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Metabolic Profiling of Healthy and Pest Infested *Solanum melongena* L. Using a Gas Chromatography-mass Spectroscopy Technique

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Authors' contributions

This work was carried out in collaboration between both authors. Both the authors are equally contributed. Both authors read and approved the final manuscript.

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ABSTRACT

A comparative metabolic profiling of healthy and pest infested *Solanum melongena* (egg plant) have been carried out. The egg plant was infested by *Helicoverpa armigera.* Total thirty non polar metabolites were detected from the leaf and stems extract, by gas chromatography-mass spectroscopy (GC-MS) technique, which includes large variety of compounds like phytosterols, diterpene, alkane hydrocabon, n-alkanoic acid and terpene etc. Significant variation on metabolites has been detected in both leaf and stem extract. Metabolites such as benzoic acid (9.13±0.71%), pentadecane (2.83±0.13%), hexadecane (1.68±0.09%), squalene (1.28±0.08%), triterpene (3.97±0.14%), linoleic acid (1.32±0.06%), linolenic acid (2.46±0.14%), stearic acid (4.69±0.38%), dodecanoic acid (0.83±0.10%), myristic acid (1.25±0.05%), palmitic acid (1.32±0.06%), linoleic acid (3.33± 0.13%), linolenic acid (2.06±0.14%), stearic acid (4.83±0.14%), lactic acid (0.50±0.09%), tetradecene (0.93±0.07%), hexadecane (1.30±0.06%), octadecane (1.19±0.05%), cholesterol (0.48 ±0.05%), stigmasterol (0.57±0.06%), (octadecene 6.90±1.58%) and tetradecane (0.84±0.10%) were

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detected. Alteration in amount of above major metabolites was observed under biotic stress condition. It concludes that, these metabolites might have played an important role in pest infested stress tolerance. This study will be helpful for the better understanding of overall biotic stress tolerance mechanism.

Keywords: Solanum melongena; H. armigera, metabolites; pest infestation; gas chromatography-mass spectrometry.

1. INTRODUCTION

Biotic stresses such as pest infestation are considered as a severe threat to plant growth and crop production [1]. In response to biotic stress, plants produce a diverse range of primary and secondary metabolites which play an important role in plant defence [2]. Consequently, there is a re-allocaton of resources to leaf and stem storage tissues which increase the plant's defence mechanism [3]. There are reports on the metabolites, indicating a rapid and significant plant response due to herbivory damage [4,5,6 and 7]. Among the most serious and widely distibuted polyphagous insect pest is *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). Its larvae stage causes severe damage to the reproductive and vegetative tissues of agricultural and horticultural crops, such as egg plant, tomato, cotton, chickpea, pigeonpea, tobacco, maize, sorghum, wheat, groundnut, sunflower and chillies [8]. In this connection the mass spectrometry based metabolic profiling is being used as the one of the widely accepted advance techniques in studies pertaining to insect herbivory attack [9], to bacterial pathogens [10] and to nematode parasitism [11]. The recent development in variety of analytical platforms, including GC-MS has enabled high throughput, non-biased analysis of thousands of metabolites from plants and other organisms.

The understanding of metabolic responses or changes to these stresses is essential for a holistic perception of plant resistance mechanisms to pest infestation conditions. Therefore this research work involves the metabolic profiling of the non-polar metabolites from healthy and *H. armigera* infested egg plants. Further, the finding of this study will helpful for agriculture researchers in better understanding of metabolic pathways during biotic stress.

2. MATERIALS AND METHODS

Egg plants (*S. melongena* BCB-11) seeds were sown in trays (52 cm x 27 cm) placed in a cultivation chamber at 24°C. Later, the seedlings were transplanted into pots. On fully matured brinjal plants (after 60 days), the larvae of *H. armigera* two per plant was inoculated. After one month, the egg plants were completely infested by larvae. The leaves and stems were collected after 30 days of the inoculation for the extraction process. Dried samples of 3 g each leaves and stems were taken for extraction by hexane (1:10 w/v). The solvent portion were collected by filtration and repeated five times until the hexane layers to become almost colourless. The separated solvent layer was concentrated under reduced pressure. The resulting hexane extracted sticky mass was stored at -5ºC and it was further used for derivatization prior to GC-MS analysis.

Volatile trimethylsilyl (TMS) derivatives of the samples were prepared by using 3.6 mg of the sample, 40 µl of methoxylamine hydrochloride in GC grade pyridine (20 mg/ml). The mixture was shaken for 2 h at 37°C in a temperature controlled vortex, followed by the addition of 70 µl of the N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and followed by continuous shaking for 30 minutes. The GC-MS analysis was performed using a GCs-Agilent 7890 A coupled with a 5975 C MS: MS detector and Electron Impact Ionization to generate mass spectra. The scan mass range was 30 m/z to 600 m/z. The total run time was 69 minutes. The resulting GC-MS profile was analyzed using the NIST mass spectral library and by matching the chromatogram with appropriate standards. The estimation of the metabolites was done using the percentage peak area that appeared at the total ion chromatogram in the GC-MS analysis. The molecular weights and fragmentation patterns were ascertained by use of the NIST library and the Duke phytochemical data base [12].

3. RESULTS AND DISCUSSION

Total 30 major non-polar metabolites were detected by gas chromatography - mass spectroscopy (GC-MS) in both healthy and pest-infested leaves and stems of *S. melongena*

Table1. Mass data of GC-MS identified metabolites of healthy and pest-infested *S. melongena* leaves and stems

Sl. No	tR (min)	Metabolite	Molecular weight	MS data (m/z)
1.	15.05	Tetrasiloxane, decamethyl	310.68	310(M^+), 295(19.60%), 209(11.87%), 208(20.46%), 207(100%), 73(82.79%)
2.	20.56	Pentasiloxane,decamethyl	416.84	416(M^+), 369(25.27%), 282(27.65%), 281(100%), 147(77.57%), 73(69.74%)
3.	20.88	Benzoic acid	282.48	282(M^+), 180(14.90%), 179(100%), 135(51.54%), 105(72.43%), 77(53.62%)
4.	24.48	Tetradecene	196.37	196(M^+), 69 (77.86%), 55(100%), 43(83.07%), 41(97.76%)
5.	25.17	Tetradecane	198.39	198(M^+), 73(80.88%), 71.10(65.62%), 57(100%), 43.10(75.27%)
6.	27.74	Pentadecane	212.41	212(M^+), 85(44.33%), 71(66.80%), 57(100%), 43(71.67%), 41(40.50%)
7.	29.98	Hexadecene	224.43	224(M^+), 83(82.75%), 57(78.14%), 55(100%), 43(83.87%), 41(92.94%)
8.	30.15	Hexadecane	226.44	226(M^+), 85(45.10), 71(67.93%), 57(100%), 43(71.16%), 41(40.36%)
9.	31.32	Dodecanoic acid	272.49	272(M^+), 257(94.66%), 129(42.86%), 117(91.70%), 73(100%), 75(86.94%)
10.	34.46	Octadecene	252.48	252(M^+), 83(86.37%), 57(83.78%), 55(100%), 43(86.58%), 41(86.79%)
11.	34.61	Octadecane	254.49	254(M^+), 85(51.52%), 71(71.31%), 57(100%), 55(25.93%), 43(61.58%)
12.	35.58	Myristic acid	300.55	300(M^+), 285(98.47%), 129(48%), 117(98.03%), 75(82.59%), 73(100%)
13.	38.53	Eicosene	282.54	282(M^+), 97(88.02%), 83(90.75%), 57(92.31%), 55(100%), 43(92.11)
14.	39.27	Lactic acid	234.44	234(M^+), 119(81.41%), 103(49.51%), 75(83.44%), 73(100%)
15.	39.49	Palmitic acid	328.60	328(M^+), 313(95.72%), 129(49.23%), 117(100%), 75(76.67%), 73(97.20%)
16.	41.82	Phytol	296.53	296(M^+), 144(12.79%), 143(100%), 123(11.75%), 75(22.70%), 73(27.56%)
17.	42.25	Docosene	308.59	30 (M^+), 97(94.11%), 83(92.09%), 57(99.91%), 43(94.34%)
18.	42.48	α Linoleic acid	352.62	352(M^+), 337(47.23%), 81(51.82%), 75(100%), 73(99.4%), 67(61.81%),
19.	42.59	α Linolenic acid	350.61	350(M^+), 95(42.31%), 79(72.79%), 75(100%), 73(94.37%), 67(47.10%)
20.	43.08	Stearic acid	328.60	328(M^+), 341(99.32%), 129(49.43%), 117(100%), 75(72.57%), 73(97.12%)
21.	45.66	Cyclotetracosane	336.63	336(M^+), 97(87.24%), 57(100%), 55(92.10%), 43(91.05%)
22.	47.82	Benzenedicarboxylic acid	278.34	278(M^+), 167(30.31%), 149(100%), 71(17.20%), 70(15.23%), 57(26.26%)
23.	48.84	Hexacosene	364.69	364(M^+), 97(88.46%), 83(83.88%), 57(100%), 55(87.33%), 43(88.95%)
24.	52.01	Squalene	410.71	410(M^+), 95(13.65%), 81(49.85%), 68(13.15%), 69(100%), 41(25.89%)
25.	53.23	Nonacosane	408.6	408(M^+), 85(51.19%), 71(71.16%), 57(100%), 55(30.67%), 43(65.71%)
26.	55.90	Hentricontane	436.85	436(M^+), 85(51.52%), 71(71.31%), 57(100%), 55(25.93%), 43(61.58%)
27.	56.28	Tocopherol	502.88	502(M^+, 100%), 503(40.30%), 237(63.90%), 73(55.52%)
28.	56.38	Cholesterol	458.83	458(M^+), 129(74.07%), 73(78.85%), 71(60.72%), 57(100%), 43(83.27%)
29.	58.02	Stigmasterol	484.87	484(M^+), 218(100%), 203(41.44%), 189(18.87%), 75(20.16%), 73(31.08%)
30	59.08	β Amyrin	440.43	440(M^+), 218(100%), 203(41.36%), 189(18.55%), 75(20.48%), 73(32.90%)

Table 2. Variation of non-polar metabolites of healthy and pest-infested *S. melongena* leaves and stems.

Sl. No.	Metabolite	Healthy leaves (Area %)	Peat infested leaves (Area %)	Healthy Stem (Area %)	Peat infested Stem (Area %)
1.	Tetrasiloxane,decamethyl	3.56 ± 0.07	ND	4.97± 0.11	ND
2.	Pentasiloxane, decamethyl	1.56 ± 0.08	ND	2.74 ± 0.10	ND
3.	Benzoic acid	9.13± 0.71	ND	7.83± 0.06	1.42 ± 0.07
4.	Tetradecene	ND	1.26 ± 0.09	ND	0.93 ± 0.07
5.	Tetradecane	1.31 ± 0.08	2.22 ± 0.19	0.55 ± 0.12	0.84 ± 0.10
6.	Pentadecane	ND	2.83 ± 0.13	0.50 ± 0.07	0.73 ± 0.06
7.	Hexadecene	ND	2.55± 0.13	0.75 ± 0.11	4.28 ± 0.07
8.	Hexadecane	ND	1.68 ± 0.09	ND	1.30 ± 0.06
9.	Dodecanoic acid	ND	ND	0.83 ± 0.10	0.76 ± 0.11
10.	Octadecene	ND	3.76 ± 0.27	1.05 ± 0.05	6.90 ± 1.58
11.	Octadecane	ND	ND	ND	1.19 ± 0.05
12.	Myristic acid	1.63 ± 0.12	ND	1.25 ± 0.05	0.82 ± 0.10
13.	Eicosene	ND	3.61± 0.34	1.00 ± 0.11	5.56 ± 0.13
14.	Lactic acid	ND	ND	ND	0.50 ± 0.09
15.	Palmitic acid	5.84 ± 0.20	3.72 ± 0.19	7.62 ± 0.11	3.17 ± 0.06
16.	Phytol	1.53 ± 0.07	11.56 ± 0.14	ND	0.61 ± 0.08
17.	Docosene	ND	2.74 ± 0.14	0.73 ± 0.10	4.29 ± 0.07
18.	α Linoleic acid	1.32 ± 0.06	ND	3.33 ± 0.13	0.50 ± 0.08
19.	α Linolenic acid	2.46 ± 0.14	ND	2.06 ± 0.14	0.52 ± 0.04
20.	Stearic acid	4.69 ± 0.38	ND	4.83± 0.14	1.48 ± 0.05
21.	Cyclotetracosane	ND	1.85 ± 0.12	ND	3.08 ± 0.05
22.	1,2-Benzene dicarboxylic acid	ND	3.75 ± 0.09	1.64 ± 0.07	1.70 ± 0.08
23.	Hexacosene	ND	ND	ND	1.67 ± 0.06
24.	Squalene	ND	1.28 ± 0.08	ND	ND
25.	Nonacosane	ND	ND	ND	0.61 ± 0.03
26.	Hentricontane	1.72 ± 0.22	7.32 ± 0.09	ND	7.12 ± 0.17
27.	α Tocopherol	ND	2.23 ± 0.15	ND	ND
28.	Cholesterol	ND	ND	ND	0.48 ± 0.05
29.	Stigmasterol	ND	ND	ND	0.57 ± 0.06
30.	β Amyrin	ND	3.97 ± 0.14	1.23 ± 0.06	3.36 ± 0.08

Where ND = not detected, Mean values ± SD (standard deviation)

(Table 1). A large variation in non-polar metabolites has been detected (Table 2). Variations of metabolites were considered based on area %. Carboxylic acids like benzoic acid detected only in healthy leaves (9.13± 0.71) where as benzenedicarboxylic acid (3.75±0.09), α - olefins like tetradecene (1.26±0.09), hexadecane (1.68±0.09), octadecene (3.76±0.27), alkane hydrocarbons like pentadecane (2.83±0.13), hexadecane (1.68±0.09), squalene (1.28±0.08), vitamin E (tocopherol) (2.23±0.15) and triterpene (β Amyrin) (3.97±0.14) were detected only in pest-infested leaves. A concentration of tetradecane was higher (2.22±0.19) in pest infested leaves and in compare to healthy leaves (1.31±0.08). Palmitic acid was present in higher concentration (5.84±0.20) in healthy leaves compare to pest infested (3.72±0.19). Fatty acids like linoleic acid (1.32±0.06), linolenic acid (2.46±0.14) and stearic acid (4.69±0.38) were present in higher concentrations in healthy leaves in compare to infested leaves.

Higher amount of benzoic acid (7.83±0.06%), dodecanoic acid (0.83±0.10%), myristic acid (1.25±0.05%), palmitic acid (1.32±0.06%), linoleic acid (3.33±0.13%), linolenic acid (2.06±0.14%) and stearic acid (4.83±0.14%) was present in healthy stems. Some other non-polar metabolites like lactic acid (0.50±0.09%), tetradecene (0.93±0.07%), hexadecane (1.30± 0.06%), octadecane (1.19±0.05%), cholesterol (0.48±0.05%) and stigmasterol (0.57±0.06%) were detected only in pest-infested stem. Concentrations of octadecene (6.90±1.58%), tetradecane (0.84±0.10%) and pentadecane (0.73±0.06%) were higher in pest-infested stems in compare to healthy stems.

4. CONCLUSION

Metabolic profiling of healthy and pest infested *Solanum melongena* using a gas chromatography-mass spectroscopy technique demonstrated that the infestation by *H. armigera* causes significant alterations in metabolism. It was observed that the amount of major metabolites such as octadecene, hexadecane, eicosene, phytol, docesene, squalene, hentricontane, α tocopherol, cholesterol, stigmasterol and β amyrin increases, while the amount of benzoic acid, myristic acid, palmitic acid, stearic acid, α linoleic acid and α linolenic acid decreases during pest infestation. These metabolites might have played an important role in biotic stress tolerance. Moreover, this finding can be used for the better understanding of various metabolic pathways during biotic stress in *S. melongena*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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