Inhibition effects of aqueous extracts of coir pith and composted coir pith on germination and seedling growth of rice (*Oryza sativa*), black gram (*Vigna mungo*), and green gram (*Vigna radiata*)

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ABSTRACT

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Classification: FOR Code: 291899p

Language: English

London Journals Press

LJP Copyright ID: 925652
Print ISSN: 2631-8490
Online ISSN: 2631-8504

London Journal of Research in Science: Natural and Formal

Volume 21 | Issue 3 | Compilation 1.0

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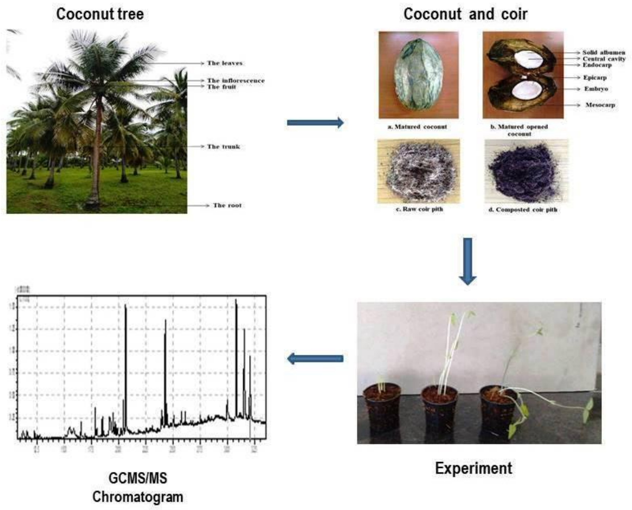
Results: The phytotoxic substances are present in coir pith, which can be exterminated by composting the coir pith for better growth and development of seedlings.

Interpretation: The negative response index (RI), high C: N ratio and chemical compounds like Tocopherol, Fucoxanthin, Tetramethyl Heptadecane, Dichloroacetamide, Tetrazole, Hydroxyethyl palmitate, Neocurdione, and Uridine derivations present in raw coir may have the phytotoxic effect of yellowing symptoms in young plants compared to composted coir pith. This is exterminated by composting the coir pith for better growth and development of seedlings as well as used for various agricultural and horticultural nurseries.



Graphical abstract

Keywords: Inhibition effect, Coir pith, Composted coir pith, Response index, C: N ratio.

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I. INTRODUCTION

Coir is a 100% natural happening fiber got from an inexhaustible asset of coconut husk. Coir strands take after the wood filaments regarding actual properties and compound organization. Coconut strands are seen firmly stuffed alongside non-sinewy, cushioned, and lightweight corky material known as coir substance or coir dust, which comprises around 50-70 percent of the husk. The elastic material that ties the coir fiber in the husk is the coir pith. Coir pith is currently utilized as a medium for seedling nurseries, for bedding plants, for preparing blend supplies for arranging, and for aqua-farming creation of blossoms, vegetables, trees, bushes, manure receptacles, compartment cultivating, packaging layer for mushroom. As of now, coir pith stands apart as the eco-accommodating and more dependable swap for the sphagnum peat greenery, rock fleece, and sawdust. Coir pith based items give a superb developing and pulling mechanism for hydroponics or container-based plant development. Wide variations in the C: N ratio of coir pith from 58:1 to 112:1 has been accounted for. Coir pith obtained from fully mature nuts has higher amounts of lignin and cellulose and a lesser amount of water-soluble salts compared to younger nuts. The coir pith is used as a medium of mat nursery for the germination of paddy seeds and seedling production. Mat nursery seedlings are used for machine transplanting of rice cultivation. Using coir pith affected seed germination and seedling growth and had the least values for germination, growth, and physiological parameters in rice by mat nursery. The application of raw coir pith in nursery preparation of rice and pulses to inhibit plant growth due to the wide C: N ratio, polyphenols, and phenolic acids can be made plants develop toxic yellowing phytotoxic symptoms.

II. MATERIALS AND METHODS

The inhibition effects of aqueous extract of coir pith have experimented at the Department of Agronomy, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, Tamil Nadu, India located at 9˚54'N latitude and 78˚54'E longitude.

The coir pith and composted coir pith soaked separately in distilled water at a weight/volume ratio of 1:10 for 24 hrs. This ratio produces low osmolality. After 24 hours, the aqueous extracts were filtered through Whatman No.1 filter paper. The aqueous extracts were filtered and tested for inhibition of germination and seedling growth of Rice (*Oryza sativa* L.), Black gram (*Vigna mungo* (L.) Hepper), and Green gram (*Vigna radiata* (L.) R. Wilczek). Twenty-five seeds were sown in poly pots of 25 × 25 cm size, filled with coir pith. The poly pots were added with the aqueous extracts frequently to avoid drying up. Distilled water served as control. The germination (%), shoot length (cm), root length (cm), fresh weight (mg), and dry weight (mg) in rice, black gram, and green gram were recorded after two weeks. The magnitude of inhibition versus simulation in the bioassay was compared through the Response Index (RI) (Richardson and Williamson, 1988) is determined as follows,

$$\text{if } T > C, RI = 1 - (C/T)$$

$$\text{if } T = C, \text{ then } RI = 0$$

$$\text{if } T < C, \text{ then } RI = (T/C) - 1$$

Where T is the treatment mean and C is the control mean. A negative RI reflects the proportional disparity in output (germination (%), shoot length (cm), root length (cm), fresh weight (mg), and dry weight (mg)) of test crop in the treatment relative to output in the control. The results were subjected to an analysis of variance (Ayeni *et al.*, 1997), and mean RI values were tested for standard error.

The total organic carbon content in coir pith was analyzed through Dry combustion method at 540°C for 4 hr and total nitrogen content by the Kjeldahl method (Bremmer and Mulvaney, 1982).

For analyzing the chemical compounds present in the coir pith, GCMS/MS analysis was done. A volume of 1 micro lit of clear extract was injected into GCMS/MS with an oven programming of 80°C @ 5°C/min to 250°C (10 min). The injector temperature was maintained at 220°C with the detector temperature of 250 °C. The carrier gas used in the analysis was helium which had a flow rate of 1ml/min. A 30 m length of capillary column HP5 Polar type was used. The GCMS/MS analysis was done at the Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai.

For identification of the components, interpretation on mass spectrum GCMS/MS was conducted using the database of national institute standard and technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, and structure of the component of the test materials were ascertained.

The Coir pith was composted by inoculating with a proprietary bio-formulation, such as PITH PLUS (*Pleurotus sajor caju*), and enriched with urea show a definite reduction in lignin and cellulose contents with an increase in total nitrogen and other nutrient elements after 30 days.

III. STATISTICAL ANALYSIS

The results for each characterization data were obtained from the mean procedure of three replicates and statistical analysis was performed in a complete randomization design. The data on various parameters studied during the investigation were statistically analyzed by applying the technique of standard error deviation (±), as suggested by Gomez and Gomez, (1984).

IV. RESULTS AND DISCUSSION

Carbon to Nitrogen ratio (C: N) is a ratio of the mass of carbon to a mass of nitrogen in a substance. For example, a C: N of 10:1 means there are ten units of carbon for each unit of nitrogen in the substance. Since the C: N ratio is the key factor that decides the decomposition of the organic material which can have a significant effect on the rate of decomposition, crop nutrient cycling (predominantly nitrogen), and soil nutrient availability. In the raw coir pith C: N ratio of more than 40:1 cause's nutrition disorder thus becoming yellowing of young plants. This is also invigorated by coir pith dust chemical composition. In forestry products (bark, sawdust, and woodchips) as well as compost container substrates can invite problems of phytotoxicity, which is largely depending on the chemical composition of the substrate, which in turn can cause salinity, nutritional disorders, and enzymatic or hormonal metabolic alterations (Ortega *et al.*, 1996). High potassium and manganese content (Maher and Thomson, 1991) and the presence of phenolic compounds are terpenes, organic acids, and fatty acids (Morel and Guillemain, 2004) can also be the cause of such problems (Gruda *et al.,* 2009).

Whereas in coir pith composted using PITH PLUS (*Pleurotus sajor caju*), the C: N ratio 19:1 were balanced and nutrients are available to the plants (Table 1) as it was inoculated with bio-formulations involving e addition of supplemental substances to substrates to eliminate the "weaknesses" of natural wooden materials like coir pith, hydrolysis of woodchips under pressure in the presence of acids (Lemaire *et al.,* 1989). Using this method, the lignin-cellulose ratio was changed from 1: 2-3 to 1: 1-2. The supply of nitrogen and other mineral additives before manufacturing fiber substrates under high pressure and heat in the presence of water vapor, to improve substrate properties, is called "impregnation (Penningsfeld, 1992).

The germination and seedling growth of rice, black gram, and green gram were inhibited by coir pith and composted coir pith aqueous extracts compared to the distilled water. Raw coir pith aqueous extracts had a more inhibitory effect on rice, black gram, and green gram when compared to the composted coir pith aqueous extracts. The inhibition is due to phytotoxins present in the extracts, instead of osmotic inhibition because the use of 10 % extract ensures low osmolality (Orwa *et al.,* 2009). The readily visible effects include inhibited or retarded germination rate (Williamson *et al.*, 1992), seeds darkening and swelling, reduced root or radicle and shoot or coleoptile extension (Turk and Tawaha, 2003; Bhatt and Todaria, 1990), swelling or necrosis of root tips, curling of the root axis, discoloration, lack of root hairs, reduced dry weight accumulation and lowered reproductive capacity (Ayeni *et al.,* 1997).

Table 1: The C: N ratio of coir pith and compost coir pith

Parameters	Coir pith	Compost coir pith
TOC (%)	59.39	30.80
Total N (100 g)	0.84	0.94
C:N ratio	40:1	19:1

The inhibition measured by RI means ranged from percentage (Figure 1). The coir pith aqueous extracts had highest suppression of germination (65.00, 46.00 and 51.00 %), root length (10.31, 5.06 and 7.55 cm), shoot length (9.21, 11.07 and 13.60 cm) Fresh weight (0.073, 0.169 and 0.332 mg) and Dry weight (0.012, 0.010and 0.018 mg) of rice, black gram and green gram respectively. It was compared to aqueous extracts composted coir pith and distilled water. (Table 2).

Table 2. Inhibition effect of rice, black gram, and green gram germination and seedling growth

Coir pith					
	Germination %	Root length (cm)	Shoot length (cm)	Fresh weight (mg)	Dry weight (mg)
Rice	65.00±0.428	10.31±1.175	9.21±1.909	0.073±0.006	0.012±0.001
Black gram	46.00±0.173	5.06±1.703	11.07±3.707	0.169±0.058	0.010±0.003
Green gram	51.00±0.690	7.55±1.090	13.60±2.716	0.332±0.042	0.018±0.004
Composted coir pith					
Rice	69.00±0.823	12.76±1.470	11.76±1.670	0.089±0.010	0.013±0.001
Black gram	51.42±0.884	5.47±1.519	11.59±3.938	0.247±0.061	0.015±0.005
Green gram	60.00±0.730	7.91±0.936	17.99±2.294	0.392±0.053	0.016±0.005
Distilled water					
Rice	84.00±0.163	10.430±1.357	8.55±0.915	0.079±0.006	0.013±0.001
Black gram	80.00±0.149	7.470±1.306	18.56±2.843	0.307±0.058	0.014±0.003
Green gram	70.00±0.843	9.970±1.455	15.30±3.133	0.263±0.064	0.014±0.004

Data are the mean values of three replicates with±standard error

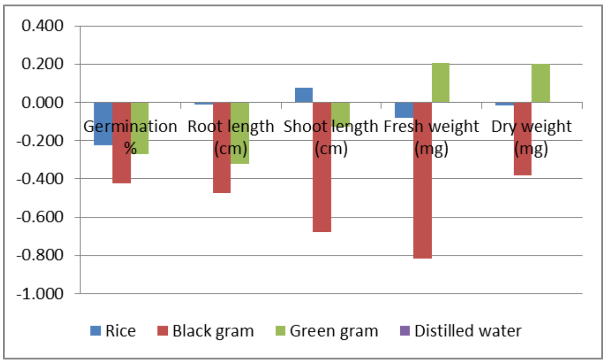

Figure 1: Response index values of coir pith on rice, black gram, and green

The RI indicated the highest negative (-) values coir pith aqueous extracts of germination (-0.226, -0.425, and -0.271), root length (-0.012, -0.476, and -0.321) of rice, black gram, and green gram respectively. Whereas, the negative RI of black gram (-0.677) and green gram (-0.125) of shoot length, rice (-0.080 and -0.016), and black gram (-0.817 and -0.384) of fresh weight and dry weight registered respectively. With regards to the highest RI of positive (+) values registered in the composted coir pith compared to the raw coir pith RI. (Table 3 and (Figure 2)

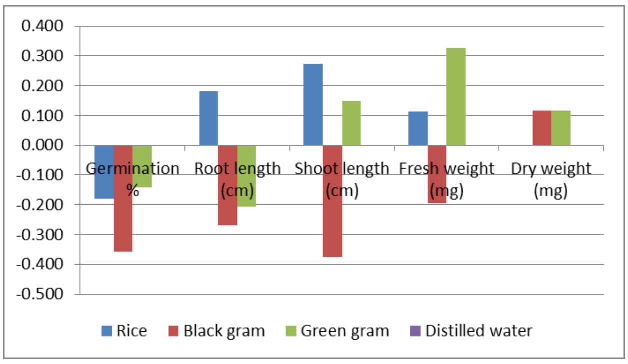

Figure 2: Response index values of composted coir pith on rice, black gram, and green

Table 3: Response index values for inhibition effect of rice, black gram, and green gram germination and seedling growth

Response Index of coir pith					
Coir pith RI	Germination %	Root length (cm)	Shoot length (cm)	Fresh weight (mg)	Dry weight (mg)
Rice	-0.226	-0.012	+0.077	-0.080	-0.016
Black gram	-0.425	-0.476	-0.677	-0.817	-0.384
Green gram	-0.271	-0.321	-0.125	+0.207	+0.203
Response Index of composted coir pith					
Rice	-0.179	+0.183	+0.273	+0.113	+0.000
Black gram	-0.357	-0.268	-0.376	-0.196	+0.117
Green gram	-0.143	-0.207	+0.150	+0.328	+0.116
Distilled water	0.00	0.00	0.00	0.00	0.00

The chemical class distributions of the coir pith extracts are summarized in Table 4 and the GCMS/MS Chromatogram in fig 3. The compounds were separated into eight classes: Acid, Alcohol, Alkane, Amide, Azo compound, Ester, Ketone, and Pyrimidine. Among the 29 chemical compounds, the alcohol class of Tocopherol showed severe seedling growth inhibition phenotype, suggesting that PC-8 functions as a lipid antioxidant in early plant development (Mène-Saffrané *et al*., 2010), and Fucoxanthin derived of strigolactone, has recently identified phytohormone involved in the inhibition of shoot branching of young plants, is also derived from carotenoids (Umehara *et al*., 2008; Gomez- Roldan *et al*., 2008; Seto *et al*., 2012). The alkane class of Tetramethyl Heptadecane and Dichloroacetamide inhibited leaf growth as induced by auxin was found to be independent of ethylene in common bean (Phaseolus vulgaris) plants (Keller, *et al*., 2004). The inhibition of ethylene by applying 1 mM ethylene synthesis inhibitor aminooxy acetic acid (AOA) with 1 mM IAA did not affect auxin-induced inhibition of leaf growth. The Azo compound of Tetrazole has been found useful as inhibitors of top growth for vegetables, fruit trees, cereals, and canes. Application of 5-amino tetrazole to the soil at planting time has caused temporary albinism in some plants and reduction of tetrazolium salts to red colored formmazans has been used to detect the activity of reducing enzymes in seeds. The Ester class of Hydroxyethyl palmitate is the derivation of N-Acylethanolamines (NAEs). The new phenoxyacylethanolamides may be able to enhance endogenous FAAH activity in wild-type seedlings, to confer some tolerance to the growth inhibition by NAE (Lionel Faure *et al*., 2014). The Ketone class of Neocurdione is a development of amine oxidase in the apical part was inhibited by administration of neocuproine, and this effect was markedly reversed by the addition of copper disodium ethylene diamine tetraacetate (EDTA-Na_2-Cu) (Yonezo Suzuki and Hiroshi Yanagisawa, 1976) and the Pyrimidine class of Uridine is inhibition of potato tuber of de novo pyrimidine synthesis leads to a compensatory stimulation of the pyrimidine salvage pathway (Geigenberger *et al.,* 2005). The presence of the above bioactive compounds in the coir pith may act as an inhibitory agent for young plants like rice, black gram, and green gram.

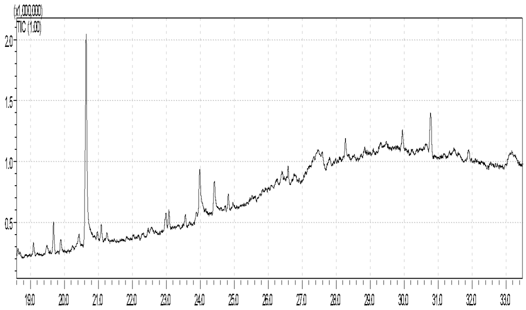

Figure 3: GCMS/MS Chromatogram of coir pith

Table 4: Chemical compounds in coir pith by GCMS/MS

S.No	Retention time	Area / Height (%)	Compound name	Compound class
1	20.643	3.82	n-Hexadecanoic acid	Acid
2	23.984	4.52	9-Octadecenoic acid, (E)-	Acid
3	24.419	5.45	Octadecanoic acid	Acid
4	27.935	4.51	Succinic acid, 1,1,1-trifluoropro	Acid
5	28.385	3.19	2-Bromopropionic acid, 6-ethyl	Acid
6	28.565	2.56	Doconexent, TMS derivative	Acid
7	29.586	5.27	.alpha.-Hydroxystearic acid	Acid
8	30.584	2.29	Fumaric acid, dodecyl 2-hexyl e	Acid
9	27.472	4.08	.alpha.-Tocopherol-.beta.-D-ma	Alcohol
10	27.655	2.49	Cholest-7-en-3-ol, 4,4-dimethyl	Alcohol
11	29.280	1.46	Fucoxanthin	Alcohol
12	29.759	2.62	Isofucosterol, O-TMS	Alcohol
13	22.992	3.88	Eicosane	Alkane
14	26.588	5.09	Octacosane, 1-iodo-	Alkane
15	27.591	7.42	4,8,12,16-Tetramethylheptadeca	Alkane
16	28.278	5.57	Eicosane	Alkane
17	29.952	6.30	Hexadecane, 2,6,10,14-tetramet	Alkane
18	27.804	4.06	Dichloroacetamide, N-nonyl-	Amide
19	28.620	2.85	Butyramide, 2-bromo-N-octyl-	Amide
20	27.780	2.28	Tetrazole, 5-[2-(1-perhydroazep	Azo compound
21	29.852	2.05	Methyl 2,3-dihydroxybenzoate,	Ester
22	30.046	4.01	Valtrate	Ester
23	26.864	2.87	2-Hydroxyethyl palmitate, TMS	Ester
24	26.029	4.00	Neocurdione	Ketone
25	27.185	1.77	Norbolethone	Ketone
26	29.255	2.22	2-Methyl-6-(5-methyl-2-thiazolidone	Ketone
27	29.479	2.06	Geldanamycin, 18,21-didehydro	Ketone
28	30.937	3.35	Dihydroartemisinin, 5-de hydro	Ketone
29	30.185	2.64	Uridine, 2',3'-O-(1-methylethyli	Pyrimidine

The chemical class distributions of the composted coir pith constituents extracts are summarized in Table 5 and the GCMS/MS Chromatogram of fig 4. The compounds were separated into seven classes: Acid, Alcohol, Aldehyde, Alkane, Amide, Ester, and Guanidine. Among the seven class compounds that mostly come under the characteristic feature of fatty acids is the carboxyl function. The composted coir pith while decomposition polyphenol content contains and phenolic compounds is less by degraded. The microorganisms can utilize and degrade polyphenols (Chan, 1986), and as many polyphenols are water-soluble nature coir pith. The composted coir pith has a low C: N ratio, which provides nutrients for crops (Bollen and Lu, 1957).

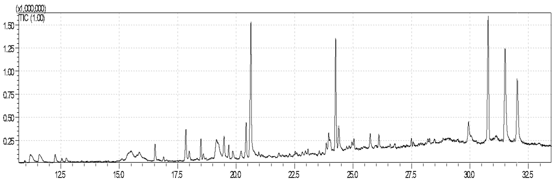

Figure 4: GCMS/MS Chromatogram of composted coir pith

Table 5: Chemical compounds in composted coir pith by GCMS/MS

S.No	Retention time	Area / Height (%)	Compound name	Compound class
1	12.256	4.13	Dodecanoic acid	Acid
2	16.532	3.06	Tetradecanoic acid	Acid
3	17.848	3.29	Pentadecanoic acid	Acid
4	18.494	2.74	1,2-Benzenedicarboxylic acid, b	Acid
5	20.211	3.25	Oleic Acid	Acid
6	20.630	3.56	n-Hexadecanoic acid	Acid
7	21.832	2.82	Eicosanoic acid	Acid
8	23.960	3.53	9-Octadecenoic acid (Z)-, 2,3-di	Acid
9	24.392	3.70	Octadecanoic acid	Acid
10	24.733	2.27	Acetic acid, chloro-, octadecyl e	Acid
11	27.900	3.45	Hydroxyvalerenic acid	Acid
12	30.615	6.04	Retinoyl-.beta.-glucuronide 6',3'	Acid
13	22.825	4.17	Behenic alcohol	Alcohol
14	22.966	3.13	7-Hexadecyn-1-ol	Alcohol
15	30.000	1.59	Perillaldehyde-O-methyloxime	Aldehyde
16	17.991	3.78	Docosanoic acid, 1,2,3-propane	Alkane
17	20.965	2.85	9-Hexacosene	Alkane
18	24.820	6.60	Decane, 5-ethyl-5-methyl-	Alkane
19	28.267	2.35	Hexacosane, 1-iodo-	Alkane
20	31.139	3.43	5-(7-Isopropyl-10-methyl-1-oxo	Alkane
21	24.030	4.01	Cyclohexanol, 1R-4cis-acetamid	Amide
22	29.946	3.90	Nonivamide	Amide
23	19.684	2.50	Hexadecanoic acid, methyl ester	Ester
24	20.425	2.93	Dibutyl phthalate	Ester
25	25.037	2.56	Eicosyl acetate	Ester
26	30.779	3.40	Bis(2-ethylhexyl) phthalate	Ester
27	26.790	3.06	n-Octyl guanidine	Guanidine

V. CONCLUSIONS

Despite numerous advantages and accessibility in huge amounts, coir substance is not completely used for gainful purposes due to the high C: N proportion (112: 1) and presence of the high amount of lignin. The use of raw coir pith with a wide C: N proportion can bring about immobilization of plant supplements. Also, polyphenols and phenolics acids can be phytotoxic and repress plant development, and hence it can be used for moisture conservation and other related works while composted coir pith can be used for various agricultural and horticultural works including nurseries. Besides, composted coir pith is also a good source of organic matter for agricultural use for soil health building.

ACKNOWLEDGMENTS

The authors wish to thank the Core Projects for research activities at College and Research stations of TNAU - Phase III (Post Doctoral Fellowships), funded by Agriculture Department, Govt. of Tamil Nadu, 2018 - 2021, Tamil Nadu Agricultural University, Coimbatore, India. Also, wish to thank the Department of Entomology, AC & RI, Madurai, TNAU for the laboratory facilities.

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