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A Comparative Study of Phytochemicals in Black Gram Treated with Manures.

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ABSTRACT

Legumes are excellent source of medicinal property for the majority of the world population. It is a good challenge for scientists to provide efficient, safe and cheap medications. Black gram can be used as an antioxidant in treating several diseases infection. The present investigation was carried out to determine the phytochemicals present in the root nodules of *vigna mungo.*L which was grown with different types of organic manures. GC-MS analysis led to identification of specific chemical compounds released from plants using two different organic manures which show antimicrobial activity.

Keywords: Black gram, organic manure, GC-MS, phytochemicals

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INTRODUCTION

Black gram (*Vigna mungo.L*) is an important legume occupying important position in Indian agriculture. It is under cultivation in India is about 3.25 million hectares and an annual production is 1.45 million tons [1]. Black gram is used in the treatment of liver diseases, rheumatism, diabetes, heart diseases and central nervous system infection. The root nodule of black gram is said to be narcotic, diuretic and is used as remedy for aching bones, dropsy and cephalgia [2,10,11]. Legume nitrogen fixation starts with the formation of a nodule. Rhizobium invades the root and multiples within cortex cells. The plant supplies necessary nutrients and energy for the bacteria [3]. Organic manure contributes to soil fertility due to addition of organic matter and nutrients such as nitrogen that is trapped by bacteria in the soil [4]. Organic manures which breakdown or decay is available to the plant faster than those which decay slowly [5]. Two different types of organic manures such as cowdung and coir waste treated with *anabaena azollae* sp were used for the growth of black gram plants. Gerald (2004) who reported that addition of organic manures in soil enhanced the symbiotic relationship between phyto chemical compounds[6].

MATERIALS AND METHODS

Plant materials

Black gram seeds *Vigna mungo.L* (ADT Mash 3) collected from Tamil Nadu Rice Research station, Aduthurai were used in the present study.

Growth conditions

The Seeds were sown in pots containing 5kg of sand and fertile clay soil mixture in the ratio of 1:2(w/w). They were amended with commercial cowdung and coirwaste. Cowdung was collected from the cattleshed. Coirwaste manure was prepared by mixing log phase culture of *anabena azollae*(ML-2) with coir waste in the ratio1:5 and composting it for six months[7,8]. The amount of manure added to the pots was 3 gms. The root nodules were collected from 52nd day old black gram plants grown with both organic manures. The experiment was done in triplicates.

Preparation of root nodule s extract for GC-MS analysis

Root nodules of black gram (vigna mungo.L) were shade dried.2g of the powdered leaves was soaked in 95% ethanol for 12 hour. The extracts were then filtered through whatmann filter paper No 41along with 0.2gm of sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper was wetted with 95% ethanol for 12 hours. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents of the plant material used. 2µl of this solution was employed for GC-MS analysis [9].

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GC-MS analysis

GC-MS analysis was carried out on a GC clarus 500 perkin elmer system comprising a Aoc-20i auto sampler and gas chromatograph interfaced to a mass spectrometer.(GC-MS instrument employing the following conditions: column Elite-1 fused silica capillary column(30mm x0.25mm ID x1µMdf, composed of 100% Dimethyl poly siloxane),operating in electron impact mode at 70 eV, helium (99.999%) was used as carrier gas at constant flow of 1ml/min and an injection volume of 0.5µl was employed(split ratio of 10:1) injector temperature250^{0}c ; ion source temperature 280^{0}c. The oven temperature was programmed from 110^{0}c (isothermal for 2 min) with an increase of 10^{0}c/min to 200^{0}c, then 5^{0}c/min to 280^{0}c ending with a 9min isothermal at 280^{0}c. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450Da. Total GC running time is 36 min.

RESULTS AND DISCUSSION

The GC-MS study of root nodules of black gram plants have shown many phytochemicals which contributes to the medicinal activity of the plant (Table1). In root nodule of black gram treated with cowdung manure (Table 2) the content of Ethylbenzene, Pentane, 1,1-diethoxy ,octanoic acid ethyl ester and Phthalicacid butyl isohexyl were identified. The root nodule of black gram treated with coir waste (Table 3 and Fig.2) had a high concentration of Oleic Acid, 9,12-Octadecadienoic acid and Phthalic acid butyl undecyl ester. This was due to supply of phyto chemicals from coir waste. The coconut contain high amount of oleic acid and octanoic acid. The specific phytocomponent, octanoic acid was identified only in coir waste treated black gram. Propane-1, 1, 3 triethoxyl derivatives were found in all samples. The maximum peak area (6.81) was observed in Coir waste and cowdung treated sample. High concentration of 1,2 benzene dicarboxylic acid(10.5) was observed in control plants(not shown). Benzene dicarboxylic acid (phthalic acid) was observed indifferent forms. The maximum peak of Phthalic acid, butyl undecyl ester (9.13) was observed in coir waste treated sample. The cow dung treated sample and contains phtyalic acid butyl isohexyl ester form. 9,12- Octadecadienoic acid (Z,Z)- was found to be very high (15.99) in coir waste treated sample when compared with other samples. Lauric acid (dodecanic acid) was found to be low peak in control (0.11) when compared with cowdung sample B(Table2,Fig1). Hexanoic acid was observed in coirwaste treated sample. 3-0-methyl D-glucose(71.55) was observed only in cowdung treated sample. The saturated fatly acid, oleic acid was observed in all the samples. The maximum peak (7.04) was observed in coir waste treated plants. Hexanoic acid ethyl ester was present in the cowdung and coir waste treated samples. Ethylbenzene, propane 1,1, 3 triethoxy pentane1,1-diethoxy, octanoic acid ethylester, Decanoic acid ethylester , phthalic acid butyl isohexy ester, 9-12 octadeca dienoyl chloride, oleic acid, octadecanoic acid ethyl ester were observed in cowdung and coirwaste treated samples. The maximum amount of Ethylbenzene, pentane, 1, 1-diethoxy and octanoic acid ethyl ester were found in cowdung treated sample when compared with control. n-hexadecanoic acid ethyl ester was found to be low (4.86) in control sample when compared with cowdung treated sample and coir waste sample.

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Table 1: Activity of some phytocomponents identified in root nodules of black gram plants by GC-MS

RT	Compound name	Activity
6.66	Octanoic acid ethylester	Antibacterial
9.74	Dodecanoic acid	Antimicrobial
14.07	3-O-Methyl-D-Glucose	Preservative
17.75	n-Hexadecanoic acid	Antioxidant
20.55	Oleic acid	Anti-inflammatory, anti-androgenic, cancer preservative and hypocholesterolemic
26.38	1,2 benzene dicarboxylicacid diisooctylester	Antimicrobial

Table 2: GC-MS analysis of phytochemicals identified from black gram treated with cowdung manure

No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	2.76	Ethylbenzene	C_8H_{10}	106	3.27
2	3.55	Pentane, 1,1-diethoxy-	$C_9H_{20}O_2$	160	2.21
3	4.98	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	6.81
4	6.66	Octanoic acid, ethyl ester	$C_{10}H_{20}O_2$	172	0.26
5	9.40	Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	200	0.65
6	10.55	Lactose	$C_{12}H_{22}O_{11}$	342	3.47
7	10.75	3-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324	4.35
8	11.70	D-Glucose, 4-O-à-D-glucopyranosyl-	$C_{12}H_{22}O_{11}$	342	1.13
9	14.07	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	71.55
10	15.83	Phthalic acid, butyl isohexyl ester	$C_{18}H_{26}O_4$	306	3.78
11	16.19	Ethanol, 2-(9-octadecenyloxy)-,(Z)-	$C_{20}H_{40}O_2$	312	3.91
12	17.41	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.80
13	17.75	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.21
14	20.22	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298	3.09
15	20.55	Oleic Acid	$C_{18}H_{34}O_2$	282	2.21
16	20.89	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.40

Table 3: GC-MS analysis of phytochemials identified from black gram treated with coir waste manure

No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	3.54	Butane, 1,1-diethoxy-3-methyl-	$C_9H_{20}O_2$	160	5.61
2	3.97	Hexanoic acid, ethyl ester	$C_8H_{16}O_2$	125	1.15
3	4.98	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	6.81
4	5.64	Octanoic acid	$C_8H_{16}O_2$	144	2.20
5	9.40	Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	200	0.65
6	10.55	Lactose	$C_{12}H_{22}O_{11}$	342	3.47
7	10.75	3-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324	4.35
8	11.70	D-Glucose, 4-O-à-D-glucopyranosyl-	$C_{12}H_{22}O_{11}$	342	1.13
9	15.83	Phthalic acid, butyl undecyl ester	$C_{23}H_{36}O_4$	376	9.13
10	16.20	Ethanol, 2-(9-octadecenyloxy)-,(Z)-	$C_{20}H_{40}O_2$	312	3.91
311	17.42	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	37.29
12	17.76	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	2.42
13	20.13	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	15.99
14	20.55	Oleic Acid	$C_{18}H_{34}O_2$	282	7.04

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CONCLUSION

In this study, nearly twenty chemical constituents have been identified from ethanolic extract of the root nodules of black gram plant by GC-MS analysis. It is observed that there is a linear relationship between black gram and organic manures. This enabled the translocation of nutrients from manure to the plant. Organic manure also releases certain specific phytochemicals that might be beneficial to root nodulation thereby nitrogen fixation.

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