



Electrophysiological and Behavioral Responses of Cabbage Aphid (*Brevicoryne brassicae*) to Rosemary (*Rosmarinus officinalis*) Volatiles, a Potential push Plant for Vegetable push-pull Cropping System

Bretor Katuku Mutua^{1,2} · Thomas Dubois¹ · Komivi Senyo Akutse^{1,3} · Benjamin Muli² · Edward Nderitu Karanja¹ · Daniel Munyao Mutyambai^{1,4}

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Abstract

The cabbage aphid (*Brevicoryne brassicae*) is a major pest of kale (*Brassica oleraceae* var. *acephala*), an important vegetable that is grown worldwide due to its high nutritional and economic value. *Brevicoryne brassicae* poses a great challenge to *B. oleraceae* var. *acephala* production, causing significant direct and indirect yield losses. Farmers overly rely on synthetic insecticides to manage the pest with limited success owing to its high reproductive behavior and development of resistance. This necessitates a search for sustainable alternatives to mitigate these challenges. This study assessed behavioral responses of *B. brassicae* to odors from rosemary (*Rosmarinus officinalis*) and *B. oleraceae* var. *acephala* headspace volatiles in a Perspex four-arm olfactometer. We identified and quantified volatiles emitted by each of the two plants and those eliciting antennal response using coupled gas chromatography-mass spectrometry (GC-MS) and GC-electroantennographic detection (GC-EAD), respectively. Our findings revealed that *B. brassicae* spent more time in the arms of the olfactometer that contained *B. oleraceae* var. *acephala* volatiles compared to the arm that held *R. officinalis* volatiles. Additionally, *B. brassicae* spent more time in the olfactometer arms with *B. oleraceae* var. *acephala* compared to the arms holding *B. oleraceae* var. *acephala* and *R. officinalis* enclosed together and clean air. GC-MS analysis revealed diverse and higher quantities of volatile compounds in *R. officinalis* compared to *B. oleraceae* var. *acephala*. GC-EAD analysis showed that antennae of *B. brassicae* detected Linalool, α -Terpineol, Verbenone, Geraniol, Camphor, and Borneol from the volatiles of *R. officinalis*, and Sabinene, γ -Terpinene, and β -Caryophyllene from *B. oleraceae* var. *acephala* volatiles. Our findings demonstrate the potential of *R. officinalis* as a repellent plant against *B. brassicae* and could be utilized as a ‘push’ plant in an intercropping strategy against this pest.

Keywords Agroecology · Cabbage Aphid · Integrated pest Management · Kale · Rosemary

Introduction

Kale (*Brassica oleracea* L. var. *acephala*) is a leafy vegetable of global importance, primarily cultivated by small-scale farmers for both subsistence and income generation, particularly in tropical and subtropical regions (Mutiga et al. 2011; Peris and Kiptoo 2017; Šamec et al. 2019). According to the Center for Disease Control (CDC), *B. oleracea* var. *acephala* was ranked 15th of the 47 powerhouse fruits and vegetables, producing more than 17 essential nutrients (CDC 2014). *Brassica oleracea* var. *acephala* has garnered significant attention recently owing to its notable health advantages. It contains phytochemicals that have been linked to reduced risk of cancer and other chronic diseases,

✉ Daniel Munyao Mutyambai
dmutyambai@icipe.org

¹ International Centre of Insect Physiology and Ecology, P. O. Box 30772-00100, Nairobi, Kenya

² Department of Agricultural Sciences, South Eastern Kenya University, P.O Box 170-90200, Kitui, Kenya

³ Unit for Environmental Sciences and Management, North-West University, Potchefstroom 2520, South Africa

⁴ Department of Life Sciences, South Eastern, Kenya University, P.O Box 170-90200, Kitui, Kenya

due to antioxidant properties and high dietary fiber content (Šamec et al. 2019). Additionally, *B. oleracea* var. *acephala* is known for its resilience to adverse effects of climate change, rendering it adaptable to extreme climatic conditions (Lagerkvist et al. 2012). In Kenya, *B. oleracea* var. *acephala* has become increasingly popular due to its ability to maximize land use and address food security and nutrition concerns amidst challenges such as land degradation and population pressure (Mutiga et al. 2011; Olwande et al. 2015; HCD 2019). Due to the low input and labour requirements for *B. oleracea* var. *acephala* production, the crop stands out as one of the most accessible vegetables to cultivate (Lans et al. 2012; Canwat et al. 2021). Its cost-effective production methods contribute to relatively low market prices, ensuring affordability for consumers. Consequently, it is widely consumed in households and extensively sold in urban areas (Ngolo Otieno 2019).

Despite these benefits, the successful production and productivity of *B. oleracea* var. *acephala* face various constraints such as pests and disease pressures, poor soils, limited market access, climate change and inadequate production techniques (Mutiga et al. 2010). The cabbage aphid, *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) is one of the most destructive insect pest that affects production of *B. oleracea* var. *acephala* and other *Brassica* sp. crops worldwide (Cole 1994; Gill et al. 2013). The pest is native to Europe but has been reported in many parts of the world (Gill et al. 2013; Munthali and Tshegofatso, 2013). The adults feed on the sap of plant tissues using their piercing-sucking mouthparts, causing direct crop damage through wilting, stunted growth and deformation, and transmission of diseases such as mosaic virus and ring necrosis, which eventually result in plant death (Powell et al. 2006; Mutiga et al. 2010; Chalise and Dawadi 2019). *Brevicoryne brassicae* has a wide host range of crops belonging to Brassicaceae family such as kale (*Brassica oleracea* var. *acephala*), cabbage (*Brassica oleracea* var. *capitata*), Brussels sprout (*Brassica oleracea* var. *gemmifera*) and Broccoli (*Brassica oleracea* var. *italica*) (Douloupaka and Van Emden 2003; Van Emden and Harrington 2007; Döring 2014).

Smallholder farmers with limited resources have resorted to indiscriminate use of synthetic insecticides to control the pest (Badenes-Perez and Shelton 2006; Ngolo Otieno 2019). The repeated use of these chemical insecticides has resulted in additional economic costs to farmers, insecticide resistance and pests resurgences, and has proven detrimental to agrobiodiversity, human and environmental health (Kianmatee and Ranamukhaarachchi 2007; Macharia and Afr 2009; Ngolo et al. 2019; Ricupero et al. 2020). There is therefore an urgent need to develop alternative control options which will be ecologically friendly, cost-effective,

sustainable and suitable for resource-limited vegetable farmers in Africa.

The push pull cropping system is one of such sustainable management options that has been successfully used in cereal pests control (Khan et al. 2001). This is a habitat management strategy that uses plant allelochemicals to manipulate the distribution of pests and their natural enemies through the production of volatile organic compounds (VOCs) in the natural ecosystem, which play an important role in communication, defense and response to biotic stresses (Khan et al. 2001). The push/repellent plant produces VOCs that have the ability to mask the host plant volatiles, attract natural enemies or deter the pest from landing on the host plant (Cook et al. 2007). Host location by insects involves the perception of specific or a blend of VOCs naturally emitted into the ecosystem which induces either an attraction or avoidance behavior (Zhang and Chen 2015). For example, the spring weevil, *Sitona humerali* was attracted to alfalfa flowers in a laboratory setting (Lohonyai et al. 2019). Field studies showed that intercropping cabbage with other repellent plants such as clover, pepper, basil and onions reduced the oviposition and damage caused by *Plutella xylostella* due to their repellence (Finch and Kienegger 1997; Yarou et al. 2017). In addition to host location, plants produce specific or a blend of VOCs in response to an attack by pest to defend themselves. Many of these VOCs act as direct repellents to pest thereby deterring feeding and oviposition, and tend to be highly concentrated compared to the naturally emitted blends (Piesik et al. 2016). For instance, in an attempt to test herbivore induced plant volatiles (HIPVs), Piesik et al. (2016) found out that weevil (*Hypera rumicis*) herbivory on mossy sorrel plant induced ten VOCs majority of which were repellent to the pest. Furthermore, VOCs play a crucial role mediating plant-pest-natural enemy interaction (Mutymbai et al. 2015; Ben-Issa et al. 2017; Sisay et al. 2023). As such, plant volatiles can be used to modify the host selection by insect pests and their natural enemies. Previous studies have demonstrated the potential of rosemary (*Rosmarinus officinalis*), an aromatic perennial herb of Lamiaceae family, as an insect repellent plant, showcasing its effectiveness against a wide range of insects pests (Cloyd et al. 2009; Cook et al. 2007; Dardouri et al. 2019; Elhalawany et al. 2019; Li et al. 2021; Waithaka et al. 2017; Zhang and Chen 2015). For example, applications of different doses of *R. officinalis* leaf extracts and essential oils have demonstrated their efficacy as repellents against the two-spotted spider mite (*Tetranychus urticae* Koch (Trombidiformes: Tetranychidae) and the citrus brown mite (*Eutetranychus orientalis* Klein (Trombidiformes: Tetranychidae) (Elhalawany et al. 2019). Similarly, laboratory bioassays with different *R. officinalis* species have demonstrated their ability to exhibit repellent

properties towards green peach aphid (*Myzus persicae* Sulzer (Hemiptera: Aphididae) through the production of different VOCs (Dardouri et al. 2019). Moreover, intercropping *R. officinalis* with sweet pepper (*Capsicum annuum* L. Solanaceae) in a greenhouse experiment in China was found to suppress the population of *M. persicae*, thrips (*Frankliniella intonsa* Trybom (Thysanoptera: Thripidae), and silverleaf whitefly (*Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) without affecting the population dynamics of natural enemies (Li et al. 2021). The repulsive effect of *R. officinalis* in these studies have been attributed to the emission of VOCs which are repellent to the pests. Despite these studies demonstrating the repellence ability of *R. officinalis*, its activity against *B. brassicae* has not been investigated.

Therefore, in this study, we (1) investigated the behavioral response of *B. brassicae* to *R. officinalis* and *B. oleracea* var. *acephala* headspace volatiles; (2) identified and compared the discriminant VOCs in the two plants; and (3) used gas chromatography- electroantennographic detection analysis to determine the responses of *B. brassicae* antenna to *R. officinalis* and *B. oleracea* var. *acephala* headspace volatiles.

Materials and methods

Plants

Brassica oleracea var. *acephala* (var. simlaw select) seeds were purchased from Simlaw Seeds Company Limited, Nairobi, Kenya. The seeds were sown in a 2 × 1 m nursery bed at 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) and allowed to grow for three weeks, after which the seedlings were transplanted individually into 5 L plastic pots. The pots were filled with soil and organic manure mixed in a ratio of 2:1 and were maintained in an insect-proof screenhouse in the same location. Irrigation was done manually once a day. Plants did not receive any synthetic insecticides or fertilizer inputs. *Rosmarinus officinalis* (var. Tuscan Blue) was propagated vegetatively through stem cuttings obtained from Kimplanter Seedlings and Nurseries located at Thika, Kenya and received the same treatment as *B. oleracea* var. *acephala*. Six weeks old *B. oleracea* var. *acephala* and eight weeks old *R. officinalis* plants were used for experiments.

Insects

An initial colony of *B. brassicae* was established with insects obtained from smallholder *B. oleracea* var. *acephala* farms in Limuru, Kenya (1° 10' 9.13" S, 36° 41' 25.18" E,

2500 m above sea level). Adult *B. brassicae* were reared on *B. oleracea* var. *acephala* plants in 50 × 80 × 40 cm Perspex rearing cages in the laboratory and maintained at the temperature of 25 ± 1 °C, 65 ± 5% relative humidity (RH) and 12 L: 12D h photoperiod as a modification of Chalise and Dawadi (2019) who reared the aphids at 25 ± 2 °C and 55 ± 5% RH. Freshly potted *B. oleracea* var. *acephala* plants were provided after every three days for feeding and reproduction. After 10 days, the newly emerged adults were transferred to a separate rearing cage, fed with *B. oleracea* var. *acephala* plants and later used for bioassays. After every two weeks, field collected insects were infused into the laboratory colony to maintain behavioral characteristics and avoid genetic decay. Insects were reared on *B. oleracea* var. *acephala* for 10 generations prior to the bioassays. All the aphids used for bioassays were 14 days old.

Chemicals

Authentic standards of α -Pinene (97%), β -Pinene (99%), Myrcene (90%), Limonene (97%), γ -Terpinene (97%), Linalool (97%), α -Terpineol (96%), Verbenone (94%), Citronellol (96%), Geraniol (97%), β -Caryophyllene ($\geq 80\%$) and Caryophyllene oxide (95%), all sourced from Sigma Aldrich (Gillingham, UK) were used for quantification and confirmation of the GC-MS tentative identifications. Dichloromethane (99.9% purity) was purchased from Merck (Darmstadt, Germany).

Collection of Volatiles Using Headspace Technique

Headspace sampling technique was used to collect volatiles from experimental plants (*B. oleracea* var. *acephala* and *R. officinalis*) and a control (empty polyethylene terephthalate bag) for 24 h, starting at the last 2 h of the photophase as described by Mutyambai et al. (2015). The aerial parts of the plants were gently enclosed inside polyethylene terephthalate (PET) bags (~12.5 mm thickness, volume 3.2 L), heated to 150 °C for 30 min before use, and fitted with Swagelok inlet and outlet ports (Mutyambai et al. 2015). Charcoal-filtered air was passed through the inlet port at a flow rate of 600 mL min⁻¹. VOCs were collected on Charcoal filters (0.05 g, 60/80 mesh, Supelco, USA) inserted into the outlet through which air was drawn at 400 mL min⁻¹. After trapping, the entrained volatiles were eluted using 250 μ L of dichloromethane (DCM) (Merck, Germany) in 2 mL micro vials (Agilent Technologies, Warsaw, Poland) and stored in a -40 °C freezer before further chemical analysis and bioassays. Entrainments from each host plant were replicated four times and each plant was used only once.

Olfactometer Bioassay

Three separate sets of experiments were carried out to evaluate the olfactory response of *B. brassicae* using a Perspex four-arm olfactometer as described by Mutyambai et al. (2015). In the first experiment, the two opposite arms of the olfactometer were directly connected to *B. oleracea* var. *acephala* and *R. officinalis* plants respectively, while the remaining two arms were connected to empty bags (control arms). Charcoal-filtered air at a rate of 300 mL min⁻¹ was pumped into the headspace of the test plants enclosed in heat-sterilized PET bags (control), positioned away from the olfactometer arena to prevent any visual cues. In order not to contaminate the headspace plant volatiles, the pots were wrapped with aluminum foil leaving only the aerial part exposed. A suction tube was used to simultaneously draw air from the plants to the olfactometer at a rate of 100 mL min⁻¹ to enable the movement of the plant volatiles (25 mL min⁻¹ per arm), and the outgoing air was then exhausted from the laboratory (Lohonyai et al. 2019). Fourteen-day-old *B. brassicae* were individually placed in Petri dish (90×20 mm) and kept in the laboratory for 1 h to acclimatize prior to the bioassay. They were then introduced individually at the center of the olfactometer and allowed to make a choice for 20 min.

In the second experiment, a choice test was conducted to determine the response of *B. brassicae* to constitutive test plant-derived volatiles and solvent (DCM) control. Each of the two opposing arms held 10 µl aliquots of the respective plants' headspace samples while the other two opposing arms held 10 µl of solvent as controls. The headspace samples were applied to a filter paper (4×25 mm) using a micropipette (Drummond Scientific, Broomall, USA) and placed at the inlet of the olfactometer arms. *Brevicoryne brassicae* were then introduced at the center of the olfactometer with a fine camel hairbrush and allowed to make a choice. To enable the insect to detect the volatiles, a suction pump was connected to the olfactometer, facilitating the suction of air containing the volatiles from the arms to the center of the olfactometer at a rate of 25 mL min⁻¹.

In the third experiment, one arm of the olfactometer held *B. oleracea* var. *acephala* plant while the opposing arm held both *B. oleracea* var. *acephala* and *R. officinalis* enclosed in a heat-sterilized PET bags together. The other two opposing arms contained empty bags as controls. Charcoal-filtered air at a rate of 300 mL min⁻¹ was pumped into the headspace of these test plants after which the procedure in experiment one was repeated. In all experiments, the duration of time spent by the insect in each arm of the olfactometer was recorded using Olfa- (F. Nazzi, Udine, Italy) (Mutyambai et al. 2015). Twelve aphids were tested and each insect was used only once. The olfactometer was rotated every 4 min to avoid

positional and directional bias. The insects were observed for 20 min and each olfactometer was used only once. If an aphid remained stationary for a consecutive duration of 2 min at the center of the olfactometer, it was regarded as inactive, leading to the rejection of that particular replicate.

Analyses of Volatiles

The headspace volatiles from *B. oleracea* var. *acephala* and *R. officinalis* were analyzed using gas chromatograph-mass spectrometry (GC-MS; 7890 A GC and MSD 5975 C triple-axis; Agilent Technologies, Palo Alto, USA). The GC-MS was configured to operate in an electron impact ionization mode of 70 eV. A HP5-MSI low-bleed capillary column with dimensions of 30 m length × 0.25 mm inner diameter × 0.25 µm film thickness (J & W Scientific, Folsom, USA). A flow rate of 1.2 mL min⁻¹ of helium gas was employed as the carrier gas. The oven temperature was initially set at 35 °C for 5 min and then increased at a rate of 10 °C min⁻¹ until reaching a final temperature of 280 °C and held for 10.5 min. The headspace samples were injected into the GC-MS using an autosampler in measured aliquots (1 µL). The identification of compounds was accomplished by comparing their mass spectra with those obtained from authentic standards where available, as well as utilizing mass spectra databases of the National Institute of Standards and Technology chemistry webbook (NIST11, Gaithersburg, Maryland). Additionally, retention indices were determined by comparing the retention times of a mixture of n-alkanes ranging from C8 to C23. To ensure further confirmation, a co-injection with available authentic standards was performed under the same experimental conditions. For quantification of identified VOCs (ng), external calibration curves of selected compounds β -Pinene and β -Caryophyllene were made from 1000 ng µL⁻¹ of their stock solution and with the concentrations ranging from 1 to 1000 ng/µL (supplementary Fig. 1). The concentration of compounds were determined by extrapolating the peak area of the unknown concentration in comparison to those of the known concentration (standards), and subsequently expressed in ng/plant/h. All compounds detected in the control group were deemed contaminants and subsequently disregarded during the identification process. The MSD Chemstation software (v F.01.00.1903; Agilent Technologies, Palo Alto, USA) was employed to analyze the data.

Coupled Gas Chromatography-Electroantennography

Adult *B. brassicae* were individually collected from the Perspex rearing cage into a 100 mm x 15 mm plastic petri dish. Antennae were prepared by separating the head of ice-chilled

B. brassicae from the rest of the body using a scalpel. Two silver-silver chloride (Ag-AgCl) borosilicate glass micro electrodes, 2 mm o.d. X 1.16 mm i.d. with an inner filament (INR-II, Syntech, Hilversum, the Netherlands) filled with Ringer saline solution (7.5 gl⁻¹ sodium chloride, 0.7 gl⁻¹ potassium chloride, 0.2 gl⁻¹ calcium chloride, 0.2 gl⁻¹ magnesium chloride) as in Maddrell (1969) but without glucose were used for electroantennogram recordings. With the help of an electrode holder, the head was placed at the indifferent electrode with the tip of the antenna touching the recording electrode.

The glass tube featured a side hole through which the column effluent was introduced. The splitter used in this setup was made of glass-lined stainless-steel tubing and deactivated fused silica tubing. VOCs to which *B. brassicae* antenna responded to were identified on GC. One μ l of the concentrated entrainment sample was injected onto a non-polar column (HP5-MSI, 30 m $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness), (Agilent Technologies, California, USA) in a HP7890 GC (Agilent Technologies, Palo Alto, USA) equipped with a cool on-column injector and a flame ionization detector (FID). The oven temperature was programmed at 35 °C for 2 min and then programmed at 10 °C min⁻¹ to 280 °C. Nitrogen was used as the carrier gas. Simultaneous recordings of the EAG and FID responses were obtained with specialized software (Electroantennographic Detection 2015 version 1.2.6, Syntech, Hilversum, The Netherlands). The EAD outlet contained an uninterrupted airflow filtered through charcoal at a rate of 200 mL min⁻¹ directed to the *B. brassicae* antenna as described by Fombong et al. (2016). A total of six coupled runs were completed. Only FID peaks which corresponded to an EAG peak in at least 3 replicates were considered electro-physiologically active.

Statistical Analyses

Data was analyzed using R statistical software version 4.2.3 (Core Team 2022). The duration of time spent by *B. brassicae* in each arm of the olfactometer was first converted into proportions to address dependence of visiting time and log₁₀-ratio transformations to allow for analysis of compositional data (Mutiyambai et al. 2015; Piepel and Aitchison 1988). To test for the normal distribution of the data, Shapiro-Wilk test (Shapiro and Wilk 1965) was performed ($P > 0.05$). Data on the time spent by *B. brassicae* in the *R. officinalis* versus *B. oleracea* var. *acephala* arms of the olfactometer and their respective volatile extracts was analysed using Kruskal Wallis test followed by Dunn's post-hoc for mean separation, following its abnormal distribution. Time spent in the olfactometer arms with *B. oleracea* var. *acephala* and *R. officinalis* versus *B. oleracea* var. *acephala* was subjected to analysis of variance (ANOVA), followed

by the Student-Newman-Keuls (SNK) test for mean separation. All P values ≤ 0.001 were considered statistically significant. The emission of compounds from all test plants underwent non-parametric statistical test, the Kruskal Wallis test following the abnormal distribution of the data as determined by the Shapiro-Wilk test ($P < 0.05$). Subsequently, Dunn's multiple pairwise comparison of the means was utilized to differentiate means between the two groups. Furthermore, to assess the contribution of various VOCs to dissimilarities among the test plants, their abundance was compared using a heatmap.

Results

Olfactory Response of *Brevicoryne brassicae* to *Brassica oleracea* var. *acephala* and *Rosmarinus officinalis* Plants and Their Headspace Volatiles

In the first experiment with individual plant odors from *B. oleracea* var. *acephala* and *R. officinalis* plants, versus clean air, *B. brassicae* spent more time in the arm containing odor source from *B. oleracea* var. *acephala* over the arms with odor source from *R. officinalis* or the clean air (Kruskal–Wallis $\chi^2 = 20.38$, df = 2, $P < 0.001$, Fig. 1A). Additionally, *B. brassicae* spent more time in the arm containing odor volatile of *B. oleracea* var. *acephala* when compared to clean air (Kruskal–Wallis $\chi^2 = 9.36$, df = 1, $P < 0.001$, Fig. 1A). In the second experiment with extracts of *B. oleracea* var. *acephala* and *R. officinalis* headspace volatiles, *B. brassicae* spent less time in the arm containing *R. officinalis* volatiles than the arms containing *B. oleracea* var. *acephala* volatiles or clean air (Kruskal–Wallis $\chi^2 = 13.42$, df = 2, $P < 0.001$). However, the time spent in the arm with *B. oleracea* var. *acephala* extracts was not different from the time spent in the clean air (Kruskal–Wallis $\chi^2 = 4.56$, df = 2, $P > 0.001$, Fig. 1B).

In the third experiment, *B. brassicae* spent more time in the olfactometer arms with *B. oleracea* var. *acephala* compared to the arms holding *B. oleracea* var. *acephala* and *R. officinalis* enclosed together and clean air ($P < 0.001$). The time spent in clean air controls was different from the time spent in *B. oleracea* var. *acephala* arm ($P < 0.001$, Fig. 1C).

Volatile Profiles

The GC-MS analysis detected 22 major compounds from the plant headspace samples belonging to three chemical classes: monoterpenes (17), ketones (1) and sesquiterpenes (4) (Table 1; Fig. 2A, B). Of the identified compounds, nine were detected from *B. oleracea* var. *acephala* (Fig. 2B) and 19 from *R. officinalis* (Fig. 2A). Common volatiles between

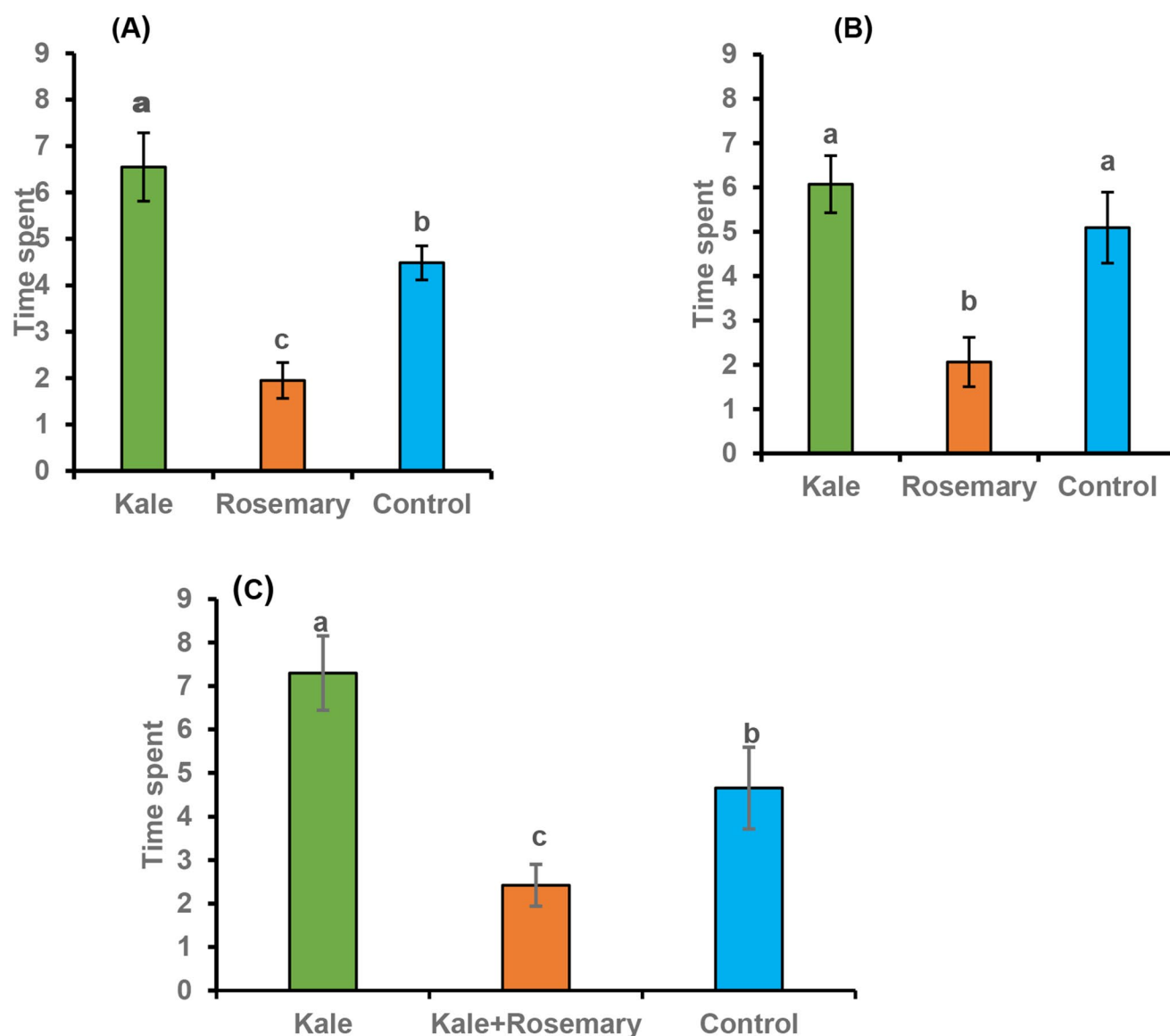


Fig. 1 Behavioral response of *Brevicoryne brassicae* to naturally emitted constitutive volatiles from *Brassica oleracea* var. *acephala* and *Rosmarinus officinalis* plants (A), their headspace volatiles (B) and a combination of the two plants (C) in a four-arm olfactometer. Time

spent by *Brevicoryne brassicae* was observed for 20 min ($N=12$). Means ($\pm$ SE) with different letters above the bars are significantly different

the two plants included α -pinene, β -pinene, myrcene, 1,8-cineole, γ -terpinene, camphor and β -caryophyllene with *R. officinalis* producing 57, 61, 6, 36, 10, 106 and 274 times more the amount produced by *B. oleracea* var. *acephala* respectively ($P < 0.001$) (Table 1). The VOCs that were detected in *R. officinalis* but not in *B. oleracea* var. *acephala* included camphene, α -phellandrene, δ -2-carene, (Z)-sabinene hydrate, linalool, borneol, α -terpineol, verbenone, citronellol, geraniol, bornyl acetate, α -humulene and caryophyllene oxide. Compounds detected in *B. oleracea* var. *acephala* but not in *R. officinalis* included sabinene and limonene (Table 1).

Heatmap clustering showed volatiles obtained from *R. officinalis* were more concentrated than those obtained from *B. oleracea* var. *acephala* (Fig. 3). It also showed that 1,8-cineole, β -pinene, myrcene and sabinene were the most abundant volatiles in *B. oleracea* var. *acephala* whereas γ -terpinene, camphor, limonene, α -pinene were the least abundant in that order. Additionally, 1,8-cineole, α -pinene, β -caryophyllene, camphor, bornyl acetate and verbenone were the most abundant VOCs in *R. officinalis*.

* Indicates compounds confirmed with authentic standards. Means ($\pm$ SE) with different superscript letter(s) within the rows are significantly different at the $P < 0.05$ level. “nd” indicates not detected.

Table 1 Mean amount (ng/plant/h) of volatile organic compounds identified in headspace collection of *Brassica oleracea* var. *acephala* and *Rosmarinus officinalis* plants (n = 4)

No	RT (min)	Compound Name ¹	RI _{alk} ²	RI _L ³	<i>Brassica oleracea</i> var. <i>acephala</i>	<i>Rosmarinus officinalis</i>	P-value ⁴
1	9.74	α -Pinene*	931	934	410.54 $\pm$ 159.48 ^b	23,465.15 $\pm$ 4393.73 ^a	0.002
2	10.03	Camphene	945	944	nd	5,914.963 $\pm$ 607.05	-
3	10.55	Sabinene	969	974	1,274.64 $\pm$ 746.55	nd	-
4	10.61	β -Pinene*	972	978	87.00 $\pm$ 50.44 ^b	5,379.80 $\pm$ 496.49 ^a	< 0.001
5	10.93	Myrcene*	987	981	953.28 $\pm$ 527.07 ^b	5,983.69 $\pm$ 831.85 ^a	0.002
6	11.17	α -Phellandrene	998	1005	nd	1,429.39 $\pm$ 253.42	-
7	11.39	δ -2-Carene	1011	1011	nd	1,555.28 $\pm$ 289.47	-
8	11.65	Limonene*	1026	1030	1,457.36 $\pm$ 854.58	nd	-
9	11.79	1,8-Cineole	1032	1036	1,232.56 $\pm$ 622.18 ^b	40,197.45 $\pm$ 14,913.86 ^a	0.009
10	12.29	γ -Terpinene*	1061	1060	381.39 $\pm$ 359.18 ^b	3,907.73 $\pm$ 632.33 ^a	< 0.001
11	12.44	(Z)-Sabinene hydrate	1069	1092	nd	3,955.11 $\pm$ 1072.60	-
12	12.92	Linalool*	1096	1101	nd	7,470.31 $\pm$ 2507.75	-
13	13.73	Camphor	1146	1146	118.25 $\pm$ 53.30 ^b	12,642.43 $\pm$ 3081.30 ^a	0.007
14	14.11	Borneol	1167	1167	nd	9,645.39 $\pm$ 1169.14	-
15	14.66	α -Terpineol*	1204	1189	nd	3,666.03 $\pm$ 1261.86	-
16	14.85	Verbenone*	1218	1209	nd	11,939.37 $\pm$ 2333.98	-
17	15.00	Citronellol*	1228	1230	nd	1,143.08 $\pm$ 347.97	-
18	15.45	Geraniol*	1259	1253	nd	4,401.97 $\pm$ 1092.20	-
19	15.90	Bornyl acetate	1290	1295	nd	12,775.81 $\pm$ 2801.34	-
20	17.79	β -Caryophyllene*	1428	1430	69.90 $\pm$ 26.39 ^b	19,141.41 $\pm$ 3947.36 ^a	< 0.001
21	18.17	α -Humulene	1462	1465	nd	4,706.05 $\pm$ 1147.31	-
22	19.77	Caryophyllene oxide*	1593	1588	nd	2,971.13 $\pm$ 491.12	-

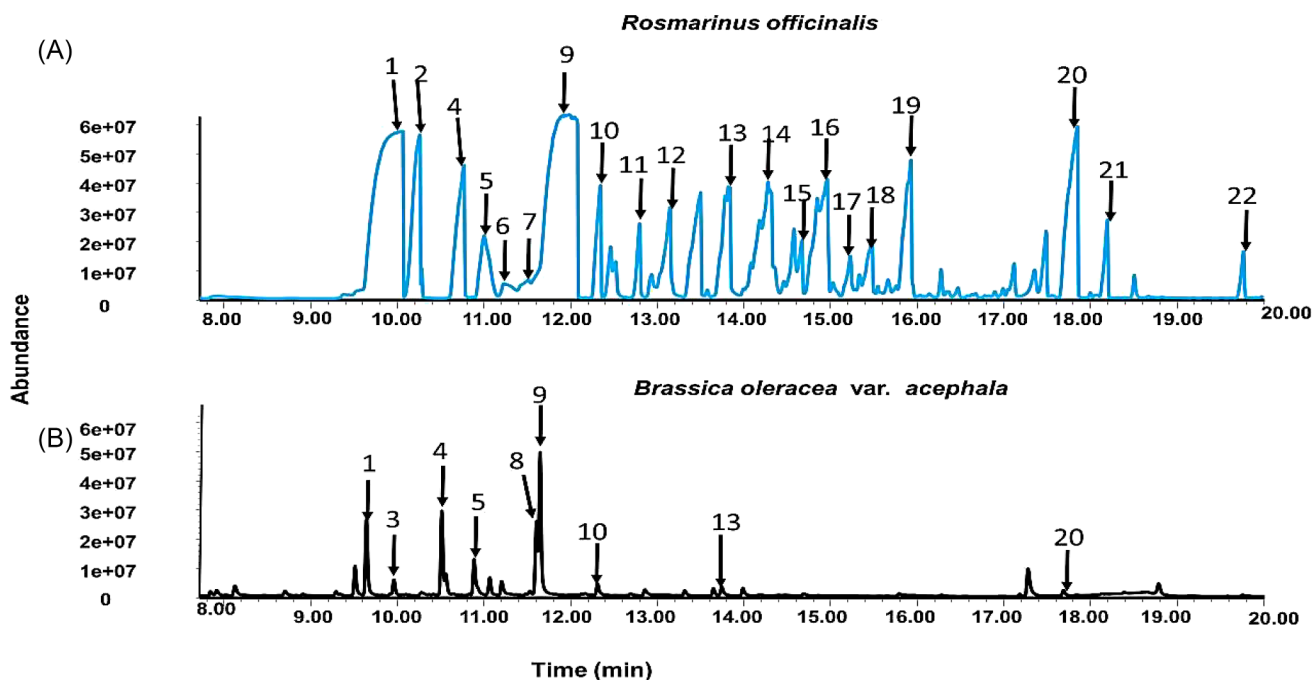
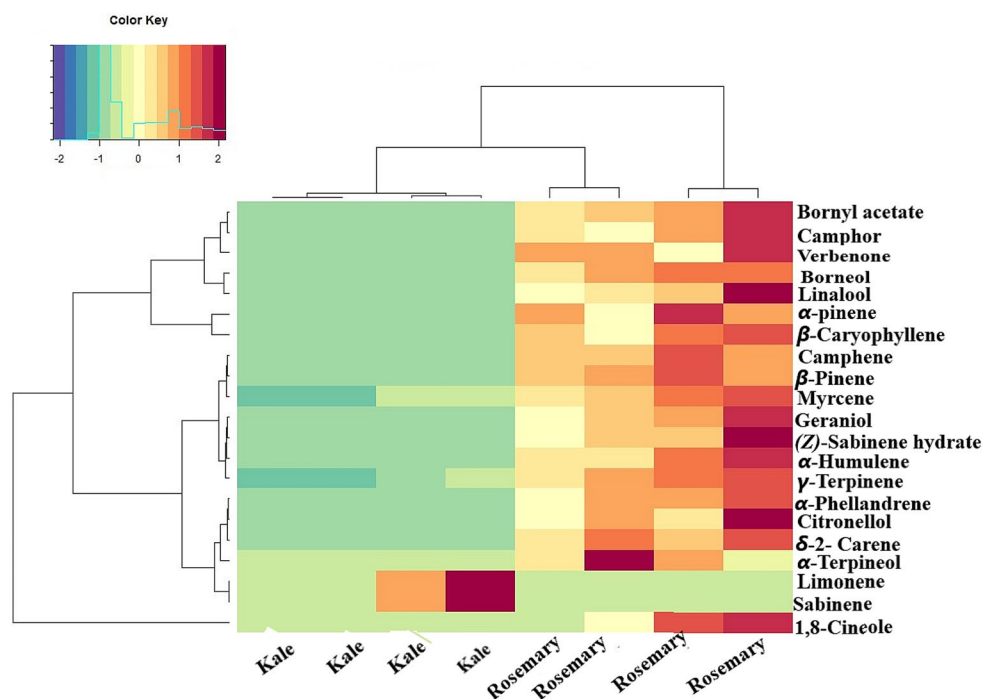
**Fig. 2** Representative gas chromatography-mass spectroscopy chromatogram of *Rosmarinus officinalis* (A) and *Brassica oleracea* var. *acephala* (B) plants. Identities of labelled peaks are represented in Table 1 above

Fig. 3 Heatmap clustering showing the abundance (in decreasing color intensity) of volatile organic compounds across replicates of *Brassica oleracea* var. *acephala* and *Rosmarinus officinalis* plants



Gas chromatography-electroantennography Responses of *Brevicoryne brassicae* to *Rosmarinus officinalis* and *Brassica oleracea* var. *acephala* Headspace Volatiles

The flame ionization detector (FID) and electroantennographic detector (EAD) were used to detect volatile compounds from *R. officinalis* and *B. oleracea* var. *acephala* plants by *B. brassicae* antennae. The GC-EAD recordings showed that *B. brassicae* elicited antennal response to three compounds from *B. oleracea* var. *acephala* namely sabinene, γ -terpinene and β -caryophyllene (Fig. 4A) and six active compounds from *R. officinalis* namely linalool (12), camphor (13), borneol (14), α -terpineol (15), verbenone (16) and geraniol (18) (Fig. 4B; supplementary Fig. 2).

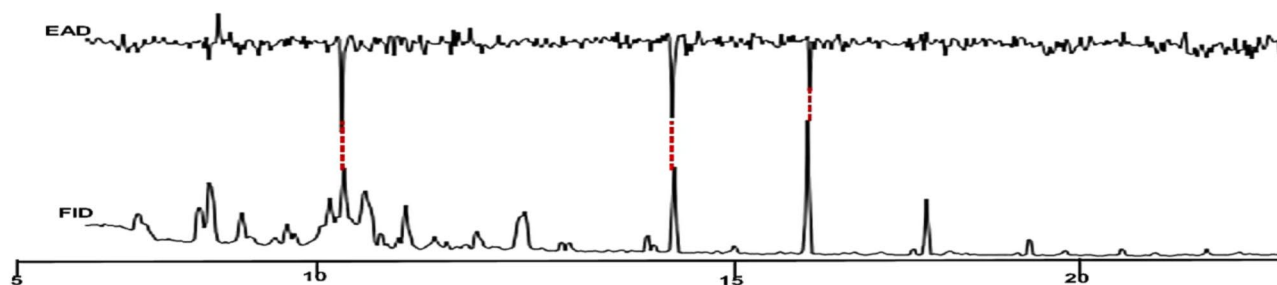
Discussion

Our findings from the current study revealed that *B. brassicae* were more attracted to constitutive volatiles of *B. oleracea* var. *acephala* (their main host) and were repelled by volatiles of *R. officinalis* when using either the whole plant or its headspace extract volatiles. These observations align with the results reported by Cai et al. (2018) where *M. persicae* were found to be attracted by cabbage (*Brassica oleracea* var. *capitata*) volatiles, one of their major host. The reduced attraction of *B. brassicae* to *R. officinalis* plant volatiles as demonstrated in the current study is in agreement with the results reported by Cai et al. (2018) and

Dardouri et al. (2019), who demonstrated that *M. persicae* preferred a blank chamber over the ones containing *R. officinalis*, which emitted VOCs in relatively higher amounts. Non-host plant odors contribute to the repellent and deterrent effects observed in push plants such as the greenleaf desmodium (*Desmodium uncinatum*) and molasses grass (*Melinis minutiflora*) used in cereal push pull cropping systems leading to reduced pest infestation and plant damage (Khan et al. 2001). Similarly, *R. officinalis* volatiles could mask the host plant attractive VOCs from *B. oleracea* var. *acephala* when tied together, given its higher emission of potential repellent volatile compounds making it difficult for *B. brassicae* to perceive its host plant chemical cues in presence of the repellent *R. officinalis* volatiles. A similar setting of a cropping system would establish an environment that can mask the host plant, reducing the chances of infestation (Mutiga et al. 2010).

Chemical analysis of headspace volatiles showed that *R. officinalis* produced more terpenes as compared to *B. oleracea* var. *acephala* (Table 1). The most abundant VOCs in *R. officinalis* included 1,8-cineole, camphor, verbenone, bornyl acetate, linalool and citronellol. Majority of these compounds have been associated with repellence properties against different insects species when used as plant extracts and essential oils (Miresmailli and Isman 2006; Cloyd et al. 2009; Webster 2009; Dayaram and Khan 2016). Comparable results on *R. officinalis* essential oils were reported by Elhalawany et al. (2019), who observed that the major constituents of *R. officinalis* oil was mostly made of linalool, α -pinene, limonene, bornyl acetate and β -caryophyllene.

(A)



(B)

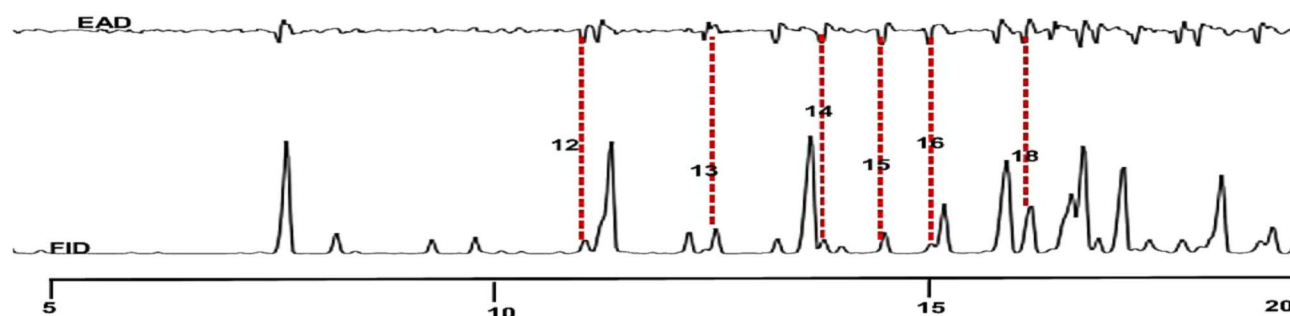


Fig. 4 Gas chromatography-electroantennography active compounds from *Brassica oleracea* var. *acephala*(A) and *Rosmarinus officinalis*(B) plant volatiles

A previous study reported verbenone, 1–8 cineole and linalool as major constituents of *R. officinalis* volatiles and its oil extracts (Hori 1998). *Rosmarinus officinalis* emitted a higher quantity of volatiles as compared to *B. oleracea* var. *acephala*, which are responsible for its characteristic aroma. *Rosmarinus officinalis*, being an aromatic herb produces such compounds in very high amounts, to mask *B. oleracea* var. *acephala* volatiles making the insect to perceive *R. officinalis* volatiles from a far and avoid them.

Host location by *B. brassicae* involves the perception of the volatiles by the sensilla of the insect's antenna. The GC-EAD gives an opportunity to utilize these antennae to determine which of the volatiles are responsible for the behavior of the insect. The findings from this study indicate that *B. brassicae*'s antenna detected to sabinene, γ -terpinene and β -caryophyllene from *B. oleracea* var. *acephala* (Fig. 4A). Sabinene was one of the major constituent volatiles in *B. oleracea* var. *acephala* but was not observed in *R. officinalis*. Additionally, despite γ -terpinene and β -caryophyllene being found in both plants, *B. brassicae* antennal detection were not observed to these compounds when *R. officinalis* volatiles were used. However, *B. brassicae* antenna showed antennal detection to linalool, camphor, borneol, α -terpineol,

verbenone and geraniol from *R. officinalis* (Fig. 4B). Among the *R. officinalis* compounds that elicited antennal detection, camphor and α -terpineol have been reported to reduce the activities of *M. persicae* and other insects such as mosquitoes (Dardouri et al. 2019). The insect's antenna did not detect 1,8-cineole despite it being a major constituent of *R. officinalis* oil. However, some studies have reported its insecticidal activity against onion aphid, *Neotoxoptera formosana* Takahashi (Hemiptera: Aphididae) (Hori 1998; Elhalawany et al. 2019). Camphor, citronellal and geraniol, have also been reported to have high insecticidal activity against aphids by disrupting their digestive and neurological enzymes hence leading to death (Chalise and Dawadi 2019). Therefore, the presence of these compounds in the volatiles emitted by *R. officinalis* could have contributed to the observed repellence behavior exhibited by *B. brassicae*.

Our electrophysiological study may confirm the results of laboratory bioassays with *R. officinalis* volatiles which showed that linalool, camphor, and α -terpineol were repellent to tea geometrid *Ectropis obliqua*, as opposed to other compounds in *R. officinalis* bouquet (Zhang et al. 2015). Similar results were obtained with *M. persicae* (Hori 1998). Li et al. (2021) reported the presence of monoterpenes such

as α -pinene, 1,8-cineole, camphor, camphene, and verbenone as the most abundant repellent compounds in *R. officinalis*. Bruce et al. (2005) and Dardouri et al. (2019) documented that α -pinene, camphene, limonene, γ -terpinene, linalool, borneol, and verbenone lack repellent properties against *M. persicae*. Our study shows that *B. brassicae* antennae detected linalool, borneol, and verbenone, an indication that these VOCs might elicit species-specific responses among different species of aphids. Camphor, verbenone and linalool have been found to be the major constituents of *R. officinalis* volatiles and its oil extracts, responsible for repellence properties against different pests. For instance, they were found to not only repel and induce an anti-appetizing effect on *M. persicae* but also on the onion aphid *N. formosana*, mosquitoes and lesser grain borer *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae) (Hori 1998; Dardouri et al. 2019).

In conclusion, this study demonstrates that *R. officinalis* emits VOCs which possess strong repellent properties against *B. brassicae* as observed in the behavioral responses. It also reveals that volatiles emitted by *R. officinalis* have a masking effect over *B. oleracea* var. *acephala* volatiles. The repellency of *R. officinalis* is attributed to the production of distinct VOCs, which elicit antennal detection when the insect is exposed to them. It therefore provides insights on the use of *R. officinalis* as a potential repellent plant in the management of *B. brassicae* through an intercropping strategy. Such an approach would be a promising strategy towards the reduction of synthetic pesticides in the management of *B. brassicae* in smallholder *B. oleracea* var. *acephala* production systems. We recommend laboratory bioassays using synthetics and blends of the reported compounds for confirmation. Field evaluation trials are also needed to validate these findings using *B. oleracea* var. *acephala* and *R. officinalis* intercropping on the *B. brassicae* infestation, damage, reproduction rate, interactions with its associated natural enemies and crop yield.

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Author Contributions DMM and TD conceived the idea, BKM, DMM, KSA, and ENK designed the study; BKM collected data; BKM, DMM, and BM analysed data; BKM and DMM led the drafting of the manuscript; TD supervised the work and DMM provided resources. All authors critically reviewed and approved the final version.

Data Availability The data that support the findings of this study are available up on request from the corresponding author.

Declarations

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References

- Badenes-Perez FR, Shelton AM (2006) Pest management and other agricultural practices among farmers growing cruciferous vegetables in the Central and Western highlands of Kenya and the western himalayas of India. *Int J Pest Manage* 52(4):303–315. <https://doi.org/10.1080/09670870600819169>
- Ben-Issa R, Gomez L, Gautier H (2017) Companion plants for aphid pest management. *Insects* 8(4). <https://doi.org/10.3390/insects8040112>
- Bruce TJA, Wadhams LJ, Woodcock CM (2005) Insect host location: a volatile situation. *Trends Plant Sci* 10(6):269–274. <https://doi.org/10.1016/j.tplants.2005.04.003>
- Cai T, Wang L, Zhang K, Lin Z, Wang R (2018) The influence of Rosemary plants (*Rosmarinus officinalis*) volatiles on Aphid (*Myzus Persicae*). *120 (Jfeesm)*, 1198–1202. <https://doi.org/10.2991/ifeesm-17.2018.220>
- Canwat V, Oelofse M, Onakuse S, de Neergaard A (2021) Agroecological intensification: can organic conversion improve the production efficiency? A perspective from smallholder kale production systems Kenya. *Clean Environ Syst* 3(July):100048. <https://doi.org/10.1016/j.cesys.2021.100048>
- CDC (2014) Defining powerhouse fruits and vegetables: a nutrient density approach. *Prev Chronic Dis* 11(6):3–7. <https://doi.org/10.5888/pcd11.130390>
- Chalise PP, Dawadi S (2019) Efficacy of plant essential oils on black bean aphid (*Aphis fabae*) and cabbage aphid (*Brevicoryne brassicae*) under laboratory condition storage insect pest management view project traditional beekeeping with Apis cerana F. in Nepal View project. *January*. <http://www.entomoljournal.com>
- Cloyd RA, Galle CL, Keith SR, Kalscheur And NA, Kemp KE (2009) Effect of commercially available plant-derived essential oil products on arthropod pests. *J Econ Entomol* 102(4):1567–1579. <https://doi.org/10.1603/029.102.0422>
- Cole RA (1994) Locating a resistance mechanism to the cabbage aphid in two wild brassicas. *Entomol Exp Appl* 71(1):23–31. <https://doi.org/10.1111/j.1570-7458.1994.tb01766.x>
- Cook SM, Khan ZR, Pickett JA (2007) The use of push-pull strategies in integrated pest management. *Ann Rev Entomol* 52:375–400. <https://doi.org/10.1146/annurev.ento.52.110405.091407>

- Core Team R (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing (4.2.3). <https://www.r-project.org/>
- Dardouri T, Gomez L, Schoeny A, Costagliola G, Gautier H (2019) Behavioural response of green peach aphid *Myzus persicae* (Sulzer) to volatiles from different rosemary (*Rosmarinus officinalis* L.) clones. *Agric for Entomol* 21(3):336–345. <https://doi.org/10.1111/afe.12336>
- Dayaram L, Khan A (2016) Repellent, Fumigant and Contact Toxicity of *Salvia Officinalis*, *Rosmarinus Officinalis* and *Coriandrum Sativum* Against *Callosobruchus Maculatus* (Fab.) (Coleoptera : Bruchidae). *National Academy of Agricultural Science*, 34(4), 893–902
- Döring TF (2014) How aphids find their host plants, and how they don't. *Ann Appl Biol* 165(1):3–26. <https://doi.org/10.1111/aab.12142>
- Douloumpaka S, Van Emden HF (2003) A maternal influence on the conditioning to plant cues of *Aphidius Colemani* Viereck, parasitizing the aphid *Myzus Persicae* Sulzer. *Physiol Entomol* 28(2):108–113. <https://doi.org/10.1046/j.1365-3032.2003.00323.x>
- Elhalawany A, Abou-Zaid A, Amer A (2019) Laboratory Bioassay for the efficacy of Coriander and Rosemary extracted essential oils on the Citrus Brown Mite, *Eutetranychus Orientalis* (Actinidida: Tetranychidae). *Acarines: J Egypt Soc Acarology* 13(1):15–20. <https://doi.org/10.21608/ajesa.2019.164149>
- Finch S, Kienegger M (1997) A behavioural study to help clarify how undersowing with clover affects host-plant selection by pest insects of brassica crops. *Entomol Exp Appl* 84(2):165–172. <https://doi.org/10.1046/j.1570-7458.1997.00211.x>
- Fombong AT, Mutunga JM, Teal PEA, Torto B (2016) Behavioral evidence for olfactory-based location of Honeybee colonies by the Scarab *Oplostomus Haroldi*. *J Chem Ecol* 42(10):1063–1069. <https://doi.org/10.1007/s10886-016-0748-1>
- Gill HK, Garg H, Gillett-Kaufman JL (2013) Cabbage aphid *Brevicoryne brassicae* Linnaeus (Insecta: Hemiptera: Aphididae)1. *Edis* 2013(10):1–5. <https://doi.org/10.32473/edis-in1014-2013>
- HCD (2019) Horticultural Crops Directorate.2017–2018 Validated Horticulture Data Report. 1–89. <http://horticulture.agricultureau-authority.go.ke/index.php/statistics/reports>
- Hori M (1998) Repellency of rosemary oil against *Myzus persicae* in a laboratory and in a screenhouse. *J Chem Ecol* 24(9):1425–1432. <https://doi.org/10.1023/A:1020947414051>
- Khan ZR, Pickett JA, Wadhams L, Muyekho F (2001) Habitat management strategies for the control of cereal stemborers and striga in maize in Kenya. *Insect Sci Its Application* 21(4):375–380. <https://doi.org/10.1017/S1742758400008481>
- Kianmatee S, Ranamukhaarachchi SL (2007) Pest repellent plants for management of insect pests of Chinese. *Int J Agric Biology* 9(1):64–67
- Lagerkvist CJ, Ngigi M, Okello JJ, Karanja N (2012) Means-End Chain approach to understanding farmers' motivations for pesticide use in leafy vegetables: the case of kale in peri-urban Nairobi, Kenya. *Crop Prot* 39:72–80. <https://doi.org/10.1016/j.cropro.2012.03.018>
- Lans C, Van Der, Snoek H, Boer F, De, Elings A (2012) Vegetable chains in Kenya: production and consumption of vegetables in the Nairobi Metropolitan. Wageningen UR Greenh Hortic, 88p. <https://edepot.wur.nl/216710>
- Li X-W, Lu X-X, Zhang Z-J, Huang J, Zhang J-M, Wang L-K, Hafeez M, Mandela Fernández-Grandon G, Lu Y-B (2021) Intercropping Rosemary (*Rosmarinus officinalis*) with Sweet Pepper (*Capsicum annuum*) reduces major Pest Population densities without impacting natural enemy populations State Key Laboratory for managing biotic and chemical threats to the Quality and Safety. 12:74
- Lohonyai Z, Vuts J, Kárpáti Z, Koczor S, Michael J, Fail J, Birkett MA (2019) Benzaldehyde: an alfalfa-related compound for the spring attraction of the pest weevil *Sitona humeralis* (Coleoptera: Curculionidae). *March*. <https://doi.org/10.1002/ps.5431>
- Macharia M, Afr W (2009) Potential environmental impacts of Pesticides Use in the Vegetable Sub-sector in Kenya. *J Hort Sci* 2:138–151
- Maddrell SHP (1969) Secretion by the Malpighian tubules of *Rhodnius*. The movements of ions and water. *J Exp Biol* 51(1):71–97. <https://doi.org/10.1242/jeb.51.1.71>
- Miresmailli S, Isman MB (2006) Efficacy and persistence of rosemary oil as an acaricide against twospotted spider mite (Acari: Tetranychidae) on greenhouse tomato. *J Econ Entomol* 99(6):2015–2023. <https://doi.org/10.1093/jee/99.6.2015>
- Munthali DC, Tshogofatso AB (2014) Factors Affecting Abundance and Damage Caused by Cabbage Aphid, *Brevicoryne brassicae* on Four Brassica Leafy Vegetables: *Brassica oleracea* var. Acephala, *B. chinense*, *B. napus* and *B. carinata*. *The Open Entomology Journal*, 8(1), 1–9. <https://doi.org/10.2174/1874407901408010001>
- Mutiga SK, Gohole LS, Auma EO (2010) Effects of integrating companion cropping and nitrogen application on the performance and infestation of collards by *Brevicoryne brassicae*. *Entomol Exp Appl* 134(3):234–244. <https://doi.org/10.1111/j.1570-7458.2009.00952.x>
- Mutiga SK, Gohole S, L., Auma O, E (2011) Agronomic performance of collards under two intercrops and varying Nitrogen application levels as assessed using Land Equivalent Ratios. *J Agric Sci* 3(1). <https://doi.org/10.5539/jas.v3n1p22>
- Mutyambai DM, Bruce TJA, Midega CAO, Woodcock CM, Caulfield JC, Van Den Berg J, Pickett JA, Khan ZR (2015) Responses of Parasitoids to Volatiles Induced by *Chilo partellus* Oviposition on Teosinte, a Wild Ancestor of Maize. *J Chem Ecol* 41(4):323–329. <https://doi.org/10.1007/s10886-015-0570-1>
- Ngolo P, Nawiri M, Machocho A, Oyieke H (2019) Pesticide Residue Levels in Soil, Water, Kales and Tomatoes in Ewaso Narok Wetland, Laikipia, County, Kenya. *J Sci Res Rep* 1–11. <https://doi.org/10.9734/jsrr/2019/v24i530165>
- Ngolo Otieno P (2019) Pesticide application, their residue levels in the environment, kales and tomatoes in ewaso narok wetland, laikipia county, kenya. *Kenyatta University*
- Olwande J, Smale M, Mathenge MK, Place F, Mithöfer D (2015) Agricultural marketing by smallholders in Kenya: A comparison of maize, kale and dairy. *Food Policy* 52:22–32. <https://doi.org/10.1016/j.foodpol.2015.02.002>
- Peris N, Kiptoo J (2017) Potential of Botanical Extracts in the Control of Kale Aphids (*Brevicoryne brassicae*) and Their Effect on the Parasitic Wasp (*Aphidius ervi*). *Asian Res J Agric* 4(3):1–6. <https://doi.org/10.9734/arja/2017/29849>
- Piepel GF, Aitchison J (1988) The Statistical Analysis of Compositional Data. *Technometrics* 30(1):120. <https://doi.org/10.2307/1270335>
- Piesik D, Wenda-Piesik A, Krasinska A, Wrzesinska D, Delaney KJ (2016) Volatile organic compounds released by *Rumex confertus* following *Hypera rumicis* herbivory and weevil responses to volatiles. *J Appl Entomol* 140(4):308–316. <https://doi.org/10.1111/jen.12238>
- Powell G, Tosh CR, Hardie J (2006) Host plant selection by aphids: Behavioral, evolutionary, and applied perspectives. *Ann Rev Entomol* 51(May):309–330. <https://doi.org/10.1146/annurev.ento.51.110104.151107>
- Ricupero M, Desneux N, Zappalà L, Biondi A (2020) Target and non-target impact of systemic insecticides on a polyphagous aphid pest and its parasitoid. *Chemosphere* 247. <https://doi.org/10.1016/j.chemosphere.2019.125728>
- Šamec D, Urlić B, Salopek-Sondi B (2019) Kale (*Brassica oleracea* var. acephala) as a superfood: Review of the scientific evidence behind the statement. *Crit Rev Food Sci Nutr* 59(15):2411–2422. <https://doi.org/10.1080/10408398.2018.1454400>

- Shapiro S, Wilk M (1965) An analysis of variance test for normality (Complete samples). *Biometrika* 52(3–4):591–611. <https://doi.org/10.1093/biomet/52.3-4.591>
- Sisay B, Sevgan S, Weldon CW, Krüger K, Torto B, Tamiru A (2023) Responses of the fall armyworm (*Spodoptera frugiperda*) to different host plants: Implications for its management strategy. *Pest Manag Sci* 79(2):845–856. <https://doi.org/10.1002/ps.7255>
- Van Emden HF, Harrington R (2007) Aphids as crop pests. Aphids as Crop Pests 1–717. <https://doi.org/10.2135/cropsci2008.02.0001br>
- Waithaka PN, Gathuru EM, Githaiga BM, Kimani SN (2017) Control of Passion Fruit Fungal Diseases Using Essential Oils Extracted from Rosemary (*Rosmarinus officinalis*) and Eucalyptus (*Eucalyptus agglomerata*) in Egerton University Main Campus Njoro, Kenya. *Int J Microbiol* 2017. <https://doi.org/10.1155/2017/2814581>
- Webster B (2009) Olfactory basis of host-recognition in the black bean aphid, *Aphis fabae*. Imperial College London
- Yarou B, Assogba Komlan F, Tossou E, Mensah A, Simon S, Verhaegen D, Francis F (2017) Efficacy of Basil-Cabbage intercropping to control insect pests in Benin, West Africa. *Commun AgriCult Appl Biol Sci* 82:157–166
- Zhang Z, Chen Z (2015) Non-host plant essential oil volatiles with potential for a push-pull strategy to control the tea green leafhopper, *Empoasca vitis*. *Entomol Exp Appl* 156(1):77–87. <https://doi.org/10.1111/eea.12310>
- Zhang Z, Bian L, Sun X, Luo Z, Xin Z, Luo F, Chen Z (2015) Electrophysiological and behavioural responses of the tea geometrid *Ectropis obliqua* (Lepidoptera: Geometridae) to volatiles from a non-host plant, rosemary, *Rosmarinus officinalis* (Lamiaceae). *Pest Manag Sci* 71(1):96–104. <https://doi.org/10.1002/ps.3771>

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