

Effect of Cold Treatment as a Post-Harvest Treatment for Killing Immature Stages of the Peach Fruit Fly, *Bactrocera zonata* (Diptera: Tephritidae) and its Effect on Fruit Quality of Pomegranate

Abd EL-Maaboud, A. S.¹; Samia M. El-Oraby² and Amal M. Hassan²

¹ Plant Protection Research Institute, Agriculture Research Center

² Horticulture Research Institute, Agriculture Research Center



ABSTRACT

Pomegranate fruits were artificially infested with immature stages (eggs and larval instars) of peach fruit fly *Bactrocera zonata* and stored at 1.7°C for 14 days. Results showed that the effectiveness of cold treatment against *B.zonata* was demonstrated by 100% mortality in all immature stages. Also the results indicated that wonderful pomegranate fruits could be stored for 8 weeks (two weeks at 1.7°C plus six weeks at 10°C and RH 85-90%) as storage or shipping temperature. Fruits transferred directly from quarantine treatment to room temperature 25±2°C and RH 45-50% had shelf life of two weeks without chilling injury symptoms plus two weeks at 1.7°C. Concerning the data dealing with packing methods, carton box (5kilo) lined with perforated lifespan was the best in comparison with other methods.

Keywords: Cold treatment, *Bactrocera zonata*, pomegranate, Fruit quality.

INTRODUCTION

The peach fruit fly, *Bactrocera zonata* (Saunders) is considered as one of the most serious pests of fruits. As with many tropical tephritids, *B. zonata* has a wide host range including apple, guava, mango, peach, and orange (Steck, 2010). *B. zonata* was recorded for the first time in Egypt in 1998 and established rapidly in most Egyptian provinces causes great losses in all fruit crops (Mahmoud 2004, Khan *et al.* 2005). The percentages of apricot and citrus infested with *B. zonata* were higher than those infested with *Ceratitis capitata* and reached 20% (Saafan *et al.*, 2005a, and b). The establishment of the peach fruit fly in Egypt has been costing over \$177 million annually (Joomaye 1999).

Phytosanitary treatments are often required to export potential commodities that may carry invasive species to ecosystems where the species are not endemic but could become established (Heather and Hallman 2008). The Plant Protection and Quarantine Treatment Manual, Treatment Schedule T107(a) (USDA 1994), provides a cold treatment against Mediterranean fruit fly for fruits, including apple, apricot, grapefruit, kiwi, loquat, nectarine, orange, peach, persimmon, plum, pomegranate, and tangerine from many different countries. The cold treatment schedule consists of holding fruit at 0, 0.6, 1.1, 1.7, or 2.2°C for 10, 11, 12, 14, or 16d, respectively.

The number of days required for Phytosanitary cold treatments for *C. capitata* and *Anastrepha ludens* (Loew) at 1.7°C are 17 and 22, respectively (Animal and Plant Health Inspection Service [APHIS] 2013b).

The main problem associated with export and prolonged storage of pomegranate fruit are weight loss, shrinking, chilling injury and maintaining fruit quality during transport and storage El Oraby *et. al* (2009).

Lower temperature and high relative humidity has been reported to play a major role in maintaining the produce quality by reducing its rate of water loss Mahajan *et. al* (2008) Caleb *et.al* (2013b). Muluaem *et.al* (2014) found that packaging and cooling maintained the chemical quality of papay fruit towards the end of storage periods. LifeSpan modified atmosphere, modified humidity was the best treatment for maintaining fruit quality of pomegranate during storage Mshraky *et.al* (2017). Miquel *et.al* (2004) found that variation of red color of pomegranate seeds during storage period at different storage temperatures may

be due to the effect of storage temperature on the activity of the enzymes of the anthocyanin and biosynthetic pathway.

This research aims to study the effect of cold treatment at 1.7°C for 14 days on pomegranate fruits artificially infested with immature stages of *B. zonata* and on fruit quality before and after transferred to marketing and storage.

MATERIALS AND METHODS

1-Rearing of the peach fruit fly *B .zonata*

The peach fruit fly *B. zonata* used in this research was obtained from Horticultural Insects Research Department (PPRI). Larvae were reared on artificial diet. Adults flies kept in cages (35×35×35cm) under laboratory conditions at 25±0.5°C, 65±5% RH and a photoperiod 12:12 (L:D) and fed on a diet composed of sugar and enzymatic yeast hydrolysate at rate of 3:1, respectively. Eggs were collected from mature females (10-14days old) during one hour period before all treatments from a plastic orange, punctured from upper part and contained water inside to keep moisture for eggs.

2-Artificial Infestation of Pomegranate:

Freshly harvested pomegranate fruits variety 'Wonderful' from local orchards were cleaned, washed thoroughly with tap water, immersed in a disinfectant solution and allowed to air dry.

Fifty fresh eggs for each fruit were counted under stereoscope and arranged on black filter papers cards (1cm×1cm) wetted with distilled water.

Small cuts were conducted in pomegranate peels using sterilized cutter and cavities were made beneath using sharp spatulas.

Black filter paper cards loaded with eggs were transferred using sterilized forceps, inserted into fruit cavities, covered by pomegranate peel and secured with adhesive tape.

Forty pomegranate fruits were artificially infected by black filter paper cards loaded with eggs one week prior entering into cold chamber to obtain 3rd larvae during treatment. After 2days a new 40 fruits were infected with eggs to obtain 2nd larvae during treatment. Same procedure was repeated after another 2 days to obtain the 1st larvae during treatment. The above three groups of infected fruits were incubated under laboratory condition at 25±1°C and 65±5%RH to allow development all immature stages of *B.*

z. zonata. The fourth and last group of fruits was infected by eggs on the day determined of entering fruits to cold chamber.

On the other hand, twenty pomegranate fruits (5 replicates each immature stages) as control were artificially infected by black filter paper cards loaded with eggs to obtain the all immature stages and incubated under laboratory condition at 25±1°C and 65±5%RH to allow development all immature stages of *B. zonata* to compare with cold treatment.

3-Cold Treatment Chamber:

The inner dimensions of cold chamber were 14.4 by 4.4 by 5m.

Plastic boxes loaded with infected fruits were distributed randomly in 8 sites inside of cold chamber representing different places and heights. Each site contained 20 fruits representing the four immature stages of *B. zonata* (5fruits loaded with eggs, 5 fruits contain 1st, 5 fruits contain 2nd and 5 fruits contain 3rd larval instars). At each site where infected fruits were placed thermographs which were fixed. The temperature inside cold chamber was observed by an internal sensor connected to an electronic panel fixed outside the chamber. When temperature reached 1.7°C, the internal thermographs were set to start recording. Fruits were moved out cold chamber after 14 days of exposure and kept at 25°C for 24h before being dissected and inspected under magnification to determine number of survived or killed larvae.

Effect of cold treatment of fruit quality:

Fruits were harvested when total soluble solid contents for juice attained level of 12-13% according to Arie *et. al* (1984). Fruits divided to three groups and packed as follows:

- 1- Fruits packed in carton boxes (5kilo) (control).
- 2- Fruits packed in carton boxes lined with perforated Lifespan (modified atmosphere packaging and modified humidity)(5 kilo)
- 3- Fruits packed in plastic boxes lined with Bargamin papers (5 kilo).

All these treatments were held at 1.7°C for 14 days as quarantine treatments, then three replicates from each treatment were transferred to room temperature 25±2°C and RH 45-50% and other replicates were stored in a cold room (10°C and 85-90% RH) as shipping period.

Physical and chemical properties were determined (fruit weight loss – peel and seed color susceptibility to chilling injury, total soluble solids and titratable acidity percentage).

Chemical and physical properties were examined every 15 days for cold storage and every week for room temperature until the end of storage period.

Physical properties:

Fruit weight loss percentage:

Fruits were periodically weighed and the percentage of weight loss was calculated by the difference between the initial weight and that recorded at the date of sampling.

Peel and seeds color:

It was measured by using a hunter colorimeter type (dp 9000) for the estimation of a value (red color) according to Me gjuire (1992).

Chilling injury studies:

To check the susceptibility of pomegranate fruit to chilling injury, fruits were evaluated for external and internal symptoms (brown discoloration) during storage period at two storage temperatures.

Chemical properties:

Total soluble solids:

Percentage: was determined by Abbe, digital – refractometer. The titratable acidity as citric acid were determined according to A.O.A.C (1990)

Data obtained were statistically analyzed according to (Snedecor and Cochran, 1972) means for treatments were compared by L.S.D at 5% level.

RESULTS AND DISCUSSION

Results obtained from Table (1) showed that cold treatment of pomegranate fruits at 1.7°C for 14days as post-harvest treatment was effective as it caused 100% kill to all immature (eggs, 1st larval instar, 2nd larval instar and 3rd larval instar) *B. zonata*.

Table 1. Total number of immature stages of *B. zonata* for control compared with cold treatment.

Stage	Control treatment			Cold treatment	
	No. eggs treated	No. live larvae recovered	Mortality (%)	No. live larvae recovered	Mortality (%)
Eggs	250	234	6.4	0	100
1st larval	250	200	20	0	100
2nd larval	250	158	36.8	0	100
3rd larval	250	143	42.8	0	100

These results agree with Hill *et.al* (1988) who stated that storage of orange for 16 days at 1.0± 0.5°C resulted in 100% mortality of the most tolerant stage of *D. tryoni* and *C.capitata*.

Jessup *et al.* (1993) found that first instar of *Bactrocera tryoni* and the second instar of *Ceratitis capitata* were the most tolerant to 1 ± 0.2°C for 14days in two cultivars of lemons. While the third instar was the most cold-tolerant stage at 1.7 and 4°C when *C. capitata* was reared in guava, mango, and orange (“Navel” and Valencia) Hashem *et al.* (2004). Hallman *et al.* (2011) found that third instar of *B. invadens* was no more cold tolerant than third instar of *Anastrepha ludens* (Loew), *Bactrocera dorsalis* (Hendel), and *Ceratitis capitata* (Wiedemann) in vitro at 0.94 ± 0.65 °C. Hallman *et al.* (2013) represented that *B. invadens* is not more cold tolerant than *C. capitata* and *B. zonata* at 1.0 ± 0.1°C but it cannot be concluded that *B. zonata* is not more cold tolerant than *C. capitata* in oranges.

Fruit weight loss percentage:

The data presented in tables (2.3.4) indicated that weight loss increased gradually and significantly after 2 weeks at 1.7°C and with advancing storage period at both storage temperatures 10°C and 25± 2°C. On the other hand the mean of fruit weight loss stored 3 weeks at room temperature 25±2°C had approximately 6 times at weight loss of fruit stored 6 weeks at 10°C. Concerning the effect of packing method on fruit weight loss, treatment 2 tended to the lowest percentage in comparing with the other treatments at three storage temperatures.

The previous results were supported by Mahajan *et.al* (2008) and Caleb *et.al* (2013b) who reported that

lower temperature and high relative humidity play a major role in reducing rate of water loss. Also, Santos *et.al* (2004) Malgarim *et.al* (2006) and El Etreby (2010) reported that polyethylene and polypropylene film minimized the weight loss.

Table 2. Effect of cold treatment at 1.7°C for two weeks on fruit quality of pomegranate fruit.

Treatment	Quarantine period		Mean
	Initial	2 weeks	
Fruit weight loss%			
1	0	1.40	0.070
2	0	0.60	0.30
3	0	0.87	0.43
Mean	0	0.96	
LSD A =2.27		LSD B=1.85	LSD A*B=3.22
Rind color			
1	34.84	36.10	35.47
2	34.84	34.10	34.47
3	34.84	36.77	35.81
Mean	34.84	35.66	
LSD A = 1.86		LSD B=1.52	LSD A*B=2.62
Seed color			
1	5.53	12.47	9
2	5.53	13.07	9.30
3	7.93	9.23	8.58
Mean	6.33	11.59	
LSD A =4.69		LSD B=3.83	LSD A*B=6.64
Total soluble solids %			
1	12.73	14.53	13.63
2	12.73	14.27	13.50
3	12.73	13.90	13.32
Mean	12.73	14.20	
LSD A =0.27		LSD B=0.22	LSD A*B=0.38
Total acidity %			
1	1.40	1.23	1.32
2	1.40	1.23	1.32
3	1.40	1.20	1.30
Mean	1.40	1.22	
LSD A =0.13		LSD B=0.11	LSD A*B=0.19

A= Treatment B= Storage period A*B=Interaction

Rind and seed color:

The data in Tables (2.3.4) showed that no differences were found in rind color parameter (a) red color of fruits before and after quarantine period. The same results were found in fruits held at shipping (10°C) or room temperature (25± 2°C). The results dealing with seed color revealed that parameter (a) increased significantly after quarantine period and during storage period at 10°C or at room temperature 25±2°C with some fluctuated at the last week of storage.

Data showed also that there was no obvious difference as means among treatments on rind and seed color during storage at the three different temperatures.

These results are in harmony with those found by Hamauzu and kumc (2005) who observed that phenolic and anthocyanin content increased at 1°C after storage 15 days in prunes fruit. Miquel *et.al* (2004) found that variation of red color during storage period at different temperatures may be due to the activity of the enzymes on the anthocyanin biosynthetic pathway.

T.S.S:

Data represented in Tables (2.3.4) showed that T.S.S of fruits stored 2 weeks at 1.7°C increased significantly in comparison with the beginning of storage.

After fruits were transferred to 10°C or 25±2°C the increase was slight till the end of storage period at 25± 2°C

but T.S.S decreased at the last week of cold storage at 10°C. The means among treatments showed that no difference on T.S.S during storage at three tested temperatures.

The pervious results were supported by Prasad and Mail (2000) Mshraky *et.al* (2017) who found that total soluble solids and total sugars increased with increased period of storage both at room as well as at low temperature.

Titratbale acidity:

With regard to the periodical changes in acidity it was evident from Tables (2.3.4) that acidity of fruits stored 2 weeks at 1.7°C had slight decrease in comparison with the beginning of storage. After fruits transferred to 10°C or 25±2°C the decrease was significant after two weeks from transferring. No obvious differences in means of acidity among packing treatments during storage at the three temperatures.

The decrease of acidity at the three temperatures may be due to the effect of temperature on respiration rate of fruits stored at 0°C or 10°C and ambient temperature (El Oraby *et. al* 2009). The decrease of acidity may be that organic acid are important as source of respiratory Zagory and kade (1989).

Susceptibility to chilling injury and decay:

No external or internal chilling injury symptoms or decay were observed on pomegranate fruits variety wonderful after quarantine period or on fruits stored at 10°C or at room temperature till the end of storage period. This result agree with data found by El Oraby *et. al* (2009) on pomegranate variety manfaloti.

Table 3. Effect of cold treatment on fruit quality after transfer to shipping or storage temperature 10 °C and 85- 90 RH.

Treatment	Storage periods (Week)				Mean
	After quarantine period	2	4	6	
Fruit weight loss%					
1	0	2.13	3.86	4.0	2.50
2	0	0.66	1.06	1.9	0.90
3	0	1.73	2.96	4.43	2.28
Mean	0	1.51	2.63	3.44	
LSD A =0.31		LSD B =0.35		LSD A*B=0.61	
Rind color					
1	36.10	35.53	35.50	34.67	35.45
2	34.17	37.30	36.10	35.17	35.68
3	36.77	32.47	36.20	32.10	34.38
Mean	35.68	35.10	35.93	33.98	
LSD A = 1.34		LSD B= 1.55		LSD A*B=1.55	
Seed color					
1	12.47	18.07	16.07	16.47	15.77
2	13.07	19.97	29.57	19.97	20.64
3	9.20	12.20	17.57	16.30	13.82
Mean	11.58	16.74	21.07	17.58	
LSD A=3.79		LSD B=4.38		LSD A*B=7.59	
Total soluble solids %					
1	14.97	14.50	15.17	13.37	14.50
2	14.27	14.90	13.80	11.97	13.73
3	14	12.77	14.50	11.60	13.22
Mean	14.41	14.06	14.49	12.31	
LSD A = 0.74		LSD B=0.85		LSD A*B=1.5	
Total acidity %					
1	1.23	0.80	0.70	0.70	0.86
2	1.23	0.70	0.67	0.80	0.85
3	1.2	0.87	0.80	0.057	0.86
Mean	1.22	0.79	0.72	0.069	
LSD A =0.059		LSD B=0.069		LSD A*B=0.12	

Table 4. Effect of cold treatment on fruit quality after transfer to room temperature 25± 2°C and 45-50 RH.

Treatment	Storage periods (Week)				Mean
	After quarantine period	1	2	3	
Fruit weight loss%					
1	0	11.43	18.60	25.27	13.82
2	0	7.90	12.27	17.30	9.37
3	0	1037	17.70	23.73	12.95
Mean	0	9.90	16.19	22.10	
LSD A =0.89		LSD B=1.03		LSD A*B=1.78	
Rind color					
1	33.20	35.60	30.20	33.27	33.07
2	33.20	31.95	31.67	31.63	32.11
3	33.53	24.10	33.90	30.60	30.53
Mean	33.31	30.55	31.92	31.83	
LSD A =2.43		LSD B=2.80		LSD A*B= 4.86	
Seed color					
1	5.47	12.47	11.97	17.70	11.90
2	5.47	13.07	16.30	15.57	12.60
3	5.47	10.20	12.20	15.07	10.73
Mean		11.91	13.49	16.11	
LSD A =1.99		LSD B=2.31		LSD A*B= 3.99	
Total soluble solids %					
1	14.53	14.93	15	15.17	14.91
2	14.27	14.17	14.87	15.67	14.74
3	13.90	14	14.53	15.10	14.38
Mean	14.23	14.37	14.80	15.31	
LSD A = 0.017		LSD B=0.020		LSD A*B=0.351	
Total acidity %					
1	1.23	0.06	0.67	0.77	0.82
2	1.23	0.67	0.73	0.77	0.85
3	1.20	0.67	0.70	0.66	0.81
Mean	1.22	0.64	0.70	0.73	
LSD A = 0.06		LSD B=0.07		LSD A*B=0.12	

CONCLUSION

Cold treatment of pomegranate fruits for 14 days at 1.7°C resulted in 100% mortality of all immature stages (eggs, 1st larval instar, 2nd larval instar and 3rd larval instar) *B. zonata*. So, this treatment was an effective a quarantine to kill both egg and larvae of *B. zonata*. According to fruit weight loss and quality the results indicated that pomegranate fruit variety wonderful could be stored for 8 weeks (two weeks at 1.7°C plus six weeks at 10°C and 85 -90% RH) and 4 weeks (two weeks at 1.7°C) plus two weeks at 25±2 °C and RH 45-50% . These results are in agreement with those reported by Sercan *et.al* (2015) who found that pomegranate cultivars were monitored at refrigeration temperature for two months. Concerning the data dealing with packing methods, carton box (5kilo) lined with perforated lifespan was the best in comparison with other methods.

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تأثير المعاملة بالتبريد كاحد المعاملات ما بعد الحصاد لقتل الاطوار غير الكاملة لذبابة الخوخ وتأثير ذلك على جودة ثمار الرمان

أكرم شوقي عبد المعبود¹، سامية محمد العرابي² و امل مصطفى حسن²

¹ معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الجيزة - مصر

² معهد بحوث البساتين - مركز البحوث الزراعية - الجيزة - مصر

اجريت هذه الدراسة على ثمار الرمان صنف وندرقل لمعرفة تأثير المعاملة بالتبريد على درجة 1.7م° لمدة 14 يوم على الاطوار غير الكاملة (بيض - عمر اول - عمر ثاني - عمر ثالث) لذبابة الخوخ وايضا تأثير هذه المعاملة على صفات الرمان (الفقد في الوزن- لون القشرة والبذور- الحموضة الكلية-المواد الصلبة الذاتية الكلية واضرار البرودة بعد نقل الثمار الى درجة التسويق او التخزين والشحن) كما تم دراسة ثلاث طرق تجارية من التعبئة على صفات الثمار أثناء التخزين. ووضحت النتائج المتحصل عليها ان المعاملة بالتبريد ادت الى قتل جميع الاطوار غير الكاملة لذبابة الخوخ بنسبة 100%. كما اوضحت النتائج ايضا ان ثمار الرمان صنف وندرقل يمكن تخزينها لمدة 8 اسابيع منها اسبوعين على درجة 1.7م° بالاضافة الى 6 اسابيع على درجة 10م° ورطوبة 85-90% كفترة تخزين او شحن بحرى والثمار التي تم نقلها مباشرة بعد المعاملة على درجة 25±2م° ورطوبة 45-50% لها عمر تسويقي اسبوعين على هذه الدرجة بالاضافة الى اسبوعين على درجة 1.7م° بدون حدوث اضرار البرودة. أما بالنسبة الى النتائج المتعلقة بطريقة التعبئة كانت العبوات الكرتون سعة 5 كجم والمبطنة باكياس Life Span هي الافضل بمقارنتها بباقي الطرق الاخرى.