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Study of mineral and nutritional components of some leguminous herbaceous and shrubs species in Tunisia

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Abstract

Chemical and mineral compositions, during four seasons, of aerial parts of five leguminous herbaceous species (*Anthyllis vulneraria, Hedysarum coronarium, Medicago italica, Trifolium repens* and *Trifolium subterraneum*) and seven leguminous forage shrubs (*Acacia cyanophylla, Anthyllis barba jovis, Chamaecytisus albidus, Colutea arborea, Cytisus villosus, Leucaena leucocephala, Medicago arborea*) grown in Tunisia were investigated. The main constituents of mineral elements determined spectrophotometrically were Zinc (120.95 ppm and 54.5 ppm), Phosphorus (0.46% and 0.3%), Sodium (2.05% and 0.24%), Potassium (1.66% and 2.25%), iron (480.35ppm and 570.11ppm) and copper (11ppm and 8ppm) in herbaceous species and shrubs, respectively. Fat contents obtained by Soxhlet varied between 3.24% and 6.3%. Both herbaceous and shrubs species had the same values of neutral detergent fiber (54%) and crude protein contents (20%) analyzed by (Van Soest, 1982) and Kjeldahl methods, respectively. These results were found to be influenced by their developmental stage and the maximum values were observed in spring. These leguminous, rich based on fiber, crude proteins, sodium, potassium and iron, might play an important role for animal nutrition. The *Medicago italica* and *Anthyllis barba jovis* were selected as good nutritive species. It had a potential for integration into livestock feeding agro-sylvo-pastoral culture.

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Introduction

Arid and semi-arid areas were characterized by low erratic rainfall of up to 700 mm per annum and periodic droughts. These conditions were suitable for the development of the leguminous species. These vegetations played a major role in keeping the ecological diversity, in limiting desertification and in production wood of height quality. Although these roles, some species have been disappeared, others were endangered (Basly, 2001). In Tunisia, these species become increasingly important as protein-rich forages to supplement a basal diet of either grass or poor quality roughage for ruminant livestock. For smallholder milk production in particular, legumes gives handsome economic returns even under constraints of irrigation and fertility and on marginal lands. In fact, they had two principal roles: they produced forage of height quality rich on proteins and they assured the fixation of the atmospheric nitrogen (Duthil 1976, Raumbaugh and Johnson 1986, Mezni et Sifi, 1995). *Medicago truncculata* Gaertn. Var. Jamalong, for example, can fix 122 Kg N. ha^{-1} (Zouari, 1996). Moreover, numerous authors detailed the importance of legumes species in many medicinal fields (systematic, genetic, physiology, etc). However, there was a lack of information on the nutritive value of these species especially in Tunisia.

The aim of this study is to characterize and compare the chemical and mineral compositions of some forage leguminous shrubs and herbaceous grown in Tunisia.

Material and methods

Stations

Plant materiel were collected from different leguminous cultivated in kroumerie and Mogod (S1), the experimental station of "Rouhia" (S2) and the arboretum of National Institute for Research in Rural Engineering, Water and Forests (INRGREF) in Tunisia (S3) (Fig. 1).

Sampling

Plant materiel was collect four times a year for shrubs (winter, spring, summer and autumn) in order to follow the effect of seasonal nutritional quality. For herbaceous, sample collection was done during the growing stage. They were placed in plastic bags and transported to the laboratory of National Institute for Research in Rural Engineering, Water and Forests (INRGREF) where species were identified and a voucher sample was deposited at the Herbarium of INRGREF in Tunisia.

Chemical analysis

Dry weight

After collect, the fresh samples were weighed, dried at 105°C till the constant weight, grounded and placed in plastic bags until analysis.

Mineral content

Plant material (0.5 g of plant powder) was calcinated at 550°C for 4 hours. The ash already cooled, was wetted with 3 mL of distilled H_2O. It was added with 10 ml of hydrochloric acid and then filtered into vials of 50 mL. The solution thus obtained was stored in vials until analysis. The mineral elements were determined using a flame spectrophotometer (Genway, Model PSP7). The phosphorus (P) content was determined by calorimetry at vanadomolybdate.

Crude protein (CP)

Crude protein content of samples was determined based on Kjeldahl method. This method consisted in the mineralization of organic nitrogen in the sample treated inorganic nitrogen (ammoniac) and the acid-base titration of the ammoniac.

Fat content (FAT)

The fat content was estimated by the Soxhlet method. Extraction was carried out from 10g of ground material and 200ml Hexane. The extraction was continued until exhaustion of the vegetable material.

Vegetable fiber (NDF)

NDF were determined by methods of (Van Soest, 1982). Theses assays were performed by adding of 100 mL of the NDF (detergent base EDTA) solution. After one hour at the boiling, the reagents were removed by suction and the residue was thoroughly rinsed with boiling water until the disappearance of foam.

Statistical Analysis

The parameters measured were subjected to analysis of variance two parameters (species and period), studying the Fisher test (F). Means were separated using the least significant difference (LSD). Only significant effects at 5% were considered.

Results

Chemical compositions of leguminous herbaceous species

The largest dry matter (33%) was observed in *Anthyllis vulneraria* and *Trifolium repens* (Table 1). *Hedysarum coronarium* and *Medicago italica* had the lowest values (23%). This variation became less important for crude protein content. In particular, *Trifolium repens* had the highest value (20.40%), but *Antyllis vulneraria* had the lowest value (12.60%).

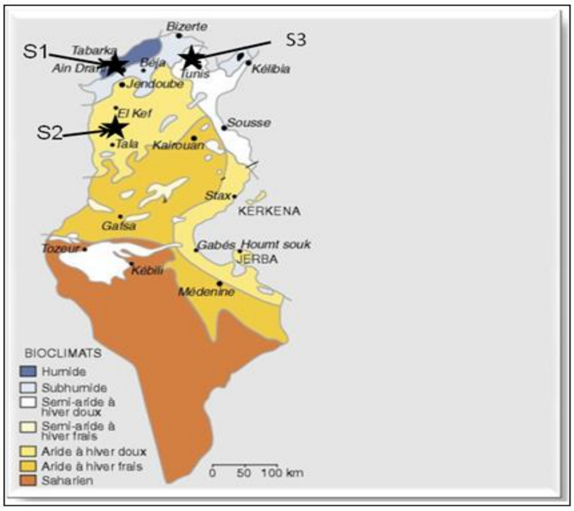

Fig. 1. Localization of leguminous herbaceous and forage shrubs species on bioclimatic map of Tunisia (Elaloui, 2013).

For fat analysis, *Hedysarum coronarium* had the highest content (3.24%), but *Anthyllis vulneraria* had the lowest level (1.25%). The NDF contents were very high and varied between 34% and 56% (*Medicago italic*). The lowest values (34.66% and 35.77%) were observed in *Trifolium subterraneum* and *Trifolium repens* respectively. *Trifolium subterraneum* had the highest mineral value (15.70 %). Potassium and calcium were the major macro elements of forage species. The potassium ranged from 1.25 g 100 g^{-1} (*Hedysarum coronarium*) to 1.66 g 100 g^{-1} (*Trifolium subterraneum*). The calcium ranged between 1.2 g 100 g^{-1} (*Trifolium repens*) and 2.44 g 100 g^{-1} (*Hedysarum coronarium*). The rate of Iron contents was statistically higher in all species (Table 2). It can rich the value of 480.35ppm in *Medicago italica*. Magnesium and zinc were also being the highest mineral salts in these species. The copper was observed the lowest contents and did not exceed 11.09 ppm in all species.

According to this table, the chemical composition had highlighted significant heterogeneity between the studied species. *Medicago italica* was the richest specie in mineral contents (Na, Mg and Ca).

Chemical composition of leguminous forage shrubs

Chamaecytisus albidus had the highest significant dry weight value (43.24%). The *Colutea arborea* had the smallest dry weight production (31.35%), while this value did not exceed 31% for *Anthyllis barba jovis*. *Anthyllis barba jovis*, *Medicago arborea* and *Acacia cyanophylla* had comparable dry matter values respectively 39%, 38% and 36% (Table 3).

Table 1. Chemical composition (g 100 g^{-1} dry weight) of some leguminous herbaceous in the region of Mogods and Kroumirie

	Dry weight	Crude protein	FAT	NDF
Anthyllis vulneraria	33.53 c	12.60 a	1.25 a	46.45 c
Hedysarum coronarium	24.07 a	17.40 b	3.24 c	40.42 b
Medicago italica	23.10 a	16.26 b	2.22 b	55.95 d
Trifolium repens	33.30 c	20.40 c	2.32 b	35.77 a
Trifolium subterraneum	27.27 b	15.70 b	2.18 b	34.66 a

Means followed by different letters (a, b, c, d) in the same row are significantly different ($p < 0.05$).

Table 2. Macroelements (g 100 g^{-1} dry weight) and microelements (ppm) of some leguminous herbaceous s from Mogods and Kroumirie region.

	Anthyllis vulneraria	*Hedysarum coronarium*	*Medicago italica*	*Trifolium repens*	*Trifolium subterraneum*
Mineral content	13.13 a	13.25 a	13.37 a	12.60 a	15.68 b
Phosphorus	0.20 b	0.29 c	0.46 d	0.17 a	0.21 b
Sodium	0.53 a	2.05 c	1.27 b	0.64 a	1.41 b
Potassium	1.48 b	1.25 a	1.54 b	1.46 b	1.66 c
Magnesium	0.22 b	0.31 c	0.32 c	0.18 a	0.24 b
Calcium	1.28 a	2.44 d	1.57 b	1.20 a	1.91 c
Copper	5.09 a	9.62 c	5.41 a	11.09 d	7.47 b
Zinc	47.07 c	38.90 b	17.54 a	60.88 d	120.95 e
Iron	357.4 c	252.30 b	480.35 d	118.80 a	256.30 b
Manganese	37.66 c	17.98 b	53.78 d	13.81 a	18.60 b

Means followed by different letters (a, b, c, d) in the same row are significantly different ($p < 0.05$).

Leucaena leucocephala, Colutea arborea and *Medicago arborea* were characterized by high crude protein amounts of (resp. 20%, 19% and 18%). *Anthyllis barba jovis* presented the lowest level (12%). NDF analysis showed that *Cytisus villosus* had the highest value (54.85%). While *Colutea arborea* had the lowest amounts (33.79%).

Table 3. Chemical compositions (g 100 g^{-1} dry weight) and Fisher values of leguminous forage shrubs during four seasons.

	Dry weight	Crude protein	Fat	NDF
Anthyllis barba jovis	39.62	12.87	5.73	44.17
Acacia cyanophylla	36.32	15.45	4.59	42.53
Medicago arborea	38.86	18.64	4.66	40.89
Leucaena leucocephala	32.34	20.54	4.90	40.06
Cytisus villosus	33.53	17.47	6.12	54.85
Chamaecytisus albidus	43.25	16.78	6.30	52.47
Colutea arborea	31.35	19.42	6.05	33.79
$PPDS_{(0,05)}$	2.74	1.48	1.13	2.29
Period				
Spring	31.36	19.96	5.65	43.69
Summer	42.45	16.36	3.79	49.34
Autumn	37.2	15.67	5.7	43.29
Winter	34.86	17.31	6.78	40.20
$PPDS_{(0,05)}$	1.8	0.97	0.74	1.5
Fisher value				
Specie (E)	46.97	56.13	7.99	190.72
Period (P)	94.01	53.07	39.48	90.83
E*P	24.04	14.46	9.27	13.89

For Fat analysis, the highest levels were estimated to 6% in *Colutea arbora, Cytisus villosus, Anthyllis barba jovis* and *Chamaecytisus albidus*. This value did not exceed 4% for *Acacia cyanophylla, Medicago arborea* and *Leucaena leucocephala* (table 4). The mineral content was comparable to *Anthyllis barba jovis* (10.58%), *Acacia cyanophylla* (9.80%), *Medicago arborea* (8.03%), *Leucaena leucocephala* (9.92%) and *Colutea arborea* (9.87%).

These leguminous forage shrubs had the lowest Phosphorus and Potassium contents and the value did not exceed 0.3% (*Colutea arborea*). Magnesium contents varied from 0.20% to 0.41% with thesuperiority of *Anthyllis barba jovis* (1.62%).

Table 4. Mineral compositions and Fisher values of leguminous forage shrubs during four seasons.

	Macro-elements (g 100 g⁻¹ MS)						*Oligo-elements (ppm)*		
	MM	P	Na	K	Mg	Ca	Cu	Zn	Fe
Anthyllis barba jovis	10.58	0.17	0.14	1.22	1.62	2.70	4.02	33.38	554.17
Acacia cyanophylla	9.8	0.15	0.15	1.19	0.35	2.14	4.64	54.57	264.63
Medicago arborea	8.03	0.21	0.21	1.82	0.22	1.25	4.20	28.31	570.11
Leucaena leucocephala	9.92	0.25	0.23	2.25	0.39	1.99	4.88	34.15	290.54
Cytisus villosus	5.03	0.22	0.19	1.22	0.20	0.65	8.05	48.21	219.26
Chamaecytisus albidus	4.56	0.18	0.17	0.96	0.29	0.72	7.81	40.92	230.90
Colutea arborea	9.87	0.3	0.24	1.94	0.41	1.69	5.62	32.15	405.68
$PPDS_{(0,05)}$	1.15	0.05	0.07	0.2	0.08	0.27	1.86	8.12	92.18
Period									
Sping	9.24	0.24	0.22	1.69	0.36	2.14	7.62	43.98	430.57
summer	8.27	0.17	0.17	1.74	0.26	1.04	6.97	43.39	323.21
Autumn	8.07	0.28	0.16	1.33	0.24	1.59	1.37	26.68	300.03
Winter	7.49	0.16	0.21	1.30	1.13	1.6	6.45	41.20	394.93
$PPDS_{(0,05)}$	0.75	0.03	0.04	0.13	0.06	0.18	1.21	5.31	
Fisher value									
Species (E)	87.97	22.40	5.63	106.7	0.90	149.8	15.17	25.87	49.27
Period (P)	13.19	53.15	6.74	42.9	1.13	91.15	77.71	33.22	14.29
E*P	5.42	6.34	3.64	10.8	0.96	10.19	3.77	3.86	9.06

There was a significant difference ($P < 0.05$) for the interaction of the species with the period. In fact, the percentage of dry weight increased in summer and reached a very high content (42%) whereas in spring, this content decreased to 31% (Fig. 2A).

The same results were observed for NDF analysis that attempt his maximum (49.34%) (Fig 2B). This phenomenon can be explained by the growth of bud that began in winter and reached the maximum in spring, whereas in summer and autumn the consumable part shrubs were lignified, so the dry weight increased in summer.

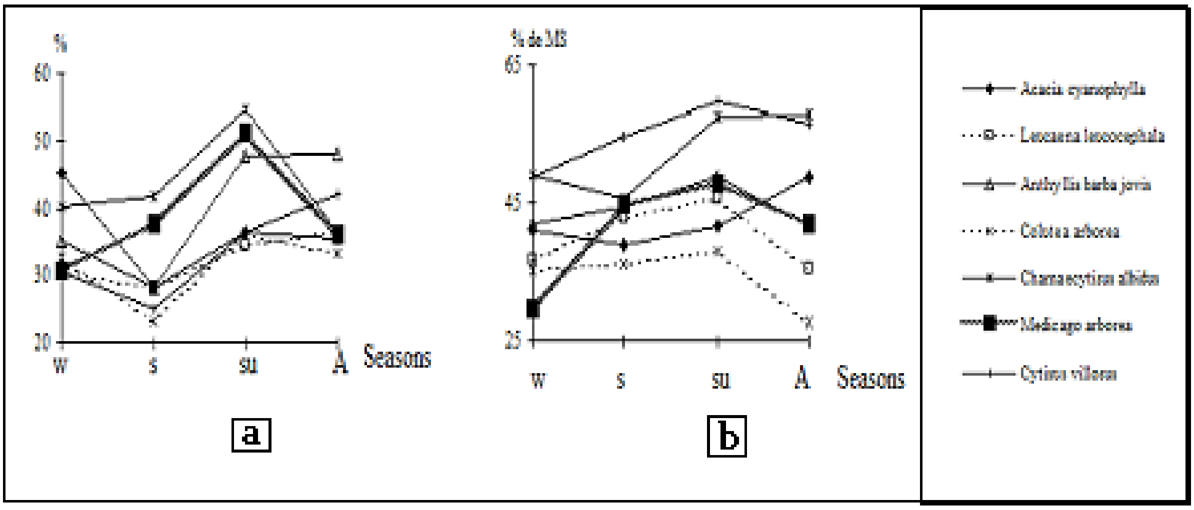

Fig. 2. Seasonal variation of dry weight (a) and NDF contents (b) in forage shrubs
W: winter, S: spring; Su: summer; A: autumn.

The Iron content was almost comparable or identical to the majority of species and exceeded 550ppm. The Zinc contents varied between 28 ppm (*Medicago arborea*) and 54 ppm (*Acacia cyanophylla*). These salts, as can be observed for fat values, crude proteins and mineral contents, being high in spring and decreased in summer (Fig. 3).

Discussion

This study showed that these forages were rich in crude protein (12% to 21%) which allowed to use these plant as a good source of proteins (Ben Salem *et al.*, 2001). The chemical composition varied widely between species. This variability can be attributed in part to the geographical origin, bioclimatic and genetics of the species.

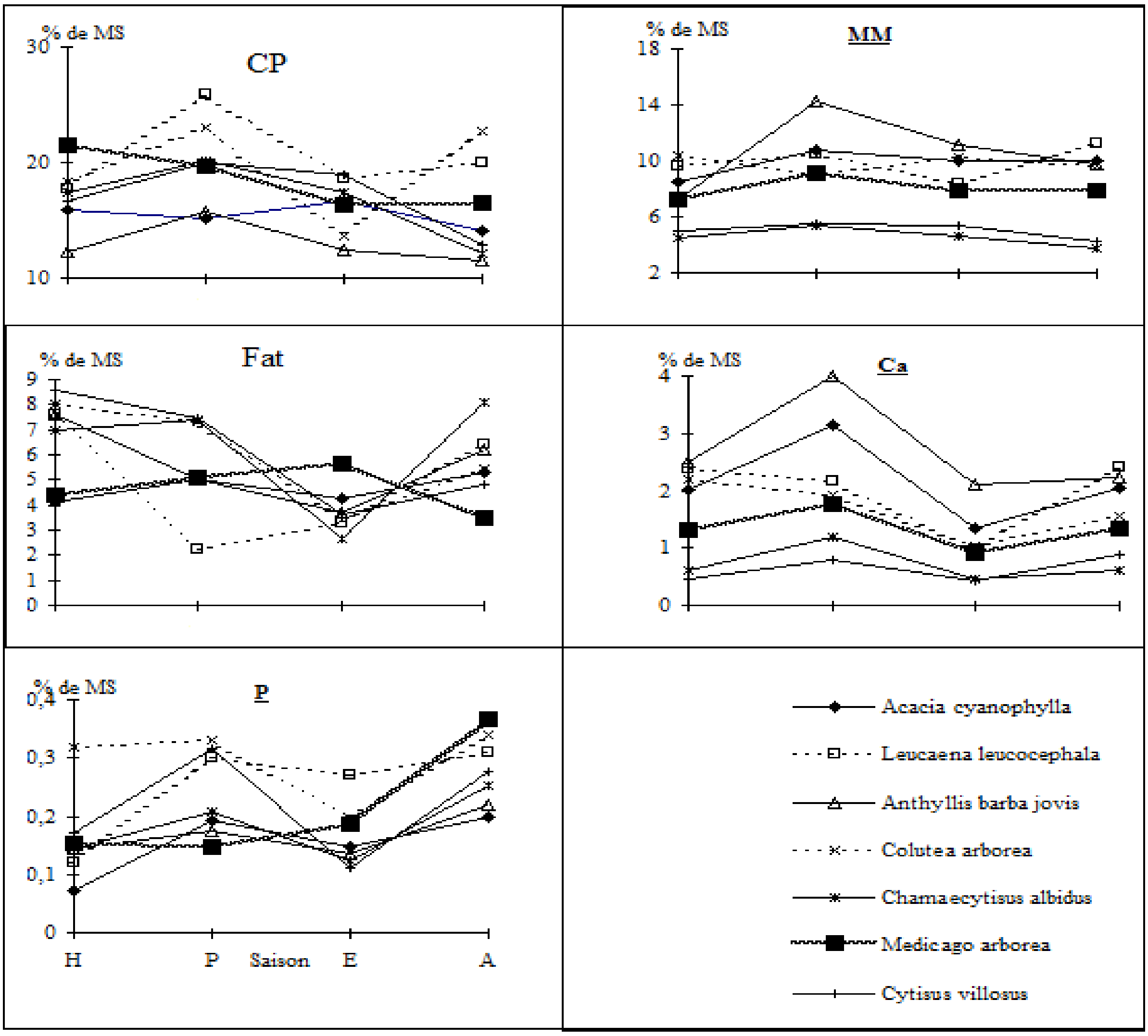

Fig. 3. Seasonal variations of crude proteins (CP), Fats, mineral (MM), Calcium (C) and Phosphorus (P) contents in leguminous forage shrubs.

These results were similar to these observed in Mediterranean (Khazaal *et al.*, 1993) and tropical species (Aletor, 1994) Seasonal variability in crude protein content was confirmed by several authors (Nastis 1982, Papachristou *et al.* 1993, Papachristou and Papanastis 1994, Ben Salem *et al.* 2001). This idea can be explained that during the growing season, the formation of crude protein peaked in the plant. The gradual increase in the NDF contents was due to the progressive lignifications and fiber formation of cell walls consumable parts of woody shrub species depending on the season. As all mature plants, carbohydrates structures increased with the season and decreased crude protein.

Conclusion

The five leguminous species studied in Mogods and Kroumirie (Tunisia) were rich in Zinc, Phosphorus, Sodium, Potassium, fats and crude proteins, provided a good source to be used as the nutrient source of ruminant animal feed along the year. Similarly, the development of rural areas by the management based on seven forage shrubs allowed the establishment of productive and sustainable systems. The effect of season on the chemical and mineral compositions let us identify the suitable periods of the most favorite use of these species.

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