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Content of polyphenolic compounds in *Melilotus officinalis* ecotypes from Morocco

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Abstract. Twenty ecotypes of yellow *Melilotus officinalis* from the north-west pastures of Morocco have been cultivated in the experimental station of the National Institute of Agronomic Research of Tangier. The samples were collected during the vegetative, budding and flowering stages in order to evaluate the phenolic content. Phenolic content of the ecotypes collection varied significantly with the maturation stage and the ecotype origin. Total phenols (TP) increased significantly from 2.14% dry mater (DM) at the vegetative stage to 2.37% DM at the flowering stage ($P < 0.01$). Total tannins (TT) increased from 0.71 % DM at vegetative stage to 0.78% DM at the budding stage and then decreased to 0.67% DM in the flowering stage. In contrast, the condensed tannins content (CT) of the collection decreased significantly from 0.10 at the vegetative stage to 0.04% DM ($P < 0.001$) at the flowering maturation stage. In addition, the content of TP and TT varied significantly between ecotypes, from 2.01 to 2.69 % DM ($P < 0.001$) and from 0.44 to 1.15% DM ($P < 0.001$), respectively. The high content of CT was obtained at the vegetative stage with 0.10% DM.

Keywords. *Melilotus officinalis* – Ecotype – Phenols – Tannins.

Teneur en composés phénoliques d'une collection d'écotypes de *Melilotus officinalis* de la région Nord-Ouest du Maroc

Résumé. Vingt écotypes de Mélilot (*Melilotus officinalis*) des pâturages du Nord-Ouest du Maroc ont été installés dans la station expérimentale de l'INRA à Tanger. Des fauches ont été réalisées, au stade végétatif, bourgeonnement et floraison, afin d'évaluer la composition en phénols et tanins. Le résultat montre que *Melilotus officinalis* est caractérisé par des teneurs modérées en composés phénoliques extractibles. La concentration de ces composés varie significativement avec le stade de maturation et aussi en fonction de l'origine de l'écotype. En effet, en allant du stade végétatif à la floraison, la teneur en phénols totaux (PT) augmente de 2,14% à 2,37% MS, alors que la teneur en tanins totaux (TT) augmente de 0,71% MS à 0,78% MS au stade bourgeonnement puis elle chute à la floraison 0,67% MS. En revanche, la teneur en tanins condensés (TC) diminue de 0,10 %MS à 0,04% MS. Selon les écotypes, la teneur en PT et TT varie significativement de 2,69% MS à 2,01% MS et 1,15% MS à 0,44% MS respectivement. Les teneurs maximales obtenues en TC sont obtenues au stade végétatif avec 0,10% MS. Ces concentrations sont au-dessous de la limite minimale (2% en TC) qui pourrait affecter la valeur nutritive du fourrage et la santé de l'animal.

Mots-clés. *Melilotus officinalis* – Écotypes – Phénols – Tanins.

I – Introduction

Technical, economic and environmental pressures limit goat's production in Morocco; therefore, the valorization of not exploited locally feeding resources is a necessary option to mitigate these issues.

The prospects of *Melilotus officinalis* (yellow sweet clover) in the Moroccan North-West has led to a collection of 20 ecotypes. This study helped afterward to analyze the content of phenolic compounds according to the genetic variation of these ecotypes. The phenolic compounds are known for their

anti-nutritional proprieties, which influence the production of milk and meat; but in exchange, they are able to improve quality of the unsaturated fatty acids (Ayadi, 2014). A study revealed that *Melilotus* sp. contain higher contents of phenols and flavonoids than condensed tannins (Olgica *et al.*, 2015).

II – Materials and methods

Twenty ecotypes of *Melilotus officinalis* were established at the experimental field of Boukhalef. Green matter samples were collected at the vegetative, budding and flowering stages. Samples were dried at 60°C then ground through a 1 mm sieve (AOAC, 1997). The samples were analyzed for total phenols (TP), total tannins (TT) and condensed tannins (CT). The determination of phenols and total tannins was accomplished according to the method of Makkar *et al.* (1993) by using the Folin Ciocalteu's reactive. Wave's length of 725 nm was used on a visible spectrophotometer to measure the absorbance of the samples. The condensed tannin's absorbance was measured at 550 nm following the method of HCl-Butanol (Porter *et al.*, 1986). The determination of the phenolic compounds content was carried out by using the linear calibration curve ($y=ax$) which was drawn according to the spectral answer of solutions composing different concentration of tannic acid.

The effect of the growth plant and ecotype on studied parameters was analyzed with software SAS (version 9, 2004) by using GLM's procedure. The multiple comparison of average was carried out using the test "Last Square Deviation".

III – Results and discussion

The ecotypes studied differed significantly ($P<0.001$) on TP contents. E19 was associated with the higher value at 2.69% DM, while E17 and E18 with the lowest values at 2.04% and 2.01%DM respectively (Fig. 1).

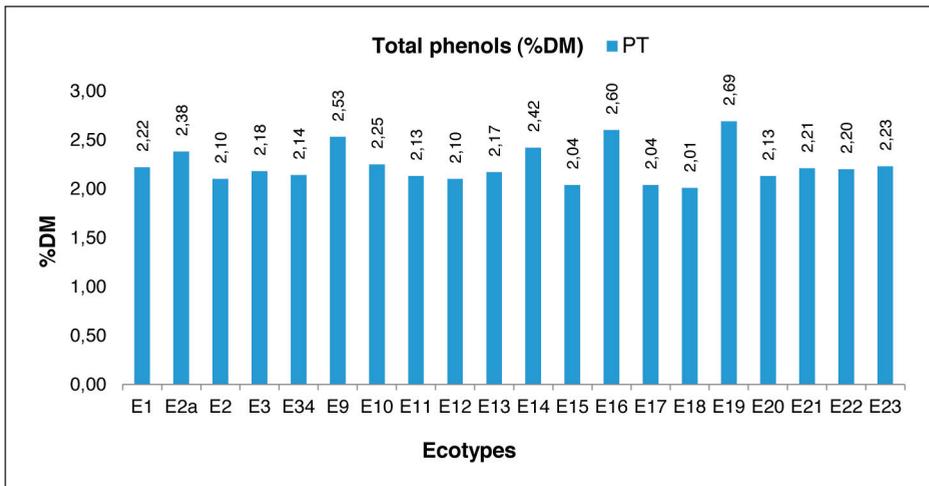


Fig. 1. Total phenols (TP) content of a collection of yellow sweet clover ecotypes.

The effect of ecotype on TTs content was also highly significant ($P<0.001$). The highest values were recorded for E14 and E16 at 1.01% DM and 1.14% DM respectively, while the lowest values were for E17 and E20 at 0.49% DM and 0.44%DM respectively (Fig. 2).

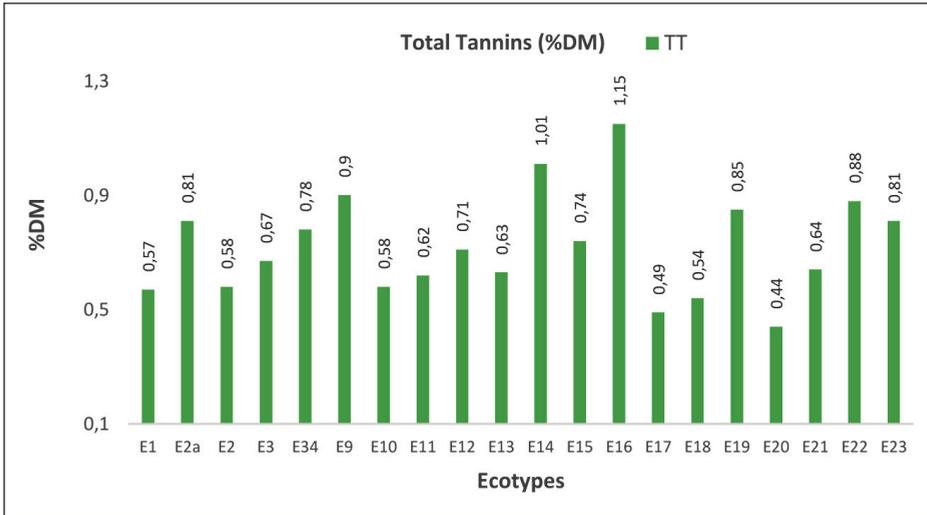


Fig. 2. Total tannins (TT) content of a collection of yellow sweet clover ecotypes.

The yellow sweet clover ecotypes studied had low CT contents (0.4 to 0.14% DM), but the variability of the CT's content between ecotypes was significant ($P < 0.05$). Indeed, three ecotypes stand out according to their CT's content. The majority (45% of the studied ecotypes) had a medium CT's content, which varied between 0.07 and 0.08% DM. The highest CT content (0.1 to 0.14% DM) was represented by a 15% of the ecotypes (Fig. 3).

The average content CT obtained was 0.07% DM, a similar value to that recorded by Hamacher *et al.* (2013) which reported a close CT value (0.06% MS). The ecotypes which recorded the lowest values are E18 and E21 with 0.04 and 0.05% DM respectively. The maximum contents of CT were obtained with only 3/20 ecotypes (E15, E22, E23) with an average of 0.12% DM.

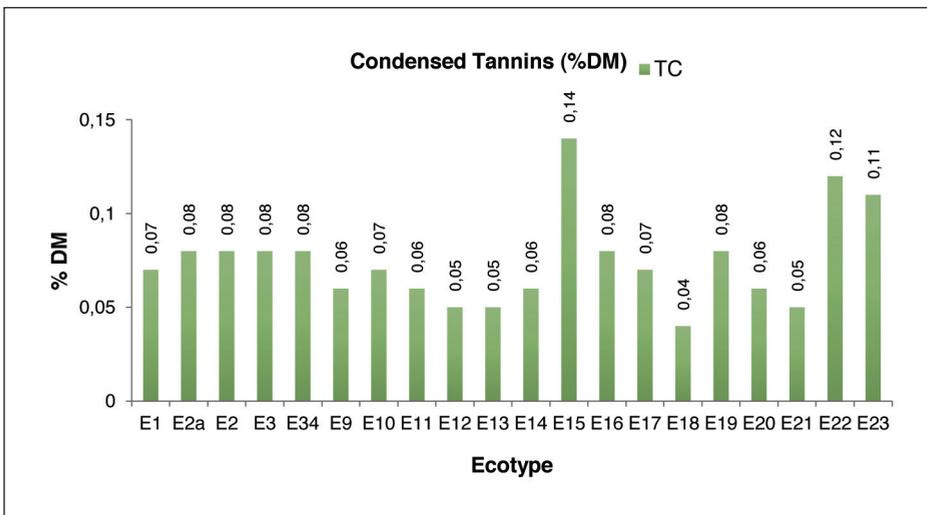


Fig. 3. Condensed tannins (CT) content of a collection of yellow sweet clover ecotypes.

The growth stage of plants had a significant effect on TP and CT ($P < 0.001$). The TP followed an ascending trend according to the development stage; they increased from 2.14% DM at the vegetative stage up to 2.37% DM at the flowering stage. TT increased from the vegetative (0.71 %DM) to the budding stage (0.78% DM) and then decreased to 0.67 %DM at flowering. However, the CT decreased continuously with the development of the plants, where contents dropped from 0.10% DM at the vegetative stage up to 0.04% DM at the flowering stage. Macheix *et al.* (1991) reported that the flowering stage had the lowest CT value, since the phenolic compounds are more important in the young plants. In general, whatever the stage of growth, the level of the CT amount recorded by *Melilotus officinalis* cannot generate a negative effect on the digestibility of proteins. Indeed, the study made by Jayanegara *et al.* (2012) revealed that a CT content lower than 2% DM does not have any effect on rumen microbial activity (anti-methanogenic effects).

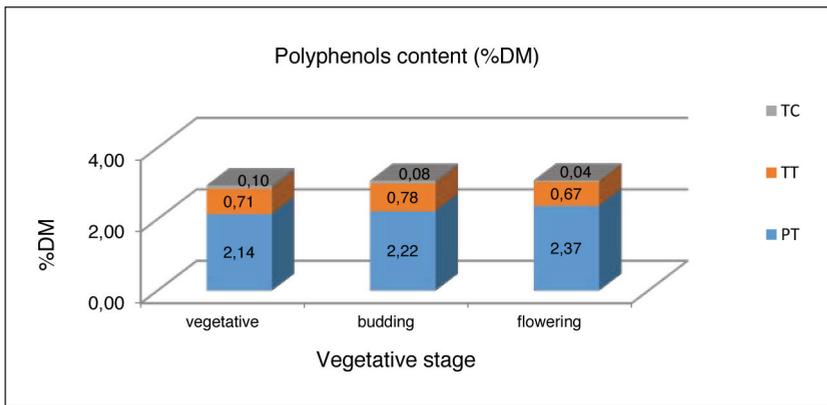


Fig. 4. Polyphenols content of yellow sweet clover by stage of growth.

The average content total phenols in the *Melilotus* ecotypes studied was 2.24% DM. This content is higher than the one found by Hamacher *et al.* (2013) who reported a content of 1.59% DM. However it remains lower than that recorded in Sulla, whose content reaches 2.6% DM (Errassi *et al.*, 2018). The increase of total phenols from the vegetative to the flowering stage can be explained by the decline to the soil nitrogen contents (Mohd *et al.*, 2011).

IV – Conclusions

The phenolic compounds are known for their anti-nutritional properties, but the collection of the yellow *Melilotus* ecotypes from the Moroccan North-West is characterized by relatively low contents. The maximum contents of condensed and total tannins of the studied ecotypes hardly exceed 0.14 and 1.15% DM respectively. Most ecotypes contained low condensed tannins varying from 0.06 to 0.08% DM. This result supports the interest on the quality of yellow sweet clover as a fodder resource in terms of contents in anti-nutritional substances.

The concentration of the total phenolic compounds in the *Melilotus* ecotypes studied showed a decline with the advance of the growth stage. For the condensed tannins, the variation of their content is negligible whatever the stage of development or the source of the seed.

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