RESISTANCE OF ALPHONSO MANGO-CALTIVER TO THE MARGARDOID MEALYBUG, *ICERYA SEYCHELLARUM* (WESTWOOD) IN RELATION TO LEAF QUALITY: I. LEAF SECONDARY METABOLITES.

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Abstract

The generalist insect herbivore, *Icerya seychellarum* (Westwood) heavily infests mango trees (*Mangifera indica*) and can complete its entire life cycle on this plant. However, we observed that mango cultivars are not equally susceptible to *I. seychellarum* infestation. While mango trees of Sultani cv are heavily infested and severely damaged, trees of Alphonso cv are completely free of *I. seychellarum* infestation. Comparison between the two cultivars leaves and their total extracts in chemotaxis, feeding and toxicity assays revealed that leaf material of Alphonso cv. contained *I. seychellarum* repellant, feeding deterrent and toxic substance. The leaf components of secondary metabolites were screened using solvent/solvent extraction technique followed by gas chromatographic/mass spectrometric (GC-MS) analysis to determine which compound(s) best explained the repellent and toxic properties of Alphonso mango cv. The results suggested that *p*-cymene, camphene, and limonene play an important role in resistance of Alphonso mango cv. to *I. seychellarum* infestation.

Key words: *Mangifera indica*, Cultivars, *Icerya seychellarum*, Resistance, Secondary metabolites

INTRODUCTION

Mango, *Mangifera indica*, is considered one of the most economic crops in Egypt. Mango trees are liable to be infested with several serious pests during their growth stages including the margarodid mealybug, *Icerya seychellarum* (Westwood). *I. seychellarum* severely damages mango trees foliage, especially in heavy infestation (Assem *et al.*, 1991). However, studies showed that different mango cultivars are differ in their susceptibility to the attack by several insect pests (Hennessey and Schnell 20Ci). We observed that Alphonso mango cv. is much less susceptible to most of insect pests than the other Egyptian mango cvs. such as Sultani and Baladi. Moreover, Alphonso mango trees were completely free *of I. seychellarum* infestation even in tree closely adjacent to *I. seychellarum* heavily infested Sultani mango trees. This observation led to hypothesize that leaf quality (concentration of secondary metabolites, nutrients, morphology and anatomy) could be of a great importance in

mango cultivar resistance to *I. seychellarum*. The association between resistance and secondary metabolites of host trees to insect herbivores has been investigated in many studies (Chen *et al.*, 2002). Peña and Moyhuddin (1997) reviewed mature mango fruit resistance to *Anas-trepha obliqua* (Macquart) and infestability differences among cvs were suggested to be caused by differences in fruit contents of secondary metabolites (repellant, attractant or toxic chemicals), nutrients, or resin ducts. However, to the best of our knowledge, there have been no studies to determine the relation between variation of mango leaf secondary metabolites and its resistance to insect pests. Accordingly, this study was conducted to determine whether the variation in secondary metabolites could explain the resistance of leaves of Alphonso mango cv. to *I. seychellarum* in comparison with Sultani cv. A better understanding of the cues that elicit or inhibit host-plant selection by *I. seychellarum* could lead to increase the efficacy of its control strategies.

MATERIALS AND METHODS

Sample collection. Leaves of Alphonso and Sultani cvs. of mango (*M. indica*) were collected from a Fisher mango orchard located in El-Saff, Giza Governorate, Egypt in August 2003. Fresh mature leaves were hand-plucked from three for each cv, packed in plastic bags, hermetically sealed, labeled, and transported in icebox to the laboratory. Leaves were carefully examined and old, insect damaged, and infected leaves were removed. Healthy leaves were maintained at –20°C until extracted (within one week).

Sample extraction. Two hundred grams from leaves of each cultivar were homogenized in a Tempest homogenizer, and were extracted with organic solvents of increasing polarity at room temperature (20°C). Extraction was performed in a Romo shaking apparatus initially with hexane then with acetone, each for 48 hrs and with methanol for additional 48 hrs. All the three extracts from each sample were filtered using Whatman filter paper no. 2 in a Buchner funnel and the solvents removed by vacuum distillation in a rotary evaporator under reduced pressure at 45°C. Extracts were later re-dissolved in the minimum amount of particular solvent of which it would dissolve and combined together for Chemotaxis assays, or re-suspended in 100 ml distilled water for feeding and toxicity assays. In the later case, Tween-60 was added as a non-ionic surfactant in concentration of 1% (v/v).

Laboratory bioassays:

Insect collection. Twigs bearing heavily *I. seychellarum* infested leaves of Baladi cv mango trees were collected from the previously mentioned orchard and sent immediately to the laboratory for performing behavioral and toxicity test at the same day. Newly hatched Crawling nymphs were collected from leaves under desiccating microscope using a fine brush.

Behavioral assays:

a) Chemotaxis assay. The experiments were designed to determine the attractiveness (or repellency) of Sultani and Alphonso mango leaves and their total extracts to *I. seychellarum* nymphs. Fresh leaves of Sultani mango cv. were cut to small pieces (~1 cm^2 each) and 10.0 leaf pieces were placed near the edge of 10-cm diameter Petri dish. Ten pieces (~1 cm^2 each) of filter paper were placed on the opposite side of the dish to serve as control. *I. seychellarum* nymphs were collected from Baladi mango leaves before the experiment and were starved for 6 hrs. Ten nymphs were placed in the center of the dish, 4 cm away from both leaf and paper pieces. Dish was covered and maintained at room temperature. After 24 hours, number of nymphs on Sultani leaf pieces and on the filter paper pieces was counted. Insects within 5 mm of the tested material were counted as being attracted to the source. The same experiment was conducted but with (a) Alphonso mango leaves, (b) total Sultani extracts and (c) total Alphonso extracts. In the case of leaf total extracts, 10 filter paper pieces (~1 cm^2 each) were impregnated by 0.2 ml of each extract, allowed to dry and were placed near the edge of a separate Petri dish opposing to 10 pieces of filter paper that was previously impregnated by 0.2 ml of the corresponding solvent and dried. A control was prepared with two clean sets of filter paper pieces placed opposite to each other near the edge of a separate Petri dish. A chemotaxis index (CI), for each test dish was calculated after 24 hours according to Thurston *et al.* (1994) as follows:

$$\mathbf{CI = (NT-NC)/(NT+NC)}$$

where CI = chemotaxis index, NT = number of nymphs counted on and near the target side of the Petri plate arena and NC = number of nymphs on and near the non-target side. CI value can range from +1.0 to −1.0; values close to 0 indicate that the test material had no effect on *I. seychellarum* chemotaxis, positive values indicate attraction to, and negative values indicate repellence by the test material. Significance of the CI values from control was determined by t-test.

b) Feeding assay. A laboratory bioassay was established to determine feeding deterrent effects of Alphonso leaves on *I. seychellarum* nymphs. The experimental arena consisted of 10-cm diameter Petri dishes containing: (1) one Sultani leaf (2) one Alphonso leaf, (3) one Alphonso leaf that was previously sprayed with Sultani total extract (4) one Sultani leaf that was previously sprayed with Alphonso total extract and (5) one Alphonso leaf that was previously sprayed with 1% Tween-60 in water to serve as control. In all cases, the sprayed extracts were allowed to dry at room temperature before transferring 10 starved nymphs on each leaf. The behavior of nymphs in each dish was observed regularly during the first 6 hours. After 24, and 48 hrs numbers of alive and dead nymphs on or away from each leaf were recorded.

Toxicity bioassay. The toxicity bioassay was conducted to evaluate toxicity of Alphonso and Sultani total leaf extracts to *I. seychellarum*. For each extract, ten infested leaves (each bearing not less than 10 *I. seychellarum* insects) of Baladi mango cultivar were sprayed for five seconds and were kept at room temperature. Control insects were sprayed with 1% Tween-60 in distilled water. Mortality of insects was assessed at 24 and 48 hrs by touching each insect with a blunt probe under dissecting microscope.

Identification of mango leaf secondary metabolites:

a). Group separation. The purpose of this procedure is to simplify extracts for Gas chromatographic-mass spectrometric (GC-MS) analysis by fractionating the chemical compounds into broad groups based on their solubility. The solvent/solvent group separation procedure used by the USA National Cancer Institute as described by Marinia (2001) was applied with some modification. Briefly, the concentrated extracts of each sample were combined together, dissolved in 1:1 mixture of chloroform and water and the two phases were separated in a separatory funnel. The water fraction was mixed with an equal volume of n-butanol in a separatory funnel to yield the water (W) and Butanol (B) fractions. The chloroform fraction was taken to dryness in a rotatory evaporator under reduced pressure as described above and extracted with an equal volume of hexane and 10% water/methanol mixture to yield the hexane (H) fraction and 10% water/methanol (WM) fraction. In all cases, equal volumes of solvents were used and the process repeated until the extracting solution was light in color. Solvents were removed from each fraction under vacuum in the rotatory evaporator. Each fraction was later re-dissolved in the minimum amount of particular solvent of which it would dissolve for gas chromatographic analysis

b) Gas chromatographic-mass spectrometric (GC-MS) analysis. GC-MS analysis were used to determine identities of compounds in fractions that showed activity against *I. seychellarum* in the laboratory bioassays. The procedure followed here was similar to that described by Marinia (2001). A weight of 0.1 gram of each concentrated fraction was re-dissolved in the minimum amount of particular solvent of which it would dissolve and 2 ul of each fraction was injected. GC-MS was carried out with a HP 5972A mass spectrometer coupled to HP-6890 GC equipped with a split-splitless injector and a HP-5MS capillary column (30 m x 0.32 ID, 0.25 µm film thickness). Electron impact (EI) mass spectra were obtained at 70 eV and the instrument scanning from 35 to 700 amu. Helium was used as the carrier gas at a flow rate of 1 ml/min. Injector temperature was 250°C; detector temperature was 280°C, and split was 20:1. Oven temperature was programmed from 35°C (5 min) to 80°C at 10°C/min and to 250°C at 4°C/min. Integration of peaks, drawing calibration table, and standard curve were performed using HP-Chemstation software. Data were collected with HP Chemstation software (A.03.00) and searched against the Wiley registry of mass spectral data (7th edition, Palisade Corp., Newfield, NY). Compounds were identified by library search and the identity of all compounds reported was confirmed by comparison of their fragmentation patterns with those cited in the literature. All chemical analyses were conducted by the Unit of pheromone analyses, Plant protection Research Institute.

Statistical Analysis. The entire assays were repeated three times each with three replicates and the results were combined for statistical analysis. The results are presented as percentage, although actual number of insects was used for statistical tests. Statistical significance was determined by analysis of variance (T-test at $P<0.05$) using the software package Costat, 1992 (Cohort Inc., Berkeley, CA, USA). Results are recorded as mean ± standard deviation (SD)

RESULTS

Chemotaxis Assays. The results of chemotaxis assays Table 1 showed that 64.82±14.79% and 82.50±5.0% of *I. seychellarum* nymphs had migrated to Sultani leaves and their total extract, respectively. On the other hand, 75.97±15.79 and 92.50± 9.57of nymphs had moved to the opposite side of Alphonso leaves and their total extract, respectively. The calculated chemotaxis indices indicated that there was a significant attraction to Sultani leaves and their total extract and repellence from Alphonso leaves and their total extract to *I. seychellarum* nymphs ($P< 0.05$, T- test).

Table 1. Response of *I. seychellarum* nymphs to Sultani and Alphonso mango leaves and their extracts.

Test material	Mango	% Nymphs moved to: (Mean ± SD)		Chemotaxis indices (CI)†
	Cultivar	Leaf material	Opposite side	(Mean ± SD)
Leaves	Alphonso	12.25±11.27	75.97± 15.79*	(-) 0.73 ± 0.20*
	Sultani	64.82±14.79*	20.89±17.08	0.58 ± 0.28*
Total extract	Alphonso	0	92.50± 9.57*	(-)1.0 ± 0.0*
	Sultani	82.50±5.0*	16.25± 5.85	0.65 ± 0.1*
Control		14.5 ±6.4	12.5±5.0	0.05 ± 0.19

† Negative CI values indicate repellence by and positive values indicate attraction to the test material.

* Means are significantly different from control, $P \leq 0.05$, t-test.

Feeding assay. During the observation period, nymphs on Sultani leaves were slowly moving on the leaves searching for a suitable place to attach themselves. The same behavior was observed with nymphs on Alphonso leaves that were sprayed with Sultani extract. On the other hand, nymphs on Alphonso leaves, Sultani leaves that were sprayed with Alphonso extract and Alphonso leaves that were sprayed with Span-60 were actively moving away from the leaves. Results of feeding assays are summarized in Table 2. After 24 hours, 96.0 ±5.4% of nymphs on Sultani leaves were already attached themselves to the leaves. There were no mortality among nymphs on Sultani leaves after 24 hrs and only 4.0 ± 5.4% of nymphs were died after 48 hrs. None of the nymphs on Alphonso leaves, Sultani leaves that were sprayed with Alphonso extract, and Alphonso leaves that were sprayed with Span-60 were able to attache themselves to the leaves. However, after 24 hrs 0.0, 8.0±8.3, and 8.0± 8.3% of nymphs were still moving on the leaf surface respectively, and the reminder nymphs were migrated away from the leaves to the dish bottom and walls. Percentage of 42± 8.4, 38±16.4, 0.0 and 30±15.8 of migrated nymphs were died, respectively. On the other hand, 82.0± 14.8 of nymphs were still moving on Alphonso leaves that were sprayed with Sultani extract and some of them were attached themselves to the leaves. However, after 48 hours, 22.0± 8.3% and 72.0±14.8% of nymphs were died on the leaf surface and on the dish bottom, respectively.

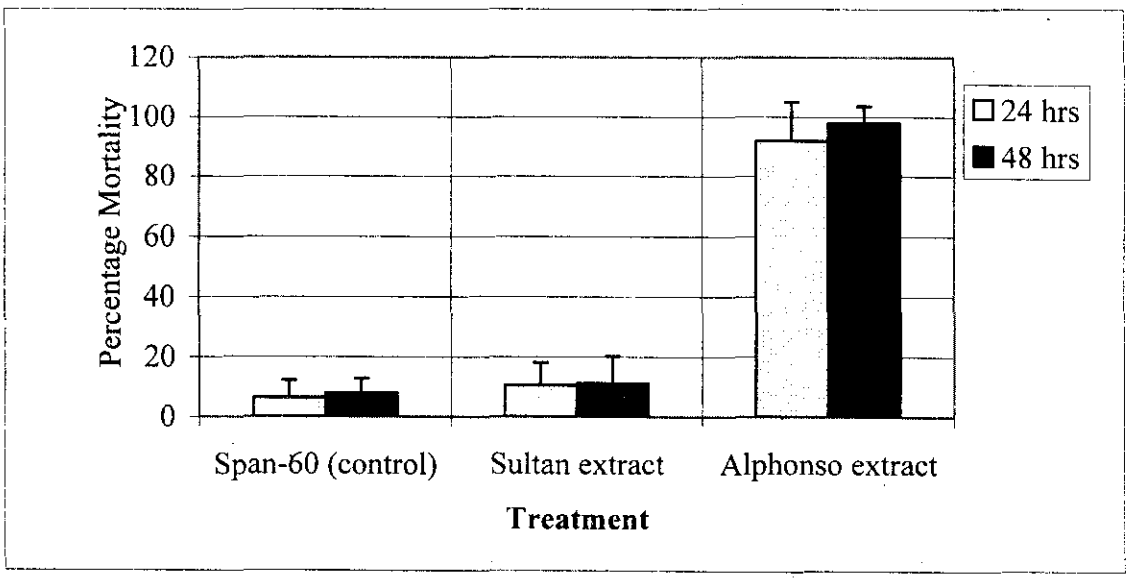
Table 2. Feeding behaviour of *I. seychellarum* nymphs on leaves of Sultani and Alphonso mango cultivars.

Leaf Materials*	24 hours				48 hours			
	% On the leaf		%Away from the leaf		% On the leaf		%Away from the leaf	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
SLT	96.0 ±5.4	4.0±5.4	0	0	96 ±5.4	4.0±5.4	0	0
ALPH	0	0	58.0±8.4	42± 8.4	0	0	34±8.9	66.0±8.9
ALPH/SLT	8.0±8.3	2.0±4.4	52.0± 14.8	38±16.4	0	4.0± 5.4	42± 14.8	54.0± 16.7
SLT/ALPH	82.0± 14.8	4.0± 6.0	14.0 ± 8.8	0	0	22.0± 8.3	6.0±8.9	72.0±14.8
Span/ALPH	8.0± 8.3	0	62.0±8.3	30±15.8	2.0±4.4	0	38± 8.3	60.0±7.1

* SLT = Sultani leaves, ALPH = Alphonso leaves, ALPH/SLT = Sultani leaves sprayed with Alphonso extract, SLT/ALPH = Sultani leaves sprayed with Alphonso extract, Span/ALPH = Alphonso leaves sprayed with Span-60.

Toxicity assay. The results of toxicity assay as represented in Fig. 1. showed that treatment of *I. seychellarum* with total extract of Alphonso leaves resulted in significant high mortality percentages than control, reached 92±13.0 and 98.0± 5.6% after 24, and 48 hours of treatment, respectively. On the other hand, mortality percentage did not differ significantly between *I. seychellarum* treated with Sultani extracts and Control.

Figure 1. Effects of leaf extracts of Sultani and Alphonso mango cultivars on survival of *I. seychyllarum* after 24 and 48 hours of treatment.



Identification of mango leaf secondary metabolites:

Table 3 shows that mango leaves contained 19 main compounds, of which 10 were identified in hexane fraction (H)and 9 were identified in butanol fraction, while there were no detected compounds in both water and water/methanol fractions. Sixteen of the detected compounds were positively identified by the GC/MS, and 4 were unknown (their match quality was less than 95% and their fragmentation patterns did not match well with the suggested compounds by GC/MS-Willy library). Table 3 shows also that there are important and appreciable differences between percentages of the leaf secondary metabolite contents of the two cvs. Leaves of Alphonso cv. were rich in Unknown compound A, menthatriene, trimethylcyclohexan2-en-1-one, methyl benzoic acid and 1-methyl-ethyl cyclopentane, while they presented as traces (their percentage less than 0.1%) in Sultani cv. Percentages of limonene in Alphonso leaves was approximately 6-fold higher than that in Sultani leaves (5.82 vs 1.0%, respectively). Percentage of Unknown compound-B in Alphosno leaves was also higher than that in Sulatn leaves (4.28 vs 0.23%, respectively). Moreover, percentages of *p*-Cymene and camphene in Alphonso leaves were 2.42 and 13.53, respectively, while they were not detected at all in Sultani leaves. Sultani leaves produced mainly *cis*-ocimene and β-ocimene, as their major constituent of secondary metabolites. These two compounds represented 82.17 % of the total Sultani leaf secondary metabolites compared with only 9.23% for Alphonso leaves. Also, percentage of unknown compounds-C and -D were higher in Sultani leaves than in Alphonso leaves.

Table 3. Percentage composition of the detected secondary metabolite compounds in leaves of Sultani and Alphonso mango cultivars

	Component	Identified In*	R_t (min)	Percentage in mango leaf	
				Sultani	Alphonso
1	*Cis*-Ocimene	H	6.88	7.762	0.21
2	β-Ocimene	H	6.7	74.41	9.02
3	Phenol	B	7.04	1.64	2.74
4	Unknown compound A	H	7.08	Tr	12.22
5	Menthatriene	B	7.46	Tr	1.63
6	*p*-Cymene	B	7.53	Nd	2.42
7	Limonene	B	7.57	1.0	5.82
8	n-Decan	H	7.68	3.76	8.50
9	Camphene	B	7.84	Nd	13.53
10	3-Methyl benzoic acid	B	8.16	Tr	3.35
11	2,6-dimethyl undecane	H	9.2	3.25	6.96
12	Tetracosane	H	10.61	1.48	3.15
13	trimethylcyclohexa2-en-1-one	B	12.05	Tr	6.39
14	B-caryophyllene	H	12.40	2.50	5.36
15	Alpha-humulene	H	12.83	1.0	2.9
16	1-methyl-ethyl cyclopentane	B	13.44	Tr	7.17
17	Unknown compound B	B	13.73	0.19	9.20
18	Unknown compound C	H	17.56	1.0	0.21
19	Unknown compound D	H	18.17	1.44	0.21

*H = Hexane fraction, B = Butanol fraction, Tr= Trace (< 0.1%), Nd= not detected,

R_t = retention time

DISCUSSION

The generalist insect herbivore *Icerya seychellarum* readily consumes mango leaves and can complete its entire life cycle on this plant (Assem *et al.*, 1991). However, we observed that mango cvs are not equally susceptible to *I. seychellarum* feeding. While Sultani cv is heavily infested and severely damaged, Alphonso cv is completely free of *I. seychellarum*. We used chemotaxis and feeding assays to confirm our observation and to test whether this preference for Sultani leaves and avoidance of Alphonso leaves is due to leaf secondry metabolities or other factors. Chemotaxis indices indicated that there was a moderate significant attraction to Sultani leaves and their total extract and a highly significant repellence from Alphonso leaves and their total extract to *I. seychellarum* nymphs. The results of feeding assay were in harmony with that of chemotaxis assay. While *I. seychellarum* nymphs accepted Sultani leaves and feed successfully on them, they refused to feed on Alphonso leaves moving away from them and died of starvation. They also refused to feed on Sultani leaves that were sprayed with Alphonso extract. In contrast, when Alphonso leaves were sprayed with the total extract of Sultani leaves to mimic the natural odor of Sultani leaves, most of *I. seychellarum* nymphs accepted Alphonso leaves, began to explore their surface in their way to inject their mouth parts and some of nymphs began to feed on the false Sultani leaves. However, after 48 hrs most of the nymphs were died either on the leaf surface or after migration away from the leaf material suggesting that there are certain substance(s) toxic to *I. seychellarum* nymphs in Alphonso leaves. The results of toxicity assay, which showed that total extract of Alphonso leaves are highly toxic to *I. seychellarum*, confirm this suggestion.

Overall results indicated that there are active substances (attractive, repellent and/or toxic to *I. seychellarum*) in Sultani and Alphonso leaves. Groysman and Shani (2003) mentioned that the scarab beetle *Maladera matrida* Argaman avoid mango trees due to the presence of feeding deterrent substances in their leaves. Peña and Moyhuddin (1997) also observed that fruits of different mango cultivars are differ in their susceptibility to *Anas-trepha oblique.* They suggested that the infestability differences among cultivars were caused by differences in fruit contents of secondary metabolites. Plant secondary metabolites are most often insect deterrent but stimulate phagostimulatory cells if they serve as host-indicating sign stimuli, or if they are sequestered for defense or used as pheromone precursors (Chapman, 2003).

Preliminary trails were conducted in order to identify the probable attractive, repellent and/or toxic secondary metabolite compounds of Sultani and Alphonso leaves using solvent/solvent extraction and GC/MS analysis. The results indicate that Alphonso and Sultani mango cultivers are differ in their percentage contents of several

secondary metabolites. These results are supported by the findings of Andrade *et al.* (2000) who mentioned that secondary metabolite concentration and composition in mango are influenced by tree genotype and environmental factors.

Alphonso leaves contain menthatriene, *p*-cymene, camphene, limonene, methyl benzoate, unknown compound-A, and unknown compound-B in relatively high percentage. Menthatriene, *p*-cymene, camphene, and limonene are related to a class of compound called monoterpene. Monoterpene group (i.e., C10 hydrocarbons) is one of the major terpene groups and it's compounds are widespread in plants. Their function is either deterrents or attractants to herbivores. The association between terpenes and resistance of host trees to insect herbivores has been investigated in several plant and insect species. High concentrations of camphene, limonene and *p*-cymene are well documented in the literature for their high insect toxicity (Cook 1992 and Ibrahim *et al.*, 2001). Camphene has been reported to adversely affect western spruce budworm performance on Douglas fir (Zou and Cates, 1995). Limonene is found to be repellent against several pest insects (Ibrahim *et al.* 2001). It has also been reported to be toxic against the spruce spider mite (Cook 1992), honey bees, *Apis mellifera* L. (Ellis and Baxendale 1997), *Rhyzopertha dominica* (F.); *Sitophilus oryzae* (L.); and *Tribolium castaneum* (Herbst) (Tripathi *et al.*, 2003). *p*-cymene has been reported to be toxic to various stages of western flower thrips, *Frankliniella occidentalis* (Janmaat *et al.*, 2001).

Accordingly, the repellence and toxic effects of Alphonso leaves on *I. seychellarum* could be related to their highly contents of camphene, limonene and *p*-cymene. Camphene and *p*-cymene were not detected at all in Sultani leaves while percentage of limonene in Alphonso leaves was approximately 6-fold higher than that detected in in Sultani leaves, that may explain the susceptibility of Sultani leaves to *I. seychellarum* infestation. On the other hand Sultani leaves contain β-ocimene as a major secondary metabolites with a relatively very high percentages in comparison with Alphonso leaves. β-ocimene is regarded as an important component of an indirect defense system in plants against insect attack. Several investigators reported that, in response to insect herbivory, plants synthesize and emit β-ocimene, in addition to blends of volatile compounds from, their damaged and undamaged tissues, which can serve as a chemical signal that attracts natural enemies of the herbivore to the infested plant (Maes, 2002). Sultani cv. appears to call for help – attract predator for its predator.

In conclusion we propose that the repellence and the toxic effects of *p*-cymene, camphene, and limonene play an important role in resistance of Alphonso mango cultivar to *I. seychellarum* infestation without ignoring the probable role of the

unidentified compounds and the other leaf components such as leaf nutrients, morphology and anatomy. Further work is in progress to study the implication of such factors in both *I. seychellarum* preference to Sultani mango cv and their avoidance of and Alphonso mango cv.

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العلاقة بين مقاومة أشجار المانجو من صنف ألفونسو للبق الدقيقى "*Icerya seychellarum*" وخصائص أوراقها:
١- محتويات الاوراق من مركبات الأيض الثانوية

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يصيب البق الدقيقى المعروف باسم *Icerya seychellarum* أشجار المانجو بكثافة عالية حيث يمكنه أن يكمل دورة حياته بنجاح متغذيا على عصارة أوراق المانجو. إلا أنه لوحظ أن قابلية أشجار المانجو للإصابة بهذه الآفة تختلف حسب صنف المانجو. وفى الوقت التى تصاب فيه أشجار المانجو من صنف سلطانى بكثافة، تخلو أشجار المانجو من صنف ألفونسو تماما من هذه الآفة. وقد أشارت اختباري التغذية والسمية التى أجريت باستخدام كلا من أوراق هذين الصنفين من المانجو ومستخلصاتهما إلى أن أوراق أشجار المانجو من صنف ألفونسو تحتوى على مواد طاردة ومانعة للتغذية وسامة لذلك النوع من البق الدقيقى. وعلى هذا فقد تم الكشف المبدئي عن مركبات الأيض الثانوية الموجودة فى الأوراق باستخدام طريقة الاستخلاص بالمذيبات ثم التحليل باستخدام جهاز الكروموتوجراف الغازى المزود بجهاز مطياف كتلى للتعرف المبدئى على المواد التى يمكن أن يعزو أليها سبب الخصائص الطاردة والسامة للبق الدقيقى. وقد أظهرت النتائج أن مركبات البارا-سيمين والكامفين والليمونين توجد بكميات كبيرة فى الأوراق من صنف ألفونسو مقارنة بالأوراق من صنف سلطانى. وبالأخذ فى الاعتبار أن الأبحاث السابقة تظهر أن هذه المواد طاردة وسامة للحشرات المختلفة يمكن القول بأن خليط مركبات البارا سيمين والكامفين والليمونين يلعب دورا كبيرا فى مقاومة أشجار المانجو من صنف ألفونسو للإصابة بالبق الدقيقى (*I. seychellarum*).