Influence of post-harvest chilling treatment on *Ceratitis capitata* and its impact on physical and chemical parameters of Valencia orange

Faten A. A. Badr[1], Magda H. Naroz[2], Hamdy A. El-Shabrawy[2] and Taher M. Amara[1]

ABSTRACT

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is found in most tropical and subtropical areas of the world. Therefore, the aim of this study was to evaluate the chilling treatment method to reduce the cooling time and the cost. This investigation evaluated post-harvest cooling treatment at 1 and 2°C on the immature stages of *C. capitata* in Valencia orange and the effect of these temperature degrees on certain physical and chemical parameters of Valencia orange fruits. The result revealed that the mortality percentage of immature stages increased by exposure time. The egg recorded 100% mortality after 8 days of exposure at 1°C, whereas the time of exposure was extended to reach 10-12 days in the case of larval instars. Cooling time was extended for the tested stages; egg stage and the three larval instars to 10, 12, 14, and 14 days after post-treatment at 2°C, till the mortality reached 100%. The data clarified that the mortality percentage increased with decreasing the temperature and the exposure time which decreased by decreasing temperature in all immature stages. This investigation clarified that the third larval instar of *C. capitata* was the most tolerable in Valencia oranges. The results showed that 12 days were enough time for killing all immature stages of *C. capitata* after treatment at 1°C and 14 days of exposure to cold treatment at 2°C in Valencia orange were sufficient to achieve this infestation. There are no significant differences in both physical (weight loss, color, and hardness) and chemical parameters (acidity and soluble solid) of Valencia orange fruits except vitamin C.

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INTRODUCTION

Citrus is one of the most important fruit crops in the world, and these fruits are regarded as important common foods in more than 100 countries (Yesiloglu *et al.*, 2017). Egypt is regarded as one of the world's most significant citrus-producing nations. About 317 thousand Feddans, or 69.5% of the entire citrus region, are covered by the orange area, which produces 3.1 million tons annually. (CAPMAS, 2021). Furthermore, Egyptian citrus is favored due to its good quality, affordable prices, and high production rate per unit area.

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is found in most tropical and subtropical areas of the world, *C. capitata* is an endemic African species, with worldwide distribution (Malacrida *et al.*, 2007) which considered one of the most deleterious agricultural pests. It attacks a wide range of fruits and vegetables; the damage makes crops inedible and unmarketable.

The repeated use of insecticide from different groups to control fruit flies on citrus affects the exportation of citrus due to the remarkable limit of insecticidal residues. So, alternative methods for controlling these pests. Integrated pest

management (IPM) should take place to control this pest. Post-harvest cooling treatment is a very important safe acceptable method as a quarantine treatment (Hashem *et al.,* 2004).

Cooling method considered a friendly environment, safe for consumers and an effective treatment (Heather and Hallman, 2008). This method increases fresh plant product- shelf life and maintain fruit quality (Ghafir, 2009). Low temperatures ranges from –0.6°C to 3.3°C for 7 to 90 days used as effective method for quarantine pests (Vincent *et al.,* 2003). The immature stage of the Mediterranean fruit fly can be effectively killed in a variety of fruits by a cold-quarantine treatment that lasts 14–18 days at 1.1–2.2°C. (Palou *et al.,* 2008).

In this study, we use low-temperature cooling treatment methods at different temperatures to decrease the cooling treatment time and reduce *C. capitata* immature stages infestation. Therefore, the purpose of this study was to evaluate the cooling treatment method at 1 and 2^{o}C to reduce the cooling time and the cost.

MATERIALS AND METHODS

Insect rearing

The experimented insects were reared and established at a laboratory found in the Horticultural Insects Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt. Eggs used in the current investigations were obtained from a culture reared at 25 ± 1°C and 70% ± 5% RH. The adult feed-on diet consisted of protein hydrolysate and sugar (1:10 gm). The artificial diet for larvae (consisted of wheat bran + sugar + dried yeast + sodium benzoate + citric acid + water) at a rate of 1mL egg /kg medium (Manoukas, 2004). Newly laid eggs used in this investigation were collected for one hour. The collected eggs were put on black filter paper that was cut into small pieces (1 × 1 cm) using scissors transferred using a fine water brush and arranged in rows eliminating cracked, crushed, damaged, or unhealthy eggs.

Fruit preparation

Valencia orange fruits were used in these experiments. 20 Valencia orange fruits were used for each temperature. The fruits were washed thoroughly with tap water and dried. Five orange fruits were artificially infected with Medfly eggs (for control). The experiments were carried out at two temperatures 1 and 2^{o}C. Small cuts were made in orange peels using sharp sterilized cutters and cavities of 1.5 x 1.5 x 0.5 cm (length, width, and depth) were induced in fruit flesh using sharp spatulas. Black filter paper cards loaded with 100 fresh eggs that transferred by sterilized forceps into fruit cavities and covered by the orange peel, then wrapped thoroughly with adhesive tape.

Valencia orange fruits are injected with eggs every other day to ensure that the fruits contain all stages of eggs, 1st, 2nd, and 3rd instar larvae when the treatments begin, then put at 1^{o}C. The same number of oranges inoculated with eggs were put at 2^{o} C. Five fruits from each stage every two days were brought out and incubated at 25^{o}C till pupation. Mortality percentage was calculated and corrected according to Abbott's formula (Abbott's, 1925). Conducting such an experiment enabled us to determine the most tolerable stage in cool treatment.

Physical Parameters of citrus

Weight Loss

The calculated weight loss percentage was determined by the following equation:

Weight loss percentage = (the initial weight before treatment - the final weight after treatment ÷ the initial weight before treatment) ×100.

$$\%WL=(IW-FW/IW) \times 100.$$

Surface color

The citrus fruit color was determined by a hand-held tri-stimulus reflectance colorimeter (model CR-400, Minolta Corp., Newburgh, NY, USA) which calibrated with a white plate. The measurements were performed on fruit surfaces before and after treatment. Measurements were taken on the shoulder of each fruit that was previously labeled as most expanded; to avoid heterogeneity on the fruit surface, a* (-greenness to + redness) was recorded (Del-Valle *et al.,* 2005).

Fruit Firmness

The measurements of citrus firmness before and after treatment were detected by a digital force gauge (model M4-200, MARK, Copiague, NY, USA) (Velickova *et al.,* 2013).

Chemical Parameters of Citrus Fruits

Soluble Solid Content (SSC)

The total soluble content (SSC) in citrus juice was measured by a digital refractometer (model PR101, Co. Ltd., Atago, Tokyo, Japan) that expressed as a percentage on the Brix scale.

Titratable Acidity and Ascorbic Acid

Titratable acidity (TA) was determined according to Horwitz and Latimer (1975). The ascorbic acid content was assessed according to the method of Horwitz and Latimer (2000).

RESULTS AND DISCUSSION

Tables 1 and 2 reveals the influence of low-temperature treatment on the mortality percentage of egg and larval instars of *C. capitata* exposed to different cooling periods at 1 and 2 in Valencia orange. Results clarified that the mortality percentage of immature stages increased with increasing exposure time. Exposure to 1 for 2 days recorded 48.46, 39.20, 37.01, and 33.14% mortality for egg stage, first, second, and third larval instars, respectively. The egg stage recorded 100% mortality after 8 days of exposure at 1^{o}C, whereas the exposure time was extended to reach 10-12 days in the case of larval instars. The exposure time to (2^{o}C), they have differed from one stage to another to reach 100% mortality, where the mortality percentages recorded 41.10, 32.38, 28.18, and 20.93 after 2 days of exposure for the egg stage, the first, second and third stadium larvae, respectively (Table, 2). The cooling time was extended in the case of the egg stage and the three larval instars to 10, 12, 14, and 14 days after cooling treatment, respectively.

The results clarified that the mortality percentage increased with temperature decrement whereas the time of exposure decreased with temperature decrement for all immature stages (egg and larval instars). Respecting the recovered pupae as an endpoint for mortality modeling of *C. capitata* immature stages. The results in Table 3 and Figures 1 and 2 indicate that there was a high response of all stages to low temperature. The cooling treatment at 1^{o}C showed median lethal time (LT_{50}) values of 2.12, 2.54, 2.87, and 3.19 days for egg stage, 1st, 2^{nd,} and 3rd larval instar, respectively.

Table 1. Corrected percentage mortality of egg and larval instars of *C. capitata* in Valencia orange fruits exposed to 1℃±0.5 after different exposure periods

Exposure period (Day)	Egg	1st instar	2nd instar	3rd instar
2	48.46 d	39.2 d	37.01	33.14
4	77.31 c	69.32 c	59.67	54.65
6	95.71 b	85.8 b	76.79	70.92
8	100 a	97.15 a	95.57	92.45
10	100 a	100 a	100	98.83
12	100 a	100 a	100	100

Table 2. Corrected percentage mortalityof egg and larval instars of *C. capitata* in Valencia orange fruits exposed to 2℃±0.5 after different exposure periods

Exposure period (Day)	Egg	1st instar	2nd instar	3rd instar
2	41.1	32.38	28.18	20.93
4	68.71	56.25	49.16	34.29
6	89.56	81.81	78.45	69.18
8	97.55	94.88	92.82	84.88
10	100	98.86	98.34	91.28
12	100	100	99.45	97.68
14	100	100	100	100

The exposure of the egg stage and the three larval instars to 2^{o}C recorded LT_{50} values of 2.49, 3.04, 3.35, and 4.17 days, respectively. The results showed a high increase in time with increasing temperature. The results clarified high differences between the LT_{99} values in all immature stages after exposure to both low temperatures (1 and 2^{o}C). The results showed that the 3rd instar larvae were the most tolerant instar to the cooling treatment in Valencia orange.

Table 3. LT_{50}, LT_{90}, and LT_{99} values of egg and larval instars of *C. capitata* in Valencia orange due to exposure to chilling at 1 and 2°C for different periods

Stage	Degree (°C)	LT_{50} (Day)	LT_{99} (Day)	Confidence limits (h)				Slope ± SE	Chi-square χ^2
				LT_{50}		LT_{99}			
				Lower	Upper	Lower	Upper		
Egg	1	2.12	10.71	1.75	2.43	8.14	16.90	3.3092+/-0.4428	2.53
	2	2.49	13.79	2.12	2.81	11.02	19.05	3.1263+/- 0.3124	2.24
1st	1	2.54	17.69	2.11	2.92	12.92	29.07	2.7613+/- 0.3340	0.16
	2	3.04	14.45	2.00	3.78	12.25	34.22	3.4371+/- 0.2929	9.14
2nd	1	2.87	19.25	2.35	3.48	16.44	28.85	2.8150+/- 0.3160	7.89
	2	3.35	14.64	2.47	4.05	12.36	25.28	3.6339+/- 0.2709	13.39
3rd	1	3.19	20.11	1.75	4.08	19.42	91.62	2.9107+/- 0.2758	11.01
	2	4.17	20.00	3.21	4.99	16.74	33.97	3.4174+/-0.2416	12.64

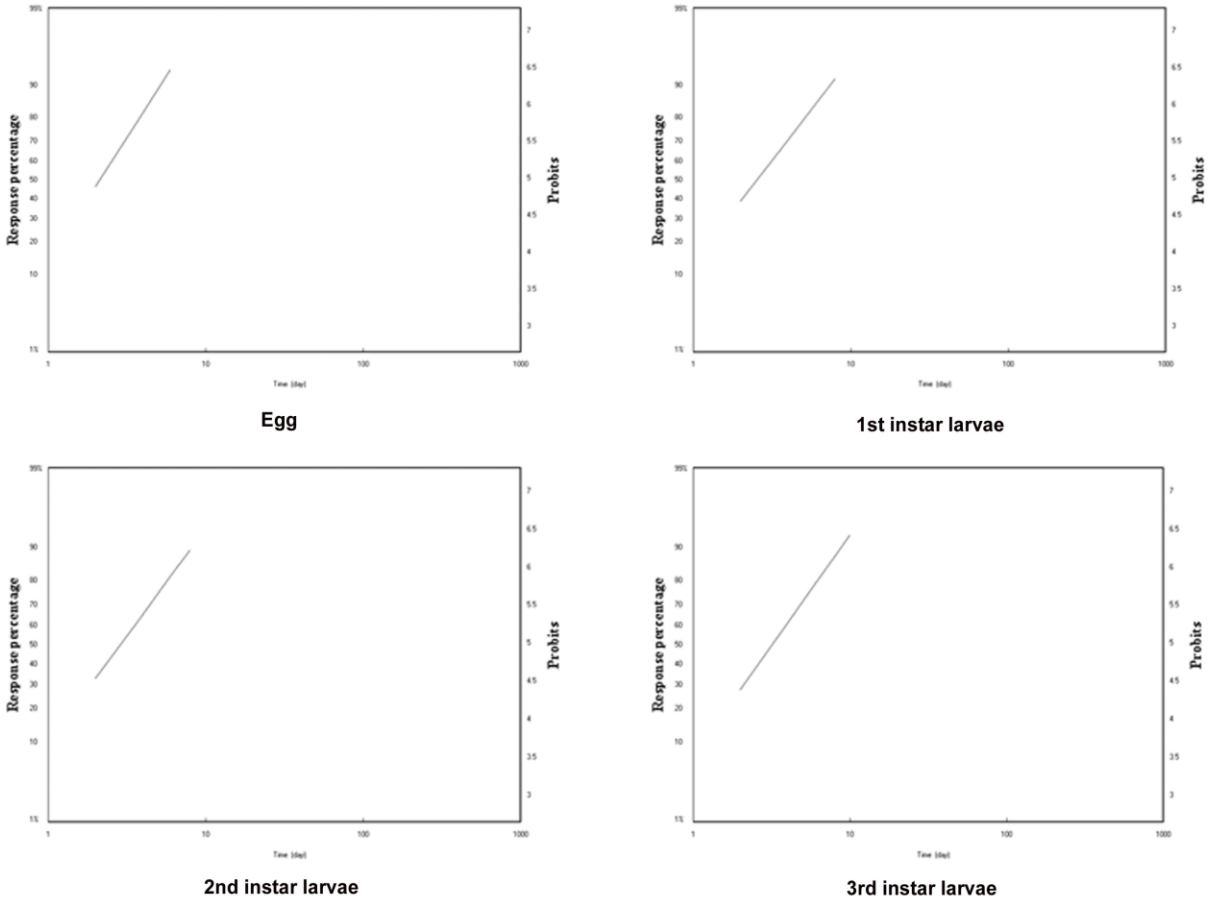

Figure 1. LT-p lines of egg stage and larval instars of *C. capitata* exposed to 1°C in Valencia orange

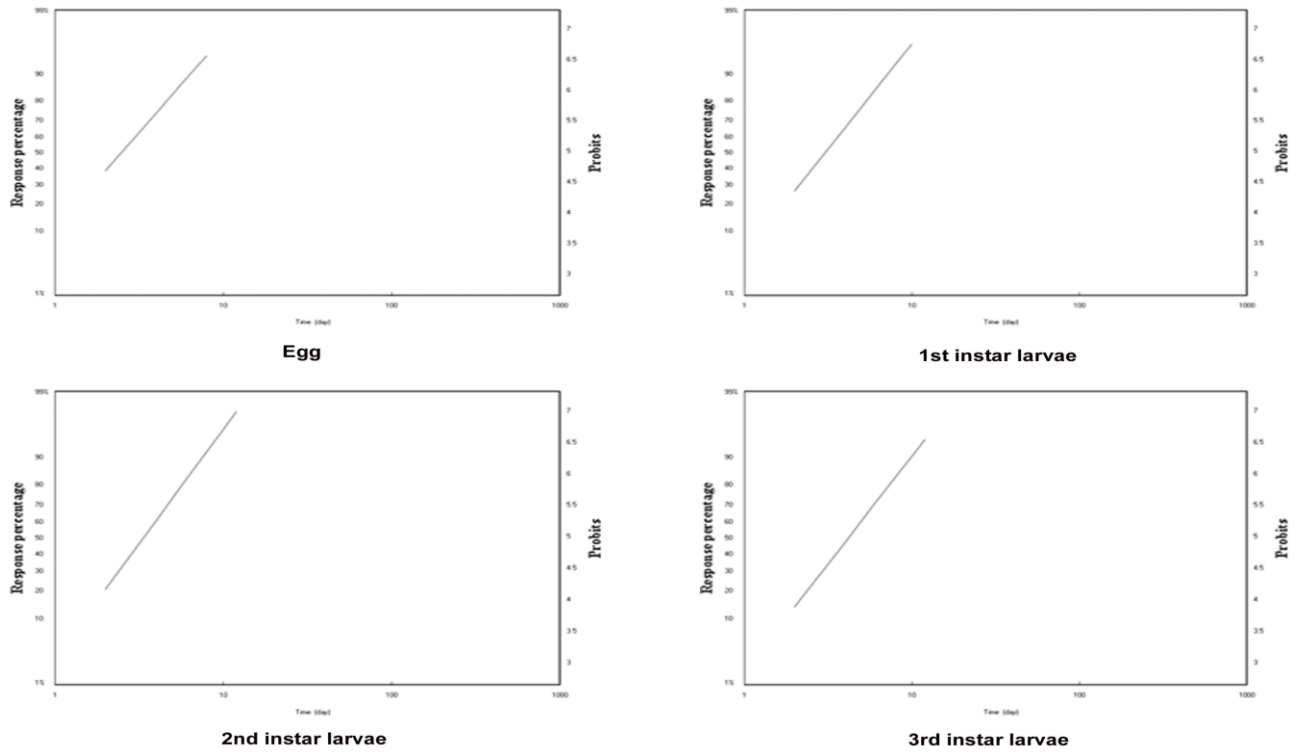

Figure 2. LT-p lines of egg stage and larval instars of *C. capitata* exposed to 2 ^{o}C in Valencia orange

The results in Table (4) showed that the physical properties (weight loss, color, and hardness) and the chemical characteristics (acidity, soluble solids, and vitamin C) of Valencia orange have no significant differences in weight, color, and hardness were found. No statistically significant differences were found in chemical parameters like acidity, or soluble solid concentrate, except vitamin C which was significantly decreased.

DISCUSSION

Low temperatures over specified periods have been used for controlling insect pests in fruits after harvest. Cold treatments method was required by many countries to prevent development of specified quarantine insect pests (Sharp 1993). The temperature degrees range from –0.6°C to 3.3°C for 7 to 90 d used for quarantine pests typically (Vincent *et al.*, 2003). Also, cold treatment protocols target citrus fruit fly pest: *C. capitata* (Wiedemann) (FAO 2016, USDA 2016).

Table 4. Effect of cold treatment on certain physical and chemical parameters of Valencia orange fruits

Parameters	Before treat	After treat	F test	LSD
Weight loss	1.2 a	1.8 a	0.07	0.92 ns
Color	75.47 a	76.01 a	0.2	3.52 ns
Firmness	3.25 a	3.6 a	3.56	0.54 ns
SSC	12.2 a	11.8 a	6	0.45 ns
Titratable acidity	3.0a	3.2 a	6	0.23 ns
Vitamin C	643.9 a	542.6 b	2749	5.36 *

Research conducted by various authors reinforces the conclusions drawn from this study. The results suggest that the third larval instar of *C. capitata* was the most tolerance instar in Valencia oranges after exposure to different low temperatures, specifically at 1 and 2 °C.

Our results fully agreement with those obtained by Willink *et al.* (2006), who studied sensitivity to cooling and showed that mature larvae (L3) of *C. capitata* were the most tolerant stage. In this field of investigation, Hallman *et al.* (2013) proved that the third instar larvae of *C. capitata* were in the coldest tolerant stage in citrus. Highly support our data approved by El-Abbassi *et al.* (2017) who stated that the 3rd larval instars of the Egyptian strain of *C. capitata* were the most tolerable stage to low temperature (1.5 ^{o}C) in Valencia oranges.

Hallman *et al.* (2019) recorded that cold treatment at 1.1°C for *C. capitata* generally increased as the insect developed; therefore, the third instar was the most tolerant instar. Al-Behadili (2020) stated that the third instar larvae of *C. capitata* were the most tolerant instar in blueberries cold treatment.

On the contrary Grout *et al.* (2011) revealed that the 1st instar was more susceptible to cooling than the 2nd and 3rd ones in oranges. Hashem *et al.* (2004) found that the storage of fruits infested with *C. capitata* at 1.7℃ for 16 days would be acceptable as recommended treatment. Also, Palou *et al.* (2008) stated that cold-based quarantine treatments (exposure to 1.1–2.2 ^{o}C for 14–18 d) against the Mediterranean fruit fly were suitable for Spanish citrus exports as a pest-free market such as the United States. The data obtained in this investigation revealed that 12 days were enough time to kill all immature stages of *C. capitata* after treatment at 1^{o}C and 14 days of exposure to cold treatment at 2^{o}C in Valencia orange was sufficient to achieve this infestation. The current results are agree with El-Abbassi *et al.* (2017) who suggested that eleven days is a suitable time for exporting sweet oranges after a cold treatment at 1.5± 0.5 ℃. The standard cold treatment against *C. capitata* was conducted by Gazit and Kaspi (2017) at 1.5 ℃, for 16 days. Follett *et al.* (2018) suggested that the exposure to temperature ≤1.5°C for about 14 days were sufficient to kill all immature stages of *C. capitata*. Al-Behadili (2020) reported that eleven days of exposure to cold treatment at 1.0 ± 0.2 ℃ were enough to eradicate all four immature stages of *C. capitata* in blueberries.

Our results showed that cold treatments of Valencia orange, have no effects in the qualitative characteristics of fruits. (Santaballa *et al,* 2009) Support our result, they treated more than 30,000 *C. capitata* in Clementine mandarin under industrial conditions and found that there were no substantial modifications in the qualitative characteristics and physiological alterations of the fruits. Lanza *et al.* (2005) found that color parameter values significantly decreased, no significant difference in hardness was found, and no statistically significant differences were found in acidity and vitamin C.

Therefore, it is recommended to exposure Valencia orange to a cold treatment at 1±0.5^{o}C for 12 days or 2±0.5^{o}C for 14 days to ensure free consignment from any immature stages of the Egyptian *C. capitata* which have no significant differences in fruit quality.

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Faten A. A. Badr[1], Magda H. Naroz[2], Hamdy A. El-Shabrawy[2] and Taher M. Amara[1*]

[1]Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

[2]Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt.

*Communication author

Mobile: +20 1061627300

E-mail: taheramara@yahoo.com