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RESEARCH ARTICLE

Volatile constituents of tea stems (*Camellia sinensis* L.O. Kuntze) as semiochemicals to attract low country live wood termite, (*Glyptotermes dilatatus* Bugnion & Popoff) in Sri Lanka

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Abstract: Low country live wood termite (LCLWT), *Glyptotermes dilatatus* Bugnion & Popoff is one of the major insect pests of low grown tea in Sri Lanka. The present study was aimed at investigating the chemical constituents and the attractant effects of tea stem volatiles isolated from different tea cultivars on the behaviour of alates of *G. dilatatus*. Volatile extracts of decayed and healthy stems of susceptible cultivars (TRI 2023, TRI 4042) and resistant cultivars (TRI 2027, TRI 4049) were collected. The attractant effects of stem extracts on termite alates were evaluated using the olfactometer bioassay. Volatile extracts of decayed stems obtained from the four tea cultivars were more attractive to alates than that of the healthy stems. The responses of alates to volatile extracts from decayed stems of TRI 2023, TRI 2027, TRI 4042 and TRI 4049 were 37 ± 0.04, 34 ± 0.04, 36 ± 0.035 and 32 ± 0.04, respectively whereas the responses of alates to volatile extracts from healthy stems TRI 2023, TRI 2027, TRI 4042 and TRI 4049 were 16 ± 0.03, 13 ± 0.03, 14 ± 0.38 and 16 ± 1.5, respectively.

The analysis of volatile extracts from decayed stems using Gas Chromatography-Mass Spectrometry led to the detection of 96 compounds in the four tea cultivars. Among them 15 compounds were common in all four tea cultivars and n-hexadecanoic acid and 9,12-octa decadienoic (Z,Z) acid were identified as the major constituents of decayed tea stems. Compound mono(2-ethylhexyl) phthalate was identified as the major constituent in healthy stem volatiles. Mono(2-ethylhexyl) phthalate and n-hexadecanoic acid were present in both types of tea cultivars and they can be developed as an alternative method to control the test insect.

Keywords: Decayed stems, *Glyptotermes dilatatus*, olfactometer, volatile extracts.

INTRODUCTION

Plant semiochemicals are small organic compounds that act as chemical signals by producing a wide range of behavioural responses in insects. It is reported that volatile plant constituents influence the behaviour of the larvae or adult or both stages of insects. Volatile chemical compounds commonly act within a long distance from the emission source, while less volatile compounds act in a close range (Dethier *et al*., 1960). Plants may release hundreds of different compounds but only a very few trigger a behavioural response in insects and help to identify their host plants. A large number of attractive volatiles released by plants, and their combinations constitute a major challenge for herbivore insects to navigate towards their host plants. Interactions between the insect pests and semiochemicals of the host plants have been studied well and recognised as a communication system within insect species (Norduland & Lewis, 1976). The use of host plant volatiles and insect pheromones have been successfully developed as an environmentally friendly pest control strategy to control economically important insect pests. It has been reported that volatile compounds produced by the host plant coconut attracts the red weevil (*Rynchophorus ferrugineus*), and this strategy is now employed in combination with the aggregation pheromone of red weevil as an alternative, eco-friendly method to control red weevil of coconut plantations in Sri Lanka (Gunawardana & Swarnakanthi, 1995). Gunawardana and Dissanayake (2000) reported the isolation of volatiles, n-hexanal, n- hexanol, n-pentanol and cis-3-hexanol from the steam distillate of banana

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stems and investigated the synergistic effect of these compounds when blended with the pheromone. A trap has been developed to control the banana weevil *Odoiporus longicollis*.

Degradation of wood by termites is a continuing problem in many tropical and even some temperate regions of the world, resulting in serious economic impact on agriculture (Sands, 1977). Termites consume essentially the cellulosic material and wood decay fungi may provide nutritional benefits to termites by increasing the availability of cellulose and other nutrients (Getty & Haverty, 1998). In a previous study, it was reported that Formosan subterranean termite, *Coptotermes formosanus* showed a strong preference for spruce saw dust infected with *Marasmiellus troyanus* (Murrill) Singer, over uninfected saw dust (Cornelius *et al*., 2002). An attractive substance (*Z, Z, E*)-3, 6, 8-dodecatrien-1-ol to the eastern subterranean termite, *Reticulitermes flavipes* was identified from the Western pine wood (*Pinus monticola* Dougl.) subjected to decay by the fungus, *Lenzites trabea* Pers. (Esenther *et al*,. 1961); it was found that this compound was analogous to the 'trail forming pheromone' of termites belonging to the Family Rhinotermitidae (Smythe *et al*., 1967). Amarasinghe *et al.* (2007) have reported that the bark of young tea stems produced six major volatile compounds namely, linalool, linalool oxide, phenyl acetaldehyde, methyl salicylate, geraniol and trans 2-hexanal, which act as semiochemicals towards *Xyleborus fornicatus* (Coleoptera; Scolytidae). Holighaus and Schütz (2006) have reported the identification of thirteen volatile compounds of beech wood (*Fagus sylvatica*) decayed by the fungus, *Nectria coccinea* and several white rot fungi, which were semiochemicals towards *Trypodendron domesticus* L. (Col., Scolytidae).

Low country live wood termite (LCLWT), *Glyptotermes dilatatus* Bugnion & Popoff is one of the economically important major insect pests of tea in low country tea plantations (Cranham, 1966; Sivapalan & Senaratna, 1981; Vitarana & Mohotti, 2008) and it has been estimated that the yield loss due to LCLWT was 3000 kg/ha made tea when the termite infestation reached up to 50 % and stand for 10 years (Sands, 1977). The alates of LCLWT are attracted to decayed stumps, which is a result of invasion by the wood rot fungi through pruned stems of the tea plant, *Camellia sinensis* L.O. Kuntze (Balasooriya, 1998). A pair of alates first colonise in the decayed stump and continue feeding the healthy heartwood. The host plant does not show any external symptoms until the main trunk is fully colonised and the second generation alates start to emerge in dispersal (Vitarana & Mohotti, 2008). High yielding tea varieties planted in low grown areas consisting of soft wooded frames that suffer extensive die back and rot after pruning increase the termite problem. Of the two types of high yielding tea cultivars, the susceptible cultivar is destroyed by termites within 10 – 15 years, while the resistant cultivars survive about 30 – 40 years (Sivapalan & Senaratna, 1981).

The control of this pest is extremely difficult because of their concealed habit and social organisation, despite various integrated pest management (IPM) practices inclusive of agronomic, cultural and biological methods. Current recommendations for managing the LCLWT are restricted only to planting of tolerant cultivars, application of a wound dressing with fungicidal properties to protect prune cuts from decay and the removal of decayed wood at pruning to remove initial colonies (Advisory Circular, 2003). However, chemical and classical biological control methods are not efficient in the field (Dantanarayana & Fernando, 1970; Vitarana, 1989). Hence, there is an urgent need to develop an environmentally friendly method that can be used to control the LCLWT in tea plantations.

Previous work carried out on the behaviour of LCLWT revealed that semiochemicals of the LCLWT have not been studied extensively. Among the few published reports, Samarasinghe *et al.*(1999) have reported the effect of volatile and non-volatile extracts of the decayed stems of tetmite resistant cultivar TRI 2027 and termite susceptible cultivars (TRI 2023 and 3063) against alates of LCLWT. The results indicated that susceptible cultivar TRI 2023 and resistant cultivar TRI 2027 were similarly attractive to termites. Highly susceptible cultivar, TRI 3063 was more attractive to alates than TRI 2023 and TRI 2027. A recent study was carried out on the behaviour of LCLWT against extracts of decayed and healthy stems of susceptible tea cultivars (TRI 2023, TRI 4042) and resistant cultivars (TRI 2027, TRI 4049) separately, and the results indicated that extracts obtained from decayed stems of each tea cultivar attracted the alates of LCLWT. Fractionation of the extract obtained from rotten tea stems has indicated that the chemical constituents in hexane fraction attract the alates of LCLWT than other polar fractions (*personal communication*). Hence the present study was aimed at analysing the volatile chemical constituents present in decayed and healthy stems of the resistant (TRI 2027, TRI 4049) and susceptible tea cultivars (TRI 2023, TRI 4042) in low country tea plantations, and compare the chemical constituents of the volatiles present in decayed and healthy stems of the four tea cultivars. Subsequently, the effects of host plant volatiles obtained from decayed and healthy stems of tea cultivars on the behavioural responses of LCLWT were explored.

METHODS AND MATERIALS

Insects

Tea bushes infected with LCLWT were collected from St. Joachim Estate, Rathnapura (latitude 6° 40'58" N and longitude 80° 23'57" E and elevation 128 m amsl.) during the period 15th May 2011 to December 2011. Termite colonies in tea bushes were maintained in the laboratory in plastic boxes (Ca.12 L) at room temperature (28 ± 2 °C) at humidity conditions (80 %) under 12:12 L: D photoperiod to collect alates for bioassays.

Collection and preparation of plant materials

Samples of decayed and healthy stems from susceptible tea cultivars TRI 2023, TRI 4042 and resistant tea cultivars TRI 2027, TRI 4049 were collected separately and transported to the laboratory in clean polythene bags. Decayed tea stems and healthy tea stems were washed with distilled water to remove soil particles and air dried for 4 h. Air dried samples were cut into small pieces before steam distillation.

Extraction of volatiles

Steam distillation was used for the extraction of volatile constituents in healthy and decayed stem pieces of tea cultivars TRI 2023, TRI 2027, TRI 4042 and TRI 4049 separately. The air dried pieces of tea stems (500 g) were steam distilled for 3 h and subsequently the condensed solution (1L) was extracted with CH_2Cl_2 (3 × 100 mL). Anhydrous Na_2SO_4 was added to the organic layer to remove water and evaporated using a rotary evaporator (IKA® RV 10 Basic) at 35 °C under reduced pressure. Dried extracts were transferred into pre weighed clean vials (2 mL) and the remaining solvent was removed under a stream of nitrogen. Dried extracts were stored in the refrigerator at 5 °C in glass vials until use for olfactometer bioassays and gas chromatography-mass spectrometry (GC-MS) analysis (Gunawardana & Dissanayake, 2000).

Evaluation of behavioural responses of LCLWT to volatile extracts in stems of tea cultivars

The Y-tube olfactometer consists of a 12 cm long stem and two 10 cm long arms, each with a 2 cm inner diameter (Hoballah *et al.,* 2002; Paranagama *et al*., 2002). The ends of the two tubes of the olfactometer were connected with perforated, transparent, wide mouthed glass bottles (20 mL) through the lids and the stem of the Y- tube was connected to a round bottom flask (100 mL), which was used to introduce the test insects. A constant airflow was maintained and the opening of the intersection of the arms facilitated the air circulation in the olfactometer. Two Whatman No. 1 filter papers (1.5 cm × 5 cm) were used, one treated with a known amount of the extract dissolved in ethanol and the other treated with equal amount of ethanol (100 µL). Both filter papers were air dried for 10 min to evaporate the solvent. The air dried filter papers were placed in the two plastic containers separately and the olfactometer was placed horizontally on a white background in day light. After switching on the vacuum pump, twenty test insects were introduced into the olfactometer. The number of alates that moved into the baited and unbaited containers within 30 min was recorded. At each trial the olfactometer was thoroughly cleaned with detergent, distilled water and acetone prior to use. Placement of the extract and the ethanol baited filter papers were interchanged randomly in subsequent replicates. The olfactometer was rotated 90° at each replicate in a clockwise direction to control any directional effect. Test insects, filter paper strips and olfactometer were changed after each replication. All bioassays were carried out between 17 – 19 h and all experiments were replicated five times. From each extract 100 µg doses were tested separately against alates of LCLWT. The responses of the alates were tested in the following volatile combinations.

(1) The extract of decayed stem of TRI 2023 *vs.* ethanol
(2) The extract of healthy stem of TRI 2023 *vs.* ethanol
(3) The extract of decayed stem of TRI 2027 *vs.* ethanol
(4) The extract of healthy stem of TRI 2027 *vs.* ethanol
(5) The extract of decayed stem of TRI 4042 *vs.* ethanol
(6) The extract of healthy stem of TRI 4042 *vs.* ethanol
(7) The extract of decayed stem of TRI 4049 *vs.* ethanol
(8) The extract of healthy stem of TRI 4049 *vs.* ethanol.

To certify that ethanol has no effect on the behaviour of the test insect, a bioassay was performed with ethanol treated filter paper strip *vs* a filter paper strip without any treatment.

Gas chromatography-mass spectrometry (GC-MS) analysis

The extracts obtained from steam distillation of the decayed and healthy tea stems of four tea cultivars TRI 2023, TRI 2027, TRI 4042 and TRI 4049 were used for the GC-MS analysis. The volatile extract of each tea cultivar was subjected to GC-MS analysis to identify the constituents in the extract. A Hewlett Packard 6890 series II chromatograph with a splitless injector (220 °C), flame ionisation detector (FID) (270 °C), and a Rtx-Wax

cross bond carbowax polyethylene glycol capillary column (0.25 mm × 0.25 µm × 30m) was used under the following conditions: initial temperature, 35 °C (5 min), 35 – 280 °C at 5 °C/ min and held for 10 min at 280 °C; the carrier gas used was Helium at a flow rate of 0.9 mL/min. The scan time and acquisition mass range of MS are 0.002 s/mass and 35 – 500 amu, respectively. For peak identification the mass spectral library Wiley W9N08 was used.

Data analysis

Data obtained from bioassays were transformed to Arc Sin values before performing ANOVA. Mean separation was performed by Tukey's Studentised test.

RESULTS AND DISCUSSION

Extraction of volatiles

Steam distillation of decayed and healthy stems of four tea cultivars gave a pale yellow oil with pleasant odour and the weight of volatile extracts obtained from the decayed stems of cultivars TRI 2023, TRI 2027, TRI 4042 and TRI 4049 were 16.2, 12.3, 16.8 and 12.0 mg, respectively. The weight of volatile extracts obtained from the healthy stems of cultivars TRI 2023, TRI 2027, TRI 4042 and TRI 4049 were 16, 13, 18 and 13 mg, respectively.

Evaluation of behavioural responses of LCLWT to volatile extracts in stems of tea cultivars

Alates of the LCLWT were exposed to a dose of 100 µg volatile extract obtained from decayed and healthy tea stems separately and the results are shown in Table 1. Statistical analysis of the results revealed that the responses of alates to extracts obtained from decayed and healthy cultivars were significantly different ($F = 93.61$, $df = 3$, $p < 0.0001$) than that of ethanol. Mean separation among the extracts showed that volatile extracts of decayed stems were significantly higher than that of healthy stem extracts ($p < 0.05$, Tukey's mean comparison). There is no significant difference of the responses of alates among the tea cultivars of decayed or healthy stems ($F = 0.34$, $df = 3$, $p = 0.7931$) and also between the two categories, resistant and susceptible ($F = 0.000$, $df = 1$, $p = 0.9897$). Interaction effect between the cultivar and extract was not significant ($F = 0.79$, $df = 9$, $p = 0.6289$) on the response of alates. Interaction effect between the two categories (resistance/susceptibility) of cultivars and extracts was also not significantly different ($F = 0.18$, $df = 3$, $p = 0.1510$) on responses of alates, suggesting that volatile compounds of decayed stems contain compounds that attract alates of LCLWT irrespective of the resistance or susceptibility of the tea cultivar. These findings are in agreement with the findings of Samarasinghe *et al*. (1999), which revealed that the extract of decayed stems of cultivars TRI 2023 (susceptible cultivar) and TRI 2027 (resistant cultivar) are equally attractive to alates and the highly susceptible cultivar TRI 3063 is more attractive than TRI 2023 and TRI 2027. However in the present study, the volatile extract of highly susceptible tea cultivar TRI 3063 was not tested since it is no longer cultivated in low country tea plantations. The attractive effect of TRI 4042 and TRI 4049 has not been studied previously and the present study was focused on evaluating the effect of volatile extracts of the two new cultivars TRI 4042 and TRI 4049 against LCLWT in addition to TRI 2023 and TRI 2027. The responses of alates to healthy stem extract are significantly higher than the untreated control ($p < 0.05$, Tukey's mean separation). It could be due to the fact that some of the common compounds present in both decayed and healthy stems of tea and those compounds may be more attractive to alates than ethanol.

Table 1: Response of *G. dilatatus* to volatile extracts of tea stem of different cultivars of *Camellia sinensis*

Tea cultivar	Percentage response ± SE		
	Decayed stems	Healthy stems	EtOH
TRI 2023		16 ± 0.03^{b}	3 ± 0.02^{c}
	37 ± 0.04^{a}		7 ± 0.02^{c}
TRI 2027		13 ± 0.03^{b}	7 ± 0.12^{c}
	34 ± 0.04^{a}		10 ± 0.02^{c}
TRI 4042		14 ± 0.38^{b}	3 ± 0.12^{c}
	36 ± 0.035^{a}		4 ± 0.12^{c}
TRI 4049		16 ± 1.5^{b}	6 ± 0.29^{c}
	32 ± 0.04^{a}		8 ± 0.02^{c}

Each data point represents the mean of five replicates. Mean numbers of insects response (%) followed by same letters are not significantly different ($p < 0.05$) according to the Tukey's mean separation.

GC-MS analysis of volatiles of decayed stem of tea

Since the volatile extracts of decayed stems were more attractive to alates than that of healthy stems, an attempt was made to identify the chemical constituents of volatiles of the tea cultivars using GC-MS. Volatile compounds identified in decayed stems of tea cultivars TRI 2023, TRI 2027, TRI 4042 and TRI 4049 are presented in Table 2. GC-MS data comparison between the recorded and library mass spectra with similarity index higher than 95 % and relative retention times were used for the identification of compounds in the four tea cultivars. At least 96 compounds were detected in decayed tea stems of which 43, 50, 36 and 46 were identified as chemical constituents

of volatiles present in TRI 2023, TRI 2027, TRI 4042 and TRI 4049, respectively (Table 2). The results indicated that a vast range of chemical constituents are present and among them long chain fatty acids, alcohols, carbonyls, esters, monoterpenes and sesquiterpenes are present as prominent group of compounds. Comparison of GC profiles of the decayed tea stem volatiles revealed that 15 compounds are common in all four tea cultivars (Table 3).

The constituents, *n*-hexadecanoic acid (palmitic acid) and 9,12-octadecadienoic acid (Z,Z) (linoleic acid) were identified as the two major constituents in decayed stems and the highest amounts of *n*-hexadecanoic acid (20.4 %) and 9, 12-octadecadienoic acid (Z,Z) (15.6 %) were observed in the decayed stem of TRI 4049. In addition to the presence of two major compounds in the volatiles of decayed tea stems, a higher percentage of mono(2-ethylhexyl) phthalate was present only in the decayed tea stems TRI 2023 (8.76 %) and TRI 4042 (9.7 %).

Volatiles of the healthy stems of tea cultivars TRI 2023, TRI 2027, TRI 4042 and TRI 4049 were also

Table 2: Chemical constituents and percentage of volatiles of the decayed stem of TRI 2023, TRI 2027, TRI 4042 and TRI 4049

Retention time	Compound	% Total			
		TRI 2023	TRI 2027	TRI 4042	TRI 4049
9.720	2-Furan carboxaldehyde	0.81	1.92		1.48
13.059	2, propenoic acid, butyl ester	1.2	1.07		1.09
14.496	Benzaldehyde		0.69		
14.775	2-Furan carboxaldehyde,5-methyl		0.69		0.58
15.563	Phenol	0.86	0.95		0.66
15.702	Ethanol, 2-[2-(2-methoxy ethoxy) ethoxy]-acetate			1.62	
17.371	Benzeneacetaldehyde		1.13		0.97
17.511	Iso cineole	1.22			
17.928	1,8-Cineole	0.65	0.7		
18.206	2-azabicyclo(2.2.1) heptanes		0.78		1.53
18.299	5,6-Dihydro-2Hpyran-2-one	1.33			
18.994	Alpha terpinolene	0.65			
19.922	n-Undecane	0.67			
20.246	D-fenchyl alcohol	0.68		0.51	
20.803	Terpenene 1-ol	2.45	1.26	1.45	1.4
21.081	Beta terpineol	0.96	0.51	0.64	0.54
21.545	Benzene acetic acid, methyl ester		1.65		
21.638	Bicyclo[2.2.1]heptan- 2-ol,1,7,7-trimethyl-exo	1.28			0.84
21.684	Bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethylEndo			1.61	
21.870	2-Butyl -1-octanol		1.23		
22.194	Alpha terpineol	3.22	1.7	2.3	1.71
22.472	1-Dodecene	1.63			
22.658	n-dodecane	1.78	0.58	0.55	0.67
23.353	Benzaldehyde 4 methoxy		0.5	0.71	0.78
23.446	2,6 -dimethyl undecane	0.6			
24.466	Nonanoic acid		1.23	1.01	0.92
24.513	2- methyl-dodecane	1.92			
24.930	2-methoxy-4-vinylphenol	2.05	1.76	1.28	2.36
25.208	n –Tridecane	2.66	0.99	1.25	1.03
26.182	Phenol,2-methoxy-4-(2-propenyl)			2.85	
26.692	Formamide,N-(2-methylphenyl)	0.75			
26.924	Decanoic acid		1.05		
27.017	4- hydroxy-3-methoxy-Benzaldehyde (Vanillin)	5.5		2.35	3.31
27.434	1- Tetradecene	3.15	0.96	2.2	1.21
27.620	n-tetradecane	1.2		0.95	0.83
28.269	Phenol,2-methoxy-4-(1-propenyl)- (Z)	0.55			0.56
28.773	1,2,3,4-Tetramethoxybenzene		1.53		
29.196	Ethanone,1-(4-hydroxy-3-methoxy phenyl)-	1.36	0.58	1.51	1.55

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Retention time	Compound	% Total TRI 2023	TRI 2027	TRI 4042	TRI 4049
33.694	2,6-dimethyl-7-octenylacetate				0.66
33.741	2',3',4',Trimethoxy acetophenone			1.54	
33.973	4-Butoxy-2,5,6,-trimethylpyrimidine		2.74		0.68
34.112	Octadecanal		1.72		
34.436	Tricyclo[4.3.1.1 (3,8) undecan-1-ol	1.07		0.55	
34.946	Tricyclo[4.3.0.0(7,9) nonene,2,2,5,5,8,8, -hexamethyl 91.alpha.,6.beta., 7.A		0.71		
35.317	n-tetradecanoic acid	1.55	5.24	4.82	3.55
35.549	5'5-Epoxymethano-2,2,6-trimethyl-7 -oxabicyclo[4.3.2]non-9-en-8-one	2.05			
35.735	1-Octadecene	1.16		0.74	0.53
36.059	Tetradecanal		1.2		
36.106	Dyhydrocarvyl acetate			1.33	
36.569	n-Pentadecanoic acid		0.76		0.7
36.755	Caffeine		0.59		
36.987	2-Pentadecanone,6,10,14-trimethyl		0.86		
37.636	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	0.69	2.3	1.33	0.64
38.331	4-[(1Z)-(N-hydroxyethanimidoyl) -2-methylpyridazin-3(2H)one/		1.22		0.88
38.563	7,9-Di-tert-butyl-1-oxopiro(4,5) deca-6,9-diene-2,8-dione	1.82	2.96	3.3	
38.656	Cis-9-hexadecenoic acid				2.75
38.888	n-hexadecanoic acid	12.47	12.06	14.2	20.4
39.120	1,2 Benzenedicarboxylic acid ,di butyl ester	6.19		8.62	
41.392	Octadecanoic acid methyl ester	0.92		0.61	0.65
41.809	9,12-Octadecadienoic acid(Z,Z)	9.76	9.58	6.74	15.6
43.386	Hexadecane,2,6,10,14,-tetramethyl		3.22		0.94
43.850	Heneicosane		1.81		
44.453	n-pentacos-3-ene		1.21		
45.334	Benzyl butyl phthalate				1.09
45.380	1,2 -Benzenedicarbxylicacid, butyl phenyl methyl ester			1.34	
45.797	Hexanedioic acid, bis(2-ethylhexyl)ester			4.65	
46.632	n-pentacosane				1.02
47.096	12 -Methyl tricosane		1.09		
47.142	n-heptadecane				0.71
47.745	1,2-Benzenedicarboxylic acid, dicyclohexyl ester	2.99	1.2	1.89	1.39
48.116	mono(2-ethylhexyl) phthalate	8.76	2.61	9.7	3.91

analysed by GC-MS and are shown in Table 4. Of the 55 compounds detected in healthy tea stems, 33, 15, 08 and 15 were identified from TRI 2023, TRI 2027, TRI 4042 and TRI 4049, respectively. According to the GC-MS analysis under the conditions described above, mono(2-ethylhexyl) phthalate was detected as the major component in TRI 2023 (27.77 %), TRI 2027 (56.62 %), TRI 4042 (82.2 %) and TRI 4049 (72.89 %). The results showed that the volatiles of healthy tea stems contained a complex mixture of numerous compounds, many of which are present in trace amounts. Unlike decayed tea stem volatiles, only three compounds, mono(2-ethylhexyl) phthalate, 1-hexadecene and n-hexadecanoic acid were common in all healthy stems of the four tea cultivars. Although TRI 2027 showed the lowest percentage of the major constituent (27.77 %), it had higher amounts of *n*-heptadecane (4.15 %), *n*-octadecane (5.26 %), nonadecane (9.22 %), eicosane (4.76 %), heneicosane (5.19 %), hexanedioic acid, bis 2-ethylhexyl ester (17.4 %) and benzenamine, 4-octyl-n-(4-octyl phenyl) (4.62 %), and there were comparatively small amounts in the volatile compounds present in healthy stem of other three tea cultivars. A comparison of decayed and healthy tea stem volatiles indicated that there is great variation in the chemical compositions of decayed and healthy tea stems as *n*-hexadecanoic acid is the major component in decayed tea stem and mono(2-ethylhexyl) phthalate is the major compound in healthy tea stem. The highest

Table 3: Volatile constituents common in decayed stem extracts of the four tea cultivars TRI 2023, TRI 2027, TRI 4042 and TRI 4049

Retention time	Compound	% Total TRI 2023	TRI 2027	TRI 4042	TRI 4049
20.803	Terpenene 1-ol	2.45	1.26	1.45	1.4
21.081	Beta terpineol	0.96	0.51	0.64	0.54
22.194	Alpha terpineol	3.22	1.7	2.3	1.71
22.658	n-dodecene	1.78	0.58	0.55	0.67
24.930	2-Methoxy-4-vinylphenol	2.05	1.76	1.28	2.36
25.208	n–tridecane	2.66	0.99	1.25	1.03
27.434	1-Tetradecene (n-Tetradecene)	3.15	0.96	2.2	1.21
29.196	Ethanone,1-(4-hydroxy-3-methoxy phenyl)-	1.36	0.58	1.51	1.55
31.329	n-dodecanoic acid	1.08	5.32	4.29	1.53
35.317	n-tertradecanoic acid	1.55	5.24	4.82	3.55
37.636	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	0.69	2.3	1.33	0.64
38.888	n-hexadecanoic acid	12.47	12.1	14.2	20.4
41.809	9,12-Octadecadienoic acid(Z,Z)	9.76	9.58	6.74	15.6
47.745	1,2-Benzenedicarboxlic acid,dicyclohexyl ester	2.99	1.2	1.89	1.39
48.116	mono(2-ethylhexyl) phthalate	8.76	2.61	9.7	3.91

Table 4: Volatile constituents and percentage in healthy stems of tea cultivars TRI 2023, TRI 2027, TRI 4042, TRI 4049

Retention time	Compound	% Total TRI 2023	TRI 2027	TRI 4042	TRI 4049
14.265	Ethanol, 2,2'-oxybis		2.06		1.85
21.406	beta sinensal	0.54			
23.353	Phenol, 2-methyl-5-(1- methyl ethyl)	0.61			0.75
25.765	1-(prop-1-enyl)-2-oxabicyclo[3.1.0] hexane	1.45			
25.579	Benzaldehyde, 4 –hydroxy-3-methoxy (vanillin)		0.51		
25.997	2-Tetradecene,(E)				3.08
25.991	2-Butanone,4-(4-methoxyphenyl)-				0.41
26.785	Cis –Isoeugenol	0.54			
29.243	Phenol, 2, 4-di-tert-butyl-		1.3		2.41
29.892	Dodecanoic acid	0.82	1.34		
30.124	3-methyl -5-(1,4,4-trimethyl-cyclohex -2-enyl-pent-2-enoic acid, ethyl ester	1.22			
30.402	1- Hexadecene	0.82	3.48	1.4	3.41
31.654	n-heptyl cyclohexane	0.57			
31.839	1-(3,5-Dimethoxy phenyl)pent 1 ene				0.53
32.071	Sulfurous acid, octadecylpentyl ester	0.65			
32.303	3-Heptadecene, (Z)	0.68			
32. 581	n-heptadecane	4.15			
33.230	Tetracosane,2,6,10,15,19,23-hexa methyl/Octyl phenol	1.33	0.57		
33.648	2-propenyl-cyclohexane	2.58			
33.231	Phenol-4-octyl		0.57		
33.833	Tetradecanoic acid		6.46		
33.880	Heptadecane, 2-Methyl	0.86			
34.251	9-Eicosane (E)		3.64		
34.436	n-Octadecane	5.26			2.39
35.085	Caffeine		0.64		
35.132	Hexadecane,2,6,10,14-tetramethyl	3.8			
35.549	Cyclohexane,2-propenyl-	2.58			

continued -

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Retention time	Compound	% Total TRI 2023	TRI 2027	TRI 4042	TRI 4049
36.152	Phthalic acid, isobutyl nonyl ester		0.67		
36.198	Nonadecane	9.22			
36.569	n-hentriacontane	1.61			
37.404	n-hexadecanoic acid	11.01	16.2	6.7	1.45
37.590	1,2-Benzenedicaboxylic acid , butyl 2–methylpropyl ester				2.01
37.868	Eicosane	4.76			2.03
39.246	1,2-Benzenedicaboxylic acid , di butyl ester			5.8	
39.491	Heneicosane	5.19			
40.418	9,12-Octadecadienoic acid (Z,Z)		4.15		0.96
40.511	Nonahexacontanoic acid	4.04			
40.975	1-Docosene				1.24
43.850	Cyclotetracosane				0.66
44.360	Hexane dioic acid, bis (2-ethylhexyl)ester	17.4			
45.241	n-tetratetracontane	1.77			
46.493	1,2-Benzenedicaboxylic acid , mono (2-ethylhexyl) ester	27.77	56.62	82.2	72.89
46.539	n-hexatriacontane	1.58			
47.745	Pentatriacontane	1.98			
48.950	Docosane	1.25			
51.362	Benzenamine,4-octyl-n-(4-octyl phenyl)	4.62			
52.196	Aspidofractinine,17-methoxy-3-methylene-,(2.alpha.alpha)	0.54			
56.045	Alpha spinasterone		0.74		
60.822	(2R*,8aS)-1-Benzyl-3(E) benzalidene-4-methyl -2-phenyl 1,2,3,5,67,8,8a-octa.	0.91			
61.244	Unknown 1			0.7	
61.540	Unknown 2			0.5	

Table 5: Common compounds present in the volatiles of healthy stems of TRI 2023, TRI 2027, TRI 4042, TRI 4049

Compound	% Total TRI 2023	TRI 2027	TRI 4042	TRI 4049
mono(2-ethylhexyl) phthalate	27.77	56.62	82.2	72.89
1- hexadecane	0.82	3.48	1.4	3.41
Hexadecanoic acid	11.01	16.2	6.7	1.45

amount of mono(2-ethylhexyl) phthalate was reported in the tea cultivar TRI 4042 (82.2 %). A different composition of compounds was detected in decayed and healthy stem volatiles. *n*-hexadecanoic acid was identified as the common compound in decayed and healthy volatiles of tea stems. The identification of chemical composition of volatiles in tea stem has not been reported before. Although Samarasinghe *et al*. (1999) had obtained the GC profiles of stem volatiles of TRI 2023 and TRI 2027, the chemical compositions of volatile fractions were not identified. Hence this is the first report on the identification of chemical composition of volatiles isolated from decayed and healthy stems of TRI 2023, TRI 2027, TRI 4042 and TRI 4049. The bioassay carried out with the volatiles of tea stem indicated that decayed tea stem volatiles are more attractive to the alates than healthy tea stem volatiles suggesting that *n*-hexadecanoic acid, linoleic acid and other compounds in decayed tea stem volatiles may be responsible for the attractant effect of the volatiles. It has been reported that Z, Z, E-3, 6, 8-dodecatrien-1-ol is an attractant substance to the eastern subterranean termite *Reticulitermes flavipes*; it was isolated from the Western pine wood (*Pinus monticola* Dougl.) decayed by the fungus *Lenzites trabea* (Esenther *et al*., 1961). The volatile

Table 6: Common compounds present in decayed and healthy stems of cultivars TRI 2023, TRI 2027, TRI4042, TRI 4049

	% Total							
	Decayed stems				Healthy stems			
Compound	TRI 2023	TRI 2027	TRI 4042	TRI 4049	TRI 2023	TRI 2027	TRI 4042	TRI 4049
Terpenene 1-ol	2.45	1.26	1.45	1.4				
Beta terpineol	0.96	0.51	0.64	0.54				
Alpha terpineol	3.22	1.7	2.3	1.71				
n-dodecene	1.78	0.58	0.55	0.67				
2-methoxy-4-vinylphenol	2.05	1.76	1.28	2.36				
n tridecane	2.66	0.99	1.25	1.03				
1- Tetradecene (n-Tetra decene)	3.15	0.96	2.2	1.21				
Ethanone ,1-(4-hydroxy-3-methoxy phenyl)-	1.36	0.58	1.51	1.55				
n-dodecanoic acid	1.08	5.32	4.29	1.53				
n-tertradecanoic acid	1.55	5.24	4.82	3.55				
1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	0.69	2.3	1.33	0.64				
n-hexadecanoic acid	12.47	12.1	14.2	20.4	11.01	16.2	6.7	1.45
9,12-Octadecadienoic acid(Z,Z)	9.76	9.58	6.74	15.6				
1,2-Benzenedicarboxlic acid,dicyclohexyl ester	2.99	1.2	1.89	1.39				
mono(2-ethylhexyl) phthalate	8.76	2.61	9.7	3.91	27.77	56.62	82.2	72.8

compounds linalool, linalool oxide, phenyl acetaldehyde, methyl salicylate, geraniol and trans 2-hexanal, which was reported in the bark of the young stems of tea was totally different from the decayed stems and healthy stems detected in the present study (Amarasinghe *et al*., 2007).

CONCLUSION

The results of the present study revealed that *n*-hexadecanoic acid and 9,12-octa decadienoic (*Z,Z*) are the major chemical constituents of decayed tea stem volatiles in all four tea cultivars, and there were several minor compounds present (Tables 2 and 3). The comparison of the volatiles of the four tea cultivars indicated that fifteen volatile compounds were common in decayed stems of tea cultivars. The volatiles of healthy tea stems show mono(2-ethylhexyl) phthalate (27 – 82 %) as the major constituent in healthy stems, and it is also present in decayed stems of cultivars but in small quantities (< 10 %). The number of volatile compounds present in healthy tea stems were less than that in decayed tea stems. The Y-tube olfactometer bioassay results revealed that the alates of LCLWT were more attracted to the volatiles of decayed stems than that of the healthy tea stems. The results indicated that attractant effect of the volatile sample is not dependent on the tea cultivar. The present study suggests that gas chromatography electroantegram (GC-EAG) studies can be carried out to isolate and identify the attractant chemical compounds in the volatiles of decayed tea stems. Successful identification of the active compounds with standard chemicals would enable the formulation of attractants to develop a new environmentally friendly attractant-based bait trap. Successful development of the bait trap techniques holds potential to improve integrated pest management (IPM) strategies for LCLWT.

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