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Review Article

Major Fungal Contaminants of Mushrooms and Their Management

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Abstract

Mushrooms are known for several nutritional and medicinal benefits and are cultivated worldwide. Several fungal contaminants of mushrooms have been serving as the major restraining factor in the growing mushroom industry for a long time. Fungal contaminants like *Trichoderma* spp., *Mycogone* spp., *Lecanicillium* spp., *Cladobotryum* spp., *Coprinus* spp., *Sependonium* spp., *Sclerotium rolfsii*, and *Cephalothecum roseum* among many, are found to infect mushroom crops at different stages from spawn run period to maturation of fruiting bodies. These contaminants may reduce yield and/or degrade the quality of fruiting bodies of the mushroom causing economic losses. These contaminants are usually peculiar in terms of their symptomatology on the substrates, disease cycle, epidemiological requirements, and yield losses. Most of these contaminants come from poorly sterilized substrates. Several sterilization techniques like steam sterilization, hot water sterilization, alkalinization, bleaching, and chemical sterilization can be employed to eliminate pre-existing contaminants and each technique has its own relative advantage over others. Besides, biological control involving botanicals and live antagonists can also be used as prophylactic sterilant or as therapeutic sprays. Biological control measures are friendly to the environment and human health. Unlike chemical fungicides (used as sterilant or spray), biological control measures don't inhibit mushroom mycelial growth and even don't raise the problem of pesticide resistance in pathogens. Roguing out of infected mushroom fruiting bodies or beds, mushroom house sanitation, and management of vector population are also equally important in preventing the spread of the fungal diseases of mushrooms.

Keywords: Biological control; Fungal contaminants; Mushroom; Mycogone; Trichoderma; Sterilization

Introduction

Mushrooms are defined as the macrofungi with a distinctive fruiting body; hypogenous or epigenous and large enough to be seen with naked eyes and picked by hand (Chang and Miles, 1992). Among 110,000 species of fungi about 16,000 species include mushrooms (Wasser, 2010). Known for good quality amino acids, vitamin B complexes, sodium, potassium, iron, dietary fibers and also considered as the primary natural source of ergosterol or provitamins, mushrooms possess high nutritional and medicinal value and are being widely domesticated these days (Jr, 2005). From various studies, edible mushrooms are also found to have many pharmacological effects like antiviral, antioxidant, anti-tumoral, hypo-cholesterolemic and hypoglycemic (Cheung, 2010). Edible mushrooms are also found to be very effective in reducing stress, cholesterol, asthma, diabetes, cancer, insomnia etc. (Wani *et al*., 2010).

The history of consumption of mushrooms is believed to have started during the hunting and gathering period (Wani *et al*., 2010). However, the first mushroom cultivation is believed to have started from China around 600BC and now China is the world's largest producer and consumer of

mushrooms (Zhang, 2014). Wild mushrooms, edible mushrooms and medicinal mushrooms are considered as the major categories of Global Mushroom Industries (Royse *et al*., 2017). Most popular edible mushrooms of the world are Cremini mushroom, Morel mushroom, Shiitake mushroom, Oyster mushroom, White button mushroom, and Straw mushroom (Joseph, 2021). Out of the total mushroom species, more than 3000 species of mushrooms are considered edible. Out of these 3,000 species of edible mushrooms, about 100 species are under economic cultivation, among them only about 60 species are commercially cultivated worldwide (Chang and Wasser, 2017). Economically mushroom farming is being done in more than 100 countries (Gupta *et al*., 2018). The trend in production (in tonnes) of mushrooms and truffles in the world from 2010 to 2019 is depicted in Fig. 1 (FOASTAT (4/26/2021)).

Among different genera of mushroom, *Lentinula* is the most widely grown mushroom worldwide followed by *Pleurotus* spp. *and Auricularia* spp. However, among the cultivated mushrooms in Nepal, Oyster mushroom occupies 86% of the total production, followed by button mushroom (10%), shiitake mushroom (2%) and other mushrooms (2%) (Raut, 2019).

Growing completely disease-free mushrooms is a daunting task since many microbes may contaminate mushrooms from substrate preparation to fruiting, causing huge loss. Contaminants like virus, bacteria, fungus, insects, nematodes etc. annually cause huge losses in the mushroom industry, among which fungal contaminants being the major and widespread problem (Fletcher and Gaze, 2008).

The fungal contaminants impede the development of mushrooms through competition with them for space and nutrients and could also produce metabolites harmful to the mushroom mycelium (Mumpuni *et al.*, 1998). *Trichoderma* spp., *Aspergillus niger*, *Coprinus* spp., *Penicillium* spp., *Sclerotium rolfsii*, *Mycogyone perniciosa*, *Lecanicillium fungicola*, *Cladobotryum* spp. are some of the important fungal contaminants of mushrooms that are associated with several economically important diseases like green mould, dry bubble, wet bubble, cobweb, etc. (Biswas and Kuiry, 2013; Fletcher and Gaze, 2008). These contaminants deteriorate quality and damage basidiomes ultimately leading to reduced production and sometimes complete failure of the crop (Gea *et al.*, 2021).

Fungal Contaminants

1. Trichoderma spp. (Green Mould)

Several researches from all around the world have illustrated the devastating effects of green mould disease in mushroom production caused by *Trichoderma* species like *T. citrinoviride, T. harzianum, T. aggressivum, T. pleuroti, T. viride, T. polysporum, T. longibrachiatum, T. koningii, and T. pleuroticola* (Kim *et al.*, 2012; Kumar *et al*., 2017; Hatvani *et al*., 2017; Innocenti *et al.*, 2018; Choi *et al*., 2010). Singh *et al*. (2006) recognized *T. harzianum* as the most important species of *Trichoderma* capable of causing green mould disease in many instances and resulting in potential yield losses. *Trichoderma spp*. are very dominant to all fungus and possess high outbreak capacity (Bhandari *et al.*, 2021). According to Kosanović *et al*. (2013), the morphological characters of *Trichoderma* influenced colonies on substrates were white in the beginning, then became yellow and green and finally dark green.

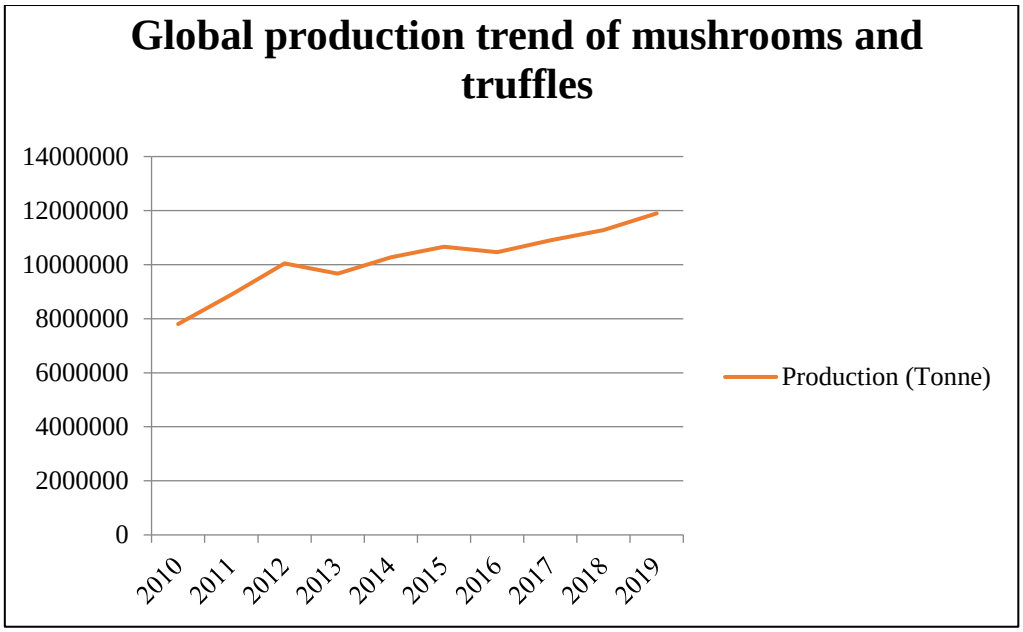

Fig. 1: Production/Yield of Mushrooms and truffles in the world (2010—2019). FOASTAT (4/26/2021)

According to Jandaik and Guleria (1999), *Trichoderma* is often observed in the early stages especially during the spawn run period. Nagy *et al.* (2012) reported 20-30% yield loss due to *Trichoderma* during the second flush of oyster mushroom production. Massive green mould epidemics occured in Hungary in 2015 resulting 100% crop loss in button mushrooms from the infection of *T. aggressivum* f. *aggressivum* (Hatvani *et. al.*, 2017). In-vitro experiments in Turkey, two isolates (Th4 and Th1) of *Trichoderma* aggressivum f. *aggressivum* were found to inhibit the mycelial growth of *A. bisporus* by 71.99% and 58.71% respectively (Aydoğdu *et. al.*, 2020).

Woo *et al.* (2004) observed the presence of *Trichoderma* species at the initial phase of substrate preparation which then disappeared during pasteurization and again seen in the substrate after spawning, during spawn run (incubation phase) and harvesting periods too. Mwangi *et al.* (2017) reported the appearance of the main symptom i.e, greenish mycelium in the compost after 2–5 weeks of beginning of production cycle of *P. ostreatus*.

Yu (2002) examined the effect of substrate moisture content on the growth of mushroom (*Pleurotus* sp.) and *Trichoderma* spp. The study showed the optimum growth of oyster mushrooms occurred at 60-70% substrate moisture content and inhibited at 80%, whereas, the mycelial growth of *Trichoderma* had direct relationship with substrate moisture content reaching its maximum at 80%.

According to Chen and Moy (2004) as cited in (Mwangi *et al.*, 2017), the parameters (such as carbon and nitrogen sources, high relative humidity, warm temperatures) favoring mushroom cultivation becomes ideal for moulds growth after a little fluctuation (including the absence of light during spawn run) that ultimately leads to contamination. The optimal temperature of *Trichoderma* spp. varies among different species generally from 20°C to 28°C (Choi et al., 2003) and no growth at 15°C (Yu, 2002). Woo *et al.* (2004) reported that *Trichoderma* species possess the ability to grow well in acidic-neutral pH (5-7) conditions when provided with low nitrogen levels. Also, the mycelial growth of Trichoderma was found to exceed by 10 times that of mushroom when the infection was initiated as a result of high compost temperature (28°C) and favored by C/N ratio of around 22-23 (Gea *et al.*, 2017).

Through laboratory experiments, Mazin *et al.* (2019) confirmed that mushroom sciarid fly, *Lycoriella ingenua, acts* as a vector for spreading pathogen *Trichoderma aggressivum f. aggressivum,* which was also supported by the experiments by (Coles *et al*., 2021).

Besides, *Aspergillus spp*. and *Penicillium spp*. also are known to cause the Green mould disease but quantitatively leading to much lesser loss than *Trichoderma* spp. (Sharma *et al.*, 2007).

2. Lecanicillium spp. (Dry Bubble)

Lecanicillium is a widely distributed fungal genus belonging to division Ascomycota and includes both entomogenous and fungicolous species (Berendsen *et al*., 2010; Amey *et al.*, 2007). *Lecanicillium fungicola (*previously described as *Verticillium fungicola*) is the causative agent of one of the most devastating diseases in mushroom crops known as dry bubble disease (Berendsen *et al*., 2010). According to Zare and Gams (2008), two varieties namely *Lecanicillium fungicola, var. fungicola* and *var. aleophilum* were found to be associated and responsible for the global spread of dry bubble disease in mushrooms. *L. fungicola* is reported to parasitize several mushroom hosts such as *Pleurotus ostreatus* (Marlowe, 1982), *Agaricus bitorquis* (Gea *et al*., 2003) and *Agaricus bisporus* (Berendsen *et al.*, 2010). *L. fungicola* doesn't have a wide host range and is not found in wild mushrooms, instead more often found to infect already decaying mushrooms (Zare and Gams, 2008).

Holmes (1971) in an experiment to study the relation between time of infection and disease severity found out that the lowest disease incidence of the pathogen (*L. fungicola*) was obtained when inoculated during casing application and maximum incidence was observed when the pathogen was inoculated after 14 days of casing application. According to Berendsen *et al.* (2010), generally three types of symptoms are observed when *A. bisporus* is infected with *L. fungicola* i.e, necrotic lesions, stipe blow-out referring to partial deformation of sporocarps or fruiting bodies and dry bubble (heterogeneously discoloured or homogeneously discoloured white undifferentiated mass of amorphous tissue). Necrotic lesions are brown or grey discolorations on stipe which later cause warty outgrowths on mushroom surface. Largeteau and Savoie (2008) observed the symptoms in infected host mushrooms as undifferentiated spherical masses resembling bubbles, spotty caps and bent or split stipe referred as stipe blowout.

The dry bubble disease is found to have potential of causing crop losses up to 20 % or even more when the disease is out of control, but 1-5 % losses are commonly observed (Grogan *et al*., 2009). Piasecka *et al.* (2021) found casing material, especially peat as the main primary source of *L. fungicola* infection in mushrooms. Apart from casing material and peat, phorid flies and contaminated equipment can serve as a primary source of infection (Wong and Preece, 2007; Kumar *et al*., 2014). Kumar and Sharma (1998) observed sciarid and phorid flies transmitting 84-100% and 76-100% of *V. fungicola* (*syn L. fungicola*) respectively under laboratory conditions. Sohi (1988) reported that minimum of 10 days is required after the infection of pathogen for the distortion symptoms to appear and 3-4 days for cap spotting and found optimum temperature for disease development to be 20°C. The study also reported that lack of proper air circulation, high

humidity, late harvesting and temperature above 16°C favored pathogen growth, the best growth of pathogen noticed at 24°C.

3. Mycogone perniciosa (Wet Bubble Disease)

Mycogone perniciosa, an ascomycotan mycoparasitic fungus is the causative agent of wet bubble disease, affecting commercial cultivation of different mushrooms (*A. bisporus*, *Pleurotus ostreatus*, *P. citrinopileatus*, *Volvariella volvacea*, etc.) in most of the major mushroom growing countries worldwide (Gea *et al*., 2010; Carrasco *et al*., 2019; Fletcher *et al*., 1995; Li *et al*., 2021).

The main symptoms of Wet bubble disease include the presence of deformed tissue called as sclerodamoid mass (result of an early infection at pin head stage) and development of white mycelial growth on fruiting bodies of button mushroom masses (Fletcher *et al*., 1995; Umar *et al*., 2000; Amin *et al*., 2021). Initially the bubbles formed are reported to be white, fleecy (feathery) and spongy which later acquire a brown color and decay (Fletcher and Gaze, 2008). Munshi *et al*. (2010) reported the presence of amber liquid droplets containing bacteria and spores which released an unpleasant smell after rotting.

Sharma and Kumar (2000) in northern India, reported the loss in yield of A. bisporus ranging from 15.72% to 80.13% in artificially inoculated conditions and upto 100% in natural incidence of the disease. Zhou et al. (2015) also observed the devastating nature of Wet bubble disease in A. bisporus and found yield losses of 15–30% under favorable conditions and even total crop loss in the most severe cases. Chulan *et al*. (2017) studied the nature of damage of *M. perniciosa* and found that it only affected the morphogenesis of *A. bisporus* fruiting bodies whereas vegetative mycelium was not affected at all.

According to Fletcher and Gaze (2008), casing material is the main source of infection of *Mycogone perniciosa* to cultivated mushrooms. Also, the pathogen was found spreading through water splashing, aerial transfer of conidia and operators like contaminated tools, clothes, etc. Siwulski *et al*. (2011) observed that the fastest growth of M. perniciosa occurred at an incubation temperature of 25°C and significant growth inhibition occurred at 15°C whereas the best mycelial growth was obtained at pH 5.5 of the agar medium which later got restricted at pH 8.4.

4. Cladobotryum spp. (Cobweb disease)

Cobweb disease caused by several species of fungus belonging to *Cladobotryum*, is considered as one of the most infectious disease of white bottom mushroom which leads to the formation of patches of white cobweb-like mycelium (Carrasco *et al*., 2017; Royse, 2014). During disease development, at first white patches appear on the basidiome and spread quickly by means of fine gray white mycelium resembling spider web (Carrasco *et al*., 2015). Severely infected mushroom bodies show discoloration and rotting. After the advancement of this disease dry spores starts to release from mycelium and spreads to other basidiomes with the help of various agents (Adie *et al*., 2006).

As a soil borne disease, mycelium or conidia from soil or debris are major sources of contamination. High relative humidity and temperature are found to be very favorable conditions for disease development accompanied by sciarids and phorids as vectors (Sharma *et al*., 2007). Back *et al*., (2012) concluded that at a temperature range of 10-22^{0}C, *Cladobotryum* species are found to have more vigorous growth.

The symptoms caused due to different fungal contaminants on substrate and mushroom mycelium are shown in Fig. 2 (Gea *et al*., 2021).

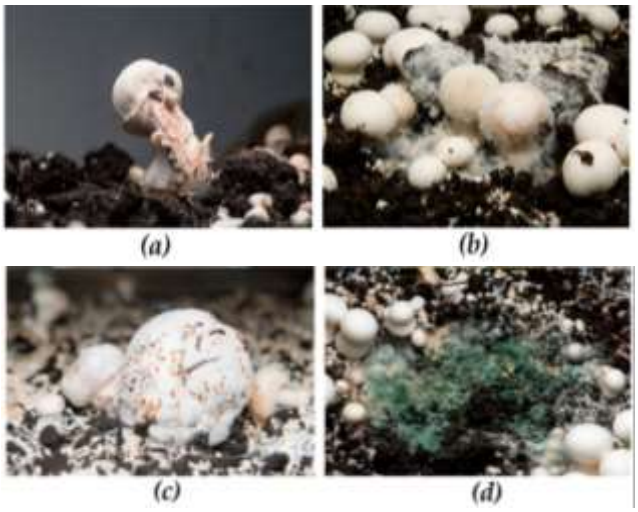

Fig. 2: Disease symptoms in *Agaricus bisporus* commercial crops (**a**) *Lecanicillium fungicola*/dry bubble disease (DBD); (**b**) *Cladobotryum* spp./cobweb disease; (**c**) *Mycogone perniciosa*/wet bubble disease (WBD); (**d**) *Trichoderma* spp./green mould disease

5. Sepedonium chrysoporium (Yellow mould)

Yellow mould disease is characterized by initially having white mycelium which later turns yellow to tan colored. Yellow brown corky mycelium on the interphase of compost and casing are initial symptoms of yellow mould disease which emit a strong metallic smell similar to carbide (Tsarev, 2021).

A yield loss of 5-20% has been recorded in button mushroom production due to yellow mould. Spores formed on yellow mould surface spreads to compost with the help of wind flies, human activities etc. This disease is found very severe at 70% moisture and 19-20^{0}C temperature of compost (Sharma *et al*., 2007).

6. Sclerotium rolfsii

Sclerotium rolfsii is one of the major fungal contaminants in mushroom cultivation. This fungus is characterized by profuse production of cottony aerial mycelium; rapid aerial mycelium growth), forming abundant light brown colored

sclerotia; 1.0-1.5 mm in diameter, globose to subglobose, smooth surface, glossy and compacted (Watanabe, 2002).

Rajarathnam *et al*. (1992) reported *Sclerotium rolfsii*. as a serious substrate-borne fungal contaminant found during commercial cultivation of *Pleurotus flabellatus* on unfermented rice straw coming from rice fields. Depending on the degree of contamination, the loss due to *S. rolfsii* in mushroom yield ranges from 80% to 96%), several folds higher than that of the mushroom (Rajarathnam and Zakia, 1987).

An investigation carried out by Rajarathnam and Zakia (1987) demonstrated that *S. rolfsii* had higher capacity to degrade, bio-convert and grow on rice straw as compared with that of the mushroom and it resulted in greatest drop in substrate pH caused by the secretion of oxalic acid during its growth on rice straw which is harmful to the hydrolyzing enzymes of *P. flabellatus.* Oxalic acid secretion by *S. rolfsii* possibly fixes with Ca^{2+} and Mg^{2+} ions, thus resulting in the shortage of these crucial metal ions necessary for the principal growth and development of the mushroom (Rajarathnam *et al*., 1992).

S. rolfsii requires high temperature for its growth and sclerotial production (Punja, 1985). The temperature range of 8-40°C is optimal for its hyphal growth and dry mass production while maximum growth and sclerotial development require a temperature of 27-30°C (Mathur and Sarbhoy, 1976). Mycelial growth of the pathogen was progressively reduced with increment of moisture content (Mustafee and Chaltopadhyay, 1971), and in well-drained and dry condition, disease incidence was found relatively greater (Wecrapat and Schroeder, 1966). The growth and sclerotial formation in *S. rolfsii* both are better under continuous light (especially blue light) than in continuous darkness (Humpherson-Jones and Cooke, 1977).

7. Coprinus spp. (Ink caps)

Coprinus spp. is also one of the most predominant fungal contaminants of mushroom beds of *Pleurotus spp.* (Biswas and Kuiry, 2013). *Coprinus comatus*, a member of the Agaricales family, is a common fungus found frequently in lawns, in waste areas and along gravel roads all over the world (Park and Lee, 2005). They auto-digest themselves with the help of sporophores as they age, from the bottom of the cap upwards, eventually turning into black ink (Jang *et al.*, 2009). *Coprinus spp*. of mushroom shows the features including the presence of a ring on the stem, pinkish young gills along with a string-like strand of fibers in the hollow stem (Kuo, 2008). They appear cream-colored at first, followed by bluish black later and are usually covered with scales (Sharma *et al*., 2007). *Coprinus spp.* are seen in the composts or substrates during spawn run or casing beds and outside the manure heaps during fermentation process (Maurya *et al*., 2020; Yang and Xue, 2000). Artificial inoculations of *C. fimetarius* has led to a reduction of weight of fruiting bodies of cultivated mushrooms by upto 94.43% (Sharma, 1992).

The incidence of the fungal contaminants is minimum during the month of January (2.87 %) while reaching its peak during the month of June (32.8 %) depending upon the climatic conditions (Biswas, 2016). The infection of *Coprinus* spp. in oyster mushrooms is favored by ammonia, present in compost during peak heating. Presence of ammonia in the compost is confirmed with the appearance of black inky liquid (Maurya *et al*., 2020). Jang *et al*.(2009) reported that the optimal temperature for the mycelial growth of *C. comatus* was found at 23-26^{o}C while the pH range of 6-8 favored the mycelial growth of this fungus.

8. Cephalothecum roseum (Pink Mould)

Pink mould is one of the competitor fungal contaminants during white button (*Agaricus spp*.) mushroom cultivation. It appears as white growth during an earlier stage of contamination on the casing soil and later it turns pink (Sohi and Upadhyaya, 1989; Singh and Singh, 2012). According to Sharma *et al.* (2007), around 90% of yield loss or even complete crop failure have also been recorded by this fungus. It reproduces asexually by the formation of conidia without known sexual stage (Batt and Tortorello, 2014).

The optimum conditions for the growth and development of the fungus are 25 °C and pH 6.0. The combination of low temperature (15 °C) and the pH of 4.0-6.5 support rapid sporulation. Higher concentrations of glucose can escalate the size of conidia, which are found floating in the air and moving places to places making air the primary route of fungal infection during mushroom cultivation (Domsch *et al.*, 1980).

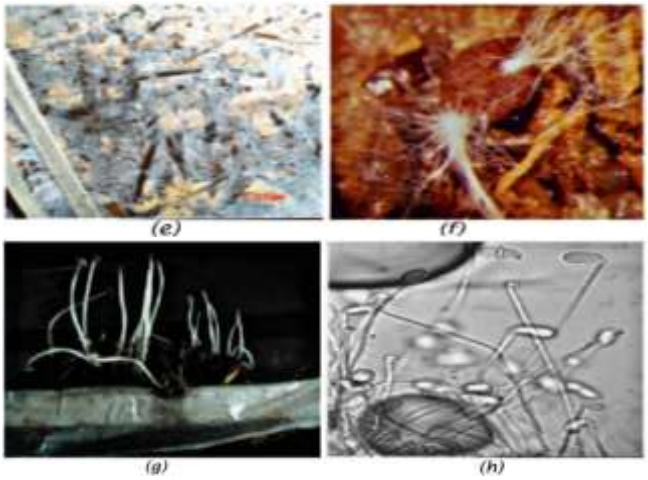

Fig. 3: Infection caused by various fungal moulds during button mushroom cultivation: ***(e)*** *Sepedonium chrysosporium (*Yellow mould) ***(f)*** *Sclerotium rolfsii* ***(g)*** *Coprinus* spp. (Ink caps) ***(h)*** Microscopic view of spores of *Trichothecium roseum* causing Pink Mould.

Infection caused by various fungal moulds during button mushroom cultivation and microscopic view of spores of

Trichothecium roseum causing Pink Mould are shown in Fig. 3 (Sharma *et al.*, 2007; Fichtner, n.d.; McPartland and Hillig, 2008).

Management

1. Sterilization

Sterilization of substrates is a very appropriate method of removing the existing microbes (Khan *et al*., 2011; Kalita, 2015). Several methods like steam pasteurization, hot water immersion, and chemical treatment are used for the preparation of substrate for mushroom cultivation (Atila, 2016). Before spawning, the vegetative forms of microbes present in substrates should be eliminated or reduced (Mejía and Albertó, 2013).

Steam Pasteurization:

Steam pasteurization is one of the most commonly used and effective substrate preparation techniques in mushroom cultivation across several parts of the world. Pasteurization helps in eliminating microbial contaminants and competitor weed fungi, in addition to enhancing mushroom mycelial growth (Atila, 2016; O'Neill *et al*., 2015; Sharma *et al.*, 2007). Autoclaving is reported to yield better in many substrates compared to chemical method (Dinesh *et al.*, 2013) and most of the experiments on mushroom are found to prefer substrates sterilized in an autoclave due to its superiority in terms of biological efficiency and reduction in contamination (Bonatti *et al*., 2004; Shah *et al.*, 2004; Khan *et al*., 2011). However, this method is not very feasible for small scale growers in rural areas (Atila, 2016).

Pasteurization is comparatively inexpensive compared to sterilization in terms of eliminating a variety of pathogens (Sánchez, 2010; Jandaik and D.S., 1999). However, pasteurization of substrates at lower temperatures (60°C) increases the risk of contamination, particularly from *Trichoderma* species since chlamydospores of several *Trichoderma* species are found to survive the exposure at 60°C for 9 hours (Atila, 2016; Sharma *et al.*, 2007). However, an aerated steam at 54.4°C for 15 minutes can eliminate *M. perniciosa* from casing soil (Wuest and Moore, 1972) as cited in (Sharma *et al.*, 2007).

In addition to prevention of contamination, different periods of pasteurization also contribute to difference in yield. (Khan *et al.*, 2011) found out that the use of lab autoclave for 1 hour gave the highest yield of *Pleurotus ostreatus* (122.61% higher than control treatment i.e. soaking of substrate in ordinary water for 30 minutes) followed by country style autoclaving for 2 hours (118.39% higher yield over control) and finally country style autoclaving for only one hour (38.17% more yield).

Chemical Sterilization:

Only a few chemicals are advised to be used in mushroom cultivation against competitive fungi. Mushrooms themselves being fungi and very sensitive to fumes, gases, and several chemicals, makes it difficult to choose effective fungicides with lower inhibitory effect on mushrooms. In addition, the short cropping cycle of mushrooms raises the concern of residual toxicity and pesticide resistance (Sharma *et al*., 2007).

Chemical sterilization involves eliminating or diminishing contaminants present in substrates through chemicals with pesticidal ability. Some chemicals that are used for substrate sterilization include formaldehyde, formalin, and carbendazim (Dinesh *et al*., 2013).

In-vitro experiments by (Kosanović *et al*., 2015) and (Gea *et al.*, 2003) found carbendazim, chlorothalonil, and, Iprodine to be very effective against several *Trichoderma* species along with *Cladobotryum dendroides.* Chlorothalonil and Carbendazim were also found effective against *Lecanicillium fungicola.* Bitertanol, an alcohol derived fungicide was also found to inhibit *Trichoderma harzianum* in vitro (Shah *et al*., 2013). Some studies have also suggested a strong fungicidal property of Prochloraz manganese and Trifloxystrobin against *Trichoderma* spp. (Kosanović *et al*., 2015).

Chlorothalonil has also been found effective as a drench against contaminants like *Mycogone* and *Verticillium*. *Verticillium fungicola*, tolerant to certain benzimidazole fungicides, have been found to be eliminated by Chlorothalonil. Similarly, *Verticillium* and *Mycogone* can also be controlled with the spray of carbendazim or Thiabendazole (Sharma *et al.*, 2007). Zaayeb (1979) as cited in (Sharma *et al*., 2007) achieved the highest yield of *Agaricus* with chlorothalonil at 3 g/ litre water/m^2 applied directly after casing and again 2 weeks later. Benomyl, Carbendazim, Chlorothalonil, Prochloraz manganese, and Thiophanate methyl have been found effective against *M. perniciosa,* Diethane Z-78 and Hexathane for sterilization of casing materials against *Verticillium sp., and* PCNB (Pentachloronitrobenzene) against the cobweb disease even after the establishment of disease (Sharma *et al*., 2007). Pink mould can be controlled by spraying Thiram or Captan (0.04%) twice on casing soil at 10 days interval (Guleria and Seth, 1977).

Most of the competitor moulds infecting oyster mushrooms have been reported to be completely inhibited by Benomyl 50%, Carbendazim (100ppm) + Blitox (100 ppm), Thiram (100 ppm) under both in vitro and in vivo conditions (Sharma *et al*., 2007). Sprays of Mancozeb (0.2%) or Bavistin (0.1%), TBZ (0.2%) or treatment with Zineb dust or calcium hypochlorite (15%) at weekly basis has been found very effective against a wide range of fungal contaminants including Green Mould (Maurya *et al*., 2020).

Aqueous solution of formaldehyde (2 litres formaldehyde per 100 litres water/ 100 m^3 immediately after casing) is recommended as a disinfectant to eliminate the spores of fungi and bacteria (Sharma *et al*., 2007). However, this toxic feature of formaldehyde can even hinder the growth

of mushroom mycelium (Atila, 2016). Alheeti *et al*. (2013) also reported that sawdust substrate treated with antifungal and antibacterial chemicals can inhibit fruiting body formation. Also, the frequent use of formalin tends to promote the development of green moulds (Sharma *et al.*, 1999) and thus not more than 2% concentration is recommended (Sharma *et al*., 2007).

In addition, most of the chemicals used in mushroom sterilization are specific to only certain contaminants and chemical disinfection also requires a longer period for colonization and harvest (Mejía and Albertó, 2013; Atila, 2016). *Pleurotus* treated with formalin was found to take a longer period for colonization (Ali *et al*., 2007). Continuous use of chemical fungicides leads to pathogenic resistance to those chemicals urging new pesticides to be tried against the pathogen similar to the case of resistance development in *Trichoderma* spp. against Benomyl and thiophanate methyl intensively used earlier (Kosanović *et al.*, 2013; Grogan, 2008). Thus, the chemical pesticides should be used in moderation if not possible to be completely discouraged (Kosanović *et al*., 2015).

Hot Water Treatment:

Hot water treatment is an inexpensive and easy method of substrate sterilization and is used across several parts of the world including India, Bangladesh, China, Taiwan, Indonesia, Africa and Latin America. For this method, substrates are treated for 10 minutes to 1 hour with hot water heated in tanks through gas, electricity or fire wood or charcoal and spawn is finally inoculated after cooling and draining of excess water from substrate (Atila, 2016; Mejía and Albertó, 2013; Akhter and Aminuzzaman, 2017). Different temperatures and times of immersion have been recommended by different experimenters. For example, Guzmán-Huerta (1983) as cited in (Mejía and Albertó, 2013) recommended 85 ° C for 40 minutes whereas (Ficior *et al*., 2006) proposed 100 ° C for 1 hour. In an experiment by (Akhter and Aminuzzaman, 2017), the prevalence of contaminants was greatly reduced when substrates were treated with hot water at 80 ° C for 3 hours compared to the maximum contamination at 60°C.

In an experiment by Mejía and Albertó (2013), it was found that no contamination was observed when the substrates were immersed in hot water (80°C for 90 minutes). However, the yield was reduced by at least 20% due to the loss of some important nutrients like N, P, and K during immersion in hot water, which was confirmed through the assessment of nutrients in the excess water extract removed from substrates and thus considering the economic loss in terms of higher water consumption and reduced yield, alternative methods over the use of hot water immersion can be considered.

Alkalization:

In the alkalization process, substrates are sterilized with the help of alkalis like calcium oxide or calcium carbonate. Microbes present in substrate get deactivated at basic pH. Adjusting the pH of substrate to alkaline level is found to be inhibitory for the growth of competitive fungi without severely lowering the growth of desired fungus (Stolzer and Grabbe, 1991). Alkali method of sterilization reduces energy use as compared to other methods and is also considered as best among other sterilization methods. Contreras *et al.* (2004) reported yield increment by 37-126% when sterilized with alkali, so it is preferred over other sterilization methods. Alkaline pH inhibits growth of harmful microbes for edible mushrooms. The research on the efficiency of contaminant control by 0.5% lime by Contreras *et al.* (2004) concluded that alkaline pH inhibits common deuteromycetes contaminants like *Monilia, Trichoderma, Penicillium* and *Aspergillus.*

Bleaching:

Bleaching is the process of disinfecting mushroom substrate by the use of various chemical agents, among which Chlorine dioxide and Sodium hypochlorite are most commonly used. Chlorine dioxide forms free chlorite ions when dissolved in water, which has a strong oxidizing and biocidal property and kills bacteria, fungi and viruses (Atila, 2020). It has a high oxidative attack on cell surface, cell proteins, enzymes etc. which ultimately kills the microorganisms (Jeny and Woodworth, 1990). Also, it has a high penetrability and dissolubility property by which it kills microbes (Du *et al*., 2002).

Chlorine dioxide is found to be very effective and widely used against some of the diseases of mushrooms *whose effectiveness mainly depends on its concentration and time of contact with the substrates* (Szumigaj-Tarnowska *et al*., 2012; Han *et al*., 2001). Another bleaching agent named sodium hypochlorite is also effectively used in mushroom cultivation against contamination, by spraying since mushroom initiation to picking (Oh *et al.*, 2000). Atila (2020) conducted an experiment on different concentrations of ClO_2 (0, 2, 4, and 8 ml/lit.) along with various immersion times (10, 20, 30, and 40 minutes) of two substrates and observed that disinfecting the substrates in 4ml/lt concentration ClO_2 for 20 and 30 minutes respectively for cottonseed hull and wheat straw substrate significantly reduced contamination.

2. Biological Control

Chemicals are very popular among mushroom farmers all over the world. However, considering the harmful impacts of chemicals to environment and human health in addition to the emerging problem of evolution of pathogenic resistance to chemicals, biopesticides should be encouraged (Potočnik *et al*., 2015).

Botanicals:

Soković and Van Griensven (2006) reported strong activity of phenolic compounds *carvacrol* and *thymol* obtained from essential oils of oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) respectively against *T. harzianum, T. aggressivum f. europaeum, and T. atroviride.* Plant extracts and essential oils extracted from several medicinal plants like *Lippia citriodora, Bunium persicum, Thymus vulgaris, and Mentha piperita* were successful in preventing mycelial growths of *Trichoderma harzianum* under both in vitro conditions (Tanhaeian *et al*., 2020) and in vivo conditions in the *Agaricus bisporus* and *P. ostreatus* farms (Hatvani *et al*., 2012). Also, the essential oils extracted from *Cinnamomum, Mentha,* and *Pelargonium* were found to inhibit the growth of *Trichoderma aggressivum f. aggressivum* (Geösel *et al*., 2006). In an experiment by Shamoli *et al.* (2016) among several phytoextracts used against *Trichoderma* spp., *Aegle marmelos, Eclipta alba*, and *Diospyros cordifolia* were found to effectively inhibit the mycelial growth. Satisfactory results were also obtained from *Lantana camara*, *Curcuma longa*, and *Cassia tora*.

Dos Santos *et al*. (2017) estimated the effect and efficacy of various essential oils extracted from various aromatic plants against *Lecanicillium fungicola* and found cinnamon oil (0.4%) as good inhibitor of pathogenic mycelium and conidial germination germination was inhibited at all concentrations. Also, clove oil and thyme oil at 0.4% and 0.8% respectively, were effective in inhibiting both the pathogenic mycelium and conidial germination of *L. fungicola*.

According to Glamoclija *et al*. (2009), essential oil from Savory (*Satureja thymbra*) was found to express antifungal activity against *M. perniciosa*. Soković *et al*. (2013) informed about the nontoxic and easily biodegradable nature of essential oils and also recommended the application of 2% Oregano oil for disinfection of commercial casing soil to prevent Wet bubble disease.

Geösel *et al*. (2014) reported the complete inhibition of growth of *Cladobotryum dendroides* by the use of essential oils from Cinnamon, Geranium, and Spearmint. Similarly, Dianez *et al*. (2018) reported about the high antifungal activity of EOs extracted from Peppermint, Patchouli, Clove, and Rose geranium against cobweb disease pathogen, *C. mycophilum* under in vitro conditions. Idrees *et al*. (2019) in an in vitro examination, found aqueous extracts obtained from dried clove seeds/buds (*Syzygium aromaticum*) exhibiting maximum inhibition (99.48%) against *C. mycophilum*.

Biological Antagonists:

Krupke *et al.* (2004) suggested the effectiveness of bacterial strains that produce *lactonase*, for controlling the infestations of green mould in mushroom farms. Few bacterial strains belonging to genus *Bacillus* showed noticeable action against *T. aggressivum* (Savoie *et al*., 2001). In addition to *Bacillus subtilis*, *Pseudomonas fluorescens* was also found effective in-vitro against *Trichoderma spp*. inhibiting its growth by 35.2-41.8% (Shaiesta and Sahera, 2011). In an experiment conducted by Milijašević-Marčić *et al.* (2016), it was found that among 50 isolates of *Bacillus subtilis* tested against green mould pathogen, Isolate B-38 and QST 713 significantly reduced incidence of *T. harzianum* and *T. aggressivum f. europaeum* respectively.

Similarly, Potocnik *et al.* (2018) found the efficacy of B. subtilis QST713, coated on mushroom grain spawn, to be 53.09% against *T. aggressivum f. europaeum* T77. In further experiments, Potocnik *et al.* (2019) studied the impact of a bio-fungicide *Bacillus subtilis* Ch-13 on mushroom yield along with its efficacy in suppressing *Trichoderma aggressivum f. europaeum* T77 in Serbia. The study showed the efficacy of *B. subtilis* Ch-13 along with an increase in mushroom yield by 12%. The extracellular antifungal metabolites produced by *B. pumilus* were examined against mushroom pathogenic fungus and was found to inhibit mycelial growth of many fungal species of *Aspergillus, Penicillium* and *Fusarium* (Munimbazi and Bullerman, 1998).

Stanojevic *et al.* (2016) reported the antagonistic activity of different strains of *Bacillus amyloliquefaciens* against different species of *Trichoderma*. Mwangi *et al.* (2017) also described about the effectiveness of *Bacillus amyloliquefaciens* strain isolated from groundnuts in antagonizing the oyster mushroom pathogens like *T. harzianum* and *T. asperellum* without affecting the growth of *P. ostreatus* mycelium. The authors also concluded that suppression of mushroom pathogenic fungi was due to the production of diffusible, volatile and non-volatile organic compounds by isolated strains of *Bacillus amyloliquefaciens*.

Different strains of actinomycetes have also been found to possess antagonistic activities against few fungal contaminants like *M. perniciosa* (Sharma *et al*., 2007). *P. fluorescens* and *P. putida* are found to be very effective bio control agents in controlling cobweb disease (Bora and Ozaktan, 2000).

3. Sanitation

Sanitation and fumigation are the two lines of defense in the control of diseases in mushroom cultivation. House sanitation is of utmost importance, and without it, fumigation is only half-effective (Charles, 1928). All diseased materials should be detached, disinfected, and destroyed as soon as fungus appears, whereas the young fruiting bodies of invasive weed mushrooms in addition to fruiting bodies of the invasive weed mushrooms should be rogued out in order to prevent their reproduction and spread. Also, the practice of placing diseased mushrooms

near the entrance of a mushroom house should be discouraged since the spores are carried into the house by the wind and insects. Moreover, soil containing spores of the fungus readily adheres to tools, hands, boots or even clothing. Thus, tools and people carrying fungal spores should be prohibited from entering into a healthy house, or at least not before disinfection (Charles, 1928; Sharma, 1994; Sharma *et al*., 2007).

4. Management of insect vectors and improved management practices

There are mainly three pests which are risky for mushroom cultivation, out of which sciarid and phorid are disease vectors while cecid declines its yield by damaging mushroom stems. *Hypoaspis miles* are the potential predators to control the above-mentioned pests (Rinker, 2002). Neem products were found effective in control of phorid (Erler *et al.*, 2008). When the flies infestation is recorded to be high, chemically they can be controlled by Dichlorovus 76EC @0.5 ml at the interval of 3-4 days on the panel of mushroom house (Raypuriya *et al.*, 2018). Nematode infestation was found in paddy straw mushroom, which was controlled by proper pasteurization and sustaining compost moisture 60-65% (Ahlawat and Tewari, 2007).

5. Selection of resistant species/ cultivars

Screening and selection of disease resistant strains of mushrooms are important to manage contaminants (Sharma *et al*., 2007). Different techniques like Hybrid breeding, Marker Assisted Selection (MAS) breeding, transgenic breeding are being studied and even employed to develop disease resistant strains of mushrooms (Chakravarty, 2011).

Conclusion

Several fungal contaminants are found to reduce mushroom yield and quality either through competition for food and space or due to the release of metabolites harmful to the mushroom. Contaminants could lead to mouldy growth like the green mould, yellow mould, and pink mould or distortion and discoloration of fruiting bodies as in the case of Dry bubble disease, wet bubble disease, and cobweb disease. These contaminants in addition to competitor fungi like *Coprinus* spp. and *Sclerotium rolfsii* can be eradicated from the substrates through various sterilization techniques. Therapeutic sprays of botanicals, biological antagonists, and chemicals are also being adopted to eliminate the contaminants. Besides, mushroom house sanitation, roguing, varietal selection, and vector management are a few more economic measures to prevent infection.

Authors' Contribution

All authors have contributed equally to the manuscript. Final form of manuscript was approved by all authors.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

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