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Lipophilic metabolite profiling of maize and sorghum seeds and seedlings, and their pest spotted stem borer larvae: A standardized GC-MS based approach

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In order to better understand the biochemical interactions and to identify new biomarkers for plant resistance against insects, we proposed a suitable lipophilic profiling method for insects and their host plants. The critical components of GC-MS based analysis are: sample amount, extraction, derivatization, temperature gradient, run time, and identification of peaks. For lipophilic metabolite profiling of maize and sorghum, and their insect pest, spotted stem borer larvae, we recommend 100 mg sample weight for seeds and insect samples (whole insect body), and 200 mg for seedlings. Maize and sorghum seeds required less time for fat extraction in comparison to their seedlings and the pest fed on these seedlings. GC-MS was standardized for better separation and intensity of peaks using different temperature gradients in the range of 180-300 C. A total of 48 lipophilic compounds encompassing various classes based on their functional groups such as fatty acids, fatty alcohols, hydrocarbons, sterols and terpenoids, vitamin derivative, etc. were separated in the seedlings (30), seeds (14), and the pest (26) in the retention time range of 3.22 to 29.41 min. This method could be useful to study nutritional aspects of different field crops in relation to various stresses apart from the analysis of lipophilic compounds for better understanding of insect-plant interactions.

Keywords: *Chilo partellus*, Insect-plant interactions, Maize, Metabolomics, Sorghum, Spotted stem borer larvae, Stress

Metabolomics is a relatively new technology that enables the simultaneous identification of hundreds of metabolites within a given system. It has rapidly become a profiling technique of choice for biomarker elucidation whether related to biotic or abiotic stresses, and nutritional or industrial aspects. The metabolite profiling has been used for various purposes such as display of gene functions[1], genetically or environmentally modified plant systems[2], biochemical phenotyping[3], phenotyping of inter-specific introgression lines[4], metabolome compartmentation[5], metabolic Quantitative Trait Loci[6], mutation studies[7], varietal differences[8], quality improvement[9], physiological processes[10], impact of diet on insect fatty acids[11], etc.

Host plant allelochemicals, specific anatomical characteristics and nutrients have all been invoked as determinants of host plant quality, and the variation in abundance and performance of herbivorous insects is host plant quality-dependent[12,13]. However, poor understanding of biochemical mechanisms of insect-plant interactions has been the biggest bottleneck in the development and deployment of insect-resistant crop plants. This has been mainly because the host plants have been the major focus for biochemical profiling, while the biochemical profile/nutritional requirements of the target insects received little attention. Knowledge on biochemistry of host plants *vis-à-vis* target insect pests in response to feeding on diverse foods could be helpful in better understanding the insect-plant interactions. Also, efficient and economic mass rearing of any insect requires an in-depth understanding of the dietary components which influence insect quality[14]. Hood-Nowotny *et al.*[11] determined the fatty acid profiles and *de-novo* or direct assimilation pathways in mosquitoes reared on various larval diets using pyrolysis GC-MS and found that diet had great impact on fatty acid profile of the insects which exhibited a high degree of dietary routing along with *de-novo* synthesis of a number of important fatty acids.

Dietary quality plays a significant role in regulating ecosystem energy and nutrient transfers in primary

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consumers. It could be used as a predictive tool for population response. To determine the role of different biochemical components of host plants and their bioavailability to the target insect pests for different life processes, an accurate and reliable method of quantitative analysis is essential. It is difficult to study nutritional impacts on small insects and generally methods of analysis are laborious and complex. Mapping the biochemical affiliation and asynchrony in host plants *vis-à-vis* their associated insect pests, require a common, standard and repeatable biochemical method. In the present study, we explored a simple, reproducible and robust method for profiling of lipophilic compounds from seeds and seedlings of maize and sorghum, and the spotted stem borer [*Chilo partellus* (Swinhoe)] larvae, a common pest of these crops.

Materials and methods

Sample collection and processing—Samples of seeds and seedlings of maize and sorghum and the spotted stem borer larvae fed on the seedlings of these crops were collected from the Division of Entomology, ICAR-IARI, New Delhi, India and were stored at −20 ^{0} C for further analysis. The preparation of samples was carried out at the Biochemistry Laboratory, Germplasm Evaluation Division, ICAR-NBPGR, New Delhi, India.

Sample amount—Appropriate sample quantity depends on the nature and type of the study as well as the test samples. Here, 100, 200 and 500 mg of each of the seed and seedling samples; and 50, 100 and 150 mg of insect (using whole insect larvae) were used.

Extraction of total fat and conversion of fatty acids to methyl esters—The above mentioned samples were ground to extract the total fat in mortar and pestle with 10 ml solvent mixture consisting of chloroform:hexane:methanol (8:5:2 v/v/v). The seedling and insect samples were kept dissolved in extraction solvent overnight for the better extraction of lipophilic compounds. The filtered extracts obtained were dried at 60 °C in nitrogen gas for 30 min. Methyl esters of the extracts were prepared following Neff *et al*.[15] with slight modifications.

Separation of compounds—Test samples (1.0 µl each) were injected with a split ratio of 20:1 and gas chromatography was performed using Rtx®-5MS column (30 m length, 0.25 mm diameter and 0.25 µm thickness). The GCMS-QP2010 Ultra system with autosampler AOC-20i, from Shimadzu (Japan) was used for separation of different lipophilic compounds. The injection, interface, and ion source temperatures were set at 250, 270 and 230 °C, respectively. Helium gas was used as carrier with a pressure of 123.5 kPa set at a total flow rate of 28.2 ml/min and column flow rate of 1.2 ml/min. The temperature program was standardized to 2 min isothermal heating at 180 °C, followed by an increase with ramping rate of 5 °C min^{-1} up to 280 °C, hold for 5 min, and again increased to 300 °C with a ramping rate of 20 °C min^{-1} with hold time of 10 min. Oven was equilibrated for 1.0 min prior to injection of next sample. Mass spectra were recorded between 2.8-30.0 min run time at 2 scans/s with an *m*/*z* 50–650 scanning range. The chromatograms and mass spectra were evaluated using the Labsolutions GCMSsolution software version 2.71 (Shimadzu, Japan).

Data analysis—Specific ion characteristic of each metabolite were selected and time windows were defined for compound detection in processing methods created using the above mentioned software. Processed data were checked manually and need based corrections were made. Compounds were identified using MS libraries (NIST08, Wiley8). The individual standard fatty acid methyl esters (FAMEs; 99.9% pure) of palmitic, stearic, oleic, linoleic, linolenic, lignoceric and erucic acids obtained from SUPELCO Analytical, Bellefonte, PA, USA were also used as retention markers. The lipophilic metabolite quantities were expressed as percentage of the whole lipophilic composition.

Results

Standardization of sample amount and preparation protocols—Determination of optimum sample quantity and extraction period prior to separation of lipophilic compounds is desirable as the lipophilic content varies across sample types, and other compounds present in the test samples which interfere in their separation. Based on sample quantities used for standardization, 100 mg was found suitable for seeds and 200 mg for the seedlings of maize and sorghum crops. In case of insects, whole insect(s) comprising ~100 mg were found suitable for better separation of different lipophilic metabolites. The seedlings and insect samples when dissolved in extraction solvent overnight yielded better extraction of lipophilic compounds as they required more time in comparison to seeds. The fatty acids present in the extract were derivatized to respective methyl esters to convert them volatile.

Standardization of GC-MS—GC-MS based method was optimized for better separation of maximum peaks. Column Rtx®-5MS, being suitable for large range of metabolites, was used for separation. The temperature gradient in the range of 180-300 °C was found suitable for separation of different compounds up to 30 min. However, an underivatized part in case of insect or plant samples was eluted after 30 min of sample run. Further increase in sample run time of 8 min at higher temperature, i.e., 300 °C was found optimum for eliminating the underivatized sample parts to get rid of the interference in separation of different components during the next sample run.

Separation of lipophilic compounds in different plant parts and insect samples—The current method separated a total of 48 lipophilic compounds in both the plant and insect test samples in the retention time range 3.22-29.41 min (Table 1). In general, 30, 14 and 26 compounds were separated in seedling, seed and the insect samples, respectively. The complete lipophilic compound profile was divided into various classes based on their functional groups including fatty acids, fatty alcohols, hydrocarbons, sterols and terpenoids, vitamin derivative, and others. A total of 17 fatty acids, 7 fatty alcohols, 6 hydrocarbons, 14 sterols and terpenoids, one vitamin derivative, and 3 other compounds were detected in the test samples. Fatty acids shared major fraction of lipophilic profile being 42.49-71.49%, followed by vitamin derivative 6.42-27.63%, sterols and terpenoids 1.87-23.30%, fatty alcohols 0.31-21.87%, hydrocarbons 1.26-2.22%, and others 0.01-1.97 %.

Comparative analysis of lipophilic compounds in test samples—The composition of lipophilic profiles and the concentration of respective compounds varied among the seeds and seedlings of maize and sorghum as well as the test insect, spotted stem borer larvae (Figs. 1 and 2; Table 2). Among fatty acids, the concentration of oleic acid was higher in seeds and insects, whereas linolenic acid was higher in seedlings. Linoleic acid was higher in maize seeds (23.08%) compared to sorghum seeds (8.98%). Stearic acid was higher in stem borer larvae fed on sorghum (19.64%) than on maize (4.58%) seedlings. Similar variation in concentrations of other fatty acids was also recorded in seeds and seedlings of maize and sorghum, and the *C. partellus* larvae fed on their seedlings (Table 2). Among fatty alcohols, only Phytol and 1-Heptacosanol were present in maize (20.92 and 0.95%) and sorghum (20.70 and 0.34%) seedlings, respectively; while absent from the seeds and test insect samples. Fatty alcohols contributed 0.31 and 4.20% to the whole profile of stem borer larvae fed on maize and sorghum seedlings,

Table 1—Retention time of different lipophilic compounds from both plants (maize and sorghum) and insect (*C. partellus* larva) samples

S. No.	Compounds	Retention Time	S. No	Compounds	Retention Time
1	n-Pentadecanol	3.222	25	9-Hexacosene	15.537
2	Methyl 3-methoxytetradecanoate	3.641	26	Erucic acid	15.659
3	Myristic acid	4.403	27	Behenic acid	16.019
4	1-Octadecanol	5.149	28	(Z)-14-Tricosenyl formate	18.016
5	2-Pentadecanone, 6,10,14-trimethyl-	5.821	29	1-Heptacosanol	18.419
6	Cinnamic acid	5.92	30	Lignoceric acid	18.924
7	Palmitoleic acid	6.615	31	Squalene	20.311
8	Palmitic acid	6.866	32	Hexacontane	21.2
9	l-(+)-Ascorbic acid 2,6-dihexadecanoate	7.378	33	1-Triacontanol	22.615
10	1-Nonadecene	7.818	34	Tetrapentacontane	24.021
11	Methyl 14-methylhexadecanoate	7.895	35	Cholesterol	24.599
12	Margaric acid	8.313	36	alpha.-Tocopherol	24.811
13	Methyl 16-methyl-heptadecanoate	9.25	37	Campesterol	26.66
14	Linoleic acid	9.391	38	Stigmasterol	27.323
15	Oleic acid	9.475	39	gamma.-Ergostenol	27.933
16	Linolenic acid	9.505	40	Chondrillasterol	28.215
17	Phytol	9.677	41	gamma.-Sitosterol	28.216
18	Stearic acid	9.843	42	Stigmastanol	28.34
19	9-Octadecen-1-ol	11.857	43	Fucosterol	28.464
20	Tetracosane	12.492	44	beta.-Amyrin	28.722
21	Methyl 11-eicosenoate	12.571	45	22-Dihydrochondrillasterol	29.002
22	Eicosanoic acid	12.964	46	Lathosterol	29.028
23	Oleamide	13.529	47	Cycloartenol	29.295
24	1,16-Hexadecanediol	15.115	48	alpha.-Amyrin	29.407

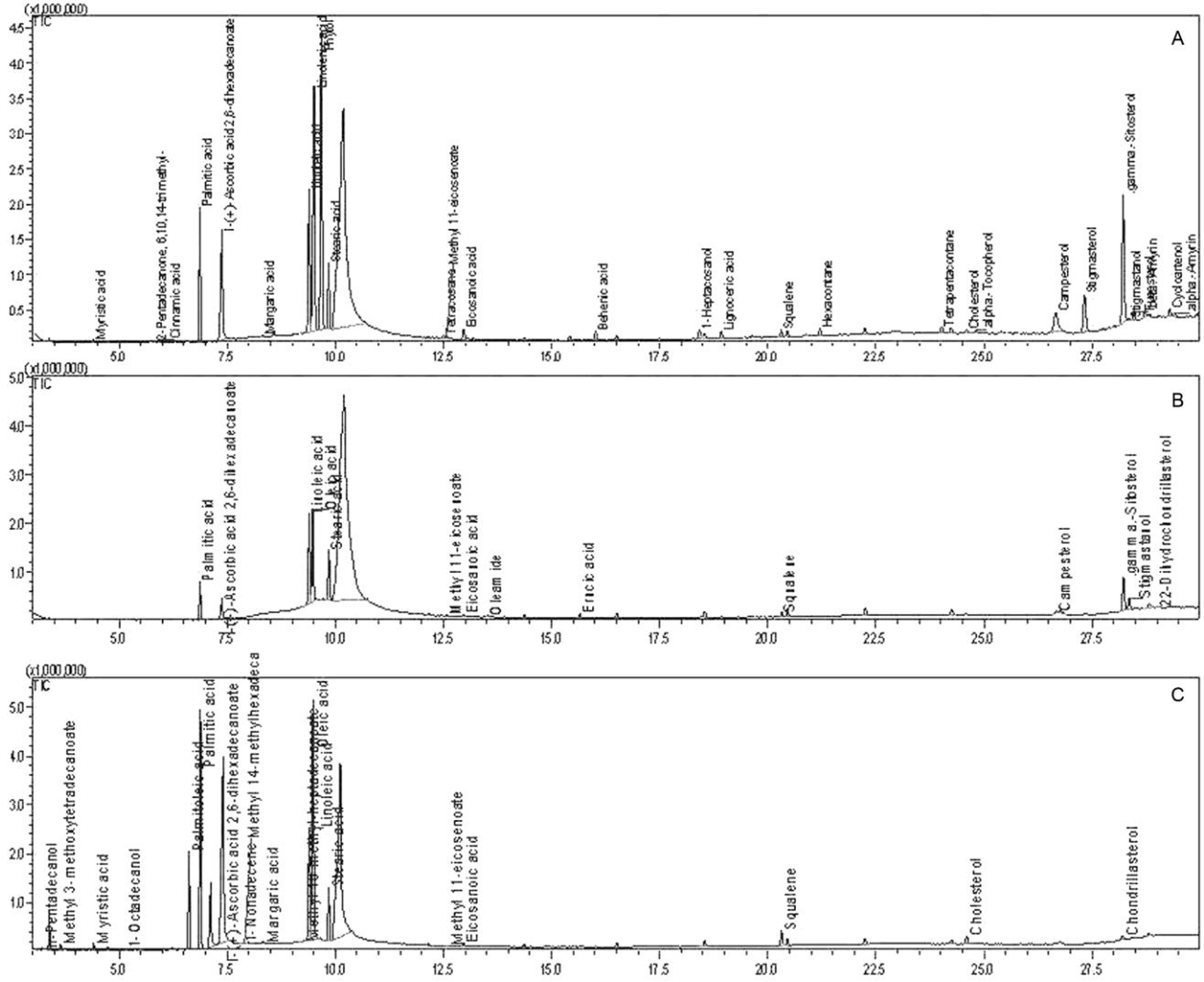

Fig. 1—Chromatogram showing lipophilic metabolite profiling of maize seedlings (A), maize seeds (B) and stem borer larvae fed on maize seedlings (C).

respectively. Squalene reported as the major hydrocarbon, was present in varying concentrations (0.45-2.22%) across plant and insect samples. The hydrocarbon, 1-Nonadecene was present only in insect samples. In general, the concentration of hydrocarbons was higher in the seedlings of maize than that of sorghum. Among sterols and terpenoids, their presence and concentrations were variable across the insects and plant parts (Table 2). Campesterol, Gamma-sitosterol and stigmastanol were found in the seedling and seed samples, whereas Gamma-ergostenol, chondrillasterol and lathosterol were present only in insect samples. Vitamin C derivative l-(+)-Ascorbic acid 2,6-dihexadecanoate was present in higher concentration in all the test samples with 27.63 and 22.2% in insects, 6.42 and 19.52 % in seeds, and 10.25 and 11.15% in seedlings of maize and sorghum, respectively. Other compounds constituted <1% of the total lipophilic metabolite profile across the plant and insect samples (Table 2).

Discussion

Several modern instruments such as GC-MS, GC-MS-TOF have been deployed to study lipophilic compounds using different separation methods for plant and insect samples which deem cumbersome to elucidate biochemical interactions between insects and their host plants. Standardization of a common method for analysis of lipophilic compounds from host plants *vis-a-vis* their insect pests is a paramount to better understand the plant-insect interactions and engineer nutritional manipulation based strategy for

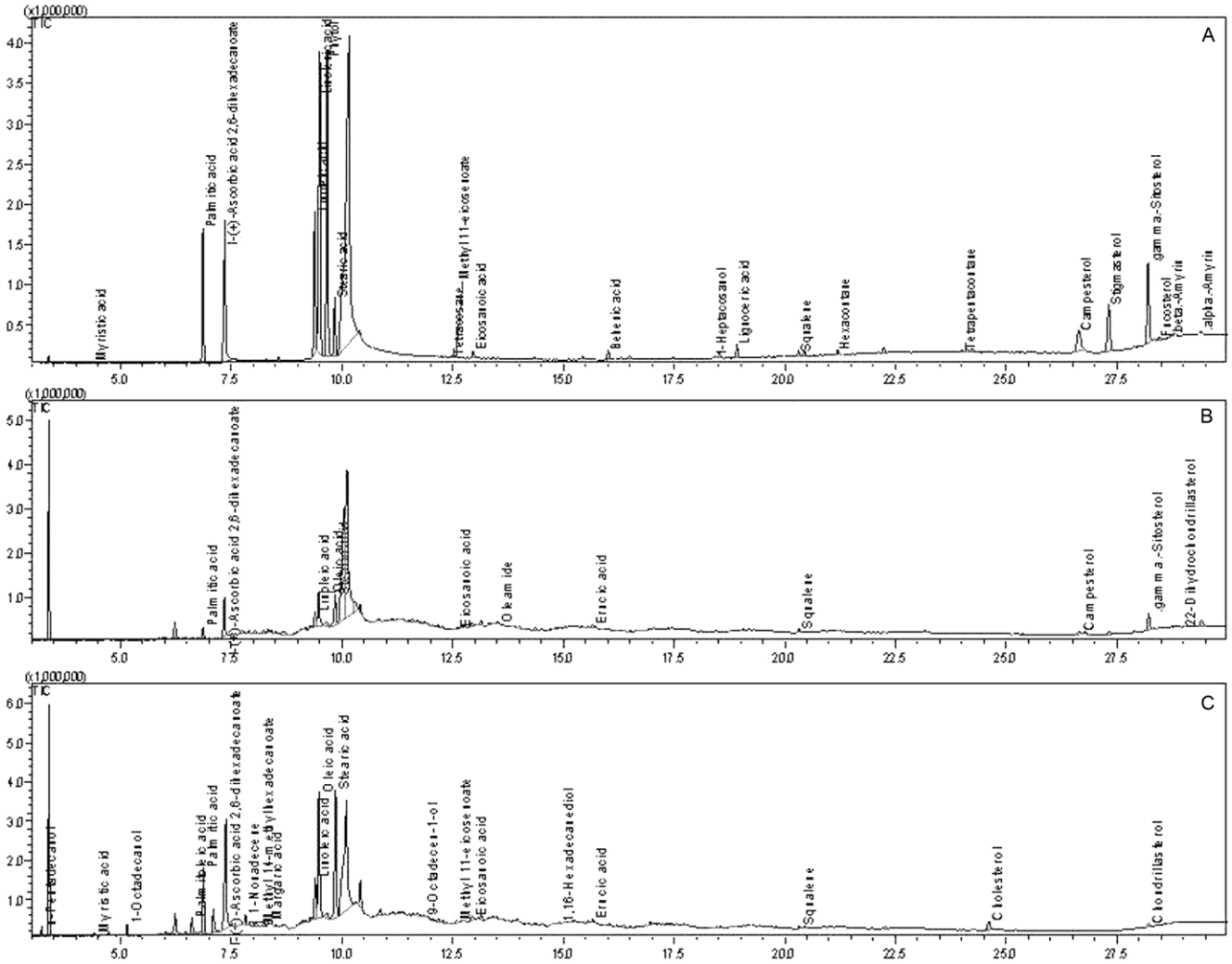

Fig. 2—Chromatogram showing lipophilic metabolite profiling of sorghum seedlings (A), sorghum seeds (B) and stem borer larvae fed on sorghum seedlings (C).

the management of insect pests. The sample amount, extraction, derivatization, temperature gradient, run time, and identification of peaks are some of the important and critical components of the GC-MS based analysis. The present study has demonstrated that the sample quantity around 100 mg for seeds and insect samples, and 200 mg for seedlings of maize and sorghum were suitable for lipophilic metabolite profiling. However, dissection of the insect for weight quantification might result in disproportionate distribution of lipophilic compounds in different pieces of the dissected insect body. Hence, it is suggested to use whole insect body as test sample. Maize and sorghum seeds were found to contain fewer number of lipophilic metabolites and require less time for their extraction in comparison to the seedlings and the stem borer larvae fed on them (Table 2). Crop seedlings and insects synthesize large number of lipophilic and other metabolites including interfering ones as a result of various metabolic activities, while such activities are minimal in case of seeds, thus require more time for their complete extraction.

All organic compounds are not suitable for direct GC-MS analysis due to their in-volatile nature. Many important biological compounds such as fatty acids, flavonoids, alkaloids, carbohydrates, amino acids and terpenoids are polar, and have limited volatility. Therefore, they were derived into related compounds for their suitability to gas chromatography. The standards fatty acid methyl esters (FAMEs) were also used as retention markers as suggested by Fiehn *et al*.[1]. The present study which adopted the GC-MS method standardized with different

Table 2—Concentration and categorization of different lipophilic compounds from insect and plant samples

Compound	Maize			Sorghum		
	Seedling	Seed	Insect*	Seedling	Seed	Insect*
Fatty acids						
Methyl 3-methoxytetradecanoate	-	-	0.33	-	-	0.15
Myristic acid	0.10	-	0.43	0.11	-	0.30
Cinnamic acid	0.14	-	-	-	-	-
Palmitoleic acid	-	-	7.35	-	-	2.63
Palmitic acid	7.17	8.32	19.53	7.34	5.35	8.80
Methyl 14-methylhexadecanoate	-	-	0.05	-	-	0.49
Margaric acid	0.19	-	0.13	0.12	-	1.14
Methyl 16-methyl-heptadecanoate	-	-	0.22	-	-	-
Linoleic acid	9.24	23.08	10.49	9.34	8.98	8.40
Oleic acid	-	24.90	24.92	-	20.27	23.90
Linolenic acid	19.28	-	-	25.11	-	-
Stearic acid	4.20	13.09	4.58	2.74	17.79	19.64
Methyl 11-eicosenoate	0.22	0.33	0.07	0.18	0.77	0.30
Eicosanoic acid	0.78	0.59	0.39	0.64	0.77	1.40
Erucic acid	-	1.18	0.09	-	2.46	0.92
Behenic acid	0.64	-	-	0.66	-	-
Lignoceric acid	0.53	-	-	1.11	-	-
Total	42.49	71.49	68.58	47.35	56.39	68.07
Fatty alcohols						
n-Pentadecanol	-	-	0.11	-	-	0.96
1-Octadecanol	-	-	0.11	-	-	1.41
Phytol	20.92	-	-	20.70	-	-
9-Octadecen-1-ol	-	-	-	-	-	0.94
1,16-Hexadecanediol	-	-	0.05	-	-	0.72
1-Heptacosanol	0.95	-	-	0.34	-	-
1-Triacontanol	-	-	0.04	-	-	0.17
Total	21.87	-	0.31	21.04	-	4.20
Hydrocarbons						
1-Nonadecene	-	-	0.08	-	-	1.53
Tetracosane	0.28	-	-	0.15	-	-
9-Hexacosene	0.15	-	-	0.08	-	
Squalene	0.49	1.66	1.53	0.45	2.22	0.58
Hexacontane	0.57	-	-	0.35	-	-
Tetrapentacontane	0.55	-	-	0.23	-	-
Total	2.04	1.66	1.61	1.26	2.22	2.11
Sterols and terpenoids						
Cholesterol	0.38	-	1.10	0.04	-	2.51
alpha.-Tocopherol	0.32	-	-	0.04	-	-
Campesterol	4.06	1.14	-	4.54	4.46	-
Stigmasterol	4.34	-	-	5.19	-	-
gamma.-Ergostenol	-	-	0.05	-	-	0.10
Chondrillasterol	-	-	0.62	-	-	0.83
gamma.-Sitosterol	11.15	12.52	-	7.51	13.24	-
Stigmastanol	0.45	4.92	-	0.29	1.54	-
Fucosterol	0.68	-	-	0.29	-	-
beta.-Amyrin	0.73	-	-	0.34	-	-
22-Dihydrochondrillasterol	-	1.19	-	-	0.66	-
Lathosterol	-	-	0.10	-	-	-
Cycloartenol	0.75	-	-	0.43	-	-
alpha.-Amyrin	0.44	-	-	0.43	-	--
Total	23.30	19.77	1.87	19.10	19.90	3.44
Vitamin derivative						
l-(+)-Ascorbic acid 2,6-dihexadecanoate	10.25	6.42	27.63	11.15	19.52	22.20
Others						
2-Pentadecanone, 6,10,14-trimethyl-	0.07	-	-	0.08	-	-
Oleamide	-	0.64	-	-	1.97	-
(Z)-14-Tricosenyl formate	-	-	0.01	-	-	-
Total	0.07	0.64	0.01	0.08	1.97	0.00

*spotted stem borer larvae

parameters produced dependable results in terms of separation and number of peaks. Moreover, it could overcome the problem of eluting the underivatized sample part from the column to prevent column contamination. Furthermore, the suitability and sensitivity of the current method is evident from the number of peaks and their intensity as shown in figures 1 and 2, and the detection limit being as low as 0.01% from different test samples (Table 2). The column Rtx®-5MS was found suitable for lipophilic metabolite profiling of insect and plant samples during the present studies, which has also been used earlier by Lytovchenko *et al*.[16] for metabolic profiling of Arabidopsis and tomato.

A total of 48 lipophilic metabolites having fatty acids, fatty alcohols, hydrocarbons, sterols and terpenoids, vitamin derivative and few others were separated from the samples of maize and sorghum seeds and seedlings and the spotted stem borer larvae. Similar metabolite functional groups have also been reported as components of lipophilic profile from various plant sources[16]. In case of insects, GC-MS has only been used for fatty acid profiles[11,17]. Recently, metabolite profiling in combination with transcriptomics has also been used to study the plant-pathogen interactions in rice[18]. Keeping in view the broad applications of GC-MS, present method of lipophilic profiling of insects and their host plants in combination with other genomic tools could be highly useful for better understanding of the biochemical and molecular interactions between the insects and their host plants, and to identify new biomarkers for plant resistance against insects. This method could also be useful for studies on lipophilic compounds from various field crops from nutritional point of view and in relation to various stresses.

References

1 Fiehn O, Kopka J, Dormann P, Altmann T, Trethewey RN & Willmitzer L, Metabolite profiling for plant functional genomics. *Nat Biotechnol*, 18 (2000) 1157.

2 Roessner U, Luedemann A, Brust D, Fiehn O, Linke T, Willmitzer L & Fernie AR, Metabolic profiling allows comprehensive phenotyping of genetically or environmentally modified plant systems. *The Plant Cell*, 13 (2001) 11.

3 Roessner U, Willmitzer L & Fernie AR, Metabolic profiling and biochemical phenotyping of plant systems. *Plant Cell Rep*, 21 (2002) 189.

4 Schauer N, Semel Y, Roessner U, Gur A, Balbo I, Carrari F, Pleban T, Perez-Melis A, Bruedigam C, Kopka J, Willmitzer L, Zamir D & Fernie AR, Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. *Nat Biotechnol*, 24 (2006) 447.

5 Benkeblia N, Shinano T & Osaki M, Metabolite profiling and assessment of metabolome compartmentation of soybean leaves using non-aqueous fractionation and GC-MS analysis~~,~~. *Metabol*, 3 (2007) 297.

6 Naturalium R, *Identification and characterization of metabolic Quantitative Trait Loci in Arabidopsis thaliana*, Dissertation, Max-Planc Institute for Molecular Plant Physiology, Germany, 2008.

7 Frank T, Yuan F, Shu QY & Engel KH, Metabolite profiling of induced mutants of rice and soybean. In: *Induced plant mutations in the genomics era*, edited by Shu QY (Food and Agriculture Organization, United Nation, Rome) 2009, 403.

8 Heuberger AL, Lewis MR, Chen MH, Brick MA, Leach JE & Ryan EP, Metabolomic and functional genomic analyses reveal varietal differences in bioactive compounds of cooked rice. *PLOS One*, 5 (2010) e12915.

9 Thissen U, Coulier L, Overkamp KM, Jetten J, van der Werff BJC, van de Ven T & van der Werf MJ, A proper metabolomics strategy supports efficient food quality improvement: A case study on tomato sensory properties. *Food Qual Pref*, 22 (2011) 499.

10 Jom KN, Frank T & Engel KH, A metabolite profiling approach to follow the sprouting process of mung beans (*Vigna radiata*). *Metabol*, 7 (2011) 102.

11 Hood-Nowotny R, Schwarzinger B, Schwarzinger C, Soliban S, Madakacherry O, Aigner M, Watzka M & Gilles J, An analysis of diet quality, How it controls fatty acid profiles, isotope signatures and stoichiometry in the malaria mosquito *Anopheles arabiensis*. *PLOS One*, 7 (2012) e45222.

12 Agrawal AA, Mechanisms, ecological consequences and agricultural implications of tri-trophic interactions. *Curr Opin Plant Biol*, 3 (2000) 329.

13 Baldwin IT, Halitschke R, Kessler A & Schittko U, Merging molecular and ecological approaches in plant-insect interactions. *Curr Opin Plant Biol*, 4 (2001) 351.

14 Benedict MQ, Hood-Nowotny RC, Howell PI & Wilkins EE, Methylparaben in *Anopheles gambiae* s.l. sugar meals increases longevity and malaria oocyst abundance but is not a preferred diet. *J Insect Physiol*, 55 (2009) 197.

15 Neff WE, Adlof RO, List GR & EL-Agaimy M, Analysis of vegetable oil triglycerols by silver ion high performance liquid chromatography with flame ionization detector. *J Liq Chromatogr*, 17 (1994) 3951e68.

16 Lytovchenko A, Beleggia R, Schauer N, Isaacson T, Leuendorf JE, Hellmann H, Rose JKC & Fernie AR, Application of GC-MS for the detection of lipophilic compounds in diverse plant tissues. *Plant Methods*, 5 (2009) 4.

17 Paiko YB, Dauda BEN, Salau RB & Jacob JO, Preliminary data on the nutritional potentials of the larvae of edible dung beetle consumed in paikoro local government area of Niger state, Nigeria. *Continent J Food Sci Technol*, 6 (2012) 38.

18 Sana TR, Fischer S, Wohlgemuth G, Katrekar A, Jung KH, Ronald PC & Fiehn O, Metabolomic and transcriptomic analysis of the rice response to the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae*. *Metabol*, 6 (2010) 451.