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Studies on Effect of Phyto-Extracts for Control of *Trichoderma* Mould in Oyster Mushroom Cultivation

A.C. Jadhav and N.V. Kokate
All India Coordinated Research Project on Mushroom, College of Agriculture,
Pune-411 005 (MS), India

Abstract:- The studies on effect of phyto-extracts for control of *Trichoderma* mould in oyster mushroom cultivation was conducted to study the efficacy of different phytoextracts against *Trichoderma* mould. Oyster mushroom is also affected by various diseases caused by fungal and bacterial agents. Among the fungal agents, *Trichoderma* mould is major mushroom infecting pathogen. Looking to the seriousness of infecting pathogens to the oyster mushroom, present investigation was undertaken for isolation and identification of *Trichoderma* mould and its management by using botanicals and chemicals.

Phytoextracts *viz Lantana camera, Azadirachta indica, Ocimum sanctum, Eucalyptus* sp and chemical were screened *in vitro* by poisoned food technique against *Trichoderma* mould. The treatment of Carbendazim + Formalin found to be the best treatment while among the phytoextracts neem (*A. indica*) @ 6% proved to be excellent in inhibiting mycelial growth of *Trichoderma* mould showing 75.19% and 32.99% per cent inhibition respectively. The least inhibition was observed in treatment T_7 *Ocimum sanctum* @ 2% (6.02%) over control.

The observation on per cent contamination index revealed that treatment T_{13} Carbendazim + Formalin recorded minimum contamination index per cent (6.66%) while among the phytoextracts treatment T_6 *Azadirachta indica* @ 6% recorded minimum contamination index per cent (33.33%) followed by T_5 *Azadirachta indica* @ 4% (40.33%) whereas T_7 *Ocimum sanctum* @ 2% recorded maximum contamination per cent (86.66%).

Keywords:- *Phyto-extracts, Trichoderma mould, oyster mushroom cultivation*

I. INTRODUCTION

Oyster mushroom (*Pleurotus* sp.) popularly known as '*dhingri'* in India and grows naturally in temperate and tropical forests on dead and decaying wooden logs or sometimes on dying trunks of deciduous or coniferous woods. It may also grow on decaying organic matter. The most well known species of oyster mushroom are *P. ostreatus, P. florida, P. eryngii, P. cystidiosis, P. flabellatus, P. cornucopie,* and *P. sajor-caju. P. sajor-caju* recognized as an excellent mushroom. The oyster mushroom is grown under natural conditions on living trees as parasite or dead woody branches of trees as saprophyte and act as primary decomposer.

Mushroom farming in India is becoming successful and also popularized day by day because of its very low input, which can bring a significant change in rural economy. The climatic conditions of the region have been found to be ideal for such an attempt. Research and field experiments on production and marketing of several varieties of mushrooms have proved its significant potentiality as a major source of income for rural people.

Oyster mushroom is the third most popularly grown mushroom in the world and ranks second in India. In India, *P. sajor-caju* is one of the commonly grown species. *P. sajor-caju* has been shown to exhibit hypotensive activity, reduce the rate of nephron deterioration which may be useful for chronic renal failure patient. Oyster mushroom (*P. ostreatus)* has also been found to have hypocholesterolemic effects in rats. *P. tuberregium* also shows some antimicrobial activity.

Like all other crops, mushroom cultivation (from spawn preparation to harvesting) is also affected adversely by a large number of biotic and abiotic agents/ factors. Among the biotic agents, fungi, bacteria, viruses, nematodes, insects and mites cause damage to mushrooms directly or indirectly. A number of harmful fungi are encountered in the cultivation of oyster mushroom. Many of these act as competitor moulds thereby adversely affecting spawn run whereas others attack the fruit bodies at various stages of crop growth producing distinct disease symptoms. At times there is complete crop failure depending upon the stage of infection, quality of spawn and environmental conditions.

During the preparation of grain spawn, it is infected by many pathogens. The worst contaminants are usually moulds *viz., T. harzianum, T. viride, A. niger, A. flavus, Fusarium* sp., *Penicillium* sp. etc. They found to infect the grain spawn. However bacteria and yeasts can also be a problem, especially when attempting to isolate pure culture from natural population of mushroom. *Trichoderma* and *Pseudomonas* spp. are important causal agents and incur significant yield losses (Badham 1991). *Aspergillus flavus* grows on practically all types of grain. This species is of serious concern to mushroom spawn producers. Careful handling of any molds, particularly those of the genus *Aspergillus* and *Trichoderma* should be a primary responsibility of all managers and workers in mushroom farms.

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For avoiding this type of contaminants, management of contaminants is necessary. Management of contaminants *in vitro* and *in vivo* can be carried out by two ways, by using chemical and using botanicals. Hence the present research was planned to isolate and identify *Trichoderma* mould infecting oyster mushroom cultivation and to evaluate the inhibition efficiency of different plant extracts and chemical against the *Trichoderma* mould with the objectives as to survey for *Trichoderma* disease incidence in different oyster mushroom farms in Pune district, to isolate *Trichoderma* sp. infecting oyster mushroom and i*n-vitro* evaluation of different phyto-extracts against *Trichoderma* spp. by poison food technique.

II. MATERIAL AND METHODS

The present study was carried out at All India Coordinated Research Project on Mushroom, College of Agriculture, Pune (MS) and the pure quality spawn of *Pleurotus sajor caju* (DMRP-112) was also obtained from this centre. The phytoextracts of *Lantana camera* (Ghaneri), *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi), *Eucalyptus sp* (Nilgiri) were used for screening against the test fungus. These botanicals were obtained from the farm area of College of Agriculture, Pune.

➢ *Methods*

The mushroom beds of *Pleurotus sajor-caju* bearing typical green mould contamination of *Trichoderma* were collected from ten oyster mushroom projects nearby Pune district. The *Trichoderma* mould fungi were collected from the contaminated oyster mushroom beds in sterilized petriplates with the help of a sterile forceps and thereafter transferred on to ten PDA plates under *in-vitro* conditions. Inoculated PDA plates were incubated at 27^0 C 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 4^0 C for preservation. Identification of the pathogens was carried out by studying the cultural and morphological characters, which were recorded right from initiation of mycelial growth till the period of 15 days. The morphological characters were studied under low (10X) and higher (40X) power magnification from 10 days old culture of pathogens and were confirmed with those given in literature.

➢ *Preparation of phyto-extracts*

For preparation of phyto-extracts, 100 g plant products were collected, washed in distilled water, air dried and homogenized with equal amount of distilled water (100 ml) by crushing 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*

The extracts of 4 botanicals were evaluated in the laboratory for their efficacy against the pathogens. The plant extracts were evaluated in *in vitro* by poisoned food technique. Test concentration of 2%, 4% and 6% were obtained by adding appropriate amount of sterile distilled water to standard solution (100%). Two mili litre of each extract (2%, 4% and 6%) was dispensed in petri plates (9cm) and then 20 ml of molten PDA was poured gently in Petri plates containing the extract solution. After solidification, inoculation was done with 5 mm dia mycelial disc cut from 6 days old culture of pathogen . The medium without the botanical extracts served as check. The plates were incubated at 27 ±1 ºC till the complete growth was observed in control plates (Shah *et al*., 2011). Observations were recorded in three repetitions. Data were analyzed statistically using completly randomized design.

The per cent inhibition of growth of the fungus in each treatment was calculated by using following formula (Vincent, 1927).

$$I = \frac{dc - dt}{dc} \times 100$$

Where, I = Per cent inhibition
dc = Colony diameter in control (cm)
dt = Colony diameter in respective treatment (cm)

III. RESULTS AND DISCUSSION

During present study, the efficacy of different phyto-extracts and chemical against *Trichoderma* were evaluated under *in vitro* conditions.

➢ *Survey for Trichoderma disease occurrence in different oyster mushroom farms*

The oyster mushroom beds of *Pleurotus sajor-caju* bearing typical green mould contamination of *Trichoderma* mould were collected from about ten oyster mushroom project nearby Pune. In all, 1860 oyster mushroom beds were observed, of which 218 beds were observed to be infested with *Trichoderma* mould from these ten different locations. About 18.39% disease incidence of *Trichoderma* mould was noticed over the different locations surveyed nearby Pune. (Table 1)

The results obtained during survey are comparable to the findings of Krzyszt *et al.* (2012) who investigated yield levels of *P. ostreatus* growing on substrates infected with *Trichoderma* isolates and found that the substrate infections with *T. pleurotum* and *T. pleuroticola* isolates caused significant yield reductions of the examined strains of oyster mushrooms.

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Sr. No.	Mushroom farms	Location	No. of beds observed	No. of beds infected with mould	Per cent disease incidence
1	Neelai oyster mushroom farm	Atulnagar, Warje, Pune	120	14	11.66
2	Silver flower oyster mushroom farm	Matalwadi, Panshet road, Pune	160	11	6.88
3	Himgiri oyster mushroom farm	Ramwadi, Pune	55	8	14.54
4	M K oyster mushroom project,	Moshi, Pune	80	22	27.50
5	Bhagwat oyster Mushroom Farm	Patas, Dist-Pune	850	42	4.94
6	Sathe oyster Mushroom Farm	Ambethan, Chakan, Dist-Pune	110	12	10.91
7	KNT oyster Mushroom Farm	Solu, Pune	200	17	8.50
8	Bhandare oyster Mushroom Farm	Tulapur, Pune	75	25	33.33
9	Kadam oyster Mushroom Farm	Vishrantwadi, Pune	150	46	30.67
10	Sourabh oyster Mushroom Farm	Hinjawadi, Pune	60	21	35.00
Total			**1860**	**218**	**-**
Average per cent disease incidence					**18.39**

Table 1:- Locations of infected oyster mushroom beds collected during survey and per cent disease incidence

➢ *Isolation of Trichoderma mould associated with oyster mushroom*

Isolation was made from the infected oyster mushroom beds. Twelve isolates were obtained from 218 beds. The fungus growth developed within 3-4 days after inoculation from infected pieces which was purified and used for further studies.

The fungus was re-isolated from the inoculated oyster mushroom bed showing typical *Trichoderma* mould symptoms. The culture was identical and similar to that of original culture in all respects, and Koch's postulate were established.

The symptoms were confirmed by pathogenicity test. The colonies had a smooth edge and aerial mycelium, initially thick and whitish. From the fourth day onwards when sporulation began, the colony turned green colour and the periphery of colonies remained greenish white. The conidia were small (3.0-3.5 µm) round, smooth and thin walled.

➢ *In vitro test*

The effect of four phytoextracts @ 2, 4 and 6% and chemicals on the mycelial growth of test fungus was investigated and data were statistically analyzed. The observations for their colony diameter were recorded on 6th day and are presented in Table 2, Fig 1 and Plate 1.

➢ *Influence of phyto extracts on radial growth of Trichoderma mould mycelium*

The phytoextracts were evaluated in *in vitro* by poison food technique at different concentrations. The phytoextracts and chemicals were poured in the petri plates with molten PDA media and observation were recorded on growth of *Trichoderma* mould mycelium.

The treatment T_{13} Carbendazim + Formalin was found significantly superior as it inhibited the maximum mycelial growth of *Trichoderma* mould over control (2.18 cm). Among the phytoextracts, treatment T_6 *Azadirachta indica* @ 6% was found to be more effective and recorded minimum mycelium growth (5.89 cm) followed by treatment T_5 *Azadirachta indica* @ 4% (7.16 cm), T_{12} *Eucalyptus* sp. @ 6% (7.27 cm), T_3 *Lantana camera* @ 6% (7.36 cm) which were found to be at par with each other. While treatment T_7 *Ocimum sanctum* @ 2% was found to be less effective to inhibit growth of mould and recorded maximum mycelium growth (8.26 cm) which was found to be at par with rest of the treatments (Table 2).

➢ *Influence of phyto extracts on per cent inhibition of Trichoderma mould*

The phytoextracts and chemical were poured in the petri plates with molten PDA media and the observation were recorded on inhibition of *Trichoderma* mould. The data presented in Table 2 revealed that the chemical treatment was found to be more effective than phytoextracts in per cent disease inhibition.

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The treatment T_{13} Carbendazim + Formalin was found more effective in reducing the disease inhibition over control (75.19%). Among the phytoextracts, the treatment T_6 *Azadirachta indica* @ 6% recorded significantly highest per cent inhibition (32.99%) followed by the treatment T_5 *Azadirachta indica* @ 4% (18.54%), T_{12} *Eucalyptus* sp.@ 6% (17.29%), T_3 *Lantana camera* 6% (16.26%). The treatments T_9 *Ocimum sanctum* @ 6% (12.51%) and T_{11} *Eucalyptus* sp. @ 4% (12.17%) were found to be at par with each other. While the treatment T_8 *Ocimum sanctum* @ 4% (9.32%), T_4 *Azadirachta indica* @ 2% (9.10%), T_2 *Lantana camera* @ 4% (8.75%) were found to be at par with each other. The treatment T_7 *Ocimum sanctum* @ 2% was found less effective (6.02%) in inhibition of *Trichoderma* mould (Table 2).

Sr. No.	Treatments	Radial growth of mycelium (cm)*	Per cent inhibition over control*
1	*Lantana camera* (Ghaneri) @ 2%	8.20	6.71
2	*Lantana camera* (Ghaneri) @ 4%	8.02	8.75
3	*Lantana camera* (Ghaneri) @ 6%	7.36	16.26
4	*Azadirachta indica* (Neem) @ 2%	7.99	9.1
5	*Azadirachta indica*(Neem) @ 4%	7.16	18.54
6	*Azadirachta indica*(Neem) @ 6%	5.89	32.99
7	*Ocimum sanctum*(Tulsi) @ 2%	8.26	6.02
8	*Ocimum sanctum*(Tulsi) @ 4%	8.04	9.32
9	*Ocimum sanctum*(Tulsi) @ 6%	7.69	12.51
10	*Eucalyptus* sp. (Nilgiri) @ 2%	8.15	7.28
11	*Eucalyptus* sp. (Nilgiri) @ 4%	7.72	12.17
12	*Eucalyptus* sp. (Nilgiri) @ 6%	7.27	17.29
13	Carbendazim @7.5g+ formalin @125 ml per 100 L water	2.18	75.19
14	Control	8.79	0.00
SE±		0.12	0.29
CD (0.05)		0.34	0.84

*=Mean of three replication

Table 2:- Influence of phyto extracts on radial mycelium growth and per cent inhibition of *Trichoderma* mould

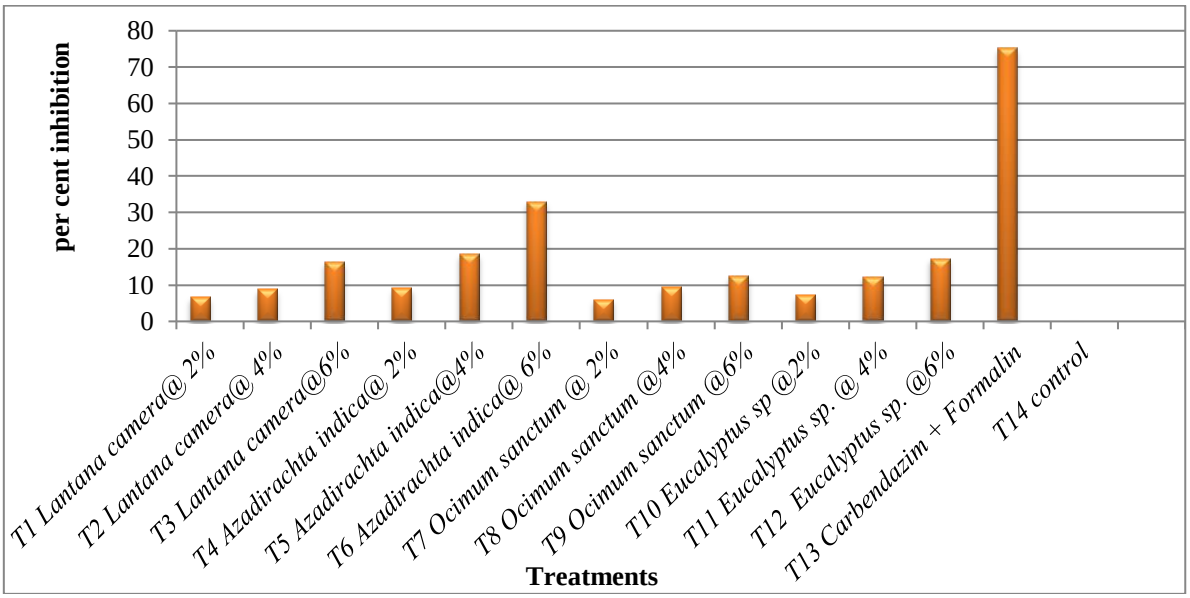

Fig 1:- Influence of phyto extracts on per cent inhibition of *Trichoderma* mould

During the present investigation, it was noticed that the treatment T_{13} Carbendazim + Formalin was found to be more effective in per cent inhibition (75.19%) and among the plant extracts, T_6 *Azadirachta indica* @ 6% (32.99%) was found to be more effective in disease inhibition. While treatment T_7 *Ocimum sanctum* @ 2% found less effective in reducing the disease inhibition. The results obtained are in conformity with the results obtained by Sharma and Iandiak (1980) who reported that neem leaves extract *(Azadirachta indica)* was found to inhibit the mycelial growth of *Trichoderma viride* by 0.8-29.6% and Pervez (2012) who reported that the percent inhibition produced by extracts against *Trichoderma* recorded *in vitro*, was *Azadirachta indica* (47.75%), *Lantana camara* (51.25%) and *Ocimum sanctum* (17.2%).

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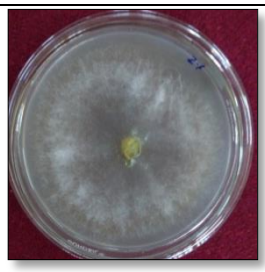
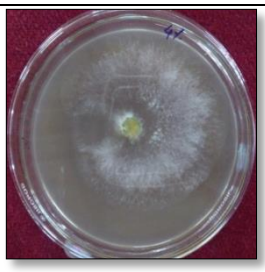


T-1 *Lantana camera* @ 2%



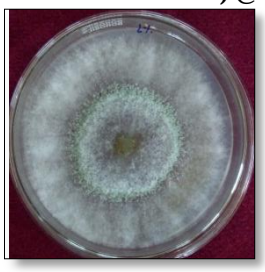
T-2 *Lantana camera* @ 4%

T-3 *Lantana camera* @ 6%

T-4 *Azadirachta indica*) @ 2%

T-5 *Azadirachta indica* @ 4%

T-6 *Azadirachta indica* @ 6%



T-7 *Ocimum sanctum* @ 2%

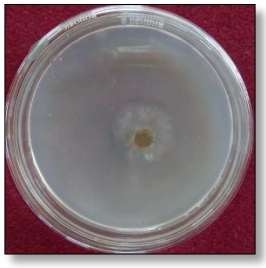
T-8 *Ocimum sanctum* @ 4%

T-9 *Ocimum sanctum* @ 6%

T-10 *Eucalyptus* sp @ 2%

T-11 *Eucalyptus* sp @ 4%

T-12 *Eucalyptus* sp @ 6%



T-13 Carbendazim 50WP(7.5 g) + Formalin (125 ml in 100 lit. of water)

T-14 Control

Plate 1:- Influence of phyto extracts on radial mycelium growth and per cent inhibition of *Trichoderma* mould

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➢ *Influence of phyto extracts on per cent contamination index*

The contamination index (%) was calculated by using scale given by Biswas *et al.*,(2018) and the data is presented in Table 3 and Fig 2.

Sr. No.	Treatment	Contamination index (%)*
1	*Lantana camera* (Ghaneri) @ 2%	77.33
2	*Lantana camera* (Ghaneri) @ 4%	66.66
3	*Lantana camera* (Ghaneri) @ 6%	60.66
4	*Azadirachta indica* (Neem) @ 2%	46.66
5	*Azadirachta indica*(Neem) @ 4%	40.33
6	*Azadirachta indica*(Neem) @ 6%	33.33
7	*Ocimum sanctum*(Tulsi) @ 2%	86.66
8	*Ocimum sanctum*(Tulsi) @ 4%	73.33
9	*Ocimum sanctum*(Tulsi) @ 6%	66.66
10	*Eucalyptus* sp. (Nilgiri) @ 2%	66.66
11	*Eucalyptus* sp. (Nilgiri) @ 4%	60.33
12	*Eucalyptus* sp. (Nilgiri) @ 6%	53.33
13	Carbendazim @7.5g+ formalin @125 ml per 100 L water	6.66
14	Control	100
SE±		0.95
CD (0.05)		2.75

*=Mean of three replications

Table 3:- Influence of phyto extracts on per cent contamination index

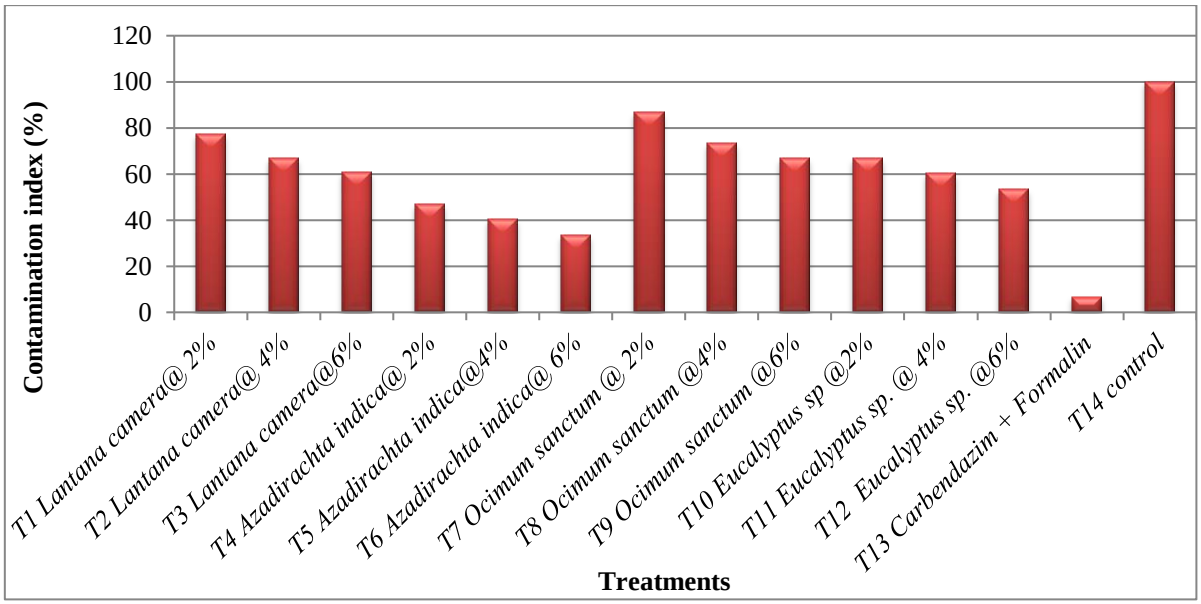

Fig 2:- Influence of phyto extracts on per cent contamination index

It was observed that the treatment T_{13} Carbendazim + formalin recorded minimum contamination index per cent (6.66%) while among the phyto-extracts, the treatment T_6 *Azadirachta indica* @ 6% recorded minimum contamination index (33.33%) followed by treatment T_5 *Azadirachta indica* @ 4% (40.33%). Whereas treatment T_7 *Ocimum sanctum* @ 2% recorded maximum contamination per cent (86.66%) (Table 3).

The observations are in agreement to the finding of Young Choi *et.al.* (2003) who reported that *T. longibrachiatum* caused the greatest damaged (78.6%) on *P. eryngii.* Krzyszt *et al.* (2012) who investigated yield levels of *P. ostreatus* growing on substrates infected with *Trichoderma* isolates and found that the substrate infections with *T. pleurotum* and *T. pleuroticola* isolates caused significant yield reductions of the examined strains of oyster mushrooms.

IV. CONCLUSION

The continuous use of chemicals to control different diseases is hazardous to human health as it retains residues, so it is important to control the disease with eco-friendly management. Hence, the study was undertaken to find out the effect of some phytoextracts and chemicals on *Trichoderma* mould contaminant in oyster mushroom cultivation.

From the above summarized results of present investigation, it could be concluded that the per cent *Trichoderma* mould inhibition due to different treatments varied from 6.02 to 75.19 %.over control. The per cent contamination index ranged from 6.66 to 86.66 % for different treatments. Thus, it could be concluded that the chemical treatment (Carbendazim + Formalin) was found to be the best for management of *Trichoderma* mould contamination in oyster mushroom cultivation and among the phytoextracts evaluated, the treatment *Azardirachta indica* @ 6% was found to be the best for management of *Trichoderma* mould in oyster mushroom cultivation.

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