DOI: 10.36036/MR.30.1.2021.116225
***Mushroom Research* 30 (1) : 69-75, 2021**

Estimation of residue of carbendazim in harvested mushroom fruiting bodies form the treated substrate in *Pleurotus* spp.

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ABSTRACT

An experiment was conducted to assess the amount of residue present in the mushroom fruiting body harvested from the beds treated with formaldehyde and carbendazim against microbial contaminants. Different species of *Pleurotus viz*., *P. sajor-caju, P. sapidus* and a commercial hybrid was used for the experiment. The different concentration levels of formaldehyde and carbendazim was at the rate of 500 ppm formaldehyde + 10 ppm carbendazim, 750 ppm formaldehyde + 20 ppm carbendazim, 1000 ppm formaldehyde + 30 ppm carbendazim, 1250 ppm formaldehyde + 40 ppm carbendazim, 1500 ppm formaldehyde + 50 ppm carbendazim, 1750 ppm formaldehyde + 60 ppm carbendazim, 1000 ppm formaldehyde, 40 ppm carbendazim were applied for managing the contaminants. The investigation revealed that the residue of carbendazim present in mushroom fruiting body was least in the concentration at the rate of 40 ppm carbendazim in *P. sajor-caju*, 60 ppm carbendazim combined with 1750 ppm formaldehyde in *P. sapidus*, 70 ppm carbendazim combined with 2000 ppm formaldehyde and 40ppm carbendazim in commercial hybrid. The maximum residue was found in the concentration at the rate of 30 ppm carbendazim combined with 1000 ppm formaldehyde in *P. sajor-caju* and *P. sapidus* and 40 ppm carbendazim combined with 1250 ppm formaldehyde in commercial hybrid.

Keywords: Carbendazim, chemical sterilization, oyster mushroom, residue analysis

Mushroom belongs to the class: Basidiomycetes, sub-class: Holobasidiomycetes, and order: Agaricales. Earlier the mushrooms are used to be overlooked as it was considered to be a low-calorie, low- nutrition food, but in fact many of them are full of nutrients. Now-a-days, mushrooms are commercially cultivated by the people for consumption. From the various research works by the researchers on the mushroom, it is reported that mushrooms are rich in protein, vitamins, minerals, and chitin. It contains a low amount of fats with a very high proportion of unsaturated fatty acid as gamma-linoleic acid, a low amount of calories and also it contains no cholesterol (Gupta *et al.,* 2018). The mushrooms are rich in vitamins like- Vitamin-B, Vitamin-D, which are not available in other food supplements (Pehrsson *et al.,* 2003). For growing of oyster mushroom, there is a major problem caused by the different contaminants on mushroom beds. On the beds of the mushroom, the fungal contaminants are the most problematic organisms, which inhibit the growth of the mycelium of mushroom. Not only they inhibit the growth of the mycelium but also they compete with them for space and nutrition. Because of that, the yield of the mushroom became reduced. Various workers had undertaken studies on various aspects of fungal contaminants and diseases of

Recieved on 08-04-2021; Accepted on 26-06-2021
http://epubs.icar.org.in/ejournal/index.php/MR/article/view/116225

Pleurotus spp., and it is reported that *Trichoderma harzianum, Aspergillus* spp., *Penicillium* spp., *Monilia stophila, Stemonities* spp. and *Coprinus* spp., were the major contaminants of *Pleurotus* spp. (Castle *et al*., 1998; Hermosa *et al*., 1999; Mamoun *et al*., 2000). These species become more prevalent in the beds of *Pleurotus* spp. that are not properly pasteurized. Among these contaminants, *Trichoderma harzianum* was reported to be the most damaging one, competing aggressively with the mycelium of *Pleurotus pulmonarius* and *Pleurotus ostreatus in-vitro*, while the *Aspergillus niger, Coprinus* spp., *Penicillium* spp. and *Sclerotium rolfsii* were the most predominant fungal contaminant of mushroom beds of *Pleurotus florida* (Shin, 1987). Spilman (2002) recognized *Trichoderma* as green mould on the production beds of oyster mushroom.

Because of the contaminants on the mushroom beds, the farmer has to face a huge amount of loss. Sometimes the contaminants cause a total loss in mushroom production. Therefore, to avoid such kind of situation in mushroom cultivation, the farmers are bound to apply different chemicals to control the contaminants on the mushroom bed. To control the contaminants, growers used to follow some pasteurization techniques like hot water treatment and chemical sterilization of substrate, etc. In case of hot water treatment, improper pasteurization of substrate can cause contamination on the beds of mushroom. Because of that chemical sterilization is more efficient than hot water treatment. But, in the chemical sterilization technique, the residue of the chemicals which are used against the contaminants may be present in harvested mushroom fruit bodies, which may become harmful for human health. According to the Office of Chemical Safety and Environmental Health Office of Health Protection (2009), the current Australian Acceptable Daily Intake (ADI) for carbendazim is 0.03 mg/kg/d. And the acceptable daily intake (ADI) of carbendazim is established as 0.03mg/day in India (Sharma, 2007).

MATERIALS AND METHODS

Source of culture and spawn preparation

The Pure culture of *Pleurotus sajor-caju* and *Pleurotus sapidus* was collected from ICAR-DMR, Solan, Himachal Pradesh. It was maintained on Potato Dextrose Agar by periodic sub culture. The rice grain was soaked overnight and washed with clean water and boiled it till 25% of grains split. After boiling grains were allowed to dry and filled in conical flasks and sterilized in the autoclave at 20 lb/sq for 1hr. 30 minutes consecutively for 2 days. After that inoculation of mushroom mycelium was done in an aseptic condition in the laminar airflow cabinet.

Preparation of mushroom bed

Fresh well-dried rice straw was chopped into 3-5 cm long pieces. The chopped straw was soaked in water treated with carbendazim and formaldehyde for 12 hrs with concentrations as shown below. The soaked straw was taken out from the water and left it for a day to facilitate the draining out of the excess water from the straw. After that, the straw was allowed to dry under the sun so as to retain approximately 60% of moisture in the straw. After drying, calcium carbonate was added to the straw at the rate of 20 g per kg of straw. During the chemical sterilization of the straw, the following treatments were given to the substrate. The chemicals, used for the treatment was added into 100 L of water. On a dry basis, 1000 g of straw was used for each bed of mushroom. The size of the polythene bags was 40x60 cm, which was used for the cultivation. Twenty to twenty-five numbers of the hole of 5 mm diameter were punched at a distance of 5 cm. On each of the mushroom bed, 100 g of spawn was used. Chemically sterilized straw was spread at the bottom of the polythene bag upto 8-10 cm depth. A layer of the spawn of about 25g was evenly spread on the first layer of straw. The process was repeated till the bag was filled with 4 layers of spawn and five layers of

straw. After that, the bag was loosely tied and tagged. The prepared mushroom beds were kept in the cropping room.

Details of treatments

T_1**:** 500 ppm formaldehyde +10 ppm carbendazim

T_2: 750 ppm formaldehyde + 20 ppm carbendazim

T_3**:** 1000 ppm formaldehyde + 30 ppm carbendazim

T_4**:** 1250 ppm formaldehyde + 40 ppm carbendazim

T_5**:** 1500 ppm formaldehyde + 50 ppm carbendazim

T_6**:** 1750 ppm formaldehyde + 60 ppm carbendazim

T_7**:** 2000 ppm formaldehyde + 70 ppm carbendazim

T_8**:**1000 ppm formaldehyde

T_9**:** 40 ppm carbendazim

T_{10}**:** Control

Residue estimation

The mushroom samples were extracted and cleaned up following a modified QUECHERS (JAOAC Int. 2003, 2007) extraction and clean up method and estimation of the residues by triple quad LC-MS/MS developed and validated by TLabs, Tea Research Association, 113 Park Street, Kolkata which is NABL accredited. Ten gram homogenized mushroom sample was taken in a 50 mL centrifuge tube and rehydrated with 10 mL water for 30 min prior to extraction. Then 10 mL ACN, 1 g NaCl and 3 g MgSO4 added and vortexed for 1 min followed by homogenized at 15000 rpm, for 2 min and centrifuged the contents at 5000 rpm, for 5 min. After that 6 mL supernatant was taken out and added 50 mg of PSA and 400 mg MgSO4 in a 15 mL centrifuge tube. It was mixed well by vortex for 1 min and centrifuged at 10000 rpm, for 5 min. Then taken out 2 mL supernatant from the mixture and added 200µL 10% Diethyl glycol (DEG) in Methanol and concentrated to dryness (N2 evaporator, 400C). Re-dissolved the residues in mobile phase filtered (0.2 µm) into auto sampler vials and injected into the LC-MS/MS.

Instrument conditions

Instrument: LC-MS/MS

Make & Model: 6460 A by Agilent technologies, USA

Column: Eclipse plus C18 (100X 4.6 mm; 3.5 µm)

Column oven temp: 40oC, Injection volume: 5 µL

Mobile Phase A: Water + 5 mM Ammonium formate + 0.1% Formic acid

B: MeOH+ 5 mM Ammonium formate + 0.1% Formic acid

Calculation of residue

The residues present in different unknown samples were calculated as:

$$\text{Residues} = \frac{\text{Peak height (area) of the sample}}{\text{Volume of the sample injected}} \times \frac{\text{Quantity of standard injected}}{\text{Peak height (area) of standard}} \times \frac{\text{Final volume of sample}}{\text{Sample weight}} \times \frac{100}{\text{Percent mean recovery}} \times$$

Calculation of Percent Dissipation

$$\frac{\text{Total amount of applied chemical - Amount of residue present in the sample}}{\text{Total amount of applied chemical}} \times 100$$

RESULTS AND DISCUSSION

Chemical treatment is a method of sterilization of the substrate, which helps in the eradication of harmful microorganisms from the substrate that makes it free from contamination. But the use of chemicals for substrate sterilization may be harmful for the human health after consumption of the mushroom fruiting body harvested from the chemically treated beds due to the presence of the residue of chemicals. Following tables explains about the residual presence of the chemicals in the fruiting bodies harvested from the treated beds.

The estimation of the residue present in the harvested fruiting body was done by LC-MS/MS. The estimated residue, presented in the Table 1 was obtained from the mushroom fruiting body, harvested from the substrate which was treated with carbendazim during the preparation of the mushroom beds for the sterilization of the substrate. It was observed from the Table 1 that the maximum residue in *Pleurotus sajor-caju* with 0.40 mg/kg of carbendazim was present in the treatment with the concentration of 30 ppm carbendazim, whereas the least residue was 0.12 mg/kg in treatment T_9 (40 ppm Carbendazim) followed by T_1, T_2, T_3, T_7, T_6, T_5 and T_4. The amount of dissipation of the carbendazim was least in T_3 at the rate of 99.96%, whereas the maximum dissipation was observed in T_9 at the rate of 99.998%.

It was observed from the Table 2 that the maximum residue in *Pleurotus sapidus* with 0.30 mg/kg of carbendazim was present in the treatment T_3 with the concentration of 30 ppm carbendazim, whereas the least residue was 0.02 mg/kg in treatment T_6 with the concentration of 60 ppm carbendazim followed by T_1, T_7, T_9, T_2, T_8, T_4, T_5. The amount of dissipation of the carbendazim was least in T_3 at the rate of 99.96%, whereas the maximum dissipation was observed in T_6 at the rate of 99.99%. It was observed from the table 3 that the maximum residue in Commercial hybrid with 0.88 mg/kg of carbendazim was present in the treatment T_4 with the concentration of 40 ppm carbendazim, whereas the least residue was 0.06 mg/kg in treatment T_7 with the concentration of 70 ppm carbendazim and T_9 with the concentration 40 ppm carbendazim followed by, T_3, T_1, T_6, T_2, T_5, T_8. The amount of dissipation of the carbendazim was least in T_4 at the rate of 99.912%, whereas the maximum dissipation was observed in T_7 and T_9 at the rate of 99.994% in both of the treatment. Although, carbendazim was applied in higher doses of concentration on the substrate of mushroom beds, the residue present in the fruiting body was found to

Table 1. Residue present in *Pleurotus sajor-caju*

Treatments	Carbendazim (mg)	Parts used for analysis	Residue (mg/kg)	Percent dissipation	Residue present in 100 g of fruiting body (mg)
T_1	1000	Fruiting body	0.13	99.987	0.013
T_2	2000	do	0.13	99.987	0.013
T_3	3000	do	0.40	99.96	0.04
T_4	4000	do	0.36	99.964	0.036
T_5	5000	do	0.26	99.974	0.026
T_6	6000	do	0.25	99.975	0.025
T_7	7000	do	0.20	99.98	0.02
T_8	-	-	-	-	-
T_9	4000	do	0.12	99.988	0.012
T_{10}	-	do	0.00	0.00	0.00

Table 2. Residue present in *Pleurotus sapidus*

Treatments	Carbendazim (mg)	Parts used for analysis	Residue (mg/kg)	Percent dissipation	Residue present in 100 g of fruiting body (mg)
T_1	1000	Fruiting body	0.08	99.992	0.008
T_2	2000	do	0.20	99.980	0.02
T_3	3000	do	0.30	99.970	0.03
T_4	4000	do	0.21	99.979	0.021
T_5	5000	do	0.22	99.978	0.022
T_6	6000	do	0.02	99.998	0.002
T_7	7000	do	0.09	99.991	0.009
T_8	-	-	-	-	-
T_9	4000	do	0.15	0.985	0.015
T_{10}	-	do	0.00	0.00	0.00

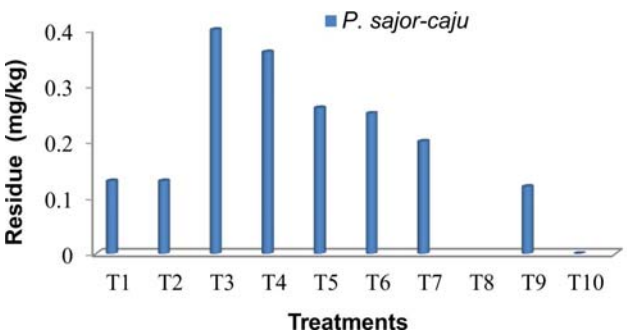

Fig. 1. Effect of treatments on residue present in harvested fruiting body of *Pleurotus sajor-caju*

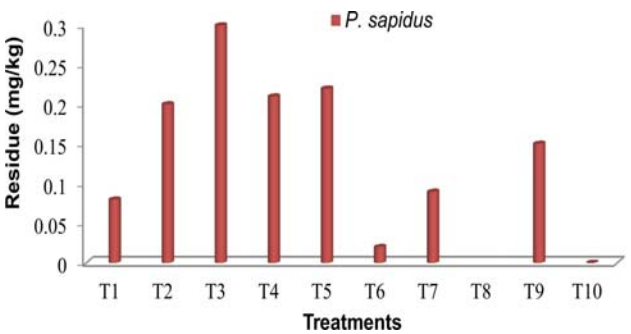

Fig. 2. Effect of treatments on residue present in harvested fruiting body of *Pleurotus sapidus*

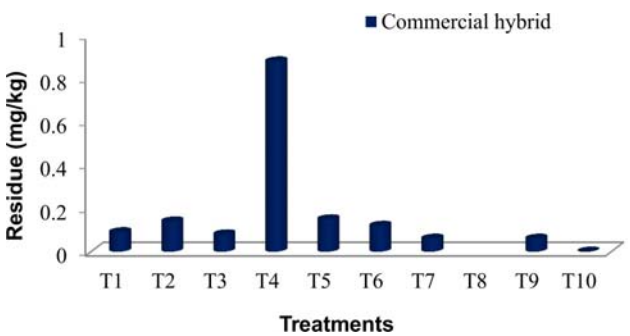

Fig. 3. Effect of treatments on residue present in harvested fruiting body of commercial hybrid

Table 3. Residue present in commercial hybrid

Treatments	Carbendazim (mg)	Parts used for analysis	Residue (mg/kg)	Percent dissipation	Residue present in 100 g of fruiting body (mg)
T_1	1000	fruiting body	0.09	99.991	0.009
T_2	2000	do	0.14	99.991	0.014
T_3	3000	do	0.08	99.992	0.008
T_4	4000	do	0.88	99.912	0.088
T_5	5000	do	0.15	99.985	0.015
T_6	6000	do	0.12	99.988	0.012
T_7	7000	do	0.06	99.994	0.006
T_8	-	-	-	-	-
T_9	4000	do	0.06	99.994	0.006
T_{10}	-	do	0.00	0.00	0.00

be less than the residue present in T_3: 1000 ppm formaldehyde + 30 ppm carbendazim and T_4: 1250 ppm formaldehyde + 40 ppm carbendazim in the higher concentrations. The residue in the fruiting body was observed as increasing upto a certain level of carbendazim concentration.

CONCLUSION

Although, the residue was exceeded the Acceptable Daily Intake only in Commercial hybrid, it would be better if the particular concentration (T_4: 1250 ppm formaldehyde + 40 ppm carbendazim) is avoided for all the species.

ACKNOWLEDGMENT

The authors are thankful to Tocklai Tea Research Institute, Jorhat, Assam for providing necessary facilities to conduct the study.

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