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General Part-1

Technology Code:-	:	201636692164718
Organization Details...		
Subject Matter Division	:	Horticultural Science
Organization Name	:	ICAR-Directorate of Mushroom Research ,Solan
AICRP name if any (AICRP)	:	AICRP Mushroom, Solan
Details of Inventors..		
Principal Inventor	:	Dr. VP Sharma
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Technology Name	:	Easy, Reliable and Safe Substrate Sterilization Technique for Oyster Mushroom
Technology Details..		
Major resource	:	Microbes

Minor Subject Classification	:	Agriculture
Minor Subject Sub Classification	:	Agricultural research
Technology Group	:	Production technology
Technology Related To	:	Potential yield Improvement

Complete Details of Technology: :

Cultivation of edible mushrooms represents one of the most efficient biotechnological processes for lignocellulosic organic waste recycling. Oyster mushroom (Pleurotus ostreatus var. florida) is a lignocellulolytic fungus that can be cultivated on varieties of agricultural wastes including banana leaves, sugarcane bagasse, tea wastes, pine needles, coconut leaves, wheat straw, rice straw, etc. Oyster mushrooms are rich in protein plentiful in B vitamins, have no cholesterol, and have significant levels of the cholesterol-lowering molecule lovastatin. Because of their native lovastatin content, oyster mushrooms have been studied for their benefits in modulating blood cholesterol levels. However, the mycelial growth of Pleurotus spp. can take place on a simple water-treated straw but cellulolytic molds present on straw can compete with its mycelium during spawn run and may release toxic metabolites affecting growth of the mushroom. The major competitor molds of oyster mushroom are cobweb (Cladoboyrum spp.) and green mold (Trichoderma spp.). Various methods have been employed to treat the substrate for cultivation of oyster mushroom such as steam pasteurization, hot water treatment, chemical sterilization, sterile technique, and fermentation or composting to kill undesirable microorganism present in the straw to favor the growth of Pleurotus mycelium. Among all, chemical sterilization technique is very popular due to low input cost and is being adopted by many farmers. However, in India, none of the fungicides have the label claim for mushroom cultivation. All the fungicides used in European countries or elsewhere are used on mushrooms in India. Benzimidazole fungicides, viz, benomyl, carbendazim, and thiophanate-methyl, are the most utilized fungicide for substrate sterilization of mushroom. Benzimidazoles are broad spectrum systemic fungicides, commonly used under mush-room cultivation to prevent competitor molds; although, their residue is detected in the mushrooms intermittently, which is a serious matter of concern for human health and environmental safety. The present study was aimed to detect thiophanate-methyl and its metabolite carbendazim in oyster mushroom fruit bodies after sterilizing the substrate with thiophanate-methyl and to identify safe optimum concentration of thiophanate-methyl that can be used in oyster mushroom cultivation without any harmful effect on human health. Residue analysis to detect thiophanate-methyl and its primary metabolite (carbendazim) during oyster mushroom (Pleurotus ostreatus var. florida) cultivation was done for two consecutive years 2017 and 2018. Wheat straw substrate was chemically treated with different treatments of thiophate-methyl,

viz, thiophanate-methyl 30 ppm + formalin 500 ppm (T1), thiophanate-methyl 40 ppm + formalin 500 ppm (T2), thiophanate-methyl 50 ppm + formalin 500 ppm (T3), thiophanate-methyl 60 ppm +formalin 500 ppm (T4), and formalin 500 ppm (T5 as control and recommended concentration), and utilized for cultivation of oyster mushroom. Treatments T3 and T4 exhibited significant difference in pH levels during both the trials. Minimum spawn run, pinhead formation, and fruit body formation time were recorded in treatments T3 and T4. Significantly higher biological efficiency (%) was recorded in treatments T3 and T4 as compared with all other treatments. No incidence of competitor molds was recorded in T3 and T4. Pesticide residue analysis for detection of thiophanate-methyl and its metabolite (carbendazim) was done in the fruit body produced in T3 and T4 treatments using liquid chromatography with tandem mass spectrometry method. No residue of thiophanate-methyl and carbendazim was detected at 50 ppm concentration of thiophanate-methyl during both the trials. However, in trial II, residue of carbendazim (5.39μg/kg) was detected at 60 ppm. Based on the findings of the trials I and II,T3 (thiophanate-methyl 50 ppm + formalin 500 ppm) may be utilized for substrate sterilization for oyster mushroom cultivation and Pleurotus ostreatus var. florida could be recognized as microorganism which could play a role in degradation of thiophanate-methyl.

Brief Description of Technology Including Salient Features:

Authenticated culture of the oyster mushroom (Pleurotus florida) having accession number DMRP-136 was procured from the culture bank of ICAR-Directorate of Mushroom Research, Solan. Spawn was prepared by using healthy wheat grain following standard package of practice. Freshly grown culture on malt extract agar medium was utilized for mass multiplication. Mycelial growth on grains was completed in10–15 days. Five different treatments of thiophanate-methyl, viz, thiophanate-methyl 30 ppm + formalin 500 ppm (T1), thiophanate-methyl 40 ppm + formalin 500 ppm (T2), thiophanate-methyl 50 ppm + formalin 500 ppm (T3), thiophanate-methyl 60 ppm + formalin 500 ppm (T4), and formalin 500 ppm (T5 as control and recommended concentration), were used for substrate sterilization for oyster mush-room cultivation. Thiophanate-methyl 70% WP (dimethyl-4,4-0-phenylenebis-3 thioallophanate) with trade name Ditto, manufactured by Coromandel Agrochemicals Private Limited, India, was used in the present investigations. One hundred liter solution of each treatment was prepared and100 kg of wheat straw was soaked into it (1:1). The substrate was left as such for 12 h. The straw was then sieved to drain off the excess water and air dried till the substrate attained the desired moisture level (65%). The spawning was done in pre-fumigated room. A total of 30 g spawn was mixed in 1 kg wet-treated substrate. The spawn was mixed thoroughly and spawned substrate was filled in polythene bags of 10 kg capacity. Ten to fifteen small holes (0.5–1.0 cm dia) were made on all sides especially in the bottom for leaching of excess water and maintain desirable level of CO2 (15000–20000 ppm). The spawned bags were kept on the shelves for mycelial colonization of substrate. During spawn run, the temperature of growing room was

maintained in the range of 22–26 °C. During spawn run, bags were neither opened and nor any ventilation was given. After spawn run, the room temperature was reduced to 16–20 °C, and relative humidity was maintained between 75 and 85%. Light of 200 lux intensity for 8–12 h was given in the room for fruit body initiation. Experiment was conducted in randomized block design (RBD) with 10 replications. Ten bags were kept under each replication. Data were subjected to statistical analysis using the SPSS software for Duncan's multiple range test. Under present investigations, two trials were conducted. Trial I was conducted during October–November (autumn) and trial II was conducted during December–January (winter). Environmental conditions, materials, and methods were kept same under both the trials. Observations were recorded on number of days required for spawn run, pin head formation, fruit body formation, presence/absence of green mold, and biological efficiency (%). The fresh mushroom yield obtained was converted into percent biological efficiency (kg q−1 dry substrate). Pesticide residue sample of dried samples of mushroom was done through Punjab Biotechnology Incubator (PBTI), SAS Nagar (Mohali), Punjab. PBTI is a State Government Undertaking registered as a "Society for Biotechnology Incubator" under the Society Registration Act 1961 and is professionally governed by the Governing Council of the Society under the Government of Punjab (India). Samples for pesticide residue analysis were harvested at crop maturity stage. Samples were air dried at room temperature and the dried samples were packed in polybags. Then, the samples were taken to the PBTI laboratory for further analysis (www.pbtilabs.com). Liquid chromatography with tandem mass spectrometry (LC-MS/MS) method was utilized for pesticide residue analysis. A finely grounded sample weighing 5.0 ± 0.1 gm was taken in a 50-mL centrifuge tube. Water, MgSO4, and NaCl was added with continuous stirring to avoid the lump formation and finally vortexed. Ten milliliter acetonitrile was added to each tube and centrifuged. Supernatant was taken and evaporated completely under gentle stream of nitrogen, reconstituted with water: acetonitrile (80:20) and injected into LC-MS/MS (Q-Trap 4000 (AB Sciex)). The unknown con-centration of the sample was calculated. Generally, oyster mushroom require pH near to neutral or slightly basic. In our studies, under effective treatments of thiophanate-methyl (50 and 60 ppm), pH of the substrate ranged from 7.3 to 7.5. However at this pH, oyster mushroom grew well, and positive effect on mycelial growth, pinhead formation, fruit body formation, and biological efficiency was recorded. Under chemical sterilization of the substrate, it is important that the optimum concentration of fungicides should reach each and every corner of the substrate because method of substrate sterilization could also affect the final crop yield. It was also recorded that in the end of process of biodegradation of thiophanate-methyl, abundant carbon dioxide gas is liberated which positively increased the spawn run rate under thetreated substrate as evidenced by our data also. Oyster mush-room requires high CO2 concentration during spawn run fortheir growth and development. No colony of competitor molds like Trichoderma sp. (green mold) was observed in the bags treated with effective concentrations of thiophanate-methyl. Spores of Trichoderma might not germinate

undersuch conditions and crop was found completely free from competitor mold (Trichoderma sp.) in the treatments T3 and T4. Undoubtedly, thiophanate-methyl (60 ppm) under T4 showed best results in all respect; however, the residue of its primary metabolite (carbendazim) was detected at this con-centration. Previously also, it has been reported that thiophanate-methyl encouraged the mycelial growth of button mushroom (Agaricus bisporus) at 12 mgl−1 but suppressed the growth when applied at higher concentration, i.e., 25 mgl-1.

Benefits/Utility :

Chemical sterilization of oyster mushroom substrate with thiophanate-methyl at 50 ppm concentration is safe to use because no residue of either thiophanate-methyl or its primary residue carbendazim was detected at this concentration. This treatment may be used by the mushroom growers while they grow the crop for drying purpose. Pesticide degradation through microbial activities is considered an important mode of their dissipation. In our studies, it is also evident that during the course of growth and development of Pleurotus ostreatus var. florida, it transformed thiophanate-methyl to carbendazim. Therefore, it would be unbiased to recognize Pleurotus ostreatus var. florida as microorganism which could participate in biodegradation of thiophanate-methyl to carbendazim. It might be utilized for bioremediation of thiophanate-methyl and fungicides with similar chemistry. The importance of fungicides of the benzimidazole group in oyster mushroom could be sensed in our findings from their role in mycoparasitism management and crop yield enhancement.

Precaution With The Technology : 1. Quality substrate materials must be utilized. 2. Recommended concentration of fungicides and method of application must be used. 3. Substrate must be exposed to the fungicide solution for a recommended period of time 4. Environmental conditions must be maintained as per the prescribed limits.

Impact, If Adopted :

Increases the adoption of oyster mushroom cultivation in India which helps to diversify the mushroom production and in turn higher economical returns to the mushroom growers.

Social Impact :

Mushrooms are increasingly considered as a high value crop, and consumer demand for mushrooms markedly expanded in the recent years. Although requiring different conditions and practices compared with traditional field crops, mushrooms are a viable option for the small and marginal scale growers. However, merely after 2-3 initial years of adoption of commercial mushroom cultivation, growing units are ravaged by various biotic stresses. Among them fungal pathogens are the common contaminants and production lowering agents. Many chemical disease management technologies are available in India, however due availability of incomplete information about many especially about the residue level and safety of human farmers hesitate or refuse to adopt such imperfect technologies. Viewing the mentioned fact and farmers' perception, in our technology all such aspects especially safe for human consumption were critically considered at the time of planning the experiments and their execution. Since the technology is easy to use, reliable and safe, therefore it is perfect option for the farmer to gain the higher profits.

TargetUsers/Stake holders	:	Mushroom growers and entrepreneurs

Technology Contact..

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Keyword for Technology	:	Pleurotus ostreatus var. florida, Thiophanate-methyl, LC-MS/MS, Pesticide residue, Disease management

Technology Development Details Part-2

Project Details (Through which technology was developed)	:	Substrate formulation and standardization of cultivation technology of different mushrooms
Time of Initiation Technology Development	:	4-2015
Time of Completion Technology Development	:	3-2021
Technology Validated by	:	Within ICAR

Technology Validation Details..

Subject Matter Division	:	Horticultural Science

Organization Name(if within ICAR)	:	ICAR-Directorate of Mushroom Research,Solan
Organization Name(if outside ICAR,Please enter)	:	
Year of Validation(YYYY)	:	12-2019
Year of Release/Adoption(YYYY)	:	12-2019
Country	:	India
Through Technology Transfer	:	YES

Applies To(Regional Differentiation)Inform Part-3

Location...

Zone(As per the planning commission)	:	All
Sub zone(As per the planning commission)	:	All
AgroEcological Zone(NBSS & LUP)	:	All
AgroEcological Sub Zone(NBSS & LUP)	:	All
State Name	:	All
District Name	:	All

Soil Type/Resource Type..

Soil Order	:	
Soil Sub Order	:	
Soil great group	:	
Soil great sub group	:	

Commodity Details..

Commodity	:	
Commodity Type	:	
Commodity Name	:	

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