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# BOTANY

## Some Metabolic Changes of Chlorotic and Green Leaflets of Date Palm Tree

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**Abstract.** Leaflet samples of two date palm (*Phoenix dactylifera* L.) trees showing either infection by green scale insects or unknown pathogen, but yellow leaflets only were compared with other healthy green leaflets. As expected, analyses showed a significant decrease of chlorophyll a and the total, but not chlorophyll b. Also, a significant increase in the dry weight/fresh weight ratio was observed in the infected trees. Total and reducing sugars were significantly higher in the infected leaflets than the healthy ones. Phenolic compounds increased significantly in the tree infected by the green scale insect only. Elemental contents of total N, Ca, K, Cu, Mn and Cd were different in diseased and healthy leaflets, but P, Mg, Zn, Fe, Sc and B contents have not changed. Total RNA was found to be significantly greater in the diseased leaflets $D_2$, which could be an indication of possible infection by virus, viroid or mycoplasma-like organisms, but some fungi were deteced after culturing, which could be secondary invaders.

### Introduction

The date palm (*Phoenix dactylifera* L.) groves and orchards grow in the phytogeographical region known as the Saharo-Sindian region as well as some places in the United States. It is very important tree in Saudi Arabian Kingdom for its fruit (dates) where threre are more than 400 different cultivars [1]. Like other plants, these cultivars are attacked by various pests and diseases. These pests and diseases range from insects, mites, nematodes, fungi, bacteria, viruses, to myoplasma like organisms. These organisms cause a wide range of damage and even death of the tree which results in great economical losses [2-10].

Few comparisons have been made of the gross biochemical changes in the infected date palm tissues harboring these pests and diseases. For example, basalah [11] found that the kinds of soluble sugars changes and protein content increased significantly in the infected leaf discs with scale insect *Parlatoria blanchardi* Targ. Therefore, it is the objective of this work to estimate these changes between the healthy and appearing diseased date palms. This kind of change has been studied in other systems [e.g., 12].

## Materials and Methods

### Plant Material

Leaflets of date palm (*Phoenix dactylifera* L.) were sampled on 14 December 1992, from both chlorotic (designated as D, about 2 years old) and green, healthy (designated as H, about 1 year old) fronds of the same tree for analysis. These were grown in different locations on the University Campus. One group was on the main road, with trees planted in the isle between the two sides (samples $D_1$, for diseased leaflets and $H_1$, for healthy ones). The pest infesting these trees was identified as the green scale insect, *Asterolecanium phoenicis* (Romachandra Rao) (Homoptera, Asteroleaniidae) (identified by Dr. J. Al-Saaday of King Faisal University at Al-Hassa). The other location was a batch of palm trees beside the department building, which is relatively far from the main road (samples $D_2$ for chlorotic leaflets and $H_2$ for green healthy ones). No pests could be isolated, but when leaflet discs from the yellow fronds, were cultured some fungi were growing on the media as will be mentioned in the results.

### Chemical analysis

Dry weight: fresh weight ration was determined by drying the entire leaflet at 60°C until constant dry weight was reached. Total and both a and b chlorophylls were estimated according to the method of Inskeep and Bloom [13]. Total sugars were determined by the method of Dubois *et al.* [14], while reducing sugars by Somogyi procedure [15]. Starch was estimated according to the methods of A.O.A.C. [16]. Folin-Denis test was employed to estimate phenolic compounds [17]. Orcinol method was used for estimating the tissue content of RNA with the use of a standard curve [18]. Elemental contents were analyzed by a Pye Unicam Atomic Absorption Spectrophotometer Sp9 (equipped with SP9 computer), except B, P and Se were estimated colorimetrically by the methods reported by A.O.A.C. [16], Watanabe and Olsen [19] and Al-Showiman *et al.* [20], respectively. All estimations were of at least three replicates. One way analysis of variance was followed, as described by Steel and Torrie [21] and when F-value was significant, Duncan's New Multiple-Range Test was used to compare content means of Healthy leaflets (H) with those of diseased ones (D).

## Results and Discussion

One of the most obvious features of plant diseases is the decoloration on necrosis of Infected tissues. Figure 1 shows the differences in chlorophyll contents (Chl a, b and total) between diseased and healthy leaflets of date palm. No significant differences (all statistical anlyses are at 0.01% probability, unless stated otherwise) in Chl b between all samples (healthy or diseased), but a very significant decrease in Chl a of both diseased leaflets was found. This decrease is reflected on the total Chl where the differences were very signficant. This is very obvious for diseased tissues and in agreement with the findings in other plants [2], but the changes were in Chl a, but not Chl b. This would suggest a reduction in photosynthesis, as Chl a is a component of photosystem centers, not an accessory pigement as Chl b.

Dry weight: Fresh weight ratio (on a unit leaflet basis), as shown in Fig. 1, indicates that there is a significant difference between infected and healthy leaflets, where the

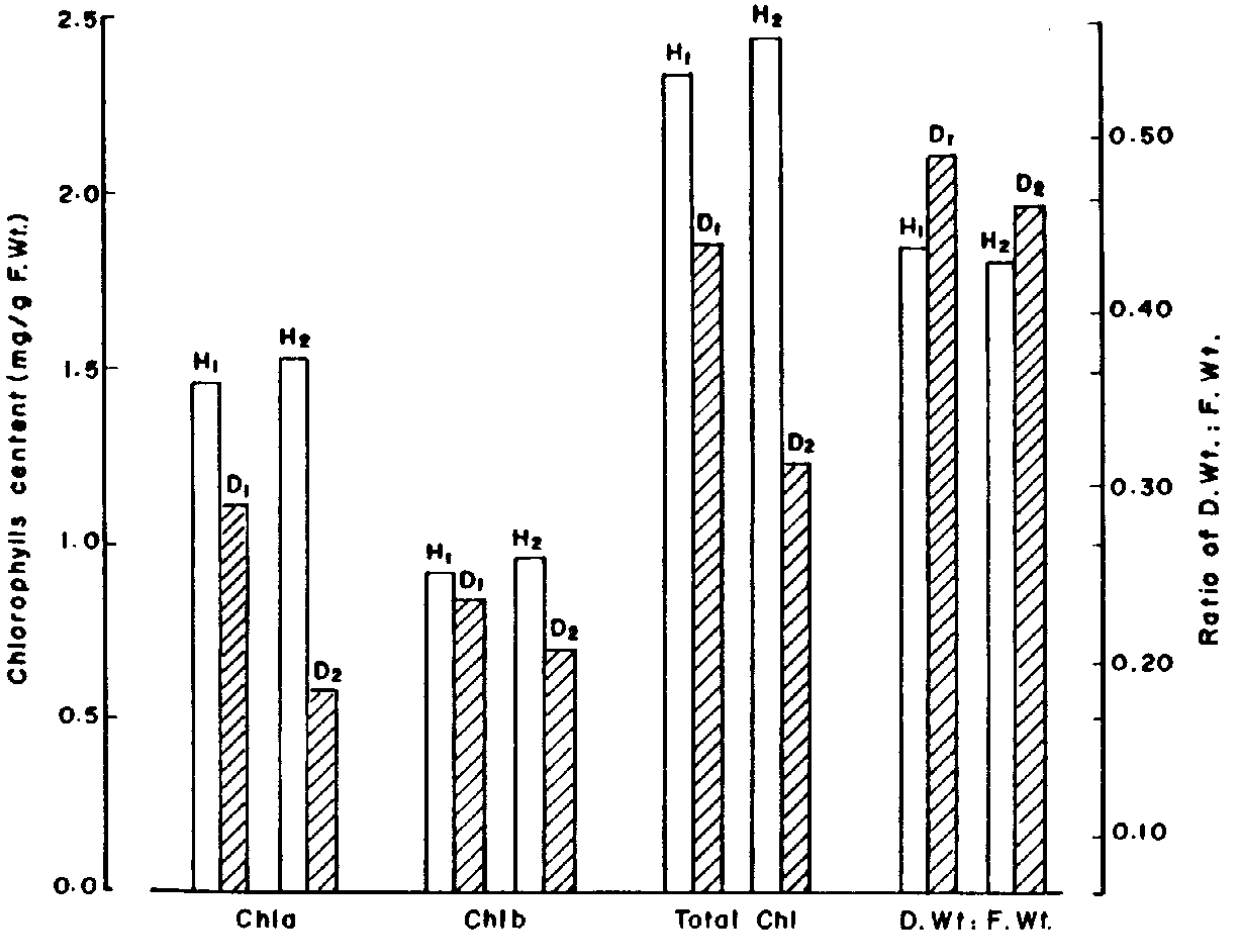


**Fig. 1. Contents of chlorophyll (a, b and total) and fresh weight (F.wt.): dry weight (D.wt.) ratio of healthy (H) and diseased (D) leaflets of both trees (1 and 2).**

infected ones have a higher weight than healthy ones. The increase was by 10% in the case of D1 and by 9% in the case of D2. This might indicate a pathogen resistant to water movement in the plant, as suggested earlier [22].

In relation to total sugars (Fig. 2), they were significantly higher in the diseased leaflets, which is due to an increase in the reducing sugars as it is obvious from Fig. 2. However, the magnitude of increase of reducing sugars was very high in the case of D2 (the date palm tree with yellow symptoms and no pest could be identified except after culturing as mentioned later). It is appropriate to mention that this tree has not produced any spathe for almost 10 years). These findings for the tree infected by insect (H2 and D2) are in agreement with the results of date palm infected with the scale insect *Parlatoria blanchardi* Targ. [11]. No specific interpretation could be drawn. However, infection by parasites alter the metabolism of tissues so that respiratory substrates move towards the site of infection from elsewhere in the plant, as was suggested earlier by Wood [19] and the discussion of other results from plants infected by different diseases.

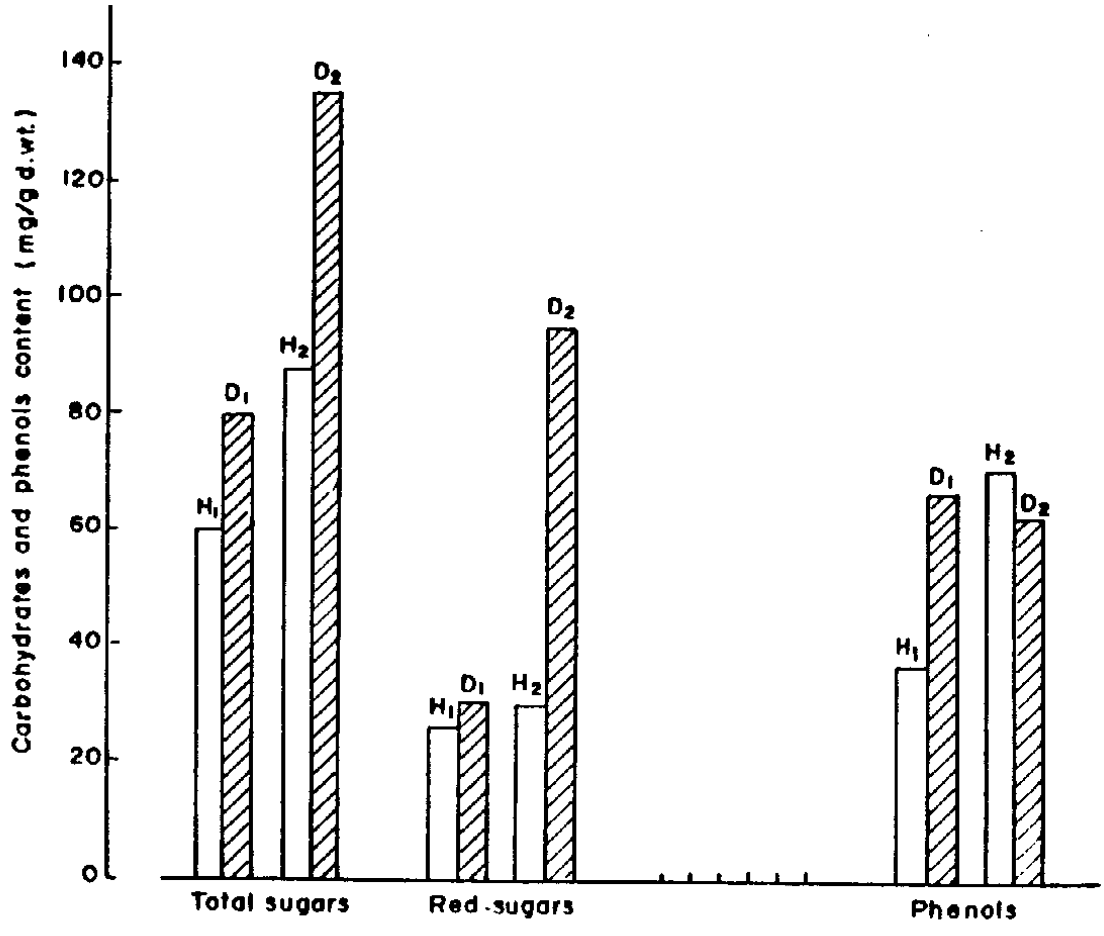


**Fig. 2. Carbohydrates (total and reducing sugars (red. sugars) and total phenols of the leaflets of the two trees**

Alternatively, it is possible that the responses of date palm tree to different diseases are not the same as shown in Fig. 2, for the two diseased plants (D1 and D2). The results of this study show similar trends as that reported by Long and Cooke [23]. Testing the carbohydrate changes in the extracts of both healthy and diseased leaflets by GLC after derivatization (data not included), it seems that there are several differences between healthy and diseased ones. Arabinose ($\propto$ and $\beta$), for example, disappeared from leaflets infected by the insect (D1) but not in the case of D2. There are also some differences in the amouts of different sugars (estimated by the peak area) between healthy and diseased leaflets, as was reported earlier for other diseases [23]. These findings need further confirmation. Starch could not be detected in all samples.

Phenolic compounds increased significantly in the case of D1 (infected by the green insect), but not in D2 (Fig. 2). This is possibly another indication of the different response of date palm to different diseases. However, this is in agreement with what has been reported earlier in virus-infected cucumber leaves [24], although other reports [e.g., 25] have different results. The increase of phenolic compounds is a characteristic of a large number of plant diseases or wounds [26]. This is likely to support the assumption that phenolic compounds or their derivatives contribute to the resistance of plants against pathogens or pests.

Table 1 shows the elemental composition of date palm leaflets (both infected and healthy ones). Infection resulted in a significant difference in the content of elements such as total N, Ca, K, Cu, Mn and Cd. Other elements such as P, Mg, Zn, Fe, Se and B did not change significantly in both healthy and infected plants. Total N was significantly lower in both diseased leaflets than the healthy ones. Reduction in total nitrogen and protein had been reported for other diseases such as the infection of potato tubers by *Phytophthora infestans* [Lepik 1939, cited by 27], celerly plants infected by *Cercospora apii* [Coons and Klotz, 1925, cited by 27] and rice infected by *Gibberella fujikuroi* [Andal and Subba Rao, 1956, cited by 27]. On the other hand, date palm leaflets infected by the insect *Parlatoria blanchardi* Targ. have shown an appreciable increase in protein content [11] which the author speculated that the source might be the insect itself. Calcium was higher in D1 (plant infected with the green scale insect) than healthy one but not D2 (plant that did not have an obvious pathogens or pest). On the other hand Mn was significantly lower (at 0.05% probability) in diseased leaflets of D2 only. Cadmium was significantly higher in healthy plants than diseased one. The magnitude of Cd content was higher (about 1.5 times) in the plant infected with the green scale which could be a reflection of the location of the tree and of pollution from the traffic.

Foliar analysis of tomato plants locally infected with "streaks" (caused by a virus or mixture of viruses) showed that they had high concentrations of N and low concentrations of K in the leaves [28]. Other variations of ion content between healthy and diseased plant leaflets is probably due to infection, but no explanation, as yet, could be speculated because

**Table 1. Elemental composition of date palm diseased (D) or healthy (H) leaflets (Total N as mg N/g d.wt., while others as umole/g d.wt.)(Mean ± SD)**

| Element | Status of leaflets H1 | D1 | H2 | D2 |
|---|---|---|---|---|
| Total N | $12.69 \pm 1.08^{a}$ | $8.00 \pm 0.82^{b}$ | $10.28 \pm 1.31^{ab}$ | $6.93 \pm 1.58^{c}$ |
| P | $2.39 \pm 0.31$ | $1.89 \pm 0.26$ | $2.31 \pm 0.45$ | $2.20 \pm 0.1$ |
| Ca | $77.41 \pm 4.00^{b}$ | $104.62 \pm 4.89^{a}$ | $41.47 \pm 3.03$ | $47.41 \pm 2.23^{c}$ |
| Mg | $298.56 \pm 33.83$ | $308.45 \pm 34.83$ | $261.96 \pm 57.11$ | $280.46 \pm 28.25$ |
| K | $430.25 \pm 31.66^{a}$ | $266.17 \pm 15.50^{b}$ | $428.76 \pm 21.93^{a}$ | $519.78 \pm 70.27$ |
| Cu | $1.20 \pm 0.09^{b}$ | $0.66 \pm 0.13^{c}$ | $1.80 \pm 0.18^{a}$ | $1.60 \pm 0.13^{a}$ |
| Zn | $2.38 \pm 0.29$ | $2.35 \pm 0.23$ | $2.24 \pm 0.29$ | $2.32 \pm 0.41$ |
| Mn | $1.34 \pm 0.07^{a}$ | $1.38 \pm 0.24^{a}$ | $1.25 \pm 0.85^{a}$ | $0.87 \pm 0.04^{b}$ |
| Fe | $14.19 \pm 1.21$ | $14.91 \pm 2.55$ | $18.11 \pm 2.14$ | $19.85 \pm 5.74$ |
| B | $0.21 \pm 0.03$ | $0.12 \pm 0.03$ | $0.14 \pm 0.04$ | $0.16 \pm 0.07$ |
| Cd | $0.15 \pm 0.02^{c}$ | $0.14 \pm 0.03^{d}$ | $0.23 \pm 0.03^{a}$ | $0.22 \pm 0.02^{b}$ |
| Se | $3.31 \times 10^{-7} \pm 0.27$ | $3.64 \times 10^{-7} \pm 1.17$ | $4.94 \times 10^{-7} \pm 0.80$ | $4.30 \times 10^{-7} \pm 0.67$ |

*Elemental contents within each row having common letter do not differ significantly at 99% propablity levels except Mn, it is for 95% probability level.

as far as known, no other reports have been reported on this subject. However, mobility of elemental nutrients in the phloem from other parts of the plant due to the presence of a sink (infected organs needing a higher supply of these ions for their development) could explain, equivocally, however, the increase of Ca (non-mobile ion), Mn, Cu, (intermediate mobile ions), but not for Cu, Zn, Fe, Mg (mobile ions) in infected tissues.

Estimation of the nucleic acid RNA (Table 2) indicates a very significant increase in RNA content of the diseased tree (D2) growing beside the Department but without obvious pathogen or pest. These results are in agreement with what has been reported, that RNA increased in wheat plants infected by *Puccinia graminis* var. *tritici*, or *P. recondita* [Rohringer and Heitefuss, 1961, cited by 29] and also in barley leaves infected with *Erysiphe graminis* [Millerd and Scott, 1963, cited by 29]. Since most of the plant viruses, viroid and mycoplasma-like organisms are of RNA nature, it is possible that this tree shows the lethal yellowing disease reported to infect coconut tree (*Coccos nucifera* L.) and date palm in some parts of the world [5-8]. To test this speculation, investigation by the author is going on.

**Table 2. The RNA content of both healthy (H) and diseased (D) leaflets of date palm ($mg.g^{-1}$.F.Wt.)**

| Sample | RNA content |
|---|---|
| $H_1$ | $9.43 \pm 1.95^{b}$ |
| $D_1$ | $12.46 \pm 1.59^{ab}$ |
| $H_2$ | $12.04 \pm 2.79^{ab}$ |
| $D_2$ | $14.83 \pm 1.40^{a}$ |

Finally, discs of the leaflets infected by unknown pathogens (D2) were surface sterilized and cultured on an agar medium, and it was found that at least seven organisms grew. They were identified primarily in the laboratory of Professor H.A. Bokhary, in King Saud University as the species of *Alternaria alternata, Drechslera australiensis, Bipolaris* sp., *Fusarium oxysporium, Drechslera* sp., *Ulocladium tuberculatum and Alternaria chl aydspora.* We would think that these organisms are secondary invaders and not the cau e. However, this is not a final conclusion.

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# بعض التغيرات الأيضيّة للوريقات الخضراء والصفراء لنخلة التمر

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(استلم في ١٤ رمضان ١٤١٥هـ؛ قبل للنشر في ١٨ جمادى الثانية ١٤١٦هـ)

**ملخص البحث**. تم مقارنة عينات من وريقات نخلة التمر *Phoenix dactylifera* L. لشجرتين إحداهما مصابة بحشرة والأخرى ذات كائن ممرض غير معروف لكنها مصفرة وذلك بوريقات سليمة خضراء. كما هو متوقع، فقد أوضح التحليل انخفاضًا معنويًا في كمية كلورفيل ا والكلوروفيل الكلي ولم يتغير كلوروفيل ب. أما نسبة الوزن الجاف إلى الوزن الرطب فقد ازدادت معنويًا في الوريقات المصابة. ازدادات أيضًا كمية المركبات الفينولية في وريقات النخلة المصابة بالحشرة فقط، لكن السكريات الكلية والمختزلة ازدادت في كلتيهما زيادة معنوية. من ناحية أخرى، كانت هناك اختلافات في الكمية الكلية لعناصر النيتروجين، الكالسيوم، البوتاسيوم، النحاس، المنجنيز والكادميوم بين الوريقات المصابة والسليمة ولم تتغير كمية عناصر الفوسفور، المغنيسيوم، الخارصين، الحديد، السلينيوم والبورون. تدل هذه الدراسة أيضًا على زيادة كمية الحمض النووي الريبوزي معنويًا في الوريقات الصفراء ذات الممرض غير المعروف، والذي قد يشير إلى احتمال الإصابة بالفيروسات و/ أو أشباه الفيروسات أو الكائنات الشبيهة بالميكوبلازما، لكن تم التعرف مبدئيًا على بعض الفطريات بعد استزراعها على منابت، والتي قد تمثل إصابة ثانوية.