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Phytotoxicity Assessment of Coir pith Effluent Generated during Lignin Recovery Process

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Abstract

Research programs are advancing for tackling the pollution risks of coir pith by converting the substrate into valuable products. The product development process shall generate byproducts or effluents that may be toxic in nature. An efficient oxidative delignification process was developed followed by lignin recovery from the process effluent. The toxicity assessment of effluent generated during lignin recovery process is of utmost importance for the safe disposal plans and the present study aims at assessing the phytotoxicity of the effluent. The test involved 96 hour exposure of Oryza sativa to 0.0% to 100% effluent concentrations. The parameters of analysis included root length (RL), shoot length (SL), seedling length (SDL), seed germination (SG), germination index (GI) and seed vigour index (SVI). The effluent produced significant inhibitory effect on growth of the plant. Root length (EC_{25}=3.05, EC_{50}=5.14) and germination index (EC_{25}=2.78, EC_{50}=4.78) were the most sensitive endpoints, whereas seed germination was the least sensitive (EC_{25}=36.63, EC_{50}=45.45). Based on sensitivity, different endpoints were arranged in decreasing order of sensitivity as: RL=GI>SDL=SVI>SL>SG. The outcome of this study points out the importance of further characterisation of the effluent generated during lignin recovery and adoption of suitable detoxification methods.

Keywords: Phytotoxicity, *oryza sativa*, bioassay, toxic unit, coir pith, lignin recovered effluent.

Introduction

Ninety percent of world's total coir production is contributed by India and Sri Lanka, whose output is estimated to be 135,000 tons per annum[1]. Coir production process involves defibering of coconut husk which releases coir pith as residue that are normally heaped near the coir defibering units leading to environmental deterioration. High lignin content[2] and structural binding of lignin-cellulose complex[3] of coir pith makes its natural degradation much slower. Traditional coir defibering process involves coir retting, a natural biochemical process which leads to the release of toxic organic compounds like polyphenols into the environment[4]. The ongoing concern regarding the pollution caused by coir pith made researchers think of alternatives which lead to the development of advanced processing methods and conversion of coir pith into value added products like biochar[5], biomanure[6] etc. Coir pith is also being utilised for production of commercially valuable enzymes[6]. Coir pith is a rich source of cellulose, a best substrate for bioenergy production. It is possible to produce ethanol from lignocellulosic biomass if the tightly bound lignin is removed from coir pith[7].

Successful delignification of coir pith through oxidative delignification process and subsequent recovery of lignin from the effluent generated (termed "Coir Pith Black Liquor - CBL") has been attained in our laboratory and was reported[8,9]. Adoption of the above mentioned technology in an industrial scale shall lead to the generation of effluent in huge volumes and hence assessment of effluent toxicity prior to its release into open environment is of much importance. The synergistic, antagonistic and additive actions of the compounds present in the effluent may have effect on toxicity. A preliminary study on CBL before lignin recovery had demonstrated its phytotoxic nature. Since the lignin recovery involved additional chemical manipulations, the characteristics of CBL are likely to be different after lignin recovery than it was before.

Plants are important component of a food chain and the sensitivity of plants to different compounds can be used in toxicity tests to identify toxicants. Phytotoxicity tests are gaining significant attention as an efficient method of environmental analysis to assess and characterise the extent of impact of various compounds or pollutants on seed germination and subsequent growth parameters. *Oryza sativa* is one of the several standard test species recommended by OECD[10]. Reports are available on the phytotoxicity assessment of various industrial effluents[11,12]. Phytotoxicity of black peat:sawdust (1:3) was studied by Politycka[13] who reported that seven phenolic acids (ferulic, p-hydroxybenzoic, p-coumaric, protocate-chuic, salicylic, syringic, and vanillic) were the cause of phytotoxicity. Phenolic acids at concentrations below 7.1 mg L were sufficient enough to inhibit root elongation of rice in paddy soils[14]. The phytotoxicity of coir dust has been reported recently[15].

This paper reports the phytotoxicity of coir pith effluent generated by oxidative delignification and lignin recovery process. Toxicity test was conducted using *Oryza.sativa*, a standard test species.

Material and Methods

Effluent Generation from the Substrate: Coir pith was obtained from a coir de-fibering unit at Alleppey district, Kerala, India. Substrate was washed in tap water, air dried and stored for further experiments. The effluent for the present study was generated in two steps. The first step involved oxidative delignification of coir pith by treating it with 2 % hydrogen peroxide at pH 11.5 with substrate to solution ratio 3g : 100 ml. The residue was removed by filtration and the filtrate collected as 'Coir pith Black Liquor' (CBL). In the second step 80 ml of CBL was added to centrifuge tube and pH lowered to 1.7 using conc. H_2SO_4.The solution was then centrifuged at 4000 rpm for 15 min at 4^0C. Lignin was obtained as residue and the filtrate collected in amber coloured bottle for toxicity test.

Toxcicity test: The seeds of *Oryza sativa* L. cv Jyothi were provided by Regional Agricultural Research Station, Pattambi, Kerala, India. The toxicity test involved static, non-renewal type of bioassay. Before beginning the toxicity test the pH of the effluent was brought to 7.5 and kept for one hour to equilibrate. The test was carried out in triplicates in glass petri plates. 10 seeds and 10 ml of lignin recovered effluent at 0, 6.25, 12.5, 25, 50 and 100 % concentrations were added and kept for 96 hours. Distilled water was used as dilution water. The seeds were exposed to 16:8 Light:dark photoperiod at temperature 28±3°C. Root length, shoot length, seeding length, seed germination, germination index (GI) and seed vigour index (SVI) were the parameters studied. Root length (RL) and seedling length (SDL) were measured using 'Fiji'[16], image analysis software. Shoot length (SL) was calculated by subtracting root length from seedling length. Germination index (GI) and seed vigour index (SVI) where calculated as follows:

$$\text{Germination Index (GI)} = \frac{RSG \times RRG}{100} \quad (1)$$

Where, RSG (Relative Seed Germination) = Seed germination as % of control, RRG (Relative Root Growth) = Root length as % of control.

SVI (Seed Vigour Index, SVI) = Germination (%) × Mean Seedling length (mm) (2)

EC_{25} and EC_{50} were computed using 'R' software[17,18]. The data were fitted using log-logistic (3 parameter) nonlinear regression method. EC_{25} and EC_{50} represent the concentrations at which 25 % and 50 % inhibition in response occurs respectively. These values were converted to Toxic Units[19] (Effective Concentration expressed in %) as shown below.

$$\text{Toxic Unit}_{acute}\ (TU_a) = \frac{1}{EC_{50}} \times 100 \quad (3)$$

$$\text{Toxic Unit}_{chronic}\ (TU_c) = \frac{1}{EC_{25}} \times 100 \quad (4)$$

Results and Discussion

All parameters of study showed a clear cut dose response relationship. Root length and germination index were the most sensitive among the parameters studied. The response at lowest effluent concentration (6.25 % effluent) was 102.4 % and 62.7 % of control (figure 1) for shoot and seedling respectively, whereas it was 40.8 % for root and germination index (figure 2). The EC_{25}s were 3.05 (TU_C=32.79) and 2.78 (TU_C=35.97) for root length and germination index respectively (table 1). Up to 90 % of seeds germinated at 25 % effluent. EC_{25} for seed germination was 36.63 (TU_C=2.73) and was the least sensitive among the parameters studied. At 50 % effluent concentration, the percentage of germination decreased to 40.0 % of control. It should be noted that none of the seeds germinated at 100 % effluent. The SVI ranged from 2954.7 to 97.7 (62 % and 2.1 % of control respectively) at 25 % and 50 % effluent concentrations respectively. Seedling length and seed vigour index (figure 3) demonstrated more or less similar response pattern with an EC_{25} of 4.13 (TU_C=24.21) and 4.35 (TU_C=22.99) respectively.

Table-1
96 h EC_{50} (95 % Confidence Interval) and Toxic Units of different parameters of *Oryza sativa*. Parameters followed by the same letter are not significantly different (based on calculated EC values) from each other

Parameter	EC_{25} (95% CI)	EC_{50} (95% CI)	Toxic Units		TU_c / TU_a
			TU_c	TU_a	
Root Length (RL) [a]	3.05(2.16-3.94)	5.14(4.39-5.89)	32.79	19.46	1.69
Shoot Length (SL) [c]	9.57(6.15-12.99)	16.21(11.91-0.51)	10.45	6.17	1.69
Seedling Length (SDL) [b]	4.13(2.99-5.26)	7.95(6.66-9.23)	24.21	12.58	1.92
Seed Germination (SG) [d]	36.63(25.60-47.67)	45.45(37.49-53.4)	2.73	2.20	1.24
Germination Index (GI) [a]	2.78(1.90-3.66)	4.78(4.00-5.56)	35.97	20.92	1.72
Seed Vigour Index (SVI) [b]	4.35(3.20-5.49)	7.78(6.57-8.99)	22.99	12.85	1.79

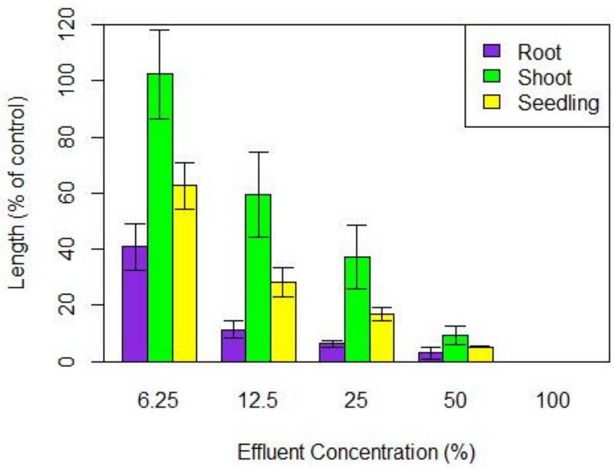

Figure-1
Bar plot showing the effect of various concentrations of coir pith effluent on root length, shoot length and seedling length of *O. sativa* at 96 h

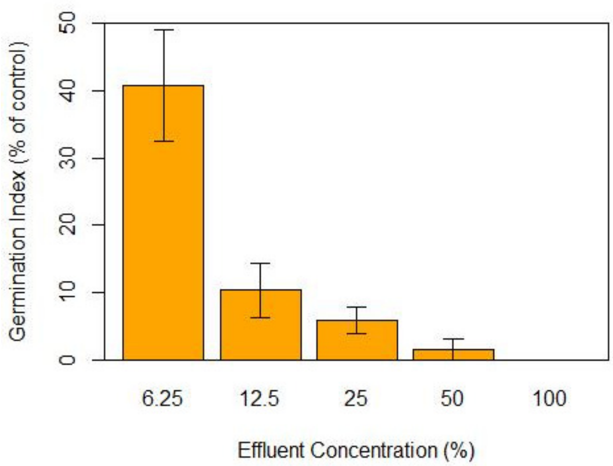

Figure-2
Bar plot showing the effect of various concentrations of coir pith effluent on germination index of *O. sativa* at 96 h

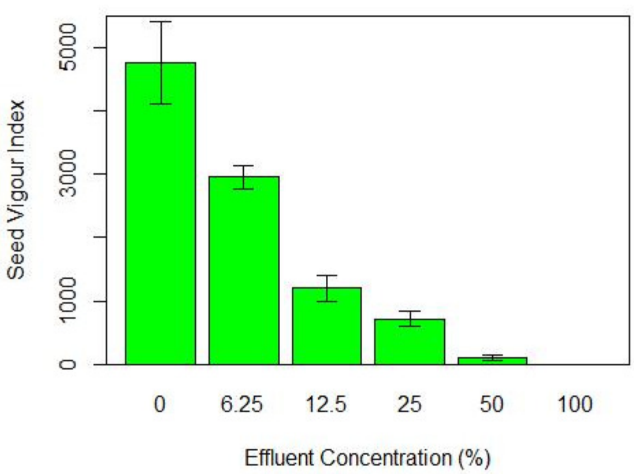

Figure-3
Bar plot showing the effect of various concentrations of coir pith effluent on seed vigour index of *O. sativa* at 96 h

The EC_{50} for root length was 5.14 (TU_a=19.46) which indicated its high sensitivity to the effluent. For seed germination EC_{50} was 45.45 (TU_a=2.2) meaning that a high dosage (closer to 50 %) of effluent, when compared to other parameters, was required to produce 50% reduction in seed germination (figure 4). This value was 1.2 times than that required to produce a 25 % reduction in seed germination. For 50 % reduction in root growth it required 0.6 times the effluent concentration than that required to produce 25 % reduction. TUc/TUa values for germination index and seed vigour index were 1.72 and 1.79 respectively. Based on effective concentrations (EC_{25} and EC_{50}) the endpoints were arranged in decreasing order of sensitivity as RL=GI>SDL=SVI>SL>SG.

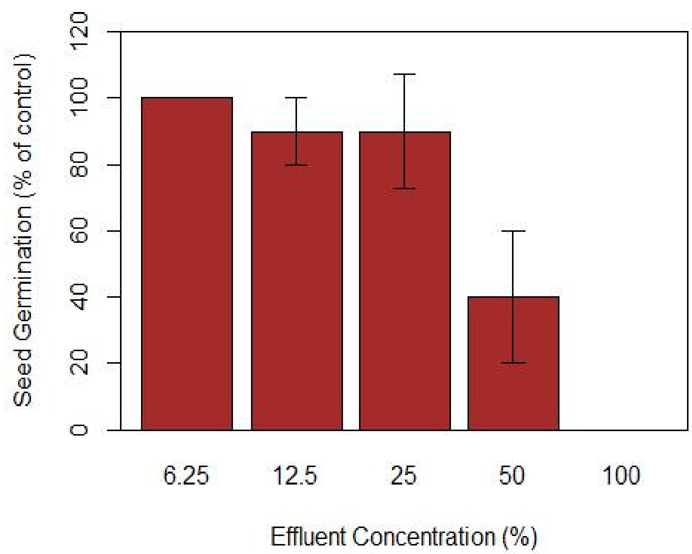

Figure-4
Bar plot showing the effect of various concentrations of coir pith effluent on seed germination of *O. sativa* at 96 h

Delignification process of coir pith involves methods similar to those followed in paper and pulp mill processing and hence effluents may have some common characteristics. However, only a limited amount of data regarding effluents from paper and pulp mill industries is available from Asian region[20]. It was reported in a study that a moderate phytotoxic residual phenolic fraction was extracted from the superficial soil layer even after one year of the application of olive mill waste waters[21]. Though lignin is a major component of such effluents, it is not known to cause phytotoxicity. Some studies have even shown the beneficial effect of lignin on heavy metal detoxification [22]. Lignin recovery from coir pith black liquor is a feasible step as the recovered lignin can be developed further for several industrial applications. However toxicity of the resultant effluent is of concern and proper detoxification methods have to be developed prior to discharge into open environment. Aeration and dilution may have impact on reduction in toxicity levels of effluents. At present several biological methods are also adopted in detoxification of industrial effluents. Experiments using white rot fungus have been initiated in our laboratory to detoxify the coir pith effluent.

Toxicity of effluents may be due to the presence of several compounds and identification of the exact compounds that may contribute to the phytotoxicity is a challenging task. Knowledge about the chemical changes that occur during effluent is generation is important to develop a proper detoxification method. Oxidative delignification is the major process involved in generation of the coir pith effluent. Chemical characteristics of coir pith changes during delignification and lignin recovery process as it involves changing of pH levels at intermediate steps. Investigations have to be carried out further to assess whether the lignin derivatives present in the coir pith effluent may have contributed towards phytotoxicity.

Possibility of utilisation of effluents for irrigation purpose has been widely investigated and studies have shown that effluents, irrespective of the type, can effectively be utilized in the improvement of crops if diluted properly to eliminate toxicity[23]. It should be noted that in the present study, none of the effluent concentrations caused stimulatory effect as it was usually reported in similar studies[24, 25].

Conclusion

The outcome of the study revealed that the effluent generated during lignin recovery process was toxic even at lower concentrations and its disposal to open environment may induce several pollution threats and hence proper detoxification method has to be developed. For development of proper detoxification method, characterisation and identification of the toxicants has to be done. Finding out the ecologically safe concentration of effluent at which it can be released to the open environment should be the primary concern before any advanced process is utilised at industrial level. In the present study, though different parameters showed varied sensitivity to the effluent, all of them were efficient enough to detect and quantify the phytotoxicity of lignin recovered coir pith effluent. The study also highlights the importance of integration of toxicity assessment practices in any industrial process, which is not commonly seen in the present Indian scenario. This is the first report of toxicity assessment of coir pith effluent produced by lignin recovery process.

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