao (1989) observed that when young pin heads are infected they develop monstrous shapes which often do not resemble mushrooms. Fletcher and Ganney (1969) have reported about 31% infection at the base of the stipe in apparently healthy sporophores in the form of black streaks. Sharma and Kumar (2000) described the symptoms as short, curly, pure white fluffy mouldy growth of the pathogen on malformed mushrooms, which can be easily observed by nacked eyes. Cross section of deformed sporophores without cottony growth showed black circular area just beneath the upper layer. Umar *et al.*, (2000) described dramatic cytological changes as a result of infection when young (up to 6mm) pin heads were infected. Large, very

Symptoms of wet bubble disease

irregular, nodular and tumorous fungal masses are formed and no differentiation or organogenesis of the cell mass takes place. Mycopathogen grew on the surface as fluffy mycelium but was absent deep on the lesions. Transmission EM revealed two kinds of cell wall reactions, either focal swelling like cushion at the site of adhesion of *M. perniciosa* or focal lytic changes with swollen mitochondria.

Nielson (1932) stated that wet bubble caused heaviest losses among all diseases in mushroom beds in France, England and United States. In USA, *M. perniciosa* was isolated from 3.7 per cent samples collected from various mushroom farms. Forer and his associates (1974) while estimating the qualitative and quantitative losses caused by wet bubble and dry bubble in Pennsylvania (USA), reported that these two diseases induced 2.2 million lbs. as quanlitative and 19.7 million lbs as quantitative loss of mushrooms. Nair (1977) conducted a survey of 24 mushroom farms in

New South Wales during 1975-76 and observed that the most economically important diseases in these farms include wet bubble. Sharma and Kumar (2000) reported that the natural incidence of wet bubble disease of button mushroom ranged from 1 to 100 per cent in northern India. Loss in yield in *A. bisporus* (S-11) due to this disease under artificial inoculation conditions has been reported to vary from 15.72 to 80.13 per cent. Bhatt and Singh (2000) have reported the yield loss up to 100 per cent as a result of artificial inoculation of *M. perniciosa*.

Etiology : The disease, wet bubble, is caused by *Mycogone perniciosa* Magn. and the perfect stage is *Hypomyces perniciosa*. Mycelium of the pathogen is white, compact, felt-like. Hyphae branched interwoven, septate, hyaline, 3.5m broad. Conidiophores short, slender, branched, hyaline measuring 200 x 3-5m and having sub-verticillate to verticillate brances which bear thin walled, one-celled conidia measuring 5-10 x 4-5m. Large two-celled chlamydospores present; upper cell warty, thick walled, globose, bright coloured measuring 15-30 x 10-20m, lower cell hyaline, smooth and measure 5-10 x 4-5m.

Host Range : *Mycogone perniciosa*, though a major pathogen of *Agaricus bisporus*, is also capable of infecting other mushroom species. Figueiredo and Mucci (1985) revealed that *M. perniciosa* can infect *A. campestris*. Sisto *et al*., (1997) have reported *Pleurotus eryngii* and *P. nebrodensis* susceptible to *M. perniciosa*. Sharma and Kumar (2000) reported all the strains of *A. bisporus* (U-3, S-11, 791, S-910) and *A. bitorquis* (NCB-6, NCB-13) susceptible to *M. perniciosa* under *in vivo* conditions.

Spread : Spread of *M. perniciosa* occurs primarily through casing soil but the introduction of pathogen through other agencies, like spent compost and infected trash, is not ruled out. The infection can be air-borne, water borne or may be mechanically carried by mites and flies (Garcha, 1978). Hsu and Han (1981) reported water splash as an important factor for wet bubble spread on the beds. Bech *et al*., (1982) reported that spread through contact occurred readily during watering and especially harvesting. They also observed that contaminated containers can be a source of spread over greater distances. Contrary to other reports it was also suggested that spores of *M. perniciosa* can also be spread by

air current (Tu and Liao, 1989). Kumar and Sharma (1998) reported that transmission percentage of *M. perniciosa* under *in vitro* conditions, by sciarid and phorid flies was 100 per cent on MEA medium and 4-12 per cent on compost. Chlamydospores have been reported to survive for a long time (upto 3 years) in casing soil and may serve as the primary source of inoculum. The aleurospores produced on the surface of monestrous structures are probably responsible for secondary infection.

Biology / Physiology : Lambert (1930) revealed that *Mycogone perniciosa* is quite sensitive to prolonged exposure to moderately high temperature. The cardinal temperatures for growth of the organism on Thaxter's agar are 8°C, 24°C and 32°C. He also reported that in agar cultures *M. perniciosa* was killed by exposures to temperatures of 42°C (106°F) or higher for 6 hr. or more. According to Zaayen and Rutigens (1981) thermal death point for *M. perniciosa* is 48°C. Bech and Kovacs (1981) reported that aqueous suspension of *Mycogone* spores can withstand 42°C and 36°C for 10 minutes and 1 hr, respectively. Hsu and Han (1981) reported that optimum temperature for mycelial growth, sporulation and conidial germination was 25°C. He also recorded pH 6.0 as optimum for conidial germination. According to Liao (1981) chlamydospore failed to germinate on various media *in vitro* even after heat (40-70°C) treatment or application of chemicals and solvent. However, germination occurred on potato dextrose agar (PDA) medium exposed to the gas produced by mushroom mycelia in compost for 36 hrs at 24°C. In anthor study Bech and Kovacs (1981) found that aleurospores are unable to germinate in water, Richard's solution, pressed mushroom juice or in PDA but verticilloid spores showed a certain degree of germination in diluted mushroom juice and on PDA. As reported by Tu and Liao (1989) the pathogen is tolerant to a wide pH range in acid side and able to grow at pH 4.4, however, the growth becomes weaker or rather restricted at pH 8.4. Holland and Cooke (1991) reported that in malt extract agar medium *M. perniciosa* formed abundant thin walled, hyaline phialo conidia and thick walled pigmented verrucose conidia. During nutrient deplection other propagules appeared, namely, lateral smooth conidia, infected interculary cells, chlamydospores and arthro conidia. Singh and Sharma (2000) have reported the maximum growth of *M. perniciosa*

on PDA. Optimum temperature and pH for growth were reported to be 25°C and 6.0, respectively. Mannose and asparagine have been reported as best sources of carbon and nitrogen, respectively. Sharma and Kumar (2000) observed compost extract agar medium as the best for the mycelial growth and malt extract peptone dextrose agar medium for spore production. A pH range 5-6 was found optimal for the mycelial growth.

Management : As the pathogen inflicts serious damage to the crop, various attempts have been made to manage the disease through various means.

a) Physical : Wuest and Moore (1972) suggested that aerated steam at 54.4°C for 15 minutes can eliminate *M. perniciosa* from casing soil. Munns (1975) suggested the use of plastic pots to cover mushroom showing wet bubble symptoms during the cropping season to prevent spread of disease. Tu and Liao (1989) while working to find out an integrated approach for the management of wet bubble disease revealed that the use of clean compost, pasteurization or sterilization of casing soil, good peak heating and fumigation of mushroom house and use of benomyl or Mertect 40 per cent were effective in managing *M. perniciosa*. Zhang (1990) suggested three methods of prevention of wet bubble disease which include steam sterilization of mushroom beds, formaldehyde fumigation and fungicidal application. Another method like screening and selection of disease resistant strains should also be exploited.

b) Biological : Jhune *et al*., (1990) screened 12 isolates of bacteria and 71 isolates of actinomyectes isolated from mushroom compost and casing mixture and observed AJ-117, AJ-136 and AJ-139 as promising bioagents. Though, almost negligible attempts have been made to control *M. perniciosa* through botanicals but the inhibition of fungal growth by plant extracts is not uncommon and has been reported earlier by a number of workers (Flierman, 1973; Michal and Judith, 1975). Gandy (1979) made an interesting observation that *Acremonium strictum* produces a heat stable antibiotic compound possibly a cephalosporin, which is inhibitory to *M. perniciosa* but no attempts have been made to explore this approach as both

fungi are pathogenic to mushrooms.

c) Chemicals : Benomyl spray at 0.5-4g/m^2 immediately after casing has been reported very effective for protecting the crop (Gandy, 1974; Stanek and Vojtechovska, 1972). Fletcher (1975) advised that adequate control of wet bubble was obtained by benomyl or Thiophanate methyl at 10g a.i. at casing while TBZ was less affective. Kim (1975) recorded satisfactory control of wet bubble by spraying benomyle @ 0.5g a.i/ m^2, 3 days after casing. Geijn (1977) suggested the control of wet bubble disease by spraying the crop with carbendazim, benomyl or thiophanate methyl at 100-150 litre water immediately after casing. Basamid (Dazomet) and Vapam (Metham sodium) applied @ 100ppm to casing has also been reported very effective (Kim *et al.*,1978). Application of carbendazim, benamyl, chlorothalonil, TBZ, prochloraz manganese complex (Sportak 50 WP) into casing mixture have been reported very effective for the management of wet bubble by several workers (Hsu and Han, 1981, Zaayen and Adrichem, 1982; Fletcher, 1983; Zaayel *et al.*, 1983; Eicker, 1984; Jhune *et al.*, 1991; Sharma and Kumar, 2000). It was reported that if casing is contaminated control can be achieved by treating it with 1 per cent formalin. Alternatively, a spray of 0.8 per cent formalin on to casing surface, immediately after casing, can be effective. However, this concentration can be injurious if used at a later stage in crop development. Sharma *et al.*, (1999) have reported 62.5-100 per cent inhibition of *M. perniciosa* in culture when inoculum discs were drenched in 0.5-2% formalin solution for 5 seconds. Exposure of *M. perniciosa* cultures to vapours of 1-4 per cent formalin for 6-24 hrs also resulted on 100 per cent inhibition of fungal growth on sub-culturing.

3. COBWEB

Pathogen : *Cladobotryum dendroides*

Common Name : Mildew, Soft decay, Hypomyces mildew disease, Dactylium disease.

This disease renders extensive damage either by causing soft rot or

decay of fruiting body. Merat (1821) described this disease as *Botrytis dendroides* and transferred it in to the genus *Cladobotryum* by making a combination *C.dendroides* (Bull : Merat) W.Gams et Hoozem. Salman and Ware (1933) were the first to report *D.dendroides* being parasitic to mushrooms. According to Fletcher and Atkinson (1977) mushroom of any age of development would be attacked by this fungus. This disease causes great damage to mushroom houses where humidity is high (Bozhkor, 1975). Forer *et al*. (1974) isolated *C.dendroides* from 0.6% of mushroom sampled from commercial mushroom houses in Pennsylvania. In India it was first recorded in Chail and Shimla (HP) (Seth, 1977) and later from Solan and Kasauli with natural incidence ranging from 8.17 - 18.83% in 1982 and 1.93-25.63% (Seth and Dar, 1989). Under artificial inoculation conditions with different levels of inocula, the loss in marketable mushrooms has been estimated at 66.6% (Sharma and Vijay, 1996) and 21.95 - 48.95% at different temperatures (Seth and Dar, 1989). Sharma *et al*. (1992) recorded *C.verticillium* a new pathogen of *A.bitorquis* in Himachal Pradesh.

Symptomatology : Cobweb appears first as small white patches on the casing soil which then spreads to the nearest mushroom by a fine grey white mycelium. A floccose white mycelium covers the stipe, pileus and gills, eventually resulting in decomposition of entire fruit body. As the infection develops, mycelium becomes pigmented eventually turning a delicate pink cover (Lane *et al*. 1991). In severe attacks, a dense white mould develops over

Symptoms of cobweb

casing and mushrooms change from a fluffy cobweb to a dense mat of mycelium. The white colour can turn pink or even red with age. One symptom which can appear and which is generally not associated with the disease is cap spotting. The spots can be brown or pinkish brown (Sharma, 1994). On inoculated fruit bodies, characteristic symptoms appeared within 24 hours of inoculation when mycelial + spore suspension were applied, symptoms appeared 4-12 days after infestation. Younger mushrooms are more susceptible than fully developed ones. Tuffs of conidiophers develop on all sides of the web and growth of engulfed mushroom is arrested. On removal of mycelial felt from affected mushroom, drops of dark brown coloured fluids exudes emitting bitter foul smell (Seth and Dar, 1989).

Causal organism : *Cladobotryum dendroides* (*Dactylium dendroides*) imperfect state of *Hypomyces rosellus*. Sterile hyphae form a turf and are prostrate, branched, septate and hyaline with approximately opposite branches, which divide above into usually three pointed branchlets. Conidiophers are erect, similar or branched in many whorls. Conidia single, elongate pointed at the base, 2-3 septate, slightly constricted at the septa and measure 20-30 x 10-12.5 u. It produces sexual stage belonging to *Hypomyces rosellus*, which has been observed on decaying dried fruit bodies of wild mushrooms in HP.

Epidemiology : High relative humidity and temperature encourage the disease. Spread is mainly by conidia. The pathogen is a soil inhabiting fungus and is normally introduced into the crop by soil contamination, spores, mycelium on crop debris or by farm workers. Spores are easily spread by air movement, workers hands, tools and clothing and by water splash (Sharma, 1994). Under laboratory conditions, sciarids and phorid flies were found to transmit 4-100% of the disease in to two different media (Kumar and Sharma, 1998). A high RH and temperature range of 19-22°C and 12-15°C resulted in maximum loss in yield (Seth and Dar 1989). Optimum temperature for growth is 20°C and for spore germination is 25°C. *C.dendroides* has been isolated from woodland soil (Canada) moss (*Polytrichum* sp.) (UK), a bracket fungus *Stereum* sp. (UK), dead wood (*Pinus* sp.) and mushroom farms (Lane *et al*. 1991). On the other hand disease caused by *C. verticillatum* on *A. bitorquis* was favoured by RH 90% and

temperature of 25-30°C (Sharma *et al*., 1992).

Management

Physical : Through disinfection of casing soil with live steam or sterilization of casing mixture at 50C for 4 hours effectively eliminates the pathogen. Regular cleaning, removal of cut mushroom stems and young half dead mushrooms after each break and controlling temperature and humidity helps in controlling the disease (Sharma, 1994).

Biological : Under laboratory conditions, leaf extract of *Cannabis sativus*, *Ricinus cummunis*, *Callistemon lanceolatus*, *Citrus* sp., *Euclyptus* sp., *Dhatura* sp. and *Urtica dioica* were found to cause 5.55%, 10.55%, 18.55%, 26.11%, 34%, 19.07% and 23.33% inhibition of *C.dendroides* (Sharma and Kumar, 1988).

Chemical : Terraclor (pentachloronitrobenzene) can eradicate *Dactylium* mildew even after the well establishment of the disease (Stoller *et al*., 1956). Bozhkor (1975) suggested annual disinfection of houses and surrounding areas with 2% bordeaux mixture or with 5% formation solution at 0.5-1.0 l/m^2 or fumigation with 2.0-2.5 l formation ands 0.5-1.0 kg chlorinated lime/100 m^3 for controlling disease. He further suggested that immediate spray after casing with benomyl at 1g in 0.5 -1.0 l water/m^2 also controls the disease. According to Russell (1984) single application of prochloraz manganese complex (sporogon) at 1.5g a.i./m^2 of bed 9 days after casing gives satisfactory control of the diseases. Seth and Dar (1989) obtained best control of disease by applying bavistin + TMTD at 0.9 and 0.6g/m^2 followed by TBZ and benlate (0.9g/m^2). Effective control of *C. verticillatum* was obtained by spraying with 0.05% carbendazim at spawning followed by 0.25% mancozeb at casing and carbendazim again 15 days later (Sharma *et al*. 1992).

4. GREEN MOULD

Pathogen : *Trichoderma viride, T. hamatum, T. harzianum, T. koningii, Penicillium cyclopium, Aspergillus* spp.

Common names : *Trichoderma* spot, *Trichoderma* blotch, *Trichoderma* mildew, Green mould

One of the most common and destructive diseases in mushroom cultivation is the green mould which

is mainly caused by different species of *Trichoderma, Penicillium* and *Aspergillus*. Among these moulds, *Trichoderma* spp. induce significant quantitative and qualitative losses in the yield of *Agaricus bisporus, Pleurotus* spp., *Auricularia, Calocybe indica* and *Lentinula edodes*. Kligman (1950) was the first to report the presence of *Trichoderma* in mushroom compost. Different species of *Trichoderma* which have been reported as competitors and / or pathogenic on button mushroom include, *T. viride, T. koningii, T. hamatum, T. harzianum, T. atroviride, T. pseudokoningii, T. logibrachiatum*. Among all these species, *T. harzianum* is recognised as causing the most severe problems (Morris *et al.*, 1991; Seaby, 1996). Seaby (1989) recorded 9 distinct groups of *Trichoderma* species and strains which had almost similar spore bearing structures as described by Rafai (1969). These included, *T. viride, T. harzianum* (Th-1, Th-2, Th-3), *T. koningii, T. pseudokoningii* and *T. longibrachiatum*. Genetically distinct biotype Th-4 of *T. harazianum* has been responsible for serious outbreak in USA. In India, *T. viride* was first reported by Thapa and Seth (1977) on *A. bisporus,* and *T. hamatum* and *T. harzianum* by Seth and Bhardwaj (1986-87).

Economic Importance : Green moulds caused by *Trichoderma* species were once recognised as indicators of poor compost quality and were of minor significance. The devastating nature of *T. harzianum* was undocumented in mushroom industry until 1985 when it was first observed in Ireland and resulted in losses estimated at 3-4 million pounds to the U.K. and Irish mushroom industries. The second wide spread epidemic occurred in early 1990's in Ireland (Seaby, 1996). Green mould epidemics have been reported from the USA, Canada, South America, Asia, Australia and European countries. *T. harzianum* biotype Th-2 was responsible for severe epidemics in Europe and biotype Th-4 in America (Mamoun *et. al.*, 2000). Crop losses to green mould are variable, however, since the onset of the disease in Pennsylvania crop losses have been estimated in excess of $30 million (Anderson *et al.*, 2000). Yield losses in first flush of *A. bisporus* by artificial inoculations have been up to 8% for *T. pseudokoningii* and 26% for *T. atro viride* (Grogan *et al.*, 2000). Sharma and Vijay (1996) have reported yield loss in *A.*

bisporus from 12.5-80.8% by artificial inoculation of *T.viride* at different inoculum loads and at different stages of crop growth. Jandaik and Guleria (1999) reported 5-46.87% and 6.25-50.0% yield losses due to *T. viride* and *T. harzianum*, respectively under artificial inoculation conditions. Anderson *et al*., (2000) recorded significant differences among hybrid mushroom strains in response to *T. harzianum* biotype 4 (Th-4) infestation. Hybrid white strains were the least resistant to green mould, sustaining yield losses upto 96%. Hybrid off-white strains exhibited intermediate susceptibility with yield losses of 56-73%. Brown strains were significantly resistant to green mould, sustaining yield losses of only 8-14%.

Symptomatology : Different species of *Trichoderma* have been reported to be associated with green mould symptoms in compost, on casing soil, in the spawn bottles and on grains after spawning. A dense, pure white growth of mycelium may appear on casing surface or in compost which resembles to mushroom mycelium. Later on mycelial mat turns to green colour

Symptoms of green mould

because of heavy sporulation of causal agent which is a characteristic symptom of the disease. Thereafter, the mould creeps to surface of casing layer and infects the new parts and developing newly borne primordia. Mushrooms developing in or near this mycelium are brown, may crack and distort, and the stipe peels in a similar way to mushrooms attacked by *Verticillium fungicola* causing dry bubble disease. Some species induce brownish lesions / spots on caps which may cover the entire cap surface under congenial conditions.

Causal organism : Several species of *Trichoderma* are associated with green mould disease complex of *A. bisporus*. The taxonomy of this genus has caused confusion and a succession of mycologists have investigated it since the turn of the century. In 1939, A. R. Bisby reviewed the literature and concluded that although there were differences in morphology between types these were not consistent and distinct. He, therefore, classified all types as *T. viride* whilst recognizing that considerable but inconsistent variability existed. In 1969, Rifai revised the genus and proposed nine species aggregates. His classification is now the one that is generally accepted. The species recorded in mushroom culture include *T. viride*, *T. harzianum* and *T. koningi*. *T. viride* is said to be weed mould, *T. koningi* a pathogen whilst *T. harzianum* has been ascribed to a variety of roles including pathogen and agent for biological control. Four biotypes namely, Th-1. Th-2, Th-3, and Th-4 have been further characterized in *T. harzianum* on the basis of occurrence, symptoms, morphological characters and physiological requirements (Seaby, 1989).

***T. viride* (*T. lignorum*) :** This is widespread in soil. Spore are ovoid, rough walled, green and measure 2.8-5x2.8-4. The colony emits coconut odour. This fungus grew slowly at 27°C but faster at 20°C.

***T. koningi* :** This is common inhabitant of soil. Spores are smooth walled, cylindrical, green and measure 3-4.8x1.9-2.8m. Colony emits no odour. Spores germinate faster than other species and growth rate is 1-1.2mm/hr.

***T. harzianum* :** Common in soils. Colonies growing rapidly, most isolates 7-9cm in diameter after 3-4 days, aerial mycelium floccose, white to greyish. Conidiation on MEA initially as compact and produce a flat postule often concentric that is green whitish and later turn to dark

green colour. Chlamydospores fairly abundant, intercalary or terminal, solitary, smooth walled, mostly 6-12 mm. in diameter. Macronematous conidiophores highly branched. Conidia smooth walled, ovoid, green in colour and measure 2.4-3.2x2.2-2.8m. Four biotypes, Th-1, Th-2, Th-3 and Th-4 have been further characterized in *T. harzianum* on the basis of morphological, cultural, physiological and genetical variations.

Epidemiology : Green mould generally appears in compost rich in carbohydrates and deficient in nitrogen. If the compost is tampled too hard in the beds, or the filling weight is too high, this can make the peak heating difficult. This is certainly the case with compost which has a short texture and which might also have too high moisture content, resulting in improper pasteurization and conditioning of compost. Frequent use of formalin also tends to promote the development of green moulds (Sharma *et al*., 1999). Different sources of primary inoculum of *Trichoderma* spp. could be dust particles, contaminated clothings, animal vectors especially the mite, *Pygmephorus mesembrinae*, mice and sciarid flies, air-borne infection, infected spawn, surface spawning, contamination of compost by handling and machinery and equipments at the mushroom farm (Seaby, 1987). Spore concentration less than $1x10^2$ was unable to cause infection (Grogan *et al*., 2000). Benomyl treated grain spawn or compost spawn in normal compost had less *T. harzianum*. High relative humidity accompanied by a low pH in the casing soil also promotes the development of *Trichoderma* spp. (Sharma and Jandaik, 1999). Chlamydospores produced by *T. harzianum*, *T. viride*, *T. longibrachiatum* and *T. pseudokoningii* survived the exposure of 9 hours at 60°C (Morris *et al*., 2000). *T. harzianum* induced significant yield reductions at 30°C than at 20°C (Seaby, 1986).

Control : Green moulds can be prevented by

a) Very good hygiene

b) Proper pasteurization and conditioning of compost.

c) Sterilizing the supplements before use and mixing them throughly preferably after spawning.

d) Using the correct concentration of formalin (maximum 2%)

e) Weekly sprays of mancozeb(0.2%) or bavistin (0.1%) TBZ(0.2%) or treatment with zineb dust or Calcium hypochlorite (15%) have given effective control of the disease.

b. Competitor moulds

5. FALSE TRUFFLE

Pathogen : *Diehliomyces microsporus*

Common name : Truffle disease

This is the most dreaded competitor in mushroom beds. It was first reported by Lambert (1930) from Ohio, USA during 1929 and described by Diehl and Lambert (1930) as *Pseudobalsamia microspora*. Glasscock and Ware (1941) observed it in UK and studied its invasion in mushroom beds. False truffle incidence in the Netherlands was reported by Bels-Koning and Bels (1958) but its serious incidence was noticed in the crops of *Agaricus bitorquis*, grown at higher temperature (Zaayen and Pol-Luiten, 1979). Gilkey (1954) reclassified the fungus from Tuberales to the Eurotiales and named the genus *Diehliomyces*. In India, Sohi *et al*. (1965) observed false truffle causing serious losses to mushroom crops when the compost temperature in the trays reached beyond 22-24C. The natural incidence of false truffle in *A. bisporus* grown under natural climatic conditions has been reported from 1-80% in the States of Himachal Pradesh, Haryana, Punjab and Uttar Pradesh (Sharma and Vijay, 1996). False truffle is a limiting factor in the production of *A. bitorquis* in India because of its higher temperature requirements. The disease is of common occurance during February or early March in *A. bisporus* in the plains of the Northern India and during summer months in *A. bisporus* and *A. bitorquis* in hilly regions of the country. Sharma and Jandaik (1996) reported 66-88 per cent incidence of this competitor in Himachal Pradesh during 1993-1996 resulting in 58-80% yield loss.

Symptoms : The coulour of the fluffy mycelium is white to start with and turns a creamy yellow at a later stage. It appears as small wefts of white cream coloured mycelium in compost and casing soil, usually more conspicuous in the layer where compost and casing mixture meet and also on casing. Gradually the mycelial growth become thicker and develops into whitish, solid, wrinkled, rounded to irregular fungal masses resembling small

Symptoms of false truffle

brains (ascocarps of the fungus), looking like peeled walnuts. They vary appreciably in size ranging from 0.5 to 3cm in diameter. At maturity they become pink, dry and reddish and finally disintegrating into a powdery mass emitting a chlorine like odour. The fungus does not allow the mushroom mycelium to grow and compost turns dull brown. The spawn in affected patches turns soggy and disappears.

Causal Organism : *Diehliomyces microsporus* (Diehl and Lambert) Gilkey, ascocarps are formed from the dense tangled hyphal knots singly or several knots coalesce to form large ascocarp. Ascocarps are fleshy, at first white then brownish and finally reddish brown containing numerous sac like asci which are oval, sub-spherical, short or long-stalked, with 3-8 ascospores, 19-27x10.5-15m. Ascospores are spherical, sulphur coloured with one distinct oil drop and measure 6.5m in diameter. Chlamydospores may be noticed in the hyphal web of ascocarp.

Epidemiology : Ascospores develop in the truffles in 3 to 6 weeks and are released when the truffle disintegrates. Ascopore production is abundant at 25 and 30°C but not at 15 or 37°C (Wood and Fletcher, 1991). Ascopore germination upto 70% has been recorded at 27°C after giving heat stimulus at 40-50°C for half an hour. (Zaayen and Pol-Luiten; Sharma 1998). The major sources of infection are casing soil and surviving ascospores/mycelium in wooden trays from the previous crops. Ascopores can survive for a periods of 5 years in soil and spent compost and mycelium for 6 months (Sharma, 1998) and thus serve as the major source of primary inoculum.

Stage of infection and temperature are important factors for determining the severity of the disease. Optimum growth of the fungus has been recorded at 26-28°C. False truffle seems to depend either on mushroom metabolities or on depletion of inhibitory factor by mushroom mycelium. It is mainly a disease of *A. bitorquis* wherein crop is raised at 25±1°C but it also develops very fast in *A. bisporus* when crop is taken under natural climatic conditions and temperature rises above 20°C.

Control

1. Compost should be prepared on a concrete floor and never on uncovered soil. Because during composting there is rise in temperature which activates the ascospores present in the soil.

2. Pasteurization and conditioning of the compost should be carried out carefully. Maszkiewiez and Szudyga (1999) observed that pasteurization of compost under optimum condition completely eliminated the false truffle incolum in the compsot.

3. Temperature above 26-27°C during spawn run and after casing should be avoided. During cropping, temperatures should be kept below 18°C . Under such conditions, it is practically impossible to grow *A. bitorquis* but disease can be managed effectively in *A. bisporus.*

4. Casing soils known to harbour traces of spores should not be used. Young truffles must be picked and buried before the fruit bodies turn brown and spores are ripe.

5. Woodwork, trays or side-boards of shelf-beds should be treated with a solution of sodium-pentachlorophenolate at the end of the crop which was infected with the truffle disease. Air-drying of wood-work for 2-3 months may also eradicate the pathogen.

6. Good cooking out (compost temperature 70°C for 12h.) at the end of the crop should be carried out which will kill mycelium and spores of the pathogen in the compost. Wooden trays should be separately chemically sterilized. Thermal death point of ascospores and mycelium has been reported to be 70°C for 1 hr. and 45°C for 30 minutes, respectively (Sharma, 1998).

7. Initial infection can be checked by treating the affected patches with formaldehyde (2%) solution (Sohi, 1988).

6. OLIVE GREEN MOULD

Pathogen : *Chaetomium olivaceum*, *C. globosum*

The first evidence of the occurrence of *C. olivaceum* in India was provided by Gupta *et al*., (1975) at the mushroom farm at Chail, Kasauli and Taradevi. Another species, *C. globosum*, was later reported from mushroom farms in HP, Delhi and Mussorie (Thapa *et al*., 1979). Yield losses ranging from 12.8-53.65% have been reported in *A. bisporus* (Sharma and Vijay, 1996).

Symptoms : The earliest signs of the fungus consist of an inconspicuous greyish-white fine mycelium in the compost or a fine aerial growth on the compost surface 10 days after spawning. Frequently initial spawn growth is delayed and reduced. By late spawn run, fruiting structures that look like gray-green cockle-burns-1/16 inch in diameter, develop on straw in isolated spots of the affected compost. The compost will have a musty odour. Compost

Symptoms of olive green mould

not supporting spawn growth generally supports the growth of *Chaetomium* and other several moulds and hence olive green mould is not the exclusive colonizer of black compost. Spawn usually grows into areas occupied by *Chaetomium*, although normal spawn growth is delayed. *C. globosum* is also noticed on spawn bottles.

Causal organism : *Chaetomium olivaceum* Cooke and Ellis, *C. globosum* Kunze ex Steudel

The fungus consists of a grayish white mycelium which later produces perithecia. Perithecia of *C. olivaceum* are superficial, opaque, globose, thin, membranous with an apical tuft of dark bristles of setae. Asci clavate and evanescent. Ascospores dark brown, broadly ovoid, umbonate at both ends and measure 9-12.5x7-9.5m. Perithecia of *C. globosum* are scattered or gregarious, broadly ovate or ellipsoid, often pointed at the base, thickly and evenly clothed with slender flexuous hairs. Asci oblong-clavate and evanescent. Ascospore dark, broadly ovoid, faintly apiculate at both ends and measure 8-9.5x6-8m.

Epidemiology : The infection usually comes through air, compost and casing soil. It appears due to defective composting in phase-II because of improper pasteurization accompained by high temperatures in the absence of adequate fresh air. Improper stacking of the compost trays in the pasteurization room which do not allow proper circulation of the air or overfilling of the room causes intensive condensation when wet steam is introduced, result in non-selective compost which harbours *Chaetomium* and other moulds. Spores are resistant to heat and are probably not killed easily during pasteurization. It is also well known that spores of *Chaetomium* are already present in the compost (Munjal *et al*., 1977, Sharma, 1992) which are activated by bad peak heating control. When compost is too wet, penetration of air is less which results in the conversions of nitrogenous compounds in wrong direction. Unfavourable conversions often results in renewed production of anhydrous ammonia which prompts the growth of ammonia. Sometimes the temperature is too high in certain spots of a room, or may be less of oxygen which often results in olive-green mould appearance. Ascospores are spread by air flows, clothes and other materials used in mushroom farm.

Control

1. The fermentation period of the compost should not be too short. It is essential to provide an active compost that is not too wet and has a good structure.

2. Do not add nitrogen, ammonium sulphate, urea, chicken manure or similar materials just before filling.

3. There should be sufficient time for peak-heating and sufficient supply of fresh air during pasteurization. Higher temperatures (above 60°C) for longer time should be avoided.

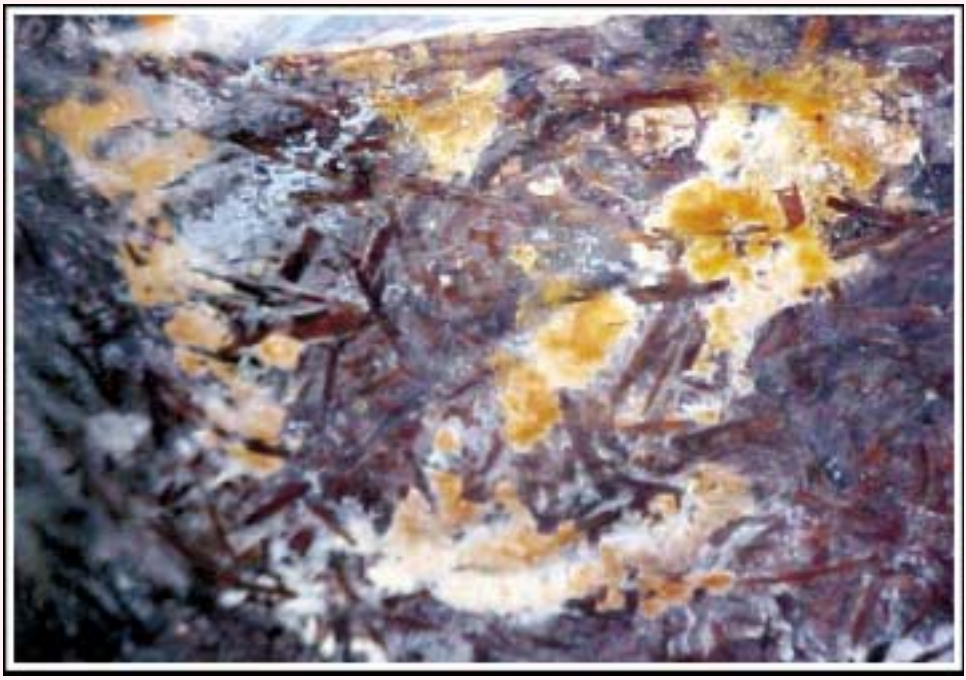
4. Large number of fungicides including Benomyl, Thiophanate methyl, TBZ, Vitavax, Dithane Z-78, Dithane M-45, Thiram and Captan have been found effective under *in-vitro* conditions (Thapa *et al*., 1979) and sprays of Dithane Z-78 (0.2%) have been recommended for checking the secondary spread (Sohi, 1986).

8. BROWN PLASTER MOULD

Pathogen : *Papulaspora byssina* Hots.

Papulaspora byssina was first reported on horse dung compost from Missouri (Hotson, 1917). Charles and Lambert (1933) later reported its occurrence on mushroom beds and recorded delayed yields in the presence of this mould. This disease has also been reported from India (Munjal and Seth, 1974) causing 90-92% yield loss in *A. bisporus*. This mould has also been reported to cause complete crop failure in oyster mushrooms in Kasuali, HP (Dar and Seth, 1981). This fungus now is frequently found at almost all the mushroom farms in India appearing usually during spawn run (Garcha *et al.* 1987; Kaul *et . al*. 1978; Sharma, 1992). This mould has invariably been isolated from different compost and casing samples collected from mushroom farms in northern India and the incidence of the disease has been recorded from 5 to 9%. (Sharma and Vijay, 1996). Loss in number and weight of fruit bodies as a result of artificial inculation of the mould has been found 7.7-53.5% and 3.0-50.7% respectively (Sharma, 1990; Sharma and Vijay, 1993).

Symptoms : It is first noticed as whitish mycelial growth on the exposed surface of compost and casing soil in trays as well as on sides in bags due to moisture condensation. This develops further into large dense patches gradually



Symptoms of brown plaster mould

changing colour through shades of tan, light brown to cinnamon brown; ultimately becoming rust coloured. No mushroom mycelium grows on places where plaster mould occurs.

Causal Organism : *Papulospora byssina*

The mycelium is brownish, septate; later produces clusters of brown coloured many celled, spherical bulbils measuring 60-130x30-190µ. These are inter-woven with a net work of hyphae, are set free later with the death of the mycelium.

Epidemiology : Primary infection comes through air-borne bulbils or containers, compost and casing soil and workers. Its development is favoured by wet, soggy and wrongly prepared compost. Higher temperature during spawn run and cropping favours the disease development. In wet, greasy compost which had not received enough oxygen during fermentation and many of amines, development of the disease is greatly favoured. Addition of less quantity of gypsum and more greasiness favour the disease development.

Control

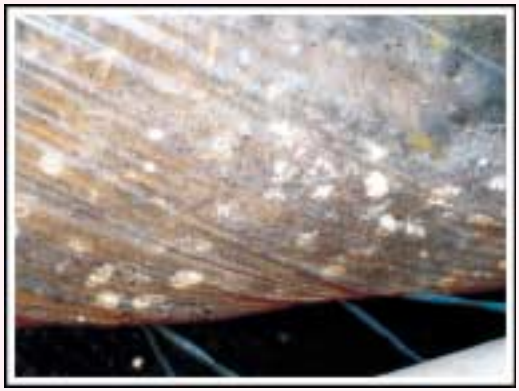
Composting should be carried out carefully, using sufficient gypsum and not too much water. Peak heating should be of sufficient duration and at proper temperatures. The compost should not be too wet before or after peak heating.Munjal and Seth (1974) recommended localized treatment of infected patches with 2% formalin while Seth and Shandilya (1978) recommended 4% formalin for its control.Large number of fungicides namely, benomyl, carbendazim, thiophanate methyl, vitavax, daconil, MBC, dithane Z-78, dithane M-45, captan, thiram and copper fungicides have been screened under *in vivo* and *in vitro* conditions by various workers (Thapa *et al.* 1979; Kaul *et al*., 1978; Dar and Seth, 1981). Spraying of systemic fungicides at 0.1% concentration has also been recommended.

9. YELLOW MOULD : (Mat disease; Vert-de. gris)

Pathogen : *Myceliophthora lutea, Chrysosporium luteum, C. sulphureum*

All these fungi produce yellow mycelial growth in the compost. Constantin (1892) reported *M. lutea* from French mushroom caves for the first time. Yellow mould inducing mat disease has been reported from Jammu & Kashmir (Kaul *et al.*, 1978), Punjab (Garcha *et al.*, 1987) and Himachal Pradesh (Thapa & Seth, 1986-87; Seth & Bhardwaj, 1989) inducing 5-20% loss on the yield of button mushrooms under natural conditions. Artificial inoculations with *M. lutea* at different stages caused 27-89% loss in yield. The incidence of the disease in HP has been reported from 20-60% during 1981-83 and 10-70% during 1985-86. Recently, the disease has also been noticed in Distt. Sonepat in Haryana State.

Symptoms : The yellow moulds may develop in a layer below the casing (Mat disease), form circular colonies in the compost (confetti) or they may be distributed throughout the compost (Vert-de-girs). In India, *M. lutea* has been reported to induce mat disease. This fungus forms a yellow brown corky mycelial layer at the interphase of compost and casing which is difficult to detect during the impregnation of casing layer by the spawn and even during the first break. It becomes apparent when it develops its stroma like morphology and mushroom production is severely inhibited.



Symptoms of yellow mould

Causal organism : *Myceliophthora lutea*

The mycelium is whitish at first then yellow to dark tan with restricted growth and creamish or dull white sporulation. Hyphae septate, hyaline, branched. It produces three kinds of spors, (i) Smooth, ovoid terminal condia borne singly, (ii) Smooth, thick walled chlamydospores, terminal or intercalary and (iii) thick walled spiny chlamydospores.

Epidemiology : The major sources of primary inoculum are air, chicken manure, spent compost and defectively sterilized wooden trays (Seth & Bhardwaj, 1989).The secondary spread is mainly through mites followed by flies, water splashes, picking and tools. The fungus survives easily through thick walled chlamydospores. Disease severity is generally more at 70% moisture content of the compost and 19-20°C temperature.

Control

1. Proper pasteurization of the casing mixture is very essential. Fungus does not survive the exposure for 6 hrs. at 51°C or 4 hrs at 54°C.

2. Benomyl (400-500ppm) and blitox (400ppm) sprays have been found effective to control the disease and increase the yield (Seth and Bhardwaj, 1989). Spraying with calcium hypochlorite solution (15%) is effective for eradication of the mould growth (Sohi, 1986).

10. SEPEDONIUM YELLOW MOULD

Pathogen : *Sepedonium* spp.

Yellow mould disease induced by *Sepedonium* has been reported in India by Thapa *et al.*, (1991) and the incidence of the mould has been reported by vary from 5-20% with insignificant reduction in yield except in extreme cases. One more species, S. *maheshwarinum*, has also been reported by Vijay *et al.*, (1993) with very high incidence causing severe losses or even complete crop failures. Bhatt and Singh (2000) have recorded 1.6 to 8% incidence of yellow mould in Haryana and UP States and 32 to 64% loss in yield under artificial inculations.

Symptoms : This mould is mainly observed in the compost and is initially white in colour turning to yellow or tan at maturity. It is generally present in the lower layers of the compost or at bottom of the cropping bags. Various types of distortions in fruit bodies are commonly observed, probably due to the production of volatile substances or toxins. These toxins inhibit the spawn and ultimately mushroom mycelium disappears from the compost.

Causal organism : *Sepedonium chrysosporium* (Bull.) Fries., *S. maheshwarianum* Muker. (*Hypomyces chrysosporium* Tull.)

Mycelium is white initially, turns yellow to tan with age. Hyphae septate, brenched, hyaline, moderately thick, 3-5mm wide. Conidiophores erect, bear lateral simple or botryose cluster of branches, 4-4.5mm wide, usually septate, bearing spores singly and terminally on the branches. Two types of spores are produced in large numbers. Conidia are hyaline, thin walled, ellipsoid or pyriform, produced singly from the tips of the phialids. Second type of spores are like chlamydospores which are globse, warted, dark yellow, thick walled and 13-21mm in diameter.

Epidemology : Primary source of inculum are probably, soil, spent compost, air or improperly sterilized wooden trays. The chlamydospore are thick-walled and resistant to heat and in this spore form, the fungus may survive peak-heat. Spores can be spread to the compost by air currents prior to or during filling operation, during the spawning operation or with unpasteurized or spent compost sticking to wooden

trays. Conditions favourable for button mushroom cultivation also favour the *Sepedonium* mould. Higher N content, especially in the form of chicken manure, have been reported to favour the mould development (Vijay *et al.*, 1993). Its appearance in the lower layers of the compsot has been linked with more wetness. Sharma and Sharma (2000) have reported very high population of *Sepedonium* spp. in 3-12 months old chicken manure which may serve as the primary source of inoculum in long method of compost.

Control

Strict temperature monitoring and control during compost pasteurization and an adequate post-crop cooking out are essential to eliminate the threat of infestation. Preventing the entry of spores during spawning and spawn-running by installing high-efficiency air filters are essential. Incorporation of 0.5% carbendazim in compost and sterilizing the chicken manure (for long method of composting) with 2% formalin and 0.5% carbendazim has given good results (Vijay *et al*., 1993).

11. INK CAPS

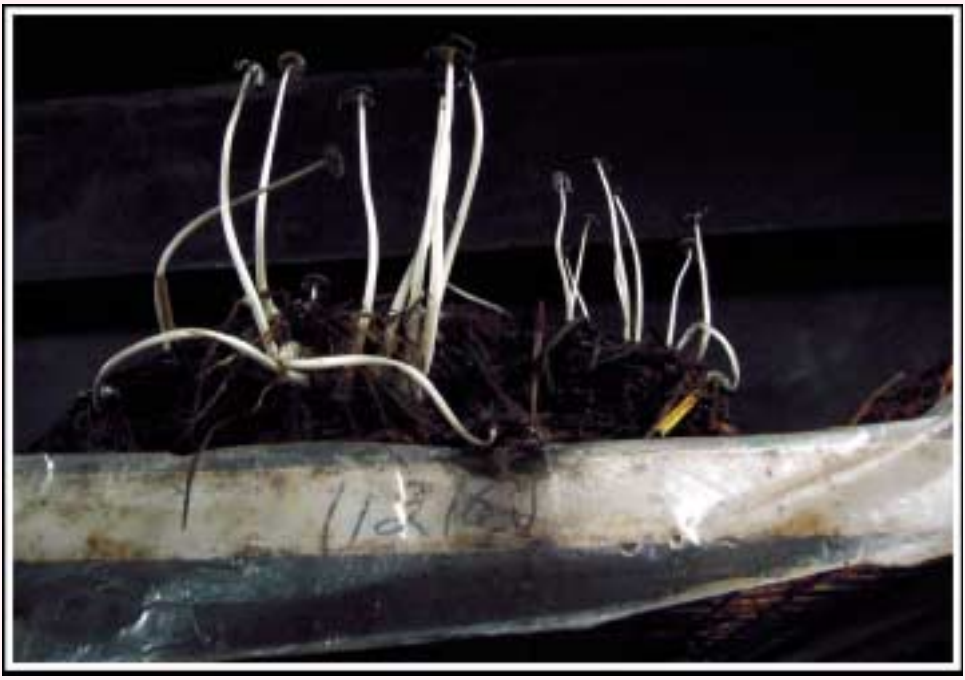
Pathogen : *Coprinus* spp.

Common names : Ink weed, wild mushrooms

The appearance of inky caps during spawn run is commonly observed on the mushroom beds in northern India (Kaul *et al*., 1978; Garcha 1984; Sohi, 1986). Artificial inculations of *C. fimetarius* at different loads of inoculum at spawning has resulted in 20.14-94.4% reduction in the number of fruit bodies and 14.68 to 94.43% reduction in the weight of fruit bodies (Sharma 1992).

Symptoms

Ink caps appear in the compost during spawn run or newly cased beds and outside the manure piles during fermentation. They are slender, bell-shaped mushrooms. Cream coloured at first, blueish-black later and are usually covered with scales. This fungus sometimes grows in clusters in beds and has a long sturdy stem which often reaches deep into the compost layer. Several days after their appearance ink caps decay and form a blackish slimy mass due to autodigestion.



Symptoms of Ink caps

Causal organism : *Coprinus atramentarium, C. lagopus, C. commatus, C. fimetarious*

Caps are 1.5-4cm wide, at first elongated oval, later conical, then companulate; surface white and covered with small white scales that disappear within a few hours, margin splitting as cap expands, turning into umbrella shape at dissolution. Gills 6-10cm long, upto 1 mm wide, free; first white, soon turn black on liquifying stem 2-4" long, 2-3mm thick, white, shinning, hollow, fragile, tapering upwards with a small bulb at the base. Spores 8-12x3-5mm, elliptic and black (Kaul *et al*., 1978).

Epidemiology

The infection generally comes through unpasteurized or partially pasteurized compost or casing soil or air. Ink caps appear if the compost contains too much N, so if too much chicken manure is used, or if the peak heating period is too short. These are therefore, genuine indicator moulds which are benefited from insufficiently converted N containing constituents like NH_3. Ink caps can also develop

if insufficient gypsum is added to the compost or if peak heating has taken place at too low a temperature or if the compost is too wet and poor in texture. Ink caps can directly use free NH_4 + and can also decompose cellulose very well, in addition to lipids and lignin. They are genuine coprophillic fungi which have an optimum pH of around 8. The large masses of spores released through inking of the caps can very easily infect freshly prepared compost.

Control : Use properly pasteurized compost and casing soil. Avoid excessive watering. Rogue out young fruit bodies of the weed fungus to avoid its further spread.

12. CINNAMON MOULD

Pathogen : *Chromelosporium fulva, C. ollare*

Common name : Cinnamon brown mould, brown mould

The occurrence of this could has been reported in mushroom beds from J&K (Kaul *et al*., 1978) Punjab (Garcha *et al*., 1987) and different parts of HP (Sohi, 1988).

Symptoms : Although *Chromelosporium fulva* (*Ostracoderma fulva*) has been called cinnamon brown mould, its colour ranges from yellow gold to golden brown to cinnamon brown. The mould first appears as large circular patches of white aerial mycelium on the compost or casing. Within few days the spores are formed and the colour changes from white to light yellow or to light golden brown. As the spores mature, the colour changes to golden brown or cinnamon and the colony develops a granular appearance. The fungus produces numerous cup-like fleshy fruit bodies on beds.

Causal organism : *Chromlosporium fulva, C. ollare*

Perfect statge : *Peziza ostracoderma*

Apothecium discoid, varying in size from a few mm when young to 1-2cm wide when mature; cup shaped, margin wavy, often splitting, tapering to a stem like base. Stem 5-9mm long. Asci cylindrical measuring 80-160x8-12. Ascospores 8 in number, arranged in a single row, ellipsoid, hyaline 8-12x4-8m. Paraphyses present, hyaline.

Epidemiology : Soil, casing mixture and damp wood are the sources of primary inoculum. Inoculum can blow through open doors or splash

from floor during cleaning. The spores of the fungus are easily air-borne. Over pasteurized compost, over-heated patches during spawn run, high moisture content of the compost and excess of ammonia present in the compost favour the disease development.

Control : Casing soil should not be made completely sterile by steam or formaldehyde. Newly cased beds should be sprayed with dithane Z-78 and maintain proper moisture content in casing layer.

13. LIPSTICK MOULD

Pathogen : *Sporendonema purpurescens*

Common name : Lipstick, Red lipstick

This disease has been reported from mushroom farms in Punjab (Garcha *et al*.,1987) and HP (Sohi, 1986, 1988).

Symptoms : The disease first appears in spawned compost as a white crystalline-like mould, rather

Symptoms of lipstick mould

nondiscernable from spawn. As the spore of the mould mature, the colour changes from white to pink, to cherry red and then to dull orange or buff. White mycelial growth is more in loose areas of casing and can colonize well conditioned compost. In crops where there is a serious virus disease, lipstick mould usually occurs as a secondary disease.

Causal organism : *Sporendonema purpurescens*

Mycelium whitish at first, often taking on a "frosty" appearance and then forming whitish balls of mycelium. Hyphae septate and become segmented into chains of l-celled, short cylindric spores with truncate ends. Spores have reddish pigment which gives the whitish mould a cherry red colouration.

Epidemiology : Soil, casing mixture and spent compost are the sources of primary inoculum. It is further disseminated by water splashes or pickers. The mould is reported to be associated with the use of chicken manure in the compost formula; the litter is said to carry the lipstick fungus.

Control : Good hygiene is essential. Good pasteurization and conditioning of the compost will eliminate the pathogen.

14. LILLIPUTIA MOULD

Pathogen : *Lilliputia rufula* (Berk & Br.) Hughes

This competitor mould has been reported from HP and Delhi (Seth and Munjal, 1981) with an incidence of 1-40% during 1975-1979, maximum being in Chail (HP). It seriously restricts the spawn spread in the compost resulting in poor yields. The sexual stage has been identified as *Gliocladium prolificum* Bainer. Chicken manure, horse manure as well as casing mixture are the primary sources of infection. Mycelium is viable upto 3 months (at 10°C) and cleistothecia upto 9 months under room temperature. Use of dithane Z-78 at 20ppm concentration has been recommended for the control of the mould. (Seth and Munjal, 1981).

15. PINK MOULD

Pathogen : *Cephalothecium roseum* Corda

This mould has been observed in J&K (Kaul *et al.*, 1970) and Chail and Solan in HP as a white growth on the casing soil which turns pink in due course (Seth, 1977; Sohi, 1986). Yield loss upto 90% or even complete crop failures have also

been recorded. Hyphae are septate and branched. Conidiophores erect, usually branched and slightly swollen at the tip. Conidia acrogenous, single, pear shaped, 2-celled, the apical cell being larger, hyaline to pink, 11-18x7.5-9.5m. Infection generally comes through air. Mould can be checked by spraying twice thiram or captan (0.04%) on casing soil at 10 day intervals (Guleria and Seth, 1977).

16. OEDOCEPHALUM MOULD

Pathogen : *Oedocephalum fimetarium, Oedocephalum* spp.

This is a common mould observed on mushroom beds in HP and incidence upto 60% has been observed in a farm at Solan during 1991 (Sharma, 1991). Artificial inoculation of casing layer with *O. fimetarium* @ 5g inoculum per 10kg compost bag has reduced the number and weight of fruiting bodies by 19.9% and 11.63%, respectively (Sharma, 1991; Sharma and Vijay, 1993). The mould forms irregular, light silver gray patches on the compost surface during cool down before spawning. After spawning, the mould is light gray but changes to dark tan or light brown as the spore mature. Similar growth is also recorded on casing layer. Conidiophores of the fungus are erect with a spherical cluster of large spores at its tip end. *Oedocephalum* sp. in compost indicates that ammonia and amines were not completely eliminated during pasteurization and conditioning. Spraying or swabbing locally with 2% formalin controls the mould.

17. WHITE PLASTER MOULD

Pathogen : *Scopulariopsis fimicola*

This disease has been reported to occur commonly in different parts of India by several workers (Garcha, 1978; Kaul *et al*. 1978; Sohi, 1986; Bhardwaj *et al*. 1989) causing about 37% loss in yield. The disease appears as white patches on the compost or casing soil. These patches or mycelial mats may be more than 50cm under favourable conditions. The white growth changes to light pink after a week of the formation of the spot. Spawn run is reduced significantly and under severe conditions complete crop failure are also recorded. Mycelium of the pathogen is septate, conidiophore short, branched, borne irregularly as lateral branches of hyphae. Annellospores ovate, globose, round or showing truncation, buff to avellaneous in mass, occur in chains or clusters,

measure 4.8-9 x 4.8 µm. The pathogen is favoured by under or overcomposted compost which still retains the smell of ammonia and has high pH (more than 8). Proper composting and addition of optimum quantities of water and gypsum are recommended. Sprays of benomyl (0.1%) and local application of formalin (4%) after the removal of the mat are helpful in controlling the disease.

B. OYSTER MUSHROOM (*Pleurotus* spp.)

a. Diseases

There are four fungal diseases reported on oyster mushroom from India. Their causal agents, symptoms and control measures are presented in Table 1.

b. Competitor moulds/weed moulds

Compared to white button mushroom, the information on diseases and competitor moulds occurring in or on oyster mushrooms is less. Several competitor moulds have been reported occurring in the substrate used for oyster mushroom cultivation. Variations in the number and types of moulds are mainly due to the use of a variety of substrates, different methods of substrate preparation and the conditions and containers used for cultivation.

Competitor moulds: Different fungi occurring in the substrate and competing with mushroom mycelium for space and nutrition are: *Arthrobotrys* sp., *Aspergillus niger, A.flavus, A.fumigatus, Alternaria alternata, Cephalosporium aspermum, C.acremonium, Chaetomium globosum, Cladosporium cladosporoides, Coprinus retirugis, C.sterguilinus, Coprinus* spp., *Cochliobolus specifer, Drechslera bicolor, Furarium moniliforme, f.moniliforme* var. *ferbolutinans, F.moniliforme* var. *subglutinans, F.graminearum, Momniella echinata, Mucor* sp., *Penicillium* sp., *Rhizopus oryzae, Rhizopus* spp., *R.stolonifer, Stachybotrys chartarum, Stilbum nanum, Stysanus medius, Sclerotium rolfsii, Sordaria fimicola, Oedocephalum globerulosum, O.lineatum, Trichoderma viride, Trichothecium roseum, Trichurus terrophilus* and *Phialospora* sp. (Sharma and Jandaik, 1980, 1981; Singh and Saxena, 1987; Doshi and Singh, 1985; Vijay and Sohi, 1989; Das and Suharban 1991). Loss in yield in

Table-1: Fungal diseases of oyster mushrooms in India

SN	Casual organism	Symptoms	Control	References
1.	*Cladobotrym apiculatum* *C.verticillatum* *C.variospermum*	White cottony growth on the substrate; small brown irregular sunken spots or fluffy growth on fruit bodies; soft rot and decay of sporophores emitting foul smell.	Spray bavistin 50ppm	Upadhyay *et al*; 1987;Sohi and Upadhyay 1980; Goltapeh *et al*. 1989
2.	*Gliocladium virens* *G.deliguescens*	Fruit bodies covered by mycelium and green spots; young pin-heads become soft, brown, pale yellow and decay. Mature fruit bodies show brown spots enclosed by yellow halo.	Spray 100ppm bavistin or benomyl	Bhardwaj *et al*. 1987; Sharma and Jandaik, 1983
3.	*Arthrobotrys pleuroti*	Fluffy growth on substrate and fruit bodies; infected tissues turn yellow, water logged and rot.	Spray 50ppm bavistin	Ganeshan, 1987
4.	*Sibirina fungicola*	Powdery white growth on stipe, gills and the primordia; primordia show brownish discolouration and soft rot and mature fruit bodies turn fragile.	Proper aeration and RH essential; spray benomyl twice	Sharma and Jandaik, 1983, Jandaik and Sharma, 1983.

different *Pleurotus* spp. by these competitor moulds has been reported upto 70%. In addition to these moulds being competitive some have been shown to produce metabolites which directly inhibit the growth of mushroom mycelium. However, detailed information about these competitor moulds especially on their relative importance, epidemiology and management is not yet available. Most of the competitor moulds have been reported to be completely inhibited under *in vitro* and/or *in vivo* conditions by benomyl (50 ppm),

carbendazim + blitox (100ppm each) and Thiram (100ppm) (Bano *et al*., 1975, Doshi and Singh, 1985; Sharma and Jandaik, 1980).

C. PADDY STRAW (*Volvariella* spp.)

Though paddy straw mushroom (*Volvariella* spp.) was the first to be cultivated in India as early as 1943 by Thomas and his associates at Coimbatore yet very little information is available on the diseases of this mushroom. This is still being cultivated outdoors in India following primitive production technology with very low biological efficiency. Paddy straw mushrooms are subject to a number of destructive diseases/competitor moulds like *Mycogone perniciosa, Scopulariopsis fimicola* and *Verticillium* spp. in other countries. In India, large number of competitor moulds and few diseases have been reported on this mushroom. *Chaetomium* spp., *Alternaria* sp. and *Sordaria* sp. have been commonly observed as contaminants on wheat, kans, maize, barely and jowar beds but not only paddy straw bundles (Gupta *et al*. 1970). A 'button-rot' disease caused by *Sclerotium* sp. has been reported by Muthukrishnan (1971) and bacterial 'button-rot' by Kannaiyan (1974). Combination of insecticide, fungicide and antibiotic (Malathion 0.025% + dithane Z-78 or benomyl 0.025% + tetracycline 0.025%) are recommended for the management of pests and diseases (Kannaiyan and Prasad, 1978). Several other competitor moulds namely, *Coprinus aratus, C.cinereus, C.lacopus, Psathyrella* sp., *Penicillium* spp., *Aspergillus* spp., *Rhizopus* sp., *R.nigricans* and *Sclerotium* spp. have been reported from the substrate (Munjal, 1975; Bahl, 1984; Purkayastha and Das, 1991, Rangaswami, 1978). Partial sterilization of the straw and sprays on the beds with captan and zineb (0.2%) have been recommended for reducing the damage. Bahl and Chowdhry (1980) have reported *Podospora favrelii* as a serious competitor and inhibits the growth of mushroom mycelium completely. Bhavani Devi and Nair(1986) have also recorded *Rhizoctoria solani* on the substrate which reduces the sporophore formation and causes malformation of fruiting primordia. A serious effort is urgently needed to investigate the diseases of paddy straw mushroom and recommend the package of practices to be followed to the growers to achieve good yields.

D. OTHER MUSHROOMS

Sporadic attempts have been made to cultivate few other mushrooms like giant mushroom (*Stropharia rugoso-annulata*), black ear mushroom (*Auricularia polytricha*), shiitake (*Lentinula edodes*) and milky mushroom (*Calocybe indica*) in different parts of the country and the competitor moulds/diseases recorded on them are briefly mentioned below:

Sohi and Upadhyay (1989) have reported *Mycogone rosea* parasitizing *S.rugoso-annulata* under natural conditions. The main symptoms are white cottony growth on gills, light brown spots on stipe and deformity of the sporophores. *Cladobotryum verticillatum* has been reported on *Auricularia polytricha* (Goltapeh *et al*. 1989) producing white fluffy growth on substrate and fruit bodies resulting in 9-96% yield loss. Spraying carbendazim (50ppm) has been reported effective for controlling the disease. *Trichoderma viride*, *Trichoderma* sp., *Aspergillus* spp. and *Fusarium* sp. have been commonly recorded as competitors (Sharma and Thakur, unpublished) during the cultivation of winter ear mushroom. During the cultivation of *C.indica*, several competitor moulds namely, *Aspergillus niger, A.flavus, A.fumigatus, Rhizopus stolonifer, Mucor* sp., *S.rolfsii, T.viride, T.haematum, Fusarium* spp. and *Coprinus* spp. have been isolated from the substrate (Doshi *et al.* 1991). In addition Sharma and Thakur (unpublished) have also recorded very high incidence of *Cladobotryum* and *Oedocephalum* spp. from the casing mixture. Incidence of *T.viride* has been recorded from 15-25% in the supplemented bags as compared to 5-10% in unsupplemented ones in *L.edodes* cultivation (Thakur and Sharma, 1992).

GENERAL GUIDELINES

In order to decide the most effective measures for controlling a disease in mushroom, it is necessary to understand the size of the initial inoculum, density, the rate at which the disease develops and spreads and the time when the infection takes place.

Based on these, the following preventive and/or eradicative control measures are necessary for the management of these diseases:

- **Ecological**-by manipulations of environmental factors such as temperature, humidity and ventilation.

- **Biological**-by use of antagonistic organisms through incorporation of biocontrol agents and organic amendments.

- **Chemical**-by use of safe and minimum doses of specific fungicides, antibiotic etc.

A close relationship exists between crop management practices and some endemic disease problems like dry bubble, brown blotch and truffle. Biological agents are being increasingly tried throughout the world but with a limited application on commercial scale. Sanitation and hygienic measures are most essential to manage the disease particularly under Indian conditions although under certain situations use of chemicals is also inevitable.

Sanitation and hygiene

Hygiene covers all the measures which are necessary to allow as little chance as possible to the pests and pathogens to survive, develop and spread. Thus hygiene and sanitation go hand in hand at all stages of growing mushrooms. Farm hygiene is the best defense a mushroom grower has against mushroom pests and diseases particularly during the present days, when use of chemicals on food crop is being discouraged.

After having gone through the details of different diseases discussed earlier we know that mushroom pathogens gain entry to a mushroom farm in a variety of ways. They can fly in, drift in on the wind and crawl in. Also they can be carried on people, on the vehicles and in the raw materials. What makes matter worse is that they are usually difficult or impossible to be seen with the naked eyes. Based on the critical observations during all the stages of mushroom production, the following steps have become a routine practice for successfully cultivating mushroom.

- The location of mushroom unit should be in such an area where effluents of chemical industries do not pollute the water and also the air is free from toxic fumes or gases.

- Floor for the preparation of compost should be cemented/ tiled and covered with a roof.

- Substrates used for compost preparation should be fresh, protected from rain and mixed in exact proportion.

- Pasteurization and conditioning of the compost should be for optimum duration at right

temperatures as over/under pasteurization may not produce quality compost and invite many disease problems.

- Do not allow free access of persons working in composting yards to spawning and other cleaner areas without changing the dress and foot-dip. Similarly, all machinery including tractors and fork-lift trucks should not be moved to the cleaner areas. After filling, all equipment and machinery should be thoroughly cleaned.
- Spawn should be fresh and free from all the contaminants.
- All equipments used for spawning, floor and walls of spawning area must be washed and disinfected.
- The fresh air should be filtered before it enters the growing rooms to exclude all particles of 2 micron and above.
- Casing mixture should be properly pasteurized (60-65C for 5-6 hours).
- Casing mixture should be stored in a clean and disinfected place. All the containers, equipments and machinery used for casing should be thoroughly washed and disinfected. Keeping dust to a minimum and not to have dusty operations going on at the same time elsewhere on the farm is also very helpful.
- The pickers should use clean overalls and gloves. Picking should start from new or cleaner crop towards older crops.
- Waste from picking, chogs, trash, stems, unsaleable mushrooms should be carefully collected not allowing to fall on the floor, and be disposed off carefully.
- Avoid surface condensation of water on developing mushrooms.
- Add bleaching powder (150ppm) at every watering to manage bacterial disease.
- Remove heavily infected bags from the cropping rooms or treat the patches by spot application of 2% formalin or 0.05% Bavistin.
- Maintain optimum environmental conditions in the cropping rooms to avoid abiotic disorders.

- Control insect-pests well in time to avoid the spread of pathogen by them.

- At the end of crop, cooking out at 70C for 12 hours is very essential to eliminate all pests and pathogens.

Use of Chemicals

It is advisable to manage the disease in mushrooms through hygienic measures listed above. There are only a limited number of pesticides registered for use on mushrooms. This is because mushrooms themselves are fungi and most of the pathogens are also fungi thereby making the choice of fungicides very difficult. Moreover, because of short cropping cycle, residual toxicity of different chemicals is of great concern and it must be kept below the tolerance limit. Mushrooms are very sensitive to fumes, toxic gases and several chemicals. This also limits the frequent use of chemicals in mushroom industry. Equally important factor which limits the use of fungicides for the management of diseases in mushrooms is the problem of resistance. Repeated and regular applications of the same chemical greatly increase the chance of resistance. If equally effective alternate fungicides are available the problem of pesticide resistance can be minimized. On the other hand there are, unfortunately only a few pests or diseases that can be controlled satisfactorily by environmental manipulation alone. Some of the most common fungicides recommended for the control of major fungal pathogens of *A.bisporus* (Fletcher *et al*. 1986) and used in mushroom industry are:

- Benomyl(Benlate 50wp)- For control of *Dactylium, Mycogone, Trichoderma, Verticillium,* mix 240g/100m² with casing or dissolve in water at 240g/ 200litres/100m² during first watering.

- Carbendazim (Bavistin) same as for benomyl.

- Chlorothalonil(Bravo or Repulse) - to control *Mycogone* and *Verticillium*. Apply as spray 2 week after casing and repeat not less than 2 weeks later @ 200ml in 100-200litre water/100m².

- Prochloroz Manganese (Sporgon)- to control *Mycogone, Verticillium, Dactylium*, give a single application of 300g/ 100litres/100m², 7-9 days after

casing. For double application, use 113g/100litres/100m², 7-9 days after casing and repeat again between second and third flushes. For triple application, use 57g/100litres/100m², 7-9 days after casing and after first and third flushes.

- Thiabendazole(Tecto)- to control *Dectylium*, *Mycogone*, *Verticillium*, apply at the same rate as Benomyl.
- Zineb(Zineb Tritoftoral)- to control *Dactylium*, *Mycogone*, Red *Geotrichum* and *Verticillum*, Use 7% dust, at 350g/100m² every week after casing or 140g/100m² before watering. For wettable powder, 1kg/1000 litres @ 5 litre/100m² after casing and between flushes. For Tritoftoral, 5kg/100m² at 4.5m² between flushes.

III. VIRAL DISEASES

a) Button Mushroom

INTRODUCTION

In recent years, viruses have increasingly been found in association with fungi, an association that has taken one of the two forms. In the first, the fungus is the vector of the virus and in the second, fungus is the host of the virus. Here only the second form of association i.e. fungi, especially the mushrooms, as hosts of viruses will be considered in detail which has been reviewed earlier by Raychaudhury (1978), Sharma (1991) and Sharma and Kumar (2000). Although the presence of viruses in fungi has long been suspected (Sinden and Hauser, 1950) experimental evidence was not forthcoming until 1962 when virus particles were demonstrated in diseased mushroom (Gandy and Hollings, 1962; Hollings, 1962). To date viruses or virus-like particles (VLPs) have been reported to occur in over 100 species from 73 genera of fungi, but only a small number of them have been isolated and characterised. Several terms have been used for the viruses of fungi including mycoviruses, fungal viruses, mycophages, double stranded RNA (dsRNA) plasmids and virus-like particles (VLPs). The term mycophage is clearly unsuitable since virus infection has very rarely been associated with lysis in fungi. Although mycoviruses may share some of the characteristics of plasmids, their morphology, nucleoprotein composition and the possession of virion-associated RNA polymerase activity are consistent with a viral nature. The term plasmid has already been abused in current literature as pointed out by Reanney (1976) and to denote the viruses of fungi as plasmids would not find ready acceptance. The term VLPs and mycoviruses have been used by some authors (Bozarth, 1972; Saksena and Lemke, 1978), with the understanding that the first term applies to those particles occurring in fungi and having a virus-like appearance in electron-micrographs but which have not been isolated and characterised, whereas the second term denotes those which have been isolated and shown to have the morphology and nucleoprotein composition generally

attributed to viruses. This distinction offers an operational convenience and has been widely adopted. Since mycoviruses have not conclusively proven to be infectitious as purified particles, some workers prefer to apply the term VLPs in all cases.

HISTORY AND GEOGRAPHICAL DISTRIBUTION

In 1948 a very serious infectitious disease of white button mushroom (*Agaricus bisporus* (Lange) Sing.) was observed in the United States of America on a farm in Pennsylvania run by the La France brothers, and thus became known as La France disease (Sinden and Hauser, 1950). In England, a disease inducing brown staining on the stipe was named as 'brown disease' by Storey (1958). Gandy (1958) observed the most common symptom in the form of large water logged patches on stipes of mushroom from diseased beds and proposed the name 'watery stipe'. A similar, possibly the same disease was observed in the mushroom industry throughout Pennsylvania. The cause of this disease was unknown and no existing description appeared to fit the disorder, hence the name 'X-disease' was coined by Kneebone and co-workers (1961). Since 1959 a similar disease 'mushroom-die-back' has been studied in England wherein degeneration of mycelium rather than the symptoms on fruit bodies were more predominent and has been attributed to a complex of atleast three different viruses (Gandy and Hollings, 1962). Schisler and co-workers (1967), reported that one of these viruses had been isolated from a white isolate of La France. They advocated the name X-disease and die-back be dropped. In the Netherlands, disorders of this type were not reported until 1964 when a heavy outbreak occurred causing significant yield losses (Dieleman Van Zaayen and Temmink, 1968). In Austrailia, mushroom diseases of viral nature probably dated back to the early days of mushroom growing in open ridge and disused railway tunnels in the 1930s but confirmation of existence of die-back was reported in 1968 (Paterson, 1968). Virus disease in button mushroom has been reported from India by Tewari and Singh (1984; 1985) and have also been described from New Zealand, Polland, German Democratic Republic, China, Denmark, Sweden and Canada. More recently La France disease has also been reported from Spain.

Viral particles have been reported, recently in *Pleurotus ostreatus* and *P.sapidus* from China and in *P.pulmonarius, P.ostreatus* and *P.columbinus* from France (Table-2). A double stranded RNA polyhedral virus has also been reported in *Volvariella volvacea* from China, Virions of different shapes and sizes have been detected in *Lentinus edodes* from Japan and USA. In India, virus and virus like disease have been on button mushroom (Tewari and Singh, 1984; 1985; Gottapeh and Kapoor, 1990) and oyster mushroom (Krishna Reddy *et al*. 1993). General symptoms, transmission and control

Table -2: Virus and VLPS reported in different mushrooms

S. No.	Host/Disease	Shape	Size	Country
I	*Agaricus bisporus* La France, Watery stipe, X-Disease, Die-back, mushroom disease	Spherical	25nm 29nm 35nm 40-50nm	Australia, England, Holland, America, France GDR, India
		Bacilliform	18x50nm	U.K.
		Club shaped	60-70nm dia or 120-170 long with a spherical body of 40-50 nm & a cylindried tail 20-30nm in dia	France, W. Germany S. Africa
		Rods of varing longth	19x9-90nm 19x35nm 20x130nm	Poland GDR China
II	*Pleurotus* spp.			
	P. colombinus	Spherical	26+2nm	France
	P. ostreatus			India
	P. pulmonarius	Spherical	24nm	China
	P. sapidus			China
	P. florida	Flexuousrods	40-600nmlong	
III	*Volvariella volvacea*	Spherical	35nm	China
IV	*L.edodes*	Spherical	20nm, 23nm 36nm, 45nm 30nm	China, Japan
		Stiff or	17x200x1200nm 15x700-900nm 18x1500nm 15x16x200-300nm	Japan China

measures of button mushroom viruses are discussed below:

SYMPTOMS

The various early names coined for mushroom virus disease give some indication of the diversity and variation of symptoms caused by viral infection. The symptoms, which have frequently been described may be expressed individually or in various combinations and in a wide range of severity. The symptoms of virus disease vary from reduced yield to distorted mushrooms. During the spawn run period, there is no visible indication of the disease however, once casing is applied, distinctive symptoms may be restorted when symptomless or slightly affected mycelial isolates are grown in compost and induced to fruit. The full range of symptoms that are encountered in non-hybrid mushrooms are also seen in hybrid mushrooms. In extreme cases all sporophore initiation is inhibited and the vigour of the mycelium is severely reduced while in other cases it is difficult to detect these symptoms. This variation depends upon a number of factors which include virus concentration, time of infection, strain of spawn used and cultural conditions. The general symptoms observed are as below:

1. Mycelium does not permeate or hardly permeates the casing layer or disappears after the normal spread. Mushrooms appear only in dense clusters, maturing too early.

2. Mycelium isolated from diseased sporophores on agar shows a slow and degenerated growth as compared with healthy mycelium.

3. The delayed appearance of the pinheads of the first flush can be an important indication of the disease as well as the formation of fruiting primordia below the surface of the casing layer. As soon as these mushrooms appear above the casing soil, their pilei are already opened.

4. Symptoms of sporophores are highly variable. The following abnormalilties can be found separately or in combination:

   a) Slow mycelial growth, development of abnormal mushrooms.

   b) Slow development of pinheads, dwarfing.

c) Delay in appearance of sporophores, reduced yield.

d) Off-white colour of the cap and early maturity.

e) Sporophores with elongated stems and small caps.

f) Elongated slightly bent stipes, sometimes with small early maturing pileus.

g) Premature opening of veil.

h) Mushrooms are loosely attached to the substrate and at the slightest touch are pushed over.

i) Accelerated post-harvested deterioration.

j) Watery stipes, streaking in the stipes

k) Stipes are spongy and quickly turn brown on cutting and show an abnormal structure.

l) Thickened barrel-shaped stipes; the veil is attached to the thickest part of the stipe, thus lower than usually. Pilei are small and fat.

m) Brown, slimy, cap occur owing to a secondary bacterial rot, stipes are sometimes tapering downwards during the first flush, sometimes a few light brown caps can be observed.

n) Veils abnormal or absent, hard gills are common.

5) A specific musty smell can be perceived in a growing room infested with the disease. Whereas in *Pleurotus*, virus infection causes dwarfing or elogation of stipe. However, no distinct symptoms are visible in *Volvariella*.

Severe and total crop losses have been reported due to club-shaped virus reported in *A.bisporus* (Albouy *et al*. 1973). It has been shown to be very difficult to produce spawn from mushroom infected with this club-shaped virus. The symptoms induced by virus disease in *L.edodes* include dwarfing, early maturity, hardened gills and thickened, elongated or barrel shaped stipes (Deahl *et al*. 1986).

Causal organism

Several viruses of different shapes and sizes have been reported on different mushrooms. In India, virions measuring 29nm and 35 nm in diameter have been found associated with a virus disease of

button mushroom. Virus like particles measuring 29nm in diameter have also been reported in button mushroom as revealed by immunosorbent electron-microscopy (Goltapeh and Kapoor, 1990).

CHARACTERIZATION OF VIRUSES AND VLPs

Morphology of Viruses

Experiments by Gandy and Hollings (1962) and Hollings (1962) demonstrated the presence of three types of virus particles associated with the diseased mushrooms having die-back symptoms. Two of the viruses had isometric particles with a diameter of 25nm and 29nm while the third was bacilliform with a diameter of 18nm and a length of 50nm. A fourth virus type was later reported from England (Hollings *et al*., 1968) and Holland (Dieleman-van Zaayen and Temmink, 1968) having 35nm diameter. Another spherical virus having a diameter of 40 to 50 nm was later reported from England by Hollings and co-workers (1968). Thus, five different types of viruses have been reported in England and the accepted nomenclature for these viruses in UK is as below:

MV-1 : Spherical particles, diameter 25nm.

MV-2 : Spherical particles, diameter 29nm.

MV-3 : Bacilliform particles, 19x50nm.

MV-4 : Spherical particles, diameter 35nm.

MV-5 : Spherical particles, diameter 50nm.

In addition to these five viruses, two more types having club-shaped and rod-shaped particles have been reported in *A.bisporus* from different parts of the world.

Recently, spherical viral particles of 24 to 26nm in diameter have been shown to exist in *Pleurotus ostreatus, P.sapidus, P.columbinus* and *P.florida* from China and France (Liang *et al*. 1987, 1990; Liu and Liang, 1986; Molin and Lapierre, 1989) and flexuous rods measuring 40-600 nm long from China (Liang *et al*. 1990). From China, polyhedral virus measuring 34 nm in diameter has been reported in *Volvariella volvacea* (Chen *et al*., 1988). Rods as well as spherical types of viruses have also been reported in *Lentinus edodes*

from China, USA and Japan. Different viruses and VLPs reported from different parts of the world have been summarised in Table-2. In India, viruses and VLPs have been reported infecting *A.bisporus* (Tewari and Singh, 1984; 1985; Goltapeh and Kapoor, 1990) and *P.florida* (Krishna Reddy *et al*., 1993).

Physico-chemical Properties

As is evident from the reports that several viruses having various shapes and sizes have been found associated with diseased mushrooms. However, the role of individual virus or VLPs in inducing the typical symptoms of the disease has proved inconclusive. Recent biochemical studies have significantly advanced our understanding of the viral nature of the diseases or VLPs. Further, the widespread occurrence of VLPs in healthy basidiocarps and mycelium (Passmore and Frost, 1974, 1979) has raised questions concerning the etiological role of viruses in disease (Frost and Passmore, 1980). Because mycoviruses typically possess double stranded RNA (ds RNA) genomes, the discovery of discrete ds RNA molecules in diseased tissues constitutes the most convincing evidence for the viral etiology of La France disease (Hicks and Haugton, 1986; Lomke, 1976; Marino *et al*., 1976; Ross *et al*., 1986; Wach, *et al*., 1987).

It was also reported that a viral complex (Sonnenberg and Griensven, 1991; Romaine and Schlagnhaufer, 1991) involving a ss RNA virus and unrelated ds RNA virus (es) plays a role in etiology of La France disease. *A.bisporus* fruit bodies affected by La France disease contain the specific set of 9 ds RNA molecules which is genome of 36nm isometric virus (Van der Lende *et al*., 1994; Revill *et al*., 1994; Zobalgogeazcoa *et al*., 1995; Goodin *et al*., 1992). The nucleotide sequence of dsRNAs M2 (1.3kb) and L3 (2.8 kb) is invariably associated with the disease. The average G+C content of these ds RNAs was 43 percent close to that of *A.bisporus* nuclear DNA. S3 ds-RNA (0.39 kb) is occasionally found in large amounts in diseased mushrooms (Harmsen *et al*., 1991). Harmsen and Wessels (1991) reported that La France disease was associated with 10 differently sized dsRNAs, which appeared to be encapsidated by virus particles of 25 and 34 nm. One of these dsRNAs was also present in healthy mushrooms. Recently, it has also been shown that dsRNAs L5 and M2 are encapsidated by 34 nm

particle (Ven der Lende *et al*., 1994). Reverse transcription-polymerase chain reaction assay (RT-PCR) showed the diseased mushrooms to be either singly infected by La France isometric virus (LIV) or doubly infected by La France isometric virus and mushroom bacilliform virus (MBV). La France disease is associated with the infection by two autonomously replicating viruses in which LTV is the primary causal agent and MBV, possibly pathogenic, capable of modulating symptoms, is not required for pathogenesis (Romaine and Schlagnhaufer, 1995). MBV was found to have a monopartite ssRNA genome of positive sense. The putative RNA-dependent RNA polymerase and coat protein displayed homology with protein encoded by plant viruses particularly luteoviruses and carmoviruses.

Transmission of LIV during basidiosporogenesis together with spore-borne nature of causal agent playes etiologic role of virus in La France disease (Romaine *et al*., 1993). In two separate trials an average of 75 and 65 per cent of the viable basidiospores discharged from diseased basidiocarp were infected by LIV. Basidiocarp showing the presence of dsRNAs in the stipe tissue produce LIV infected basidiospores. Double stranded RNA having molecular weight of 3.2x10 dalton has been demonstrated with 35nm virus particles in *Volvariella volvacea* (Chen *et al*., 1988) and 0.85x10 daltons with 24nm particles in *P.sapidus* and *P.ostreatus* (Liange *et al*., 1987). In *A.bisporus*, dsRNA has been demonstrated with 25 and 34nm particles (Hicks and Haughton, 1986; Romaine and Schlagnhaufer, 1989) whereas with bacilliform particles measuring 19x50nm, single sRNA has been reported (Molin and Lapierre, 1973; Tavanizis *et al*., 1980). In *L.edodes* dsRNA has been demonstrated with 39nm spherical particles. Most of the spherical VLPs or viruses are isometric, with sizes between the limits of 25 and 45nm diameter. Many have never been transmitted even by hyphal anastomosis to a healthy mycelium and nothing is known about their sedimentation characteristics, number of components or the composition of the viral nucleic acid and polypeptide moieties. They are still only the VLPs. The status of other particles is uncertain for different reasons. These are regarded by some workers as artifacts, fragments from 19x50nm, MV-3 virions, seen transversely (Hollings *et al*., 1971).

These particles, derived only from preparations containing MV-3, had a UV absorption spectrum lacking the 260nm peak of nucleoprotein virions and could not be transmitted. However, some workers regarded these as virions of a distinct type.

From *L.edodes* isometric particles 25, 30 and 39nm (Ushiyama and Nakai, 1975) and 30, 36 and 45nm (Yamashita *et al*., 1975) have been recorded in Japan, but whether these refer to the same three viruses or to four different viruses, is not known. Too little is known about the rods from *L.edodes* measuring 38x300nm in dip preparation and 15x200nm in thin sections to decide whether or not these could be tobamovirus particles.

Purification Procedures

It has proved much difficult to obtain consistently good preparations of mushroom viruses. In repeated tests mycelium from agar or from liquid cultures has been reported wholly unsatisfactory as the source of virus (Hollings and Stone, 1971) for virus extraction. Most of the virus is lost during the different steps in purification and therefore, sporophores with higher concentrations of the virus should be taken for grinding. Mushrooms contain powerful polyphenols oxidase system and often copious amount of polyphenolic complexes in virus extraction. Several methods of extraction, clarification and purification of viruses or VLPs have been tried in *A.bisporus* with varying degree of success. Some of these procedures are:

1. Hollings and co-workers(1971) attempted extraction with phosphate buffer and precipitation of virus with citric acid which gave best yield of viruses MV-1, MV-3 and MV-4. Precipitation of virus by ammonium sulphate or by sodium chloride plus polyethylene glycol(PEG) gave unsatisfactory results(Hollings and Stone, 1971).

2. Extraction in borate or phosphate buffer and clarification with butanol gave preparation of viruses MV-1, MV-2, MV-3 and MV-5 and proved very satisfactory for virus 2 but virtually destroyed MV-4(Hollings, 1962).

3. Extraction in phosphate buffer, clarification with ethoxy and butoxy and butoxy-ethanols has yielded MV-1, MV-3 and MV-4 (Kitano *et al*., 1961)

4. Dieleman-van Zaayen and Temmink(1968) used the methods of Hollings and co-workers (1965) and Kitano and co-workers (1961) with slight modifications followed by differential centrifugation and obtained good yields of MV-1, MV-3 and MV-4.

5. In case of *Pleurotus ostreatus* and *P. sapidus*, Liang and co-workers (1990) used 0.03 M phosphate buffer (pH 7.0) for extraction followed by low speed centrifugation and density gradient centrifugation and a fairly high concentration of both spherical as well as rod shaped virions was obtained. Molin and Lapierre (1973) have also used a similar method for purifying spherical virus from *P. pulmonarius*.

6. Chen and co-workers (1988) used Tris-HCl buffer (pH 7.6) for extracting the spherical virus from *V.volvacea* followed by three centrifugation at 5000g each for clarification. PEG 6000 and 0.1M sodium chloride were used for precipitation and pellets were again suspended in 0.05M Tris-HCl and 1M and NaCl buffer. Concentrated preparations of the virus were obtained by further differential centrifugation.

It can not be disputed that very pure virus preparations are essential for chemical, physical and biochemical studies and that many biological investigations are dependent on the availability of atleast partially purified preparations. It must be stressed here that no two viruses are exactly alike and consequently there are about as many purification procedures as there are viruses which have been purified. To achieve a purified virus preparation one has to take into account several factors like selection of propagating host, conditions affecting virus multiplication, selection of proper host tissue for extraction, extracting media (buffers, pH and molarity) method of extraction, clarification procedures and methods of isolation, concentration and further purification.

EPIDEMIOLOGY

The wide variation in symptoms reflect the variation in the economic impact of viruses on mushrooms. It is possible to have yield losses so slight that they are masked by other factors and the growers remain

unaware of them. Alternatively, the infection may be so severe that virtually no marketable mushrooms are produced. The first appearance of pinheads is delayed by several days and they remain as a small grey-fawn clump without further growth, although some may shed spores. Loss of crop varies from slight to 95 per cent (Barton, 1985; Dieleman-van Zaayen, 1970; Hollings *et al*., 1963; Rasmussen *et al*., 1969; Schisler *et al*., 1967). Various factors like time of infection, cultural conditions and the strains of the spawn used greatly affect the loss in yield. Dieleman-van Zaayen (1972) reported that when artificial inoculation was done from 0 to 12 days after spawning, the extent of loss due to dieback varied from 37.5 to 95.6 per cent over uninoculated control. He also concluded that: a) the time of infection is much more important than the amount of inoculum; b) with early infection, the amount of inoculum is of no consequence, and c) the amount of inoculum has a slight negative influence with later infection, which, by itself, causes a small loss in yield. A survey among more than 1000 Dutch growers showed that in 1967 and in the first half of 1968, one out of three mushroom farms was contaminated and on these farms average yield loss was 15 per cent. Thus in 1967, in the Netehrlands, 4.5 per cent or about 7,90,000 kg of mushroom were lost (Dieleman-van Zaayen, 1972a).

Detection Methods

Diagnosis of virus infection in mushrooms is not easy because of two reasons. The first reasons is that mushroom being an anatomically simple organism, responds to a range of adverse stimuli in only a limited number of ways. For example systems like elongation of the stipe, water logging of stipe, general loss in yield and bare patches in the mushroom beds may be induced by virus infection as well as a variety of other biotic and abiotic factors. The second difficulty with diagnosis is generally the low virus concentration. The different approaches adopted for the diagnosis of virus infection in mushroom are:

1. Symptoms on the bed.
2. Comparative growth rates of mycelium on agar.
3. Direct electron-microscopic examination (EM).

4. Immunosorbent electron-microscopy (IEM or ISEM).

5. Polyacrylamide gel electrophoresis (PAGE).

6. Enzyme-linked immunosorbent assay (ELISA).

7. Reverse transcription-polymerase chain reaction assay (RT-PCR).

Symptoms on the beds

Symptomatology has been discussed in detail earlier and they certainly indicate that something is wrong. However, it is difficult to conclude with authenticity that a particular abnormally in mushroom is only due to virus. Moreover, viruses have been detected by other methods in apparently healthy mushrooms.

Agar growth test

This was the first test to be devised and depends upon the facts that affected mushroom mycelium has a slower growth rate than otherwise identical healthy mycelium. Tissue cultures of healthy mushrooms in 2.5 per cent malt agar at 25°C grow aggressively and achieve a diameter of 80 to 100mm in 21 days. The periphery of the colony will have bare dense aerial hyphae which are white or pale cream in colour and resemble cotton wool. In the centre of the colony, the aerial hyphae largely disappear as they amalgamate to form rhizomorphs which have the appearance of fine white threads on the surface of the agar. In comparison, virus-infected mushrooms cultured in the same manner will achieve a colony diameter of 5 mm upto 80 to 100 mm. If the mushroom is severely infected colony will be flat and slightly waxy in appearance with few aerial hyphae. The colour is usually a deep cream or even light brown and can sometimes be dark in the Centre. Rhizomorphs do not generally form on diseased colonies and are often replaced by very flat aggregates of tissue which give a speckled or pepper and salt appearance to the centre of the colony (Gandy and Hollings, 1962, Nair, 1973). The advantage of this system is that it does not require expensive equipment. The disadvantages are, firstly, the long time needed to obtain a result and, secondly, the relative insensitivity of the test.

Electron microscopy

Gandy and Hollings (1962) first observed virus-like particles in

transmission electron microscope while examining purified and concentrated sap from mushrooms exhibiting dieback symptoms. However, virus purification and concentration was a complex and time consuming process which was not suitable for large number of diagnostic tests and Hollings and co-workers (1965) found that virus particles could be detected more quickly if the juice from diseased mushrooms, disrupted by ultra-high frequency sound waves, was examined under the electron microscope. Hollings and co-workers (1967) modified that detection procedure further wherein the juice from the suspected sporophore was squeezed through a piece of fine cloth. The juice thus expressed was mixed with 2 per cent PTA (pH 7) and mounted on carbon coated grids for examining in electron microscope. Thereafter the use of electron-microscopy allowed several viruses to be found in either purified preparations or ultrathin sections of diseased mushrooms (Barton, 1985; Barton and Hollings, 1979; Chen *et al*., 1988; Dieleman-van Zaayen, 1972b; Dieleman-van-Zaayen and Igesz, 1969; Dieleman-van Zaayen and Temmink, 1968; Hollings *et al*., 1968; Koons *et al*., 1983; Leistner, 1980; Lesemann and Koenig, 1977; Liang *et al*., 1990; Molin and Lapierre, 1989; Mori and Mori, 1974; Mori *et al*., 1978; Passmore and Frost, 1979; Tavanizis *et al*., 1980; Tewari and Singh, 1984; Ushiyama, 1975). Virus particles (MV-1, MV-2 and MV-3) could be detected with reasonable certainly in severely affected mycelium in as little as 1mg (fresh weight) of mycelium disrupted by sonication (Hollings *et al*., 1965). One of the advantages of this technique is the speed in the detection in samples when large number of virus particles are present. The disadvantage is its uncertainty of detecting levels of virus too low to cause disease at the time of examination but which may indicate a potential problem.

Immunosorbent electron microscopy

ISEM, originally developed by Derrick (1973) is a rapid method of detection and cheap to perform. Although it is a serological method, monospecific sera are not necessary its first use with mushrooms was reported by Del Vecchio and co-workers (1978). In this procedure, electron microscope grids are coated with carbon which behaves much like activated charcoal. It strongly absorbs proteins and by floating

these grids on antiserum droplets they become coated with antibody molecules. This coating can then selectively adsorb virus from mushroom extract and the antigen-antibody aggregates can be easily seen in EM. ISEM is almost 5000 times more sensitive than direct EM.

Polyacrylamide gel electrophoresis

This is a highly sensitive and specific detection technique, and is used for detecting double stranded RNA (dsRNA) in diseased mushrooms (Marino *et al*., 1976). In order to use this technique, the viral RNA must be extracted from diseased mushrooms. This can be identified by applying the preparation to an agar gel column which is subjected to an electric field. By staining the column after a predetermined time, the RNA, if present, can be identified. This technique is about 20 times more sensitive than direct EM and can also be used for detecting specific viruses. However, the drawback is that it is dependent upon the stability of the virions during the extraction of the dsRNA. This technique has been widely used in detecting dsRNA in virus infected *A.bisporus*, especially *Lentinus edodes* (Ushiyama *et al*., 1977), *Pleurotus ostreatus*, *P.sapidus* (Liang *et al*., 1990) and *Volvariella volvacea* (Chen *et al*., 1988).

Enzyme-linked immunosorbent assay

Virus detection by ELISA (Voller *et al*., 1976) had become widespread among plant and animal virologists and was a simple and fairly rapid (1-2 days) detection method. Its drawback was that a monospecific (perfectly pure) antivirus serum was required.

Any antibody to normal mushroom constituents gave very strong, nonspecific, false positive tests. Mushroom was detected in preference to or as well as, virus. The great difficulty in adequately purifying most of the mushroom viruses for antiserum production resulted in limited use of this technique for detecting MV-3 and a spherical virus in *P.pulmonarius* (Barton, 1985; Liu and Liang, 1986).

Tests have shown that direct electron microscopy can detect MV-1 at a concentration of 1mg/ml (micro-gram per ml). A little better is dsRNA at concentration 250mg/ml. Most ELISA tests with plant and animal viruses can detect down to 2ug/ml, an increase in sensitivity

over direct electron microscopy of 5,000 times (Barton, 1985).

Reverse transcription-polymerase chain reaction assay (RT-PCR)

Harmsen (1990) described RT-PCR detection method for the presence of dsRNA in spawn run compost. This is a sensitive and reliable test available for detection of dieback disease virus at any stage of cultivation of *A.bisporus*. This method, in principle, could be applied to mycelium in the compost since dsRNA L3 encodes for one of the coat protein of 34nm particle. However, it is especially important to test atleast two dilutions of each compost extract in a range equivalent to 0.5-5 ug freeze dried compost per RT-PCR reaction. Low dilution of the samples inhibit the RT-PCR by the presence of inhibitory compounds and high dilutions lower the concentration of dsRNAs beyond the detection limit.

Transmission and spread

Through mycelium

It was revealed at an early stage that viable mycelium could transmit the 'dieback' disease (Gandy, 1960). Viable diseased mycelium would remain behind in trays after a crop and after inadequate disinfection, would anastomose with healthy mycelium in the following crop and thus transmit the virus.

This is the most common method of transmission and has been confirmed by several workers (Dielman-van Zaayen, 1986; Hollings, 1962, 1972, 1982; Hollings *et al*., 1963). However, this is possible only among the compatible strains and not in others. For example, *A.bitorquis* was not infected after exposure to diseased *A.bisporus* mycelium and spores (Van Zaayen, 1976). Although *A.bitorquis* has been regarded as highly resistant or immuse to mushroom viruses, it may escape infection by incompatibility with *A.bisporus*, for heterokaryosis did not occur between the two species (Raper, 1976). *Mycogone perniciosa* heterokaryosis did not occur between the two species (Raper, 1976). *Mycogone perniciosa* and *Verticillium fungicola*, both parasitize *A.bisporus* and their mycelial strands penetrate the intercellular spaces in mushroom sporophores, the known viruses of *M.perniciosa* and *V.fungicola* are serologically unrelated to any of the known viruses of *A.bisporus* and there is no evidence of any virus

transmission occurring between them. Tubular virus particles were found in *Plicaria* sp., a weed mould growing among the mushrooms in trays, and in very low concentration in the mushroom sporophores (Dielman-van Zaayen, 1967) but there is no evidence to suggest that any transfer of the virus took place between the two fungi.

Through spores

This was first demonstrated for mushroom virus (MV-1) by Schisler and co-workers (1963, 1967) and subsequently for mushroom viruses 2,3 and 4 (Hollings *et al*., 1971). Mushroom virus 4 particles have also been visualized in thin sections of mushroom spores and germtubes (Dielman-van Zaayen, 1972b). Last and co-workers (1967) isolated 25nm virus particles from some of Schisler's spore-derived cultures and confirmed the transmission of the disease. Spores can infect the compost at any stage before and/or after spawning and after germination the mycelium anastomose with disease-free mycelium thereby resulting in virus transmission. Infected mushrooms usually mature too early and growers can not pick them all before they open and release the spores. Spores from diseased mushrooms often germinate better and faster than uninfected spores (Dielman-vaan Zaayen, 1970, Schisler *et al*., 1967). Over 40 per cent of spores from infected mushrooms germinate within a week on agar medium compared with none of the healthy spores. The spore load within a mushroom house fluctuates greatly; over 3 million per minute were recorded in the exhaust air from a mine shaft with an accumulation of unpicked mushrooms (Schisler *et al*., 1967) under ordinary cropping conditions, 1000 to 10000 spores per m3 were estimated from cascade impactor and volumetric spore traps (Gandy, 1971). Spores were detected 5cm away from exit ventilators but further away no spores were trapped (Gandy, 1971) but Frost and Passmore (1979) have reported that daily mean concentration of order of 10-10 were present in the compost yard at distances of 10 to 20m from the nearest growing room exhaust. Mushroom spores have been detected in fairly good concentration in air samples taken from the pasteurized filtered air-zone of the farm where peak heating, spawning, spawn run and casing were done (Frost and Passmore (1979). Gandy (1971) also detected basidiospores

upto 16 per m3 in a spawn run room at GCRI where the air was filtered to exclude particles greater than 2 um and detected similar concentrations on a commercial mushroom farm with a similar air-filtration system. Since minimum dose of basidiospores necessary for transmission of the disease lay between 1 to 10 spores per tray (Schisler *et al*., 1967) the efficiency of transmission of virus diseases in mushrooms through spores will be very high. Thirty years or more is the estimate given by Schisler and co-workers (1967) as the life of healthy spores although they did not specify the conditions of storage. Van Zaayen (1979) claimed a life of 14 years of spores stored at 4C. Atkey and Barton (1978) found that virus-infected spores stored under more stringent conditions of normal room-temperature (20C) on a window sill in full sunlight were viable and able to transmit 25 nm and 35 nm viruses after 6.5 years although the efficiency of transmission had declined somewhat compared with that of fresh spores. Nair (1976) observed that infected basidiospores were smaller and had thin walls but this was not verified by other workers (Stalpers and Van Zaayen, 1981). Isometric virus particles measuring 25, 30 and 39 nm in diameter have also been transmitted through basidiospores of *Lentinus edodes* (Ushiyama and Nakai, 1975).

Transmission through vectors

There is no any report about the involvement of any vector for the transmission of mushroom viruses. However, a very low level of transmission of MV-1 by mushroom phorid fly (*Megaselia halterata*: Diptera) was obtained when aseptically reared insects were allowed to feed first on purified virus from a sucrose density-gradient and then on healthy mushroom mycelium. There is no evidence, however, that *M.halterata* can acquire the virus from infected mushroom mycelium. Hollings and Gurney (1973) also failed to transmit MV-1 and MV-4 from sterile virus-infected mycelial cultures to healthy ones, using aseptically reared mites(*Tarsonemus myceliophagus*). However, both phorid flies and mites do carry the mushroom spores from one place to another within a tray or from tray to tray, thereby resulting in introduction of virus inoculum. Such agencies may be important carriers but not vectors.

Mechanical transmission

Many workers have attempted to infect mycelial cultures by applying

cell-free virus preparations but none has succeeded so far even when the cultures were abraded or shaken with carborundum powder or glass blades. Very low transmission has been obtained when purified preparations of mushroom viruses 1, 2 and 3 were hypodermically injected into sporophore initials of healthy *A.bisporus* grown in screened isolation chambers (Hollings, 1962; Holling and Stone, 1971). The viruses were not subsequently detected in the injected sporophores but were confirmed in the mycelium growing beneath them. This has been confirmed with MV-4 with very low levels of transmission (Dieleman-van Zaayen and Temmink, 1968).

Temperature and time of inoculation had great effect on symptom development in inoculations at casing the cream X-disease and the La France isolates produced more severe symptoms when held at a cropping temperature of 20 to 21°C than when held at 15 to 16°C (Hager, 1968). Disease severity was also observed to be correlated with time of inoculation. Inoculations at spawning were more damaging than inoculations at casing (Hager, 1968; Last *et al*., 1967; Schisler *et al*., 1967). Contamination of trays or shelves with fragments of mycelium provides a very important means of spread of all the viruses to the next crop causing maximum damage. For detecting viral infections and predicting percentage of yield losses on the basis of dsRNA bands, Batterley and Olson (1989) have standardized the sampling technique from the mushroom beds/ cropping rooms. It is not unusual to get positive detection by PAGE or ELISA tests for mushroom viruses without observing any virus symptoms in the sporophores. But positive detection results using agar growth test and direct EM are almost always associated with yield reduction or symptoms of the disease.

MANAGEMENT OF MUSHROOM VIRUSES

For adopting suitable management strategies for mushroom viruses, one has to keep in mind that the disease is spread by viable mycelium and spores of diseased mushrooms; early infection is dangerous, especially an infection simultaneous with or shortly after spawning. Upto the time of casing, the compost and mycelium must be protected. Owing to the lack of useful resistance with the species, control of the disease is based largely on the use of hygienic practices

directed at the elimination of diseased mycelium and basidiospores from the production (Schisler *et al*., 1967, Van Zaayen, 1976). Dieleman/van Zaayen (1970, 1986) has suggested various approaches to reduce the spread of mushroom virus diseases which have been summarized below:

When the disease is not present

1. Steam the compost for 12 hours at a temperature of 70°C. At emptying, remove the compost quickly.

2. Spray the wood with 2 per cent sodium pentachlorophenate to which 0.5-1.0 per cent soda (sodium carbonate) has been added, after drying spray with water.

3. Disinfect doors, little holes in the floor, shutters, racks, floors and walls with formaldehyde (not with sodium pentachlorophenate). Also clean the manure yard and adjacent patches of ground with formaldehyde.

4. Before filling, fit spore filters, during growing time these spore filters should be replaced once or twice according to the amount of dust in the air. Use a fan for extracting air.

5. Immediately after spawning, use a pesticide against flies and cover the compost with paper. Keep the paper moist. Wet the paper twice a week with a 2 per cent solution of the 40 per cent commercial formaldehyde. Repeat till a few days before casing. Never use sodium pentachlorophenate here. Moisten the paper before removing it carefully.

6. Quickly remove cuttings and litter and destroy.

7. The entire farm and its surroundings should be maintained very clean and stay so. In the working corridor formaldehyde should be sprayed. Machines, refrigerator and other utilities should be disinfected with a formaldehyde solution.

8. At the first sight of contamination, the disease can be controlled best by immediately steaming out the concerned room.

When the disease is already present

1. Adopt practices 1,3 and 4 mentioned under when the disease is not present.

2. Immerse the wood in a 4 per cent sodium pentachlorophenate solution to which 0.5-1 per cent sodium carbonate has been added.

3. Pick the mushrooms when still closed.

4. Keep each room as a separate entity with separate clothes, shoes, steps, buckets, picking knives, picking racks, fans etc. Kill off diseased patches with salt and cover with plastic, make the limits of the patches rather big. First pick from the healthy parts then from the diseased patches. Wash hands often.

5. Admit as few visitors in the diseased rooms as possible and keep the door towards the working corridors closed. Kill off pests in particular. Have a short picking period only (not more than 4 weeks).

Heat Therapy

When infected cultures were grown at 33C for 2 weeks, and hyphal tips then sub cultured and returned to 25C, many of the latter showed normal growth and did not contain virus (Gandy and Hollings, 1962). However, these findings were not conclusively proved by Dieleman-van Zaayen (1970). Rasmussen and co-workers(1972) also obtained increased sporophore yields when tissue and spore cultures derived from symptomatic sporophores of white and two cream strains were incubated at 32C for 2 weeks. Wuest and Mataka (1989) have observed more extensive spawn run on horse manure compost with the symptomatic spawn incubated at 30C than the spawn incubated at 23 or 27C.

Spawn Strains

Immunity to the virus disease of the cultivated mushroom, *A.bisporus* has been found in several strains of the white mushroom, *A.bitorquis*, collected from nature. Some strains of *A.bisporus* do not show symptoms as markedly as others. These are the brown, cream and off-white strains, or some smooth-white strains known to anastomose less frequently with others, or *A.bitorquis* can help to reduce the general virus inoculum and can enable economically worthwhile crops to be grown. Hybrid strains can anastomose with both white and off-white strains and therefore, their widespread culture may reduce the effectiveness of strain alteration as a means of virus control (Fletcher *et al.*, 1989; Romaine, 1987).

b) OYSTER MUSHROOM

A mycovirus affecting *P.florida* has been detected by immunodiffusion and ELISA tests and found related to *P.ostreatus* virus (Krishna Reddy *et al*. 1993). But varions measuring 26±2nm and 21nm in diameter have been reported associated with virus disease of *P.ostreatus* and it is not clear as to which virus is affecting *P. florida* in India.

Symptoms induced in *P.florida* include; pileus curling upwards, swollen stalks and greatly distorted basidiocarps. Premature spore shedding and elongation of stalk are typical symptoms of the disease. Management practices are almost same as described in white button mushroom.

IV. ABIOTIC DISORDERS

In addition to biotic agent which adversely affect the mushrooms, there are a large number of abiotic agents which create unfavourable environment for the proper growth of mushrooms resulting in the quantitative as well as qualitative loss. These abiotic agents include low or high moisture in the substrate, pH, temperature, CO_2 concentration in the room, wind velocity, fumes and relative humidity. Many of these agents make the substrate non-selective for mushroom mycelium and encourage other moulds and pests while some interfere with the normal mushroom production. Management of environment is of great significance in mushroom cultivation and any deviation from the optimum requirements may lead to various kinds of abnormalities. Since a major proportion of button mushrooms is being produced under natural climatic conditions in India, the following abiotic disorders are quite frequently observed.

1. Storma

Common name : Storma, Sectors, Sectoring.

Stroma are noticeable agreegations of mushroom mycelium on surface of spawned compost or the casing. Discrete aerial patches of white mycelium form a dense tissue layer on the substrate surface. Stroma can be easily peeled from the surface of compost or casing. Stroma form on the compost in small localized areas and the smaller patches can coalesce into larger areas. After casing, stroma may form on the casing above a patch of compost-borne stroma or on casing where stroma does not exist in the compost. Stroma on casing developes in advance of pinning but rapidly putrefies once watering begins. Mushrooms can develop on stroma, but this is somewhat unusual.

A sector is a portion of spawn that is distinctive when compared to the general appearance of spawn. A sector may be extra-white, extra-dense or extra-ordinary fluffy and is always different from the normal spawn. Sectors appear on or in the compost and on the casing, and tend to disappear as the crop ages.

Stroma and sectors are related to the genetic character of the

spawn but are sometimes induced if spawn is mishandled or exposed to harmful petroleum based fumes or chemicals or certain detergents during preparation, storage, transit or at the farm. Production practices during cropping also affect the appearance of these abnormalities but specific relationship has not been elucidated. Excessive CO_2 and prolonged spawn run period also result in stroma formation. A few sectors will not affect yield adversely but the presence of excessive stroma may reduce yield. Large patches of stroma 8 to 12 inches are often removed from the compost or casing surfaces with the hope that next growth of spawn will be normal and bear mushrooms. Removing patches of stroma does not ensure growth of mushrooms in these areas, so removal of stroma is a matter for each farmer to decide. This disorder has been commonly observed in seasonal farms in HP where proper aeration is lacking.

2. Weepers

Common names : Strinkers, Leakers

Mushrooms described as being 'Weepers' typically exude considerable amount of water from mushroom cap. When small water droplets exude from stem or cap, the mushrooms are called leakers. These water droplets may be few in number and relatively isolated from each other or may be sufficiently numberous to cover the mushrooms. The distinction between a 'leaker' and 'weeper' is that the water droplets remain as droplets on the leaker mushrooms while it actually falls or flows from a weeper.Weepers are usually noticed since they are quite unusual. A weeping mushroom can dissolve into a white foam. Water collects on the casing surface beneath a weeper and the area developes a putrid odour becoming a 'stinker'.

Factors that induce a mushroom to become a weeper are not known but low-moisture compost-less than 64% coupled with high moisture casing is where weepers are frequently seen. The combination of these two conditions often foster weeper mushrooms prior to and during the first break.

Smooth white mushrooms seems to have some sort of protection against leakers and weepers. Other major types-off-white, cream, golden white are susceptible to this malady. The disease has been recorded in the seasonal farms in Himachal Pradesh.

3. Flock, Hard cap and Open veil

Common names : Flock, Hard cap, Open veil, Saggine socks.

Flock is a physiologically induced malformation of the mushroom's cap and gill tissue. The cap opens pre-maturely and the gills of the affected mushrooms are rudimentary, poorly developed and have little pigmentation. The flocked mushrooms generally appear in first flush and may disappear in subsequent flushes but in some cases it continues increasing in subsequent flushes.

The mechanism that causes the mushrooms to be flocked is genetic and certain strains have a greater tendency to develop the abnormality. Environmental conditions including diesel exhaust, oil-based point fumes and certain anticorrosive chemicals in steam boilers or certain diseases like die-back, brown plaster mould and false truffle induce flock symptoms. Hard cap is a variation of flock syndrome. With hardcap, cap and gills are as described for flock and the cap tends to be disproportionately small in relation to stem diameter. Hard cap mushrooms are restricted to a limited area on the casing but at times 30% areas may produce hardcaps. Hard cap means a loss of harvestable mushrooms. Open veil is the premature opening of veil with abnormal gill development. Open veil sometimes occurs when a period of water stress of 1 to 3 days - is followed by a generous watering. It also occurs when fumes of certain organic chemicals drift into or are released in a growing room. Overall, if open veil appears, it is safe to conclude that the mushroom had been under stress during its development. This abnormality is of common occurrence in H.P. and Haryana, especially during the termination of the crop or under high temperature conditions.

4. Hollow core and Brown pith

These two disorders seem to afflict cream strains much more than other strains, although off-white strains can have hollow core. When the bottoms of the stems are trimmed after harvesting, a circular gap is seen in the centre of the stem. This hole may extend the length of the stipe or it may be shorter. When the hollow cut end portion is brown in colour the sale price is considerably reduced. This abnormality seems to be related to watering and water stress.

5. Purple stem

Common names : Purple stem, Black leg, Storage bum.

Cut stems of the mushrooms develop a deep purple colour within few hours of harvest or after being in cold storage (36F) overnight. At times colour is closer to black than purple and it occurs in all strains-smooth white, off-white, cream and brown. Generally mushrooms from 3rd break to the end of the crop are most susceptible. Polyphenol oxidase, an enzyme increases in later-break mushrooms and this enzyme influences pigment formation. Conditions that predispose mushrooms to this phenomenon are unknown but the frequency and the amount of water applied before harvest seems to affect its occurrence.

6. Rose Comb

Large lumps and swelling are visible on the mushroom cap. The gills often grow in the top of the cap tissue and even on the top of the cap. These mishappen gills make the swellings look spongy. The mushrooms can even burst or split and then turn brown.

The abnormality is caused by gases or vapours coming from solvents, paint or oil products and polluted casing soil.

7. Scales or crocodiles

Scales arise through the surface tissue failing to grow while the cap develops further. The main reason for scales being formed is poor climate control, in particular too much drying out or too great air velocities. Strong vapours of formaldehyde or pest-control products in excess can also cause the outer layer of the skin of half-grow mushrooms to tear off. As the mushroom continues to grown, the skin bursts and so-called 'crocodile' skin is formed. The off-white and cream mushroom strains are more sensitive to scalyness than white mushrooms. This is the most common and serious malady affecting button mushroom in seasonal farms in HP.

8. Long stemmed mushrooms

The presence of long stems in combination with a number of other symtoms can indicate virus diseases but it is often the result of too high CO_2 concentration so that the stems

extend more (drumsticks). With the improvement of aeration such conditions can be avoided.

9. Brown Discolouration

Browning of small pin heads or half grown mushrooms is very common on seasonal mushroom farms. This may be caused by high temperature, sprinkling at high water pressure(maximum pressure is 0.4 atm), chlorinating with too high a chlorine rate [maximum rate is 500ml (10%) per 100 litre of water per 100m²] or incorrect use of formalin, e.g. by spraying the mushrooms with a formalin solution.

10. Oyster Mushroom

As compared to white button mushroom, there are few physiological disorders recorded in oyster mushrooms. Reduced light in the cropping room results in longer and thicker stipes and pileus is partly reduced. Insufficient ventilation (1-2% carbon dioxide) and low light exposure induce bunched growth regeneration.

V. BACTERIAL DISEASES

Broadly, the mushroom is defined as macro-fungus with distinctive fruiting body which can be either epigeous or hypogeous. In this article the term mushroom has been used to denote edible cultivated mushrooms. More than 2,000 species of fungi are reported to be edible throughout the world (Chang and Miles, 1982). Out of these about 16 genera representing more than 25 species have been successfully domesticated.

In India, three mushrooms namely white button mushroom (*Agaricus bisporus*), dhingri or oyster mushroom (*Pleurotus* species) and paddy straw mushroom (*Volvariella volvacea*) are being exploited for commercial cultivation. In addition to this, recently *Calocybe indica* which is commonly known as milky mushroom is also gaining popularity in some parts of the country and is suited for cultivation in warmer areas where *A. bisporus* can not be cultivated. These mushrooms like any other living organism are attacked by several pathogens. The present chapter deals mainly with the bacterial pathogens which produce recognizable symptoms and cause significant crop losses. The expression of disease symptoms in mushroom depends upon the stage of development of the fruit body at the time of infection and cause of the disease/inoculum potential present.

The bacterial diseases have been reported from all over the world on fruit bodies of *A. bisporus, A. bitorquis, Pleurotus* species, *Volvarella* species, *Lentinus edodes, Flammulina velutipes* and *Auricularia* species and are given along with their causal organism(s) and distribution in Table 1.

The bacterial pathogens induced varieties of symptoms like blotch, mummy, pit, drippy gill, soft rot, yellowing and immature browning but in India, bacterial diseases has been reported only on fruit bodies of *A. bisporus* and species of *Pleurotus* and *Auricularia*. The various bacterial diseases reported from India are discussed as under:

Table 3: Bacterial diseases of edible cultivated mushrooms

Mushroom	Disease	Causal organism	Distribution	Reference
Agaricus bisporus	Bacterial blotch	*Pseudomonas tolaasii* *P. fluorescens*	Worldwide	Fletcher *et al.* (1986)
	Ginger blotch	*P. gingeri***	UK, Netherlands	Fletcher *et al.* (1986)
	Drippy gill**	*P. agarici*	UK, Netherlands	Fletcher *et al.* (1986)
	Mummy	*P. aeruginosa*	UK	Wuest and Zarkower (1991)
A. bitorquis	Bacterial blotch	*P. tolaasii*	Worldwide	Fletcher *et al.* (1986)
	Soft rot	*Bukholdria gladioli pv.* agaricicola	Worldwide	Guleria *et al.* (1987)
Oyster mushroom (*Pleurotus* spp.)	Bacterial rot	*P. alcaligens***	India	Biswas *et al.* (1983)
	Brown blotch	*P. tolaasii*	Japan, Australia Netherlands	Fermor (1986) Ferri (1985)
	Yellow blotch	*P. agarici*	India, USA	Jandaik *et al.* (1993b) Bessette *et al.* (1985)
	Fist-shaped Fruit bodies*	*P. fluorescens*	Belgium, Italy and Europe	Poppe *et al.* (1985)
Other mushrooms				
Volvariella spp.	Bacterial rot	*Pseudomonas* sp.	India Indonesia	Kannaiyan (1974) Fermor (1986)
Lentinus edodes	Browning*	*P. fluorescens*	Japan	Komatsu and Goto (1974)
Flammulina velutipes	Brown soft rot*	*Erwinia* sp.	Japan	Phawicit (1985)

* Not recorded from India

** Invalid names

Bacterial disease(s) of *Agaricus* species

Bacterial blotch

Bacterial blotch of mushrooms is also known as brown blotch and bacterial spot.

Occurrence and losses

Blotch is one of the most common and serious diseases of *A. bisporus* and is responsible for considerable losses. The disease also affects. *A. bitorquis*. The disease was first described by Tolaas (1915) from America and later Paine (1919) identified the organism as *P. tolaasii*. From India, it was first reported in 1976 (Guleria, 1976). Bacterial blotch disease reduces crop yield because lesions develop on the surface of mushroom caps making the mushrooms unmarketable. The disease has been reported from almost all mushroom growing countries of the world. The disease causes 5 to 10 per cent losses in yield (Fermor, 1986; Vantomme *et al*., 1989). In Australia, bacterial blotch is second in economic importance only to the virus disease complex (Nair, 1969) and substantial losses occurred particularly after harvest and overnight storage of mushrooms at low temperature.

Symptoms

Bacterial blotch of white button mushroom is characterized by brown spots or blotches on the pilei and in more severe cases, on the stipes. Circular or irregular yellowish spots develop on or near the margins of the cap which enlarges rapidly under favourable conditions and coalesce to form rich chocolate brown blotches that are slightly depressed. The most characteristic symptom of bacterial blotch is the occurrence of dark brown areas of blotches on the surface of the cap. These may be initially light in colour but may eventually become dark brown. Severely affected mushrooms may be distorted and the caps may split where the blotch symptoms occur. Brown and slightly concaved spots appeared on the surface of the diseased fruit bodies. Light infection of mushroom caps produced a yellow light brown spotting on the surface, but the common symptom associated with infection was appearance of brown, slightly sunken lesions of variable size and mushroom tissues were usually affected to a depth of 1 to 3 mm. Mushrooms often become infected at a very early stage in their development. The enlargement of the spots on the cap surface is dependent upon environmental conditions and is favoured by

temperatures of at least 20°C together with the presence of water film.

Casual organism

Tolaas (1915) described a causal organism as a pathogenic strain of *Pseudomonas fluorescens*, but Paine (1919) while working with other isolates found differences in their action on nitrates and starch and as such proposed the name *P. tolaasii* Paine. Lelliot *et al*. (1966) showed that *P. tolaasii* was indistinguishable from some isolates of *P. fluorescens* and suggested that *P. tolaasii* could be considered as one of the natural constituents of microflora of mushroom beds. Fahy (1981) observed that members of *P. tolaasii* contained both pathogenic and non-pathogenic strains which were common on mushroom.

Olivier *et al*. (1978) reported the appearance of both smooth and rough forms of *P. tolaasii* and claimed that the smooth form was non-pathogenic. Wong and Preece (1979) proposed the white line in agar and mushroom tissue rapid pitting tests for the identification of *P. tolaasii*. They observed that a sharply defined white line of precipitate was formed in *Pseudomonas* agar F between the opaque white colonies of *P. tolaasii* and translucent colonies of certain unidentified pseudomonads. The visible interaction has been utilized a s specific and reliable method for the identification of *P. tolaasii*.

Epidemiology

Casing and airborne dust are the primary means of introducing the blotch pathogen into a mushroom house. Even after pasteurization the bacterial pathogen is present in most casing materials. Occurrence of the disease is associated with the rise in the bacterial population on the mushroom cap rather than in the casing. Blotch can develop on cap, stipe or both at any stage of mushroom development. Bacteria present on mushroom surface reproduce in moist conditions especially when moisture or free water film persists for more than 3 hours. Once the pathogen has been introduced at the farm, it may survive between crops on the surfaces, in debris, on tools and various other structures. It is also a natural inhabitant of both peat and chalk. When the disease is present on the farm, its secondary spread may take place through workers, implements, ingredients, mushroom spores, debris etc. Sciarids and mites are also important carriers of the pathogen beside water splashes.

Management

Ecological management : Manipulation of relative humidity, temperature, air velocity and air movements are of great significance in managing the disease. Temperature above 20°C and relative humidity of more than 85 per cent should be avoided. Additional ventilation and air circulation after watering can ensure the quick drying of mushrooms. Temperature fluctuations at higher relative humidity leading of water condensation must be avoided.

Biological management : Isolates of *P. fluorescnes* and other antagonistic bacteria have resulted in 30 to 60 per cent control of bacterial blotch. Many selective bacteriophages have also been found effective against *P. tolaasii* without any significant effect of *P. fluorescens*. Spraying the casing soil with a mixture of *P. fluorescens* and bacteriophage has resulted in more than 80- per cent control of blotch symptoms.

Chemical management : Application of terramycin 9 mg per square foot, streptomycin (200 ppm), oxytetracycline (300 ppm), kasugamycin and kanamycin has been found effective in managing the disease.

Physical management : Pasteurization of casing soils by steam/air mixture and short wave length irradiation have been reported effective in eliminating the bacterial pathogen but over-heating should be avoided otherwise biological vacuum will be created and successive invasion of moulds would be very high. The introduction of water retentive acrylic polymers as a component of casing soil mixture is also claimed to reduce the disease.

Other bacterial diseases of *Agaricus* species

Bacterial pathogen other than *P. tolaasii* recorded on *Agaricus* species are *P. agarici*, *P.aeruginosa* and *Bukholder gladioli* pv. *agaricicola*. However, *P. gingeri* is considered to be a part of the *P.tolassii* (Miller and Spear, 1995).

Bacterial disease(s) of oyster mushroom

Till date, four bacterial pathogens namely, *Pseudomonas alcaligens*, *P. tolaasii*, *P. agarici* and *P. fluorescens* have been reported parasitising *Pleurotus* fruit bodies

and causing considerable economic losses to the growers. Among these, *P.agarici* and *P. alcaligens* (not valid name) have been reported from India and are described as under:

Yellow blotch

Occurrence and losses

In India, heavy incidence of yellow blotch was reported (Jandaik *et al*., 1993) which resulted in complete failure of crop in some of the mushroom units.

Symptoms

The disease appears as blotches of varying sizes on pilei sometimes depressed, yellow, hazel-brown, fawn or orange in colour. When the disease appears at primordial formation or pinhead stage, it affects the total group of early fruit bodies or only a part of them. Infected fruit bodies turn yellow and remain stunted. The slimy appearance of the infected fruit bodies under high relative humidity (more than 90%) is a common symptom. If the relative humidity is less than 75 per cent, the blotched fruit bodies give appearance of burnt ulcers.

Causal organism

Pseudomonas agarici is a gram negative rod shaped and motile. The colony is buff, circular, pulvionate, semiopaque and 2 to 6 mm in diameter. Oxzidase and catalase tests were positive and starch hydolysis and nitrate reduction were negative. The bacteria can utilize benzoate, citrate and gluconate efficiently. In carbohydrate media, acid was produced from glucose, maltose and fructose. There was no acid production in sucrose, sorbitol, inositol and cellobiose.

Epidemiology

The disease incidence is more under warm and humid conditions. The pathogen is easily spread inside the mushroom farm through water splashes, workers, tools and mushroom flies. When the humidity is more than 90 per cent the fruit bodies gave slimy appearance and finally fruit bodies start rotting and smelling foul within next twenty four hours. Presence of water film on the surface of fruit bodies is quite favourable for earlier appearance of symptoms.

Management

Environmental manipulation : High relative humidity and continuous persistence of water film on the surface of pilei enhance bacterial multiplication. Hence, proper ventilation and careful

watering coupled with monitoring f temperature in the mushroom unit help in limiting the disease incidence.

Use of chemicals : The regular application of chlorinated water containing 100-150 ppm of freely available chlorine (FCA) at 3 to 5 days interval help in minimizing losses due to bacterial pathogen, Use of oxytetracycline and streptocycline have also been reported.

Biological management : Biocontrol of yellow blotch of oyster mushroom appears to offer a viable proposition, especially with the increasing awareness among consumers about the use of chemicals in mushroom units. The possibility of using bacteriophages as control agent for plant diseases caused by various bacterial pathogens including *Pseudomonads* has been reported and it may have application in mushroom industry as well.

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