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Antioxidant compounds and nutritional quality of eight Tunisian populations of echinus medic (*Medicago ciliaris* L.)

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Abstract. Analysis of the nutritional value of dry biomass, pods and straw of eight populations of *Medicago ciliaris* L. was undertaken. Classic composition, total phenol and tannins, condensed tannins and antioxidants compounds were analyzed. Results showed significant differences for crude protein content in biomass and pods, chlorophyll b in pods and lycopene in straw. Crude protein content varied from 7 to 24 % in dry biomass and from 9 to 21 % in pods. Chlorophyll b in pods varied between 0.6 and 4.6mg/g DM while lycopene in straw ranged from 0.1 to 0.3 mg/g DM. Populations 9144, 9140, 9142 and 9153 are the most interesting for their nutritional values and their antioxidants compounds. These results showed the importance of this species in animal nutrition and allowed us to exploit the populations according to their vocation (green or dry pastures).

Keywords. *Medicago ciliaris* L. – Local populations – Antioxidants compounds – Phenols and tannins.

Composés antioxydants et qualité nutritionnelle de huit populations tunisiennes de* Medicago ciliaris *L.

Résumé. *L'analyse de la valeur nutritionnelle de la biomasse sèche, des gousses et de la paille de huit populations de* Medicago ciliaris *L. a été étudiée dans le présent travail. La composition classique, les tanins et les phénols totaux, les tanins condensés ainsi que les antioxydants ont été analysés. Les résultats ont montré des différences significatives pour les protéines brutes dans la biomasse sèche et les gousses, la chlorophylle b dans les gousses et le lycopène dans la paille. La teneur en protéines brutes varie de 7,5 à 23,9 % dans la biomasse sèche et de 9 à 21,7 % dans les gousses sèches. La chlorophylle b varie de 0,6 à 4,6 mg / g MS dans les gousses alors que le lycopène varie de 0,1 à 0,3 mg / g MS dans la paille. Les populations 9144, 9140, 9142 et 9153 sont les plus intéressantes pour leurs valeurs nutritionnelles et leurs composants antioxydants. Les résultats ont montré aussi l'importance de cette espèce dans l'alimentation des animaux et nous permettent d'exploiter ces populations selon leur vocation (pâturage vert ou sec).*

Mots-clés. Medicago ciliaris *L. – Populations locales – Composés chimiques antioxydants – Phénols et tanins.*

I – Introduction

Tunisian flora is particularly rich in species, some are proving of great agricultural value, as they are used as fodder for livestock or as food plants and others have medicinal application. In order to enhance plant genetic resources in Tunisia, knowledge on forage and pastoral species is an essential concern. This work focused on chemical and nutritional analysis of the dry biomass, pods and straw of eight local populations of *M. ciliaris* belonging to the genus *Medicago*. The evaluation of forage quality is a very important factor affecting both animal health and animal products. In vetches, phenolics may act as antioxidants and they have an important role in the defense system of the seeds (Stanisavljević *et al*., 2014).

II – Materials and methods

Eight populations of *M. ciliaris* L. collected from different regions of the country and stored at INRAT were used in this work. The ecological characteristics of collection sites of the studied populations are given in Table 1.

Table 1. Main characteristics of the collection sites of *M. ciliaris* L. populations

Code population	Site name	Bioclimate	Direction	Altitude (m)	Rainfall (mm)	Max T (°C)	Min T (°C)
9140	Siliana	Semiarid	NW	575	341.1	38.6	1.6
9141	OuedMessouge	Semiarid	NW	500	367.1	38.6	1.6
9142	Dougga	Sub humid	NW	320	493.5	21.7	9.9
9144	Ghzala	Humid	NE	5	554.2	31.8	6.7
9153	Ghar El Melh	Humid	NE	6.19	497.8	32.9	6.7
9150	Zana	Humid	NE	10	507.0	31.8	6.7
9151	Raoued El Hessiane	Semiarid	NE	5.75	398.5	35.1	5.4
9152	KalaatAndalous	Semiarid	NE	5.85	398.5	35.1	5.4

Only ground samples of dry biomass and pods were analyzed for dry matter (DM), organic matter (OM) and crude protein (CP) according. They were also analyzed for secondary compounds (total phenol and tannins and condensed tannins) according to Makkar (2003) and saponins according to Hiai *et al.* (1976). However, antioxidants components (β- carotene, lycopene and chlorophylls a and b) were determined on dry biomass, pods and straw using the method of Nagata and Yamashita (1992). All pigments in sample were extracted with acetone – hexane mixture (4:6) at once, the absorbance of the filtrate was measured at λ = 453, 505, 645 and 663 nm by spectrophotometer at the same time. Contents of β- carotene and lycopene were calculated according to the following equations: β- carotene (mg/100ml) = $0.216A_{663}$ – $0.304A_{505}$ + $0.452A_{453}$; lycopene (mg/100ml) = - $0.0458A_{663}$ + $0.372A_{505}$ + $0.0806A_{453}$; chlorophyll a (mg / 100 ml) = $0.999A_{663}$-$0.0989A_{645}$; chlorophyll b (mg / 100ml) + = -$0.328A_{663}$+$1.77A_{645}$.

Data were subjected to analysis of variance with two factors of classification (population, replication) using the GLM produced by SAS (1998). Duncan's test for comparison was applied to the measured parameters.

III – Results and discussion

1. Classic chemical composition and secondary compounds

DM, OM, CP and secondary metabolites contents of dry biomass and pods are presented in Table 2. Results show that OM and DM contents varied significantly among populations while the secondary metabolites are not variables. CP of the dry biomass ranged from 12 to 24 % with an average of 17%, similar to that of sulla (16%) and slightly lower than that of *Scorpiurus* (22%) (Zoghlami *et al.,* 2008). CP content is higher in biomass than in pods (17 % *vs* 14% DM, respectively).The contrary was showed by Fois *et al.,* (2000) in *M. polymorpha*. Total phenols and tannins, condensed tannins and saponins did not vary significantly between populations. Total phenols varied between 3.6 and 4.2 g eq.ac.tann/kg DM with an average of 3.9 g eq. ac. tann/kg DM. Total tannins varied from 1 to 3 g eq. ac. tann/kg DM with an average of 2 g eq. ac.tann/kg DM; condensed tannins is very low for all accessions and saponins varied from 6 to 12 g/kg DM with an average of 9 g/kg DM).

Table 2. Chemical composition of dry biomass and pods

Populations	Dry biomass							Pods
	DM%	OM%	CP%	TPh*	TT*	CT*	SAP*	CP%
9140	13.8	80.5	22.6a	4.24	3.3	0.003	9.3	13.3b
9153	14.9	80.8	18.7ab	4.21	2.8	0.004	9.2	13.1b
9144	15.5	78.5	23.9a	4.2	1.2	0.006	5.8	14.9ab
9142	14.1	72.9	11.8dc	4	3.1	0.015	6.4	19.5a
9151	15.1	80.3	12.8c	3.8	3.2	0.002	9.3	13.8b
9141	16.5	79.2	13.0c	3.7	3	0.006	11.9	-
9152	14.1	72.4	17.5ab	3.6	2.5	0.013	8.3	10.9b
9150	14.4	70.3	16.3bc	3.7	2.6	0.005	9.9	12.4b
2	*14.7*	*77.4*	*17*	*3.9*	*2.2*	*0.006*	*8.9*	*14*
Standard error	-	*4.9*	*2.7*	*0.3*	*1.7*	*0.005*	*3.7*	*2.9*
Pr	*0.44*	*0.19*	*0.0002*	*0.25*	*0.17*	*0.31*	*0.64*	*0.09*

DM: dry matter content; CP: crude protein; OM: organic matter; TPh : total phenols ; TT: total tannins; SAP : saponins; *: g tannic acid equivalent/kg of DM.

2. Antioxidants components

Levels of antioxidants compounds are presented in Table 3. Results showed that only chlorophyll b in pods and lycopene in straw varied significantly among populations. Chlorophyll a is higher than chlorophyll b in dry biomass while chlorophyll b is higher in dry pods and straw. Same result was obtained by Haffani Ksontini (2015) in fresh biomass of vetches. For our populations, chlorophyll a varied from 0.6 mg/g DM in straw to 3.7 mg/g DM in dry biomass. Low content of β-carotene (0.05, 0.1 and 0.01 mg/g DM) in pods, biomass and straw, respectively could be attributed to its degradation by light and/or high temperature (Ilahy, 2015, personal communication). These values are higher than those found by Karadas *et al.* (2006) in alfalfa concentrate (0,005mg/g DM). Lycopene content varied from 0.2 mg/g DM in straw to 0.6mg/g DM in both dry biomass and pods.

Significant correlations were found between chlorophyll b and CP, β- carotene and lycopene in dry pods (r=-0.59**, n= 19; r=0.64**, n=20; r=0,74***, n=20, respectively) and between chlorophyll b β- carotene (r=0.86***, n=22) in straw.

Table 3. Antioxidants compounds analyzed in different parts of the plant (dry biomass, pods and straw) (mg/g DM) in *Medicago ciliaris* L.

Population	Pods				Biomass				Straw			
	β-carot	Lyco	Chla	Chlb	β -carot	Lyco	Chl a	Chl b	β-carot	Lyco	Chla	Chlb
2	0	1.1	0.7	3.6a	0a	0.5	3.7	0.5	0	0.1c	0.4	0.3
9153	0.1	1	1.4	4.6a	0.1	0.4	3.8	1.3	0.05	0.2bc	0.3	4.4
9144	0	0.9	1.6	2.3ab	0.4	0.4	3.4	1.4	0a	0.34a	0.3	1.5
9142	0	0.4	0.7	0.6b	0.1	0.7	4.2	0.9	0a	0.3ab	1.3	0.9
9150	0	0	0.8	2.6b	0.3	0.4	4.6	1.9	0a	0.2abc	0.	0.5
9141	0.1	0.5	0.5	0.8b	0	0.8	3.9	2	0a	0.2abc	0.7	0.5
9152	0.1	0.2	0.5	1.4ab	0	0.6	3.2	2.5	0.03	0.2bc	0.7	0.6
9150	0	0.5	1.1	1.7ab	0	0.3	1.3	0.3	0	0.1c	0.4	0.4
Mean	*0.05*	*0.6*	*0.9*	*2.2*	*0.1*	*0.6*	*3.7*	*1.5*	*0.01*	*0.2*	*0.6*	*1.2*
Standard error	*0.1*	*0.6*	*0.6*	*0.1*	*0.3*	*0.4*	*1.8*	*1.1*	*0.04*	*0.1*	*0.5*	*2.5*
Pr	*0.8*	*0.4*	*0.5*	*0.16*	*0.79*	*0.86*	*0.82*	*0.52*	*0.74*	*0.03*	*0.6*	*0.5*

β-carot= β- carotene, lyco=lycopene; Chl a= chlorophyll a; Chl b= chlorophyll b.

IV – Conclusion

The study of nutritional value of dry biomass, pods and straw of eight populations of *M. ciliaris* showed significant variation among populations for crude protein, organic matter, lycopene and chlorophyll b. Populations 9144, 9140, 9142 and 9153 are the most interesting for their nutritional value and antioxidant composition. Based on the positive correlations between chlorophyll b and β- carotene in straw and chlorophyll b and lycopene in pods, summer grazing of dry pods and straw of echinus medic is recommended for the growth of sheep.

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