International Journal of Aquatic Science
ISSN: 2008-8019
Vol 12, Issue 03, 2021

Investigation Of Novel Biological Pretreatment For Microbial Infection Control In Organic Mushroom Cultivation

Karunakaran S[1], Prabhu N[2], Gajendran Tholan[3], Manivasagam Veerasamy[4].

[1]*Karunakaran S*,Department of Chemical Engineering, KPR Institute of Engineering and Technology,Coimbatore-641 407,Tamilnadu,India.*
[2]*Prabhu N, Department of Biotechnology, Vivekanandha College of Engineering for Women, Elayampalayam, Tiruchengode-637 205, Tamilnadu, India*
[3]*Gajendran Tholan, Department of Biotechnology, Bharathidasan Institute of Technology, Anna University, Tiruchirappalli-620 024, Tamilnadu, India.*
[4]*Manivasagam Veerasamy, Department of Biotechnology, Adhiyamaan College of Engineering, Hosur - 635 109, India.*

Abstract
Mushrooms are affected by a large number of biotic and abiotic agentsdirectly or indirectly. Fungal pathogens like *Mycogene perniciosa, Lecanicillium fungicola, Cladobotryum* causes Dry bubble, Wet bubbleand Cob web disease in mushroom respectively. *Trichoderma* is the other species responsible for green mould (*T.aggressivm* in button mushroom, *T.pleurotum* in oyster mushroom & *T.harzianum* in shitake mushroom). *Pseudomonas tolaasii* is the bacterium that is responsible for causing Bacterial brawn blotch disease in Mushroom. The activities of these microbes against mushroom are controlled by usingchemical fungicides that includesBitertanol, Captan, Carbendazium, Hexaconazole and Mancozeb. Unfortunately, the usage of these fungicidescauses negative impact on human health and environment. The aim of our study is to utilize the natural plant extracts as an antifungal for treating the fungal pathogens affecting mushroom. It is observed that these extracts used exhibit the maximum activity in controlling the pathogens.

Keywords: *T.aggressivm, T.pleurotum, T.harzianum, Pseudomonas tolaasii, Carbendazium*

1. INTRODUCTION

Mushroom cultivation is a new horticultural enterprise developing in India. India produces about 40,900 tons of mushrooms per annum (Karuppuraj *et al.*,2014).With growing awareness of nutritive and quality food for health conscious among population, the demand of mushrooms will increase among global needs and it will be of 8.3 million by extended population by 2025 with expandable income (Arya & Arya, 2003). An highly efficient *Pleurotus*is, lignin-degrading mushroom can grow well on different types of lingo cellulose materials (Yildiz*et al*., 2002). Various species of *Pleurotus*can grow at different temperate conditions and having high gastronomic value (Erkel, 1992). Mushrooms are cultivated in faster and cheaper methods. On comparing numerous *Pleurotus*species, *Pleurotusflorida*having an extra over other types in terms of easy cultivation, role in

extracellular enzymes production and neutraceuticals production, biodegradation and bioremediation, (Sivrikaya&Peker, 1997)

317 million metric tons of fresh mushrooms can be cultivated per year using 25% of cereal straws (Chang and Miles, 1991). The *Pleurotus* genes have favourable organoleptic and medical properties, low cost production technology and bio efficiency (Chirinang*et al.,* 2009). *Pleurotus* mushrooms grow in tropical, subtropical and temperate regions and are easily artificially cultivated (Akindahunsi and Oyetayo, 2006). Mushroom is a Basidiomycetes fungus, which is a popular bio-remediate. Mushroom‟s efficiency is that producing food proteins from different agro wastes (Kathiravan Subramanian *et al.,* 2014).India is a heavily populated country and this is the only reason for enormous wastes produced regularly out of household & industrial activities like peeling and cutting of raw FVW (Food and vegetable waste) prior to processing, eating, cooking. Serious environmental and health problems related to inadequate solid waste disposal (Rashad*et al.,* 2009). Fruit and vegetable wastes (FVW) are produced in large quantities in markets and constitute a source of nuisance in municipal landfills because of their high biodegradability FVW is generally stale or spoilt, not fit for human consumption (Obodai*et al.,* 2003). These resources are usually high in fibre content and are of dissimilar sizes and forms. Vegetable wastes usually have a high moisture content of 80-89%. *P.florida* commonly called as oyster mushroom (Zhu *et al*., 2014). It is one of the most commonly cultivated mushrooms. They know for their nutritive values and medicinal properties (Karuppuraj*et al.,* 2014). It belongs to the Class Basidiomycetes. Using this substrate *P.florida* can be cultivated. The substrates that used are paddy straw, cauliflower waste, onion peel, potato peel, vegetable and fruit waste, banana leaves,Garlic peel, banana leaf, sugarcane bagasse (Chumpoo*et al*., 2010) and *Thespesiapopulnea*, used as substrate for mushroom cultivation.

1.1 Pretreament Of Substrates:

The lignocellulosic substrates treated by hot water with different temperature and time duration to optimize the effectiveness to avoid contamination. Finally, they reported substrates pre-treated with hot water at 80ºC for 3hrs would be a good technique to adapt to produce a good yield in oyster mushroom (akhter*etal*.,). While soaking the substrates in alkaline water as the pre-treatment for themushroom cultivation indicates it has a valuable potential and it also controls the moisture of the substrates(Contreras *et al*.,).

1.2 Chemical Fungicide:

During the cultivation process, chemical fungicides applied directly or indirectly into the compost during the spawn production and chemical sterilization of substrate to protect the mushroom from fungal pathogens. Phyto-pathogenic fungi are introverted by synthetic fungicides (Obodai*et al.,* 2003). Therefore, the use of these is increasingly restricted due to the destructive effects of pesticides on the environment and human health. Alternatively, this can replace by some plant extract as biological fungicide. Some plants, which have strong fungicidal property, are neem, *Euphorbia hirta*, *Paneerdodi*, turmeric, ginger, clove, mint leaves and garlic. Use of these fungicidal plants will reduce the rate of contamination as like chemical fungicide (kosinkova*et al.,* 2017). One of the major problems faced by our world is the management of wastes and energy crisis because of population. Mushroom cultivation has showeda little attention in the developing countries; mostly in the tropical regions where many cellulose wastes are remain unused. In this, many of the studies directed towards the development of alternative substrates for the growing of oyster mushroom. The main aim of the study is the growth of mushroom using different substrates and the sterilize using

biological fungicides (Philioppoussis*et al*., 2001).Generally, the systemic fungicides mainly control fungal contamination in mushroom bed (kosinkova*et al*., 2017). The use of these is increasingly restricted because of its harmful effect on human health and environment. Fungi are ubiquitous in the environment, and fungal infections are more common.

1.3 Plants As Fungicide

The neem has played an important role in Ayurveda medicine and agriculture. The earliest documentation of neem mentioned the fruits, seeds, oil, leave roots and bark for their medicinal properties. The antifungal activity of neem leaves against the pathogenic fungi was analysed (D.K.Shrivastava & KshmaSwarnkar, 2014).

The extracts *Calotropisprocera* inhibited the growth of the dermatophytes. They consider *Calotropisprocera* extract as a new source of developing local antifungal agents (S.Goyal*et al.,* 2013). *Nerium oleander* was screened for antifungal activity against three economical important fungi *Sclerotiumrolfsii, Macrophominaphaseolina,* and *Fusariumoxysporum*(Siddiqui *et al.,* 2016). The antifungal activity of *Pongamiapinnata* evaluated against clinical isolates of *Candida albicans and C. tropicalis (*PatilUsha, 2017).

The in-vitro potential of antifungal activity against the contaminated fungal species and the field trial of using those medicinal plants as a bio fungicide. The main work is to calculate the yield efficiency among comparing the growth of fruit body by time, weight, withstand, etc.Practically, farmers facing a lot of difficulties while on undergone a farm scale level of mushroom cultivation. Their difficulties mainly focus on pure culture growth, spawn run and bed production without contamination, long term withstand on fresh mushrooms, etc. On term concluding pre-treatment of substrates in spawn run, bed production is a major conquer among mushroom farming. To avoid this lots of scientist undergone a different pre-treatment like boiling off substrates with water, alkaline soaking, acid pre-treatment and usage of autoclave, then they grew up with a lit bit advancement of using chemical fungicide as a sterilizing agent. It has been commonly used because of its less work and less contaminates observance. However, it causes many disadvantages mainly focusing on mushroom that grown from chemically pre-treated shows a presence of toxic compound that can cause several adverse effects on human health and ailments. Because of mushroom needs, we cannot able to get move from chemical pre-treatment. To enclose a proper solution for this, we aimed on organic mushroom farming on using plant extracts as a bio-fungicide for substrate pre-treatment that may not be a best effect like chemical so as its reduces the toxicity of mushroom and proves to be less contaminative mushroom farming.

2. MATERIALS AND METHOD

2.1 Substrates Collection:

Different substrates were collected in and near Tiruchengode and from Vivekanandha college canteen, mess. These materials were washed using tap water and which was sun dried.

2.2 Plant Materials:

Different plant species collected from natural habitat near Tiruchengode in 2017. The collected plant identified at the Vivekanandha institutions in TamilNadu.

collected plant identified at the Vivekanandha institutions in TamilNadu.

International Journal of Aquatic Science
ISSN: 2008-8019
Vol 12, Issue 03, 2021

Figure 3.1 a) Euphorbia hirta b) Gutterda speciosa` c) Azadirachta indica

2.3 Preparation Of Plant Extract:

Matured leaves was collected up to 250gms were washed thoroughly in running water and shade dried. The plant leaves are finally grinded and convert into paste. Then it mixed with 6 liters of normal tap water.

Figure3.2 Plant Extract

2.4 Collection Of Different Substrate And Inoculation:

Different substrates collected from near Tiruchengode and from Vivekanandha college canteen, mess and market. The substrates were soaked for 4 hours in water and drained later.

2.5 Spawn Production:

Pleurotus florida spawn produced using, 2% calcium carbonate and water respectively. Then mixed rice husk and it filled in polypropylene bags of size 11^{x} 5 inches. It was autoclaved at 121° C for 20 min after which the bags were cooled at room temperature. In addition, the mother spawn was inoculated into the spawn bags and keep undisturbed for 20-25 days. The mycelium covers entirely. Using that spawn, the beds were prepared in alternate spawning methods. It was spawn again and the whole procedure was repeated until four to six layers are piled and the temperature (35° C) and humidity at 70-85% was recorded

2.6 Fruiting And Harvesting:

Pin heads appeared after about 20 days, and after 2-3 days, they matured into mature fruit bodies, which were harvested 2-3 times a day for the next three days. Other crops are due for harvesting after 5-6 days of rest, and the average harvesting period was 2-3 months. At each harvest, the total weight of the fruit was taken and measured on a weighing scale.

$$\text{Biological Efficiency} = \frac{\text{Total weight of fruit bodies}}{\text{Total weight of substrate of spawning}} * \frac{100}{1}$$

2.7 Phytochemical Screenings (Pooja Shah Et Al., 2014):

Using various reagents and chemicals, the crude extract examined for the presence of chemical constituents. The extracts obtained through the successive extraction process subjected to a variety of qualitative tests using the methods mentioned, as well as preliminary phytochemical screening for the identification of various phyto-constituents such as alkaloids, carbohydrates, steroids, cardiac glycosides, and flavonoids. (Harborne *et al.,* 1998)

2.7.1 Test For Alkaloids

Wagner'stest: Sample(2-3ml),treatedwithfewdropsofWagner's reagent. Appearance ofreddishbrownprecipitateindicatedthepresenceof alkaloids**.**

2.7.2 Test For Anthraquinone(Adebayo Et Al., 2009):

Sample (1ml), treated with few drops of 10% potassium hydroxide.Appearance of red color conform the test.

2.7.3 Test Of Glycosides(Kokate Et Al., 2002):

Test sample (2ml), dissolved in 1ml of water and add few drops of sodium hydroxide formation of yellow color indicates the glycosides.

2.7.4 Test For Proteins:

Biuret test: Test sample (3 ml) mixed with 4% NaOH and few drops of 1% CuSO4solution added. Violet or pink color not appeared. Few drops of 10% sodium chloride and 1% copper sulphate applied to 3 ml of the extract to produce a violet or purple colour. It turns dark violet when alkali is added.

2.7.5 Test For Amino Acids:

Ninhydrin test: Test sample (3 ml) and 3 drops of 5% ninhydrin solution have heated in boiling water for 10 mins. Purple color appeared.

2.7.6 Test For Flavanoids:

Shinoda test: Sample extract (1ml), treated with 5 drops of 5%NAOH yellow color was observed. 2% HCl added as 2 to3drops the yellow colour will be disappear.

2.7.7 Test For Phenols:

0.1g of lead acetate dissolved in 10ml of distilled water with few drops of sample has added. Formation of precipitate indicates the presence of phenols.

2.7.8 Test For Terpenoids:

Salkowski's test: Sample (2 ml), mixed 2 ml of concentration sulphuric acid, it well shaken then chloroform layer appeared red and acid layer shown greenish yellow fluorescence.

2.7.9 Test For Saponins:

Foam test: To 1 ml of the extracts 5 ml distilled water had added and shaken vigorously. Formation of foam indicated presence of saponins.

2.8 Fungal Pathogen From Contaminated Mushroom Bed:

Mushroom beds contaminated by various types of fungal pathogens. Some of the pathogens are collected and cultured in the PDA. By comparing with some research paper, its colour and microscopic morphological characteristics of the pathogens assumed. As previously mentioned plants, extracts could use as steriliser of mushroom substrate. In vitro analysis of antifungal activity of plant, extracts against the isolated fungal pathogens are analysed by well diffusion method (Rao *et al.,* 2010).

2.9 Selection Of Plant Species:
Plant species selected based on the fungal activity, easy availability and cost efficient. We selected five types of plant species

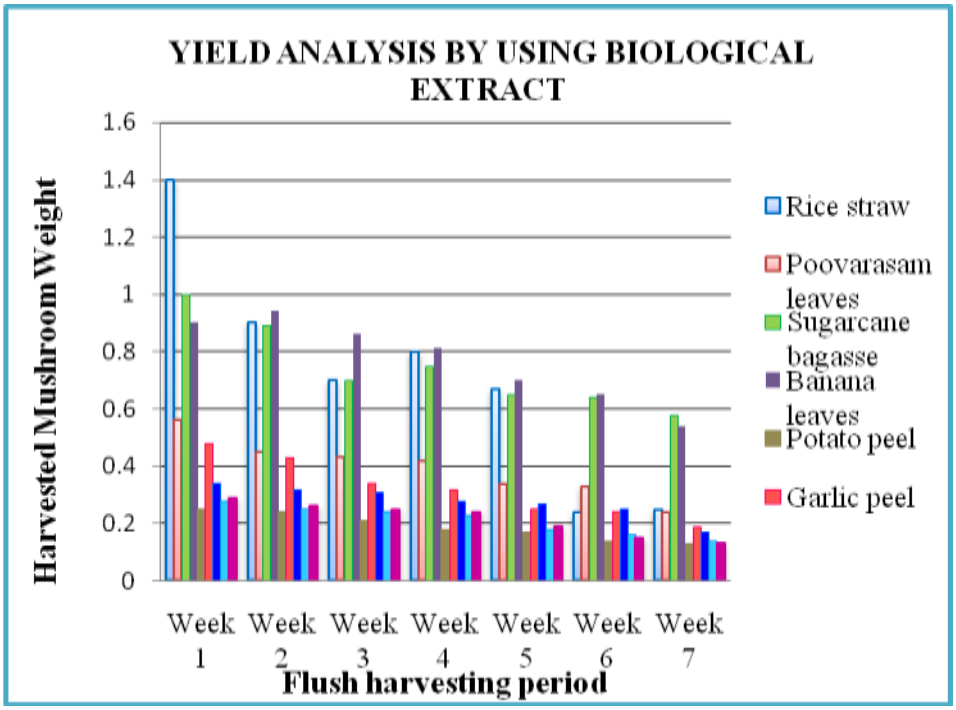
3. RESULT AND DISCUSSION

3.1 Growth Analysis Of Mushroom Cultivation:
Moreover, all substrates showed good result, after analyzing all the substrates paddy straw showed best result when compare to others. From the below table it is clear that sugarcane bagasse and banana leaves exhibit best results like rice straw for the cultivation of mushroom.

Table 4.1 Yield Analysis by Using Biological Extract on Different Substrates
A. Rice straw B. Poovarasam leaves C. Sugarcane bagasse
D. Banana leaves E. Potato peel F. Garlic peel
G. Onion peel H. Vegetable waste I. Cauliflower waste

No.of Weeks	**Number of fruiting bodies**								
	A (kg)	**B (kg)**	**C (kg)**	**D (kg)**	**E (kg)**	**F (kg)**	**G (kg)**	**H (kg)**	**I (kg)**
Week 1	1.4	0.56	1	0.9	0.25	0.45	0.34	0.28	0.29
Week 2	0.9	0.45	0.89	0.94	0.24	0.43	0.32	0.25	0.26
Week 3	0.7	0.43	0.7	0.86	0.21	0.34	0.31	0.24	0.25
Week 4	0.8	0.42	0.75	0.81	0.18	0.32	0.28	0.23	0.24
Week 5	0.67	0.34	0.65	0.7	0.17	0.25	0.27	0.18	0.19
Week 6	0.24	0.33	0.64	0.65	0.14	0.24	0.25	0.16	0.15
Week 7	0.25	0.24	0.58	0.54	0.13	0.19	0.17	0.14	0.13

Moreover, all substrates showed good result, after analyzing all the substrates paddy straw showed best result when compare to others. From this table Sugar bagasse and banana leaves shows the second place in the yield of mushroom.



4.2 Cultivation of mushrooms using biological extracts:

Table 4.2 Stages of Growth Analysis in Paddy Straw

STAGES OF GROWTH ANALYSIS	**BIOLOGICAL EXTRACT**		
	Azadirachta indica	***Euphorbia hirta***	***Gutterda Speciosa***
	(DAYS)	(DAYS)	(DAYS)
Spawn running	10	15	-
Pin-headed formation	14	20	-
Flush	17	24	-
Harvest	19	26	-
Total yield	1.344 kg	1.006 kg	-

From the beginning to end of the cultivation process, proper sterility should be maintained to avoid contamination. Among the three plant species, *Azadirachta indica* and *Euphorbia hirta* shows good fungicidal property, where *Gutterda speciosa* showed negative result.(Khan *et al*., 2009) reported thatExtracts of neem leaf, neem oil are effective against certain human fungi, including, *Candida*, *Geotricum* and *Trichosporon.*

Maragatharavlli *et al*., (2012) said that the antifungal activity of neem has been attributed to volatile sulfides and the limonoid gedumin and reported that inhibition of the protease activity of *Trichophyton* species by neem. Mahmood *et al.,* (2010) and Mosaddek & Rashid, (2008) have demonstrated that addition of neem leaf extract effectively inhibited aflatoxin production by *A.parasiticus. In vitro* antifungal activity of aqueous neem leaf extract against *Penicillium expansum* has also been documented by Mossini *et al*., (2013).

The antimicrobial activity leaves of *E. hirta* was premeditated for its by agar well diffusion method against: *Staphylococcus aureus* (MTCC 2940), *Bacillus ceresus*, *Aspergillus fumigatus* (MTCC 343 *Klebsiella pneumoniae* (MTCC139), *Salmonella typhi* (MTCC 733), , *Aspergillus flavus* (MTCC 418) in the methanol extract (Quy *et al.*, 2014). The ethanol extract of the leaves of *Euphorbia hirta* showed significant antimicrobial effects (Rao *et al*., 2007).

While analysing the yield, the bed prepared by using neem extracts shows high yield. It took 10 days to get yield after first yield. Among the three beds, the mycelium running time is fast in neem while comparing to the other two species. Among the three, *Azadirachta indica* gives good result.

Gutterda speciosa showed the negative result. There is not evenmycelium coverage. By using the neem extract different substrates was sterilizing and kept for observation. Among them, only sugar bagasse, paneer dodi leaves and banana showed a good result while comparing the other. (Belewu & Belewu, 2005) reported that the mycelia covered in the banana leaves is about 12 days as full colonization of the substrate was experiential in 15 days. The total number of fruits and the total weight of the fruits was 2.5 kg. Biological value was observing at 15.21% in (*Volvariella volvacea*) species. This is same as the yield observed in this study approved with the observation of (Oei, 2003) for similar species. Nevertheless, we only observed mycelium growth in 14 days but did not get fruit body.

5. REFERENCES

[1]. Adebayo E A, Ishola O R (2009), " Phytochemical and antimicrobial screening of crude extracts of *Terminalia gaucescens*", African Journal of Pharmacy and Pharmacology, Vol 3(5), pp. 217-221.

[2]. Akindahunsi A A and Oyetayo (2006), "Nutrient and Antinutrient Distribution of Edible Mushroom, *Pleurotus Tuber – Regium* (Fries)", Food Science and Technology, Vol 39, pp. 548-53.

[3]. Anjana Sharma, Juhi Sharma and Pooja Thakrele (2016). "Screening, Localization and Activity of Mushroom Tyrosinase from Various Developmental Growth Phases of *Pleurotus florida*". International Journal of Current Microbiology and Applied Science, Vol 5(1), pp. 565-575.

[4]. Asmamaw Tesfaw, Abebe Tadesse, Gebre Kiros (2015), "Optimization of oyster (*Pleurotus ostreatus*) mushroom cultivation using locally available substrates and materials in Debre Berhan, Ethiopia", Journal of Applied Biology and Biotechnology, Vol 3(01), pp. 015-020.

[5]. Arya C, Arya A (2003), "Effect of Acid Hydrolysis of Substrate on Yield of Oyster Mushroom *Pleurotus Sajor-Caju (Fr) Singer'*, Mushroom Research, Vol 12, pp.35-38.

[6]. akhter.K, Salahuddin N, Chowdhury M, Aminuzzanan (2017), "Effect of sterilization of substrate by hot water treatment on prevalence of contaminants and yield attributes of oyster mushroom (*Pleurotus ostreatus*)". Scholars journal of agriculture and veterinary sciences, Vol 4(11), pp.464-471.

[7]. Bano Z, Srivastava H C (1962), "Studies in the Cultivation of *Pleurotus Sp.* on paddy straw", Food Science, Vol 12, pp. 363-368.

[8]. Belewu M A and Belewu K Y (2005), "Cultivation of mushroom (*Volvariella volvacea*) on banana leaves", African Journal of Biotechnology, Vol 4(12), pp. 1401-1403.

[9]. Bernardo E, Lechner and Santiago Monaldi (2011), „Utilization of garlic and maize wastes supplemented with olive mill waste water for *Pleurotus ostreatus* cultivation", Revista Mexicana De Micologia, Vol 34, pp.17-22.
[10]. Karunakaran S, Gurusamy R, Senbagam D, Anarasu K,Senthilkumar B. 2018. Evaluation of the saccharification and fermentation process of two different seaweeds for ethanol production. Journal of Biocatalysis and Agricultural Biotechnology.44-49.
[11]. Chang S T, Miles P G, (1991), „Recent Trends in World Production of Cultivated Edible Mushroom", Mushroom Journal, Vol 50, pp.15-18.
[12]. Chirinang P and Intarapichet K (2009), „Amino Acids and Antioxidant Properties of the Oyster Mushrooms, *Pleurotous Ostreatus* and *Pleurtous Sajor-Caju*", Science Asia, Vol 35, pp.326-331.
[13]. Chumpoo, Jade, Prasassarakich and Pattarapan (2010), „Bio-Oil from Hydro-Liquefaction of Bagasse in Supercritical Ethanol", Energy Fuels, Vol 24(3), pp. 2071–2077.
[14]. EI-Mahmood A M, Ogbonna O B and Raji M (2010), „The antibacterial activity *Azadirachta indica* (Neem) associated with eye and ear infections", Journal of Medical Research, Vol 4(14), pp.1414-1421.
[15]. Karunakaran S, Gurusamy R.2011.Bioethanol Production as Renewable Biofuel from Rhodopyhtes Feedstock. International Journal of Biological Technology.2(2):94-99.
[16]. Elaine Marshaii and (Tan) Nair N G (2009), „Make Money by Growing Mushrooms", Rural Infrastructure and Agro-Industries Division. FAO. Rome, Vol 25, pp.43-56.
[17]. Goyal S, Kumar S, Rawat P and Dhaliwal N (2013), „Antifungal Activity of *Calotropis procera* Towards *Dermatoplaytes*", International Journal of Advances in Pharmacy, Biology and Chemistry, Vol 2(3), pp. 470-472.
[18]. Harborne J B (1998), "Phytochemical methods", A Guide to modern techniques of plant analysis. 3rd ed., Chapman and Hall International, New York, Vol 29, pp. 199-203.
[19]. Hayes W A and Haddad S P (1976), „The Nutritive Value of Mushrooms", Mushroom Journal, Vol 30, pp.204-209.
[20]. Holger B. Deising, Sven Reimann and Sergio F. Pascholati (2008), „Mechanisms and significance of fungicide resistance", Brazilian Journal of Microbiology, Vol 39, pp. 286-295.
[21]. Ivana Potonik, jelena vukojevic, mirjana stajic, dejana kosanovic, emil rekanovic, milos stepanovic and Svetlana milijasevic-marcic (2012), "impact of fungicides used for wheat treatment on button mushroom cultivation", pesticide in phytomedicine,Vol 27(1), pp. 9-14.
[22]. Kathirava Subramanian, Krishanakumari Shanmugasundaram and Nagalakshmi Muthu (2014), „Spawn and Cultivation Strategies for *Pleurotous Eous* (Pink Oyster Mushroom)", World Journal of Pharmacy and Pharmaceutical Sciences, Vol 3, pp. 915-924.
[23]. Khan M and Wassilew S W (1987), „Natural Pesticides from the Neem Tree and Other Tropical Plants", GTZ, Eschborn, Germany, Vol 13, pp. 645–65.
[24]. Kosinkova, Jana, Ramirez, Jerome, Jablonsky, Michal, Ristovski, Zoran, Brown, Richard, Rainey and Thomas (2017), „Energy and chemical conversion of five Australian lignocellulosic feedstocks into bio-crude through liquefaction", Research and Science of Chemical Advances, Vol **7** (44), pp. 27707–27717.
[25]. Lintzel (1943), Ueber Den Nahrwest Des Eiwesses Essbarrer Plize, Chemical engineering, Vol 67, pp. 33-34.

[26]. Mane V P, Patil S, Syed A A, and Baig M M V (2007), „Bioconversion of Low Quality Lignocellulosic Agricultural Waste into Edible Protein by *Pleurotus Sajor-Caju (Fr) Singer*", Journal of Zhejiang University of Science, Vol 8(10), pp.745-751.
[27]. Manson J C, Sims P G G and Broad P (1989), „Biological routes to improve digestibility of animal feeds In: Biotechnology in livestock in developing countries", World Journal of Pharmacy and Pharmaceutical Sciences, Vol 34(6), pp. 78-98.
[28]. Maragatharavlli S, Brinda S, Kaviyaarshi N S and Gangwar S R (2012), „Antimicrobial activity in leaf extract of neem (*Azadirachta indica Linn*.)", Internal national Journal of Science and Nature, Vol 3(1), pp.110-113.
[29]. Karunakaran S,Saravanan A, Dhansekaran S,Senbagam D, Senthil Kumar B,2014.Xylanase production from Aspergillus niger.International Journal of Chemtech Research.6(9),4207-4211.
[30]. Monali Bhakta and Prasant Kumar (2013), „Mushroom Polysaccharides as a Potential Probiotics", International Journal of Health Science and Research, Vol 4(5), pp. 189-200.
[31]. Monika Komon-Zelazowska, John Bissett, Doustmorad Zafari, Lo ra nt Hatvani, La szlo Manczinger, Sheri Woo, Matteo Lorito, La szlo Kredics, Christian P. Kubicek, and Irina S. Druzhinina (2007), „Genetically Closely Related but Phenotypically Divergent *Trichoderma* Species Cause Green Mold Disease in Oyster Mushroom Farms Worldwide", Applied and environmental microbiology, Vol, 73, pp.7415–7426.
[32]. Mosaddek A S M and Rashid M M U A (2008), „Comparative study of the anti inflammatory effect of aqueous extract of neem leaf and dexmethasone", Banglech Journal of Pharmacology, Vol 3, pp. 44-47.
[33]. Mossini S A, de Oliveira K P and Kemmelmeirer C J (2009), „Chromatographic Evaluation and Antimicrobial Activity of Neem (*Azardirachta Indica A. Juss., Meliaceae*) Leaves Hydroalcoholic Extracts" Revista Brasileira De Farmacognosia, Vol 19, pp. 1981-528.
[34]. Obodai M, Cleland-Okine J and Vowotor K (2003), „Comparative study on the growth and yield of *Pleurotuostreatus* mushroom on different lignocellulosic by-products", Journal of Industrial Microbiology and Biotechnology, Vol 30(3), pp.146-9.
[35]. Oei P (2003), „Mushroom cultivation, appropriate technology for mushroom growers", Backhugs Publishers, Leiden. The Netherlands, Vol 12(30), pp.128-130.
[36]. PayalMago, IshaGunwale, Lata Singh., and Deekshant Awasthi., (2014), „Commercial Production of Oyster Mushroom", Vol 8, pp. 2319-2399.
[37]. Philioppoussis A, Zervakis G and Diamantopoulou P (2001), „Bioconversion of Agricultural Lignocellulosic Wastes through the Cultivation of the edible Mushrooms *Agrocybe Aegerita, VolvariellaVolvacea* and *Pleurotous Spp'*, World Journal of Microbiology & Biotechnology, Vol 172, pp.191-200.
[38]. Pooja Shah H A, Modi M D, Shukla Suman K (2014), „Preliminary Phytochemical Analysis and Antibacterial Activity of *Ganoderma lucidum* collected from Dang District of Gujarat", India. Int J Curr Microbiol App Sci, Vol 3(3), pp. 246-255.
[39]. Patil usha (2017), „antifungal activity of karanja (pongamia glabra) on medically important clinical isolates of candida fungi", international journal of applied ayurved research, Vol 3, pp. 43-47.
[40]. Karunakaran S, Dhanasekaran S, RojaBlessy R, RavulathuNisha A,2015. Production and

[41]. Purification of Cellulase from Trichodermaviridae by Optimized Medium using various Agrowaste for Poultry Feed Supplement. International Journal of Engineering Research and Management, 2(1),2349-2058.
[42]. Rainey and Thomas J (2009), „A study of the permeability and compressibility properties of bagasse pulp", Brisbane: Queensland University of Technology, Vol 56(78), pp. 456-500.
[43]. Ranjit R, Raut Ajit R, Sawant and Bhagyashree B. Jamge (2014), „Antimicrobial activity of *Azadirachta indica* (Neem) against Pathogenic Microorganisms", Journal of Academia and Industrial Research, Vol 3, pp.213-218.
[44]. Rao B, Loganatan K, Elumalai E K and Kumar G (2010), „Antibacterial and antifungal activity of *Euphorbia hirta* l. Leaves", A comparative study. Journal of Pharmacy Research, Vol 3(3), pp.548.
[45]. Rashad M, AbdouH M, Mahmuoud A E, Nooman M U (2009), „Nutritional analysis and enzymes activites of *Pleurotus ostreatus* cultivated on Citrus limonium and Carica papaya wastes", Austrian Journal of Basic Applied Science, Vol 3(4), pp.3352-3360.
[46]. Rathi Sanjesh G, Patel Kanu R and Bhaskar Vaidhun H (2012), „Isolation of Herbal Plants: Antifungal and Antibacterial Activities", Journal of Pharmacological Science and Biotechnological Research, Vol 2, pp.25-29.
[47]. Shuping D S S and Eloff J N (2017), „The use of plants to protect plants and food against fungal pathogens", Shuping and Eloff, African Journal of Traditional Complement Alternative Medicine, Vol 14 (4), pp.120-127.
[48]. Sivrikaya H and Peker H (1997), „Cultivation of *Pleurtous Flurioda* on Forest and Wood Waste", Turkian Journal of Agriculture and Forest, Vol 23, pp.585-596.
[49]. Subapriya R and Nagini S (2005), „Medicinal Properties of Neem Leaves", Current Medical and Chemical and Anti-Cancer Agents, Vol 5, pp.149-156.
[50]. Syed Abrar Ahmed J A, Kadam V P and Mane S S (2009), „Biological Efficiency and Nutritional Contents of *Pleurotus Florida* Singer Cultivated on Different Agro-Wastes", Nature and Science, Vol 7(1), pp.122-125.
[51]. Siddiqui N A, Bokhari And K Perveen (2016), „Antifungal Ability of *Nerium Oleander* Against *Fusarium oxysporum, Sclerotium rolfsii* and *Macrophomia phaseolina'*, The Journal of Animal & Plant Sciences,Vol 26(1), pp. 269-274.
[52]. Shrivastava D K and Kshma Swarnkar (2014), „Antifungal Activity of Leaf Extract of Neem (*Azadirachta indica* Linn)", International Journal of Current Microbiology and Applied Sciences, Vol 3, pp. 305-308
[53]. Van Soest P J (2006), „Rice straw, the role of silica and treatments to improve quality", Animal Feed Science Technology, Vol 130 (3-4), pp.137-171.
[54]. Karuppuraj V , Chandra Sekarenthiran S and Perumal K (2014), „Yield Improvement of *Pleurotus Florida* Fruting Bodies from Locally Available Unexplored Lignocellulosic Substrates", International Journal of Current Microbiology and Applied Sciences , Vol(3), pp.985-990.
[55]. Yashvant Patel, Ram Naraian and V K Singh (2012), „Medicinal Properties of *Pleurotus Species* (Oyster Mushroom)", Fungal and Plant Biology, Vol 3(1), pp. 2219-4312.
[56]. Yildiz S, Yildiz E D, Gezer and Temiz (2002), „Some lignocellulosic wastes used as raw material in cultivation of the *Pleurotus ostreatus* culture mushroom", Process Biochemistry, Vol 3, pp. 301-306.

[57]. Zhu, Beibei, Miao and Xiaoping (2014), „Allium vegetables and garlic supplements do not reduce risk of colorectal cancer, based on meta-analysis of prospective studies", Clinica Gastroenterology and Hepatology, Vol 12(12), pp.1991–2001.