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Original article (Orijinal araştırma)

Behavioral responses of *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) to hydrolyzed yeast and different types of sugars[1]

Ceratitis capitata (Wiedemann, 1824) (Diptera: Tephritidae)'nın hidrolize maya ve farklı şeker türlerine davranışsal tepkileri

Gülsevim TİRİNG[2*] **Serdar SATAR[2,3]**

Abstract

Ceratitis capitata (Wiedemann, 1824) (Diptera: Tephritidae) is a major pest of fruits and vegetables worldwide. This study was conducted at Çukurova University (Türkiye) in 2021. In this study, different types of sugar and hydrolyzed yeast were evaluated to determine the behavioral response of adult *C. capitata* using a four-arm olfactometer and wind tunnel. Some of the most attractive sugars to *C. capitata* were combined with hydrolyzed yeast, to check whether their attractiveness could be further improved. The sugars used in the study were alpha glucose, arabinose, fructose, galactose, maltose, melibiose, ribose, sucrose and trehalose. The results showed that *C. capitata* had a significantly higher attraction to arabinose, fructose, melibiose, ribose and trehalose than the other four sugars. The number of adults that responded to trehalose was higher than the other sugars, so behavioral responses of *C. capitata* to hydrolyzed yeast, trehalose and hydrolyzed yeast + trehalose were tested in comparison to a control group. This study demonstrated that *C. capitata* was more attracted to the combination of hydrolyzed yeast + trehalose than to each of these alone or to the control.

Keywords: Attractant, medfly, olfactometer, wind tunnel

Öz

Ceratitis capitata (Wiedemann, 1824) (Diptera: Tephritidae) dünya çapında meyve ve sebzelerin önemli bir zararlısıdır. Bu çalışma 2021 yılında Çukurova Üniversitesi'nde (Türkiye) yürütülmüştür. Bu çalışmada, dört kollu olfaktometre ve rüzgâr tüneli kullanılarak *C. capitata* erginlerinin davranışsal tepkilerini belirlemek amacıyla farklı tipte şekerler ve hidrolize maya değerlendirilmiştir. İleriye yönelik bir adım olarak, cezbediciliğin daha da gelişip-gelişmediğini kontrol etmek için en çok yönelimin görüldüğü şekerlerden biri hidrolize maya ile kombine edilmiştir. Çalışmada kullanılan şekerler alfa glikoz, arabinoz, fruktoz, galaktoz, maltoz, melibioz, riboz, sakkaroz ve trehalozdur. Sonuçlar, arabinoz, fruktoz, melibioz, riboz ve trehalozun *C. capitata* için diğer dört şekerden önemli ölçüde daha yüksek bir çekiciliğe sahip olduğunu göstermiştir. Trehaloza tepki veren ergin sayısı diğer şekerlerden daha fazla olduğu için *C. capitata'*nın hidrolize maya, trehaloz, hidrolize maya + trehaloza karşı davranışsal tepkileri kontrol grubuna göre test edilmiştir. Bu çalışma, *C. capitata*'nın hidrolize maya + trehaloz kombinasyonun, bunların her birine veya kontrole göre daha fazla çekici olduğunu göstermiştir.

Anahtar sözcükler: Cezbedici, Akdeniz meyve sineği, olfaktometre, rüzgâr tüneli

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[2] BIOTEK Scientific Services, Agricultural R & D and Consultancy Company, Çukurova Techno city, 01330, Adana, Türkiye.
[3] Çukurova University, Faculty of Agriculture, Department of Plant Protection, 01330, Adana, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: glsvm.trng@gmail.com.tr
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Introduction

Tephritids (Diptera: Tephritidae) comprise some of the most destructive pests of fruit and vegetable crops worldwide (Tiring & Satar, 2021). Crop losses due to fruit flies have been predicted to cause annual economic damage of 1 billion USD worldwide. The most noxious species belong to the genera *Anastrepha, Bactrocera, Ceratitis, Dacus, Rhagoletis* and *Zeugodacus* (White & Elson-Harris, 1992).

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) is one of the most devastating and economically significant pests worldwide (Elekçioğlu, 2013; Tiring & Satar, 2017, 2021). Feeding on more than 300 different hosts and having a cosmopolitan geographic distribution that is ever-expanding, it exerts a direct economic loss to growers such as 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 highly susceptible hosts such as persimmon (Tiring & Satar, 2017, 2021; Kouloussis et al., 2022). Growers are very concerned about the high reproductive potential and adaptability of *C. capitata*, combined with the low effectivity of natural enemies and their wide range of hosts (Castillo et al., 2000).

New techniques for medfly control are being developed to replace the commonly-used organophosphate insecticide applications. Recently, insecticidal bait sprays have been used against *C. capitat*a. Mass trapping with liquid or dry food-based baits offers promising medfly control within integrated pest management (IPM) programs (Navarro-Llopis et al., 2011). The new attractants are very significant for food-based baits, so the studies related to lures need to continue.

Recently, some control of *C. capitata* has been achieved by mass trapping without bait spray application. However, protein hydrolysates and commercially fermented compounds are generally used as attractants to lure medfly and other fruit flies for monitoring and mass trapping. These attractants contain significant food resources required by the adults for egg development and sexual maturation and frequently consist of compounds such as sugar baits and yeast (Heath et al., 1997; Plácido-Silva et al., 2005; Epsky et al., 2014).

The high cost of commercial mass trapping products, especially attractants has prevented their use in medfly control in Türkiye. As alternatives, some farmers have used monitoring practices that depend on materials including fermented products, sugars, vinegar and diluted molasses as lures. However, the efficiency of these techniques has not been evaluated. Therefore, olfactometer and wind tunnel experiments were conducted to evaluate new and low-cost lures as tools to support sustainable *C. capitata* IPM.

Materials and Methods

Insects

Infested fruits were collected from a mixed fruit orchard at Çukurova University Research and Application Farm located in the southeast Mediterranean Region of Türkiye. Adults of *C. capitata* were cultured under laboratory conditions (25 ± 2°C, 60-70% RH and 12 h photoperiod). Adults were provided a solid diet consisting of sucrose and hydrolyzed yeast (Condolab, Laboratorios Conda S.A., Madrid, Spain) (3:1 w/w). Adults were kept in plexiglass cages. Eggs of *C. capitata* were collected through a fine-meshed tulle on the front wall of a 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, hydrochloric acid (37%) 4 ml, sodium benzoate 1 g and tap water 127 ml). Individuals of the last larval stage were then placed in cages containing moist perlite to pupate.

Test insects were sexed and kept separate until use in olfactometer and wind tunnel studies. Virgin adults were used in the experiment. Bioassay studies were conducted between 10:00 and 16:00.

Compounds

Alpha glucose, arabinose, fructose, galactose, hydrolyzed yeast, maltose, melibiose, ribose, sucrose and trehalose were purchased from Sigma-Aldrich (Adana, Türkiye).

Four-arm olfactometer bioassay

Attraction of *C. capitata* to sugars and yeast was tested in a four-arm olfactometer. The olfactometer consisted of a central glass area (20 x 20 cm) with four arms. Each arm was connected via silicon tubing to gas-washing bottles that contained the odor source. Silicon tubes were used to connect the vacuum pump, activated carbon filter bottle, flow meter and gas-washing bottle containing water and compounds, respectively. To prevent visual disturbance, a 20 W light was placed above the olfactometer in a room at 25 ± 2°C and 70% RH. The bioassay studies were conducted using three-day-old adults. Test insects were unfed for 24 h before the bioassays. A piece of filter paper containing samples diluted to 5% (20 µl) or the control (fresh air) was placed into each of the gas-washing bottles. For each assay, one group of 10 adults (5 females and 5 males) was introduced into the release portion and observed for 10 min using a stopwatch. These assays were replicated four times. Flies entering an arm within this time were deemed to be responders. Olfactometer was cleaned thoroughly with 70% ethanol and distilled water before each use. Also, arms were rotated (90°) to minimize positional effects.

Wind tunnel bioassays

This study was conducted in a wind tunnel (45 × 80 × 220 cm). Charcoal-filtered air was passed through the chamber at 0.20 cm/s^{-1} with at 24 ± 1°C and 70% ± 5% RH. To avoid bias caused by light, the wind tunnel was lit from above by LED lights set to 10 lux. Test insects were unfed for 24 h prior to use in the assays. Samples for odor delivery were prepared at a concentration of 5% and transferred to a 20 ml polypropylene vial before testing. This vial was placed on the tripod in front of a 15 cm fan. For each treatment, we tested the landing rate of 10 separately released *C. capitata* that were given 10 min to respond to the volatile chemicals. These assays were repeated four times. If the adult did not take off, we terminated the test and deemed it to be a non-responder. Each adult was tested only once. At the end of each treatment, the wind tunnel was cleaned with 70% alcohol and distilled water.

Statistical analysis

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 log10(x + 1) to satisfy normality assumptions prior to analysis of variance (ANOVA). Olfactometer bioassays were conducted 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 a two-way (sex and chemicals as factors) 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 P = 0.05. Also, the behavior of the adults in the wind tunnel were analyzed using the Chi-square goodness-of-fit test. Multiple comparisons were performed using Chi-squared tests with a Bonferroni correction. All data in this study are given as mean ± standard error.

Results

Fructose and galactose attracted significantly more females than alpha glucose and the control, but males were not significantly different (Figure 1) (female, F = 4.00; df = 3, 15; P = 0.035; male, F = 1.73; df = 3, 15; P = 0.214). Also, two-way analysis of the data showed that there was no significant interaction between sex and sugars in bioassay studies (Table 1).

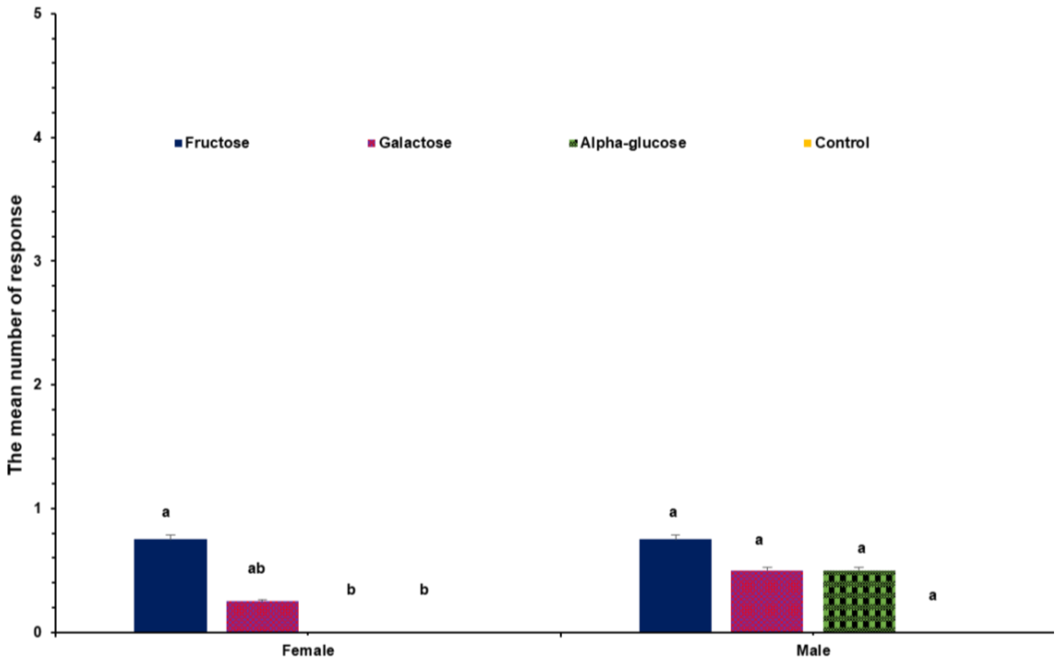

Figure 1. Mean number of *Ceratitis capitata* attracted to different types of sugar in a four-arm olfactometer. The data shows the attraction of *Ceratitis capitata* to sugars for each of the female and male adults listed on the x-axis. Means with the same letter are not significantly different (Tukey's test, P: 0.05).

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
Attractant	Compounds	3	4.750	0.010	143.597	0.000	143.597	0.000	114.535	0.000
	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 did not indicate a significant effect of interaction between the sugars and sex for the following test compounds: arabinose, ribose, sucrose and the control (Table 1). Both females and males were significantly more attracted to the olfactometer arm containing sugars with arabinose and ribose in comparison to those containing sucrose and the control (female, F = 72.5; df = 3, 15; P = 0.000; male, F = 72.5; df = 3, 15; P = 0.000) (Figure 2).

Two-way analysis of variance indicated a significant interaction between the sugars and sex on the following test compounds: maltose, melibiose, trehalose and the control (Table 1).

Olfactometer experiments showed that adults were significantly more attracted to the sugars melibiose and trehalose compared to maltose and control (female, F = 9.33; df = 3, 15; P = 0.002; male, F = 11.3; df = 3, 15; P = 0.001) (Figure 3).

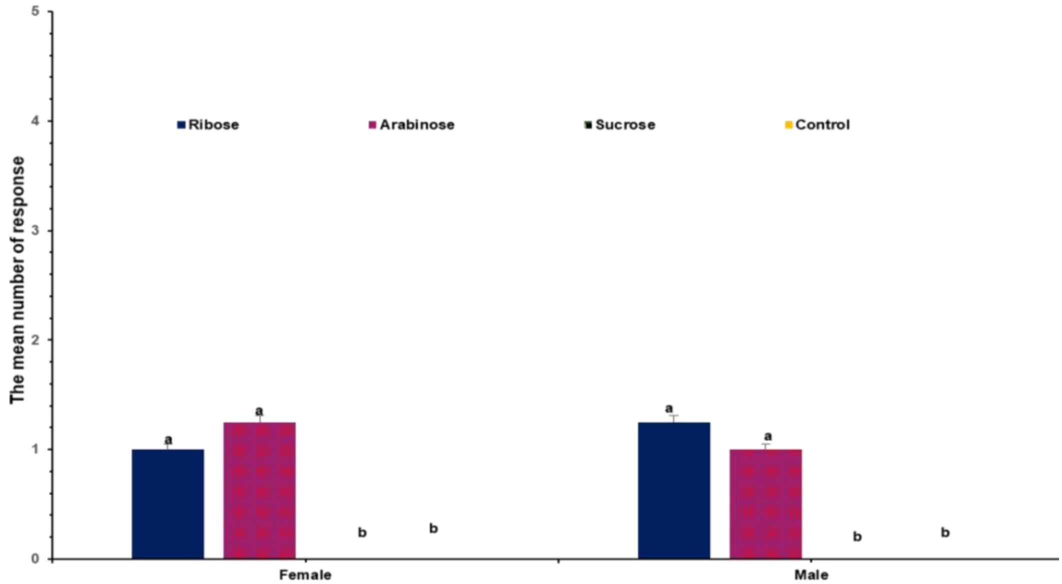

Figure 2. Mean number of *Ceratitis capitata* attracted to different types of sugar in a four-arm olfactometer. The data shows the attraction of *Ceratitis capitata* to sugars for each of the female and male adults listed on the x-axis. Means with the same letter are not significantly different (Tukey's test, P: 0.05).

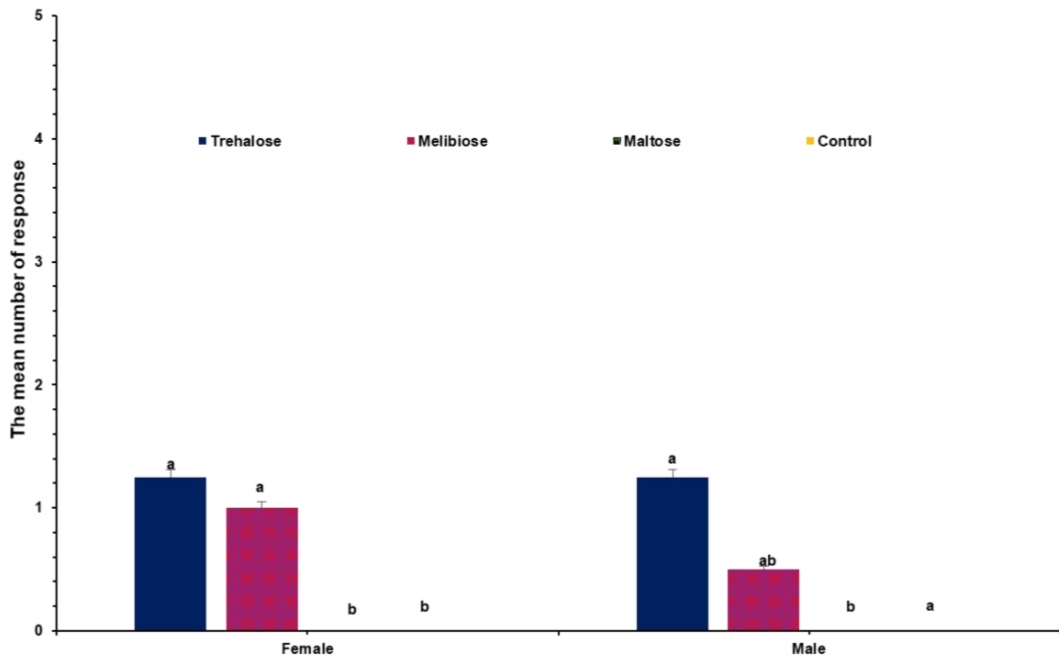

Figure 3. The mean number of *Ceratitis capitata* attracted to different types of sugars in a four-arm olfactometer. The data shows the attraction of *Ceratitis capitata* to sugars for each of the female and male adults listed on the x-axis. Means with the same letter are not significantly different (Tukey's test, P: 0.05).

Trehalose attracted more *C. capitata* than other sugars. Therefore, the response to trehalose, yeast, and the combination of both was also tested in the four-arm olfactometer (Figure 4). Adults showed significantly different responses to the treatments with trehalose, yeast, yeast + trehalose, and control (female, F = 92.5; df = 3, 15; P = 0.000; male, F = 39.7; df = 3, 15; P = 0.000). In addition, two-way analysis of the data showed that there was no significant interaction between sex and sugars (Table 1).

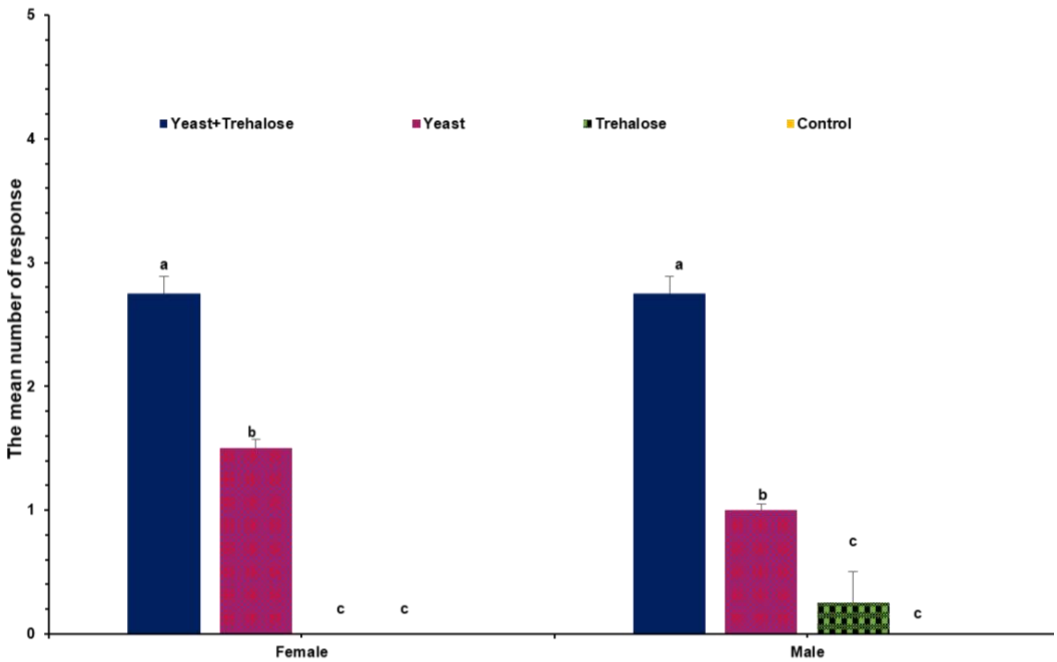

Figure 4. Mean number of *Ceratitis capitata* attracted to yeast, trehalose, and both in a four-arm olfactometer. The data shows the attraction of *Ceratitis capitata* to yeast, trehalose, and both for each of the female and male adults listed on the x-axis. Means with the same letter are not significantly different (Tukey's test, P: 0.05).

The percentage of upwind-oriented flights differed among different types of sugar and yeast (x^2 = 3.68; P = 0.000). Wind tunnel experiments confirmed that trehalose was more attractive than the other sugars. Also, the percentage of *C. capitata* attracted to yeast + trehalose was consistently higher than the others (Figure 5).

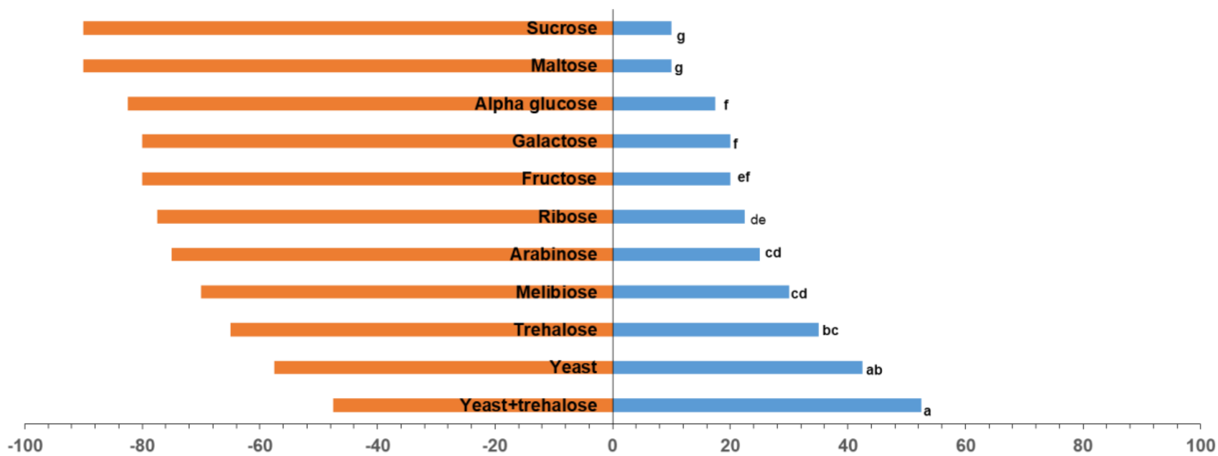

Figure 5. Response of *Ceratitis capitata* to different types of sugar and yeast in a wind tunnel. Horizontal bar plots with positive values represent the percentage of flies responding (take off) to the compounds. If the adult did not take off, we considered it a non-responder. Means with the same letter are not significantly different according to Chi-squared tests (P = 0.05).

Discussion

The results of the study revealed that trehalose is more attractive to *C. capitata* than the other sugars tested*.* Also, hydrolyzed yeast + trehalose is strongly attractive to medfly adults. The strong olfactometer and wind tunnel response suggest that the compounds contained in this mixture could influence the behavior of the insect in the field.

Food-based attractants are similar with 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). The results of the present study were consistent with hydrolyzed yeast is more attractive to significantly more females than the others.

By the mid-1990s, an aqueous solution of torula yeast borax (TYB) pellets (Lopez et al., 1971) was a food lure used in fruit fly mass trapping systems worldwide (Heath et al., 1995) and is widely used still (Enkerlin & Reyes-Flores, 2018). For example, five TYB-baited traps per 2.59 km^2 are used as a component of a fruit fly detection network that covers ca. 64,750 km^2 in California (Vargas et al., 2013). The present study supported that hydrolyzed yeast is more attractive to *C. capitata* than the tested sugars.

With attractants for *C. capitata* now including both protein and sugar, different formulations of protein hydrolysates are commercially available for *C. capitata* control. Biodelear, 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 the present study, fructose attracted significantly more females than alpha glucose and the control in a four-arm olfactometer. However, the adults responded significantly more to trehalose than fructose in the wind tunnel experiments. Also, wind tunnel experiments showed that arabinose, melibiose and ribose were more attractive to *C. capitata* than fructose.

Various formulations of protein hydrolysates are commercially available for *C. capitata* control. GF-120 Naturalyte is a formulated mixture that contains spinosad (0.02%) in a non-toxic bait (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* spp. in Türkiye (Başpınar et al., 2013) and Nigeria (Ekesi & Tanga, 2016), and *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) 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 confirms that the attraction of different types of sugars and yeast 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, this study found that yeast + trehalose was more attractive to *C. capitata* than the others test substances.

The present study confirmed that the attraction of *C. capitata* to some sugars and hydrolyzed yeast particularly trehalose and yeast. This combination, therefore, has potential as a novel monitoring tool. Finally, further 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 these types of odor alone.

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