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Evaluation of phytotoxicity of coir pith black liquor generated by oxidative delignification process to *Oryza sativa* L

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ABSTRACT

Coir pith is a polyphenol containing agroindustrial residue which causes land and water pollution. Research programs are advancing by utilising coir pith as a substrate for bio energy production, and oxidative delignification was reported as an effective pretreatment method. Oxidative delignification process generates highly coloured 'Coir pith Black Liquor' (CBL) as effluent and its release to open environment poses pollution risks. Evaluation of toxicity of CBL is of significance for its safe disposal plans. In the present study, we investigated the phytotoxicity of CBL to a standard test species *Oryza sativa*. A 96 hour bioassay was conducted. Root length, shoot length, seed germination, Germination Index (GI), Seed Vigor Index (SVI) and Shoot to Root ratio (S:R) were the parameters studied. Data acquisition was done with the help of image analysis software 'Fiji'. Statistical analysis was carried out using 'R' software. The EC_{50} (Median Effective Concentration), the concentration at which 50% reduction in growth, compared to control, occurred was calculated for each parameter. The EC_{50}s were converted into Toxic Units (TU) for comparison. All parameters studied were significantly affected by the CBL. The study revealed that CBL was acutely toxic to *Oryza sativa*. Root length and Germination Index (GI) were the most sensitive endpoints (TUs = 8.9 and 8.8 respectively) whereas seed germination was the least sensitive endpoint (TU = 1.73). Based on sensitivity, the parameters were arranged as : Root length=GI>Seedling length=SVI>Shoot length>Germination. The results points towards the necessity of further characterization of toxicants present in the CBL and treatment procedures to eliminate the toxicity.

KEYWORDS

Coir Pith Black Liquor;
Phytotoxicity;
Oxidative delignification;
Toxic Unit;
Dose response.

INTRODUCTION

Coir pith is an agroindustial byproduct produced during the extraction of fiber from coconut husk. Million tons of coir pith are generated annually and besides its present application major percentage of coir pith are heaped near coir processing units leading to environmental deterioration. Coir pith degrades slowly due to its high lignin content[19] and structural binding of lignin-cellulose complex[13]. Lignin is considered as the second most abundant group of biopolymers in biospere [2]. Coir pith contains polyphenols, the leaching of which pollutes the natural water bodies. Some polyphenols are known to have carcinogenic/genotoxic effects[3] or interference with thyroid hormone biosynthesis[7].

Considering the above issue, research programs are advancing by utilizing coir pith in the manufacture of economically important products such as briquettes[6], biomanure[10], biochar[16], etc. Cellulosic ethanol can be produced from lignocellulosic residues and composition of coirpith makes it a suitable substrate for Biofuel production via pretreatment, enzymatic saccharification and fermentation. Lignocellulosic materials can be delignified using hydrogen peroxide treatment[18] or sodium hydroxide treatment[1]. Effective pretreatment of coir pith using hydrogen peroxide has been developed and reported with 53 % delignification efficiency[15]. Highly coloured filtrate generated by hydrogen peroxide pretreatment termed as 'Coir pith Black Liquor' (CBL) contains solublised lignin and its derivative. In industrial level production perspective, the adoption of hydrogen peroxide pretreatment process shall generate huge volume of CBL as effluent. Release of CBL to open environment without toxicity analysis may lead to pollution risks to the receiving environment. Hence to ensure safe discharge of CBL, to assess the need for effluent treatment, toxicity analysis is of high significance.

Though several studies focus on the product diversification of coir pith and coir pith derived materials, the byproducts produced from such practices has never been evaluated for toxicity. At present no reports are available on the toxicity characterization of byproducts formed during the processing of coir pith. Hence the present study aims at characterizing the phytotoxicity of Coir pith Black liquor (CBL) produced during the delignification procedure of coir pith.

MATERIALS AND METHODS

The coir pith black liquor (cbl)

Coir pith was collected from a coir defibering unit at Alleppey district, Kerala, India. Collected coir pith was washed in tapwater dried and stored in air tight containers. Oxidative delignification was performed by 2 % hydrogen peroxide treatment on coir pith at pH 11.5 with substrate to solution ratio 3g :100 ml. The residue was filtered and filtrate collected as 'Coir pith Black Liquor' (CBL) was used for toxicity test.

Test species

Standard test species *Oryza sativa* (OECD, 1984) was used to conduct toxicity test. The seeds were obtained from Rice Research Institute, Pattamby, Kerala.

Experiment design

A static bioassay was conducted in glass petri dishes, containing 10 seeds and 10 ml of CBL dilution series at various concentrations for 96 hours. The test concentrations selected were 0, 6.25, 12.5, 25, 50 and 100 % of CBL dilution series. All dilutions were prepared in distilled water. The experiment was conducted in triplicates at 16:8 Light:dark photoperiod. The temperature was maintained at 28±3 °C. Root length and shoot length were measured using the software 'Fiji' (Johannes, 2012). Root length, shoot length, seeding length, seed germination (count), germination index (GI) and shoot to root ratio (S:R) and Seed Vigor Index (SVI) were the parameters studied. Germination Index (GI) and Seed Vigor Index (SVI) where calculated as follows:

$$\textbf{Germination Index (GI)} = \frac{\textbf{RSG} \times \textbf{RRG}}{\textbf{100}}$$

where

RSG (Relative Seed Germination) =

$$\frac{\textbf{Number of seeds germinated in test solution}}{\textbf{Number of seeds Germinated in control}} \times 100$$

RRG (Relative Root Growth) =

$$\frac{\textbf{Mean root length in test solution}}{\textbf{Mean root length in test solution}} \times 100$$

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

S:R (Shoot to Root ratio) = Shoot length / Root length

'R' software[12,14] was used to compute EC_{50}s. EC_{50} (Median Effective Concentration), the concentration at which 50% reduction in growth compared to control occurred was calculated for each parameter. EC_{50} of each parameter was converted to Toxic Units (TU) for comparison. Toxic Unit is the inverse of Effective Concentration expressed in % and is calculated as follows:

$$\textbf{Toxic Unit (TU)} = \frac{1}{EC_{50}} \times 100$$

The Toxic Units of each endpoint is classified into different categories[11] as follows:

No acute toxicity	=TU < 0.4
Slight acute toxicity	=0.4<TU< 1
Acute toxicity	=1 ≤TU< 10
High acute toxicity	=10≤TU< 100
Very high acute toxicity	= ≥TU100

RESULTS AND DISCUSSION

The present study revealed that CBL was acutely toxic *Oryza sativa.* All the parameters studied showed concentration dependent reduction in response. In general, among the parameters studied, root length and germination index (GI) were found to be the most sensitive with a 50 % growth reduction (EC_{50}) at 11.18 and 11.3 CBL concentrations respectively (TABLE.1, Figure 1 and Figure3). The root length was 39.68 mm and 1.39 mm at the lowest and highest concentrations of CBL respectively (TABLE 2). Seed germination was least sensitive (EC_{50} = 57.91). Less than 50 % seeds germinated at 50 % CBL concentration. Though shoot to root ratio (S:R) did not show any significant reduction when compared to control, it showed a steep increase up to 50 % CBL followed by a sudden drop at 100 % CBL (Figure5). Shoot to root ratio was less than 1 at 6.25 % CBL whereas it was greater than 1 at 12.5 % CBL. Based on the Toxic Units (TABLE.2) the root elongation and germination index were found to be 5.18 and 5.12 times sensitive than germination respectively (ie, TUs 8.95, 8.85 and 1.73 respectively). It should be noted that all the seeds germinated in 6.25 % CBL. A comparison of dose response curves of root and shoot depicted in Figure1 reveals that the rate of decrease in growth, as the CBL concentration increased, was high for root when compared to shoot. This indicates that root elongation was approximately twice as sensitive as shoot elongation (TUs 8.95 and 4.05 respectively). The confidence interval (TABLE.1) of root elongation and Germination Index indicated that they were not significantly different from each other. The same was true for seedling length and Seed Vigor Index as well. It is not surprising that root elongation and Germination Index showed similar response since the later was derived from the root length. Similarly Seed Vigor Index and seedling length showed similar response since the SVI was a parameter derived from seedling length. Weibull (3 parameter) model was found to be the most suitable model used to fit all the parameters except shoot to root ratio for which hormetic model was used. Toxic Units calculated for all the six parameters ranged between 1.73 and 8.94 and this indicated that CBL was acutely toxic to all the parameters studied, except shoot to root ratio. Based on calculated EC_{50}s, the parameters can be arranged as: Root length=GI >Seedling

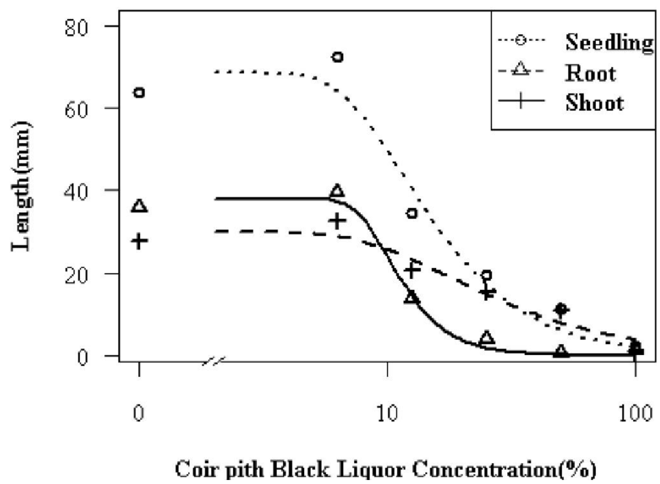

Figure 1 : Response of seedling, root and shoot of *O. sativa* to various concentrations of CBL at 96 h

TABLE 1 : 96 h EC_{50} (and 95 % Confidence Interval) and Toxic Units of different parameters of *Oryza sativa*. Symbols *a*, *b*, *c* and *d* represent parameters significantly different from each other. Param = Parameter.

Parameter	Model	EC_{50} (% CBL v/v)	Slope	CI (95%)	Toxic Units
Root Length ^{a}	Weibull (3 param)	11.18	-3.23882	9.36 – 13	8.944544
Shoot Length ^{c}	Weibull (3 param)	24.71	-1.15697	12.59 – 36.82	4.046945
Seedling Length ^{b}	Weibull (3 param)	14.69	-1.63438	11.42 –17.97	6.807352
Seed Germination ^{d}	Weibull (3 param)	57.90712712	-1.046292	38.13 – 77.69	1.726817
Germination Index (GI) ^{a}	Weibull (3 param)	11.3	-3.6498	9.12 – 13.47	8.849558
Seed Vigour Index (SVI) ^{b}	Weibull (3 param)	13.76	-2.12768	11.02 – 16.5	7.267442
Shoot to Root ratio (S:R)	Hormetic	nd	nd	nd	nd

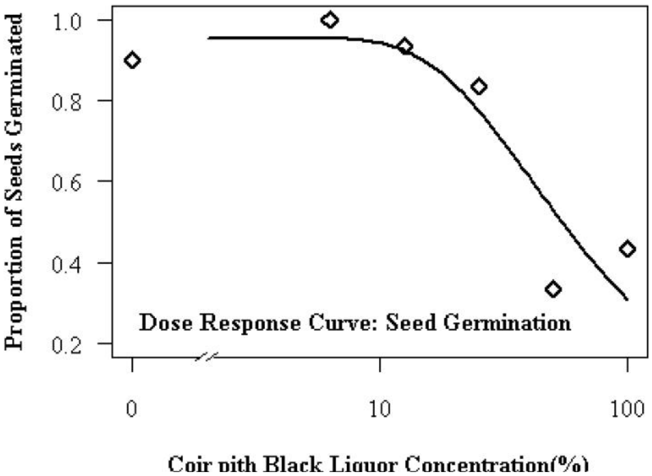

Figure 2 : Effect of various concentrations of CBL on seed germination of O.sativa at 96 h

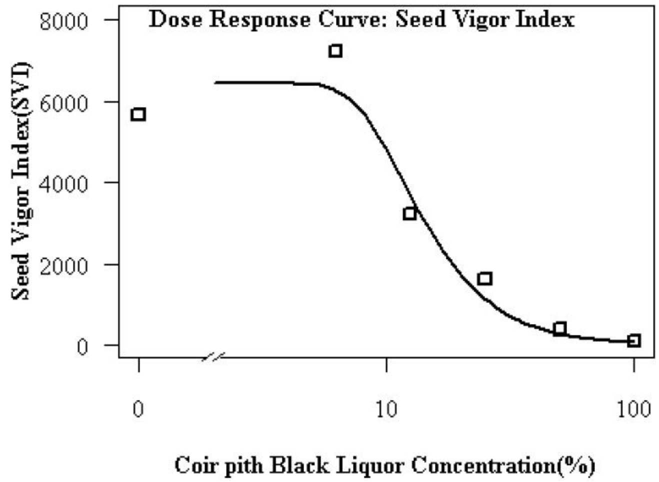

Figure 4 : Effect of various concentrations of CBL on Seed Vigor Index (SVI) of *O.sativa* at 96 h

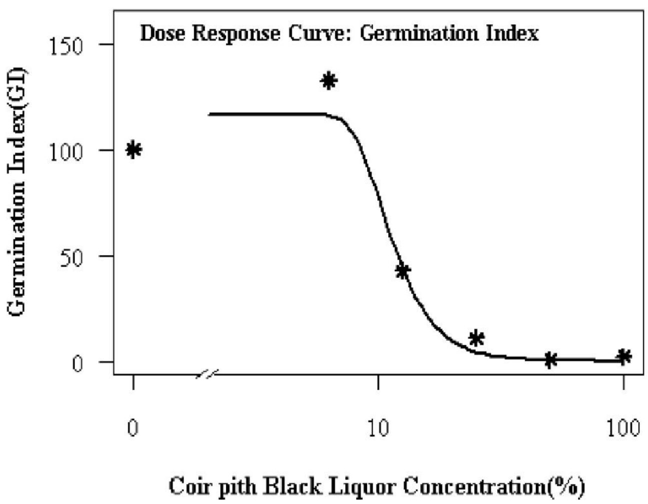

Figure 3 : Effect of various concentrations of CBL on Germination Index (GI) of *O.sativa* at 96 h

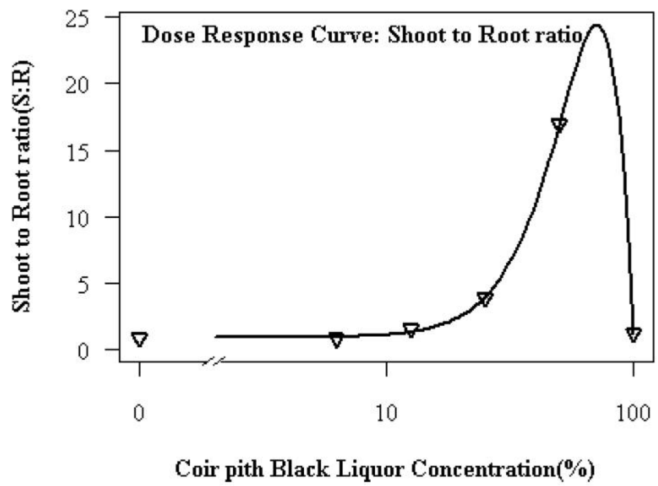

Figure 5 : Effect of various concentrations of CBL on Shoot to Root ratio of *O.sativa* at 96 h

TABLE 2 : Effect of CBL on Root Length (mm), Shoot Length (mm), Seedling length (mm), Germination (count), Germination Index, Seed Vigor Index and Shoot to Root ratio of *Oryza sativa.* Data represents mean±SD (n=3).

CBL Concentration(% v/v)	0	6.25	12.5	25	50	100
Root Length	35.75±10.77	39.68±4.26	13.78±3.79	3.96±0.5	0.63±0.15	1.39±0.8
Shoot Length	27.8±11.23	32.63±7.8	20.68±1.82	15.36±0.6	10.27±3.36	0.94±0.62
Seedling Length	63.54±10.25	72.3±9.81	34.46±3.26	19.32±1.09	11.35±3.48	2.33±0.72
Germination	9±1	10±0	9.33±0.58	8.33±0.58	3.33±0.58	4.33±2.08
Germination Index (GI)	90±0	116.76±42.52	37.16±17.7	9.77±3.67	0.65±0.41	1.84±1.77
Seed Vigour Index (SVI)	5685.17±805.99	7230.48±981.13	3228.85±511.72	1611.94±173.22	383.33±148.04	105.21±62.45
Shoot to Root ratio (S:R)	0.85±0.43	0.82±0.19	1.61±0.6	3.91±0.37	16.94±3.57	1.23±1.47

length=SVI>Shoot length>Germination.

There was a slight stimulatory effect (except for shoot to root ratio), though statistically not significant, at 6.25 % CBL when compared to control. Shoot to root ratio was greater (1.61) than that of control at 12.5 % CBL. The stimulatory effect might be due to the presence of organic and inorganic nutrients which stimulated the growth at lower concentration. Further bioassay at much lower dilutions may reveal if there is a significant stimulatory effect at lower concentrations[20] have already demonstrated stimulatory effect at lower concentrations of effluents on plumule growth of *Lactuca sativa*. Kaliselvi *et al*. (2010) noted that effluents, irrespective of the type, can effectively be utilized in the improvement of crops if diluted properly to eliminate toxicity.

The byproducts, especially CBL, derived during the processing of coir pith is supposed to be more or less similar to the effluent released from paper pulp mill or related industries. The coir pith contains lignin, the decomposition of which produces toxic phenolic compounds[8] was able to extract a moderate phytotoxic residual phenolic fraction from the superficial soil layer 1 year after the application of olive mill waste waters. This residual fraction had a phytotoxic potential comparable to that of olive mill waste waters at a 25-fold dilution. Though oxidative delignification of coir pith was carried out under high pH[15], it may not be the only factor affecting toxicity since the Coir pith Black Liquor is supposed to contain poly phenols and chemical residues derived from the delignification procedure. It is likely that the pH of CBL may modify the toxicity by influencing the synergistic, antagonistic and additive actions (if any) of CBL components. In general, the results from the present study suggest that Coir pith Black Liquor is acutely phytotoxic and it is not safe to release it to open environment without proper detoxification. Phytotoxicity of CBL may depend on many parameters and further experiments have to be carried out to detoxify CBL.

All industrial effluents are supposed to be toxic, but 'how much toxic' is the question to be answered before any effective effluent treatment method is developed or procedural modification in product development is achieved. The work presented here points towards the need for more efficient and eco-friendly method of detoxification of Coir pith Black Liquor for which protocol has to be developed. The study is also the first of its kind regarding the toxicity of Coir pith Black Liquor.

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