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EFFECT OF BOTANICALS ON THE INCIDENCE OF COMPETITOR MOULDS AND BIOLOGICAL EFFICIENCY OF GREY OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*)

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ABSTRACT
The main obstacle for increased production of oyster mushroom(*Pleurotus* spp.) in West Bengal isthe frequent contamination of the mushroom growing beds with micro flora. An experiment was conducted with a view to develop a suitable management practice against the competitor moulds of *P.ostreatus* in an eco- friendly manner. Eight different botanicals were tested along with the most popular chemical treatment (carbendazim 75 ppm +formalin 500 ppm) against the competitor mould fungi *in vitro* and in *vivo*. Chemical treatment was found to be most effective in reducing the mycelial growth of five contaminants (65.4 to 86.6%) *in vitro* and checked the incidence of competitor moulds 81.36 % *in vivo,* which increased the yield of mushroom up to the tune of 35.20 % (106 % B.E.).*A. indica* (neem) showed its supremacy among all the botanicals tested and gave minimum effect on the growth of mushroom mycelium (4%) and exhibited maximum inhibitory effect (54.1 to 71.6 %)against *Aspergillus* spp., *Trichoderma* spp., *Coprinus* spp., and *Penicillium* spp. which was and found to be less effective against Sclerotium *rolfsiiin vitro* followed by extracts of *Pongamia pinnata* (6.7 %) and (42.4 to 61.3%) inhibition respectively against the mycelium of *P. ostreatus* and mould fungi. A range of 35.3 to 62.4% reduction in the incidence of inky caps (*Coprinus sp.*) and 26.3 to 68.4% reduction in green moulds(*Trichoderma* spp.) were recorded with different phyto-extracts. All the botanicals except *Acacia nilotica* reduced the incidence of competitor moulds (18.18 to 70.91%) in mushroom beds which resulted in an increaseof yield up to the level of 21.3 %. The information provides an alternative method of surface sterilization of substrates under the agro-ecological conditions of lateritic belt of West Bengal, India, which can minimize the use of fungicides in mushroom cultivation.

INTRODUCTION

Oyster mushroom (*Pleurotus* spp.) 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 and Nandi, 2004 and Gregori *et al.*, 2007). Cultivation of mushroom helps in removing the toxicity produces by the agro wastes (Fan *et al.* 2000a; Fan *et al.*, 2000b and Murthy and Manonmani, 2008). Microbial contamination of oyster mushroom bed is one of the major hindrance in increased yield in West Bengal (Biswas *et al.*, 1997; Sharma *et al.*, 2013). Studies on various aspects of fungal contaminants and diseases of *Pleurotus* spp. were undertaken by different workers (Castle *et al.*, 1998; Hermosa *et al.*, 1999; Mamoun *et al.*, 2000, Neelam *et al.*, 2014) and they reported *Trichoderma harzianum*, *Aspergillus* spp., *Penicillium* spp., *Moniliasi tophila*, *Stemonitis* spp. and *Coprinus* spp. were the major contaminants of *Pleurotus* spp. These species become prevalent in *Pleurotus* cultures when the substrate has not been uniformly or properly pasteurized. Among these contaminants, *Trichoderma harzianum* was reported to be most damaging one, competed aggressively with the mycelium of *Pleurotus pulmonarius* and *Pleurotus ostreatus in- vitro* and reducing the production surface from 30 to 50% (Shin, 1987) .While, *Aspergillus niger*, *Coprinus* sp, *Penecillium* sp and *Sclerotium rolfsii* were the most predominant fungal contaminant of mushroom beds of *P. florida* (Biswas and Kuiry, 2013). Spilman (2002) recognized *Trichoderma* as green mould on the production bed of oyster mushroom. *Trichoderma*, *Aspergillus* and *Rhizopus* on oyster mushroom bed were predominant microorganisms and especially occurrence of these was severe in summer and spring seasons than autumn and winter (Jaivel and Marimuthu, 2010). The use of fungicides for controlling the competitor moulds and diseases in oyster mushroom cultivation is very common in India (Singh and Kumar, 2011 and Jain and Vyas, 2002, Debata *et al.*, 2014). The hazardous effects of chemicals in human health and environmental aspect are known. Apart from these problems continuous usage of same chemicals may leads towards pest's resistance.The growth of competitor moulds in mushroom beds can be checked by using different plant extracts (Singh, 1999 and Patra *et al.*, 1998). These plant extracts offer a viable choice which are non persistent in the environment and safer to use. Considering the above, an attempt was made to develop a suitable management practice against the competitor moulds of *Pleurotus ostreatus in* an eco- friendly manner under the agro-ecological condition of lateritic belt of West Bengal.

MATERIALS AND METHODS

Preparation of beds

Chopped paddy straw was soaked into a solution containing the requisite amount of sterilizing agent for 16-18 hours. Thorough spawning @ 4% by wet weight basis was followed. The spawned substrate was filled in polypropylene bags (45x30 cm). A unit of 2 kg of dry straw was used for each treatment, which was equally distributed in four bags representing each as a replication. The moisture content of the straw at the time of spawning was kept around 72-75%. The filled bags were incubated in a dark room at a temperature ranging between 24-30ºC, 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 cut open and compacted mass of aggregated straw called, as "bed" was ready for cropping. The beds were hanged by nylon string at a distance of 60 cm. Harvesting was done when the small primordial converted into a full grown sporophore.

Isolation and purification of competitor moulds

Competitor moulds fungi were collected from the damaged beds in sterilized petriplates with the help of a sterile forceps and thereafter transferred into PDA plates under *in vitro* conditions. Inoculated PDA plates were incubated at 27ºC (+ 2ºC) for 3 to 4 days. 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 4ºC for preservation.

Preparation of photo-extracts

For preparation of phyto-extracts, 100 gram plant products were collected, wash in tap water, air dried and homogenized with equal amount of distilled water (100mL) by crashing them with electric grinder machine. The extract was filtered through double - layered muslin cloth and centrifuged at 4000 rpm, for 10 minutes. The supernatant was collected and filtered through Whatman No.1 filter paper which was considered as standard solution.

In vitro study

Different eco-friendly botanicals *i.e. Azadirachta indica, Lantana camera, Pongamia pinnata, Acacia nilotica, Clerodendron indicum, Eucalyptus hybrid, Datura metal, Cassia tora*and chemicals (carbendazim 75 ppm + formalin 500 ppm) were evaluated individually against competitor moulds and oyster mushroom (*P. ostreatus*). Poisoned food technique (Grove and Moore, 1962) was carried out to evaluate the inhibitory effect of fungal mycelia. For one competitor mould, four mL of each plant extract (standard solution) was incorporated in 100 mL of potato dextrose agar medium (PDA) and autoclaved for 20 minutes at 1.41 kg/ cm^2 pressure. The molten media were poured into four sterilized glass petriplates (90 mm) considering each as a replication. After solidification of the agar plates were inoculated with 5 mm diameter mycelial cut from 6 day old culture of competitor moulds *i.e. Aspergillus* spp., *Trichoderma* spp., *Coprinus*spp., *Penicillium* spp and *Sclerotium rolfsii* and mushroom *P. ostreatus*. The petriplate without any treatment was served as control. The plates were incubated at room temperature till the complete growth observed in control plate.

The mycelial inhibition was calculated with the following formula

$$\text{Mycelial inhibition} = \frac{dc - dt}{dc} \times 100$$

Where,

dc = Colony diameter in control

dt = colony diameter in treatment

In vivo study

Phyto-extracts and chemicals were further evaluated *in vivo* to see their inhibitory effect against the major contaminants, *i.e. Trichoderma* sp., *Aspergillus* sp., *Coprinu s*sp., *Sclerotium rolfsi* and *Penicillium* spp. Paddy straw was dipped into the solution containing appropriate concentration of phyto-extracts and chemicals separately for 16-18h and spawning was done @ 4% dry weight basis. The polythene bags were cut open when colonization was completed. Untreated paddy straw was used as control. Data on different parameters were collected on a regular basis during the cropping.Percent contamination index was calculated for the test and control beds depend upon the following scale

Grade 0: 0% –incidence of contaminants

Grade 1: >0 – 20% coverage by the contaminants

Grade 2: >20 – 40% coverage by the contaminants

Grade 3: >40 – 60% coverage by the contaminants

Grade 4: >60 – 80% coverage by the contaminants

Grade 5: >80 – 100% coverage by the contaminants

$$\text{Percent contamination index} = \frac{\text{Sum of the total scores}}{\text{Maximum rating x total number of observations}} \times 100$$

RESULTS

In vitro study

The extent of inhibition of mycelium growth of*P. Ostreatus* and different competitor moulds varied considerably with different botanicals and chemicals used (Table 1). Significant differences were obtained among all the treatments. Chemical treatment (bavistin 75 ppm + formalin 500 ppm) was proved its superiority among all the treatments and found most effective in inhibiting the mycelial growth of five contaminants (65.4 to 86.6%). Among the botanicals, *A. indica* (neem) showed maximum inhibitory effect (54.1 to 71.6 %) against the growth of four competitor moulds fungi *i.e. Aspergillus niger, Trichoderma viride, Coprinus*spp. and *Penicillium* sp., and found less effective against the mycelium growth of *P. ostreatus* (4.4%). This was followed by extracts of *Pongamia pinnata* (karanja) 42.4 to 61.3% (mould fungi) and 6.7 % (*P. ostreatus*) and *Clerodendron indicum* (clerodendron) which inhibited 40.0 to 53.8 % and 8.9 % mycelium growth of mould fungiand *P. ostreatus* respectively. Among the five contaminants, *Sclerotium rolfsii* was reported to be more resistant against most of the botanicals used. However, the growth of the same fungi was reduced considerably with the extract of *Pongamia*

Table 1: *In vivo* evaluation of different phytoextracts and chemicals against major competitor moulds of oyster mushroom

S.N.	Treatments	Dose	Radial growth of mycelium and percentage growth inhibition 8 days after inoculation{mycelium growth in (mm) and growth inhibition in (%)}											
			Pleurotus ostreatus		*Coprinus sp* .		*Aspergillus niger*		*Penicillium sp.*		*Sclerotium rolfsii*		*Trichoderma* spp		Sem ± Treatment mean	CD at 5% (mycelium growth)
1	*Azadirachtaindica*		mm	%	mm	%	mm	%	mm	%	mm	%	mm	%		
		4 %	86.0	4.4	29.3	62.4	31.2	54.1	22.4	71.6	68.0	24.4	27.2	68.4	0.899	2.671
2	*Lantana camera*	4 %	73.0	18.9	54.0	30.8	*45.0*	33.8	50.0	36.7	62.0	31.1	61.0	29.1	1.067	3.170
3	*Pongamiapinnata*	4 %	84.0	6.7	30.2	61.3	35.0	48.5	43.0	45.6	42.0	53.3	49.5	42.4	0.853	2.536
4	*Acacia nilotica*	4 %	67.0	25.6	50.5	35.3	52.0	23.5	54.0	31.6	73.0	18.9	63.4	26.3	1.106	3.286
5	*Clerodendronindicum*	4 %	82.0	8.9	36.0	53.8	36.5	46.3	38.0	51.9	54.0	40.0	51.0	40.7	1.02	3.032
6	*Eucalyptus hybrida*	4 %	80.0	11.1	39.0	50.0	42.0	38.2	33.0	58.2	51.0	43.3	46.0	46.5	1.027	3.052
7	*Datura metal*		71.0	21.1	37.0	52.6	35.0	48.5	46.0	41.8	57.0	36.7	55.0	36.0	1.130	3.358
8	*Cassia tora*	4 %	75.0	16.7	41.0	47.4	49.0	27.9	58.0	26.6	64.0	28.9	59.0	31.4	0.971	2.887
9	Bavistin + Formalin l	75ppm + 500ppm	83.0	7.8	15.0	80.8	23.5	65.4	15.5	80.4	25.0	72.2	11.5	86.6	0.824	2.450
10	Control		90.0	0.0	78.0	0.0	68.0	0.0	0.0	90.0	0.0	86.0	0.0	0.726	2.158	
	Sem ± Treatment mean		1.125		0.887		0.965		1.037		0.953		0.826			
	CD at 5%		3.250		2.563		2.787		2.997		2.754		2.387			

pinnata (53.3%) and *Eucalyptus hybrida* (43.3 %). The extracts of *Acacia nilotica* showed minimum inhibitory effect (18.9 to 35.3 %) against the contaminants tested and also exhibited adverse effect on the growth of mushroom mycelium (25.6%) Fig. 3.1

***In vivo* study**

The response of eight plant extracts and chemicals used as surface sterilizing agent for cultivation of P. *ostreatus* are presented in Table 2. Data indicated the supremacy of chemical treatment over the botanicals used. An increase of 35.20 % in biological efficiency and 81.36 % reduction in the incidence of competitor moulds were observed from chemically treated substrate. Similar trends were also noticed with the botanicals *in vivo*, where maximum yield of mushroom (95.10 % B.E.) was obtained from the substratetreated with *A. indica* which checked 70.91 %. incidence of competitor moulds in beds. This was followed by *Pongamia pinnata* 92.20 % (B.E.) and 61.36 % (reduction in mould incidence) and *Clerodendron indicum* treated substrate89.00 % and 56.82 % respectively.

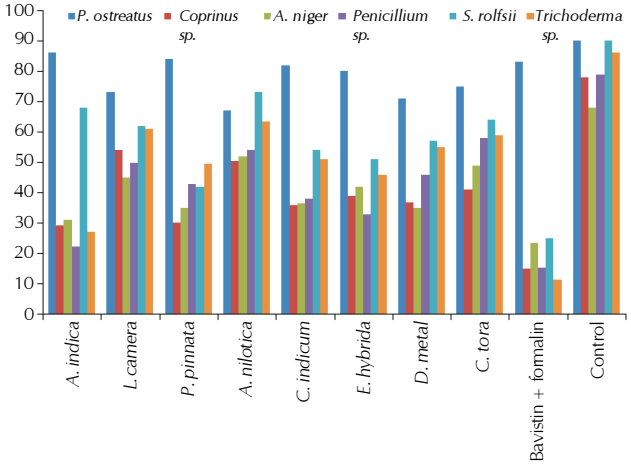

Figure 3.1: *In vitro* evaluation of botanicals against competitor mould fungal

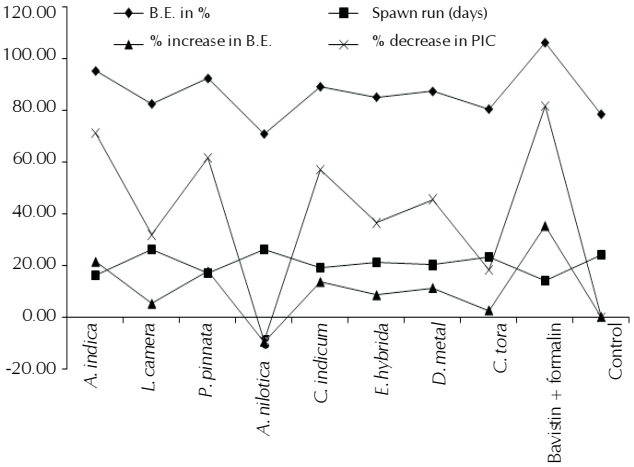

Figure 3.2: *In vivo* evaluation of different botanicals against competitor mould fungi and B.E.

Table 2: *In vitro* evaluation of different phytoextracts and chemicals against major competitor moulds of oyster mushroom

Sl No.	Treatment	Dose	Average yield from 500 g substrate(g))	Biological Efficiency %	Average weight of sporophore (g)	Days to emergence of Pinhead	% increase/ decrease in B.E.	% incidence of CM	% (+) /(-) in incidence of CM	Remarks
1	*Azadirachtaindica*	4.0%	475.5	95.10	6.9	16	21.30	4.1	-70.91	-
2	*Lantana camera*	4.0%	412	82.40	6.4	26	5.10	7	-31.82	*Cs, An, Sr.*
3	*Pongamiapinnata*	4.0%	461	92.20	6.85	17	17.60	5	-61.36	-
4	*Acacia nilotica*	4.0%	354	70.80	5.32	26	-9.69	24	+9.09	*Cs, An, Ps, Sr*
5	*Clerodendronindicum*	4.0%	445	89.00	6.75	19	13.52	6	-56.82	*Sr, Cs*
6	*Eucalyptus hybrida*	4.0%	425	85.00	6.2	21	8.42	7.8	-36.36	*Sr, Tr*
7	*Datura metal*	4.0%	436	87.20	6.1	20	11.22	6.5	-45.45	
8	*Cassia tora*	4.0%	402	80.40	5.9	23	2.55	8.5	-18.18	
9	Bavistin + Formalin	75ppm +500ppm	530	106.00	7.3	14	35.20	0	-81.36	
10	Control		392	78.40	5.8	24	0.00	22	0.00	*Cs, Ps, Sr,Tr*
	SE (treatment mean)		4.251	0.850	0.235	0.695	0.683			

Cs - Coprinus sp. , *As - Aspergillusniger*, *Ps - Penicilliumsp.*, *Sr - Sclerotiumrolfsii*, *Tr - Trichoderma spp.*, *CM - Competitor moulds*

The substrate treated with chemicals (bavistin 75 ppm + formalin 500 ppm) has taken minimum period (14 days) for completing the spawn run where, no moulds attack have been noticed. The extract of *A. indica* showed excellent response towards the growth of mushroom mycelia and completed the spawn run within 16 days. It also reduced the incidence level of competitor moulds (4.1% PCI). However, no correlation has been exist between the treatments in terms of average weight of sporophores (Fig. 3.2)

DISCUSSION

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 and Sohi, 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 (Doshi and Singh, 1985; Sharma and Jandaik, 1980). In the present study the inhibition patterns of carbendazim 75 ppm + formalin 500ppm against the competitor moulds *in vitro* were further support the findings of Jain and Vyas (2002). Carbendazim show high affinity for tubulin protein in fungi,a heterodimetric protein with subunits as alternating helices of á- and â-tubulin, which forms an essential part of fungal cytoskeleton as well as are active in spindle formation. Thus it primarily acted upon cell and nuclear division of fungi which inhibited the mould growth completely onthe beds and hence increased the biological efficiency of mushroom (106 %). Phyto-extract of *A. indica* (neem) showed maximum inhibition against the competitor moulds was due to the presence of antifungal and antibacterial molecules azadirachtin, limonoid sandterpinoids (Nathan *et al.*, 2005 and Jarvis and Morgan, 2000). Leaf extracts of*A. Indica* having antifungal properties against *Aspergillus parasiticus* an aflatoxin producer (Allameh *et al.*, 2002), it's azadirachtin and meliantrioletc. Interfered with the release of ammonium during the early stages of fruiting body formation of *Coprinus* spp (Morimoto *et al.*, 1981). The above mentioned facts can be attributed to the higher biological efficiency of *P. ostreatus*. Extract of *Pongamia pinnata* (karanj) leaf and oil contain karanj in, oleic acid, karanjic acid and their three esters, karanj ketone and its oxime derivaties. All these compounds have outstanding antifungal activity (Tirkey, 2006) The karanj based products exhibited outstanding antifungal activity against the soil-borne phytophagous fungus like *Sclerotium rolfsii* (Sacc.) (Kesari *et al.*, 2010). Excellent response of *Pongamia pinnata*l extract against *Sclerotium rolfsii* was probably due to the detrimental effect of one or more above mentioned antifungal molecules, which not only minimize the growth of fungal contaminants but also contributed to spawn run period (17 days) and biological efficiency (92.20%). Good response of leaf extractof *Eucalyptus hybrida* against *Sclerotium rolfsii* was probably due to the effect of 2', 6'-dihydroxy-3'-methy l-4'-methoxy-dihydrochalcone, eucalyptinand 8-desmethyl-eucalyptin which have strong anti antifungal and antibacterial activity (Takashi *et al.*, 2004; Ranaware *et al.*, 2010)

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First page of the paper should be headed with the title page, (in capital, font size 16), the names of the authors (in capitals, font size 12) and full address of the institution where the work was carried out including e-mail address. A short running title should be given at the end of the title page and 3-5 key words or phrases for indexing.

The main portion of the paper should be divided into Abstract, Introduction, Materials and Methods, Results, Discussion (or result and discussion together), Acknowledgements (if any) References and legends.

Abstract should be limited to 200 words and convey the main points of the paper-outline, results and conclusion or the significance of the results.

Introduction should give the reasons for doing the work. Detailed review of the literature is not necessary. The introduction should preferably conclude with a final paragraph stating concisely and clearly the aims and objectives of your investigation.

Materials and Methods should include a brief technical description of the methodology adopted while a detailed description is required if the methods are new.

Results should contain observations on experiment done illustrated by tables and figures. Use well known statistical tests in preference to obscure ones.

Discussion must not recapitulate results but should relate the author's experiments to other work on the subject and give their conclusions.

All tables and figures must be cited sequentially in the text. Figures should be abbreviated to Fig., except in the beginning of a sentence when the word Figure should be written out in full.

The figures should be drawn on a good quality tracing/ white paper with black ink with the legends provided on a separate sheet. Photographs should be black and white on a glossy sheet with sufficient contrast.

References should be kept to a minimum and listed in alphabetical order. Personal communication and unpublished data should not be included in the reference list. Unpublished papers accepted for publication may be included in the list by designating the journal followed by "in press" in parentheses in the reference list. The list of reference at the end of the text should be in the following format.

1. **Witkamp, M. and Olson, J. S. 1963.** Breakdown of confined and non-confined Oak Litter.*Oikos*. **14**:138-147.
2. **Odum, E.P. 1971.** *Fundamentals of Ecology*. W. B. Sauder Co. Publ. Philadelphia.p.28.
3. **Macfadyen, A.1963.** The contribution of microfauna to total soil metabolism. In:*Soil organism*, J. Doeksen and J. Van Der Drift (Eds). North Holland Publ. Comp., pp 3-16.

References in the text should be quoted by the **author's name and year** in parenthesis and presented in year order. When there are more than two authors the reference should be quoted as: first author followed by *et al.,* throughout the text. Where more than one paper with the same senior author has appeared in on year the references should

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