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Original Article

Fumigant toxicity and biochemical properties of (α + β) thujone and 1, 8-cineole derived from *Seriphidium brevifolium* volatile oil against the red imported fire ant *Solenopsis invicta* (Hymenoptera: Formicidae)

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ABSTRACT

The excessive use of chemical insecticides has led to negative effects on human health and the environment. Volatile oils are one of the possible potential alternatives to chemical insecticides. Traditionally *Seriphidium brevifolium* (Wall. ex DC.) Ling & Y.R.Ling, Asteraceae, powder from its leaves is used to treat gastric problems and expel intestinal worms by local peoples, but yet there is no literature available regarding its insecticidal activity. In this study fumigant toxicity and enzyme inhibition activities of the *S. brevifolium* volatile oil collected from the highlands of Skardu Baltistan, Pakistan, was evaluated against the red imported fire ant *Solenopsis invicta*. The phytochemical studies indicated that monoterpenes were the most abundant constituents, accounting for 88% of the total oil. The major dominant constituents were 2-bornanone (28.2%), 1,8-cineole (19.9%), α-thujone (7.5%), β-thujone (6.7%) which accounts for 62.3% of total constituents identified, with volatile oil yield of 4.11% (w/w). The fumigation assay indicated that the volatile oil was acutely toxic to fire ants, with an LC_{50} of 16.47 µl/l. Among the constituents tested, only (α + β) thujone and 1,8-cineole were toxic, with LC_{50} of 17.68 and 30.66 µl/ after 12 h of exposure. The volatile oil, (α + β) thujone and 1, 8-cineole showed strong fumigant activity against the red imported fire ant workers in a time- and dose-dependent manner. The volatile oil caused 100% mortality of the red imported fire ant workers, even at the lowest concentration of 20 µl/l after 24 h of exposure. In addition, the volatile oil and 1,8-cineole inhibited acetylcholinesterase activity, while (α + β) thujone inhibited carboxylesterase activity in the fire ant workers. It has been concluded that the volatile oil and some of the compounds from *S. brevifolium* might be developed as eco-friendly approaches for the control of red imported fire ants.

Introduction

In recent decades due to insecticide resistance, pest resurgence, ground water pollution, and environmental hazards interest in the development of botanical pesticides has increased (Isman, 2017; Pavela and Sedlák, 2018). Among botanicals pesticides, plant volatile oils are gaining interest as deterrent, contact, fumigant, and ingestion toxicity against numerous pests of economic importance (Isman, 2006; Benelli et al., 2018b). The most important factor regarding the utilization of volatile oil appears to be safety in humans and the environment and their multiple modes of action against insect pests (Benelli and Pavela, 2017, 2018). Presently, more than 300 plant volatile oils are used worldwide on an industrial scale (Khan and Abourashed, 2011; Rizvi et al., 2018a). These economically important volatile oils are mostly obtained from several plant families, including Asteraceae, Geraniaceae, Lamiaceae, Verbenaceae, Annonaceae, and Meliaceae (Benelli and Pavela, 2017; Benelli et al., 2019).

The red imported fire ant, *Solenops isinvicta* Buren, is a fierce and invasive medical and agricultural pest (Ascunce et al., 2011; Bockoven et al., 2017). These ants sting humans, domesticated pets and wildlife, and they also damage electrical equipment and irrigation channels by making their nests in them. These ants are omnivorous pests that not only feed on agricultural commodities but also feed on fruits, seeds and plant roots (Cheng et al., 2008). The rapidly increasing populations and invading trends of red imported fire ants drastically affect other arthropods, including the beneficial insects that they prey on at all life stages, such as eggs, larvae, pupae and adults (Gifford et al., 2017; Tschinkel and King, 2017). To over-

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come the threats of the red imported fire ant, effective chemical and bio-control agents must be developed. Mostly synthetic insecticides of different groups including pyrethroids, organophosphates, and carbamates have primarily been used to control fire ants in the form of baits and contact insecticides (Hara et al., 2014; Guo et al., 2017). However, the massive use of synthetic insecticides causes ground water contamination and human health impacts, as well as a reduction in natural enemies' population and the development of resistance (Dey, 2016; Dawoud et al., 2017).

Volatile oils (VO) are widely used in the cosmetic, scent and beverage industries; during the last decade, most research has focused on VO as alternatives to synthetic insecticides (Benelli et al., 2018; Rizvi et al., 2019). To avoid the excessive use of synthetic insecticides and to instead use rapidly degradable, environmentally friendly compounds that are nontoxic or less toxic to non-targeted organisms, this approach emphasizes the development of novel and environmentally friendly natural pesticides from plant sources. Among the plant-derived products, volatile oils attain much attention due to the presence of significant numbers of potent molecules with different modes of action, *i.e.*, fumigant, contact, antifeedant, deterrent and growth regulator (Afshar et al., 2017; Isman and Tak, 2017). Recently, several plant products from different species have been tested against red imported fire ant workers. Some good examples include *Cinnamomum osmophloeum* VO and sweet orange volatile oil fractions that showed fumigant activity against *S. invicta* (Cheng et al., 2008). *Cedrus deodara* significantly increased foraging time and blocked the trophallaxis by the worker (Wang et al., 2010). Similarly, *Artemisia annua* and *Cinnamomum camphora* and their major volatile constituents showed strong fumigant toxicity (Tang et al., 2013; Zhang et al., 2014). Several plant families have been reported to have attractant, repellent and insecticidal activities against a number of agricultural and household pests, including the Asteraceae, Labiateae, Rutaceae, Annonaceae and Meliaceae (Akhtar and Isman, 2004; Pavela, 2014; Pavela and Sedlák, 2018).

Seriphidium brevifolium (Wall. ex DC.) Ling & Y.R. Ling, commonly known as short-leaved wormwood, locally called (Bursay), belongs to family Asteraceae and is an annual plant. The short-leaved worm wood is a strong aromatic plant less preferred by herbivores. Locally, the powder from its leaves is used to treat gastric problems and intestinal worms (Shah and Thakur, 1992; Hayat et al., 2009). Recent studies found that the genus *Asteraceae* contains biologically active monoterpene, a sesquiterpene, and volatile acetylene components as well as biological properties such as repellent, antifeedant, insecticidal, acaricidal and antifungal activities (Benelli et al., 2019; Mihajilov-Krstev et al., 2014; Pavela and Benelli, 2016). The extracts and volatile oils from the family Asteraceae have been reported to show insecticidal activities against a number of agricultural and household pests, including the pea-leaf-weevil *Sitona lineatus* L. (Rusin et al., 2016) and the lesser mulberry pyralid *Glyphodes pyloalis* (Khosravi et al., 2010), the housefly *Musca domestica* (Akhtar and Isman, 2004), the white fly *Bemisia tabaci* (Gennadius) (Soliman, 2007) and the tomato leafminer *Tuta absoluta* (Abad et al., 2012), as well as ticks and mites (Benelli and Pavela, 2017).

There is an abundant natural resource of *S. brevifolium* in Skardu Baltistan region, this plant wildly grown on barren lands, where rainwater is the only water source (Hayat et al., 2009). The phytochemical composition of the volatile oil from *S. brevifolium* has been poorly investigated, while its bioactivity against all organisms remains unknown. Ancient local people used powder from its leaves for gastric problems, intestinal worms and as insect repellent, but due to lack of study every year this plant is wasted. Therefore, in this study we evaluate its fumigant and enzyme inhibitory activities against red imported fire ant workers. This study provides information about the chemical composition of the *S. brevifolium* volatile oil and allows for the development of novel and effective control candidates for fire ants.

Materials and methods

Insects

The red imported fire ant workers were collected from the university campus of South China Agriculture University, Guangzhou P. R. China. The ants were reared in plastic boxes at 25 ± 2 C and 50–80% RH. The boxes were coated from the upper side with Teflon emulsion to prevent the escape of the ant workers. The ants were fed with grasshoppers purchased from a local market. A 10% sucrose solution in partially filled glass tubes (25 by 200 mm^2) capped with cotton plugs were used as a water source.

Plant material and volatile oil extraction

The leaves and flowers of *Seriphidium brevifolium* (Wall. ex DC.) Ling & Y.R.Ling, Asteraceae, were collected from Skardu Baltistan, Pakistan (35°16.775″N 75°38′40″E, elevation 2396 m) in the middle of August 2016. The plant species were identified by Prof Dr. Amir Sultan National Agriculture and Research center Islamabad, Pakistan (NARC). The collected leaves and flowers were sheltered and dried. The dried plant materials were ground into powder using an electric grinder and were then sieved to avoid unwanted granules in the powder. Plant material (50 g) was hydro-distilled in a Clevenger-type apparatus for 5 h. The oil was collected and dried over anhydrous sodium sulfate and filtered (CCAA-104, 0.22 μm). The filtered oil was then stored in transparent glass vials (1.5 ml) (ANPEL Laboratory Technologies Shanghai Inc. China) and was kept at 4 °C for subsequent use.

Gas chromatography-mass spectrometry analysis (GC–MS)

GC–MS (Agilent 6890 N GC, Agilent 5973 N MS) was used to analyze the volatile oil. The GC–MS was equipped with an HP-5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness). The injector and detector temperatures were 250 °C and 250 °C. The oven temperature was programmed from 60 °C (3 min) to 220 °C (3 °C/min and held for 2 min) and then was increased to 230 °C (3 °C/min, held for 10 min). The injection size was 0.1 ml of a 1% solution prepared in hexane, and the split ratio was 1:10. The MS was taken at 70 eV, with a mass scan range of 50–800 amu, and helium was used as a carrier gas at 1 ml/min. The chemical constituents were identified by comparing their mass spectra with those of the computer mass libraries (08, N., 2008; NCBI, 2017) and by their retention index (RI), which was determined relative to the homologous series of *n*-alkanes C_7-C_{40} Sigma–Aldrich (St. Louis, Missouri) under identical experimental conditions (08, N., 2008; Adams and Sparkman, 2007). The individual peaks were computer also accorded with NIST 05 spectral library and their disintegration arrangements were also compared with the previous literature.

Fumigant toxicity

The fumigant toxicity of the volatile oil and its dominant constituents against red imported fire ant workers (3.5–3.7 mm body length, 0.8–0.9 mm head width) was measured as described by (Seo et al., 2009), with slight modifications. Briefly, the undiluted oil and its major constituents were pipetted into 2 ml centrifuge tubes. The tubes were drilled with eight pinholes to vaporize the oil or its dominating constituents. The centrifuge tube was tapped with the inside wall of a 250 ml glass beaker. The vertical wall inside each glass beaker was coated with Fluon emulsion and allowed to dry for 24 h to prevent the ants from escaping. Forty fire ant workers were placed inside the glass beaker and were provided with food and water, and the glass beakers were covered with rubber lids to make them airtight. The flask was placed in an incubator at 27 ± 2 °C and 75 ± 5% RH. Preliminary tests were performed to find the appropriate dosage range to determine the LC_{50} values. Five concentrations of the volatile oil were prepared (1, 3, 5, 7 and 10 μl/tube), which corresponded to dosages of 4, 12, 20, 28 and 40 μl/l of air based on the flask volume, and the percent mortalities were assessed after 6, 12, 18 and 24 h of treatment. Similarly, the dominant constituents (α + β) thujone and 1,8-cineole were prepared (1, 3, 5, 7 and 10 μl/tube) and (3, 5, 7, 10 and 20 μl/tube), which corresponded to the dosages of 4, 12, 20, 28 and 40 μl/l and 12, 20, 28, 40 and 80 μl/l, respectively. Each flask was considered as a single treatment, and each treatment was replicated five times. To determine the LC_{50} values, after 12 h exposure, the insects were moved into clean vials, and their mortality was immediately determined.

Enzyme activity analysis procedures

Enzyme extraction procedure

The fire ant workers that survived 24 h after the fumigation assay were homogenized in buffer A [100 mM phosphate buffer (pH 7.2), containing 1 M of DTT, 100 mM of 4-(2- aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) and 0.5 M of EDTA. The homogenates were centrifuged (Eppendorf 5804R, Eppendorf International) at 1000 *g* for 5 min at 4 °C, and the resultant supernatants were used for the glutathione *S*-transferase (GST), carboxylesterase (CarE) and acetylcholinesterase (AChE) activity analyses. For the carboxylesterase (CarE) and glutathione *S*-transferase (GST) analyses, the enzyme sources were collected from the whole bodies of the ant workers (Zhang et al., 2007), and for acetylcholinesterase (AChE) activity was obtained from the heads of the ant workers (Gorun et al., 1978).

Enzyme assay procedure

The acetylcholinesterase (AChE) activity was measured using the head homogenates as the enzyme source, as described (Ellman et al., 1961), with acetylthiocholine (ASCh) as the substrate. The incubation of the enzymes was conducted in TpS [10 mM of DTNB, 0.1 mM of EDTA, 100 mM of ASCh and 100 mM phosphate buffer (pH 7.2)] for 30 min at 30 °C. The optical density was measured with a Biotek Synergy H1 microplate reader at 412 nm. The AChE activity was converted to nM of acetylthiocholine hydrolyzed per min (3412 nm = 1.36 × 104 M^{-1} cm^{-1}).

The glutathione-*S*-transferase (GST) activity was measured as described (Oppenoorth et al., 1979). The reaction solution contained 100 μl of enzyme solution, 200 μl of 50 mM potassium phosphate buffer, and 10 μl of 150 mM CDNB (1-chloro-2,4-dinitrobenzene), with pH 7.3. The GST activity was recorded with a microplate reader at 340 mm at intervals of 30 s for 3 min at 37 °C. The total GST activity was determined from the extinction coefficient of CDNB (0.0096).

The carboxylesterase (CarE) activity was measured as described (Bullangpoti et al., 2012). Enzyme solution (40 μl) were mixed with *p*-nitrophenyl acetate (pNPA) (40 μl; 10 mM in DMSO) and (200 μl; 50 mM, pH 7.4) phosphate buffer. The optical density was measured with a microplate reader at 410 nm and 37 °C in kinetic mode. The carboxylesterase activity was measured by the extinction coefficient of *p*NPA (176.4705). The protein content of each fraction utilized as the enzyme source was determined by the Bradford method (Bradford, 1976). In all of the enzyme analyses, the fire ant workers were fumigated with the LC_{30} concentrations of the volatile oil, (α + β) thujone and 1,8-cineole. Three biological replicates were conducted for every treatment, and the means were separated by Tukey's test using SPSS 17.0.

Chemical reagents

Thymol (99%) was obtained from Shanghai (China) Macklin Biochemical Co., Ltd, 1,8-cineole (99%), 2-bornanone (96%) and (*R*)-(+)-limonene (97%) from Alfa Aesar (China) Chemical Co. Ltd, (α + β) thujone (70%) TCI (Shanghai) Development (China) Co., Ltd. and chloro-2,4-dinitrobenzene (CDNB) (97%), 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) (97%), 1,4-dithiothreitol (DTT) (97%), *p*-nitrophenylacetate (pNPA) (99%), ethylenediaminetetraacetic acid (EDTA) (99.4%), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and acetylthiocholine (ASCh) (98%) and carvacrol (98%) were purchased from Sigma Aldrich (St. Louis, Missouri) United states.

Data analysis

Statistical analysis of the fumigant toxicity data was performed using Probit analysis to determine the 50% lethal concentration (LC_{50}) using SPSS 17.0. For enzyme inhibition analysis every treatment, three biological replicates were made, and means were separated by Tukey's test using SPSS 17.0. All statistical analyses were carried out with the software package SPSS 17; *p*-values of less than 0.05 were considered significant.

Table 1
Chemical composition of *Sephredium brevifolium* volatile oil.

PK	Library/ID[a]	KI[Exp][b]	KI[Lit][c]	Area %	ID[d]
1	α-Thujene	928	919	1.5	MS,RI
2	α-Pinene	934	926	1.9	MS,RI
3	Camphene	949	955	1.8	MS,RI
4	Sabinene	975	978	0.7	MS
5	β-Myrcene	995	999	1.2	MS,RI
6	δ-3-Carene	1016	1023	0.8	MS
7	*p*-Cymene	1026	1027	2.9	MS,RI
8	Limonene	1030	1030	1.9	MS,RI
9	β-Phellandrene	1032	1034	1.7	MS,RI
10	1,8-Cineole	1036	1037	19.9	MS,RI
11	(Z)-β-ocimene	1040	1043	0.8	MS,RI
12	γ-Terpinene	1054	1052	0.9	MS,RI
13	Linalool	1094	1094	0.8	MS,RI
14	α-Thujone	1109	1109	7.5	MS,RI
15	β -Thujone	1119	1122	6.7	MS,RI
16	*Trans*-*p*-Menth-2-en-1-ol	1144	1144	2.0	MS,RI
17	2-Bornanone	1160	1164	28.2	MS,RI
18	Terpinen-4-ol	1174	1170	0.9	MS,RI
19	Myrtenol	1200	1203	1.0	MS,RI
20	Carvone	1239	1229	2.9	MS,RI
21	Thymol	1292	1286	1.6	MS,RI
22	*n*-Tridecane	1300	1307	3.0	MS,RI
23	β-Elemene	1395	1389	2.0	MS,RI
24	Isocaryophyllene	1407	1409	3.0	MS
25	β-Caryophyllene	1431	1421	2.0	MS,RI
26	α-Humulene	1450	1455	2.5	MS,RI
	Monoterpenes			88.0	
	Sesquiterpenes			9.0	
	Others			3.0	
	Total			99.9	

[a] Compounds are listed in order of their elution from a HP-5MS column.

[b] KI(Exp) retention indices (Kovats) relative to C_7-C_{40} *n*-alkanes on a HP-5MS column.

[c] KI(Lit) retention indices of reference compounds from literature (Bertella et al., 2018; Bossou et al., 2013; Cheng et al., 2008; Goodner, 2008; Herrera et al., 2014; Joshi, 2013; Nezhadali et al., 2008; Rajeswara Rao et al., 2014; Sonboli et al., 2013; Vieira et al., 2017).

[d] ID = Identification methods; MS = comparison of the mass spectrum with those of the computer mass libraries, and in Adams or NIST 08.

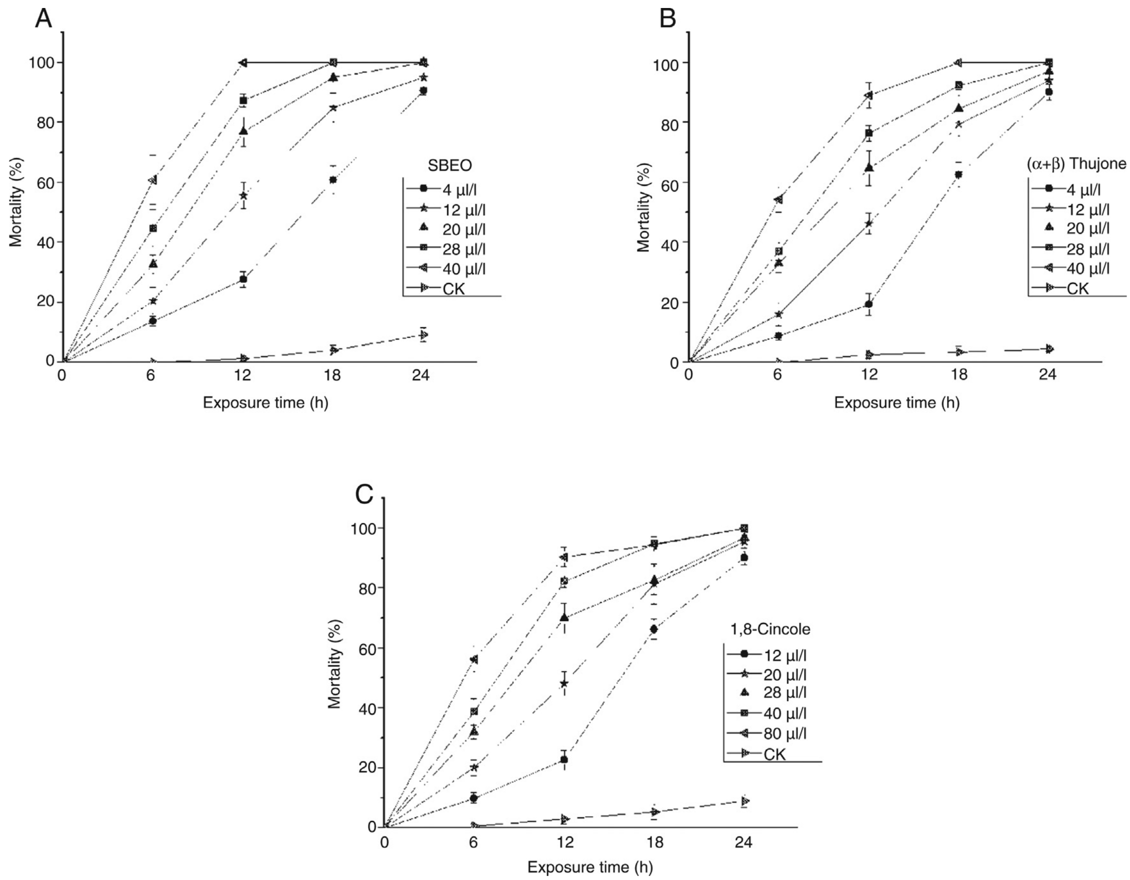

Fig. 1. Mortality of medium workers of red imported fire ant caused by the *Seriphidium brevifolium* volatile oil and its dominant constituents, (α + β) thujone at 4, 12, 20, 28 and 40 μl/l and 1,8-cineole at 12, 20, 28, 40, 80 μl/l in the fumigation bioassay, where CK is stands for control.

Results

The chemical composition of volatile oil

Volatile oils are a mixture of different groups of compounds; however, they are mostly dominated by monoterpenes and sesquiterpenes (Couladis and Koutsaviti, 2017). The chemical composition of SBVO is presented (Table 1). Upon hydro-distillation, SBVO produced very strong fragrant light yellow color oil with a yield content of 4.11% (w/w) (based on sheltered dried aerial parts). A total of twenty-five chemical constituents were identified by the GC—MS analysis, accounting for 99.99% of the total constituents identified. The oil was dominated by monoterpenes 88% and sesquiterpenes 9%, respectively. The primary dominating constituents were 2-bornanone (28.2%), 1,8-cineole (19.9%), α-thujone (7.5%) and β-thujone (6.7%) (Table1).

Fumigant toxicity

The volatile oil was acutely toxic to the red imported fire ant workers, with an LC_{50} of 16.47 μl/l and LC_{90} of 74.52 μl/l after 12 h of exposure. Among the constituents tested, only (α + β) thujone and 1,8-cineole proved to be toxic against the fire ant workers, with an LC_{50} of 19.11 and 30.04 μl/l and LC_{90} 89.03 and 103.03 μl/l, respectively. There was no activity observed against fire ant workers in the glass jars fumigated with 2-bornanone, (*R*)-(+)-limonene and thymol (Table 2). The volatile oil and its constituents (α + β) thujone and 1,8-cineole showed strong fumigant activity against the workers of red imported fire ants in a time- and dose-dependent manner (F = 895.40, df = 4, 76; $p = 0.0003$). The fumigant toxicity of the volatile oil, (α + β) thujone and 1,8-cineole against the workers of red imported fire ants is shown (Fig. 1). The volatile oil showed a strong fumigant activity against the workers of red imported fire ants, with percentage mortalities reaching 36.62, 76.81, 94.95 and 100% at 20 μl/l after 6, 12, 18 and 24 h exposure times, respectively, followed by (α + β) thujone, with percentage mortalities reaching 37.08, 76.35, 92.27 and 100% at a concentration of 28 μl/l and 1,8-cineole, with mortality percentages of 56.53, 90.23, 94.47 and 100% at a concentration of 80 μl/l after 6, 12, 18 and 24 h exposure times, respectively. However, the fumigant toxicity of SBVO, (α + β) thujone and 1,8-cineole is relatively low as compared to dichlorvos with an LC_{50} and LC_{90} of 96.77, 1018.55 ng/l concentrations.

Enzyme inhibition activities

The *in vivo* AChE activity of the head homogenates indicated that only the volatile oil and 1,8-cineole significantly inhibited the AChE activity in the red imported fire ant workers ($p < 0.05$, Tukey), while (α + β) thujone also showed a decreasing trend in AChE activity, but this trend was not statistically significant compared to the control (Fig. 2A).

The *in vivo* GST activities of the red imported fire ant worker homogenates are shown in Fig. 2B. Only (α + β) thujone significantly inhibited GST activity ($p > 0.05$, Tukey), while the volatile oil and 1,8-cineole showed decreased GST activity, but these differences were not statistically significant compared to the control.

The results of the *in vivo* CarE activity of the red imported fire ant worker homogenates are shown in Fig. 2C. The results indicated that the volatile oil, (α + β) thujone and 1,8-cineole all showed decreased CarE activity, but these changes were not statistically significant compared to the control ($p > 0.05$, Tukey). In all the enzymatic assays, we used the LD_{30} concentration of the volatile oil and

Table 2
Fumigant toxicity of *Seriphidium brevifolium* volatile oil and its major dominant constituents against *Solenopsis invicta* 12 h after exposure.

Treatments	Concentration (μl/l)	Mortality (%) ± SD[a]	N[b]	LC50 (μl/l) (95% LCL-UCL)[c]	LC90 (μl/l) (95% LCL-UCL)[d]	Slope ± SE	X^2, (d.f)[e]	p-Value
SBEO	0	1.9 ± 4.77	942	16.472 (12.23 - 26.57)	74.52(39.84-245.22)	1.95 ± 0.19	3.8(3)	0.21n.s[f]
	4	16.4 ± 1.34						
	12	31.30 ± 1.23						
	20	52.23 ± 1.04						
	28	65.44 ± 0.97						
	40	85.43 ± 0.56						
(α + β) Thujone	0	2.56 ± 3.23	949	17.68 (10.71-29.59)	89.03(44.92-268.19)	1.82 ± 0.17	4.4(3)	0.32 n.s
	4	16.49 ± 2.23						
	12	30.33 ± 1.98						
	20	51.23 ± 1.31						
	28	60.44 ± 1.11						
	40	82.43 ± 0.56						
1,8-Cineole	0	3.46 ± 3.71	935	30.66 (26.281-33.037)	103.03(82.33-143.22)	2.44 ± 0.23	4.5(3)	0.67 n.s
	12	15.46 ± 3.36						
	20	30.22 ± 2.34						
	28	49.23 ± 2.11						
	40	63.44 ± 1.56						
	80	83.24 ± 0.87						
Dichlorvos (ng/l)[h]	0	1.46 ± 3.43	867	96.776 (77.38-121.13)	1018.55(667.29-1244.12)	1.25 ± 0.11	4.44(3)	0.21 n.s
	10	14.33 ± 2.36						
	50	29.33 ± 2.43						
	100	51.53 ± 1.43						
	200	62.88 ± 1.03						
	500	85.26 ± 0.51						
(R)- (+)- Limonene		NA[g]						
Limonene		NA						
Thymol		NA						
2-Bornanone		NA						

[a] Values are mean ± SD of five replicates.
[b] Total number of fire ant workers treated.
[c] LC_{50}: lethal concentrations (μ-/l).
[d] LC_{90}: lethal concentrations (μl/l).
[e] *χ2* chi square, *d.f.* degrees of freedom.
[f] n.s. = not significant ($p > 0.05$).
[g] No activity was observed against fire ant workers during fumigation assay.
[h] Dichlorvos was used as positive control (ng/l).

its constituents, which may be another reason for the few significant differences observed.

Discussions

Botanical insecticides have attracted increasing attention as alternatives to chemical insecticides because of their environmentally friendliness, rapid biodegradability and low toxicity to human and animals (Afshar et al., 2017; Isman, 2006, 2017). Researchers seek toxicants from plant sources to control agricultural and household pests, and thousands of research papers have been published since 2000 on toxicants from plant sources (Isman, 2017). The phytochemical composition of the *S. brevifolium* volatile oil has been poorly investigated, while its toxicity against insect pests remains unknown. To our knowledge, only one study has been reported about the chemical composition of the *S. brevifolium* volatile oil from India, with α-thujone (60.2%), β-thujone (5.5%) and α-pinene (1.5%) as the dominant constituents (Shah and Thakur, 1992). The chemical composition and volatile oil content within the same species were greatly influenced by the geographical distributions (Okut et al., 2017), climate conditions and times of harvest (Joshi, 2013; Afshar et al., 2017). The SBEO was dominated by (+)-2-bornanone (28.18%), 1,8-cineole (19.90%), α-thujone (7.54%) and β-thujone (6.54%) with volatile oil yields 4.11% (w/w). Similarly, the chemical constituents and the yield of the volatile oil from *A. absinthium* collected from Iran by (Rezaeinodehi and Khangholi, 2008) was dominated by *trans*-thujone (18.6%) and guaiol (19.33%) from Pakistan (Rizvi et al., 2018c). The climatic conditions of Skardu Baltistan, Pakistan also affect the chemical composition and volatile oil yield of *S. brevifolium*.

Volatile oils have been the subject of much research due to their numerous pharmacological and biological activities, including anti-inflammatory, anti-cancer, anti-microbial, antioxidative, and hypolipidemic effects (Guo et al., 2018; Li et al., 2018); volatile oils also have insecticidal (Hu et al., 2017; Benelli et al., 2018a) and acaricidal activities (Benelli and Pavela, 2017). Volatile oils consist of a mixture of volatile substances that are mostly dominated by terpenoids and are used for their aromatic qualities (Isman, 2017). The compositions of terpenoids (monoterpenes and sesquiterpenes) among the plant species are highly variable, and some of the terpenoids show significant toxicity against insect pests with low mammalian toxicity (Ortiz de Elguea-Culebras et al., 2018). Monoterpenes and sesquiterpenes have been reported as having toxicity against various insects in different ways. For example, α-pinene, (-)-carvone, and (-)-limonene showed fumigant and antifeedant activities (Ibrahim et al., 2018). Thyme showed contact toxicity against *Trichoplusia ni* (Tak and Isman, 2017), and (-)-carvone, geraniol, 1,8-cineole and cuminaldehyde were active against Sitophilus oryzae and *Tribolium castaneum* (Abdelgaleil et al., 2009). In this study, the fumigant activity of the volatile oil and its dominant constituents against the fire ant workers was evaluated, and the results indicated that the intact oil exhibits strong fumigant activity against fire ant workers. Among the constituents tested, only (α + β) thujone and 1,8-cineole exhibited fumigant activities. From the fumigation bioassay, it is confirmed that (α + β) thujone and 1,8-cineole were the main active constituents of *S. brevifolium* having formicidal activity. (α + β) thujone is a monoterpene found in many plant species, including *A. absinthium* (Juteau et al., 2002), *Salvia officinalis* (Raal et al., 2007), *Artemisia herba-alba* (Mighri et al., 2010), and *Melissa officinalis* (Couladis and

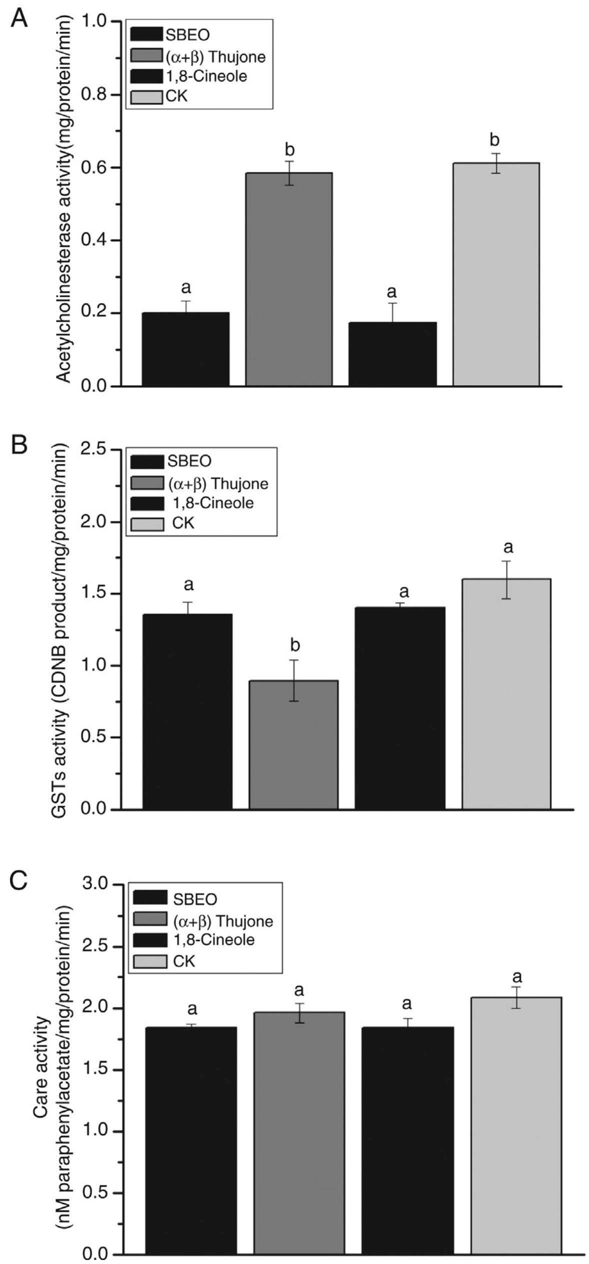

Fig. 2. Enzyme inhibition activities in red important fire ant workers 24 h after fumigation of *Seriphidium brevifolium* volatile oil and its dominant constituents, (α + β) thujone and 1,8-cineole (A) Acetylcholinesterase activity (AChE) (acetylcholinesterase activity/mg/protein/min) (B) Glutathione s-trasferase activity GST activity (CDNB product/mg/protein/min) (C) Carboxylesterase activity (CarE) (nM paraphenylacetate/mg/protein/min). The bars labelled with the same letters are not significantly different (Tukey's, $p < 0.05$).

Koutsaviti, 2017). Thujone has been reported to show toxicity against *Drosophila melanogaster* (Höld et al., 2000) and the red poultry mite Dermanyssus gallinae (Tabari et al., 2017), while in rats, thujone inhibits the γ-aminobutyric acid A ($GABA_A$) receptor (Pelkonen and Ahokas, 2017). However, thujone is reported to be toxic to brain, kidney, and liver cells and could cause convulsions if used in too high a dose (Kolassa, 2013), the possible reason behind this is interaction of thujone with GABA-receptors (Lachenmeier et al., 2006), and antagonistic effect of thujone on the γ-aminobutyric acid receptor(Dettling et al., 2004).

Furthermore, 1,8-cineole is also a monoterpene primarily found in plants, including Angophora floribunda, Callistemon citrinus, Eucalyptus dives (Lee et al., 2013) and Mentha longifolia (Asekun et al., 2007). This monoterpene has been reported as having strong fumigant activity against store pests, including Sitophilus oryzae, Tribolium castaneum and Rhyzopertha dominica (Jayakumar et al., 2017). In our current study, SBEO, (α + β) thujone and 1,8-cineole showed fumigant activity in a time- and dose-dependent manner, and 100% mortality of red imported fire ant workers was obtained when the exposure time reached 24 h, even at the low concentrations tested. Similarly, *Cinnamomum osmophloeum* showed fumigant activity against red imported fire ants (Cheng et al., 2008), and *Cedrus deodara* significantly increased foraging time, interfered in the recruitment of fire ant workers, and blocked the trophallaxis by the workers (Wang et al., 2010), in contrast, the volatile oils from *Artemisia annua* and *Cinnamomum camphora* showed a strong fumigant toxicity (Tang et al., 2013). In addition, sweet orange volatile oil fractions showed very strong fumigant toxicity against red imported fire ant workers (Hu et al., 2017). Similarly, many volatile oils have been reported have fumigant toxicity against store pest including *Petroselinum sativum, Eucalyptus obliqua* and *Rosmarinus officinalis* against *Callosobruchus maculatus* (Kamanula et al., 2017; Massango et al., 2017), *Lippia javanica* against *Sitophilus zeamais* (Kamanula et al., 2017). Therefore, due to the high volatile oil yield and acute fumigant toxicity, *S. brevifolium* volatile oil is a potential candidate for control of fire ants and store pests.

In arthropods, detoxification enzymes, including glutathione-*S*-transferase and carboxylesterase, cytochrome P450 monooxygenases and carboxyl/cholinesterases, play key roles in maintaining physiological functions by detoxifying xenobiotic compounds. These xenobiotic compounds include toxic secondary metabolites from host plants and pesticides (Oakeshott et al., 2005; Bullangpoti et al., 2012). Volatile oils possess several insecticidal compounds that cause primarily neuroexcitation with hyperactivity, tremor, and paralysis. This neuro-inhibition results in paralysis and immobility because of oxygen deprivation that eventually leads to death (Song and Scharf, 2008; Yeom et al., 2012). Similarly, in our study, SBEO and 1,8-cineole significantly inhibited the acetylcholinesterase activity in fire ant workers compared to the control, while (α + β) thujone also showed decreased acetylcholinesterase activity, but this difference was not statistically significant. The volatile oils mainly dominated by monoterpenes and sesquiterpenes and their insecticidal activities are primarily neurotoxic (Hummelbrunner and Isman, 2001; Park and Tak, 2016). Due to their high volatility and low weight, they have strong fumigation action and gaseous action that may be important for ants, termites, and store product insects (Rattan, 2010). Similarly, many monoterpenes, including carvacrol, 1-8-cineole, (-)-limonene and (-)-carvone, showed inhibition of acetylcholinesterase activity in *Sitophilus oryzae* and *Tribolium castaneum* (Abdelgaleil et al., 2009; Tak et al., 2016). Therefore, it is also suspected that, in addition to the inhibition of AChE activity, the volatile oils and monoterpenes may act on other vulnerable sites, including cytochrome P450 monooxygenases and carboxylesterases (Bullangpoti et al., 2012; Tong and Bloomquist, 2013; Rizvi et al., 2018b). However, in our study, only (α + β) thujone significantly inhibited GST activity in the fire ant workers, while SBEO and 1-8-cineole showed decreased GST activity that was not statistically significant. While SBEO, 1-8-cineole and (α + β) thujone all showed decreased CarE activity compared to the control, none of these differences were statistically significant. These results indicate that the insecticidal mode of action of SBEO and its dominant constituents (α + β) thujone and 1,8-cineole may be largely attributable to fumigant action; these substances may be toxic by penetrating the insect body via the respiratory system.

To the best of our knowledge, this is the first report on the bioactivity of *S. brevifolium* volatile oil. Our study provided important baseline information for the potential use of *S. brevifolium* volatile oil and its dominant constituents (α + β) thujone and 1,8-cineole as potential candidates for the development of safe and eco-friendly formicidal agents against red imported fire ants. Our results suggest that the fumigant activity of the SBEO has some promise as a possible novel fumigant/insecticide for the control of red imported fire ants and grain storage insects. Currently used fumigants are mostly synthetic in nature including aluminium phosphide, dichlorvos, acrylonitrile, carbon disulfide, ethylene, paradichlorobenzene, sulfur dioxide, and sulfuryl fluoride which are highly toxic to humans, environment and other non-target organisms (Zettler and Arthur, 2000). However, for the development of volatile oil as a natural fumigant/insecticide, further research should be focus into the safety of the volatile oil in humans is needed. Similarly, we know that pests including, fire ants, mosquitos and store grain pests, are frequently in human contacts, use of synthetic pesticides *i.e*. contact, repellent and fumigants will ultimately cause health effects on humans, therefore, there is need to develop toxicants from natural sources to minimize the health risks are one of the major concerns in the present era. For the practical application of volatile oil-based formulations to improve efficacy and stability as well as to reduce cost further research should be focus on its formulation and application techniques. However, the efficacy and persistence of volatile oils can be enhanced using some techniques including encapsulation, cyclodextrins and nanoparticle synthesis. In this study we reported that *S. brevifolium* volatile oil and some of its constituents showed toxic effect against red imported fire ants, and this plant have the potential to be developed into eco-friendly approaches for control fire ants.

Author contributions

Conceived, designed, and performed the experiments by XF and RSAH. ZXN gives the direction of research and reviewed the manuscript. RSAH and XF help in writing and analyzing the manuscript.

Conflict of Interest: The author declared no conflict of interest

Informed consent: Informed consent was obtained from all authors included in the study.

Research involving human participants and/or animals: This article does not contain any studies with human participants or animals performed by any of the authors.

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