



**Leaf morphological characters can be a factor for intra-varietal preference of whitefly  
*Bemisia tabaci* (Hemiptera: Aleyrodidae) among certain cantaloupe cultivars**

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**Abstract:**

Cantaloupe, *Cucumis melo* L. (Family: Cucurbitaceae) is commercially one of the most importantly tasty, nutritional summer vegetable crops cultivated in Egypt and many countries worldwide. The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most serious pests attacking cantaloupe, causing direct and direct damage and consequently a significant reduction in plant production. To search for alternative methods of chemical control, the relative susceptibility of four tested cantaloupe cultivars; Arava, Majus, Darvina, and Royal 481 was assessed in field trials over 2015, 2016, and 2017 summer plantations. Arava and Majus hosted the lowest *B. tabaci* eggs and nymphs. Darvina was the most susceptible cultivar, especially to the nymph population. Royal 481 hosted the highest deposited eggs number. The morphological cultivars traits by scanning electron microscope clarified the rejection or attraction features to *B. tabaci* found in leaves over cantaloupe growth. The lowest infested cultivar "Majus" had high trichomes density. Royal 481 had the lowest density and longest trichomes, which facilitate adults landing to lay eggs and feed. Long trichomes can act as shelters for *B. tabaci* immature stages.

**Introduction**

Cantaloupe, *Cucumis melo* L. (Family: Cucurbitaceae) is commercially one of the most importantly tasty, nutritional, and exportable summer cucurbitaceous vegetable crops. It is adapted to be cultivated to several types of soils (FAO, 2006) in tropical and temperate climates on all continents in dry and irrigated conditions, directly seeded or transplanted in the open field or under plastic tunnels (Dogimont and Boissot, 2016). In Egypt, the cultivated areas of cantaloupe in old and new land reached about 42,037 feddans and produces about 475,817 tons of fruits in summer

plantations (Anonymous, 2015). Cantaloupe contains vitamins viz., vitamin A, and different groups of vitamin B such as Thiamin (Vit. B<sub>1</sub>), Riboflavin (Vit. B<sub>2</sub>), Niacin (Vit. B<sub>3</sub>), and Ascorbic acid (Watt and Merrill, 1963). Tomato and cotton whitefly; *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) found to be one of the most serious pests attacking cantaloupe (Metwally *et al.*, 2013).

*B. tabaci* is a major pest invading the world, especially in tropical and subtropical regions (Toscano *et al.*, 1994 and Denholm *et al.*, 1996). The worldwide spread of *B. tabaci* continues to cause severe crop

losses on many crops (tomato, cucurbits, beans, cotton, potatoes, and sweet potatoes). It has an extensive host range covering 118 species of plants in 79 genera belonging to 28 families in Egypt (Abd-Rabou and Simmons, 2010). *B. tabaci* has become the most important pest for melon crops in several countries, causing losses of millions of dollars/years (Perring *et al.*, 1993; Polston and Anderson, 1999 and Azevedo and Bleicher, 2003). Nymphs and adults cause direct damage by removing phloem nutrients and inoculating salivary toxic enzymes, which weakens plants and reduces crop yield and quality (Inbar and Gerling, 2008). The indirect damage arises from sooty mold fungi proliferation on whitefly excrements of honey-dew deposited on leaves and impairing respiration and photosynthesis and development of disorders including silver leaf and irregular ripening of fruit (Oliveira *et al.*, 2001 and Byrne *et al.*, 2003).

*B. tabaci* transmits more than 100 virus species the majority of which belong to Begomovirus genus such as the tomato yellow leaf curl virus (Jones, 2003). Insecticides can affect pollinators which are essential to the successful production of cantaloupe crops. The development of new control tactics and viable alternatives to conventional pesticides for population management is crucial (Attia *et al.*, 2012). Whitefly control using resistant cultivars may be considered an ideal option in integrated pest management programs (Alves *et al.*, 2005). So, selecting resistant cultivars to be included in the control program is a must from the morphological point of view. The highest density of trichome was found in the resistant cultivar (Baldin *et al.*, 2012).

This work aimed at selecting low-infested cultivars with high production to be involved in the IPM

program. Moreover, recommendation their morphological characters in the breeding program.

## **Materials and methods**

### **1. Field tests:**

All field trials were carried out in the Experimental Farm of Plant Protection Research Institute at Qaha region, southeast of Qalyubiya Governorate (30°17'00 "N, 31° 12'00 "E of 133 meters (436 ft) below sea level) in Egypt over three successive growing seasons; 2015, 2016 and 2017 plantation summer. The four tested cantaloupe cultivars i.e., Arava, Majus, Darvina and Royal 481 were assessed. Cantaloupe seeds were obtained from Horticultural Research Institute Agricultural Research Center (ARC), Ministry of Agriculture and land Reclamation.

### **2. Experimental design and cultivation method:**

An area of about 350 m<sup>2</sup> was used to establish the field experiments. It was divided to three plots. Each cultivar is represented by three plots (Replicates) running in 9 rows laying out using statistical design in a complete randomized block (CRBD). Each plot (replicate) measured 11.9 m<sup>2</sup> represented by three rows. seeds were directly sown in the field on Mar.16 in successive single rows on the southern edges. Rows are designed as 7 plants per row spaced 0.30 m apart. All the recommended agronomic practices for cantaloupe cultivation including irrigation, hoeing land, pruning, recommended fertilization, and harvesting adopted keeping the whole experimental plot area free from employing any insecticidal, fungicidal, and herbicidal plant protection measures during the whole period of study in summer (starting on Mar. until Jul.). The natural infestation of whiteflies was evaluated in the experiments.

### **3. Whitefly sampling procedures:**

During the three vegetative growing seasons (2015, 2016, and 2017) assessments were conducted 15 days following plant settlement in the field and continued weekly. Concerning the susceptibility of different cultivars to *B. tabaci* infestation, one hundred and twenty leaves (10 leaves \* 3 plots (Replicates) \* 4 cultivars) for 15 weeks were randomly picked up from the three plant levels (upper, middle, and lower) in the morning before noon.

All leaf samples of each experiment were kept separately in polyethylene bags to be transferred to the laboratory. The presence of whitefly *B. tabaci* eggs and nymphs was examined with the aid of a binocular stereomicroscope. The number of eggs and nymphs was assessed on the abaxial leaf surface. Then, the number of eggs and nymphs per the three replicates in each experiment was estimated.

#### 4. Laboratory tests:

##### Morphological aspects as shown by scanning electron microscope:

Morphological aspects such as trichomes density and length were generally correlated with the variations in oviposition on the four tested cultivars.

Sampling of young leaves were successfully taken 21 days after cultivation in the vegetative growth stage. However, older leaves were taken in interfering between the flowering and development of fruit stages 54 days after cultivation in the summer plantation of 2017. Leaf samples of each cultivar were picked randomly up early in the morning from the same place of each plant and were of the same age to obtain an accurate comparison between assessments. The chosen leaves were then transferred to the icebox on the same day in order to be scanned in the Central Laboratories Sector, The Egyptian Mineral Resources Authority, The Ministry of Petroleum, Egypt.

To obtain the number and length of leaf trichomes present in 1 mm<sup>2</sup> of the abaxial leaf surface (Where whiteflies usually feed and lay eggs), leaves of each material (Cultivar) were scanned immediately using Scanning Electron Microscope Model Quanta 250 FEG (Field Emission Gun attached with EDX Unit (Energy Dispersive X-ray Analyses), with accelerating voltage 30 K.V., magnification 14x up to 1000000 and resolution for Gun.1n). Trichomes density and length were estimated using Compu Eye, according to Bakr (2005).

#### 5. Data analysis:

All the obtained data during the trials over the three growing seasons were subjected to analysis by using SAS Institute (1988) program. Duncan's multiple range test was used to obtain the mean separation and arrange cultivars according to their degree of infestation by *B. tabaci* eggs and nymphs at the level of 5 % of probability.

#### Results and discussion

##### 1. Preference of cultivars for oviposition over 2015:

Results of mean weekly numbers of *B. tabaci* eggs/leaf in the field on four different cantaloupe cultivars (i.e., Arava, Majus, Darvina, and Royal 481) in the 2015 summer season were illustrated in Fig. 1. Darvina and Royal 481 were the most preferred and *B. tabaci* adults oviposited the high number of eggs. They harbored during the whole season total mean numbers (Mean  $\pm$  SD) of 10.02  $\pm$  2.42 and 10.3  $\pm$  3.4, respectively. Arava and Majus were the least preferred cultivars and had mean counts of 5.6  $\pm$  1.54 and 4.94  $\pm$  1.5, respectively ( $P = 0.006$ ).

##### 2. Preference of cultivars for nymphal infestation over 2015:

The mean population density of *B. tabaci* nymphs on different tested cantaloupe cultivars revealed that there

were significant differences among nymphs infesting tested cultivars. The highest mean counts of *B. tabaci* nymphs were recorded on leaves of Darvina and Royal 481 ( $17.4 \pm 4.6$  and  $16.45 \pm 3.1$ , respectively), followed by

Arava and Majus cultivars ( $9.76 \pm 2.35$  and  $9.3 \pm 1.83$ , respectively) ( $P < 0.05$ ) (Figure 1).

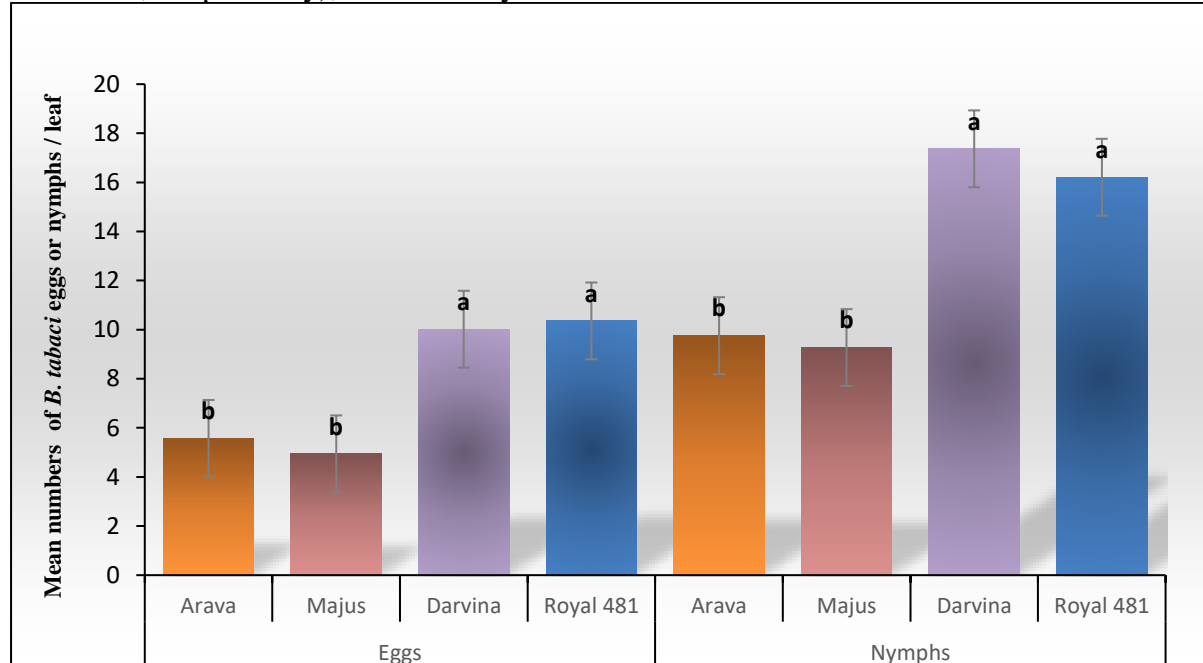


Figure (1): Mean numbers ( $\pm$ SE) of *Bemisia tabaci* eggs and nymphs per leaf on the four cantaloupe cultivars over 2015 summer plantation season. Bars topped with different letters are significantly different by Duncan’s multiple range test ( $P < 0.05$ ).

### 3. Preference of cultivars for oviposition over 2016:

The preference for oviposition by *B. tabaci* can be categorized into the following groups; the first group of higher oviposition preference is represented by Darvina and Royal 481 with seasonal mean counts of  $6.2 \pm 2.35$  and  $6.8 \pm 2.3$ , respectively. The second group of lower preference cultivars is represented by Arava ( $3.02 \pm 1.01$ ) and Majus ( $2.02 \pm 0.6$ ).

### 4. Preference of cultivars for nymphal infestation over 2016

Data illustrated in Figure (2) clearly indicated different levels of attractiveness for *B. tabaci* nymphs depending on the different cantaloupe cultivars throughout 13 weeks in 2016. Darvina had the same trend as in egg numbers. It still suffered from the severe infestation and it was significantly the most infested cultivar with mean population of  $18.3 \pm 4.3$ . Darvina significantly followed by Royal 481 ( $13.2 \pm 1.95$ ). Arava and Majus were recorded with low number of *B. tabaci* nymphs ( $6.8 \pm 1.6$  and  $6.34 \pm 1.3$ , respectively) [LSD= 4.1].

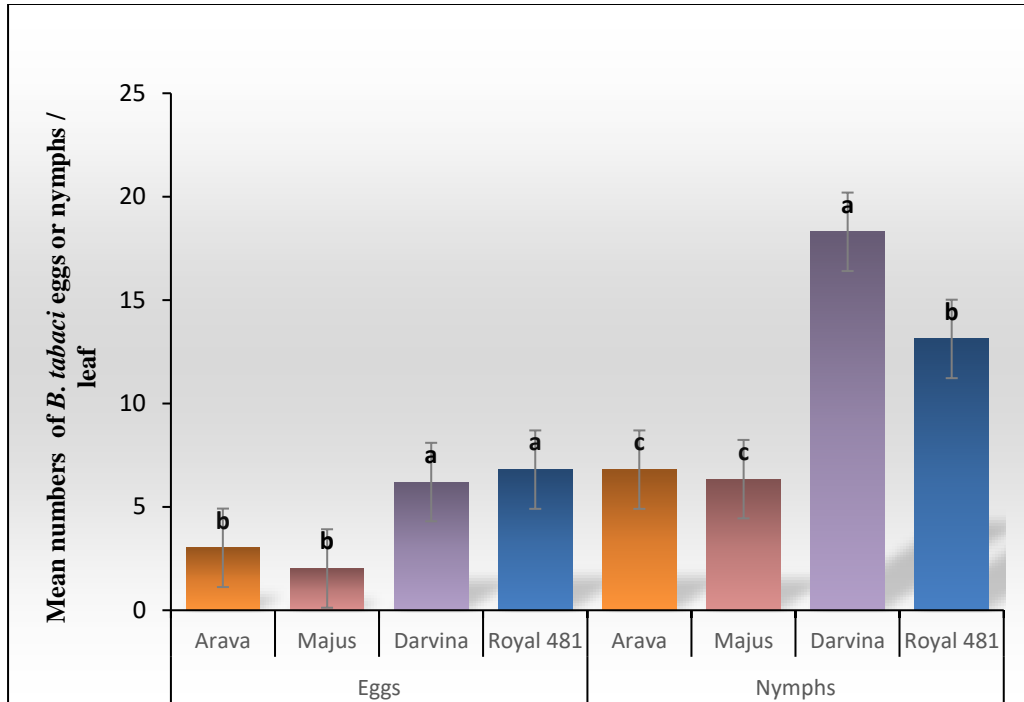


Figure (2): Mean numbers ( $\pm$ SE) of *Bemisia tabaci* eggs and nymphs per leaf in the four cultivars over 2016 summer plantation season. Bars topped with different letters are significantly different by Duncan’s multiple range test ( $P < 0.05$ ).

**5. Preference of cultivars for oviposition over 2017:**

Results illustrated in Figure (3) revealed the variations in the oviposition preference throughout the whole season of study (Apr. 16 throughout Jul. 10) over the third season of 2017. Concerning the mean of eggs laid/leaf, "Majus" cultivar was the

least oviposited by females of *B. tabaci* adult ( $3.91 \pm 1.3$ ) (Figure 3) followed by Arava cultivar ( $5.2 \pm 2.15$ ) but it didn’t differ significantly from Majus [LSD=7.1]. The highest preferred cultivars for egg oviposition were "Royal 481" ( $14.12 \pm 7.5$ ) ( $P < 0.0001$ ) and "Darvina" ( $12.45 \pm 4.4$ ).

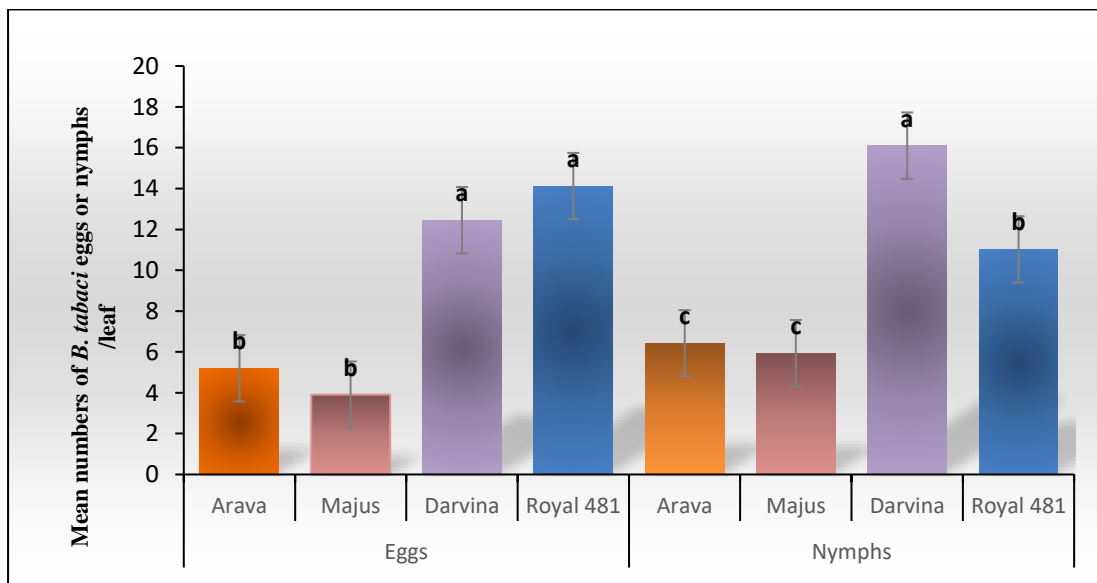


Figure (3): Mean numbers ( $\pm$ SE) of *Bemisia tabaci* eggs and nymphs per leaf in the four cantaloupe cultivars over 2017 summer plantation season. Bars topped with different letters are significantly different by Duncan’s multiple range test ( $P < 0.05$ ).

**6. Preference of cultivars for nymphal infestation over 2017:**

The overall mean of the four tested cantaloupe cultivars revealed that the highest infestation rate of *B. tabaci* nymphs was also recorded on Darvina and the mean count was  $16.1 \pm 3.01$ . On the contrary, the Royal 481 cultivar ( $11.02 \pm 2.7$ ) which had the highest mean number of eggs harbored a moderate number of nymphs. However, Arava ( $6.42 \pm 1.81$ ) or Majus cultivar ( $5.94 \pm 1.5$ ) expressed slight infestation [LSD= 3.8].

**7. Effect of leaf trichomes density and length in cantaloupe cultivars on laying *Bemisia tabaci* eggs in the early stage:**

Data in Figure (4) clearly indicated that, the mean number of cantaloupe trichomes in the early stage. Majus had the highest mean number of trichomes ( $47.7$  trichome/ $1\text{mm}^2$ ). However, Darvina and Arava had a moderate leaf trichome density ( $31.1$  and  $26.2$  trichome/ $1\text{mm}^2$ , respectively). The lowest mean number of trichomes was recorded in the highest infested cultivar Royal 481 ( $20.5$  trichome / $1\text{mm}^2$ ). As shown in Figure (4) the trichome density in cantaloupe leaves had a linear

relationship with egg numbers deposited

Concerning mean length, the longest trichomes were recorded in Royal 481 ( $377.7\ \mu\text{m}$ ), followed by the moderate length in Arava ( $272.5\ \mu\text{m}$ ) and Majus ( $209.8\ \mu\text{m}$ ). On the other hand, Darvina had the shortest trichome length ( $115.1\ \mu\text{m}$ ). Royal 481 was the most infested cultivar with eggs ( $14.2$  eggs/leaf in the early stage) and had the lowest mean number of trichomes ( $20.5$  trichome/ $1\text{mm}^2$ ) and the longest length of trichomes ( $377.7\ \mu\text{m}$ ) (Figures 4 and 5).

On the contrary, the tolerant cultivar Majus ( $6.8$  eggs/leaf) was found to have the highest mean number of trichomes ( $47.7$  trichome /  $1\text{mm}^2$ ) and shorter trichome length ( $209.8\ \mu\text{m}$ ). However, the shortest trichome length ( $115.1\ \mu\text{m}$ ) was recorded in the susceptible cultivar Darvina cultivar ( $12.9$  eggs/leaf). Statistical analysis of these data indicated a polynomial relationship concerning the length of trichomes (Figure 4). Figures (6-9) show scanning electron micrographs of cantaloupe leaf showing trichomes density and length in the four cultivars under consideration during the early stage.

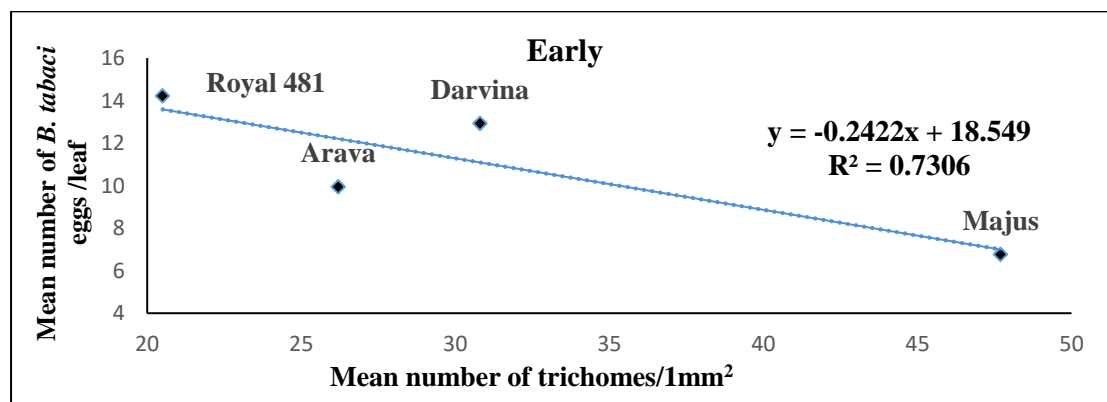


Figure (4): Correlation between mean number of trichomes/ $1\text{mm}^2$  and eggs on Arava, Majus, Darvina and Royal 481 cultivars in the early stage.

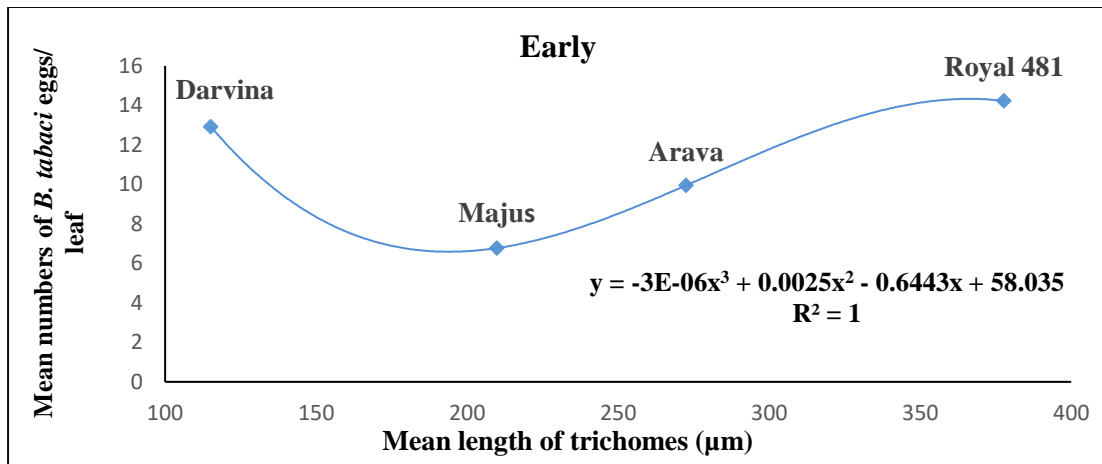


Figure (5): Correlation between mean trichomes length (µm) and eggs on Arava, Majus, Darvina and Royal 481 cultivars in the early stage.

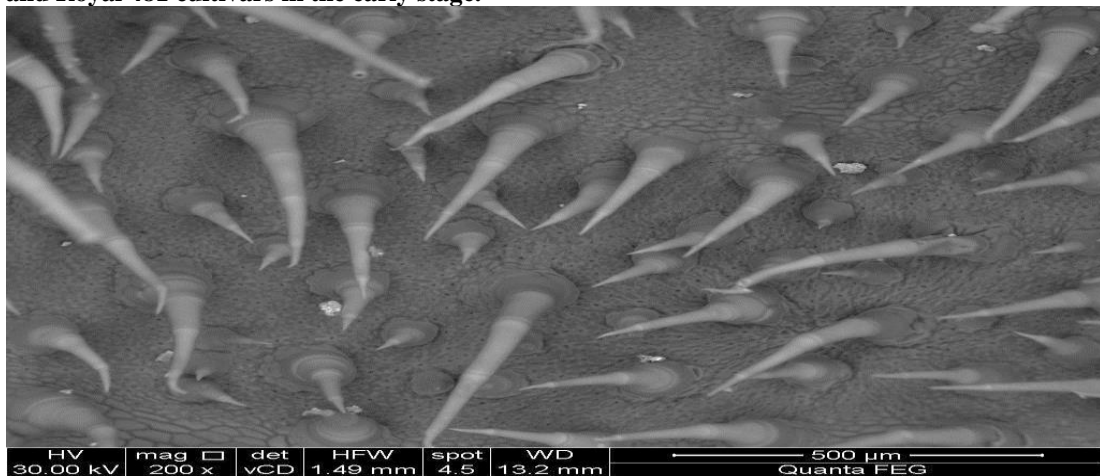


Figure (6): Scanning electron micrographs of cantaloupe leaf showing trichomes density and length in Arava cultivar during the early stage.

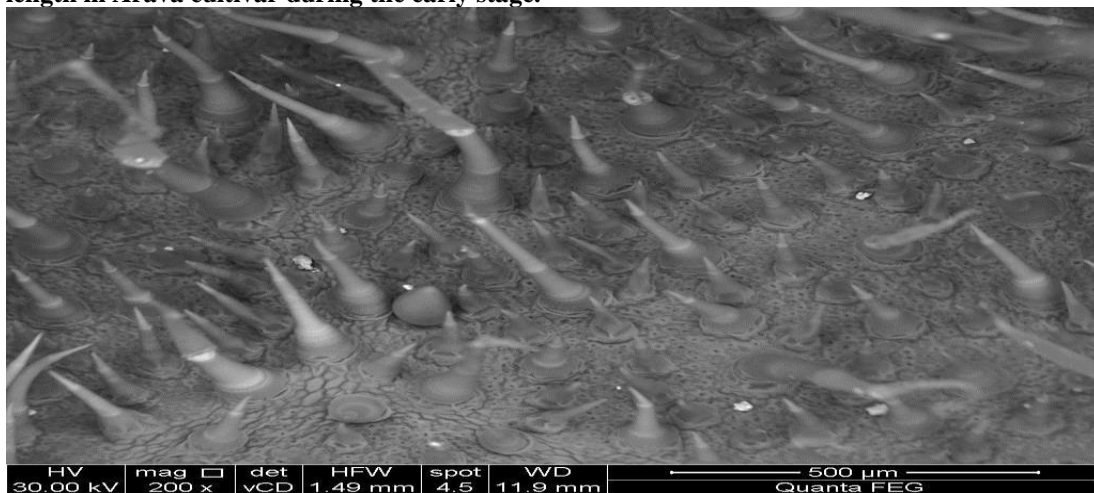
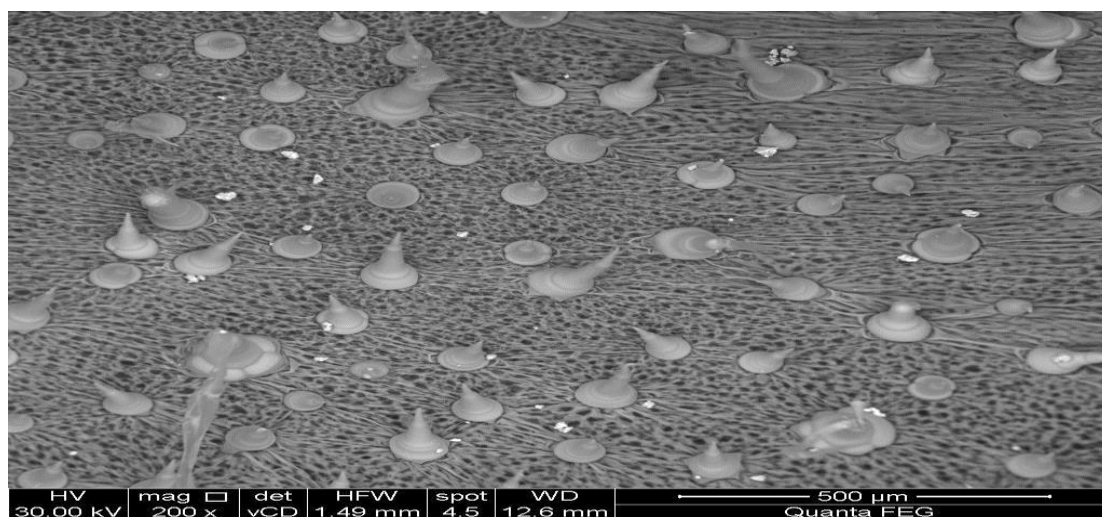


Figure (7): Scanning electron micrographs of cantaloupe leaf showing trichomes density and length in Majus cultivar during the early stage.



**Figure (8):** Scanning electron micrographs of cantaloupe leaf showing trichomes density and length in Darvina cultivar during the early stage.



**Figure (9):** Scanning electron micrographs of cantaloupe leaf showing trichomes density and length in Royal 481 cultivar during the early stage.

**8. Effect of leaf trichomes density and length in cantaloupe cultivars on laying *Bemisia tabaci* eggs in the late stage:**

The highest mean number of leaf trichomes in the older leaves is still recorded in Majus (79.5 trichome/1mm<sup>2</sup>), followed by the moderate mean which was represented by Royal 481 and Darvina (43.6 and 38.5 trichome/1mm<sup>2</sup>, respectively). The lowest mean number of leaf trichomes was recorded in Arava (27.7 trichome/1mm<sup>2</sup>).

As shown in Figure (10) trichomes density in the late stage had polynomial relation with eggs number. Regarding the mean length of trichomes, the longest trichome was found in Majus cultivar (266

μm) followed by Royal 481 (227 μm). Darvina had short trichomes (173.1 μm). However, the shortest leaf trichome in this stage was recorded in Arava (164.3 μm).

Data in Figure (11) revealed that the trichome length had polynomial relation with egg numbers in this stage. This means *B. tabaci* eggs didn't increase or decrease according to change in trichome length. One can conclude that the lowest mean number of eggs in the late stage was recorded in Majus leaves (3.7 eggs/leaf) corresponding to the highest mean of trichomes density and length.

On the contrary, the susceptible cultivars, "Darvina and Royal 481" showed a moderate mean number of trichomes.



Figures (12-15) show scanning electron micrographs of cantaloupe leaf showing trichomes density and length in

the four cultivars under consideration during the late stage.

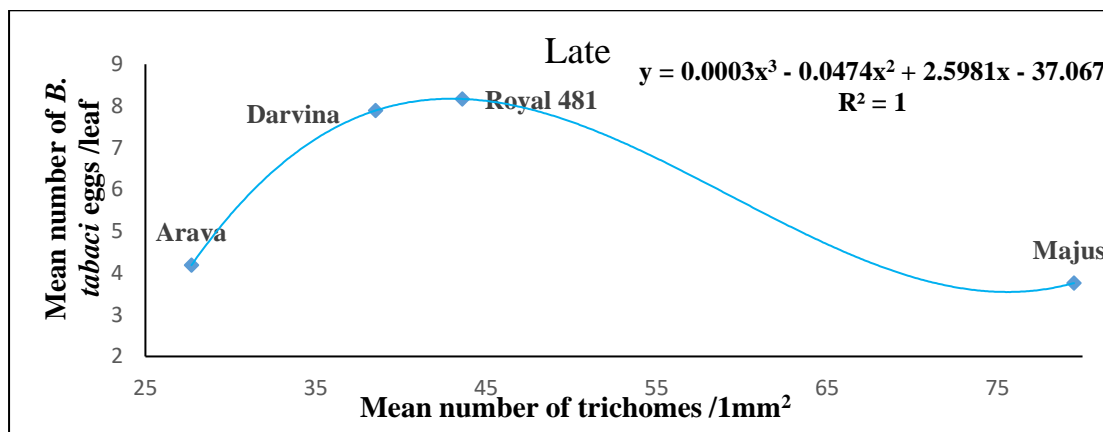


Figure (10): Correlation between mean number of trichomes/1 mm<sup>2</sup> and mean number of eggs on Arava, Majus, Darvina and Royal 481 cultivars in the late stage.

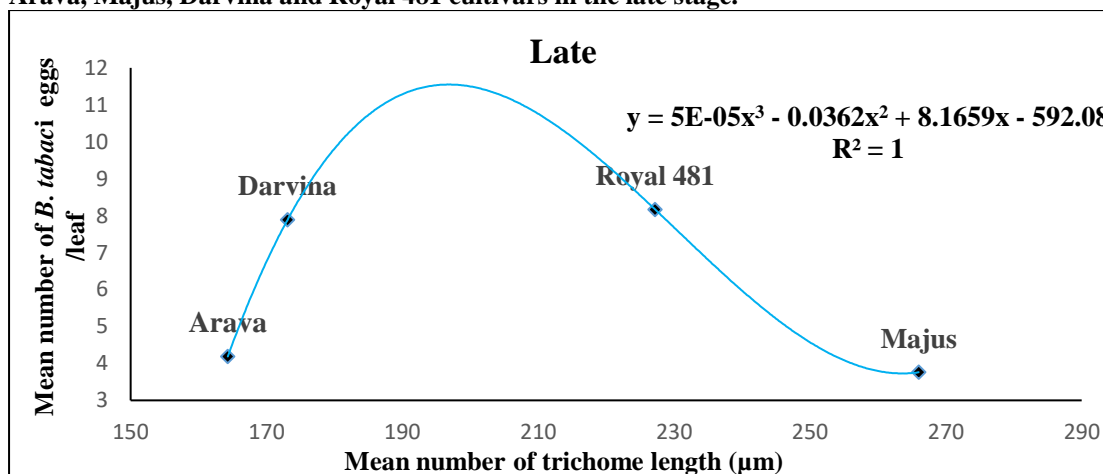


Figure (11): Correlation between mean of trichomes length (µm) and mean number of eggs on Arava, Majus, Darvina and Royal 481 cultivars in the late stage.

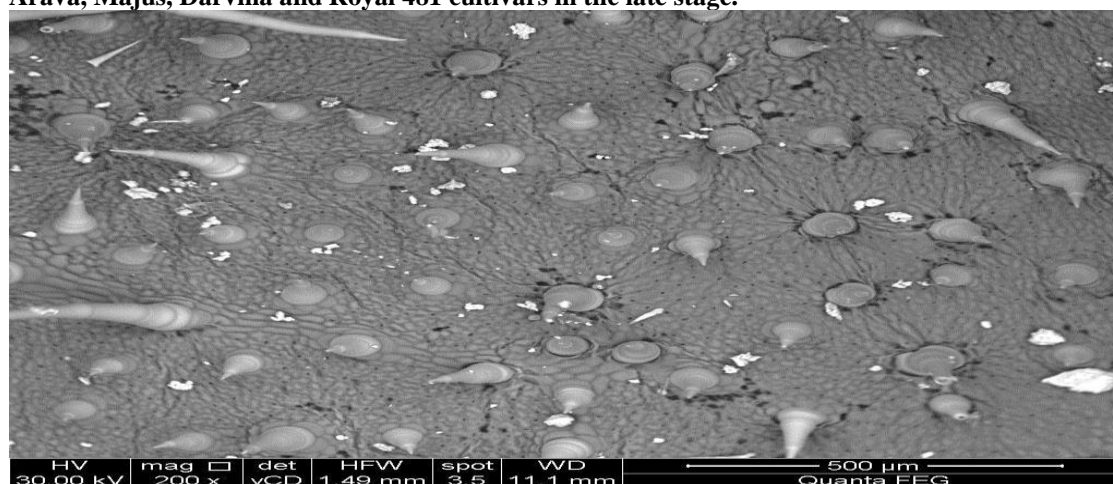


Figure (12): Scanning electron micrographs of cantaloupe leaf showing trichomes density and length in Arava cultivar during the late stage.

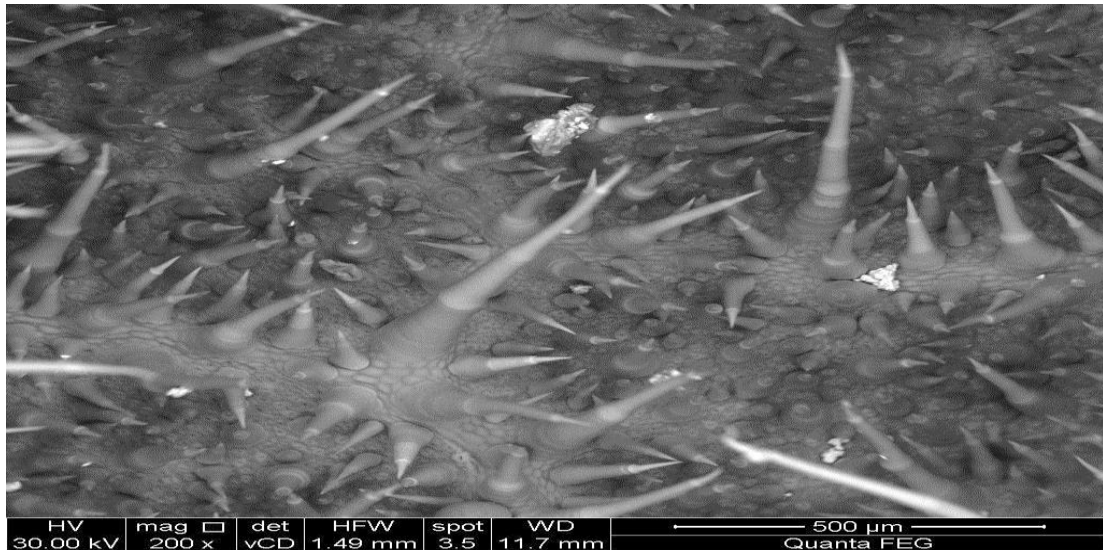


Figure (13): Scanning electron micrographs of cantaloupe leaf showing trichomes density and length in Majus cultivar during the late stage.

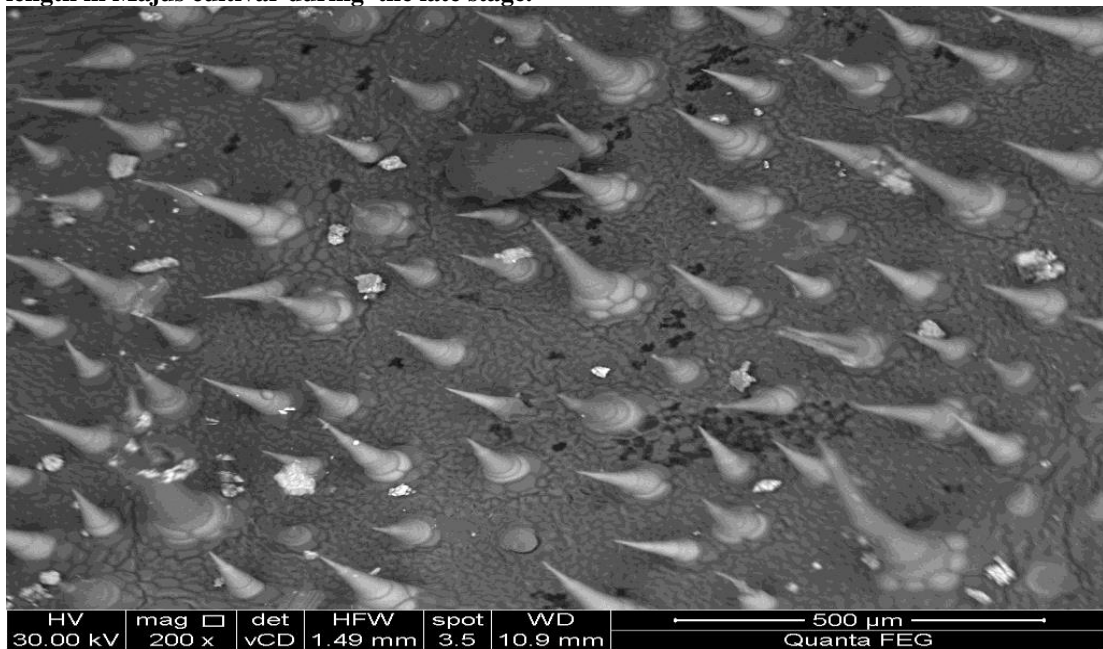


Figure (14): Scanning electron micrographs of cantaloupe leaf showing trichome density and length in Darvina cultivar during the late stage.

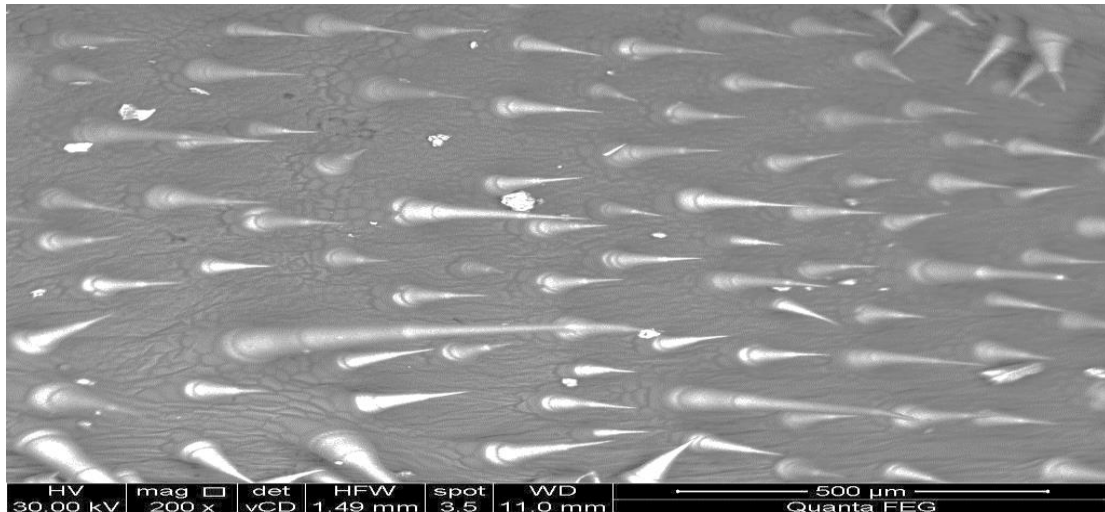


Figure (15): Scanning electron micrographs of cantaloupe leaf showing trichome density and length in Royal 481 cultivar during the late stage.

### 9. Oviposition preference in relation to trichomes in cantaloupe early and late stages:

The data in Figure (16) demonstrated that the preference of *B. tabaci* adults to lay eggs decreased as cantaloupes grew (10.97 and 6 eggs/leaf, respectively). However, the mean number of leaf trichomes increased with the growing cantaloupe (31.4 and 37.3 trichome/1mm<sup>2</sup>, respectively) and this may explain decreasing laying eggs by *B. tabaci*

adults in the late stage than the early one because of the high numbers of leaf trichomes which might prevent adults to lay its eggs in an easy way.

On the other hand, the mean length of leaf trichomes hadn't significantly affected by plant ageing (243.8 and 207.7 μm, respectively). In other words, trichome length became shorter in the older leaves compared to the younger ones but this decrease didn't be significant (Figure 17).

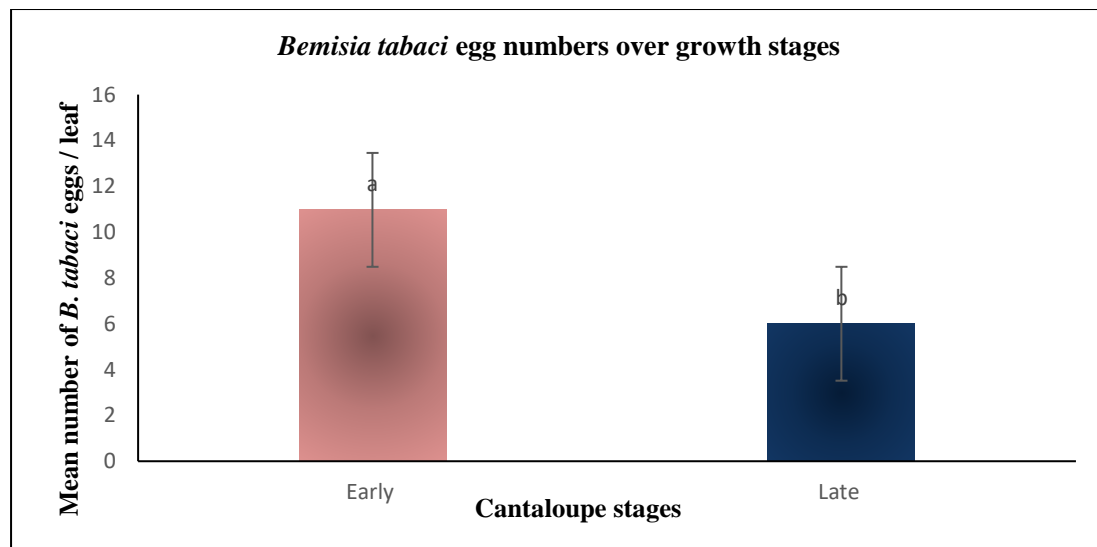
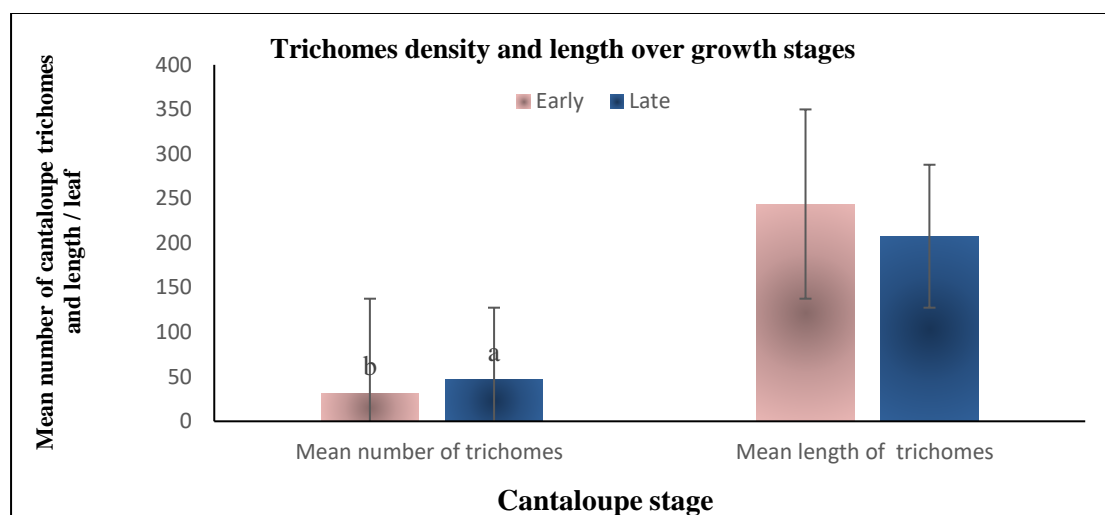


Figure (16): Difference between mean number of eggs in cantaloupe early and late stages. Bars topped with different letters are significantly different by Duncan's multiple range test ( $P < 0.05$ ).



**Figure (17): Difference between trichomes density and length in early and late stages. Bars topped with different letters are significantly different by Duncan's multiple range test ( $P < 0.05$ ).**

The obtained results revealed that, the four tested cantaloupe cultivars varied in their susceptibility to *B. tabaci* eggs and nymphs. Regarding, the mean number of eggs laid/leaf in the cantaloupe cultivars, the eggs were recorded in the highest significant numbers in 2015, 2016, and 2017 in leaves of Darvina and Royal 481 cultivars. Antibiosis symptoms, expressed as the failure of some whitefly eggs to hatch to nymphs (Less number of eggs) was on Royal 481 leaves. The data obtained over the years 2016 and 2017, that, Royal 481 was more attractive to *B. tabaci* to lay eggs in high numbers but the population of nymphs was low on the leaves of this cultivar as the plant grows. This may indicate less egg hatching. This result was in agreement with the results of Coelho *et al.* (2009) who investigated the resistance of melon cultivars to *B. tabaci* biotype B. The authors suggested that the antibiosis was the mechanism of resistance. Vieira *et al.* (2011) observed the high number of eggs on two soybean genotypes and a reduced number of nymphs, indicating antibiosis. Silva *et al.* (2019) depicted resistance expression in some commercial common bean cultivars to the whitefly. The resistance was

expressed as low egg deposition on certain cultivars and others showed lower adult infestation.

On the other hand, Arava and Majus showed a low preference for *B. tabaci* in this investigation, this could be attributed to antibiosis or antixenosis, or other physiological traits. Baldin *et al.* (2012) recorded that, the four cultivars Vereda, 'Amarelo Ouro' and 'AF646' were least infested with whitefly. They suggested that regardless of the chemical components or morphological characters the non preference of melon cultivar to *B. tabaci* infestation is good management that prevents oviposition, feeding, physiological disorders, and virus transmission. Royal 481 cultivar, which showed high infestation with *B. tabaci* eggs, had a high yield which didn't differ significantly from the resistant cultivar Majus. The susceptible cultivar Darvina had a lower yield.

Arava cultivar had lower *B. tabaci* colonialization and had the lowest weight cantaloupe yield. In a similar study, Baldin *et al.* (2009) found that the lowest deposited squash cultivar with *B. tabaci* eggs didn't give the highest weight of fruits.

Cultivation whitefly-resistant or tolerant cantaloupe cultivars is now one

of the most environmentally safe strategies in Integrated Pest Management. The search for resistant cultivars for the control of whiteflies can easily be combined with other control techniques that are environmentally friendly, such as the application of biological pesticides or biological agents (Stansly and Natwick, 2010; Vieira *et al.*, 2011 and Baldin *et al.*, 2012). Host plant resistance is characterized by the use of cultivars that have chemical, physical, and/or morphological mechanisms acting alone or in combination to reduce insect infestation by affecting herbivore preference (Antixenosis) and performance (antibiosis) or by keeping or promoting plant fitness after herbivory (Tolerance) (Emden, 2002 and Mitchell *et al.*, 2016). The assessment of novel cultivars for whitefly resistance traits is fundamental to providing farmers with options for pest control (Silva *et al.*, 2019). The development of sustainable methods for crop production and pest management such as organic agriculture and biological control is necessary (Togni *et al.*, 2019).

The scanning electron micrographs of the Royal 481 leaves revealed that the early stage had the low trichome density and the longest trichomes these may explain the preference of *B. tabaci* to lay their eggs on Royal 481 leaves. This is in agreement with Sulistyono and Inayati (2016) found that the resistant soybean genotype to *B. tabaci* infestation had the longest trichomes and the lowest mean trichomes number. In contrast, high density and short trichomes were recorded in leaves of Majus, which might alter egg laying and acts as protection means against *B. tabaci*. This is in agreement with War *et al.* (2012) who reported that leaf trichomes disrupt the movement of insect on the leaf surface, and thus prevents the insect to reach the leaf

epidermis. The high infested cultivar Darvina had short trichomes, which may explain egg preference and high nymphal infestation recorded in this cultivar. Generally, plant growing stages may alter whitefly egg deposition. One factor was the trichomes numbers and shapes. Our speculations are also supported by several other investigators e.g. Baldin *et al.* (2012) who found that the highest density of trichome was recorded in the resistant melon (*C. melo*) cultivar compared to other tested cultivars. Neiva *et al.* (2013) found that the high trichomes density in tomato lines expressed resistance to *B. tabaci* nymphs. On the other hand, Chu *et al.* (2000) reported that the top young leaves of cotton cultivar contain more stellate trichomes than the older leaves and suggested other factors in addition to trichomes affecting *B. tabaci* population development.

Some cantaloupe cultivars have shown levels of resistance to *B. tabaci*, these cultivars provide farmers options for pest control. Arava and Majus cultivars showed low infestation. The lowest infested cultivar "Majus" had the highest trichomes density this information should be involved in genetic breeding programs for cantaloupe, targeting the development of commercial cultivars less susceptible to whitefly.

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