

Review Article

Agriculture Crop Residues Disinfection Methods and Their Effects on Mushroom Growth

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Agriculture crop residues have been effectively utilized as substrates for mushroom cultivation from ancient times. For cultivation of edible mushrooms from these substrates requires various pre-treatments in order to promote the growth of mushroom mycelium by excluding other competitor microorganism. The various pre-treatments such as hot water boiling, pasteurization by hot water and chemical, sterilization, steam treatments etc. are employed cross the world. However, pre-treatment specifically the sterilisation is not an ideal disinfection method for mushroom substrate, since it kills both beneficial and harmful organisms in the substrate. In this context, the pasteurization will not only arrests the growth of competitor fungi such as *Coprinus cinereus*, *Trichoderma harzianum* and *Coprinus comatus* but also breakdown the lignin and cellulose compounds in substrate for enzymatic degradation and make the substrate more favourable for mycelial growth. Studies have proven that pasteurisation of the substrate seems to be an ideal disinfection method which permits re-growth of beneficial organisms during the cooling period. This paper presents an outline on the utilisation of agro residues as substrate in mushroom cultivation; various chemical and non-chemical sterilization and pasteurisation methods with their benefits and drawbacks in mushroom cultivation. The information generated in this paper shows the lack of knowledge on pasteurization equipment which offers a great potential to develop simple and low cost pasteurization equipments for a successful long term mushroom cultivation business. More policies are required to promote the mushroom cultivation which is an eco-friendly disposal of agriculture crop wastes. This would inturn positively influence the overall human nutrition, and improve the economic standards of families.

Keywords: Agro Residues; Mushroom; Substrate; Disinfection; Pre-Treatment; Pasteurization

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 (Satyendra *et al.*, 2013). Overall, India produces annually about 500-550 MT of agricultural crop residues, of which nearly 85-100 MT of crop residues are burnt on the field itself (MNRE, 2009; Devi *et*

al., 2017). 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 (Satyendra *et al.*, 2013).

The total un-utilized residue available in India is shown in Fig. 1. The annual estimated surplus residues availability for bio-energy generation was 234 MT, of which 92.8MT residues are burnt in the field with intent of making the field free from straw and stubble after the harvest of crop (Pathak *et al.*, 2010; Hiloidhari *et al.*, 2014). However, in case of rice and

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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 86.87MT which had produced nearly 233.55 of rice straw and 130.3 MT of wheat straw (Anon, 2013).

The main reason which makes farmers to burn the residue on 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 difficult for farmers to utilize or dispose these residues within a short period of time. *In situ* incorporation of residues takes longer duration for decomposition and hence burning facilitates farmers for early land preparation for sowing next crop. And also these residues are of high moisture content, low bulk density, irregular shape and size, and high carbon and nitrogen content, it is difficult to handle, transport, store, dispose and utilize residues appropriately (Sokhansanj *et al.*, 2005).

Utilization of Agro Residues for Mushroom Cultivation

The crop residues management is vital for long-term sustainability of Indian agriculture. Hence, burning of residues must be avoided and should be used positively so as 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 immediate benefit to the community; enrichment the human diet and fitness with high nutrition and nutraceutical compounds; improvement of economic standards of 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 combination of several residues in order 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 single substrate. This is because these supplements favour quick mycelia impregnation of substrate as well as an earlier primordial initiation with good number of fruiting bodies (Pani *et al.*, 1997). Mushroom can be grown on almost all types of organic wastes available. Some of the successful mushroom cultivations from several residues are presented in Table 1.

Bioconversion of Agro Residues into Mushroom

Mushrooms are macro fungus with 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 (reproductive phase that bear 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 a lesser extent pectin, starch and other polysaccharides (Thomsen, 2005). The seligno cellulosic materials are converted into substrate by mushroom fungi through enzymatic degradation and there conversion productivity is expressed as its biological efficiency (Chang *et al.*, 1981). The mycelium growth, mushroom quality and crop yield are mainly affected by nutrient composition (Philippoussis *et al.*, 2003).

Table 1: Mushroom grown on various agricultural residues in India

Mushroom variety	Residues	Reference
<i>Pleurotus species</i>	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
<i>Volvariella species</i>	Barley straw; coconut coir, fibre & husks; cotton wastes; wheat straw; paddy straw; oil palm fibre; sawdust; banana leaves; rice husk etc.	Kwon and Kim, 2004
<i>Calocybeindica</i>	Rice straw; wheat bran; sawdust; coir pith; paddy straw; maize stalk; sunflower stalk; sesame stalk; sugarcane bagasse; cotton waste etc.	Kwon and Kim, 2004

For an instance, (Zadrazil 1975a) have shown that cellulose, hemicellulose and lignin are the major constituents of plant waste and have direct impacts on the growth and development of mushroom fungi. Singh *et al.* (1989) observed that 75% of cellulose, hemicellulose and lignin are degraded during the growth period. 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. It is said that for successful mushroom cultivation, disinfection of the substrate prior to inoculation with spawn is a crucial task (Chang and Hayes, 2013).

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 same till substrate preparation, later few varieties needs additional casing material after spawn running period during mycelium growth. The most important step in the mushroom substrate preparation is its disinfection, which has some relation to the pasteurization or sterilization used for milk and other liquids. Thus

substrate preparation by disinfection of agro residues is difficult and most expensive operation since it requires fuel-consumption for boiling or steaming. Nevertheless this is so important for obtaining high yield, it is a critical, tricky and labour-intensive.

There are several reasons which make the disinfection process a critical step in mushroom growth. Another study reported by Chang (2008) showed that the substrate pre-treatment for cultivating edible mushrooms (*Pleurotostreatus*), would promote 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 (Lampthey *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 substrate more accessible to enzymes that converts 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 absolutely necessary which permits mycelia to grow better and faster, as the yield from substrate without disinfection was less than half of the yield from disinfected substrate. A similar case was reported by Muhammad *et al.*, 2007, which showed the importance of cotton waste disinfection on *Pleurotus* yield. The pasteurization of substrate at 65°C for 6-8 hour was done to soften the texture of substrate and kill

mesophilic microorganism, but mould spores are stable at 65°C which can be killed at temperature above 80°C (Choi, 2004). Most essentially, disinfection of substrate is done to kill the competitor moulds such as *Trichoderma* spp., *Coprinus* spp., *Penicillium* and *Aspergillus* appear during colonisation and beginning of fruiting. These species cause green mould 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 recognised 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 moulds are common and causes green mould disease which hampers the growth mycelium (Lopez, 1996). According to Balasubramanya & Kathe (1996), the fungi *Penicillium* sp. and *Trichoderma* sp., are the competitors with *Pleurotus* sp. after pasteurization with hot water at 80°C for 2 hour, this was probably due to the partial breakdown of cellulose and hemicellulose, making them available to competitors. These competitor moulds will grow because of insufficient substrate pasteurization or excessive use of nitrogen will inhibits the synthesis of lignin degrading enzyme (Bisaria *et al.*, 1997). Another reason behind heat treatment above 60°C will immediately melts 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 disease 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 vary with respect to 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, superiority of these methods and their inference are briefly discussed.

Conventional Methods of Substrate Disinfection

Substrate disinfection before spawning is 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 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 type of pathogen present inside substrate. Hence, selection of right pasteurization time and temperature is always a challenging task to retain the beneficial organism in 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”, “cook out”, or “Phase-II” of mushroom substrate. All these terms refer to blowing steam or pour hot water into substrate at 60°C for few hours to achieve any one of these terms (Denham, 1975).

Few methods for substrate pasteurization and sterilization such as (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 were proposed by Laborde and Delmas (1974) and Valencia and Lopez (2005). According to Sinden and Hauser, (1953) the best way to eliminate the 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 Sterilization 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 large amount of straw with less expensive, since it does not require additional machinery. Several chemicals are used for sterilization of straw, however only few chemicals have shown better results, which includes formaldehyde and hydrogen peroxide are most commonly used. Indrea and Apahidean (1995) has suggested chemical disinfection can also be done with diluted solutions of fungicide. The sterilization of 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 for 12 to 48 h 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 are: 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. (De Leon-Monzon *et al.*, 2004; Hernandez and Sanchez, 2013).

Non Chemical Sterilization of Substrate

The sterilization of substrate can be achieved either by hot water, steam or chemical method. Generally boiling water and hot steam is useful for sterilization of substrate in small scales, while for large scale disinfection it is difficult, hence growers prefer chemicals such as Bavistin, Formaldehyde, Carbendazim, etc. Chemical sterilization is easy operation and cost effective method for disinfecting large volume of substrate. The first attempt for *Pleurotus* cultivation in sterilized substrate was

reported by Falck (1917), during early decades of 20th century. Oei (1996) recommended the sterilization of substrate as appropriate method for mushroom cultivation. Zanetti and Ranal (1996) showed the substrate sterilization can also be done using autoclaves. Kwon and Kim (2004) sterilized substrate bags in two ways. Firstly, in normal pressure sterilizer at temperature of either 90 to 95°C for 5 to 8h or 100°C for 1h is maintained. Secondly, in high pressure autoclave temperature of 121°C for 60 to 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 pressure. The different hot and steam sterilization techniques employed within the last few decades were compiled and shown in Table 3.

Sterilization of substrate was found to have more drawbacks than pasteurized substrate, some of the drawbacks reported by few researchers includes. Quimio *et al.* (1990) observed the substrate sterilisation is not an ideal disinfection solution, since it kills both beneficial and harmful organisms in the substrate. Maintenance of the substrate at 70°C for 24 h in hot water was recommended by Miroslawa (1991). The pasteurization will selectively kills only temperature sensitive micro-organisms, while permitting remaining population to grow with less competition against mycelia growth and also provide an ample opportunity for the mushroom mycelium to colonize (Stamets, 1993). Similarly, Diana *et al.* (2006) have suggested before spawning, the disinfection of substrate should only destroy only the competitive fungi, but not the beneficial microorganisms. Since beneficial microorganisms do not compete against mycelia growth but they interrupt the development of competitive microorganisms (Apahidean, 2006). This suggests that it is better to pasteurize the substrate rather than sterilization. In addition, sterilisation of substrates is not an easy job for mushroom growers, since determining the right sterilisation time and temperature depend on type of pathogens present in the straw material (Kwon and Kim, 2004). The oyster mushrooms substrate does not requires sterilization, simple pasteurization would be sufficient to reduce the pathogenic damage (bacteria, mould and insect pests) caused on mycelia development and yield (Sanchez, 2010).

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

Chemical	Inference	Reference
100 kg of substrate treated in 150 ml of Formalin + 5g of Bavistin solution	Effective in controlling yellow molds (<i>M. Lutea</i> and <i>S. chrysospermum</i>) in <i>Agaricusbisporus</i>	Vijay and Sharma, 1996
1m ³ of cotton waste treated in formalin (0.5l) in water (10l)	Resulted poor yields in <i>Pleurotus</i> sp. and took longer duration for the completion of mycelial growth due to unavailability of carbon sources	Muhammad <i>et al.</i> , 2007
Paddy straw soaked in water for 12 h and treated with 1. Formaldehyde (500ppm) + Bavistin (75ppm) 2. Hydrogen peroxide solution at 1.5%, 3.0%, 6.0% and 8.0% (v/v) 3. Bleaching powder solution (33% chlorine) at 2%, 4%, 6%, 8%, and 10% (w/v)	Complete prevention of competitor molds, with highest yield of 129.5g/1kg 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 <i>Pleurotus</i> sp. varied between 681.72 to 742.98 g/kg substrate and <i>H. Ulmariusn</i> was 855.52g/kg substrate	Shukla and Jaitly, 2011; Rajak <i>et al.</i> , 2011
Paddy straw sterilized with Carbendazim (75 ppm) + Formalin (500 ppm)	Highest yield of <i>Pleurotus</i> sp. (1700g/2kg with 85.9% B.E)	Rajak <i>et al.</i> , 2011
Soaked for 18-24h in solution (10l of water + 12.5ml Formaldehyde + 0.7g Bavistin per kg substrate)	Average biological efficiencies of all seven <i>Pleurotus</i> sp. cultivated varied from 35% to 85.2%	Chandravanshi <i>et al.</i> , 2012
Different substrates treated with solution of Carbendazim (75ppm) + Formalin (500ppm) for 18hr	Substrate supplemented with 5% soybean flour produced highest yield of 675g/2kg substrate for <i>P. sajor-caju</i> and 670 g 2 kg substrate for <i>P. florida</i>	Singh and Prasad, 2012
Paddy straw, wheat straw, apple leaves and chinara leaves treated with formalin solution (36:1 ratio of water to formaldehyde) and carbendazim solution (1gm Carbendazim in 10l 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 chinara leaf (426.8/500g with 85.3% B.E)	Pala <i>et al.</i> , 2012
Wheat straw, grounded pulse powder and rice bran treated with solution (100l water + 5kg 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 (113g/1kg). Protasan-DC yielded nothing, because of 100% contamination by <i>Coprinuss</i> pp.	Saritha and Pandey, 2010
Alkaline immersion technique 70 kg substrate immersed in solution (2% calcium hydroxide + 350l water) for 12h	Average mushroom yield was 684.7g/3.4 kg bag with B.E of 57.6 %	Hernandez and Sánchez, 2013
Substrate disinfected with alkaline solution for 12h	62% of biological efficiency was obtained and study also showed the drawbacks as reported by De León-Monzón <i>et al.</i> , 2004	Barrios-Espinoza <i>et al.</i> , 2009

Hernandez *et al.* (2003) developed self-heating method of disinfection without using any heating source, stacking the substrate and allowing it for 2 to 3 days that build-up heat inside straw bulk. Though this method was feasible in experimental condition, but it failed to pasteurize entire volume of substrate except centre 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 m³) containing 230 kg substrate at 65% moisture. Under experimental conditions this method showed positive result in pasteurising the whole mass of substrate. Barrios-Espinoza *et al.* (2009) achieved highest BE of 112 % from self-heated substrate. Hernandez and Sánchez, (2013) conducted study on self-heating pasteurization of Pangola grass by developing similar setup as suggested by Sanchez

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 <i>Pleurotussajor-caju</i>	Krishnamoorthy, 1981
Paddy straw	Steam sterilization at 121°C for 1h	Undue method for industrial scale with devastating effect on competitor molds and pathogens	Rajarithnum and Bano, 1987
Sunflower thrash	Cold water immersion for 8, 16, 24 and 32 hr; Hot water immersion for 30 minutes; Boiling in water for 30 minutes; Autoclaved at 121°C at 15 psi pressure for 30 minutes	<i>Pleurotussajor-caju</i> offered highest yield when substrate was autoclaved for 30 minutes at 15 psi	Balakrishna <i>et al.</i> , 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/1 kg 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 <i>et al.</i> , 2012
Coir pith, maize straw, paddy straw, sugarcane bagasse, sugar-cane leaves and vetivera leaves	1. Steam treatment in autoclave for 15, 30, 45, 60, 75 and 90 minutes 2. Substrate soaked for 6h and steamed at 121°C for 60min	Substrate soaked for 6h and steamed at 121°C for 60 min produced maximum yield of <i>Calocybe indica</i> with 0.6% contamination; projecting the economic viability	Lakshmipathy <i>et al.</i> , 2012
Mixture of wheat straw (94%), wheat bran (5%) and gypsum (1%)	1. Autoclave sterilization at 121°C for 1.5 h 2. 1%, 3% Formaldehyde (v/v)	<i>Pleurotus</i> sp. yield was found to vary with the disinfection method; highest yield was found from sterilized substrate	Kibar and Peksen, 2008

et al. (2011). Five different *Pleurotus* species were grown from substrate drawn from top, middle and top layers after 48h of self-heating. The yields obtained were in the range of 896 to 1331 g/3.4 kg bag with B.E of 75.3 to 111.9 %.

Pasteurization of Substrate

Substrate pasteurization can be done either by steam or hot water at a temperature range of 80°C for 2h (Bahukhandi and Munjal 1989; Balasubramanya and Kathe, 1996). However, pasteurization of agro residues is done at wide range of time and temperature combinations. The substrate pasteurization using hot water and steam and the yields of various mushrooms obtained are shown in Table 4. Bulk pasteurization is also another common practise followed by large scale mushroom growers in order to disinfect large volume of 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 is an inexpensive method. Though this method is inefficient and unhygienic the method has become usual from decades. These drum 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 was covered with plastic sheet or tarpaulin to form a tent like structure. Hot steam from steam boiler is directly allowed into tent for required time and temperature of pasteurization. Ukoima *et al.* (2009) disinfected substrate by filling in to plastic bags and

Table 4: Studies conducted on substrate pasteurization

T-t combination	Inference	Reference
Hot water immersion at $65 \pm 5^\circ\text{C}$ for 10 min to 1h	Averts the competitor organisms and provide favorable environment for growth of <i>Pleurotus spp.</i> mycelium	Kurtzman and Zadrazil, 1982
Hot water treatment at $55\text{-}58^\circ\text{C}$ 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 <i>Pleurotus</i> in polyethylene bags	Dravininkas, 1997
Hot water treatment at $70\text{-}80^\circ\text{C}$ for 4-6h 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 to 80°C for 24, 48, 72 and 96h (wheat & rye straw)	Highest yield of <i>Pleurotus</i> was obtained from straws pasteurized for 48 hours	Ziombra and Fiedorow, 1998
Cereal straw as disinfected in hot water at $60\text{-}65^\circ\text{C}$ for 2h and steam at 60°C for 2-3h 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 1h; substrate scalded with boiled water (100°C); substrate disinfected with a fungicide solution (Derosal 0.01%); and substrate soaked in normal water for 24h	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 <i>et al.</i> , 2006
Cotton waste disinfected in boiling water for 30 minutes; steam pasteurization at 80°C for 1h; and chemical sterilization with formalin	The steam pasteurization produced best results for all three <i>Pleurotus</i> species cultivated, followed by formalin, hot water and control (without pasteurization)	Muhammad <i>et al.</i> , 2007
Paddy straw was pasteurized by ten different methods which include steaming (80°C for 2h in autoclave), hot water (80°C for 60min) 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 (<i>Trichoderma</i> and <i>Coprinus spp</i>)	Saritha and Pandey, 2010
Paddy straw was pre-soaked for 2h and pasteurized in hot water (80°C for 60min)	<i>Calocybe indica</i> yield of 450.6g/1 kg substrate was obtained	Senthilnambi <i>et al.</i> , 2011

then placing bags inside a drum with hot water at $60\text{-}62^\circ\text{C}$ for 3h. 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 wire basket with a holding time of 30 to 60 min. Later wire basket containing substrate can be pulled out from drums by using chain and pulley assembly which can be handled by single operator. It is possible to pasteurize one ton of dry substrate per batch in 8-10 hour. Even FAO suggested similar technique, using

200l oil drum with bamboo screen at bottom of drum with 4 inches of clean water. Water is heated until steam rises up through the bags and temperature of $90\text{-}100^\circ\text{C}$ is maintained for 3-4h (Anon, 2001). All these methods are unhygienic, inefficient, difficult to handle, and high chances of re-contamination.

Gowda and Kumar (2014) have developed a hot water substrate pasteurizer having capacity of 25 kg dry substrate per batch. The system have facility to disinfect the substrate at a temperature between 50°C to 80°C for required time period without any risk of contamination. This setup is an inexpensive,

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 h	The pathogenic fungi and bacteria in substrate mixture will be killed making more favorable for mycelial running of <i>Pleurotus</i> spp.	Zadrzil and Schneidereit, 1972
Heating substrate for 6h at 60°C	Kills green molds present in straw, while to kill flies & nematodes mild time-temperature treatments are sufficient	Overtijns,1981
Substrate pasteurized by pouring hot water into straw at 60°C and held for 30-60min	Few years later the conditions changed and reorganized which seems to be more favourable 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
1. Short fermentation 2. Steam pasteurization (60-70°C for 12h) of various agro-residues	The B.E. of <i>P. Ostreatus</i> was 59.52% for short composting and 61.75 % for steam treatment	De Siqueira <i>et al.</i> , 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 <i>et al.</i> , 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, 2014
Paddy straw pasteurized at 60°C for 4h, 70°C for 3h and 80°C for 2h under three different compressed levels (without compression, 50% compressed & maximum compressed)	The highest yields of <i>H. ulmariusn</i> , <i>P. sajor-caju</i> and <i>C. indica</i> were obtained from substrate pasteurized at 80°C for 2h under maximum compressed level during pasteurization	Gowda <i>et al.</i> , 2014

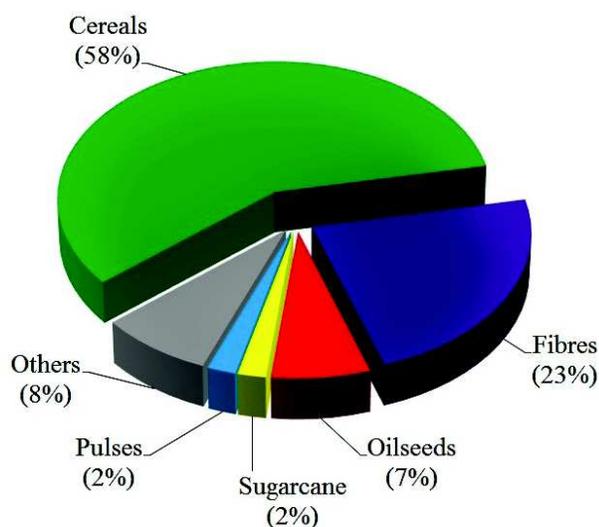


Fig. 1: Surplus crop residues available in India (MNRE, 2009)

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 were shown. All the above reported methods for boiling, pasteurization, sterilization and fermentation of agricultural residues are not easy, high energy input, labour 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 lot of drawbacks in this technique which include: use of carcinogenic chemicals effect 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 development of resistance among competitor moulds. Most importantly, it was evident from many studies that chemical method is not effective in killing all the competitors which leads to the 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, favouring substrate contamination. Another benefit of 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 farm yard manure, which can be utilized as fertilizer for growing cash crops by farmers. This creates solutions for both agro residues utilization and eco-friendly disposal of those wastes generated from mushroom cultivation.

During pasteurization, the substrate temperature will remain around 60°C which needs almost 16 h for cooling to 25°C before spawning. Cooling period is the time where most of beneficial organisms will grow. Thus if straw is sterilized at 121°C, it takes even more time for cooling which arises the contamination problems in 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 crucial role in C:N ratio of substrate. The growth of contaminants consumes hemicellulose during their growth hence growth of mushroom will be restricted. 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, labour intensive, energy input, and the 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 remains contradictory most of time. Even though the experiments are conducted under similar conditions, results will vary widely depending upon several factors such as quality of spawn, 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 is 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 ideal to tackle energy crisis. Though there are few trials been conducted to disinfect the substrate by ionising radiation, still there are no considerable results been reported. So disinfection of soaked substrate by radiation can be studied and optimised. 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 equipments is the need of hour for mushroom growers to solve the most critical problems encountered in mushroom cultivation.

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