The behavioral responses of Ceratitis capitata (Diptera: Tephritidae) to hydrolyzed yeast and some sugars

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Research Article

Keywords: Attractant, Medfly, olfactometer, toxic bait

Posted Date: May 25th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1656667/v1

Abstract

Ceratitis capitata Wiedemann (Diptera: Tephritidae) is a major pest of fruits and vegetables worldwide. The nine different sugars and yeast were evaluated to determine the behavioral response of adult *C. capitata* using a four-arm olfactometer. The sugars used in the study are fructose, alpha glucose, galactose, ribose, arabinose, sucrose, trehalose, melibiose, and maltose. The results showed that *C. capitata* had a significantly higher attraction to ribose, arabinose, fructose, melibiose, and trehalose than the other four sugars tested. The number of *C. capitata* that responded to trehalose was higher than the other sugars, so we tested the behavioral response of adult *C. capitata* to hydrolyzed yeast, trehalose, hydrolyzed yeast + treholese, and control. Our study demonstrated that in four-arm olfactometer bioassays, *C. capitata* was more attracted to treated arms with hydrolyzed yeast + treholese than those treated with hydrolyzed yeast, trehalose, and untreated arm. These findings can provide insights into the development of more effective sugar-based toxic baits and attractants for traps for the operational application in *C. capitata* control programs.

Introduction

Tephritids (Diptera: Tephritidae) comprise some of the most destructive pests of fruit and vegetable crops worldwide (Tiring & Satar, 2021). The fruit flies of losses have been predicted to cause annual economic damage of US$1 billion worldwide. The most noxious species belong to the genera *Rhagoletis, Anastrepha, Dacus, Bactrocera*, and *Ceratitis* (White et al., 1994). *Ceratitis* is the predominant genus among these species.

Ceratitis capitata Wiedemann (Diptera: Tephritidae) is one of the most devastating and economically significant pests worldwide (Tiring & Satar, 2017; 2021). Feeding with more than 300 different hosts and having a cosmopolitan geographic distribution that is ever-expanding, *C. capitata* exerts a direct economic loss to growers and dramatically affects national and international vegetable-fruit commerce (Liquido et al., 1990; 1991). If *C. capitata* populations are not managed, the percentage of damage often exceeds 50% of the total fruit production, and the infestation may reach 80–100% in ‘preferred’ hosts such as persimmon (Tiring & Satar, 2017; 2021; Kouloussis et al., 2022).

Commercially-available fermentation substrates and protein hydrolysates are commonly used to attract it and other fruit fly species for monitoring or pest control. These attractants contain significant food resources and amino acids needed by the flies for sexual maturation and egg development and often are comprised of substrates such as fermenting sugar baits, yeast, by-products from the brewing industry, and proteins modified by hydrolyzed proteins singly or in combination (Heath et al., 1997; Placido-Silva et al., 2005; Epsky et al., 2014).

The high cost of commercial monitoring and mass trapping products in Turkey has prevented their use in Medfly control. As an alternative, some farmers have developed monitoring practices that rely on locally available materials including diluted molasses, sugars, vinegar, fermented products as lures. Although, the efficiency of these techniques has not been evaluated.

In our study, we carried out olfactometer experiments, to evaluate new and low-cost lures as tools to support sustainable *C. capitata* Integrated Pest Management.

Materials And Methods

Insects

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 cages containing moist perlite.

Compounds

Fructose (Cas no: 57-48-7), alpha glucose (Cas no: 492-62-6), galactose (Cas no: 59-23-4), ribose (Cas no: 50-69-1), arabinose (Cas no: 5328-37-0), sucrose (Cas no: 57-50-1), trehalose (Cas no: 6138-23-4), melibiose (Cas no: 585-99-9), maltose (Cas no: 69-79-4), and hydrolyzed yeast were purchased from Sigma-Aldrich (Adana, Turkey).

Four-arm olfactometer bioassay

The response toward sugars and yeast 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.

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). Olfactometer bioassays were carried out as completely randomized designs with the 4 test dates as replicates. For olfactometer 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 separate a one-way ANOVA (chemicals as factors). Significant ANOVAs were followed by TUKEY’s test at the $P < 0.05$ level. All data in this study are shown as mean ± standard error (SE).

Results

Fructose and galactose attracted significantly more females than alpha glucose and control, but males were not importantly different (Fig. 1) (female, $F = 4.000$; $df = 3, 15$; $P = 0.035$; male, $F = 1.727$; $df = 3, 15$; $P = 0.214$; both, $F =$

6.630; df = 3, 15; P = 0.007). Furthermore, two-way analysis of the data showed that there was no significant interaction between sex and sugars in bioassay studies (Table 1).

Table 1
The result of the two-way analysis of the variance test for the number of behavioral responses in a four-arm olfactometer

			Fructose, galactose, alpha glucose, and control		**Ribose, arabinose, sucrose, and control**		**Maltose, melibiose, trehalose and control**		**Trehalose, yeast, trehalose + yeast and control**	
		df	F	P	F	P	F	P	F	P
	Compounds	3	4.750	0.010	143.597	0.000	143.597	0.000	114.535	0.000
Attractant	**Sex**	1	0.750	0.395	1.013	1.000	1.331	0.285	0.016	0.900
	Compounds *Sex	3	0.750	0.533	1.000	0.287	1.333	0.048	1.749	0.184

Two-way analysis of variance showed a not significant effect of interaction between the sugars and sex on the following tested compounds of the behavioral responses of *C. capitata*: ribose, arabinose, sucrose, and control (Table 1). Both female and male *C. capitata* were importantly more attracted to the olfactometer arm containing sugars with arabinose and ribose in comparison to those containing sucrose and control (female, F = 72.463; df = 3, 15; P = 0.000: male, F = 72.463; df = 3, 15; P = 0.000; both F = 176.592; df = 3, 15; P = 0.000) (Fig. 2).

Two-way analysis of variance showed a important effect of interaction between the sugars and sex on the following tested compounds of the behavioral responses of *C. capitata*: maltose, melibiose,trehalose and control (Table 1).. Olfactometer experiments confirmed that adults were significantly attracted to sugars from melibiose and trehalose compared to maltose and control (female, F = 9.333; df = 3, 15; P = 0.002: male, F = 11.285; df = 3, 15; P = 0.001. both F = 78.083; df = 3, 15; P = 0.000) (Fig. 3).

The mean number of attracted *C. capitata* with trehalose in the olfactometer was higher than other sugars. For these reasons, the response to trehalose, yeast, and both of *C. capitata* was tested in a four-arm olfactometer, and the results showed in Fig. 4. Adults showed significantly different responses to the treatments with trehalose, yeast, yeast + trehalose, and control (female, F = 92.470; df = 3, 15; P = 0.000: male, F = 39.737; df = 3, 15; P = 0.000; both F = 81.194; df = 3, 15; P = 0.000). Also, two-way analysis of the data showed that there was no significant interaction between sex and sugars in bioassay studies (Table 1).

Discussion

New techniques for Medfly control are being developed to replace traditional organophosphate insecticide applications. Insecticide bait sprays have been used against *C. capitata*, recently. Mass trapping with liquid or dry food-based baits offers promising medfly control within Integrated Pest Management (IPM) programs (Navarro-Llopis et al., 2011). Food-based lures primarily mimic nitrogen sources that provide the protein required by adults to reach sexual maturity. The female-biased attractants are generally food lures. The reason for this is that females have higher needs for protein acquisition than males for egg development (Christenson & Foote, 1960; Kouloussis et al., 2017). Hydrolyzed yeasts contain high protein (San Martin et al., 2020). Our study supported that hydrolyzed yeast is more attractive to significantly more females than others.

By the mid-1990s, an aqueous solution of torula yeast borax (TYB) pellets (López et al., 1971) was the standard food attractant used in fruit fly trapping systems worldwide (Heath et al., 1995) and is still widely used (Enkerlin & Reyes, 2018). California, for instance, recently deploys five TYB-baited traps per mi^2 (2.59 km^2) as a component of a fruit fly detection network that covers approximately 25,000 mi^2 (64,750 km^2) in the southern part of the state (Vargas et al., 2013). Our study supported that hydrolyzed yeast is more attractive to *C. capitata* than trehalose.

The attractants of *C. capitata* have been used protein and sugar recently. Different formulations of protein hydrolysates are commercially available for fruit fly control Biodelear is a patented, female-specific attractant produced by the Maillard reaction of fructose, urea, and water at a ratio of 3:1:1 (Kouloussis et al., 2022). In our study, the fructose attracted significantly more females than alpha glucose and control.

Various formulations of protein hydrolysates are commercially available for C. capitata control. GF-120 Naturalyte is a formulated bait mixture that contains the toxicant spinosad and 99.8% inert ingredients (including water, different types of sugar, and maize protein). The M3 bait station comprises a protein attractant and insecticide housed in a plastic device. The flies feed on the bait and die soon afterward (Ware et al., 2003).

In a mass trapping control of *Ceratitis* species in Turkey and Nigeria,(Ekesi & Tanga, 2016) and B. dorsalis in Kenya and Uganda, (Umeh & Garcia, 2008) baits based on brewers-waste are used as a commercial hydrolyzed protein bait (e.g. NuLure).

Our study supports the attraction of some of the sugars and yeast that can be used in mass trapping and insecticide bait sprays to manage *C. capitata*. The present study demonstrates that trehalose is more attractive to *C. capitata* than other sugars. Also, our study supported that yeast + trehalose is more attractive to *C. capitata* than others.

In the present study, the behavioral response of *C. capitata* to some sugars and yeast was confirmed. Our conclusions support the conjecture that *C.capitata* responds to trehalose and yeast. These could be novel monitoring tools. Finally, research is needed to determine whether a combination of sugar, yeast, and ammonium odors is a more effective and species-specific novel monitoring tool than the type of odor alone.

Declarations

Acknowledgments

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

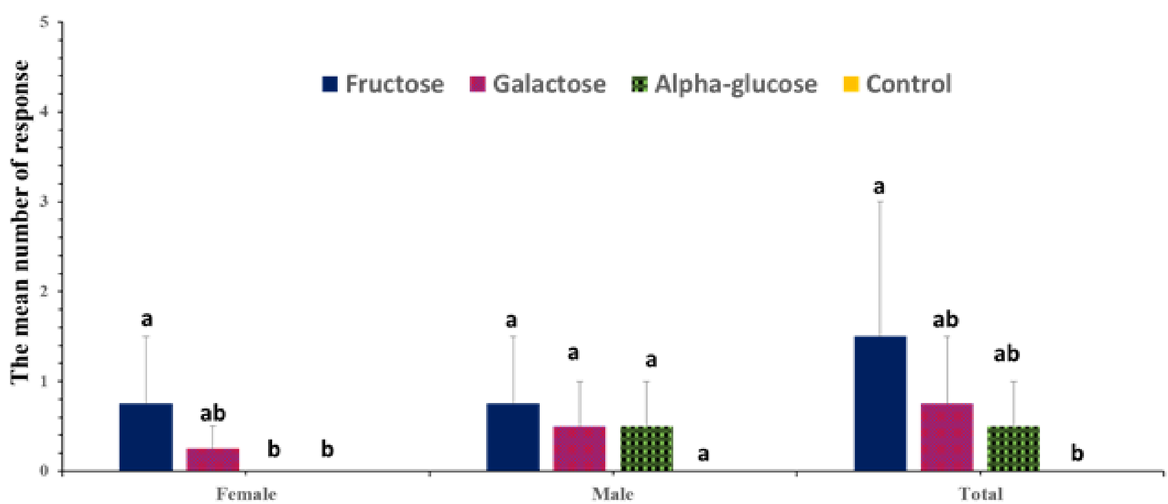

Figure 1

The mean number of *Ceratitis capitata* attracted to some sugars in a four-arm olfactometer. The data shows the preferences of *Ceratitis capitata* to sugars 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).

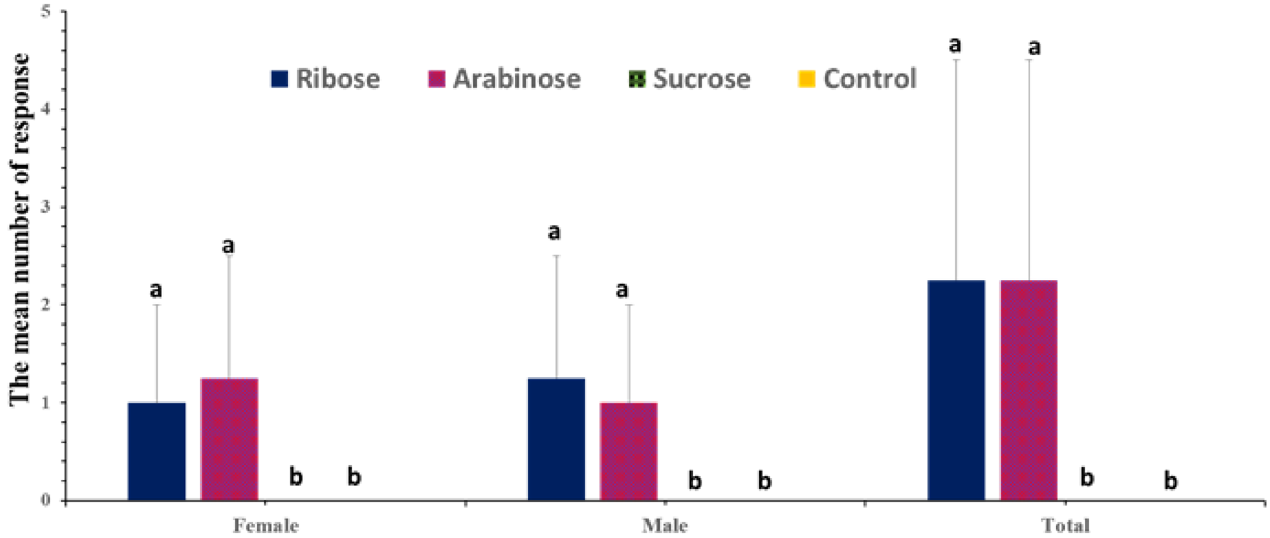

Figure 2

The mean number of *Ceratitis capitata* attracted to some sugars in a four-arm olfactometer. The data shows the preferences of *Ceratitis capitata* to sugars 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, $P < 0.05$).

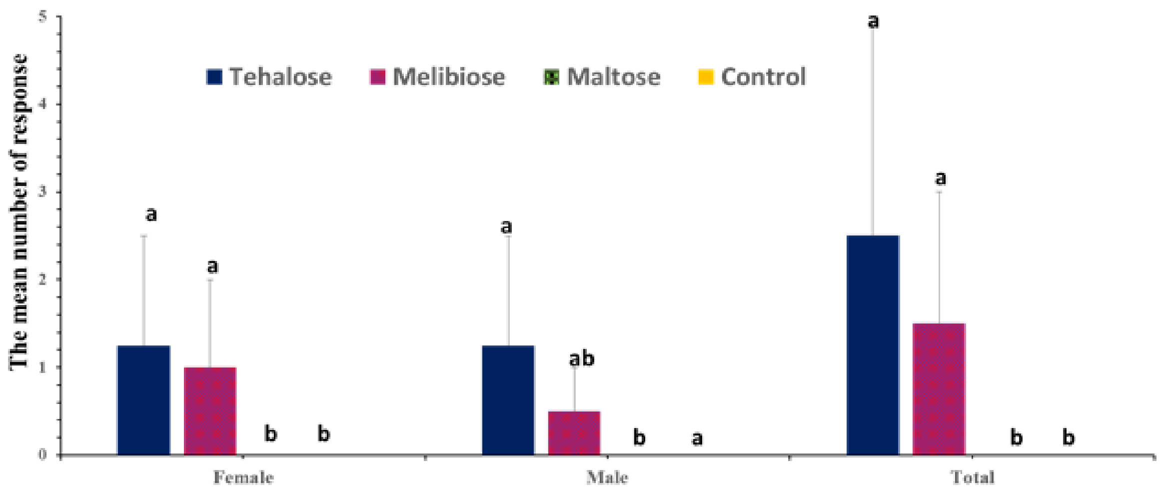

Figure 3

The mean number of *Ceratitis capitata* attracted to some sugars in a four-arm olfactometer. The data shows the preferences of *Ceratitis capitata* to sugars 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, $P < 0.05$).

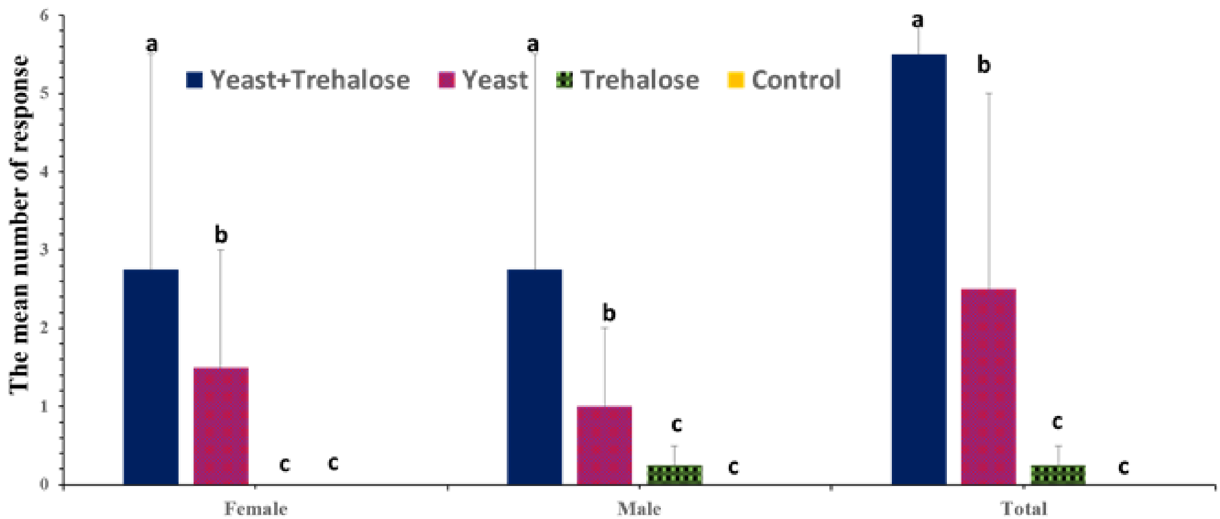

Figure 4

The mean number of *Ceratitis capitata* attracted to yeast, trehalose, and both apricots in a four-arm olfactometer. The data shows the preferences of *Ceratitis capitata* to yeast, trehalose, and both 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, $P < 0.05$).