Degradation of Tendu leaf Extract using Pleurotus djamor

K. Parani and M.Sivasankari

Department of Botany, Sri Parasakthi College for Women (Autonomous), Courtallam, Tirunelveli (Dt.), TamilNadu.

E.mail ID: barani12101975@gmail.com

Abstract: **The radial growth of Pleurotus djamor was faster in the malt extract agar medium as compared to the different concentration of supplemented tendu leaf waste (5%, 10%, and 15% agar medium). As the concentrations of tendu leaf waste increased to 15% , the growth rate was reduced as prolonged incubation period in the plate culture and liquid medium. Thereby, at very high concentration of tendu leaf extract (15%) was toxic for the growth of mushroom, where protein content and sugars were declined. The antibacterial activity was tested to various concentrations of tendu leaf extract degraded by Pleurotus djamor against human pathogens. E.coli was more susceptible at 5% tendu leaf extract showed maximum zone of inhibition (19mm) and less inhibition zone (12mm) by Bacillus subtilus as compared to 10 % and 15% extracts respectively.**

Key words: **Pleurotus djamor, Tendu leaf extracts, zone of inhibition**

I. INTRODUCTION

Kendu / Tendu *(Diospyros melanoxylon* Roxb.) leaves belongs to Ebenaceae family. It is native to India and Srilanka. Its trade name is Ebony tendu / kendu and is also called as coromandel ebony and contributes to social economic livelihood of tribal people in India. The leaf and fruit of the kendu plant have also been used for traditional medicines. The leaves are commonly used for making bidis (an indigenous traditional cigarette, which uses the kendu leaf for rolling instead of paper [1].

The waste generated from bidi industry is about 12 to 15 thousand tons per annum. In the absence of proper scientific method the leaf waste is thrown on the streets and the improper disposal of leaf garbage leads to various environment al hazards .The volume of waste generated is posing great threats to the environment as no proper disposal mechanism is practiced by the workers [2] .

Mushrooms such as *Pleurotus* and various other varieties are a promising possibility to recycle nutrients a they have been used for centuries to produce protein rich food from solid waste. This simple biotechnological process can provide a solution for safe handling of organic biodegradable soiled waste as well as the most needed plant nutrients for sustainable productivity [3]. In order to solve this problem of leaf waste disposal is an eco-friendly and economically beneficial manner the present work has been designed to convert this biomass into biofertilizer using mushrooms and physico chemical parameters are analyzed.

This simple biotechnological process can provide a solution for safe handling of organic biodegradable soiled waste as well as the most needed plant nutrients for sustainable productivity[4]. In order to solve this problem of leaf waste disposal is an eco-friendly and economically beneficial manner. Hence, the present work was undertaken to see 1. the growth in culture plates and flasks, 2. To study the physico – chemical tests of biomolecules in fermented tendu leaves and 3. To check the antibacterial activity against the human pathogens .

II. MATERIALS AND METHODS

Tendu leave wastes was procured from Local houses in and around Tenkasi, Tirunelveli (Dt.), Tamil Nadu. The waste was collected in the gunny bags and dried under shade. Further the waste was used for the biodegradation studies. Pure culture of oyster mushroom *viz*., *Pleurotus djamor* [Fr] Singer (CO 1) variety was obtained from the Agricultural Research station, T. Kallikulam, Tirunelveli (Dt.), Tamil Nadu. The culture was maintained on Potato Dextrose Agar (PDA) slants and stored at 4ºC and the slant was sub cultured once in a month.

Biodegradation studies

Various amounts of Tendu leaves (5%, 10%, 15% w/v) were weighed and boiled in 100ml of hot water (90°C) for 15 min prepared. The supernatant of tendu leaf was used to prepare 100ml of modified tendu leaf extract medium by adding 1.5g of agar. This medium was sterilized at 121°C for 15min and dispersed into Petri plates (20ml / plate). Culture was inoculated at the centre with a mycelial agar block taken from the margin of 7 days- old fungal colony growing on PDA medium. The plates were incubated at 28 ± 2°C in the dark for 6 to 8 days. The radial growth of white rot fungi was measured on Malt extract agar medium (control) and modified tendu leaf extract agar medium on the 6th day after inoculation. The radial growth rate was calculated by noting the days

required by the fungi for completely covering the plate. The bleaching of the colour at the reverse of the individual plates was observed on the 21st day after inoculation [5].

Compositional Changes

Biodegradation of tendu leaves at various proportions (5%, 10% and 15% W/V) were studied in liquid state of Erlenmeyer flasks (250ml) using the known mushroom fungi and Malt extract broth culture used as control. Visual observations were made on the colonization and substrate colour removal by the known monoculture treatment. After 40 days interval of study, the contents of each flask was used in the analyses for change in pH, reducing sugars [6], protein [7], tannins, flavonoids and alkaloids [6] and antioxidant activity [8] and Antibacterial activity using well diffusion method [9] .

III. RESULT AND DISCUSSION

Most agricultural residues are rich in lignocellulosic compounds whose handling and disposal are often problematic, due to their chemical structure and decomposition properties [10].

Growth rate of *Pleurotus djamor* in plate culture:

The radial growth of *Pleurotus djamor* was faster in the malt extract agar medium as compared to the different concentration of supplemented tendu leaf waste (5%, 10%, and 15% agar medium). The mushroom *Pleurotus djamor* took 6 days to cover the malt extract agar medium and also produces dense biomass in the solid medium and liquid medium. Where as the mushroom took 10-12days in the 5%,and 10% of supplemented tendu leaf waste agar medium to covered the 9cm of Petri plate. As the concentrations of tendu leaf waste increased to 15% , the growth rate was reduced as prolonged incubation period in the plate culture and liquid medium. There by it also favours in the contamination of *Aspergillus niger* and *Pencilium* species. And also simultaneously the biomass also reduced in the plate culture and liquid culture (Table .1, 2).

In the qualitative screening , sugars, proteins, alkaloids, phenols and flavonoids are present in different concentrations of tendu leave extract inoculated with *Pleurotus djamor* after 40 days of incubation respectively.Similar to our observations, considerable variation among different *Pleurotus sp*., in colonizing different spawn bases had been reported [11]. *Lentinus edodes* strain growing on coffee pulp based on similar parameters used in this study and that *Pleurotus* species scored over *Ganoderma lucidum* coffee pulp degradation [12, 13]. Similarly our results showed the higher ability of the *Pleurotus* species to grow on tendu leaf extract as the sole carbon source (Table .3).

Physico – chemical parameters:

In the present investigation, the pH content of Tendu leaf waste from different experimental groups (malt extract as control, 5%, 10% and 15%) were variable during their fermentation period and were found to be respectively.The change in colour of the substrate from dark to yellow brown, an indicator of the efficiency of biodegradation, was found to be pronounced and small primordia were observed in 5% , 10% extracts respectively. Similar results are in accordance with Mata and Savoie, 1998 [14].

The enchancement of ash content may be due to faster and consistent increased microbial activity at the time of fermentation. It can also predicted that increasing ash content indicates faster consumption of available tendu leaf litter because of increased palatability of waste after initial decomposition. It is concluded the higher biomass content indicates larger quantity of ash which result in greater utilization of tendu leaf waste [15]. Moisture is an important factor for the mobility of water. The increased moisture level in the tendu leaf waste was attributed to the fact that for the enhanced contents of nitrogen, phosphrous, potassium, and other secondary micro nutrient. Ash is important indicative parameter for decomposition and mineralization of the substrate.

Then polyphenol content as the concentration increased of tendu leaf waste increased (10%). But there was no reduction in polyphenol content at the concentration of 15% of tendu leaf waste was supplemented after 40 days of fermentation on inoculated with *Pleurotus djamor*. Where as malt extract broth showed a megor amount in production of polyphenol content due to the presence of yeast extract, maltose in the medium composition. Our results are positively correlated with the N,N-Dimethyl Form amide (DMF 50%) extract showed the highest polyphenol content of 167.4 mg GAE/g and provided the greatest antioxidant activity for the tobacco leaf [16]. Similar values of phenolic content were found for guava, plum and star fruit and beta carotene content was found similar to different variety of mangoes [17].

The tendu leaf waste extract with higher antioxidant activity also had higher polyphenol content. These findings are positively correlated in accordance with where the polyphenol extracted from tobacco leaf had great potential as antioxidant and antimicrobial activity for textile application, similar findings has been reported for kiwi, guava, red apple, banana [16, 14]. The different concentration (5%,10% and 15%) of tendu leaf waste was high content of calcium, phosphorus, potassium, nitrogen. Similar trend of mineral composition can be found in mango and passion fruit (Table 5) [18] .

The physical chemical parameters such moisture content, ash, crude fibre, protein, carbohydrate, pH and colour importance for further processing and value addition of kendu/tendu leaf. As the concentration of tendu leaf extract increases, the protein and sugar content, tannins, phenols, flavonols were also increased. At high concentration of tendu leaf extract was toxic for the growth of mushroom, thereby protein content and sugars were declined.

Similar to this observation, *P.djamor* which produced dense mycelial growth on both tendu leaf extract agar medium and tendu leaf extract broth cultures did not produce fruiting bodies as in coffee pulp bags. As suggested that use of tannase producing fungal strains to degrade tannins might be an alternative to use tendu leaf waste as an animal feed [19].

Antibacterial activity of various concentrations of tendu leaf extract degraded by *Pleurotus djamor* against human pathogens *viz.*, *E.coli*, *Bacillus subtilus*, *Staphylococcous aureus.*. *E.coli* was more suscetiple to 5% tendu leaf extract showed maximum zone of inhibition (19mm dia.) and less inhibition zone (12mm) by *Bacillus subtilus* but resistant at high concentration of tendu leaf extract (15%) (Table 4). Similar results was observed the inhibition activities on *Escherichia coli* and *Staphylococcus aureus* were also measured for evaluating the antimicrobial activity of cotton fabric treated with the tobacco leaf extract [16].

References

[1]. Behera , M.C and Nath, M.R., (2014). Financial valuation of non-timber forest products flow from tropical dry deciduous forests in Boudh district, Orissa, *International Journal of farm sciences*: 2(2): 83-94.

[2]. Bhonsle RD, Muri PR, Gupta PC: 1990. Tobacco habits in India.In: Gupta PC, Hamner JE, Murti PR, (Eds) . Control of tobacco related cancers and other diseases. Proceedings of an International symposium: Jan15-19; Bombay;: Oxford university press 1992: 25-46.

[3]. Dhaliwal, H.P.S., H.S. Garcha and R.P. Phutela, 1992. Early fruiting and improved yields by laccase mutants of *Pleurotus florida*. *Mush. Res.*, 1: 73-78.

[4]. Hecht,S.B., Anderson,A.B.and May,P., (1988). The subsidy from nature ;shifting cultivation successsional palm forests,and rural development.Human organ ,47 :25-35.

[5]. Soccol, C.R., 1994. New potentialities and uses of coffee agro-industrial wastes for production of bioproducts in Brazil. In: *International Conference on New Horizons in Biotechnology* (Abs.), Trivandrum, India, April, 18-21, p.26.

[6]. Harbone, J.B.1998. Methods of extraction and isolation. In Phytochemical methods. Chapman and Hall, Lonon

[7]. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. *J.Biol. Chem.*, 193: 265 - 275.

[8]. Usman H, Abdulrahman, F.I. and Usman, A.(2009): Qualitative phytochemical screening and *in vitro* antimicrobial effects of methanol stem bark extract of *Ficusthonningii* (Moraceae). Afr J Tradit Complement Altern Med. 6(3):289–295.

[9]. Aneja, K.R., 1996. Experiments in Microbiology, Plant Pathology, Tissue culture and Mushroom cultivation, Wishwa Prakashan (New Age International (Pvt.Ltd), New Delhi. p.192-195.

[10]. Buswell, J.A., Y.J.Cai, S.T. Chang, J.F. Peberdy, S.Y.Fu and H.S.Yu, 1996. Lignocellulolytic enzyme profiles of edible mushroom fungi. *World J. Microbiol. Biotechnol*., 12: 537 - 542.

[11]. Rafique, A., 1998. Preparation and evaluation of spawn for the cultivation of *Pleurotus. J.Sci.Ind. Res*., 57: 143 -147.

[12]. Leifa, L., A.Pandey and C.R.Soccol, 2000. Solid state cultivation an effficient method to use toxic agro-industrial residues. *Basic Microbiol*., 40: 187 - 197.

[13]. Sobal, M., D.Martinez-Carrera, B.Rio and S.Roussos, 2001. Screening of edible mushrooms for polyphenols degradation and tannase production by solid state fermentation. In: *International Conference on New Horizons in Biotechnology* (Abs), Trivandrum, India, April, 18-21, p. 109.

[14]. Mata, G.and J.M.Savoie, 1998. Extracellular enzyme activities in six *Lentinula edodes* strains during cultivation in wheat straw. *World J.Microbiol.Biotechnol*.,14: 513 - 519.

[15]. Gupta,V.,Maithili,V.,Vishwakarma,P.K.,comparat ive study of analgesic activity of *Diospyros melanoxylon* (Roxb). Bark and root bark. *Journal of National Remedies*: 13(1): 15-8: (2013).

[16]. Dunangsri, P. K. Juntarapun and C.Satirapipathkul, 2012. The tobacco lea extract and antibacterial activity in textile. *RMUTP International Conference: Textiles and Fashion*, Bangkok, Thailand, July 3 - 4.

[17]. Sahr , U.C., Panda, S.K.,Mohapatra, U.B and Ray, R.C., : (2012). Preparation and evaluation of wine from tendu (Diospyros melanoxylon L) Fruits with antioxidants, *Internation Journal of Food Fermatation Technology*: 2(2): 167.

[18]. Aguilar, C., C.Augur, G.Viniegra and E.Favela, 2001. Production of fungal tannase in a model system by solid state fermentation. Paper presented at III SIBAC, Londrina, Brazil, May 24-28.

[19]. Utsunomiya, N., Subhadrabandhu, S.Yonemori, K., Oshida, M., Kanzaki, S., Nakatsubo, F.and Sugiura, A.,Diospyros species in Thailand: Their distribution, Fruit Morphology and uses. *Economic Botany*: 52(4): 345-351: (1998).

Table -1 Growth rate of *Pleurotus djamor* in supplemented Tendu leaf waste in plate culture.
4+ =Excellent; 3+ =Good; 2+ =Moderate ; 1+ =Not bad

Table: 2 Visual observations in flask studies by *Pleurotus djamor*

S.No	Agar medium	Radial growth	Growth rate per day	Bio mass	Decolourization
1	Tendu leaf waste 5%	35mm	3mm	+++	++++
2	10%	20mm	2mm	++	++
3	15%	9mm	0.6mm	++	+
4	Malt extract (control)	45mm	20mm	++++	++++

S.No	Liquid medium	Mycelial Biomass	Colour change in broth medium	pH
1	5%	More Dense growth	Mycelium turns to brown colour	6.5-5.8
2	10%	Dense growth	Mycelium turns to pale yellow colour	6.5-6.0
3	15%	Less growth favours contamination	Dark Brown colour	6.5
4	Malt extract((control)	Profuse growth	Brown to yellow colour	6.5-5

Table:3 Changes in components of supplemented tendu leaf waste by *Pleurotus djamor*

S.no	components	Malt extract (control)	Tendu leaf waste 5%	10%	15%
1	pH	6.5	6.1	5.8	5.8
2	Sugar	+	+	+	+
3	Protein	+	+	+	+
4	Alkaloids	-	+	+	+
5	Flavonoids	+	+	+	+
6	Phenols	+	-	-	-s

7	Tannin	-	+	+	-

Table.4 : Antibacterial activity of different concentration of tendu leaf waste in well diffusion method

Table : 5 Qualitative analysis of minerals in Tendu leaf extracts using *Pleurotus djamor*

S.NO	Tendu leaf supplemented	*E.coli*	*Bacillus subtilis*	*Staphylococcus aureus*
1	5%	19 mm	12 mm	14 mm
2	10%	15 mm	17 mm	17 mm
3	Malt extract (control)	17 mm	18 mm	16 mm
4	Streptomycin	14 mm	14 mm	16 mm

S.No	**Minerals**	**Malt extract (control)**	**Tendu leaf waste 5%**	**Tendu leaf waste 10%**
1.	Nitrogen	+	+	+
2.	Phosporus	-	-	-
3.	Potasium	+	+	+
4.	Calcium	+	+	+
5.	Magnesium	+	+	+
6.	Sulphur	+	+	+
7.	Iron	+		+

