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Research Article

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GC-MS study of phytochemicals in black gram using two different organic manures

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ABSTRACT

Plants are the almost exclusive source of drugs for majority of the world population. The phyto components in black gram help in treating aliments like liver diseases, cancer, diabetes, kidney diseases by functioning as antioxidants. 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 using GC-MS analysis. The analysis revealed the presence of nearly 15 components of pharmaceutical importance in the root nodules of vermicompost and coir waste manure treated plants.

Key words: Black gram, organic manure, GC-MS, phytochemicals.

INTRODUCTION

Black gram (*Vigna mungo.L*) is an important pulse crop occupying unique position in Indian agriculture. It is under cultivation in India in about 3.25 million hectares and its annual production is 1.45 million tons [1]. Legumes are a rich source of antioxidants in treating various ailments like liver diseases, rheumatism, diabetes, heart diseases and infections in the central nervous system [2]. The root nodule of black gram is said to be narcotic, diuretic and is used as remedy for aching bones, dropsy and cephalgia [3, 4, 5]. Legume nitrogen fixation starts with the formation of a nodule. *Rhizobium* invades the root and multiples within the cortex cells. The plant supplies necessary nutrients and energy for the bacteria [6]. 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 [7, 8]. The addition of organic manures in soil enhanced the symbiotic relationship between phyto chemical compounds [9].

Organic manures which breakdown or decay is available to the plant faster than other manure which decay slowly [10]. GC-MS analysis of phyto constituents in plants gives a clear picture of the pharmaceutical value of that plant. There are very less reports on the analysis of phytochemicals in plants that are grown after the addition of organic manures. In the present study the phytochemicals present in the root nodules of black gram (*Vigna mungo* L) treated with various soil amendments were analyzed using GC-MS.

EXPERIMENTAL SECTION

Plant Material:
Black gram seeds *Vigna mungo* L. (Var. Adt Mash3) purchased from the Tamil Nadu Rice Research Station, Aduthurai, Tamil Nadu was used as the plant material in the present study.

Growth conditions:
The seeds were sown in pots containing a pot mixture comprising of 5 Kg of sand and fertile clay soil mixture in the ratio 1:2 (w/w). They were amended with 3 gm of either commercial vermicompost or coir waste manure. Commercial vermicompost was purchased from the local market. Coir waste manure was prepared by mixing log phase culture of *Anabaena azollae* (ML-2) with coir waste in the ratio 1:5 and composting it for six months [11, 12]. The root nodules were collected from 52nd day old plants for further analysis. The experiment was done in triplicates.

Preparation of root nodule extract for GC-MS analysis:
Root nodules of black gram were shade dried and 2 g of the powdered leaves was soaked in 95% ethanol for 12 hr. The extracts were then filtered through Whatman No 41 filter paper along with 0.2 g of sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper was moistened with 95% ethanol for 12 hr. 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. An aliquot of 2µl of this solution was employed for GC-MS analysis [13].

GC-MS analysis:
GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising of a Aoc-20i auto sampler and gas chromatograph interfaced to a mass spectrometer. GC-MS instrument employed the following conditions: column-Elite-1 fused silica capillary column (30 mm x 0.25 mm ID x1 µMdf), composed of 100% dimethyl poly siloxane, operating in electron impact mode at 70 eV; carrier gas- helium (99.999%) at a constant flow of 1 ml/min; injection volume- 0.5 µl (split ratio of 10:1); injector temperature- 250°C and an ion source temperature of 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min to 200°C, then 5°C/min to 280°C ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min.

RESULTS AND DISCUSSION

The present study was carried out to identify the phytochemicals present in the root nodules of black gram plants grown in the presence of commercial vermicompost manure and blue green algae decomposed coir waste manure. The result of the GC-MS analysis of root nodules of black

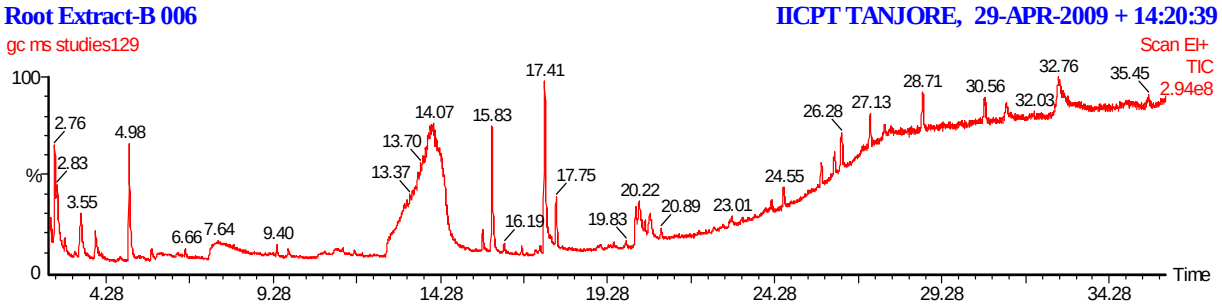

Figure 1: GC–MS spectra of phytochemicals in root nodule of black gram treated with vermicompost

gram amended with commercial vermicompost is depicted in Fig-1. About seventeen peaks were identified and tabulated in Table-1. The maximum peak area was shown by 3-O-Methyl-d- glucose (71.55%) followed by n-hexadecanoic acid (5.80%), Propane, 1, 1, 3-triethoxy- (4.07%), phthalic acid, butyl isohexyl ester (3.78%) and ethylbenzene (3.27%). The other peaks indicated a peak area less than 3%.

Fig-2 shows the results of the GC-MS analysis of root nodules of black gram amended with coir waste manure. The fourteen major peaks are tabulated in Table-2. The most prominent peak area was depicted by n-hexadecanoic acid (37.29%) followed by 9, 12-octadecadienoic acid (15.99%), pthalic acid (9.13%) and oleic acid (7.04%).

Table -1: GC-MS analysis of phytochemicals identified from black gram treated with vermicompost manure

S.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	3.98	hexanoic acid, ethyl ester	$C_8H_{16}O_2$	125	1.15
4	4.98	propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	4.07
5	6.66	octanoic acid, ethyl ester	$C_{10}H_{20}O_2$	172	0.26
6	9.40	decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	200	0.26
7	9.74	dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	216	0.35
8	11.38	[1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	322	0.27
9	11.71	desulphosinigrin	$C_{10}H_{17}NO_6S$	279	0.13
10	14.07	3-O-methyl-d-glucose	$C_7H_{14}O_6$	194	71.55
11	15.83	phthalic acid, butyl isohexyl ester	$C_{18}H_{26}O_4$	306	3.78
12	16.19	ethanol, 2-(9-octadecenyloxy)-,(Z)-	$C_{20}H_{40}O_2$	312	0.20
13	17.41	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.80
14	17.75	hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.21
15	20.22	9,12-octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298	3.09
16	20.55	oleic Acid	$C_{18}H_{34}O_2$	282	2.21
17	20.89	octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.40

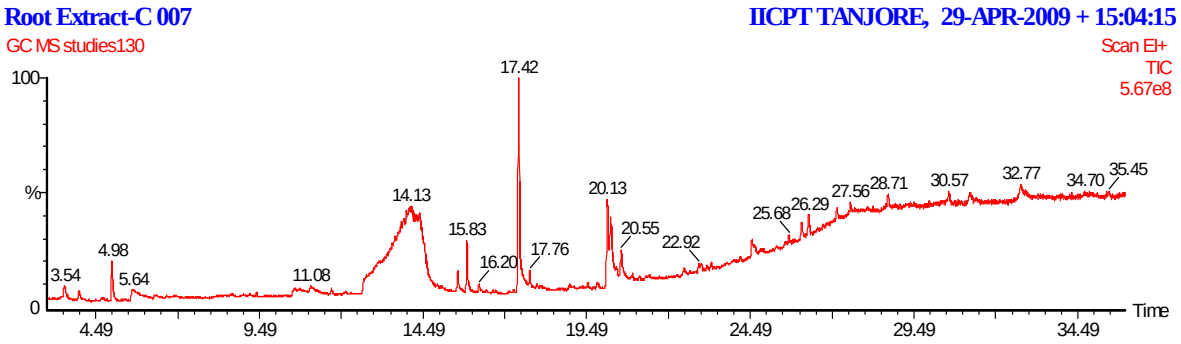

Figure 2: GC–MS spectra of phytochemicals in root nodule of black gram treated with coir waste manure

Table- 2: GC-MS analysis of phytochemials identified from black gram treated with coir waste manure

S. 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

Coir waste manure treated plants showed elevated levels of all phytochemicals when compared to vermicompost treated plants. Compounds like hexanoic acid, octanoic acid, decanoic acid etc. are present in both the manure

treated plants but were at higher levels in coir waste manure treated plants. This might be due to the supply of phytochemicals from the manure. Coconut contains high amounts of oleic acid and octanoic acid. Coir waste manure also helps in the increased translocation of nutrients from the soil [11, 12].

Some of the phytochemicals detected in the present study are of pharmaceutical importance Table-3. The data in Table-3 was derived from Dr. Duke's phytochemical and ethnobotanical database. Hence the phytochemicals observed in the present study were found to possess antimicrobial, anti-inflammatory, anticancer properties etc. The present study emphasizes more on the pharmaceutical value of black gram which might have been enhanced by the addition of these manures.

Table-3: Properties of some phytocomponents identified in root nodules of black gram plants by GC-MS

S.No	RT	Compound name	Activity
1	6.66	octanoic acid ethylester	Antibacterial
2	9.74	dodecanoic acid	Antimicrobial
3	14.07	3-O-methyl-D-glucose	Preservative
4	17.75	n-hexadecanoic acid	Antioxidant
5	20.55	oleic acid	Anti inflammatory, anti androgenic, anticancer, preservative and hypocholesterolemic
6	26.38	1,2 benzene dicarboxylic acid diisooctylester	Antimicrobial

Source: Dr. Duke's phytochemical and ethnobotanical database.

GC-MS analysis to detect phytochemicals has been done in *Delonix regia* [14] and *Mussaenda frondosa* [15]. In both the cases the presence of antioxidants was highlighted. There are reports on the changes in metabolites and antioxidants in *Vigna* species that are induced by NaCl stress [16, 17]. Hence there is a strong correlation between the abiotic stress factors and the change of phyto components in plants.

CONCLUSION

The phyto components identified in the present study add to the nutritional value of the black gram treated with various manure. It is observed that there is a linear relationship between black gram and organic manures used. There is a possibility of translocation of nutrients from manure to the plant. Organic manure also releases certain specific phytochemicals that might be beneficial to root nodulation thereby aiding in nitrogen fixation. Further work to emphasis more on this concept of correlating the nutritional value of pulses with the amendments in soil is needed.

REFERENCES

[1] FAlqurainy. *Res.J.Agric.Biol.Sci.*, **2007,** 3:714-723.
[2] G Battu; ChKVLSN Anjana Male; T. Hari Priya; Veena Naga Malleswari; SK Reeshma. *Pharmanest*., **2011**, 2 (1): 62-67.
[3] T Pullaiah. *Encyclopedia of world medicinal plants*, **2000,** 1045-1046.
[4] RN Chopra. *Glossary of Indian medicinal plants*, 1 Ed. CSIR, **1956,** 113
[5] SN Yoganarasimhan. *Medicinal plants of India*, **2000,** 2,194.
[6] R Saxena Rai. Crop Res, **2002,** 21(2),153-156.
[7] R Haynes; J Pressey; N Jen and Mondy. *J.food sciences*, **1986,** 54(3):64-69.
[8] PK Chauhan; V Singh; Vinod Kumar Dhatwalia; B Abhishek. *Journal of Chemical and Pharmaceutical Research*, **2011**, 3 (3): 799-804.
[9] W Gerald. *Journal of medical science*, **2004,** (13):81-83
[10] E Boller; F Hani..*Ideal book on functional biodiversity at the farm level*, **2001,** 34-40.
[11] S Anbuselvi; Jeyanthi Rebecca. *International Journal of Applied Agricultural Research*, **2009**, 4(2):173-178.
[12] S Anbuselvi; Jeyanthi Rebecca. *Journal of Applied sciences Research*,**2009**,5(12):2369-2374.
[13] MJ Merlin; V Parthasarathy; R Manavalan; S Kumaravel. *Pharmacognosy Res*, **2009,** (3):152-156.
[14] P Maria Jancy Rani; PSM Kannan; S Kumaravel. *African Journal of Biochemistry Research*, **2011**, 5 (12): 341-347.
[15] S. Gopalakrishnan; E. Vadivel. *International journal of pharma and Biosciences*, **2011**, 2: 313-320.

[16] D Arulbalachandran; K Shankar Ganesh; A Subramani. *American Eurasian Journal of Agronomy*, **2009**, 2(2): 109-116.
[17] Alok Bharadwaj; Vishal Khandelwal; Pradeep Choudhary ; AK Bhatia. *Journal of Chemical and Pharmaceutical Research*, **2011**, 3 (6): 997-1003.