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
International Centre of Insect Physiology and Ecology
Thomas Dubois
International Centre of Insect Physiology and Ecology
Komivi Senyo Akutse
International Centre of Insect Physiology and Ecology
Benjamin Muli
South Eastern Kenya University
Edward Nderitu Karanja
International Centre of Insect Physiology and Ecology
Daniel Munyao Mutyambai
dmutyambai@icipe.org

International Centre of Insect Physiology and Ecology

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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 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 behavioral response using coupled gas chromatography-mass spectrometry (GC-MS) and gas chromatography-electroantennogram (GC-EAG), 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. GC-MS analysis revealed diverse and higher quantities of volatile compounds in *R. officinalis* compared to *B. oleraceae* var. *acephala*. GC-EAG showed that *B. brassicae* was responsive to linalool, camphor, borneol, α-terpineol, verbenone, geraniol and bornyl acetate from *R. officinalis* and sabinene, γ-terpinene, and β-caryophyllene from *B. oleraceae* var. *acephala*. Our findings demonstrate that *R. officinalis* is repellent against *B. brassicae* and could be utilized as a ‘push’ plant in an intercropping strategy against this pest.

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, 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, 2014). 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 transmits 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*) (Douloumpaka 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 semiochemicals to manipulate the distribution of pests and their natural enemies through production of volatile organic compounds (VOCs) in the natural ecosystem, which play an important role in communication, defense and response to abiotic 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 involves perception of specific or a blend of VOCs naturally emitted into the ecosystem which determine attraction or avoidance (Zhang and Chen, 2015). As such, non-host plant volatiles can be used to modify the behavior of pests by interfering with their host selection and orientation. 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 two-spotted spider mite (*Tetranychus urticae* Koch (Trombidiformes: Tetranychidae)) and 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 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- electroantennography 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 (01º 13’ 25.6” S 036º 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 the *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 x 40 cm Perspex rearing cages in the laboratory and maintained at the temperature of 25 ± 1 ºC, 65 ± 5% relative humidity (RH) and 12L: 12D hrs photoperiod as a modification of Chalise and Dawadi (2019) who reared the aphids at 25 ± 2°C and 55 ± 5% Relative Humidity. 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 fourteen days old.

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 first two hours of the photo phase 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 µL dichloromethane (analytical grade, Sigma-Aldrich, USA) 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

Two 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 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 choice.

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. The two opposite arms held 10 µl aliquots of each of the 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 both 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. In the event that 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; 7890A GC and MSD 5975C 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, 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 the amount (in ng) of identified VOCs, the peak areas were divided by the known quantities of external standards. The emission rate, expressed as $ng^{-1}plant^{-1}h^{-1}$, was determined by multiplying the reciprocal of the proportion of the total headspace utilized and subsequently dividing it by the number of hours in the sampling period. 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 15mm 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 µl of the concentrated entrainment

sample was injected onto a nonpolar column (HP-1, 50 m × 0.32 mm i.d.× 0.52 µm film thickness, (Agilent Technologies, California, USA) in a HP5890 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. Hydrogen was used as the carrier gas. Simultaneous recordings of the EAG and FID responses were obtained with specialized software (Electro Antenno Detection 2015 version 1.2.6, Syntech, Hilversum, The Netherlands). The EAD outlet contained an uninterrupted airflow filtered through charcoal at a rate of 400 mL min^{-1} directed to the *B. brassicae* antenna. A total of six coupled runs were completed. Only FID peaks which corresponded to an EAG peak in 3 or more replicates were considered electro-physiologically active.

Statistical analyses

Data was analyzed using R statistical software version 4.2.3 (R 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 log_{10}-ratio transformations to allow for analysis of compositional data (Mutyambai et al. 2015; Piepel and Aitchison, 1988). For the normal distribution of the data, Shapiro-Wilk test (Shapiro and Wilk, 1965) was performed before being subjected to analysis of variance (ANOVA), followed by the Student-Newman-Keuls (SNK) test for mean separation whenever treatments were found to be significantly different at $P < 0.05$. All P values ≤ 0.05 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, or clean air, *B. brassicae* showed more preference to the arm containing *B. oleracea* var. *acephala* over the arms with *R. officinalis* or clean air ($P < 0.001$) (Fig. 1A). In the second experiment with odour sources from *B. oleracea* var. *acephala* and *R. officinalis* headspace volatiles, or clean air, *B. brassicae* showed less preference to the arm containing *R. officinalis* volatiles than the arms containing *B. oleracea* var. *acephala* volatiles or clean air ($P < 0.001$) (Fig. 1B).

Volatile profiles

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 and 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 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). VOCs that were detected in *R. officinalis* but not detected 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. Those 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 was the most abundant volatile in both *B. oleracea* var. *acephala* and *R. officinalis*, while 1,8-cineole, α-pinene, β-caryophyllene, camphor, bornyl acetate and verbenone were the most abundant VOCs in *R. officinalis*.

Table 1
Mean amount (ng/plant/h) of volatile organic compounds (VOCs) 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]	*Brassica oleracea* var. *acephala*	*Rosmarinus officinalis*	*P*-value[4]
1	9.74	*α*-pinene*	931	934	410.54 ± 159.48[b]	23,465.15 ± 4393.73[a]	0.002
2	10.03	Camphene	945	944	nd	5,914.963 ± 607.05	-
3	10.55	Sabinene	969	974	1,274.64 ± 746.55	nd	-
4	10.61	*β*-pinene*	972	978	87.00 ± 50.44[b]	5,379.80 ± 496.49[a]	< 0.001
5	10.93	myrcene*	987	981	953.28 ± 527.07[b]	5,983.69 ± 831.85[a]	0.002
6	11.17	*α*-phellandrene	998	1005	nd	1,429.39 ± 253.42	-
7	11.39	*δ*-2-carene	1011	1011	nd	1,555.28 ± 289.47	-
8	11.65	Limonene*	1026	1030	1,457.36 ± 854.58	nd	-
9	11.79	1,8-cineole	1032	1036	1,232.56 ± 622.18[b]	40,197.45 ± 14,913.86[a]	0.009
10	12.29	*γ*-terpinene*	1061	1060	381.39 ± 359.18[b]	3,907.73 ± 632.33[a]	< 0.001
11	12.44	*(Z)*-sabinene hydrate	1069	1092	nd	3,955.11 ± 1072.60	-
12	12.92	Linalool*	1096	1101	nd	7,470.31 ± 2507.75	-
13	13.73	Camphor	1146	1146	118.25 ± 53.30[b]	12,642.43 ± 3081.30[a]	0.007
14	14.11	Borneol	1167	1167	nd	9,645.39 ± 1169.14	-
15	14.66	*α*-terpineol*	1204	1189	nd	3,666.03 ± 1261.86	-
16	14.85	Verbenone*	1218	1209	nd	11,939.37 ± 2333.98	-
17	15.00	Citronellol*	1228	1230	nd	1,143.08 ± 347.97	-

No	RT (min)	Compound Name[1]	RI_{alk}[2]	RI_L[3]	*Brassica oleracea* var. *acephala*	*Rosmarinus officinalis*	*P*-value[4]
18	15.45	Geraniol*	1259	1253	nd	4,401.97 ± 1092.20	-
19	15.90	Bornyl acetate	1290	1295	nd	12,775.81 ± 2801.34	-
20	17.79	*β*-caryophyllene	1428	1430	69.90 ± 26.39 [b]	19,141.41 ± 3947.36 [a]	< 0.001
21	18.17	*α*-humulene	1462	1465	nd	4,706.05 ± 1147.31	-
22	19.77	Caryophyllene oxide*	1593	1588	nd	2,971.13 ± 491.12	-

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

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 seven active compounds from *R. officinalis* namely linalool (12), camphor (13), borneol (14), α-terpineol (15), verbenone (16), geraniol (18) and bornyl acetate (19) (Fig. 4B)

DISCUSSION

Our findings from the current study revealed that *B. brassicae* were more attracted to constitutive and headspace volatiles of *B. oleracea* var. *acephala* (their main host) and were repelled by *R. officinalis* as a whole plant and its headspace 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), where the authors 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* given its

higher emission of some of the repellent volatile compounds making it difficult for *B. brassicae* to perceive its host in presence of the repellent *R. officinalis* volatiles.

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. *Rosmarinus officinalis* produced 1,8-cineole 36-fold the amount produced by *B. oleracea* var. *acephala*. Additionally, verbenone, linalool and β-caryophyllene were found to be the other two most abundant VOCs. This is in tandem with previous studies that reported verbenone, 1–8 cineole and linalool as the 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. The high abundance of these major compounds is evidence that *R. officinalis*, being an aromatic herb produces such compounds in very high amounts, which the insect can perceive from a far and avoid them, while masking the host plant volatiles.

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 and under controlled volumes, determine which among the volumes of the volatiles are responsible for the behavior of the insect. The findings of this study indicate that *B. brassicae*'s antenna responded 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* antenna didn't show any antennal response when *R. officinalis* volatiles were used. However, *B. brassicae* antenna showed antennal response to linalool, camphor, borneol, α-terpineol, verbenone, geraniol and bornyl acetate from *R. officinalis* (Fig. 4B). Among the *R. officinalis* compounds that caused antennal response, bornyl acetate, 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 show any response to 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 & Dawadi, 2019); therefore, their presence in the volatiles emitted by *R.* officinalis could have contributed to the observed repellence behavior exhibited by *B. brassicae*.

Our electrophysiological study confirms the results of laboratory bioassays with *R. officinalis* volatiles which showed that linalool, camphor, and α-terpineol were repellent to *B. brassicae* as opposed to other compounds in *R. officinalis* bouquet. 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.* Conflictingly, *B. brassicae* did not show any electrophysiological response to 1,8-cineole and camphene. 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 contradicts this as we observed that *B. brassicae* antennae detected linalool, borneol, and verbenone, an indication that these VOCs might elicit species specific response 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 are repellent to *B. brassicae*. 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 management of *B. brassicae* in smallholder *B. oleracea* var. *acephala* production systems. However, field evaluation trials are warranted to validate these findings using *B. olearacea* var. *acephala* and *R. officinalis* intercropping on the *B. brassicae* infestation, damage, reproduction rate, interactions with its associated natural enemies and yield.

Declarations

Institutional Review Board Statement

No institutional approval was required to conduct the study.

Informed Consent Statement

No Informed consent was required to conduct this study.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contribution

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.

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Data Availability Statement

The derived data that support the findings of this study will be made available without undue reservation upon request.

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Figures

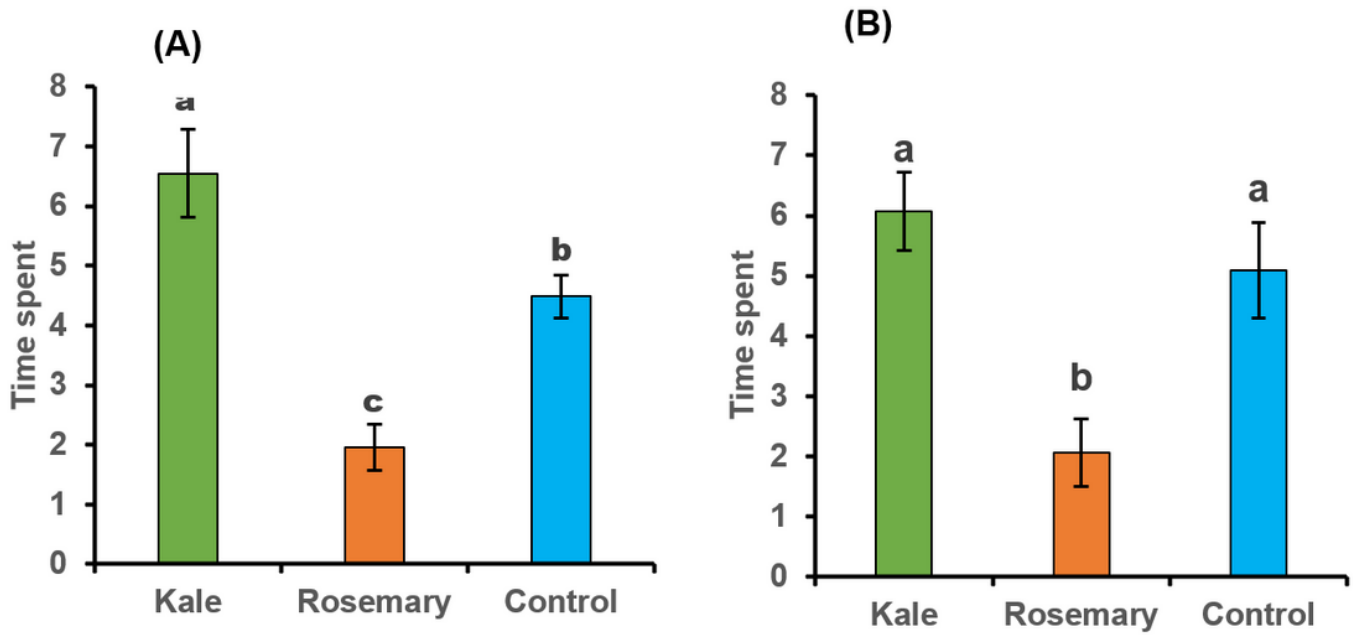

Figure 1

Behavioral response of *Brevicoryne brassicae* to naturally emitted constitutive volatiles from *Brassica oleracea* var. *acephala* and *Rosmarinus officinalis* plants (A) and their headspace volatiles (B) in a four-arm olfactometer. Time spent by *Brevicoryne brassicae* was observed for 20 min (N=12). Means (± SE) with different letter above the bars are significantly different.

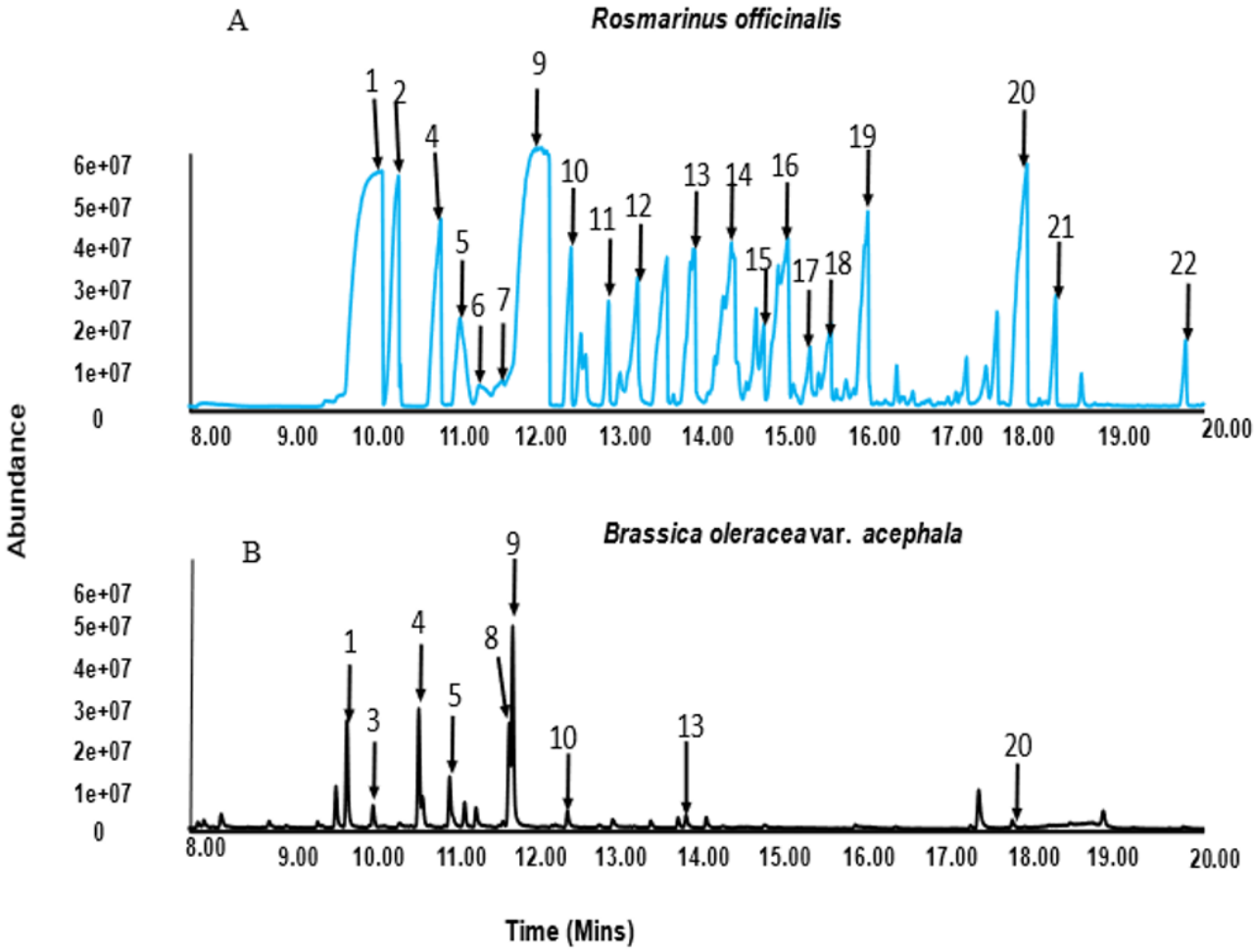

Figure 2

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 below.

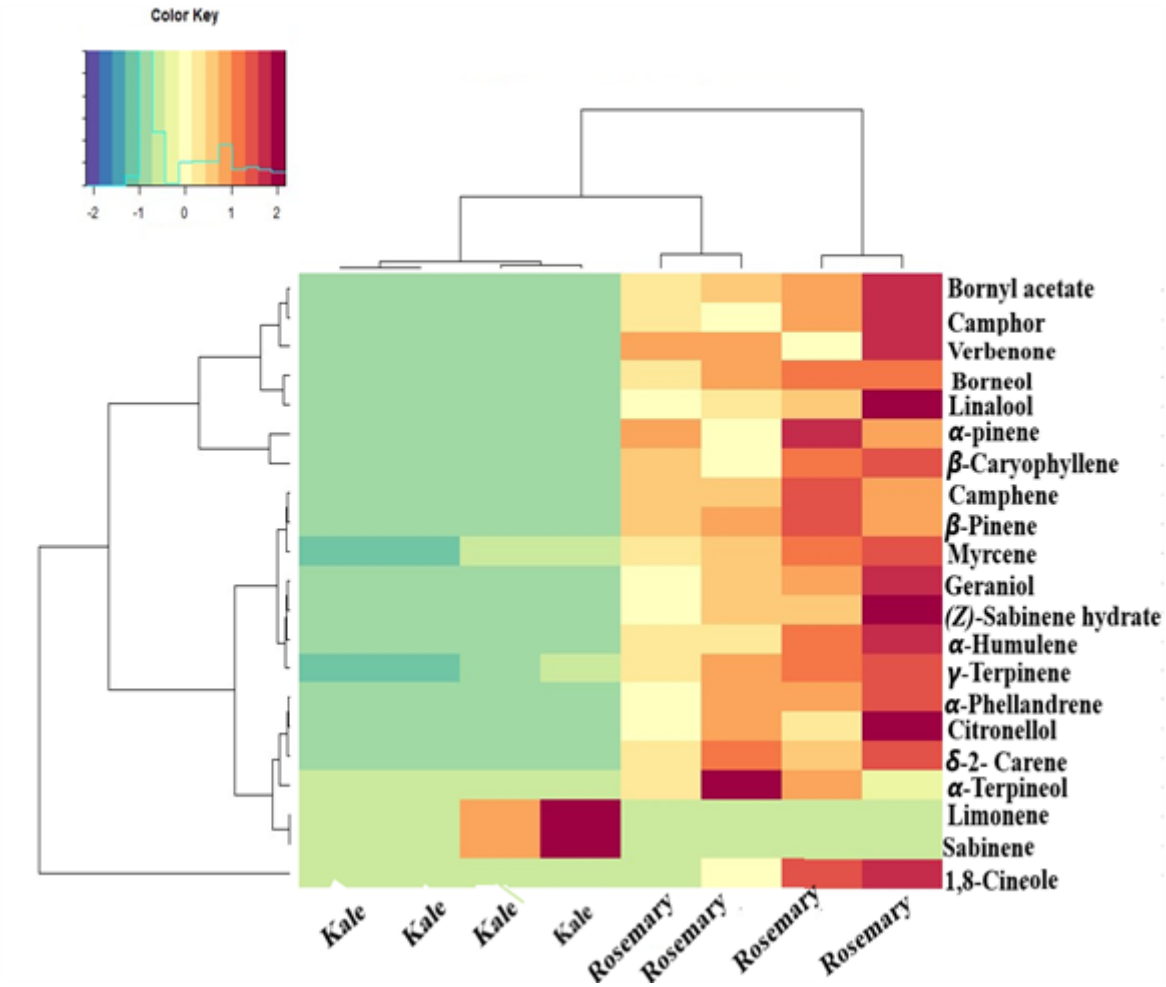

Figure 3

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

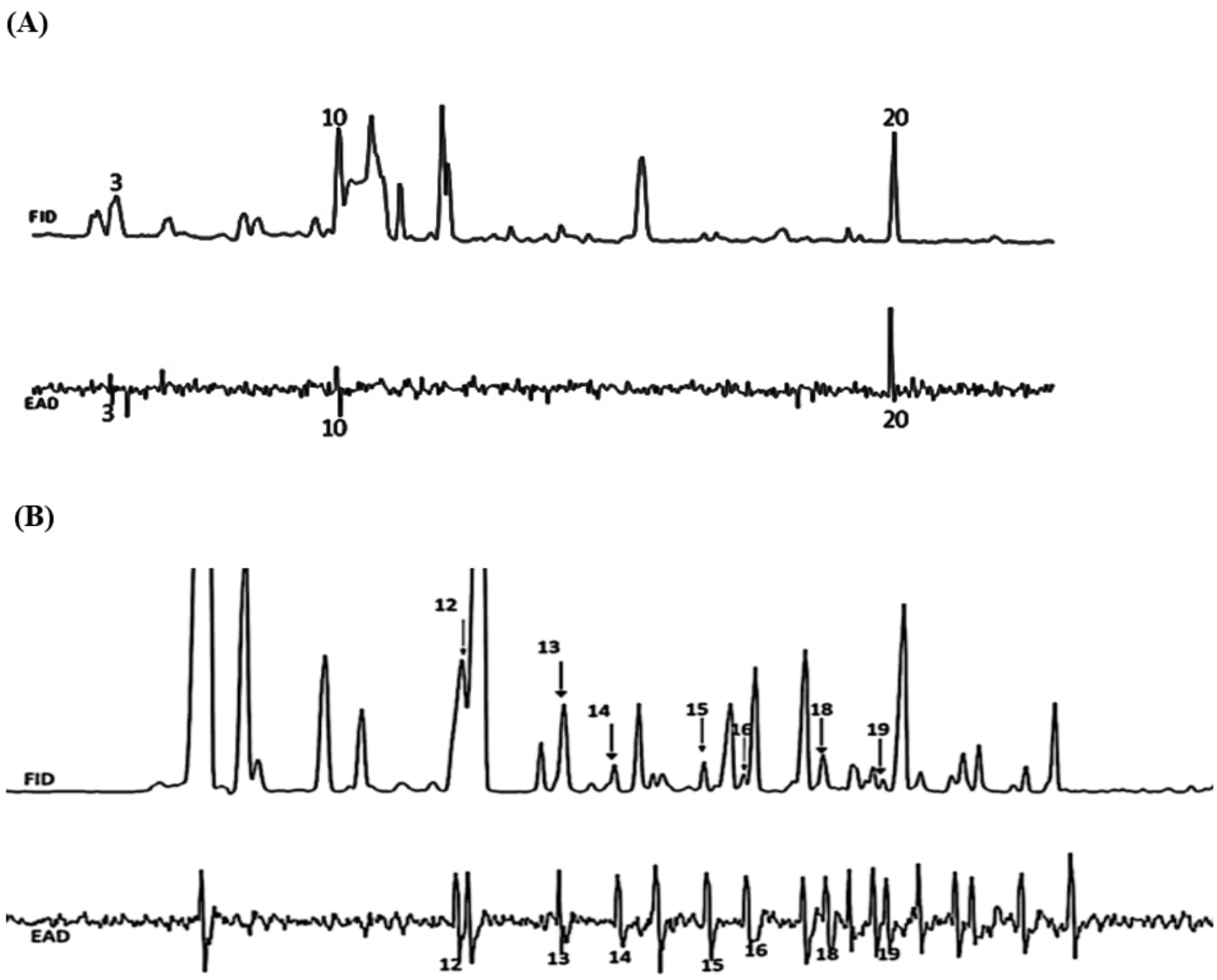

Figure 4

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