SUSCEPTIBILITY OF FIVE MANGO CULTIVARS TO SEYCHELLES FLUTED SCALE, *ICERYA SEYCHELLARUM* (WESTWOOD) IN RELATION TO LEAF QUALITY: III. LEAF TOUGHNESS AND ANATOMICAL CHARACTERISTICS.

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Abstract

The population density of seychelles fluted scale, *Icerya seychellarum* was studied in relation with the toughness and anatomical characteristics of the leaves of five mango cultivars. The order of susceptibility levels of mango cultivars to *I. seychellarum* could be arranged in descending order as follows: Sultani (highly susceptible) > Baladi > Hendi > Ewaisi >Alphonso (completely resistant). Ewaisi leaves were characterized by higher stomata density, thicker blade, as well as thicker upper and lower epidermis than those of the other studied mango cultivars. Lignin and tannins were deposited mainly in the palisade cells, phloem, pericyclic fibers, and in some cortical parenchyma cells of both Ewaisi and Alphonso leaves. Leaves of the completely resistant cultivar (Alphonso) were characterized by heavily lignified epidermis cells. In consequence, leaves of Ewaisi and Alphonso cultivars had higher toughness than the other studied mango cultivars. The overall results suggest that of the studied leaf properties, heavily lignified tissues and high contents of condensed tannins play an important role in resistance of Ewaisi and Alphonso culuvars to *I. seychellarum* infestation.

Key words: *Mangifera indica*, Cultivars, *Icerya seychellarum,* Toughness, Anatomy.

INTRODUCTION

Mango, *Mangifera indica*, is considered one of the most economic crops in Egypt, where many cultivars such as Alphonso, Baladi, Ewaisi, Hendi and Sultani, are successfully grown (El-Zohgbi and Mostafa, 2002). Mango trees are liable to be infested with many serious pests during their growth stages including the phloem-feeding mealybug, *Icerya seychellarum* (Westwood) (Assem, 1990). However, several studies conducted in different Egyptian localities showed that different mango cultivars express varying levels of resistance to scale insects infestation in general and *I. seychellarum* in particular. (Salem, 1994; Monzer *et al.*, 2006 and Salem, *et al.*, 2006). Long-term observation in Fisher mango orchards located at El-Saff, Giza Governorate, Egypt, indicated that Sultani mango cultivar was highly susceptible to *I. seychellarum* infestation followed by Baladi, Hendi then Ewaisi, while Alphonso cultivar was completely resistant (Monzer *et al.*, 2006 and Salem, *et al.*, 2006).

Influences of the environmental conditions and resource availability on the level of cultivar susceptibility to *I. seychellarum* were excluded because trees of all cultivars grow next to each other in Fisher mango orchards. Thus it was suspected that the leaf quality state of each cultivar might be the main susceptibility factor. Investigations on interaction between plants and insect pests suggest that the leaf quality in relation to insect preference could be related to: 1) its ability to produce qualitative effective secondary metabolites (toxic, repellent and attractant substances), 2) its contents and proportions of essential nutrients (e.g. carbohydrates and proteins) together with its contents of certain nutritional inhibitors (e.g. phenols, lignin or tannins) and/or 3) leaf toughness and anatomical characteristics. Differences in susceptibility to insect pests among different cultivars of a given plant species could be due to one of such factors or most likely due to interactions of the three factors (Chen *et al.*, 2002)

The present study was conducted to investigate the possible role of leaf toughness and certain leaf anatomical characteristics that related to defense against phloem feeding insects in susceptibility of five mango cultivars to *I. seychellarum* infestation.

MATERIALS AND METHODS

Study site:

Tree leaves from five Mango cultivars (Sultani, Baladi, Hendi, Ewaisi and Alphonso) were collected from Fisher mango orchards located in El-Saff, Giza Governorate, Egypt during August 2003. The threat of this orchard from the *I. seychellarum* in 2002 was assessed to be high with main population peak during August.

Infestation levels:

Population densities of *I. seychellarum* on Sultani, Baladi, Hendi, Ewaisi and Alphonso cultivar leaves were estimated on three trees for each cultivar. Leaf samples (20 leaves/tree) were taken from the four cardinal directions of the middle crown parts in August 15, 2003. Leaves of each tree were packed separately in plastic bags, hermetically sealed, labeled, and transported to the laboratory for examination in the same day. Number of insects (nymphs and adults) on each leaf was counted under a dissecting microscope.

Toughness and anatomical characteristics of leaves:

For toughness and anatomical study, leave were sampled for each cultivar using the same procedure described above at the same date. Leaves were carefully examined and old, insect damaged and infected leaves were removed. Toughness and anatomical characteristics of leaves were determined within 6 hrs of field collection.

Leaf toughness:

The toughness of leaves was determined using an in-house designed penetrometer (designed by Monzer, M.A., Plant Protection Research Institute, Egypt), which measures the threshold weight required for piercing the leaf. The penetrometer was designed following the basic criteria described by King (1988) using a simple cylindrical needle (0.9 mm in diameter). A constant needle tip surface area was used for all measurements, thus differences in toughness are expressed in units of mass (g). Toughness was measured at three different regions outside the major vein of each of 20 representative leaves for each cultivar and the mean per leaf was calculated.

Anatomical Studies:

1. Stomata density:

In order to determine stomata density, five leaves of each cultivar were evaluated. The lower epidermal peels were obtained according to the method described by Ferris and Taylor (1994) by spreading a thin layer of nail polish over the lower leaf surface. When dry, the film of polish was pulled from the leaf and mounted on a microscope slide. Mounted cuticles were examined through a light microscope containing a micrometric ocular and photographed with a digital camera. Number of stomata was counted on the computer-acquired image at the magnification of 400. The stomata counting were done in five images from each leaf and mean stomata density for each cultivar was calculated. Stomatal density is defined as the number of stomata per square millimeter of leaf surface (Ferris and Taylor, 1994)

2. Internal anatomical characteristics:

Samples of the material for slide preparations were taken from identical regions of each fresh leaf, generally from mid-way between the leaf base and apex of lamina. Five Healthy leaves for each cultivar were fixed in FAA (50 ml 95% ethanol, 5 ml glacial acetic acid, 10 ml 37-40% formaldehyde, and 35 ml H2O). After fixation they were dehydrated in 50% and 70% ethanol. Sections of about 18-20 microns in thickness were prepared and were stained with 1% safranin and 0.5% fast-green in 95% ethanol according to the procedure described by O'Bruen and McCuly (1981). In addition to its applicability for general plant tissues, this stain procedure is designed to show deposits of condensed tannin and lignified tissues. Using this stain, tannin deposits and lignified tissues stained red, while other constituents stained green or yellow (O'Brien and McCuly, 1981). Sections were microscopically examined and photographed with a digital camera. For the analysis of the transversal cuts, five measurements of each leave were performed using a micrometric ocular.

Statistical analysis

Data of all experiments were evaluated statistically using ANOVA and means compared using Duncan's Multiple Range Test at P<0.05. All statistical analyses were done using the software package Costat (Cohort Inc., Berkeley, CA, USA, Ver.1). Values were recorded as means ± Standard deviations (SD).

RESULTS

Infestation levels:

Data in Table (1) show population densities of the *I. seychellarum* on leaves of Sultani, Baladi, Hendi, Ewaisi and Alphonso mango cultivars at the sampling date (August, 15). The highest main number of *I. seychellarum* individuals per leaf was found on Sultani cultivar (28.5±1.2) flowed by Baladi (15.0±0.93), then Hendi (12.0 ± 0.77) and Ewaisi (8.5±0.65) while Alphonso leaves were clear of any *I. seychellarum* infestation. The differences in population density between all the studied mango cultivars were statistically significant (P<0.05). Accordingly, the order of susceptibility levels of mango cultivars to *I. seychellarum* could be arranged in descending order as follows: Sultani (highly susceptible) > Baladi > Hendi > Ewaisi >Alphonso (completely resistant).

Leaf toughness:

The average toughness of mango leaves belonging to different cultivars (expressed as threshold weight needed for piercing leaf) are presented in Table (١). Baladi leaves showed significantly lower toughness value than the other mango cultivars (19.32 ± 3.65g) followed by Sultani and Hendi (25.23 ± 2.68 and 29.81 ± 2.00g, respectively), then Ewaisi and Alphonso leaves (36.81 ± 2.49 and 38.36 ± 1.91 g, respectively). There were no significant differences in mean toughness between Sultani and Hendi leaves or between Ewaisi and Alphonso leaves (P>0.05).

Leaf anatomy:

Figure (1) shows stomata of samples of the studied mango cultivars viewed at 400x in nail polish impression from leaf underside surface. There were no significant differences in the estimated stomata density between Sultani, Baladi, Hendi, and Alphonso cultivars (96± 8, 100 ± 9, 92 ± 8 and 116 ± 12 stomata/mm^2, respectively) (Table 1). However, leaves of Ewaisi cultivar had a significantly higher stoma density (176 ± 20 stomata/mm^2, P<0.05) than the other 4 mango cultivars.

Transverse sections of mango leaves, at mid-way between the leaf base and apex of lamina (Fig.2), revealed that there were no significant differences between Sultani, Baladi and Hindi cultivars in thickness of the blade, upper and lower epidermis as well as depth of the vascular tissues. Ewaisi leaves were characterized by thicker blade, as well as thicker upper and lower epidermis than those of the other studied mango cultivars (Table 1; Fig. 2). The depth of vascular tissues from the upper epidermis was also significantly higher in Ewaisi (100± 7.5um) than the other four mango cultivars. However, the depth of vascular tissues from the lower epidermis was significantly higher in Hendi (75± 5.5µm) leaves than the other mango cultivars (Table 1). Figure (2) showed accumulation of lignin and condensed tannins mainly in the palisade cells, phloem, pericyclic fibers, and in some cortical parenchyma cells of both Ewaisi and Alphonso leaves. The cell walls of both upper and lower epidermis of Alphonso leaves were heavily lignified (Fig. 2).

DISCUSSION

To best of our knowledge, this is the first study that examines the relation between susceptibility of different mango cultivars to the scale insect; *I. seychellarum* and toughness together with anatomical characteristics of their leaves. The measured leaf characteristics in this study are potentially important determinants of leaf quality as a food source for the phloem feeding insect herbivores.

Results of the present study indicated that the order of susceptibility levels of mango cultivars to *I. seychellarum* could be arranged in descending order as follows: Sultani (highly susceptible) > Baladi and Hendi (intermediate)> Ewaisi (low) >Alphonso (completely resistant). The low susceptibility of Ewaisi cultivar to feeding by *I. seychellarum* is coincident with significantly higher blade thickness, both upper and lower epidermis thickness and vascular tissue depth than the other *I. seychellarum* susceptible cultivars. *I. seychellarum* usually feed from the underside surface of the leaf. However, in heavily infestation, portion of insects feed from the upper side of the leaf Assem, (1990). It have piercing-sucking mouthparts, formed from tubular structures called stylets which they use for piercing through leaf tissues to reach, tap into and feed upon phloem (sap) tissue of plants Assem, (1990). Sometimes the stylets go through stomata, through individual cells or between cells to reach the target tissue. Accordingly, several studies suggest that thick epidermis; deeper vascular tissues and high stomata density of the leaf are associated with reduced fitness and feeding performance of phloem feeding insects (Kuncheva, 1999, Hossain *et al.*, 2002, and Peeters, 2002).

However, Ewaisi cultivars had higher stomata density than the other more susceptible cultivars indicating that stomata density had little influence on susceptibility of mango leaves to *I. seychellarum* infestation despite the reports which mentioned that stomata density was correlated positively with susceptibility of plant to phloem feeding insects (Skorupska, 2004). This could be attributed to the future of

Mango stomata. Bally (1999) examined stomata of mango leaves and fruits with electron microscope. He reported a small stomata size with very narrow opening (between1 and 5 mm in diameter). Further more, any stress conditions can lead to quick and complete stomatal closure (Urban and Jannoyer, 2004).

On the other hand, although Alphonso cultivar was completely resistance, it had blade thickness, upper- and lower-epidermis thickness and vascular tissue depth comparable or even less than to those of the other studied cultivars indicating that it relay on other mechanism(s) of resistance. Results of this study revealed that toughness of both Ewaisi and Alphonso leaves were significantly higher than that of the other studied cultivars. High leaf toughness is considered as anti-herbivore defense by making it physically difficult or energetically costly for insect to penetrate and reach the phloem. High toughness of plant leaves could be due to the presence of condensed tannins and lignified cell wall of various leaf tissues Hossain *et al.*, (2002) and Peeters, (2002). The accumulation of such compounds in palisade cells, phloem, and pericyclic fibers in both Ewaisi and Alphonso leaves could explain the higher toughness of those two cultivar leaves than the other cultivars. Furthermore, upper and lower epidermis of Alphonso leaves were highly lignified. The previous biochemical studies of Monzer *et al.* (2006) and Salem *et al.* (2006) support our findings and showed that Alphonso and Ewaisi leaves have higher contents of condensed tannins, and other phenolic compounds could support this conclusion. The overall results suggest that of the studied leaf properties, heavily lignified tissues and high contents of tannins play an important role in resistance of Ewaisi and Alphonso cultivars to *I. seychellarum* infestation without ignoring the role of other biochemical mechanisms of resistance.

Table 1. Density of *I. seychellarum*, toughness, stomatal and anatomical measurements of leaves of the studied mango cultivars.

Cultivar	*I. seychellarum* density	Leaf toughness	Leaf anatomy					
			Stomata density	Thickness of the blade	Thickness of upper epidermis	Thickness of lower epidermis	vascular tissue depth (µm)	
	Individuals/leaf	(g)	Number/ mm^2	(µm)	(µm)	(µm)	from lower	from upper
Sultani	28.5 ± 1.2 a	25.23 ± 2.68a	96± 8a	228±5.2a	22.0 ± 3.4a	7±2.1a	45±3.6a	80± 4.8a
Baladi	15.0 ± 0.93 b	19.32 ± 3.65b	100 ± 9.a	200± 7.6b	21 ± 2.1a	7±2.3a	50± 4.2a	75± 4.5a
Hindi	12.0 ± 0.77 c	29.81 ± 2.00a	92 ± 8 a	220±5.2a	18.4 ± 2.6a	5±1.6a	75± 5.5b	55± 3.1b
Ewaisi	8.5 ± 0.65d	36.81 ± 2.49c	176 ± 20b	290±6.5d	25 ± 2.8b	12±2.4b	50± 3.8a	100± 7.5c
Alphonso	0	38.36 ± 1.91c	116 ± 12 a	230±5.3a	22.6 ± 1.8a	6±2.0a	55± 3.2a	60± 3.3b

Values (means ± SD) followed by similar letter within the same column do not differ significantly ($P < 0.05$)

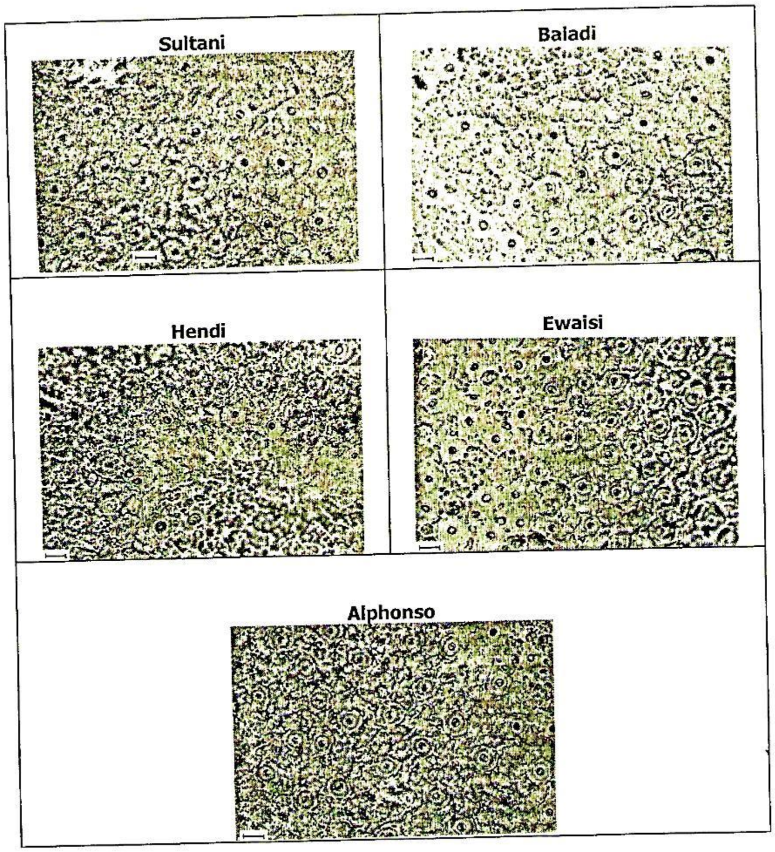

Figure 1. stomata viewed at 400x in nail polish impression from leaf underside of the studied mango cultivars (Scale bar = 25 micrometer)

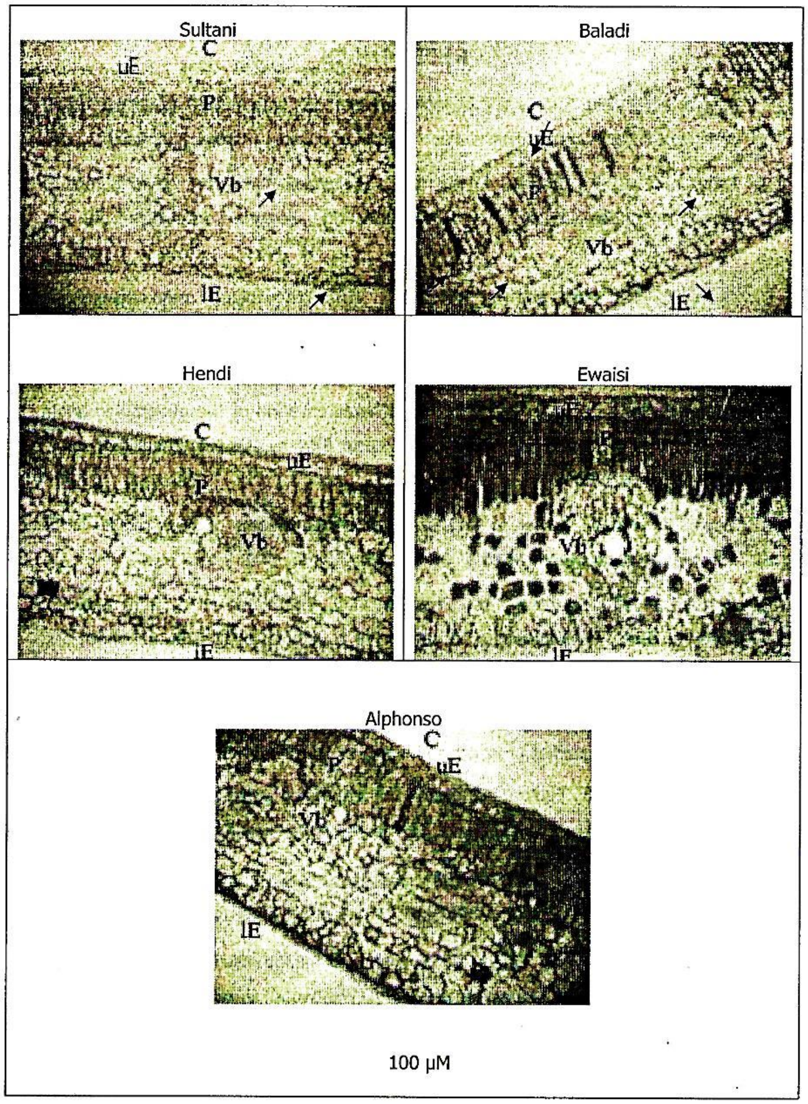

Figure 2. T.S. of the leaf of the studied mango cultivars. C: Cuticle, lE: Lower epidermis, uE: upper epidermis, P: Palisade tissues, Vb, Vascular tissues. Black arrows pointed to the red stained lignified tissues and tannin deposits (darkened colour in black and white pictures).

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قابلية خمسة أنواع من أشجار المانجو للإصابة بالحشرة القشرية ***Icerya seychellarum*** وعلاقتها بنوعيةِ الأوراق: صلابة الأوراق وخصائصِها التشريحيةِ.

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تم دراسة العلاقة بين كثافة تعداد الحشرة القشرية *Icerya seychellarum* والخصائص التشريحية لأوراقِ خمسة أنواع من أشجار المانجو. وقد أظهرت الدراسة انه يمكن ترتيب أنواع أشجار المانجو من حيث قابليتها للإصابة بتلك الآفة ترتيبا تنازليا كالتالى: السلطانى يليه البلدى ثم الهندى ثم العويسى ثم الفونسو الذى كان مقاوما تماما. وقد أظهرت الدراسات التشريحية تميز أوراق العويسى عن بقية الأنواع التى تم دراستها بأكبر كثافة للثغور وأعلى سمك للنصل وأعلى سمك لكل من طبقتي البشرة العلوية والسفلية. وقد اشارت الدراسة الى وجود كلا من التانين واللجنين بشكل رئيسي في خلايا طبقة البلاستيدات، واللحاء والألياف المحيطة به وأيضا في بَعْض خلايا النسيج الحشوى فى كلا من أوراق العويسى وألفونسو. وقد تميزت أوراق أشجار الفونسو بخلايا بشرةِ تحتوى جدرانها على مادة اللجنين. وقد أظهرت القياسات أن صلابة أوراق كلا من العويسى والفونسو كانت الأعلى من بين أنواع أشجار المانجو التى تم دراستها. وبناء على ذلك يمكن استنتاج أن محتوى أنسجة أوراق المانجو من اللجنين والتانين لهما دور مهم في مقاومةِ أوراق أشجار العويسى والفونسو للإصابة بذلك النوع من الحشرات القشرية الثاقبة الماصة بما لهما من تأثير على صلابة الأوراق ومقاومتها للثقب.