



# Allelopathic Chemical Screening of Aqueous Extracts of Coir Pith on Seedling Growth of Black Gram (*Vigna mungo* L.) and Green Gram (*Vigna radiata* L.)

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## ABSTRACT

**Background:** Black gram (*Vigna mungo* L.) and green gram (*Vigna radiata* L.) were mostly used as chemical residue indicators for testing the persistence of pesticides, fungicides and herbicides, etc. in the soil. Presently, the coir pith was used as a medium of nursery for many seeds and seedling production. This study evaluated the inhibition effects and screening of allelopathic chemicals from aqueous extracts of coir pith and composting coir pith on the black gram and green gram by response index method and GCMS/MS analysis.

**Methods:** The coir pith and composted coir pith soaked the ratio of 1:10 for 24 hrs, filtered through Whatman No.1 filter paper. The inhibition effect tested for germination and seedling growth of black gram and green gram was sown in poly pots. The carbon: nitrogen ratio and the total organic carbon were analyzed by dry combustion method, Kjeldahl method and GCMS/MS analysis.

**Result:** The phytotoxic substances are present in coir pith, which can be exterminated by composting the coir pith for better growth and development of seedlings. The negative response index (RI), high C: N ratio and chemical compounds like tocopherol, fucoxanthin, tetramethyl heptadeca, dichloroacetamide, tetrazole, hydroxyethyl palmitate, neocurdione and uridine derivations present in raw coir may have the phytotoxic effect and produced 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.

**Key words:** Allelopathic chemical, C: N ratio, Coir pith, Composted coir pith, Inhibition effect, Response index.

## 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 per cent 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 aqua-farming, production of blossoms, vegetables, trees, bushes, manure receptacles, vertical farming and packaging layer for mushrooms. 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 by the different scientists. 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. Using coir pith affected seed germination and seedling growth and had the least values for germination, growth and physiological

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parameters in nursery plants by mat nursery. The application of raw coir pith in nursery preparation may inhibit plant growth due to the wide C: N ratio, polyphenols and phenolic acids that made plants develop toxic yellowing phytotoxic symptoms. With this proof, the coir pith extracts were prepared and tested for their inhibitory effect and screening of allelopathic chemicals based on germination, shoot and

root length (cm) as well as fresh and dry weight of Black gram and Green gram by Response Index method.

## MATERIALS AND METHODS

The inhibition effects of aqueous extract of coir pith have experimented during January 2021 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 were 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 black gram [*Vigna mungo* (L.)] and green gram [*Vigna radiata* (L.)]. Twenty-five seeds were sown in poly pots of 25 × 25 cm size, filled with coir pith (Anbarasu and Swaminathan, 2021). The poly pots were added with the aqueous extracts frequently to avoid drying up. Distilled water was served as control. The germination (%), shoot length (cm), root length (cm), fresh weight (mg) and dry weight (mg) of 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,

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

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

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

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.

## 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 ( $\pm$ ), as suggested by Gomez and Gomez, (1984).

## 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 might have caused 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 the 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

**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

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 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 the 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 (Parafiniuk and Czerwińska, 2019) 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 (Aveni *et al.*, 1997).

The coir pith aqueous extracts had the highest suppression of germination (46.00 and 51.00 %), root length (5.06 and 7.55 cm), shoot length (11.07 and 13.60 cm) Fresh weight (0.169 and 0.332 mg) and dry weight (0.010 and 0.018 mg) of black gram and green gram respectively. It was compared to aqueous extracts composted coir pith and distilled water (Table 2).

The RI indicated the highest negative (-) values coir pith aqueous extracts of germination (-0.425 and -0.271), root length (-0.476 and -0.321) of black gram and green gram respectively. Whereas, the negative RI of black gram (-0.677) and green gram (-0.125) of shoot length. The black gram (-0.817 and -0.384) of fresh weight and dry weight were 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, Fig 1 and 2).

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 Tetramethylheptadeca 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 aminoxy 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 an inhibitor of top growth for vegetables, fruit trees, cereals and canes.

**Table 2:** Inhibition effect of black gram and green gram germination and seedling growth.

	Germination %	Root length (cm)	Shoot length (cm)	Fresh weight (mg)	Dry weight (mg)
<b>Coir pith</b>					
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
<b>Composted coir pith</b>					
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
<b>Distilled water</b>					
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.

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

Coir pith RI	Germination %	Root length (cm)	Shoot length (cm)	Fresh weight (mg)	Dry weight (mg)
<b>Response index of coir pith</b>					
Black gram	-0.425	-0.476	-0.677	-0.817	-0.384
Green gram	-0.271	-0.321	-0.125	+0.207	+0.203
<b>Response Index of composted coir pith</b>					
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

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 formazans 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 phenoxyacyl-

ethanolamides 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- $\text{Na}_2$ -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 black gram and green gram.

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

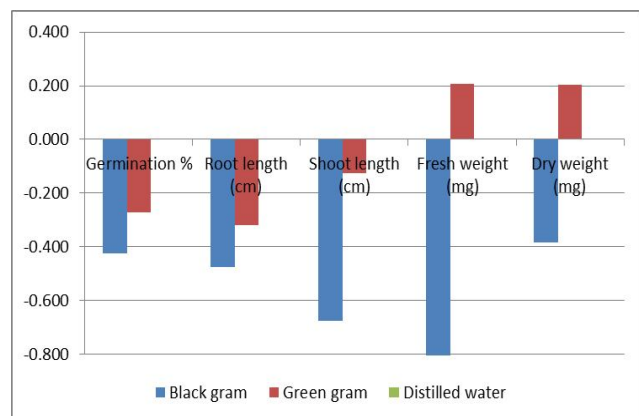


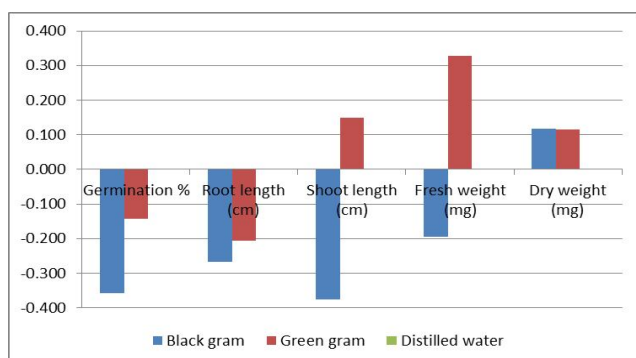
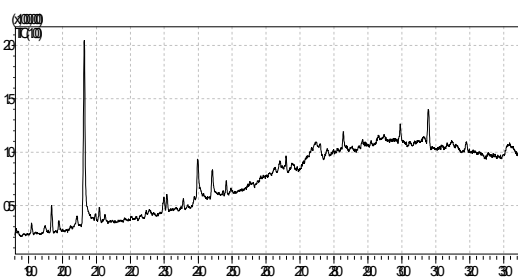
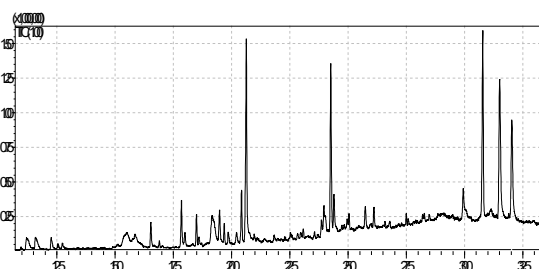
Fig 1: Response index values of coir pith on black gram and green gram.

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

Retention time	Area/Height (%)	Compound name	Compound class
20.643	3.82	n-Hexadecanoic acid	Acid
23.984	4.52	9-Octadecenoic acid, (E)-	Acid
24.419	5.45	Octadecanoic acid	Acid
27.935	4.51	Succinic acid, 1,1,1-trifluoropro	Acid
28.385	3.19	2-Bromopropionic acid, 6-ethyl	Acid
28.565	2.56	Doconexent, TMS derivative	Acid
29.586	5.27	.alpha.-Hydroxystearic acid	Acid
30.584	2.29	Fumaric acid, dodecyl 2-hexyl e	Acid
27.472	4.08	.alpha.-Tocopherol-.beta.-D-ma	Alcohol
27.655	2.49	Cholest-7-en-3-ol, 4,4-dimethyl	Alcohol
29.280	1.46	Fucoxanthin	Alcohol
29.759	2.62	Isofucosterol, O-TMS	Alcohol
22.992	3.88	Eicosane	Alkane
26.588	5.09	Octacosane, 1-iodo-	Alkane
27.591	7.42	4,8,12,16-Tetramethylheptadeca	Alkane
28.278	5.57	Eicosane	Alkane
29.952	6.30	Hexadecane, 2,6,10,14-tetramet	Alkane
27.804	4.06	Dichloroacetamide, N-nonyl-	Amide
28.620	2.85	Butyramide, 2-bromo-N-octyl-	Amide
27.780	2.28	Tetrazole, 5-[2-(1-perhydroazep	Azo compound
29.852	2.05	Methyl 2,3-dihydroxybenzoate,	Ester
30.046	4.01	Valtrate	Ester
26.864	2.87	2-Hydroxyethyl palmitate, TMS	Ester
26.029	4.00	Neocurdione	Ketone
27.185	1.77	Norbolethone	Ketone
29.255	2.22	2-Methyl-6-(5-methyl-2-thiazolidone	Ketone
29.479	2.06	Geldanamycin, 18,21-didehydro	Ketone
30.937	3.35	Dihydroartemisinin, 5-de hydro	Ketone
30.185	2.64	Uridine, 2',3'-O-(1-methylethyl)	Pyrimidine

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

Retention time	Area / Height (%)	Compound name	Compound class
12.256	4.13	Dodecanoic acid	Acid
16.532	3.06	Tetradecanoic acid	Acid
17.848	3.29	Pentadecanoic acid	Acid
18.494	2.74	1,2-Benzenedicarboxylic acid, b	Acid
20.211	3.25	Oleic Acid	Acid
20.630	3.56	n-Hexadecanoic acid	Acid
21.832	2.82	Eicosanoic acid	Acid
23.960	3.53	9-Octadecenoic acid (Z)-, 2,3-di	Acid
24.392	3.70	Octadecanoic acid	Acid
24.733	2.27	Acetic acid, chloro-, octadecyl e	Acid
27.900	3.45	Hydroxyvaleric acid	Acid
30.615	6.04	Retinoyl-.beta.-glucuronide 6',3'	Acid
22.825	4.17	Behenic alcohol	Alcohol
22.966	3.13	7-Hexadecyn-1-ol	Alcohol
30.000	1.59	Perillaldehyde-O-methoxime	Aldehyde
17.991	3.78	Docosanoic acid, 1,2,3-propane	Alkane
20.965	2.85	9-Hexacosene	Alkane
24.820	6.60	Decane, 5-ethyl-5-methyl-	Alkane
28.267	2.35	Hexacosane, 1-iodo-	Alkane
31.139	3.43	5-(7-Isopropyl-10-methyl-1-oxo	Alkane
24.030	4.01	Cyclohexanol, 1R-4cis-acetamid	Amide
29.946	3.90	Nonivamide	Amide
19.684	2.50	Hexadecanoic acid, methyl ester	Ester
20.425	2.93	Dibutyl phthalate	Ester
25.037	2.56	Eicosyl acetate	Ester
30.779	3.40	Bis(2-ethylhexyl) phthalate	Ester
26.790	3.06	n-Octyl guanidine	Guanidine

**Fig 2:** Response index values of composted coir pith on black gram and green**Fig 3:** GCMS/MS Chromatogram of coir pith.**Fig 4:** GCMS/MS Chromatogram of composted coir pith.

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).

## CONCLUSION

Despite numerous advantages and accessibility in huge amounts, the coir substance is not completely used for gainful purposes due to the high C: N proportion (112: 1) and the presence of a 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

phenolic 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.

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