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RESEARCH ARTICLE

Studies on weed fungi encountered in the cultivation of *Pleurotus eous* (Berk.) Sacc.

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SUMMARY

A sum of 6 fungal species belonging to 6 different genera was found associated with the contaminated beds of *Pleurotus eous.* These were: *Aspergillus niger, Alternaria alternata, Fusarium semitectum, Rhizopus* sp., *Sclerotium rolfsii* and *Trichoderma viride.* Formaldehyde at the concentrations of 1, 2 and 4 per cent caused cent-per-cent inhibition of *Pleurotus eous* and *S. rolfsii* even after 12 hours of exposure period. Fungicide bavistin at the concentrations of 25, 50, 75 and 100 ppm exerted total inhibitory effect on all the contaminants. However, all the fungicides tested in the investigation *viz.*, captan, bavistin and kavach were toxic to *Pleurotus eous* even at the lowest concentration of 25 ppm. There was no antagonistic reaction between *Pleurotus eous* and various weed fungi *viz., A. niger, A. alternata, F. semitectum, Rhizopus* sp., *S. rolfsii* and *T. viride.*

Key Words : *Pleurotus eous,* Contaminants, Weed fungi

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As mushroom cultivation is a controlled biological activity, which depends upon many biotic and abiotic factors, contamination by several microorganisms during cultivation is manifested (Liao, 1993; Singh *et al.*, 2006). Among the biotic agents, fungi, bacteria, viruses, nematodes, insects and mites cause damage to mushrooms directly or indirectly (Sharma *et al*., 2007). Fungi, both parasitic and competitor are the most important group, amongst the biotic factors, which adversely affects both quality and quantity of sporophore. In addition to these moulds being competitive, some have been shown to produce metabolites, which directly inhibit the growth of mushroom mycelium. Contaminants could reduce the yield of mushroom up to 70 per cent (Sharma, 1995).

In the present investigation, attempts were made to isolate microbial contaminants associated with *Pleurotus eous* cultivation, to find out suitable concentration of formaldehyde and fungicides for inhibiting the growth of contaminants and, to study antagonism between weed fungi and *Pleurotus eous.* Moreover, adverse effect of formaldehyde and fungicides on the mushroom fungus was also studied.

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MATERIAL AND METHODS

Isolation of mycoflora :

Ten pieces (1.0 cm long) of substrate from contaminated mushroom beds of *Pleurotus eous* were introduced separately in 250 ml flasks having 100 ml sterilized water. The flasks were shaken vigorously for 30 minutes to get homogenous suspensions. Suspension of 0.5 ml was poured in each Petridish (90mm) having 20 ml potato dextrose agar (PDA) medium. Three replications were maintained. Petridishes were incubated at 26 ± 2^0C for 6 days and observed on third to sixth day for fungal flora. The fungi were isolated on PDA slants and identified. Percentage frequency of occurrence of each of the taxonomic entities of fungi was calculated for substrate colonization as per Eicker (1980) as follows:

$$\text{Per cent frequency occurrence} = \frac{\text{Number of dishes colonized by species}}{\text{Total number of dishes prepared}} \times 100$$

Four categories of frequency were recognized, each

with a quantitative significance: 0-25 per cent, rare; 26-50 per cent, frequent; 51-75 per cent, common; 76-100 per cent, dominant.

Effect of formaldehyde on growth of *Pleurotus eous* contaminants :

Cultures of *Alternaria alternata, Aspergillus niger, Fusarium semitectum, Rhizopus* sp., *Sclerotium rolfsii* and *Trichoderma viride* isolated from mushroom beds during the cultivation of *Pleurotus eous*, were maintained on potato dextrose agar (PDA) medium.

Different concentrations of formaldehyde (37-41% w/v of E Merck (India) Ltd.) *viz.*, 1.0, 2.0 and 4.0 per cent were prepared in sterilized water. Twenty ml of each concentration was poured in uncovered Petridish and the same was put at the bottom of desiccators.The lids of Petridishes containing 7 days old cultures of different weed fungi and *Pleurotus eous* were removed and placed inside the desiccators in upside down condition so that cultures faced formaldehyde fumes directly for 12 to 48 hours depending upon the treatment. The desiccators were kept at 25^0C and sealed with grease to prevent the escape of formaldehyde fumes. After the desired exposure, the Petridishes were taken out and 5 mm (dia.) bits were picked with the help of inoculating needle and inoculated centrally in Petridishes containing PDA medium. In control, the culture plates were exposed to sterilized water inside the sealed desiccators for the same corresponding duration. The Petridishes were incubated at 25 ± 1^0C for 7 days and their radial growth was compared with that of control. Four replicates were maintained in each treatment.

Relative effect of fungicides on weed fungi and *Pleurotus eous* :

In the present investigation, three fungicides were evaluated under laboratory conditions to see their comparative efficacy on the growth of weed fungi as well as on the mushroom fungus.Weed fungi only whose frequency of occurrence in the mushroom bed was more than 75 per cent *viz., Aspergillus niger, Rhizopus* sp. and *Trichoderma viride* were selected for the study alongwith the mushroom fungus *Pleurotus eous.* The fungicides selected for this investigation were: bavistin (methyl benzimidazole-2-ylcarbamate), captan (N-trichloromethylthio-4-cyclohexene-1) and kavach (2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile).

In the laboratory assay of the fungicides against the weed fungi and the mushroom fungus, poisoned food technique was used. Fungicidal solutions of 10,000 ppm were prepared. From that, required amount of solutions were added to molten PDA to get the final concentrations of active ingredient of 25, 50, 75 and 100 ppm level and 20 ml of such medium was poured into sterilized Petridishes (90mm). These were then seeded with 0.5 cm discs of mycelial felt from 7 days old culture on PDA. The fungal colony diameter in different treatments was measured after 48 hours in case of *Rhizopus* sp. and *T. viride* and, after 144 hours in case of *A. niger* and *Pleurotus eous.* Inhibition co-efficient for each fungicide with respect to each fungus was calculated by using the formula of Chakravarty *et al.* (1982): Inhibition co-efficient of the fungicide with respect to each fungus = 1 - (Growth of fungus in presence of fungicide / growth of fungus in control)

Antagonism between weed fungi and *Pleurotus eous* :

To study the interaction between weed fungi *viz., Alternaria alternata, Aspergillus niger, Fusarium semitectum, Rhizopus* sp., *Sclerotium rolfsii* and *Trichoderma viride* and a mushroom fungus *Pleurotus eous;* 90 mm Petridishes containing 20 ml of PDA medium were inoculated with 5 mm mycelial discs of *Pleurotus eous* and weed fungi. In case of control, mycelial disc (5 mm diameter) of *Pleurotus eous* fungus was inoculated and incubated at 26^0C in incubator. In each case three replicates were maintained. Observations were recorded after 48 h of incubation.

RESULTS AND DISCUSSION

The experimental findings obtained from the present study have been discussed in following heads:

Isolation of mycoflora :

A sum of 6 fungal species belonging to 6 different genera was found associated with the contaminated beds of *Pleurotus eous.* These entities were identified as: *Aspergillus niger, Alternaria alternata, Fusarium semitectum, Rhizopus* sp., *Sclerotium rolfsii* and *Trichoderma viride.* The mycoflora were categorized as per Eicker (1980): dominant (fungi with 76-100 per cent frequency of occurrence): *Aspergillus niger, Trichoderma viride* and *Rhizopus;* common (fungi with 51-75 % frequency of occurrence): *Alternaria alternata, Fusarium semitectum* and *Sclerotium rolfsii.*

Average occurrence of *Rhizopus* (96.7%), *Trichoderma viride* (93.3%) and *Aspergillus niger* (86.7%) was most dominant whereas, *Alternaria alternata* (66.7%), *Fusarium semitectum* (56.7%) and *Sclerotium rolfsii* (63.3%) were the common species found during *Pleurotus eous* cultivation.

Various weed fungi *viz., Trichoderma viride, Rhizopus* spp., *Aspergillus flavus, Aspergillus niger* and *Trichoderma harzianum* have been encountered in the cultivation of *Pleurotus* mushrooms by many workers (Sharma and Jandaik, 1979; Thakur and Jandaik, 2000; Anandh *et al.*, 1999). Results of the present investigation are in agreement with the results obtained by these workers.

Effect of formaldehyde on growth of *Pleurotus eous* and contaminants :

Perusal of the data (Table 1) regarding efficacy of formalin against weed fungi and *Pleurotus eous* reveals highly significant decrease in overall growth of various fungi

Table 1 : Effect of formaldehyde against some competitors and *Pleurotuseous*

Fungus	*Average diametric growth (mm) Exposure formaldehyde conc. (%)				Mean EP	Mean F
	Period (hrs)	1	2	4		
Aspergillus niger	0	90	90	90	88.5	40.2
	12	90 (0)	35 (61.1)	0 (100)	41.2	
	24	90 (0)	0 (100)	0 (100)	17.1	
	36	85 (5.6)	0 (100)	0 (100)	11.3	
	48	33 (63.3)	0 (100)	0 (100)	7.7	
Alternaria alternata	0	90	90	90		54.0
	12	90 (0)	90 (0)	90 (0)		
	24	90 (0)	90 (0)	90 (0)		
	36	0 (100)	0 (100)	0 (100)		
	48	0 (100)	0 (100)	0 (100)		
Fusarium semifectum	0	79	80	79		57.9
	12	76 (3.8)	76 (5.0)	49 (38.0)		
	24	76 (3.8)	72 (10.0)	0 (100)		
	36	76 (3.8)	76 (5.0)	0 (100)		
	48	71 (10.1)	58 (27.5)	0 (100)		
Rhizopus sp.	0	90	90	90		36.0
	12	90 (0)	90 (0)	90 (0)		
	24	0 (100)	0 (100)	0 (100)		
	36	0 (100)	0 (100)	0 (100)		
	48	0 (100)	0 (100)	0 (100)		
Fungus	*Average diametric growth (mm) Exposure formaldehyde conc. (%)				Mean EP	Mean F
	Period (hrs)	1	2	4		
Sclerotium rolfsii	0	90	90	90		18.0
	12	0 (100)	0 (100)	0 (100)		
	24	0 (100)	0 (100)	0 (100)		
	36	0 (100)	0 (100)	0 (100)		
	48	0 (100)	0 (100)	0 (100)		
Trichoderma viride	0	90	90	90		20.4
	12	36 (60.0)	0 (100)	0 (100)		
	24	0 (100)	0 (100)	0 (100)		
	36	0 (100)	0 (100)	0 (100)		
	48	0 (100)	0 (100)	0 (100)		
Pleurotus eous	0	90	90	90		18.0
	12	0 (100)	0 (100)	0 (100)		
	24	0 (100)	0 (100)	0 (100)		
	36	0 (100)	0 (100)	0 (100)		
	48	0 (100)	0 (100)	0 (100)		
Mean		43.5	34.5	26.8		

Figures in parentheses represent inhibition over control, C.D. at 5 % : Concentration = 7.4; Exposure period (EP) = 3.2; Fungi (F) = 2.0; Concentration x EP x F = 52.1, *Average of three determinations

including *Pleurotus eous*, with the increase in exposure period, irrespective of different concentrations and fungi. Irrespective of fungi and different exposure period, the overall mean growth of various fungi was 43.5, 34.5 and 26.8 mm at 1, 2 and 4 per cent concentration, respectively.

The interaction between fungi x concentration x exposure period, reveals that all the concentrations of formaldehyde tried caused cent-per-cent inhibition of *Pleurotus eous* and *Sclerotium rolfsii* even after 12 hours of exposure period. Growth of *Trichoderma viride* was observed when it was exposed to 1 per cent concentration for 12 hours and failed to grow with increase in exposure period beyond 12 hours at this concentration. This fungus did not grow at all when exposed to higher concentrations of 2 and 4 per cent even after 12 hours of exposure. *Rhizopus* indicated its sensitivity to all the concentrations only beyond 12 hours of exposure period, as it did not grow when exposed for 24, 36 and 48 hours to all the concentrations of formaldehyde whereas, *Alternaria alternata* showed its sensitivity to all the formaldehyde concentrations beyond 24 hours of exposure period. *Aspergillus niger* did not respond to 1 per cent concentration of formaldehyde even when it was exposed to this concentration for 36 hours. Its exposure to 2 per cent concentration even for 12 hours of exposure period resulted in significant growth inhibition. However, it exhibited its total sensitivity at 4 per cent concentration even for 12 hours of exposure. *Fusarium semitectum* indicated its resistance to formaldehyde at 1 and 2 per cent concentration even for 48 hours of exposure. However, it showed sensitivity to formaldehyde only at 4 per cent concentration beyond 12 hours of exposure.

Formaldehyde is most widely used biocide in mushroom industry and its use is well documented in literature. Effectiveness of formalin (800 ppm) in controlling contaminants during cultivation of *P. flabellatus* was reported by Rajarathnam *et al.* (1983). Bhandari and Singh (1981) reported that formalin soaked straw was protected from contaminants upto 10 days. The present findings support use of formaldehyde as mushroom house disinfectant at the concentration of 4 per cent, as it is effective against most of the competitors. Since *Pleurotus eous* is highly sensitive to formaldehyde, one should ensure absence of formalin fumes at the time of spawning, incubation and cropping, which otherwise would adversely affect the growth and yield of *Pleurotus eous*. Similarly, after substrate pasteurization with formalin, one should be cautious to allow the residual formalin to evaporate for at least 2 hours, as suggested by Earanna and Shivappa Shetty (1999).

Relative effect of fungicides on weed fungi and *Pleurotus eous*:

It is evident from Table 2 that irrespective of different

Table 2 : Comparative efficacy of different fungicides on the growth of contaminants and *Pleurotuseous*

Fungicide	Conc. (ppm)	Average diametric growth (mm) of different fungi			
		Aspergillus niger	*Trichoderma viride*	*Rhizopus* sp.	*Pleurotus eous*
Captan	25	90 (0.0)	90 (0.0)	90 (0.0)	0.0 (1.0)
	50	90 (0.0)	90 (0.0)	90 (0.0)	0.0 (1.0)
	75	90 (0.0)	90 (0.0)	90 (0.0)	0.0 (1.0)
	100	90 (0.0)	90 (0.0)	90 (0.0)	0.0 (1.0)
Kavach	25	86 (0.04)	81 (0.1)	90 (0.0)	0.0 (1.0)
	50	86 (0.04)	86 (0.04)	90 (0.0)	0.0 (1.0)
	75	85 (0.06)	86 (0.04)	90 (0.0)	0.0 (1.0)
	100	84 (0.07)	85 (0.05)	90 (0.0)	0.0 (1.0)
Bavistin	25	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)
	50	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)
	75	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)
	100	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)
Control		90	90	90	90
			C.D. at 5 %		
Fungicide			2.8		
Concentration			0.5		
Fungi			1.9		
Fungicide x Concentration x Fungi			10.1		

Figures in parentheses represent inhibition co-efficient of the fungicide with respect to each fungus

fungicidal concentrations and fungi, maximum inhibitory effect was recorded with bavistin followed by kavach and captan indicating that bavistin is the most toxic fungicide against all the test fungi *viz.*, *Aspergillus niger, Trichoderma viride, Rhizopus* sp. and *Pleurotus eous*. Interaction between fungicides x fungi x concentrations reveals that all the fungicides completely inhibited the growth of *Pleurotus eous* even at the lowest concentration of 25 ppm and there was no growth of all the contaminants at all the concentrations of bavistin. However, the growth of contaminants remained unaffected even at 100 ppm concentration of captan and kavach except with slight non-significant inhibition of *Aspergillus niger* and *Trichoderma viride* with kavach at all the concentrations.

Scientists worldwide have reported incidence of contaminants and their role in reduction of mushroom yield. The present findings are partially in agreement with the results obtained by Anandh *et al.* (1999), Vijay *et al.* (1986) and Chakravarty *et al*. (1982), who reported the action of carbendazim against contaminants at 25 ppm. The fungicide bavistin belongs to benzimidazole group. It is quite likely that the fungicide because of its antimiotic activity inhibited the germination process. Even though bavistin controlled the contaminants, it inhibited the mycelial growth of *Pleurotus eous*. So the mushroom could be produced without the damage by contaminants, however, the inhibitory effect of bavistin on *Pleurotus eous* should be overcome.

Antagonism between different weed fungi and *Pleurotus eous*:

The cultural studies indicated that none of the contaminating fungus and *Pleurotus eous* produced any antifungal substance, which was explicitly evident from the absence of inhibition zone between the colonies of contaminants and *Pleurotus eous* in paired culture (Table 3). These observations clearly indicate the absence of antagonistic reaction between *Pleurotus eous* and various weed fungi. However, *Rhizopus* sp. and *Sclerotium rolfsii* overran the colonies of *Pleurotus eous* within 36 and 48 hours of incubation, respectively and restricted its growth completely.

Though no inhibition zone was observed between the colonies of contaminants and *Pleurotus eous*, the diametric mycelial growth of *Pleurotus eous* was restricted to varying degrees ranging from 7.7 to 100 per cent, the maximum being with *Rhizopus* and *Sclerotium rolfsii*. Restricted growth of *Pleurotus eous* observed, was mainly due to rather expeditious growth of the contaminating fungi in comparison to that of *Pleurotus eous*. *Aspergillus niger* was the next contaminant to restrict the growth of *Pleurotus eous* to the extent of 34.6 per cent. The minimum restricted growth of *Pleurotus eous* was observed in dual culture with *Fusarium semitectum.*

In the present investigation, though no antagonistic reaction was observed between various contaminants and *Pleurotus eous*, the growth of *Pleurotus eous* was found to be restricted to some extent in the presence of contaminants which may be due to the fact that, these contaminants grew most rapidly than *Pleurotus eous*. Secondly, *Aspergillus* spp. are known to produce acids in culture (Turner, 1971). If it produces acids, it might have inhibited the mycelial growth of *Pleurotus eous* in dual culture. This is the advantageous factor for *Aspergillus* spp., which facilitates them for colonization and establishment in the beds thereby causing yield reduction.

Results of the present investigation are in agreement with the results obtained by Doshi and Singh (1985) who also observed no antagonistic effect of weed fungi on *P. sajor-caju* but they observed that *Rhizopus stolonifer, Fusarium semitectum, Fusarium moniliformae* var. *ferbglutinans, Mucor*

Table 3 : Pattern of *in vitro* antagonism in dual culture between *Pleurotuseous* and various contaminants

Characters of fungal colony	Fungal combinations*	Status of antagonistic reaction	Per cent inhibition of *Pleurotus eous* growth (%)
Colony overran	Rh (90) x Pe (0.0)	No antagonism detected. Colonies of	100.0
	Sr (90) x Pe (0.0)	*Rhizopus* sp. and *Sclerotium rolfsii* grew	100.0
		only in 36 and 48 h, respectively and	
		completely overran *P. eous* colonies	
Colonies close	Tv (69) x Pe (18)	No antagonism detected	30.8
	Fs (61) x Pe (24)		7.7
	Aa (55) x Pe (23)		11.5
	An (74) x Pe (17)		34.6
Control (*P. eous*)	Pe (26)		-
CD at 5 %			3.6

* Rh = *Rhizopus* sp., Sr = *Sclerotium rolfsii*, Tv = *Trichoderma viride*, Fs = *Fusarium semitectum*, Aa = *Alternaria alternate*, An= *Aspergillus niger*, Pe = *Pleurotus eous*, Figures in parentheses represent average diametric growth (mm) in *in vitro* dual culture

sp. and *Sclerotium rolfsii* overlapped the colonies and restricted the growth of *P. sajor-caju.*

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