

## Spawn Preparation by Irradiation of Grains

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**Abstract:** Sterilization of grains for spawn preparation is a means of contamination control in and around the media. To avoid the tedious procedure of steam sterilization,  $^{60}\text{Co}$   $\gamma$ -irradiation was used to sterilize the mature and uniform quality wheat grains var. *sharbati*. The irradiation dose was standardized in order to destroy all the spores inside the grains and any glassware or polypack contaminants. The effects of irradiation on the spawn shelf life and the yield of button mushroom (*Agaricus bisporus*) were studied. Total food reserves to sustain the mycelium for longer periods were also compared. Standard spawn as per Stoller-1962 was used as control. Samples were prepared in half-litre glass jars and 1-litre polypacks and were incubated for 14 and 21 days, respectively. An irradiation dose of 3.8 mega rads was found to be most effective for best quality (shelf life), maximum food reserves of spawn and yield of button mushrooms. Constraints of steam sterilization like overcooking, shaking of autoclaved bottles, altitude of spawn laboratory, wetting of cotton plugs, and loss of food reserves were also checked.

**Key words:** Mushroom spawn,  $^{60}\text{Co}$   $\gamma$ -irradiation, *Agaricus bisporus*, contamination control

### 1 Introduction

One of the most important requirements for the successful cultivation of any plant species is the seed of that plant species. What we call a seed in higher plants is a microscopic spore in mushrooms. Spores cannot be used for cultivation of that species because those spores germinate only when very specific 'particular sterile, conditions are provided. The vegetative mycelium instead offers a greater scope for propagation of mushrooms. However, this mycelium cannot be mixed into the substratum and needs to be carried on a proper carrier, which will not only provide nutrition to the growing mushroom mycelium but also help in its proper distribution in the substratum. Thus, the seed of a mushroom species is its vegetative mycelium grown on a suitable substrate. This is properly known as spawn. Technically, spawn is pure culture of mycelium growing on a solid substrate such as cereal grain, compost or on any other agaric residue.

In 1932, Dr. James Sinden<sup>[1]</sup> patented a spawn making process using cereal grain as the mycelia carrier. Sinden's novel approach set a new standard for spawn making and forms the basis for most modern spawn production. Most microorganisms are killed in the sterilization process. The standard time and pressure used for steam sterilization must ensure that the steam penetrates sufficiently into the small air pockets and structural cavities within the grain. The time necessary for sterilization varies at different altitudes. When a certain pressure (= temperature) is recommended, it is based on sea level standards.

During the steam sterilization process, any wetting of jars and cotton plugs leads to the emergence of a bacterial population. If this remains unchecked, it will soar to astronomical figure at the time of inoculation. Jars have to be cooled after steam sterilization. During cooling, the suction process is activated and allows the surrounding air, along with microorganisms, to enter into the jars through the cotton plugs. Sometimes, overcooking or even charring of grains takes place, leading to the failure of the spawn culture. Along with the standard time and pressure of the steam, the size of the container is also a factor: the center of the container should also be exposed to the sterilization temperature. After steam sterilization, the grains often clumped together and the containers have to be shaken in order to loosen the grains and evenly distribute the wet and dry kernels.

In an attempt to avoid such steam sterilization and establish a low level of contamination, a simple and latest irradiation technique was developed for the production of spawn. In this paper, the use of  $^{60}\text{Co}$   $\gamma$ -irradiation was used to sterilize the grains to avoid the tedious procedure of steam sterilization.

### 2 Materials and Methods

A  $^{60}\text{Co}$   $\gamma$ -irradiation source was used for irradiating samples. Glass bottles and polypropylene bags were used as spawn containers. Mature and uniform quality wheat grains var. *sharbati* were used as a carrier for spawn making. Spawn was prepared as per Stoller.<sup>[2]</sup> Wheat straw and chicken manure were procured locally. Sunflower meal (de-oiled cake) having a protein content of 40-45% (dry wt.) was obtained from Arti Traders, New Delhi. Formaldehyde-treated casing soil (2:1 mixture of two year-old farm yard manure and garden soil) was used for casing the beds. Fresh grain spawn (control) of *A. bisporus* (Lange) Sing strain S11 was obtained from Bharat Mushrooms, Delhi, India.

Compost was prepared by the short method. All the ingredients were composted for 18 days of two phase composting using a tunnel for peak heating as advocated by Shandilay *et al.*<sup>[3]</sup> and Garg.<sup>[4]</sup> Spawn was mixed with the compost at a concentration of 0.6% by the through spawning method in sixteen trays each containing 80 kg of compost (72% moisture). Spawned trays were covered with polythene sheets. After spawn run, the polythene sheets were removed and the trays were cased with formaldehyde-treated (41%) casing soil to a thickness of 4.0 cm. Regular watering and appropriate ventilation, especially at the time of pinhead formation, was provided in the growing room. Mushrooms were picked for 60 days and the weight of mushrooms recorded daily for each tray to determine yield. Yield was determined as kilogram per square meter.

### 3 Results and Discussion

Radiation doses of 3.8 mega rads were found to be most appropriate and were capable of sterilizing the entire medium and its surroundings. No sign of any contamination appeared, even after 56 days. Doses less than these invites contamination at one time or another (Table 1), and are not safe for spawn production.

Table 1. Effect of irradiation dose on contamination levels

Dose (megarads)	*Appearance of contamination (days)
2.5	4
3.0	6
3.5	9
3.8	Nil
4.0	Nil

Mean of four trials.

Total carbohydrate and protein contents were more in irradiated spawn in comparison to controls. The contents of total sugars, reducing and non-reducing were remarkably higher in radiated spawn. Colonization of irradiated grains occurred in 12 days instead of 14-16 days as in the case of control (steam sterilized) spawn. Mycelium ramification starts within 14 hrs in irradiated grains instead of 38 hrs as in case of control grains.

Ready spawn (after 14 days of inoculation) can be stored for longer periods (56 days) without any sign of degeneration and adverse affect on yield, whereas control spawn started sweating and signs of sectoring appeared. Yield was also affected (Table 2).

Table 2 Effect of spawn age on yield

Age (days) (After 14 days inoculation)	Yield (Kg)/ mT of compost	
	Irradiated spawn	Control spawn
15	228	210
30	220	190
40	217	170
56	210	140

The yield of different spawns showed that compost with irradiated spawn gives higher and more consistent yields (Table 3) whereas in other cases, the yield was lower and inconsistent. The reduced yield in the compost with control spawn is obvious due to the uneven nutritional status of the grain and sub-optimal conditions for the mycelium.

Table 3. Yield from composts after 60 days\*

Compost (kg)	Yield (kg/m <sup>2</sup> )	Yield (kg/m <sup>2</sup> )	Yield (kg/m <sup>2</sup> )	Yield (Kg)/ mT of compost
	0-30 days	30-60 days	0-60 days	
Irradiated Spawn I	9.92	8.93	18.85	236
Irradiated Spawn II	9.90	8.9	18.80	234
Control Spawn I	9.42	8.02	17.44	218
Control Spawn II	9.30	7.42	16.72	209

\* Means of four trials.

#### References

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