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Management of contaminants in mushroom spawn

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ABSTRACT

In a study carried out at Mushroom Research and Training Center, SKUAST-J during 2015-16, mushroom spawn prepared on different substrates and supplements was observed for incidence of contamination by fungal and bacterial contaminants. Contamination in spawn ranged from 12.66% (sorghum based spawn) to 20.66% (bajra based spawn). Four major types of contaminants including three fungal, viz. *Aspergillus* spp., *Penicillium* spp., *Trichoderma* spp. and one bacterial contaminant, viz. *Bacillus* spp. were observed. These contaminants were found individually as well as in combination in all the grain substrates. Management of contaminants using multiple strategies was also studied in the present research. The treatment comprising three boiling treatment showed maximum efficiency in management of fungal and bacterial contaminants that reduced the fungal contamination up to 78.33%. However, up to 98.66% reduction in fungal as well as bacterial contamination of spawn was obtained with three autoclavings of the spawn. Among the antibiotics, application of tetracycline (50 µg/kg) resulted in the reduction of 98.33% of bacterial contamination in the spawn.

Key words: *Aspergillus*, *Penicillium, Bacillus,* Mushroom, Spawn

Mushroom farming today is being practised in more than 100 countries and its production is increasing at an annual rate of 6-7% (Chang 1999). India alone produces about 600 million tonnes of agricultural by-products, which can profitably be utilized for the cultivation of mushrooms (Chadha and Sharma 1995). Spawn is the mushroom seed analog to the seed in crop plants. It plays an important role in the mushroom industry because the failure or success of mushroom cultivation depends upon the timely availability of pure culture spawn which is counted as the most important aspect of mushroom production (Goltapeh and Pujram 2003).

The problems being faced by the spawn laboratories are high cost of production and the contamination of spawn by various fungal and bacterial contaminants. Keeping in view the above cited factors, the present research was designed to study the common contaminants associated with spawn production and the strategies for their management.

MATERIALS AND METHODS

Spawn preparation: In a study carried out at Mushroom Research and Training Center, SKUAST-J during 2015-16, quality spawn of *Agaricus bisporus* (strain S-11) was prepared on different substrates, viz. wheat grain, barley grain, bajra grain, sorghum grain, oat grain and maize grains as per the method suggested by Gupta *et al.* (2016). In some treatments, the substrates were supplemented with different supplements, viz. gram husk and paddy husk in the ratio of 1:1 (v/v).

Inoculation of spawn bottles: After sterilization and cooling, the glass bottles containing different substrates and combination of substrates and supplements were shaken to remove clumps and were aseptically inoculated with small bits of fungal mycelium taken from pure culture of mushroom grown on Potato Dextrose Agar (PDA) medium. The bits were placed at the top of the substrates in the bottles containing the substrates alone and in the middle of bottles having mixture of substrates and supplements. The bottles were incubated at 22±2°C.

Incidence of contaminants: Inoculated spawn bottles were constantly monitored for appearance of any contamination or competitor moulds during the period of mycelial growth in bottles. Spawn bottles showing any type of contamination were removed from the incubation room for recording observations and identification of contaminants.

Isolation and identification of bacterial contaminants: Bacterial contaminants were isolated by streak method. Infected grains were placed on Nutrient Agar (NA) medium in petri plates and incubated at 25°C for 2-3 days. These bacterial contaminants were purified by repeated streaking and the colony characters were recorded. The isolated bacteria were identified on the basis of Bergey's Manual of Systematic Bacteriology (Sneath *et al*. 1986).

Isolation and identification of fungal contaminants: For identification of fungal contaminants, infected spawn grains were inoculated at three points on Potato Dextrose

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Agar and incubated at 25ºC. Isolations were made from the hyphal tip of the growing culture. Preliminary identification of the fungi was made on the basis of morpho-cultural characteristics. The identities were further confirmed by getting the cultures identified from Indian Type Culture Collection identification services of Indian Agricultural Research Institute, New Delhi (India).

Management of contaminants: Three types of treatments were adopted for management of contaminants. These included boiling treatments and autoclaving treatments. One, two and three boiling treatments were given with a gap of 24 h between the two subsequent treatments. The time period of boiling depended on the hardness of the grain and was adjusted in a way that the grains did not split apart. Like boiling treatments, one, two and three autoclaving treatments were also given with a gap of 24 h between the two subsequent treatments. The time period of each autoclaving was 20 minat 15 lbs psi.

Statistical analysis: The experiments were conducted in completely randomized design with three replications of each treatment. The analysis of variance was performed using SPSS version 16.0 and means were compared by Duncan's multiple range tests at 5% level of probability for interpretation of results (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Effect of grain substrates on incidence of contamination: Incidence of contamination was observed during spawn production in all the six grain substrates and supplements and the results are presented in Table 1. The contaminants were identified as *Aspergillus* spp*., Penicillium* spp., *Trichoderma* spp. and *Bacillus* spp. Spawn bottles containing different grain substrates were regularly examined for mycelial run of the mushroom fungi as well as contaminants. These contaminants were found individually as well as collectively in the spawn substrates. Percentage contamination varied significantly among different spawn substrates. Sorghum grain spawn showed the minimum contamination (12.66%) which was statistically at par with maize grain spawn (14.33%). However, maximum contamination of 20.66% was observed in spawn prepared using bajra grains. Mazumder *et al*. (2005) isolated and identified eight fungal contaminants and one bacterial contaminant (*Bacillus brevis*) from severely contaminated spawn bearing wet spot symptoms from the naturally contaminated paddy grain base spawn and reported that contamination on paddy was significantly lower than the wheat grain based spawn. High incidence of contamination in wheat grains as compared to paddy grains might be due to soft texture of wheat seeds with very thin seed coat creating portal for easy entry of any bacteria including *B. brevis* into the wheat seeds. Biswas (2016) observed that when the substrate has not been uniformly or properly pasteurized, *Trichoderma harzianum* was reported to be most damaging contaminant which compete aggressively with the mycelium of *Pleurotus pulmonarius* and *Pleurotus ostreatus* and reduce the production surface from 50-30%. Moreover, Suman and Jandaik (1992) reported that wheat grain itself could be the primary source of contamination.

Table 1 Incidence of contamination of spawn prepared using different grains and supplements

Spawn substrate	Contaminant	Contamination (%)		
		Individual	Combined	Total
Wheat	*Aspergillus* spp.	6.00	2.33	18.33[b]
	Penicillium spp.	3.33		
	Trichoderma spp.	2.66		
	Bacillus spp.	4.00		
	Total	16.00		
Wheat + Gram husk	*Aspergillus* spp.	6.33	3.66	18.00[b]
	Penicillium spp.	2.33		
	Trichoderma spp.	2.33		
	Bacillus spp.	3.33		
	Total	14.33		
Wheat + Paddy husk	*Aspergillus* spp.	5.00	3.00	18.66[b]
	Penicillium spp.	2.66		
	Trichoderma spp.	2.33		
	Bacillus spp.	5.66		
	Total	15.66		
Bajra	*Aspergillus* spp	4.00	3.00	20.66[c]
	Penicillium spp	2.66		
	Trichoderma spp	1.66		
	Bacillus spp.	9.33		
	Total	17.66		
Maize	*Aspergillus* spp	4.66	2.33	14.33[a]
	Penicillium spp	2.33		
	Trichoderma spp	2.66		
	Bacillus spp.	2.33		
	Total	12.00		
Maize + Gram husk	*Aspergillus* spp.	5.00	3.00	15.00[ab]
	Penicillium spp.	2.33		
	Trichoderma spp.	2.00		
	Bacillus spp.	2.66		
	Total	12.00		
Maize + Paddy husk	*Aspergillus* spp.	5.00	2.66	16.00[ab]
	Penicillium spp.	1.66		
	Trichoderma spp.	1.66		
	Bacillus spp.	5.00		
	Total	13.33		
Sorghum	*Aspergillus* spp	3.00	2.00	12.66[a]
	Penicillium spp	2.66		
	Trichoderma spp	2.33		
	Bacillus spp.	2.66		
	Total	10.66		

Cond.

Table 1 (*Concluded*)

Spawn substrate	Contaminant	Contamination (%)		
		Individual	Combined	Total
Sorghum + Gram husk	*Aspergillus* spp.	3.00	2.66	13.33[a]
	Penicillium spp.	2.00		
	Trichoderma spp.	1.33		
	Bacillus spp.	4.33		
	Total	10.66		
Sorghum + Paddy husk	*Aspergillus* spp.	5.33	2.00	15.00[ab]
	Penicillium spp.	1.66		
	Trichoderma spp.	1.33		
	Bacillus spp.	4.66		
	Total	13.00		
Barley	*Aspergillus* spp	5.33	4.00	19.00
	Penicillium spp	4.00		
	Trichoderma spp	2.33		
	Bacillus spp.	3.33		
	Total	15.00		
Barley + Gram husk	*Aspergillus* spp.	6.00	3.33	18.33[b]
	Penicillium spp.	2.33		
	Trichoderma spp.	1.66		
	Bacillus spp.	5.00		
	Total	15.00		
Barley +Paddy husk	*Aspergillus* spp.	4.33	2.66	16.66[ab]
	Penicillium spp.	1.66		
	Trichoderma spp.	1.33		
	Bacillus spp.	6.66		
	Total	14.00		
Oat	*Aspergillus* spp	5.00	3.33	16.66
	Penicillium spp	2.66		
	Trichoderma spp	2.33		
	Bacillus spp.	3.33		
	Total	13.33		

GH-Gram husk PH-Paddy husk. Means followed by the same letter(s) within the same column in a treatment group are not statistically significantly different at 5% level of probability using DMRT.

Differences were also observed in incidence of fungal and bacterial contaminants among the different types of grain spawn substrate. Bacterial contamination was maximum (9.33%) in case of bajra spawn and minimum in case of maize (2.33%). Among the fungal contaminants, *Aspergillus* spp. was the most prevalent ranging from 3-6% followed by *Penicillium* spp. (2.33 to 4%). The percentage of mixed contamination varied from 2-4% in different grain substrates (Table 1).

Effect of grain substrates in combination with supplements on incidence of contamination: The effect of four grain substrates, viz. wheat, maize, sorghum and barley in combination with gram husk and paddy husk on incidence of contamination was observed during spawn preparation (Table 1). The results revealed that four types of contaminants appeared individually as well as collectively in the substrates. Barley supplemented with gram husk, wheat supplemented with gram husk and wheat supplemented with paddy husk had the highest contamination of 18.33, 18.00 and 18.66%, respectively. The lowest incidence of contamination was observed in sorghum supplemented with gram husk (13.33%) which was statistically at par with maize supplemented with gram husk (15.00%), sorghum supplemented with paddy husk (15.00%), maize supplemented with paddy husk (16.00%) and barley supplemented with paddy husk 16.66%). Moreover, sorghum supplemented with paddy husk (15.00%), maize supplemented with paddy husk (16.00%) and barley supplemented with paddy husk (16.66%) were also statistically at par with barley supplemented with gram husk (18.33%), wheat supplemented with gram husk (18.00%) and wheat supplemented with paddy husk (18.66%). The incidence of fungal and bacterial contaminants also varied with the type of spawn substrates. The bacterial contamination was highest (6.66%) in case of spawn prepared with barley supplemented with paddy husk and lowest (2.66%) in case of maize supplemented with gram husk. Among the fungal contaminants, *Aspergillus* spp. was the most prevalent (3.00-6.33%), whereas the *Trichoderma* spp. was the lowest (1.66-2.33%) in almost all the substrates. Incidence of contamination showing presence of more than one contaminant ranged from 2.00-3.66% in various spawn substrates. Earlier many species of *Bacillus* were reported from contaminated spawn of oyster and button mushroom (Ahlawat *et al.* 1999, Singh *et al.* 2002). About 34 species of bacteria, mostly *Bacillus, Pseudomonas* and *Xanthomonas* spp. have been reported to be associated with cereal grains (Pepper and Kiesling 1963). Singh *et al.* (2009) isolated and identified various moulds (*Penicillium, Aspergillus, Rhizopus, Mucor, Dehliomyces*) and one bacteria (*Bacillus* spp.) from the spawn of *Agaricus bisporus*. They observed that maximum spoilage was caused by *Penicillium* spp. (39.3%) followed by *Mucor* spp. (25.9%) and *Aspergillus* spp. (14.7%). However, *Bacillus* spp. caused minimum contamination (2.6%) in the spawn bags of button mushroom. Suman (1993) also reported that the spawn spoilage by various contaminants ranged from 1.0-6.3% but in certain cases it was as high as 20.2%. Suman and Jandaik (1992) while studying the microbial contaminants of spawn of *A. bisporus* reported that the sources of contaminants are both the un-sterilized wheat grains and microbes present in spawn laboratory environment. Mazumder and Rathaiah (2001) found *Trichoderma harzianum, Aspergillus* spp. and *Penicillium* spp. as the three most dominant fungal contaminants during spawn production in oyster mushroom. Three isolates of *Bacillus subtilis* from contaminated spawn bags were isolated and characterized by Ahlawat *et al.* (1999). Mkhize *et al.* (2017) also observed that wheat bran supplementation

Table 2 Evaluation of physical treatments for management of spawn contamination

No. of boiling	Reduction in fungal spoilage symptoms (%)	Reduction in bacterial spoilage symptoms (%)
One	36.66[c]	23.33[c]
Two	58.33[b]	68.33[b]
Three	78.33[a]	88.33[a]
No. of autoclavings		
One	54.00[c]	23.33[c]
Two	89.33[b]	83.33[b]
Three	98.66[a]	98.66[a]

Means followed by the same letter(s) within the same column in a treatment group are not significantly different statistically at 5% level of probability using DMRT.

(20%) encountered higher contamination in *Pleurotus pulmonarius*.

Management of contamination: Various physical and chemical treatments for management of contamination of spawn were evaluated (Table 2). *Effect of different boiling treatments for management of contaminants*: The effect of one, two and three boiling treatments was tested for the management of contamination and significant variation was found in the results for both fungal and bacterial contaminants (Table 2). The results revealed that, the treatment comprising of three boiling showed maximum efficiency in management of fungal and bacterial contaminants and reduced the fungal contamination up to 78.33%. However, the reduction in fungal contamination was only up to 36.66% in the treatment comprising of one boiling. Similarly, the treatment of three boilings reduced bacterial contamination up to 88.33%, whereas it was up to 23.33% in case of one boiling. Kumar and Rana (2000) also reported the effectiveness of pre-soaking and boiling treatments in reducing the contaminants of spawn.

Effect of different autoclaving treatments for management of contaminants: Like boiling treatments, the effect of one, two and three autoclaving treatments were tested for the management of contamination and significant variation was observed in management of fungal and bacterial contamination (Table 2). Up to 98.66% reduction in fungal as well as bacterial contamination of spawn was observed with three autoclavings where as it was up to 54.00% in case of fungal and 23.33% in case of bacterial contamination after one autoclaving treatment.

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