SUSCEPTIBILITY OF FIVE MANGO CULTIVARS TO *ICERYA SEYCHELLARUM* (WESTWOOD) (HOMOPTERA: MARGARODIDAE)IN RELATION TO LEAF QUALITY, NUTRIENTS AND INHIBITORS

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Abstract

The susceptibility of five mango cultivars to the sap sucking insect, *Icerya seychellarum* was screened and the relation between their susceptibility differences and both leaf nutrients and inhibitors was studied. For this purpose chemical analysis of the leaves of Sultani, Baladi, Hendi, Ewaisi and Alphonso mango cultivars were conducted to estimate their contents of the two main nutritional substances, carbohydrates and proteins, and also their content of two nutrition inhibitors (phenolics and tannins). Based on the obtained results, food quality index (FQI) for each cultivar was calculated. It was found that both the level of susceptibility of mango cvs to *I. seychellarum* and the values of leaf FQI could be arranged in descending order as follows: Sultani (highly susceptible and had the highest FQI) > Baladi > Hendi = Ewaisi. The results revealed that levels of susceptibility of mango cultivars to *I. seychellarum* depend on the combined action of leaf nutrients, inhibitors and secondary metabolites which determined the food quality of their leaves. The relative role of these factors may differ among different cultivars.

INTRODUCTION

The trend for combating phytophagous insects has shifted from the use of broad-spectrum harmful insecticides to cultivating plant varieties that has low susceptibility to insect attack (Tester, 1977). Pest populations could thereby be suppressed below the level of economic damage with no added pollution and no additional cost to the producer. For determining of low susceptible cultivar, a better understanding of the factors that elicit or inhibit host-plant selection by insects is critical.

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 several serious pests during their growth stages including sap-sucking mealybug, *Icerya seychellarum* (Westwood) (Assem *et al.*, 1991). Long-term observation in Fisher mango orchards located at El-Saff, Giza Governorate, Egypt showed that different mango cultivars express varying levels of resistance to *I. seychellarum* (Maha I. El-Said, unpublished observations). This resistance approaches immunity in the case of the Alphonso mango cultivar (Monzer *et al.*, 2005). In Fisher orchards, mango trees belonging to different cultivars grow next to each other, and expose to the same environmental conditions and resource availabilities. Accordingly, the differences in *I. seychellarum* population between cultivars was obviously not regulated by such environmental factors. Thus it was suspected that the leaf quality state of each cultivar might be the main factor. Investigations on interaction between plants and insect pests suggest that the leaf quality in relation to insect preference could be determined by: 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) that determine the nutritional value of leaves to insect, 3) its contents of certain inhibitors (e.g. phenols, lignin or tannins) which determine the digestibility of the ingested food and/or 4) leaf anatomical and physical characteristics (Habermann, 2000).

In a previous study, we investigated the role of the first leaf quality factor, and showed that Alphonso mango cultivar contains high concentrations of foliar insect toxic and repellant secondary metabolites (*p*-cymene, camphene, and limonene) that could explain its resistance to *I. seychellarum* (Monzer *et al.*, 2005). Obviously, although toxic and repellent substances could explain the immunity of Alphonso mango leaves against *I. seychellarum*, they do not explain the difference in resistance levels between the susceptible cvs. After all, herbivores require nutritive compounds from their diet and insect population grows better on leaves that have an appropriate nutritional balance (Chapman, 2003).

Accordingly, in this study parallel samples from leaves of the five mango cultivars were analyzed for their contents of the two main essential nutrients (carbohydrates and proteins) and inhibitors (phenols and tannins) to determine their role in susceptibility differences among the studied mango cvs.

MATERIALS AND METHODS

Study area: Tree leaves from five Mango cultivars (Sultani, Baladi, Hendi, Ewaisi and Alphonso) were collected from Fisher mango orchard located in El-Saff, Giza Governorate, Egypt at 15 August 2003. The threat of this orchard from the *I. seychellarum* in 2002 and 2003 was assessed to be high with main population peak during August (Maha I. El-Said, unpublished results).

Infestation levels: Population densities of *I. seychellarum* on Sultani, Baladi, Hendi, Ewaisi and Alphonso cultivar leaves were estimated on 3 trees for each cv. Leaf samples (20 leaves/tree) were taken from the four cardinal directions of the middle crown parts in August, 15, 2004. 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.

Chemical analysis: For chemical analysis, leave were sampled for each cultivar using the same procedure described above. Leaves were carefully examined and old, insect damaged, and infected leaves were removed. Healthy leaves of each cultivar were dried separately at room temperature, ground to pass a 1.0 mm sieve. Dried powdered samples were then used to estimate the nutritional quality of the leaves.

Total soluble Protein: Soluble protein was extracted using the methods described by Redak *et al.* (1997). Protein concentration in the final solution was measured colorimetrically at 530 nm using Bradford reagent (Bradford, 1976) with bovine serum albumin as a standard.

Extraction and determination of total carbohydrates and phenolics:

Total soluble carbohydrates and phenolics were extracted using the methods described by Ossipov *et al.* (2001). Total carbohydrates in the purified extract were determined using phenol-sulfuric acid reagent according to the method described by Dubois *et al.* (1956). Quantity of total carbohydrates was estimated by measuring absorbance at 490 nm in spectrophotometer using glucose as standard.

A modification of the Folin-Ciocalteau's method as mentioned by Torres *et al.* (1987) was used to determine the total content of phenolic compounds. Pyrogallol was used for preparing standard curve and the leaf content of phenolics were expressed as μg pyrogallol equivalent/g dry leaf weight.

Extraction and determination of soluble condensed tannins (CT):

Soluble condensed tannins (CT) was extracted and determined according to the method described by Kouki, and Manetas (2002). Given the problems and complexities for applying an appropriate standard for the tannins method, the final absorbance of the red product at 550 nm was used as a measure of condensed tannin concentration (Kouki, and Manetas, 2002). This optical density (OD) value was appropriately corrected to derive the OD_{550} nm/g dry weight

Calculation of a food quality index: A food quality index (FQI) was calculated as described by Habermann, (2000) by dividing the sum of the concentrations of soluble carbohydrates (A) and protein (B) by the contents of condensed tannins (C).

FQI = (A+B)/C

Statistical analysis: All parameters of both of *I. seychellarum* population density on mango leaves, nutrients and inhibitors were reduced to tree-specific means, and these means were used in statistical analyses. Statistical significance was determined by analysis of variance (F-test at P<0.05). The correlations between *I. seychellarum* population density and the FQIs of leaves of the susceptible mango cultivars were tested using regression analysis. All statistical analyses were done using the software package Costat (Costat, 1992).

RESULTS

Figure (1) shows population densities of the *I. seychellarum* on leaves of Sultani, Baladi, Hendi, Ewaisi and Alphonso mango cvs. at the sampling date (August, 15). The highest main number of *I. seychellarum* infestation/leaf was found on Sultani cv. (22.89±1.34) flowed by Baladi (14.11±0.68), then Hendi (10.12±1.38) and Ewaisi (9.15±1.52) while Aphonso leaves were clear of any *I. seychellarum* infestation. The differences in population density between Sultani cv and the other mango cvs and Baladi and the other mango cvs. were significant (P<0.05, F-test), while there were no significant difference in population density between Hendi and Ewaisi mango cvs (P>0.05, F-test).

Carbohydrate contents of the studied mango cultivar leaves are shown in Fig. (2). Sultani leaves were found to contain significantly higher carbohydrates (91.70± 5.6 mg/g dry weight) than the other four cultivars, while there were no significant differences in carbohydrates between Baladi, Hendi, Ewaisi and Alphonso mango cvs. (78.1± 4.2, 80.52 ± 3.9, 78.04 ± 7.1 and 79.36±5.0 mg/g dry weight, respectively, P>0.05, F-test).

Fig. (3) shows that leaves of Sultani mango cultivar contained significantly higher concentration of total soluble proteins (122.66±5.72 mg/g dry weight) than the other four cvs., while leaves of Hendi cv. contained significantly less soluble protein (72.83 ± mg/g dry weight) then the other studied mango cvs. There were no significant differences in leaves total soluble protein between Baladi, Ewaisi and Alphonso cvs. (99.66 ± 3.71, 93.91 ± 3.22 and 103.49 ±4.61 mg/g dry weight, respectively, P>0.05, F-test).

Analysis of leaves for total phenolic compounds (Fig. 4) indicated that leaves of Sultani cv. contained the lowest concentration (62.66 ± 6.35 mg pyrogallol equivalent/g dry weight) followed by leaves of Baladi cv (72±2.1855 mg/gdry weight) then Ewaisi (85.67±3.510 mg/g dry weight) and Alphonso (92.67±4.55 mg/g dry weight), while leaves of Hendi cv. contained the highest concentration of total phenolics (104.0±3.78 mg/g dry weight). The differences in total phenolics between

Sultani, Baladi, Hendi and Ewaisi were significant (P<0.05, F-test) while there were no significant difference in total phenolics between Ewaisi and Alphonso cvs.

Concentrations of soluble condensed tannins (CT) in leaves of different Mango cvs. (as expressed in absorbance equivalents/g dry weight) are represented in Fig. (5). Ewaisi and Hendi leaves contained the highest concentration of CT followed by Baladi, then Sultani, while Alphonso leaves contained the lowest CT concentration. The differences in leaf CT between all the studied mango cvs. were significant (P<0.005, F-test) except that between Ewaisi and Hendi, and Sultani and Alphonso cvs. which were insignificant.

Table (1) shows the food quality index (FQI) for leaves of each mango cvs. Leaves of Sultani and Alphonso cvs had the highest FQI (27.62 ± 1.22 and 24.47 ± 0.90, respectively), followed, by Baladi leaves (17.11 ± 0.66) then Ewaisi (13.9 ± 0.42) and Hendi (13.08 ± 0.35). The difference in FQI between Hendi and Ewaisi was not significant (P>0.05, F-test). Statistical analysis showed that population densities of the *I. seychellarum* on leaves of the four susceptible cvs. (Sultani, Baladi, Hendi, and Ewaisi) correlated strongly with their corresponding FQIs (r = 0.83).

DISCUSSION

To best of our knowledge, this is the first study that examines the relation between the differences in susceptibility of different mango cultivars to *I. seychellarum* and concentrations of major nutritive compounds together with concentrations of total phenolics and tannins in their leaves. Long-term observation in the fisher mango orchard showed that main peak of *I. seychellarum* population was at August. Also, the susceptibility of mango trees to *I. seychellarum* infestation differs among different cultivars (Maha I. El-Said, unpublished observations). In this study, the obtained data on levels of *I. seychellarum* population density at sampling date (15 August, 2003) confirmed this observation. The order of susceptibility levels of mango cvs. to *I. seychellarum* could be arranged in descending order as follows: Sultani (highly susceptible) > Baladi > Hendi = Ewaisi > Alphonso (completely resistant). Influences of the environmental conditions and resource availability on the level of cultivar resistance to *I. seychellarum* were excluded because the sample trees of all cvs. were next to each other. These findings together with the results of the previous study (Monzer *et al.*, 2005) suggested that different mango cvs. could have different nutritional values that affecting *I. seychellarum* choice of its host trees. The measured traits in this study are potentially important determinants of leaf quality as a food for the generalist insect herbivores. Results showed that the most susceptible mango cv. (Sultani) contained the highest concentrations of both total carbohydrates and soluble proteins and the lowest concentrations of both total phenolics and tannins in comparison with the other 4 cultivars. Carbohydrates and proteins are generally assumed as the primary arrestant (chemical serving as effective phagostimulant and thereby maintaining prolonged feeding by the pest) for insects belonging to various taxa (Chapman, 2003). Carbohydrates are the main source of energy and Proteins are the major source of amino acids and nitrogen for insects (Jain *et al.*, 2000). Thus low concentrations of such essential nutrients in mango leaves may decrease the tree's suitability for *I. seychellarum*. Phenolic compounds are produced in higher plants for several functions, one of which is defence against herbivores (Riipi *et al.*, 2002). They have traditionally been considered to play an important role in plant-herbivore interactions (Woda-Lesniewska and Giebel, 2002). Condensed tannins represent a major group of plant phenolics, and their concentrations have been shown to have adverse affects on various herbivores, the effects are considered to be caused by inhibiting the herbivore's digestive enzymes and by forming strong molecular complexes with ingested proteins in the gut of insects (Ossipov *et al.* 2001 and Riipi *et al.*, 2002).

Accordingly, the relatively low level of phenolics and tannins may contribute to the high susceptibility of Sultani mango cultivar, along with the relatively high leaf concentrations of carbohydrates and proteins compared with leaves of Baladi, Hendi and Ewaisi cultivars.

However, in attempts to associate the difference in susceptibilities of Baladi, Hendi, and Ewaisi to *I. seychellarum* with their leaf chemistry, we have found some contradictory results. Although Baladi, and Hendi (or Ewaisi) cultivars were differing significantly in their susceptibility to *I. seychellarum*, they were not significantly differing in their contents of carbohydrates. Moreover, leaves of Hendi cultivar contained significantly lower protein concentration and higher phenolics concentration than Ewaisi cultivar without any significant differences between their susceptibilities. This discrepancy could be explained by results of the calculated food quality index (FQI). Leaf quality in relation to generalist insect herbivores depends largely on FQI, i.e., the relative concentrations of compounds with different physiological effects (arrestant and inhibitors) rather than on the absolute concentrations of individual leaf compounds (Lunderstadt, 1980). No phytophagous insect is known that tastes all its essential nutrients, and the ability to discriminate between nutrients is limited. The insects acquire a nutritional balance largely "adventitiously" because leaves have an appropriate chemical composition and proportions (i.e. FQI) (Chapman, 2003). Thus, population of insects feeding on leaves with higher FQI grow better than that feed on low FQI leaves. The ability of phenolics to inhibit food digestion and uptake depends on the nature of its compounds. Tannins are the main group of phenolic compounds that decreases leaf digestibility and quality as described by several studies

(Habermann, 2000, Ossipov *et al.* 2001 and Riipi *et al.*, 2002). Thus tannins, but not total phenolics, were used to calculate FQIs.

In the present study, values of FQIs displayed significant positive correlation with levels of population density on leaves of each of the studied susceptible mango cvs. Sultani cv. had the highest FQI and susceptibility, followed by Baladi then Hendi and Ewaisi, indicating that FQI plays an important role in determining suitability of mango leaves for *I. Seychellarum.* On the other hand, Alphonso cv. had relatively high FQI in spite of its complete resistance. However, we previously showed that Alphonso cv, alternatively, defined itself by producing certain insect toxic and repellent secondary metabolites (foliar *p*-cymene, camphene, and limonene) while the susceptible cvs (e.g. Sultani) lack of such compounds (Monzer, *et al.*, 2005).

Accordingly, it could be concluded that the levels of susceptibility of mango cvs. for the generalist sap sucking mealybug, *I. seychellarum* depend on the combined action of leaf nutrients, inhibitors and secondary metabolites which determined the food quality of their leaves. The relative role of these factors, however, may differ in different cultivars. Further study on the possible implication of leaf anatomy and physical properties in susceptibility of different mango cvs. to *I. seychellarum* is in progress.

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Table 1. Food quality index (FQI) for leaves of Sultani, Baladi, Hendi, Ewaisi and Alphonso mango cultivars.

Mango Cultivar	Food Quality index FQI (Mean ± SD)
Sultani	27.62 ± 1.22 A
Baladi	17.11 ± 0.66 B
Hendi	13.08 ± 0.35 C
Ewaisi	13.9 ± 0.42 C
Alphonso	24.47 ± 0.9 D

Values followed by different letters are significantly differed ($p < 0.05$, F-test)

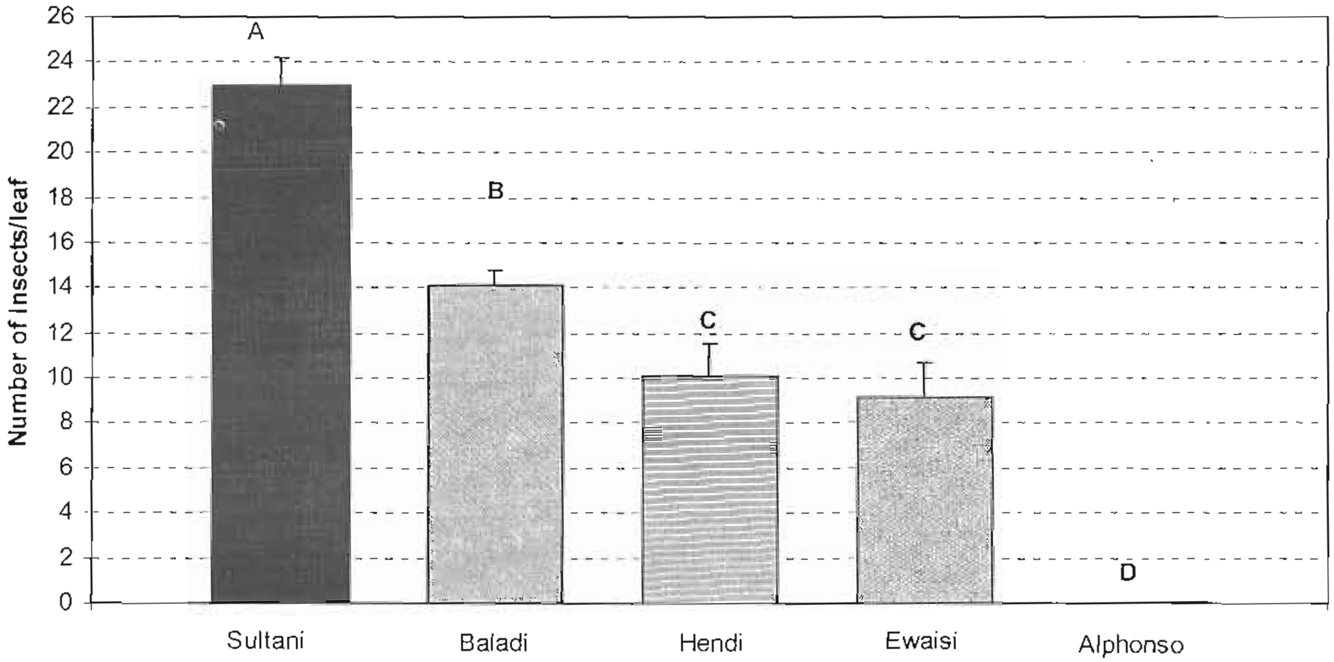

Fig. 1. Population density of *I. seychellarum* on leaves of the studied mango cultivars. Data are means ± SD. Different letters over each column indicate statistically significant differences ($p < 0.05$, F-test).

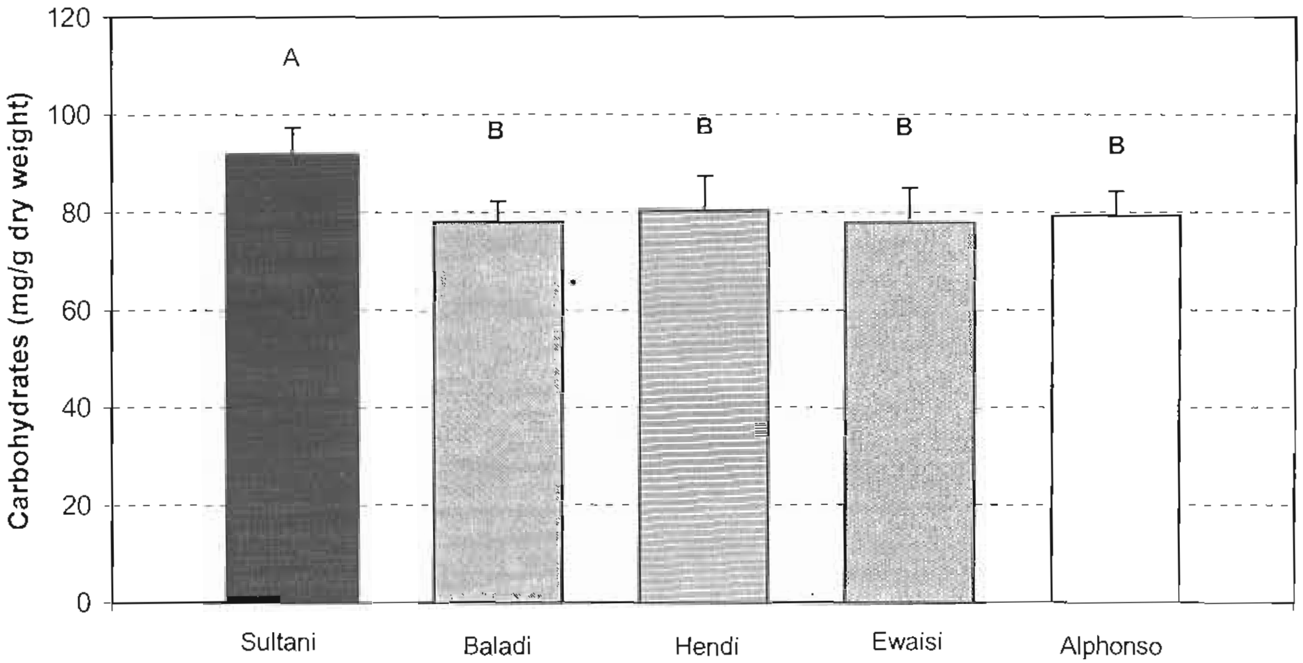

Fig. 2. Total leaf carbohydrates of the studied mango cultivars. Data are means ± SD. Different letters over each column indicate statistically significant differences ($p < 0.05$, F-test).

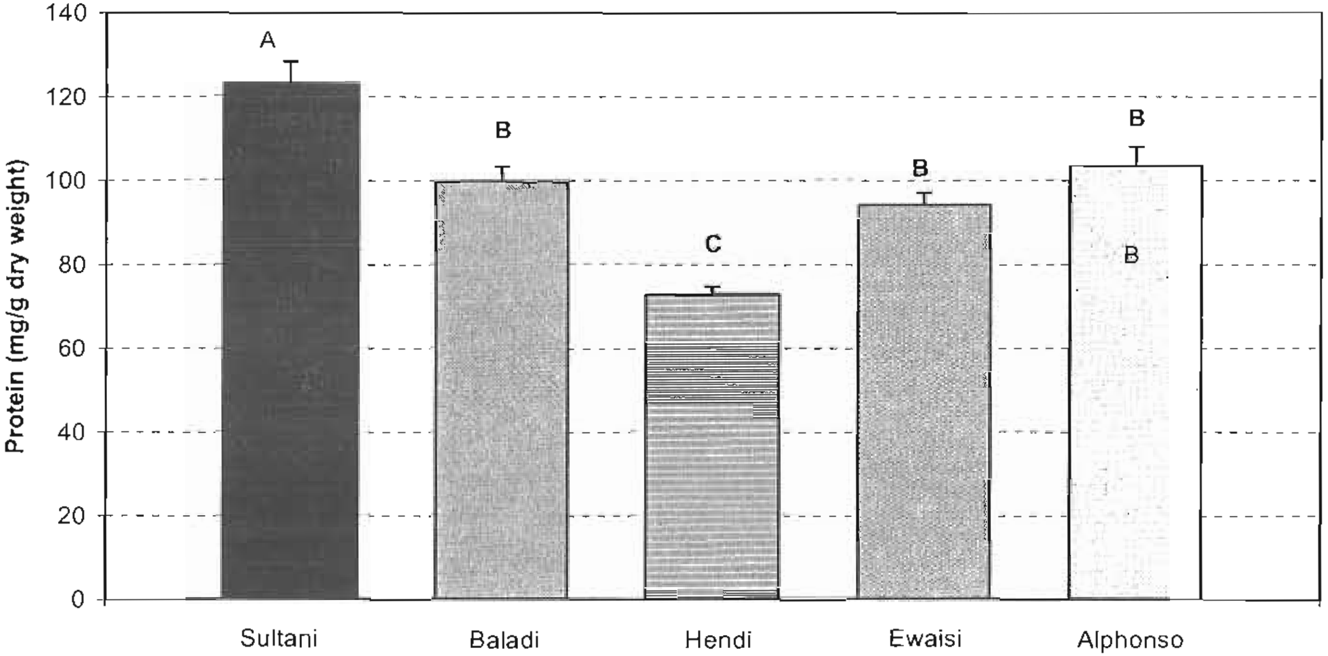

Fig. 3. Total leaf soluble proteins of the studied mango cultivars. Data are means ± SD. Different letters over each column indicate statistically significant differences ($p < 0.05$, F-test).

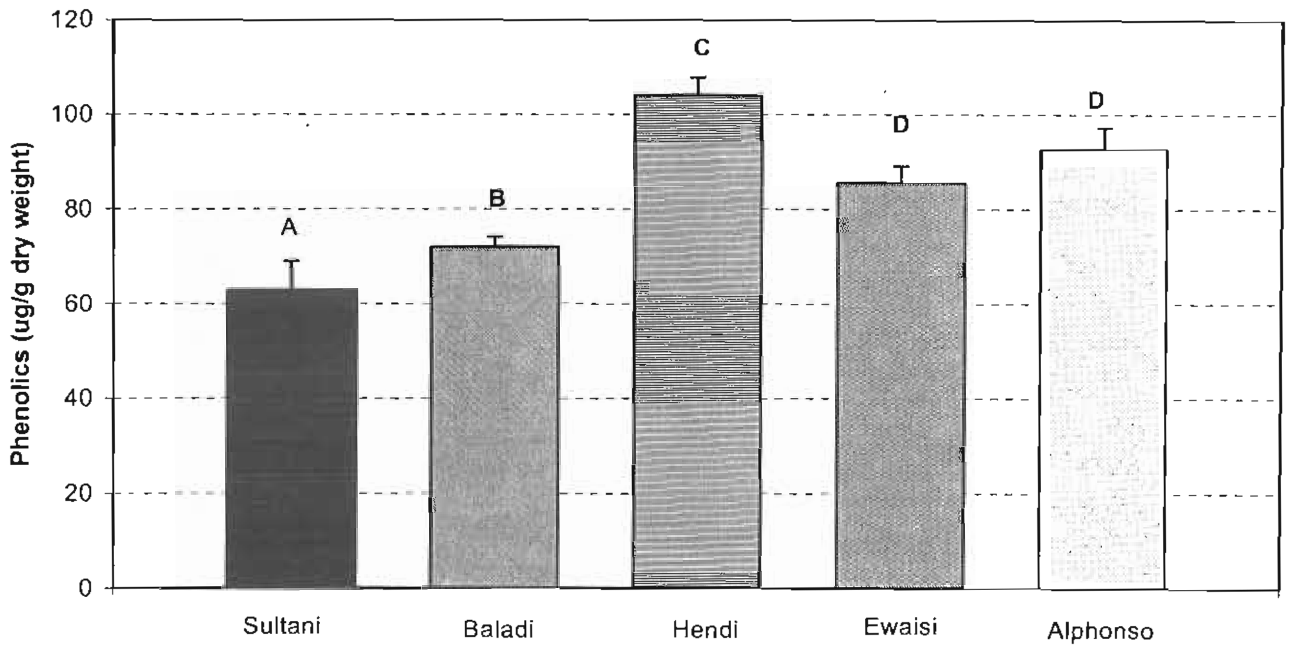

Fig. 4. Total leaf phenolics of of the studied mango cultivars. Data are means ± SD. Different letters over each column indicate statistically significant differences ($p < 0.05$, F-test).

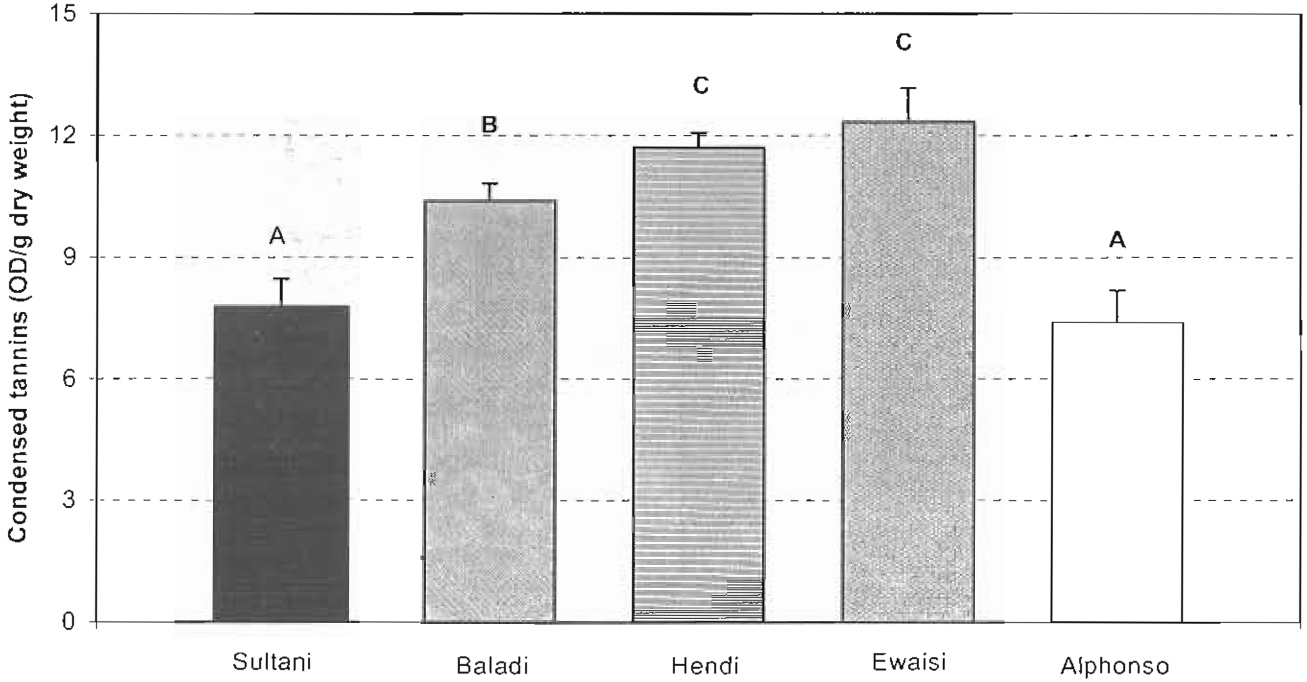

Fig. 5. Leaf condensed tannins of the studied mango cultivars. Data are means $\pm$ SD. Different letters over each column indicate statistically significant differences ($p < 0.05$, F-test)

العلاقة بين قابلية خمسة أصناف من أشجار المانجو للإصـــــــــابة بالبق الدقيقى *Icerya Seychellarum* وخصائص أوراقها ومحتوياتها من المواد الغذائية ومثبطاتها

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تم استكشـــــاف قابلية خمسة أصناف من أشجـــار المانجو للاصابة بالبق الدقيقى المعـــروف باسم *Icerya Seychellarum* ودراسة العلاقة بين التباين فى قابلية تلك الاصناف للإصـــابة ومحتـــوى أوراقها من بعض المواد الغذائية والمواد المثبطة للتغذية الرئيسية. وقد تم تحليل محتويات عينـــات مـــن أوراق المانجو من أصناف سلطانى، بلدى، هندى، عويسى و ألفونسو من حيث محتواهـــا مـــن مـــادتين غذايتين هامتين هما النشويات والبروتينات الكلية بالاضافة الى محتواها من الفينولات والتانينات الكليـــة. ومن النتائج المتحصل عليها قد تم حساب مدى جودة الأوراق كغذاء لتلك الآفة. وقـــد وجـــد أن ترتيـــب أصناف المانجو طبقا لمستوى الاصابة بتلك الآفة كان كالتالى: صنف سلطانى (الأعلى فى نسبة الإصابة) يليه صنف البلدى ثم صنفى الهندى والعويسى. وقد أظهرت نتائج حساب مدى جودة أوراق كل صنف من حيث القيمة الغذائية للحشرة أن أوراق صنف السلطانى هى الأعلى جودة يليها أوراق صنف البلدى، يليهما صنفى الهندى والعويسى. وتشير النتائج فى مجملها إلى أن مستوى قابلية أوراق أصناف المانجو المختلفة للإصابة بتلك الآفة يعتمد على الفعل المشترك لمحتواها من المواد الغذائية ومثبطاتها ومواد الايض الثانوية وهى العوامل التى تحدد مدى جودة الاوراق كغذاء للآفة، وأن الدور النسبى لكل عامل من هذه العوامـــل يختلف من صنف الى آخر.