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Effect of *Parlatoria blanchardi* Targ. *(Homoptra, Coccidae)* on the Metabolites in the Leaves of Date Palm (*Phoenix dactylifera* L.)

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ABSTRACT. The effect of *Parlatoria blanchardi* on some metabolites of healthy and injured leaf discs of date palm (*Phoenix dactylifera* L.) was studied. No significant changes were observed in the reducing and non-reducing sugars of leaf discs before and after injury by the insect. The analysis of soluble sugars by GLC revealed the presence of fructose, glucose, inositol, sucrose, and glycerol. However, the kind of soluble sugars changed in the injured leaf discs; glycerol content increased and sorbitol appeared after the injury. Organic acids did not change significantly in healthy and injured leaf discs. Soluble protein content, however, increased significantly in injured leaf discs as compared to healthy ones.

Annon (1978) stated that 400 different date cultivars are grown in the Kingdom of Saudi Arabia. Most of these cultivars are attacked by various pest species, which may cause damage (Stickney *et al.* 1950, Talhouk 1969, and Martin 1972). *Parlatoria blanchardi* is one of these insect pests to which many of the date palm varieties are susceptible (Dabbour 1981, and Dabbour and Hammad 1982). This insect is found in greater number on the dorsal side of the lower leaves than on the upper leaves (Dabbour 1981). Infestation is characterized by the white appearance of palm caused by scale population covering the tree. *P. blanchardi* spreads over the entire leaf surface and as the infestation increased, the pinnae become infected (Sachan 1976). The insect sucks the material and affect the leaf which becomes weak and decay. The insect also attacks the fruits which becomes deshaped and dry up (Dabbour and Hammad 1982).

The damage caused may be reflected in a qualitative changes in the nutrient values of the date. So, many epidemic diseases have serious economic consequences (Carpenter and Elmer 1978 and Hammad *et al.* 1981). Major date varieties grown in the Middle East have been physically and chemically characterized. Some

studies have been carried out on various metabolites of the date palm seeds (El-Shurafa *et al.* 1982). Hussain and El-Zeid (1975) showed that the flesh and the seeds of var. Khalas contained 13 essential amino acids and some reducing and non-reducing sugars.

Chemical analyses were undertaken in some plants attacked by some of the isects. Soluble carbohydrate, free amino acids and major inorganic constituents were determined in the leaves of pignut hickory, *Carya glaba* (Mill). Sweet, and southern red oak, *Quercus falcata* Michx., hosts of *Ennomos subsignarius* (Hubnew), (Clark and White 1971). The foliar feeding aphids, *Monelliopsis nigropunctata* (Granovsky), *Monellia caryella* (Fitch.), and *Melanocallis caryaefoliae* (Davis), exhibited reduction in dry weight of root and main stem, chlorophyll content of the leaves, starch content of root and starch and total carbohydrate content of the stem (Tedders *et al.* 1982).

However, to my knowledge, there is no information available on the effect of *P. blanchardi* on the metabolites in leaves of date palm. Consequently, in the present study, the effect of *P. blanchardi* on the changes and nature of metabolites has been carried out.

Material and Methods

Plant Material

Leaves of date palm (*Phoenix dactylifera* L.), healthy and injured by the insect were collected from a farm on Al-Kharj Road (Riyadh), in March 1984. There were 7 to 11 insects per 1 cm^2 of leaf. The insect was identified as *Parlatoria blanchardi* Targ. (Talhouk 1984). Leaf discs were sampled immediately after collection using a cork boarer of 10 mm diameter. Samples were taken from healthy and injured leaf discs in triplicate, each replication consisting of 20 discs.

Extraction Procedure and Analysis of Extracts

Leaf discs were extracted by boiling under reflux with 80% ethanol with three changes of the solvent as previously (Basalah 1984). The soluble extracts were fractioned into the ethanol-soluble fraction (neutral), amino acid (basic fraction), and organic acid (acid fraction) using two types of ion exchange resins [Dowex 50 × 8-200 (H^+) 100-200 mesh and Dowex 1 × 8-400 (Cl^-) 200-400 mesh], Basalah (1984).

Carbohydrate Estimation

Soluble carbohydrates were estimated by the method of Somogyi (1945) before and after hydrolysis with 1.5 N H_2SO_4. Quantitative estimation of soluble carbohydrates was done by gas-liquid chromatography (GLC), as trimethysilyl

derivatives (TMS), (Holligan and Drew 1971).

Organic Acids Estimation

Free organic acids were estimated in the acidic fraction by titration with standard NaOH. Combined organic acids were measured by further titration with standard HCl (Basalah *et al.* 1984). Both of the measurements provided a quantitative reading.

Protein Estimation

Soluble proteins were assayed in the basic fraction using the Lowry *et al.* (1951) method.

Analyses of Data

Data obtained from the experiment were analysed with the analysis of variance, except the qualitative estimation of carbohydrates by GLC.

Results

Soluble Carbohydrate Content

Table 1 shows the soluble carbohydrate content of healthy as well as injured leaves by *P. blanchardi*. It is clear from the Table that reducing sugars increased while non-reducing sugars decreased in injured leaves as compared to healthy leaves. However, the changes were slight. By analysis of variance (ANOVA) test at probability 0.05% (Table 2) for results in Table 1, shows that reducing sugars before and after hydrolysis, and non-reducing sugars in healthy and injured leaves differ non-significantly.

Table 1. Soluble carbohydrate contents of healthy, and injured leaves by *Parlatoria blanchardi* of date plam.*

Condition of the leaf	Carbohydrate content (mg/g dry weight)		
	Reducing sugars		Non-reducing sugars
	before Hydrolysis	after Hydrolysis	
Healthy	1.00	1.04	0.04
Injured	1.40	1.42	0.02

* The values are means of three estimates.

Table 2. ANOVA table for analysis of soluble carbohydrate content of healthy and injured leaves of date palm.

Source	D.F.	S.S.	M.S.	F. Value	PR F
[1] Condition of leaf	1	0.09	0.09	1.80	4.75**
[2] Parameter	2	6.52	3.26	65.20	3.89*
Parameter × condition of the leaf	2	0.15	0.08	1.60	3.89*
Error	12	0.54	0.05		
Total	17				

1 = Healthy and injured
2 = Reducing sugars (before and after hydrolysis) and non-reducing sugars
* = Significant
** = Non-significant.

Quantitative Estimation of Carbohydrates

Figure 1 shows GLC traces of the ethanol soluble fraction of carbohydrates in healthy and injured leaf discs. Identification of sugars in GLC traces was done by calculating their retention times with respect to β-glucose (RTβg values) (Table 3).

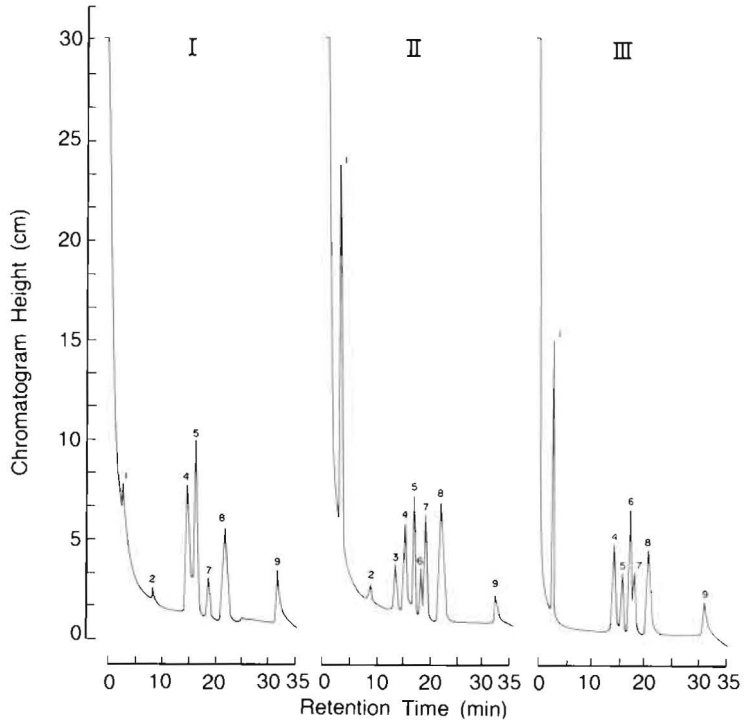
As indicated by Fig. 1 and Table 3, glycerol, an un-identified sugar (peak No. 2), fructose, α-glucose, β-glucose, inositol and sucrose were present in both healthy and injured leaf discs. Peak No. 3 and sorbitol appeared in injured leaf discs only, as well as exceedingly high amount of glycerol.

Organic Acid Content

Table 4 lists the soluble organic acid content of healthy as well as injured leaves by *P. blanchardi*. It revealed that weak organic acids decrease while strong and total organic acids increased with injury as compared with healthy leaves, but the changes were very small. ANOVA at 0.05% probability (Table 5) for these results revealed that changes in weak, strong and total organic acids due to injury were non-significant.

Soluble Protein Content

Table 6 indicates soluble protein content of healthy as well as injured leaves by *P. blanchardi*. It reveated that protein content increased within the injured leaves as compared to healthy ones. ANOVA at probability 0.05% (Table 7) for these results showed that the difference of protein content in healthy and injured leaves was significant.



Temperature programme: 140-290°C at 40°/min, attenuation 8 × 10^3. Abbreviations: I=Healthy, II=Injured, III=Marker, I=Glycerol, 2=Peak No. 2, 3=Peak No. 3, 4=Fructose, 5=α−glucose, 6=Sorbitol, 7=β−glucose, 8=Inositol, 9=Sucrose.

Fig. 1. GLC analysis of TMS derivatives of soluble carbohydrates in healthy and injured leaves of Date Palm.

Table 3. Relative retention times of peaks with respect to β-glucose peak. (RTβg) of the soluble carbohydrates present in ethanol soluble fractions of healthy, and injured leaves by *Parlatoria blanchardi* of date plam.

Possible identity of sugar with similar RT g	Mean RTβg* in		
	Marker	Healhty leaves	Injured leaves
Glycerol	0.241	0.240	0.241
Peak No. 2	0.567	0.567	0.566
Peak No. 3	0.820	0.820	0.820
Fructose	0.844	0.844	0.844
α-Glucose	0.936	0.936	0.936
Sorbitol	0.980	0.980	0.980
β-Glucose	1.000	1.000	1.000
Inositol	1.214	1.214	1.214
Sucrose	1.895	1.895	1.895

* = Values of RTβg listed are means of three estimates.

Table 4. Soluble organic acid contents of healthy leaves, and leaves attacked by *Parlatoria blanchardi** of date palm.

Condition of the leaf	Organic acid content (ml/100 ml of sample solution)						
	Weak organic acid			Strong organic acid			Total organic acids
	Free	Combined	Total	Free	Combined	Total	
Healthy	1.71	0.15	1.86	4.20	0.25	4.45	6.31
Injured	0.98	0.16	1.14	4.45	0.25	4.70	5.85

* The value are means of three estimates.

Table 5. ANOVA table for analysis of soluble organic acid content of healthy and injured leaves of date palm.

Source	D.F.	S.S.	M.S.	F. Value	PR F
[1] Condition of leaf	1	0.42	0.42	1.56	4.18**
[2] Parameter	6	199.79	33.30	123.33	2.34*
Parameter × condition of the leaf	6	1.79	0.30	1.11	2.34*
Error	28	7.52	0.27		
Total	41				

1 = Healthy and injured
2 = Weak organic acids (free, combined, total), strong organic acids (free, combined, total) and total organic acids.
* = Significant
** = Non-significant.

Table 6. Soluble protein contents of healthy leaves, and leaves attacked by *Parlatoria blanchardi* of date palm*

Condition of the leaf	Protein content (mg/g dry wt.)
Healthy	1.84
Injured	3.50

* Protein content (mg/g dry wt.)

Table 7. ANOVA table for analysis of soluble protein content of healthy and injured leaves of date palm.

Source	D.F.	S.S.	M.S.	F. Value	PR F
[1] Condition of leaf	1	4.13	4.13	13.41	7.71*
Error	4	1.23	0.31		
Total	5	5.37			

1 = Healthy and injured
* = Significant

Discussion

The GLC analysis demonstrated that glycerol, fructose, α-glucose, β-glucose, inositol and sucrose were present in leaves of date palm. These results are in agreement with Jaddou and Hakim (1980). However, their studies were carried out on semi-dry dates. There is a clear difference in the nature of the sugars of the healthy and injured leaves (Fig. 1 and Table 2). Nevertheless in the injured leaves of the present study, sugar alcohols (sorbitol and glycerol) were detected but not in the healthy ones. As far as the author aware the literature on the effect of insects on the metabolism of date plam is scarce; this may be the first report on the accumulation of sugar alcohols in the leaves of date palm injured by *P. blanchardi.* However, this is similar to the previous reports on the accumulation of sugar alcohols around the sites of the fungal infection (Holligan *et al.* 1973, 1974, and Basalah 1984); and also presence of three simple sugars and one sugar alcohol in the honeydew accumulated on the pecan leaves fed by foliar aphids (Tedders *et al.* 1982).

Although, on the other hand, no significant differences were found in the soluble carbohydrates, there was an increase in the amount of reducing sugars due to injury. The soluble protein content also increased in injured leaves whereas organic acid content did not. In the pecan leaves, the foliar feeding aphids reduced the starch content of the root as well as starch and total carbohydrate content of stem (Tedders *et al.* 1982). In the present investigations the starch content of the leaves of date palm was not studied, but it can be speculated that some insoluble carbohydrates may have been hydrolysed by certain enzymes which resulted in an increase in the amount of reducing sugars. It seems that the presence of high amounts of soluble proteins in the injured leaves may be due to more mobilization of proteins at the site of injury than the other compounds. Much of the soluble protein is probably insect enzyme injected into the leaf tissue *via* the insect feeding stylet. However, this is speculative and will require further investigation.

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تأثير حشرة بارلاتوريا بلانشاردي على الأيض في أوراق نخيل البلح

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درس تأثير حشرة بارلاتوريا بلانشاردي على بعض المركبات الأيضية في أوراق نخيل البلح. لم تلاحظ تغيرات ملموسة في محتوى السكريات المختزلة وغير المختزلة قبل وبعد حدوث الإصابة بالحشرة لأوراق النخيل. أوضح تحليل السكريات الذوّابة بواسطة جهاز الفصل اللوني الغازي وجود الفركتوز والجلوكوز والأينزتول والسكروز والجليسرول. وقد تغيرت نوعية السكريات الذوّابة في الأوراق المصابة وإزداد محتوى الجليسرول كما ظهر السربتول بعد الإصابة. لم يحدث تغير ملموس في الأحماض العضوية قبل وبعد الإصابة، بينما ازداد محتوى البروتينات الذوّابة زيادة ملموسة في الأوراق المصابة مقارنة بالأوراق السليمة.