The behavioral responses of Ceratitis capitata (Diptera: Tephritidae) to predominant volatile compounds of apricot

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Research Article

Keywords: GC-MS, field, olfactometer, wind tunnel

Posted Date: May 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1606173/v1

Abstract

Ceratitis capitata Wied. (Diptera: Tephritidae) is a significant pest of apricot (*Prunus armeniaca*) fruit in the spring and summer seasons in Turkey. New integrated pest management techniques are required for *C. capitata* to reduce dependency on insecticides used for its control that may result in negative environmental impacts. In the current study, we identified volatiles of apricot fruit by using gas chromatography-mass spectrometry (GC-MS). We tested the behavioral responses of *C. capitata* to some of the identified compounds of apricot in both field and laboratory. Our study demonstrated that in four-arm olfactometer bioassays, *C. capitata* was more attracted to treated arms with 3-hexen-1-ol, acetate, *(Z)*- and ethyl acetate than those treated with butanoic acid, 2-hexenyl ester, *(E)*- and untreated arm. In the wind tunnel studies, we found that the landing rate on 3-hexen-1-ol, acetate, *(Z)*- was higher than those on butanoic acid, 2-hexenyl ester, *(E)*- and ethyl acetate. Similar results were obtained from the field study. Also, females of *C.capitata* were more responsive to volatile compounds than males. Our study could be helpful for the development of novel control strategies against the female flies of *C. capitata* in orchards.

Introduction

Plant semiochemicals play a significant role in insect physiology and behavior. Insect interaction with pheromones and host plant semiochemicals has been known as significant communication systems within species (Tiring et al. 2021). Such interactions reflect in various insect strategies to optimize behavior, reproduction, mating, and feeding (Reddy and Guerrero 2004).

Tephritids (Diptera: Tephritidae) include some of the most destructive pests of fruit and vegetable crops worldwide (Tiring and Satar 2021). The behavior and ecology of tephritid fruit flies are connected to olfaction, including the pheromonal attraction of species during mating, and the location of host fruit by females for oviposition (Landolt et al. 1985; Liu and Zhou 2016). The management of pest tephritids relies on chemical ecology, using olfactory attractants for monitoring, lure-and-kill controls, mass trapping, and oviposition deterrents (Aluja 1996; Navarro-Llopis et al. 2013).

Ceratitis capitata Wied. (Diptera: Tephritidae) is a destructive pest of fruits and vegetables worldwide (Tiring and Satar 2017, 2021). Females lay their eggs inside the fruits and larvae develop in them. *Ceratitis capitata* necessitates insecticide sprays several times per season in commercial fruit orchards. The application of insecticides has become a serious environmental and human health issue (Costa and Klein 2006; Magana et al. 2007). Mass trapping is an environmentally safe alternative to broad-spectrum pesticide application for *C. capitata* (Navarro-Llopis et al. 2004; Piñero et al. 2009; 2011; Martinez-Ferrer et al. 2010; Satar and Tireng 2016). Also, mass trapping is a recommended pest control product in fruit groves with a low impact on non-target organisms. (Hafsi et al. 2020). In the mass trapping technique, *C. capitata* males are attracted by trimedlure, and ceralure whereas females are attracted by volatile compounds (VOCs) emitted from host fruits. Different fruits can promote oviposition in *C. capitata* (Suarez et al. 2021).

To decrease the cost, more efficient trapping tools are required that will reduce the number of attractants used. *Ceratitis capitata* prefers ripe and middle-ripe fruits. Apricot is a important host of *C. capitata* in Turkey (Tiring and Satar 2021). Thus, we presumed that this fruit contains volatile compounds that could be used as attractants for the *C. capitata*.

In this study, we first detected the volatile compounds emitted by the fruit using gas chromatography-mass spectrometry (GC-MS). Second, we tested the behavioral responses of *C. capitata* to some of the identified volatile compounds of apricot in both olfactometer and wind tunnel. Finally, we evaluated in the field the responses of *C. capitata* to these compounds.

Materials And Methods

İnsects

Infested fruits were collected from a mixed fruit orchard at Cukurova University Research and Application Farm located in the southeast Mediterranean region of Turkey. Emerged adults of *C. capitata* were cultivated under laboratory conditions (25 ± 2 C and 60–70% relative humidity (RH) and 12h photophase). Adults were provided water and a solid diet that consisted of a three-part sugar and one-part yeast diet. Adults were kept in plexiglass cages. Eggs of *C.capitata* were collected through a fine-meshed on the front wall of their cage into a trough of water. The larvae were reared on a wheat bran diet (wheat bran 65 g, sugar 30 g, yeast 20 g, HCL 37% 4ml, sodium benzoate 1g, and tap water 127 ml). Then, the last larval stage was placed into the containers containing moist perlite.

Apricot fruits

Apricot fruits were purchased from a supermarket. Fruits were inspected visually to be free from any infestation. Also, they were doused in water with vinegar for 20 min to remove residues from the surface before headspace collection.

Extraction of chemical compounds

For the extraction of compounds in apricot, the automatic HS-SPME/GC-MS technique was used. Headspace volatiles was collected by using (HS-SPME) fiber coated with a 50/30 µm DVB/CAR/PDMS and concentrated volatiles by their sorption characteristics. The flesh of each apricot was cut into cubes (0.3*0.3*0.3), and 3 g of sample were placed into a vial then 40°C held for 15 min. Solid-phase microextraction (SPME) fiber was inserted into the headspace with continuous heating and agitation for 30 min to adsorb volatile substances. Thermal desorption was conducted in the injector glass liner at 250 °C.

GC-MS analysis

Volatile compounds in the samples were analyzed using GC-MS. In the gas chromatographic system, a DB-Wax (60 m x 0.25mm i.dx 0.25 µm) capillary column was used. Helium was used as a carrier gas with

a flow rate of 1 mL/min. The split rate is 1:5. The GC oven temperature program was as follows: 40°C maintained for 4 min, increased to 90°C at 3°C/min, increased at 4°C/min to 130°C and held for 4 min, then increased to 240°C at 5°C/min. MS data were was set to scan mode from m/z = 30 to m/z = 600. The ionizing voltage was 70 eV. Volatiles were identified through mass spectral comparison with the Wiley 8 mass spectral databases.

Chemicals

3-hexen-1-ol, acetate, *(Z)*- (98%), ethyl acetate (98%), butanoic acid, 2-hexenyl ester, *(E)*- (98%) were purchased from Sigma-Aldrich (Adana, Turkey).

Four-arm olfactometer bioassay

The response toward apricot volatile odors of *C. capitata* was tested in a four-armed olfactometer. The olfactometer consisted of a central glass area with four arms, each connected to a gas cleaning bottle. Each arm was connected via silicon tubing to a gas cleaning bottle that contained the odor source. Silicon tubes were used to link the activated carbon filter bottle, vacuum pump, flow meter, and gas cleaning bottle containing the water. To prevent the occurrence of visual disturbances, a 20-W light was placed above the olfactometer in a room at 70% R.H., and 25°C ±2. The bioassay studies were conducted using three-day-old adults. Test insects were kept starved for 24 h before the bioassays. A piece of filter paper containing at 5% concentration volatile samples or the control (fresh air) was placed into each of the gas-washing bottles. For each assay, one group of 10 fresh adults (5 females+5 males) was introduced into the release portion, and they were observed for 10 min using a stopwatch. Adults were assayed separately and replicated four times. Flies entering an arm within this time were considered ‘responders’. Olfactometer was cleaned thoroughly with 70% ethanol and distilled water before use. Also, arms were rotated (90°) to minimize positional effects.

Wind tunnel bioassays

Our study was carried out in a wind tunnel with a 45 cm × 80 cm × 220 cm glass fight section. Charcoal-filtered air was passed through the chamber at 0.20 cm/s^{-1} with air temperatures of 24 ±1°C and 70% ± 5% RH. To avoid bias caused by light, the wind tunnel was lit from above by LED lights set at 10 lux. Test insects were kept starved for 24 h prior to the bioassay. Volatile samples for odor delivery were prepared at 5% concentration and transferred to a 20-ml polypropylene vial before testing. This vial was placed on the tripod ahead of 15 cm of the fan. For each treatment, we tested the landing rate of 10 separately released *C. capitata* that were given 10 minutes to respond to the volatile chemicals. This test was repeated four times. If the adult did not take off, we terminate the trial and consider it a non-responder. Each adult was used only once. At the end of each treatment, the wind tunnel was cleaned with 70% alcohol and distilled water.

Experimental field

This study was conducted in the cropping year 2021 in Adana (37°03' 98,21"N,35°36' 05.73"E), Turkey. The study was carried out in 3 da planted Clementine mandarin orchard.

Trapping

The chemicals were evaluated separately in the field to test their attractiveness to *C. capitata* adults. A 20-ml polypropylene vial with a 5-mm-diameter hole punched into the cap served as a slow-release dispenser. Dispensers contained 0.5 g of cotton and were baited with 5 ml using 5% concentrations of the chemicals to be evaluated as single compounds. The vials were placed in Lastfly traps. Traps were placed at 1.5–2 m height in the open shade of the Clementine mandarin canopy between every third row in the Clementine mandarin orchard with a distance of more than four trees apart. There were four replicates of each treatment. Traps were cleared weekly between 15 July and 10 August 2021. The captured adults were sexed. During the field studies, traps were rotated clockwise to eliminate possible bias because of uneven distributions of *C. capitata* populations. Unbaited traps were used as control.

Statistical analyses

All statistical tests were performed on IBM SPSS 23. Data were checked for homogeneity of variance (Levene test) and the normal distribution of all data (Shapiro–Wilk test; $P < 0.05$) before analysis. Data were transformed using log 10 (x + 1) to satisfy normality assumptions prior to analysis of variance (ANOVA). Wind tunnel and olfactometer bioassays were carried out as completely randomized designs with the 4 test dates as replicates. The field study was conducted as a randomized block design with four replications. The behaviors of the adults in the wind tunnel were analyzed using the Chi-square goodness-of-fit references. Multiple comparisons were performed using Chi-squared tests with a Bonferroni correction. For olfactometer and field assays, significant differences in the number of *C. capitata* were analyzed using the two-way (sex and chemicals as factors) analysis of variance test (two-way ANOVA) followed by Tukey's multiple comparison test at $P < 0.05$. Also, to further understand the effect of chemicals, data from females, males, and both were subjected to seperate a one-way ANOVA (chemicals as factors). Significant ANOVAs were followed by TUKEY's test at $P < 0.05$ level. All data in this study are shown as mean ± standard error (SE).

Results

According to odor characterization results by SPME and GC-MS, 63 compounds were isolated from apricot (Table 1). A total of 56 compounds were identified from apricot, representing 97.2% of the total composition. The predominant volatile components were found to be 3-hexen-1-ol, acetate, *(Z)-*, ethyl acetate, and butanoic acid, 2-hexenyl ester, *(E)-*. Olfactometer experiments confirmed that 3-hexen-1-ol, acetate, *(Z)-*, ethyl acetate, and butanoic acid, 2-hexenyl ester, *(E)-* attracted significantly more flies than the control (Fig. 1) ($F = 10.800$; $df = 3, 15$; $P = 0.001$). 3-hexen-1-ol, acetate, *(Z)-*and ethyl acetate attracted significantly more females than control, but males were not importantly different (female, $F = 9.333$; $df = 3, 15$; $P = 0.002$: male, $F = 9.709$; $df = 3, 15$; $P = 0.258$). Also, the two-way analysis of the data showed that there was not a significant interaction between sex and chemicals (Table 2).

Table 1
The major volatile organic compounds released by apricot fruit through headspace sampling by the SPME technique

6	Chemicals	%Area	Number	Chemicals	%Area
1	Carbon dioxide	7.48	32	1-Hexanol	2.14
2	Acetaldehyde	1.25	33	Oxalacetic acid	0.13
3	Unknown	0.37	34	4-Hexen-1-ol, (E)-	1.58
4	**Ethyl Acetate**	**23.17**	35	Propanoic acid, 4-hexen-1-yl ester	0.93
5	Etanol/Dimethyl ether	4.96	36	Butanoic acid, 3-hexenyl ester, (Z)-	0.89
6	2(3H)-Furanone, dihydro-4-hydroxy-	0.25	37	Butanoic acid, 2-hexenyl ester, (E)-	2.30
7	Propanoic acid, 2-methyl-, ethyl ester	0.23	38	Cyclopentanol, 2-methyl-, trans-	1.22
8	Acetic acid ethenyl ester	0.42	39	Butanoic acid, hexyl ester	3.25
9	Isobutyl acetate	0.55	40	Butanoic acid, 2-methyl-, hexyl ester	0.88
10	Butanoic acid, ethyl ester	3.12	41	Octanoic acid, ethyl ester	0.69
11	Butanoic acid, 2-methyl-, ethyl ester	0.95	42	Acetic acid	0.60
12	Unknown	0.15	43	Butanoic acid, 3-hexenyl ester, (E)-	2.15
13	Pyrimidine-2,4(1H,3H)-dione, 5-amino-6-nitroso-	0.13	**44**	**Butanoic acid, 2-hexenyl ester, (E)-**	**7.46**
14	Butanal, 3-hydroxy-	0.22	45	2-Pentene, 1-ethoxy-4-methyl-, (Z)-	0.88
15	Pentanoic acid, ethyl ester		46	Vanillin, TBDMS derivative	0.52
16	trans-3-Methyl-4-octanolide	0.16	47	2,5-Dihydroxybenzaldehyde, 2TMS derivative	0.34
17	Butanal, 3-hydroxy-	0.18	48	Linalool	0.88
18	D-Limonene	0.24	49	Hexanoic acid, hexyl ester	0.40
19	4-Ethylbenzamide	0.24	50	Hexanoic acid, 4-hexen-1-yl ester	0.25
20	1-Butanol, 2-methyl-	0.17	51	4-Fluorohistamine	0.22
21	Heptanal	0.22	52	Oxime-, methoxy-phenyl-_	1.99
22	2,6,6-Trimethyl-bicyclo[3.1.1]hept-3-ylamine	0.13	53	Hexanoic acid	0.79

6	Chemicals	%Area	Number	Chemicals	%Area
23	Hexanoic acid, ethyl ester	4.41	54	Bicyclo[3.2.0]heptan-2-one, 5-formylmethyl-6-hydroxy-3,3-dimethyl-6-vinyl-	0.38
24	Acetic acid, hexyl ester	2.84	55	Unknown	0.14
25	Acetoin	1.56	56	Heptanoic acid	0.13
26	**3-Hexen-1-ol, acetate, (Z)-**	**9.82**	57	d-Glycero-d-ido-heptose	0.52
27	2-Hexen-1-ol, acetate	1.22	58	2(3H)-Furanone, 5-hexyldihydro-	0.35
28	2-Octanone, 1-nitro-	0.18	59	Unknown	0.72
29	Propanoic acid, hexyl ester	0.96	60	N-2,4-Dnp-L-arginine	0.18
30	Propanoic acid, 2-methyl-, hexyl ester		61	Unknown	0.21
31	Acetoin		62	Phorbol	0.15

Table 2
The results of the two-way analysis of variance test for the number of adults captured in trap and responded in the olfactometer.

Experiments	Factor	df	F	P
Olfactometer	Chemicals	1	4.662	0.011
	Sex	3	1.056	0.314
	Chemicals* Sex	3	1.732	0.187
Field	Chemicals	1	698.631	0.000
	Sex	3	589.487	0.000
	Chemicals* Sex	3	45.180	0.000

Only 35.83% of *C. capitata* landed on the filter paper treated with infested chemicals (Fig. 2). The proportion of upwind-oriented flights did not differ among 3-hexen-1-ol, acetate, ethyl acetate and butanoic acid, 2-hexenyl ester, *(E)*- (x^2=3.683; P = 0.159).

During the field experiment, traps caught a total of 144 *C. capitata* (Fig. 3, 4). 126 females and 18 males were trapped; females made up 87.5% of the total, significantly more than males (total, df = 3, 15, F = 249.130, P = 0.000: female, F = 9.806, df = 3, 15, P = 0.000: male F = 9.709; df = 3, 15; P = 0.002). The number of trapped flies in 3-hexen-1-ol, acetate, ethyl acetate, butanoic acid, 2-hexenyl ester and control was 51, 48, 44 and 1, respectively. Statistical results showed that there were significant differences in interaction between chemicals and sex (Table 2).

Discussion

Ceratitis capitata is a significant pest of pome and stone fruits and a key pest of apricot worldwide. Control strategies mainly rely on insecticide applications and mass trapping with lures. The mass trapping strategy is affected both males and females. Because the damage is due to egg-oviposition into fruits by female flies, the catching of the female flies in the control strategy is very important. Therefore, efficient attractants which will target *C. capitata* female adults are promptly required. We have identified the volatile chemicals in the headspace of apricot fruits. Then, we evaluated the behavioral responses of *C. capitata* ethyl acetate, 3-hexen-1-ol, acetate and butanoic acid, 2-hexenyl ester from the identified compounds from apricot in the olfactometer, wind tunnel, and field. In our study, the behavioral response of *C. capitata* to some of the volatile compounds emitted from apricots was confirmed in the olfactometer, wind tunnel, and field. The female of *C.capitata* was more responsive to ethyl acetate, 3-hexen-1-ol, acetate and butanoic acid, 2-hexenyl ester than males. This situation is pretty significant for the control of medfly. Similar results have been obtained by some authors about the olfactory responses of *C. capitata* to semiochemicals emitted from host plants. Nishida et al. (2000) detected that α-Copaene is a minor component in the volatile compounds of numerous plant species, including *C. capitata* hosts such as mango, guava, and orange. *C. capitata* responded importantly to α-Copaene. Another study observed that *C. capitata* female flies responded to three compounds from mango volatiles (Cosse´ et al. 1995). Prokopy et al. (1997) determined that the odor of *Coffea arabica* fruit was more attractive than the odor of mature fruit of some other *Coffea* spp for *C. capitata*. Casaña-Giner et al. (2001) observed that γ-terpinene, α-terpinene, β-pinene, and α-phellandrene are volatile compounds with field trapping activity on both female flies and males. Fruit volatiles is important attractants not only *C. capitata* but also other Diptera species. Cunningham et al. (2016) studied the electrophysiology and preferences of *Bactrocera tryoni* (Diptera: Tephritidae) to VOCs emitted from guava fruit. Those authors identified esters attractive to *female B. tryoni*: ethyl butyrate, ethyl propionate, and ethyl acetate. Mas et al. (2020) analyzed the odors of four ripe bananas, feijoa, cherry guava, and orange. The response of virgin and mated females of *B. tryoni* to VOCs was identified with electro-antennographic detection. These authors have detected statistically important differences in the female EAD responses for three volatile compounds in cherry guava ((Z)-3-hexenyl butyrate, ethyl hexanoate, and ethyl octanoate). Liu et al. (2017) examined *Drosophila suzukii* (Diptera: Drosophilidae) behavioral bioassays to VOCs emitted by Chinese bayberry. These authors found that α-humulene, ethyl (E)-2-hexenoate and *(E)*-3-hexenoate, methyl *(E)*-2-hexenoate are important attractants for *D. suzukii.*

In the present study, the behavioral response of *C. capitata* to some of the volatile compounds emitting from apricots was confirmed. Our conclusions support the conjecture that *C.capitata* responds to some volatile compounds emitted from apricots, both in the field and laboratory. These chemicals could be a novel monitoring tool.

Further work is required to detect if the odor emitting from apricot fruit is more attractive than the odor of other host fruits of *C. capitata*. Finally, research is needed to determine whether a combination of host

fruit odor and ammonium odors is a more effective and species-specific novel monitoring tool than the type of odor alone.

Declarations

Acknowledgments

We are grateful to Dr. Osman Barış Derici and Osman Kula for helping the wind tunnel. This research was funded by the Project development and coordination unit from the Cukurova University (Grants Code: ZF202012827).

Authors' contributions

GT and SS conceived and designed the study and collected the data.GT wrote the initial draft and analyzed the data, and all authors edited and contributed to subsequent drafts.

Funding: This work was supported by the Project development and coordination unit from the Cukurova University (Grants Code: ZF202012827).

Confict of interest The authors declare that there are no interests to declare.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Figures

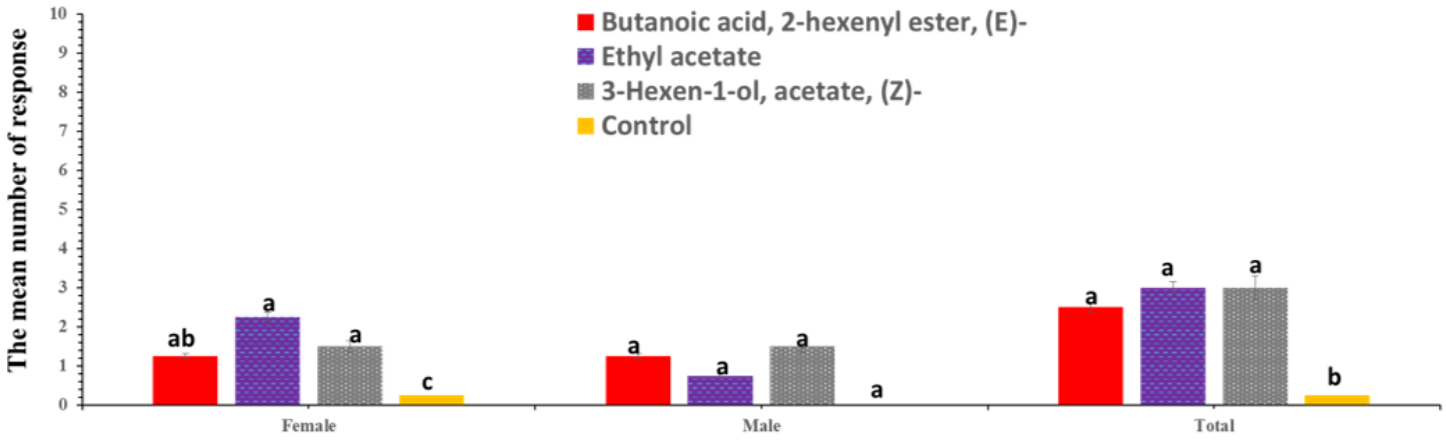

Figure 1

The mean number of *Ceratitis capitata* attracted to some volatile compounds emitted from apricots in a four-arm olfactometer. The data shows the preferences of *Ceratitis capitata* to volatile compounds for each of the female, male, and total adults listed on the x-axis. Different letters above bars indicate a significant difference among values (Tukey's test, $P < 0.05$).

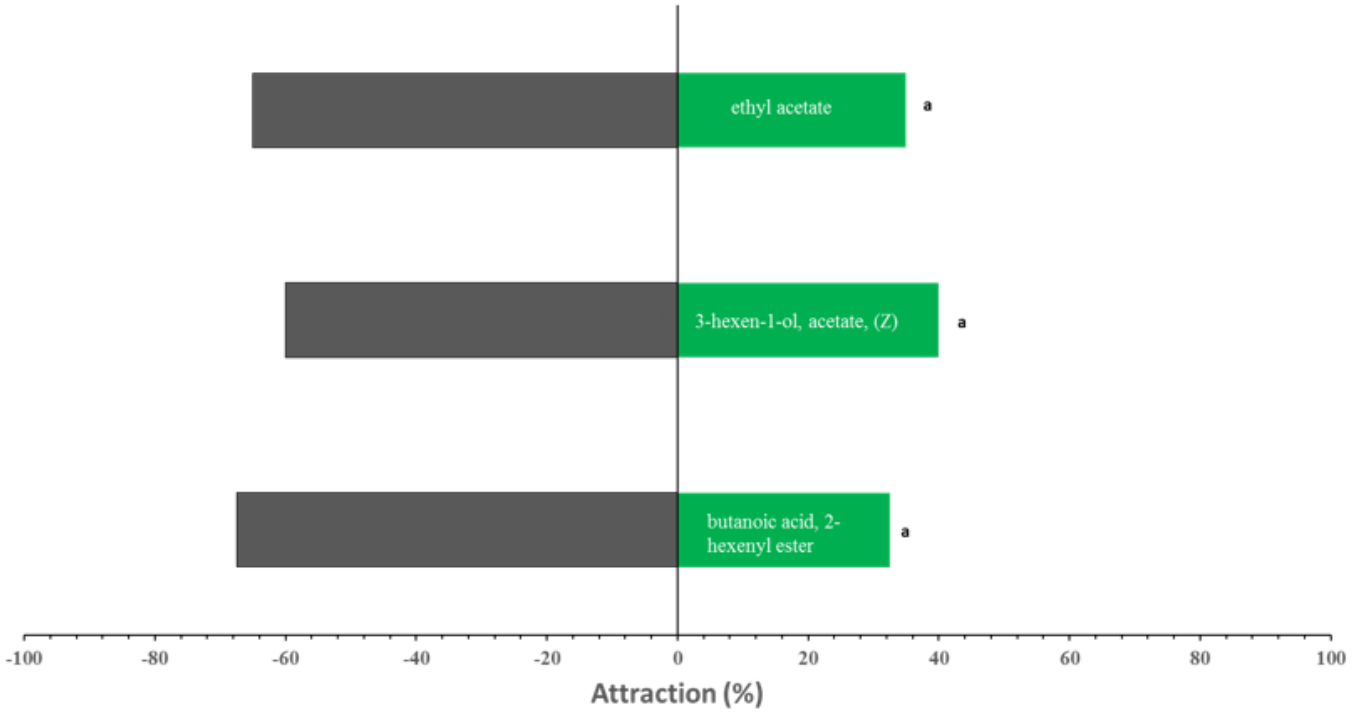

Figure 2

Behavioral responses of *Ceratitis capitata* to some volatile compounds emitted from apricots in a wind tunnel. Horizontal bar plots with positive values represent the percentage of flies responding (take off) to the volatile compounds. If the adult did not take off, we consider it a non-responder. Bars followed with the same letter indicate no significant differences according to Chi-squared tests (P>0.05).

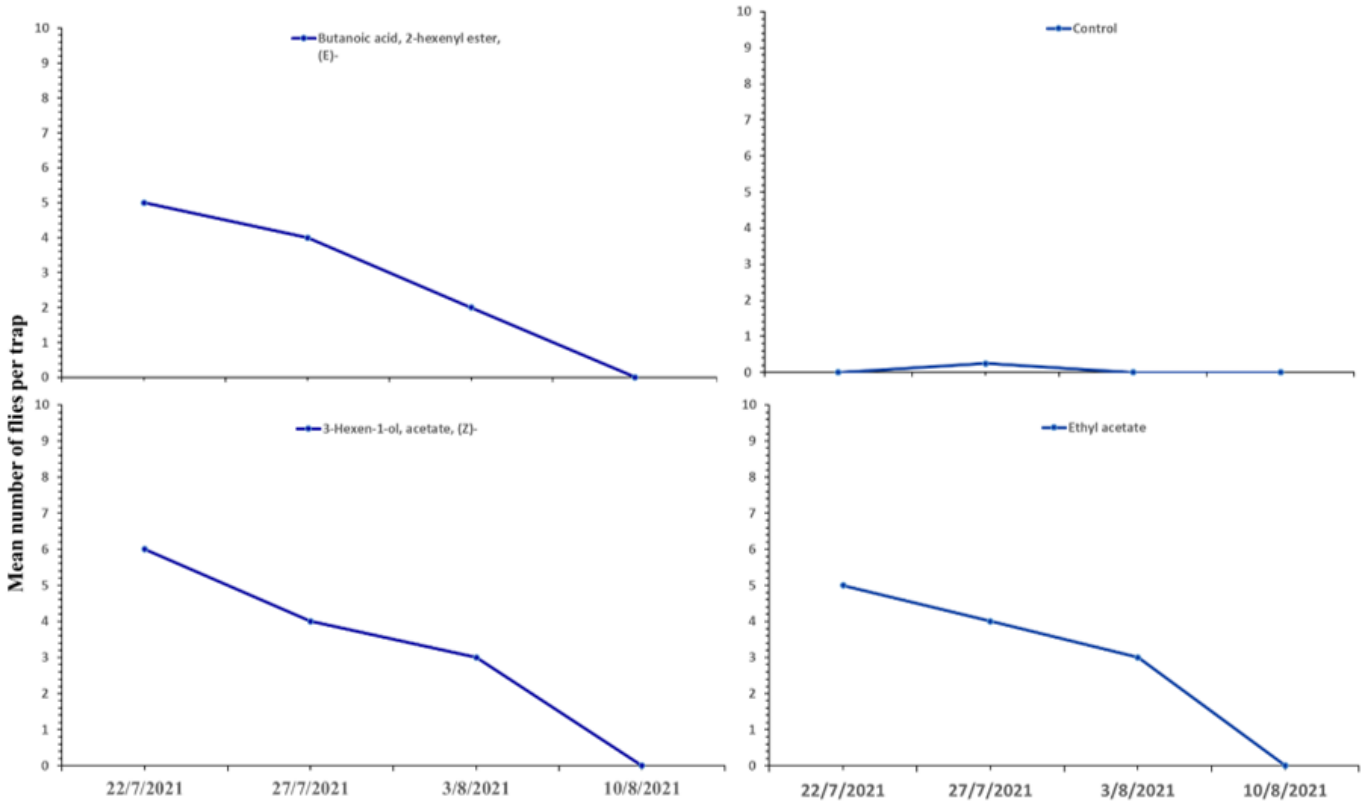

Figure 3

The weekly total catches of *Ceratitis capitata* in traps baited with 3-hexen-1-ol, acetate, *(Z)* butanoic acid, 2-hexenyl ester, *(E)*-, ethyl acetate, and control in Turkey (Adana) in 2021.

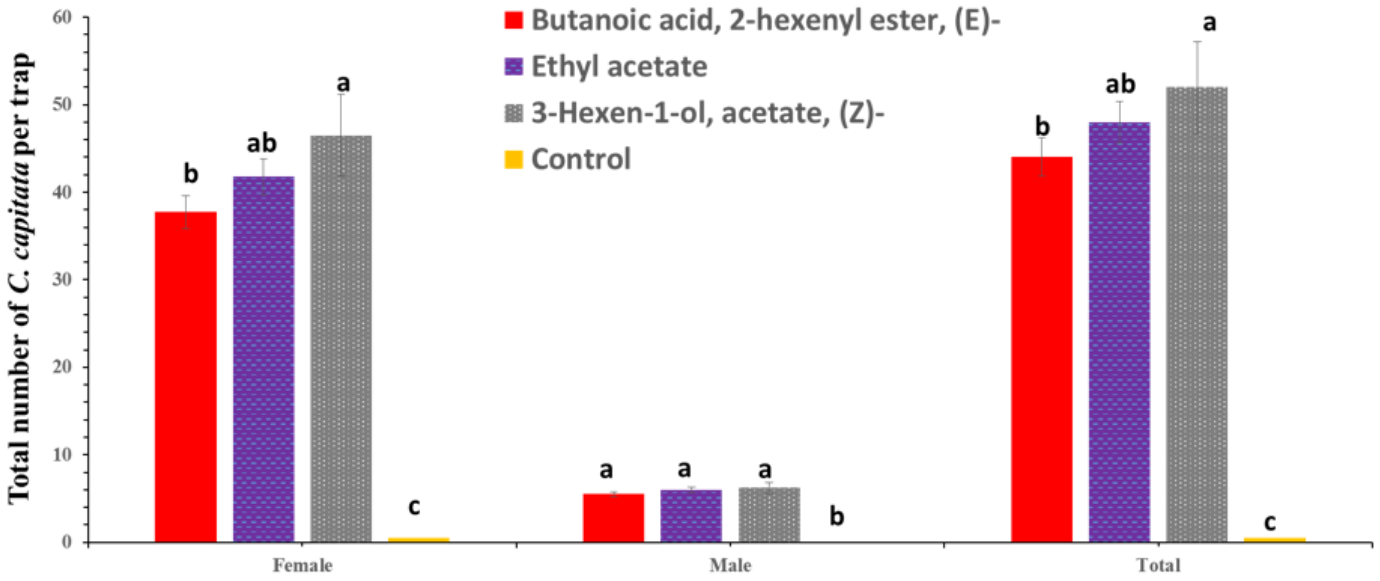

Figure 4

T The data shows total catches (± SE) of male, female, and both of *Ceratitis capitata* in traps baited with 3-hexen-1-ol, acetate, *(Z),* ethyl acetate, butanoic acid, 2-hexenyl ester, *(E)*- and control in Turkey (Adana) in 2021. Different letters indicate significant differences in the number of flies captures among volatile compounds (Tukey's test, $P < 0.05$).