



Effect of leaflets palm date biochemical composition on infestation rate by the white cochineal *Parlatoria blanchardi* (Himiptera-Diaspididae)

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Abstract

The white cochineal *Parlatoria blanchardi* is the most redoubtable pest of date palm *Phoenix dactylifera* in the palm groves of the region of Ouargla (Sahara Desert of Algeria). In order to explain the food preferences of the insect pest, this study aims to relate the variation of infestation rates caused by the white cochineal to biochemical characteristics of leaflets of two date palm cultivars Deglet-Nour and Ghars. The study of the dynamic populations of the white cochineal showed that it had the same developmental pattern in both cultivars ($P=0.107$ for the study months). Three annual generations are recorded, of which the spring generation is the most dangerous, with a mean number of individuals of 77.72 ± 6.07 at Deglet-Nour and 46.10 ± 5.14 at Ghars. Estimating infestation rates of *Parlatoria blanchardi* showed that the cultivar Deglet-Nour has higher infestation rates ($36.34\%\pm 9.92$) compared to Ghars ($7.08\%\pm 2.34$). The highest infestation rates are recorded during the months of April, May and October. Biochemical analyses were performed on leaflets of the two cultivars and the study of the principal components (PCA) shows that the Ghars cultivar has a higher cationic ratio $K^+ / Ca^{2+} + Mg^{2+}$ ($0.34\%\pm 0.11$) than the Deglet-Nour ($0.29\%\pm 0.04$) rich on Mg^{2+} (0.33 ± 0.02) and Ca^{2+} (1.03 ± 0.13), which contributes to its resistance against the pest.

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Introduction

In Algeria, the Phoenix dactylifera date palm undoubtedly constitutes significant socio-economic speculation in Saharan agriculture (Idder, 2011). It creates a microclimate favorable to crop development and is widely cultivated for these multiple uses and eco-systemic services, particularly for its edible fruits (Benameur-Saggou, 2018).

The palm groves in the Ouargla region are of considerable ecological and economic importance. In terms of varietal diversity, these palm groves contain 55 cultivars, 22 of which are rare (Idder-Ighili, 2015) among about 1000 cultivars recorded in Algeria (Bouguedoura *et al.*, 2015).

The date palm and its production are attacked by a series of diseases and pests that affect the production and quality of dates (Haddad, 2000; Idder, 2011; Benameur-Saggou, 2018).

The white cochineal insect *Parlatoria blanchardi* Targioni-Tozzetti 1892 (Hymenoptera -Diaspididae) is the most common pest of date palms. It establishes itself on all aerial parts of the palm and causes enormous problems in case of heavy crusting (Brun *et al.*, 1998). The intense population of *Parlatoria blanchardi* unbalances photosynthesis and disrupts breathing and normal perspiration. Furthermore, the scale in a continuous layer on the young tissue prevents normal bud growth. In fact, the intense *Parlatoria blanchardi* population not only hinders the normal development of the plant but also causes the premature drying out of the palms and can lead to the total loss of a plant as robust and resistant as the date palm (Smirnoff, 1957; El-Said, 2000 and El-Sherif *et al.*, 2001).

The existence of physical and chemical links between insects and plants was recognized as early as the beginning of the twenty century (Mangold, 1978). These relationships are conditioned by different physical plant characteristics such as size, shape, presence of epicuticular waxes and trichomes, phenological stage and color of the plant (Berenbaum,

1995) but also by chemical factors such as the presence of secondary metabolites (Vet and Dicke, 1992; Harborne 1993). According to Chaboussou (1975), nutritional products are mainly responsible for the insect's feeding behaviour, *i.e.*, the attack on the plant.

This study focuses on the role of the biochemical composition of the leaflets of date palm cultivars in mineral elements on the rate of white scale infestation.

Materials and methods

Field sampling

The plant material is located in the Mekhadma palm grove (31° 59' N; 5° 20' E) in the region of Ouargla. This material is represented by the two most dominant cultivars of date palm, Deglet-Nour and Ghars, with differences in the characteristics of the vegetative part and their production.

The date palm plants are randomly selected at the study sites, of which eighteen (18) plants are chosen for each cultivar. However, the palms sampled have the same age and height. Leaflets are intended for infestation rate estimates and biochemical analysis.

Estimation of infestation rate

Leaflet collection

Using a pruning shear, two leaflets were collected from the heart and the two crowns, internal and external of the date palm, in the four directions with respect to the trunk (North, South, East and West) to obtain a total of 24 leaflets at the end for each base sampled. The samples are taken bi-monthly during our experiment, from October 2016 to September 2017. Leaflets are placed in Kraft paper bags on which are noted mainly the information relating to the tree, the date and the level of sampling of the leaflets.

Scale counting using the EUVERTE method

The estimated infestation rate is established by the method of Euverte (1962). Leaflets are brought back to the laboratory for white cochineal counts. On the latter, three squares of 1 cm² each are delimited. Their

position on the leaflet depends on the density of the insect (low, medium and high density).

Using a binocular magnifier, the total population count (dead and live) was carried out, including mobile larvae, fixed larvae (stages 1 and 2), male and female adults. The values of the three cm² selected A1, A2 and A3 were then obtained for each side of the leaflet (upper and lower).

The average of which established the population density of the leaflet. Densities were then averaged for each study month over the whole plot.

Biochemical analyses of date palm leaflets

The biochemical analyses were performed on date palm leaflets of the two cultivars studied. These analyses included the determination of moisture content, mineral composition and total sugars.

Water content

The percentage moisture in the leaflets was determined by the difference between the weight of the fresh and dried leaflet at 105 °C to the constant weight using the following formula:

$$\text{Humidity (\%)} = \frac{\text{PF-PS}}{\text{PF}} \times 100 \text{ (Audigie et al., 1980).}$$

Where PF is the fresh weight and PS is the dry weight

Dosage of mineral elements

The determination of the mineral elements Ca, Mg, P, K and Na are only done by switching to mineralization of our samples using Bonvalet (1981) methods.

Calcium and magnesium are measured by the atomic absorption spectrophotometer (AA-680), while Phosphorus, potassium and sodium are by a flame photometer (410 Sherwood). For Nitrogen, we opted for the Kjeldahl method and without mineralization of the plant samples. The total sugars are colorimetric by a UV-visible spectrophotometer (WPA Biowave II).

Statistical analyses

Dynamic of the population of white cochineal data were analyzed for two ways variance analysis (ANOVA) to find the presence and absence of difference and interaction on the one hand between the cultivars and phenological stages and on the other hand between cultivar and study month.

The results obtained on the determination of chemical elements in the leaflets of the two cultivars studied are processed by using the analysis of variance (ANOVA) also. A *p*-value <0.05 was considered statistically significant.

To study the interactions that may exist between the rate of white cochineal infestation and the biochemical characteristics of leaflets (water content and mineral elements), a PCA (principal components analysis) is performed out. The software used is XLSTAT 2021.3.1.1149

Results and discussion

Population dynamics of the live white cochineal of both cultivars

The evolution of the average numbers of individuals in the different stages of development of the white cochineal indicates the population dynamics.

Table 1. ANOVA testing the effect of factors cultivar and phenological stages on dynamics of population densities of *Parlatoria blanchardi*.

Source	DF	SS	F	Pr > F
Cultivars	1	118,459	15,242	0,000
Phenological stages	3	681,886	29,246	< 0.0001
Cultivars * phenological stages	3	59,867	2,568	0,059

The results show that the same numbers of peaks exist for all stages of development of the white cochineal on the two cultivars studied (Fig. 1A, 1B).

Mobile larvae record two apparent peaks, in March (0.9±0.15) and September (1.22±1.45) for Deglet-Nour, March (0.45±0.9) and October (0.96±0.55) for

Ghars, the third peak in July is less important. The low numbers recorded are due to the mobility of these larvae and their escape during sampling.

Live fixed larvae (L1+L2) have two main peaks during the year; the periods of activity of these larvae are March-May which is the longest period and September-October, which is the shortest. Live females show three peaks. The first between March and April is the highest, followed by the lowest in July; the third is recorded mainly between September-October, according to cultivar. The highest density of females is a little late in Ghars with 8.37 ± 2.33 in October (Fig.1B) compared to Deglet-Nour (Fig. 1A.). The number of females stops increasing during the winter period (low temperature); this is due to the diapause period, which ends with the return of favorable conditions for development. According to Madkouri (1978) and

Idder-Ighili (2015), females constitute a predominant proportion of the stages of the cochineal; they are the form of resistance of *Parlatoria blanchardi* during periods of cold and high heat. Live males have the same appearance as females; their development coincides with females throughout the year.

The ANOVAs reveal that the numbers of individuals of each phenological stage differ very significantly ($P < 0.0001$) and both cultivars studied ($P=0.000$) (Table 1).

The analysis of variance shows a non-significant difference ($P= 0.107$) between the phenological stages of white cochineal of the two cultivars studies and the month studies, and the interaction between this two factors ($P= 0.995$). The duration of each phenological stage of the white scale on both cultivars is the same (Table 2).

Table 2. ANOVA testing the effect of factors cultivar and study month on phenological stage of population densities of *Parlatoria blanchardi*.

Source	DF	SS	F	Pr > F
Cultivars	1	118,459	7,687	0,007
Month	11	277,523	1,637	0,107
Cultivars * Month	11	38,569	0,228	0,995

Population's dynamic of dead white cochineal of both cultivars

Concerning dead white cochineal, three peaks are recorded for the cultivar Deglet-Nour (Fig. 1A), the most important being in June when the average number of individuals of dead white cochineal (30.67 ± 3.53) exceeds the number of live ones.

The other two peaks, in March (17.56 ± 4.48) and September (23.12 ± 2.33), are less important, but it should be noted that during this period, high levels of live scale are also recorded.

Unfavorable climatic conditions (high temperatures, low humidity and strong winds) cause a decrease in population density of the different stages of development of the white cochineal (Achoura, 2013; Idder-Ighili, 2015 and Bakry *et al.*, 2015). During the

hot period between June and July, the number of 1st instar larvae is low, with much-reduced oviposition and, therefore, a considerable drop in the other development stages of this insect (Nadji, 2011).

Similarly, Benameur-Saggou (2018) reported significant activity of two natural auxiliaries of the white cochineal during the months of March and September, namely *Pharoscygnus ovoideus* and *Pharoscygnus numidicus*. These two species of ladybirds contribute to the reduction of cochineal populations.

From our study of the population dynamics of the white cochineal, we found that there are three annual generations, a very formidable spring generation with an average number of individuals of 77.72 ± 6.07 at Deglet-Nour and 46.10 ± 5.14 at Ghars.

Table 3. Results (average of 3 replicates) leaflets of biochemical analyses of both cultivars (Deglet-Nour and Ghars).

Average rate of mineral elements (%)	biochemical analysis		Statistical analysis one factor ANOVA	
	Deglet Nour	Ghars	F	P
N	1.11±0.017 ^b	1.25±0.44 ^b	46.286 ^{***}	<0.000
Ca ²⁺	1.03±0.13 ^b	1.04±0.35 ^{bc}	3.475 [*]	<0.099
Mg ²⁺	0.33±0.02 ^{cd}	0.36±0.12 ^{cd}	10.216 ^{**}	<0.013
K ⁺	0.40±0.02 ^c	0.49±0.16 ^{cd}	27.851 ^{***}	<0.001
P ⁺	0.37±0.06 ^{cd}	0.51±0.16 ^{cd}	2.805	<0.133
Na ⁺	0.28±0.03 ^{cd}	0.36±0.11 ^{cd}	48.674 ^{***}	<0.000
K/Ca+Mg	0.29±0.04 ^{bc}	0.34±0.11 ^{cd}	23.946 ^{***}	<0.001
T.S.	0.02±0.001 ^d	0.02±0.007 ^d	0.013	<0.912
M. C.	44.24±0.15 ^a	56.37±0.31 ^a	46.111 ^{***}	<0.000

Mean ± standard deviation; *P < 0.05, **P < 0.01 and ***P < 0.001 Where N= Nitrogen ion; Ca²⁺= Calcium ion; Mg²⁺ ion= Magnesium; K⁺ = Potassium ion; P⁺ = Phosphor ion; Na⁺ = Sodium ion; K/Ca+Mg = Potassium to Calcium and Magnesium ratio; S.T = Total sugar; M.C = Moisture content.

The second is summer and the third summer-autumn, which is considered the most extended generation (Fig. 1A, 1B).

Several studies on the white cochineal announce the presence of three generations of this insect with the same dynamics encountered in our research. Citing Idder 2011 results for the Deglet-Nour cultivar, and Boughezala-Hamad (2011) for the Ghars. The similar results were found by Bakry *et al.*, 2015 for Egyptian cultivars.. The population dynamics of the white cochineal insects show the same evolution.

White cochineal infestation rates of both cultivars

The total numbers of white cochineal live and dead (L1+L2 larvae, male and female adults) on Deglet-Nour and Ghars are indicators of the rate of infestation by this pest over the course of a year. Following the densities of white cochineal insects using the method of Euverte (1962) found that there is a very highly significant difference (P = 0.000) between the two cultivars studied, where the cultivar Deglet-Nour registers a maximum infestation with 36.34%±9.92 and Ghars with 7.08%±2.34 (Fig.2).

According to several authors (Boussaid and Maache, 2000; Boughezala-Hamad, 2011 and Allam, 2013), the cultivar Deglet-Nour is still more infested than Ghars.

The most significant means of defense is the presence of chemical compounds in the plant. Reduced insect attacks may be due to a decrease in nutritional value or the presence of toxins, repellent substances, or unpleasant taste in the plant tissue (Vet and Dicke, 1992; Harborne, 1993). There are relations between the nutritional factors offered by the plant and the multiplication of phytophagous pests, especially the Himiptera order (Nadir, 1972; Chaboussou, 1975; Belguendouz, 2014 and Mehaoua, 2009).

Effect of the biochemical composition of leaflets on the rate of white cochineal infestation

Given that the white cochineal mainly attacks leaflets, the preferred organ for feeding and oviposition, its biochemical composition contributed to the choice of this diaspine from its host plant. Leaflets biochemical analyses of the two cultivars studied were carried out. The results obtained are presented in Table 3. Based on the results obtained, we find that the mineral elements show average rates that differ from one cultivar to another.

The Ghars cultivar is rich in N, K⁺ and Na⁺ compared to Deglet Nour with P respectively 0.000, 0.001 and 0.000. But in general, the Deglet-Nour cultivar is rich in Mg²⁺ with P= 0.013. The Ghars cultivar always shows richness in Nitrogen, potassium and sodium. The massive use of Nitrogen weakens plants, which

become more susceptible to diseases and attacks by pests such as aphids (Pousset, 2008).

The cationic ratio $K^+ / Ca^{2+} + Mg^{2+}$ tells us about the balance of cations in the plant. It is higher in the

Ghars cultivar (0.34 ± 0.11) compared to Deglet-Nour (0.29 ± 0.04) as this ratio is positively correlated with potassium. The analysis of variance shows a very highly significant difference between cultivars ($P = 0.001$).

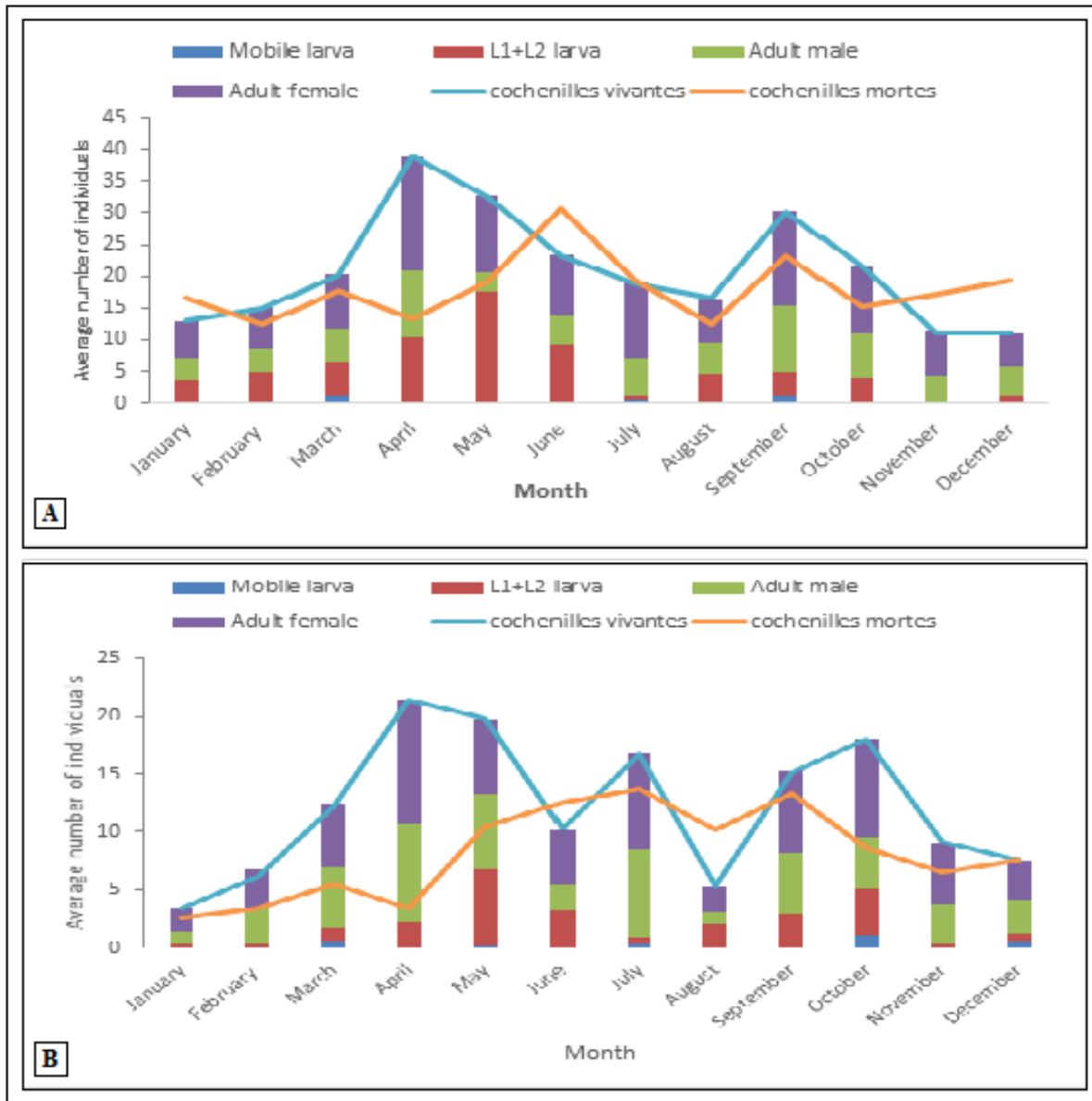


Fig. 1. Population dynamics of *Parlatoria blanchardi* (live) during one year (October 2016 to September 2017) on two date cultivars (A: Deglet-Nour cultivar - B: Ghars cultivar) in the Ouargla region.

The moisture content in the leaflets seems to be closely related to the moisture content in the date. The Ghars cultivar, considered as a soft date is very rich in water than the Deglet-Nour cultivar considered as a semi-soft date. However, the difference is very highly significant ($P = 0.000$) between the two cultivars studied, with a percentage

of 45.84 ± 0.27 for the first cultivar and 56.37 ± 0.31 for the second.

The Ca^{2+} , P^+ and total sugars contents are highly correlated in the two cultivars studied with non-significant differences. PCAs are performed in order to determine the existing correlations between the

different chemical elements of the cultivars study and the rate of infestation by the white cochineal insect (Fig. 3A, 3B).

The choice of the two axes of the plot was based on the most relevant main components. These concern the axes F1 and F3, which gave the eigenvalues 3.06 and 1.68 respectively.

They represent the highest percentages of inertia with respectively 34.08 and 18.74 for the cultivar Deglet-Nour. The Bartlett sphericity test associated with the

PCA and the significance threshold $\alpha=0.05$ allowed us to reject the null hypothesis of no significant dependence between the variables. Therefore, we accept the alternative hypothesis: the dependence between the variables is significant ($\chi^2_{Obs} = 74.227$, $\chi^2_{Cri} = 50.998$, $ddl = 36$, $P = 0.000$) (Fig. 3A).

For Ghars cultivar, these concern the axes F1 and F2, which gave the Eigenvalues 5.26 and 1.42 respectively; and the highest percentage of inertia with 58.49 % and 15.83.

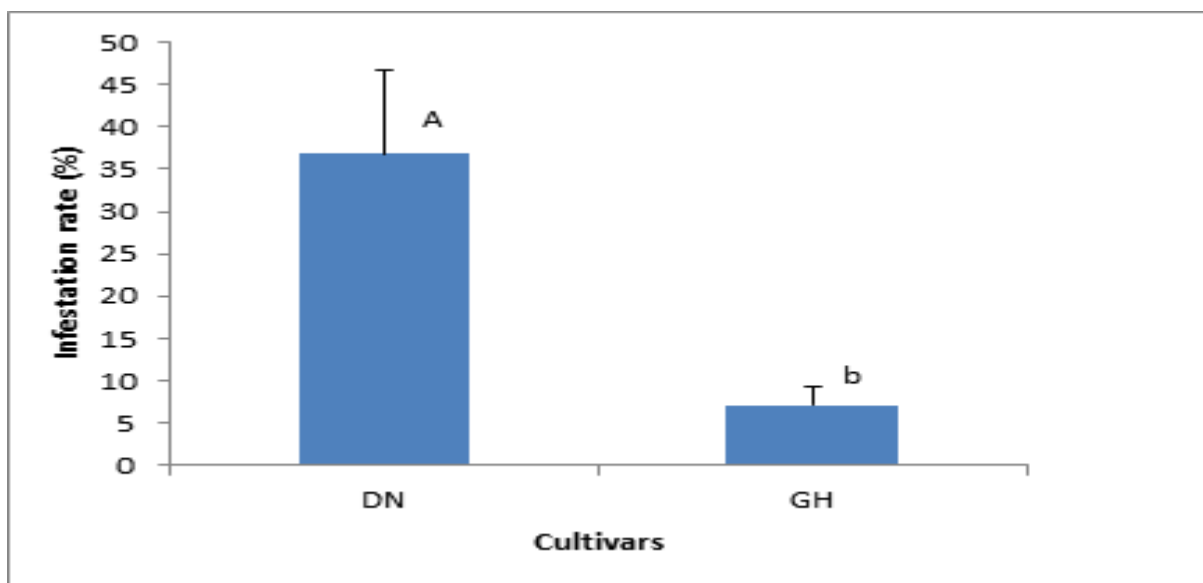


Fig. 2. White cochineal insect infestation rate of the two cultivars Deglet-Nour and Ghars in the Ouargla region. Different letters indicate a significant difference between treatments ($\alpha = 0.05$). Vertical bars indicate \pm SE for means.

At a significance level of $\alpha = 0.05$, Bartlett's test of sphericity associated with the PCA allowed us to reject the null hypothesis of no significant dependence between variables (infestation rates vs. biochemical characteristics). Therefore, we accept the alternative hypothesis, where the dependence between variables and infestation is significant ($\chi^2_{Obs} = 134.422$, $\chi^2_{Cri} = 50.998$, $ddl = 36$, $P < 0.0001$) (Fig. 3B). The projection of the biochemical variables of the leaflets of the two cultivars allowed us to draw the following correlations: There is a strong negative correlation between the white cochineal infestation rate and potassium (K^+) for the two cultivars studied. Potassium is an essential element in

plants; its presence increases the resistance of plants against diseases and pests (Hellali, 2002). In the cultivar Ghars, this negative correlation is also recorded with sodium (Na^+), and with Nitrogen in Deglet-Nour. According to Jabnour (2008), the beneficial effect of sodium can be observed in particular under conditions of K^+ deficiency. In these conditions, a controlled accumulation of this element helps to regulate the pressure of cell turgidity. For the other chemical elements (P^+ , Ca^{2+} , Mg^{2+} and water content) the correlations are positive with the white cochineal infestation rate. Also, this correlation was negative between K^+ , Ca^{2+} and Mg^{2+} , and positive between total sugar and water content (Fig. 3A, 3B).

In the trophic relationship between the pest and its host plant, the cationic ratio has a major role to play in the resistance phenomenon. There is an interaction between the three cations K^+ , Ca^{2+} and Mg^{2+} . The cationic ratio $K^+ / Ca^{2+} + Mg^{2+}$ indicates the balance of

cations in the plant (Nadir, 1972). According to Mehaoua (2009), this ratio is strongly influenced by the high foliar contents of potassium and the low foliar contents of calcium and magnesium in date palms.

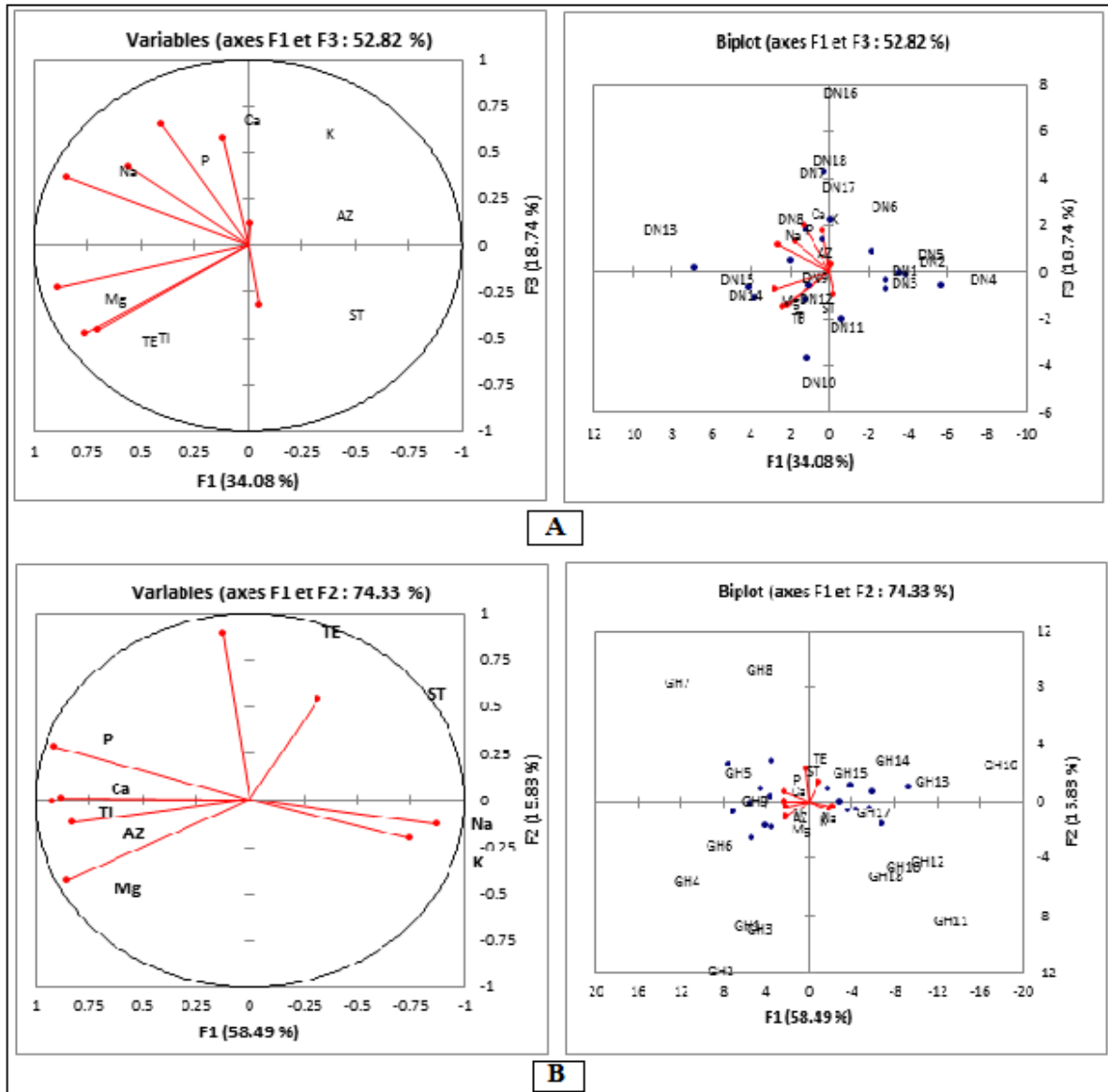


Fig. 3. Factorial plot 1–2 of the principal components analysis (PCA) projecting leaflets biochemical variables, infestation rate of date palm cultivars (A: Deglet-Nour, B: Ghars) (solid circles). (TI= rate of infestation, N= Nitrogen ion; Ca^{2+} = Calcium ion; Mg^{2+} ion= Magnesium; K^+ = Potassium ion; P^+ = Phosphor ion; Na^+ = Sodium ion; S.T = Total sugar; M.C = Moisture content).

The low cationic ratio in the Deglet-Nour cultivar compared to Ghars indicates that the concentration of potassium is lower compared to calcium and magnesium. According to Chaboussou (1975), the optimal presence of this element would be doubly beneficial. On the one hand, by ensuring maximum

plant growth and yield, and on the other hand by causing a reduction in the multiplication of cochineal insects by reducing the amino acid content. However, Mehaoua (2009) announces an antagonism between K^+ and the contents of date palm leaflets in water, N, Ca^{2+} , Mg^{2+} and total sugars.

Indeed, Bruning and Uebel (1971) in Chaboussou (1975) point out that cochineal insects show a clear preference for leaves that are both rich in Nitrogen and low in potassium, also the multiplication of the California red louse *Aonidiella aurantii* (Himiptera - Diaspididae) is favored by an excess of calcium and magnesium in relation to potassium.

Conclusion

The white cochineal of the date palm always remains a very serious pest. The presence of three annual generations still causes the weakening of the date palm and the deterioration of its date production. The cultivar Deglet-Nour, which has the highest market value, is more infested than Ghars. The biochemical composition of these leaflets is a determining factor in the establishment of the white cochineal. The high potassium content of the Ghars cultivar and its low content of calcium and magnesium make it resistant to this diaspine. Correct fertilization control with good irrigation can improve the resistance of Deglet-Nour by increasing its cationic balance.

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