European Journal of Medicinal Plants

22(4): 1-8, 2018; Article no.EJMP.40411
ISSN: 2231-0894, NLM ID: 101583475

Use of Plant Extracts for Substrate Sterilization and Its Effect on Competitor Moulds and Biological Efficiency of Oyster Mushroom

M. K. Biswas[1*], Sanjib Kuiry[2] and Tanmay Ghosh[3]

[1]Department of Plant Protection, P.S.B., Visva-Bharati, Sriniketan, W. B. 731236, India.
[2]Department of Plant Pathology, BCKV, Kalyani, W.B., India.
[3]Department of Microbiology, Rabindra Mahavidyalaya, Champadanga, Hooghly, W.B., India.

Authors' contributions

This work was carried out in collaboration between all authors. Author MKB executed the plan of the study and layout the design of work and checked the data collected and applied the methodology. Author SK conducted the experiment and collected the data and reviews, prepared the pure cultures and author TG prepared the manuscript initially, made statistical analysis of the data. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2018/40411
Editor(s):
(1) Marcello Iriti, Professor, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Jihan Seid Hussein, Egypt.
(2) Fatih Kalyoncu, Manisa Celal Bayar University, Turkey.
(3) Esraa Ashraf Ahmed ElHawary, Ain Shams University, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/23677

Original Research Article

Received 29th November 2017
Accepted 15th March 2018
Published 17th March 2018

ABSTRACT

Mushrooms are recognized as nutritionally functional food and a source of physiologically beneficial and nontoxic medicines. Oyster mushroom (*Pleurotus* spp.) is an efficient lignin degrading mushroom and can grow well on different types of lignocellulosic materials including agricultural and forest waste. Cultivation technique for oyster mushroom is very simple and the production cost is low, which gives consistent growth with high biological efficiency. Plant derivatives have shown considerable promise as an effective alternative of chemicals used in surface sterilization. To develop a suitable method for substrates treatment, six different plants extract were evaluated along with most popular chemical treatment (bavistin 75 ppm + formalin 500 ppm) for cultivation of oyster mushroom *(Pleurotus florida)*. Chemical treatment (bavistin 75 ppm + formalin 500 ppm) was found to be most effective among all the treatments and exhibited 120.50% Biological Efficiency (B.E.). Among the phyto-extracts, *Zingiber officinale* was found to be excellent in

**Corresponding author: E-mail: mkb.psb@gmail.com;*

controlling the growth of competitor mould fungi (114% B.E.) followed by *Azadirachta indica* (109.25%) and *Allium cepa* (98.75%). Chemically treated substrate was taken minimum (20 days) for spawn run and gave 7.10 gm average weight of sporophore followed by *Zingiber officinale* (22 days and 6.740 gm). *In vitro* study revealed the superiority of chemicals and reduced 61.80 to 70.67% mycelium growth of four contaminants. Extract of *Zingiber officinale* was found excellent in inhibiting the mycelium growth of *Penicillium* sp., *Aspergillus niger* and *Coprinus* sp. but, reported to be less effective against *Sclerotium rolfsii*. While, *Azadirachta indica* seed oil was found very effective against the mycelium growth of *Sclerotium rolfsii*, *Penicillium* sp, and *Coprinus* sp. Extract of *Allium cepa, Lantana camera, Eucalyptus hybrida* and *Allium sativum* showed moderate effects on the mycelium growth of competitor moulds.

Keywords: Oyster mushroom; competitor moulds; plant- extracts; substrate sterilization.

1. INTRODUCTION

Mushrooms are popular for their delicacy and flavored food value. It has been accepted as a nutritionally rich substance having reasonable amounts of protein, carbohydrate, minerals and vitamins. FAO recognizes mushroom as a proteinaceous food for the poor people in underdeveloped countries. Mushrooms are useful against diabetes, ulcer and lungs diseases [1] and are good source of protein, vitamins and minerals [2]. Oyster mushroom (*Pleurotus* sp.) is the third most important edible mushroom cultivated worldwide [3]. It can efficiently decompose lingo-cellulose without chemical or biological pre-treatment because it possesses an enzymatic complex system that includes phenol oxidises and peroxidises [4]. Oyster mushroom (*Pleurotus* spp.) is popularly known as 'dhingri' in India, has attained popularity because of its ability to grow in wide range of growing conditions, low cost of production and excellent in recycling of agricultural waste like paddy straw and wheat straw [5,6]. China, the world leader in oyster production, contributes nearly 85% of the total world production. India's contribution is only1.5% with a production of 2500 MT. Paddy straw was reported to be the best substrate for the cultivation of oyster mushroom [7]. The main obstacle for increased production in India is the frequent contamination of the mushroom growing beds with competitor moulds, diseases, insects and nematodes. Various techniques were followed by different workers to minimize the competitors moulds in oyster mushroom beds [8,9]. The use of chemical fungicides for disease management is discouraged as it cause environmental pollution and leave toxic residues to the soil, water and thus effect on non target organisms leading to an ecological imbalance. With the development of resistance to chemical fungicides and the demand for safer product, alternative biotic pesticides such as phyto-extracts offer a viable choice which are non persistent in the environment and safer to use various plant products like gum, oil, resins etc. are used as fungicides [10,11]. Plant derivatives have shown considerable promises as an effective alternatives for minimizing the infection of competitor moulds and diseases of oyster mushroom [12,13]. Biotic fungicides, being plant product are easily convertible into a common organic material and create fewer hazards to mankind than the synthetic one. In view of the above, an effort has been made to identify the competitor moulds and diseases of oyster mushroom beds responsible for the loss in biological efficiency and also to minimize their incidence with the help of plant extracts.

2. MATERIALS AND METHODS

2.1 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 27°C (+ 2°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°C for preservation.

2.2 Identification of Competitor Moulds

Identification of various microbial contaminants were done through microscopic study where various morphological parameters were viewed under a high level of magnification of different microscopes. Expert suggestions were also taken for further confirmation.

2.3 Sources of Plant Materials

Zingiber officinale (Ginger), *Allium sativum* (Garlic), *Allium cepa* (onion), *Azadirachta indica*

(neem seed oil), *Eucalyptus hybrida* and *Lantana camera* were collected from the nearby forest and Agricultural Farm of Palli Siksha Bhavana, Visva-Bharati, Sriniketran.

2.4 Preparation of Photo-Extracts

For the preparation of phyto-extracts, 100 gram plant products were collected, washed in tap water, air dried and homogenized with an equal amount of distilled water (100 ml) 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 a standard solution.

2.5 Evaluation of Phyto-extracts and Chemicals

Phyto-extracts of different plant products i.e. *Zingiber officinale* (Ginger), *Allium sativum* (Garlic), *Allium cepa* (onion) all at the rate of 4%, *Azadirachta indica* (neem seed oil) 0.05%, *Eucalyptus hybrida* and *Lantana camera* (leaf) both at the rate of 4% were evaluated along with chemicals like, Bavistin 50 ppm +formalin 500 ppm and $CaCo_3$ (lime) 0.1% for cultivation of oyster mushroom (*Pleurotus florida*). The extracts were mixed with water (20 litres for each treatment) at the rate of aforementioned percentages. The paddy straw was immersed in suspension for 18 hours. Layering method of spawning at the rate of 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 and 500g of dry straw was taken for each replication. After harvesting of the first flush of mushroom, one final spray of photo-extracts was further given on beds to reduce the competitor's molds. Paddy straw immersed in plain water without chemicals and photo- extracts was served as control.

2.6 *In vitro* Evaluation of Phyto-extracts, and Chemicals against Major Competitor Moluds of Oyster Mushroom (*Pleurotus florida*)

The fungitoxicity of phyto-extracts i.e. *Zingibner officinale*, *Allium sativum, Allium cepa, Lantana camera, Azadirachta indica, Eucalyptus hybrida*, and chemicals bavistin + formalin and $CaCo_3$ were tested against the major competitor molds of oyster mushroom(*Pleurotus florida*) namely *Coprinus* sp, *Aspergillus niger*, *Penicillium* sp. and *Sclerotium rolfsii* through poisoned food technique [14]. For one competitor mould, four ml of each plant extract (standard solution) was incorporated in different 100 ml of potato dextrose agar medium (PDA) and autoclaved for 20 minutes at 1.41 kg/ cm^2 pressure. After sterilization, the molten media were poured into four sterilized glass petriplates (90 mm) considering each as a replication. After solidification, all the plates were inoculated individually with a 3 mm diameter culture disc of one contaminant. A similar technique was also applied to other mold fungi (contaminants) and *Pleurotus florida*. Neem seed oil (*Azadirachta indica*), Bavistin 50 ppm + formalin 500ppm, and $CaCo_3$ 0.10% were dissolved in 100 ml of sterilized molten PDA prior to inoculation of competitor mold fungi and *Pleurotus florida*. The plates inoculated only with mold fungi (contaminants) and *Pleurotus florida* with out any phyto-extract and chemicals were served as control. Four replications were maintained for all the treatments and plates were incubated in BOD incubator at a temperature of 22-25^{0}c. for 8 days (when mycelial growth of any treatment fully covered the surface of petriplates). The colony diameter of the test fungus in treatments and control sets was measured and fungitoxicity in terms of % mycelial growth inhibition was calculated.

3. RESULTS AND DISCUSSION

3.1 Evaluation of Phyto-extracts, and Chemicals for the Cultivation of Oyster Mushroom (*Pleurotus florida*)

The effects of different phyto-extracts and chemicals on biological efficiency and other parameters are presented in Table.1. It was evident from the table that, the majority of phyto-extracts i.e. *Zingiber officinale* (Ginger), *Allium sativum* (Garlic), *Allium cepa* (onion) and *Azadirachta indica* were proved their efficiency in suppressing the growth of competitor mould and hence, increasing the biological efficiency of oyster mushroom over control. However, chemical method of substrate sterilization was proved its superiority among all the treatments and gave highest yield and biological efficiency (120.50%) of *Pleurotus florida*. Among the phyto-extracts, *Zingiber officinale* (Ginger), was reported to be excellent in controlling the growth of mould fungi and exhibited 114% biological efficiency, which was found statistically at par with chemical treatment, followed by *Azadirachta*

indica (109.25% B.E.), *Allium cepa* (98.75% B.E.) and *Allium sativum* (86.25% B.E.). Various antagonistic *microorganisms viz. Coprinus sp., Aspergillus niger, Penicillium* sp. *Sclerotium rolfsii* etc. were noticed on the beds treated with the extract of *Lantana camera*, *Eucaluptus hybrida* and *Allium sativum* which exhibited 80.25% and 77.00% biological efficiency respectively (plate no.1-4). Spawn run period was found minimum (20) days in the substrate treated with bavistin 75ppm + formalin 500 ppm, followed by *Zingiber officinale* and lime($CaCo_3$) both 22 days, *Azadirachta indica* 23 days, *Allium cepa* and control both 24 days. The substrates treated with *Eucaluptus hybrida*, *Allium sativum* and *Lantana camera* took maximum 28 and 26 days respectively, for completing the spawn run. The effect of various photo-extracts and chemicals on the average weight of sporophore was also recorded. Maximum average weight of sporophore (7.1 gm) was observed from chemically treated substrates, which was significantly superior than control. The average weight of sporophore recorded from the beds treated with *Azadirachta indica* and *Zingiber officinale* and *Allium cepa* were 6.750g, 6.740 g and 6.660 g respectively (Fig. 1). Similar observations of chemical treatment were also made by [8,9] with *Pleurotus sajor-caju* and *Pleurotus florida* respectively. Fresh extract of ginger contains [6] gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one (the most abundant constituent in the gingerol series) which inhibits the growth of *Aspergillus niger*, *Penecillium* spp, *Coprinus* sp. and *Mycoderma* spp. [15] and hence, increased the biological efficiency of oyster mushroom. The effects of azadirachtin ($C_{35}H_{44}O_{16}$), the principle active ingredient of *Azadirachta indica* (neem oil) on the yield and biological efficiency (109.25%) of oyster mushroom was corroborated with the findings of Sharma and Jandaik [12,15,16].

Plate 1. *Penicillium* sp.

Plate 2. *Coprinus* sp.

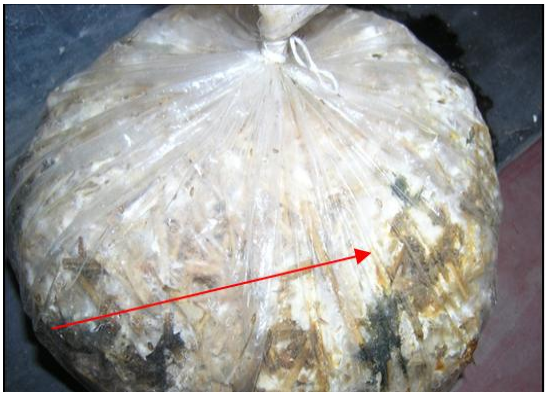

Plate 3. *Aspergillus niger*

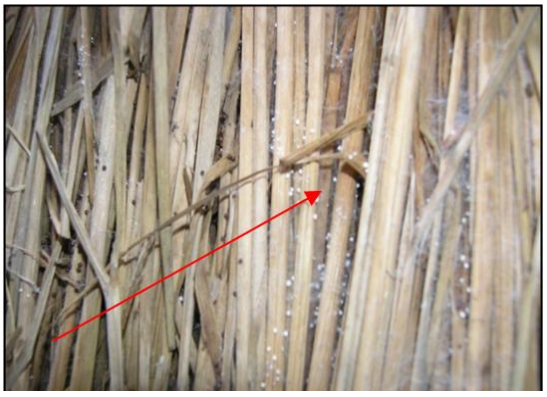

Plate 4. *Sclerotium rolfsii*

Plate (1-4): Different types of mould fungi observed during the cultivation of oyster mushroom

Table 1. Evaluation of phyto-extracts, and chemicals for the cultivation of oyster mushroom (*Pleurotus florida*)

Sl No.	Treatment	Dose	Average yield in gm from 500 gm substrate	Total yield in gram from 2 kg. substrate	Biological eefficiency %	Average weight of sporophore in gram	Number of days taken for complete spawn run	Associated competitor mould
1	*Zingiber officinale*	4.0%	570.00	2280	114.00	6.740	22	_
2	*Allium sativum*	4.0%	431.25	1725	86.25	5.820	26	*Cs, An, Sr.*
3	*Allium cepa*	4.0%	493.75	1975	98.75	6.660	24	-
4	*Lantana camera*	4.0%	401.25	1605	80.25	5.320	26	*Cs, An, Ps, Sr.*
5	*Azadirachta indica*	0.05%	546.25	2185	109.25	6.750	23	_
6	*Eucalyptus hybrida*	4.0%	385.00	1540	77.00	5.200	28	*Sr, Cs*
7	Bavistin + Formalin	75ppm+500ppm	602.50	2410	120.50	7.100	20	_
8	Lime($CaCo_3$)	0.10%	502.50	2010	100.50	6.750	22	*Sr*
9	Control		412.50	1650	82.50	6.500	24	*Cs, Ps, Sr,*
SE(treatment mean)				20.768	4.417	0.228	1.186	
CD at 5%				60.270	12.818	0.664	3.442	

** Cs = Coprinus sp., As = Aspergillus niger, Ps = Penicillium sp., Sr = Sclerotium. Rolfsii*

Table 2. *In vitro* evaluation of phyto-extracts, and chemicals against major competitor molds of oyster mushroom (*Pleurotus florida*)

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 florida		*Coprinus sp.*		*Aspergillus niger*		*Penecillium sp.*		*Sclerotium rolfsii*		Sem± for mycelium growth	CD at 5% for mycelium growth
			mm	%	mm	%	mm	%	mm	%	mm	%		
1	***Zingeber officinale***	4%	82	8.89	29	54.69	28	54.84	31	58.67	54	39.33	0.98	2.963
2	***Allium sativum***	4%	73	18.89	45	29.69	*45*	*27.42*	67	10.67	78	12.36	1.914	5.770
3	***Allium cepa***	4%	76	15.56	34	46.88	42	32.26	62	17.33	67	24.72	1.570	4.733
4	***Lantana camera***	4%	72	20.00	48	25.00	47	24.19	47	37.33	64	28.09	1.643	4.951
5	***Azadirachta indica***	0.05%	84	6.67	32	50.00	36	41.94	37	50.67	40	55.06	1.437	4.332
6	***Eucalyptus sp.***	4%	74	17.78	48	25.00	49	20.97	52	30.67	71	20.22	1.264	3.812
7	**Bavistin+Formalin**	50ppm+ 500ppm	81	10.0	19	70.31	22	64.52	22	70.67	34	61.80	1.402	4.226
8	**Calcium Carbonate**	0.10%	75	16.67	38	40.63	50	19.35	53	29.33	61	31.46	1.449	4.367
9	**Control**	-	90	0.00	64	0.00	62	0.00	75	0.00	89	0.00	2.000	6.027
	Sem± Treatment mean		1.563		1.763		1.312		1.672		1.269			
	CD at 5%		4.537		5.118		3.808		4.852		3.689			

3.2 *In vitro* Evaluation of Phyto-extracts, and Chemicals against a Major Competitor Moulds of Oyster Mushroom (*Pleurotus florida*)

The inhibitory effect of different phyto-extracts in terms of radial growth of mycelium was recorded and the data obtained are presented in Table 2. Chemical treatment (Bavistin 75ppm + Formalin 500 ppm) was found to be most effective in reducing the mycelial growth of four contaminants (61.80 to 70.67%), and reported less effective on mycelium growth of *Pleurotus florida* (10.00%). Treatment of bavistin 75ppm + formalin 500 ppm was reported to be better against *Penecillium* sp. and *Coprinus* sp., reduced mycelium growth 70.671% and 70.31% respectively. Among the phyto-extracts, *Zingiber officinale* was reported to be excellent in inhibiting the mycelium growth of *Penicillium* sp., *Aspergillus niger*, and *Coprinus* sp. 58.67 %, 54.84% and 54.69 % respectively. Whereas, *Azadirachta indica* seed oil was noticed very effective against the mycelium growth of *Scleroum rolfsii*, *Penicillium* sp, and *Coprinus* sp., and reduced 55.06 %, 50.67 % and 50.00 % growth respectively. The average radial growth of mould fungi in PDA amended with various extracts and chemicals was greatly influenced and found significant over control (Fig. 2 & Fig. 3). Similar observations on fungicidal effects of bavistin 75ppm + formalin 500 ppm against *Trichoderma* sp., *Coprinus* sp. and *Penecillium* sp. were also reported by [12,17]. Presence of azadirachtin ($C_{35}H_{44}O_{16}$), desacetylnimbin, nimbidol, and meliantriol in neem seed oil might have determined the the C/N ratio of the medium and interfared with the release of ammonium during the early stages of fruiting-body development of *Coprinus* sp which resulted in poor growth of mycelium [18,19,20,21]. The anti-fungal activity of *Allium cepa, Lantana camera, Eucalyptus hybrida* and *Allium sativum* against pathogenic fungi and competitor moulds was further confirmed the findings of [22-25].

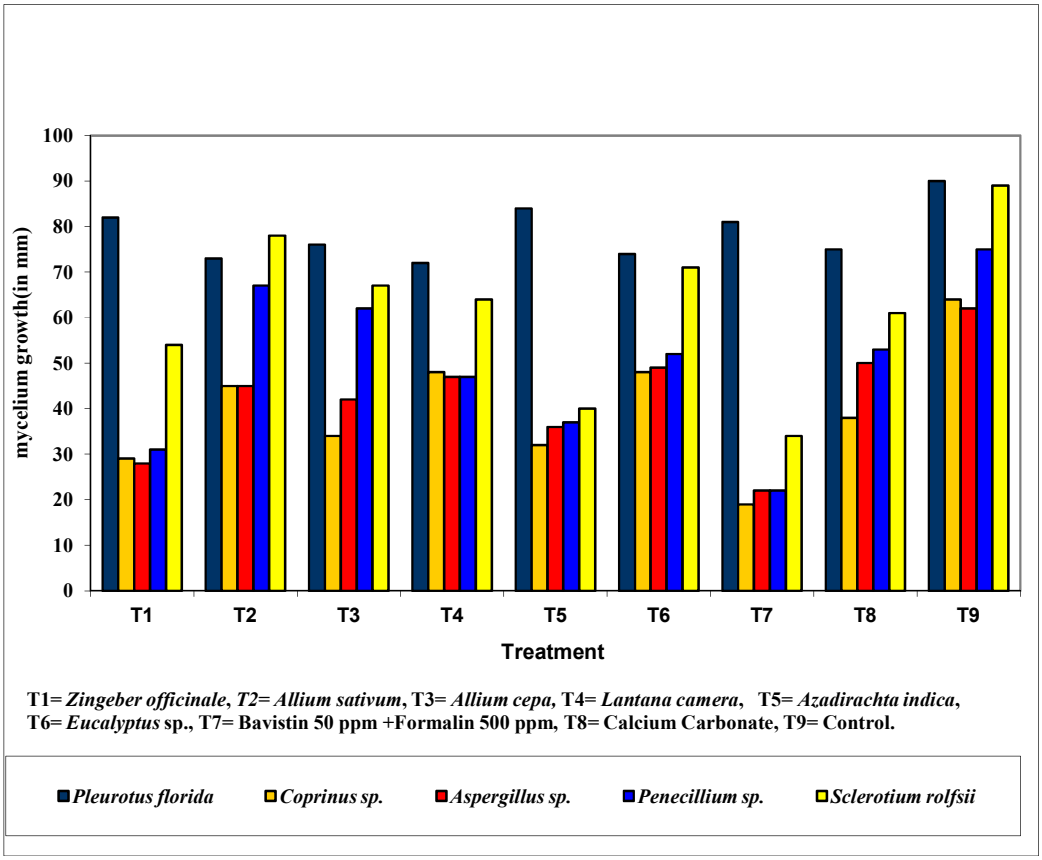

Fig.2: Effect of Photo-extracts and chemicals against the radial growth of mycelium (in mm) of *Pleurotus florida* and four competetor moulds 8 days after inoculation

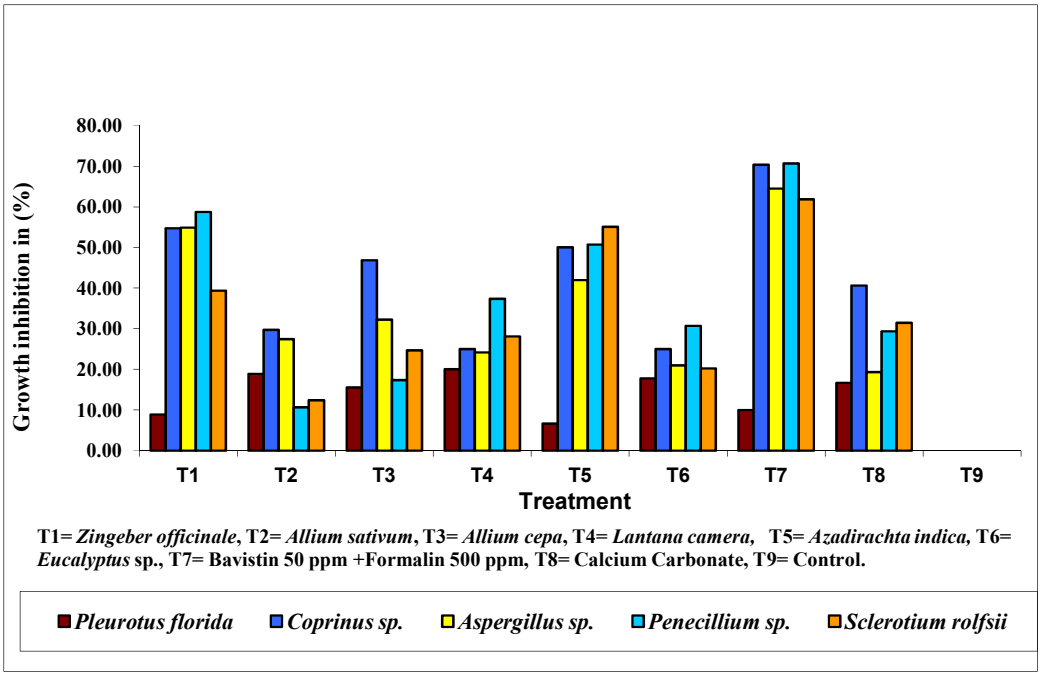

Fig. 3. Growth inhibitory effect (in %) of *Pleurotus florida* and four different moulds against the phyto-extracts and chemicals 8 days after inoculation

4. CONCLUSION

Plant extract has shown considerable promises as an effective alternative for minimizing the infection of competitor moulds and diseases of oyster mushroom under the agro-ecological condition of the lateritic belt of West Bengal, India. Extract of *Zingiber officinale* was reported to be excellent in inhibiting the mycelial growth of *Penicillium* sp., *Aspergillus niger* and *Coprinus* sp. 58.67 %, 54.84% and 54.69 % respectively. However, Chemical treatment (Bavistin 75ppm + Formalin 500 ppm) was found to be most effective in reducing the mycelial growth of four contaminants (61.80 to 70.67 %), and reported less effective on mycelial growth of *Pleurotus florida* (10.00 %).Extract of *Zingiber officinale* could be used as an alternative source for substrate sterilization which has the potentiality to suppress the mycelium growth of competitor moulds. Extract of these plants not only protect the environment and human health from hazardous effects of chemicals but also minimize the cost of cultivation.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Quimio TH. Cultivation ganoderma the *"Pleurotus-way"* mushroom. In: Newsletter for Tropics. 1976;121-130.
2. Khan SM, et al. Yield performance of different stains of oyster mushroom (*pleurotus spp.)* on paddy straw. In: Pakistan. Mush. Sci. X1 Sydney. 1981;1: 675-679.
3. Das M, Kalita MC. Value addition of mushroom. Sci Tech. Entrepreneur. 2006; 1-5.
4. Leonowicz A, et al. M. Biodegradation of lignin by white rot fungi. Fungal Gen. Biol. 1999;27:175−185.
5. Verma RN. Indian mushroom industry - past and present. Bulletin 8, World Society for Mushroom Biology and Mushroom Products. 2013;1-16.
6. Thakur MP. Present status and future prospects of tropical mushroom cultivation in India: A review. Indian Phytopath.2014; 67(2):113-125.

7. Bano Z, Srivastava HC. Cultivation of *Pleurotus* species on paddy straw. Food Sci. 1962;11:363-365.
8. Vijay B, Sohi HS. Effect of different sterilants and farm wastes on yield of Pleurotus citrinopileatus. Mushroom Journal Tropics. 1987;7:67-75.
9. Shukla CS, Biswas MK. Evaluation of different techniques for oyster mushroom cultivation. Journal of Mycology and Plant Pathology. 2000;30(3):431-4326.
10. Dwivedi SK, Kishore N, Dwivedi SK. Fungitoxicity of some essential oils against Macrophomina phaseolina. Indian Perfumer. 1990;34:20-21.
11. Daoud AS, Qasim NA, Al-Mallah NM. Comparison study on the effect of some plant extracts and pesticides on some phytopathogenic fungi. Mesopotamia J Agric. 1990;22:227-235.
12. Sharma VP, Jandaik CL. Effect of some plant materials in controlling different moulds in *Agaricus bisporus*. Indian Journal of mycology and Plant pathology. 1994;24:183-185.
13. Patra A, Pani BK. Yield response of different species of oyster mushroom (*Pleurotus*) to paddy straw. Current Agricultural Research. 1995;8:11-14.
14. Grove, Moore. Toximetric studies of fungicides against brown rot organism. Sclerotia fruveticola and S.laxa. Phytopathology. 1962;52:876–880.
15. Kapoor A. Antifungal activities of fresh juice and aqueous extracts of turmeric and ginger (*Zingiber officinale*). Journal Phytological Research. 1997;10:59.
16. Biswas MK. Effect of botanicals on the incidence of competitor moulds and biological efficiency of grey oyster mushroom (*Pleurotus ostreatus*). The Bioscan. 2015;10(2):511-515.
17. Thakur MP, Ram RN, Shukla CS. Mycoflora associated with paddy straw substrate during different stages and cropping months of oyster mushroom (*Pleurotus florida*). J Mycol Plant Patho. 2001;31(1):59-63.
18. Morimoto N, Suda S, Sagara N. Effect of ammonia on fruit body induction of *Coprinus cinereus* in darkness. Plant Cell Physiology. 1981;22:247-254.
19. Biswas MK. Microbial contaminants in oyster mushroom (*Pleurotus ostreatus*) cultivation their management and role of meteorological factors. Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMB MP8). 2014;567-575.
20. Godfrey EZ, Siti MK, Judith ZP. Effects of temperature and hydrogen peroxide on mycelial growth of eight *Pleurotus* strains. Scientia Horticulturae. 2010;125(2):95-102.
21. María EV, Juan ER, Carmen SR, Rosa O, Domingo B. Microbiological quality and safety of fresh cultivated and wild mushrooms commercialized in Spain. Food Microbiology. 2011;28(8):1492-1498.
22. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants (vol-Iand vol-II). Central Drug Research Institute, Lucknow. Publication and information Directorate, New Delhi; 1993.
23. Meena PD, Meena RI, Chattopadwyay C, Kumar A. Identification of critical stage for disease development and biocontrol of *Alternaria* blight of Indian mustard *(Brassica juncea)*. Journal of Phytopathology (Berlin). 2004;152 (4):204-209.
24. Naik BM, Sabalpara AN. Bio-efficacy of botanicals against *Alternaria tenvissima* causing leaf spot of Indian Bean. Journal of Mycology and Plant Pathology. 2004;34(3):972-938.
25. Raina R, Tikoo ML, Kalha CS. *Trichoderma* green mold in the white button Mushroom *Agaricus bisporus* (Lange) sing. With botanical extracts. Mushroom Research. 2003;12:39-42.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23677