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Comparative Analysis of Phytochemical compounds in Normal and root gall of Okra plant

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Abstract: *Okra, Abelmoschus esculentus (L.) Monech belongs to family Malvaceae and is widely cultivated in Tropical and Subtropical countries. In India, okra has been ranked first in its consumption. A multitude of threats on stable and secure yields of this crop exists including losses caused by pathogens like bacteria, virus, fungi and nematode. The root knot nematode (Meloidogyne incognita) infects root part of okra plant which leads to gall formation on root. The plant – pathogen interaction leads to production of increased secondary metabolites owing to the stress conditions. The secondary metabolites are supposed to provide resistance against pathogen. GC-MS analysis of the normal and galled root of Abelmoschus esculentus (L.)Monech leads to the finding that under stressed conditions larger no. of secondary metabolites were produced. Further studies on the efficacy of these secondary metabolites can result into various findings and discovery of novel and useful secondary metabolites resulting in increased resistance against pathogen to host plant.*

Keywords:*Abelmoschus esculentus, GC-MS, Galled root, Host-pathogen interaction, Meloidogyne incognita.*

I. Introduction

Okra, Abelmoschus esculentus (L.) Monech is annual member of the Malvaceae family. It is native plant to tropical Africa, Asia and northern Australia. Okra has high fiber vitamin C and mucilage content [1]. Okra is also known for being high in antioxidants [2, 3]. The fruit of okra is extensively used as vegetables in tropical and subtropical countries. Mucilage content is also found in root of plants which have a strongly demulcent action [4]. The infusion of root is used for treatment of syphilis. The juice of root is used externally to treat cut, wounds and boils. Mucilage found in okra, is responsible for washing away toxic substances and bad cholesterol which loads the liver. Mucilage is supposed to be replacement of plasma. Due to having many medicinal and nutrition quality okra is widely cultivated in tropical and subtropical countries [5, 6, 7]. This crop is also attacked by various pathogens like bacteria, virus and root knot nematode. These pathogens cause biotic stress to the plant. A stress can lead into various results. Stress can have a devastating impact on plant growth and yield [8] or can result into enhancement of production of secondary metabolites [9]. These secondary metabolites are capable of triggering changes into plants cell which helps to overcome the stress [10]. Present study reveals the comparative analysis of stress (Galled Root) and non-stressed (Normal Root) condition of Okra Plant.

II. Material And Methods

2.1 Dry powder preparation

Plants were collected from field area of greenhouse of Department of Botany. The roots were separated from plants and washed with tap water to remove soil particle followed by distilled water. Normal and infected roots were cut into small pieces and were shade dried separately. Dried roots were pulverized to powder using mechanical grinder**.**

2.2 Preparation of Extract

About 5 gm powder of normal and infected root was weighed and was extracted with methanol (70-80ºC) by hot continuous percolation method in soxhlet apparatus for 24 hours. The extract was taken and filtered through whatmann filter paper. Then extract was concentrated by rotary evaporator to obtain extract.

2.3 GC-MS analysis

The GC-Ms analysis of methanolic extract of normal and galled root of Abelmoschus esculentus was carried out on Shimadzu QP-2010 plus with thermal desorption system TD 20. It includes auto sampler and a gas chromatograph which interfaced to a mass spectrophotometer. The column size of this system is 30m × 0.25mm i.d × 0.26µm with a film thickness of 0.26mm, composed of 5MS (5% diphenyl/ 95% dimethyl poly siloxane). Helium gas (99.999%) was used as carrier gas at constant flow rate of 1ml/min. The 2µl injection volume of sample was utilized with split ratio of 10:1. The injector temperature was programmed initially at 280 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C (for 4 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 analyzed using electron impact ionization at 70 eV. The total running time for each sample was 45 min.

2.4 Identification of phytochemical

Interpretation of phytochemical present in the sample was conducted using NIST, having more than 62,000 patterns and Wiley8 Library. The comparison of unknown spectrum with known spectrum of various components was done by stored spectrum of NIST library and Wiley8 Library. The name, molecular weight and structure of the components were ascertained**.**

III. Result

GC-MS is a combined technique of Gas Chromatography with Mass Spectrophotometry. MS is wide ranging analytical technique, which identify the charged species according to their mass to charge ratio (M/Z). GC-MS is one of the best techniques to identify the constituents of volatile compounds. The GC-MS analysis of normal and galled root (infected root) of Abelmoschus esculents showed the presence of eight (Fig 1) and thirty five (Fig 2) phytochemical compounds respectively. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their retention time (RT), area %, compound name, of normal and galled root are presented in Table 1 and 2 respectively.

IV. Discussion

The GC-MS analysis showed that the methanolic extract of normal root had fewer compounds than the galled root extract of okra plant. Normal root extract showed the major compound present as 3-deoxy-D-mannoic acid with 66.91% peak area. The next highest found compound was Hydroxy methyl furfural (HMF), retention time is 6.156 with 22.78% peak area. This compound is derivative of furan, which has potential to be sustainable substitute for petroleum [11]. HMF is obtained from sugars (carbohydrates) so that carbohydrates can be transformed into HMF [12]. This compound can be converted into DMF (2, 5-dimethyl furan), a liquid that is potential biofuel with greater energy content bioethanol [13]. Whereas GC-MS analysis of the extract of galled root showed 35 compounds. The major compound was Octdec-9-enoic acid with 37.21 peak area. It is monounsaturated fatty acid which decreases LDL cholesterol and blood pressure [14]. Second major compound is hexadecanoic acid whose consumption increase the risk of cardiovascular disease. Other compound such as various fatty acids, thymol, and methyl stearate were also found.

V. Conclusion

This study showed that the Galled root (under Stressed condition) led to production of more phytochemical compounds than the normal root (under non-stressed condition) of Okra plant.

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Figures And Tables

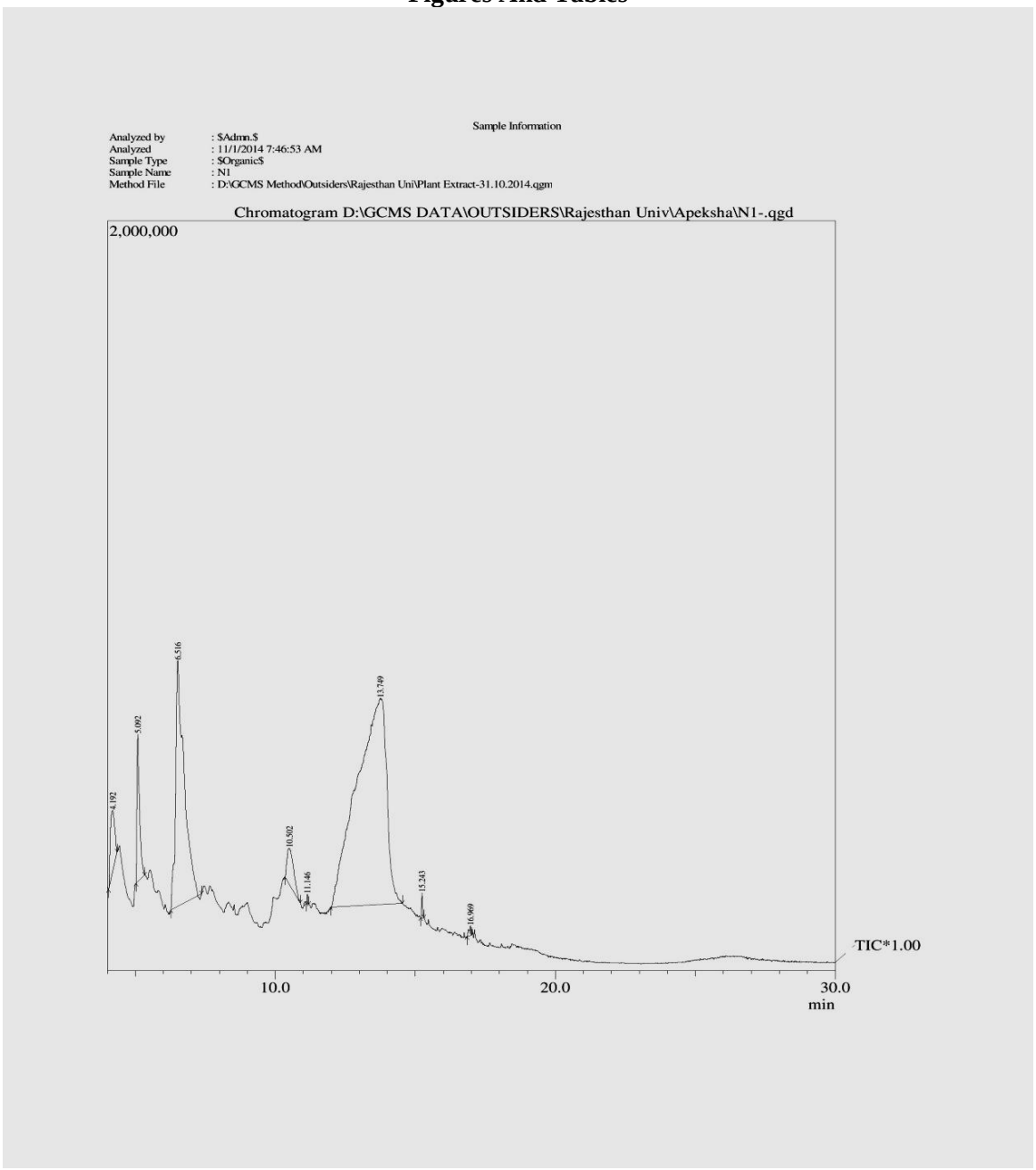

Figure 1:- Shows GC-MS Chromatogram of Normal Root Part of *Abelmoschus esculentus*

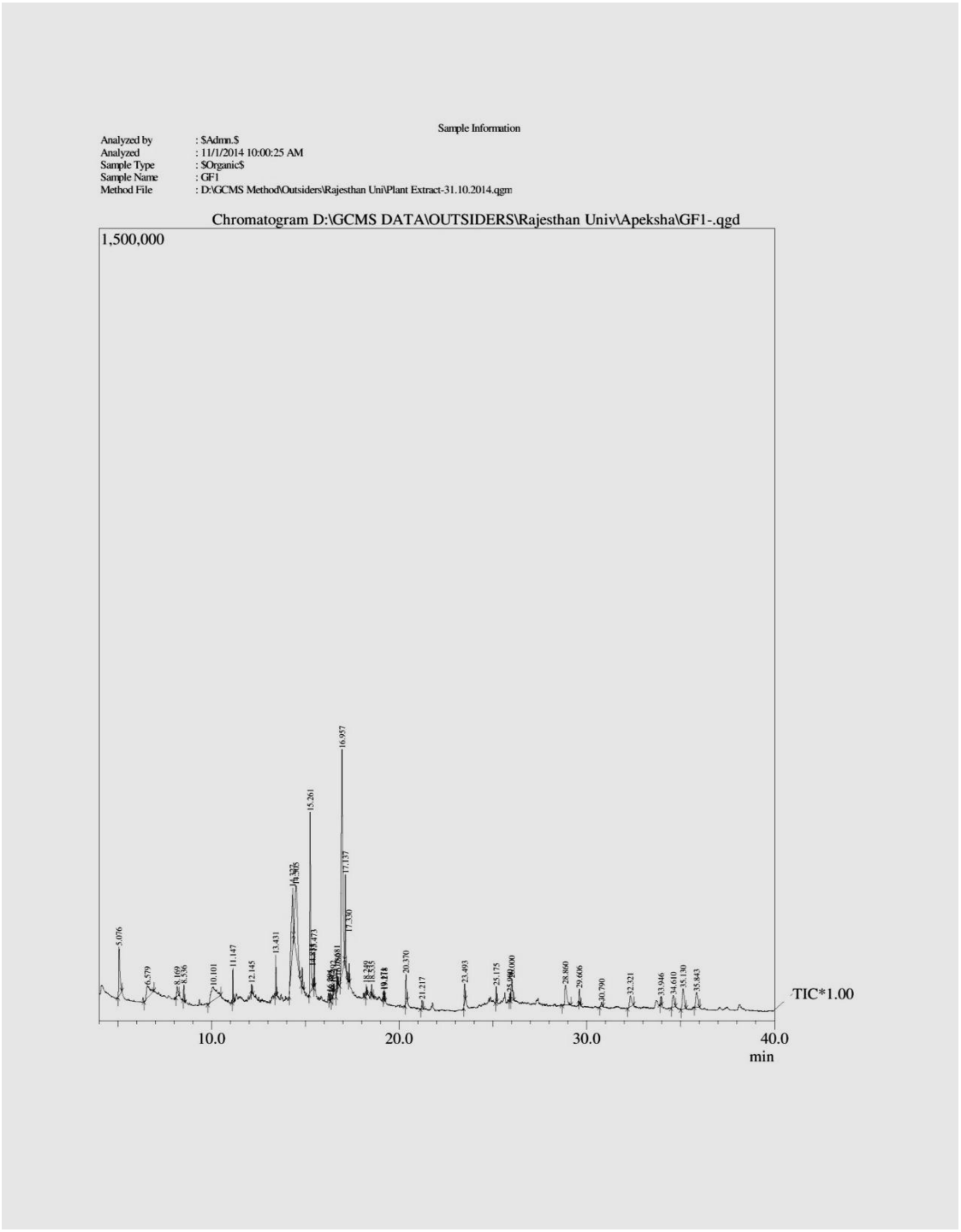

Figure 2:- Shows GC-MS Chromatogram of Galled Roots of *Abelmoschus esculentus*

Peak	R.Time	Area	Area%	Name Of Compounds
1	4.192	1810819	2.96	Cyclopentane, 1-Acetyl-1,2-Epoxy-
2	5.092	2564436	4.20	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-
3	6.516	13913901	**22.78**	**Hydroxy Methyl Furfural**
4	10.502	1600026	2.62	D-Allose
5	11.146	47913	0.08	3-Hexadecene, (Z)-
6	13.749	40866860	**66.91**	**3-Deoxy-D-Mannoic Acid**
7	15.243	148648	0.24	9-Octadecenoic Acid (Z)-
8	16.969	121207	0.20	7-Tetradecenal
		61073810	100.00	

Table 1:- Compounds identified from methanolic extract of Normal Root Part of *Abelmoschus esculentus* using GC-MS analysis.

Peak	R.Time	Area	Area%	Name Of Compounds
1	4.489	1536610	0.91	Butanedioic Acid, Monomethyl Ester
2	5.099	2637618	1.56	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-
3	5.792	374533	0.22	Pyridine, 1-Acetyl-1,2,3,4-Tetrahydro-
4	6.899	280440	0.17	Nonanoic Acid
5	7.258	3108191	**1.84**	**Thymol**
6	8.334	237029	0.14	Decanoic Acid
7	10.325	4555412	2.69	1,3-Propanediol
8	10.925	476945	0.28	N,N-Bis(2-Hydroxyethyl)Dodecanamide
9	12.716	1097046	0.65	Tetradecanoic Acid, Methyl Ester
10	13.269	4378070	2.59	Tetradecanoic Acid
11	13.410	785199	0.46	Pentadecanoic Acid, Methyl Ester
12	13.907	2246318	1.33	Pentadecanoic Acid
13	14.332	549732	0.32	8-Octadecanone
14	14.648	1372959	0.81	9-Hexadecenoic Acid, Methyl Ester, (Z)-
15	14.858	9052909	5.35	Hexadecanoic Acid, Methyl Ester
16	15.197	2402527	1.42	Cis-9-Hexadecenoic Acid
17	15.439	24611629	**14.54**	**Hexadecanoic Acid <N->**
18	15.822	245662	0.15	Octadecanoic Acid, Methyl Ester
19	15.926	1226226	0.72	Heptadecanoic Acid
20	16.066	297333	0.18	Hexadec-(9Z)-Enal
21	16.515	2896832	1.71	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester
22	16.648	24462940	**14.45**	**11-Octadecenoic Acid**
23	16.784	5264190	3.11	Methyl Stearate
24	17.264	62991483	**37.21**	**Octadec-9-Enoic Acid**
25	17.353	6745045	3.98	Stearic Acid
26	17.967	307133	0.18	Heptadecene-(8)-Carbonic Acid-(1)
27	18.092	652289	0.39	5,8,11,14,17-Eicosapentaenoic Acid, Methyl Ester
28	18.227	284393	0.17	2,4,4-Trimethyl-3-(3-Oxo-But-1-Enyl)-Cyclohex
29	18.317	157394	0.09	9-Hexadecenal
30	18.414	887002	0.52	13-Docosenoic Acid, Methyl Ester, (Z)-
31	18.579	1317555	0.78	Methyl 10,12-Pentacosadiynoate
32	18.860	948034	0.56	Cis-11-Eicosenoic Acid
33	19.006	393140	0.23	Eicosanoic Acid
34	20.839	253703	0.15	Heneicosanoic Acid, Methyl Ester
35	24.015	255667	0.15	Heneicosanoic Acid, Methyl Ester
		169289188	100.00	

Table 2:- Compounds identified from methanolic extract of Galled Root Part of *Abelmoschusesculentus* using GC-MS analysis