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EFFECT OF SUBSTRATE DISINFECTION ON THE BIOLOGICAL EFFICIENCY OF *PLEUROTUS SAJOR-CAJU* (FR.) SINGER

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Abstract

Various disinfection techniques, *viz.*, Autoclaving, Hot water application, Radiation and Chemical treatment were assessed for the yield potential of *Pleurotus sajor-caju*. The appearance of competitor fungi was also noticed during the cultivation period. The crop of mushroom was harvested in three flushes. Amongst different techniques employed, yield and biological efficiency ranged in between 305-360 g and 61-72%, respectively. All of them showed highly significant yield and biological efficiency over control (unpasteurized sets). These were observed maximum in chemically treated substrate (360 g, 72%) followed by autoclaving (340 g, 68%), hot water (335 g, 67%) and Radiation treatments (305 g, 61%). The unpasteurized sets gave negligible result in aforesaid manifestations (10 g, 2%). Two fungal species belonging to Ascomycetes (*Peziza* sp.) and Basidiomycetes (*Coprinus* spp.) were detected from mushroom beds. The pasteurized beds showed lesser incidence of competitors than unpasteurized ones.

Key words : Biological efficiency, fungal competitors, *Peziza* sp., *Coprinus* sp., pasteurization techniques, *Pleurotus sajor-caju,* yield loss.

Introduction

Pleurotus sajor-caju, an edible mushroom belonging to order Agaricales of class Basidiomycetes is most promising mushroom, coming next to button mushroom in respect of its production at global level sharing 25 per cent of total world production. Although, it is not valid oyster species (Guzmán, 2000), even then it is as nutritious as other valid taxa which makes it an ideal food for human consumption. In India, it is admired especially due to its excellent flavour, taste and above all, easy method of growing without any sophisticated infrastructure. For the growing of *Pleurotus* spp. paddy straw is found most common and efficient substrate (Ram, 1995; Dubey, 1999; Gupta *et al*., 1999 and Siddhant *et al*., 2009), which is accompanying indigenous micro flora. The antagonistic interaction between these micro flora and desired fungus contributed the low productivity of mushroom (Bhandari and Singh, 1983; Sharma and Jandaik, 1980, 1981a, b and 1982; Shiddique *et al*., 2004). Therefore, proper substrate disinfection is pre-requisite to eliminate weed and obtained good yield. It can be achieved by various methods (Arya and Arya, 2003; Bahukandi, 1990; Champawat and Chitale, 2003; Kumar *et al*., 1990; Ram and Thakur, 2005; Tewari and Pandey, 1988).

In present communication, six months stored paddy straw was used for the cultivation of *Pleurotus sajor-caju* to evaluate the effect of substrate preparation methods on the biological efficiency of this mushroom species.

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Materials and Methods

Mushroom culture

The pure culture of *Pleurotus sajor-caju* was obtained from the Mushroom Section of Plant Pathology Department, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (U.P.), India. It was maintained on Potato Dextrose Agar medium (peeled, sliced and boiled potato, 200g; dextrose, 20g; agar, 20g l^{-1}) by using serial subculture method (Naraian *et al.*, 2009).

Spawn strategy

a) Spawn substrate

Wheat grain (*Triticum aestivum*) was used as a spawn substrate, which was purchased from the seed market of Faizabad. The spawn was prepared by the conventional method (Naraian *et al*., 2009).

b) Spawn dose

Inoculation of substrate was made with spawn of *P. sajor-caju* @ 5% w/w on dry weight basis under aseptic conditions.

Substrate and its preparation

Six month stored paddy straw was selected as a substrate for the cultivation of *Pleurotus sajor-caju*. It was chopped into 1-2 cm long pieces and filled (500 g) in polypropylene bags (42 × 30 cm size) and washed separately in fresh water. When excess water was drained off, it was disinfected by following treatments:

a) **Autoclaving (T_1) :** This method included sterilization of substrate at 15 lbs pressure for 60 minutes (Tewari and Panday, 1988).

b) **Hot water application (T_2) :** In this method, paddy straw was boiled in the water for one hour at 100°C (Diana *et al*., 2006).

c) **Radiation treatment (T_3) :** In this treatment, the straw substrate was exposed to U.V. light for 1 hour 30 minutes (Ram and Thakur, 2005).

d) **Chemical treatment (T_4) :** Paddy straw substrate was pasteurized in the solution of Formaldehyde (500 ppm) and Bavistin (75 ppm) for 18 hours as suggested by Vijay and Sohi (1987).

e) **Control (T_5) :** Substrate was not pasteurized at all and it was only soaked in water for 24 hours.

Method of Cultivation

Plastic bag technology was used in this experiment. The beds were prepared from pasteurized substrate by multilayered (3) spawning. The mouth of bag tightened with fibre thread. 4-5 holes were made in the bottom of each bag. These were incubated in cultivation room at 22-30°C temperature for spawn run. When mycelia had completely covered the beds, the polythene covering were turned off and relative humidity was maintained 85-95 per cent with the help of humidifier.

Concerning data

a) Data regarding yield parameters

It included time lapsed in spawn run, pin head initiation and maturity of fruit bodies, number of flushes, total yield and biological efficiency on the substrate subjected to different pasteurization techniques. The biological efficiency of mushroom was worked out as percentage yield of fresh mushroom in relation to dry weight of the substrate as suggested by Chang and Miles (1989).

b) Encountered microorganism

The competitors encountered on the mushroom beds were examined under light microscope. These were identified on the basis of morphological characters.

Statistical analysis

Completely Randomized Design (CRD) was employed for yield data of the experiments. These were statistically analysed. The critical difference (CD) was worked out at five per cent probability level.

Results

The effect of different disinfection techniques on cultivation statistics of *Pleurotus sajor-caju* is presented in table 1, fig. 1 and plate 1.

Table 1 : Effect of pasteurization techniques on yield performance and biological efficiency of *Pleurotus sajor-caju* on stored paddy straw.

Pasteurization Technique	Spawn run (Days)	Primordial development (Days)	First harvest (Days)	Total yield from three flushes [g/500 gm dry substrate]	Biological Efficiency (%)
Autoclaving (T_1)	14	15	20	335	67
Hot water (T_2)	14	15	19	340	68
UV-Radiation (T_3)	15	17	22	305	61
Chemical treatment (T_4)	14	15	19	360	72
Water (T_5)	30	37	46	10	02
SE	—	—	—	**8.14**	**1.62**
CD (P=0.05)	—	—	—	**18.15**	**3.75**

Data presented is mean of three replications

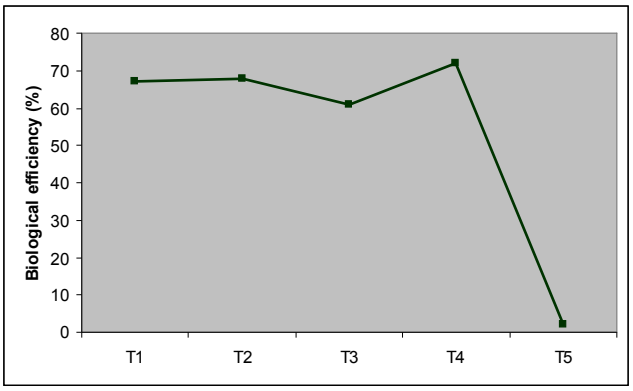

Fig. 1 : Comparison of biological efficiency among the treatments; T_1 : Autoclaving, T_2 : Hot water application, T_3 : Radiation treatment, T_4 : Chemical treatment and T_5 : Control.

which was pat par to each other (table 1).

Appearance of competitors

During the course of study, two fungal species belonging to Ascomycetes (*Peziza* sp.) and Basidiomycetes (*Coprinus* spp.) were encountered on the mushroom beds. The unpasteurized beds, used in triplicate were severely infected by *Coprinus* spp. and *Peziza* spp. (plate 1), which resulted in great loss in mushroom yield. The bed contaminated with *Peziza* spp. could not produced even a single fruit body. The higher decomposition of substrate was also noticed in unpasteurized beds.

The results revealed that disinfection techniques played a crucial role in minimizing the loss of production

(A) *Coprinus* sp.

(B) *Peziza* sp.

Plate 1 : Fungal competitor appeared on unpasteurized mushroom beds (A) *Coprinus* spp (B) P*eziza* spp.

Effect of substrate preparation techniques

The substrate disinfected by employed techniques, *viz.*, T_1, T_2, T_3 and T_4 showed quick spawn run, primordial initiation and fruit body maturation than control [T_5] (table 1). The crop of *P. sajor-caju* was harvested in three flushes where yield and biological efficiency ranged 10-360 g and 02-72%, respectively. All the substrate preparation methods showed highly significant yield and biological efficiency over control (unpasteurized sets). It was recorded the maximum in chemical pasteurization (360 g, 72%), followed by autoclaving (340 g, 68%), hot water treatment (335 g, 67%) and radiation treatments (305 g, 61%). The unpasteurized sets gave negligible result (10g, 2%) in aforesaid manifestations (fig. 1). Among the treatments, chemical pasteurization was found most significant. The hot water treatment and autoclaving was considered as second best treatment and biological efficiency (fig. 1) this also protects the beds from competitors.

Discussion

The disinfection methods reduce the natural micro flora of substrate and improve substrate colonization and yield of mushroom. Due to this reason, pasteurized substrates showed quick mycelial run and higher sporophore formation. In respect of yield performance, chemical treatment gave significant response. This was because of the fact that chemical treatment permitted a minimum number of bacterial population, which favoured the growth and production of more cellulase by *Pleurotus* spp. and thereby increased the yield (Krishnamooethy *et al*., 1991). Production of this enzyme is directly proportional to the yield of Pleurotus *in vitro* (Kochuthresiamma *et al*., 1991; Nallathambi and

Marimuthu, 1994). Besides this, bacterial population actively corrodes the surface of the substrate providing more suitable site for the colonization of fungus. *Pleurotus* spp. are also reported to utilize the nitrogen fixed by N-fixing bacteria present in the substrate. In addition, chemical treatments slightly modified the nature of substrate and favoured the growth of fungal mycelium. As compared to chemical treatment, autoclaving and hot water treatments gave insignificant response. This was due to higher temperature which eliminated all the beneficial microorganisms present in the substrate (Bano *et al*., 1979). Unpasteurized beds gave highly insignificant result in respect of reduction in yield and biological efficiency than pasteurized ones. It is well known that stored straw is an easy source of contaminants which competes with mushroom mycelium for the available nutrients. The loss in yield was either due to depletion of food material from the substrate required for growing mushroom mycelia or through the production of toxic substances by the microorganisms (Atkins, 1949). Although, Doshi and Singh (1991) stated that toxic metabolites produced by microorganisms are very mild and has little inhibitory effect on growth of *Pleurotus sajor-caju* and whatever effect is seen was because of depletion of nutrients from the medium due to contaminants. The higher decomposition of substrate was also visible in unpasteurized beds. It might be the combined action of contaminating microflora and cultivated fungus.

During the cultivation of *Pleurotus sajor-caju* two types of competitor were encountered on mushroom beds. This result was supported by the conclusions given by different workers which revealed the presence of weeds associated with oyster mushroom cultivation (Vijay and Sohi, 1987, Siddique *et al.,* 2004). No or lesser appearance of competitors in pasteurized substrate revealed the efficiency of treatments. Chemical treatment was effective against fungal competitors which is agreement with the finding of Champawat and Chitale (2003) and Pervez *et al*. (2009), who reported formaldehyde and bavistin combination as a best pasteurization practice. The effectiveness of hot water and autoclaving methods was due to the high temperature that kills the foreign inoculums from the substrate while ultraviolet light in the range from 200 to 300nm affects the growth of the competitors. Direct exposure of UV radiation is lethal to their DNA.

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