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Full Length Research Paper

Chemical diversity analysis of Tunisian *Lawsonia inermis* L. populations

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***Lawsonia inermis* L. (commonly known as henna) is a cosmetic and medicinal plant cultivated from North-east Africa to India. The objective of this study was to evaluate the diversity of 25 *L. inermis* Tunisian populations, based on chemical markers. The populations were collected from the region of Gabès. The leaves and stems macro elements (Na, K, Ca, Mg and P) and trace elements (Cu, Zn, Fe and Mn) were analyzed for each population using spectrophotometry, and the nitrogen content was measured by Kjeldahl technique. The results showed that the leaves had a level of Ca, Na, P and K contents, ranging between 0.2 and 4%. Mg content was less than 0.2%, Cu, Zn and Fe contents were above 0.5, 1.1 and 15%, respectively, Mn content was less than 1.5% and nitrogen matter (NM) content was less than 1.5%. In the stems, P and K contents were respectively, above 5.12 and 0.5%, Mg content was less than 0.08%, Na and Ca contents were less than 0.2%, Cu, Zn, Fe and Mn contents were less than 0.95, 1.7, 4 and 0.5%, respectively and NM contents was less than 0.2%. However, the statistical analysis structured the populations based on the contents of their leaves and stems, in five groups.**

Key words: *Lawsonia inermis*, chemical diversity, macro elements, trace elements, nitrogen content.

INTRODUCTION

Lawsonia inermis L. (vernacular name: henna) is a shrub of the *Lythraceae* family that exists in wild and cultivated forms. This plant is cultivated in North-east Africa and India. In Tunisia, it has been known since 1400 to 500 BCE. Henna has been widely used over the centuries for medical and cosmetic purposes (Al-Tufail et al., 1999) and it contains the active dye (red-orange pigment) lawsone (2-hydroxy-1,4-naphthoquinone) (Lekouch et al., 2001), which makes it useful for dying of hair, as well as coloring of palms, fingers, fingernails and soles (Cartwright-Jones, 2006; Hanna et al., 1998). Besides its use in cosmetics, their leaves are used as a prophylactic against skin diseases (Ahmed at al., 2000) and their stem is reported to be useful for jaundice, enlargement of the spleen (Kirtikar and Basu, 1956), calculus affliction and skin diseases (Satyavati et al., 1987). It is used also in therapy with oral administration, where the infusion of leaves is used against diarrhea, renal lithiase (Bellakhdar, 1997) and gastric pains (Lahsissene and Kahouadji, 2010). Seeds have been reported to possess deodorant action and are used in cases of menorrhgia, vaginal discharge and leucorrhoea (Nawagish, 2005; Zafar et al., 2006).

Differences in proportions of the chemical quantity, within the same medicinal plant species and between different species of the same genera, direct the preference over their demand (Kala et al., 2006). On many occasions, the wild medicinal plants are preferred by manufacturers as compared to the cultivated ones, as there is a general feeling that wild plant species contain better chemical contents (Kala, 2004). The variation in chemical contents also depends on the harvesting seasons of species and different stages of species' growth. Chemical markers can be investigated in biovariability studies (Marzougui et al., 2007). Such studies which are both quantitative and qualitative, based on the chemical parameters of medicinal plants, are needed in developing countries to conserve plant species before any destruction by advancing civilization (Small and Catling, 1999). The aim of this study was to evaluate the diversity of 25 cultivated populations of *Lawsonia inermis* based on their leaves and stems composition in macro and trace element

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Table 1. Location, latitude, longitude and altitude of the *Lawsonia inermis* populations in Gabès oases.

Code	Population	Locality	Latitude (N)	Longitude (E)	Altitude (m)
1, 2, 3	Bou Said I, II and III	Bou Said	34°06´	9°59´	11
4, 5	Mdou I and II	Mdou	33°48´	10°04´	46
6, 7, 8, 9	Bsissi I, II, III and IV	Bsissi	33°59´	10°01´	3
10, 11, 12, 13, 14, 15, 16, 17, 18, 19	Awled Elhej I, II, III and IV; Chatt Elfarik I, II, III, and IV, and Ghassena I and II	Chenini	33°53´	10°03´	24
20, 21, 22, 23, 24, 25	Chatt Essalem I, II, III, IV, V and VI	Chatt Essalem	33°53´	10°06´	3

trace elements and nitrogen contents.

MATERIALS AND METHODS

This study was conducted with 25 populations of henna, collected from the coastal oases of Gabès in the South-east of Tunisia (Table 1). The different populations were cultivated in an experimental field at Elfejeh, located in the South-east of Tunisia (Latitude 33°35' N, Longitude 10º48'3''E, Altitude 105 m). The climate in this region is arid inferior with a mild winter, while the soil type is diversified, ranging from sandy to loamy and to clayey, although, red Mediterranean soil is largely present. The leaves and stems of 3 random plants were harvested 3 times per year from the second year of its culture. Harvesting was done when the flowers used for floral buttons germinated.

Minerals analysis

Five grams of each powdered dry leaves and stems sample, placed in a porcelain capsule, were calcined at 550°C for 4 h. After cooling, ashes were attacked by a mixture of 5 ml ultrapure water and 2.5 ml hydrochloric acid and were subjected to boiling. The capsule content was filtered and adjusted to 100 ml by ultrapure water. This solution will be used for mineral analysis. A Shimadzu AA 6800 was used to determine the sodium, potassium, calcium, magnesium, zinc, copper, manganese and iron contents with the flame atomic absorption method.

For the macro-elements, concentrations of Na, K, Ca and Mg were calculated according to the equation:

$$\% \text{ macro-elements (Na, K, Ca and Mg)} = C \times V / (10^4 \times m)$$

Where, C, V and m are featured values (ppm), extract volume (ml) and mass (g), respectively. The total phosphorus (P) was determined by an Anthelie Advanced spectrophotometer (SecoNMm, Domont, France). The standard solutions of P (0, 2, 4 and 8 mg/l) were prepared by diluting the basic solution of P (100 mg/l), and 10 ml of each standard and the diluted extract were placed in 25 ml tubes with 10 ml of the vanadomolybdic reagent. The solution comprised the following: 200 ml of a 10% ammonium heptamolybdate solution obtained with dissolving 100 g of ammonium heptamolybdate in 10 ml of ammonium hydroxide and adding distilled water until a volume of 1 L was obtained; 200 ml of ammonium monovanadate solution obtained with 2.35 g of ammonium monovanadate in 400 ml of hot distilled water, 20 ml of 35% diluted nitric acid and distilled water until a volume of 1 L was obtained; 135 ml of concentrated nitric acid and distilled water until a volume of 1 L was obtained. After 10 min, the absorbances were measured at 430 nm, and extracts were diluted if needed. P concentrations were calculated according to the formula:

$$\% P = (C \times DF) / (100 \times m)$$

Where, C, DF and m are P content (mg/l), dilution factor and extract mass (g), respectively. The trace elements concentrations were calculated according to the equation:

$$(\% Zn, \%Fe, \% Mn \text{ and } \% Cu) = C \times V / (10 \times m)$$

Where, C, V and m are featured values (ppm), extract volume (ml) and mass (g), respectively.

The determination of nitrogen content was realized by Kjeldahl technique, that is, the method commonly used for nitrogen analysis in food products (AOAC, 1995). Samples were first digested, using concentrated sulfuric acid in the presence of an inorganic catalyst, which accelerated the reduction of all the organic nitrogen present in an ammonium salt. The second step was a separation of the formed ammonium, using distillation, and its capture with boric acid. The third step was ammonium quantification with hydrochloric acid titration. The nitrogen content was calculated as a percentage using the following equation:

$$\% NM = VHCl * AWA * (CF / W) * \%DW$$

Where, %NM, VHCl, AWA, W, %DW and CF are nitrogen matter percentage, volume of HCl used for digestion, atomic weight of nitrogen, weight of used dry pulp (mg), percentage of dry weight and conversion factor of 6.25, respectively.

Statistical analysis

For each of the chemical elements, a one-way variance analysis (ANOVA) was performed to compare the variations between the studied populations using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). As a consequence, their variations were significant when $p < 0.05$. A hierarchical classification analysis, based on macro and trace elements and nitrogen contents variations between the populations, was carried out by SPSS 16.0

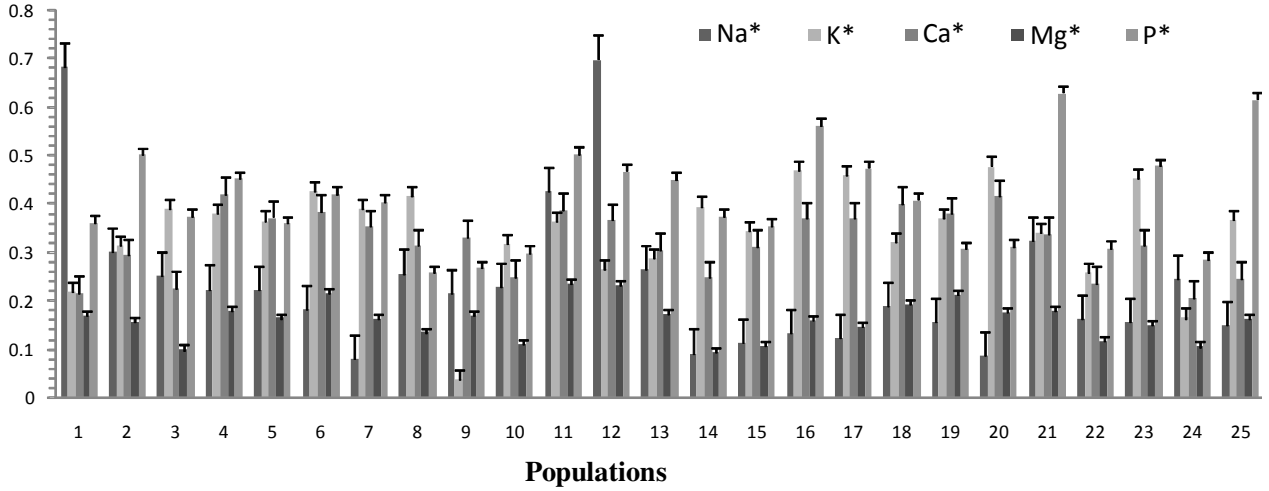

Figure 1. Macro elements contents in the leaves of *Lawsonia inermis* studied populations.*Significant variation (ANOVA). Standard deviation corresponds to three replicates for each population.

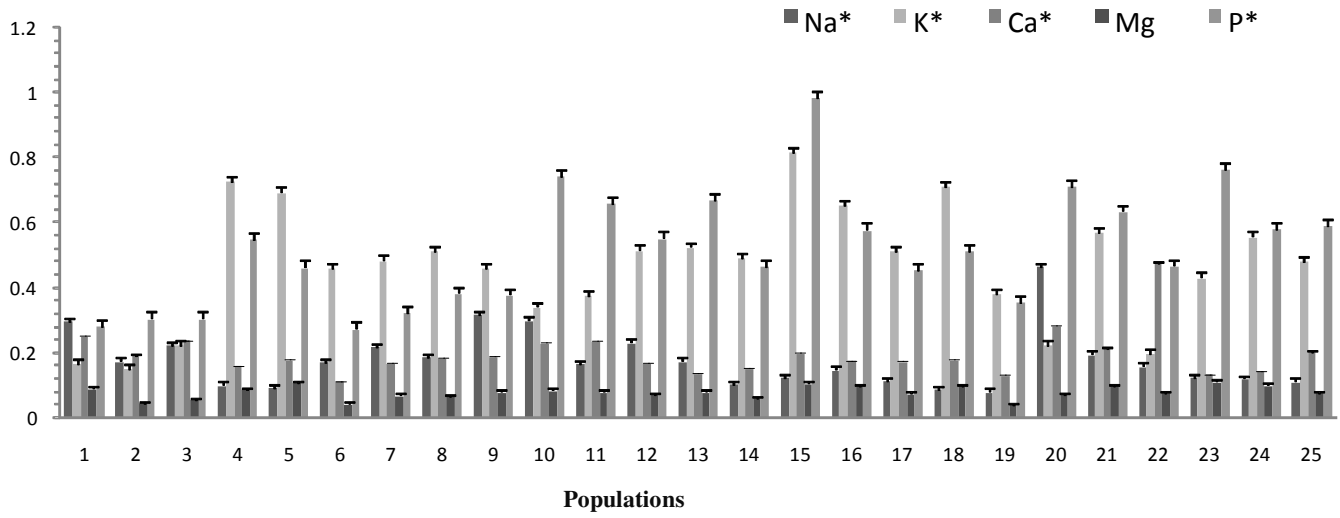

Figure 2. Macro elements contents in the stems of *Lawsonia inermis* studied populations.*Significant variation (ANOVA). Standard deviation corresponds to three replicates for each population.

to organize the different populations in related groups and to better visualize the differences and similarities between them. Results were also subjected to a principal component analysis (PCA) using StatBox Version 6.4 (Grimmersoft, Paris, France). This test was done to evaluate the overall structure of populations and ensure the grouping obtained by the hierarchical classification.

RESULTS

Macro elements contents

The macro elements analysis revealed significant variations between the populations in the majority of the studied leaves and stems' nutrients ($p<0.05$). As such, no significant variations were observed for Mg stems' concentrations ($p=0.26$). The sodium content in leaves varied from 0.08 (Bsissi II) to 0.69% (Wled Elhej III) (Figure 1), while in stems, it varied from 0.08 (Ghassana II) to 0.46% (Chatt Essalem I) (Figure 2). The potassium content varied from 0.16 (Chatt Essalem V) to 0.47% (Chatt Essalem I) in the leaves (Figure 1) and from 0.15 (Bou Said II) to 0.81% (Chatt Elfarik III) in the stems (Figure 2). For the calcium content, there was content variation from 0.2 (Chatt Essalem V) to 0.41% (population) in the leaves (Figure 1) and from 0.11 (Bsissi I) to 0.47% (Chatt Essalem IV) in the stems (Figure 2). The magnesium content varied from 0.09 (Chatt Elfarik I) to 0.23% (Wled Elhaj III) in the leaves (Figure 1) and from 0.03 (Ghassena II) to

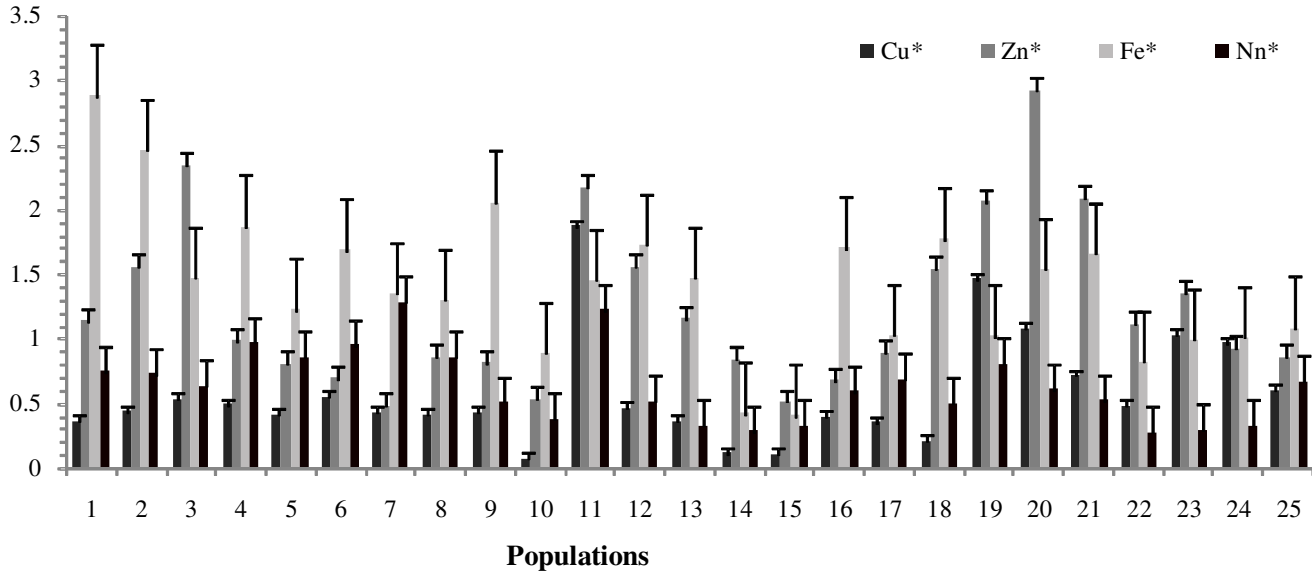

Figure 3. Trace elements contents in the leaves of *Lawsonia inermis* studied populations. *Significant variation (ANOVA). Standard deviation corresponds to three replicates for each population.

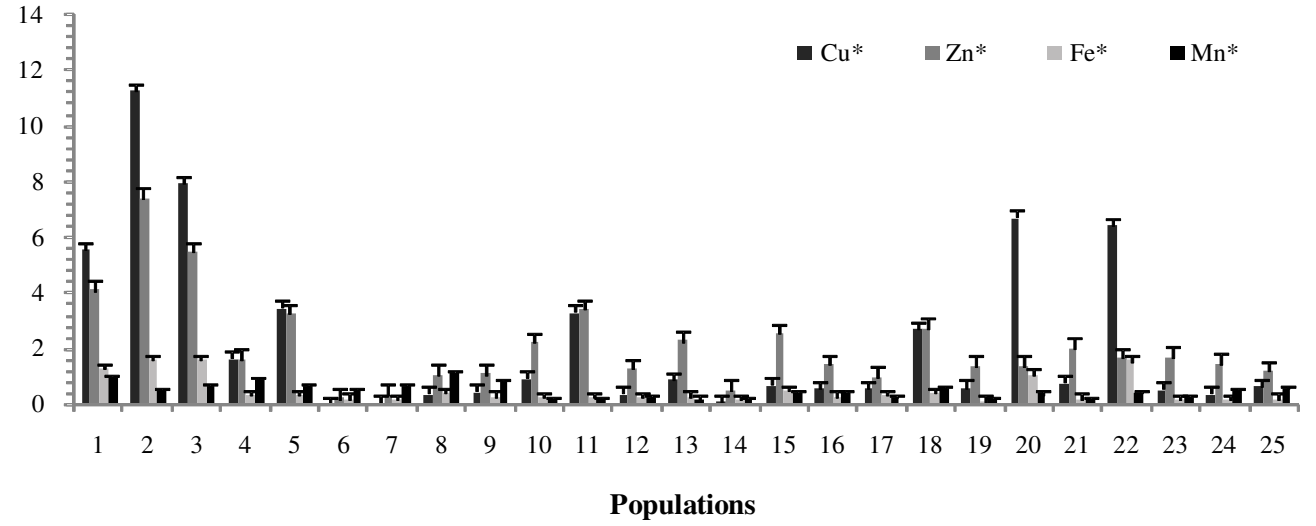

Figure 4. Trace elements contents in the stems of *Lawsonia inermis* studied populations. *Significant variation (ANOVA). Standard deviation corresponds to three replicates for each population.

0.11% (Chatt Essalem IV) in the stems (Figure 2). For the phosphorus content, there was content variation from 2.57 (Bsissi III) to 6.29% (Chatt Essalem II) in the leaves (Figure 1) and from 2.73 (Bsissi I) to 9.84% (Chatt Elfarik II) in the stems (Figure 2).

Trace elements contents

The trace elements' analysis revealed significant variations between the populations in the leaves and the populations in the stems ($p<0.05$). The copper content in the leaves varied from 0.06 (Awled Elhej I) to 1.87% (Awled Elhej II) (Figure 3), while in the stems, it varied from traces (Bsissi I) to 11.27% (Bou Said II) (Figure 4). The zinc content in the leaves varied from 0.47 (Bsissi II) to 2.92% (Chaat Essalem I) (Figure 3) and in the stems, it varied from 0.2 (Bsissi II) to 7.39% (Bou Said II) (Figure 4). In the leaves, the iron content varied from 4.03 (Chatt Elfarik II) to 28.77% (Bou Said I) (Figure 3) and in the stems, it varied from 1.17 (Ghassena II) to 15.85% (Bou Said II) (Figure 4). For manganese, there was content

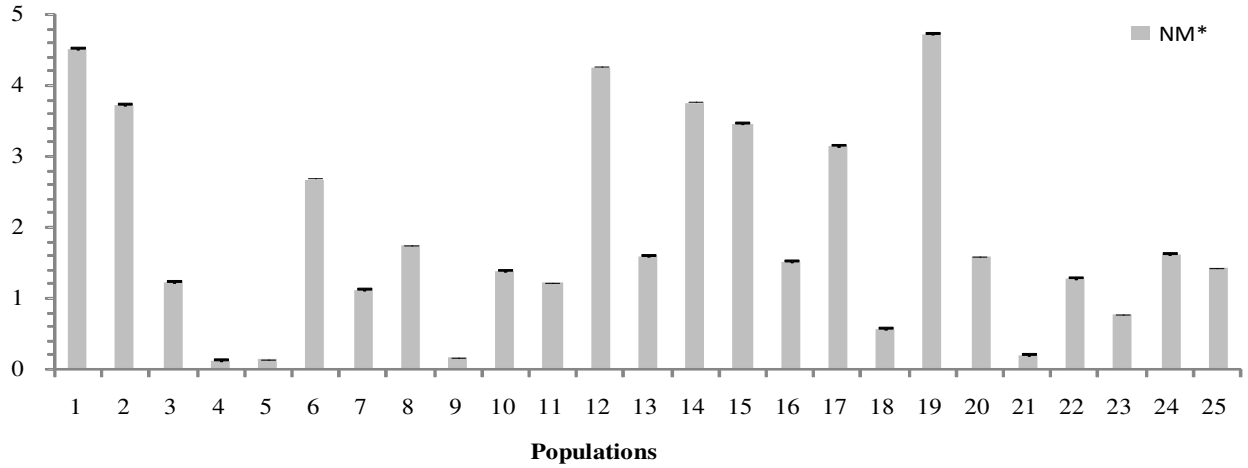

Figure 5. Nitrogen matter content in the leaves of *Lawsonia inermis* studied populations. *Significant variation (ANOVA). Standard deviation corresponds to three replicates for each population.

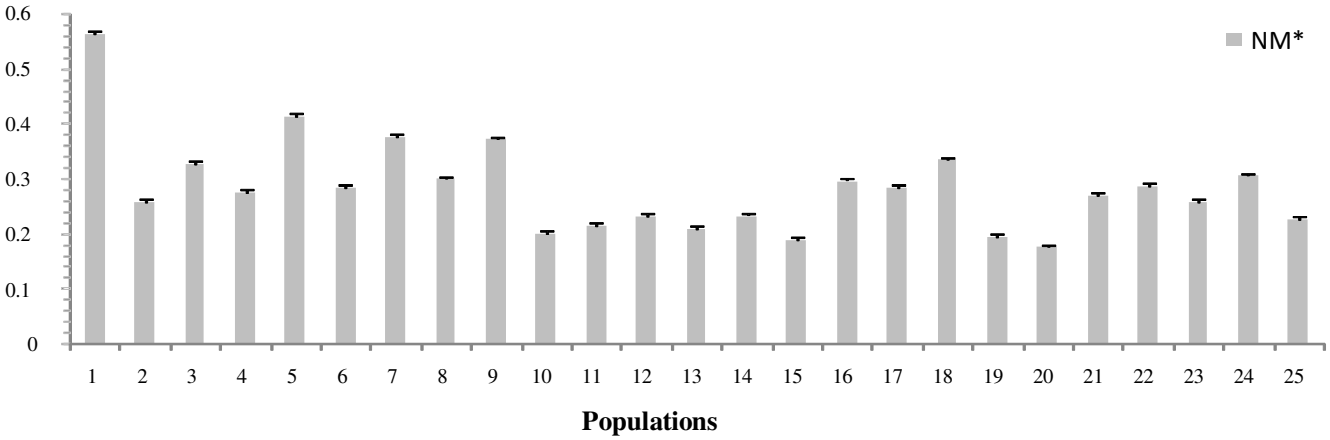

Figure 6. Nitrogen matter content in the stems of *Lawsonia inermis* studied populations. *Significant variation (ANOVA). Standard deviation corresponds to three replicates for each population.

variation from 0.27 (Chatt Essalem III) to 1.28% (Bsissi II) in the leaves (Figure 3) and from 0.14 (Chatt Essalem IV) to 0.95% (Bou Said I) in the stems (Figure 4).

Nitrogen content

The nitrogen matter content (NM) variations between the populations were significant in leaves and stems ($p<0.05$). The results revealed that NM varied from 0.14 (Mdou I) to 4.72% (Chenini X) in the leaves (Figure 5) and from 0.17 (Chatt Essalem I) to 0.56% (Bou Said I) in the stems (Figure 6).

Chemical structure

The hierarchical classification, based on macro elements contents, trace elements contents and nitrogen content, structured the studied populations in five groups (Figure 7). The first group contains the majority of populations: Mdou (4 and 5), Bsissi (6, 7, 8 and 9), Chenini (12, 13, 16, 17 and 18) and Chatt Essalem (21 and 25). The second group is composed of populations 10, 14 and 15 of Cheneni and 22, 23 and 24 of Chatt Essalem. The third group is formed by populations 11 and 19 of Cheneni. The fourth group contains population Bou Said (1, 2 and 3), and the last group comprises population 20 of Chatt Essalem. However, variations of the macro elements contents, trace elements contents and nitrogen contents, in each group, were illustrated in Table 2.

PCA analysis confirmed the dendrogram structuring of the populations and showed that the first three axes absorbed 80% of the total variance. The plots obtained according to axes 1 and 2 (41% of the total inertia) showed the same 5 clusters (Figure 8A) that were obtained by the dendrogram (Figure 7), while the plots obtained according

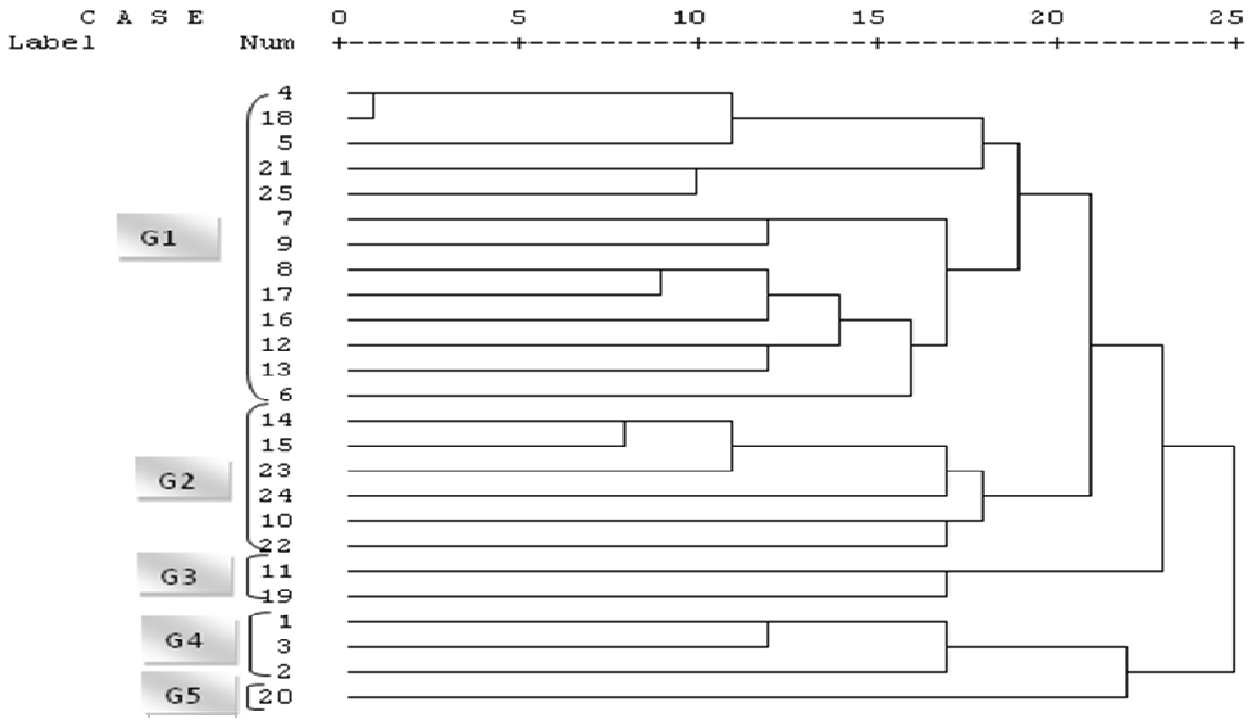

Figure 7. Dendrogram of *Lawsonia inermis* studied populations according to macro elements contents, trace elements contents and nitrogen contents (SPSS 16.0). G1: Group 1, G2: Group 2, G3: Group 3, G4: Group 4 and G5: Group 5.

Table 2. Macro elements contents, trace elements contents and nitrogen contents variations of leaves and stems in each group.

Element		Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
Macro elements	Na	Leaves	0.08 to 0.69	0.09 to 0.24	<0.42	>0.24	0.08
		Stems	0.08 to 0.31	0.1 to 0.3	<0.16	>0.17	0.46
	K	Leaves	0.03 to 0.46	0.16 to 0.45	0.36	0.21 to 0.38	0.47
		Stems	0.45 to 0.72	0.19 to 0.81	0.37 and 0.38	0.15 to 0.29	0.22
	Ca	Leaves	0.24 to 0.41	0.2 to 0.31	0.38	0.21 to 0.29	0.41
		Stems	0.11 to 0.21	0.13 to 0.41	<0.23	0.19 to 0.25	0.28
	Mg	Leaves	0.13 to 0.23	0.09 to 0.14	0.21 to 0.23	0.09 to 0.16	0.17
		Stems	0.04 to 0.1	0.06 to 0.11	<0.08	0.04 to 0.09	0.07
	P	Leaves	2.57 to 6.29	2.84 to 4.7	3.06 to 5.03	>3.61	3.11
		Stems	2.73 to 6.69	4.6 to 9.84	3.55 to 6.58	>2.86	7.11
	Fe	Leaves	10.21 to 20.25	4.03 to 10.1	10.17 to 14.47	>14.7	15.33
		Stems	1.33 to 3.85	1.65 to 15.51	1.17 to 2.31	>12.82	10.58
Traces elements	Zn	Leaves	0.47 to 2.08	0.5 to 1.35	2.05 to 2.16	>1.13	2.92
		Stems	0.2 to 3.24	0.5 to 2.53	1.38 to 3.39	>4.09	1.37
	Cu	Leaves	0.21 to 0.71	0.06 to 0.96	1.46 to 1.87	>0.36	1.08
		Stems	<3.48	0.09 to 0.94	0.6 to 3.33	>5.56	6.73

Table 2. Contiunes

	Mn	Leaves	0.32 to 1.28	0.27 to 0.37	0.8 to 1.22	>0.63	0.6
		Stems	0.14 to 0.86	0.13 to 0.48	0.18	>0.5	0.36
Nitrogen	NM	Leaves	0.14 to 4.26	0.77 to 3.76	1.22 to 4.72	>1.23	1.59
		Stems	0.2 to 0.41	0.19 to 0.3	0.19 to 0.21	>0.25	0.17

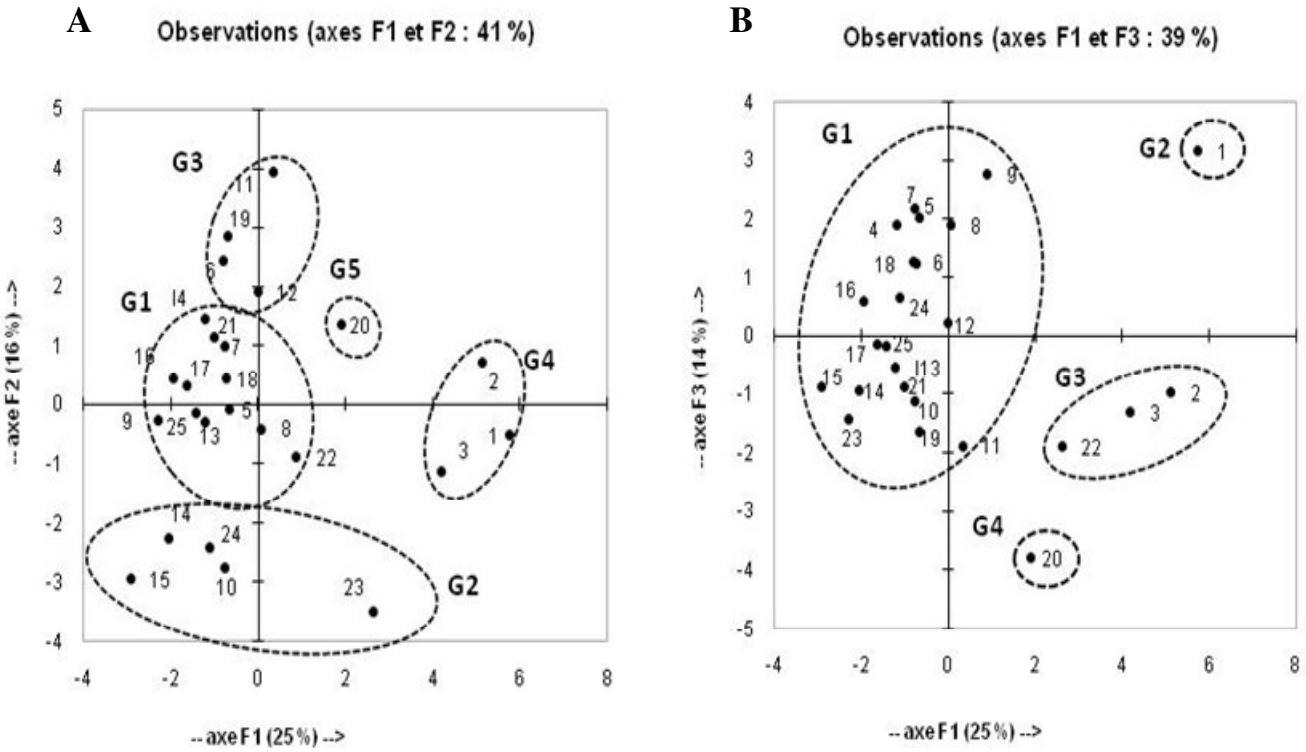

Figure 8. Dispersion of *Lawsonia inermis* studied populations in the principal components analysis. A: *Lawsonia inermis* populations according to axes 1-2; B: axes 1-3. G1: Group 1, G2: Group 2, G3: Group 3, G4: Group 4, G5: Group 5, G6: Group 6.

to axes 1 to 3 (39% of the total inertia) showed 4 clusters (Figure 8B), where the first (G1) group comprised populations 21, 23, 24 and 25 of Chatt Essalem and all the populations of Mdou, Bsissi and Chenini; the second (G2) group was formed by population 1 of Bou Said; the third (G3) group contained populations 2 and 3 of Bou Said and population 22 of Chatt Essalem; and the fourth (G4) group contained population 20 of Chatt Essalem.

DISCUSSION

The hierarchical classification, based on plants chemical constitution has been approved by some authors who studied the diversity of *Trigonella foenum-graecum* (Marzougui et al., 2007), *Medicago sativa* (Touil et al., 2009), *Euphorbia esula* (Torell and Evans, 1998), *Thymus pulegioides* (Loziene et al., 2003), *Citrus bergamia* (Statti et al., 2004) and various *Origanum* species (Schulz et al., 2005). Other authors used PCA to assess the mineral variability between the fruits of *Rhamnus alaternus* varieties (Izhaki et al., 2002) and in various organs of medicinal plant species (Wesolowski et al., 2001; Wesolowski and Konieczyński, 2003). In addition to PCA, Boubaya et al. (2010) used the UPGMA method to establish the structure of mulberry cultivars (*Morus spp*) in Southern Tunisia, based on mineral and morphological characterizations. Mukhomorov and Anikina (2009) employed another approach that may have more potential for yielding information about the diversity of chemical elements in plant tissues. They used, in fact, the principle of statistical homogeneity to classify various tomato and wheat plant organs besides the quantitative index of diversity namely information function.

There were no several studies on the mineral composition of the different organs of *Lawsonia inermis* worldwide. On the other hand, several authors were interested in studying the chemical composition of henna

products. Bernth et al. (2005) studied the chemical substances in henna hair dyeing; their study results showed that the content of manganese, in 10 out of the 17 studied products, was at the range of 0.0045 to 0.023%. These contents are clearly lower than the contents of manganese observed in this work (from 0.27 to 1.28% in leaves and from 0.14 to 0.95% in stems). Jallad and Espada-Jallad (2008) studied the mineral contents found in red and black henna. Their samples were obtained from the local products of consumers in Sharjah and Dubai in the United Arab Emirates. The contents, in these samples, were respectively 0.18 and 0.19% of sodium, 0.37 and 0.24% of magnesium, 0.22 and 0.17% of phosphorus, 1.36 and 0.55% of potassium, 2.16 and 1.02% of calcium, 0.26 and 0.25% of iron and less than 50 ppm and 0.42% of zinc. The content of sodium, detected by Jallad and Espada-Jallad (2008), was comparable to the average content of the 25 samples analyzed in this study (0.18% in stem and 0.23% in leaves), and it was higher than the contents of potassium, magnesium and calcium (0.46% in stem and 0.34% in leaves; 0.079% in stem and 0.15% in leaves; 0.19% in stem and 0.32% in leaves) and lower than the contents of phosphorus, iron and zinc (5.19% in stem and 4.08% in leaves; 4.8% in stem and 14.03% in leaves; 2.1% in stem and 1.23% in leaves), respectively in this study.

The diversity analysis of *L. inermis* populations cultivated in the oases of Gabès, based on macro and trace elements contents and nitrogen content, made it possible to distinguish 5 groups and some populations. The population of Chatt Essalem II (20) was characterized by its richness in potassium in the stem; sodium and zinc in the leaves; and calcium, phosphor, iron and copper in the leaves and stem. It was also characterized by its poverty in nitrogen. The populations of Bou Said (1, 2 and 3) were characterized by their richness in sodium, phosphorus and nitrogen in the leaves; copper in the stem; and zinc and iron in the leaves and stem. These populations were also characterized by their poverty in magnesium in leaves and stem.

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