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Companion crops alter olfactory responses of the fall armyworm (*Spodoptera frugiperda*) and its larval endoparasitoid (*Cotesia icipe*)

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Abstract

Background The fall armyworm (FAW), *Spodoptera frugiperda*, is a devastating invasive pest and a threat to food security in Africa, with yield losses of 20–50%. Recent studies highlighted the importance of cereal crops such as maize and sorghum as the most preferred host plants for FAW oviposition. In the current work, we investigated the olfactory responses of FAW and its key larval endoparasitoid *Cotesia icipe* to odours from the preferred host (maize) in the presence of six potential companion crops including beans, groundnut, sweet potato, greenleaf- and silverleaf desmodium, and cassava. We hypothesized that odours released by companion crops in maize-based intercropping systems would alter host preferences of FAW for oviposition and its parasitoid responses.

Results In dual choice oviposition bioassays, FAW laid significantly more eggs on maize than on the other plants. However, in the multiple-choice bioassays, significantly fewer eggs were laid on maize when companion plants were present except cassava. While wind tunnel bioassays confirmed the differential behavioural responses of FAW, we found that its larval endoparasitoid *C. icipe* was attracted to volatiles from the companion plants tested individually and/or when they were combined with maize. Coupled gas chromatography–mass spectrometry (GC–MS) analysis detected several potential behaviour-modifying compounds including (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene, (*E*)-4,8-dimethyl-1,3,7-nonatriene, (*E*)-β-caryophyllene, camphor, methyl salicylate and (*E, E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene.

Conclusions Our findings provide evidence supporting diversified maize cropping system could reduce FAW damage by repelling the pest while simultaneously recruiting its natural enemies. Hence, diversifying cereal cropping system with companion crops could serve as an ecologically sustainable FAW management strategy.

Keywords Crop diversification, Bioassay, Companion plants, Fall armyworm, Natural enemies, Oviposition, Plant volatiles

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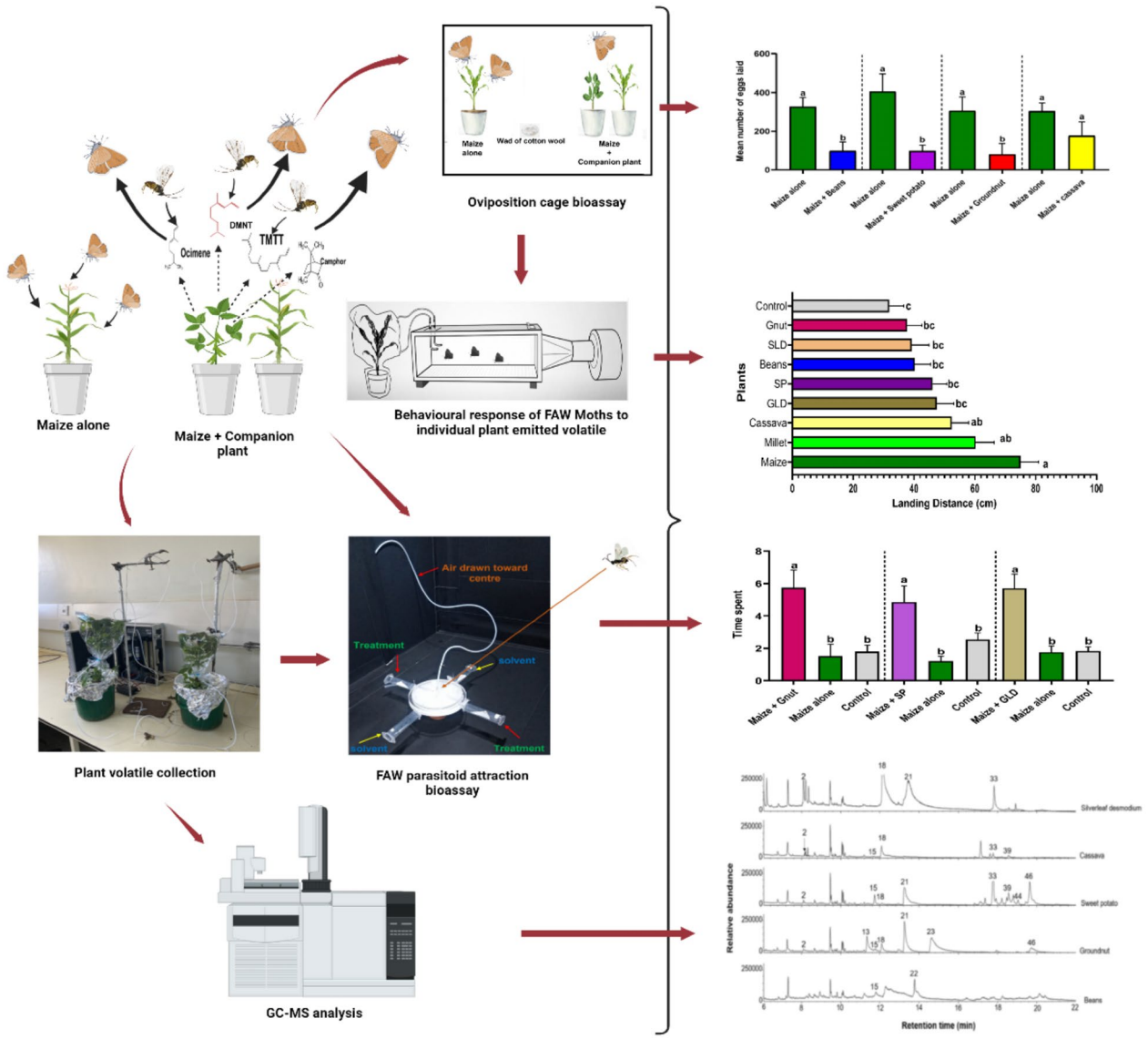
Graphical Abstract



Background

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a generalist and invasive noxious pest native to tropical and subtropical regions of the Americas [1]. The FAW has been recorded in over 350 host plants belonging to several plant families, although it prefers grasses [2, 3]. In 2016, the FAW was first reported in West and Central Africa [4, 5], from where it spread very quickly across the continent and currently the pest is present in over 45 African countries [6–8]. FAW has further spread to other regions of the world reaching Asia in January 2019 and subsequently to Korea, Japan, and other countries in Oceania and the Middle East by 2021 [8].

FAW has become a serious threat to food security in the continent due to its substantial damage on staple food crops especially maize (*Zea mays* L.). For example, estimates from 12 African countries showed that FAW can cause annually maize losses of 8.3–20.6 million metric tonnes, equivalent to $2.5–6.2 billion, and enough to feed 40–100 million people [9]. In Kenya, FAW causes yield loss of about a third of the annual

maize production, estimated at 1 million tonnes [10]. Emergency responses to counter the rapid spread and damage by FAW relied on extensive application of chemical pesticides [9, 11]. However, such approaches may have several undesirable consequences, such as development of insecticide resistance, environmental pollution and health-related risks [6, 12]. In addition, most smallholder maize farmers in Africa cannot afford the costs of repeated insecticide applications [13]. Hence, there is an urgent need to develop ecologically sustainable and cost-effective FAW management strategies suitable to African smallholder farming systems.

Increasing vegetation diversity through intercropping has been shown to suppress insect pest infestation, enhance naturally occurring pest enemies and lessen crop damage [14, 15]. Moreover, intercropping influences the rate at which the insect pest immigrates into a crop field and its population dynamics within the field [16, 17]. Smallholder farmers in Africa commonly intercrop maize with other crop species to reduce insect pest damage while enhancing crop productivity [18]. Several studies have reported reduced FAW infestation in diversified cropping systems, where maize fields are intercropped with cowpea, beans, soya bean, pigeon pea, groundnut, canavalia and desmodium [1, 16, 19–26]. A notable example is the push–pull cropping system, which involves use of pest repellent intercrop and an attractive trap plant [27]. A recent study confirmed that a reduced FAW infestation in the push–pull cropping system is due to insect pest repellent volatile organic compounds (VOCs) emitted by desmodium intercrops [28].

On the other hand, there have been cases of contrasting findings [7, 29, 30]. For example, field studies carried out in Cuba showed lower FAW infestations on maize fields intercropped with pumpkin compared to maize alone [30]. In contrast, Baudron et al. [7] reported increased FAW damage on maize crop intercropped with pumpkin in Eastern Zimbabwe [7]. Different factors including natural enemy abundance, variation in the ecosystems, and agronomic practices employed by respective farmers could contribute to the contrasting results. However, several studies from FAW native region of tropical and subtropical America have demonstrated that the combined use of diversified cropping system and natural enemies could maintain FAW populations at significantly low level for smallholder farmers [31]. Thus, the effectiveness of diversified cropping systems in mitigating FAW damage could be enhanced by selecting appropriate crop mixtures as well as better understanding the kind of tritrophic interactions between crops, pest and its natural enemies. Moreover, better insight into the ecological interactions and the underpinning mechanisms are crucial for the success of adopting crop diversification as an appropriate pest management strategy.

Recent studies from our laboratory demonstrated that FAW exhibited divergent ovipositional preferences when presented with different host plants [3]. Our current work extends that of our previous findings and investigates olfactory responses of FAW and its key larval endoparasitoid, *Cotesia icipe* Fernandez-Triana and Fiaboe (Hymenoptera: Braconidae), to diverse companion food crops commonly used in a maize-based intercropping system. We tested the hypothesis that odours released by companion crops in maize-based intercropping systems could alter FAW ovipositional responses and host finding in its larval endoparasitiod *C. icipe*. To achieve this, we: (i) compared the oviposition responses of FAW to maize with and without six potential companion plants including beans (*Phaseolus vulgaris* L.), groundnut (*Arachis hypogaea* L.), sweet potato (*Ipomoea batatas* (L.) Lam.), greenleaf desmodium (*Desmodium intortum* (Mill.) Urb.), silverleaf desmodium (*Desmodium uncinatum* (Jacq.) DC.), cassava (*Manihot esculenta* Crantz) and millet (*Panicum miliaceum* L.); (ii) assessed the role of olfaction in FAW responses and that of its endoparasitoid *C. icipe*; and (iii) used gas chromatography–mass spectrometry (GC–MS) to compare and identify the potential discriminant and behavior-modifying companion plant volatiles.

Methods

Plants

The experimental plants, including maize (*Z. mays*), variety "SC Duma 43", bean (*P. vulgaris*), groundnut (*A. hypogaea*), sweet potato (*I. batatas*), cassava (*M. esculenta*), greenleaf desmodium (*D. intortum*), silverleaf desmodium (*D. uncinatum*) and millet (*P. miliaceum* L.) were obtained from Simlaw Seeds Company, Kenya and the nursery plots of the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya [01°13′25.6″S 036°53′49.1″E, 1616 m above sea level (masl)]. The experimental plants were grown individually in plastic pots (4 L) filled with soil and organic manure mixed at a 2:1 ratio inside an insect-proof screen house. All plants were grown under natural conditions (25 ± 2 °C, 65 ± 5% RH; 12 L:12 D photoperiod) and were used in the experiments after 3–4 weeks from the date of planting.

Insects

FAW larvae used in our study were obtained from *icipe's* Animal Rearing and Quarantine Unit (ARQU). The initial insect colony at ARQU was established with specimens collected from FAW infested maize fields in Mbeere (00°42′25.1″S 037°29′0.14″E, 1091 masl), Embu County, Kenya. The larvae were reared on a

natural diet in ventilated sleeved transparent Perspex cages (60×60×60 cm). The bottom of the cage was lined with a paper towel to absorb excess moisture and provide an environment for pupation. The larvae were fed with young maize leaves that were changed and replaced with fresh ones every 2–3 days and allowed to pupate inside the same containers. Pupae were transferred into plastic containers (10 mm diameter×50 mm height) lined with cotton wool inside ventilated Perspex cages (30×30×30 cm) until adult emergence. The adults were fed on water and honey mixture (9:1) soaked in cotton wool. Butter paper was placed inside the cage as an oviposition substrate for females. Harvested eggs were placed in 8 L jars with ventilated lids till neonates emerged. At the 3rd instar stage, larvae were collected with a camel brush and were transferred into Perspex cages (60×60×60 cm) and fed with maize leaves until pupation. Pupae were transferred into Perspex cages as described above and the process was repeated. The laboratory-reared culture was infused with a field-collected insect population every 2–3 months to ensure colony vigour. The endoparasitoid *C. icipe* were reared on 2nd instar FAW larvae. The insect cultures were maintained at 25 ± 2 °C, 50–70% RH, 12L:12D photoperiod.

Oviposition bioassay

Two complementary experiments were conducted to investigate the oviposition responses of FAW moths towards test plants in dual and multiple-choice tests following previously described methods by Khan et al. [32], with some modification.

In Experiment I (Fig. 1A), a dual choice test was carried out using two potted 3–4-week-old seedlings. Each test plant was placed in the opposite corners of the oviposition cage (100×100×100 cm) covered with fine wire mesh netting. A wad of cotton wool (10 cm diameter) soaked with a 10% honey solution in a Petri dish (90×15 mm) was placed at the centre of each cage to nourish FAW moths. Thereafter, six gravid FAW females (2–3 days) were released into each cage before nightfall and allowed to oviposit overnight under natural conditions. Seven plant combinations were tested (Additional file 1: Table S1). The following day (15 h after release), the experimental plants were removed and the number of eggs and egg batches on each plant were counted under a light microscope (Leica EZ4HD, Leica Microsystems, Schweiz, Switzerland).

In Experiment II (Fig. 1B), FAW oviposition behaviour was investigated by setting up assays for different crop combinations commonly used under maize-based multiple cropping systems. Potted plants with six different plant combinations (Additional file 1: Table S1), were placed in the opposite corners of an oviposition cage (150×150×150 cm) covered with a fine wire mesh netting. Ten gravid FAW females (2–3 days) were released into the cage to oviposit overnight, and a cotton ball saturated with 10% honey solution was placed in the middle of the cage to serve as food source for the moths. The eggs and egg batches on each plant were collected and counted the following day as described above. All treatments were replicated 10 times.

Wind tunnel bioassay

Behavioural responses of female FAW to volatiles from maize, millet, cassava, sweet potato, beans, groundnut, silverleaf desmodium and greenleaf desmodium

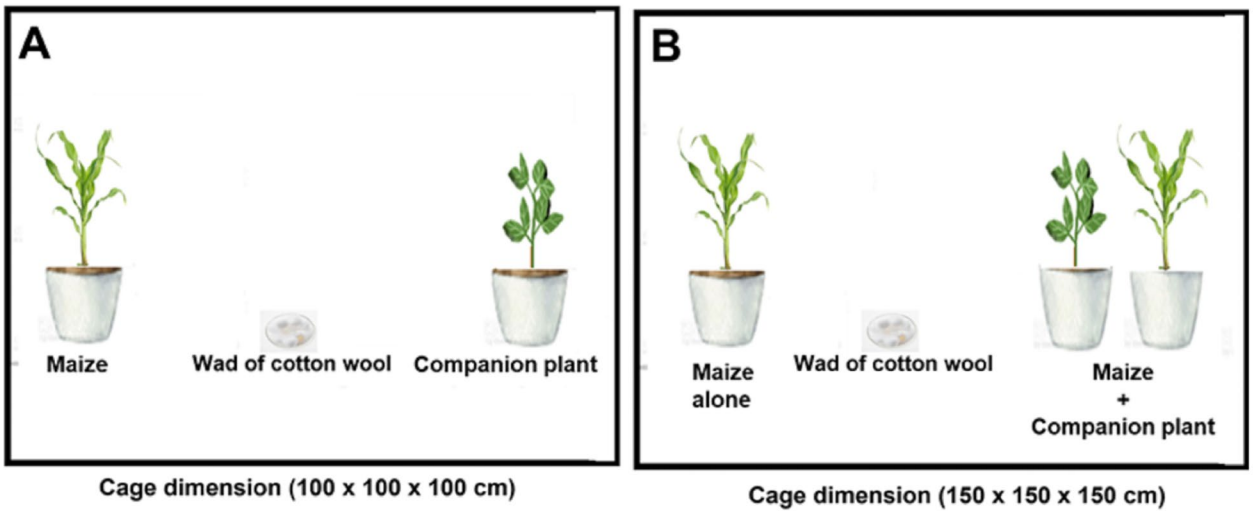


Fig. 1 Schematic representation of the FAW oviposition bioassay set up (**A**) dual choice test (**B**) multiple choice test

and control (air) were tested individually and in a combination with maize in a wind tunnel bioassay (122×32×32 cm, Analytical Research Systems, Gainesville, USA). The wind tunnel comprised a transparent glass on an aluminum frame, odour source ports and exhaust tubes [28]**.** Bioassays were conducted using 2–3-day-old gravid FAW moths. Newly emerged female moths were allowed to spend at least two nights in cages containing male FAW moths to ensure mating and females with enlarged abdomens carrying fully developed eggs were selected for the bioassay. Prior to running the wind tunnel assay, the moths were kept in the bioassay room for 1 h to acclimatize. Potted live plants were placed outside the wind tunnel as odour sources for the bioassay and avoid any visual cues bias. Volatiles from the experimental plants, enclosed with heat sterilized oven bags, were delivered into the wind tunnel chamber through the inlet ports at the upwind end of the wind tunnel at the rate of 300 mL min^{-1}. Gravid FAW moths were gently introduced individually into the wind tunnel through a side panel at the downwind end of the tunnel, 100 cm away from the odour source. The following behavioural parameters were recorded during a 5 min observation period: takeoff, wing fanning, walking, upwind flight (oriented flight to odour source), landing distance and the number of close visits to the odour source. Odour in the wind tunnel chamber was continuously exhausted (at 30 cm s^{-1}) out of the bioassay room through polyvinyl chloride (PVC) pipes fitted to a suction pump (50/60 Hz, 10A). All bioassays were conducted between 18:00 and 22:00 with reduced lighting provided by a red-light bulb (40 watts), positioned at 30 cm above the wind tunnel to provide uniform illumination of the wind tunnel. All tests were replicated 3 times with 20 insects per replicate making a total of 60 insects per treatment and each female was tested only once.

Olfactometer bioassay

Two sets of bioassays were conducted to investigate the response of *C. icipe* to constitutive plant-derived volatiles from maize, cassava, sweet potato, beans, groundnut, silverleaf desmodium, greenleaf desmodium and solvent [Dichloromethane (DCM)] control in a Perspex four-arm olfactometer as described previously by Tamiru et al. [12]. In the first experiment, a choice test compared *C. icipe* responses to constitutive volatiles from individual plants and control (solvent only). In this set up, the choice test was carried out by placing the test stimuli (10 µL aliquots of headspace volatile sample) in one arm, while the remaining three arms were the same volume of solvent (DCM) controls. The treatment stimulus was applied to a piece of filter paper (4×25 mm) using a micropipette (Drummond Scientific, Broomall, USA), and allowed to dry for 30 s, then the filter paper was placed in an inlet port at the end of each olfactometer arm. Thereafter, 2–3-day-old mated and experienced female *C. icipe,* i.e., exposed to odours from maize leaves damaged by FAW larvae [28], were transferred individually into the central chamber of the olfactometer using a custom-made piece of glass tubing. Air was drawn through the four arms towards the centre at 260 mL min^{-1}. Time spent and number of entries into different olfactometer regions were recorded and compared using Olfa software (F. Nazzi, Udine, Italy) for 12 min [33]. The olfactometer was rotated clockwise by 90° every 3 min to avoid positional choice bias. In the second experiment, a choice test was carried out, using a similar set up as described above, to compare insect responses to a combination of volatiles from maize with greenleaf desmodium, sweet potato, beans, cassava, groundnut, silverleaf desmodium against maize alone and solvent control. The two opposite arms held the test stimuli, i.e., 10 µL of maize + 10 µL of companion plants headspace volatiles in one arm and 20 µL of maize headspace sample in the opposite arm, while the remaining two arms were blank controls (solvent only). All tests were replicated 12 times and each female was tested in the olfactometer bioassay once only.

Collection of volatiles

Headspace volatiles from the experimental plants (3–4 weeks) were collected using a headspace sampling technique as described by Tamiru et al. [12]. Volatiles were collected from the test plants and control for 24 h starting at the last 2 h of photophase with four replications. Leaves of the test plants were gently enclosed inside polyethylene terephthalate (PET) oven bags (volume 3.2 L, ~ 12.5 mm thickness), heated to 150 ℃ for 1 h before use, and fitted with Swagelock inlet and outlet ports. Charcoal-filtered air was passed through the inlet port at the rate of 500 mL min^{-1}. Volatiles were trapped on Porapak Q (50 mg, 60/80 mesh; Supelco, Bellefonte, USA) packed in filters, preconditioned with dichloromethane before use, and inserted in the outlet port through which air was drawn at 300 mL min^{-1}. After entrainment, trapped volatiles were eluted with 0.5 mL DCM (analytical grade, Sigma-Aldrich, USA) into 2 mL sample vials (Agilent Technologies, Warsaw, Poland) and stored inside a freezer (− 80 °C) until required for bioassay and chemical analysis.

Analyses of volatiles

An aliquot (2 µL) of headspace samples from the experimental plants was analysed using an Agilent 7890A gas

chromatograph (GC) directly coupled to a mass spectrometer (MS) (MSD 5975C triple-axis, Agilent Technologies, Palo Alto, USA). The GC–MS was equipped with a non-polar capillary column (HP5-MSI, 30 m length × 0.25 mm i.d. × 0.25 µm film thickness) (J & W Scientific, Folsom, USA). Helium was used as a carrier gas at a flow rate of 1.2 mL min^{-1}. The oven temperature was maintained at 35 °C for 5 min and then programmed to increase at 10 °C min^{-1} to a final temperature of 280 °C and held for 10.5 min. The mass selective detector was maintained at an ion source temperature of 230 °C. Spectra was recorded at an electron impact of 70 eV, and an MS quadrupole temperature was maintained at 150 °C. Compounds were identified by comparing their mass spectra data with those of authentic standards, using reference databases (Adams2, Chemecol and NIST11) and retention indices of a mixture of n-alkanes (C8–C23). Tentative GC-MS identification of compounds was confirmed by co-injection with commercially available authentic standards. Quantification of the amounts (in nanogram) of identified volatile compounds was achieved using external calibration curves made from 1000 ng μL^{-1} stock solutions of the selected identified compounds (*Z*)-3-hexen-1-ol, α-pinene, (*E*)-β-caryophyllene with varying concentrations ranging from 1 to 500 ng/µL. The concentration of compounds was computed by extrapolating the peak area of the unknown against those of the known concentration and expressed in ng/plant/h. All peaks detected in the control (oven bag) were considered contaminants and, therefore, were not included in the volatile quantification. Data were analysed using MSD Chemstation software F.01.00.1903 (Agilent Technologies).

Chemicals

Authentic standards used for quantification and comparison of tentative GC–MS identification were (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, 2-heptanone, 2-heptanol, α-pinene, β-pinene, 1-octen-3-ol, β-myrcene, α-phellandrene, ρ-cymene, limonene, β-phellandrene, (*E*)-β-ocimene, terpinolene, linalool, methyl salicylate, β-elemene, (*E*)-β-caryophyllene, α-humulene (> 95% purity) were purchased from Sigma-Aldrich. Dichloromethane (99.9% purity) was purchased from Merck (Darmstadt, Germany).

Statistical analyses

All data analyses were performed using R statistical software [v 4.0.4; [34]]. The number of egg batches and eggs laid in both oviposition bioassays were not normally distributed (Shapiro–Wilk test: $p < 0.05$). Hence, we used the Mann–Whitney–Wilcoxon test to analyse the data collected from choice bioassays and subsequent bioassays to compare egg deposition between maize (alone) and maize combined with companion plants. Data on the behavioural response of female FAW to plant volatiles in the wind tunnel were analysed using a generalized linear model (GLM) with logistic regression and a Tukey post-hoc test. The parameters recorded were insect take-off, wing fanning, walking and oriented flight to odour source. Kruskal–Wallis tests followed by Dunn's post-hoc tests were used to analyse the data on the distance flown upwind and the number of visits to the odour source by the moths to the various treatments.

Cotesa icipe responses in the four-arm olfactometer choice tests were analysed using one-way ANOVA after converting the time spent data into proportions to address dependence of visiting time followed by $\log_{10}$-ratio transformation to allow the analysis of compositional data as described by Tamiru et al. [12] and Aitchison [35]. Significant means were separated using Student–Newman–Keuls (SNK) post hoc test ($P < 0.05$). The concentrations of volatile compounds emitted from the test plants were analysed using the non-parametric Mann–Whitney Wilcoxon (two treatments) and Kruskal–Wallis (multiple treatments) tests, because the data were not normally distributed (Shapiro–Wilk test: $p < 0.05$), followed by Dunn's post-hoc to separate means. To determine the relative contribution of different VOCs to the dissimilarity across the test plants, we subjected the peak areas of identified VOCs to similarity percentage analysis (SIMPER). The profile was visualised using the non-metric multidimensional scaling (NMDS) and the VOCs profiles of the eight test plants were compared using one-way ANOSIM with the Bray–Curtis dissimilarity matrix. Furthermore, we used Spearman's correlation analysis to elucidate the relationship between plant emitted volatiles and the behavioural activities of FAW (moths visit, landing distance and the number of eggs laid).

Results

Responses of FAW in oviposition assays

In experiment I, female FAW deposited eggs on all crop species tested, but significantly more egg batches and eggs were deposited on maize than on companion plants (Fig. 2A, B). In the dual choice oviposition bioassay, significantly more eggs were deposited on maize than on silverleaf desmodium ($W = 89.5$, $P = 0.002$), beans ($W = 11.5$, $P = 0.003$), cassava ($W = 10$, $P = 0.002$), greenleaf desmodium ($W = 23$, $P = 0.03$), groundnut ($W = 20.5$, $P = 0.025$) and sweet potato ($W = 94$, $P = 0.0007$) (Fig. 2A). A similar trend was recorded in the number of egg batches deposited on maize compared to the companion plants (Fig. 2B). However, there were no significant differences in the number of eggs ($W = 61$, $P = 0.42$) and egg batches

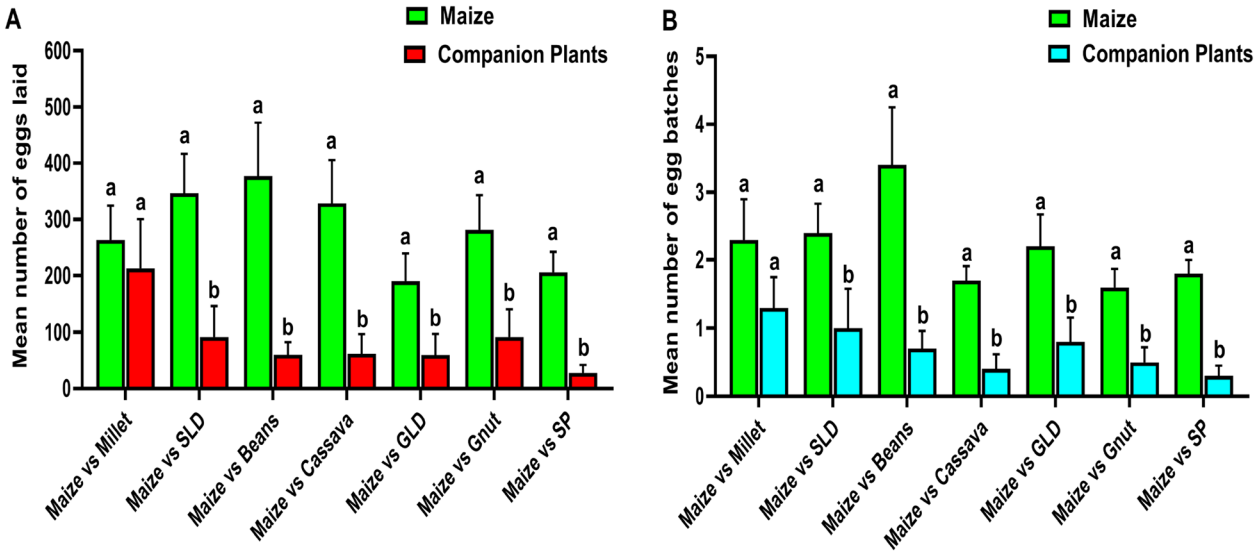


Fig. 2 Oviposition responses of gravid *Spodoptera frugiperda* moths to maize and companion plant species in a dual-choice test ($N=10$). Mean (± SE) number of (**A**) eggs and (**B**) egg batches laid on different plant species are shown. Treatments with similar letters above the bars are not significantly different. **SLD* silverleaf desmodium, *GLD* greenleaf desmodium, *Gnut* groundnut, *SP* sweet potato

($W=66$, $P=0.23$) deposited between maize and millet (Fig. 2A, B).

In experiment II, the number of eggs oviposited by gravid FAW moths on maize (alone) were significantly reduced when maize was combined with companion plants (Fig. 3). Significantly more eggs were laid on maize alone than on maize combined with beans ($W=12$, $P=0.004$, Fig. 3A), sweet potato ($W=8$, $P=0.001$, Fig. 3B), groundnut ($W=16.5$, $P=0.01$, Fig. 3C), silverleaf desmodium ($W=9$, $P=0.002$, Fig. 3E) and greenleaf desmodium ($W=1.5$, $P=0.003$, Fig. 3F). However, the number of eggs deposited on maize alone were not significantly different when maize was combined with cassava ($W=31$, $P=0.158$, Fig. 3D).

Significantly more egg batches were deposited on maize alone than on maize combined with beans ($W=15$, $P=0.007$, Fig. 4A), sweet potato ($W=21$, $P=0.03$, Fig. 4B) groundnut ($W=22$, $P=0.03$, Fig. 4C) and silverleaf desmodium ($W=14.5$, $P=0.006$, Fig. 4E). However, the number of egg batches deposited on maize alone were not significantly different from maize combined with cassava ($W=28.5$, $P=0.102$, Fig. 4D).

Responses of FAW in wind tunnel assays

In experiment I, with individual plant odour sources including control (clean air), maize volatiles elicited significantly more oriented upwind flight (Fig. 5A) from female FAW moths than volatiles from companion plants and control (GLM Likelihood Ratio (LR) $\chi^2=61.38$, $df=8$, $P<0.001$). Significantly more female moths flew upwind closer to odours from maize than companion plant species and control (Kruskal–Wallis $\chi^2=38.60$, $df=8$, $P<0.001$) (Fig. 5B). Furthermore, the moths landed significantly further up from the release point and closer to maize odour source (Fig. 5C) compared to greenleaf desmodium, sweet potato, beans, silverleaf desmodium, groundnut and control (Kruskal–Wallis $\chi^2=36.98$, $df=8$, $P<0.001$). Odours from the various experimental plants elicited significantly different behavioural responses in the female FAW moths including wing fanning (LR $\chi^2=36.75$, $df=8$, $P<0.001$, Fig. 5D), walking (LR $\chi^2=20.93$, $df=8$, $P=0.007$, Fig. 5E) and take-off flight (LR $\chi^2=20.71$, $df=8$, $P=0.008$, Fig. 5F).

In experiment II, maize odour elicited significantly less upwind oriented flights (Fig. 6A) from gravid FAW moths when presented with odours from beans, cassava, groundnut, sweet potato, groundnut and both *Desmodium* sp. compared to maize odour alone (LR $\chi^2=36.75$, $df=6$, $P<0.001$). Similarly, combining maize with other companion plant odours significantly reduced female FAW moth attraction and led to fewer closer flights to the odour sources (Kruskal–Wallis $\chi^2=47.31$, $df=6$, $P<0.001$, Fig. 6B). Furthermore, the forward landing distance of gravid moths from the release point (Fig. 6C) was significantly reduced when maize volatiles were presented in combination with companion plants compared to maize volatile alone (Kruskal–Wallis $\chi^2=49.07$, $df=6$, $P<0.001$). However, no significant differences were observed in the proportion of female moths that exhibited wing fanning (LR $\chi^2=5.53$, $df=6$, $P=0.48$, Fig. 6D),

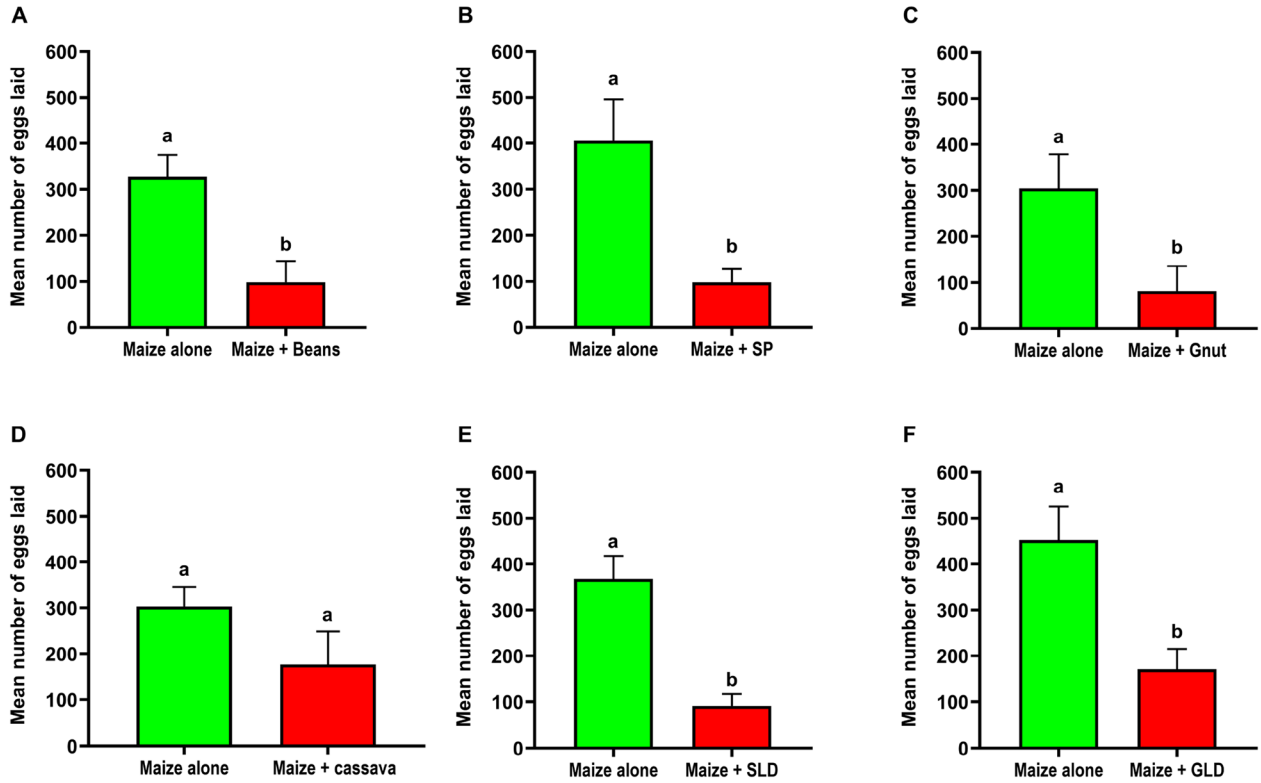


Fig. 3 Oviposition responses of gravid *Spodoptera frugiperda* moths to maize (alone) and maize combined with (**A**) beans (**B**) sweet potato (SP) (**C**) groundnut (Gnut) (**D**) cassava (**E**) silverleaf desmodium (SLD) and (**F**) greenleaf desmodium (GLD) in a multiple-choice test ($N = 10$). Mean (± SE) number of eggs deposited on different treatments is shown. Treatments with similar letters above the bars are not significantly different.

walking (LR $\chi^2 = 2.99$, $df = 6$, $P = 0.81$, Fig. 6E) and take-off flight behaviours (LR $\chi^2 = 11.91$, $df = 6$, $P = 0.06$, Fig. 6F) between treatments.

Responses of *C. icipe* in olfactometer assays

Behavioural responses of *C. icipe* to constitutive companion plant volatiles and solvent control (DCM) in a four-arm olfactometer are shown in Figs. 7 and 8. Female *C. icipe* spent significantly more time in the olfactometer region containing volatiles from greenleaf desmodium ($F_{(1, 46)} = 11.32$, $P = 0.002$), sweet potato ($F_{(1, 46)} = 25.70$, $P < 0.001$), beans ($F_{(1, 46)} = 7.393$, $P = 0.009$), cassava ($F_{(1, 46)} = 4.295$, $P = 0.044$), groundnut ($F_{(1, 46)} = 6.36$, $P = 0.015$) and silverleaf desmodium ($F_{(1, 46)} = 15.55$, $P < 0.001$) than solvent control. However, no significant differences were observed in the time spent by *C. icipe* between maize volatiles and solvent control ($F_{(1, 46)} = 0.619$, $P = 0.435$) (Fig. 7).

Interestingly, *C. icipe* showed significant attraction to odours from maize when combined with companion plant species (greenleaf desmodium, sweet potato, beans, groundnut, silverleaf desmodium) than to volatiles from maize (alone) and solvent control (Fig. 8). Female *C. icipe* parasitoids spent significantly more time in the olfactometer arm containing volatiles from maize combined with greenleaf desmodium ($F_{(2, 45)} = 10.84$, $P < 0.001$), sweet potato ($F_{(2, 45)} = 3.66$, $P = 0.03$), beans ($F_{(2, 45)} = 5.788$, $P = 0.005$), groundnut ($F_{(2, 45)} = 14.57$, $P < 0.001$) and silverleaf desmodium ($F_{(2, 45)} = 18.56$, $P < 0.001$) compared to olfactometer arms with maize (alone) volatiles and solvent control. In contrast, there was no significant difference in *C. icipe* response to volatiles of maize combined with cassava, maize alone and solvent control ($F_{(2, 45)} = 0.648$, $P = 0.528$) (Fig. 8D).

Analyses of volatiles

GC–MS analysis of headspace volatiles from companion plants detected a total of 48 compounds belonging to seven chemical classes, namely, aldehyde [1], alcohols [3], ketones [2], monoterpenes [14], esters [3], homoterpenes (2), and sesquiterpenes [23] (Table 1 and Fig. 9). Heatmap clustering revealed quantitative and qualitative variations in volatile emissions from the test plants (Fig. 9A). The monoterpene (*E*)-β-ocimene was the most abundant VOC identified in silverleaf and greenleaf desmodium (Kruskal–Wallis $\chi^2 = 18.36$, $df = 5$, $P = 0.002$) followed by (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) (Kruskal–Wallis $\chi^2 = 14.23$, $df = 5$, $P = 0.01$) and (*E*)-β-caryophyllene

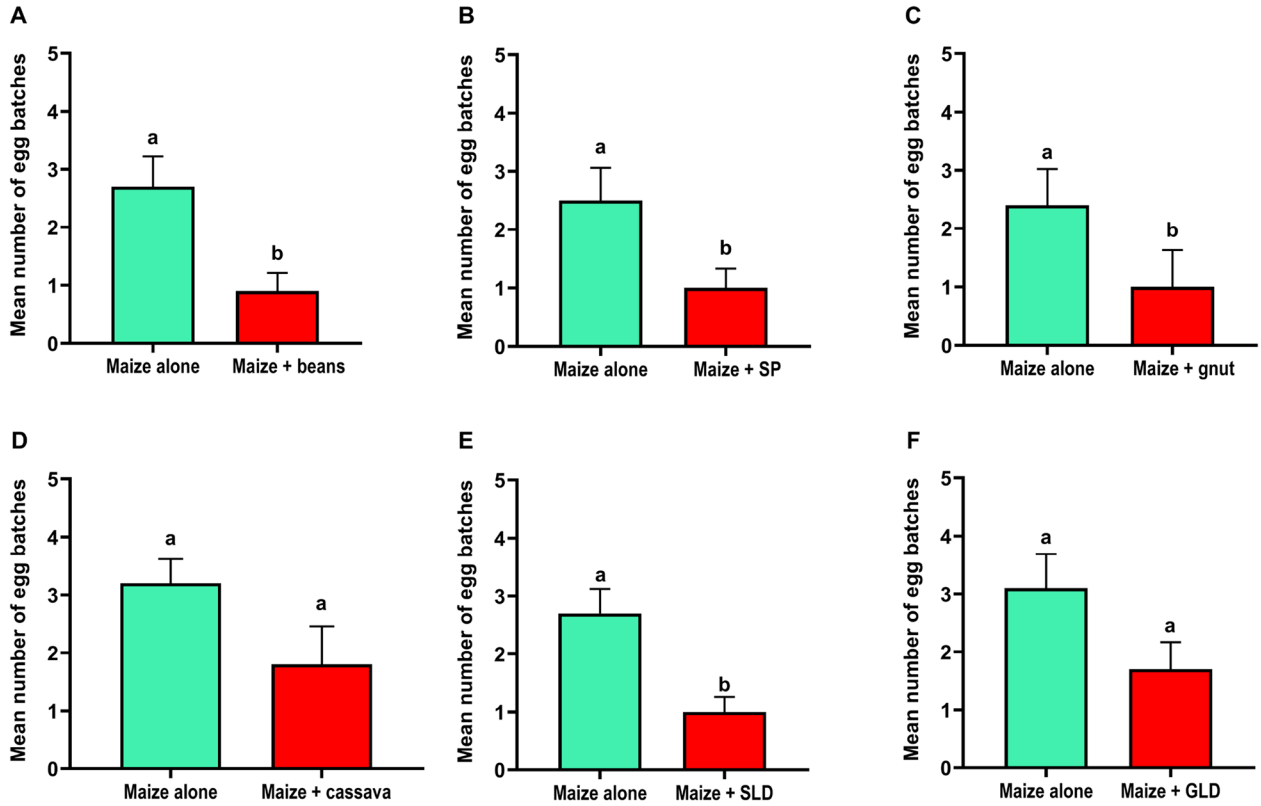


Fig. 4 Oviposition responses of gravid *Spodoptera frugiperda* moths to maize alone and maize combined with (**A**) beans (**B**) sweet potato (**C**) groundnut (**D**) cassava (**E**) silverleaf desmodium and (**F**) greenleaf desmodium in a multiple-choice test ($N = 10$). Mean (± SE) number of egg batches deposited on different treatments is shown. Treatments with similar letters above the bars are not significantly different. **SLD* silverleaf desmodium, *GLD* greenleaf desmodium, *Gnut* groundnut, *SP* sweet potato

(Kruskal–Wallis $\chi^2 = 14.56$, $df = 5$, $P = 0.01$) (Table 1). The three compounds were also detected from other companion plant species, but in relatively lower amounts than the volatiles released by desmodium spp. VOCs that were detected in companion plants but not detected or found in trace amounts in the main crop (maize) included (*E*)-2-hexenal, 1-octen-3-ol, 3-octanone, methyl salicylate (MeSA), β-selinene and (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), and hence could be of potential biological relevance. Interestingly, these compounds including DMNT were not detected in cassava except for (*E*)-2-hexenal. The monoterpenoids camphor and limonene were the main VOCs identified in headspace collection from beans, with likely impact on FAW and *C. icipe* behaviour.

Mapping volatile organic compounds using non-metric multidimensional scaling plot (NMDS) clustering demonstrated significant variation in volatile composition between the test plants (ANOSIM: $P = 0.0001$, $R = 0.85$) (Fig. 9B). Based on analysis of similarities (ANOSIM) the following compounds, namely, (*E*)-*β*-ocimene (21%), (*E*)-β-caryophyllene (15%), DMNT (10%), (*Z*)-3-hexenyl acetate (10%), TMTT (7%), camphor (7%), limonene (6%), (*Z*)-3-hexen-1-ol (4%), germacrene D (4%) and δ-cadinene (4%) contributed for most of the differences between the test plants (Fig. 9C and Additional file 1: Fig. S1).

To determine the role of plant volatile influence on the behavioural responses of FAW, we performed a correlation analysis between plant emitted volatiles and FAW moth activities such as moth visits (number of approaches), landing distance and number of eggs laid across test plants. To achieve this, we focused on predominant VOCs identified based on the analysis of similarities (ANOSIM) (Fig. 9). The result showed a significant negative correlation between the following VOCs (*E*)-*β*-ocimene ($r_s = -0.47$, $P = 0.02$), (*Z*)-3-hexenyl acetate ($r_s = -0.36$, $P = 0.01$), TMTT ($r_s = -0.82$, $P < 0.001$), (*Z*)-3-hexen-1-ol ($r_s = -0.67$, $P < 0.001$) and the number of moth visits to test plants (Table 2). In addition, there was a significant negative correlation between (*E*)-*β*-ocimene ($r_s = -0.43$,

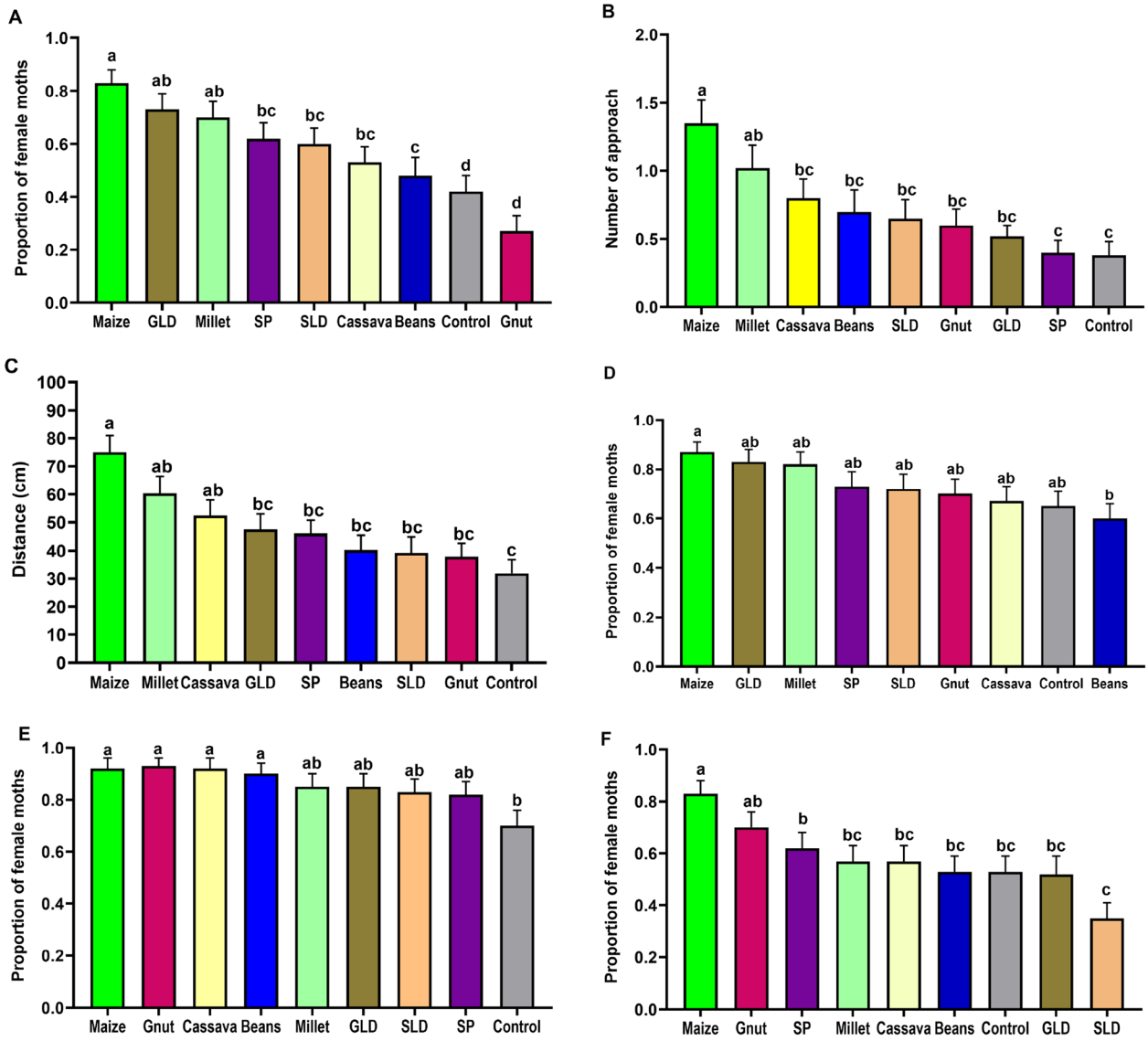


Fig. 5 Behavioural responses of gravid *Spodoptera frugiperda* moths in wind tunnel assays (**A**) oriented (upwind) flight (**B**) number of visits to odour source (**C**) landing distance from the release point (**D**) take off flight (**E**) walking and (**F**) wing fanning in a wind tunnel to volatiles emitted by individual test plants and control (clean air) (N = 60). Means (± SE) with similar letter (s) above the bars are not significantly different. **SLD* silverleaf desmodium, *GLD* greenleaf desmodium, *Gnut* groundnut, *SP* sweet potato

$P = 0.003$), DMNT ($r_s = -0.48$, $P = 0.004$), (*Z*)-3-hexenyl acetate ($r_s = -0.40$, $P = 0.001$) and landing distance of the moths, whereas a positive significant correlation was observed when compared with germacrene D ($r_s = 0.61$, $P < 0.001$) (Table 2). Moreover, we observed a significant negative correlation between TMTT ($r_s = -0.57$, $P = 0.003$), limonene ($r_s = -0.53$, $P = 0.002$) and number of FAW eggs laid (Table 2).

Discussion

Olfaction plays a crucial role in locating and discriminating between preferred host plant and non-host plants by phytophagous insects. Our results demonstrated that maize is a more preferred host plant for FAW oviposition than other tested crops confirming previous findings [3]. The presence of companion plants significantly reduced FAW oviposition on maize. This demonstrates the impact of the companion plants in disrupting olfactory responses of gravid FAW moths leading to less egg deposition, and subsequently reduced infestation and damage

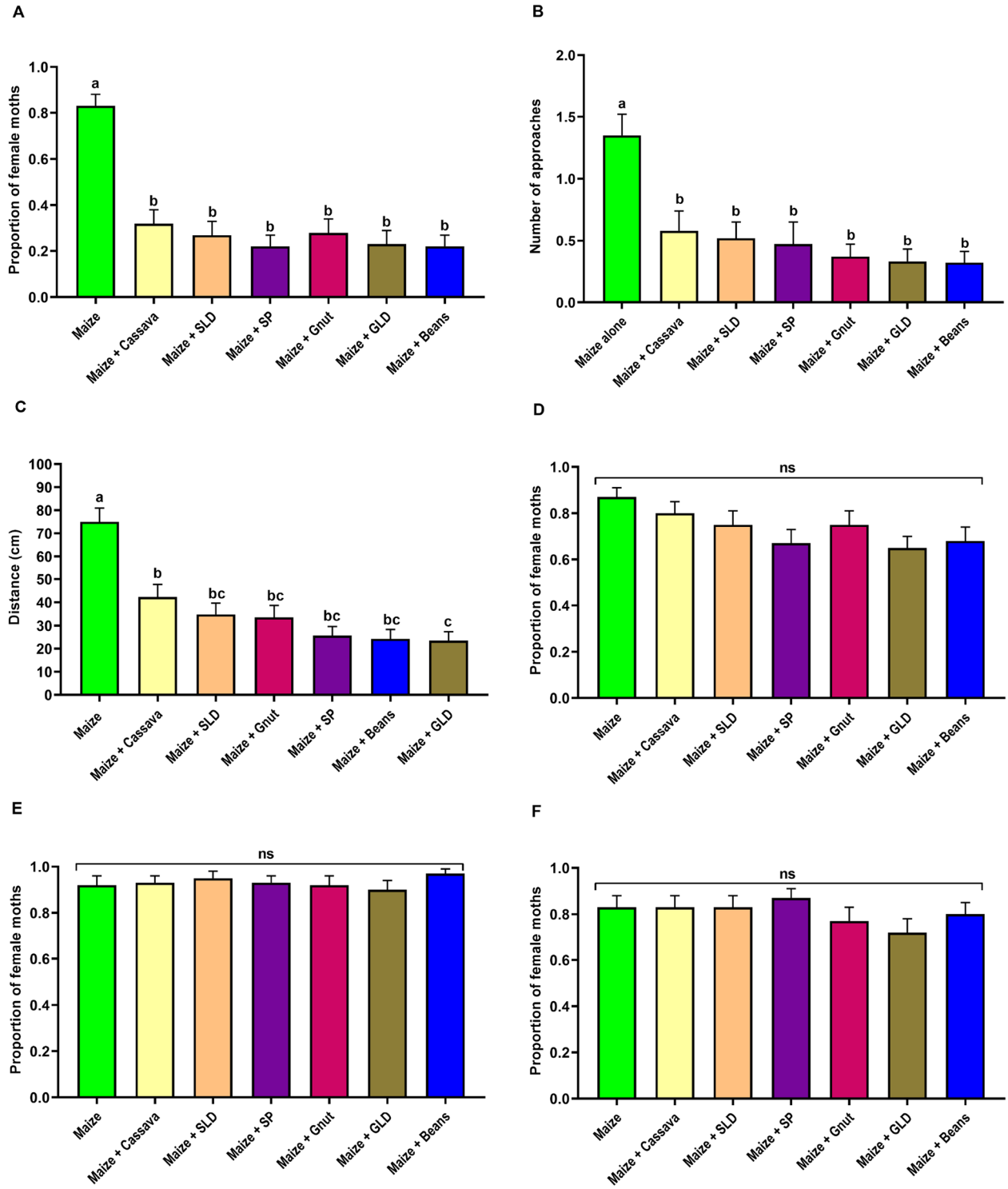


Fig. 6 Behavioural responses of gravid *Spodoptera frugiperda* moths in wind tunnel assays (**A**) oriented (upwind) flight (**B**) number of visits to odour source (**C**) landing distance from the release point (**D**) take off flight (**E**) walking and (**F**) wing fanning in a wind tunnel bioassay to volatiles emitted by maize alone and maize combined with companion plant species ($N = 60$). Means (± SE) with similar letter (s) above the bars are not significantly different. **SLD* silverleaf desmodium, *GLD* greenleaf desmodium, *Gnut* groundnut, *SP* sweet potato. *ns* no significance difference

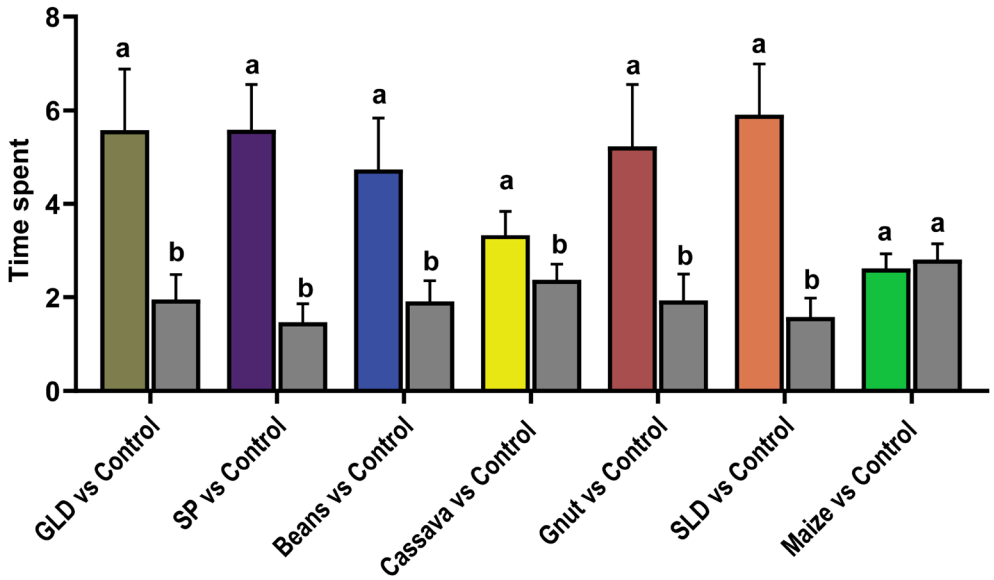


Fig. 7 Behavioural response of *Cotesia icipe* females to constitutive headspace volatiles from greenleaf desmodium (GLD), sweet potato (SP), beans, cassava, groundnut (Gnut), silverleaf desmodium (SLD), maize and solvent control (DCM) in a four-arm olfactometer. Time spent by each parasitoid was observed for 12 min ($N = 12$). Means (± SE) with similar letter (s) above the bars are not significantly different

by hatching larvae of the pest. Our findings support the results from previous studies which reported decreased FAW infestation in maize fields intercropped with companion plant species compared to sole maize cropping [24, 25, 28, 37]. Intriguingly, the presence of cassava did not affect the egg laying responses of FAW, implying odours released by cassava plants may not influence oviposition behaviour of the pest. These results support the findings by Nwanze et al. [29], who reported an extreme scenario, where the presence of cassava in maize-cassava intercropping system encouraged FAW oviposition and subsequent feeding on maize plants in Niger Delta Region.

Significant upwind flight and closer landing by FAW moths to odour sources from maize plants compared to companion plant species in the wind tunnel bioassay suggest attractiveness of maize volatiles. However, the attractiveness of maize volatiles was significantly reduced in the presence of companion plant volatiles resulting in decreased oriented (upwind) flight of FAW moths. Ovipositional preferences by insects are often modulated by chemical differences between the host plant volatiles [3, 28, 37]. Our research results corroborate the presence of FAW oviposition deterrents in the volatile chemistry of neighbouring companion plants, since the experimental plants were positioned outside the wind tunnel to avoid influence of any visual and tactile cues. The FAW moths were subjected to only naturally emitted headspace volatiles delivered through the inlet ports of the wind tunnel bioassay set up. Moreover, the findings explain the reason behind the significantly lower number of eggs deposited by gravid FAW females on maize when combined with the companion plants. Several studies reported the repellent and masking effects of companion plant odours, disrupting the host-seeking abilities of crop pest, as shown in the current study [1, 13, 28, 38–43]. Our results contrast with an inconclusive report by Rojas et al. [44] who claimed that FAW females do not rely on plant volatiles for orientation to host plants but who also suggested a more precise experiments under natural conditions to verify their results. Our findings from complementary oviposition and wind tunnel experiments provide clear evidence on the vital role plant volatiles play in the host-selection process of the FAW moths. Moreover, the nocturnal behaviour of FAW moth (active during night), necessitates the pest to rely more on olfactory cues to locate its preferred host plant for egg deposition than other cues, such as visual. Moreover, our results agree with the findings from several studies that have established the chemical basis for host-location behaviour of several lepidopterous insects [28, 45–48].

In contrast to their effects on FAW, constitutive volatiles from the companion test plants were attractive to the larval endoparasitoid *C. icipe,* one of the key biological control agents of FAW in the region. Furthermore, the parasitoids were more attracted to odours from maize mixed with sweet potato, beans, groundnut, greenleaf desmodium and silverleaf desmodium than volatiles from maize plant alone. The attraction of *C. icipe* is of interest as the parasitic wasp provides effective biological control

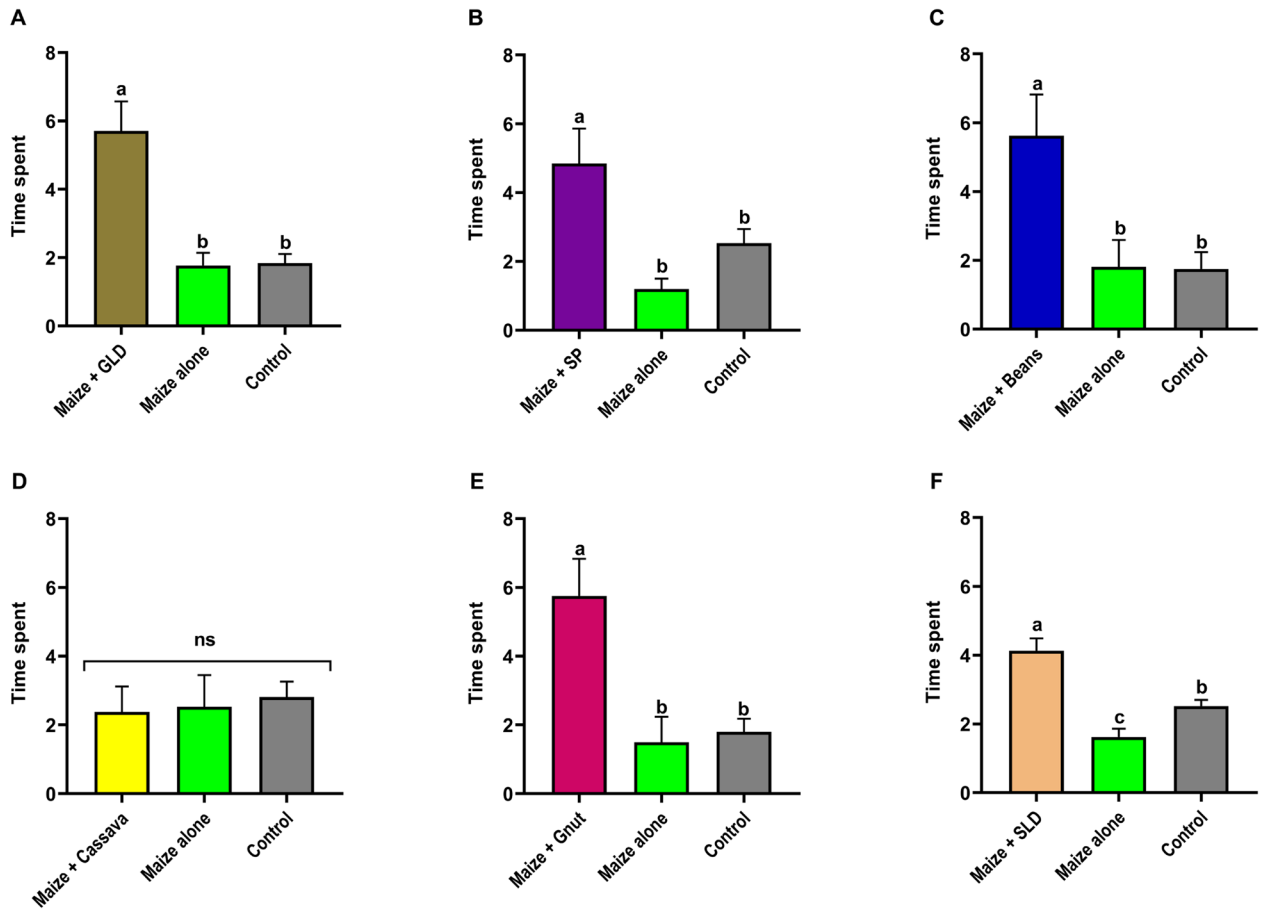


Fig. 8 Behavioural response of *Cotesia icipe* females to constitutive volatiles from maize (alone) and maize combined with greenleaf desmodium (GLD), sweet potato (SP), beans, cassava, groundnut (Gnut), silverleaf desmodium (SLD), and solvent control in a four-arm olfactometer. Time spent by each parasitoid was observed for 12 min ($N = 12$). Means (± SE) with similar letter (s) above the bars are not significantly different. **ns* no significance difference

against FAW [49]. This is an additional ecological benefit of crop diversification system, where plant damage and infestation are reduced through enhanced ecosystem service provided by natural enemies. Diversified crop fields have been shown to enhance foraging behaviour and abundance of natural enemies such as parasitoids which suppress pest population [27, 50]. Increasing number of studies reported enhanced activities of beneficial insects (parasitoids and predator) in a diversified cropping system leading to arthropod pests suppression under field conditions [14, 18, 51].

Chemical analysis of headspace samples revealed qualitative and quantitative variation in volatile profiles of companion plants. Some of the predominant volatiles identified include (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene, DMNT, camphor, (*E*)-β-caryophyllene, and TMTT. Our correlation results further demonstrated significant negative relationship between the companion plant VOCs, such as (*E*)-*β*-ocimene, (*Z*)-3-hexenyl acetate, TMTT, (*Z*)-3-hexen-1-ol, DMNT and limonene and FAW activity, implying the repellent effect of these volatiles on the pest. A recent study by Sobhy et al. [28] demonstrated that these compounds elicit electrophysiological and behavioural responses in FAW and its parasitoid (*C. icipe*). The same compounds have also been reported to be produced by maize plants when attacked by herbivores and shown to play a defence role by repelling pests and recruiting their natural enemies [33, 53–56]. Therefore, it is rational to attribute the FAW repellence effects and attraction of *C. icipe* parasitoid to these compounds emitted by the companion plants. Previous studies by Khan et al. [57, 58] and Pickett et al. [59] have documented the emission of herbivore-induced compounds, such as (*E*)-β-ocimene, (*E*)-β-caryophyllene and (*E*)-4,8-Dimethyl-1,3,7-nonatriene (DMNT), by an intact *Melinis minutiflora* and *Desmodium* sp. used in push–pull companion cropping system. Moreover, intercropping these forage crops with maize, repelled insect pests

Table 1 Mean amount (ng/plant/h) of volatile organic compounds (VOcs) identified in the headspace collections of intact test plants ($n = 4$)

No	RT(Min)	Compound[1]	RI_{alk}[2]	RI_L[3]	Maize	Millet	Sweet potato	Groundnut	Cassava	SLD	GLD	Beans	*P* value[4]
1	8.05	(*E*)-2-Hexenal*	861	856	nd	nd	nd	nd	21.84 ± 0.49^{b}	26.48 ± 0.32^{a}	nd	nd	0.02
2	8.12	(*Z*)-3-Hexen-1-ol*	863	860	26.3 ± 1.28^{bc}	nd	27.4 ± 4.24^{bc}	26.6 ± 2.1^{bc}	23.66 ± 1.07^{c}	57.62 ± 12.14^{a}	36.7 ± 3.12^{ab}	nd	0.01
3	8.93	2-Heptanone*	894	895	24.99 ± 2.62	nd	nd	nd	nd	nd	nd	nd	–
4	9.16	2-Heptanol*	904	903	23.93 ± 1.44	nd	nd	nd	nd	nd	nd	nd	–
5	9.82	α-Pinene*	935	934	24.44 ± 2.44	26.98 ± 3.83	27.04 ± 1.69	23.09 ± 0.31	22.09 ± 0.41	27.22 ± 1.9	30.82 ± 9.03	19.88 ± 2.07	0.14
6	10.67	Sabinene	975	974	nd	22.18 ± 0.4	23.23 ± 1.82	22.06 ± 0.11	nd	nd	nd	nd	0.19
7	10.71	β-Pinene*	977	978	nd	21.32 ± 0.15^{ab}	23.75 ± 0.85^{a}	21.17 ± 0.13^{ab}	nd	nd	nd	18.36 ± 1.57^{b}	0.005
8	10.82	1-Octen-3-ol*	982	981	nd	nd	nd	25.29 ± 1.61	nd	37.43 ± 8.79	35.02 ± 9.97	nd	0.47
9	10.98	3-Octanone	989	984	nd	nd	nd	nd	nd	nd	33.86 ± 8.74	nd	–
10	11.01	2,3-Dehydro-1,8-cineole	991	986	nd	22.38 ± 0.84	nd	nd	nd	nd	nd	nd	–
11	11.03	β-Myrcene*	992	992	22.81 ± 0.37	nd	23.47 ± 0.36	nd	nd	nd	nd	nd	0.11
12	11.29	α-Phellandrene	1005	1005	nd	21.68 ± 0.3	nd	nd	nd	nd	nd	nd	–
13	11.36	(*Z*)-3-Hexenyl acetate	1009	1007	26.67 ± 2.9	nd	nd	60.68 ± 8.87	nd	nd	32.54 ± 12.07	nd	0.08
14	11.66	ρ-Cymene*	1026	1020	nd	nd	nd	24.19 ± 0.95	21.92 ± 0.38	nd	nd	nd	0.20
15	11.73	Limonene*	1029	1030	31.33 ± 6.6	21.38 ± 0.32	41.04 ± 5.66	25.06 ± 0.18	21.8 ± 0.32	nd	38.27 ± 16.09	27.66 ± 4.12	0.12
16	11.76	β-Phellandrene*	1031	1032	nd	24.55 ± 2.92	nd	nd	nd	nd	nd	nd	–
17	11.78	1,8-Cineole	1032	1036	nd	22.04 ± 0.34	nd	25.58 ± 1.27	nd	nd	nd	nd	0.20
18	12.09	(*E*)-β-Ocimene*	1050	1050	nd	27.86 ± 6.43^{b}	23.9 ± 1.39^{b}	47.66 ± 3.62^{ab}	38.97 ± 9.81^{ab}	202.5 ± 35.81^{a}	153.08 ± 28.67^{a}	nd	0.002
19	12.80	Terpinolene*	1089	1090	nd	nd	21.08 ± 0.12	nd	nd	nd	nd	nd	–
20	12.99	Linalool*	1101	1101	30.01 ± 5.76	23.17 ± 1.51	nd	25.39 ± 2.76	nd	41.77 ± 4.23	nd	nd	0.06
21	13.26	DMNT	1117	1116	40.01 ± 8.06^{ab}	26.48 ± 2.21^{b}	36.91 ± 5.27^{ab}	68.24 ± 6.19^{a}	nd	97.8 ± 21.61^{a}	57.79 ± 7.25^{a}	nd	0.01
22	13.77	Camphor	1148	1146	nd	nd	nd	nd	nd	nd	nd	45.26 ± 8.37	–
23	14.62	Methyl salicylate*	1202	1199	nd	nd	nd	51.38 ± 10.79	nd	nd	23.2 ± 9.37	nd	0.11
24	15.93	Lavandulyl acetate	1293	1289	33.11 ± 4.58	nd	nd	nd	nd	nd	nd	nd	–
25	16.85	α-Longipinene	1360	1352	nd	nd	nd	nd	21.81 ± 0.58	nd	nd	nd	–
26	17.07	Cyclosativene	1377	1369	55.25 ± 8.02	nd	nd	nd	nd	nd	nd	nd	–
27	17.15	Longicyclene	1382	1376	nd	nd	nd	nd	40.8 ± 5.4	nd	nd	nd	–
28	17.16	α-Ylangene	1383	1382	nd	27.33 ± 1.51	23.61 ± 2.61	nd	nd	nd	17.73 ± 2.21	nd	0.14
29	17.17	α-Copaene	1384	1384	67.27 ± 10.88^{a}	nd	22.77 ± 0.78^{b}	nd	nd	nd	nd	nd	0.02
30	17.37	β-Elemene*	1398	1397	28.84 ± 5.63	nd	35.99 ± 2.89	nd	nd	nd	nd	nd	0.34
31	17.64	Longifolene	1419	1406	nd	nd	nd	nd	26.67 ± 0.95^{a}	nd	nd	16.87 ± 1.17^{b}	0.02
32	17.73	α-Cedrene	1426	1424	nd	nd	nd	nd	nd	nd	nd	15.93 ± 0.68	–
33	17.80	(*E*)-β-Caryophyllene*	1432	1430	83.37 ± 4.68^{a}	25.29 ± 3.52^{b}	79.22 ± 26.07^{a}	nd	29.97 ± 2.92^{b}	81.34 ± 11.8^{a}	89.04 ± 10.01^{a}	nd	0.01
34	17.92	(*E*)-α-Bergamotene	1442	1451	25.35 ± 2.22	nd	34.38 ± 2.44	28.96 ± 4.86	nd	nd	nd	nd	0.19
35	18.22	α-Humulene*	1466	1465	32.04 ± 3.2	27.32 ± 6.24	30.78 ± 3.55	nd	22.47 ± 1.11	24.02 ± 1.44	21.81 ± 0.58	20.61 ± 2.93	0.10

Table 1 (continued)

No	RT(Min)	Compound[1]	RI_{alk}[2]	RI_L[3]	Maize	Millet	Sweet potato	Groundnut	Cassava	SLD	GLD	Beans	*P* value[4]
36	18.31	β-Chemigrene	1473	1476	nd	nd	22.67 ± 0.84	nd	nd	nd	nd	nd	–
37	18.34	β-Selinene	1475	1475	nd	nd	nd	nd	nd	30.59 ± 7.45	nd	nd	–
38	18.39	γ-Gurjunene	1479	1477	nd	nd	nd	nd	nd	nd	26.59 ± 4.82	nd	–
39	18.58	Germacrene D	1494	1490	39.91 ± 8.55	25.79 ± 4.55	48.35 ± 6.04	nd	26.37 ± 3.28	nd	nd	nd	0.07
40	18.62	α-Selinene	1498	1498	nd	nd	34.81 ± 1.48	nd	nd	33.14 ± 9.79	nd	nd	0.34
41	18.79	Isodaucene	1512	1500	nd	nd	42.95 ± 8.09	nd	nd	nd	nd	nd	–
42	18.82	α-Muurolene	1514	1502	25.22 ± 3.93	nd	nd	nd	nd	nd	nd	nd	–
43	18.96	γ-Cadinene	1525	1513	23.21 ± 1.15[b]	66.11 ± 4.61[a]	nd	24.41 ± 0.82[b]	nd	nd	nd	nd	0.02
44	19.09	δ-Cadinene	1536	1525	46.66 ± 9.97	32.47 ± 11.07	33.19 ± 1.33	nd	nd	nd	nd	nd	0.27
45	19.52	unknown	1574	–	38.34 ± 7.28	24.75 ± 2.77	27.42 ± 2.55	35.43 ± 5.59	nd	nd	29.73 ± 5.48	nd	0.46
46	19.67	TMTT	1585	1584	nd	nd	58.54 ± 8.03[ab]	35.51 ± 5.18[b]	nd	nd	61.6 ± 2.58[a]	nd	0.01
47	19.84	Caryophyllene oxide	1599	1588	nd	nd	39.34 ± 8.61	nd	nd	nd	nd	nd	–
48	20.12	Epi-cedrol	1623	1625	nd	nd	nd	nd	nd	nd	nd	17.91 ± 2.09	–

[1] Tentative identification of volatile organic compounds (VOCs) was done by comparing their mass spectra with those from the authentic standards where available, mass spectra databases (Adams2, Chemecol and NIST11) and the online NIST Chemistry Webbook as well as retention index (KI). *Indicates compounds confirmed with authentic standards

[2] Retention index relative to C_8–C_{23} n-alkanes on HP-5MS capillary column

[3] Retention index obtained from literature [36]

[4] *P* value of non-parametric Kruskal–Wallis and two sample Wilcoxon tests for comparing amounts of volatile organic compounds between the test plants. Means (±SE) with different superscript letter(s) within the rows are significantly different ($P < 0.05$)

nd not detected, *SLD* Silverleaf desmodum, *GLD* Greenleaf desmodium, *DMNT* (*E*)-4,8-Dimethyl-1,3,7-nonatriene, *TMTT* (*E, E*)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene

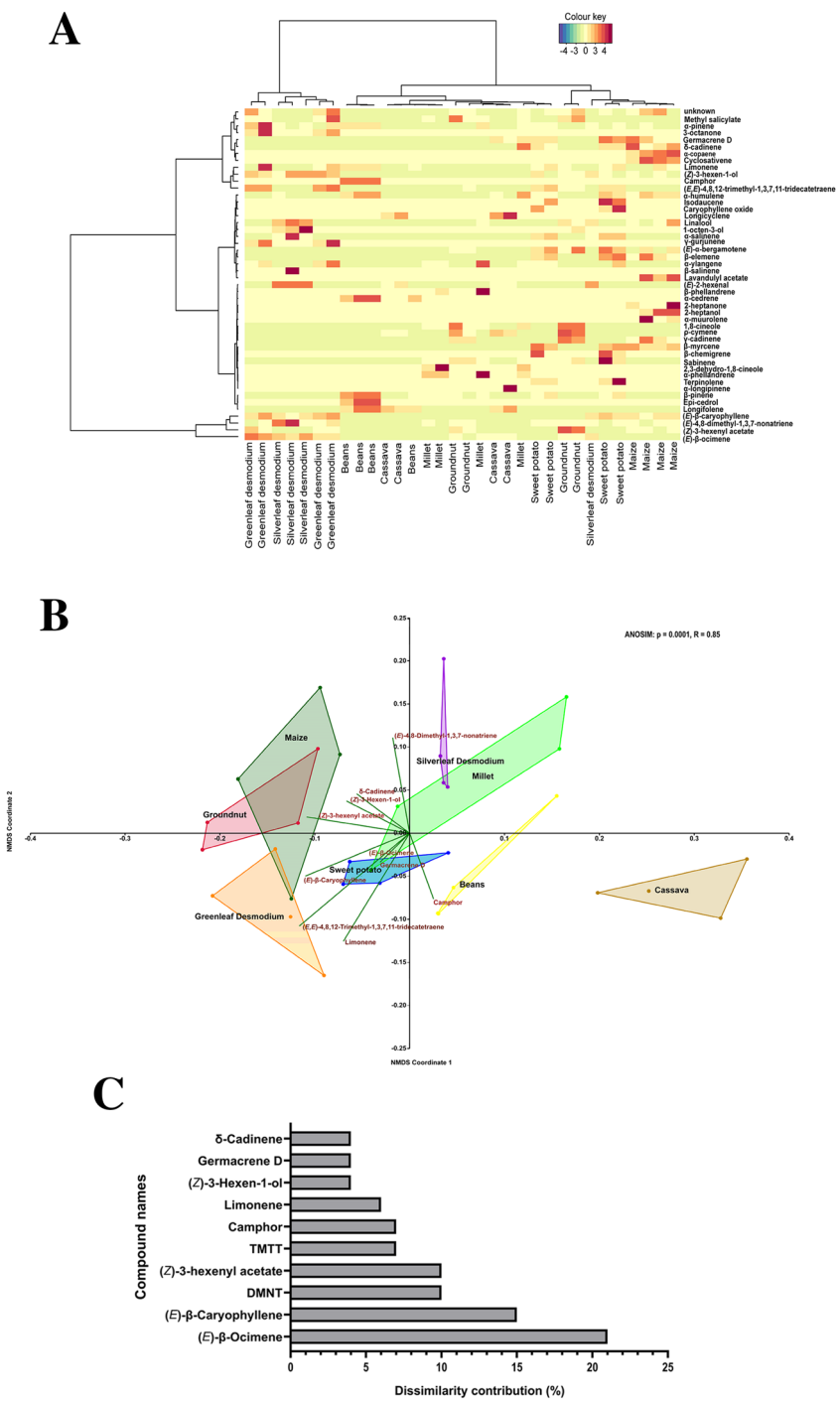


Fig. 9 Variation in volatile organic compounds identified (**A**) Heatmap clustering depicting the abundance of volatiles identified across replicates of 8 tested plants (**B**) Non-metric multidimensional scaling (distance Bray–Curtis; Stress value = 0.21) clustering showing differences in volatile patterns between test plants (**C**) Histogram depicting the percent contribution of the predominant volatiles from the 8 test plants based on analysis of similarities. *DMNT* (*E*)-4,8-Dimethyl-1,3,7-nonatriene, *TMTT* (*E, E*)-4,8,12-Trimethyl-1,3,7,11-tridecatetraen

while simultaneously recruiting more natural enemies (parasitoids) to the intercrop system compared to maize monocrop [27]. In our study, the volatile compounds (*E*)-β-ocimene, DMNT and (*E*)-β-caryophyllene were emitted in relatively large amounts by companion plants silverleaf and greenleaf desmodium while in a relatively

Table 2 Spearman's correlation analysis between the fall armyworm behavioural activity and selected predominant VOCs across test plants based on Analysis of similarities (ANOSIM)

Volatile compound	FAW behavioral activities					
	Moth visits		Landing distance		No. of eggs Laid	
	[1]r_s	[2]P	[1]r_s	[2]P	[1]r_s	[2]P
(*E*)-*β*-ocimene	− 0.47	0.02*	− 0.43	0.003**	− 0.03	0.90
(*E*)-β-caryophyllene	− 0.14	0.18	0.54	0.30	− 0.05	0.36
DMNT	− 0.33	0.06	− 0.48	0.004**	0.37	0.59
(Z)-3-hexenyl acetate	− 0.36	0.01*	− 0.40	0.001**	0.08	0.65
TMTT	− 0.82	< 0.001***	− 0.11	0.26	− 0.57	0.003**
camphor	0.21	0.11	− 0.20	0.83	− 0.20	0.97
limonene	− 0.43	0.16	0.35	0.11	− 0.53	0.002**
(*Z*)-3-hexen-1-ol	− 0.67	< 0.001***	− 0.29	0.06	− 0.14	0.39
germacrene D	0.08	0.37	0.61	< 0.001***	− 0.11	0.56

[1] Spearman's correlation coefficient (r_s) for respective parameters compared'

[2] Spearman rank-order correlations *P* values (*P*); statistically significant correlation is given in asterisks (*** for $P < 0.001$, ** for $P < 0.01$, * for $P < 0.05$)

lower amount in other companion plant species. Sometimes, lower volatile emissions could elicit a potent behavioural response in insects if the bioactive compounds are present in the right blend [60]. It was also interesting to note that most of the prominent bioactive compounds such as (*E*)-β-ocimene and DMNT were not detected in beans, though similar behavioural effects were exhibited due to the crop. The defence responses in beans could be attributed to the relatively large emission of camphor, which has been reported to have a repellent effect on most insects [61]. Other volatile compounds present in the companion plants but were not detected or found in trace amounts in the intact maize plant include (*E*)-2-hexenal, 1-octen-3-ol, 3-octanone, methyl salicylate, β-selinene and TMTT. These compounds have been reported to have ecological importance by mediating plant defence responses either as a repellent, or deterrent to herbivores and attraction of their natural enemies [28, 48, 62, 63]. Germacrene is among the volatile compounds emitted by maize plant and not detected or found in trace amount in most companion plants except in cassava and sweet potato. A significant positive correlation between the VOC Germacrene and closer FAW landing distance to maize volatiles may suggest the role of the compound in FAW attraction towards maize. Germacrene has been reported to increase attraction and oviposition by the tobacco budworm moth *Heliothis virescens* [64]. Proper knowledge about the correlation between the variations in plant derived VOCs and insect behavioural responses is particularly important to understand chemical ecology mechanisms of plant–insect interactions [65]. Our study provides insights not only into the correlation between the VOCs profiles of the various edible companion plants and behavioural responses of FAW and its parasitoid natural enemy but also paves a way for exploiting the knowledge for designing a robust pest management strategy against damaging crop pests, such as FAW. There is a great potential to improve crop yield by designing effective, affordable, and ecologically sustainable pest management strategies, such as crop diversification [14, 66].

Conclusions

There is an urgent need to redesign effective agroecological pest management strategies alternative to pesticide-intensive monoculture crop production. However, this requires a better understanding of the ecological interactions between the crops, their pests, and the natural enemies of the pests. Our findings provided evidence supporting associational resistance conferred by companion plant volatiles to the main (maize) crop. Constitutively emitted volatiles by certain companion plant had a repellent effect on FAW leading to decreased host attractiveness to FAW oviposition and fewer egg depositions on maize when combined with the companion plants. Moreover, the heterospecific plant association enhanced the attraction of the key pest's natural enemy, *C. icipe* parasitoid. A correlation analysis between the VOCs emitted by different companion plant and FAW activities demonstrated the role of plant volatiles in modifying the behavioural responses of FAW. This was further supported by volatile analyses results and a series of complementary bioassays. Exploiting defense VOCs delivered cheaply through companion plants may provide an affordable, ecologically sound, and sustainable way of protecting crops from destructive pests, such as the FAW. Our

study not only provides the scientific insights to properly understand the chemical ecology of FAW–maize plant–*C. icipe* interactions but also demonstrate the potential of using different edible companion plant species for FAW management in maize-based cropping system. Some of the companion crops with strong pest repellent and natural enemy enhancement abilities could be used in diversified maize cropping system for managing the devastating FAW pest under realistic field conditions.

Abbreviations

FAW	Fall armyworm
GC–MS	Gas chromatography–mass spectrometry
VOCs	Volatile organic compounds
ARQU	Animal rearing and quarantine unit
DCM	Dichloromethane
GLM	Generalized linear model
LR	Likelihood ratio
DMNT	(*E*)-4,8-Dimethyl-1,3,7-nonatriene
TMTT	(*E, E*)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene
NMDS	Non-metric multidimensional scaling
SLD	Silverleaf desmodium
GLD	Greenleaf desmodium
Gnut	Groundnut
SP	Sweet potato
ns	No significance difference

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00415-6.

Additional file 1: Table S1. Plant combinations used to test the preference of FAW in dual and multiple-choice oviposition assays. **Fig. S1.** Representative GC–MS profiles of companion plants. Identities of the peaks are shown in Tables 2 and 7 and depict the predominant compounds based on analysis of similarities.

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Author contributions
AT, SS, BT and EP designed the study; EP conducted the experiment; EP AT and BT wrote the main manuscript text; EP and AT prepared figures. All authors read, revised and approved the final manuscript.

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Availability of data and materials
The data sets generated during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study does not contain any experiments using any animal species that require ethical approval.

Competing interests
The authors have no relevant financial or non-financial interests to disclose.

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References

1. Harrison RD, Thierfelder C, Baudron F, Chinwada P, Midega C, Scha U, et al. Agro-ecological options for fall armyworm (*Spodoptera frugiperda* J.E. Smith) management : providing low-cost, smallholder friendly solutions to an invasive pest. J Environ Manage. 2019;243:318–30.
2. Montezano DG, Specht A, Sosa-Gómez DR, Roque-Specht VF, Sousa-Silva JC, Paula-Moraes SV, et al. Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. Afr Entomol. 2018;26(2):286–300.
3. Sisay B, Sevgan S, Weldon CW, Kruger K, Torto B, Tamiru A. Responses of the fall armyworm (*Spodoptera frugiperda*) to different host plants: implications for its management strategy. Pest Manag Sci. 2023;79(2):845–56. https://doi.org/10.1002/ps.7255.
4. Goergen G, Kumar PL, Sankung SB, Togola A, Tamò M. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in west and central Africa. PLoS ONE. 2016;11(10):1–9. https://doi.org/10.1371/journal.pone.0165632.
5. FAO. FAO advisory note on fall armyworm (FAW) in Africa. Rome: FAO; 2017.
6. Day R, Abrahams P, Bateman M, Beale T, Clottey V, Cock M, et al. Fall armyworm: impacts and implications for Africa. Outlooks Pest Manag. 2017;17:96–201. https://doi.org/10.1564/v28.
7. Baudron F, Zaman-Allah MA, Chaipa I, Chari N, Chinwada P. Understanding the factors influencing fall armyworm (*Spodoptera frugiperda* J.E. Smith) damage in African smallholder maize fields and quantifying its impact on yield. A case study in Eastern Zimbabwe. Crop Prot. 2019;120:141–50. https://doi.org/10.1016/j.cropro.2019.01.028.
8. Guimapi RA, Niassy S, Mudereri BT, Abdel-Rahman EM, Tepa-Yotto GT, Subramanian S, et al. Harnessing data science to improve integrated management of invasive pest species across Africa: an application to fall armyworm (Spodoptera frugiperda) (J.E. Smith) (Lepidoptera: Noctuidae). Glob Ecol Conserv. 2022;35:e02056. https://doi.org/10.1016/j.gecco.2022.e02056.
9. Rwomushana I, Bateman M, Beale T, Beseh P, Cameron K, Chiluba M, et al. Fall armyworm: impacts and implications for Africa evidence. Outlook Pest Manag. 2018;28(5):18–9.
10. De Groote H, Kimenju SC, Munyua B, Palmas S, Kassie M, Bruce A. Spread and impact of fall armyworm (*Spodoptera frugiperda* J.E. Smith) in maize production areas of Kenya. Agric Ecosyst Environ. 2020;15(292):1–9. https://doi.org/10.1016/j.agee.2019.106804.
11. Kumela T, Simiyu J, Sisay B, Likhayo P, Gohole L, Tefera T. Farmers' knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. Int J Pest Manag. 2018;65:1–9. https://doi.org/10.1080/09670874.2017.1423129.
12. Tamiru A, Bruce TJA, Woodcock CM, Caulfield JC, Midega CAO, Ogol CKPO, et al. Maize landraces recruit egg and larval parasitoids in response to egg deposition by a herbivore. Ecol Lett. 2011;14:1075–83. https://doi.org/10.1111/j.1461-0248.2011.01674.x.
13. Hruska AJ. Fall armyworm (*Spodoptera frugiperda*) management by smallholders. Cab Rev. 2019;14:1–11. https://doi.org/10.1002/arch.940300102.

14. Letourneau DK, Armbrecht I, Rivera BS, Lerma J, Carmona EJ, Daza MC, et al. Does plant diversity benefit agroecosystems? a synthetic review. Ecol Appl. 2011;21(1):9–21. https://doi.org/10.1890/09-202.
15. Lithourgidis AS, Dordas CA, Damalas CA, Vlachostergios DN. Annual intercrops: an alternative pathway for sustainable agriculture. Aust J Crop Sci. 2011;5(4):396–410.
16. Smith HA, Mcsorley R. Intercropping and pest management : a review of major concepts. Am Entomol. 2000;46:154–61. https://doi.org/10.1093/ae/46.3.154.
17. Han-ming HE, Li-na L, Munir S, Bashir NH, Wang Y, Yang J, et al. Crop diversity and pest management in sustainable agriculture. J Integr Agric. 2019;18:1945–52. https://doi.org/10.1016/s2095-3119(19)62689-4.
18. Rahman MM, Joaty JY, Islam MM. Intercropping for insect pest management in sustainable agriculture: a review. Bull Inst Trop Agric Kyushu Univ. 2020;43:11–22. https://doi.org/10.1118/bita.43.11.
19. Murdoch WW. Diversity, complexity, stability and pest control. J Appl Ecol. 1975;12(3):795–807.
20. Altieri MA. Diversification of corn agroecosystems as a means of regulating fall armyworm populations. Fla Entomol. 1980;63:450–6.
21. Altieri MA, Letourneau DK. Vegetation management and biological control in agroecosystems. Crop Prot. 1982;1:405–30.
22. Sekamatte BM, Ogenga-latigo M, Russell-smith A. Effects of maize-legume intercrops on termite damage to maize, activity of predatory ants and maize yields in Uganda. Crop Prot. 2003;22:87–93. https://doi.org/10.1016/S0261-2194(02)00115-1.
23. Thayamini HS, Brintha I. Review on maize based intercropping. J Agron. 2010;9:135–45.
24. Hailu G, Niassy S, Zeyaur KR, Ochatum N, Subramanian S. Maize–legume intercropping and push–pull for management of fall armyworm, stemborers, and striga in Uganda. Agron J. 2018;110:2513–22. https://doi.org/10.2134/agronj2018.02.0110.
25. Tanyi CB, Nkongho RN, Okolle JN, Tening AS, Ngosong C. Effect of intercropping beans with maize and botanical extract on fall armyworm (*Spodoptera frugiperda*) infestation. Int J agron. 2020. https://doi.org/10.1155/2020/4618190.
26. Udayakumar A, Shivalingaswamy TM, Bakthavatsalam N. Legume-based intercropping for the management of fall armyworm, *Spodoptera frugiperda* L in maize. J Plant Dis Prot. 2021;128:775–9. https://doi.org/10.1007/s41348-020-00401-2.
27. Khan ZR, Midega CAO, Bruce TJA, Hooper AM, Pickett JA. Exploiting phytochemicals for developing a "push-pull" crop protection strategy for cereal farmers in Africa. J Exp Bot. 2010;61:4185–96. https://doi.org/10.1093/jxb/erq229.
28. Sobhy IS, Tamiru A, Morales CX, Nyagol D, Cheruiyot D, Chidawanyika F, et al. Bioactive volatiles from push-pull companion crops repel fall armyworm and attract its parasitoids. Front Ecol Evol. 2022;10:1–16. https://doi.org/10.3389/fevo.2022.883020.
29. Nwanze JAC, Bob-Manuel RB, Zakka U, Kingsley-Umana EB. Population dynamics of fall armyworm [(*Spodoptera frugiperda*) J.E. Smith] (Lepidoptera: Nuctuidae) in maize-cassava intercrop using pheromone traps in Niger delta region. Bull Natl Res Cent. 2021. https://doi.org/10.1186/s42269-021-00500-6.
30. Garcia González MT, Rojas JA, Castellanos Gonzalez L, Enjamio JD. Policultivo (maíz-calabaza) en el control de *Spodoptera frugiperda* (Smith) en fomento, sancti spiritus. Cent Agríc. 2010;37(1):57–64.
31. Wyckhuys KAG, O'Neil RJ. Population dynamics of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) and associated arthropod natural enemies in Honduran subsistence maize. Crop Prot. 2006;25:1180–90.
32. Khan ZR, Midega CAO, Wadhams LJ, Pickett JA, Mumuni A. Evaluation of napier grass (*Pennisetum purpureum*) varieties for use as trap plants for the management of African stemborer (*Busseola fusca*) in a push–pull strategy. Entomol Exp Appl. 2007;124:201–11.
33. Tamiru A, Bruce TJA, Woodcock CM, Birkett MA, Midega CAO, Pickett JA, et al. Chemical cues modulating electrophysiological and behavioural responses in the parasitic wasp *Cotesia sesamiae*. Can J Zool. 2015;93:281–7.
34. R Core Team. R: a language and environment for statistical computing. Vienna: R foundation for statistical computing; 2021.
35. Aitchison J. The statistical analysis of compositional data. J R Stat Soc Ser B. 1982;44:139–77.
36. Khan M, Mousa AA, Syamasundar KV, Alkhathlan HZ. Determination of chemical constituents of leaf and stem essential oils of *Artemisia monosperma* from central Saudi Arabia. Nat Prod Commun. 2012;7:1079–82. https://doi.org/10.1177/1934578x1200700829.
37. Willey RW, Natarajan M, Reddy MS, Rao MR, Nambiar PTC, Kannaiyan J, et al. Intercropping studies with annual crops. In: Nugent J, O'Connor M, editors., et al., Ciba foundation symposium. London; 2008.
38. Renwick JAA. Chemical ecology of oviposition in phytophagous insects. Experientia. 1989;45:223–8.
39. Cook SM, Khan ZR, Pickett JA. The use of push-pull strategies in integrated pest management. Annu Rev Entomol. 2006;52:375–400. https://doi.org/10.1011/annurev.ento.52.110405.091407.
40. Bandara KANP, Kumar V, Ninkovic V, Ahmed E, Pettersson J, Glinwood R. Can leek interfere with bean plant-bean fly interaction? test of ecological pest management in mixed cropping. J Econ Entomol. 2009;102:999–1008. https://doi.org/10.1603/029.102.0319.
41. Sujayanand GK, Sharma RK, Shankarganesh K, Saha S, Tomar RS. Crop diversification for sustainable insect pest management in eggplant (Solanales: Solanaceae). Fla Entomol. 2015;98:305–14. https://doi.org/10.1653/024.098.0149.
42. Webster B, Cardé RT. Use of habitat odour by host-seeking insects. Biol Rev. 2017;92:1241–9. https://doi.org/10.1111/brv.12281.
43. Ahissou BR, Sawadogo WM, Bokonon-Ganta AH, Somda I, Verheggen F. Integrated pest management options for the fall armyworm *Spodoptera frugiperda* in west Africa: challenges and opportunities a review. Biotechnol Agron Soc Environ. 2021;25:192–207. https://doi.org/10.2551/1780-4507.19125.
44. Rojas JC, Virgen A, Cruz-López L. Chemical and tactile cues influencing oviposition of a generalist moth, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Environ Entomol. 2003;32:1386–92. https://doi.org/10.1603/0046-225x-32.6.1386.
45. Rembold H, Köhne AC, Schroth A. Behavioral response of *Heliothis armigera* Hb. (Lep, Noctuidae) moths on a synthetic chickpea (*Cicer arietinum* L.) kairomone. J Appl Entomol. 1991;112:254–62. https://doi.org/10.1111/J.1439-0418.1991.TB01055.X.
46. Honda K. Chemical basis of differential oviposition by lepidopterous insects. Arch Insect Biochem Physiol. 1995;30:1–23. https://doi.org/10.1002/arch.940300102.
47. Hartlieb E, Rembold H. Behavioral response of female *Helicoverpa (Heliothis) armigera* HB. (Lepidoptera: Noctuidae) moths to synthetic pigeonpea (*Cajanus cajan* L) kairomone. J Chem Ecol. 1996;22:821–37. https://doi.org/10.1007/BF02033589.
48. Signoretti AGC, Peñaflor MFGV, Bento JMS. Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), female moths respond to herbivore-induced corn volatiles. Neotrop Entomol. 2012;41:22–6. https://doi.org/10.1007/s13744-011-0003-y.
49. Mohamed SA, Wamalwa M, Obala F, Tonnang HEZ, Tefera T, Calatayud PA, et al. A deadly encounter: alien invasive *Spodoptera frugiperda* in Africa and indigenous natural enemy, *Cotesia icipe* (Hymenoptera, Braconidae). PLoS ONE. 2021;16(7):e0253122. https://doi.org/10.1371//journal.pone.0253122.
50. Quispe R, Mazón M, Rodríguez-Berrío A. Do refuge plants favour natural pest control in maize crops? Insects. 2017;8:71. https://doi.org/10.3390/insects8030071.
51. Seni A. Role of intercropping practices in farming system for insect pest management. Acta Sci Agric. 2018;2:8–11.
52. Carroll MJ, Schmelz EA, Meagher RL, Teal PEA. Attraction of *Spodoptera frugiperda* larvae to volatiles from herbivore-damaged maize seedlings. J Chem Ecol. 2006;32:1911–24. https://doi.org/10.1007/s10886-006-9117-9.
53. D'Alessandro M, Turlings TCJ. Advances and challenges in the identification of volatiles that mediate interactions among plants and arthropods. Analyst. 2006;131:24–32. https://doi.org/10.1039/b507589k.
54. War AR, Paulraj MG, Ahmad T, Ahad A, Hussain B, Ignacimuthu S, et al. Mechanisms of plant defense against insect herbivores. Plant Signal Behav. 2012;7:1306–20. https://doi.org/10.4161/psb.21663.
55. Pickett JA, Khan ZR. Plant volatile-mediated signalling and its application in agriculture: successes and challenges. New Phytol. 2016;212:856–70. https://doi.org/10.1111/nph.14274.
56. Pinto-Zevallos DM, Strapasson P, Zarbin PHG. Herbivore-induced volatile organic compounds emitted by maize: electrophysiological responses in

Spodoptera frugiperda females. Phytochem Lett. 2016;16:70–4. https://doi.org/10.1016/j.phytol.2016.03.005.
57. Khan ZR, Ampong-Nyarko K, Chiliswa P, Hassanali A, Kimani S, Lwande W, et al. Intercropping increases parasitism of pests. Nature. 1997;388:631–2.
58. Khan ZR, Pickett JA, Van Den Berg J, Wadhams LJ, Woodcock CM. Exploiting chemical ecology and species diversity: stem borer and striga control for maize and sorghum in Africa. Pest Manag Sci. 2000;56:957–62.
59. Pickett J, Bruce T, Chamberlain K, Hassanali A, Khan Z, Matthes M, et al. Plant volatiles yielding new ways to exploit plant defence. In: Dicke M, Takken W, editors., et al., Chemical ecology: from gene to ecosystem. Dordrecht: Springer, Netherlands; 2006.
60. Bruce TJA, Midega CAO, Birkett MA, Pickett JA, Khan ZR. Is quality more important than quantity? insect behavioural responses to changes in a volatile blend after stemborer oviposition on an African grass. Biol Lett. 2010;6(3):314–7.
61. Chen W, Vermaak I, Viljoen A. Camphor-a fumigant during the black death and a coveted fragrant wood in ancient Egypt and Babylon-a review. Molecules. 2013;18:5434–54. https://doi.org/10.3390/molecules18055434.
62. Binder BF, Robbins JC. Age- and density-related oviposition behavior of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). J Insect Behav. 1996;9:755–69. https://doi.org/10.1007/BF02213555.63.
63. Agbessenou A, Akutse KS, Yusuf AA, Khamis FM. The Endophyte *Trichoderma asperellum* M2RT4 induces the systemic release of Methyl Salicylate and (Z) -jasmone in tomato plant affecting host location and herbivory of *Tuta absoluta*. Front Plant Sci. 2022;13:860309. https://doi.org/10.3389/fpls.2022.860309.
64. Mozuraitis R, Stranden M, Ramirez MI, Borg-Karlson AK, Mustaparta H. (-)-germacrene D increases attraction and oviposition by the tobacco budworm moth *Heliothis virescens*. Chem Senses. 2002;27(6):505–10.
65. de Brito-Machado D, Ramos YJ, Defaveri ACAE, de Queiroz GA, Guimarães EF, de Lima MD. Volatile chemical variation of essential oils and their correlation with insects, phenology, ontogeny and microclimate: *Piper mollicomum* Kunth, a case of study. Plants. 2022;11(24):3535. https://doi.org/10.3390/plants11243535.
66. Isbell F, Adler PR, Eisenhauer N, Fornara D, Kimmel K, Kremen C, et al. Benefits of increasing plant diversity in sustainable agroecosystems. J Ecol. 2017;105:871–9.

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