REVIEW ARTICLE

Agro-residues Disinfection Methods for Mushroom Cultivation

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Abstract

Agro-residues have effectively been utilized as a substrate in mushroom cultivation. The substrates used in cultivating edible mushroom requires varying degree of pre-treatment to promote the growth of mushroom mycelium by excluding other competitor microorganisms. Sterilization using hot water, steam, or chemical methods is done to disinfect the substrate. However, sterilization is not an ideal disinfection method as it kills both beneficial and harmful organisms in the substrate. Pasteurization of the substrate seems to be the better alternative which permits re-growth of beneficial organisms during the cooling period. This paper presents an outline on the utilization of agro-residues as a substrate in mushroom cultivation. Various methods of chemical, non-chemical sterilization, and pasteurization of substrate have been discussed. It also highlights that more focus is needed to promote mushroom growers to adopt the pasteurization technique than sterilization by developing simple and low-cost pasteurization equipments for rural and small scale mushrooms growers.

Keywords: Agro-residues, Disinfection, Mushroom, Pasteurization, Pre-treatment, Substrate.

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Introduction

The agricultural residues are of two types: crop residues in the field and agro-industrial residues from processing industries. Most crop residues are plant materials like root, stem, stalk, straw, leaves, and branches which are obtained at the field. Agro-industrial residues are the by-products obtained during post-harvest processing of crops yielding residues like shell, husk, bran, cob, straw, and other fibrous materials (Thripathi *et al*., 2013). India produces about 500–550 MT of agricultural crop residues annually out of which 85MT of crop residues are burnt on the field itself (Anon, 2009). The total un-utilized residue available in India is shown in Graph 1. However these residues are widely used as packaging material, animal feed, biomass, thatching for rural homes, biomass production, domestic/industrial fuel, composting for mushroom cultivation, and *in situ* incorporation and green manure (Tripathi, 2013).

Annually about 140.8 MT of residues are available as surplus in India, out of which about 92.8 MT residues are burnt annually in the field with the intent of making the field free from straw and stubble after the harvest of the crop (Pathak *et al*., 2010). However, in case of rice and wheat fields, if combine harvesters are used for harvesting almost all the residues are left out in the field, resulting in *in-situ* burning. According to Thakur (2003), for every 4 tonnes of wheat and rice grain harvested, about 6 tonnes of straw is produced. In this context, during 2011, India's production of rice was 155.7 MT and wheat was 86.87 MT which had produced nearly 233.55 of rice straw and 130.3 MT of wheat straw (Anon, 2013).

The main reason which makes farmers burn the residue on the farm is to clean the field for sowing the next crop. In addition, decreased labour availability, increased labour wages, declining number of livestock, rapid removal of residue from the field, a fast way of controlling weeds, insects and diseases, pest and pasture management, less economically viable alternate use of residues and increased mechanisation has resulted in more usage of combine harvester leaving >80% of residue in the field. Hence it is

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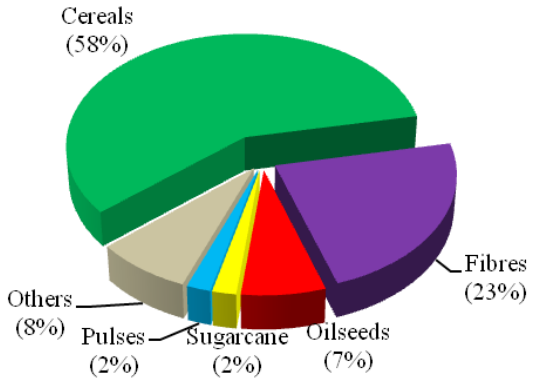

Graph 1: Unutilized residues (surplus) available in India (MNRE, 2009)

difficult for farmers to utilize or dispose of these residues within a short period.

Utilization of Agro-residues for Mushroom Cultivation

Crop residues management is vital for the long-term sustainability of Indian agriculture. Hence burning of residues must be avoided and should be used positively to improve the economic returns of farmers and reduce the environmental impacts. A potential and promising agro-residues management which can attract the farmer would be mushroom cultivation. This technique has various benefits such as the reduction in environmental pollution; generation of employment, particularly for rural women and youth; mode of short return agricultural business offering an immediate benefit to the community; enrichment the human diet and fitness with high nutrition and nutraceutical compounds; improvement of economic standards of the family. Mushroom production has a great potential for exploitation of forest and agricultural residues (Nigam *et al.,* 2009).

Mushrooms are cultivated from wide varieties of lignocellulosic residues. Paddy straw, wheat straw, maize straw, cotton waste, and sugar cane waste are the most widely used substrates. However, cereals straw has gained more importance in mushroom cultivation due to its high nutrient composition. These agricultural residues can either be used alone or a combination of several residues to enrich the nutrients required for mushroom growth. There are several studies which suggest that the supplementation of substrates would increase yield, as compared to the use of the single substrate. This is because these supplements favor quick mycelia impregnation of the substrate as well as an earlier primordial initiation with a good number of fruiting bodies (Pani *et al*., 1997). Some of the successful mushroom cultivation from several residues is presented in Table 1.

Bioconversion of Agro-residues into Mushroom

Mushrooms are macrofungus with a distinctive fruiting body that can be either epigeous or hypogeous and usually grows in decaying organic matter. Generally, mushrooms have a two-phase life cycle, the mycelium (vegetative or colonization phase) and the fruiting body (a reproductive phase that bears the spores). Mycelia secrete enzymes during their vegetative growth phase, which breakdown compounds such as cellulose and lignin present in the substrate (Wood and Smith, 1987). The carbon and nitrogen content in the substrate also plays a vital role in mycelium running and mushroom growth. Since basidiomycetes use carbon sources, the fungi during vegetative growth produce a wide range of enzymes to degrade the lignocellulosic substrates. All plant materials are mainly composed of low molecular weight substances such as organic, inorganic matter and macromolecular substances such as lignin, cellulose, hemi-cellulose, and to lesser extent pectin, starch and other polysaccharides (Thomsen, 2005). These lingo-cellulosic materials are converted into the substrate by mushroom fungi through enzymatic degradation, and their conversion productivity is expressed as its biological efficiency (Chang *et al*. 1981). For instance, Zadrazil, 1975a have reported that cellulose, hemicellulose, and lignin are the major constituents of plant waste and have direct impacts on the growth and development of mushroom fungi. The variation in the carbon and nitrogen ratio is also crucial in substrate degradation and mycelium growth. At optimum C:N ratio for best fungus growth in case of paddy straw is 82.5:1. For instance, Narain *et al*. (2008) reported that mushroom mycelia growth and development is dependent on the lignocellulosic materials especially the C:N ratio. Quimio (1981) recommended an optimum C:N ratio of 90:1 for better growth of Pleurotus spp. mushrooms. Apart from nutrient composition, mycelial growth and fruiting of fungi are also regulated by temperature, gaseous composition, water activity, light and purity of substrate that is free from competitor microorganisms.

Agro-residues Disinfection

There are several thousands of mushroom varieties available across the world, and about 2,500 varieties of mushrooms are known to be edible, out of which only 280 species are cultivated in India (Christensen and Martin, 1969). The basic procedure involved in almost all the varieties of mushrooms remains the same till substrate preparation, later few varieties need additional casing material after spawn running period during mycelium growth. The most important unit operation in the mushroom substrate preparation is its disinfection itself, which has some relation to the pasteurization or sterilization used for milk and other liquids. The mushroom substrate preparation by disinfecting the Agro-residues is a complex and laborious operation as the operation has to be carried out in a hygienic condition.

Table 1: Various agricultural residues in India for mushroom cultivation

Mushroom variety	*Residues*	*Reference*
Pleurotus species	Banana leaves; cinnamon leaves; coconut fiber pith and coir; coconut husks; coffee pulp; corn fiber, cottonseed hulls; groundnut shells; maize straw; soybean stems and husk; sunflower stipes, wheat straw; paddy straw; bajara leaves; jowar leaves; sugar cane bagasse; saw dust etc.	Kwon and Kim, 2004
Volvariella species	Barley straw; coconut coir, fibre and husks; cotton wastes; wheat straw; paddy straw; oil palm fibre; sawdust; banana leaves; rice husk etc.	Kwon and Kim, 2004
Calocybe indica	Rice straw; wheat bran; sawdust; coir pith; paddy straw; maize stalk; sunflower stalk; sesame stalk; sugarcane bagasse; cotton waste etc.	Kwon and Kim, 2004

There are several reasons which make the disinfection process a critical step in mushroom growth. Chang (2008) reported that the substrate pre-treatment for cultivating edible mushrooms (*Pleurotus ostreatus*), which promotes the growth of mushroom mycelium by eliminating other competitor microorganisms. Hydrothermal treatments of Agro-residues also have various effects, including extractive removal, hydrolysis of hemicellulose to produce sugars and also alter the cellulose and lignin properties (Lamptey *et al*. 1985). It is said that in the cell wall, both cellulose and hemicellulose are encrusted within the lignin, thus lignin acts as a barrier against the availability of carbohydrates. Hence, hydrothermal treatment is essential to obtain a good degree of fermentable sugars for mushroom growth by breaking this barrier. Since pre-treatment alters the cellular structure inside the agro-industrial residues and makes the substrate more accessible to enzymes that convert the carbohydrate polymers into fermentable sugars and to cellulase-producing microorganisms (Mosier *et al*. 2005). A study outcome by Diana *et al*., (2006) proved that disinfection of substrate (corn cob) is necessary which permits mycelia to grow better and faster, as the yield from the substrate without disinfection was less than half of the yield from the disinfected substrate. Choi (2004) stated that the pasteurization of substrate at 65°C for 6–8 hours is done to soften the texture of the substrate and kill mesophilic microorganism. But mold spores are stable at 65°C which can be killed at a temperature above 80°C. Most essentially, disinfection of substrate is done to kill the competitor molds such as *Trichoderma spp*., *Coprinus spp*., *Penicillium* and *Aspergillus* those appears during colonization and beginning of fruiting. These species cause green mold which is one of the most common and destructive diseases in mushroom cultivation.

The first person who identified the presence of Trichoderma in mushroom compost was Kligman (1950) and *T. harzianum* was recognized to induce significant quantitative and qualitative losses in the yield (Morris *et al*., 2000). The *Coprinus* spp. was found to cause inky caps during spawn run on the mushroom beds (Sharma *et al*., 2007). These two competitor molds are common and cause green mold disease which hampers the growth mycelium (Lopez, 1996). According to Balasubramanya and Kathe (1996), the fungi *Penicillium* sp. and *Trichoderma* sp. are the competitors with *Pleurotus* sp. even after hot water disinfection at 80°C for 2 hour. This was probably due to the partial breakdown of cellulose and hemicellulose in the substrate, creating more nutrients to the competitors. These competitor molds will grow because of insufficient substrate disinfection, or excessive use of nitrogen will inhibit the synthesis of lignin degrading enzyme (Bisaria *et al*., 1997). Another reason behind heat treatment above 60°C will immediately melt the natural waxy coating on straw materials. Hence this melting process permits the water to easily penetrate and wet the dry substrate material, which is difficult to achieve using cold water. Simultaneously heat inside the straw kills the pathogens present and will not give another chance for growth of new microorganisms (Kurtzman, 2010).

Only one review was written by Kurtzman (2010) on pasteurization with simple methods and good management to minimize the amount of fuel and reduce pests and diseases were discussed and effect of pasteurization and sterilization of substrate on Oyster mushroom yield were also shown in the literature. However, till date there is no review available on substrate disinfection methods, so the disinfection methods followed varies with respect to the type of residues to be disinfected and energy sources available as per several investigators. Thus the disinfection methods currently followed are those developed earlier. In this review, the various disinfection methods (chemical and non-chemical) developed and adopted from two to three decades to treat different agro-residues, the superiority of these methods and their inference are briefly discussed.

Conventional Methods of Substrate Disinfection

Substrate disinfection before spawning is an important operation to kill or reduce pests and microorganisms. Disinfection can be done in two methods: sterilization and pasteurization. Sterilization will kill both beneficial and harmful microorganisms, while pasteurization will only reduce the number of harmful microorganisms. The most common method followed is injected steam into chambers, where the substrate is packaged. The pasteurization time varies as a function of the temperature (Abe *et al*., 1992; Mansur *et al*., 1992). However, the pasteurization time-temperature will depend on the type of pathogen present inside the substrate. Hence selection of right pasteurization time and temperature is always a challenging task to retain the beneficial organism in the disinfected substrate. The methodology for substrate preparation is reported by several studies which include composting the agricultural residues, followed by disinfection of substrate which can be done in several ways (Balasubramanya and Kathe, 1996). From many decades it has been routine to "pasteurize", "decontaminate", "disinfection", "peak heat", "pre-treatment", or "phase II" of mushroom substrate. All these terms refer to blowing steam or pour hot water into the substrate at 60°C for a few hours to achieve any one of these terms (Denham, 1975).

Laborde and Delmas (1974) proposed different methods for substrate pasteurization or sterilization (1) autoclaving (axenic), (2) axenic and inoculation with thermophilic microorganisms, (3) steam treatment between 80 to 100°C for several hours, (4) NA746.DASI system: pasteurization at 72°C for 4–5 days, and (5) tunnel pasteurization by steam treatment at 60°C for several days. According to Sinden and Hauser (1953), the best way to eliminate unwanted microorganisms is to prepare substrate by short method (pasteurize). However, still, there is little confusion among the several mushroom growers weather to pasteurize or sterilize the straw for better growth and yield of mushrooms. Treschow (1944) was one who confused the meaning of "serialization" by combining the process temperatures of both pasteurization

and sterilization together. Though he treated the substrate for Agaricus at 60, 80, 100 and 120°C all for 1h, the process was termed as sterilization process for all temperature range used, rather convincing it as pasteurization based on results obtained.

Chemical Disinfection of Substrate

Mushroom growers prefer chemical sterilization to decontaminate the substrate because autoclaving, hot water, and steam pasteurization are expensive due to the high cost of the equipment. Chemical sterilization can be done easily for a large amount of straw with less expensive since it does not require additional machinery. Several chemicals are used for sterilization of straw; however, only a few chemicals have shown better results, which includes formaldehyde and hydrogen peroxide are most commonly used. The sterilization of the substrate using different chemicals is compiled in Table 2. The alkaline immersion technique (AIT) shown in Table 2 is a simple chemical disinfection method, which is inexpensive and non-thermal technique performed by soaking substrate

Table 2: Chemical sterilization of various substrates used in mushroom cultivation

Chemical	*Inference*	*Reference*
100kg of substrate treated in 150 mL of Formalin + 5 g of Carbendazim solution	Effective in controlling yellow molds (*M. Lutea* and *S. chrysospermum*) in *Agaricus bisporus*	Vijay and Sharma, 1996
1 m^3 of cotton waste treated in formalin (0.5 L) in water (10 L)	Resulted poor yields in *Pleurotus sp*. and took longer duration for the completion of mycelial growth due to unavailability of carbon sources	Muhammad *et al*., 2007
Paddy straw soaked in water for 12h and treated with: • Formaldehyde (500ppm) + Carbendazim (75ppm) • Hydrogen peroxide solution at 1.5%, 3.0%, 6.0% and 8.0% (v/v) • Bleaching powder solution (33% chlorine) at 2%, 4%, 6%, 8%, and 10% (w/v)	Complete prevention of competitor molds, with highest yield of 129.5 g/1 kg substrate from formaldehyde and bavistin treated substrate. But, up to 39.33% contamination incidence was observed; twice that of hot water treatment	Saritha and Pandey, 2010
Wheat straw sterilized with Carbendazim (75 ppm) + Formalin (500 ppm) for 18h	Average yields of *Pleurotus sp*. varied between 681.72 to 742.98 g/kg substrate and *H. ulmariusn* was 855.52 g/kg substrate	Shukla and Jaitly, 2011; Rajak *et al*., 2011
Paddy straw sterilized with Carbendazim (75 ppm) + Formalin (500 ppm)	Highest yield of Pleurotus sp. (1700g/2kg with 85.9% B.E)	Rajak *et al*., 2011
Soaked for 18–24 hours in solution (10 L of water + 12.5 mL Formaldehyde + 0.7 g Carbendazim per kg sustrate)	Average biological efficiencies of all seven Pleurotus sp. cultivated varied from 35% to 85.2%	Chandravanshi *et al*., 2012
Different substrates treated with solution of Carbendazim (75 ppm) + Formalin (500ppm) for 18hr	Substrate supplemented with 5% soybean flour produced highest yield of 675g/2kg substrate for P. sajor-caju and 670g2kg substrate for *P. florida*	Singh and Prasad, 2012
Paddy straw, wheat straw, apple leaves and chinar leaves treated with formalin solution (36:1 ratio of water to formaldehyde) and carbendazim solution (1 gm Carbendazim in10 L of water)	Highest yield of 747.1g/500g dry weight with 149.4% B.E was obtained using paddy straw followed by wheat straw (623.7/500g with 124.7% B.E), apple leaf (478.1/500g with 95.62% B.E) and chinar leaf (426.8/500g with 85.3% B.E).	Pala *et al*.,2012
Wheat straw, grounded pulse powder and rice bran treated with solution (100 L water + 5 kg limestone)	Lime stone acts as anti-bacterial agent against competitor organisms and also reduces the acidity of substrate which is not ideal for mycelium growth	Khan, 2004
Paddy straw soaked with 1%, 3%, and 5% (v/v) solution of 1kg onion + 0.5kg garlic + 10l water	Highest yield was recorded 2.5% onion-garlic solution (113 g/1 kg). Protasan-DC yielded nothing, because of 100% contamination by Coprinus spp	Saritha and Pandey, 2010
Alkaline immersion technique 70 kg substrate immersed in solution (2% calcium hydroxide + 350 L water) for 12 hours	Average mushroom yield was 684.7g/3.4kg bag with B.E of 57.6 %	Hernandez and Sánchez, 2013
Substrate disinfected with alkaline solution for 12 hours	62% of biological efficiency was obtained and study also showed the drawbacks as reported by De León-Monzón *et al*., 2004	Barrios-Espinoza *et al*., 2009

for 12–48 hours in limed water. Contreras *et al*. (2004) suggested the methodology of AIT. Later this was followed by several workers. But, the drawbacks of this method were: chances of bacterial contamination and survival of fly eggs, pre-soaking of straw overnight is necessary, longer time duration, and lesser mushroom yields and B.E (Hernandez and Sanchez, 2013).

Nonchemical Disinfection of Substrate

Sterilization of Substrate

The sterilization of the substrate can be achieved either by hot water, steam, or chemical method. Generally boiling water and hot steam is used for substrate sterilization at a temperature above 80°C in small scales, while the heat sterilization in large scale is difficult; therefore growers prefer chemicals such as bavistin, formaldehyde, carbendazim, etc. Chemical sterilization is an easy operation and cost-effective method for disinfecting a large volume of the substrate. The first attempt for *Pleurotus* cultivation in the sterilized substrate was reported by Falck (1917) during the early decades of the 20th century. Oei (1996) recommended the sterilization of the substrate as an appropriate method for mushroom cultivation. Zanetti and Ranal (1996) showed the substrate sterilization could also be done using autoclaves. Kwon and Kim (2004) sterilized substrate bags in two ways. Firstly, in normal pressure sterilizer at a temperature of either 90–95°C for 5–8 hour or 100°C for 1 hour is maintained. Secondly, in high-pressure autoclave temperature of 121°C for 60–90 minutes is maintained under pressure between 15 to 20 psi. Similarly, Villaceran *et al*., 2006 sterilized the polypropylene bags filled with 1000g of rice straw for one hour at 121°C at 15 psi. The different hot and steam sterilization techniques employed within the last few decades are compiled and shown in Table 3.

Sterilization of substrate was found to have more drawbacks than the pasteurized substrate, some of the drawbacks reported by few researchers include. Quimio *et al*. (1990) have observed that substrate sterilization is not ideal disinfection method since it kills both beneficial and harmful organisms in the substrate. Maintenance of the substrate at 70°C for 24 hours in hot water was recommended by Miroslawa (1991). The pasteurization will selectively kill only temperature sensitive microorganisms while permitting the remaining population to grow with less competition against mycelia growth and also provide an ample opportunity for the mushroom mycelium to colonize (Stamets, 1993). This suggests that it is better to pasteurize the substrate rather than sterilization. In addition, sterilization of substrates is not an easy job for mushroom growers, since determining the right sterilization time and temperature depend on the type of pathogens present in the straw material (Kwon and Kim, 2004). The oyster mushrooms substrate does not require sterilization, and simple pasteurization would be sufficient to reduce the pathogenic damage (bacteria, mold and insect

Table 3: Non-chemical sterilization of various substrates used in mushroom cultivation

Substrate	*Pre-treatment*	*Inference*	*Reference*
Paddy straw	Autoclave at 15 psi	Maximizes the biological efficiency of Pleurotussajor-caju	Krishnamoorthy, 1981
Paddy straw	Steam sterilization at 121°C for 1 h	Undue method for industrial scale with devastating effect on competitor molds and pathogens	Rajarathnum and Bano, 1987
Sunflower thrash	Cold water immersion for 8, 16, 24 and 32 hours; Hot water immersion for 30 minutes; Boiling in water for 30 minutes; Autoclaved at 121°C at15 psi pressure for 30 minutes	Pleurotus sajor-caju offered highest yield when substrate was autoclaved for 30 minutes at 15 psi	Balakrishna *et al*., 2001
Sugarcane bagasse and horse manure compost	Autoclaved at 121°C for 4h; Hot water pasteurisation at 60°C for 2h and Hot water pasteurisation at 60°C for 3h	Though, autoclaved bagasse gave best yield (410.4 g/1kg substrate with BE of 82.10%), the hot water pasteurized (60°C for 3 h) bagasse proved to be a feasible method particularly for rural areas, owing to cost and capacity considerations	Oseni *et al*., 2012
Coir pith, maize straw, paddy straw, sugarcane bagasse, sugar-cane leaves and vettivera leaves	• Steam treatment in autoclave for 15, 30, 45, 60, 75 and 90 minutes • Substrate soaked for 6h and steamed at 121°C for 60 min	Substrate soaked for 6h and steamed at 121°C for 60min produced maximum yield of Calocybe Indicawith 0.6% contamination; projecting theeconomic viability	Lakshmipathy *et al*., 2012
Mixture of wheat straw (94%), wheat bran (5%) and gypsum (1%)	• Autoclave sterilization at121°C for 1.5 h • 1%, 3% Formaldehyde (v/v)	*Pleurotus* sp. yieldwas found to vary with the disinfection method; highest yield was found from sterilized substrate	Kibar and Peksen, 2008

pests) caused on mycelia development and yield (Sanchez, 2010).

Hernandez *et al*. (2003) developed a self-healing method of disinfection without using any heating source, stacking the substrate and allowing it for 2–3 days that build-up heat inside straw bulk. Though this method was feasible in the experimental condition, it failed to pasteurize the entire volume of the substrate except for center and top layers of substrate. To overcome this drawback Barrios-Espinoza *et al*. (2009) and Sanchez *et al*. (2011) modified this method by increasing the substrate mass and volume using a wooden crate (1 m^3) containing 230 kg substrate at 65% moisture. Under experimental conditions, this method showed a positive result in pasteurizing the whole mass of the substrate. Five different *Pleurotus* species were grown from substrate drawn from top, middle and top layers after 48 hours of self-healing. The yields obtained were in the range of 896–1331 g/3.4 kg bag with B.E of 75.3–111.9 %.

Pasteurization of Substrate

Unlike the milk pasteurization at 63°C for 30min or 72°C for 15s, the heat treatment at a temperature up to 80°C is still considered as a pasteurization process in mushroom substrate preparation according to few studies. This high-temperature pasteurization can be done either by steam or hot water at a temperature range of 80°C for 2 hours (Bahukhandi and Munjal, 1989; Balasubramanya and Kathe, 1996). The pasteurization of the substrate is done at a wide range of time and temperature combinations. The substrate pasteurization using hot water and steam and the yield of various mushroom obtained are shown in Table.4. Bulk pasteurization is also another common practice followed

Table 4: Studies conducted on substrate pasteurization

T-t combination	*Inference*	*Reference*
Hot water immersion at 65 ± 5°C for 10 min to 1h	Averts the competitor organisms and provide favorable environment for growth of *Pleurotus* spp. mycelium	Kurtzman and Zadrazil, 1982
Hot water treatment at 55-58°C for 6-24 h	For better growth of mushroom with less contamination	Mateescu, 1985
Hot water treatment at 70°C for 24h	Beneficial to mushroom growth	Miroslava Ziombra, 1991
Hot water treatment at 90°C for 5h	Optimum growth of Pleurotus in polyethylene bags	Dravininkas, 1997
Hot water treatment at 70–80°C for 4–6 h and six fungicides (paddy straw substrate)	95% B.E was recorded for hot water treatment, except mancozeb at 100 ppm remaining treatments gave significantly higher yields that of untreated substrate	Pani and Das, 1998
Hot water treatment at 58–80°C for 24, 48, 72 and 96 hours (wheat and rye straw)	Highest yield of Pleurotus was obtained from straws pasteurized for 48 hours	Ziombra and Fiedorow, 1998
Cereal straw as disinfected in hot water at 60-65°C for 2 hours and steam at 60°C for 2–3 hours inside room containing 12h pre-soaked substrate	Better yields of mushrooms with less chances of contamination	Viziteu, 2004
Corn cob substrate disinfected by boiling in water for 10 min; boiling in water for 1 hour; substrate scalded with boiled water (100°C); substrate disinfected with a fungicide solution (Derosal 0.01%); and substrate soaked in normal water for 24 hours	Best spawning rate was obtained for substrate boiled for one hour, while highest and earlier yields of Oyster mushroom was obtained from substrate scalded with boiled water	Diana *et al*., 2006
Cotton waste disinfected in boiling water for 30 minutes; steam pasteurization at 80°C for 1 hour; and chemical sterilization with formalin	The steam pasteurization produced best results for all three Pleurotus species cultivated, followed by formalin, hot water and control (without pasteurization)	Muhammad *et al*., 2007
Paddy straw was pasteurized by ten different methods which include steaming (80°C for 2 hours in autoclave), hot water (80°C for 60 min) and chemical methods	The average yield obtained from hot water treatment was 51.35% more than that of chemical sterilization technique. Hot water treatment was best method even though steam treatment offered highest yield, but steam method failed to prevent the contamination (*Trichoderma* and *Coprinus* spp.)	Saritha & Pandey, 2010
Paddy straw was pre-soaked for 2h and pasteurized in hot water (80°C for 60 min)	Calocybe Indica yield of 450.6g/1kg substrate was obtained	Senthilnambi *et al*., 2011

Table 5: Studies conducted on bulk pasteurization of substrate with steam and hot water

T-t combination	*Inference*	*Reference*
Steam pasteurization of substrate between 60–100°C for 2–6 hours	The pathogenic fungi and bacteria in substrate mixture will be killed making more favorable for mycelial running of Pleurotus spp.	Zadrazil and Schneidereit, 1972
Heating substrate for 6 hours at 60°C	Kills green molds present in straw, while to kill flies and nematodes mild time-temperature treatments are sufficient	Overtijns ,1981
Substrate pasteurized by pouring hot water into straw at 60°C and held for 30–60 min	Few years later the conditions changed and reorganized which seems to be more favorable for oyster mushroom	Kurtzman, 1975; Kurtzman, 1979a; Kurtzman, 1979b
Mixer method that uses a large concrete mixer to mix substrate at 60 ± 3°C for 30–60 min	The mixer conditions differ from drum method, the dry substrate was added to the hot water, which was found to be more effective way of disinfection	Kurtzman, 2010
• Short fermentation • Steam pasteurization (60-70°C for 12 hours) of various agro-residues	The B.E of *P. ostreatus* was 59.52% for short composting and 61.75 % for steam treatment	De Siqueira *et al*., 2012
Sugarcane substrate disinfected by soaking and heating till start of boiling; soaking and boiling for 15, 30, 45, 60, 75, 90 min; and soaking for 24 hours and boiling for 30 min	The maximum percent yield (61.75%) from substrate soaked and boiled for 75 minutes and there was no yield in case of soaking till start of boiling and soaking and boiling for 15 minutes	Pathan *et al*., 2009
Bulk (20–30 kg) paddy straw pasteurizer cum excess moisture reducer, can heat water between 60–95°C for required time period	Substrate can be loaded, compressed, and pasteurized using this machine. Also offers flexibility in time-temperature maintenance, loading-unloading substrate, draining out excess substrate moisture, and hygienic operation	Gowda and Kumar, 2013
Paddy straw pasteurized at 60°C for 4 hours, 70°C for 3 hours and 80°C for 2 hours under three different compressed levels (without compression, 50% compressed and maximum compressed)	The highest yields of *H. ulmariusn*, *P. sajor-caju* and *C. indica* were obtained from substrate pasteurized at 80°C for 2 hours under maximum compressed level during pasteurization	Gowda *et al*., 2014

by large scale mushroom growers to disinfect large volume of the substrate using hot water or live steam. The different methods reported by various workers were summarized in Table 5. Majority of small scale mushroom growers uses normal oil drums as boiling equipment with little or no modifications which are an inexpensive method. Though this method is inefficient and unhygienic, the method has become usual from decades. These drums can be used to pasteurize both chopped straw and straw bags. Dewraj Taurachand (2004) showed substrate can also by pasteurized by filling into gunny bags or polyethylene bags; these bags are arranged in layers inside pasteurizer drum with enough space for steam circulation. Then heating water either by electricity or burning fuel until the temperature reaches 60–70°C and maintain it for 3–4 hours. Kwon and Kim (2004) reported another method for pasteurization of substrate in bags. Substrate bags are stacked and placed above shelves, entire shelves were covered with plastic sheet or tarpaulin to form a tent-like structure. The hot steam from the steam boiler is directly allowed into the tent for the required time and temperature of pasteurization. Ukoima *et al*., (2009) disinfected substrate by filling into plastic bags and then placing bags inside a drum with hot water at 60–62°C for 3 hours. Kurtzman (2010) developed an inexpensive method for pasteurizing substrate by using two ordinary steel drums as pasteurizing vessels. This method involved heating the water inside the drums up to 60°C and immersing the straw into hot water using a wire basket with a holding time of 30–60 minutes. Later wire basket containing substrate can be pulled out from drums by using chain and pulley assembly, which can be handled by a single operator. It is possible to pasteurize one ton of dry substrate per batch in 8–10 hours. Even FAO suggested a similar technique, using 200 L oil drum with the bamboo screen at the bottom of the drum with 4 inches of clean water. Water is heated until steam rises through the bags and temperature of 90-100°C is maintained for 3–4 hours (Anon, 2001). All these methods are unhygienic, inefficient, difficult to handle, and high chances of re-contamination.

Gowda and Kumar (2013) have developed a hot water substrate pasteurizer having a capacity of 25 kg dry substrate per batch. The system has the facility to disinfect the substrate at a temperature between 50–80°C for the required time period without any risk of contamination. This setup is an inexpensive, hygienic, energy efficient, portable, and has provision for multi-functions such as loading, compacting

the straw, substrate heating, draining excess water, substrate cooling, and unloading by a common man without drudgery. The developed setup is an ideal technology for mushroom growers, to pasteurize any agricultural residue and effectively use them for mushroom cultivation.

Summary

In this review, the most commonly used or newly developed substrate disinfection methods and their superiority in mushroom cultivation are shown. All the above-reported methods for boiling, pasteurization, sterilization, and fermentation of agricultural residues are not easy, high energy input, labor-intensive and complicated, this motivates the growers to adopt chemical sterilization. The chemical sterilization drags most of the interest since this method eases all the complication faced in other methods. However, there are a lot of drawbacks in this technique which include: use of carcinogenic chemicals affect the health of workers inhaling these fumes, create environmental pollution, chemical residues in mushrooms can cause chronic diseases, and also repeated use of chemicals may lead to the development of resistance among competitor molds. Most importantly chemical disinfection method seems to be not effective in killing all the competitors which lead to a reduction in yield and 100% loss of crop in many farms. Yield reduction is because of non-softening of straw, which prevents efficient substrate colonization and damage the mycelium growth. This is due to the partial breakdown of the lignin-cellulose bonds, favoring substrate contamination. Another benefit of the pasteurized substrate over chemical sterilization is that the generated waste after mushroom cultivation will be organically rich in nutrients such as nitrogen, phosphorus, potash, iron, zinc, manganese, and copper as high as farmyard manure, which can be utilized as fertilizer for growing cash crops by farmers. This creates solutions for both agro-residues utilization and ecofriendly disposal of those wastes generated from mushroom cultivation.

During pasteurization, the substrate temperature will remain around 60°C, which needs almost 16h for bulk substrate cooling to 25°C before spawning. Cooling period is the time where most of the beneficial organisms will grow. Thus, if the straw is sterilized at 121°C, it takes even more time for cooling which arises the contamination problems in the sterilized substrate as reported by many studies. The maintenance of large quantity of sterilized substrate free of contaminant growth until spawning is arduous. And also sterilization kills beneficial organisms which will compete against contaminant growth when those organisms are killed, thus sterilized substrate becomes free for contaminants growth. An example of beneficial organisms is nitrogen-fixing bacteria, which plays a crucial role in C:N ratio of the substrate. Thus the purpose of pasteurization is to get rid of those that compete with mushroom not to kill everything, unlike sterilization. In addition, any commercial mushroom grower needs to disinfect many tons of substrate every day which will consume a large amount of time, labor intensive, energy input, and cost of equipments makes it expensive and unaffordable for mushroom growers.

Hence it is advised to pasteurize the substrate either by steam or hot water which is more appropriate for small scale mushroom growers, as these substrates are more stable and less susceptible to contamination. The results of growth and yield of mushrooms obtained among various researchers remain contradictory most of the time. Even though experiments are conducted under similar conditions, the results will vary widely depending upon several factors such as quality of spawn, the effectiveness of pasteurization, quality substrate, substrate moisture, nutrients availability, unhygienic condition, cross-contamination, environmental factors during growth period like light intensity, temperature, aeration, and relative humidity.

Research Needs

Despite the development of several methods for substrate disinfection, pasteurization found to be the most efficient disinfection method. There is scope for developing low-cost energy efficient pasteurization methods by using solar energy to produce hot water and steam which would be idle to tackle the energy crisis. Though there are few trials been conducted to disinfect the substrate by ionizing radiation, still there are no considerable results been reported. So disinfection of soaked substrate by radiation can be studied and optimized. There are limited reports on pasteurization of substrate using microwave energy. Since the microwave is capable of heating any quantity of substance rapidly and uniformly, microwave energy can be exploited for rapid and uniform disinfection of large quantity of substrate after soaking. The development of low-cost appropriate pasteurization equipment's is the need of the hour for mushroom growers to solve the most critical problems encountered in mushroom cultivation.

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