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Screening of Competitor Mould in Oyster Mushroom (*Pleurotus florida*) Cultivation and their Management

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A B S T R A C T

Survey revealed that the occurrence of eight contaminants in mushroom beds and out of which *Trichoderma viride*, *Aspergillus niger*, *Coprinus* sp. were found to be the dominant fungal contaminants and occurrence was high during may to July (23.5 - 26.7 %) causing maximum loss to mushroom yield. The incidence of contaminants were minimum during December and January (3.60%) and maximum during the month of May (26.5 %). A good harvest of mushroom (107% Biological Efficiency) was obtained during the month of October. A range of average maximum temperature (23.5 - 34.6º C), minimum temperature (13.4 - 24.2º C) was found most appropriate for the cultivation of oyster mushroom in this region. Among the chemicals tested for management of competitors moulds Carbendazim 50% WP + formalin (T3) 0.01g+0.15 ml/100 ml showed its supremacy and exhibited maximum inhibitory effect (44.1 to 61.6 %) against *Aspergillus* sp., *Trichoderma* sp., *Coprinus* sp. and *Penicillium* sp. and was found to be less effective against *Sclerotium rolfsii in vitro.* The study will provide the idea of appropriate cultivation time as well as provide an alternative method of surface sterilization.

Introduction

Oyster mushroom (*Pleurotus* sp.) belonging to class Basidiomycetes and family Agaricaceae is popularly known as 'dhingri' in India. The popularity of oyster mushroom has been increasing due to its ease of cultivation, high yield potential and high nutritional value (Banik *et al*., 2004; Gregori *et al*., 2007). Mushrooms are now-a-days popularly known as functional foods (Liu *et al*., 2003). Bioconversion of lignocelluloses residues through cultivation of *Pleurotus* offers the best prospect to utilize renewable resources in the production of protein rich food that will sustain food security for peoples (Naraian *et al*., 2009). Oyster mushroom help to remove the toxicity produces by the agro wastes (Fan *et al*., 2000a, b). During oyster mushroom cultivation, mushroom growers facing various problems especially competitor moulds damage the mushroom beds and reduce yield. Studies on various aspects of fungal contaminants and diseases of *Pleurotus* spp. were undertaken by various workers (Castle *et al*., 1998; Mamoun *et al*., 2000) and they reported *Trichoderma harzianum*, *Aspergillus*

sp., *Penicillium* sp., *Monilia sitophila*, *Stemonitis* sp. and *Coprinus* sp. were the major contaminants of *Pleurotus* sp. These species become prevalent in *Pleurotus* cultures if the substrate has not been uniformly or properly pasteurized. Among these contaminants, *T. harzianum* was reported to be the most damaging one, competing aggressively with the mycelium of *Pleurotus pulmonarius* and *Pleurotus ostreatus in vitro* and reducing the production surface from 30 to 50%. Considering the above, an experiment was conducted to develop a suitable management practice against the competitor moulds of *P. florida* in use of various fungicides.

Materials and Methods

Screening of Natural Incidence of Competitor Moulds: Ten home scale mushroom farms of Thanjavur and its nearby areas were surveyed every month from March 2015 to February 2016 for the occurrence of contamination in mushroom beds of oyster mushroom (*Pleurotus florida)*. The incidences of different competitor moulds were recorded. Infected mushroom bags were tagged and the contaminated micro floras were identified. Total numbers of infected beds were counted from each farm. In addition, five mushroom beds were raised in first week of every month and allowed for spawning and yield at Sri Amman Biocare mushroom unit, Thirukkanurpatti, Thanjavur District.

A month wise average data on mean temperature, relative humidity were recorded. Spawn of oyster mushroom (*Pleurotus florida)* was supplied from the Sri Amman Biocare to the progressive farmers for cultivation. Paddy straw was taken as substrate and chemical sterilization technique was followed in cultivation. Container system of cultivation (polypropylene bags) was followed in all experiments. Samples of various diseases and the competitor fungi were collected on a regular basis from the cropping room and subsequently, *in vitro* studies have been carried out in laboratory.

Preparation of Beds: Chopped paddy straw was soaked into a solution containing the require amount of sterilizing agent for 10-12 hours. The substrate was filled in polypropylene bags as layer by layer and each layer spawn was inoculated. A unit of 3 kg of dry straw was used for each treatment, which was equally distributed in 3 bags representing each as a replication. The moisture content of the straw at the time of spawning was kept around 70 - 75%. The filled bags were incubated in a dark room at a temperature ranging between 24 - 30º C, where 90% relative humidity was maintained till the spawn run was complete. When the straw is fully covered with milky white mycelium in the bag, it is regarded as complete spawn run, then the bags were hanged by nylon string at a distance of 60 cm. Harvesting was done when the fruit body was mature.

Isolation and Purification of Competitor Moulds: Competitor moulds fungi were collected from the damaged beds in sterilized Petri plates with the help of a sterile forceps and thereafter transferred into PDA plates under *in vitro* conditions. Inoculated PDA plates were incubated at 27º C ± 2º C for 72 h. A single colony was isolated from the PDA plate and again transferred to PDA plates for obtaining the pure culture. All the pure cultures were kept in refrigerator at 4º C for preservation.

***In vitro* study:** Three different treatments were used to control the competitor mould in *in vitro* condition, such as treatment 1 (T1) was 0.01g/100ml of chemical fungicides (carbendazim (50% WP), mancozeb (75% WP) and zineb (75% WP)), treatment 2 (T2) was 0.005g+0.1 ml/100 ml (chemical

fungicides with formalin) and treatment 3 (T3) 0.01g+0.15 ml/100 ml (chemical fungicides with formalin) were used against competitor moulds *T. viride, Trichoderma harzianum, Penicillium notatum, Aspergillus niger, Aspergillus flavus, Mucor* sp., *Rhizopus* sp., and *Sclerotium Rolfsii* by using disc diffusion method on PDA medium (Kirby *et al*., 1966).

Results and Discussion

Role of Meteorological Factors: The survey of effect of fluctuations in temperature and relative humidity on the incidence of competitor moulds and yield of oyster mushroom was studied and the data obtained are presented in Table 1. Survey revealed the occurrence of eight contaminants i.e. *T. viride, T. harzianum, P. notatum, A. niger, A. flavus, Mucor* sp., *Rhizopus* sp., and *S. Rolfsii* out of which *T. harzianum, T. viride, A. niger* and *P. notatum* were found to be the most dominant fungal contaminants. The incidence of the contaminants were minimum during the month of December and January (3.6 %) and it increased considerably with the fluctuating climatic conditions and reached its peak during the month of May and June (26.5 %). Thereafter, a decline trend in contamination % was noticed in this region. Minimum range of contamination (3.6 to 6.8%) was observed during the period from November to January and again August, when maximum biological efficiency 107% was obtained. A range of average maximum temperature (27.4 – 34.6º C), minimum temperature (13.4-22.7º C) was found most appropriate for the cultivation of oyster mushroom in this region (Table-1,2).

***In vitro* study:** The extent of antifungal experiment carried out against different competitor moulds by use of three different treatments (Table 2). Significant differences were obtained among all the treatments. Treatment 3 (T3) 0.01g+0.15 ml/100 ml (chemical fungicides with formalin) proved its superiority among all the treatments and found to be most effective in inhibiting the mycelial growth of three contaminants (20.15, 19.20 and 17.40 mm) i.e *T. viride, Mucor* sp. and *P. notatum* respectively and treatment 2 (T2) was found less effective against the mycelium growth of *S. rolfsii, T. harzianum, A. niger* and *A. flavus.* Treatment 1 (T1) was does not form zone of inhibition against competitor mould and *Rhizopus* sp. was does not inhibited by all treatments (Table-3, 4 and 5).

Table.1 List of competitor mould isolated in oyster mushroom cultivation

S.No	Organisms isolated
1.	*T. viride*
2.	*T. harzianum*
3.	*P. notatum*
4.	*A. niger*
5.	*A. flavus*
6.	*Mucor* sp.
7.	*Rhizopus* sp
8.	*S. rolfsii*

Table.2 Month wise mean temperature, mean total contamination % and yield biological efficiency of mushroom cultivation.

Month	Mean Temperature°C	Mean Total Contamination %	Yield biological efficiency %
January	22.7	3.67	104.5
February	25.2	4.10	103.0
March	26.6	5.80	101.4
April	28.7	12.15	100.5
May	34.6	26.50	90.6
June	29.3	20.30	96.5
July	27.4	12.40	100.2
August	23.5	6.80	104.0
September	24.5	7.20	105.0
October	13.4	6.15	107.0
November	19.3	5.80	106.5
December	20.5	3.60	106.0

Table.3 Effect of chemical fungicide on the growth of competitor moulds (Treatment I)

S.No.	Organisms	Zone of inhibition in mm		
		Carbendazim	Mancozeb	Zineb
1	*A. niger*	11.57	10.33	6.35
2	*Rhizopus* sp.	Nil	Nil	Nil
3	*T. viride*	16.32	Nil	5.7
4	*T. harzianum*	Nil	Nil	Nil
5	*Mucor* sp.	17.80	8.9	Nil
6	*S. rolfsii*	13.16	12.1	8.86
7	*P. notatum*	12.10	10.60	11.5
8	*A. flavus*	10.61	9.85	10.40

Table.4 Effect of chemical fungicide on the growth of competitor moulds (Treatment II)

S.No.	Organisms	Zone of inhibition in mm		
		Carbendazim	Mancozeb	Zineb
1	*A. niger*	13.15	11.50	8.50
2	*Rhizopus* sp.	Nil	Nil	Nil
3	*T. viride*	19.75	10.40	9.65
4	*T. harzianum*	14.25	12.25	11.55
5	*Mucor* sp.	18.00	15.9	7.25
6	*S. rolfsii*	14.70	12.1	8.86
7	*P. notatum*	14.65	12.20	12.10
8	*A. flavus*	11.10	10.50	10.20

Table.5 Effect of chemical fungicide on the growth of competitor moulds (Treatment III)

S.No.	Organisms	Zone of inhibition in mm		
		Carbendazim	Mancozeb	Zineb
1	*A. niger*	14.20	11.90	10.10
2	*Rhizopus* sp.	6.40	Nil	Nil
3	*T. viride*	20.15	12.50	10.55
4	*T. harzianum*	16.15	11.75	12.50
5	*Mucor* sp.	19.20	14.10	11.70
6	*S. rolfsii*	16.80	14.20	10.00
7	*P. notatum*	17.40	14.60	15.40
8	*A. flavus*	13.20	12.50	12.00

Fig.1 Effect of chemical fungicide on the growth of competitor moulds (Treatment I)

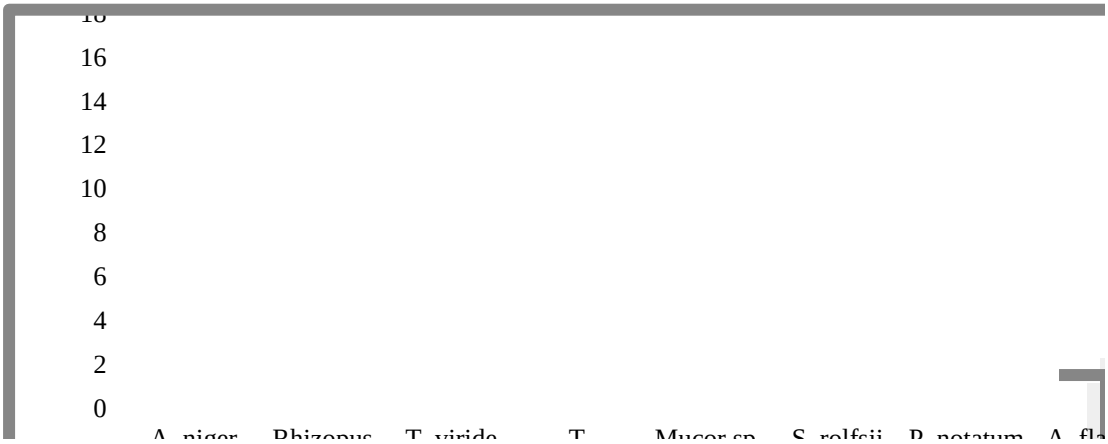
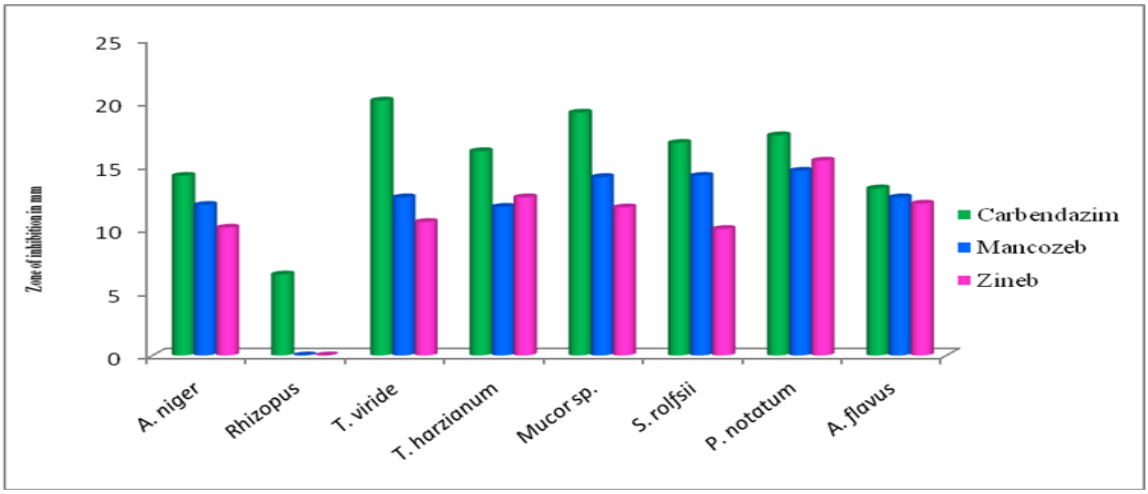


Fig.2 Effect of chemical fungicide on the growth of competitor moulds (Treatment II)

Fig.3 Effect of chemical fungicide on the growth of competitor moulds (Treatment III)



The optimal temperature for the spread of the mycelium or vegetative growth of *Pleurotus* sp. is around 25 - 28º C and for fruiting body formation the temperature requirement is nearly 2 to 4º C less than that. If there is too cold then the mycelial growth is to be arrested and at more than optimum temperature there is the risk of mould and bacterial contamination on the production beds which leads to destruction of the mushroom mycelia. In present investigation, heavy rain coupled with elevated minimum temperature (>25º C) increased the contamination in beds was probably due to the decreasing of oxygen supply and increase in CO_2 concentration in the mushroom house or growing bags which may reduce the growth rate of oyster mushroom mycelia. Different concentration of carbendazim (bavistin) and its combination with formaldehyde (formalin) were evaluated against the major contaminants of *P. sajorcaju, P. flabellatus* and *P. citrinipileatus* (Upadhyay *et al*., 1987; Vijay *et al*., 1987) and they reported complete inhibition of the mould fungi under *in vitro* and/ or *in vivo*. Complete inhibition of most of the competitor moulds of oyster mushroom was obtained with the application of 50 ppm benomyl + 100 ppm thiram. In the present study the inhibition patterns of (carbendazim (50% WP), mancozeb (75% WP) and zineb (75% WP)) against the competitor moulds *in vitro* further support the findings of Jain and Vyas (Jain *et al*., 2002).

In conclusion, survey revealed the occurrence of eight competitor moulds were identified out of which *T. harzianum, T. viride, A. niger* and *P. notatum* were found to be the most dominant fungal contaminants. The incidence of the contaminants were minimum during the month of December and January (2.60 %) and it increased considerably with the fluctuating climatic conditions and reached its peak during the month of May and June (21.5 %).

The extent of antifungal experiment treatment 3 (T3) 0.01g+0.15 ml/100 ml (chemical fungicides with formalin) proved its superiority among all the treatments and found to be most effective in inhibiting the mycelial growth of three contaminants (20.15, 19.20 and 17.40 mm) i.e *T. viride, Mucor* sp. and *P. notatum* compared with other treatments and *Rhizopus* sp. was does not inhibited by all treatments.

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