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EDITED BY
Paul O. Mireji,
Kenya Agricultural and Livestock Research Organization, Kenya

REVIEWED BY
Benson Wachira,
Pwani University, Kenya
Kounbobr Roch Dabiré,
Research Institute for Health Science-Direction Régionale de l'Ouest (IRSS-DRO), Burkina Faso

*CORRESPONDENCE
David P. Tchouassi
dtchouassi@icipe.org

†These authors have contributed equally to this work

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Enzyme-catalyzed kinetic resolution of racemic 1-octen-3-ol and field evaluation of its enantiomeric isomers as attractants of sandflies

David P. Tchouassi*, Juliah W. Jacob†, Xavier Cheseto†, Lydia S. Chepkemoi, Iman B. Hassaballa and Baldwyn Torto

International Centre of Insect Physiology and Ecology, Nairobi, Kenya

Phlebotomine sand flies are medically important as vectors of the protozoan parasites that cause leishmaniasis and other bacterial and viral pathogens. Previous work demonstrated that both sexes of certain species of sandflies are attracted to 1-octen-3-ol (octenol). Since 1-octen-3-ol exists as two enantiomeric isomers — ((*R*)-(-)- (*R*-form) and (*S*)-(+)- (*S*-form), we tested the hypothesis that the two enantiomeric forms and racemic mixture (*R/S*) attracted different sand fly species. We carried out field trials in a leishmaniasis endemic foci in Baringo County, Kenya. In a randomized design, trap captures of sandflies in CDC light traps baited with the *R*-, *S*- and racemic (*R/S*) forms of 1-octen-3-ol in hexane varied with the form and dose of the compound. Interestingly, of the captured species, only *Phlebotomus martini*, the vector of the parasite causing visceral leishmaniasis, exhibited a dose-dependent response to octenol; captures of both sexes of the species being generally 1.7-fold higher with the *R*- than *S*-form. There was no significant effect of treatment on captures of *Sergentomyia* species (*S. schwetzi, S. antennata, S, clydei*). Our findings have implications for surveillance of sandfly populations as part of leishmaniasis epidemiologic investigation.

KEYWORDS

volatile organic compounds, (R)-(-)-1-octen-3-ol, (S)-(+)-1-octen-3-ol, field evaluation, *Phlebotomus martini*, visceral leishmaniasis

Introduction

Volatile organic compounds (VOCs) serve various biological functions including roles as semiochemicals - signalling chemicals mediating intra- and inter- organismal interactions (1, 2). They are chemically diverse and mediate a wide range of behaviors in arthropods, including host seeking, reproduction, and oviposition, to name a few (3). Examples include chemicals mainly in the classes: aldehydes, carboxylic acids,

terpenes, ketones, alcohols, phenols, and sulfides (2). Their correct identification is key to as environmentally- friendly tools for monitoring and controlling vector populations.

The 'mushroom alcohol' 1-octen-3-ol, has been widely studied as an attractant for diverse blood-feeding arthropods to locate their vertebrate hosts. First demonstrated in tsetse flies (4), its kairomonal effect has also been reported in other insects such as horseflies (5), biting midges (6), and blackflies (7, 8). In mosquitoes, the dengue vector *Aedes aegypti* (9) and the malaria vector *Anopheles gambiae* (10) are attracted to octenol in combination with carbon dioxide, whereas the vector of West Nile virus vector *Culex quinquefasciatus* (11) is repelled by the blend. These findings suggest potential species-specific responses to this compound.

Notably, 1-octen-3-ol exists as two optical isomers or enantiomers: (*R*)- (–)-1-octen-3-ol (*R*-form) and (*S*)-(+)-1-octen-3-ol (*S*-form). Behavioral evidence suggests that certain insects respond differently to the different enantiomers of 1-octen-3-ol, although most studies have examined the effects of the racemic form (*R/S*). For instance, in Y-tube olfactometer assays using different mosquito species, Cook et al. (12) found higher behavioral response to the *R*- than *S*-form which translated to increased flight and attraction in *Ae. aegypti* but only increased activation in *Cx. quinquefasciatus*. Electrophysiological studies demonstrated higher sensitivity of the *R*- over the *S*-form in *Aedes* and *Culex* mosquitoes (12–15). In contrast, female beetles of the species *Cis boleti*, a known coloniser of tree fungus, exhibited greater attraction to the *S*- than *R*-form (16). In field studies, increased captures of the mosquito species *Anopheles crucians*, a malaria vector (17) and *Ochlerotatus infirmatus* an arbovirus vector (18), were found in traps baited with *R*- than *S*-forms (19). Similar discriminatory effect between the enantiomeric isomers have been reported in tsetse flies (4, 20, 21), indicating that the different enantiomers function differently in different insects.

Phlebotomine sand flies are vectors of *Leishmania* parasites and cause leishmaniasis that afflict humans besides other bacterial and viral pathogens (22, 23). To date, only a handful of studies have assessed the behavioral response of 1-octen-3-ol (racemic mixture) on sandflies. For instance, the sandfly species *Lutzomyia longipalpis* (Lutz and Neiva), and *Phlebotomus duboscqi* Neveu-Lemaire have been tested in laboratory assays (24, 25) and *Lutzomyia intermedia* (Lutz and Neiva) in field conditions (26) for their responses to the racemic mixture. However, evaluation of the individual enantiomers on sandfly attraction in the field has little been explored. In this study, we first carried out an enzyme-catalyzed kinetic resolution of racemic 1-octen-3-ol to obtain the individual enantiomeric isomers, and then tested the hypothesis that the individual enantiomeric and racemic forms differentially attracted sandflies in conventional traps.

Materials and method

Chemical analysis

Gas chromatography-Flame ionization detector (GC-FID)

Chemical profiling and enantiomeric excess (ee) of the two enantiomers; *(S)*-1-octen-3-ol and *(R)*-1-octen-3-ol were analyzed on an Agilent 6890N gas chromatography instrument equipped with 2-columns: a) chiral column, β-cyclodextrin capillary column 30 m length, 0.25 mm inner diameter, and 0.25 µm film thickness (J&W Scientific, USA), and b) achiral column HP-1 MS low bleed capillary column (30 m× 0.25 mm i.d., 0.25 µm) (J&W, USA). For all the analyses, we injected 0.2-1 µL samples, equivalent to a maximum of 100 ng on-column mass, into a cooling-on-column inlet system. The initial oven temperature was set at 30°C for 0.5 min and then ramped up to 150°C at a rate of 5°C/min. Subsequently, we increased the temperature to reach the target value of 230°C at a rate of 10°C/min, maintaining this temperature for 33 min. We used high purity nitrogen gas as a carrier gas, maintained at a constant linear velocity of 49 cm/s throughout the analysis. The Flame Ionization Detector (FID) was set at a temperature of 230°C, and data were acquired at a rate of 20 Hz.

NMR spectroscopy

Identities of the resolved enantiomeric forms, namely, (*S*)-1-octen-3-ol 1 and (*R*)-1-octen-3-ol **3**, were established using nuclear magnetic resonance (NMR) analyses. Each compound (5 mg) was dissolved in $CDCl_3$ (Cambridge Isotope Laboratories, Tewksbury, MA) and then transferred into 2.5 mm × 100 mm MATCH NMR tubes (Norell, Landisville, NJ). The NMR analyses encompassed both ^{1}H NMR and ^{13}C NMR spectroscopy. All NMR spectra were acquired at 22°C, utilizing a Bruker Avance II 600 console, which operates at 600 MHz for ^{1}H NMR and 151 MHz for ^{13}C NMR.

Enzyme-catalyzed kinetic resolution of racemic 1-octen-3-ol

An optimized lipase-mediated synthetic route (LMER) using immobilized lipase B of *Candida antarctica* (Novozym 435) was selected for multi-gram synthesis and resolution of the two enantiomers following a high reported enantiomeric excess (ee) (27) (Figures 1, 2). Briefly, racemic 1-octen-3-ol (30 g, 233.97 mmol), (Sigma Aldrich Co. Ltd, Gillingham Dorset-UK) was dissolved in vinyl acetate 30 ml (28 g, 325.45 mmol), followed by addition of novozyme 435 (6g), stirred at room temperature for 60 hr. The reaction was stopped by filtering through glass wool to separate the enzyme, then the filtrate was concentrated *in vacuo* to yield 29.7 g of clear liquid. The filtrate was purified by column chromatography on silica gel (Et_2O: pet ether 20%) to afford 'crude' *(R)*-1-octen-3-ol 1 (15 g) and *(S)* - 1-octen-3-yl acetate -2 (13 g ee 98.9%). 2 was deacetylated using KOH to yield (*S*)-1-octen-3-ol 3 (10 g) $[\alpha]_D^{20}$ + 13.6° (MeOH). 'Crude' 1 was reintroduced into the enzyme pot, and the aforementioned process was repeated for 12 hr, to yield *(R)*-1-octen-3-ol 1 (11g) $[\alpha]_D^{20}$ -15.5° (MeOH). 10 mg of compound 1 underwent acetylation, yielding compound 4 for subsequent enantiomeric excess analysis (ee > 99.9%). After testing three different substrate to enzyme ratios including 10:1, 10:2, and 10:4, the 10:2 ratio was found to be most suitable for the subsequent scale-up reaction.

1 and 3: *(R)*-1-octen-3-ol and *(S)*-1-octen-3-ol. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.36-1.42 (m,9H, CH_2CH_3), δ 1.46-1.99 (m,2H, CH_2), δ 2.06(s,1H, OH), δ 4.06-4.10 (dt,1H, J = 6.7,13.0 Hz, C-OH), δ 5.08-5.10 (d,1H, J = 10.4 Hz, $CHCH_2$), δ 5.19-5.23

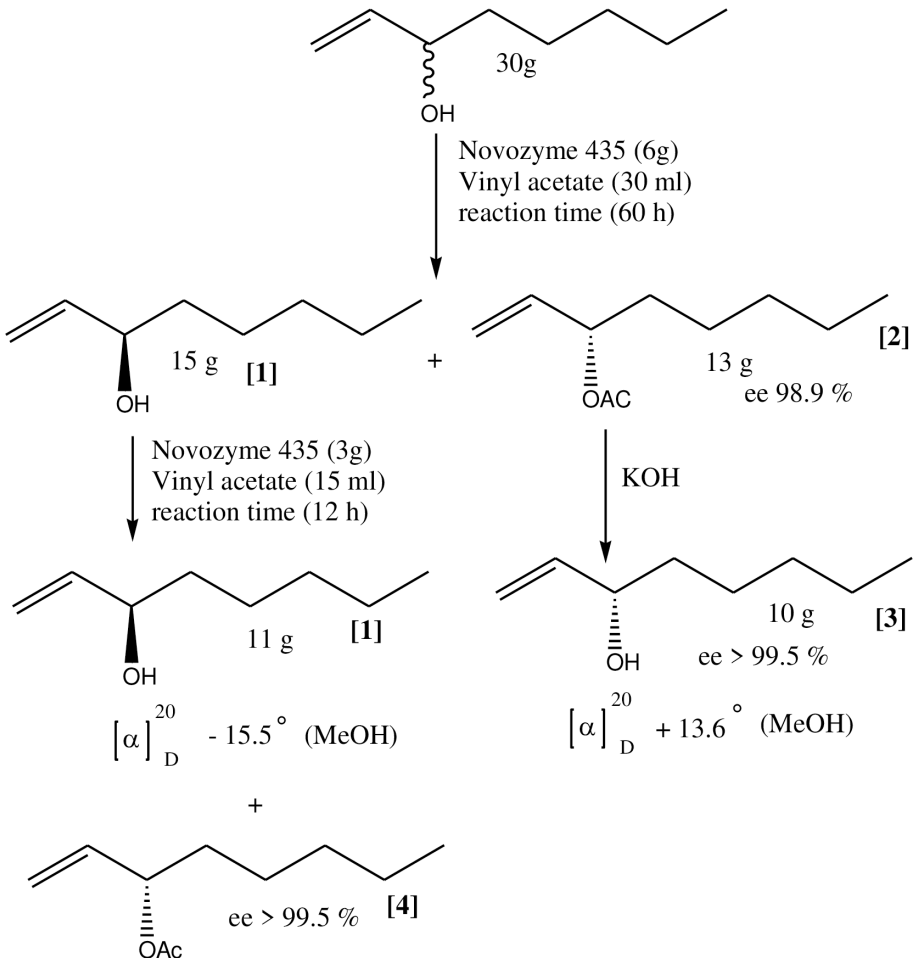

FIGURE 1
Kinetic resolution of *(S)*-1-octen-3-ol and *(R)*-1-octen-3-ol.

(d,1H, *J* = 17.3 Hz, $CHCH_2$), δ 5.83-5.88 (ddd,1H, *J* = 6.5,10.7,16.6Hz, CH).

1 and 3 *(R)*-1-octen-3-ol and *(S)*-1-octen-3-ol.^{13}C NMR ($CDCl_3$,126 MHz) δ: 14.0 (CH_3), δ: 22.6,25.0,31.8,37.0 (CH_2), δ: 73.3 (C-OH), δ: 144.5,141.4 ($CHCH_2$).

Study site

Field evaluation was carried out in the surrounding community of Rabai (0.45866 N, 35.9889 E), in Marigat town located in Baringo County in the Kenyan Rift Valley. A map of the site is provided in Hassaballa et al. (28). The semi-arid ecology that characterizes the site is endemic for visceral and cutaneous leishmaniasis with presence of driving vectors including *Phlebotomus martini* and *P. duboscqi* (29, 30). Interspersed across the landscape is abundance of termite mounds, important habitat for sandflies (28, 29). The vegetation comprises mainly acacia and *Prosopis juliflora* trees, Cactus plants, *Balanites* spp, and *Commiphora* bushes. The area is sparsely inhabited, and people commonly indulge in subsistence agriculture and predominantly livestock keeping.

Study design

Trials were conducted in February 2015 (experiment 1) and December 2018 (experiment 2) to coincide with peak sandfly activities in the dry season (28, 29). Each experiment comprised ten treatments, with the compounds formulated in hexane (31). These were racemic octenol *R/S*, *R*- and *S*- forms, all at three doses (0.1, 1 and 10 mg/ml), and a control trap baited with hexane only. The considered doses are within tested ranges of semiochemicals against other disease vectors in the field (31, 32). The treatment dose was used to bait a CDC light trap (Model 512, John W Hock, Gainesville, FL, USA) attached close to the fan (Supplementary Figure 1), and it released (1 ml solution) by diffusion from 1.5 ml polyethylene tubes with a pin hole in the center of the cap as described previously (31). CDC light trap is the conventional tool for surveillance of Phlebotomine sand flies and often without a bait (26, 28, 33). Generally, sandflies are considered poor flyers but move via jumping flights (34), although their flight ranges could vary by species. Thus, evaluation followed a randomised experiment targeting termite mounds which is a suitable sandfly microhabitat (28, 29), where treatments were individually positioned, with an

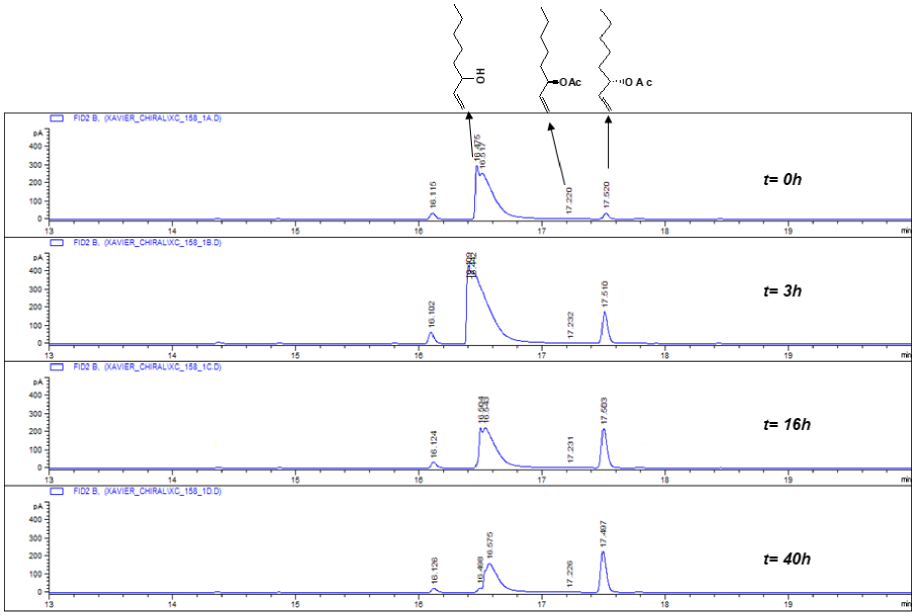

FIGURE 2
Representative GC-FID trace showing time lapse of a lipase-mediated resolution of racemic 1-octen-3-ol.

inter-trap distance ~40 m. Traps were moved daily targeting new sets of termite mounds. Lures were renewed daily, as well. Because sandflies are nocturnal, traps were set at 18:00 h and retrieved at 06:00 h the following day.

Sandfly processing and species identification

Captured sandflies were immobilised using triethylamine, sorted, and then preserved in liquid nitrogen in the field. Thereafter, they were transported to the laboratory at the *icipe* Duduville Campus, Nairobi. Here, the samples were stored at −80°C until further processing. For identification, the head and genitalia from each sandfly specimen were excised and mounted on a slide and cover slip using Berlese's medium. Species were determined by observing the dried slides (at least after a day) via microscopic examination of the cibarial armatures (*Phlebotomus* or *Sergentomyia*), male genitalia or female spermathecae and pharynx using published morphological keys of Kirk and Lewis (35) and Abonnenc and Minter (36).

Chemicals

(*R*)- (−)-1-octen-3-ol and (*S*)- (+)-1-octen-3-ol were enzymatically and kinetically resolved, and purity determined as 99.5% each and spectroscopically characterized. The racemic 1-octen-3-ol (98%, 50:50 R:S) was obtained from Sigma Aldrich (Louis, MO 63103, USA).

Data analysis

Sandflies were counted daily in each treatment and analysed using binomial regression following generalised linear models using the MASS package in R v 4.2.1. Data was analysed separately for the trial periods. The total abundance and that for species and sex encountered in adequate numbers were analysed separately and compared between the treatments. Treatment was considered the explanatory variable. Mean catches and standard errors for selected species were plotted. All results were considered significant at $p \leq 0.05$.

Results

Enzyme-catalyzed kinetic resolution of racemic 1-octen-3-ol

In GC-FID analysis on an achiral column, one peak was detected for the racemic mixture of 1-octen-3-ol. However, when the analysis was carried out on a chiral column, two peaks resolved, with compound 1 eluting at 16.58 min and compound 3 at 17.41 min (Figure 2). In our enzyme-catalyzed kinetic resolution of racemic 1-octen-3-ol, monitored by gas chromatography-flame ionization detector (GC-FID) on the chiral column with 4 hr intervals over a total of 80 hr, maximum resolution of the two enantiomeric isomers occurred at 60 hr to give a reaction mixture of 45% acetate and 55% alcohol plus, vinyl acetate, and lipase (Figure 2).

As expected, the ^{1}H NMR and ^{13}C NMR spectra were identical to those of 1-octen-3-ol (37). Notably, the singlet at δ 2.06 ppm

indicates the presence of the hydroxyl group (-OH). The multiplets at δ 1.36-1.99 ppm are consistent with methylene (CH_2) groups, while the doublets and triplets at δ 4.06-5.88 ppm indicate the presence of olefinic protons. In the ^{13}C NMR spectrum, peaks at δ 14.0 ppm correspond to methyl (CH_3) group, while peaks at δ 22.6-37.0 ppm represent methylene (CH_2) carbons. The signal at δ 73.3 ppm is associated with the carbon atom attached to the hydroxyl group (C-OH). Additionally, the peaks at δ 144.5 and 141.4 ppm correspond to the olefinic carbons (CH=CH_2).

Sandfly composition

Experiment 1, carried out in 2015 resulted in captures of 1812 sandflies (female, f=1253, male, m=559) in four replicate trials. A minor proportion of the females captured were blood-fed (4.6%). The sandfly fauna was dominated by *Sergentomyia* species (94.9%, 1720/1812), notably, *S. schwetzi* (n=1149, 63.4%), followed by *S. antennata* (244, 13.5%) and *S. clydei* (n=126, 7.0%). Other sparsely captured *Sergentomyia* species included *S. africana africana*, *S. adleri*, *S. squamipleuris* and *S.ingrami* (Table 1). Only two *Phlebotomus* species were encountered including *Phlebotomus martini* (n=185,10.2%) and a sparse representation of *P. duboscqi* (n=2, 0.1%).

The 2018 trial (Experiment 2) captured 3802 sandflies (f=2545, m=1257) in 9 replicate experiments. Among the females captures were 79 blood-fed (3.1%). The sandfly composition mirrored the pattern observed for the 2015 captures. *Sergentomyia* sandflies were most abundant (93.9% of total captures) and highly represented by the species *S. schwetzi* (60.7%), *S. antennata* (10.8%), *S. clydei* (9.5%). The sand fly species *P. martini* (11.9%) occurred in higher numbers relative to *P. duboscqi* as the only *Phlebotomus* species (Table 1).

Regardless of dose, total captures in racemic *R/S*, *S*- and *R*-form were 1621, 1646 and 1939, respectively, with control traps recording the lowest (n=408) for the combined trapping periods (2015 and 2018) (Table 1).

TABLE 1 Sandfly composition in field evaluations conducted in 2015 and 2018 in Rabai, Marigat sub-county, Kenya.

			Octenol			S-Octenol			R-Octenol			Control
Period	Sex	Treatment	0.1	1	10	0.1	1	10	0.1	1	10	Hexane
		P.martini	8	10	8	7	6	5	6	16	16	8
		P.duboscqi	0	0	0	0	0	1	1	0	0	0
		S.schwetzi	82	71	66	41	106	52	31	100	113	70
		S.antennata	16	26	15	26	24	21	7	36	33	16
		S.clydei	10	18	12	12	27	10	6	8	8	7
		S.africana_africana	3	3	5	4	8	3	3	9	3	1
		S.adleri	1	0	1	0	0	0	0	0	0	0
		S.squamipleuris	2	2	4	6	9	6	1	3	6	7
	F	*S.ingrami*	0	0	1	0	0	0	0	0	0	0
		Treatment	**0.1**	**1**	**10**	**0.1**	**1**	**10**	**0.1**	**1**	**10**	**Hexane**
		P.martini	16	6	12	7	3	6	15	8	11	11
		P.duboscqi	0	0	0	0	0	0	0	0	0	0
		S.schwetzi	30	37	46	36	75	28	25	66	39	35
		S.antennata	4	2	7	3	0	5	1	0	0	2
		S.clydei	1	0	2	2	0	0	0	1	0	2
		S.africana_africana	1	0	2	0	4	2	0	4	0	1
		S.adleri	0	0	0	0	0	0	0	0	0	0
2015	M	*S.squamipleuris*	0	0	0	0	1	0	0	0	0	0
		Treatment	**0.1**	**1**	**10**	**0.1**	**1**	**10**	**0.1**	**1**	**10**	**Hexane**
		P.martini	24	26	23	26	16	12	12	29	42	14
		P.duboscqi	1	3	0	0	2	1	1	1	0	0
		S.schwetzi	123	96	152	115	139	138	109	212	193	88
2018	F	*S.antennata*	34	43	25	46	29	35	24	64	50	34

(Continued)

TABLE 1 Continued

			Octenol			S-Octenol			R-Octenol			Control
Period	Sex	Treatment	0.1	1	10	0.1	1	10	0.1	1	10	Hexane
		S.clydei	20	36	34	53	39	28	37	41	33	14
		S.africana_africana	8	5	6	9	13	4	7	15	6	1
		S.squamipleuris	25	13	13	12	17	13	7	22	21	11
		Treatment	**0.1**	**1**	**10**	**0.1**	**1**	**10**	**0.1**	**1**	**10**	**Hexane**
		P.martini	34	20	30	21	14	11	21	23	34	19
		P.duboscqi	0	1	1	2	1	0	0	1	0	0
		S.schwetzi	72	85	102	121	85	75	101	162	80	60
		S.antennata	4	2	7	3	0	5	1	0	1	3
		S.clydei	3	2	4	8	0	0	2	2	3	2
		S. africana Africana	1	0	2	0	5	2	0	4	0	1
	M	*S. squamipleuris*	4	3	4	0	0	0	1	0	1	1

F, female; M, male; The doses indicated are in mg/ml.

Analysis between the treatments for total sandfly captures and for individual species (fairly represented) by sex was performed separately for each period. Despite a dose-response variation, total sandfly captures did not differ between the treatments both in 2015 ($\chi^2_{11,30}$ = 45.4, p=0.78) and 2018 ($\chi^2_{11,80}$ = 106.03, p=0.84). However, there were species-specific differences (Table 1).

Comparison of *P. martini* captures

Captures of female *P. martini* in 2015 were relatively highest for traps baited with the highest doses of the *R*-form (1 and 10 mg/ml) and lowest at all doses of the *S*-form. However, analysis of the 2015 data showed no significant variation in female *P. martini* captures between any of the treatments relative to the control trap that had only hexane ($\chi^2_{11,30}$ = 44.32, p=0.23). We found that traps baited with the *R*-form (0.1 mg/ml) and *R/S* (0.1 mg/ml) captured the highest number of male *P. martini*. These captures differed significantly from captures with the *S*-form (1mg/ml), which recorded the lowest captures. The mean captures are plotted in Figures 3A, B.

In 2018, the *R*-form at the dose of 10 mg/ml captured the highest number of female *P. martini*. This number was significantly different from that found for the *S*-form at the same dose, which with the control recorded the lowest captures (z=2.168; p=0.03). Likewise, as recorded in 2015, the lowest captures were recorded in traps baited with the *S*-form at the dose of 10 mg/ml. With regards to males during this period, significantly higher numbers were captured in traps baited with the *R*-form at the dose of 10 mg/ml and racemic *R/S* (0.1 mg/ml) compared to the *S*-form at 10 mg/ml which recorded the lowest captures. However, captures with the *R*-form (10 mg/ml) did not differ from captures recorded in the other treatments and control. The mean captures are plotted in Figures 3C, D.

After controlling for dose, total *P. martini* captures were 217, 134 and 233 in traps baited with *R/S*, *S*- and *R*-form, respectively. Control traps had the lowest captures (n=52). The data was for the combined trapping periods 2015 and 2018 (Table 1).

Abundance trends of *Sergentomyia* species

The most abundant species captured, *S. schwetzi*, was compared between treatments in both trapping periods. In 2015, there was no effect of treatments on captures of female *S. schwetzi* ($\chi^2_{11,30}$ = 45.88, p=0.71) and male ($\chi^2_{11,30}$ = 47.41, p=0.70). In 2018, the highest captures of *S. schwetzi* were obtained with the *R*-form (1 and 10 mg/ml) for females and at dose of 1mg/ml for males. However, no statistical significance was evident between the treatments and relative to captures recorded for females in the control ($\chi^2_{11,80}$ = 105.68, p=0.88) and males ($\chi^2_{11,80}$ = 104.98, p=0.81). The mean captures plotted for this species are indicated in Figures 4A–D.

Females of the two additional *Sergentonyia* species (*S. antennata* and *S. clydei*) encountered in relatively good numbers in 2018 were compared. Likewise, no significant difference was found between the treatments and control (*S. antennata*: ($\chi^2_{11,80}$ = 104.81, p=0.4956); *S. clydei*: ($\chi^2_{11,80}$ = 96.71, p=0.81). Nonetheless, *S. antennata* was most abundant in traps with the *R*-form (doses 1mg/ml and 10 mg/ml) and *S. clydei* with the *S*-form (0.1 mg/ml). The mean captures are represented graphically in Figures 5A, B.

Discussion

This study evaluated whether the enantiomeric isomers of 1-octen-3-ol (*R*- and *S*-forms) and the racemic mixture exhibited differential responses in sandflies with regards to trap captures. Enzymatic resolution of the enantiomeric mixtures gave highly pure

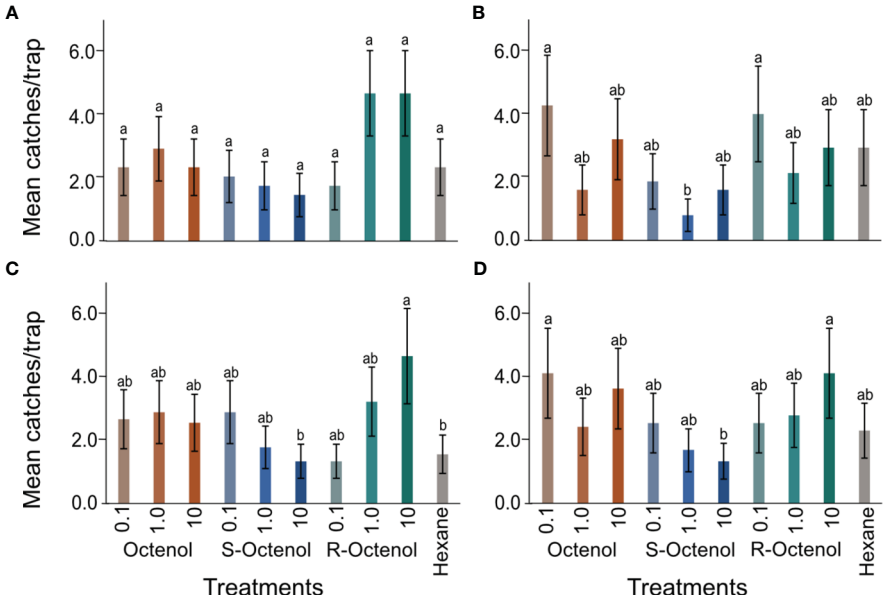

FIGURE 3
Mean (± se) captures of *P. martini* in the different treatments. **(A)** female and **(B)** male during 2015 (4 replicate trials); **(C)** female and **(D)** male in 2018 (n=9 replicate experiments). The dose for each form of octenol indicated is in mg/ml. se, standard error; Bars followed by different letters are significantly different from each other at $p \leq 0.05$.

individual components (ee, 99.5%), whose structures were determined using spectroscopic methods. This suggests presence of minimal contaminants in the final products which were field evaluated. Thus, interference on sandfly responses by possible reaction contaminants may be ruled out from the field captures.

The trap composition comprised species dominated by those in the genus *Sergentomyia*. *Phlebotomus* species were represented by *P. martini* and *P. duboscqi*, vectors of causative *Leishmania* parasites of VL and CL, respectively (30, 38). The captures appear to mirror the sandfly fauna in the geographic area as reported previously (28, 30). Higher captures were recorded in the *R*- than *S*-forms for *P. martini* but not *Sergentomyia* species. These results suggest species-specific effect in behavioral response activity between the enantiomers. Variation in behavioral impact of octenol to

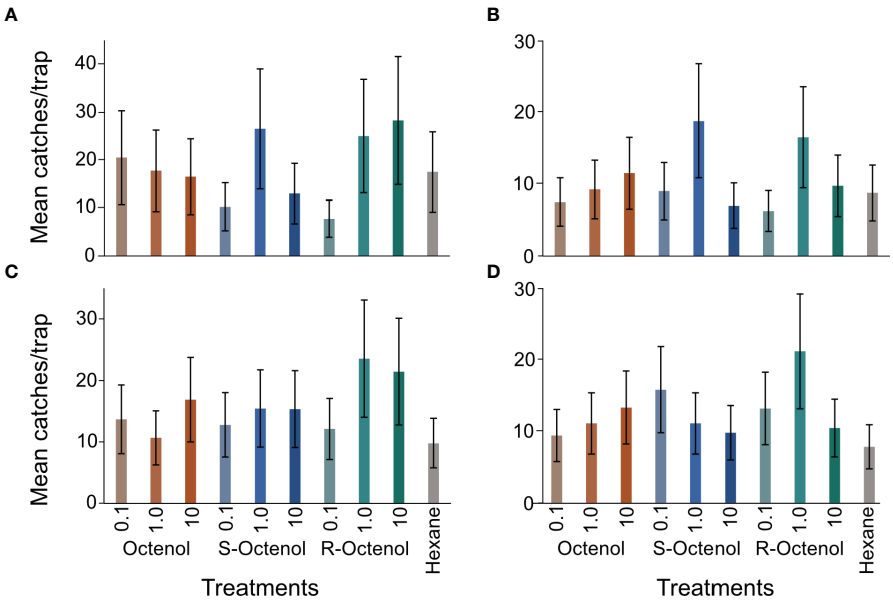

FIGURE 4
Mean (± se) captures of *S. schwetzi* in the different treatments. **(A)** female and **(B)** male during 2015 trials (4 replicate experiments); **(C)** female and **(D)** male in 2018 (n=9 replicate experiments). se, standard error; The dose for each form of octenol indicated is in mg/ml. There was no significant difference in collections of different sexes of this species among the treatments including the control at $p \leq 0.05$.

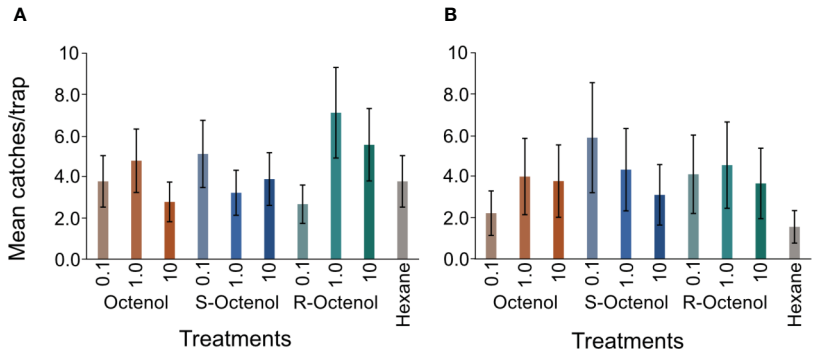

FIGURE 5
Mean (± se) captures of **(A)** *S. antennata* and **(B)** *S. clydei* in the different treatments in 2018 (n=9 replicate experiments). se, standard error; The dose for each form of octenol indicated is in mg/ml. There was no significant difference in collections of different sexes of this species among the treatments including the control at $p \leq 0.05$.

different sandfly species, for instance, *Lutzomyia intermedia* and *L. longipalpis* (26, 39) and mosquito vectors (e.g. *An. crucians*, *Oc. infirmatus* (19) have been described previously. Our findings add to the few available data on the kairomonal effect of octenol on phlebotomine sandflies (26) and for the first time, the differential behavioral effect of its enantiomers in field settings.

The performance although dose-dependent was somewhat similar between male and female *P. martini*. Whereas the lowest captures of both sexes were associated with the *S*-form at the dose of 10mg/ml, highest captures were observed at specific doses with the *R*-form or the racemic mixture (Experiment 2). Invariably, these results suggest differential response of this sandfly species, and both sexes, to the two enantiomers. Octenol is represented in the volatile emissions of plants (40) and habitats like termite mounds (28), to which male and female sandflies exploit (41–43). Octenol is present also in the volatile emissions of human skin (44). Thus, the response of both sexes to octenol is not unexpected, indicating that it is part of the odour bouquet that drives variation in attraction to these substrates. Nonetheless, the relative proportion of the isomers in different natural substrates is unknown, although literature suggests a dominance of *R*- over *S*-form (4). Electrophysiological studies are needed to corroborate the differential sensitivity of this sandfly species to the different isomers.

In experiment 1 (2015) of the present study, overall higher captures of female *P. martini* at higher doses were recorded using the *R*-form (1 mg/ml, 10mg/ml). Analysis of the data indicated that males showed an apparent preference for the *R*-form at a lower dose instead (0.1 mg/ml). Males feed on plants entirely and could adapt to probably low levels released by plants compared to vertebrates. There was no variation in female captures of this species across treatments including the control. Lack of statistical significance could have been masked by the low numbers in the trapping efforts or number of replicates during this period. Additional studies to investigate release rates, seasonal and geographic effects of the enantiomeric isomers and racemic mixture on sand fly responses would be required.

Despite the overall abundance of *Sergentomyia* sandflies, among the species caught, *S. schwetzi* showed no variation between the treatments and relative to the control. This pattern contrasts to that found for *P. martini*, indicating divergence in behavioral activity elicited to octenol among sandfly species. Understanding the basis including sensitivity threshold via electrophysiological studies could shed light on species adaptation to resources mediated by volatile organic compounds such as octenol.

In conclusion, this study investigated the effects of enantiomers and racemic mixture of 1-octen-3-ol on trap catches of sandflies with CDC light traps under field conditions. Of the species encountered only *P. martini* exhibited a dose-dependent response to octenol; Captures of both sexes of the species were generally higher in the *R*- than *S*-form (at specific doses) indicative of clear differences in behavioral activity of the isomers in this sandfly species. Biologically, the (*R*)-1-octen-3-ol could be a more important kairomone that can be incorporated into traps for *P. martini*. Despite overall increased collections, no dose of the *R*-form performed better statistically, than the racemic mixture for this and the other sandfly species caught.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

DT: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Project administration. JJ: Data curation, Formal Analysis, Investigation, Methodology,

Validation, Writing – review & editing. XC: Conceptualization, Data curation, Formal Analysis, Funding administration, Investigation, Methodology, Validation, Writing – review & editing. LC: Data curation, Investigation, Validation, Writing – review & editing. IH: Investigation, Methodology, Writing – review & editing. BT: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fitd.2023.1327349/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Trap design comprising the lure solution released from a 1.5 ml polyethylene tube with a pin hole attached close to the fan of a CDC trap.

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