Biological Forum – An International Journal **13(4): 33-38(2021)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Efficacy of selected Mizo Ethnobotanicals on the Incidence of Contaminant Mycoflora of Oyster Mushroom [*Pleurotus florida* (Mont.) Singer] Cultivation

Malsawmtluangi[1*], Abhilasha A. Lal[2] and Sobita Simon[3]
[1]M. Sc. Scholar, Department of Plant Pathology,
Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj – 211007, India.
[2]Assistant Professor, Department of Plant Pathology,
Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj – 211007, India.
[3]Professor and Head, Department of Plant Pathology,
Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj – 211007, India.

*(Corresponding author: Malsawmtluangi *)*
(Received 25 July 2021, Accepted 04 October, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: *Pleurotus florida* (Mont.) Singeris an edible mushroom and the third largest cultivated mushroom in the world. However, the limiting factor for its successful cultivation is the occurrence of weeds and competitor moulds. The crop frequently failed and fruiting bodies did not appear. But now, the knowledge derived from studies of their requirements for fruiting like nutrition, temperature, humidity, etc. has enabled their cultivation a sure and profitable industry. An experiment was conducted at the Mushroom Crop Room, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh. The main objectives were to check the effect of four selected Mizo ethnobotanicals viz. *Capsicum frutescens, Clerodendrum colebrookianum, Eryngium foetidum* and *Zingiber officinale* @ 2% along with the most popular chemical treatment (carbendazim 75 ppm + formalin 500 ppm) against the growth and yield of the mushroom as well as against the occurrence of fungal contaminants on the substrate. The study was conducted in Completely Randomised Design under the agro-climatic conditions of Prayagraj (2021). The results revealed that the treatment with *Zingiber officinale* was significantly superior over all the other ethnobotanical treatments and untreated check, as it required the minimum number of days for complete spawn run (16.25 days), primordial initiation (17.75 days), for formation of mature fruiting bodies (20.25 days) and produced maximum yield (101.50 g). *Zingiber officinale* treatment also showed significantly least number of occurrences of contaminant fungal colonies (1.838) and at par with treatments of *Capsicum frutescens* (2.008) and *Clerodendrum colebrookianum* (2.420). In conclusion, *Zingiber officinale* was found to be the most promising for substrate sterilisation as it minimised the infection of mycoflora in the mushroom bags and also increased the yield of the mushroom.

Keywords: *Capsicum frutescens, Clerodendrum colebrookianum, Eryngium foetidum, Mizo ethnobotanicals, mycoflora, Pleurotus florida, Zingiber officinale*

INTRODUCTION

Pleurotus florida (Mont.) Singer is one of the commonly known species of oyster mushroom. It is an edible mushroom with excellent flavour and taste; it has low calorie content and a high content of proteins, minerals and dietary fibre (Beluhan and Ranogajec, 2011). Oyster mushroom is currently the third largest cultivated mushroom in the world and it is popularly grown in China, India, South Korea, Japan, Italy, Taiwan, Thailand and Philippines. India produces 10,000 tons of oyster mushroom annually (Das and Sarkar, 2016).

Until recently, it was a gamble growing oyster mushrooms and mushrooms in general. The knowledge derived from studies of their requirements for fruiting like nutrition, temperature, humidity, etc. has enabled their cultivation a sure and profitable industry (Dube, 2013). However, the problem of weed and competitor moulds is one of the limiting factors in cultivation of oyster mushroom cultivation successfully in India (Singh, 1999). They reduce the mushroom yield by competing for oxygen, water, space and nutrition. In addition to mycoflora being competitive, some have been shown to produce metabolites which directly inhibit the growth of mushroom mycelia during spawn run on the substrate. Most of the competitor microflora generally results in delayed cropping, lesser yield, and production of poor-quality mushroom and even complete crop failure if the infection took place at the early stage of spawn run; which calls for effective management measures (Chhetri *et al*., 2018).

Studies on various aspects of fungal contaminants and diseases of *Pleurotus* spp. were taken up by different workers (Castle *et al*., 1998; Hermosa *et al*., 1999; Sharma *et al*., 2007; Neelam *et al*., 2014) and they reported *Trichoderma harzianum, Aspergillus* spp., *Penicillium* spp., *Moniliasitophila, Stemonitis* spp. and

Coprinus spp. were the major contaminants of *Pleurotus* spp. *Aspergillusniger*, *Coprinus* sp., *Penicillium* sp. and *Sclerotium rolfsii* were the primary fungal contaminants of mushroom beds of *Pleurotus florida* (Biswas and Kuiry, 2018). Spilman (2002) acknowledged *Trichoderma* as green mould on the production bed of oyster mushroom.

The use of fungicides for controlling the competitor moulds and diseases in oyster mushroom cultivation is very customary in India (Jain and Vyas, 2002; Debata *et al*., 2014). However, the growth of competitor moulds in mushroom beds can also be managed by using different plant extracts (Singh, 1999 and Patra *et al*., 1998). Many biologically active compounds can be procured from extracts of botanicals which include alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones (Raja *et al.*, 2009). Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds (Biswas *et al.*, 2002). Phyto-chemicals from medicinal plants serve as lead compounds in antimicrobial discovery (Ebi and Ofoefule, 2000; and Cohen, 2002).

The mushroom farmers of the country generally use synthetic chemical pesticides to save their crop from foreign microorganisms which may directly or indirectly also inhibit the growth of mushroom mycelia during spawn run on the substrate. Repeated and regular application of the same fungicide may considerably increase the chance of development of resistant mutants of microflora, resident toxicity, food poisoning, environmental pollution etc. in near future. This makes the use of ethnobotanical extracts which are locally available to even the remotest farmers a very convenient and non-sophisticated alternative for the commonly used chemical fungicides and supplements. Extracts from botanicals are a potential source of bioactive compounds and secondary plant metabolites, which may also improve the nutritional value of oyster mushroom.

MATERIALS AND METHODS

An experiment was carried out during the months of January, 2021 – April, 2021 in the Mushroom Laboratory and Mushroom Cultivation Room, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, 211007, U.P., India with a view to find the comparative efficacy of four selected Mizo ethnobotanical treatments in combination with wheat straw as substrate on the growth and yield and for managing the fungal contaminants of oyster mushroom cultivation.

A. Preparation of ethnobotanical treatments

The ethnobotanicals (Table 1) procured from Aizawl area of Mizoram were brought to Prayagraj, Uttar Pradesh which were air-dried at room temperature after which it was grinded using electrical grinder to obtain the powder form. These were kept separately in polypropylene bags, weighed to required amounts, sterilized and mixed with the substrate.

Table 1: List of Mizo ethnobotanicals used as treatments against the contaminant mycoflora of oyster mushroom cultivation.

Sr. No.	Scientific Name	Vernacular Name	Local Name	Plant Parts Used	Family
1.	*Capsicum frutescens* L.	Mizo Bird's Eye Chilli	Hmarchate	Fruit	Solanaceae
2.	*Clerodendrum colebrookianum* Walp.	East India Glory Bower	Phuihnam	Leaves	Verbenaceae
3.	*Eryngium foetidum* L.	Culantro	Bahkhawr	Leaves	Apiaceae
4.	*Zingiber officinale*	Ginger	Sawhthing	Rhizome	Zingiberaceae

B. Cultivation of oyster mushroom

The mother culture of *Pleurotus florida* (Mont.) Singer was prepared through tissue culture technique and the pure cultures were maintained. The mushroom spawn was procured from Mushroom Research Laboratory, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj (U.P.).

Fig. 1. Oyster mushroom: (A) primordial initiation and (B) mature fruiting bodies.

Chopped wheat straw was soaked for 16-18 hours and spawning was done with 2% wet weight basis. A unit of 500 g of wet wheat straw was used for each treatment.

The spawned bags were kept in a dark cultivation room where temperature was maintained at 20-25° and humidity at 70-85 % by spraying water daily on the walls and floor. The bags were cut and removed when it was completely colonized by the mushroom mycelium i.e. at complete spawn run. The substrates were sprayed with water 2-3 times a day. Harvesting was done when the primordial (Fig. 1A) gets converted into a full grown fruiting body (Fig. 1B) and readings were taken. Daily inspection was made to trace any growth of contamination during the cultivation period.

Evaluation of selected Mizo ethnobotanical treatments on the growth and yield of oyster mushroom. The ethnobotanicals *Capsicum frutescens* L. (T_1), *Clerodendrum colebrookianum* Walp.(T_2), *Eryngium foetidum* L. (T_3) and *Zingiber officinale* (T_4) @ 2% concentration was evaluated on the growth and yield of oyster mushroom. The chemical check treatments (T_5) have substrates which are soaked in carbendazim 75 ppm and formaldehyde 500ppm solution. The powdered form of the ethnobotanicals was mixed thoroughly with the substrate at the time of spawning. The substrate treated with only formaldehyde solution served as control (T_0). Four replications were maintained for each treatment. Readings for the growth parameters and yield from each treatment were recorded on a regular basis. The experiment was carried out inside the Mushroom Cultivation Room.

C. Isolation and purification of contaminant mycoflora

The pathogen was isolated from the contaminated bags. The infected portion of the wheat straw substrate (Fig. 2 & 3) was collected. The infected wheat straw was cut into small pieces (0.5 cm^2) and surface sterilized with sodium hypochlorite (0.2%) for 15-30 seconds, rinsed with three changes of sterile distilled water to remove the disinfectant and blotted dry. The sterilized pieces were placed on potato dextrose agar (PDA) medium in test tube slants and Petri dishes under aseptic conditions and were incubated at 25°C for 2 weeks. For obtaining sufficient inocula, pure cultures were obtained by sub-culturing. For this purpose, a tinyportion of the fungus were taken at the tip of a sterilized needle and transferred aseptically to the centre of the PDA medium in petri dishes. The dishes were incubated at 25°C. After 3-4 days, colonies of the fungus appeared and slides were prepared.

Visual observation on cultures in Petri dishes and micro-morphological studies in slide culture using microscope were adopted for identification of the contaminants. The mode of mycelia growth, colour, odour and changes of medium colour for each isolate were examined. For micro-morphological studies, examination of the shape, size, arrangement and development of conidia, conidiophores or phialides provided a tentative identification.

Evaluation of selected Mizo ethnobotanical treatments on the number of fungal contaminant colonies. The incidence of the contaminant mycoflora was recorded by counting the number of fungal colonies for each mushroom bag. The reading was taken by visually assessing all the bags thoroughly. Three readings were taken at seven days interval from first appearance of contamination for each treatment. For each bag, the number of fungal colonies were counted and recorded. The number of fungal contaminant colonies for every treatment was counted from the four replications.

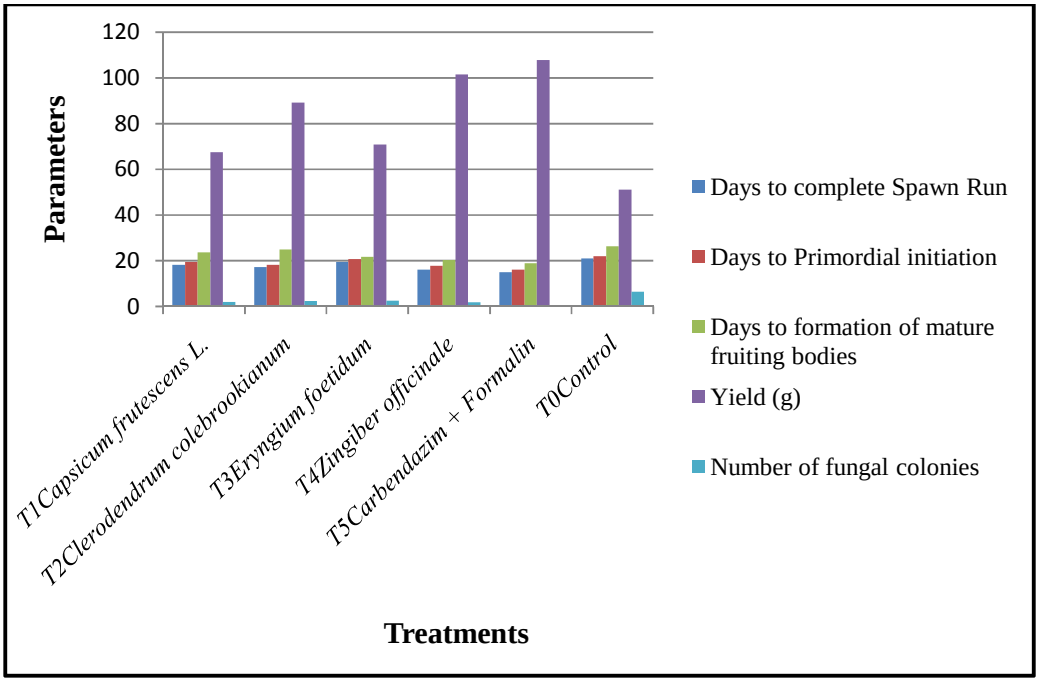

Fig. 2. Evaluation of selected Mizo ethnobotanical treatments on the growth and yield and against the contaminant mycoflora of oyster mushroom [*Pleurotus florida* (Mont.) Singer] cultivation.

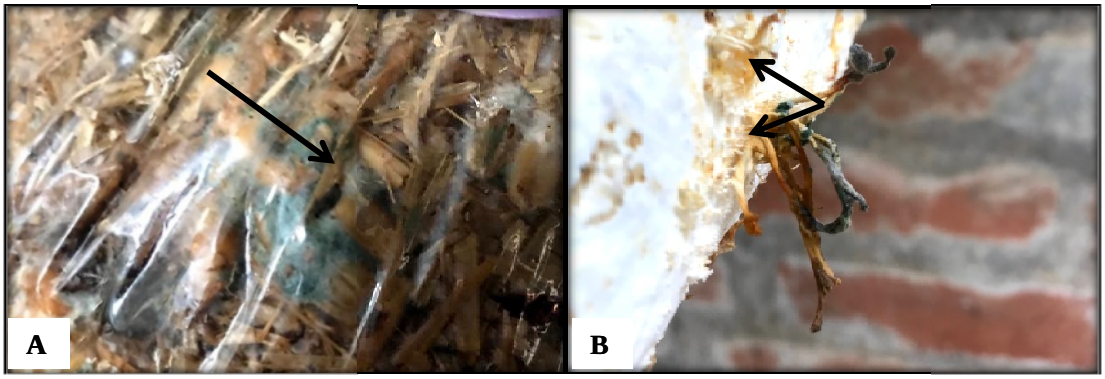

Fig. 3. Mycoflora contamination on oyster mushroom: (A) on wheat straw substrate and (B) on mature fruiting bodies.

D. Statistical Analysis

In this experiment, Complete Randomized Design (CRD) was adopted. The analysis of variance (ANOVA) technique was applied for drawing conclusion from data. The calculated values were compared the tabulated values at 5% level of probability for the appropriate degree of freedom (Fisher and Yates, 1968).

RESULTS AND DISCUSSION

A. Effect of selected Mizo ethnobotanicals on the growth and yield of oyster mushroom

The response of the four selected Mizo ethnobotanicals and chemical treatments along with wheat straw used for cultivation of *Pleurotus florida* (Mont.) Singer are presented in Table 2 and depicted in Fig. 2. Data indicated that among the ethnobotanical treatments, T_4 *Zingiber officinale* shows significantly the most effective treatment for the growth and yield as well as against the incidence of fungal contamination with complete spawn run at 16.25 days, primordial initiation at 17.75 days, formation of mature fruiting bodies at 20.25 days and also significantly reduced the occurrence of mycoflora contamination with the least number of occurrences of fungal colonies of 1.838. The probable reasons for such findings may be the presence of high carbohydrate content of 50 – 70% in ginger rhizome mentioned by Grzanna *et al.* (2005) and the high cellulose content of 88% in ginger fibres (Abral *et al.*, 2019) which could be responsible for early primordial initiation and formation of mature fruiting bodies of *Pleurotus florida* on wheat straw substrate. The high carbohydrate percentage could be the cause of higher rate of mycelial run in the wheat straw treated with *Zingiber officinale*.

Table 2: Evaluation of selected Mizo ethnobotanical treatments on the growth and yield and against the contaminant mycoflora of oyster mushroom [*Pleurotus florida* (Mont.) Singer] cultivation.

Sr. No.	Treatments	Dose	Days to Complete Spawn Run	Days to Primordial initiation	Days to Formation of Mature Fruiting Bodies	Yield (g)	Number of Fungal Colonies
1.	*Capsicum frutescens* L.	2%	18.25	19.50	23.75	67.50	2.005
2.	*Clerodendrum colebrookianum* Walp.	2%	17.25	18.25	25.00	89.25	2.420
3.	*Eryngium foetidum* L.	2%	19.50	20.75	21.75	70.75	2.503
4.	*Zingiber officinale*	2%	16.25	17.75	20.25	101.50	1.838
5.	Carbendazim + Formalin	75ppm+ 500ppm	15.00	16.25	19.00	107.75	0.00
6.	Control	-	21.00	22.00	26.25	51.25	6.505
	S. E. d (±)	-	0.559	0.577	0.645	8.533	0.350
	C.D. (at 5%)	-	1.184	1.222	1.367	18.065	0.741

B. Symptomatology and identification of the contaminant mycoflora

Cultural and micro-morphological studies show the occurrence of *Trichoderma* spp. and *Alternaria* spp. as contaminants in the mushroom bags. Distinct deep green and compact sporulation was found growing on the mushroom substrate (Fig. 3A), which is on the contrary, to the white mycelium of *Pleurotus florida*. Green sporulations were also found on the fruiting bodies (Fig. 3B). These are the characteristic symptom of green mold disease of mushroom caused by *Trichoderma* spp. Similar symptoms of *Trichoderma* spp. contamination was observed by Shamoli *et al.* (2016). Soggy patches of fungal colonies which are brown to black in colour were also observed on the mushroom substrate. Little report has been done regarding the occurrence of *Alternaria* spp. in mushroom beds. Ashraf *et al.*, (2017) reported *Alternaria* as one of the air-borne fungal contaminants of mushroom compost.

C. Effect of selected Mizo ethnobotanicals on the incidence of contaminant mycoflora of oyster mushroom

The efficacy of the four selected Mizo ethnobotanicals and chemical treatments along with wheat straw against the contaminant mycoflora of *Pleurotus florida* (Mont.) Singer cultivation are presented in Table 2 and depicted in Fig. 2. Carbendazim shows zero occurrence of mycoflora contamination by *Trichoderma* sp. and *Alternaria* sp. This is in agreement with the findings of Biswas (2015) who reported that the substrate treated with chemicals (bavistin 75 ppm + formalin 500 ppm) has complete inhibition of the competitor moulds of *Pleurotus ostreatus* under in vivo and in vitro conditions.

Among the ethnobotanical treatments, the most effective treatment against the incidence of fungal contamination was observed in T_4 *Zingiber officinale* with significantly least number of occurrence of fungal colonies of 1.838 and at par with by T_1*Capsicum frutescens* (2.005), T_2 *Clerodendrum colebrookianum* (2.420) with a contamination percentage of 12.04%, 13.13% and 15.85% respectively, compared to T_0 untreated check (6.505) which gives a contamination percentage of 45.59%. Biswas *et al*. (2018) also reported that in an *in-vivo* experiment, *Zingiber officinale* shows excellence in inhibiting the mycelium growth of the competitor moulds of oyster mushroom. The probable reasons of these findings may be due to the fact that extract of ginger contains gingerol, shagelol, curcumin, quercetin, ellargic acid which have anti-microbial properties including anti-fungal property. This is inhibits the growth of the fungal contaminants and hence, increased the yield of oyster mushroom.

CONCLUSION

As per the results obtained from this study, *Zingiber officinale* in combination with wheat straw as substrateshowed the best results for all the growth parameters of *Pleurotus florida i.e.* minimum days required for complete spawn run, primordial initiation, formation of mature fruiting bodies and produces highest yield. *Trichoderma* spp. causing green mold disease was the common contaminant mycoflora found on the mushroom bags, along with patches of *Alternaria* spp. contamination. *Zingiber officinale* also showed the least number of occurrences of fungal contaminant colonies on the wheat straw substrate with the least contamination percentage of 12.04%. However, the results of the present study are of one cropping season of January – April, 2021 under the Prayagraj agro-climatic conditions. As such to validate the findings, more such experiments should be taken up in the future.

FUTURE SCOPE

Zingiber officinale could be used as an alternative source for substrate sterilization which has the potentiality to suppress the mycelium growth of competitor moulds and also increase the mushroom yield.

Acknowledgements. This is a part of M.Sc. (Agri.) thesis work of the first author. The first author is thankful to the student advisory committee members for their technical help and critical advice during the study. The authors are also grateful to the Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, for providing necessary facilities.

Conflicts of Interest. The authors declare no conflict of interest.

REFERENCES

Abral, H., Ariksa, J., Mahardika, M., Handayani, D., Aminah, I., Sandrawati, N. And Ilyas, R. A. (2019). Transparent and antimicrobial cellulose film from ginger nanofiber. *Food Hydrocolloids*, *98*: 105226.

Beluhan, S. and Ranogajec, A. (2011). Chemical composition and non-volatile components of Croatian wild edible mushrooms. *Food Chemistry*, *124*: 1076-1082.

Biswas, K., Chattopadhyay, I., Banerjee, R. K. and Bandyopadhyay, U. (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current science*, *82*: 1336-1345.

Biswas, M. K. (2015). Effect of botanicals on the incidence of competitor moulds and biological efficiency of grey oyster mushroom. *The Bioscan*, *10*: 511-515.

Biswas, M. K., Kuiry, S. and Ghosh, T. (2018). Use of plant extracts for substrate sterilization and its effect on competitor moulds and biological efficiency of oyster mushroom. *European Journal of Medicinal Plants*, *22*(4): 1-8.

Castle, A., Speranzini, D., Rghei, N., Alm, G., Rinker, D. and Bisset, J. (1998). Morphological and molecular identification of *Trichoderma* isolates on North American mushroom farms. *Applied Environmental Microbiology*, *64*(1): 133-137.

Chhetri, K., Senapoty, D. and Sharma, D. K. (2018). Management of contaminant mycoflora of oyster mushroom (var- *Pleurotus florida*) with botanicals and GRAS chemicals. *International Journal of Current Microbiology and Applied Sciences*, *7*(2): 1972-1978.

Cohen, M. L. (2002). Changing patterns of infectious disease. *Nature*, *406*: 762-767.

Das, G. and Sarkar, S. (2016). Oyster mushroom (*Pleurotus* spp.): Adoption percentage of mushroom trainees of Cooch Behar Krishi Vigyan Kendra-A review. *World Journal of Pharmacy and Life Science*, *2*(1): 86-92.

Debata, D. K., Biswas, M. K. and Mohapatra, K. B. (2014). Management of competitor fungi in paddy straw mushroom (*Volvariella volvacea*) beds. *Journal of Plant Protection and Environment*, *11*(1): 98-102.

Dube, H. C. (2013). An Introduction to Fungi: Mushroom Cultivation. Scientific Publishers (India).

Ebi, G.C. and Ofoefule, S.I. (2000). Antimicrobial activity of *Pterocarpsosun* stems. *Fitoterapia*, *71*: 433- 435.

Fisher, R. A. and Yates, F. (1968). Statistical method for research workers.Oliver and Boyd Ltd, Edinburgh and London, 10.

Grzanna, R., Lindmark, L. and Frondoza, C. G. (2005). Ginger—an herbal medicinal product with broad anti-inflammatory actions. *Journal of Medicinal Food*, *8*(2): 125–132,

Hermosa, M. R., Grondona, I. and Monte, E. (1999). Isolation of *Trichoderma harzianum* Th2 from commercial mushroom compost in Spain. *Plant Disease*, *83*: 591.

Jain, A. K. and Vyas, D. (2002). Yield response of *Pleurotus florida* on wheat straw in combination with other substrates. *Mushroom Research*, *11*(1): 19-20.

Neelam, Upadhyay, V. and Kushwaha, K. P. S. (2014). Effect of alcali gens faecalis supplementation to different casing mixtures on its physicochemical properties and yield stimulation of *Agaricus bisporus*. *The Bioscan*, *9*(2): 659-661.

Patra, A. K., Pani, B. K. and Panda, S. N. (1998). Improvement of sporophore yield of oyster mushroom (*Pleurotus sajor-caju*) by leaf and oil cake amendments of substrate. *Environmental Ecology*, *16*(1): 15-19.

Raja, R. V., Ramanathan, T. and Savitha, S. (2009). Studies on wound healing property of coastal Medicinal plants. *Journal of Biosciences and Technology*, *1*: 39-44.

Sharma, S. R., Kumar, S. and Sharma, V. P. (2007). Diseases and Competitor Moulds of Mushrooms and their Management. National Research Centre for Mushroom (Indian Council of Agricultural Research). Chambaghat, Solan-173 213 (H.P.).

Singh, A. K. (1999). Efficacy of some plant leaf extracts against weed fungi coming up during cultivation of oyster mushroom on sugarcane bagasse. *Indian Phytopathology*, *52*(2), 179-181.

Spillman, A. (2002). What's killing the mushrooms of Pennsylvania? –A mushroom mystery. *Agricultural Research*, *26*: 112-117.

How to cite this article: Malsawmtluangi, Lal, Abhilasha A. and Simon, S. (2021). Efficacy of selected Mizo Ethnobotanicals on the incidence of contaminant mycoflora of oyster mushroom [*Pleurotus florida* (Mont.) Singer] cultivation. *Biological Forum – An International Journal*, *13*(4): 33-38.