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ORIGINAL RESEARCH ARTICLE

Chemical compositions and insecticidal efficacies of four aromatic essential oils on rice weevil *Sitophilus oryzae* L

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Abstract

Plant essential oils contained secondary metabolite chemicals showing toxicities on stored grain insects. The main aims of this study was to evaluate the insecticidal efficacies and chemical compositions of essential oil from *C. flexuosus*, *C. winterianus*, *C. martini*, and *P. cablin* against *Sitophilus oryzae* (L.). The GC-MS analysis results showed that, the essential oils of four aromatic plants are rich in citral, neral, geranyl acetate, geraniol, citronellal, geraniol, citronellol, photocitral B, azulene and α-guaiene respectively. The essential oils of *C. flexuosus* with LC_{50} values are 44.5 and 26.1 μl/L air after 24 and 48 h exposure which was found to be most potent fumigant than *C. winterianus* followed by *C. martini* and *P. cablin* respectively. Further, in contact toxicity, *C. martini* oil being the most effective against adults of *S. oryzae* with LC_{50} values of 2.6 and 1.8 μl/cm^2 after 24 and 48 h exposure respectively, whereas moderate toxicity was observed in *S. oryzae* from the essential oils of *C. winterianus* and *P. cablin*. These results suggested that the essential oils extracted from *C. flexuosus*, *C. winterianus*, *C. martini*, and *P. cablin* can be used as in the management of *S. oryzae* in order to decrease the harmful effects and risk of synthetic insecticides on human beings.

Keywords *Cymbopogon* species · *Pogostemon cablin* · Essential oils · GC-MS · Insecticidal efficacy · *Sitophilus oryzae*

Introduction

The most problem for the storage of grain is the possibility of pest infestation and it widely affects the global economy. The infestation caused by pest are mainly controlled by using chemical insecticides but prolong using of many conventional fumigants lead insects to develop resistant against these synthetic pesticides which affects health as well as environment (Isman 2006; Pimentel et al. 2007). In view of all the aspects of control of stored-product insects and raised problems have highlighted the urgent need to develop newer eco-friendly safer and effective biofumigant (Alexander 1981). Essential oils and their individual constituents have been used as potential fumigants against stored grain insect pests (Rajendran and Sriranjini 2008; Shaaya and Kostyukovsky 2011; Rajashekar et al. 2012a, 2013). The aromatic plants contain essential oils which are naturally occurring substances responsible for scent obtained from leaves, root, stem, wood, bark, flower, fruit etc. There were reported more than 17,500 aromatic species that occur in higher plants (Bruneton 1999). These essential oils contained secondary metabolites and their toxicity worked against many stored grain insects as their activity enormously differ to the secondary plant product. The essential oil obtained from different plant contained major constituents having insecticidal activity with low mammalian toxicity and biodegradation (Isman 2006). Many essential oils contained insecticidal property (Schmidt et al. 1991; Shaaya et al. 1991; Ayvaz et al. 2009). The essential oils act as repellents, fumigants, ovicides, larvicides, insecticides and insect

Key messages
• Evaluated the insecticidal efficacy and chemical composition of essential oils from *Cymbopogon flexuosus, Cymbopogon winterianus, Cymbopogon martini*, and *Pogostemon cablin*
• The essential oils of *C. flexuosus* and *C. martini* exhibited potent fumigant and contact toxicities against *Sitophilus oryzae*
• The essential oils were found to be a potent bio-fumigant and contact toxicity to stored grain pests instead of synthetic fumigant and insecticides

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growth regulators against various insect species and known as environmental friendly pesticides (Isman 2000; Cetin et al. 2004; Mondal and Khalequzzaman 2009; Kumar et al. 2011; Rajashekar et al. 2012b; Nenaah 2014; Sousa et al. 2015).

Cymbopogan spp. is very vast genus belong to poaceae family and an important essential oil bearing plants. These essential oils are extensively used in perfumeries, cosmetics and pharmaceutical applications (Kalemba and Kunicka 2003). The important chemical constituents of these essential oils are geraniol, citral, citronellal and piperiotone respectively. The essential oil from *C. flexuosus* and *C. martini* was toxic to *Tribolium castaneum* (Caballero-Gallardo et al. 2012). The plant *Pogostemon cablin* is perennial herb commonly called as patchouli. The essential oil from leaves of patchouli is widely used in perfumery and aromatherapy (Hasegawa et al. 1992; Swamy et al. 2016). This essential oil has also toxic effects on *Myzus persiae* (Chen et al. 2017). Since essential oil has toxicity properties, the present study is proposed to determine the chemical compositions of *C. flexuosus* Nees ex Steud. Wats (Poales: Poaceae), lemongrass, *C. winterianus* Jowitt ex Bor (Poales: Poaceae), citronella, *C. martini* Roxb. Wats (Poales: Poaceae), palmarosa and *P. cablin* Blanco Benth. (Lamiales: Lamiaceae) patchouli essential oils. The study further evaluates fumigant and contact toxicity of four different essential oils against highly destructive stored product insect, rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) to find a new agrochemical from aromatic plants.

Materials and methods

Insect cultures

A culture of rice weevil (*Sitophilus oryzae*) was kept on whole wheat (*Triticium aestivum*). The insect cultures were collected from warehouse of Food Corporation of India (FCI), Sangaiprou, Imphal West, India. The collected insects were placed into insect-rearing bottle at the rate of 300 adults/kg of the respective culture medium in 2 l glass jar. Two-to-five-day-old adults of *S. oryzae* were shifted from the cultures and maintained continuously at temperature 30 ± 1 °C and 65 ± 5% RH for further procedures. These populations of 2–5 d old insect was used for the experiment for toxicity tests (Rajashekar 2016). Experiments were conducted in the same environmental condition and the cultures were further maintained in the dark in growth chamber at 27 ± 2 °C and 70 ± 5% r.h.

Collection of plant materials

The plant materials like *C. flexuosus*, *C. winterianus*, *C. martini*, and *P. cablin* were cultivated by adopting the recommended agricultural practices in the research farm of National Bioresources Park of Institute of Bioresources and Sustainable Development, (latitude 24°8′N, longitude 93°9′E) Haraorou, Imphal. The matured plants (six months old) were harvested 10 cm above the ground surface and cleaned properly by removing the extraneous material.

Extraction of essential oil

The collected aerial parts of the sample were semidried in normal temperature (25 °C - 27 °C) for 2 d on laboratory benches. The semidried plant materials were chopped into 2–3 cm in length using secateurs. The essential oils were hydro-distilled separately for 4 h by using Clevenger type apparatus. The distillation flask was charged with 500 g each of the plant materials. The distillations process was carried out in triplicate for all the different parameters. After completing the distillation process, the oil samples with water were collected in clean vial (15 ml). Further the oil was transferred into 10 ml glass vial by help of micro pipette and dried over anhydrous sodium sulphate (M. wt = 142.04). The extracted oil was stored in amber vials in a 4 °C refrigerator until further analysis.

Analysis of volatile compound

The composition of the volatile constituents was recognized by gas chromatography mass spectrometry having Quadrapole detector analyses using Thermo Scientific (Trace 1300 Gas Chromatograph & TSQ 8000 DUO Mass Spectrometry) having TG-5MS fused with silica capillary column (30 m × 0.25 mm i.d. and 0.25 μm film thickness). The ionization energy was set at 70 eV for GC-MS detection. While the injector and mass transfer line were set at 250 °C and 280 °C. Helium was used as a carrier gas at a flow rate of I ml/min and the injection volume of each sample was 0.5 μl (1:100 sample oil in hexane). The initial column temperature was programmed from 40 °C for 1 min to 250 °C at a rate of 5 °C/min heating ramp and then held at 250 °C for 20 min. The identification of the components was based on comparison of their mass spectra with those of National Institute of Standards and Technology (NIST) GC-MS Libraries 2017 respectively.

Fumigant toxicity

Fumigant toxicity methodology was followed from Rajashekar et al. (2016). By using filter paper discs, the four different essential oils were infused with different doses range from 10 to 100 μl/L air by choosing effective dosages based on trial experiments. Fumigation desiccator's having one-liter capacity were used for fumigant toxicity. The essential oils were injected using syringes through rubber septum fitted to

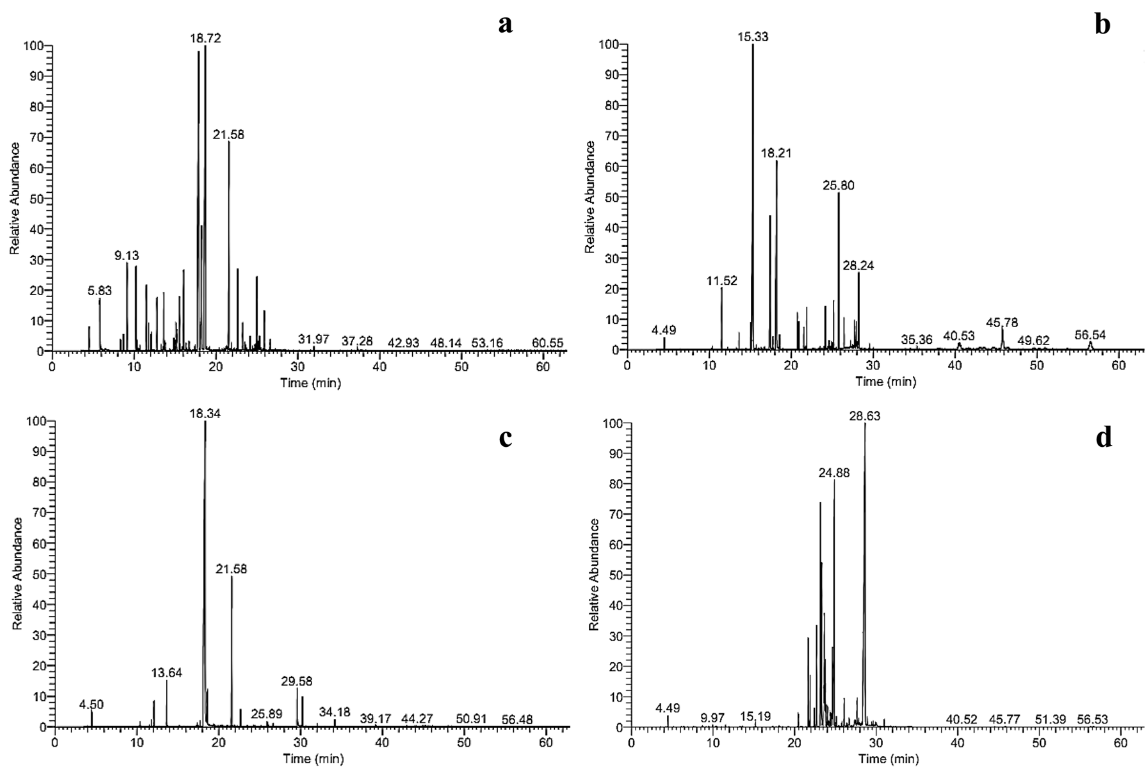

Fig. 1 Representative GC-MS total ion chromatogram of essential oils from **a**) *Cymbopogon flexuosus*, **b**) *Cymbopogon winterianus*, **c**) *Cymbopogon martini*, **d**) *Pogostemon cablin*

the desiccator's lid and the infused filter papers were placed to the under surface of the glass jars which was checked from direct contact to the insects. For each treatment, 40 individuals were tested for *S. oryzae*, both treated as well as control were four replicates were used for each dosage. Later both the live and dead adult insects were removed, and percentage of insect mortality was calculated after 24 and 48 h of treatment by using Abbott formula (Abbott 1925) and LC_{50} and LC_{90} were determined from dose response data using probit analysis (Finney 1971).

Contact toxicity

The insecticidal activity of different plant extract was studied by contact toxicity by filter paper diffusion method against *S. oryzae* adults (Broussalis et al. 1999; Rajashekar et al. 2012a). Different dosage ranges from 0.5–25 μl/cm^2 air each of essential oils were sprayed over the filter papers (Whatman N0.1; 6 cm diameter) and placed in the disposable petri dish having 6 cm diameter. Released of known age having 10 adults of *S. oryzae* was separately placed into each dish. The petri dish was reattached with adhesive tape and kept at 26 ± 2 °C and 70 ± 5% relative humidity for 24 and 48 h. Control dishes without essential oil were recorded. Each group treatment had four replicates. Insect mortality was recorded after 24 and 48 h exposure and mortality percentages were estimated while control mortality was corrected as suggested by Abbott (1925), LC_{50} and LC_{90} values were calculated as described by Finney (1971). The mortality in essential oils treatment was estimated by taking survival of adults in the control as 100% and data's were expressed as means ± Standard error (SE) of four observations ($n = 4$). Probit analysis (Finney method) was performed for calculating LC_{50} and LC_{90} (Finney 1971).

Results

Chemical constituents of four aromatic essential oils

Hydrodistillation of semi dried plant material (500 g each) yield of *C. flexuosus* (1.87%, light yellow), *C. winterianus* (1.09%, pale yellow), *C. martini* (2.17%, pale yellow in color) and *P. cablin* (0.98%, dark yellow in color) with pleasant odor. In essential oil of *C. flexuosus* nearly 46 compounds were identified amounting to 99.79% (Fig. 1a, Table 1), 44 compounds of *C. winterianus* amounting to 99.57% (Fig. 1b, Table 2), *C. martini* constitutes 23 compounds amounting to 99.69% (Fig. 1c, Table 3) and *P. cablin* having 44 compounds were identified with amounting 99.35 (Fig. 1d, Table 4). The major components of the *C. flexuosus* oil were citral (20.43%), neral (22.13%) followed by geranyl acetate (8.30%) and geraniol (7.5%); in *C. winterianus* oil, citronellal (31.4%) followed by geraniol (18.3%), citronellol (10.27%)

Table 1 Chemical composition of *Cymbopogon flexuosus* essential oil

Peak no.	RT[a]	Component	RSI[b]	%RA[c]
1	4.49	1,3,5-Cycloheptatriene	911	0.46
2	5.83	Dimethyl Sulfoxide	923	1.88
3	8.35	Tricyclo[2.2.1.0(2,6)]heptane,1,7,7-trimethyl	942	0.27
4	8.7	á-Pinene	900	0.41
5	9.13	Camphene	961	2.35
6	10.24	5-Hepten-2-one, 6-methyl	927	2.38
7	10.37	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene	887	0.48
8	10.7	Octanal	883	0.14
9	11.52	Cyclohexene,1-methyl-5-(1-methylethenyl)	907	1.85
10	11.77	á-Ocimene	923	0.79
11	12.08	trans-á-Ocimene	902	0.5
12	12.8	4-Nonanone	885	1.47
13	13.31	Cyclohexene,1-methyl-4-(1-methylethylidene)	947	0.17
14	13.55	3-Methyl-2-(2-methyl-2-butenyl)-furan	915	0.11
15	13.63	Linalool	937	1.66
16	13.79	photocitral B	982	0.39
17	14.82	Spiro[2.5]octane	840	0.38
18	14.95	6-Octenal,-methyl-3-methylene	879	0.31
19	15.13	photocitral A	990	0.73
20	15.53	3-Heptyne, 5-methyl	908	1.54
21	15.64	endo-Borneol	937	0.45
22	15.85	2-((3,3-Dimethyloxiran-2-yl)methyl)-3-methylfuran	906	0.19
23	16.06	Isoneral	915	2.41
24	16.34	à-Terpineol	913	0.25
25	16.7	Decanal	903	0.28
26	17.43	5,10-Pentadecadien-1-ol,(Z,Z)	880	0.27
27	17.87	Neral	880	22.13
28	18.22	Geraniol	935	7.1
29	18.72	Citral	899	28.43
30	21.29	1,2,4-Metheno-1H-indene,octahydro-1, 7a-dimethyl-5-(1-methylethyl)[1S-(1à,2à,3aá,4à,5à,7aá,8S*)	938	0.29
31	21.58	Geranyl acetate	943	8.38
32	21.89	,5-Cyclodecadiene,1,5-dimethyl-8-(1-methylethenyl) [S-(Z,E)]	925	0.29
33	22.66	Caryophyllene	949	2.54
34	23.27	Phenol,2-methoxy-4-(1-propenyl)	936	0.85
35	23.51	1,4,7,-Cycloundecatriene,1,5,9,9-tetramethyl-, Z,Z,Z	914	0.59
36	23.62	Precocene I	914	0.54
37	24.18	Germacrene D	937	0.53
38	24.48	cis-Muurola-4(15),5-diene	895	0.23
39	24.77	3,4-Nonadien-6-yne,5-ethyl-3-methyl	898	0.26
40	24.97	ç-Muurolene	914	2.48
41	25.16	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydro naphthalene	923	0.49
42	25.32	(E)-1-Methyl-4-(6-methylhept-5-en-2-ylidene)cyclohex - 1-ene	942	0.63
43	25.89	Neryl butyrate	936	1.05
44	26.64	10-Pentadecen-5-yn-1-ol, (E)	943	0.47
45	31.97	Neophytadiene	952	0.29
46	37.28	6-Methyl-4,6-bis(4-methylpent-3-en-1-yl)cyclohexa-1, 3-dienecarbaldehyde	896	0.1
		Total compound identified (%)	99.79	

[a] Retention time

[b] Reverse similarity index on TG-5MS capillary column

[c] Relative area (peak area relative to the total peak area)

and in *C. martini* geraniol (69.99%) followed by geranyl acetate (12.16%) and linalool (2.87%). Where as in *P. cablin*o main constituents were photocitral B (31.64%), azulene (15.22%), α-guaiene (11.46%) and seychellene (7.19%).

Fumigant toxicity of the essential oils

The insecticidal activity of the different essential oils of aromatic plants is presented in Table 5. Among the essential oils, maximum insecticidal activity was shown by the essential oils of lemongrass (*C. flexuosus*) against the adults of test insects (*S. oryzae*) followed by *C. winterianus*, *C. martini* and *P. cablin*. The LC_{50} values of essential oils are 44.5 and 26.1 μl/L air (*C. flexuous*), 84.5 and 54.4 μl/L air (*C. winterianus*), 158.5 and 137.5 μl/L air (*C. martini*), 198.9 and 142.9 μl/L air (*P. cablin*) against *S. oryzae* adults after 24 and 48 h exposure, respectively. Based on LC_{50} values of essential oils, lemongrass oil was found to be toxic

Table 2 Chemical composition of *Cymbopogon winterianus* essential oil

Peak no	RT[a]	Component	RSI[b]	%RA[c]
1	4.49	1,3,5-Cycloheptatriene	915	0.54
2	10.38	á-Pinene	913	0.12
3	11.52	Cyclohexene,1-methyl-5-(1-methylethenyl)-,(R)	903	2.78
4	12.26	5-Heptenal, 2,6-dimethyl-	956	0.09
5	13.63	Linalool	938	0.72
6	15.03	(1R,2R,5S)-5-Methyl-2-(prop-1-en-2-yl)cyclohexanol	954	1.37
7	15.32	Citronellal	930	31.4
8	15.75	(1R,2R,5R)-5-Methyl-2-(prop-1-en-2-yl)cyclohexanol	950	0.13
9	15.98	Cyclopenta[c]pyran-1,3-dione,4,4a,5,6-tetrahydro-4,7-dimethyl	892	0.17
10	16.34	à-Terpineol	928	0.1
11	16.71	Decanal	885	0.1
12	17.41	Citronellol	925	10.27
13	17.74	Citral	885	0.79
14	18.21	Geraniol	943	18.38
15	18.56	2,6-Octadienal, 3,7-dimethyl-,(E)	931	0.62
16	20.75	6-Octen-1-ol, 3,7-dimethyl-,acetate	940	1.58
17	20.92	Eugenol	939	1.29
18	21.54	Geranyl acetate	941	0.98
19	21.71	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]	900	0.29
20	21.89	1,5-Cyclodecadiene,1,5-dimethyl-8-(1-methylethenyl)-, [S-(Z,E)]	929	1.96
21	22.64	Caryophyllene	919	0.08
22	23.5	Humulene	902	0.11
23	24.03	ç-Muurolene	925	0.14
24	24.18	Germacrene D	935	2
25	24.48	(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol	925	0.22
26	24.61	1R,4aS,8aR)-1-Isopropyl-4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphthalene	938	0.45
27	24.78	à-Guaiene	878	0.18
28	24.96	(1S,4aR,8aS)-1-Isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6,8a-octahydronaphthalene	924	0.42
29	25.16	5,6-Decadien-3-yne,5,7-diethyl-	919	2.38
30	25.51	1-Isopropyl-4,7-dimethyl-1,2,3,4,5,6-hexahydronaphthalene	904	0.09
31	25.8	3,7-Cyclodecadiene-1-methanol, à,à,4,8-tetramethyl-,[s-(Z,Z)]	928	9.09
32	26.44	(2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclodeca-2,7-dienol	900	1.46
33	27.26	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	911	0.24
34	27.34	Dihydro-cis-à-copaene-8-ol	946	0.29
35	27.49	3,4-Nonadien-6-yne,5-ethyl-3-methyl	895	0.16
36	27.64	Cubenol	924	0.19
37	27.73	10-epi-ç-Eudesmol	957	1.21
38	27.94	tau.-Muurolol	918	1.9
39	28.03	1-Naphthalenol,1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-,[1R-(1à,4á,4aá,8aá)]	925	0.3
40	28.24	1-Naphthalenol,decahydro-4a-methyl-8-methy lene-2-(1-methylethyl)	944	4.42
41	29.56	2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	925	0.21
42	35.36	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene	928	0.12
43	37.9	5H-Pyrano[2,3,4,5-lmn]phenanthridin-5-one	816	0.13
44	38.12	Heptasiloxane,hexadecamethyl	759	0.1
Total compound identified (%)			99.57	

[a] Retention time

[b] Reverse similarity index on TG-5MS capillary column

[c] Relative area (peak area relative to the total peak area)

to the *S. oryzae* and toxicity was dose dependent and mortality was increased with exposure time (Table 5).

Contact toxicity of tested essential oils

The essential oils of four aromatic plants were toxic to *S. oryzae* adults. In the filter paper diffusion bioassay, the LC_{50} values essential oils of 5.0 and 3.8 μl/cm^2 (*C. flexuous*), 11.4 and 5.3 μl/cm^2 (*C. winterianus*), 2.6 and 1.8 μl/cm^2 (*C. martini*), 4.9 and 3.7 μl/cm^2 (*P. cablin*) against *S. oryzae* adults after 24 and 48 h exposure, respectively. Based on LC_{50} values of *C. martini* essential oils was found to be highly toxic to the *S. oryzae* and toxicity was dose and time dependent Table 6.

Discussion

Cymbopogon species have been widely cultivated in tropical countries. The essential oil of *C. citratus* are broadly used in flavours, fragrances, cosmetics, perfumery, and pharmaceuticals and they are also applied in aromatherapy (Shah et al. 2011). The major components of essential oils from *Cymbopogon* spp. are citral, geranial, citronellol, citronellal, geranyl acetate and geranyl formate (Prashar et al. 2003; Khanuja et al. 2005; Bhatnagar 2018). On the other hand, our results of GC-MS analysis revealed that the essential oils of *Cymbopogon* spp. are rich in citral (20.43%), neral (22.13%), geranyl acetate (8.30%), geraniol (7.5%) in *C. flexuosus*; citronellal (31.4%) geraniol (18.3%), citronellol (10.27%) in *C. winterianus* oil, geraniol (69.99%) and geranyl acetate (12.16%) in *C. martini*. Whereas photocitral B (31.64%) followed azulene (15.22%) and α-guaiene (11.46%) were in *P. cablin*. These are major components of the essential oils, which account for more than 10%. Geraniol and geranyl acetate are major component of essential oils of *C. martini* (Prashar et al. 2003), *Lippia alba* (Peixoto et al. 2015), *C. nardus* (Delespaul et al. 2000), *C. citrus* (Yen and Lin 2017), *C. flexuosus* and *C. winterianus* (Bhatnagar 2018), *C. confertiflorus* and *C. microstachys* (Khanuja et al. 2005) and *C. distans* (Zhang et al. 2011). Citral is the main constituents of essential oils from *C. pendulus* and *C. flexuosus* (Khanuja et al. 2005), *C. citratus* (Li et al. 2005). Citronellal is one of main chemicals of essential oils from *Corymbia* spp. and *Eucalyptus* spp. (Filomeno et al. 2017), *C. winterianus* Jowitt (Simic et al. 2008). In spite of geraniol, citral, citronellal or geranyl acetate are the major phytochemical compounds of essential oils from *Cymbopogon* spp., *Corymbia* spp. and *Eucalyptus* spp. but all are identified as predominant in the essential oils of a single plant of *Cymbopogon* spp. Along with photocitral B, azulene, α-guaiene and seychellene chemical components of *P. cablin* essential oil were broadly identified. In the present data percentage content of major and minor volatiles were differ from those earlier published on the essential oils of same species (Pandey et al. 2003; Raina et al. 2003) and these variations may be geographical or environmental factors. Further study needed for ecological role on presence of volatile molecules in essential oils of same species in different geographical locations of North East Region of India.

Table 3 Chemical composition of *Cymbopogon martini* essential oil

Peak no.	RT[a]	Composition	RSI[b]	%RA[c]
1	4.5	1,3,5-Cycloheptatriene	918	0.63
2	10.38	á-Pinene	903	0.38
3	11.53	1-methyl-5-(1-methylethenyl)-,(R)	906	0.12
4	11.78	Tricyclo[3.2.1.0(2,4)]octane, 8-methylene-, (1à,2à,4à,5à)	920	0.48
5	12.1	á-Ocimene	941	1.49
6	13.64	Linalool	938	2.87
7	17.38	Geraniol	850	0.56
8	17.73	trans-Verbenol	881	0.38
9	18.33	Geraniol	949	69.99
10	18.6	2,6-Octadienal, 3,7-dimethyl-,(E)	916	1.98
11	19.42	2,6-Octadien-1-ol,3,7-dimethyl-, formate, (Z)	889	0.1
12	20.34	Oxiranemethanol,3-methyl-3-(4-methyl-3-pentenyl)	857	0.38
13	21.06	2-Methyl-6-methylene-octa-1,7-dien-3-ol	904	0.19
14	21.58	Geranyl acetate	951	12.26
15	22.66	Cyclohexanemethyl propanoate	876	1.14
16	24.24	4a,8-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronapthalene	891	0.29
17	25.89	Neryl butyrate	933	0.29
18	26.01	1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-, (E)	930	0.25
19	26.65	10-Pentadecen-5-yn-1-ol, (E)	940	0.34
20	29.57	2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	934	3
21	30.22	Hexanoic acid,3,7-dimethyl-2,6-octadienylester, (Z)	954	1.86
22	32.04	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene	896	0.28
23	34.18	Geranyl caprylate	941	0.43
Total compound identified (%)				99.69

[a] Retention time

[b] Reverse similarity index on TG-5MS capillary column

[c] Relative area (peak area relative to the total peak area)

Table 4 Chemical composition of *Pogostemon cablin* essential oil

Peak no.	RT[a]	Composition	RSI[b]	%RA[c]
1	4.49	1,3,5-Cycloheptatriene	911	0.23
2	9.97	á-Pinene	932	0.05
3	15.19	6-Octenal, 3,7-dimethyl-, (S)	937	0.1
4	20.44	Cyclohexene,4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-,3R-trans)	900	0.42
5	21.7	1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-,[1S-(1à,4à,7à)]	895	2.91
6	21.9	1,5-Cyclodecadiene,1,5-dimethyl-8-(1-methylenenyl-,nyl)-, [S-(Z,E)]	933	1.62
7	22.44	(1S,1aS,1bR,4S,5S,5aS,6aR)-1a,1b,4,5a-Tetramethyldecahydro-1,5-methanocyclopropa[a]indene	946	0.63
8	22.68	Caryophyllene	955	3.65
9	23.17	à-Guaiene	938	11.46
10	23.33	Seychellene	957	7.19
11	23.44	3,4-Nonadien-6-yne,5-ethyl-3-methyl	908	0.21
12	23.56	Cyclohexene,4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methyl	720	1.43
13	23.66	1-Nitrosoadamantane5,6-Decadien-3-yne,5,7-diethyl	927	4.69
14	23.73	5,6-Decadien-3-yne,5,7-diethyl	930	3.36
15	23.89	Bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]	913	0.59
16	24.07	1,5-Cyclodecadiene,1,5-dimethyl-8-(1-methylethenyl)-, [S-(Z,E)	871	0.62
17	24.19	Germacrene D	949	0.29
18	24.38	Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)	915	0.69
19	24.54	1H-3a,7-Methanoazulene,octahydro-1,9,9-trimethyl-4-methylene-, (1à,3aà,7à,8aá)-	915	0.41
20	24.63	Aciphyllene	944	3.33
21	24.87	Azulene,1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1à,7à,8aá)]	910	15.22
22	24.95	(2R,8R,8aS)-8,8a-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,7,8,8ahexahydronaphthalene	884	0.1
23	25.13	à-Maaliene	928	0.36
24	25.77	3,7-Cyclodecadiene-1-methanol, à,à,4,8-tetramethyl-,[s-(Z,Z)]	925	0.14
25	26.08	(1R,4aS,6R,8aS)-8a,9,9-Trimethyl-1,2,4a,5,6,7,8,8a-octahydro-1,6-methanonaphthalen-1-ol	951	0.97
26	26.4	(2S,4R)-p-Mentha-[1(7),8]-diene 2-hydroperoxide	878	0.16
27	26.65	10-Pentadecen-5-yn-1-ol, (E)	939	0.3
28	26.7	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol	891	0.31
29	27.36	5,10-Pentadecadiyne, 1-chloro	890	0.39
30	27.47	5,10-Pentadecadiyn-1-ol	902	0.23
31	27.66	Dihydro-cis-à-copaene-8-ol	951	0.91
32	27.86	(1,2,2-trimethyl-3-cyclopenten-1-yl)acetaldehyde	886	0.28
33	27.97	Neointermedeol	887	0.11
34	28.1	2R-Acetoxymethyl-1,3,3-trimethyl-4 t-(3-methyl-2-buten-1-yl)-1 t-cyclohexanol	942	0.08
35	28.23	2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene	901	0.11
36	28.4	1,4-Dimethyl-7-(prop-1-en-2-yl)decahydroazulen-4-ol	918	3.03
37	28.62	photocitral B	928	31.64
38	28.89	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol	909	0.35
39	29.44	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	958	0.14
40	29.62	(2S,4R)-p-Mentha-[1(7),8]-diene 2-hydroperoxide	865	0.13
41	29.79	1-Hexyl-2-nitrocyclohexane	906	0.05
42	29.94	2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl-9-(phenylsulfonyl)-, (E,E)	911	0.12
43	30.02	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene	955	0.12
44	30.96	(1R,2R,(4A)S,(8A)R)-1,2,(4A),5,6,7,8,(8A)-Octahydro-2-methylnaphthalen-1-methanol	862	0.22
		Total compound identified (%)		99.35

[a] Retention time

[b] Reverse similarity index on TG-5MS capillary column

[c] Relative area (peak area relative to the total peak area)

Table 5 Fumigant activity of essential oils from four aromatic plants against *S. oryzae*

Essential oils	Period (h)	Toxicity/stage			
		LC_{50}[a, b]	LC_{90}[a]	Slope ± SE	X^2(df)
Cymbopogon flexuosus	24	44.5 ± 4.1 (38.0–54.6)	80.1	2.09 ± 0.8	0.405(4)
	48	26.1 ± 1.2 (23.6–28.6)	48.9	3.579 ± 1.2	1.174(3)
Cymbopogon winterianus	24	84.5 ± 3.4 (77.6–91.3)	152.1	1.320 ± 0.2	5.871(5)
	48	54.4 ± 4.7 (45.1–63.7)	98.3	1.951 ± 0.4	0.463(4)
Cymbopogon martini	24	158.6 ± 5.5 (147.8–169.4)	285.4	0.361 ± 0.1	1.983(6)
	48	137.5 ± 4.6 (128.4–146.6)	248.6	0.605 ± 0.2	1.868(6)
Pogostemon cablin	24	198.4 ± 5.6 (187.3–209.6)	357.1	0.208 ± 0.1	1.234(5)
	48	142.9 ± 4.2 (134.2–151.3)	257.2	0.378 ± 0.1	6.685(5)

[a] LC_{50} and LC_{90} = µl/L air

[b] Values in parenthesis represent Lower Confidence Limit- Upper Confidence Limit (n = 4)

The above results clearly revealed that essential oils of four aromatic plants content bioactive insecticidal components.

The present results indicate that essential oils of *Cymbopogon* spp. and *P. cablin* have fumigant and contact toxicity properties against adults of *S. oryzae*. The essential oils of Lemongrass (*C. flexuosus*) more toxic in fumigant assay against *S. oryzae* followed by *C. winterianus, C. martini* and *P. cablin*, with median lethal dosage of 44.5 & 26.1 µl/L air at 24 and 48 exposures, respectively Table 5. The LC_{50} values of *Sature jahortensis* oil is 68.7 µl/L against *Callosobruchus maculatus* after 24 h exposure (Ebadollahi et al. 2012). The essential oils of *Eucalyptus camalculensis* Dehnh., *Laurus nobilis* L., *Syzygium aromaticum* L. and *Citrus sinensis* L. were exhibited toxic against *S. oryzae* with LC_{50} values of 22.2, 41.7, 41.76 and 63.8 µl/L after 48 h exposure, respectively (El-Bakry et al. 2016). Bett et al. (2016) reported that the essential oils of *Cupressus lusitanica* and *Eucalyptus saligna* toxic effect on *Sitophilus zeamais* with LC_{50} values of 29.1 and 26.8 50 µl/L air, after 24 h exposure. *Ocimum americanum* leaf essential oil at 50 µl/L air, 7 d exposure and 120 h exposure time caused 66.9 and 100% mortality of *T. castaneum, S. oryzae* and *R. dominica*, respectively (Ogendo et al. 2008). Fumigant toxicity of essential oils of lemongrass found to be stronger as compared with above mentioned essential oils. The lemongrass essential oils contain nearly 46 compounds, among them citral, neral, geranyl acetate and geraniol were major components. These major components have insecticidal properties based on their earlier reports (Cheng et al. 2009; Jeon et al. 2009). The potent fumigant toxicity of lemongrass may be attributed to its major compounds or trace compounds and possible synergistic and antagonistic effect of compounds in the oils.

Further our work-related contact toxicity bioassay determined using filter paper diffusion assay showed that palmarosa (*C. martini*) essential oils exhibited potent contact toxicity, whereas lemongrass and patchouli essential oils have average and citronella oils revealed moderate toxicity against adults of *S. oryzae*. Bachrouch et al. (2015) reported that the essential oil of *Artemisia herba-alba* and *Artemisia absinthium* were toxic to *T. castneum* (7.4 and 2.2 µl/insect,

Table 6 Contact toxicity of essential oils from four aromatic plants against *S. oryzae* by filter paper diffusion method

Essential oils	Period (h)	Toxicity/stage			
		LD_{50}[a, b]	LD_{90}	Slope ± SE	X^2(df)
Cymbopogon flexuosus	24	5.0 ± 0.2 (4.62–5.42)	9.54	0.963 ± 0.1	2.04 (6)
	48	3.8 ± 0.26 (3.29–4.34)	6.87	1.409 ± 0.3	0.405 (4)
Cymbopogon winterianus	24	11.4 ± 0.4 (10.6–12.2)	20.5	0.356 ± 0.08	0.358 (4)
	48	5.3 ± 0.28 (4.8–5.92)	9.65	0.913 ± 0.14	0.162 (3)
Cymbopogon martini	24	2.6 ± 0.1 (2.39–2.83)	4.72	1.912 ± 0.2	2.68 (4)
	48	1.8 ± 0.13 (1.58–2.12)	3.31	2.901 ± 0.4	0.229 (3)
Pogostemon cablin	24	4.9 ± 0.13 (4.6–5.19)	8.83	0.888 ± 0.1	1.65 (7)
	48	3.7 ± 0.15 (3.4–4.0)	6.69	1.327 ± 0.14	0.511 (5)

[a] LD_{50} and LD_{90} = µl/cm^2

[b] Values in parenthesis represent Lower Confidence Limit- Upper Confidence Limit (*n* = 4)

respectively). Bett et al. (2016) revealed that *Eucalyptus* oil was toxic to both insects (LC_{50} = 0.36 ml/cm^2 for *S. zeamais* and 0.48 ml/cm^2, respectively). The bioactive compound, carvone was most toxic to stored grain pests *S. oryzae* (LC_{50} = 28.17 μg/cm^2) and *T. castaneum* (LC_{50} = 19.8 μg/cm^2) respectively (Abdelgaleil et al. 2009). The essential oils of *Corymbia citriodora* leaves (LC_{50} = 21.53 μg/mg of insect) and neem oil (LC_{50} = 30.19 μg/mg of insect) were toxic to *Plutella xylostella* (Filomeno et al. 2017). El-Bakry et al. (2016) reported that the essential oils of *E. camadulensis* Dehnh., *L. nobilis* L., *S. aromaticum* L. and *Citrus sinensis* L. were exhibited toxic against *S. oryzae* with LC_{50} values of 0.34, 0.44, 0.04 and 0.43 mg/cm^2 after 48 h exposure, respectively. Whereas the contact toxicity of palmarosa (*C. martini*) essential oils to be potent comparable with above cited or reported essential oils.

The contact and fumigant toxicity of aromatic essential oils are largely attributed to secondary metabolites or monoterpenoids which are high degradation, typically volatile, lipophilic in nature and have high penetrative character. They can penetrate insects rapidly and affect with their physiological and cellular functions (Isman 2000; Rajashekar et al. 2016; Bachrouch et al. 2015). Owing to high degradation, penetration and volatile in nature, they have fumigant and gaseous action, which are very important in grain protectants against stored -product beetles. In the similar state, Zhang et al. (2011) and Olivero-Verbal et al. (2010) reported that the insecticidal effect of *Cymobopogon* species could be attributed to its chemical constituents. However toxic effects of the essential oils cannot be explained by the action of their major constituents or single compound only, signifying that these actions may be result of a synergistic/intact interaction between major and minor constituents. Therefore, the toxicity of four aromatic essential oils can be attributed to synergistic action of its diverse major and minor components.

Conclusion

The aromatic essential oils have broad spectrum activity against stored grain insect pests, food borne fungal pathogens and mite pests and they have considerable grain protectants against stored product beetles. The present study indicates that, the GC-MS analysis for essential oils of four aromatic plants are rich in citral, neral, geranyl acetate, geraniol, citronellal, geraniol, citronellol, photocitral B, azulene and α-guaiene respectively. The essential oil of *C. flexuosus* was found to be most potent fumigant than *C. winterianus* against *S. oryzae*. Also, as in contact toxicity of *C. martini* oil is being the most toxic to adults of *S. oryzae* whereas, the essential oils of *C. winterianus* and *P. cablin* showed moderate toxicity to *S. oryzae*. These results were newly reported on cultivated *Cymbopogon* spp. and *P. cablin* from Manipur, North East India. Therefore, these essential oils may be potential candidates for their use in the development of novel biofumigants or biopesticides and it could be served as a component in an IPM strategy instead of synthetic fumigant and insecticides, further study is needed in their safety evaluation in humans. Additional research on the development of formulations is also necessary to improve effective control options, in the management of stored-product beetles.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest regarding the publication of this paper.

Ethical standard This study does not contain any studies with animals, as defined by the Institute of Bioresources and Sustainable Development, performed by any of the authors.

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