SUPPORTING INFORMATION

Freeze-thaw Sample Preparation Method Improves the Detection of Volatile Compounds in Insects Using Headspace Solid-Phase Microextraction (HS-SPME)

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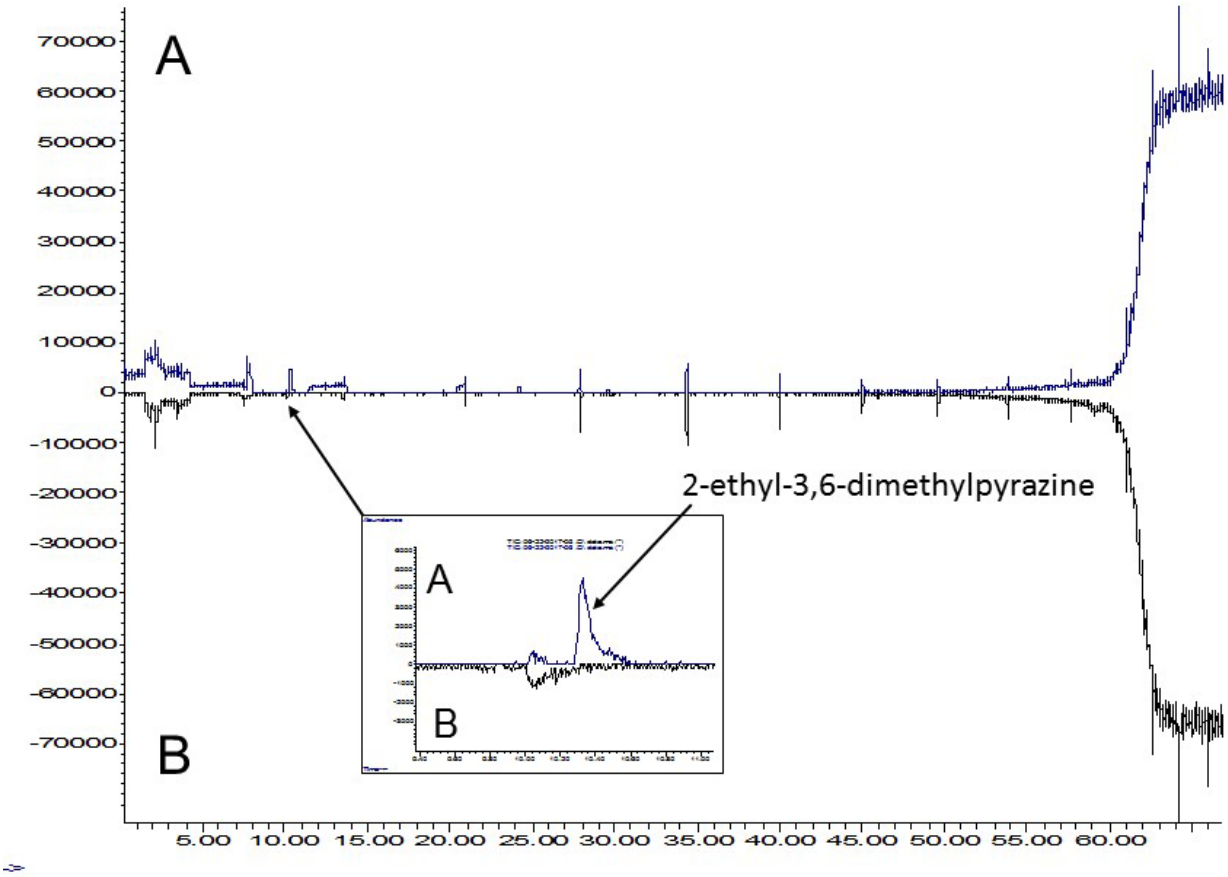

Figure S1. Overlay of GC–MS chromatograms comparing profiles of volatiles in *Solenopsis invicta* male alates between two sample preparation methods. Freeze-thaw method (A, 10min SPME time) detected 2-ethyl-3,6-dimethylpyrazine (see the close-up), but the nonfreeze method (B) failed to detect it at the 2h SPME time.

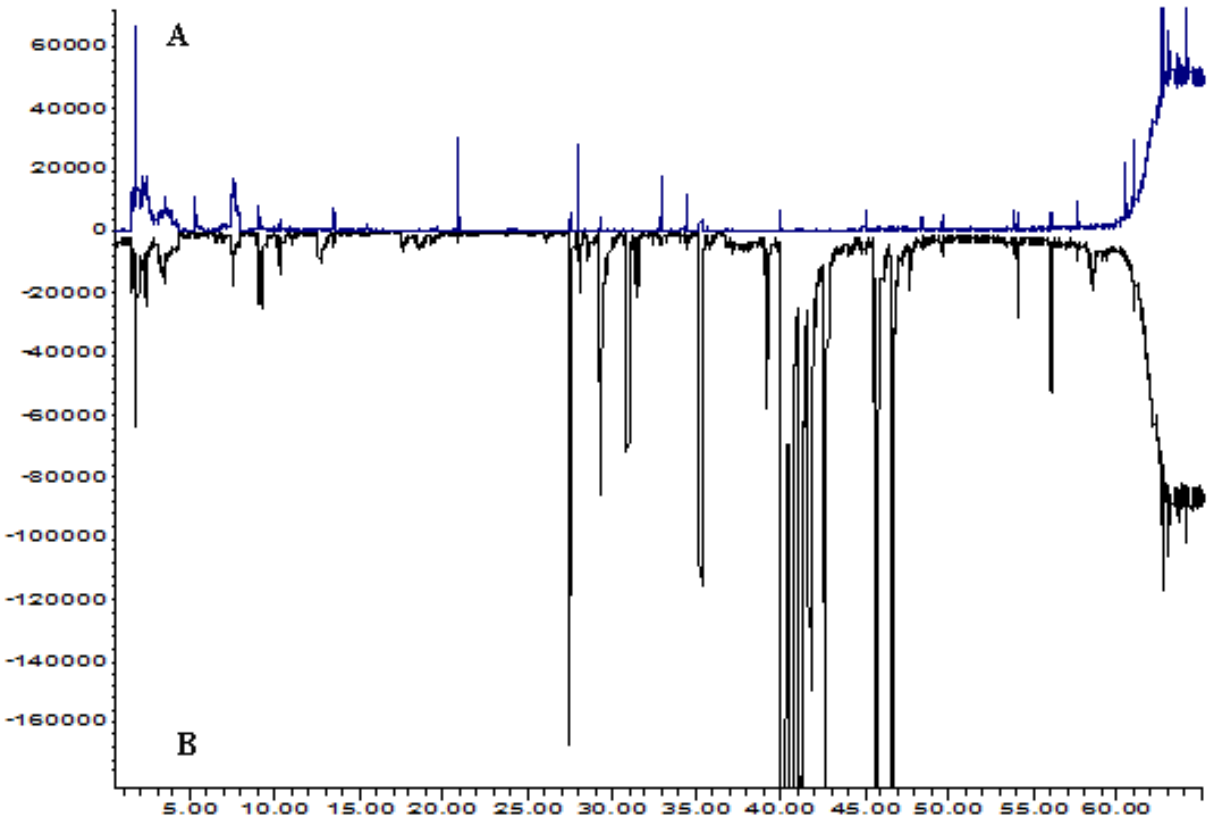

Figure S2. Overlay of GC–MS chromatograms comparing profiles of volatiles between *Solenopsis invicta* male alates (A) and female alates (B) using freeze-thaw method at 12h SPME time. Female alates produced significantly more volatiles than male alates.

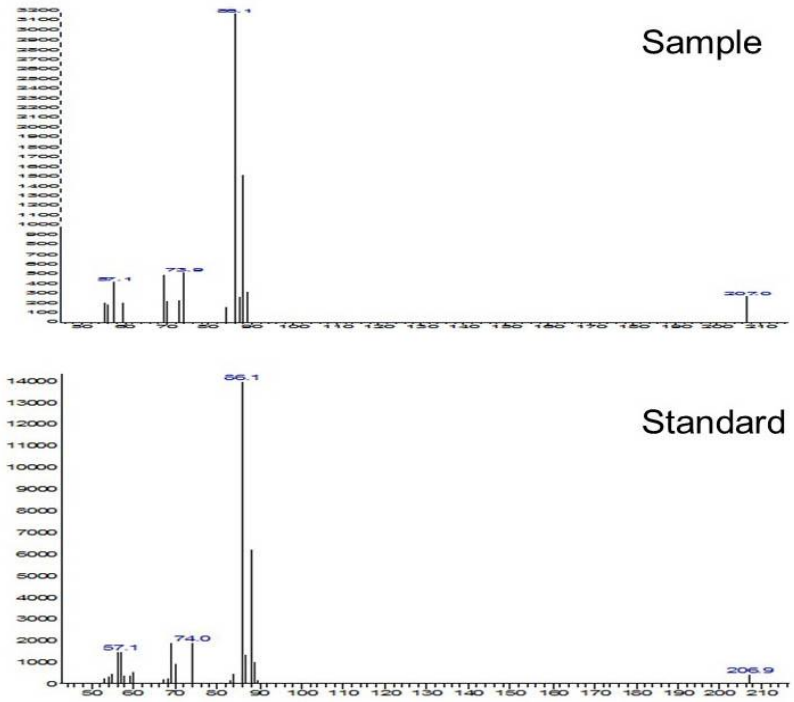

Figure S3. Mass spectra of L-isoleucine methyl ester found in workers and female alates of *S. invicta* and *S. richteri* and synthetic standard.

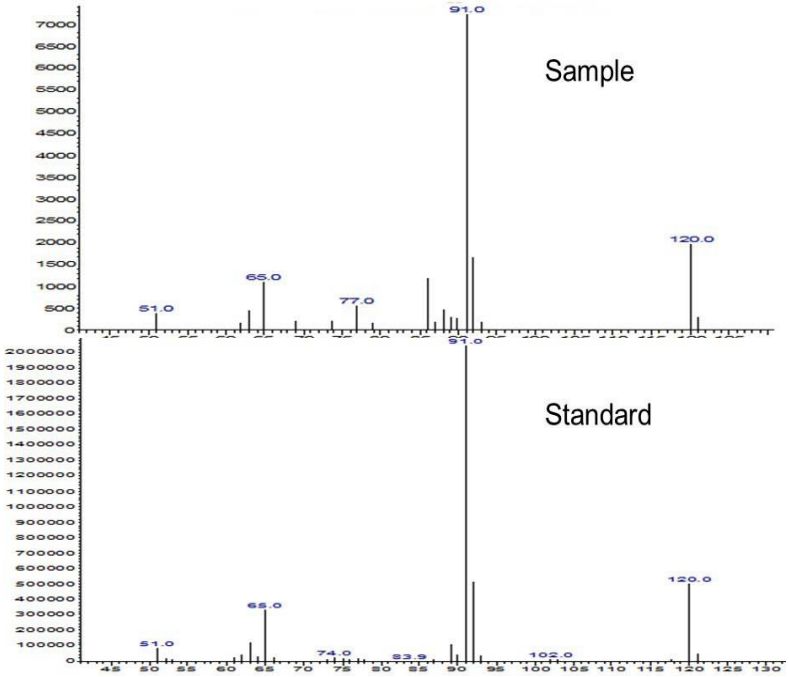

Figure S4. Mass spectra of phenylacetaldehyde found in workers and female alates of *S. invicta* and *S. richteri* and synthetic standard.

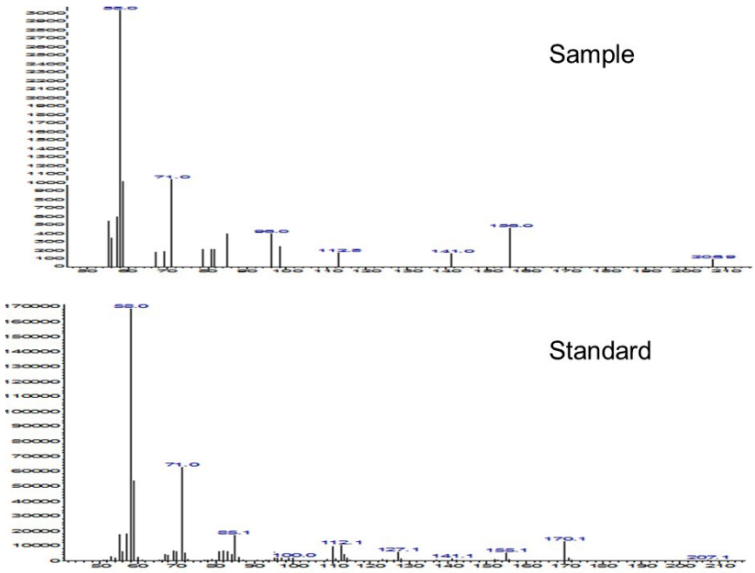

Figure S5. Mass spectra of 2-decanone found in female alates of *S. richteri* and synthetic standard.

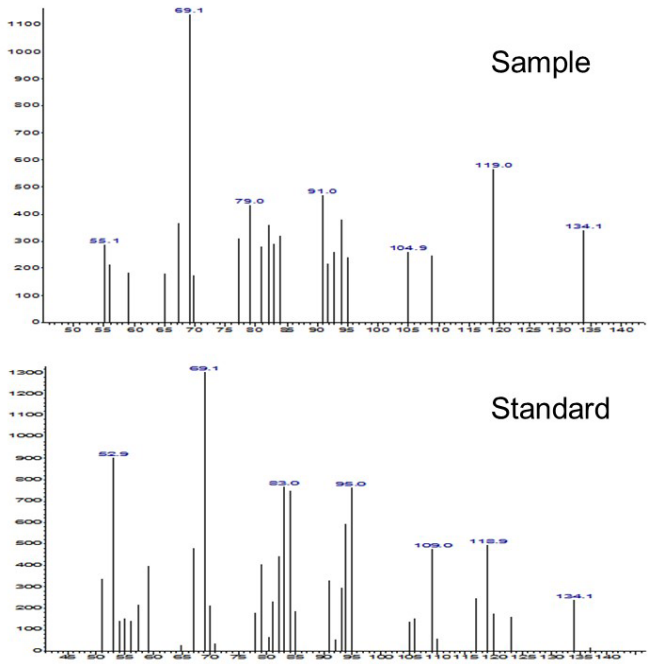

Figure S6. Mass spectra of geranial found in workers of *S. invicta* and synthetic standard.

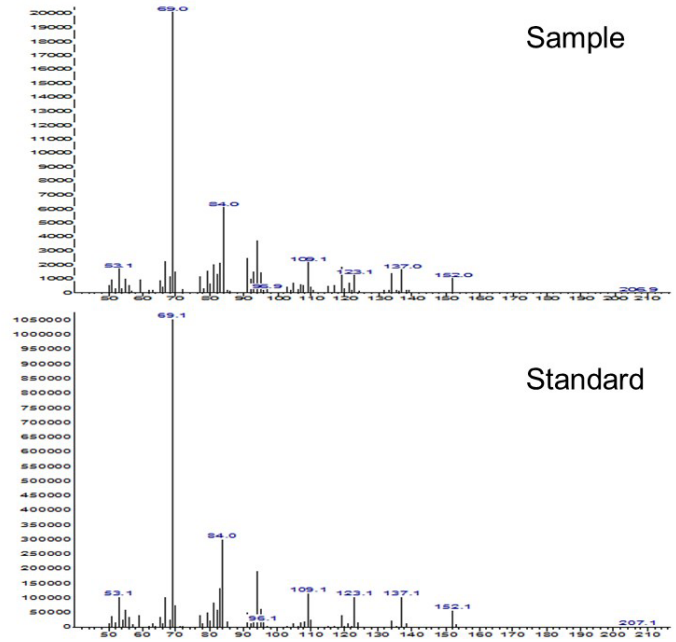

Figure S7. Mass spectra of neral found in workers of *S. invicta* and synthetic standard.

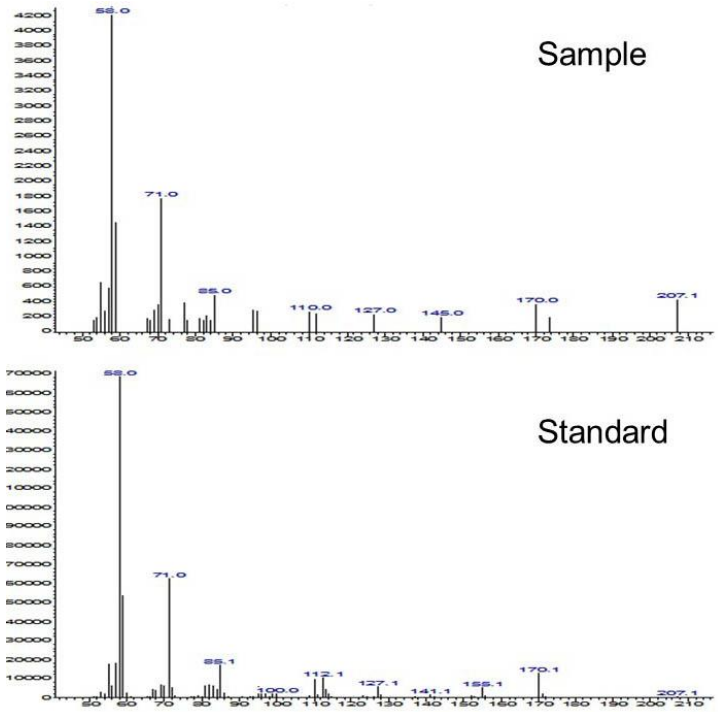

Figure S8. Mass spectra of 2-undecanone found in workers and female alates of *S. richteri* and synthetic standard.

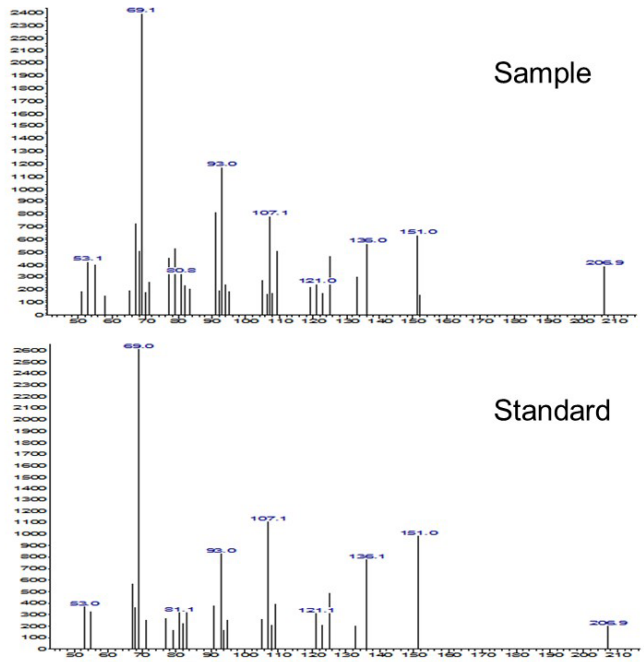

Figure S9. Mass spectra of *cis*-geranylacetone found in workers of *S. invicta* and synthetic standard.

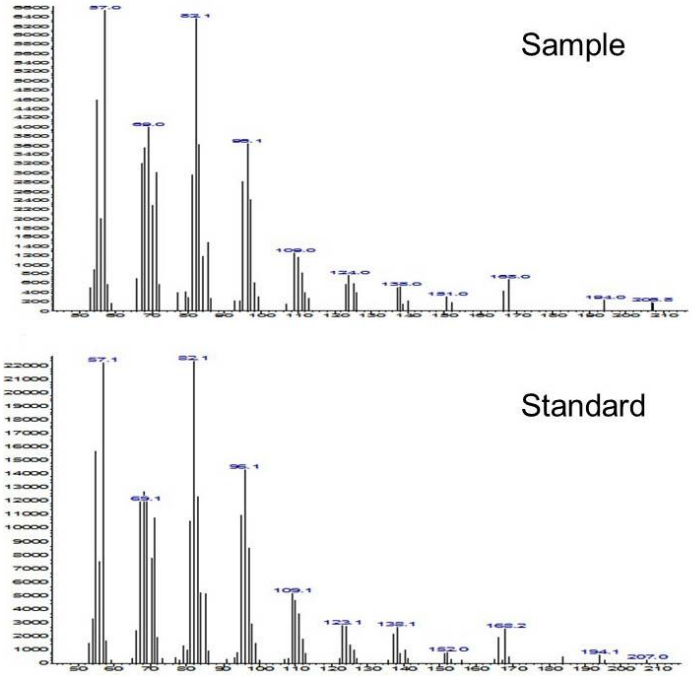

Figure S10. Mass spectra of tetradecanal found in workers of *S. richteri* and synthetic standard.

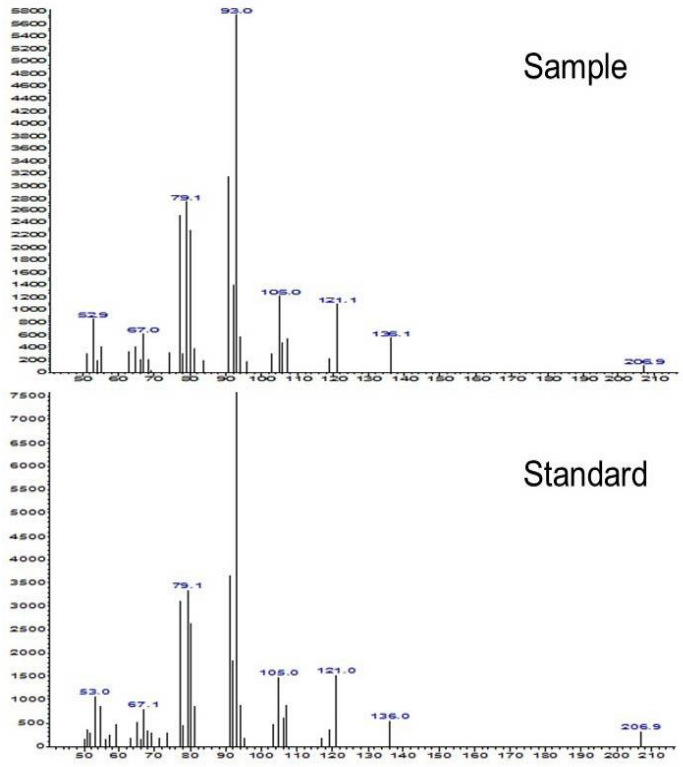

Figure S11. Mass spectra of *cis*-β-ocimene found in female alates of *S. invicta* and *S. richteri* and synthetic standard.

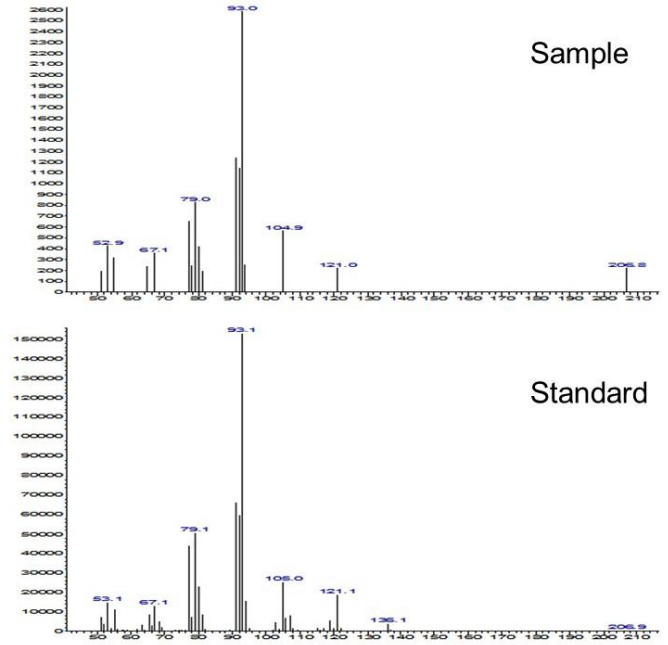

Figure S12. Mass spectra of *trans*-β-ocimene found in female alates of *S. invicta* and *S. richteri* and synthetic standard.

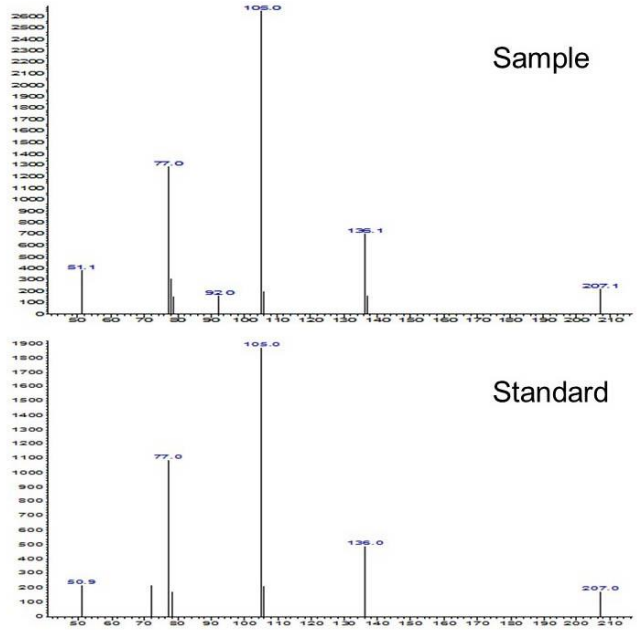

Figure S13. Mass spectra of benzoic acid methyl ester found in female alates of *S. invicta* and synthetic standard.

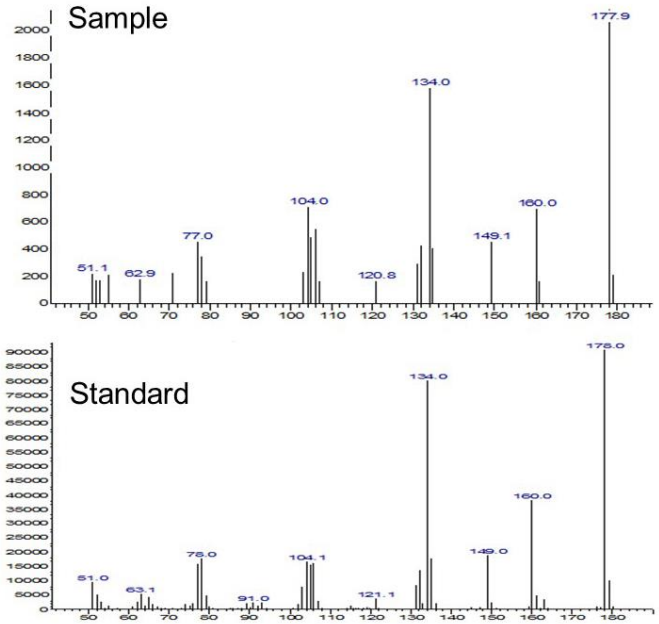

Figure S14. Mass spectra of mellein found in female alates of *S. invicta* and synthetic standard.

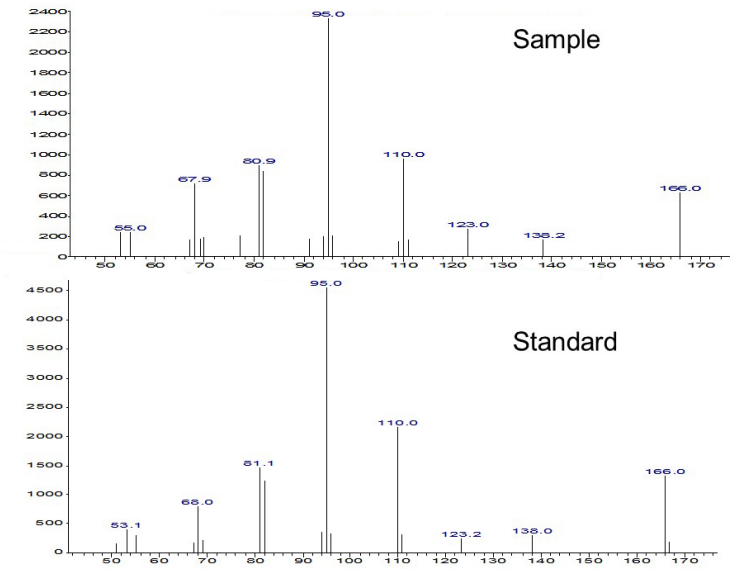

Figure S15. Mass spectra of 6-pentyl-2*H*-pyran-2-one found in female alates of *S. invicta* and synthetic standard.

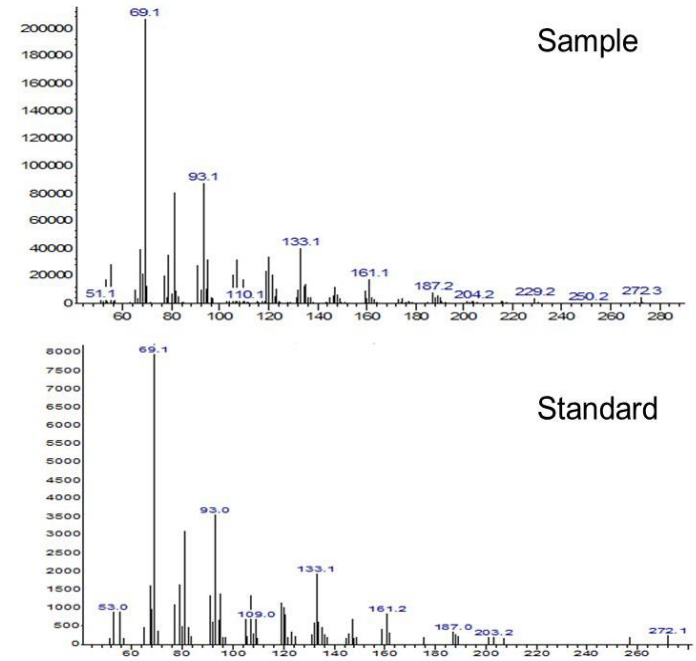

Figure S16. Mass spectra of β-springene found in female alates of *S. invicta* and *S. richteri* and synthetic standard.

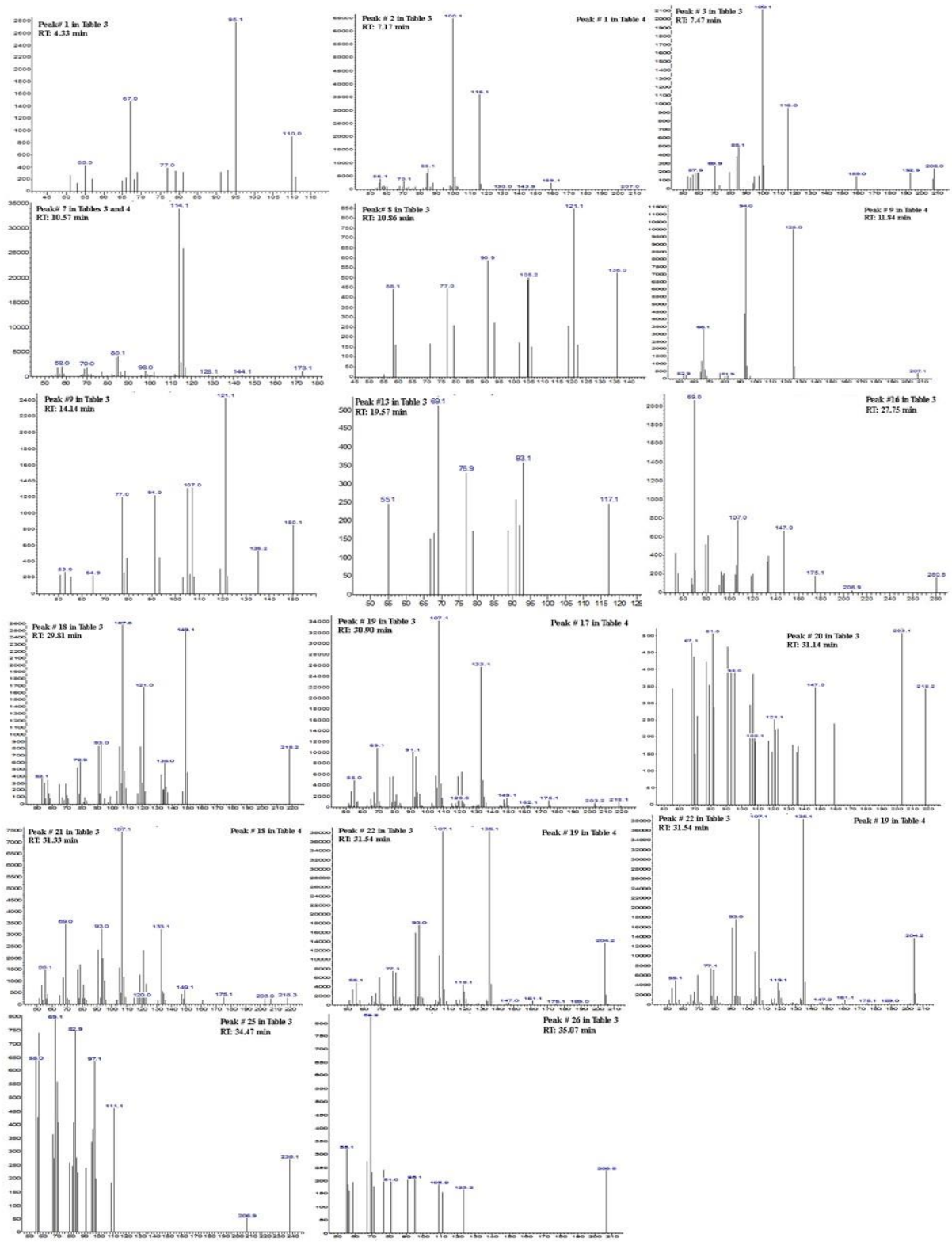

Figure S17. Mass spectra of unidentified compounds found in *S. invicta* and *S. richteri*.

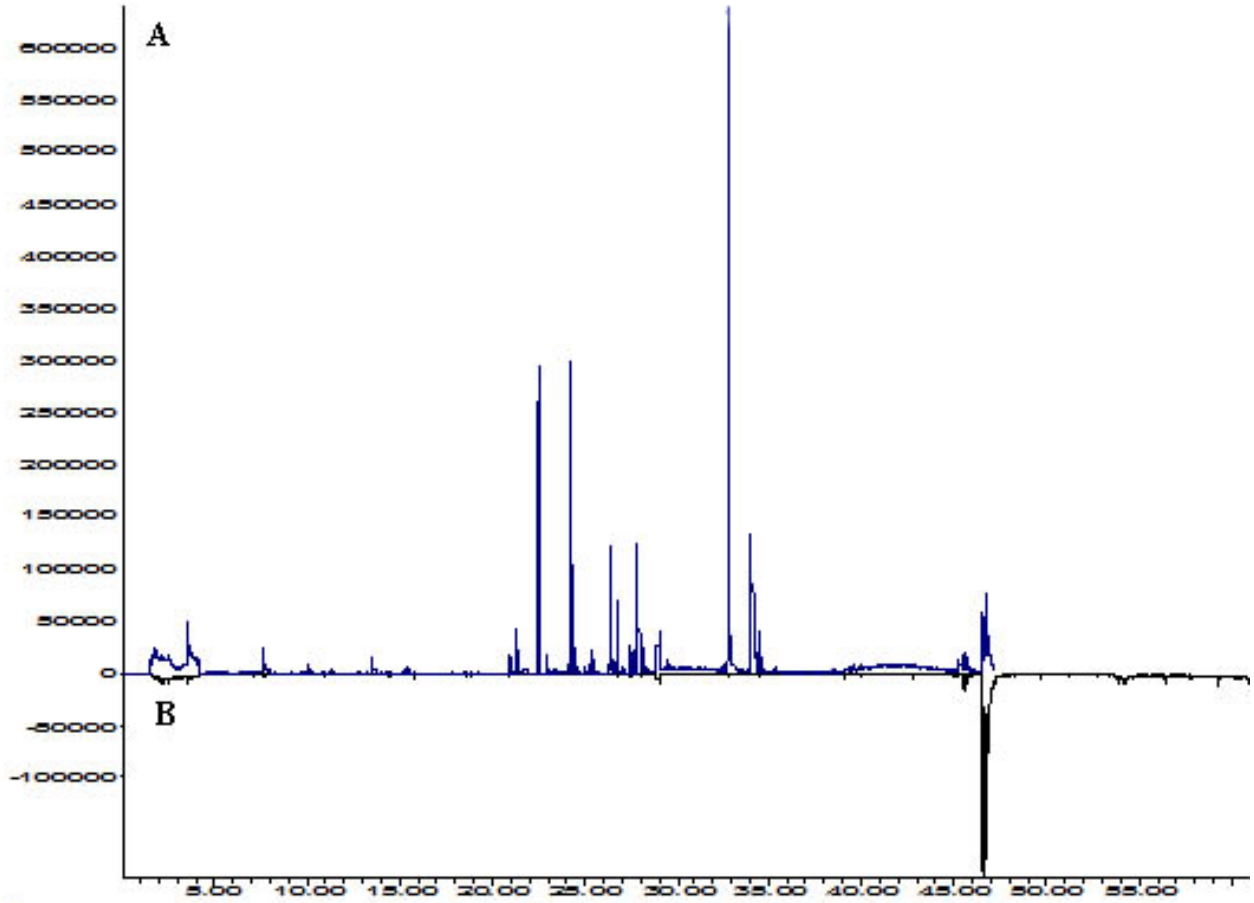

Figure S18. Overlay of GC–MS chromatograms comparing profiles of volatiles in *Monomorium minimum* workers between two sample preparation methods.
A: freeze-thaw method, SPME 2h.
B: nonfreeze method, SPME 2h.

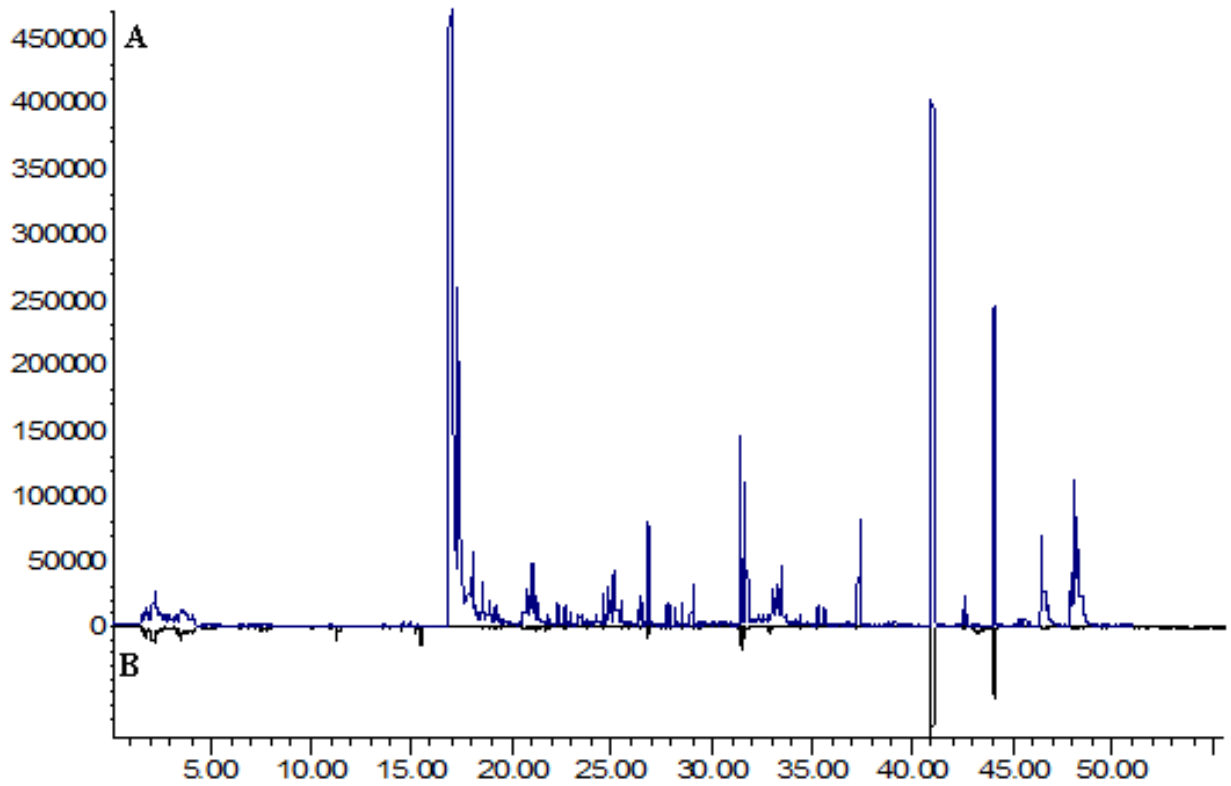

Figure S19. Overlay of GC–MS chromatograms comparing profiles of volatiles in *Monomorium minimum* queens between two sample preparation methods.
A: freeze-thaw method, SPME 2h.
B: nonfreeze method, SPME 2h.

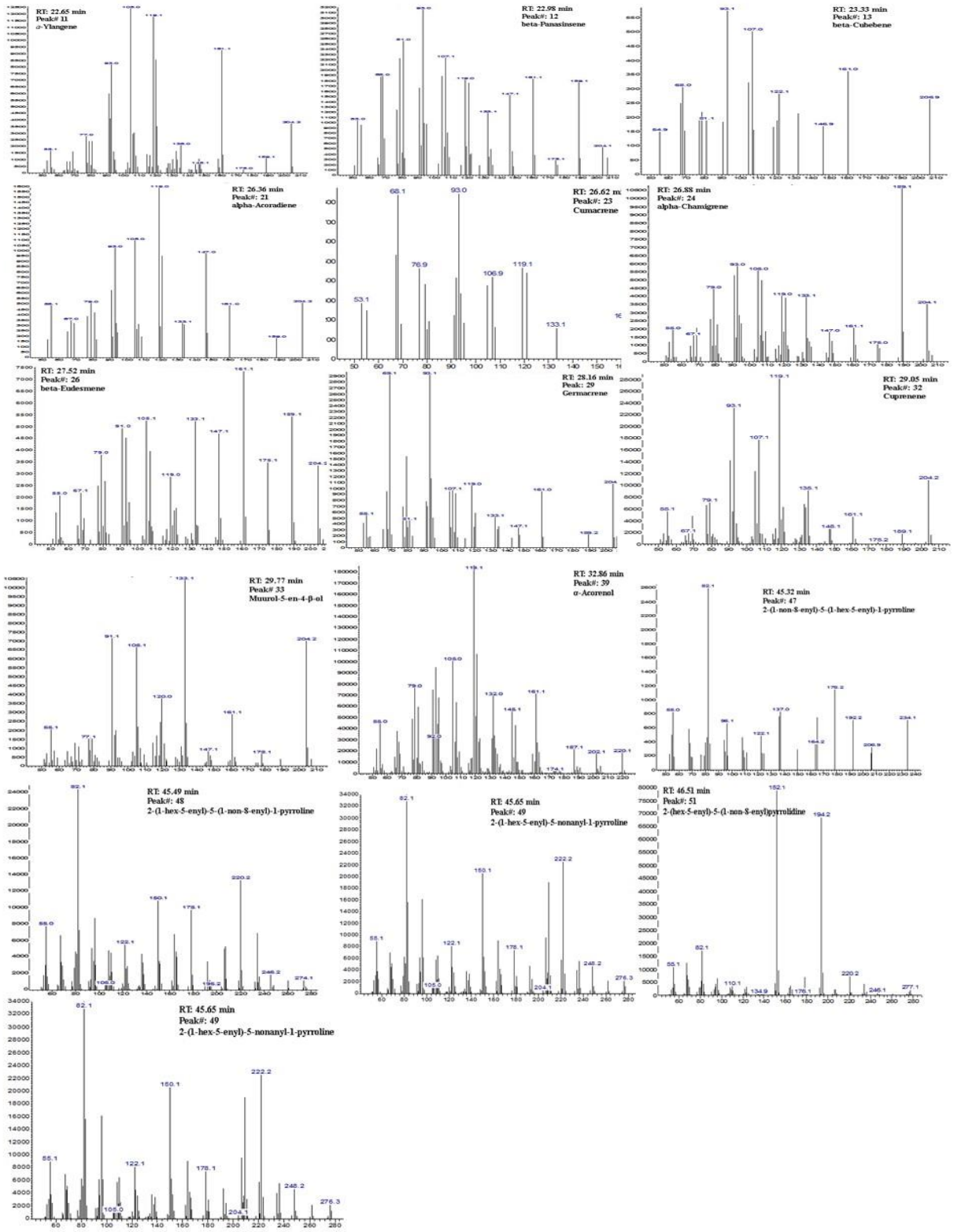

Figure S20. Mass spectra of identified compounds found in *M. minimum*.

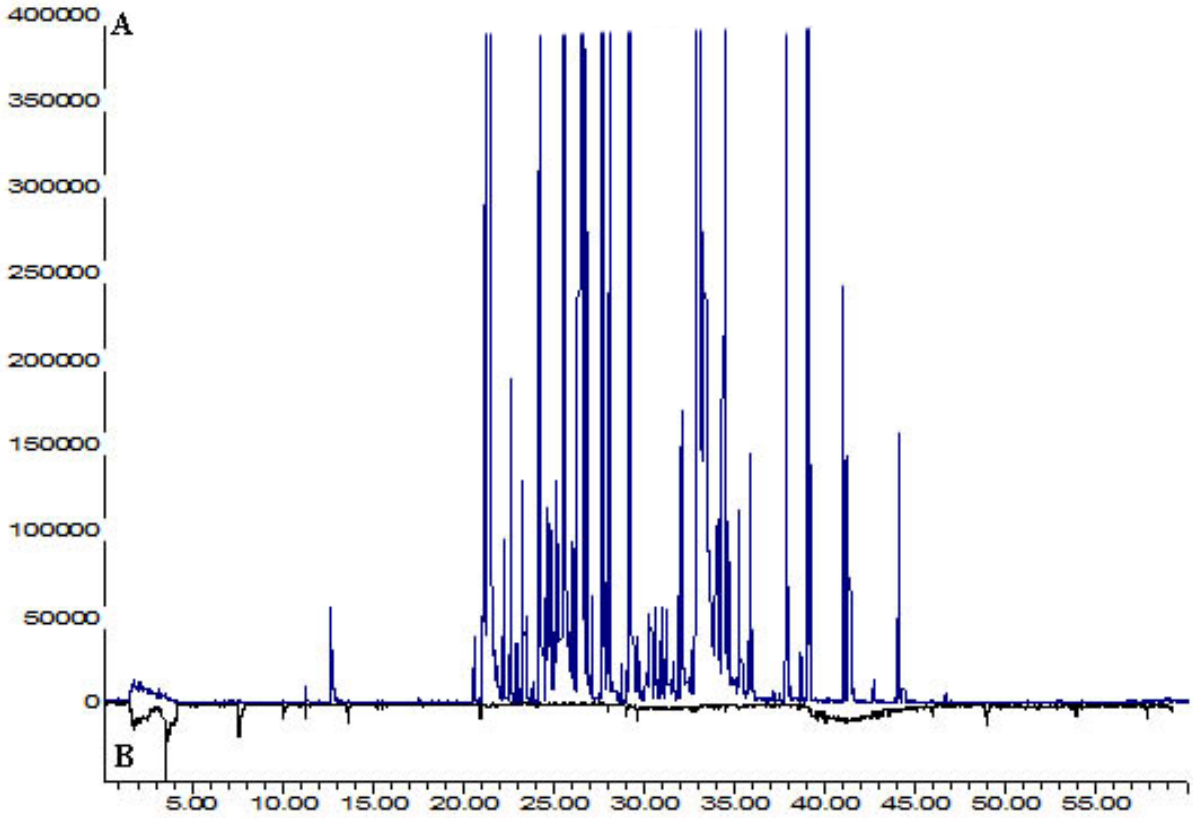

Figure S21. Overlay of GC–MS chromatograms comparing profiles of volatiles in *Monomorium pharaonis* workers between two sample preparation methods.
A: freeze-thaw method, SPME 2h.
B: nonfreeze method, SPME 2h.

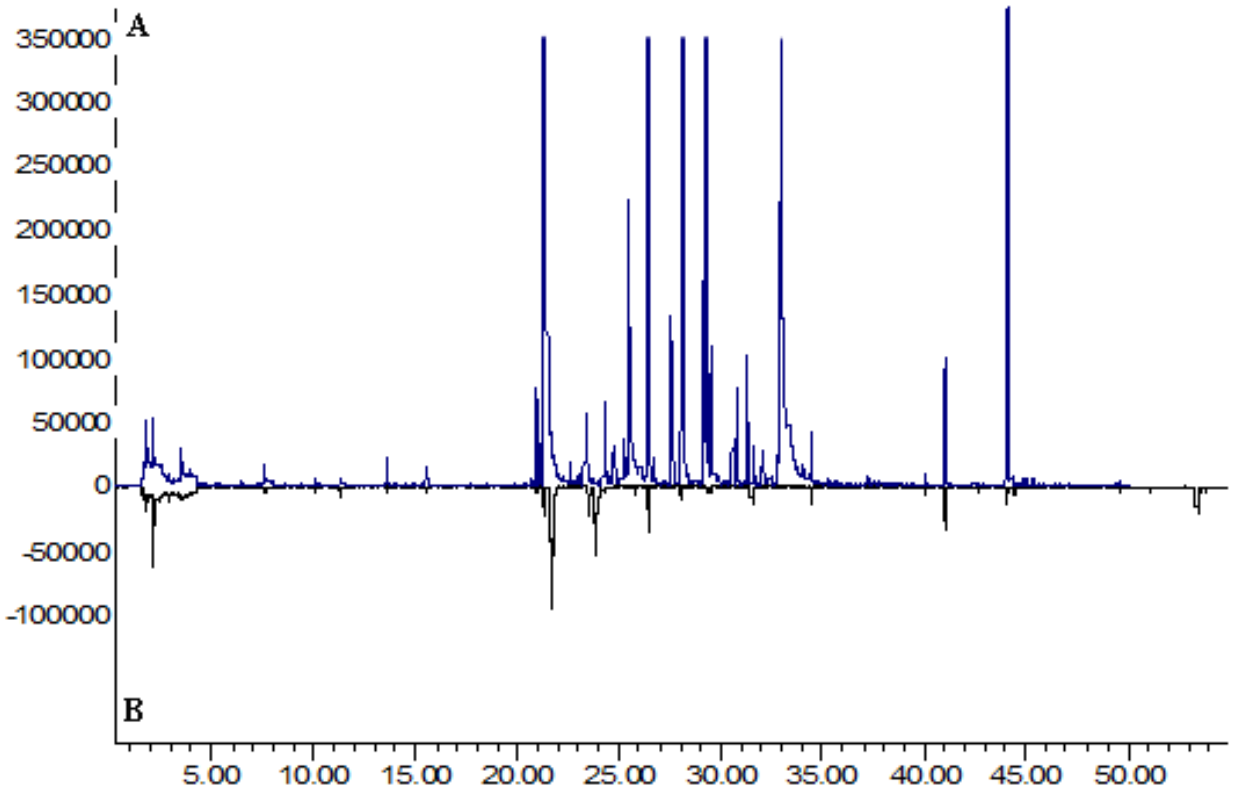

Figure S22. Overlay of GC–MS chromatograms comparing profiles of volatiles in *Monomorium pharaonis* queens between two sample preparation methods.
A: freeze-thaw method, SPME 2h.
B: nonfreeze method, SPME 2h.

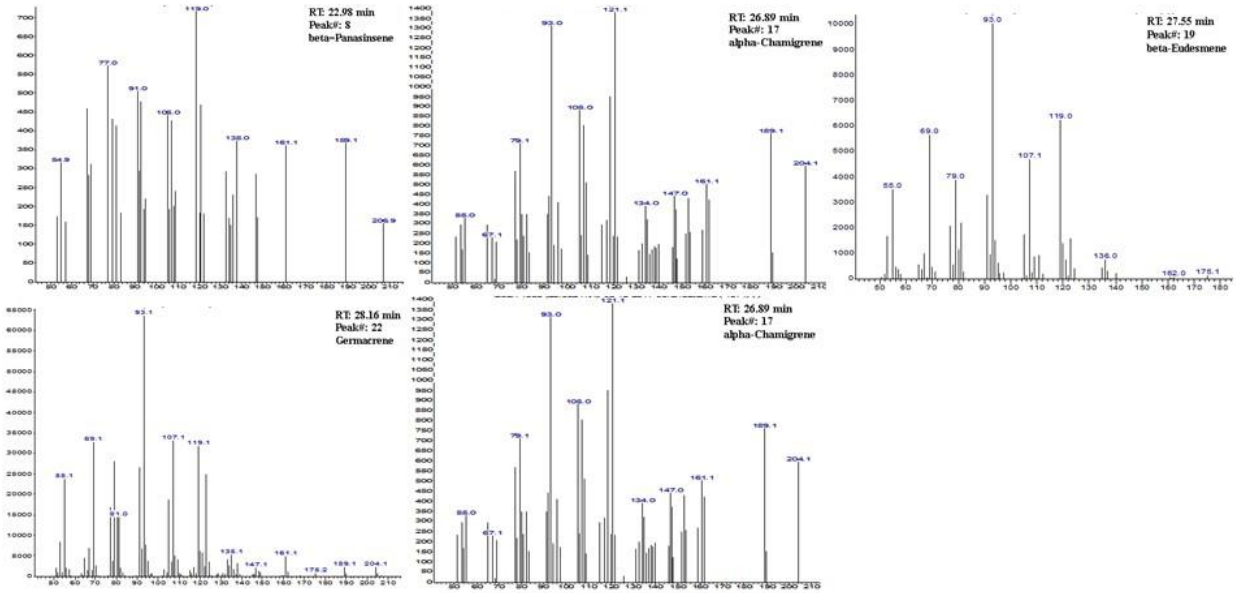

Figure S23. Mass spectra of compounds identified in *Monomorium pharaonis*.

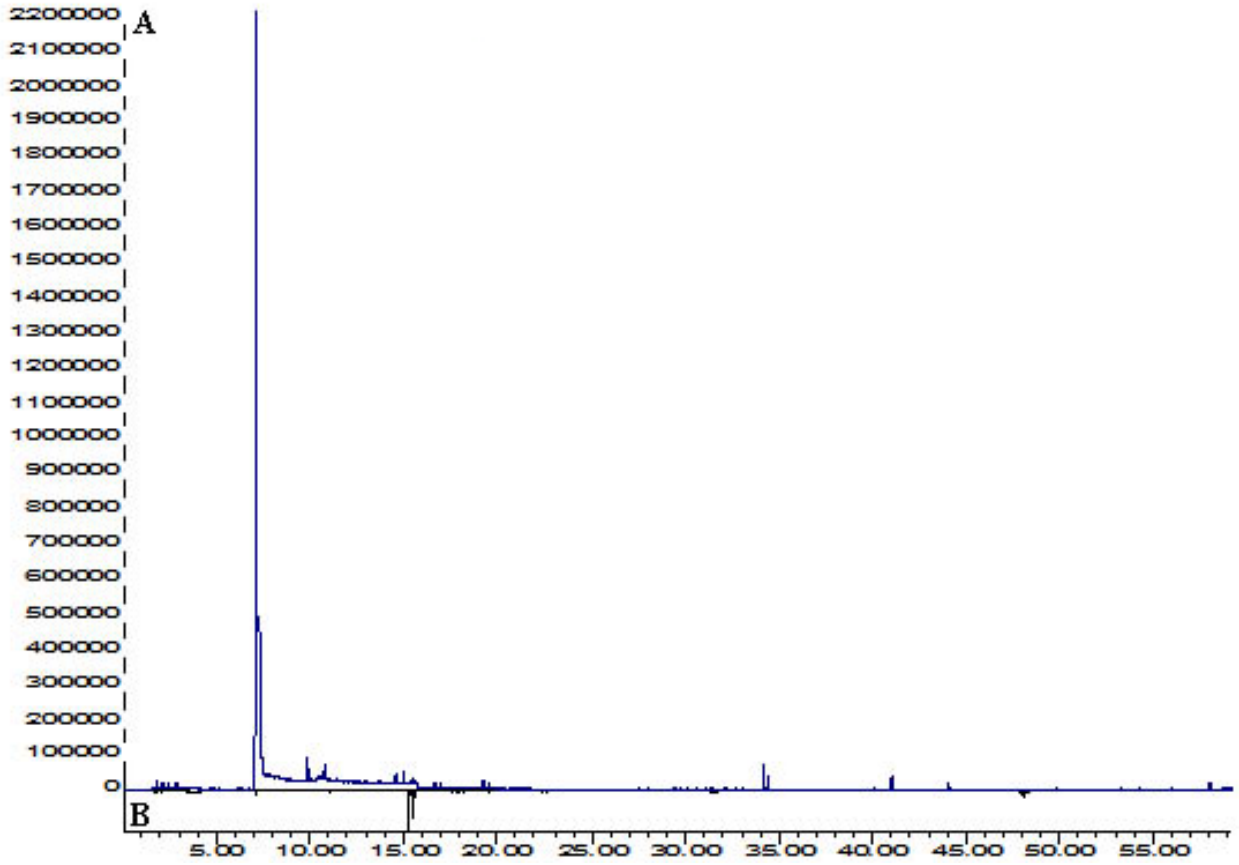

Figure S24. Overlay of GC–MS chromatograms comparing profiles of volatiles in *Reticulitermes flavipes* workers between two sample preparation methods.
A: freeze-thaw method, SPME 2h.
B: nonfreeze method, SPME 2h.

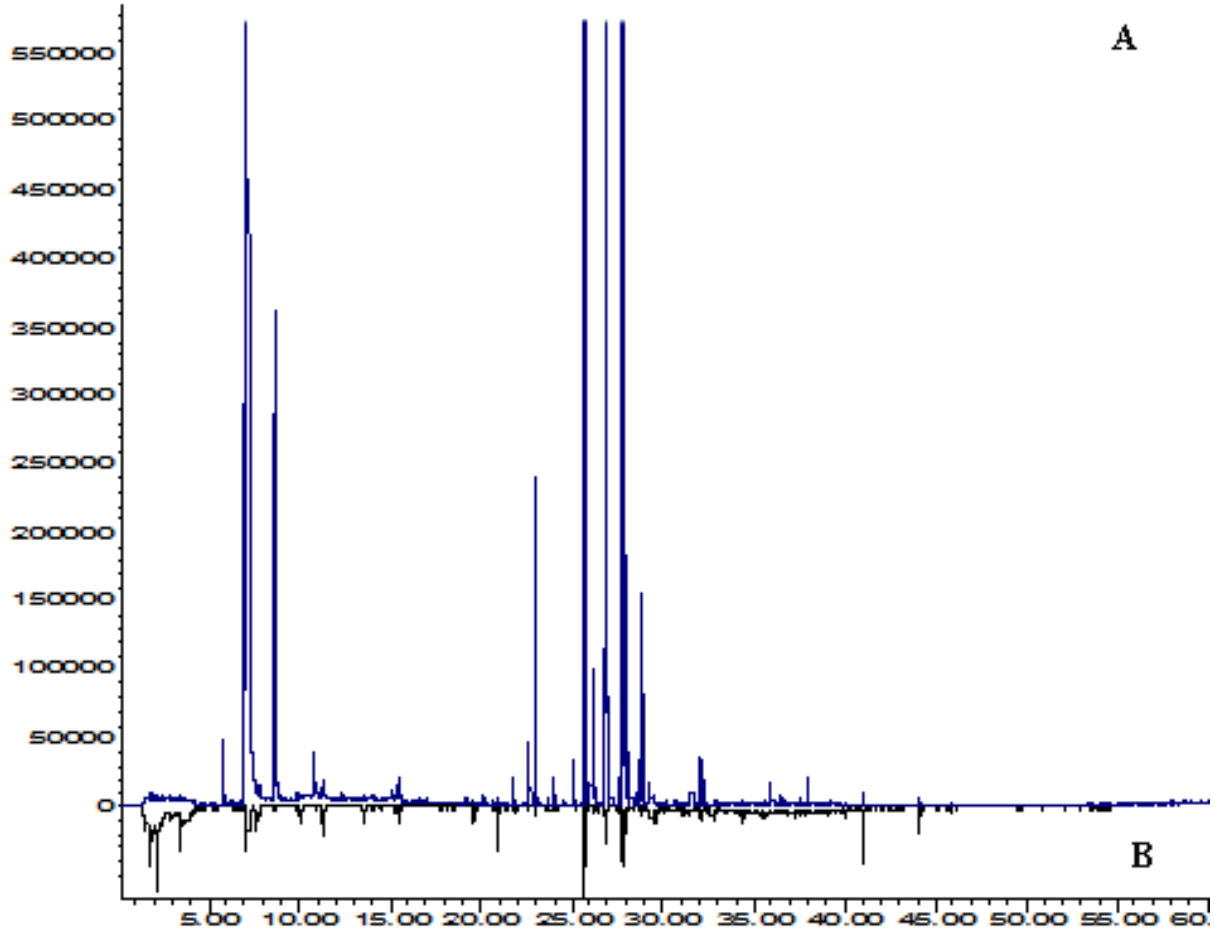

Figure S25. Overlay of GC–MS chromatograms comparing profiles of volatiles in *Reticulitermes flavipes* soldiers between two sample preparation methods.
A: freeze-thaw method, SPME 2h.
B: nonfreeze method, SPME 2h.

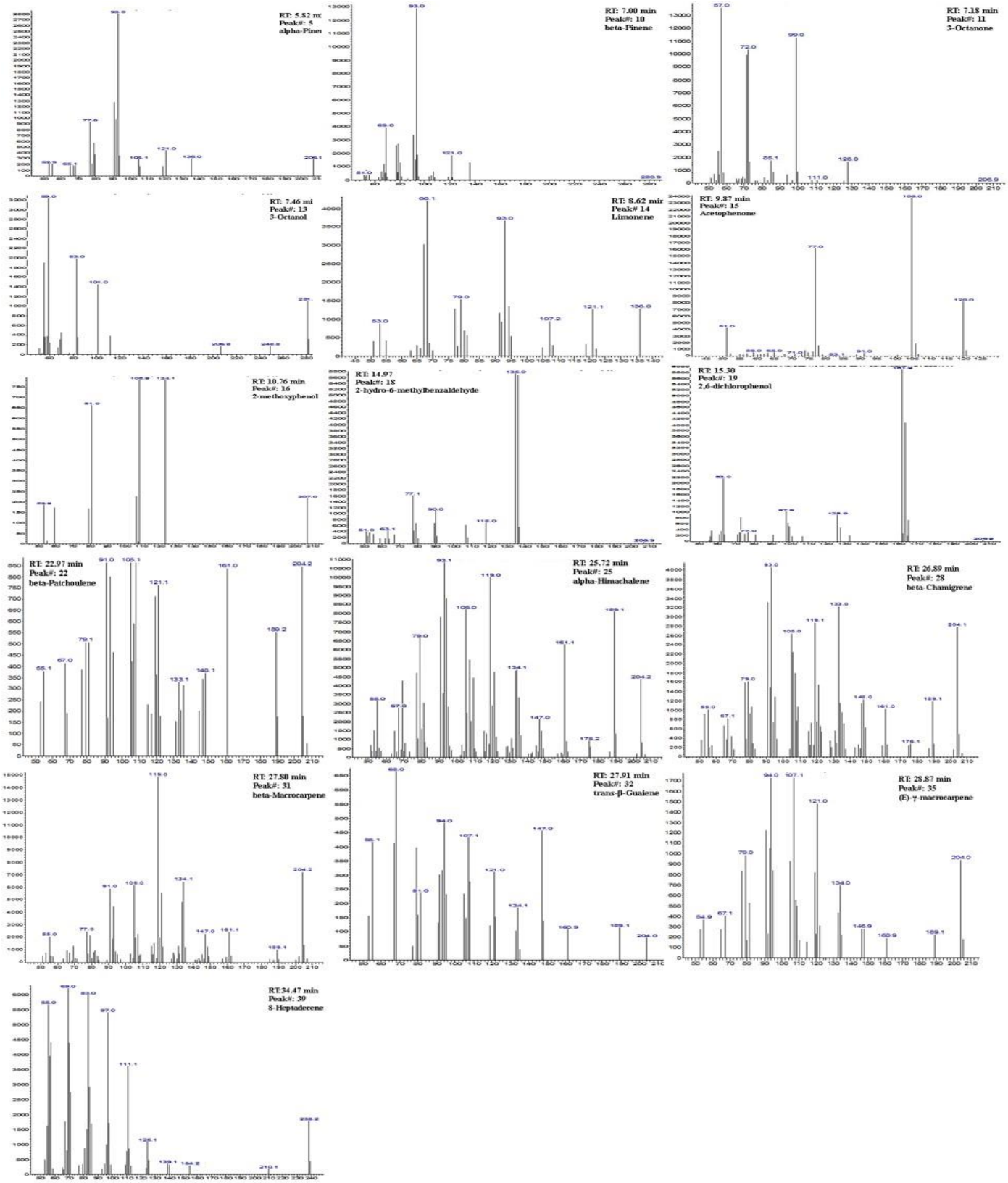

Figure S26. Mass spectra of compounds identified in *Reticulitermes flavipes*.

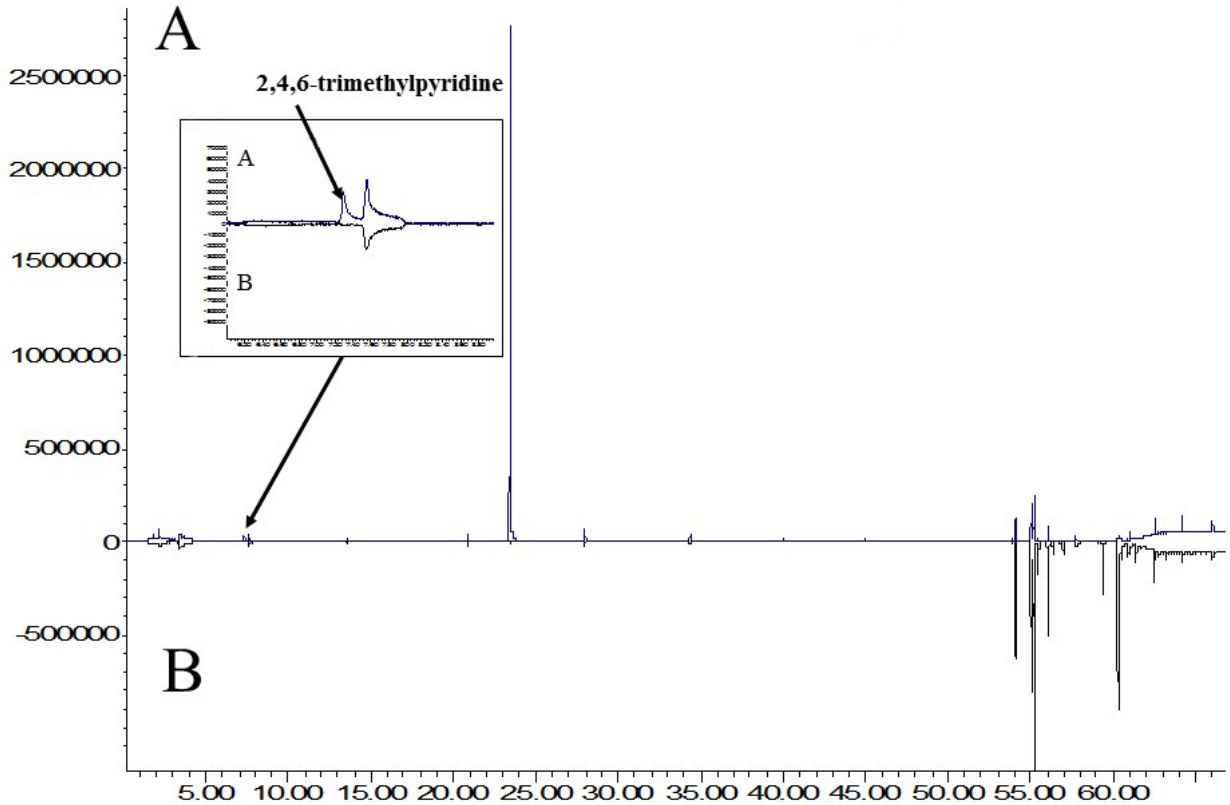

Figure S27. Overlay of GC–MS chromatograms comparing profiles of volatiles in *Coleomegilla maculata* males between two sample preparation methods. Freeze-thaw method (A, 2h SPME time) detected 2,4,6-trimethylpiridine (see the close-up), but the nonfreeze method (B) failed to detect it at the 2h SPME time.

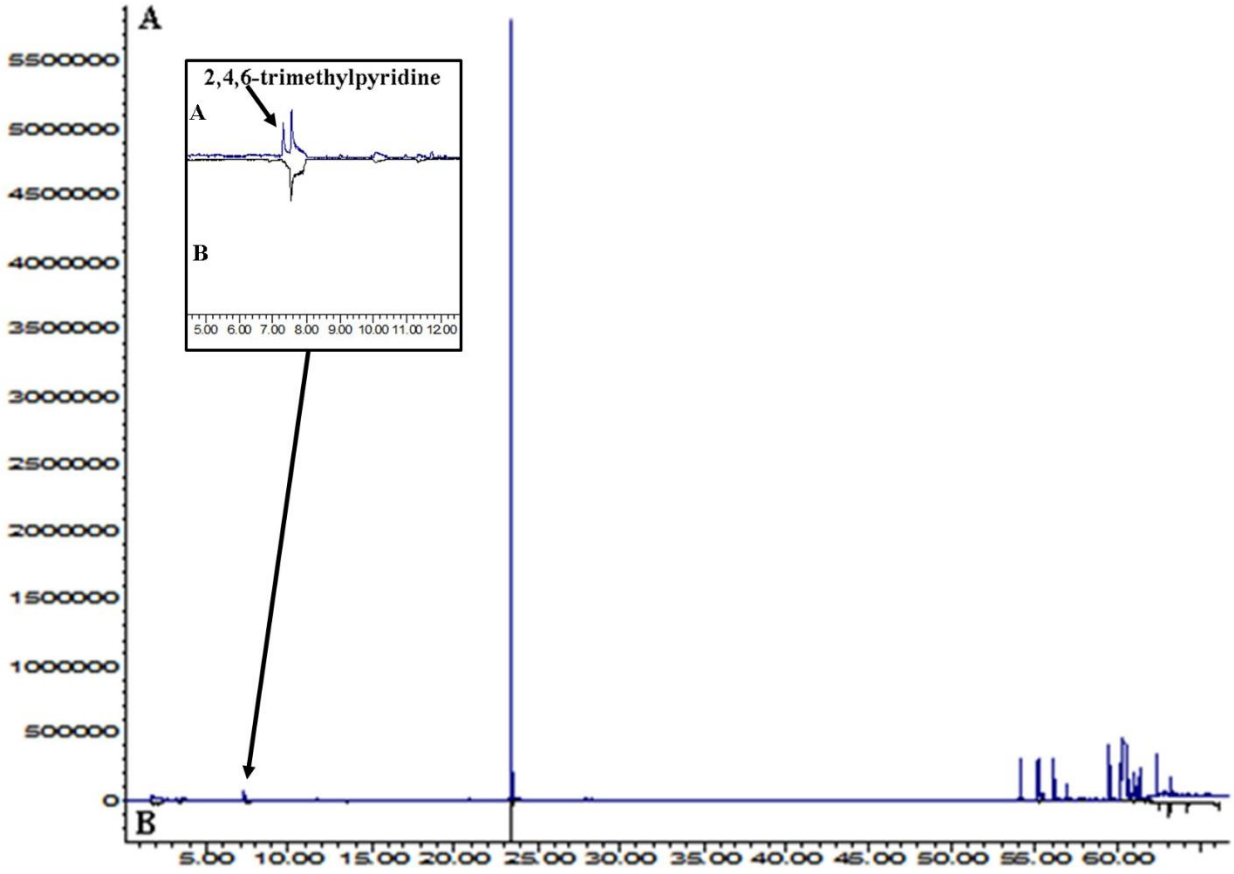

Figure S28. Overlay of GC–MS chromatograms comparing profiles of volatiles in *Coleomegilla maculata* females between two sample preparation methods. Freeze-thaw method (A, 2h SPME time) detected 2,4,6-trimethylpiridine (see the close-up), but the nonfreeze method (B) failed to detect it at the 2h SPME time.

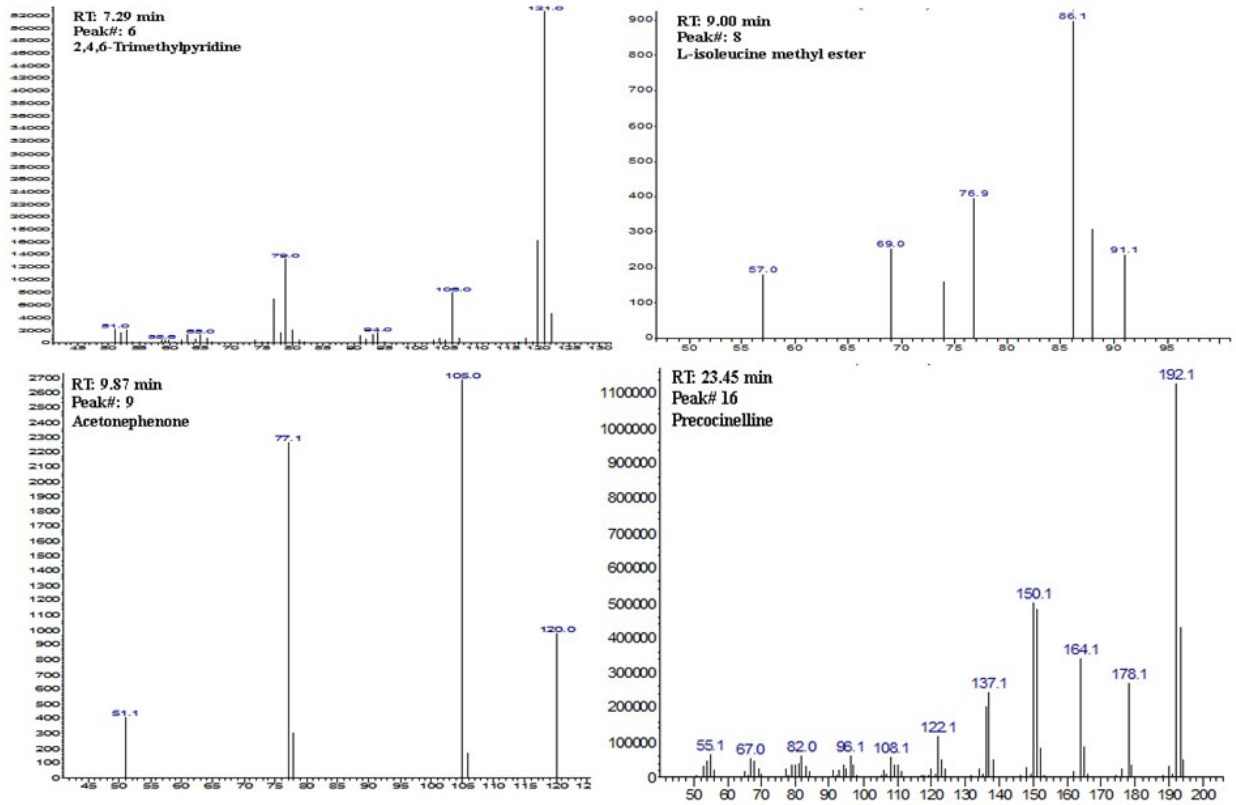

Figure S29. Mass spectra of compounds identified in *Coleomegilla maculate*.

Table S1. Retention times, retention time indices of GC-MS peaks found in *Monomorium minimum* samples using freeze-thaw method (frozen at -80 °C for 10 min, thawed at 25 °C for 5 min, and SPME at 25 °C for 2h). Peak assignment was based on the match of mass spectrum and AI with those reported in the literature[1]

Peak #	RT	Compound	AI [a]	KI [b]	Workers	Queens
1	16.96	unknown	1238	1240	–	†
2	17.32	unknown	1246	1249	–	†
3	18.04	unknown	1263	1266	–	†
4	18.58	unknown	1276	1278	–	†
5	18.64	unknown	1277	1279	†	†
6	18.83	unknown	1282	1284	†	†
7	19.20	unknown	1290	1291	–	†
8	21.04	unknown	1334	1336	–	†
9	21.14	unknown	1337	1339	†	–
10	21.31	unknown	1341	1343	†	†
11	22.65	α-Ylangene	1373	1375	†	†
12	22.98	β-Panasinsene	1381	1382	†	†
13	23.22	β-Cubebene	1387	1388	†	–
14	23.47	unknown	1392	1393	†	–
15	24.30	unknown	1413	1413	†	–
16	24.57	unknown	1419	1421	†	†
17	24.87	unknown	1427	1428	–	†
18	25.02	unknown	1430	1432	†	†
19	25.26	unknown	1436	1438	†	†
20	25.48	unknown	1442	1444	†	†
21	26.36	α-Acoradiene	1464	1465	†	†
22	26.48	unknown	1467	1468	†	†
23	26.62	Cumacrene	1470	1472	†	–
24	26.88	α-Chamigrene	1476	1478	†	†
25	27.11	unknown	1482	1483	†	–
26	27.52	β-Eudesmene	1492	1493	†	–
27	27.81	unknown	1499	1499	†	†
28	27.96	unknown	1503	1503	†	†
29	28.16	Germacrene A	1508	1509	†	†
30	28.28	unknown	1512	1512	†	†
31	28.50	unknown	1517	1518	†	†
32	29.05	Cuprenene	1532	1533	†	†
33	29.77	Muurol-5-en-4-β-ol	1550	1552	†	–
34	29.98	unknown	1556	1557	†	–
35	30.84	unknown	1578	1579	†	–
36	31.51	unknown	1595	1596	†	†
37	32.01	unknown	1609	1609	†	†

38	32.72	unknown	1628	1629	–	†
39	32.86	α-Acorenol	1632	1633	†	–
40	33.26	unknown	1643	1644	–	†
41	33.51	unknown	1650	1651	–	†
42	34.23	Unknown	1670	1671	†	–
43	34.65	unknown	1681	1682	†	–
44	35.31	unknown	1699	1699	†	†
45	35.58	unknown	1707	1707	–	†
46	37.34	unknown	1757	1758	–	†
47	45.32	2-(1-non-8-enyl)-5-(1-hex-5-enyl)-1-pyrroline	1997	1998	†	†
48	45.49	2-(1-hex-5-enyl)-5-(1-non-8-enyl)-1-pyrroline	2003	2003	†	†
49	45.65	2-(1-hex-5-enyl)-5-nonanyl-1-pyrroline	2008	2009	†	†
50	45.73	unknown	2011	2011	–	†
51	46.51	2-(hex-5-enyl)-5-(1-non-8-enyl)pyrrolidine	2037	2038	†	†
52	46.72	2-(hex-5-enyl)-5-nonanylpyrrolidine	2044	2045	†	†
53	47.93	unknown	2084	2085	–	†
54	48.13	unknown	2091	2091	–	†
55	48.30	unknown	2097	2097	–	†

a**:** AI: Arithmetic index[1] **.**
b**:** KI: Kovats index[1] .
–: Peak not detected.
†: Peak detected.

It was reported that when temperature programming is used, AI would be more appropriate than a logarithm based index, such as KI[2].

Table S2. Retention times, retention time indices (AI and KI) of GC-MS peaks found in *Monomorium pharaonis* samples using freeze-thaw method (frozen at -80 °C for 10 min, thawed at 25 °C for 5 min, and SPME at 25 °C for 2h). Peak assignment was based on the match of mass spectrum and AI with those reported in the literature[1].

Peak #	RT	Compound	AI [a]	KI [b]	Workers	Queens
1	1.94	unknown	NA [c]	NA	†	–
2	12.66	unknown	1134	1138	†	–
3	20.55	unknown	1323	1324	†	†
4	21.14	unknown	1337	1339	†	†
5	21.31	unknown	1341	1343	†	†
6	22.26	unknown	1363	1366	†	–
7	22.58	unknown	1371	1373	†	†
8	22.98	β-Panasinsene	1381	1382	†	†
9	23.47	unknown	1392	1393	†	–
10	24.15	unknown	1409	1409	†	–
11	24.26	unknown	1412	1412	†	†
12	24.57	unknown	1419	1421	†	†
13	24.74	unknown	1423	1425	–	†
14	25.21	unknown	1435	1437	–	†
15	26.43	unknown	1465	1467	†	†
16	26.75	unknown	1473	1475	†	†
17	26.89	α-Chamigrene	1477	1478	†	–
18	27.11	unknown	1482	1483	†	–
19	27.55	β-Eudesmene	1493	1494	†	†
20	27.63	unknown	1495	1495	†	†
21	27.96	unknown	1503	1503	†	–
22	28.16	Germacrene A	1508	1509	†	†
23	29.16	unknown	1535	1536	†	†
24	29.23	unknown	1536	1538	†	†
25	29.56	unknown	1545	1547	–	†
26	30.35	unknown	1565	1567	†	–
27	30.61	unknown	1572	1573	–	†
28	30.77	unknown	1576	1577	–	†
29	31.34	unknown	1591	1592	–	†
30	31.59	unknown	1597	1598	–	†
31	32.08	unknown	1611	1611	–	†
32	32.84	unknown	1632	1633	–	†
33	32.97	unknown	1635	1636	†	†
34	34.07	unknown	1665	1666	†	†
35	34.19	Acorenol	1668	1670	†	–
36	34.39	unknown	1674	1675	†	–
37	35.29	unknown	1699	1699	†	–

38	35.82	unknown	1714	1714	†	–
39	37.84	unknown	1771	1772	†	–
40	39.03	unknown	1805	1805	†	–
41	44.11	unknown	1960	1960	–	†

a**:** AI: Arithmetic index.
b**:** KI: Kovats index.
c: Not calculated.
†: Peak detected.
–: Peak not detected.

Table S3. Retention times, retention time indices (AI and KI) of GC-MS peaks found in *Reticulitermes flavipes* samples using freeze-thaw method (frozen at -80 ^{o}C for 10 min, thawed at 25 ^{o}C for 5 min, and SPME at 25 ^{o}C for 2h). Peak assignment was based on the match of mass spectrum and AI with those reported in the literature[1].

Peak #	RT	Compound	AI [a]	KI [b]	Workers	Soldiers
1	1.76	Unknown	NA [c]	NA	–	†
2	2.87	Unknown	NA	NA	†	–
3	4.59	Unknown	881	884	†	–
4	4.68	Unknown	886	887	†	–
5	5.19	Unknown	910	912	†	–
6	5.82	α-Pinene	933	938	–	†
7	6.24	Unknown	948	954	†	–
8	6.34	Unknown	952	957	†	–
9	6.72	Unknown	966	970	†	–
10	7.01	β-Pinene	976	980	†	†
11	7.18	3-Octanone	983	986	†	†
12	7.29	Unknown	987	989	–	†
13	7.46	3-Octanol	993	994	†	–
14	8.63	Unknown	1027	1031	†	†
15	9.87	Acetopnenone	1061	1066	†	–
16	10.76	2-Methoxyphenol	1086	1088	†	†
17	11.08	Unknown	1095	1096	†	†
18	14.97	2-Hydro-6-methylbenzaldehyde	1191	1192	†	†
19	15.30	2,6-dichloro phenol	1198	1199	–	†
20	21.70	Unknown	1350	1352	–	†
21	22.68	Unknown	1373	1375	–	†
22	22.97	β-Patchoulene	1380	1382	–	†
23	23.95	Unknown	1404	1404	–	†
24	25.04	Unknown	1431	1433	–	†
25	25.72	α-Himachalene	1448	1450	–	†
26	26.16	Unknown	1459	1460	–	†
27	26.55	Unknown	1468	1470	†	–
28	26.89	β-Chamigrene	1477	1478	–	†
29	26.99	Unknown	1479	1480	–	†
30	27.63	Unknown	1495	1495	†	†
31	27.80	β-Macrocarpene	1499	1499	–	†
32	27.91	*trans*-β-guaiene	1502	1502	–	†
33	28.07	Unknown	1506	1506	–	†
34	28.69	Unknown	1522	1523	–	†
35	28.87	(*E*)-γ-Macrocarpene	1527	1528	–	†
36	29.54	Unknown	1544	1546	†	–

37	30.82	Unknown	1578	1579	†	–
38	34.19	Unknown	1669	1670	†	–
39	34.47	Heptadecene	1676	1677	†	–
40	37.99	Unknown	1775	1776	–	†

a**:** AI: Arithmetic index.
b**:** KI: Kovats index.
c: Not calculated.
–: Peak not detected.
†: Peak detected.

Table S4. Retention times, retention time indices (AI and KI) of GC-MS peaks found in *Coleomegilla maculate* samples using freeze-thaw method (frozen at -80 ^{o}C for 10 min, thawed at 25 ^{o}C for 5 min, and SPME at 25 ^{o}C for 2h). Peak assignment was based on the match of the mass spectrum of the peak with that of synthetic standard or based on library search

Peak #	RT	Compound	AI [a]	KI [b]	Males	Female
1	1.79	Unknown	NA [c]	NA	†	–
2	1.82	Unknown	NA	NA	–	†
3	4.57	Unknown	880	883	–	†
4	4.78	Unknown	892	893	–	†
5	5.19	Unknown	910	912	–	†
6	7.29	2,4,6-Trimethylpyridine	987	989	†	†
7	8.63	Unknown	1027	1031	–	†
8	9.06	L-Isoleucine methyl ester	1039	1044	†	–
9	9.87	Acetophenone	1061	1066	†	†
10	9.96	Unknown	1064	1068	–	†
11	10.81	Unknown	1087	1089	–	†
12	10.97	Unknown	1092	1093	†	†
13	11.75	Unknown	1112	1114	†	†
14	13.19	Unknown	1147	1151	†	–
15	19.61	Unknown	1300	1300	†	–
16	23.45	Precocinelline	1392	1393	†	†
17	35.83	Unknown	1714	1714	†	–
18	38.23	Unknown	1782	1783	†	–
19	39.80	Unknown	1828	1829	†	–
20	42.47	Unknown	1908	1908	†	–

a**:** AI: Arithmetic index.
b**:** KI: Kovats index.
c: Not calculated.
†: Peak was detected.
–: Peak was not detected.

References

(1) Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th ed.; Allured Publ. Corp: Carol Stream, IL., 2007.
(2) Van den Dool, H.; Kratz, P. D. *J. Chromatography* **1963**, *11*, 463-471.