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Use of Locally Food Attractants for *Ceratitis capitata* (Diptera: Tephritidae)

Ilias Faiza[1,2], Gaouar Semir Bechir Suheil[2]

[1]Laboratory of applied hydrology and environment, Faculty of science and technology, University of Ain Temouchent, Algeria; Email: faizailias@yahoo.com
[2]Laboratory of Applied genetic in agriculture, ecology and public health, University of Tlemcen, Algeria. Email: suheilgaouar@gmail.com

**Corresponding author's E-mail: faiza.ilias@univ-temouchent.edu.dz*

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Abstract

The high price of commercial products against pests impose small farmers to use a locally available baits against C.capitata that is the most fruit fly in our country. The aim of this research was to evaluate the effectiveness of food-based attractants for the capture of C. capitata as well as their selectivity on the populations of beneficial arthropods. we tested some local fermentation products compared to commercial lures (CeraTrap®). Four attractants were evaluated in two peach orchads. Field evaluations show that local fermentation products, particularly the red wine solution outperformed other food-based attractants. However, the average of fruit damage was differed between the four attractants and it was high in the border than in the center of each attractants plot. These attractants also were selective, capturing few beneficial arthropods. It will be necessary to focus our research on how to optimally employ such a trap in conjunction or alternative with other control techniques to drive concerted and integrated pest management due to its low environmental impacts and selectivity toward beneficial insects.

Keywords: *Ceratitis capitata; food attractants; fruit damage; beneficial arthropods*

1. Introduction

The Mediterranean fruit fly (*Ceratitis capitata Wiedemann*) is considered one of the most important fruit pests worldwide (Flores et al., 2016) It is spread out over a wide area around the world due to its high tolerance to climatic changes compared to many other fruit fly species. With it's attacked causing a high yield losseson numerous fruit and vegetables crops (Liquido et al., 1991; Papadopoulos et al., 2001; Bali et al., 2021). Monitoring and trapping systems using attractants, are used also in early detection programs in those countries or areas where fruit fly outbreaks and invasion are subject to intense monitoring and control (Enkerlin et al., 2015; Tan et al, 2014; Candia et al., 2018).

To control medfly, and other tephritid species, fermentation substrates and protein hydrolysates are commonly used (Candia et al., 2018). These attractive substances constitute important energy and amino acid sources needed for sexual maturation and oviposition (Placido-Silva et al., 2005).

Some farmers developed a local product as attractants as red wine solution and vinegar solution that disposed in plastic bottles used as traps. They developed these products for their low-cost against the commercials products.Candia et al., (2018)reported the uses of some local products in Bolivia by the farmers and its efficacities against *C.capitata*. The red wine is considered as a specially solution used by some our farmers for the increased attacked by some insects like *C. capitata*.

A first step toward a standardized concerted action will be the development of a bait and trap setup using locally-available materials(Candia et al., 2018).Research attention should thus be given to the development of a locally available bait to provide growers with an economically affordable trap for either monitoring and direct control (Figueroa, 2015;Navarro-Llopis&Vacas, 2014).Mass trapping with bait stations can be used to reduce or eliminate the use of insecticide bait sprays in various integrated pest management programs against fruit flies (Navarro-Llopis et al., 2011; Lasa et al., 2013; Lasa et al., 2014; Yokoyama, 2014; Flores et al., 2017). Several studies demonstrate that *C. capitata* causes serious damage, synthetic female attractants used in mass trapping and bait station techniques have proved to

be more efficient than chemical treatments in reducing population level and fruit damage at harvest in orchards with minimum adverse effects on non-target arthropods (Bouagga et al., 2014; Jemâa et al., 2010; Hafsi et al., 2015; Hafsi et al., 2016).

The main advantages of mass trapping compared to other control methods is the direct assessment of efficacy. Bali et al., (2021) reported that trapped fruit flies can be spotted and even counted to confirm the effectiveness of the method or to follow the population trends. When bait stations or insecticide applications are employed, efficacy can be only indirectly assessed using population monitoring traps or fruit sampling to determine infestation levels (Navarro-Llopis & Vacas, 2014). (Manrakhan & Kotze, 2011; Piñero et al., 2011) mentioned that the type of attractant is critical in the performance of bait stations. Some attractants can be improved when combined with substances such as oils (McQuate& Peck, 2001) or antifreeze (Thomas, 2008) that increase the efficiency and long-term stability in the field (Lasa et al., 215).

The main objective of this study was to select low-cost and easy use locally trap and attractants to capture *C.capitata*adults for mass-tapping purpose.

2. Materials And Methods

Location of field trials:

Field experience were carried on the peach orchads from Mars to June 2022 in the northern area of Algeria (Ain Temouchent region). The altitude is 245 m and35°17' 22 north with longitude 1°8’ 28 west. Peach orchard in Hammam Bouhadjar (located at 35°20'47" N, 0°56'35" O, 195 m altitude), and other orchadin “Benighanem” (geographic position 35°15’11 N,1°25’34 W, 18 m altitude) were selected for attratants traps.

Food attractant:

Four products were tested as attractants from different origins were evaluated. CeraTrap® (Bioiberica, Barcelona, Spain) is a soluble concentrate of enzymatic protein hydrolysate of animal origin that releases a series of volatile compounds, mostly amines and organic acids that are highly attractive to fruit flies (Marín, 2006). Two locally produced are used also, the first based on vinegar solution (100 mL vinegar + 100gr sugar in 1 liter water), the second based on alcoholic beverage that is red wine (100 mL red wine + 100gr sugar in 1 liter water) and in the last, solution of yeast+sugar: yeast 20gr + sugar 20 gr in 1 liter water. The synthetic food-based bait formulation were applied in Ceratrap® commercial traps, which is a transparent bottle trap of 1 L volume that has five 10 mm circular holes spaced 5 cm apart. This type of traps is commercially supplied with CeraTrap® attractant and it was used in this study because of its low cost. Yeast + sugar solution was make in also in bottle traps. All the traps baited were placed in orchards at a density of 50 traps per ha. In each plot, 25 traps were placed randomly in the center (inner peach trees) and the remaining 25 traps were placed in the borders (peach trees in the perimeter of the plot) (Hafsi et al., 2016). Traps were spaced by 12–15 m and positioned at 1.5 m above the ground level, on the south eastern part of canopies of peach trees, and were distributed uniformly within each orchard. The average lifespan CeraTrap® baits is three months under field conditions as confirmed by the suppliers (Hafsi et al., 2016). Traps were thoroughly cleaned with ethanol between experiments. The captures were evaluated weekly.

Evaluation of fruit damage

Evaluation of fruit damage caused by *C. capitata* were carried out at harvest in each plot. A total of 400 fruits per plot, selected from 40 random trees (10 trees from the center and 10 from the perimeter, 10 fruits from each tree), were numbered and visually checked for oviposition punctures of *C. capitata*. For each tree, the rate of fruit damage was calculated as the number of fruits with at least one oviposition puncture over the total number of checked fruits. The rate of fruit damage was estimated for the border and the center of each treated plot.

Selectivity of protein-based baits and traps

For each food-based bait, two sampling separated by four weeks interval were performed. In each sampling, 25 out of the 50 deployed traps were randomly selected and checked. Captured insects were collected and placed in 70% ethanol, then were counted and identified in the laboratory while considering the trapped insect species and the sex of the target captured insect. In cases where “fly bodies” had decomposed, the number of remaining wings was counted to estimate the total number of flies per trap. The selectivity of each food-based bait toward non-target insects was also recorded. The non-target insects selected for this study were Diptera (all Diptera excluding *C. capitata*), Hymenoptera, Neuroptera, Coleoptera (Coccinellidae) and Hemiptera (Miridae) (Hafsi et al., 2020). Particular

attention was given to these groups of insects as they are useful for conservative biological control strategies against some orange pests (Hafsi et al., 2020).

Statistical analyses

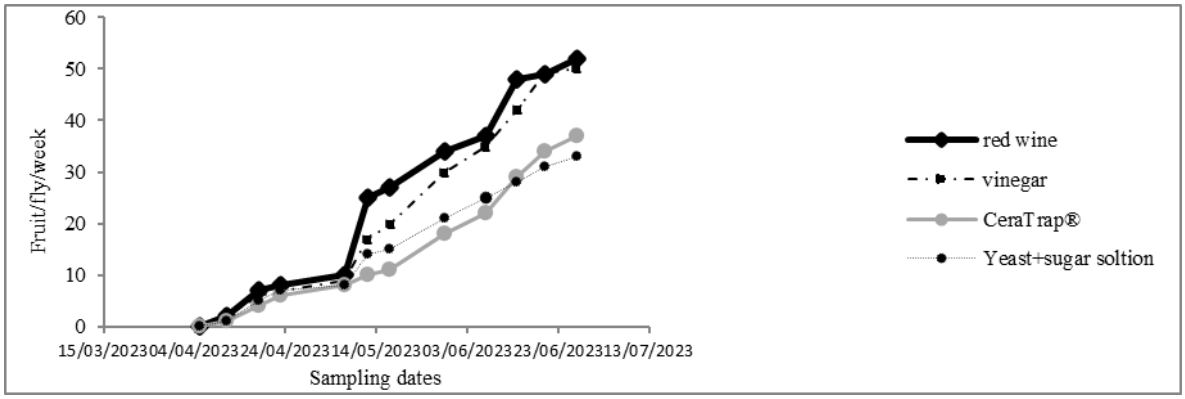
The analysis of variance (ANOVA) followed by post-hoc Tukey's or Student–Newman–Keuls test at $p < 0.05$ after a root square transformation were used to compare the number of *C. capitata* caught in traps in date and in orchad. Damag e fruit ratio (%) was determined weekly and during harvest. The impacts of the food attract on the pest population were measured according to the Abbot formula [Effectiveness % = (Infected fruit % in Control - Infected fruit % in Trials/ Infected fruit % in Control) x 100] by the infected fruit reduction between the plots (Abbott, 1925). Results were expressed as percentage of damaged fruits, using with binomial error as function of traps and tree location in the plot (Border and center of the plot). In each peach orchard, the total numbers of target and non-target insects captured in each trap was with Poisson error as function of traps. All the statistical analyses were performed using SPSS.

3. Results and Discussion

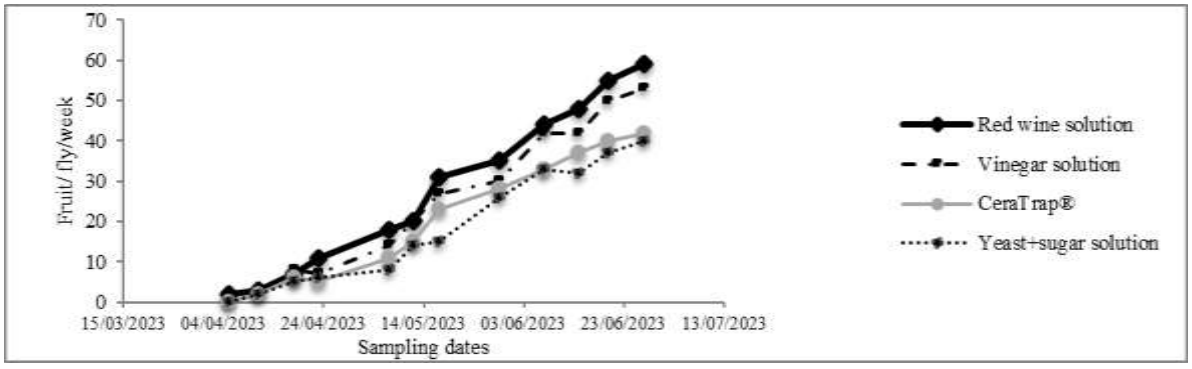
Food attractant:

Figure 1 show the mean number of the medfly trapped by red wine solution, vinegar solution, CeraTrap® and yeast + sugar solution in the two orchads. The cumulative catch (Week) during the field season, which demonstrates that the population rose rapidly in early April and peaked in the last week of June. Captures of *C. capitata* males in the different traps differed significantly as function of traps (F = 43,69; df = 3, 33; P <0.001) and sampling dates (F=19,13; df = 1.79; P <0.001) in orchard 1. *Ceratitiscapitata* captures remained significantly lower in red wine solution trap followed by vinegar solution trap during all the experience. (*Figure. 1A*). In orchard 2, (*Figure. 1B*), captures of *C. capitata* captured in all the traps differed significantly between traps (F=58,11; df =3.33; P <0.001), and sampling dates (F=24.09; df =1, 79; P <0.001).

The results shows that red wine solution and vinegar solution attracted more medfly in general. The other traps had fewer captured insect, with yeast + sugar solution being the lowest (*Figure. 1*). The number of *C. capitata* did exceed 50 flies/trap/week regardless the attractive and the sampling dates.



(A)



(B)

Fig 1: The mean number of Ceratitiscapitatatrapped by red wine solution, vinegar solution, CeraTrap® and Yeast+sugar solution located in two peach orchards; (a) orchards 1 and (b) orchards 2.

Fruit damage:

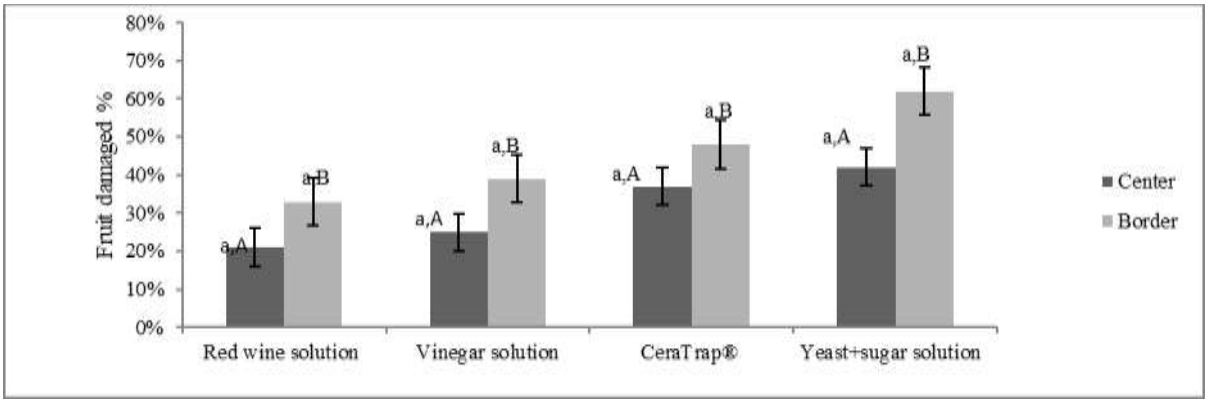


Fig2: Proportion of peach fruit damage on the center and border of red wine solution, vinegar solution, CeraTrap® Yeast + sugar solution plots located in the two orchards. Different lower-case letters indicate significant statistical differences between treatments within the same tree location. Different Upper-case letters indicate significant statistical differences between tree locations.

The number of damaged peach fruit was significant lower in the red wine solution and vinegar solution compared to the CeraTrap® and the Yeast + sugar solution.

Fruit damage differ significantly between treatments (F=104.22; df= 3, 207; P< 0.000), and differed also significantly between tree locations in the same attracted plot in orchard 1 (F= 74.11; df = 1,16; P< 0.000). In attracts food plots, fruit damage in the border was about two-times more than in the center. In orchard 2, fruit damage did not differ between attracts food (F=99.02; df = 2, 33; P < 0.116), but differed significantly between tree locations in the same plot (F=101.02; df = 1, 789; P <0.009) (*Figure. 2*). Although fruit damage was higher in the border than in the center of plot.

Efficacy of protein-based baits in capturing *C.capitata*:

The total number of *C. capitata* adults captured in red wine solution traps was significantly greater than that captured in in all other traps (F=2695,32; df =3.589; P <0.001) either in the border or the center of traps (*Table.1*). The total number of captured medflies in all study plots indicates that red wine solution was more efficient in capturing *C. capitata.*

Table 1. Total catches (mean ± SD) per trap per location within a plot (center or border) in two orchads during 2022.

		Orchad 1			
	Border traps	**Center traps**	**F**	**df**	**P**
Redwine solution	180.00±3.83bA	99.13±42.03bA	12.148	2.32	0.012
Vinegar solution	152.14±21.80bA	78.33±45.52bA	17.188	2.32	0.022
CeraTrap®	119.16±20.12bA	56.29±13.33bA	4.532	2.32	0.232
Yeast+sugar solution	98.08±2.32bA	44.12±12.28bA	0.532	2.32	0.501
	F=233.520	F= 74.143			
	df= 4.51	df= 4.51			
	P<0.001	p<0.001			
		Orchad 2			
	Border traps	**Center traps**	**F**	**df**	**P**
Redwine solution	171.78±22.01bA	82.78±22.01bA	20.456	2.32	0.412
Vinegar solution	136.34±16.09bA	65.18±72.14bA	0.273	2.32	0.354
CeraTrap®	93.18±37.19bA	52.33±02.41bA	11.231	2.32	0.780
Yeast+sugar solution	85.11±25.17bA	41.39±19.11bA	1.455	2.32	0.457
	F= 84.667	F= 30.524			
	df= 4.51	df= 4.51			
	P<0.001	P<0.001			

The number of captured females was the highest and differed significantly between food-based baits in orchard 1 and orchard 2, with respectively 1022 and 1035 for red wine solution, 801 and 846 for vinegar solution, 518 and 623 for CeraTrap® and 278 and 354 for Yeast + sugar solution (*Table. 2*). The number of captured males in red wine solution traps was significantly higher than that in vinegar baited traps, CeraTrap® and Yeast+sugar solution (*Table. 2*).

All the food-based baits captured a broad diversity of insects, representing 5 orders of arthropods (Diptera, Hymenoptera, Neuroptera, Coleoptera, and Hemiptera). The number of other insects captured in traps was affected significantly by the type of food-based bait (F= 1842.20; df =2,03; P <0.001) (*Table. 2*).

The number of other insects was significantly higher in red wine solution than in other food attracted trapson orchard 1 and orchard 2 (*Figure. 3; Table 2*). Captures were dominated by Diptera (all Diptera excluding *C. capitata*) in red wine solution followed by Hymenoptera (Formicidae), Hemiptera (Miridae), neuroptera and coleoptera (Coccinellidae). The number of Diptera (all Diptera excluding *C. capitata*) in All baited traps was, respectively, 90, 88, 41 and 32 in orchard 1, and 98, 79, 35 and 22 individuals in orchard 2 (*Table. 2*). For Hymenoptera (Formicidae), 89, 78, 41 and 20 individuals were obtained in red wine solution, vinegar solution, CeraTrap® and corn hydrolyzed protein, respectively. The number of Coleoptera (Coccinellidae) captured in red wine solution was higher four times more than in Yeast+sugar solution in orchad 1. Regarding Neuroptera, 57, 42, 13 and 13 individuals were captured in orchad 1 for red wine solution, vinegar solution, CeraTrap® and Yeast+sugar solution, respectively and 53, 46, 10, 13 for red wine solution, vinegar solution, CeraTrap® and corn hydrolyzed protein, respectively, in orchad 2 (*Table. 2*).

Table 2. Total number of Ceratitis capitata (Male and females) and other insects trapped. a, b different letters in the same row indicate significance difference between food-based bait formulations.

		Orchad 1		
Captures insects	**Redwine solution**	**Vinegar solution**	**CeraTrap®**	**Yeast+sugar solution**
C.capitata male	523^{a}	312^{b}	223^{a}	111^{b}
C.capitatafemale	1022^{a}	801^{b}	518^{b}	278^{a}
Diptera (exluding*C.capitata*)	90^{b}	88^{a}	41^{b}	32^{a}
Hymenoptera	89^{b}	78^{a}	41^{b}	20^{a}
Neuroptera	57^{b}	42^{a}	13^{b}	13^{a}
Coleoptera: Coccinellidae	35^{b}	45^{a}	9^{b}	11^{a}
Hemiptera: Miridae	82^{b}	67^{a}	15^{b}	8^{a}
		Orchad 2		
Captures insects	**Redwine solution**	**Vinegar solution**	**CeraTrap®**	**Yeast+sugar solution**
C.capitata male	585	409	222	193
C.capitatafemale	1035	846	623	354
Diptera (exluding*C.capitata*)	98	79	35	22
Hymenoptera	88	81	36	16
Neuroptera	53	46	10	13
Coleoptera: Coccinellidae	61	25	13	7
Hemiptera: Miridae	71	55	24	5

Several mass trapping systems have been developed to control *C. capitata* worldwide, depending on the strength of the attractant, this tactic targets females or males. Mass trapping with liquid or dry food-based baits can be used to reduce or, ultimately, substitute the use of insecticide bait sprays against Tephritidae species (Navarro-Llopis et al., 2011; Lasa et al., 2013Yokoyama, 2014; Hafsi et al., 2016).

Red wine solution and vinegar solution were the more effective attractants for *C. capitata* in the orchads and all dates. The captures of *C. capitata* adults in the experimental orchards started very low and increased gradually as function of maturity of peach fruits (Eskafi & Kolbe, 1990; Martinez-Ferrer et al., 2012).

Actually, many commercial products are not used because its prices. The results are that locally a low-cost alternative have been researched. In a mass trapping trial of *Ceratitis* species in sweet oranges in Nigeria, and against *B. dorsalis* in Kenya and Uganda, proteinaceous baits made from brewers waste was similarly effective as a commercial hydrolysed protein (e.g. NuLure), but less effective than torula yeast(Ekesi & Tanga, 2016; Umeh & Garcia, 208.In Spain, mixtures of corn-steep liquor with ammonia compounds and amines were the most effective attractants for female medflies (Casaña-Giner et al., 2001)In Mexico, commercial proteinaceous baits (Torula yeast and Ceratrap) were tested against grape juice for attraction of *Anastrepha* fruit flies in citrus. Low efficacy, male-biased catch, highnon-target and beneficial insect catch, and the need for frequent re-baiting were main drawbacks (Herrera et al.,

2016; Mangan & Thoma, 2014). In Bolivia, chicha appeared to be a highly favorable alternative with better effectiveness than commercial baits for small growers (Candia et al., 2018).

Our farmers developed some solutions to captured insects including *C. capitata*. Red wine solution is like chicha (Alcoholic fermentation), but in our daily works is not useful. For this reason most farmers used vinegar solution that improve also an attractive efficacity against *C.capitata.* Red wine solution constitutes an active yeast fermentation culture, which continuously produce volatiles. In contrast, most protein products are lysed through autolysis or through bacterial fermentation and are rather different in volatile profile (Candia et al., 2018). In our study red wine solution and vinegar solution were more effective at capturing *C. capitata.* Yeast+sugar solution was inefficient in capturing tephritids in all dates**.** (Delgado et al., 2022) reported that sugarcane molasses was inefficient in capturing tephritids in all field trials. Also (Malavasi et al., 1990) observes these results for *A. fraterculus* and *A. grandis*. Fermentation products and protein hydrolysates are frequently lumped together and directly compared in terms of attractiveness or potential in control (Epsky et al., 2014). (Lasa et al., 2013,2014) evaluated the capture of *A. ludens* with different mass trapping devices with several attractants. Efficacy of mass trapping technique is related to the formulation of the attractant(Mangan, 2009; Epsky et al., 2014) and to the target pest population density and geographic isolation of treated crops (El-Sayed et al., 2006).(Bali et al., 2021)reported that, there is a list of other factors that affect the performance of the traps, such as the physiological and age structure of feral flies (e.g., age, mating status, feeding history), the structure of orchards as well as the prevailing environmental conditions that may affect both dispersion of odor attractants and response of adult flies.

Fruit damage reduction remains the main objective of each control method against fruit flies (Hafsi et al., 2016). The proportion of fruit damage showed that this parameter was less in the center than in the border in the two orchads. This result is similar to results of (Hafsi et al., 2016). The number of traps baited with food-based attractants should be increased in the border of the treated orchards in order to prevent fruit fly intrusions (Hafsi et al., 2016).

All treatments evaluated had female-biased captures, which is the base of an effective mass trapping strategy. Females are lured more often to attractants than males (Manrakhan & Kotze, 2009; Jahnke et al., 2014). Females lured to these attractants mostly were sexually immature (Heath et al., 1995; Bortoli et al., 216) in stages prior to oviposition and damage to fruits.

(Delgado et al., 2022) indicate that fruits are an oviposition stimulus for sexually mature females and protein-based attractants are a stimulus for the development of the ovaries of sexually immature females, the presence of fruits in the field does not compete with traps for capturing females. Tephritid captures were concentrated in the post-harvest period in most of the trials probably due to the increase in the population of fruit flies in the crop area.

Females are often clustered around host trees bearing fruits, as opposed to males which are more randomly dispersed across orchards and the fruiting season (Liquido et al., 1991; Epsky et al., 2014; El-Sayed et al., 2006). Mated females are more likely to be attracted to traps that mimic the shape, size, and color of host fruits when searching for mates and oviposition sites (Economopoulos, 1989; Nakagawa et al.,1978). For increasing female selectivity, female-targeted traps are suggested to be placed on or near host trees bearing ripe or semi-ripe fruits (Lance & Gates, 1994).

We found that all traps in the two orchads captured more females than males. The high attractiveness of protein derivatives for *C. capitata*females may be associated with the need for the intake of protein sources and particularly of amino acids to ensure their fertility (Cangussu&Zucoloto, 1997; Oviedo et al., 2011). The high attraction of *C. capitata* females to red wine solution compared to other food attracts could be related to the variation in their chemical composition and particularly to nitrogen, ammonia, and acetic acid like reported by (Robacker, 2007).

In selecting food-based baits for deployment in mass trapping strategies, efficacy and cost are major considerations like the potential influences on attraction and capture of beneficial insects used in biological control programs Hafsi et al., 2016).

In our experiment, the food-based baits attracted a broad diversity of insects covering 5 orders (Diptera, Hymenoptera, Neuroptera, Coleoptera, and Hemiptera), dominating by Diptera (all Diptera excluding *C. capitata*), that known to contain high number of beneficial insects used in biological control programs Hafsi et al., 2016).This pattern was observed in previous studies with CeraTrap® or other food-based baits (Thomas, 2003; Martinez et al., 2007; Leblanc et al., 2010; Hafsi et al., 2015; 2016). The attraction of insects to several synthetic baits can be related to the putrescine, compound commonly found as a volatile emitted by fermentations of fruit and protein baits for fruit flies Heath et al., 1995;

Hafsi et al., 2016). Dipteran species are attracted perhaps for the presence of the ammonium acetate (Thomas, 2003; Martinez et al., 2007; Leblanc et al., 2010; Hafsi et al., 2016).

4. Conclusion

The high price of insecticides with the very high risk of reinfestation from neighbours orchads, limit options for control. The small farmers use a locally with low-cost products. The experience was conducted in two peach orchards to assess the efficacy of a locally food-based baits (Red wine solution, vinegar solution, Ceratrap® and Yeast+sugar solution) used in mass trapping. The selectivity of attractants was evaluated toward other insects. Results showed that red wine solution was the most attractive food for *C. capitata* as confirmed also by females' attractants. In all cases, the average of fruit damage was differed between food attractants and was less in the center than in the border of each treated plot. Red wine solution was found to be the most selective toward other insect groups. This study provides small farmers with a cheap, environmentally friendly and effective method to capture *C. capitata*.

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