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Resistance and Susceptibility of Two Pomegranate Varieties Fruits to Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) and Peach Fruit Fly, *Bactrocera zonata* (Saunders) Infestation

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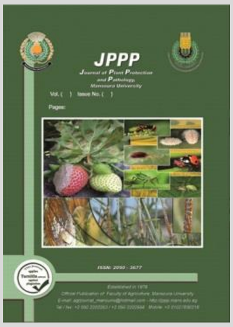

ABSTRACT

Pomegranate, *Punica granatum* L. (Punicaceae) fruits varieties (Baladi and Wonderful) were tested for their resistance and susceptibility to Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and peach fruit fly, *Bactrocera zonata* (Saunders) infestation in choice and non-choice tests. *P. granatum* L. var. Baladi fruits were resistant to both *C.capitata* and *B.zonata* infestations in choice and non-choice tests. Host preference test proved that *P.granatum* L. var. Wonderful acted as a preferred host as guava fruits, *Psidium guajava* fruits for *C. capitata* and *B. zonata*. Egg-larval durations of *C. capitata* and *B. zonata* reared on *P. granatum* var. Wonderful fruits were longer than that reared on *P.guajava* fruits. Pupal durations of *C.capitata* and *B.zonata* produced from *P.granatum* L. var. Wonderful and *P.guajava* fruits varied insignificantly. The chemical analysis of *P.granatum* fruit peels proved variation in the polyphenolics amounts in both tested varieties. The amounts of total phenols, flavonoids and tannins in *P.granatum* var. Baladi were more than var. Wonderful. Fungal infection of the tested fruits affected the polyphenolics amounts in both tested varieties.

Keywords: *Ceratitis capitata*, *Punica granatum*, *Bactrocera zonata*, resistance and susceptibility.

INTRODUCTION

The Mediterranean fruit fly, *C. capitata* and peach fruit fly, *B. zonata* hold an impressive record of successful invasions threatening the Egyptian agricultural economy, growth and development of international fruit trade. Pomegranate, *P. granatum* L. is cultivated in many countries of the world as the trees are highly adaptive to a wide range of climatic and soil conditions including the Mediterranean basin (Holland *et al*., 2009). Mostly, fruits are consumed fresh or processed for juice that rich in nutrients and for other industrial purposes. In Egypt, different varieties of pomegranate are cultivated with preference to those give enormous crop yield as Wonderful variety. Tephritids are recorded to infest pomegranate fruits in many cultivating countries. Mediterranean fruit fly, *C. capitata* and peach fruit fly, *B. zonata* are the most important fruit flies in Egypt that threaten the horticultural fresh fruit production (White and Elson-Harris, 1992). Resistance and susceptibility of *P.granatum* L. var. Baladi and var. Wonderful fruits to *C.capitata* and *B.zonata* infestation are investigated in the present work. The flies' host preference was measured by using guava fruits (*P. guajava*) as a favorable host for both fruit flies. *P.granatum* L. fruit peels contain variation of polyphenolics that reflect a distinct feature of each cultivar, so, in the present work, total phenolics, falvonoids and tannins of each tested pomegranate variety fruit peels were estimated.

MATERIALS AND METHODS

Tested fruits:

Full ripen pomegranate, *P. granatum* L. var. Baladi and Wonderful in addition to guava (*P. guajava*) non-infested fruits were obtained from a private organic farm at Maikana land, Kilo 90. Nubaria, Behira governorate, Egypt. All fruits were washed separately and well dried carefully with cotton cloth before subjected to tests.

Insects:

Ceratitis capitata and *B. zonata* flies obtained from the laboratory colony reared in the Horticulture Insects Department, Plant Protection Research Institute, Dokki, Giza, Egypt. The insect larvae reared using artificial larval rearing medium according to the technique of Tanaka *et al*., (1969). The flies were fed on the regular diet (sugar and enzymatic yeast hydrolysate in ratio 3:1, respectively) (El-Sayed, 1979) in addition to a source of water. The flies were kept in room temperature 29±3ºC, relative humidity (RH) 65-70% and photoperiod 12D:12L. The colony was provided with wild flies monthly to keep flies strong features and progeny.

Susceptibility of pomegranate fruits to *C.capitata* and *B.zonata* infestation:

The non-choice test:

A flies wire screen cage measured 35×33×30 cm contained 200 *C.capitata* flies (approximately 1♀:1♂) was set for the test. The flies were provided with the regular diet and a source of water. A 24 hrs before fruit exposure to the insects, the egg receptacles were removed from the cages. As the flies aged 13 days (matured flies and gravid females), five pomegranate Baladi fruits weighed 200-210 g were hanged inside the cage using galvanized wires on different heights to enable the gravid females to oviposit in them. The test was replicated three times. After 24 hours, the fruits were removed, examined for punctures and number was recorded. Eggs in each puncture were

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removed by a fine hairbrush then placed carefully on a moistened black cloth and counted. The same procedures were done with pomegranate variety Wonderful fruits weighed 200-230g and guava fruits weighed 180-200g, separately. The same steps were repeated with *B.zonata* except for the ages of flies that were 19 days for the hatchability test and 21 days at fruit exposure.

The choice test:

A flies cage measured 35×33×30 cm contained 200 *C.capitata* flies (approximately 1♀ :1♂) was set for the test. The same steps were done as in the non-choice test for both flies and pomegranate fruit varieties and guava fruits except for the presence of the egg receptacles inside the cages to enable flies to choose where to oviposit and lay their eggs.

Host Preference test:

A screen cage measured 90×40×50 cm contained 600 *C.capitata* flies (approximately 1♀:1♂) was set for the test. The flies were fed on the flies' regular diet in addition of a source of water. Egg receptacles were removed before the test by 24 hrs. Once the flies aged 13 days, five fruits of each pomegranate Baladi and Wonderful varieties were introduced to the gravid females in addition to five guava fruits that weighed (180-200g). The cage was divided without septors into three areas to distribute each fruit variety apart from the others. The fruits of each variety were hanged at different heights using galvanized wires to make the flies have the choice to oviposit at any place of the fruits. The test was replicated three times. The fruits were removed after 24 hours. Punctures number was recorded and fruits were kept individually in plastic containers covered with muslin cloth and rubber bands. Produced pupae were removed and counted. The same steps were done with *B.zonata* at flies' age 21 days.

Egg-larval duration inside fruits, pupal duration and sex ratio:

A day before fruits exposure to flies, a random sample of the laid eggs were taken from receptacles and tested for hatchability. A hundred eggs aged six hours were collected and placed on a moistened black tissue in a Petri dish (9cm). After four days, the eggs were examined for the hatched larvae and the dead eggs were recorded. The hatchability test was repeated three times. Five pomegranate wonderful fruits were introduced to gravid *C.capitata* females (13 days old) in a test cage for 24 hours. Each fruit was removed and placed on a sieve inside a plastic container with fine sand at the bottom to receive full-grown larvae. The containers were covered with muslin cloth for ventilation and a rubber band to protect the fruits from other insects' infestation. The containers were examined for the popping larvae in sand after ten days from fruits isolation. Dates of the first popped larva to the last one were recorded. The fruits were examined for the unhatched eggs and dead undeveloped larval stages using saline solution (0.9%). The solution was sieved and filtered using black cloth then examined by binoculars for the unhatched eggs and the undeveloped larvae. The same steps were repeated with guava fruits. The egg-larval duration of *B.zonata* inside pomegranate Baladi and Wonderful fruits and guava fruits was determined by the same steps. All containers were kept under the room temperature (29±3ºC). All produced *C.capitata* and *B.zonata* pupae from the infested fruits were kept individually in Eppendorf tubes at the room temperature (29±3ºC) until flies emerge. The flies' emergence time, percentage and the individuals' sex were recorded.

Preparation of pomegranate fruit peels:

Punica granatum L. var. (Baladi and Wonderful) fruits were washed, rinsed with distilled water then well dried manually with cotton cloth then peeled. All peel samples were cut into small pieces and placed in a drying oven at 40°C for 72h. Dried pomegranate peels were ground into powders to get 60-mesh size using a mixing grinder. Peels powders were kept in airtight plastic containers and stored at 4°C until used for extraction. Peels of inspected fruits for internal fungal infection were treated as mentioned above.

Samples Extraction preparations

Extracts of pomegranate peels were prepared according to Fischer *et al*., (2011) with some modifications. Peels powder of each variety were separately extracted with ethyl acetate. Five grams of finely-powdered dried pomegranate peels samples were added to ethyl acetate (100 ml) in conical flasks and shacked in water bath at 25°C at 20 rpm for 24h. To avoid light exposure the flask was covered with aluminum foil. Mixture was centrifuged for 15 minutes at 10000 g at 3°C using refrigerated centrifuge (Beckman, J2-MS centrifuge). The supernatant was filtered using Whatman No.41 filter paper. Extract was then stored at 4°C until used for estimation of phenolic compounds. All chemicals used were purchased from Sigma.

Total Phenolic Content

Total phenolics contents were determined according to Velioglu *et al*., (1998) and Jayaprakasha *et al*., (2001) with slight modifications. The sample extract (200 μl) was mixed with 1.5 ml of Folin-Ciocalteu reagent [previously diluted 10 times with double distilled water] and allowed to stand at room temperature for 5 min. 1.5 ml sodium bicarbonate solution (7.5%) was added to the mixture. The total volume was made up to 5 ml by adding distilled water and was mixed by vortex for one minute and then incubated for two hours in dark. Subsequently, the absorbance was measured at 765 nm using a UV-visible spectrophotometer (Beckman, DU 7400). Total phenolic were quantified by calibration curve obtained from measuring the absorbance of the known concentrations of gallic acid standard solutions [10-150 μg/ml]. The results were expressed as gallic acid equivalents (GAE) in mg/g dry weight powder.

Total Flavonoids Content

Total flavonoids content was measured by the aluminum chloride colorimetric method of Zhishen *et al*., (1999). Briefly, 1 ml of each extract was added into a 10 ml test tube containing 4ml of distilled water. Then 0.3 ml of 5% $NaNO_2$ was added to the test tube and after 5 min, 0.3 ml AlCl3 (10%) was also added. At 6 min, 2 ml of 1 M NaOH was added to the mixture and the total volume was made up to 10 ml with double distilled water. The solution was mixed completely and the absorbance of the pink colored mixture was read at 510 nm versus prepared reagent blank. Total flavonoids content was expressed as mg catechin equivalents (CE) per gram of dry powder sample (mg/g). An appropriate calibration curve was prepared using different concentrations of catechin solutions.

Determination of hydrolysable tannins content (HTs)

Hydrolysable tannins content (HTs) were determined by the method of Cam and Hişil (2010). 1 ml of 10-fold diluted extracts and 5 ml of 2.5% Potassium iodate (KIO_3) were added into a vial and vortexed for 10 seconds. In the reaction optimum, absorbance of the red colored mixture was determined at 550 nm versus the prepared blank. Optimum reaction defined as the time to gain maximum absorbance value, was determined to be 2 min for pomegranate peel extracts and 4 min for standard solutions of tannic acid. Different concentrations of tannic acid solutions (100 to 1600 mg/l) were used for calibrations. The final results were expressed as mg tannic acid equivalent per g of dry weight (mg TAE/g dry weight).

Determination of pH degree of fruits content

P.granatum var Wonderful arils were crushed and squeezed with fruit juicer without adding any additives. The pH of the squeezed fruit product was measured using pH meter Model KCB-300. The same steps were followed to measure *P.guajava* pH fruit pulp.

Statistical analysis:

Variance of means were subjected to ANOVA , MAXStat Pro v.3.6 statistics software (2015).

RESULTS AND DISCUSSION

The non-choice and choice tests revealed a variation in the ability of both *C.capitata* and *B.zonata* to infest *P.granatum* L. var. (Baladi and Wonderful) and *P.guajava* exposed fruits (Table 1).

Table 1. Susceptibility of pomegranate fruits *P. granatum* var. Baladi and Wonderful and guava fruits, *P. guajava* to *C.capitata* and *B.zonata* infestation in choice and non-choice tests

Non-Choice test			Choice test		
		C.capitata			
		Mean number of punctures/fruit ± SE			
P.granatum		***P.guajava***	***P.granatum***		***P.guajava***
Baladi	**Wonderful**		**Baladi**	**Wonderful**	
0.34±0.45	7.83±0.84	5.61±1.14	0.21±0.24	7.41±0.12	4.89±0.42
	F=104.33 , P<0.0001			F=101.17, P<0.0001	
		Mean number of eggs/puncture± SE			
0.00±0.00	12.61±0.51	8.45±0.24	0.00±0.00	12.41±0.93	6.31±0.37
	F=362.24, P<0.0001			F=523.40, P<0.0001	
		B.zonata			
		Mean number of punctures/fruit ± SE			
1.61±0.89	11.29±1.30	7.89±0.37	1.01±0.32	8.87±0.43	5.49±0.21
	F=21.92, P<0.0001			F=203.72, P<0.0001	
		Mean number of eggs/puncture± SE			
6.63±0.04	14.09±0.31	8.43±0.13	4.87±0.43	16.18±0.58	6.42±0.23
	F=163.68, P<0.0001			F=130.20, P<0.0001	

Significance level (95%) and *P*<0.05.

The non–choice test

Ceratitis capitata, gravid females showed a significant difference in number of punctures in *P. granatum* var Baladi and Wonderful fruits (F= 14.91, P<0.0001) revealing a higher susceptibility of the Wonderful fruits to puncture response even when compared to *P. guajava* fruits (F=11.09, P=0.0004). *B.zonata* females showed a similar pattern of punctures in both pomegranate varieties (F=12.29, P=0.0003) reflecting higher puncture response of Wonderful more than *P. guajava* (F=6.78, P=0.0026). Examination of the eggs in punctures proved that *C.capitata* could not oviposit in *P. granatum* var Baladi but oviposited in Wonderful fruits (F=50.62, P<0.0001) and assured that, significant difference as compared to egg oviposited in *P. guajava* (F=5.16, P=0.0048). *B.zonata* gravid females were able to oviposit in all tested fruits reflecting significant differences between numbers of eggs in *P. granatum* L. both varieties Baladi and Wonderful fruits punctures (F=3.16, P=0,0026) and between Wonderful and *P.guajava* fruits punctures (F=22.05, P<0.0001).

The choice test

Ceratitis capitata, females punctured *P. granatum* L. var. Wonderful more than Baladi fruits (F=13.98, P=0.0002) and more than *P. guajava* fruits (F=6.01, P=0.0127). Puncture response of *P. granatum* L. tested varieties to *B. zonata* females cleared a high significance (F=16.45, P<0.0001) confirming the susceptibility of the Wonderful variety fruits to their infestation even more *P. guajava* fruits (F=8.63, P=0.0010). As in the non-choice test, *C. capitata* females were not able to oviposit eggs in *P.granatum* L. var. Baladi fruits punctures but oviposited in var. Wonderful fruits while *B.zonata* females were able to oviposit eggs in all puncture in both tested varieties fruits. The number of eggs oviposited by *C.capitata* females in *P.granatum* var Wonderful showed non-significant difference in both non-choice and choice tests (F=1.27, P=0.3046). In addition, there was a significant difference between the number of eggs oviposited by *C.capitata* females in *P.guajava* fruits either in the non-choice or the choice tests (F=10.61, P=0.0004). There were slight significances between number of eggs oviposited by *B.zonata* in *P.granatum* var Baladi (F=4.52, P=0.0111), var Wonderful (F= 4.81, P=0.0086) and significance in *P.guajava* fruits (F= 9.88, P=0.0006) in both non-choice and choice tests. It is obvious that *P.granatum* var. Baladi is resistant to *C.capitata* and *B.zonata* while var. Wonderful was susceptible for both of them. *B.zonata* revealed a greater ability to infest *P.granatum* var Wonderful than *C.capitata*. Infestation of fruit flies varied among horticultural fruits depending on fruiting phenology. Fruit flies use a variety of host cues when searching for oviposition sites that may explain the ability of *B.zonata* females to oviposit in *P.granatum* L. var

Baladi while *C.capitata* females were not able to attack the fruits. Negm *et al*., (2018) studied the *C.capitata* and *B.zonata* infestation of *P.granatum* L. varieties in Assiut and Fayom governorates and found that Manfaloti and wonderful were susceptible to *C.capitata* and *B.zonata* infestation but var. Baladi was susceptible to *B.zonata* females only. Resistance or susceptibility of *P.granatum* L. fruits varieties to *C.capitata* and *B.zonata* infestation may depend on different interfering factors including climate, soil type, fertilizing programs and irrigation.

Host preference

Data obtained revealed that *P.granatum* L. var. Baladi fruits were not a preferred host for both tested fruit flies females as *C.capitata* could not oviposit in the fruits and *B.zonata* eggs did not hatch and produced no pupae (Table 2). *P.granatum* L. var. Wonderful showed susceptibility to both *C.capitata* and *B.zonata* infestation and proved that it is a preferred host for them. *C.capitata* females showed a significant difference between numbers of punctures in *P.granatum* L. tested varieties clearing preference to Wonderful variety fruits (F=10.98, P=0.0004) and revealed non-significant difference when compared to *P.guajava* fruits (F=1.63, P=0.1778). Negm *et al*., (2018) exposed wounded *P.granatum* L. var. Manfaloti and var. Wonderful fruits to *C.capitata* and *B.zonata* females and the exposed fruits produced pupae of both insects. Host selection by tephritids is determined for several factors including the chemical properties of the fruit (Papachristos and Papadopoulos, 2009). Degree of pH of the larval rearing medium (fruit pulp) plays an important role for tephritids in host selection. The suitable pH for *C.capitata* and *B.zonata* larval rearing ranges from 3-5 (Dias *et al.,* 2019). The degree of pH of *P.granatum* L. fruit pulp arils of var. Wonderful ranges from 3.28-3.62 while *P.guajava* from 4.07-4.65 that give an indication of these fruits are susceptible to *C.capitata* and *B.zonata* infestation and suitable as rearing hosts producing a new flies generation.

A similar pattern was showed by *B.zonata* gravid females, that reflected higher significance in number of punctures in both *P.granatum* varieties (F=14.51, P<0.0001) and a significant difference between Wonderful variety and *P.guajava* preferring Wonderful variety fruits (F=5.01, P=0.0074). *P.granatum* var. Wonderful fruits infested with *C.capitata* and *B.zonata* females produced more pupae than infested *P.guajava* fruits (F=14.62, P<0.0001) and (F=12.13, P=0.0003), respectively. *B.zonata* females infested *P.granatum* var. Wonderful and *P.guajava* fruits surpassed *C.capitata* and produced pupae more pupae by approximately 1.69 and 1.62 folds and reflected significance (F= 12.17, P=0.0003) and (F=11.72, P=0.0003), respectively. Fruit flies use a variety of host cues as size, shape, color and chemical structure when foraging for oviposition sites. Prokopy *et al*., (1984) suggested that fruit size play an important role in female ovipositional response independent of the taxonomic status. Fetoh and Soliman (2007) studied *B.zonata* ovipositional activity in different horticultural fruits and declared that size and color (red and yellow) act as factors in host preference.

Table 2. Host preference of *C.capitata* and *B.zonata* to *P.granatum* var Baladi and Wonderful and *P.guajava* fruits

P.granatum Baladi	*P.granatum* Wonderful	*P.guajava*
	C.capitata	
	Mean number of punctures/fruit ±SE	
0.02±0.50	5.81±0.44	5.01±0.32
	F=74.48, P<0.0001	
	Mean number of produced pupae/fruit ±SE	
0.00±0.00	45.43±2.33	21.89±1.75
	F=190.21, P<0.0001	
	B.zonata	
	Mean number of punctures/fruit ±SE	
0.83±0.44	7.03±0.32	4.87±0.37
	F=60.51, P<0.0001	
	Mean number of produced pupae/fruit ±SE	
0.00±0.00	76.69±3.79	34.73±1.91
	F=229.10, P<0.0001	

Significance level 95% (P<0.05)

Biological parameters of *C.capitata* and *B.zonata* reared on *P.granatum* and *P.guajava*

The biological parameters of *C.capitata* and *B.zonata* in *P.granatum* var. (Baladi and Wonderful) and *P.guajava* fruits were represented in Table (3).

Table 3. Biological parameters of *C.capitata* and *B.zonata* reared on *P.granatum* and *P.guajava* fruits

Tested fruits	pH range	Mean% of eggs hatchability ±SE	Egg-larval duration in days±SE	Pupal duration in days±SE	%of unhatched eggs±SE	% of undeveloped larvae±SE	%of flies emergence ±SE	Sex ratio ♀	Sex ratio ♂
					C.capitata				
P.granatum var Baladi			0.00±0.00	0.00±0.00	100.00±0.00	100.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
P.granatum var wonderful	3.28-3.62	97.13±0.49	12.03±0.32	9.63±0.40	9.26±0.73	8.55±0.44	97.86±2.81	48.87±1.72	51.23±1.25
G.psidium	4.07-4.65		9.63±0.25	9.23±0.22	2.85±0.36	6.13±0.78	94.77±1.61	58.40±1.44	41.60±0.51
	Significance level 95% P<0.05		F=9.88, P=0.0006	F=0.784, P=0.4766	F=13.50, P=0.0002	F=9.00, P=0.0008	F=27.71, P<0.0001	F=38.34, P<0.0001	F=18.20, P<0.0001
					B.zonata				
P.granatum var Baladi			0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
P.granatum var wonderful		98.04±0.31	11.48±0.45	9.21±0.20	4.94±0.71	6.42±0.58	97.21±2.58	47.56±1.53	52.44±2.73
G.psidium			9.63±0.25	9.20±0.20	5.94±0.51	5.45±0.27	94.36±1.61	52.73±0.92	46.11±0.74
	Significance level 95% P<0.05		F=4.81, P=0.0086	F=1.00, P=1.00	F=1.92, P=0.5421	F=8.50, P=0.0618	F=9.40, P=0.4054	F=8.92, P=0.0009	F=7.32, P=0.0019

In *P.granatum* var. Wonderful infested fruits, *C.capitata* spent longer egg-larval duration more than *B.zonata* and varied insignificantly (F=2.45, P=0.0705). Also, their pupal durations of the produced pupae varied insignificantly (F=1.63, P=0.1778). *C.capitata* showed more unhatched oviposited eggs in *P.granatum* var.

Wonderful fruits more than *B.zonata* reflecting a significant difference (F=21.05, P<0.0001) while the undeveloped larvae were not significant (F=2.44, P=0.0705). The sex ratio of *C.capitata* and *B.zonata* produced flies from *P.granatum* infested fruits showed that produced emerged females were lesser than males but varied insignificantly (F=1.76, P=0.1671) and (F=2.26, P=0.0865), respectively. In *P.guajava*, the egg-larval and pupal durations of *C.capitata* and *B.zonata* were not significantly different (F=2.44, P=0.701) and (F=0.00 and P=1.00), respectively. The unhatched eggs of *C.capitata* were lesser than *B.zonata* in *P.guajava* infested fruits and varied insignificantly (F=1.37, P=0.2420) and the undeveloped larvae as well (F=1.61, P=0.1761). The sex ratio of the produced *C.capitata* and *B.zonata* females and males resulted in a significant difference (F=5.93, P=0.0041) and (F=5.22, P=0.0064). Dias *et al.,* (2019) stated that the lower the pH degree of the larval rearing medium the longer the egg hatch and larval durations.

Chemical analysis of *P. granatum*, var Baladi and Wonderful peels

The phytochemicals that are present in the fruit peel layers play an important role as a factor against herbivores attack (Whitehead and Bowers, 2013b). The chemical analysis of *P. granatum* peel layers Flavedo and albedo in Baladi and Wonderful varieties showed that total phenols, total flavonoids and total tannins were present in different amounts (Table 4). The chemical analysis of *P. granatum* var. Baladi flavedo with albedo layers showed that total phenols were (146.25 mg/g) more by 2.66 times than the amounts of the same layers in var. Wonderful (55 mg/g) (F=375.00, P<0.0001). The total flavonoids in Baladi Flavedo and albedo layers were (84.33 mg/g) increased by 1.14 times than in Wonderful (74.91mg/g) (F=29.01, P=0.0012) and the total tannins were (386.80 mg/g) increased by 3.65 times more than in Wonderful (112 mg/g) (F=4124.23, P<0.0001). The chemical analysis of the flavedo layer of var. Baladi reflected that total phenols amount (120.37 mg/g) increased by 2.67 times more than in Wonderful (45.01 mg/g) (F=123.37, P<0.0001), total flavonoids (98.35mg/g) increased by 1.24 times than in var. Wonderful (79.13 mg/g) (F=58.50, P=0.0003) and total tannins (347.40 mg/g) increased by 3.62 times more than the flavedo layer in var. Wonderful (96.30mg/g) (F=290.15, P<0.0001).

Table 4. *P.granatum* var Baladi and Wonderful peel total phenols, flavonoids and tannins

Fruit peel portions	*P.granatum* varieties					
	Baladi			Wonderful		
	Total phenols	Total flavonoids	Total tannins	Total phenols	Total flavonoids	Total tannins
	mg/g dry peel powder weight					
Flavedo+Albedo	146.25±1.11	84.33±0.88	386.80±0.93	55.00±0.69	74.91±077	112.00±0.57
Flavedo only	120.37±0.60	98.35±0.91	347.40±0.67	45.01±0.51	79.13±0.71	96.30±0.38
Flavedo+Albedo of infected fruits	67.60±0.61	48.77±0.33	314.40±0.0.63	27.66±0.13	64.21±0.53	68.09±0.67

Fungal diseases infection that may start during the fruit set stage without a physical visible appearance had worked on decreasing all phytochemical constituents in flavedo and albedo layers of both Baladi and Wonderful pomegranate varieties. The total phenols in Baladi flavedo and albedo layers (67.60 mg/g) of infected fruits decreased significantly by 53.87% than in uninfected fruits (F=206.97, P<0.0001). The same happened to total flavonoids (48.77 mg/g) in the fungal infected Baladi fruits that decreased by 42.83% than the uninfected fruits (F=41.28, P=0.0006) and tannins (314.40 mg/g) decreased by18.84% (F=121.74, P<0.0001). The Wonderful variety fungal infected fruits showed amounts of total phenols (27.66 mg/g) decreased by 49.71% (F=48.57, P=0.0004), the total flavonoids (64.21 mg/g) decreased by 14.33% (F=16.20, P=0.0039) and the total tannins (68.09 mg/g) decreased by 39.21% (F=67.19, P=0.0002) less than the uninfected fruits.

From the results of the present study, it is dedicated that *P. granatum* L. peel phytochemical polyphenolic compounds play an important role in resistance or susceptibility of fruits to *C.capitata* and *B.zonata* infestation. Both flavedo and albedo rind layers are rich in polyphenolic compounds. These findings coincide with those of Hamid *et al*., (2020) who analyzed some pomegranate fruit peels and found most of the polyphenolic compounds in the flavedo rind layer. Studies reporting the effect of polyphenolics in *P. granatum* L. peels on *C.capitata* and *B.zonata* developmental stages are rare. Haldhar *et al*., (2018) studied the resistant and susceptible varieties of snapmelon to melon fly, *Bactrocera cucurbitae* infestation and found that the rich polyphenolic compounds are a feature of the resistant varieties. Sharma and Sohal (2013) studied the effect of polyphenols on melon fly, *Bactrocera cucurbitae* eggs hatchability and found that it inhibited the enzyme peroxidase that is a functional and structural component of the chorion involved in the hardening process. In the present study, the unhatched *B.zonata* eggs observed in *P.granatum* var Baladi fruits could be due to toxic effect of polyphenols especially tannins in the protein synthesis or crosslinking of proteins. In support of this point, Ageu *et al*., (2018) estimated the effect of polyphenols and tannins on egg hatching of *Aedes aegypti* eggs and proved an embryotoxic activity in relation to hatching as well as repellent action in oviposition activity. The chemical analysis of *P.granatum* L. var Baladi peels cleared that it contained a high concentration of tannins that may explain the reluctance of *C. capitata* females to oviposit eggs in the fruits. Molan *et al*., (2002) showed that tannins inhibited the development of eggs at higher concentrations and exhibited high toxicity to *Trichostrongylus colubriformis* larvae in concentrations from 200 to 500µg/mL. These findings indicated that tannins have an inhibitory effect even in higher organisms in the evolutionary scale. In addition, it may give a hint about the unhatched oviposited eggs of *B. zonata* in the same fruit variety. On contrary, Yadav (1997) reported the presence of tannins in low concentrations seems to provide chemical and nutritional conditions suitable for larval development. The chemical analysis of *P.granatum* L. var. Wonderful peels proved that total tannins are present in lower concentrations than var. Baladi and this may uncover the reason behind the susceptibility of this variety fruits as a host for both *C. capitata* and *B. zonata*. In conclusion, resistance or susceptibility of *P.granatum* L. varieties to *C.capitata* and

B.zonata seems to be dependent on the amounts of polyphenolics that are present in the fruit peels. These findings need more research work to understand how to support the fruit varieties with low polyphenolics amounts in the aim to protect them against fruit flies infestation.

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دراسة على مقاومة وقابلية صنفان من الرمان للإصابة بحشرتى ذبابة فاكهة البحر المتوسط *Ceratitis capitata* وذبابة ثمار الخوخ *Bactrocera zonata*

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تم دراسة مقاومة أو قابلية ثمار الرمان من صنف "البلدى" وصنف "واندرفول " للإصابة بحشرتى ذبابة فاكهة البحر المتوسط *Ceratitis capitata* وذبابة ثمار الخوخ *Bactrocera zonata* في إختبار الإختيارية و غير الإختيارية؛ و قد أثبتت الدراسة أن ثمار الرمان من صنف البلدى مقاومة للإصابة بكل من الحشرتين سواء في إختبار الإختيارية و غير الإختيارية. كما أوضحت تفضيل الحشرتين لإصابة ثمار الرمان من صنف "واندرفول" مقارنة بثمار الجوافة. وقد بينت الدراسة أن الفترة المستغرقة لإتمام العمر من البيضة والأعمار اليرقية للحشرتين في ثمار الرمان صنف "واندرفول" كانت أطول من نظيراتها في ثمار الجوافة؛ أما عن الفترة المستغرقة لإتمام عمر العذراء فلم تسفر عن فروق معنوية للحشرتين المنتجتين من ثمار الرمان صنف "واندرفول" والجوافة. وبدراسة النسبة الجنسية للأفراد المنتجة من الثمار المصابة، فقد كانت نسبة الذكور أكثر من نسبة الإناث للحشرتين المنتجتين من ثمار الرمان "واندرفول" أما ثمار الجوافة فقد أسفرت عن نسبة أعلى للإناث. أثبت التحليل الكيميائى لقشور ثمار صنفى الرمان محل الدراسة تباينا وفروقا معنوية في كميات مركبات البولي فينول مع تقدم الثمار من صنف البلدى في محتوى مركبات "الفينول" الكلية و "الفلافونويد" الكلية و "التانين" الكلية عن أمثالها في صنف الواندرفول؛ كما كان للإصابة بالأمراض الفطرية تأثيرا بالنقص على كميات تلك المركبات في قشور ثمار الرمان المختبرة من الصنفين.