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RESEARCH ARTICLE

Repellence and fumigant toxicity of essential oils of *Ocimum gratissimum* and *Ocimum kilimandscharicum* on *Tuta absoluta* (Lepidoptera: Gelechiidae)

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Abstract

Tuta absoluta Meyrick originates in South America and is now one of the most important insect pests of Solanaceae in different parts of the world, including Africa. Its control has relied primarily on chemical insecticides, which are associated with negative ecological effects. In the present study, essential oils of *Ocimum gratissimum* and *O. kilimandscharicum* were tested for repellence and fumigant toxicity on the adult stages under laboratory conditions. The oil of *O. gratissimum* was more repellent, but its toxicity was comparable with that of *O. kilimandscharicum*. The major constituents of *O. gratissimum* were methyl eugenol (39.5%) and eugenol (29.7%). Those of *O. kilimandscharicum* were camphor (47.1%) and 1.8-cineole (19.3%). Eugenol (LC_{50} of 0.24 μl/ml, 83.3%, $RI_{50} = 0.15$) and camphor (LC_{50} of 0.23 μl/ml, 89.5%, $RI_{50} = 0.13$) were more toxic (at 1 μl/ml for 24 h) and repellent than the other constituents. The results show potential of the essential oils for use in integrated management of the tomato pest.

Keywords Tomato leaf-miner · Essential oil · *Ocimum* species · Botanical insecticides · Repellence · Fumigant toxicity

Introduction

The tomato borer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), is an oligophagous and very harmful leaf-mining moth which feeds on Solanaceae crops, particularly tomato (*Lycopersicon esculentum* Mill.), but also on eggplant (*Solanum melongena* L.), potato (*Solanum tuberosum* L.), common bean (*Phaseolus vulgaris* L.), sweet pepper (*Solanum muricatum* L.) and *Datura* spp. (Garcia and Espul 1982). The moth was first known as a plant pest in many South American countries (Korycinska and Moran 2009). Now, it has spread rapidly throughout Afro-Eurasia and Middle Eastern countries (Sylla et al. 2017; Xian 2017; Biondi et al. 2018) and is considered as a global economic pest on tomato and other Solanaceae plants (Desneux et al. 2011). This high speed of colonisation is associated with the ability of the pest to adapt to varying climatic conditions and its high biotic potential. Each adult female of *T. absoluta* may

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lay 200 to 300 creamy coloured eggs, and 10 to 12 generations can be produced per year. After hatching, the larvae (the most destructive stage) can penetrate different parts of the whole plant, but prefer apical buds, tender new leaflets, flowers and green and ripe fruits. These larvae feed on mesophyll tissue of leaves creating mines, which affect the photosynthetic capacity of the plant. They can also form extensive galleries in the stems and fruits, which can provide entry points for secondary pathogens (Desneux et al. 2010). The pest damages tomato and other Solanaceae crops which may cause significant production losses ranging from 50 to 100% (Biratu 2018).

Control of *T. absoluta* is traditionally based on spraying crop plants with synthetic insecticides, including organophosphates, pyrethroids, thiocarbamates, diamides and acylurea growth regulators (Abbes and Chermiti 2012; Roditakis et al. 2015). However, these have low to moderate efficiency due to the high biotic potential and cryptic nature of the larvae of *T. absoluta* (Lietti et al. 2005; Haddi et al. 2012). The high reproduction of tomato borer leads to increase in spray frequency per crop cycle, which accelerates the evolution of resistance to the insecticides (Biondi et al. 2018; Roditakis et al. 2015). Moreover, these increasing and indiscriminate applications of synthetic pesticides have several environmental drawbacks and negative impact to natural biological control and disrupt pollination processes (Desneux et al. 2007).

Thus, there is an urgent need of finding new eco-friendly tools for the control of *T. absoluta*. Botanical-based insecticides have long been considered as attractive alternatives to synthetic chemicals for pest management (Zhu et al. 2008). These products are less harmful to the natural environment and human health (Isman 2006). Pyrethrum and neem are well established commercially and pesticides based on plant essential oils have recently entered the marketplace (Isman 2019). Insecticides based on essential oils and its constituents have been proved effective against many stored-grain insect pests. These have been formulated and applied variously as repellant, antifeedants, growth inhibitors, oviposition inhibitors and ovicides (Said-Al Ahl et al. 2017).

Tropical and subtropical plants such as *Ocimum* spp. belonging to the Lamiaceae family are recognised sources of bioactive essential oils (Clemente et al. 2003; Umerie et al. 1998). Essential oils of *Ocimum* spp. have been found to possess bio-pesticidal efficacy such as fungicidal, adulticidal, larvicidal, nematicidal, antihelminthic, ovicidal, oviposition deterrence, repellency, acaricidal, antileishmanial, trypanocidal and antimalarial (Bekele and Hassanali 2001; Ntonga et al. 2014; Chowdhary et al. 2018; Benelli et al. 2019). Furthermore, the essential oil of *O. gratissimum* and its constituents have demonstrated fumigant toxicity against many adults of insects (Kéita et al. 2001; Ogendo et al. 2008). Recently, researchers reported the efficacy of botanical plants against the tomato borer such as the essential oils of *Shirazi thyme* (Chegini et al. 2018) and *Citrus peel* (Campolo et al. 2017), and the oviposition deterrent activities of two basil plants (Yarou et al. 2017). In sensitive environments or enclosed areas like food processing, storage facilities and greenhouses, fumigant method is ideal to limit the residue problem, get to those hard to reach areas where pest may be hiding and acted in all life stage of the insect. For example, fumigant toxicity of cinnamaldehyde obtained from *Cinnamomum verum* J. Presl essential oil has shown potential activity against adults of *Sitophilus oryzae* L. (Lee et al. 2008). However, knowledge remains limited on how the essential oils of these aromatic plants (*O. gratissimum* and *O. kilimandscharicum*, and some of their constituents) affect the *T. absoluta* moths.

Mostly constituted with terpenoid, the essential oils of the two chosen species have shown different effects on varied insects (Cruz et al. 2017; Lima et al. 2018; Benelli et al. 2019) and post-harvest pest (Nguemtchouin et al. 2013; Bekele and Hassanali 2001). Given the use of these plants, no reports on their potential fumigant toxicity and repellency effects on *T. absoluta* have been reported so far. Thus, the paper present information on the repellent effects and fumigant toxicity of essential oil concentration of two ethnobotanical *Ocimum* plants (*O. gratissimum* L. and *O. kilimandscharicum* Gürke) and some of their constituents against *T. absoluta*.

Materials and methods

Plant materials

The aerial parts of *O. gratissimum* L. and *O. kilimandscharicum* Gürke (Lamiaceae) were collected in Naivasha (0°43′S/36°26′E) and Njoro (0°11′S/35°30′E), respectively, near Egerton University, Nakuru County, Kenya, in December 2017. The plants were identified and authenticated at the Department of Botany, University of Nairobi, where voucher specimens were deposited: EEFR-2017/1 and EEFR-2017/2 for *O. gratissimum* and *O. kilimandscharicum*, respectively. The plant materials were dried under shade for 1 week before extraction.

Insects

The insects used for all bioassays originated from a colony reared at the International Centre of Insect Physiology and Ecology (ICIPE), Duduville Campus, Nairobi, Kenya. The original population from pupae and larvae of *T. absoluta* was collected in October 2016 from tomato plants (*Lycopersicon esculentum*) in the field in Meru County, Eastern Kenya, without any history of exposure to pesticides. The insects collected were initially maintained under quarantine to identify any parasitized individuals before the establishment of the colony, which was maintained at 26 ± 2 °C, relative humidity ranging from 60 to 70% and photoperiod of

16/8 (light:day). In the rearing cages, the sex ratio was 2:1 and insects were randomly sampled.

Isolation of essential oils and preparation of different concentrations for bioassays

Each dried plant material (1000 g) was steam-distilled using a Clevenger apparatus for over 4 h. The oils obtained were separated from water, dried using anhydrous sodium sulphate and weighed. The oils were stored in closed amber-coloured vials at 4 °C in the dark until used. For bioassays, 10 μl of each essential oil was dissolved in 5 ml of acetone and successively diluted with acetone to give 0.031, 0.063, 0.125, 0.25, 0.5 and 1 μl of the oil in 1 ml of the solution.

Analyses of the essential oils

The two essential oils were analysed using a gas chromatograph (GC) (HP-7890A, Agilent Technologies, Wilmington, USA) linked to mass spectrometer (MS) operated in the electron impact mode (HP 5975 C, Agilent, Wilmington, USA). The apparatus was equipped with a non-polar HP-5MS capillary column (30 m × 0.25 mm i.d.; 0.25-μm film thickness, with 5% phenyl methyl silicone as the stationary phase; J & W Scientific, Folsom, USA). Helium (1.2 ml min^{-1}) was used as the carrier gas. The oven temperature was programmed at 35 °C (for 5 min) to 280 °C at 10 °C min^{-1} and then held isothermally at 280 °C for 10.5 min. An aliquot of 1 μl of each oil (100 mg of each sample was dissolved in 10 ml of dichloromethane) was injected in the splitless mode (column effluent was split 1:1 for simultaneous detection). The ion source temperature was 230 °C; electron ionisation mass spectra were acquired at 70 eV within a mass range of 38–550 Da (Da) during a scan time of 0.73 scans s^{-1}. Compounds were identified using ChemStation software (Agilent) by comparison of mass spectral data of their retention time with library data: Adams (Adams 2017) and NIST 05 (NIST 2008). Identities of some constituents were confirmed by co-injections with commercially available authentic standards. Quantification was based on calibration curves (peak area vs. concentration) generated from authentic standards of identified compounds and also by flame ionisation gas chromatography (CG/FID), under the same conditions as in the GC/MS analysis.

Standards of constituents of essential oils that were sourced

Authentic standards of eugenol (purity 99%), *E*-caryophyllene (purity 97%), 1,8-cineole (purity 99%), (1S) (−)-β-pinene (purity 98%), L-(−)-fenchone (purity 98%), α-terpineol (purity 97%) and (+)-limonene (purity 97%) were purchased from Acros Organics, NJ, USA. α-Pinene (purity 98%) and methyl eugenol (purity 98%) were purchased from Sigma-Aldrich, Steinheim, Germany. Acetone (GC analytical grade 97%) was purchased from Sigma-Aldrich Chemical, Milwaukee, WI, USA. Camphor (purity 97%) and (−)-camphene (purity 97%) were purchased from abcr GmbH, Karlsruhe, Germany.

Repellence assays on adults

Repellence of *O. gratissimum* and *O. kilimandscharicum* oils

The repellent effect of the essential oils and some of their constituents on adult tomato borer was evaluated in a two-choice cuboidal plexi-glass wind tunnel (25 × 25 × 200 cm), which was a slight modification of the one described by Gikonyo et al. (2003) (Fig. 1). The wind tunnel had three square windows (5 cm × 5 cm) on the top side; two rear ones were for introducing sample dispensers while the middle one, which divided the tunnel symmetrically into the left and right arms, was for introducing insect release cages. Each side of the tunnel had a tube (20 cm long and 3.3 cm internal diameter) fixed with a white plexi-glass gauze on the inner side, which prevented the moths from getting out of the tunnel. These tubes were connected to an air cylinder by rubber tubing via air flow metres (air was cleaned with activated charcoal). The middle window was covered with a white plexi-glass wire mesh to facilitate air flow from both arms. Light was supplied from fluorescent tubes suspended 2 m above the tunnel giving about 1000 lx incident light. A white sheet of paper marked with black stripes 5 cm apart was fixed on the wooden frame of the tunnel to allow correct recording of fly distances during chemical triggering. The system was tested with clean air before experiment. All assays were performed with twelve adults (more than 3-day-old males and females, which were randomly sampled from the rearing population) of tomato leaf-miner in a laboratory kept at 25 ± 2 °C. One millilitre of each concentration of each essential oil (containing 0.031, 0.063, 0.125, 0.25, 0.5 and 1 μl of the oil, prepared as outlined in "Isolation of essential oils and preparation of different concentrations for bioassays") was dispensed from a piece of clean cotton wool held in a rolled copper wire at the bottom of one of the plexi-glass rods attached to the lids covering the rear window. The other rod carried cotton wool treated with 1 ml of acetone, which served as control. The rods were covered with pieces of clean aluminium foil, which were replaced after every assay. The rear windows were closed tightly and air was allowed to flow (25 cm/s) for 2 min before the moths were released. The upwind flight behaviour of the moths was observed, and after 30 min, the number of moths in the two sides (beyond 20 cm from the centre) of the tunnel was recorded. Moths that were located closer to the centre were not classified. After each assay, air was blown into the wind tunnel at 50 cm/s to

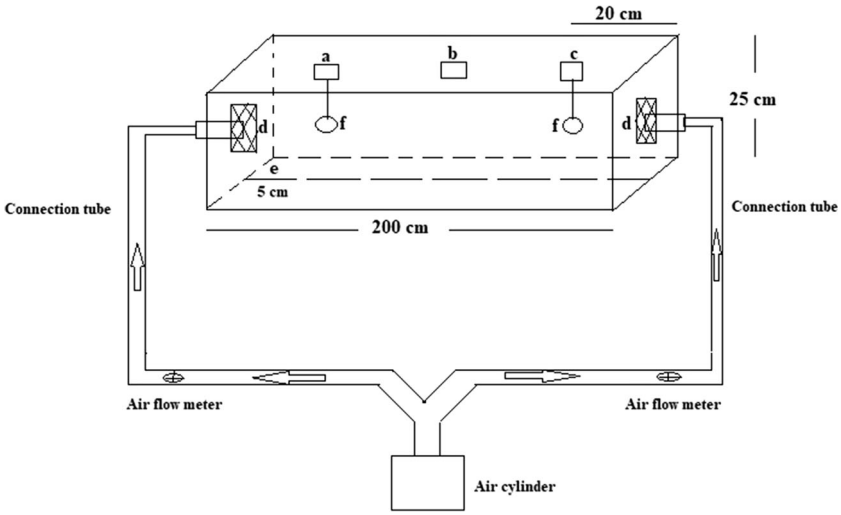

Fig. 1 Repellence tool. (a, c) Square windows for samples introduction. (b) Square window for insect introduction. (d) White plexi-glass gauze at the inner side to prevent moths from getting out to the tunnel. (e) White sheet of paper marked with black stripes of 5 cm to allow correct recording of fly distances. (f) Samples and control set; 20 cm: distance between the window side and the end of the tunnel; 25 cm: width of the tunnel; 200 cm: length of the tunnel

clear any residual odour. Four replicates were carried out for each concentrate, and the repellence index (RI) was calculated using the McDonald et al. (1970) formula, % (RI) = $[(N_c - N_t)/N_c + N_t] \times 100$.

Repellence of blends of available constituents and assessment of their relative contributions in subtractive assays

Synthetic blends of available constituents were prepared in relative amounts found in each essential oil, or with each component missing in the same oil. Each blend was tested following the experimental design outlined in "Repellence of *O. gratissimum* and *O. kilimandscharicum* oils" at the following concentrations: 0.031, 0.063, 0.125, 0.25, 0.5 and 1 μl ml^{-1}. Acetone alone was used as control. Each dose of each blend was tested in four replicates, and the repellence index of each blend was calculated.

Fumigant toxicity assays on adults

Effects of different concentrations of *O. gratissimum* and *O. kilimandscharicum* oils

The effects of exposing *T. absoluta* adults to 1 ml each of different concentrations (0.031, 0.063, 0.125, 0.25, 0.5 and 1 μl ml^{-1}) of *O. gratissimum* and *O. kilimandscharicum* oils, respectively, and some of their constituents, were studied in small cage chambers (20 × 20 × 35 cm) according to the method described by WHO (1996). The top window of the cage was covered with white plexi-glass wire gauze to facilitate the introduction of moths. Test materials

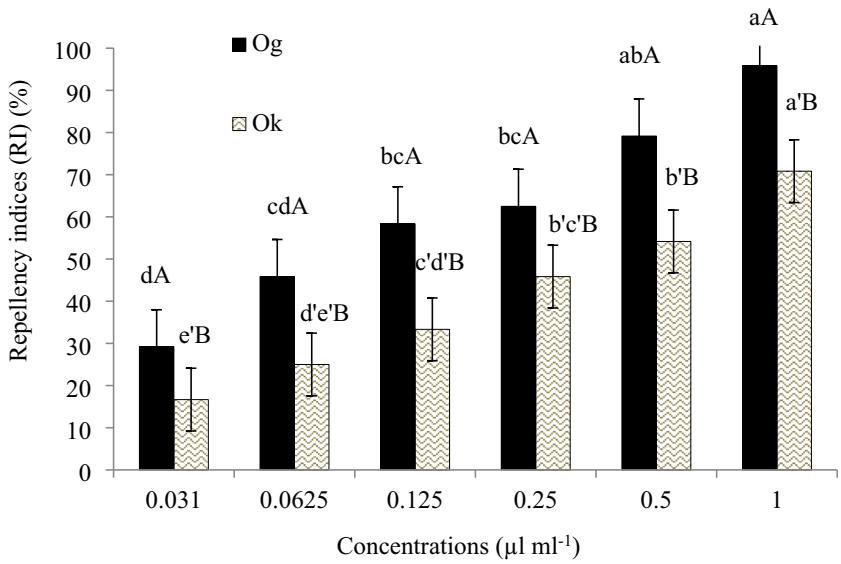

Fig. 2 Repellency indices (RI) (%) of *Ocimum gratissimum* (Og) ($df = 5$, $F = 69.28$, $P < 5$ 0.0001) and *Ocimum kilimandscharicum* (Ok) ($df = 5$, $F = 92.62$, $P < 0.0001$) essential oils on 6 adults of *Tuta absoluta*. For each oil, means at different concentrations marked by same lowercase letter(s) are not significantly different; and for each concentration of the two oils, means marked by the same uppercase letters are not significant

Table 1 Fumigant toxicity of essential oils of *Ocimum gratissimum* and *Ocimum kilimandscharicum* on *Tuta absoluta* adults after 24 h of exposure

Concentrations (μl ml^{-1})	Mortality (%), mean ± SEM	
	O. gratissimum	*O. kilimandscharicum*
Control	00^{d}	00^{d}
0.031	12.50 ± 15.96^{cA}	12.50 ± 8.33^{cA}
0.063	37.50 ± 8.33^{bA}	25.00 ± 9.62^{bcA}
0.125	41.66 ± 9.62^{bA}	29.17 ± 8.33^{bcA}
0.25	50.00 ± 13.61^{bA}	41.66 ± 9.62^{bA}
0.5	83.33 ± 13.61^{aA}	75.00 ± 16.67^{aA}
1	95.83 ± 8.33^{aA}	87.50 ± 15.96^{aA}
LC_{50}	0.24 ± 0.04	0.43 ± 0.10
LC_{90}	0.66 ± 0.10	1.83 ± 0.48
df	5	5
Slope	1.52 ± 0.55	1.57 ± 0.48
F	64.56	78.58
P	< 0.0001	< 0.0001

Mean values followed by different letter(s) in the same column (lower-case) or in the same row (upper-case) differ significantly

SEM standard error of the mean

in acetone applied to Whatman filter paper (70 mm diameter) in the Petri dish (80 mm) acted as the sources of fumigants. These Petri dishes were covered with wire gauzes to prevent the leaf-miner to directly contact the filter papers. Control cages were similarly set with acetone only. Rolled filter papers (5 × 10 cm) dipped into honey served as sources of food for the insect. In each assay, six adult male and female moths (> 3-day-old) were released in both treated and control cages, and the number of live and dead insects was counted after 24 h. Each test was replicated four times.

Time-course effects of higher concentrations of *O. gratissimum* and *O. kilimandscharicum* oils

The time-course mortality of adult *T. absoluta* exposed to three concentrations of each essential oil (1 ml of 0.25, 0.5 and 1 μl ml^{-1}) was monitored after 6, 12, 18 and 24 h. The assays were carried out in the same setups as in "Effects of different concentrations of *O. gratissimum* and *O. kilimandscharicum* oils".

Fumigant toxicity of some constituents and assessment of contribution of each to synthetic blends in subtractive assays

Dose-response effects of some constituents were assessed. Also, synthetic blends of available constituents in each essential oil and blends with missing component were made. Each of these assays was setup as outlined in "Effects of different concentrations of *O. gratissimum* and *O. kilimandscharicum* oils" at the concentration of 0.031, 0.063, 0.125, 0.25, 0.5 and 1 μl ml^{-1}, each in four replicates.

Statistical analyses

Mortality was corrected using Abbott's formula and probit analysis was used to estimate lethal concentration (LC_{50} and LC_{90}), lethal time (LT_{50}) and the repellence index at 50% (RI_{50}). A time-course effect at different exposure times was estimated using GLM model and the Log rank test was performed using the Kaplan-Meier survival method to compare

Table 2 Percentage mortality following post-exposures of adults of *Tuta absoluta* to *Ocimum gratissimum* and *Ocimum kilimandscharicum* essential oils, respectively, for 6, 12, 18 and 24 h

Plants species	Concentrations (μl ml^{-1})	% Mortality ± SEM				LT_{50}	*P* value	Log rank (X^2)
		Exposure time (h)						
		6	12	18	24			
Ocimum gratissimum	Control	00^{a}	00^{a}	00^{a}	00^{a}	–		
	0.25	4.16 ± 0.01^{c}	16.67 ± 0.01^{b}	33.33 ± 6.29^{a}	50 ± 10.61^{a}	25.68 ± 1.33		
	0.5	12.5 ± 1.67^{c}	29.17 ± 8.33^{b}	50 ± 10.61^{b}	83.5 ± 8.33^{a}	17.63 ± 1.78	0.05	6.01
	1	16.67 ± 0.01^{c}	33.33 ± 6.29^{b}	54.25 ± 9.62^{b}	92.5 ± 8.33^{a}	15.49 ± 1.29		
Ocimum kilimandscharicum	Control	00^{a}	00^{a}	00^{a}	00^{a}	–		
	0.25	00^{c}	4.16 ± 0.01^{c}	16.67 ± 0.01^{b}	38.58 ± 9.62^{a}	27.16 ± 2.02		
	0.5	8.33 ± 1.92^{c}	25.5 ± 1.67^{b}	46.5 ± 1.67^{a}	67.58 ± 6.97^{a}	19.80 ± 1.03	0.01	8.91
	1	12.5 ± 1.67^{c}	29.17 ± 8.33^{bc}	54.25 ± 9.62^{ab}	83.5 ± 8.33^{a}	17.39 ± 1.84		

Means marked with different letter(s) within the same row are significantly different. *SEM* standard error of the mean

the surviving at any specific time for each group (Kleinbaum and Klein 2012). The mean numbers of repelled or dead insects were analysed using analysis of variance and compared by the Student-Newman-Kuels (SNK) test. Results giving P value < 0.05 were considered significantly different. All statistical analyses were implemented in a custom script R 3.3.2 software (R Core Team 2015).

Results

Essential oils of *O. gratissimum* and *O. kilimandscharicum*

Repellence on *T. absoluta* adults

Figure 2 depicts the repellence effects of the two essential oils on adult leaf-miner. Both oils were found to be repellent to *T. absoluta*, with *O. gratissimum* essential oil showing higher activity than that of *O. kilimandscharicum*. The repellence index at 50% was $RI_{50} = 0.13\%$, $F = 69.28$, $df = 5$ and $P < 0.0001$ for *O. gratissimum* oil, and $RI_{50} = 0.50\%$, $F = 92.62$, $df = 5$ and $P < 0.0001$ for *O. kilimandscharicum*.

Fumigant toxicity on *T. absoluta* adults

Effects of different concentrations of the oils

Although, *O. gratissimum* demonstrated somewhat higher fumigant toxicity compared with *O. kilimandscharicum* to *T. absoluta* at most concentrations following 24 h of exposures (Table 1), the differences were not statistically significant. The lethal concentrations were $LC_{50} = 0.24$ and $LC_{90} = 0.66$ for *O. gratissimum* oil, and $LC_{50} = 0.43$ and $LC_{90} = 1.83$ for *O. kilimandscharicum* oil.

Time-course effects of selected concentrations

Table 2 summarises mortality of the adults following exposures to higher concentrations of the two oils after 6, 12, 18 and 24 h. The results show not significant effect of exposure time for *O. gratissimum* oil (with $P = 0.05$, $LT_{50} = 15.49$) and significant effect for *O. kilimandscharicum* (with $P = 0.01$, $LT_{50} = 17.39$) at 1 μl ml^{-1}.

Compositions of *O. gratissimum* and *O. kilimandscharicum* essential oils

The yields of *O. gratissimum* and *O. kilimandscharicum* essential oils that were obtained were 0.3% and 0.15%, respectively. A total of 41 and 35 constituents were identified by GC-MS in the two oils, respectively. The more abundant constituents (more than 1%) of *O. gratissimum* oil were methyl eugenol (39.5%), eugenol (29.7%), epi-β-santalene (4.7%), (*E*)–caryophyllene (3.7%), neo-allo-ocimene (3.1%), linalool formate (1.6%), (*Z*)-β ocimene (1.5%), (1S)-(−)-β-pinene (1.1%) and 1,8-cineole (1.1%) (Fig. 3(A)). Those of *O. kilimandscharicum* included camphor (47.0%), 1,8-cineole (19.3%), (−)-camphene (5.2%), L-(−)-fenchone (4.9%), terpinene-4-ol (2.7%), myrtenol

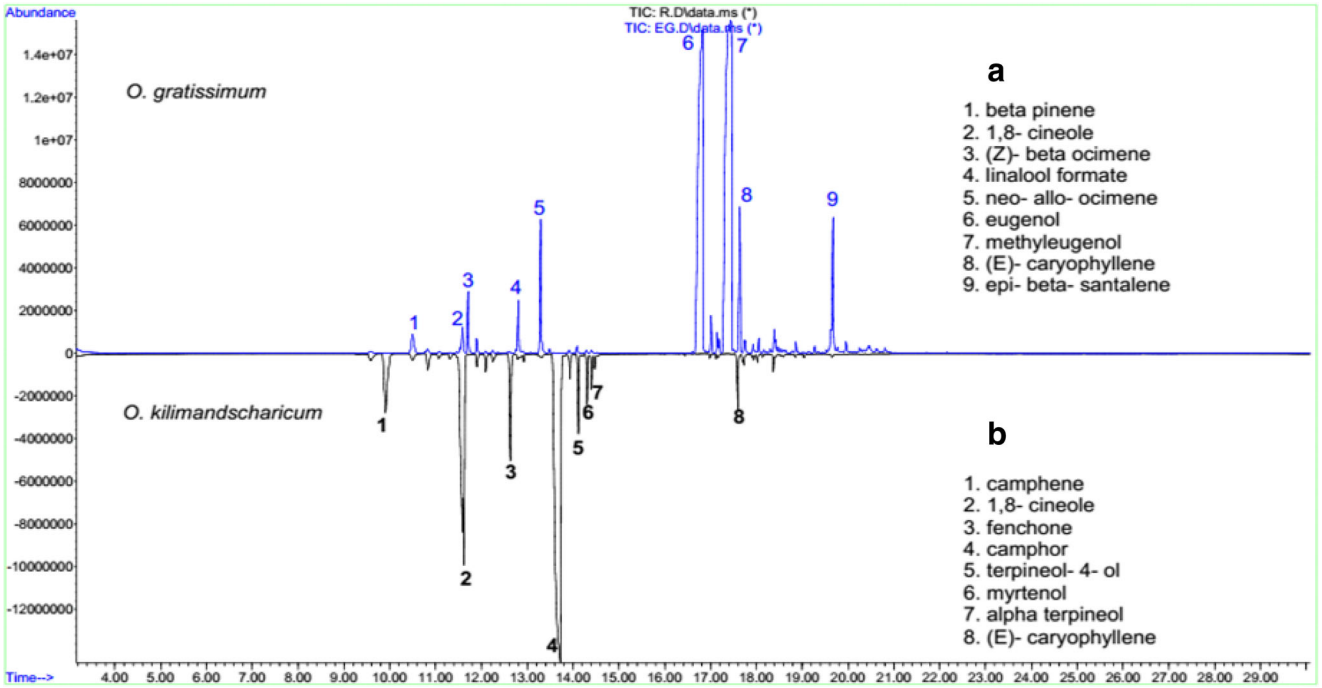

Fig. 3 GC-MS composition of *Ocimum gratissimum* (A) and *Ocimum kilimandscharicum* (B) with some assigned peaks

Table 3 Relative proportion of constituents in the pure oil of *O. gratissimum* and *O. kilimandscharicum*

Peak No.	*Ocimum gratissimum*				*Ocimum kilimandscharicum*			
	RI_c	RI_L	Compound names	Abundance (%)	RI_c	RI_L	Compound names	Abundance (%)
1	620	620	3-Hexyne	0.1	922	926	Tricyclene	0.1
2	800	780	2,6-Dimethyl-2,4,6-octatriene	0.1	936	931	α-Pinene	0.1
3	936	931	α-Pinene	0.6	945	946	Camphene	5.2
4	934	954	α-Hydroxy-benzeneacetonitrile	"-"	961	965	Benzaldehyde	"-"
5	973	972	β-Pinene	1.1	973	972	β-Pinene	"-"
6	981	982	Myrcene	0.2	981	982	Myrcene	1.0
7	990	976	Verbenene	0.1	1005	1002	α-Phellandrene	0.3
8	1011	1009	α-Terpinene	0.1	1011	1009	α-Terpinene	0.8
9	1030	1026	1,8-Cineole	1.0	1025	1023	Limonene	0.6
10	1033	1022	(*Z*)-β-Ocimene	1.4	1030	1026	1,8-Cineole	19.3
11	1041	1037	(*E*)-β-Ocimene	0.4	1041	1037	(*E*)-β-Ocimene	0.6
12	1051	1050	γ-Terpinene	0.1	1051	1050	γ-Terpinene	0.8
13	1061	1068	α-Methyl-α-(4-methyl-3-entenyl) oxiranemethanol	0.1	1056	1071	β-Terpinene	0.3
14	1078	1089	α-Terpinene	0.1	1083	1087	Fenchone	4.9
15	1099	1101	Linalool formate	1.6	1092	1096	*p*-Mentha-1(7),8-diene	0.9
16	1103	1104	4,8-Dimethyl-1,3-(*Z*)-7-nonatriene	0.1	1109	1112	6-Camphenol	0.5
17	1108	1110	1,3,8-*p*-Menthatriene	"-"	1144	1141	Camphor	47.1
18	1110	1115	Neo-allo-ocimene	3.1	1148	1150	Borneol	0.9
19	1118	1120	Allo-ocimene	0.2	1164	1162	Terpinen-4-ol	2.6
20	1132	1148	1-Methyl-1,4-cyclohexadiene	0.1	1174	1176	α-Terpineol	1.5
21	1164	1162	Terpinen-4-ol	0.2	1186	1188	Myrtenol	2.1
22	1264	1261	(*E*)-Anethole	0.3	1192	1198	Dehydro-sabina ketone	0.5
23	1323	1318	γ-Elemene	0.1	1346	1345	α-Cubebene	0.3
24	1330	1329	Eugenol	29.6	1359	1356	Eugenol	"-"
25	1373	1374	α-Copaene	0.8	1373	1374	α-Copaene	0.1
26	1386	1384	β-Bourbonene	0.8	1386	1384	β-Bourbonene	0.3
27	1411	1403	Methyl eugenol	39.5	1409	1417	(*E*)-Caryophyllene	1.9
28	1409	1417	(*E*)-Caryophyllene	3.7	1410	1422	7-epi-Sesquithujene	0.7
29	1411	1418	β-Cubebene	0.5	1451	1458	(*E*)-β-Farnesene	0.7
30	1448	1436	(*Z*)-Isoeugenol	0.3	1464	1470	*trans*-Muurola-4(14),5-diene	0.1
31	1455	1452	α-Humulene	0.3	1479	1474	Germacrene D	0.8
32	1469	1460	trans-Muurola-4(14),5-diene	0.1	1484	1490	Isoledene	0.3
33	1479	1474	Germacrene D	1.5	1506	1503	δ-Amorphene	0.2
34	1523	1516	*trans*-Cadina-1(6),4-diene	0.4	1532	1529	(*Z*)-α-Bisabolene	0.3
35	1548	1528	α-Calacorene	0.1	1595	1600	*trans*-Muurola-3,5-diene	0.1
36	1581	1582	Caryophyllene oxide	0.3	–		–	–
37	1589	1585	epi-β-Santalene	4.7	–		–	–
38	1646	1666	2,2,3-Trimethyl-bicyclo[2.2.1]heptane	0.5	–		–	–
39	1696	1680	Germacra-4(15),5,10(14)-trien-1-α-ol	1.0	–		–	–
40	1702	1699	14-Hydroxy-(*Z*)-caryophyllene	0.3	–		–	–
41	1717	1725	Amorpha-4,9-dien-2-ol	0.5	–		–	–
Total			96%	95.9%				
Monoterpene hydrocarbons			8.2%	10.7%				
Oxygenated monoterpene			3.1%	78.9%				
Sesquiterpenes			13%	5.8%				
Oxygenated sesquiterpenes			2.1%	–				
Phenylpropanoids			69.4%	–				
Others			0.2%	0.5%				

RI_c retention index calculated, RI_L retention index from literature

"-": value < 0.1%

(2.1%) and α terpineol (1.5%) (Fig. 3(B)). The identified constituents of the two oils are listed in Table 3.

Some commercially available constituents (methyl eugenol, eugenol, *E*-caryophyllene, 1,8-cineole, (1S)-(−)-β-pinene and α-pinene of *O. gratissimum* oil, and (−)-camphene, (%), L-(−) fenchone, α-terpineol, 1,8-cineole, *E*-caryophyllene and limonene of *O. kilimandscharicum*) were purchased and tested on *T. absoluta*. These compounds were prepared separately and also blended to obtain a mixture in relative amounts found in each oil.

Table 4 Repellency indices (RI) (%) of six constituents of *O. gratissimum* against *T. absoluta* adults

Constituents	Concentrations (μl/ml)						RI_{50}
	0.031	0.063	0.125	0.25	0.50	1.00	
Methyl eugenol	25.0 ± 4.8^{aF}	31.8 ± 1.5^{aE}	44.6 ± 3.9^{aD}	61.7 ± 3.9^{aC}	74.2 ± 5.2^{aB}	95.8 ± 4.1^{bA}	0.20 ± 0.02
Eugenol	10.6 ± 3.9^{bcF}	33.3 ± 6.8^{aE}	48.8 ± 1.1^{aD}	62.5 ± 4.1^{aC}	78.7 ± 4.0^{aB}	100 ± 0.0^{aA}	0.15 ± 0.03
E-Caryophyllene	$9.0 \pm 0.0b^{cF}$	30.4 ± 3.1^{aE}	38.6 ± 6.5^{abD}	57.5 ± 4.4^{aC}	65.9 ± 0.7^{bB}	75 ± 4.8^{cA}	0.26 ± 0.05
1,8-Cineole	6.4 ± 4.0^{cE}	14.7 ± 1.8^{bD}	29.1 ± 4.1^{bC}	35.0 ± 4.5^{bC}	57.5 ± 4.4^{cB}	69.4 ± 5.4^{cA}	0.43 ± 0.03
β-Pinene	4.1 ± 4.1^{cD}	6.4 ± 4.0^{cD}	13.2 ± 5.7^{cCD}	25.0 ± 4.6^{cB}	54.1 ± 4.1^{cdA}	58.3 ± 4.8^{dA}	0.49 ± 0.02
α-Pinene	0 ± 0.0^{dF}	4.1 ± 4.1^{cE}	6.4 ± 4.0^{cD}	29.1 ± 4.1^{cC}	45.8 ± 4.1^{dB}	56.4 ± 5.1^{dA}	0.86 ± 0.05

Mean values followed by different letter(s) in the same column (lowercase) or in the same row (uppercase) differ significantly

Contribution of some constituents of *O. gratissimum* oil to fumigant toxicity and repellence

Repellence of six compounds of *O. gratissimum* essential oil

Table 4 summarises the individual repellence of six constituents *of O. gratissimum* oil after 30-min exposure. Significant differences ($P < 0.0001$) were found between the compounds, and the most repellent was eugenol with RI_{50} of 0.15.

Repellency of six-component blend and blends with each constituent subtracted

The effect of subtracting each of the six constituents on the repellence of the resulting blend is summarised in Table 5. Subtraction of eugenol (RI_{50} of 0.44) gave a blend with the lowest repellence at all concentrations. However, subtraction of *α*-pinene (RI_{50} of 0.07) showed a minimum reduction of repellence.

Fumigant toxicity of the six constituents

The dose-response fumigant toxicities of the six constituents are depicted in Fig. 4. The constituents showed varied level of mortality, with eugenol being most lethal (LC_{50} of 0.24; LC_{90} of 1.77 μl/ml and 83.3% at 1 μl/ml) followed by methyl eugenol (LC_{50} of 0.32; LC_{90} of 1.79 μl/ml and 79.1% at 1 μl/ml). The lowest mortality was recorded with *α*-pinene (LC_{50} of 1.17; LC_{90} of 3.09 μl/ml and 31.2% at 1 μl/ml). No mortality was observed in the control assay.

Effect of each constituent of the 6-component blend in subtractive fumigant toxicity bioassays

Effects of the synthetic blend of the six constituents of *O. gratissimum* (MOG) and those of five-component blends with each constituent subtracted are depicted in Fig. 5. A significant difference ($P < 0.0001$) was found between the treatments and the most lethal was the five-component blend with *α*-pinene missing (MOG-*α*-pinene) with LC_{50} of 0.19,

Table 5 Repellence indices (RI) (%) of synthetic blends of six constituents of *O. gratissimum* and those with each component subtracted on *T. absoluta* adults

Constituents	Concentrations (μl/ml)						RI_{50}
	0.031	0.063	0.125	0.25	0.50	1.00	
MOG	13.3 ± 2.2^{bF}	33.6 ± 3.5^{bE}	52.7 ± 4.1^{abD}	63.6 ± 0.0^{bC}	70 ± 3.3^{cB}	82.5 ± 0.4^{cA}	0.10 ± 0.02
MOG-Methyl eugenol	12.8 ± 2.1^{bF}	16.4 ± 2.5^{eE}	42.7 ± 1.5^{cD}	57.5 ± 4.4^{eC}	73.3 ± 3.7^{bcB}	79.8 ± 0.8^{dA}	0.24 ± 0.01
MOG-Eugenol	4.1 ± 4.1^{cF}	10.6 ± 3.7^{fE}	28.7 ± 1.5^{dD}	42.4 ± 3.1^{eC}	56.8 ± 3.8^{eB}	75.4 ± 1.6^{eA}	0.44 ± 0.05
MOG-*E*-Caryophyllene	13.3 ± 8.1^{abF}	32.9 ± 3.0^{bcE}	50.2 ± 4.5^{bD}	62.7 ± 0.9^{bC}	72.5 ± 4.3^{bcB}	81.2 ± 0.5^{cdA}	0.13 ± 0.05
MOG-1,8-Cineole	14.7 ± 1.7^{bF}	20.8 ± 4.1^{dE}	40.9 ± 4.5^{cD}	49.1 ± 3.6^{dC}	60 ± 0.0^{dB}	70.7 ± 6.5^{fA}	0.27 ± 0.01
MOG-*β*-Pinene	18.3 ± 2.5^{aF}	27.0 ± 2.1^{cE}	47.7 ± 4.3^{bcD}	62.7 ± 0.9^{bC}	78.7 ± 4.0^{bB}	95.8 ± 4.1^{bA}	0.22 ± 0.03
MOG-*α*-Pinene	19.6 ± 3.1^{aF}	41.6 ± 4.8^{aE}	57.7 ± 1.2^{aD}	70.8 ± 4.1^{aC}	87.5 ± 4.1^{aB}	100 ± 0.0^{aA}	0.07 ± 0.01

Mean values followed by different letter(s) in the same column (lowercase) or in the same row (uppercase) differ significantly. *MOG* blend of the six constituents of *O. gratissimum*

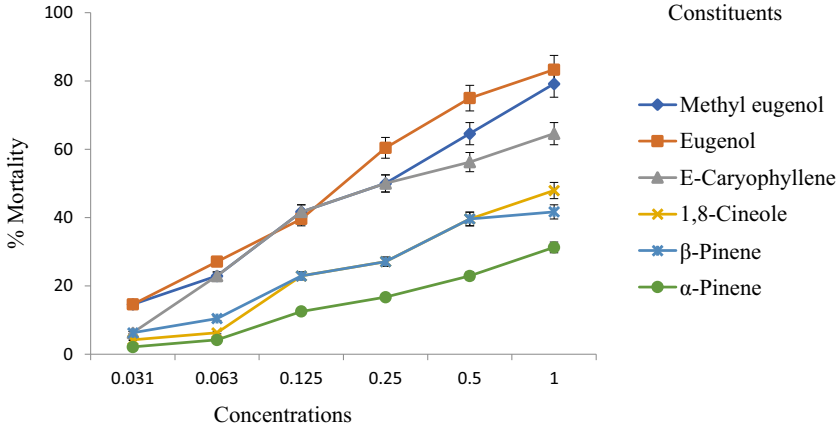

Fig. 4 Fumigant toxicities of six constituents of *O. gratissimum* after 24 h on *T. absoluta* adults

LC_{90} of 1.82 μl/ml and 77.0% at 1 μl/ml. Subtraction of methyl eugenol led to the highest drop in the activity of the resulting blend (LC_{50} of 0.68, LC_{90} of 2.89 μl/ml, 54.16% at 1 μl/ml). No mortality was observed in the control assay (acetone alone).

Contribution of seven constituents of *O. kilimandscharicum* oil to fumigant toxicity and repellence

Repellence effect of each of the seven constituents

The repellence effects of the seven purchased constituents of *O. kilimandscharicum* oil are outlined in Table 6. Camphor was the most repellent compound followed by camphene and 1,8-cineole with RI_{50} of 0.13, 0.17 and 0.19, respectively.

Repellence effect of seven-component blend and blends with each constituent subtracted

The synthetic blends prepared with that seven compounds of *O. kilimandscharicum* oil were found to have varying levels of repellence against *T. absoluta* (Table 7). The repellencies of different blends were highly significant ($P < 0.0001$) and the interaction between the constituents of the blends was also significant ($P = 0.002$). Subtraction of limonene (MOK-limonene) with 100% at 1 μl/ml (RI_{50} of 0.09) gave the six-component blend with minimum reduction in repellence. Subtraction of 1,8-cineole or camphor led to significant drops (78.3%, RI_{50} of 0.23 and 79.8%, RI_{50} of 0.21 at 1 μl/ml, respectively) in the repellence.

Fumigant toxicity of each constituent

Significant differences ($P < 0.0001$) were also found between camphor, 1,8-cineole, (−)-camphene, L-(−)-fenchone, *E*-caryophyllene, α-terpineol and limonene present in *O. kilimandscharicum* oil (Fig. 6). The most toxic constituent was camphor (LC_{50} of 0.23; LC_{90} of 1.69 μl/ml and 89.5%) followed by 1,8-cineole (LC_{50} of 0.26; LC_{90} of 1.99 μl/ml and 72.9%), with limonene (LC_{50} of 1.10; LC_{90} of 3.05 μl/ml and 33.3%) being least effective at 1 μl/ml.

Contribution of each constituent to fumigant toxicity in subtractive assays

Fumigant toxicities of the synthetic blend of the seven compounds in proportion found in *O. kilimandscharicum* oil

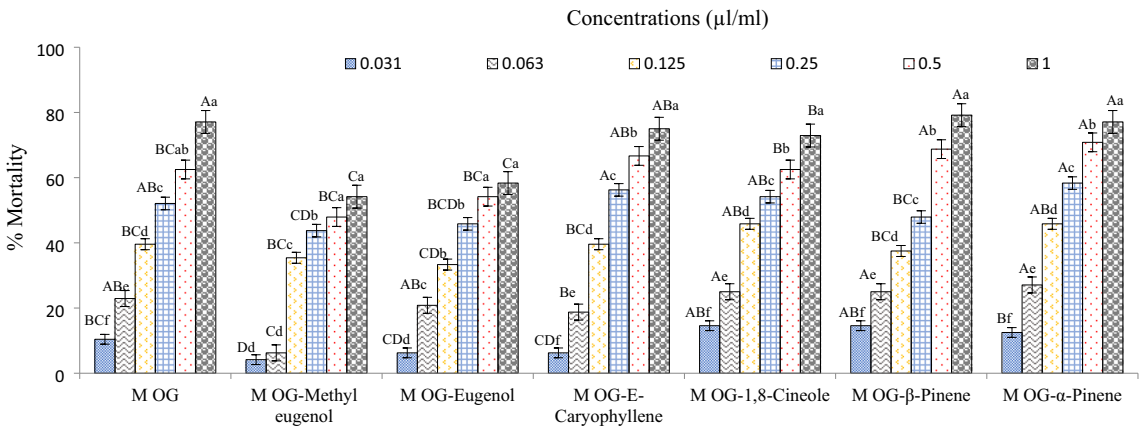

Fig. 5 Mean mortality (%) of a blend of 6 constituents of *O. gratissimum* oil and blends with each compound missing on *Tuta absoluta* adults after 24 h

Table 6 Repellency indices (RI) (%) of seven constituents of *O. kilimandscharicum* oil on *T. absoluta* adults

Constituents	Concentration (μl/ml)						RI_{50}
	0.031	0.063	0.125	0.25	0.50	1.00	
Camphor	31.8 ± 1.5^{aF}	40.5 ± 4.2^{aE}	48.8 ± 1.1^{aD}	70.0 ± 4.4^{aC}	91.2 ± 5.0^{aB}	100 ± 0.0^{aA}	0.13 ± 0.02
1,8-Cineole	11.8 ± 2.7^{bcF}	20.0 ± 4.4^{bE}	41.6 ± 4.8^{bcD}	58.3 ± 4.8^{bC}	76.5 ± 3.4^{bB}	84.4 ± 5.2^{cA}	0.19 ± 0.05
Camphene	12.8 ± 2.1^{bcE}	25.7 ± 5.6^{bD}	46.5 ± 1.1^{bC}	73.8 ± 5.1^{aB}	90.9 ± 5.2^{aA}	95.8 ± 4.1^{bA}	0.17 ± 0.03
Fenchone	4.5 ± 2.6^{dE}	10.9 ± 1.8^{cD}	31.8 ± 6.9^{cC}	51.8 ± 5.0^{cdB}	73.8 ± 5.1^{bA}	78.3 ± 3.9^{deA}	0.21 ± 0.02
E-Caryophyllene	4.5 ± 2.6^{dF}	10.9 ± 1.8^{cE}	26.1 ± 3.4^{dD}	37.8 ± 4.5^{eC}	55.0 ± 6.1^{dB}	71.8 ± 4.7^{eA}	0.45 ± 0.02
α-Terpineol	6.4 ± 4.0^{cdF}	21.9 ± 3.1^{bE}	36.8 ± 3.2^{cD}	53.1 ± 6.1^{bcC}	65.1 ± 0.8^{cB}	70.3 ± 3.9^{eA}	0.24 ± 0.01
Limonene	0 ± 0.0^{eE}	4.5 ± 2.6^{dD}	21.9 ± 3.1^{dC}	43.1 ± 6.7^{dB}	58.1 ± 4.3^{dA}	60 ± 0.0^{fA}	0.47 ± 0.01

Mean values followed by different letter(s) in the same column (lowercase) or in the same row (uppercase) differ significantly

(MOK) and blends with each constituent missing are summarised in Fig. 7. Significant difference was found between the blends ($P < 0.0001$). The most lethal blend was one without limonene (MOK-limonene) (LC_{50} of 0.22; LC_{90} of 1.76 μl/ml and 83.3%) and the less one was MOK (LC_{50} of 0.92; LC_{90} of 2.86 μl/ml and 58.3%) at 1 μl/ml. No effect was found in the control set.

Discussion

The results showed significant levels of repellence and fumigant toxicity of the essential oils of both *O. gratissimum* and *O. kilimandscharicum* to the adults of *T. absoluta*. The repellence and fumigant toxicity of some individual constituents that were assayed, and subtractive assays with synthetic blends of these, showed that the activities of both oils are due to additive/synergistic effects of different constituents. However, these assays also showed differential intrinsic activities of the constituents. The leaves' essential oils of the two oils were analysed using GC-MS method and methyl eugenol (39.5%), eugenol (29.7%) (for *O. gratissimum*), camphor (47.0%) and 1,8-cineole (19.3%) (for *O. kilimandscharicum*) were found to be the major constituents. Base on the GC profile, these oils belonged to methyl eugenol-eugenol and camphor-1,8-cineole chemotype, respectively. These major constituents were the most repellent and toxic, and the subtraction of them in the artificial blend leads to significant drop of effect which showed that they contributed most to the activity in both oils. *O. gratissimum* and *O. kilimandscharicum* plants growing in different agro-ecological areas have also been investigated by many researchers and variations associated with epigenetic, the different seasons and the extraction method may impact the composition of the oil and may significantly affect their activities (Beatovic et al. 2015; Hussain et al. 2007). In fact, a literature survey revealed that, in *O. gratissimum*, eugenol, thymol, citral, geraniol, ethyl cinnamate, linalool, linalool-methyl chavicol, methyl eugenol-eugenol and methyl cinnamate chemotype were found (Matasyoh et al. 2007; Asawalam et al. 2008; Faria et al. 2006; Vieira et al. 2001; Padalia et al. 2014). Likewise, in *O. kilimandscharicum*, camphor, linalool-camphor-1,8-cineole, camphor-limonene, camphor-1,8-cineole, 1,8-cineole, linalool, 1,8-cineole-eugenol, 1,8-cineole-methyl chavicol-

Table 7 Repellency indices (RI) (%) of subtractive synthetic blends of seven constituents of *O. kilimandscharicum* on *T. absoluta* adults

Constituents	Concentrations (μl/ml)						RI_{50}
	0.031	0.063	0.125	0.25	0.5	1	
MOK	8.3 ± 4.8^{cF}	16.6 ± 0.6^{dE}	31.8 ± 1.5^{dD}	45.4 ± 0.8^{dC}	63.6 ± 0.6^{eB}	79.4 ± 0.5^{dA}	0.30 ± 0.05
MOK-Camphor	4.5 ± 2.6^{dF}	20.0 ± 4.4^{cE}	40 ± 0.0^{cD}	55.9 ± 3.4^{cC}	67.8 ± 1.6^{dB}	79.8 ± 0.8^{dA}	0.21 ± 0.02
MOK-1,8-Cineole	2.7 ± 2.7^{dF}	10.7 ± 3.5^{eE}	28.5 ± 7.5^{dD}	55.5 ± 0.1^{cC}	69.4 ± 5.4^{dB}	78.3 ± 0.5^{dA}	0.23 ± 0.03
MOK-Camphene	8.3 ± 2.7^{cF}	23.6 ± 4.6^{bcE}	41.6 ± 4.8^{bcD}	59.7 ± 1.5^{bC}	70.5 ± 4.3^{cdB}	78.1 ± 1.1^{dA}	0.19 ± 0.02
MOK-Fenchone	16.6 ± 0.6^{aF}	20.8 ± 4.1^{cE}	41.6 ± 4.8^{bcD}	58.3 ± 4.8^{bcC}	75 ± 4.8^{bcB}	86.7 ± 4.4^{cA}	0.17 ± 0.01
MOK-*E*-Caryophyllene	16.6 ± 0.6^{aF}	25 ± 4.8^{bE}	45.8 ± 4.1^{bD}	58.3 ± 4.8^{bcC}	66.6 ± 0.6^{dB}	91.6 ± 4.8^{bA}	0.15 ± 0.01
MOK-α-Terpineol	10.6 ± 3.8^{bcF}	29.1 ± 4.1^{abE}	45.8 ± 4.1^{bD}	58.3 ± 4.8^{bcC}	70.4 ± 3.7^{cdB}	91.6 ± 4.8^{bA}	0.14 ± 0.02
MOK-Limonene	15 ± 5.0^{abF}	33.6 ± 3.6^{aE}	58.1 ± 4.2^{aD}	65.1 ± 0.9^{aC}	83.3 ± 0.2^{aB}	100 ± 0.0^{aA}	0.09 ± 0.01

Mean values followed by different letter(s) in the same column (lowercase) or in the same row (uppercase) differ significantly. *MOK O. kilimandscharicum* blend of the seven constituents

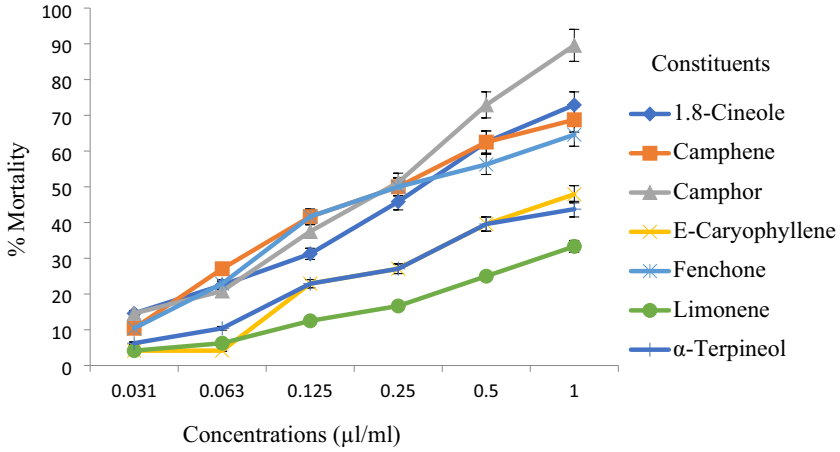

Fig. 6 Fumigant toxicity at different concentrations of seven constituents of *O. kilimandscharicum* on *T. absoluta* adults after 24 h of exposures

eugenol, 1,8-cineole-β-bisabolene-(*E*)-α-bisabolene and 1,8-cineole-methyl chavicol-β-bisabolene chemotype were found (Lawal et al. 2014; Sarin et al. 2013; Ram et al. 2013; Ntezurubanza et al. 1984; Charles and Simon 1992).

Phenylpropanoids (69.4%) and oxygenated monoterpenoids (82%) are found to be the main compounds in the oils and could be responsible for the activities. Moreover, methyl eugenol was found to be more effective in term of knockdown activity, as well as repelling and killing effects (Ngoh et al. 1998), apart from larvicidal activity against *Spodoptera litura* F., (Bhardwaj et al. 2010). It is a potent inhibitor of the enzyme acetylcholinesterase (Lee et al. 2001), responsible for the hydrolysis of the neurotransmitter acetylcholine, which can eventually lead to paralysis in insects. Eugenol has been shown to exhibit insecticidal property toward *Sitophilus zeamais* Motsch (Huang et al. 2002) and be toxic and repellent to the beetle *Dinoderus bifoveolatus* Wollaston (Ojimelukwe and Adler 2000) and tick (*Ixodes ricinus* L.) (Bissinger and Roe 2010) and a fumigant toward *Callosobruchus maculatus* F. (Ajayi et al. 2014); it is also used in insect attractant formulation developed for oriental fruit flies, *Bactrocera dorsalis* Hendel, and melon flies, *Bactrocera cucurbitae* Coquillett (Gomez and Coen 2013; Hausen et al. 1999). Camphor has been found to possess insecticidal activity against wheat weevil, *Sitophilus granarius* L. (Mossa et al. 2017), and sheep bot fly, *Oestrus ovis* L. larvae (Mazyad and Soliman 2001), and fumigant and contact toxicity against cigarette beetle *Lasioderma serricorne* F. adults (Chen et al. 2014), red flour beetle *Tribolium castaneum* Herbst (Khiyari et al. 2014), armyworm *Pseudaletia unipuncta* Haworth (Isman et al. 2008) and the cowpea weevil, *Callosobruchus maculatus* (Khani and Asghari 2012). 1,8-Cineole is used as an insect repellent and insecticide to the American cockroach (Sfara et al. 2009); as a mosquito larvicide (Corbet et al. 1995); as a mosquito ovipositional repellent (Klocke et al. 1987) and as a fumigant toward adults of *Callosobruchus maculatus*, *Rhyzopertha dominica* F. and *S. oryzae* (Aggarwal et al. 2001), and has toxic effect on the two-spotted spider mite, *Tetranychus urticae* Koch (Isman et al. 2008), and inhibitor of the enzyme

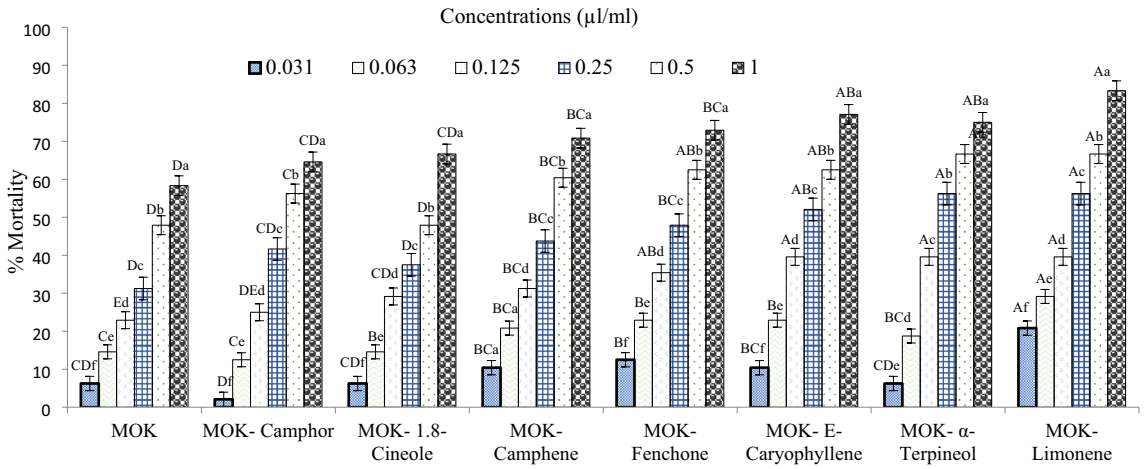

Fig. 7 Means mortality (%) of a blend of 7 constituents of *O. kilimandscharicum* oils and blends with each compound missing on of *Tuta absoluta* adults after 24 h

acetylcholinesterase (Ryan and Bryan 1988). Nevertheless, the presence of minor components might also play a role in the activities of the oils.

However, a number of research questions and challenges would need to be addressed in the downstream development and evaluation of the oils. The relatively high volatility of the essential oils means that they would have to be nano-encapsulated in a suitable inclusion complex like Alginate microsphere, which can facilitate their controlled-release and their performance over longer periods of time in the field (Soliman et al. 2013). Recently, micro- and nano-emulsions studies have shown capability of decreasing volatility and improving stability, water solubility and efficacy of essential oil formulations (Pavoni et al. 2020; Mossa et al. 2017). Also a number of reports to optimise the control released dynamic using cyclodextrins to enclose essential oils and other volatile compounds or blends have significantly controlled the rate of evaporation of volatiles with good efficacy in the field (Marques 2010; del Toro-Sánchez et al. 2010; Junnila et al. 2015; Khoshtinat et al. 2017). One of our follow-up objectives is to pursue this line of research to find out if the oils can be effectively deployed using the host plant. Further tests will have to be done to explain the mechanism of action of the oils and their effect on non-target organisms. However, tests on sublethal doses of the oils on fecundity, longevity of the next generation and vitality could justify the higher values of LC_{90} due to the long-term effect. In fact, previous reports of Benelli et al. (2018) and Pavela et al. (2020) have shown how sublethal concentrations can decrease longevity, fecundity, fertility and natality of insects. Also, the effect of the environmental factor like temperature when applied in field conditions could play a major role in the oil efficacy. The low or high temperature of both *Ocimum* spp. during application could eventually modify the action of the different constituents inside the oils. For example, Pavela and Sedlak (2018) reported the influence of temperature on three major constituents (thymol, carvacrol and *p*-cymene) on *Spodoptera littoralis* Boisd larvae mortality. In addition, it will be interesting to see if these perennial *Ocimum* plants naturally emit volatiles at sufficient quantities to have significant negative effects on the different stages of *T. absoluta*.

In conclusion, this study has shown potential effect of *O. gratissimum* and *O. kilimandscharicum* essential oils on *T. absoluta*. Follow-up studies highlighted above could shed more light on their full potential in their downstream applications.

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