

Variability of overhead volatile organic compounds in clonal tea (*Camellia sinensis*) and their influence on red crevice mite (*Brevipalpus phoenicis* Geijskes) infestations

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ABSTRACT: Tea production in Kenya is under threat due to red crevice mites (*Brevipalpus phoenicis*) infestations during droughts. Cultural pests control practices, e.g. use of resistant/tolerant cultivars are used in their control since pesticide use is prohibited. Plants release volatile organic compounds (OVOCs) that may influence susceptibility/resistance to pest infestations. OVOCs profiles released by 11 tea cultivars were evaluated to assess relationship between OVOCs and cultivar tolerance/susceptibility to *B. phoenicis*. Five clones were susceptible, with high *B. phoenicis* infestations while four clones were resistant, exhibiting low infestation levels. The infestations were linearly correlated to (*E*)-2-hexenal, (*Z*)-3-hexenal ($p \leq 0.001$), (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, linalool, germacrene D, sum of green leaf volatiles (GLVs) ($p \leq 0.01$), 1-pentene-3-ol, hexanal, indole and (*E*)- β -ocimene ($p \leq 0.05$) levels. Most of aromatic compounds, some terpenoids compounds and sum of aromatic compounds were inversely ($p \geq 0.05$) correlated with *B. phoenicis* infestations. Susceptible varieties to *B. phoenicis* emitted high amounts of GLVs, especially (*E*)-2-hexenal, (*Z*)-3-hexenal, (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate. Results demonstrate that OVOCs profile may provide selection criteria for cultivars resistant to *B. phoenicis* infestations. Resistant cultivars are recommended for commercial exploitation in red crevice mites prone areas while breeding/selection programmes should incorporate OVOCs profiles to develop tea cultivars that resist red crevice mites attack.

KEYWORDS: Volatile organic compounds, tea varieties, red crevice mites, *Brevipalpus phoenicis*.

RUNNING TITLE: Clonal tea overhead volatile organic compounds and mites infestations.

Introduction

Pest infestations are a major problem in tea (*Camellia sinensis*, (L) O. Kuntze) growing areas causing yield losses, with losses up to 100% having been reported in India (1). While pest infestations are relatively rare in Kenya, mite infestations especially red crevice (*Brevipalpus phoenicis* Geijskes) mites causing yield losses estimated to be up to 50%, during prolonged drought around the Mount Kenya region (2-4). When red crevice mite infestations are high, their management in tea is usually through use of synthetic

pesticides. However, heavy use of organosynthetic pesticides leads to undesirable pesticide residues in made tea, in addition to proliferation of pesticide-resistant pests and devastation of natural enemies (5). Pesticide residues in tea is a serious global concern, necessitating several regulatory bodies to set maximum residue limits (MRL) (6). For the Kenya tea industry, use of pesticides on tea is prohibited and control of pests is through use of cultural and agronomic practices, including resistant/tolerant tea cultivars and agronomic inputs that deter pests attack (2, 3).

The tea breeding/selection research programme has released tea varieties to the Kenyan tea industry some of which are resistant/tolerant while others are susceptible to red crevice mite infestation. For

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example clone TRFK 54/40 is susceptible to *B. phoenicis* while TRFK 303/1199 is resistant (3, 4). Resistance/susceptibility can also be influenced by abiotic factors (7-10) such as nitrogen fertilizers (3, 4) and biotic factors (2, 3, 8, 10, 11). In Bangladesh (7, 8) red crevice mite infestations in tea were influenced by temperature, rainfall and relative humidity among other climatic factors. At high temperature (during drought) mites took shorter period to complete the life cycle (9) leading to very high rate of population increase and high densities (12). Normally mites attack the upper surface of mature leaves, feeding on chlorophyll of the maintenance foliage, but in severe cases young leaves are also attacked causing defoliation and occasional death of tea bushes (8, 13). These patterns of variations in attack could be related to herbivore-plant interactions that are influenced by the plant defence mechanisms. Plants produce and emit metabolites that influence predators attack or repulsion (14). The changes in mite infestations could in part be related to the variations in the levels of the overhead volatile organic compounds (OVOCs) emitted by the tea cultivars. For example, lipoxygenase activity responsible for production of green leaf volatile compounds (14, 15), increases during water deficit periods (16). Plant emission of mono, homo and sesquiterpenes rise with temperature (17) and changes with weather conditions (18).

An alternative method to reduce pest pressure is to identify key plants that deter or repel the herbivores (19). Plants use indirect defences such as OVOCs to repel herbivores or attract predators and parasitoids of the herbivores feeding on them (20). Some OVOCs including hexanal, (E)-2-hexenal, (Z)-3-hexen-1-ol and (Z)-3-hexenyl acetate attract insect pests while (E)-ocimene, β -caryophyllene, humulene, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) are repellents (21). OVOCs are emitted in small quantities from unstressed plant tissues but are released in large amounts during or after stress factors including drought, herbivore infestation, etc (22). All plant species typically respond to stress by triggering emissions of a variety of characteristic stress volatiles (22). Typical stress emissions in various plant species consist of green leaf volatiles such as (E)-2-hexenal, (Z)-3-hexenal, (Z)-3-hexen-1-ol and (Z)-3-hexenyl acetate, volatile mono-, homo- and sesquiterpenoid compounds including linalool, (E)- β -ocimene, (Z)- β -ocimene, DMNT, (E)- β -farnesene, (E)- β -caryophyllene, and aromatic compounds such

as indole, methyl salicylate and benzaldehyde (22-24). The four major VOC biosynthetic pathways, namely the shikimate, the mevalonic acid and the methylerythritol phosphate and lipoxygenase release aromatic, mono-, homo- and sesquiterpenoids and green leaf volatiles, respectively. Tea plant produces OVOCs belonging to monoterpenes, sesquiterpenes, homoterpenes, GLVs and aromatics (25) which have been implicated in plants defense mechanism against pests and diseases (21, 22, 26, 27). While much is known about how volatile organic compounds influence quality of tea varieties (15, 28-33) due to location of production (34-36), agronomic (37, 38) and processing (39-41) practices, little is known on emissions of overhead volatile organic compounds from tea varieties and particularly their specific variability in relation to mite infestations levels. In this study, we report the clonal tea variations in the OVOCs and their relationship to red crevice mites (*B. phoenicis*) attack.

Materials and methods

Plant materials for use

Trials were carried out in Kangaita Tea Research Institute Sub-station, at Kangaita Tea Farm in Kerugoya, Mount Kenya region (37° 7.8'E and 0° 19.8'S, 2130 m above mean sea level). The red crevice mites sampling was superimposed on on-going Clonal Field Trials at the site established in the year 2005 to evaluate agronomic performance of the newly developed Kenyan (TRFK) clones, imported Tanzania (TRIT) clones relative to some commercial clones in production in Kenya (42). The plots were arranged in a randomized complete block design with three replicates, each plot consisting of 20 plants, planted at 1.22 m by 0.75 m planting spacing. The clones used were:- TRIT 201/16, TRIT 201/50, AHP S15/10, TRFCA SFS150, TRFK 31/8, TRFK 6/8, TRFK 18/3, TRFK 301/4, TRFK 303/1199, TRFK 54/40 and STC 5/3. After bringing into bearing, the clones received recommended agronomic and cultural management inputs (43).

Determination of red crevice mite infestations levels

Ten mature leaves per bush were plucked randomly then the mites were brushed using mite brushing machine (Model-Leedom Engineering, USA) and the number counted under dissecting microscope (2-4).

Volatile Collection in the field

The OVOCs collection was carried out according to the method of Chen et al. (44). Shoots of the 11 tea varieties were individually enclosed in large oven bags (355 mm x 508 mm) (that had been conditioned in an oven at 170°C) together with two Teflon pipes and tied with elastic bands. Activated charcoal-purified air generated by a pump entered the bag through one pipe flowing at 100 ml min⁻¹. The air exiting the bag passed through an adsorbent trap consisting of a 200 × 7-mm glass tube containing 30 mg Alltech Super-Q, 80/100 mesh (Alltech Associates Inc., Deerfeld, IL) adsorbent material. Collected volatiles were eluted from the Super-Q adsorbent with 250 µl dichloromethane (HPLC grade; purity: 99%) and 10 µl of cumene internal standard and then concentrated to 20 µl with a stream of nitrogen gas while cooling under ice. VOCs from the control (without tea shoots) were collected simultaneously. Replicates were made on different days but at the same time of day i.e. from 0800 to 1200 h.

Volatile analysis and identification

Both GC and GC-MS were used for analysis. The GC analysis was carried on a Shimadzu model GC-2010 equipped with flame ionization detector. A 50 m silica gel capillary column (film thickness 0.20 µm, 0.25 mm inner diameter) was used. Oven temperature was programmed from 35 to 230°C with the initial temperature maintained for 5 min then 5 °C/min to 190 °C, then at 50°C/min to 230 °C, and then 5 min hold. The flow rate for the carrier gas (N₂) was 3.0 ml/min and for detector gases 40.0 ml/min hydrogen and 400.0 ml/min air, respectively. The detector temperature was set at 230.0°C. A sample volume of 1 µl was injected in splitless mode. Analysis on GC-MS was conducted using an Agilent 7890A Series gas chromatograph coupled to a mass spectrometer (Agilent 5973 quadrupole detector). The gas chromatographic conditions were as follow: helium was used as the carrier gas at a flow rate of 1.25 ml/min; Inlet temperature 270°C, transfer line temperature of 280°C, and column (HP-5MS 30 m×0.25 mm×0.25 µm film) oven temperature programmed from 35 to 280°C with the initial temperature maintained for 5 min then 10 °C/min to 280 °C for 10.5 min and the final temperature for 29.9 min 50 °C/min to 285 °C. Parameters for electron impact sample ionization

were as follow: mass selective detector maintained at an ion source temperature of 250°C and quadrupole temperature of 180°C, electron energy, 70 eV; source temperature, 250°C. Fragment ions were analyzed over 40-550 m/z mass range in the full scan. The compounds were quantified as ratio of the peak area of each compound to that of cumene (internal standard). Identification of compounds was by comparing the fragmentation pattern with mass spectral data in mass spectral library and literature.

Statistical Analysis

The obtained red crevice mites data were transformed, log e (x+1) and then subjected to analysis of variance (ANOVA) as randomised complete block design using MSTAT-C statistical package. Excel Microsoft Office was used to perform regression analyses.

Results and Discussion

Susceptibility/resistance of the tea cultivars to red crevice mite

Red crevice mites population varied significantly ($p \leq 0.05$) with clones (Table 1). Five clones (TRFK 18/3, TRIT 201/16, AHP S15/10, STC 5/3 and TRFK 54/40) had high ($p \leq 0.05$) red crevice mite infestations levels ranging from 36 for TRFK 18/3 to 112 for TRFK 54/40. These clones were classified as susceptible to red crevice mites attack. Clones TRFK 6/8, TRIT 201/50, TRFK 303/1199 and TRFK 301/4 had very low levels of red crevice mites, ranging from 8-13 and were considered resistant to red crevice mites. However, two clones TRFCA SFS150 and TRFK 31/8 had levels of red crevice mites that were of moderate infestation levels. Previously, clones TRFK 6/8, TRFK 303/1199, TRFCA SFS150 and TRFK 301/4 exhibited resistance against *B. phoenicis*, STC 5/3 and TRFK 54/40 were susceptible while AHP S15/10 was moderately susceptible (2, 10, 11, 45). The present study corroborates those observations except on clones TRFCA SFS150 and AHP S15/10 that were moderately resistant and susceptible, respectively. The resistant cultivars are recommended for commercial exploitation in the areas prone to red crevice mite infestations. TRIT 201/50, TRIT 201/16 and TRFK 18/3 were screened for susceptibility/resistance to *B. phoenicis* for the first time. Both TRIT 201/16 and TRFK 18/3 were susceptible to *B. phoenicis* infestation. The two cultivars may not be suitable

TABLE 1: The changes in red crevice mite infestations and overhead greenleaf volatile compounds (GLVs) and their relationships in tea cultivars.

Clone	Overhead green leaf volatile compounds										Red crevice mite infestations	
	1-Penten-3-ol	4-Penten-1-ol	Hexanal	E-2-Hexenal	Z-3-Hexenal	Z-3-Hexenol	Z-3-Hexenyl acetate	Nonanal	Sum of GLVs	Mites	Log (1+x) mite	
TRFK 6/8	0.135	0.080	0.267	0.758	0.029	0.575	4.195	0.000	6.039	8±1.53	2.20±0.42	
TRFK 18/3	0.250	0.112	0.353	0.792	0.115	1.611	6.558	0.062	9.853	36±4.58	3.61±1.52	
TRFK 31/8	0.225	0.112	0.260	0.684	0.019	0.675	4.905	ND	6.880	15±3.79	2.77±1.33	
TRFK 54/40	0.242	0.189	0.480	1.466	0.308	1.954	8.509	0.065	13.213	112±13.11	4.73±2.57	
TRIT 201/16	0.254	0.059	0.373	1.050	0.106	2.025	6.272	0.099	10.238	74±23.26	4.32±3.15	
TRIT 201/50	0.020	ND	0.050	0.163	ND	0.269	0.869	ND	1.371	9±2.00	2.30±0.69	
TRFK 301/4	0.059	0.053	0.153	0.149	ND	0.543	3.283	ND	4.240	9±2.00	2.30±0.69	
TRFK 303/1199	0.067	0.054	0.140	0.142	ND	0.475	1.464	ND	2.342	13±0.58	2.64±0.55	
AHP S15/10	0.270	0.105	0.293	1.084	0.171	2.000	6.965	0.115	11.000	59±15.89	4.09±2.77	
TRFCA SFS150	0.142	0.132	0.379	0.138	0.093	1.438	6.051	0.147	8.520	17±3.46	2.89±1.24	
STC 5/3	0.229	0.043	0.457	0.849	0.128	1.188	5.745	0.142	8.781	49±8.72	3.91±2.17	
Mean	0.172	0.085	0.291	0.661	0.088	1.159	4.983	0.057	7.498	36.45± 5.36	3.25±1.55	
STDEV	0.091	0.052	0.135	0.458	0.095	0.677	2.346	0.061	3.585	33.89±7.30	0.91±0.97	
CV (%)#	52.880	60.310	46.420	69.19	107.32	58.43	47.080	105.880	47.813	92.979	27.93	
r _(mite)	0.698*	0.537	0.720*	0.8643***	0.924***	0.831**	0.772**	0.492	0.828**			
r ² _(mite)	0.487	0.288	0.519	0.747	0.854	0.690	0.596	0.242	0.686			
CV (%)##										22.73	2.75	
LSD, (p≤0.05)										15	0.47	

ND = not detected, a value of 0.000 has been used in the statistical calculations

*, **, *** significant at p≤0.05, 0.01 and 0.001, respectively

As $\frac{STDEV}{Mean} * 100$

From ANOVA

for commercial exploitation in geographical regions prone to red crevice mite infestation.

Variations in volatile organic compounds emitted by the cultivars and their relationship with red crevice mite infestations.

All chemical groups (GLVs, aromatics, homoterpenes, monoterpenes, and sesquiterpenes) were simultaneously emitted by the eleven tea cultivars thereby giving rise to unique pattern of OVOCs (Tables 1-4) Such emission of a complex OVOCs had been reported in plants, and implicated in plant-herbivore interactions (20). A total of 8 GLVs were released by the tea varieties. The major GLVs identified were (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate. All GLVs were directly correlated ($p \leq 0.05$) with red crevice mites levels ($r = 0.698$ to 0.924) except nonanal and 4-penten-1-ol (Table 1). (*Z*)-3-hexenyl acetate was released in the highest quantities with $r=0.772$ ($p \leq 0.01$). High amounts were emitted by clones STC 5/3, TRFCA SFS150, TRIT 201/16, TRFK 18/3, AHP S15/10 and TRFK 54/40 while TRIT 201/50, TRFK 303/1199, TRFK 301/4, TRFK 6/8 and TRFK 31/8 released relatively lower amounts. (*Z*)-3-Hexenal levels had the highest relationship with red crevice mite infestations ($r=0.924$, $p \leq 0.001$). However, it was not detected in TRFK 201/50, TRFK 303/1199 and TRFK 301/4. The total amounts of the GLVs compounds emitted by the cultivars were in the following ascending order TRIT 201/50 < TRFK 303/1199 < TRFK 301/4 < TRFK6/8 < TRFK 31/8 < TRFCASFS150 < STC 5/3 < TRFK 18/3 < TRIT 201/16 < AHP S15/10 < TRFK 54/40. Cultivars TRFK 54/40, TRIT 201/16, AHP S15/10, STC 5/3 and TRFK 18/3 that released high amounts of GLVs had high levels of red crevice mite infestations. In contrast clones TRIT 201/50, TRFK 6/8, TRFK 301/4 and TRFK 303/1199 that emitted low amounts of GLVs had low red crevice mite infestations. Insect pests prefer plant cultivars that emit large amounts of GLVs. For example, flea beetles (*Epitrix hirtipennis*) were more abundant on GLV-producing wild type plants compared to plants with reduced hydroperoxide lyase activity (46). Hydroperoxide lyase catalyse the cleavage of fatty acid hydroperoxides to aldehydes and oxo acids(47), the aldehydes being part of and precursors of the GLVS (15). *Uschisus heros* preferred soybean pods

that released high amounts of GLVs for feeding and oviposition over deficient cultivars (48). The GLVs serve as feeding stimulants to pests (49). Tea cultivars that release low amounts of GLVs are likely to suffer less when there is an outbreak of *B. phoenicis* and can be commercially exploited in red crevice mites prone areas.

Unlike the GLVs, the aromatic compounds (Table 2) were released in smaller quantities ranging from below detection limit to 0.421. Except indole, phenyl acetaldehyde and methyl salicylate, the levels of the aromatic compounds inversely correlated with red crevice mite levels. The correlation between the red crevice mite infestations and indole level was significant ($r=0.650$, $p \leq 0.05$). The direct relationship between indole and the mites levels corroborated earlier studies (14, 50) that indole induced herbivore attack of plants. Methyl salicylate (51, 52) and phenyl acetaldehyde (53, 54) have also been associated with insect attraction in plants. Tea plants that emit high levels of overhead indole, methyl salicylate and phenyl acetaldehyde are therefore likely to be susceptible to red crevice mite infestations. The level of indole was high in clones STC 5/3, TRFK 18/3, AHP S15/10 and TRFK 54/40 that had high red crevice mite numbers. The cultivars that emitted the aromatic compounds in large quantities, e.g. TRIT 201/50, were resistant while clones releasing low quantities of the aromatic compounds, e.g. TRIT 201/16 were susceptible to red crevice mite attack. Indeed, there was inverse relationship between most of the aromatic compounds, although the relationships were insignificant. A number of aromatic compounds have been implicated in plant defence against insect pests which may contribute to resistance observed. For example, acetophenone causes acute insecticidal activities (55), anethole is an effective insect repellent while benzothiazole exhibit a wide range of biological properties including antimicrobial activities (56). TRIT 201/50 produced the highest amounts of total aromatic compounds, dominated by acetophenone and had the least red crevice mite infestations while TRIT 201/16 produced the least amount of aromatic compounds and was one of the clones with the highest population of red crevice mites. Although, clone TRFK 54/40 also emitted high amounts of aromatic compounds, it still had high levels of red crevice mites. These results suggest that the amounts of aromatic compounds released/emitted by the tea cultivars could be a good

TABLE 2: Clonal variations and the relationships between red crevice mite infestations and overhead volatile aromatic compounds.

CLONE	Aromatic compounds														Red crevice mites
	Phenyl ethyl alcohol	Methyl salicylate	Ethyl benzene	p-Xylene	Indole	Benzaldehyde	Phenyl acetaldehyde	Benzyl alcohol	Acetophenone	Z-Anethole	Benzothiozole	Benzophenone	Sum aroma		
TRFK 6/8	0.145	0.194	0.268	0.168	0.236	0.161	0.050	0.079	0.103	0.242	0.074	0.072	1.792	8±1.53	
TRFK 18/3	0.093	0.139	0.149	0.073	0.421	0.054	0.038	0.089	0.115	0.182	ND	ND	1.353	36±4.58	
TRFK 31/8	0.153	0.153	0.197	0.21	0.260	0.087	0.059	0.094	0.146	0.137	ND	ND	1.496	15±3.79	
TRFK 54/40	0.112	0.238	0.158	0.202	0.395	0.150	0.136	0.150	0.110	0.112	ND	ND	1.763	112±13.11	
TRIT 201/16	0.057	0.001	0.061	ND	0.306	0.019	0.054	0.059	0.161	0.164	0.050	ND	0.932	74±23.26	
TRIT 201/50	0.142	0.088	0.261	0.093	0.300	0.144	0.063	0.064	0.368	0.258	0.114	0.192	2.089	9±2.0	
TRFK 301/4	0.125	0.073	0.135	0.236	0.140	0.128	0.033	ND	0.122	0.190	ND	0.141	1.323	9±2.0	
TRFK 303/1199	0.262	0.065	0.118	0.266	0.152	0.122	0.074	0.119	0.270	0.213	ND	0.236	1.897	13±0.58	
AHP S15/10	0.088	0.054	0.124	ND	0.350	0.192	0.025	0.044	0.190	0.189	ND	ND	1.256	59±15.89	
TRFCA SFS150	0.121	0.114	0.103	ND	0.284	0.21	0.050	0.163	0.109	0.085	ND	ND	1.239	17±3.46	
STC 5/3	0.099	0.182	0.263	0.069	0.346	0.115	0.099	0.108	0.161	0.091	ND	ND	1.533	49±8.72	
MEAN	0.127	0.118	0.173	0.120	0.290	0.126	0.062	0.088	0.169	0.169	0.022	0.058	1.522	36.45±5.36	
STDEV	0.053	0.07	0.069	0.100	0.090	0.560	0.032	0.047	0.082	0.058	0.040	0.090	0.346	33.89±7.3	
CV (%)#	41.800	59.53	39.730	83.790	30.990	44.940	51.530	53.360	48.66	34.22	184.13	153.77	227.33	92.979	
r _(mite)	-0.477	0.190	-0.302	-0.249	0.650*	-0.161	0.561	-0.243	-0.265	-0.449	-0.248	-0.550	-0.261	-	
r ² _(mite)	0.228	0.030	0.091	0.062	0.428	0.026	0.315	0.059	0.070	0.202	0.062	0.303	0.068	-	

ND = not detected, a value of 0.000 has been used in the statistical calculations

*, significant at p≤0.05

As $\frac{STDEV}{Mean} * 100$

TABLE 3: Changes and relationships in clonal overhead mono and homo terpenes and red crevice mite levels.

CLONE	Mites														Mites
	p-Mentha-1,3,8-triene	Sabinene	β -Phellandrene	Limonene	(Z)- β -Ocimene	(E)- β -Ocimene	Linalool oxide (Cis) Furanoid	Linalool oxide (trans) Furanoid	Linalool	Terpinen-4-ol	Geraniol	4,8-Dimethyl-1,3(E),7-nontriene	Jasnone	Sum of Mono and homo terpenes	
TRFK 6/8	0.194	0.066	0.4	0.097	0.548	0.884	0.100	0.047	0.377	0.103	0.100	0.154	0.009	3.079	8 \pm 1.53
TRFK 18/3	0.139	ND	ND	0.113	0.312	0.75	0.054	0.018	0.563	0.159	ND	0.109	ND	2.217	36 \pm 4.58
TRFK 31/8	0.153	0.047	0.308	0.165	0.474	0.741	0.114	0.037	0.412	0.233	0.009	0.138	0.004	2.835	15 \pm 3.79
TRFK 54/40	0.238	0.035	0.467	0.104	0.586	1.186	0.106	0.043	0.719	0.181	ND	0.181	0.013	3.859	112 \pm 13.11
TRIT 201/16	0.001	ND	ND	0.093	0.361	0.99	0.094	0.026	0.552	0.132	ND	0.137	0.00	2.386	74 \pm 23.26
TRIT 201/50	0.088	0.071	0.393	0.136	0.439	0.754	0.107	0.046	0.258	0.041	0.025	0.127	0.013	2.498	9 \pm 2.0
TRFK 301/4	0.073	0.052	0.327	0.122	0.44	0.875	0.102	0.025	0.316	0.07	0.087	0.112	ND	2.601	9 \pm 2.0
TRFK 303/1199	0.065	0.082	0.433	0.125	0.502	0.765	0.1	0.037	0.304	0.103	0.018	0.113	0.047	2.694	13 \pm 0.58
S15/10	0.054	ND	ND	0.039	0.346	0.658	0.053	0.024	0.602	0.072	0.07	0.124	ND	2.042	59 \pm 15.89
TRFCA SFS150	0.114	ND	ND	0.134	0.388	0.91	0.089	0.028	0.615	0.124	ND	0.168	ND	2.57	17 \pm 3.46
STC 5/3	0.182	0.035	0.205	0.178	0.398	0.903	0.111	0.048	0.468	0.185	0.005	0.179	0.011	2.908	49 \pm 8.72
Mean	0.118	0.035	0.230	0.119	0.856	0.094	0.034	0.471	0.187	0.029	0.140	0.009	0.088	2.699	36.45 \pm 5.36
STDEV	0.070	0.031	0.195	0.037	0.147	0.021	0.011	0.150	0.066	0.038	0.026	0.014	1.09	0.497	33.89 \pm 7.3
CV (%)#	59.530	88.67	84.72	31.46	17.18	22.52	31.23	31.73	35.23	133.42	18.85	157.86	23.62	184.14	92.979
r _(mite)	0.190	-0.481	-0.152	-0.356	0.045	0.626*	-0.141	-0.032	0.785**	0.342	-0.378	0.442	-0.145	0.369	-
r ² _(mite)	0.036	0.231	0.023	0.127	0.002	0.392	0.020	0.001	0.616	0.117	0.143	0.195	0.021	0.136	-

ND = not detected, a value of 0.000 has been used in the statistical calculations

*, ** significant at p \leq 0.05 and 0.01, respectively

As $\frac{STDEV}{Mean} * 100$

TABLE 4: The variations and relationship between sesquiterpenes released by 11 tea varieties and red crevice mite infestations levels.

CLONE	Sesquiterpenes											Red crevice mite
	α -copaene	α -cedrene	(E)- β -Caryophyllene	(E)- β -Farnesene	(E)- γ -Muurolene	Humulene	Germacrene D	Calamene	Nerolidol	Cedrol	Sum of sesquiterpenes	
TRFK 6/8	0.225	0.798	0.568	0.955	ND	0.034	0.121	0.323	0.116	1.968	5.108	8 \pm 1.53
TRFK 18/3	ND	0.809	0.346	1.183	ND	0.124	0.233	ND	0.193	1.844	4.372	36 \pm 4.58
TRFK 31/8	0.210	0.844	0.546	0.735	ND	0.255	0.147	ND	0.126	2.041	4.904	15 \pm 3.79
TRFK 54/40	0.151	1.269	0.655	0.066	ND	0.341	0.277	0.047	0.320	3.384	7.510	112 \pm 13.11
TRIT 201/16	ND	0.588	0.416	1.051	ND	0.112	0.271	ND	0.240	1.315	3.993	74 \pm 23.26
TRIT 201/50	0.284	0.474	0.479	0.578	0.036	0.377	ND	ND	0.067	1.347	3.642	9 \pm 2.00
TRFK 301/4	ND	0.553	0.514	0.730	ND	0.199	ND	0.223	ND	1.501	3.720	9 \pm 2.00
TRFK 303/1199	0.193	0.573	0.503	0.670	ND	0.304	ND	ND	0.105	1.654	4.002	13 \pm 0.58
AHP S15/10	ND	0.564	0.359	1.043	ND	0.132	0.229	ND	ND	1.581	3.908	59 \pm 15.89
TRFCA SFS150	ND	0.755	0.299	0.632	ND	0.132	0.229	ND	0.287	2.375	4.709	17 \pm 3.46
STC 5/3	0.062	0.750	0.681	0.934	0.019	0.259	0.217	ND	0.113	1.908	4.943	49 \pm 8.72
Mean	0.102	0.725	0.488	0.871	0.005	0.206	0.157	0.054	0.142	1.902	4.619	36.45 \pm 5.36
STDE	0.111	0.220	0.124	0.207	0.012	0.109	0.111	0.111	0.107	0.585	1.091	33.89 \pm 7.3
CV(%)#	108.99	30.38	25.39	23.82	236.50	52.75	70.62	206.68	74.93	30.77	23.62	92.979
$r_{(mite)}$	-0.292	0.570	0.210	-0.266	-0.187	0.114	0.742**	-0.311	0.515	0.505	0.534	
$r^2_{(mite)}$	0.086	0.326	0.044	0.071	0.035	0.013	0.550	0.097	0.265	0.258	0.285	

ND = not detected, a value of 0.000 has been used in the statistical calculations

** significant at $p \leq 0.01$

As $\frac{STDEV}{Mean} * 100$

indicator for selection for resistance/susceptibility to red crevice mite infestations.

The monoterpenes (Table 3) were produced in the largest number compared to the other groups of compounds. The predominant monoterpenes were (*Z*)- β -ocimene, (*E*)- β -ocimene, and linalool. The relationship between the mono and homo terpenes levels and red crevice mite infestations were, however, variable. Large differences in the terpenoid compounds emissions had been reported among 15 susceptible and resistant mango cultivars (57). Similar variations were observed in the 11 cultivars. The low emission of mono terpenoids by susceptible tea clones compared to those that were resistant was in agreement with findings amongst 6 peach cultivars (58). The responses of the red crevice mites to the mono terpenes varied. Whereas high levels of sabinene, β -phellandrene, limonene, linalool oxides (cis and trans furanoid) geraniol and jasmone reduced the red crevice mite infestations, there was increase in the infestation with increase in p-mentha-1,3,8-triene, (*Z*)- β -ocimene, (*E*)- β -ocimene, linalool, terpine-4-ol and 4,8-dimethyl-1,3(*Z*),7-nonatriene. However, only (*E*)- β -ocimene ($r=0.626$, $p\leq 0.05$) and linalool ($r=0.785$, $p\leq 0.01$) levels were significantly correlated with red crevice mite levels. In chilli (59) and tomato (60) high levels of monoterpenes repelled insects. The repellency was attributed to the mono terpenes being toxins and feeding deterrents (61) that interfered with acetyl cholinesterase enzyme activity in insects (62). The dominance of the monoterpenes complex with OVOCs that repel the red crevice mites could therefore be selection criteria for selecting red crevice mite resistant tea cultivars. The significant attraction of red crevice mites by (*E*)- β -ocimene and linalool were similar to previous observations. (*E*)- β -ocimene was attractant to insects in wheat (63) and oats (64). Contrary to results in this study, linalool was repellent to insects (65, 66). However, linalool has also been reported as a male pheromone attractant to bee *Colletes cunicularius* (67) and aphid (14). Thus, the ability of linalool to attract or repel insects maybe species dependent. The levels of (*E*)- β -ocimene and linalool were elevated in most of the clones that were susceptible to red crevice mites (TRFK 18/3, TRFK 54/40, TRIT 201/16, AHP S15/10 and TRFCA SFS150) compared to those with low red crevice mites levels (TRFK 6/8, TRFK 301/4, TRFK 303/1199 and TRIT 201/50). Clones TRFK 6/8, TRFK 301/4,

TRFK 303/1199 and TRIT 201/50 released the highest amounts of total monoterpenes and had low levels of red crevice mites while clones TRFK 18/3, TRFK 54/40, TRIT 201/16, AHP S15/10, TRFCA SFS150 and STC 5/3 released lower total monoterpenes levels and had high red crevice mites levels. Although clone TRFK 54/40 emitted high amount of monoterpenes, it still had high levels of red crevice mites. Overall, the total mono terpenes correlated directly with red crevice mite infestations levels although the relationship was not significant.

The levels of the overhead volatile sesquiterpenes emitted are presented in Table 4. High number of sesquiterpenes observed in this study are in agreement with earlier findings on African grass (26) and other plants (68). The levels ranged from below detection limit to 1.90. Cedrol, α -cedrene, and (*E*)- β -farnesene were released in large quantities especially by susceptible clones (TRFK 54/40, TRFK 18/3, TRIT 201/16 and AHP S15/10). In contrast (*E*)- β -caryophyllene was released in large amounts by all resistant clones (TRFK 6/8, TRIT 201/50, TRFK 301/4 and 303/1199) and small amounts by most susceptible clones. There were mixed responses of the red crevice mites to the sesquiterpenes. Whereas red crevice mite infestation levels declined with increase in α -copaene, (*E*)- β -farnesene, (*E*)- γ -muurolene and calamenene, the infestation levels increased with increases in α -cedrene, (*E*)- β -caryophellene, humulene, germacrene D, nerolidol and cedrol amounts. The relationship was significant for germacrene D ($r=0.742$, $p\leq 0.01$). Overall, total levels of sesquiterpenes directly correlated ($r=0.534$) with red crevice mite infestation levels. However, the relationship was insignificant. The result concurred with increased attraction of heliothine moths by germacrene D (69, 70) and the terpene is a useful marker for insect attraction (71). Indeed sesquiterpenes have been associated with attraction of insects to plants (72), although some sesquiterpenes are repellents (73). Sesquiterpenes not only defend plants against pest attack by attracting natural enemies but also possess repellency and toxicity properties (74).

Generally (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*E*)- β -farnesene, cedrol, α -cedrene, (*E*)- β -caryophyllene, linalool, (*E*)- β -ocimene, (*Z*)- β -ocimene were released in high but different amounts by the different tea cultivars. Cultivars that released high amounts of (*E*)-hexenal, (*Z*)-3-hexenal, linalool, germacrene D, (*Z*)-3-

hexenol, (*Z*)-3-hexenyl acetate, 1-penten-3-ol, hexanal and (*E*)- β -ocimene were susceptible to red crevice mite infestations. On the other hand, high levels of total GLVs increased while that of aromatic compounds reduced red crevice mite infestations. These VOCs had been identified as volatile semiochemicals involved in plant defence against insect pests (22). Identifying the variations of these defence VOCs can form the basis of breeding and clonal selection programmes.

In previous studies, the ratio of linalool to linalool plus geraniol (terpene index) in macerated tea leaves was shown to be cultivar specific (75, 76). The ratios were confirmed to be maintained in processed black tea (77, 78) in which clones TRFK 6/8 and AHP S15/10 were shown to have low terpene indices. Although these cultivars expressed high linalool levels in the OVOCs composition, the levels of geraniol were very low. The results demonstrate that there are volatile compounds in tea leaves which are not easily released to the atmosphere. The released volatile compounds could be responses to environmental stress and are released as a mechanism of responding or overcoming such stresses. Only necessary volatiles are released for these purposes.

Conclusions

Cultivars TRIT 201/50, TRFK 301/4, TRFK 303/1199 and TRFK 6/8 were resistant to *Brevipalpus phoenicis* while TRFK 54/40, TRFK 18/3, TRIT 201/16, AHP S15/10, and STC 5/3 were susceptible. Susceptibility of the cultivars was correlated ($p \leq 0.05$) with the quantities of the GLVs released. In contrast, resistance was determined by the low amounts of GLVs and the amount of both terpenes and aromatics the cultivar emitted. This implies that breeding efforts should be focused on cultivars that produce low levels of GLVs and high levels of both terpenes and aromatics in order to develop tea cultivars that resist *B. phoenicis* attack.

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