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## Seasonal Chemical Composition of Leaves of Three *Atriplex halimus* (Chenopodiaceae) Natural Populations Grown in a Common Garden

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**Abstract:** Compared analyses of the chemical composition of the ramets of three natural populations of *Atriplex halimus* placed in a common garden were carried out. The study showed a significant variability of chemical and nutritive value between the three Moroccan populations originating from three bio-climate contexts (semi arid, arid and saharan). This variability appears within and between populations according to the sampling date. Ramets from Safi (semi arid bio-climate) and Marrakech (arid bio-climate) showed high contents of crud proteins (21.59 to 25.07% and 19.56 to 25.48% during the humid period and 19.12 to 20.64% and 17.42 to 19.94% during the dry period, respectively). As for the total phosphorus contents, the two populations reached 0.12 to 0.17% and 0.12 to 0.19% during the dry period and 0.25 to 0.31% and 0.21 to 0.32% during the humid period, respectively. Lipid matter levels were high during the autumnal period and estimated respectively at 10.14 to 10.87% and 10.27 to 12.66% for the same populations. The highest contents of fiber (acid detergent fiber: ADF), of crud ash, sodium and calcium were observed during the dry period (June, July, August and October). A highly significant negative correlations ( $P < 0.01$ ) were found between crud protein, fibers (ADF), crud ash, sodium, potassium, calcium and iron. Whereas, a highly significant positive correlation ( $P < 0.01$ ) was detected between crud proteins, phosphorus and lipid fraction.

**Key words:** *Atriplex halimus*, chemical composition, leaves, common garden

### INTRODUCTION

Soil salinisation is one of the major problem that limit the agricultural productivity and intensify desertification particularly in arid and semi-arid Mediterranean regions. In this area, about 20 million ha of agricultural lands are adversely affected by soil salinity<sup>[1]</sup>. The introduction of species with a high socio-economic value and tolerance to the salinity and aridity conditions constitutes one of the rational solutions for the rehabilitation and the restoration of these degraded lands. Among these species, *Atriplex halimus* constitutes an excellent choice as vegetable material for several reasons. The species is a xerohalophyte, perennial, native of the arid and semi arid Mediterranean areas. In addition, it presents a very satisfactory appetability and palatability constituting an appreciated fodder of camels, sheeps and caprines particularly during the dry period<sup>[2]</sup>. Endowed with a complex root system and a considerable air biomass, *A. halimus* is an efficient and a relatively cheap means against erosion and desertification<sup>[1,3,4]</sup>. Its ligneous wood is an interesting source of energy<sup>[2]</sup>. Indeed, the species was planted on several thousands of hectares in several

Mediterranean areas<sup>[5]</sup>. It represents for the livestock an important source of minerals, vitamins and proteins<sup>[6,7]</sup>. However, *A. halimus* is characterized by a high polymorphism due to its large ecological amplitude<sup>[2,8,9]</sup>. This situation leads to heterogeneous plantations and a high difference in their productivity. Few studies were carried out to determine the seasonal variation of the chemical composition and the feed value of the species in relation to its polymorphism. The aim of this study was to analyse the seasonal variation of the chemical composition and the feed value of leaves of three natural ecotypes of *A. halimus*, placed in a common garden. Only leaves were taken into the account because they constitute the richest part in nutritive substances and the most digestive at the fodder plants<sup>[10]</sup>.

### MATERIALS AND METHODS

**Sampling and chemical analysis:** For the sampling an aridity gradient was followed from the south-eastern to the north-western orientation through three stations: (I) a coastal station with a semi-arid bioclimat (Sidi Bouzid, region of Safi) ; (ii) a semi continental station with an arid

Table 1: Chemical and textural characteristics of soil of the experimental parcel

	Samples	
	1	2
pH	8.05	8.08
P <sub>2</sub> O <sub>5</sub> (ppm)	19.83	27.30
Ca <sup>2+</sup> (ppm)	15580.00	21080.00
Na <sup>+</sup> (ppm)	4761.00	5267.00
Cl <sup>-</sup> (ppm)	21655.00	35500.00
Mg <sup>2+</sup> (ppm)	3119.04	4158.72
K <sup>+</sup> (ppm)	6906.90	14718.60
Soluble salts (meq/100g)	94.98	134.19
Organic matter (%)	1.20	0.62
Total nitrogen content (%)	0.09	0.12
Sand (%)	77.00	57.30
Silt (%)	19.46	14.20
Clay (%)	5.10	6.17
CaCO <sub>3</sub> (%)	33.20	35.10

Data were recorded during the summer 1999. Samples were from the first 40 cm of soil

bioclimat (region of Marrakech) and (iii) a continental station with a saharan bioclimat (Idelssen, region of Ouarzazate). In each station, a natural population of *A. halimus* has been localised and semi ligneous cuttings were sampled in March 1999. The cuttings were treated by steeping in a indole-3-butyric acid solution (IAB, 0.4%) for 5 second, then placed in the greenhouse of the Institut National de Recherches Agronomiques (INRA, Marrakech-Morocco) during the rhizogenesis phase. After two months, the young seedlings were transplanted into polyethylene bags containing sand and peat (2: 1, v/v) and placed in the nursery for an acclimatization phase. In September 1999, the young individuals were transplanted randomly in a common parcel arranged for this purpose at the Faculté de Sciences Semlalia, Marrakech-Morocco. The physicochemical characteristics of the parcel ground are summarized in Table 1. The common garden was chosen for the examination of chemical composition of these 3 populations in order to discard age and environmental differences. During years 2001/2002 and 2002/2003, 7 shrubs were randomly selected for each population being composed of 30 individuals. Two branches emerging of the base of each shrub were cutted taking into the account the four cardinal expositions. This operation was repeated in the end of April, June, July, August, October, November, January and February. For each sample, the leaves were separated from the branches and were dried at 70°C during 72 h. 100 g of leaves pulverized and milled to pass a 0.4 mm screen were used for the chemical analyses. Magnesium and iron were determined by atomic absorption spectrophotometer. Calcium, sodium and potassium by flame spectrophotometer.

**Statistical analysis:** Data were analysed according to a factorial randomised complete design with seven replicates. Mains effects were populations (3), years (2) and months (8). The interactions between effects were sounded at the level of significance of  $P < 0.05$ . Means were compared using the least significant difference (LSD) at  $P < 0.05^{[11]}$  and their relationships were determined by the Pearson's correlation at variable levels of significance.

The total variance analyses showed highly significant differences ( $P < 0.001$ ) of the chemical composition and the feed value of the species. This variability is mainly related to the populations, the sampling date (month) and their interaction effect (populations x months), however, the year generates significant variations only for the fiber contents, crud proteins, sodium, magnesium calcium and iron. In fact, for the compared analysis between individuals of the three populations, values of chemical composition of leaves were pooled over years.

## RESULTS AND DISCUSSION

For the whole ramets of the three populations, seasonal variations were raised concerning the leaves chemical composition. The highest values of crud ash, fiber, calcium and sodium were recorded during June, July, August and October (Table 3, 4, 5 and 6), which correspond to the dry period of the two years studied (Fig. 1). Thus, the ramets of Idelssen population show the maximum contents reaching 34.58% of crud ash, 19.03% of fiber, 2.76% of calcium and 18.23% of sodium. These high contents might affect the palatability of the three populations and particularly that of Idelssen. Indeed, the concentrations of these lignocellulosic component (fiber) and minerals in leaves goes against the nutrition of the livestock<sup>[12-14]</sup>. These values decrease significantly during the end of the autumnal period (November) which coincide with the beginning of rainfall period (Fig. 1) to reach minimal contents during the winter period (January and February). The lowest values are recorded in the ramets of Sidi Bouzid population (Safi) and Marrakech. The total contents of phosphorus and crud proteins vary also within and between the three populations studied according to the sampling date. The three populations present the lowest contents of crud proteins during the dry period (Table 4 and 7), which remained relatively important (19.12 to 20.64% for the ramets of the Sidi Bouzid population, 17.42 to 19.94% for those of Marrakech population and 14.84 to 15.86% for those of Idelssen population). These values are higher compared to those obtained for the same species in Jordan<sup>[7,15]</sup>

Table 2: Global analyses of variance

Source of variance	df	F									
		ADF	Lip	C.P.	C.A.	Na	K	Ca	Mg	P	Fe
Population	2	94.062***	155.19***	262.29***	128.97***	31.29***	58.65***	185.01***	269.98***	94.44***	8.31***
Year	1	24.26***	0.75	15.68***	0.702	52.02***	1.037	16.69***	82.55***	3.2	5.85*
Months	7	27.03***	279.1***	106.03***	95.1***	66.9***	40.49***	19.59***	17.18***	134.9***	71.54***
Pop x Year	2	0.07	5.74**	0.468	4.01*	7.17***	12.29***	3.5*	21.93***	15.53***	3.009
Pop x Months	14	5.8***	6.21**	4.62***	12.83***	2.17**	6.4***	6.41***	5.64***	3.54***	2.04*
Year x Months	7	0.24	0.17	0.15	1.7	0.36	0.187	0.54	0.42	0.119	0.06
Pop x Year x Month	14	0.39	0.24	0.133	1.044	0.254	0.156	0.36	0.313	0.143	0.127

(\*\*\*) P<0.001 ; (\*\*) P<0.01 ; (\*) P<0.05. df: degree of freedom. ADF: Acid Detergent Fiber ; Lip: Lipid fraction ; C.P.: Crude protein ; C.A.: Crude Ash ; Ca: Calcium ; Na: Sodium ; Mg: Magnesium ; P: Phosphorus ; K: Potassium ; Fe: Iron

Table 3: Seasonal fiber (ADF) and lipid fraction contents of leaves of three natural populations of *Atriplex halimus* during 2001/2002 and 2002/2003

Months	ADF (%)			lipid fraction (%)		
	Sidi Bouzid (Safi)	Marrakech	Idelssen (Ouarzazate)	Sidi Bouzid (Safi)	Marrakech	Idelssen (Ouarzazate)
April	11.25±1.45f	15.15±1.946cd	13.01±1.59de	5.63±0.52gh	5.56±0.7gh	4.54±0.45i
June	13.09±1.57de	13.88±1.317cde	14.2±1.21cde	5.9±0.36fgh	6.29±0.78fg	5.11±0.74hi
July	13.1±1.16de	15.45±1.39c	14.39±0.75cde	6.21±0.71fg	6.62±0.62f	5.13±0.59hi
August	13.83±1.02cde	15.06±2.25cd	15.13±1.46cd	7.43±0.64e	8.47±1.45d	5.56±0.69gh
October	13.5±2.22cde	17.28±2.23b	19.03±2.7a	10.14±0.96bc	10.27±0.6bc	8.927±0.73d
November	13.16±1.15de	14.48±2.22cde	14.71±2.9cde	10.87±0.85b	12.66±1.21a	9.7±0.66c
January	10.14±1.45fg	13.85±1.83cde	15.46±0.89c	10.126±0.43bc	9.63±1.08c	7.69±1.01e
February	9.71±1.01g	12.83±1.27e	13.78±0.94cde	9.82±0.66c	8.74±0.88d	7.26±1.08e

Means of each component with a common letter do not differ significantly (P>0.05). ADF: Acid detergent fiber

Table 4: Seasonal crude protein and crude ash contents of leaves of three natural populations of *Atriplex halimus* during 2001/2002 and 2002/2003

Months	Crude protein (%)			Crude ash (%)		
	Sidi Bouzid (Safi)	Marrakech	Idelssen (Ouarzazate)	Sidi Bouzid (Safi)	Marrakech	Idelssen (Ouarzazate)
April	22.94±0.99c	20.71±0.66de	18.18±1.49fg	26.67±2.21ij	26.93±2.15ij	32.11±1.03cd
June	20.18±1.15de	18.02±1.1fg	15.86±1.25h	29.83±2.15efg	27.45±2.55hij	33.22±2.05abc
July	20.21±2.28de	18.27±1.39fg	15.37±1.09h	29.87±1.00efg	30.54±1.73defg	34.58±2.14a
August	19.12±1.78ef	17.42±0.99g	14.84±0.7h	31.61±1.48cde	30.46±1.68defg	34.05±1.61ab
October	20.64±1.9de	19.94±2.32de	15.12±0.71h	30.79±1.51def	32.43±2.32bcd	28.49±1.98ghi
November	21.59±2.92d	19.56±1.19ef	18.28±1.18fg	24.71±0.87kl	23.43±2.32l	27.3±1.47hij
January	25.07±1.09ab	23.84±1.88bc	21.54±0.98d	26.2±1.73jk	24.28±2.79l	28.96±1.78fgh
February	24.56±1.23ab	25.48±1.41a	19.13±1.64ef	24.62±1.77kl	22.89±1.01l	29.29±1.53fg

Means of each component with a common letter do not differ significantly (P>0.05)

Table 5: Seasonal Sodium and Potassium contents of leaves of three natural populations of *Atriplex halimus* during 2001/2002 and 2002/2003

Months	Sodium (%)			Potassium (%)		
	Sidi Bouzid (Safi)	Marrakech	Idelssen (Ouarzazate)	Sidi Bouzid (Safi)	Marrakech	Idelssen (Ouarzazate)
April	14.2±2.5cde	13.71±2.48def	14.72±1.78bcde	6.32±1.02ij	6.94±0.76bc	8.02±0.88a
June	16.96±2.49ab	14.29±1.73cde	14.8±2.01bcde	7.16±1.03b	6.49±0.49bcd	6.54±1.41bcd
July	16.32±1.78abc	16.46±1.18abc	17.67±1.84a	4.71±0.35bcdefg	5.81±1.46cdefgh	6.28±1.54bcde
August	18.41±2.46a	15.98±1.05abcd	18.22±2.84a	4.63±0.56b	5.49±0.47defghi	5.21±1.13efghij
October	18.09±1.9a	17.15±2.65a	18.23±2.49a	3.86±0.4bcde	5.54±0.67defghi	5.46±0.82defghi
November	13.06±2.08efg	10.17±0.91h	11.71±1.25fgh	3.88±0.54efghij	5.72±0.94defghi	5.06±0.57fghij
January	13.86±2.27def	11.01±1.85gh	13.75±2.57def	4.42±0.72hij	6.16±0.88bcdefg	4.96±0.69ghij
February	13.07±2.38efg	9.84±0.61h	12.81±3.26efg	3.83±0.59j	6.2±0.84bcdef	5.04±0.89fghij

Means of each component with a common letter do not differ significantly (P>0.05)

Table 6: Seasonal calcium and magnesium contents of leaves of three natural populations of *Atriplex halimus* during 2001/2002 and 2002/2003

Months	Calcium (%)			Magnesium (%)		
	Sidi Bouzid (Safi)	Marrakech	Idelssen (Ouarzazate)	Sidi Bouzid (Safi)	Marrakech	Idelssen (Ouarzazate)
April	2.3±0.25cd	1.82±0.23gh	2.11±0.49defg	1.8±0.25abc	1.31±0.11ef	1.81±0.08abc
June	2.59±0.23ab	2.14±0.19def	2.25±0.35cde	1.98±0.36a	1.17±0.15ef	1.66±0.18bcd
July	2.7±0.15ab	1.98±0.29efgh	2.64±0.23ab	1.59±0.23cd	1.13±0.22ef	1.64±0.15bcd
August	2.85±0.14a	1.91±0.27fgh	2.76±0.28ab	1.4±0.23de	1.13±0.11ef	1.31±0.29ef
October	2.7±0.21ab	1.9±0.2fgh	2.74±0.37ab	1.63±0.31bcd	1.13±0.18ef	1.62±0.31bcd
November	2.31±0.23cd	2.0±0.23efgh	2.71±0.14ab	1.65±0.29bcd	1.07±0.23f	1.9±0.09ab
January	2.29±0.18cd	1.85±0.24fgh	2.47±0.28bc	1.78±0.35abc	1.27±0.12ef	1.95±0.19a
February	2.06±0.17defg	1.71±0.17h	2.33±0.37cd	1.78±0.27abc	1.14±0.16ef	1.93±0.32a

Means of each component with a common letter do not differ significantly (P>0.05)

Table 7: Seasonal phosphorus and iron contents of leaves of three natural *Atriplex halimus* populations during 2001/2002 and 2002/2003

Months	Phosphorus (%)			Iron (ppm)		
	Sidi Bouzid (Safi)	Marrakech	Idelssen (Ouarzazate)	Sidi Bouzid (Safi)	Marrakech	Idelssen (Ouarzazate)
April	0.27±0.05abc	0.26±0.05bcd	0.16±0.028ghi	303.0±77.51fg	325.9±142.51efg	313.21±71.42fg
June	0.17±0.04fgh	0.19±0.03fg	0.15±0.06ghij	364.6±62.44def	454.71±55.61cd	427.64±169.37cde
July	0.16±0.029ghi	0.17±0.05fgh	0.11±0.01jk	381.28±52.46def	524.71±58.25c	448.85±99.98cd
August	0.12±0.017ijk	0.13±0.03ijk	0.09±0.009k	474.35±49.91cd	472.21±84.44cd	517.92±67.65c
October	0.14±0.014hij	0.12±0.04jk	0.09±0.01k	652.21±127.63ab	723.14±143.3a	635.92±182.76b
November	0.25±0.04cd	0.21±0.04ef	0.14±0.01ghij	334.42±53.18efg	374.57±46.4def	431.71±112.5cde
January	0.31±0.042a	0.3±0.045ab	0.23±0.047de	334.28±102.14efg	290.0±80.06fg	306.92±82.36fg
February	0.31±0.079a	0.32±0.032a	0.25±0.041cd	222.5±46.75g	322.64±103.14efg	264.07±54.92fg

Means of each component with a common letter do not differ significantly ( $P>0.05$ )

Table 8: Correlation coefficients among some chemical components of *A. halimus* leaves

	ADF	LIP	CP	CA	Na	K	Ca	Mg	P	Fe
ADF	1	0.014	-0.479**	0.250**	0.295**	0.120*	0.079	-0.150**	-0.463**	0.426**
LIP		1	0.337**	-0.473**	-0.274**	-0.423**	-0.117*	-0.132*	0.206**	0.106
PB			1	-0.609**	-0.477**	-0.141**	-0.351**	0.028	0.732**	-0.397**
CB				1	0.716**	0.096	0.372**	0.070	-0.639**	0.376**
Na					1	-0.105	0.471**	0.098	-0.575**	0.409**
K						1	-0.188**	-0.008	-0.046	-0.063
Ca							1	0.426**	-0.444**	0.162**
Mg								1	0.109*	-0.248**
P									1	-0.541**
Fe										1

Significant correlations at  $P<0.05$  (\*) and  $P<0.01$  (\*\*) and ADF: Acid detergent fiber; Lip: Lipid fraction; CP: Crude protein; CA: Crude ash; Ca: Calcium; Mg: Magnesium; P: Phosphorus; Fe: Iron; Na: Sodium; K: Potassium

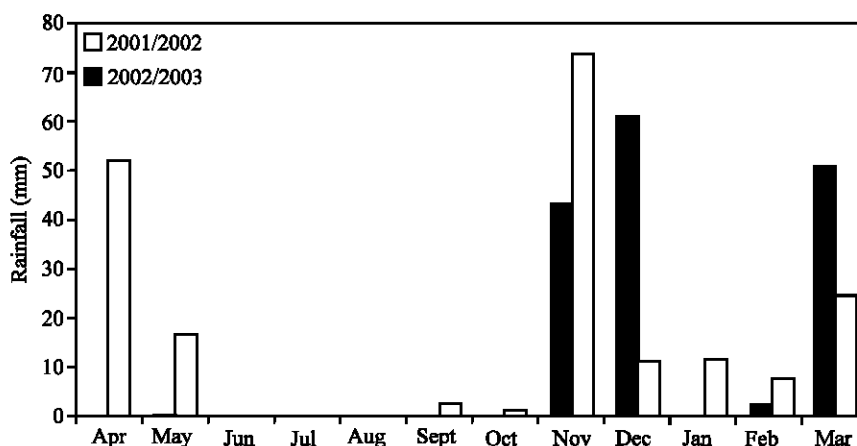


Fig. 1: Monthly rainfall (mm) for 2001/2002 and 2002/2003 at the meteorological station of Marrakech

and in Island<sup>[3]</sup>. As the ewes require 7 to 9% of crude proteins for their maintenance and 10 to 12% for their lactation<sup>[16]</sup>, the three studied populations can ensure an interesting source of nitrogen matter for the dry period and the feed shortage with an advantage for the Sidi Bouzid population. In fact, protein is one of the most limiting nutrients for range livestock production and its supplementation is cost effective, because it improves forage intake and digestibility<sup>[17]</sup>. For the dry period, phosphorus contents which constitute one of the important minerals for the microbial synthesis in the rumen of the animals<sup>[18]</sup> vary also between the ramets of the three populations (0.12 to 0.17% for the ramets of the Sidi Bouzid population against 0.12 to 0.19% for those of

Marrakech population and 0.09 to 0.15% for those of Idelssen population). Only the phosphorus contents recorded in the ramets of the populations of Sidi Bouzid and Marrakech were similar to those obtained by El-Shatnawi and Mohawesh<sup>[7]</sup> and El-Shatnawi and Turuk<sup>[15]</sup> and those advanced by Holechek *et al.*<sup>[16]</sup> to ensure the maintenance of the ewes (0.25 to 0.3% of total Phosphorus). During the humid period (January and February), these two populations present interesting contents of crude proteins (23 to 25%) and of total phosphorus (up to 0.3%). They also show in autumnal period (October, November) a significant contents of lipid matter (10.14 to 10.87% for the ramets of the Sidi Bouzid population and 10.27 to 12.66% for those of the

Marrakech population (Table 3). The high percentages of iron are raised during October with a non significant variation between the three populations (Table 7) and decrease gradually to reach minimal values during April. For magnesium, the maximum values were observed in the ramets of the Idelssen population during November, January and February, in those of the Sidi Bouzid population during April and June and in those of Marrakech during February and April. Potassium present very high values during April and June with maximum contents in the ramets of the Idelssen population was 6.54 to 8.02% (Table 5). These values were much higher than that required for the nutrition of sheep<sup>[19,20]</sup>.

The correlation analysis showed a highly significant negative relationships ( $P < 0.01$ ) between crude proteins, fibers (ADF), crude ash, sodium, potassium, calcium and iron, whereas crude protein exhibit a significant positive correlation with phosphorus and lipid fraction (Table 8).

The *A. halimus* presents very interesting fodder and environmental potentialities. It is a valuable source of crude proteins, phosphorus and lipid during dry seasons and feed shortage times. However, the polymorphism which characterize the species imposes a pre-selection of populations with an interesting fodder value. In our case, the populations of Sidi Bouzid and Marrakech showed a very interesting chemical composition and food value. The widening of the study on several natural populations of *A. halimus* in Morocco will make it possible to set up populations adapted to a particular environment with a high fodder value. This work pointed out that the Sidi Bouzid population could be planted in the Marrakech region and may be in the Ouarzazate region. Further studies should be carried out to make a national program of *A. halimus* plantation depending on the soil and climates of several regions. Within such program, studies will be carried out also to analyse the nutritive value of *A. halimus* natural populations. The use of *in vitro* and *in sacco* techniques will be helpful to predict the energy value and the rumen degradable protein content.

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