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Headspace Solid Phase Micro Extraction and GC/MS Analysis of the Volatile Components in Seed and Cake of *Azadirachta indica* A. juss.

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Abstract: The present investigation was carried out to identify the components present in the headspace volatiles of neem seed and cake as these have been found to exert insect repellant action on many pests of horticultural crops. The composition of volatiles emitted by neem seed (*Azadirachta indica* L.) and its cake obtained after extraction of oil was analyzed by headspace solid-phase micro extraction (HS-SPME) combined with gas chromatography and gas chromatography- mass spectrometry (GC-MS). Major differences were found in the volatile composition of seed and cake. A total of 71 and 101 volatile compounds representing 83.47 % and 85.62 % of the total quantum of volatiles present in seed and cake, respectively, could be identified. These compounds were found distributed in several chemical classes namely, alcohols, carbonyl compounds (ketones, aldehydes, and esters), fatty acids, terpenes, nitrogenous and sulphur compounds among others. Of the various classes of compounds identified, fatty acids constituted the major group accounting for 24.82 % and 16.97 % of the total volatile concentration followed by aldehydes and ketones representing 26.49 % and 17.53 % and sulfur compounds contributing 12.94 % and 9.32 % of seed and cake volatiles, respectively. The most abundant volatile components were (Z)-9,7-octadecadienal and palmitic acid, a medium chain fatty acid comprising 25.47 % and 14.97 % of the total in seed and cake respectively.

Keywords: *Azadirachta indica*, neem seed and cake, neem volatiles , SPME, GC-MS.

1. Introduction

The neem tree, *Azadirachta indica* A.Juss.(Meliaceae), is indigenous to India and Southeast Asia. Extracts of neem fruit, seeds, seed kernels, twigs, stem bark and root bark have been shown to possess insect antifeedant, insecticidal, insect growth disrupting, nematicidal, fungicidal, bactericidal, anti-inflammatory, antitumour, immuno-stimulating and many other biological activities [1]. In India, neem cake, a byproduct produced during extraction of oil from neem seed, is used as a biopesticide. The use of neem cake as an amendment to agricultural soils to control plant parasitic nematodes under both greenhouse and field conditions is a well known practice in India [2, 3, 4]. Field studies conducted by us showed that the volatiles emitted from neem cake were effective in controlling diamondback moth (DBM), *Plutella xylostella* of cabbage, a notorious insect pest known to develop resistance against most of the commercially available pesticides and other insects affecting vegetable crops [5]. Although several reviews have been published on the chemical constituents of neem seed [6], the chemical profile of volatiles emitted by both neem seed and cake have not received much attention excepting a report [7] on the volatile organosulfur compounds of neem seed in which it was shown that the major component, di-n-propyl disulfide exhibited larvicidal properties. Subsequently, the presence of certain volatile compounds in neem leaf having fungicidal properties were reported [8]. Neem seed volatiles extracted by organic solvents were also found to be effective against the eggs and adults of pulse beetle, *Callosobruchus maculatus* [9].

Keeping in view, the ecological safety and possible commercialization potential of neem volatiles for insect pest control in horticultural crops, the present work was carried out with the aim of separating and identifying the entire range of volatile compounds emitted by seed and cake using GC-GCMS. Previous studies on the head space volatiles of other seeds reported advantages of using SPME [10, 11] to avoid interferences from nonvolatile matrix components. Based on these reports, headspace sampling was employed for the extraction of volatiles in the present study.

2. Experimental

2.1. Plant material

Neem trees growing near the experimental farm of the institute were used for the study. The experimental site was characterized by a mild tropical weather throughout the year with a mean maximum temperature of 28-30 ^{0}C and a

minimum of 17-20 ^{0}C during the fruit development phase. Mature neem fruits were harvested during the month of August and ripened under ambient conditions. Seeds from ripe fruits were separated from the pulp, washed with water and dried in the shade for a week. The kernel obtained after decorticating the seeds was crushed in a hydraulic press at a high pressure in a single step to extract the oil. The resulting neem cake containing 8 % oil was ground into a fine powder and used for the experiment. Neem seed powder was prepared by finely pulverizing the seeds in a tissue grinder immediately before use.

2.2. Isolation of volatile components

Extraction of head space volatiles of neem seed and cake was performed as described earlier [12] with some modifications. The SPME fibre coated with carboxan-polydimethylsiloxane-divinylbenzene (50/30µm, CAR/PDMS/DVB) (Supelco, Bellefonte, PA, USA) was used for the analysis, due to its high sensitivity for aroma compounds and excellent reproducibility. 50 g each of seed and cake were homogenized with 100 mL double distilled water using a commercial blender. The slurry was transferred to a 250 ml conical flask to which 5 g of NaCl was added. Subsequently, the flask was sealed with a teflon-lined septum and the samples were kept stirred at 37±1°C. After 20 min of equilibration between the solution and the headspace, the fibre was exposed to the headspace of the sealed flask for 60 min. Prior to sampling, the fibre was preconditioned for 1hr at 260°C in the GC injection port as per the manufacturer's instructions.

2.3. Gas chromatography

GC / FID analysis was carried out using a Varian-3800 Gas chromatograph system with SPME sleeve adapted to injector on a VF-5 column (Varian, USA), 30 m x 0.25 mm i.d, and 0.25 µm film thickness. The carrier gas was helium at a flow rate of 1ml min^{-1}; injector temperature 250 °C and detector temperature 270°C. The temperature program for column was as follows: The initial oven temperature was 50 °C for 2 min , increased by 3 °C /min up to 200 °C, held for 3 min, increased further at 10 °C/min up to 220 °C and maintained constant for 8 min. For desorption, the SPME device was introduced in the injector port for chromatographic analysis and remained in the inlet for 12 min. Initially injection mode was splitless followed by split mode (1:20) after 1.5 minutes. For the qualitative identification of volatile substances and computation of retention time and index, the following standards, ethyl acetate, propanol, isobutanol, hexanol, 1-octene-3-ol and eugenol were co-chromatographed.

2.4. Gas chromatography/mass spectrometry (GCMS)

GC-MS analysis was performed on Varian-3800 gas chromatograph coupled with Varian 4000 GC-MS/MS mass selective detector. Volatile compounds were separated on VF-5MS (Varian, USA) column (30 m x 0.25 mm ID with 0.25 µm film thickness) by applying the same temperature program as described above for GC-FID analysis. Mass detector conditions were: EI-mode at 70 eV, injector temperature, 250 °C; ion source-temperature, 230 °C; trap temperature, 220 °C; transfer line temperature 250 °C and full scan range, 50–450 amu. The carrier gas was helium at a flow rate of 1 ml.min^{-1}.

2.5. Identification of components

Volatile compounds were identified by comparing the mass spectra with the data system libraries (Wiley and NIST-2007) and by Kovats indices (KI) computed in accordance with modified Kovats method [13,14] using a homologous series of n-alkanes (C_5 to C_{32}). The Kovats index, for the unknown was calculated from the formula:

$$KI = 100[n + (N - n) \times \{\log t_r(\text{unknown}) - \log t_r(n)\}/\{\log t_r(N) - \log t_r(n)\}]$$

where (n) is the number of carbon atoms in the shorter chain alkane, N is the number of carbon atoms in the longer chain alkane, (t_r) is the adjusted retention times for unknown compounds, t_r (n) , shorter-chain and t_r (N), longer-chain alkanes.

The total volatile production was estimated as the sum of all GC-FID peak areas in the chromatogram and individual compounds were quantified as relative percent area. All data are means of three independent determinations expressed as percentage of total peak area.

3. Results and Discussion

A total of 71 and 101 volatile compounds could be separated and identified from the headspace samples of neem seed and cake powder respectively (Table 1). Of these, 57 compounds were common to both. The number of sulfur compounds was 20 in both neem seed and cake accounting for 12.94 % and 9.32 % of the total amount of volatiles respectively. The number of hydrocarbon compounds in cake was higher at 22 as against 17 in seed but their proportion increased from 2.74 % in seed to 18.33 % in cake. The relative proportion of oxides, esters and miscellaneous compounds in cake increased appreciably as compared to those in seed. On the other hand, the relative contribution of sulfur compounds, acids, aldehydes and ketones showed a marked fall in cake as compared to seed (Table 1). There were no marked differences in either the number or proportion of nitrogen compounds present in seed and cake. Of the various groups of compounds identified, aldehydes and ketones constituted the major part (26.49 %) of seed volatiles followed by acids accounting for 24.82 %. On the contrary, the proportion of acids was higher in cake volatiles (18.54 %) followed by aldehydes and ketones (17.53 %). Palmitic acid was the most abundant fatty acid compound in both seed (24.34 %) and cake (14.97 %) followed by (Z)-9, 17-octadecadienal which constituted 25.47 % and 10.57 % of seed and cake respectively. Besides these two compounds, other major

volatiles found in seed were, 5-chloro-1H-indole (10.27%), 2-methoxythiophene (3.44 %), 4-benzylfuro [3, 2-c] pyridine (3.16 %), 2-furylmethylsulfide (1.57 %) and 2,5-dimethylthiophene (3.06 %). Similarly, the major volatile compounds found in neem cake included, 3, 5-diethyl-1, 2, 4-trithiolane (2.25 %), 1, 5-dithiaspiro [5,7] tridecan-7-ol (1.79 %), 4-benzylfuro(3, 2-c) pyridine (5.74 %), 5-chloro-1H-indole (2.73 %), (E)-3-(2-Quinolyl) propenenitrile (2.80 %), 3-furaldehyde (2.29 %), 1-methylene-1H-indene (6.59%),4-hexyl-2,5-dihydro-2,5-dioxo-3-furanylaceticacid (1.28 %), 1-undecyne (1.28 %), zingiberene (1.49 %) and bisabolene (2.01 %).

TABLE 1. SPME headspace analysis of volatile compounds of neem seed and cake

Sl. No.	Class and compound	Kovats Index (KI)		*Area (%)		Detector used
		Calculated	Reported	Neem Seed	Neem Cake	
	Sulfur compounds					
1	Carbon disulfide	569	568	0.064	0.123	FID
2	Methyl-thiirane	652	650	0.404	0.113	FID
3	(1E)-1-(Methylsulfanyl)-1-propene	684	678	0.297		FID
4	Methyl-3-thiophene	788	794	0.096	0.082	FID
5	Thiophene, 2-methoxy-	878	NA	3.441	0.390	MS
6	Thiophene, 2,5-dimethyl-	882	882	3.059	0.431	FID
7	Thiophene,3,4-dimethyl-	889	888	0.659	0.113	FID
8	2-Furylmethylsulfide	902	894	1.572	0.359	FID
9	2,3-Butanedithiol	910	902	0.085	0.082	FID,MS
10	(1E)-1-(Methyldisulfanyl)-1-propene	922	922	0.149	0.092	FID,MS
11	1,2-Dithiacyclopentane	985	978		0.216	FID
12	Isothiazole, 4,5-dimethyl-	988	974	0.340		FID
13	1,3-Dithiane	1031	1027	0.202	0.082	FID,MS
14	2-Ethyl[1,3]dithiane	1076	1063	0.836	0.195	MS
15	2-Thiaadamantan-4-ol	1222	NA	0.372		MS
16	3,5-Dimethyl-2-(methylsulfanyl)thiophene	1230	NA	0.276	0.113	MS
17	1-Methoxy-4-(methylthio) benzene	1248	NA		0.257	MS
18	5-methyl-4,7-dithiadeca-1,9-diene	1256	NA		0.955	MS
19	Propenyl propyl trisulfide	1311	1318	0.085	0.534	FID
20	1,2,4-Trithiolane, 3,5-diethyl-	1359	NA	0.096	2.248	MS
21	[4-(Methylsulfanyl)phenyl]methanol	1419	NA		0.267	MS
22	2,3-Thiophenedicarboxylic acid	1618	NA	0.127	0.873	MS
23	2,3,4,5-Tetrathiabicyclo[4,4,0]decane	1682	NA	0.542		MS
24	1,5-Dithiaspiro[5.7]tridecan-7-ol	1961	NA	0.234	1.796	MS
	Nitrogen compounds					
25	3,4-Dihydro-2H-pyran	668	NA		0.062	MS
26	1H-Pyrrole-2-carboxaldehyde	1012	988		0.667	FID,MS
27	1H-Pyrazole, 3,5-dimethyl-	1024	1014		0.062	FID,MS
28	4-benzyl furo[3.2-c] pyridine	1112	N.A	3.165	5.738	MS
29	2-Ethyl-3,5,6-trimethylpyrazine	1139	1120		0.092	MS
30	1H-Indole, 5-chloro-	1361	N.A	10.271	2.731	MS
31	7-Chloro-s-trizolo(pyrimidine-5(1H)-one	1669	N.A	0.329	0.749	MS
32	2-Propenenitrile, 3-(2-quinolinyl)-, (E)-	1686	N.A	0.276	2.803	MS
33	(9Z)-octadec-9-enenitrile	2126	NA	0.446	0.626	MS
	Alcohol Compounds					
34	4-Methyl-1-penten-3-ol	708	704	0.064	0.082	FID
35	3-Hexyn-2-ol	796	797		0.062	FID,MS
36	3-Hexen-1-ol, (Z)-	853	851	0.096	0.092	FID,MS
37	5-Hexen-1-ol	858	NA		0.113	FID,MS
38	2-Cyclohexen-1-ol	890	887	0.202	0.082	FID,MS
39	Cyclohexanol, 4-methyl-	926	929		0.092	FID,MS
40	6-Methyl-6-hepten-4-yn-3-ol	972	NA	0.096	0.195	MS
41	(2E,6E)-2,6-Octadiene-4,5-diol	1186	NA	0.085		MS
42	α-Methyl-1,4-benzenedimethanol	1422	1411	0.096		FID,MS
43	(E)-Isoeugenol	1455	1459		0.729	FID,MS
44	Nerolidol	1572	1564		1.006	FID,MS
45	Spathulenol	1581	1576	0.149	0.842	FID,MS
46	Ledol	1621	1608		0.390	FID,MS

47	1-(1-Naphthyl)-2-propanol	1712	NA		0.113	MS
48	1,2-Benzenediol, 3,5-bis (1,1-	1788	NA	0.117	0.267	MS
49	(6Z,9Z)-6,9-Pentadecadien-1-ol	1792	1771	0.096	0.082	FID,MS
	Aldehydes and ketones					
50	Furfural	842	831		0.062	FID
51	3-Furaldehyde	846	832		2.290	FID
52	4,6-Heptadiyn-3-one	868	868	0.276		FID,MS
53	1-Methyl-1-cyclopenten-3-one	885	887	0.127		FID,MS
54	2,4-Hexadienal, (E,E)-	909	908		0.082	FID,MS
55	Octenal	1059	1060		0.216	FID,MS
56	1-(1H-pyrrol-2-yl)-Ethanone	1065	1063		0.113	FID,MS
57	(2E,4E)-2,4-Nonadienal	1235	1218	0.191	0.955	FID,MS
58	4-(1,2-Dimethyl-cyclopent-2-enyl)-butan--2-	1265	1240		0.226	FID,MS
59	Ethanone, 2-hydroxy-1-phenyl-	1277	NA		0.082	MS
60	10-Undecenal	1288	1278	0.149	0.832	FID,MS
61	Dihydro-β-ionone	1433	1432		0.708	FID,MS
62	1H-2-Benzopyran-1-one, 3,4-dihydro-8-	1532	1514		0.734	FID,MS
63	Benzophenone	1628	1612	0.096		FID,MS
64	Isolongifolen-5-one	1678	NA	0.117		MS
65	1,4-cis-1,7-cis-Acorenone	1698	NA		0.636	MS
66	(E,E)-10, 12-Hexadecadienal	1702	NA	0.064	0.123	MS
67	(Z)-9,17-Octadecadienal	2018	1997	25.469	10.574	FID,MS
	Acids					
68	2-Cyclopentene-1-carboxylic acid, 1-methyl-	1085	1073		0.287	FID,MS
69	Dodecanoic acid	1569	1566	0.191	0.770	FID,MS
70	Tetradecanoic acid	1772	1760	0.127	0.903	FID,MS
71	Pentadecanoic acid	1822	1806	0.159	0.327	FID,MS
72	4-Hexyl-2,5-dioxo-2,5-dihydro-3-	2118	NA		1.283	MS
73	Palmitic acid	1978	1961	24.340	14.970	FID,MS
	Esters					
74	Phenethyl acetate	1251	1260		0.062	FID,MS
75	Methyl (6E)-3,7,11-trimethyl-6,10-	1698	1685		0.709	MS
76	Benzyl Benzoate	1695	1693	0.266		FID,MS
77	Methyl hexadecadienoate	1869	1881	0.085	0.113	FID,MS
78	Methyl hexadecanoate	1878	1890	0.085	0.595	FID,MS
79	Ethyl palmitate	1991	1991		0.873	FID,MS
80	Methyl (7E,10E)-7,10-octadecadienoate	2101	2093	0.096	0.226	MS
81	Methyl linolenate	2108	2098	0.191		FID,MS
82	Ethyl linoleate	2165	2155	0.085	0.154	FID,MS
	Hydrocarbons					
83	Toluene	764	759	0.159	0.257	FID
84	1,3,7-Octatrien-5-yne	828	822	0.096	0.062	FID
85	4-Nonene, 5-methyl-	1015	1002		0.051	FID
86	1-Methylene-1H-indene	1098	1095		6.589	FID
87	1-Undecyne	1109	1096	0.266	1.282	FID
88	Naphthalene	1186	1179	0.223		FID
89	α-Longipinene	1371	1353		0.185	FID,MS
90	Ylangene	1381	1371	0.085		FID,MS
91	β-Elemene	1386	1391	0.191	0.431	FID,MS
92	Copaene	1388	1376	0.096	0.283	FID,MS
93	α-Gurjunene	1436	1403	0.159	0.915	FID,MS
94	Elixene	1449	1431	0.191	0.749	FID,MS
95	Acoradiene	1479	1471	0.159	0.606	FID,MS
96	β-Eudesmene	1488	1483		0.287	FID,MS
97	α-Calacorene	1493	NA		0.133	MS
98	α-Muurolene	1498	1495	0.117	0.400	FID,MS
99	δ-Cadinene	1522	1536	0.127	0.082	FID,MS
100	Valencene	1512	1499	0.329	1.039	FID
101	Zingiberene	1515	1500	0.212	1.486	FID,MS
102	α-Amorphene	1518	NA		0.216	MS
103	Bisabolene	1521	1507	0.191	2.013	FID,MS

104	1-Pentadecyne	1526	1510	0.064	0.257	FID
105	Juniper camphor	1688	1675		0.780	FID,MS
106	Podocarpa-8,11,13-triene, 13-isopropyl-	2078	2054	0.074	0.226	FID,MS
	Oxides					
107	1,2-Dimethoxybenzene	1150	1150		0.113	FID
108	2,3-Dimethoxytoluene	1169	1172		0.113	FID,MS
109	1,4-Dimethoxybenzene	1172	1186		0.257	FID
110	Caryophyllene oxide	1579	1582		0.287	FID,MS
	Others					
111	2,5-Dimethyl furan	718	695		0.062	FID
112	2,4-Dimethyl furan	729	708		0.051	FID
113	2-Ethenylfuran	765	761	0.085	0.060	FID
114	2-Pentylfuran	996	991		0.154	FID
115	2,6-di-tert-Butyl-p-benzoquinone	1482	1462	0.117	0.287	MS
	Total identified constituents			**83.475**	**85.617**	

*Data are expressed as percentage of the total peak area

Earlier attempts in our laboratory to extract the volatile compounds of neem cake by steam distillation followed by partition with organic solvents and concentration under vacuum gave extracts in which many volatile constituents were identified [15]. However, based on the previous reports [16, 17] in which it was shown that certain compounds added to neem leaf could not be detected after boiling of neem leaves for extraction of volatiles, it was suspected that many volatile components might have either escaped due to their high volatility or had undergone changes in their structure and activity due to the extreme conditions of temperature and pressure employed in the study. Therefore, SPME was used in the present analysis as it facilitated rapid sample preparation from a small amount of sample and with little or no change in structure and composition in comparison to cold press or steam distillation [18, 19]. GC MS analysis of SPME samples gave 20 organosulfur compounds from seed and 21 from cake which were chemically distinct from those reported earlier [7]. It must be noted that the above study reported the presence of 22 volatile organosulfur compounds from the headspace samples of neem seed collected by purging with a continuous stream of nitrogen gas followed by extraction using diethyl ether solvent. It was apparent therefore, that the differences in the nature and composition of separated volatiles were the result of the differences in the extraction protocols. On the contrary, the differences in the composition of sulfur compounds of neem cake samples from those reported by Krist et *al.*,[20] could have arisen from the enzymatic degradation of sulphur-containing compounds of seed .

The two most abundant individual components of seed were (Z)-9,7-octadecadienal and palmitic acid, a medium chain fatty acid comprising 25.47% and 24.34% of the total respectively. On the other hand, palmitic acid was the most abundant individual component of cake (14.97%) followed by (Z)-9,7-octadecadienal forming 10.57% of the total. Incidentally, a recent study [21] also reported that the most abundant fatty acid present in *Azadirachta indica* and *Azadirachta siamensis* was palmitic acid. Palmitic acid has been reported to be active in *Aedis aegypti* biting deterrent assays [22]. The levels of acids, aldehydes and ketones were lower in cake as compared to seed while there was an increase in the levels of esters in cake. This could perhaps be due to the action of seed enzymes on the cellular substrates which are released due to cellular damage occurring during the extraction of oil from seed. The marked increase in the levels of oxides and miscellaneous compounds in neem cake indicated increased oxidation and hydrolysis of seed components by hydrolytic and oxidative enzymes released due to tissue damage during cake formation.

In an earlier study, we had observed that the volatiles from neem cake exhibited insect repellant activity on the diamondback moth (*Plutella xylostella*) of cabbage (Unpublished). Based on the data obtained in the present study, it should be possible to identify the components of volatiles of neem cake responsible for the insect repellant activity. It has been reported that volatile compounds emitted from neem leaf exert significant inhibitory effects on fungal growth and aflatoxin production in aflatoxigenic *Aspergillus parasiticus* cultures [8]. Short term exposure to neem volatiles was found to impair the gonotrophic cycle and suppress oviposition of female *Anopheles* while long term exposure impaired vitallogenosis irreversibly [23]. It is noteworthy that diamondback moths exposed to a mixture of neem compounds for 35 successive generations did not show any signs of incipient resistance [24]. Volatiles from neem seed have also been reported to be effective against stored grain pests [9, 25, 26]. Several studies have established the distinctive activity and function of neem components in either altering insect behavior or life processes in such a subtle manner that the insect would be unable to feed, breed, metamorphose or cause damage to plants [24]. Based on these findings, it is imperative that the volatiles of neem seed and cake reported in this paper could have potential application for management of insect pests of crops and mosquito control. Neem seed and cake being natural products with a long recorded history of use in agriculture in India would be ideal candidates for successful mosquito control and eco-friendly management of insect pests of crops.

Supplemental information on the MS spectra analysis

I= 878, [M^+] 114, *m/z* 114(100) 99(97)55(55) 71(42). I= 1076, [M^+] 146, *m/z* 146(100)72(65.5)118(15). I= 1222,

[M^+] 170, *m/z* 70(100) 79(56)99(41)142 (35)67(20). I= 1230, [M] 158, *m/z* 143(100) 158(90) 99(19) 59(11). I= 1248, [M^+] 154, *m/z* 139(100) 154(97.5) 111(12) 77(8)95(7). I= 1256, [M^+] 188, *m/z* 73(38)59(33)115(18)147(17)101(11)188(20). I= 1359, [M^+] 180, *m/z* 74(79) 180(26) 106(18)59(18)116(12). I= 1419, [M^+] 154, *m/z* 154(100)107(35) 79(34) 137(29) 109(24)125(20). I= 1618, [M^+] 172, *m/z* 111(100) 128(62) 172(32) 83(21) 155(14) 82(13) 57(11). I= 1682, [M^+] 210, *m/z* 210(100) 81(60) 146(14) 113(10) 82(7) 145(4)67(3). I= 1961, [M^+] 232, *m/z* 145(100) 125(80) 106(52) 119(28) 232(27) 95(27) 55(23)132(17)73(16). I= 668, [M^+] 84, *m/z* 55(100)84(51)56(32)54(26)83(26). I= 1112, [M^+] 209, *m/z* 207 (100) 194(20) 180(6) 134(5). I= 1139, [M^+] 150, *m/z* 49(100) 150(83)53(25)54(21)122(18)67(14)56(13). I= 1361, [M^+] 151, *m/z* 151(100) 89(26)116 (19)124(11)75(9)63(7)115(5). I= 1669, [M^+] 170, *m/z* 170(100) 172(35) 107(15) 129(10) 142(8). I= 1686, [M^+] 180, *m/z* 180(100)154(24)179(24)181(14)155(4). I= 2126, [M^+] 263, *m/z* 55(100) 69(50)122(48)136(40)83(35)97(28)150(20)220(15)234(8)2 63(7). I= 972, [M^+] 124, *m/z* 95(100) 67(25)65(23)109(15)51(13)91(9)77(8)66(7). I= 1186, [M^+] 142, *m/z* 71(100)72(10) 53(10) 57(6) 69(5)55(4)125(4)70(3). I= 1712, [M^+] 186, *m/z* 42 (100)141(59)186(17)115(16.8) 143(12) 139(6) 63(4)128(3). I= 1788, [M^+] 222, *m/z* 207(100)222(20)57(19)208(15)179(5)91(5)77(4)191(4). I= 1277, [M^+] 136, *m/z* 105(100)77(60)51(16)106(8)136(6)78(5)50(4). I= 1678, [M^+] 218, *m/z* 175(100) 218(69)147(68)119(66)105(43)162 (35)133(34)91(29)134(28). I= 1702, [M^+] 236, *m/z* 55(100) 67(80)81(60)96(35)236(25). I= 2018, [M^+] 264, *m/z* 67(100)81(72) 55(58) 95(45) 264(20). I= 2118, [M^+] 240, *m/z* 126(100)98(21)140(16)95(12). I= 1978, [M^+] 256, *m/z* 73(99)60(84) 57(63) 55(62) 69(31)71(28)61(22). I= 1698, [M^+] 252, *m/z* 69(100)109(89)209(48)123(37)95(22)81(18)67(17) 55(14)177(14). I= 2101, [M^+] 294, *m/z* 67(100)81(90)55(70)95(67)82(54)79(48)96(45)68(43). I= 1493, [M^+] 200, *m/z* 157(100)142(52)141(35)156(24)158(14)115(14)200(11)14 3(9)128(9). I= 1518, [M^+] 204, *m/z* 61(100)105(72)119(56)91(52)93(46)79(41)133(31)77(30)2 04(28). I= 1433, [M^+] 194, *m/z* 121(100)161(43)136(36)123(36)93(33)95(27)81(25)119(2 3). I= 1698, [M^+] 220, *m/z* 82(100)109(56)135(45)123(40)220(36)55(31)136(28)121(26.5)|93 (25). I= 1482, [M^+] 220, *m/z* 177(100)220(73)67(37)135(33)57(33)149(30) 205(28)163(24).

4. Conclusion

The present work on separation and characterization of a large number of volatiles from both neem seed and cake has established that the neem seed cake is a better source of volatiles than the seed *per se.* Since these volatiles were found effective in controlling insect pests of horticultural crops, it would be worthwhile extending the study further to identify the specific components which are effective against insect pests. It is likely that detailed studies on the structure and mode of action of the biologically active volatile components would open up a new generation of ideal pesticides for the future**.**

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