



# Population Fluctuation of Mediterranean Fruit Fly, *Ceratitis capitata* Wied (Diptera: Tephritidae) on Different Pomegranate (*Punica granatum* L.) Varieties by Pheromone Traps and Associated Loss Assessment

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## Background

Mediterranean fruit fly, *Ceratitis capitata* Wied (Diptera: Tephritidae) is considered as one of the most deleterious fruit pests which may cause high pomegranate, *Punica granatum* L. yield losses in Turkey. Monitoring population density of herbivore insects is a significant element of integrated pest management strategies. Therefore, *C. capitata* population fluctuation was monitored via a SEDQ yellow trap + pheromone in a pomegranate orchard consisted of three cultivars, Acco, Hicaz, and Wonderful, in Seyhan, Adana in 2015. In addition, yield losses were calculated for all cultivars. There was a significant role of pomegranate cultivar on *C. capitata* catches by traps. Wonderful has the highest number of *C. capitata* following by Hicaz while traps in Acco caught the least number of fly adults. Peel thickness was 3.15, 3.05, and 2.80 mm while cracking level was 10.8%, 13.2%, and 15.1% for Wonderful, Hicaz, and Acco, respectively. In addition, infestation ratios of Wonderful, Hicaz, and Acco were 3.8%, 4.6%, and 5.2%, respectively. There was a significant relationship between fruit peel thickness and fruit cracking level as well as population growth and yield losses. Further efforts should be devoted to cultivar preference of and cultivar resistance against *C. capitata* for sustainable pest management practices.

**Keywords:** Cracking; Fruit fly; Generalized linear mixed model; Infestation; Population monitoring

## Introduction

Pomegranate is native to the region from Iran to the Himalayas in northern India and cultivated in Turkey, Caucasus, Western Asia, Mediterranean Europe, India, Saharan Africa, North and South America (EPP0, 2019). Turkey is one of the leading countries of pomegranate production in the world. Thanks to increasing awareness of nutritional values, easy to grow and cost-effective care conditions, resistant to long-term storage and export conditions, marketing and processing facilities, pomegranate production has exceeded 500.000 tons in recent years in Turkey (TUIK, [1]). Mediterranean region (53%) of Turkey has more than half of production while Aegean (33 %) and Southeastern Anatolia (12 %) have substantial contributions to Turkish pomegranate production. Accordingly, Turkey hosts many local pomegranate varieties while some other foreign varieties are present in Turkey either for research purposes or commercially. The varieties employed in our study are among the most commonly available commercial varieties in Turkey.

Hicaz variety is the most widely planted, preferred, and exported variety in Turkey. Size of flattened fruits ranges from the middle to large. The peel is thick and its color is red. Grain size is small, grain yield is medium and its juice yield is low to middle. It tastes sour and has hard grains. The variety Wonderful tastes sweet and is rich in the juice which contains the highest rate of anti-oxidants. It has red colored peels and seeds. The fruit size is relatively large. Originally from Israel, Acco variety was introduced many countries including Turkey. Fruits of Acco are early available to consume which lead the increase in preference by farmers. It has a sweet taste with a low acidity level and deep red color in both the peel and the grains. This variety is hard peeled and has smooth seeds. Pomegranate production may be qualitatively and quantitatively restricted by many pests, diseases, and natural enemies. Among diverse pomegranate pests are aphids (Hemiptera: Aphididae), mealybugs (Hemiptera: Pseudococcidae), whitefly (Hemiptera: Aleyrodidae), leafhoppers (Hemiptera: Cicadellidae), scale insects

(Hemiptera: Coccidae), leafrollers (Lepidoptera: Tortricidae), fruit borers (Lepidoptera: Pyralidae and Noctuidae), fruit sucking bugs (Hemiptera: Coreidae and Lygaeidae) and fruit flies (Diptera: Tephritidae). The Mediterranean Fruit Fly, *Ceratitis capitata* Wied (Diptera: Tephritidae) is considered as the most deleterious agricultural pest in the world, has a wide range of cultivated and uncultivated host plants including subtropical fruits, some vegetables, and some ornamental plants (CABI, [2]). The damage of *C. capitata* to fruits may reach 100%. Besides the feeding damage, *C. capitata* may transmit fruit-rotting fungi which increase qualitative and quantitative yield losses (Cayol et al., [3]).

As in many other economically important crops, control of *C. capitata* in pomegranate orchards mostly relies on insecticide applications in Turkey. However, due to resistance and non-target effects of insecticides on organisms, alternative sustainable control strategies are required. Host plant resistance to herbivory is a very important component of integrated pest management strategies especially in horticulture to prevent possible long-term damage. Therefore, population density of *C. capitata* on Acco, Hicaz and Wonderful varieties of pomegranate were monitored starting from late-May until mid-November of 2015. We employed yellow traps impregnated with pest's pheromone for weekly samplings. In addition, some pest-associated damage parameters such as dent rates on the fruits and were evaluated.

## Material and Method

### Study location

The experimental orchards were in Seyhan district of Adana. Population densities of *C. capitata* were monitored on three varieties of pomegranate, Hicaz, Wonderful, and Acco. Hicaz and Wonderful trees were 9 years old while Acco trees were 5 years old. The distance between trees was  $5 \times 3$  in all orchards. The orchards of Hicaz and Wonderful varieties were 30 da and the orchard of Acco variety was 15 da. Conventional agricultural practices were performed in all orchards and pesticides were used against diseases and pests at certain periods and it was detected that the number of *C. capitata* decreased as a result of pesticide application.

### Population monitoring and sampling procedure

*Ceratitis capitata* population densities were monitored via pheromone traps which consisted of yellow bucket type traps, traps apparatus and sex pheromone (Ammonium salts (7.8 g Ammonium acetate + 1,04 Triethylamine hydrochloride) + 0.034 g Cadavarin (1,5-diamino pentane) 18 g Attractive feed + 15 mg Deltamethrin disc + trap, Spain) of the pest. Thirteen traps were placed in both orchards of Hicaz and Wonderful varieties and 6 traps were placed in Acco variety orchard with a range of 4 traps per decare in each orchard. The traps were placed on external branches of healthy pomegranate trees at south-southeast direction at a height ranging from 1.40 to 2.00 meters close to fruits. The traps were placed on May 13, 2015 (Blooming period, BBCH scale 61) and removed on November 25, 2015 (leaf fall, BBCH scale 93) (Melgarejo et al., [4]; Meier et al.,[5]). Traps were checked daily to detect first *C. capitata*

adult. *C. capitata* adults were counted weekly until leaf fall stage. The alive flies in traps were killed and traps were cleaned after sampled.

### Fruit Characteristics and *C. capitata* Associated Loss Assessment

A total of 200 pomegranate fruits were randomly checked visually via a hand magnifier (10×) in each sampling date. Dented fruits were counted and denting direction of fruit was recorded. Damage rate of fruits was calculated the proportioning number of dented fruits to total fruits (200, dented and healthy). The phenological periods and pomological characteristics of fruits were recorded. A caliper compass was employed to calculate pomegranate peel thickness for all varieties.

### Statistical Analysis

Weekly insect data were checked for normality (Shapiro - Wilk's test) and homogeneity of variances (Levene's test) after log ( $\times+1$ ) transformation prior to the data analysis. Because weekly insect count data do not meet assumptions of linear models, the data were analyzed by fitting generalized linear mixed models (GLMM, lme4 package) with Poisson error distribution (log-link function) to indicate the significance of pomegranate variety, sampling date (Diggle et al., [6]; Bolker et al., [7]; Bates et al., [8]). In the model, variety the collection (sampling) dates were fixed and trap id was a random factor (Bayram and Tonga, 2018 a and b). Additionally sampling over month effects was tested in the same way that allowed monthly and whole season population density comparisons among tested varieties. A further GLMM analysis was performed for each variety separately (sampling date as fixed and trap id as a random factor) to test the effect of sampling date over the growing season. The fixed factor effects were tested using Wald tests ( $p < 0.05$ ). Tukey's post hoc tests (contrasts) were used to compare the means at the 95% confidence level using the glht function of the multcomp package. All statistical computations were done with R software, version 3.4.0 (R Core Team, 2017).

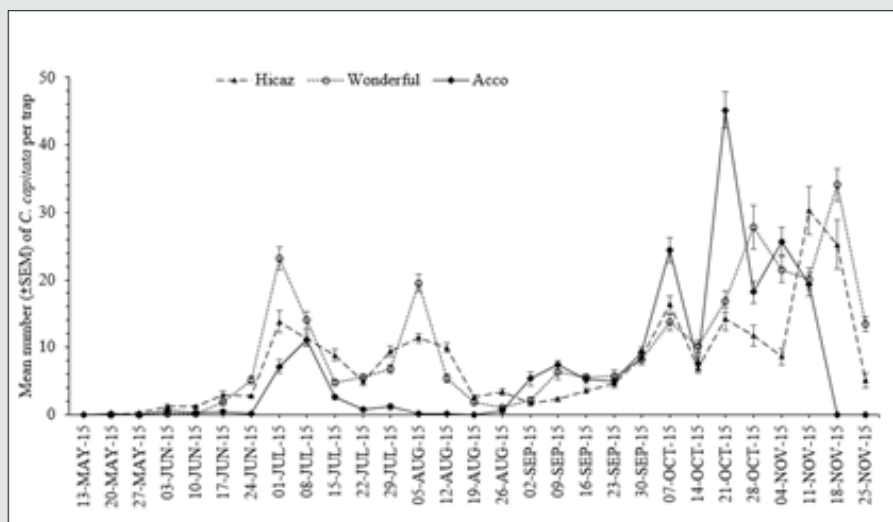
## Results

### *Ceratitis capitata* Population Fluctuation on different pomegranate varieties

A total number of 3572, 2900, and 1189 *C. capitata* adults were caught in traps in pomegranate orchards of Wonderful, Hicaz and Acco varieties, respectively. The first adults of *C. capitata* were trapped on June 3, May 26, and May 20, 2015, in Wonderful, Hicaz, and Acco varieties respectively (Figure 1). Inconsistent *C. capitata* population fluctuations were recorded via pheromone traps in all three pomegranate orchards, namely; Hicaz, Wonderful, and Acco throughout 2015 growing season (Figure 1). Flies were present until harvest in orchards of all varieties. Generalized linear mixed model results have revealed that effects of variety, sampling date and their interaction (variety x sampling date) on *C. capitata* population fluctuations were statistically significant ( $F_{\text{variety}}= 34.93$ ,  $df= 2$ ,  $P<0.001$ ;  $F_{\text{date}}= 111.33$ ,  $df= 28$ ,  $P<0.001$ ;  $F_{\text{variety} \times \text{date}}= 17.35$ ,

df= 54, P<0.001). In total, fly density caught in pheromone traps in Hicaz orchard was significantly lower than flies caught by traps in Wonderful orchard (Figure 1A and B). The mean number of flies caught in traps in Hicaz and Acco varieties throughout the season were not significantly different (Figure 1A and B). The number of

flies caught in traps set in Acco orchard was significantly lower than those in Wonderful variety in the whole season (Figure 1A and B). Thus, the highest density of *C. capitata* flies was recorded from traps located in Wonderful orchard (Figure 1A and B).



**Figure 1:** Population fluctuation of *Ceratitis capitata* on three different pomegranate varieties (Hicaz, Wonderful, and Acco) as captured by pheromone traps in Seyhan, Adana in 2015.

**Table 1:** Some morphological characteristics of the pomegranate varieties, Hicaz, Wonderful and Acco.

Characteristics	Hicaz	Wonderful	Acco
Origin	Turkey	Florida	Israel
Fruit size (g)	300-450	350-600	250-450
Fruit skin color	dark red	purple-red	pinkish
Seeds	hard	soft	very soft
Ripening brix (d)	16-18	17-21	17-18
From flowering to ripening (d)	170-190	160-180	130-150

**Table 2:** ANOVA table of effects of variety on *C. capitata* monthly population

		F	df	P
Variety	May	1.612	2, 125	0.207
	June	11.557	2, 157	<0.001
	July	25.037	2, 157	<0.001
	August	41.006	2, 125	<0.001
	September	8.64	2, 157	<0.001
	October	9.64	2, 125	<0.001
	November	21.619	2, 125	<0.001

To test differences between *C. capitata* adult numbers in traps from different pomegranate varieties per month, we considered sampling-associated each month as replications. Monthly fly densities in pomegranate varieties were found statistically different throughout the season except for May (Table 2). Very few number flies were recorded in Hicaz and Acco varieties while no Medfly was caught in traps located and Wonderful in May. The first *C. capitata* individuals were caught in traps in Wonderful variety in June

(Figure 1, Table 2). The mean fly density was lower in Acco than in Hicaz and Wonderful varieties in June, July, and August (Figure 1 and 2a). In September and October, mean *C. capitata* density was higher in Acco than those of other varieties (Figure 1 and 2a). Hicaz variety had the lowest number of flies in September (Figure 1 and 2a). Number of flies was lowest in Acco variety following by Hicaz while Wonderful had the highest number of *C. capitata* adults (Figure 1 and 2a).

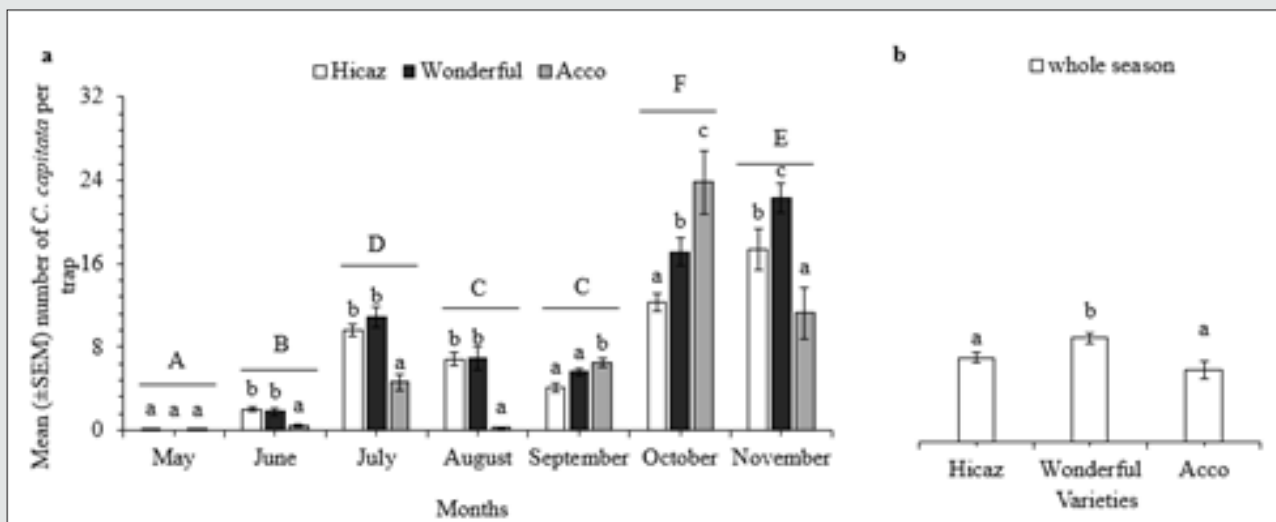


Figure 2: Monthly (a) and whole season (b) mean (± SEM) number of *Ceratitis capitata* per pheromone trap on different pomegranate varieties (Hicaz, Wonderful, and Acco).

### Ceratitis capitata-Associated Loss Assessment

Dates of first dent detection were different on all varieties. For example, first dents were detected on September 09, September 02, and August 19 on Wonderful, Hicaz, and Acco varieties, respectively. Percent of Acco dented fruits was 1.9, 24.0, and 74.0% in August, September, and October, respectively. Percent dented Hicaz variety

fruits were recorded as 16.3, 58.6, and 25.0% in September, October, November, respectively. Percent dented fruit numbers of Wonderful variety were 13.1, 59.2, and 27.6% September, October, and November, respectively (Figure 3). Mean damage rates of *C. capitata* were different on all varieties, for example, the highest damage rate was on Acco variety (5.2%) followed by Hicaz variety (4.6) while the lowest damage rate was on Wonderful variety (3.8%).

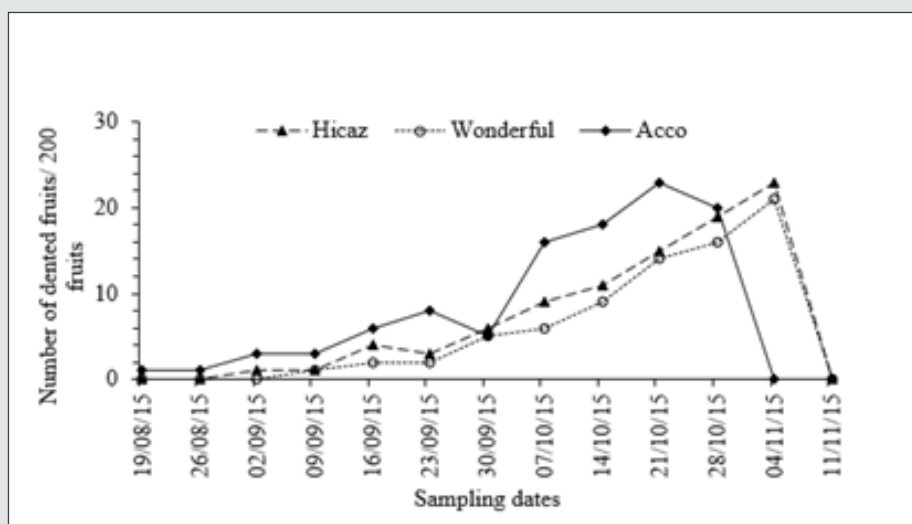


Figure 3: The number of pomegranate fruits dented by *Ceratitis capitata* on the pomegranate varieties (Hicaz, Wonderful and Acco varieties).

### Pomegranate Fruit Peel Thickness

Acco, Hicaz, and wonderful varieties had 2.80, 3.05, and 3.15 mm of peel thickness respectively (Figure 4). Cracks occur on

pomegranate depending on the climate, environmental conditions, and variety characteristics; and it has been observed that cracking rate is 15,1% for Acco, 13,2% for Hicaz and 10,8% for Wonderful (Figure 5).

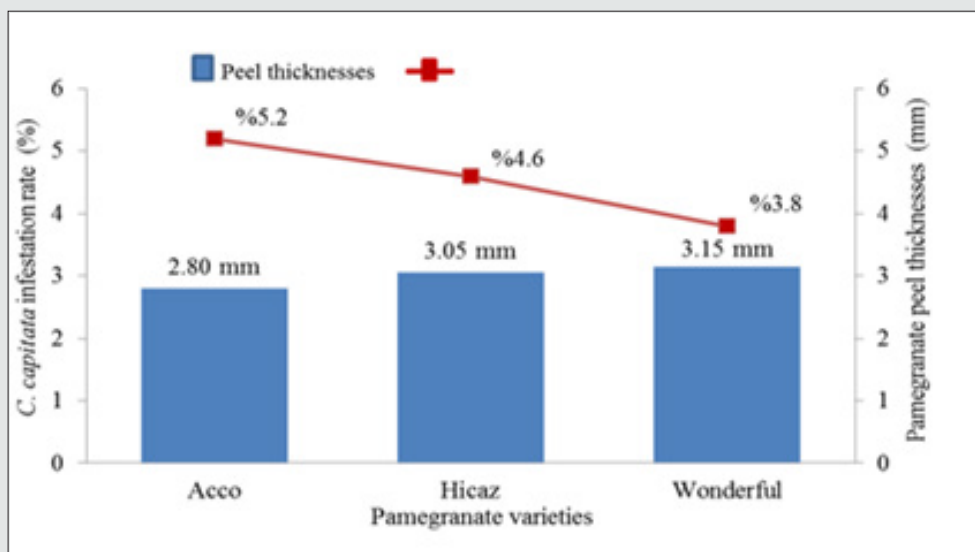


Figure 4: Peel thicknesses and infestation rates for Acco, Hicaz and Wonderful varieties.

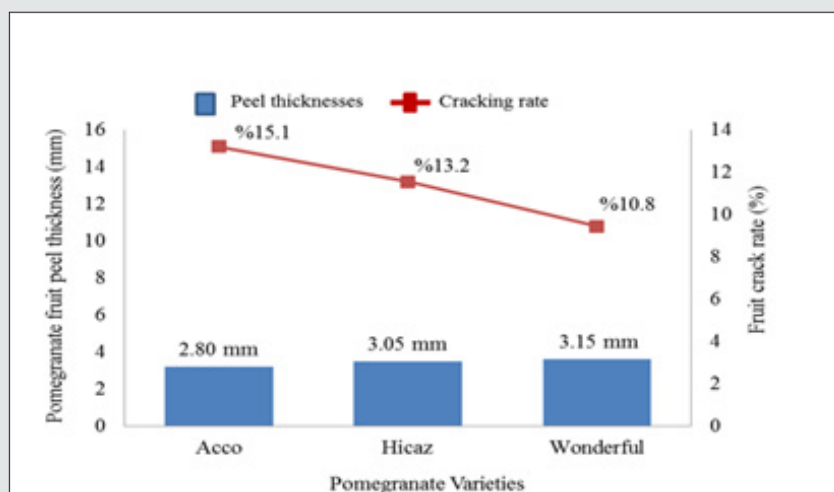


Figure 5: Peel Thicknesses of Acco, Hicaz and Wonderful varieties and fruit crack rates.

### Discussion

During the growing season, inconsistent population fluctuations of *C. capitata* were recorded by pheromone traps on Hicaz, Wonderful, and Acco pomegranate cultivars starting from late May until end of the harvest lasting. Presence of *C. capitata* was reported for the same period in pomegranate and persimmon plants in previous work, but the population fluctuations were still inconsistent (Kasap and Aslan). Variations occurred in fly catches on different cultivars although population dynamics were not constant throughout the growing season. Mediterranean fruit fly did not constitute as a threat for early peach varieties which harvesting started at the beginning of May in the Çukurova Region, but it is threat for late maturing varieties, which was harvesting time started at middle of the June (Tiring and Satar, [9]). Population fluctuation of *C. capitata*

adults monitored by pheromone traps relies on factors affecting adult flight. For example, the low density of *C. capitata* population August and September was likely a result of insecticide treatments. Insecticide treatments were performed since pheromone traps were not efficient enough to control the pest and were employed for population monitoring purposes (Broughton and De Lima, [10]). In addition, the longevity of pheromone releasing sachets which is highly dependent on climatic conditions is another factor requiring special attention since it may influence both numbers of catches over time and insecticide application decision periods (Levinson and Buchelos, [11]; Vanaclocha et al., [12]).

The results of pheromone traps and infestation ratios were inconsistent. Although Wonderful variety had the lowest infestation ratio pheromone traps caught the highest total number of flies in

this variety. The reason was probably possible migration of flies from surrounding cultivated *C. capitata* host fruits such as Japanese plum, apple, blackberry, mandarin, sweet lemon, jujube and fig fruits around wonderful variety (Thomas et al., [13]). Combined with varying infestation ratios, therefore, population monitoring may not reflect cultivar-dependent host preference since infestation ratios highly rely on oviposition preference of the pest. Therefore, the number of flies responding to mate cues and host plant cues may vary as expected. The highest infestation ratio of *C. capitata* was recorded for Acco cultivar which had highest cracking rate and lowest peel thickness. The cultivar was reported having softest seeds and brightest red color among employed cultivars (Kahramanoğlu and Usanmaz, [14]). Cultivar-dependent physical characteristics such as cracking rate, peel thickness, color, and seed softness may play important roles in host preference (McInnis [15]; Levinson et al. [16]). The studies which have tested the preference of host or host-like experimental tools by *C. capitata* demonstrated varying results (Katsoyannos et al., Katsoyannos, Prokopy et al.,). The experimental designs of flies' choice tests were different; therefore, field studies might better represent the fly's choice in a complex environment.

Date factor (developmental stages, fruit ripening) influenced *C. capitata* population densities on all pomegranate varieties since fruits ripen in October and November when the greatest population densities were observed. Joachim-Bravo et al. [13] recorded higher oviposition incidence on ripest parts of papayas plants, *Carica papaya* L. The ripening period of different fruits and its cultivars may differ and, thus, affects population fluctuation of pests. For example, studies conducted in another Mediterranean province, Hatay reported higher population density of *C. capitata* on different pomegranate varieties in September, October, and November depending on variety (Demirel [17] Demirel, [18]). Further research is required to reveal whether the differences in developmental periods of pomegranate varieties could be benefited as a pest management component. Cracking rate, having possible roles in flies host preference, varied between employed pomegranate varieties. Such morphological characteristics pomegranate fruits may vary depending on variety, climatic conditions or agricultural practices (Kasap ve Aslan, [14]; Singh et al., [19]). Previous research demonstrated that cracking rate may also vary depending on irrigation and kaolin could balance internal water capacity of pomegranate fruits, reduced peel temperature by reflecting ultraviolet lights and, accordingly, reduced cracking rates (Ghanbarpour et al., [20]). Therefore, inducing physiological processes of pomegranate fruits by chemical substances or other stress-related compounds may contribute to fruit protection against *C. capitata* (Yılmaz and Özgüven, [21]; Khalil and Aly, [22-35]).

Pest management practices against *C. capitata* highly depend on cultivar characteristics and success in population monitoring. However, still, further research is required to contribute pomegranate cultivar preference of *C. capitata* involving host plant physical and chemical properties (host plant volatiles) to construct an ideal pest management tactics [35-44].

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## Conflict of Interest

The authors declare no conflict of interest.

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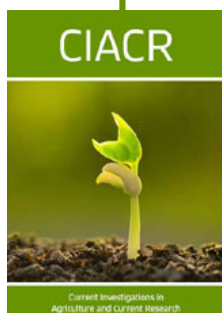
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