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Allelopathic effect of *Festuca rubra* on perennial grasses

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Abstract

The allelopathy research can contribute to protecting the biodiversity and may develop new strategies for sustainable ecosystems controlled by allelochemicals. The present investigations have estimated the allelopahtic features at *Festuca rubra* and the ability of the allelopathic compounds to affect the germination and the quality of perennial grasses (*Dactyilis glomerata, Lolium perenne* and *Poa pratensis*). These perennial grasses were treated with alcolic extracts obtained from dry aerial parts of Festuca Rubra. Were quantified and also physico–chemical characterized the polyphenolic compounds and the alkaloids from the chemical structure of plants. The extracted compounds from the dry aerialparts of F. Rubra were quantified using HPLC method. In the aqueous extracts were quantified the following alkaloids: N–formyl–loline (NFL), N–acetyl–loline (NAL) and ergovaline (EGV). The results of the research have showed that the effect of the alkaloids lead to modifications in the quality index by reducing of the crude protein content and thus lead to lower feed value of these plants.

Key words: lolinic alkaloids, chemical composition, quality, *Festuca rubra*

Introduction

Due to the advances in physiology and vegetal biochemistry research, in the field of plant research it is becoming more and more feasible the possibility to explain the mechanism of association of plants in a phytocoenosis (the allelopathy phenomenon). [Weckwerth *et al.*, 2011; Jovanović Onć *et al.*, 2010]. The importance of allelopathy resides in a series of bioprocesses which take place at inner molecular level of the organisation of the vegetal unit, being simultaneously projected at the external level by a particular physiological behaviour. [Morris *et al*., 2009; Bozinovic *et al*., 2006]. The term "allelopathy" was first introduced in 1937 by Molisch, defining the chemical interactions (both stimulation and inhibition) between all types of plants, including microorganisms. [Azim 2008, Murrell *et al*., 2011]. Observations regarding the allelopathic interactions between the plant species have a history of centuries, but the transition from observation to scientific certitude and demonstration has been achieved recently. [Wilkinson *et al.*, 2000; Tharayil *et al.*, 2009]. The allelopathic inhibition is often generated and amplified by the association with one or more abiotic or biotic stress. [Kaur *et al*., 2009]. On the background of the geochemical configuration of the biotope, for each ecosystem is realised a particular biochemical structure, produced by the plants metabolism. [Viard-Crétat *et al.*, 2009].

Biochemical changes of the environment provides competitive advantages to the species that are allelochemical compounds donors. [Stoyanova, 2010].

On the background of the existing trend to meet the chalenges of sustainable ecosystems development [*Macias et al., 2003*] and the rising need to offer products from organic agriculture, with no use of synthetic chemicals in weed and pest management of the crops, the allelopathy concept gained an increasing attention of the researchers.

The aim of this work was to identify and quantify the allelochemical potential of the substances extracted from *F. Rubra* on selected perennial grasses (*Dactylis glomerata, Lolium perenne, Poa pratensis*).

Materials and methods

Vegetal material: For the laboratory tests, were taken into consideration four plant species: three perennial grasses species, used as receiver plants, respectively, *Dactylis glomerata, Lolium perenne, Poa pratensis* and the donor plant *Festuca rubra.* The plants were cultured in pots under vegetation house conditions.

Bioassay of the plant growth: Was realised in vegetation house under standard temperature and humidity conditions (25°±27°C and 45%). The research design was conceived in three replications. The perennial grasses species *D. glomerata, L. perenne* i *P.pratensis* were treated with alcoholic extract obtained from aerial parts of *F. rubra*. The experiment was achieved using three different concentration samples of extract (D1=10mL/pot, D2=40mL/pot, D3=80mL/pot) and a control sample.

The obtaining and analysis of plant extract. Extraction of phenolic compounds. Dried aerial parts of *F. rubra* were minced and a quantity 20 g of the obtained powder was further used in a Soxlet apparatus under reflux conditions (extraction time 4 hours), for alcohol (80%) extraction of phenolic acids and total fenolic compounds. The analysis of the total phenolic compounds was realised by Folin-Ciocalteu method. [*Zadra et al., 2012*]. The phenolic acids were qualitatively and quantitatively determined by HPLC method. [*Gooneratne* et al*., 2012; Iwasa* et al*., 2012*].

Results and discussions

The allelopathic phenomenon is a cause-effect type of biological interaction between the plants allelopathic chemicals and the physiological responses developed by the target plant.[*Kruse et al. 2000;* Basuny *et al.*, 2011].

Allelopathy mechanism can be described as a chain of biochemical and physical processes, between the allelochemicals donor and the acceptor species.

The obtained results presented in Table 1 demonstrate that lolinic alkaloids can be found in high concentrations in extracts of *F. rubra*.

Table 1. Alkaloids content in the low–alcohol vegetal extracts *F. rubra*.

Species	NFL [µg/g]		NAL [µg/g]		EGV [µg/g]	
	$\bar{x} \pm s_{\bar{x}}$	S %	$\bar{x} \pm s_{\bar{x}}$	S %	$\bar{x} \pm s_{\bar{x}}$	S %
Festuca rubra	1550±65.8	7.34	771±23.5	5.27	0.149±0.00061	0.71
Note: NFL–N–formyl loline; NAL–N–acetyl loline, EGV–ergovaline.						

NFL concentration exceeded NAL corresponding concentration, the differences between the concentrations of the two alkaloids being significant.

The analysis of experimental data presented in Table 2 have shown that the plants treatment with *F. rubra L.* extract, leads to changes in chemical composition of *L. perenne L.* plants, which varies with the applied dose.

Table 2. *Lolium perenne* chemical composition after spraying with *Festuca rubra* extract.

	Ash %			**CP %**		
Version	Average	%	Diff./Significance	Average	%	Diff./Significance
Blank sample L.p.	11.10	100.00	–	17.78	100.00	–
D1	11.05	99.55	–0,05	17.54	98.65	–0.24
D2	12.25	110.36	1.15 *	16.04	90.21	–1.74 ^{0}
D3	11.95	107.66	0.85	15.22	85.60	–2.56 ^{00}
	$DL_{5\%}$=1.10(%);$DL_{1\%}$=1.67(%);$DL_{0.1\%}$=2.68(%)			$DL_{5\%}$=1.45(%);$DL_{1\%}$=2.19(%);$DL_{0.1\%}$=3.52(%)		
	NDF %			**ADF %**		
Blank sample L.p.	69.35	100.00	–	29.75	100.00	–
D1	68.78	99.18	–0.57	28.21	94.82	–1.54
D2	67.30	97.04	–2.05	27.11	91,13	–2.64
D3	64.22	92.60	–5.13 ^{0}	26.51	89,11	–3.24 ^{0}
	$DL_{5\%}$=4.90(%); $DL_{1\%}$=7.42(%); $DL_{0.1\%}$=11.92(%)			$DL_{5\%}$=3.11(%); $DL_{1\%}$=4.71(%); $DL_{0.1\%}$=7.57(%)		
	NFL µg/g			**NAL µg/g**		
Blank sample L.p.	541.00	100.00	–	127.00	100.00	–
D1	572.00	105.73	31.00	137.00	107.87	10.00
D2	602.00	111.28	61.00	187.00	147.24	60.00 *
D3	741.00	136.97	200.00 *	327.00	257.48	200.00 ***
	$DL_{5\%}$=139(µg/g);41$DL_{1\%}$=211.1(µg/g);$DL_{0.1\%}$=339.12(µg/g)			$DL_{5\%}$=53,31(µg/g);$DL_{1\%}$=80,73(µg/g);$L_{0.1\%}$=129,69(µg/g)		
	EGV µg/g					
Blank sample L.p.	0.071	100.00	–			
D1	0.086	121.13	0.015			
D2	0.156	219.72	0,085 ***			
D3	0.184	259.15	0.103 ***			

$DL_{5\%}$=0.034(µg/g);$DL_{1\%}$=0.052(µg/g);$DL_{0.1\%}$=0.083(µg/g)

Note: NFL–N–formyl loline; NAL–N–acetyl loline, EGV–ergovaline, NDF–neutral detergent fibers, ADF–acid detergent fibers, CP–crude protein.

Thus the NFL, NAL, EGV concentrations increase with the increasing of the applied extract dose. At a dose of 40 mL (D2), the N–acetyl loline (NAL) content in *L. perenne L.* treated plants, has an increase significantly higher compared to the blank sample and very significantly higher at a dose of 80 mL (D3). N–formyl loline (NFL) has an increasing very significantly higher only in the plants treated with dose 3.

Instead, ergovaline (EGV) presents an increasing very significantly higher at *L. perenne L.* treated plants with 40 mL of the extract (D2) and 80 mL of the extract (D3).

The application of *F. rubra L.* extract doses caused a decrease of the crude protein content at *L. perenne*, thus at the dose application of 40 mL (D2) the crude protein content is significantly lower, and the dose application of 80 mL (D3) is distinct significantly lower than to the blank samples plants. In order to realize a better characterization of the inhibitory effect of the lolinic alkaloids, were calculated the regression curves between dose and response.

All the three allelopathic substances represented by the NFL, NAL and EGV have a negative regression with crude protein, thus the increasing of the allelopathic substances amount causes a protein decrease [Zhang *et al.*, 2010].

This phenomenon occurs at *L. perenne* plants treated with extracts of *F. rubra,* therefore it can be concluded that *F. rubra* extract presents allelophatic potential, which influence the quality of the treated plants with this extract, meaning that there is a decrease in crude protein. (Fig. 1, 2, 3.)

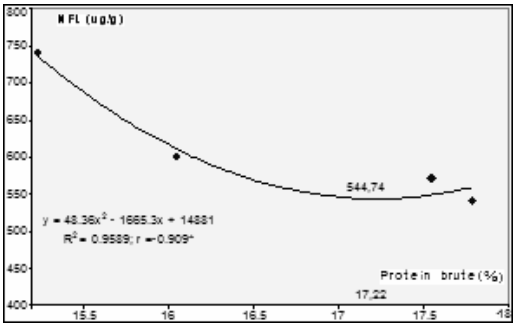

Figure 1: Regression curve between NFL and crude protein at *L. perenne* plants after spraying with *F. rubra* extract.

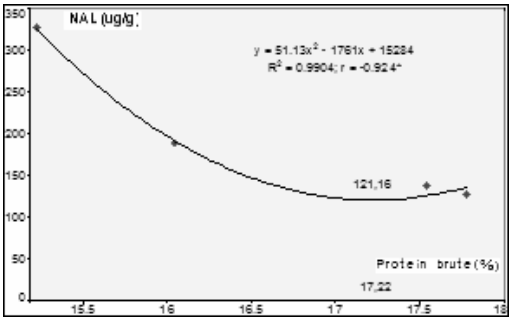

Figure 2: Regression curve between NAL and crude protein at *L. perenne* plants after spraying with *F. rubra* extract.

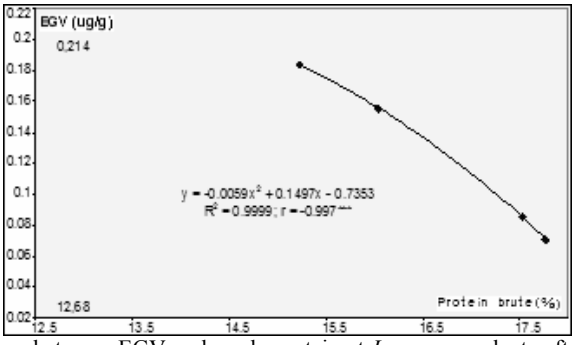

Figure 3: Regression curve between EGV and crude protein at *L. perenne* plants after spraying with *F. rubra* extract.

The NFL and NAL concentration at *D. glomerata* treated plants increase with the increasing of the applied extract dose. Compared with the concentrations of the lolinic alkaloids from the blank samples [Arafat, *et al*., 2010], the applying of a dose of 40 mL (D2) determines a significant increase of NFL content and an increase very significantly higher by the application of a dose of 80 mL (D3). It can be noticed a decrease of crude protein content for *Dactylis glomerata L.* when it was treated with *F. rubra* extract in dose 3. (Table 3).

Tabel 3. *Dactylis glomerta* chemical composition after spraying with *F. rubra* extract.

	Ash %			**CP %**		
Version	Average	%	Dif./Semnif	Average	%	Dif./Semnif
Blank sample D.g.	10.80	100.00	–	18.78	100.00	–
D1	10.33	95.65	–0.47	18.65	99.31	–0.13
D2	11.43	105.83	0.63	17.35	92.39	–1.43
D3	11.23	103.98	0.43	16.55	88.13	–2.23 ^{0}
$DL_{5\%}$=0.98(%);$DL_{1\%}$=1.48(%);$DL_{0.1\%}$=2,38(%)				$DL_{5\%}$=1.59(%);$DL_{1\%}$=2.41(%);$DL_{0.1\%}$=3.87(%)		
	NDF %			**ADF %**		
Blank sample D.g.	74.89	100.00	–	31.45	100.00	–
D1	74.34	99.27	–0.55	29.02	92.27	–2.43
D2	72.54	96.86	–2.35	28.22	89.73	–3.23
D3	70.14	93.66	–4.75	27.46	87.31	–3.99 ^{0}
$DL_{5\%}$=5.27(%);$DL_{1\%}$=7.98(%);$DL_{0.1\%}$=12.81(%)				$DL_{5\%}$=3.45(%);$DL_{1\%}$=5.23(%);$DL_{0.1\%}$=8.40(%)		
	NFL µg/g			**NAL µg/g**		
Blank sample D.g.	0.090	100.00	–	0.050	100.00	–
D1	0.109	121.11	0.019	0.090	180.00	0.040 *
D2	0.157	174.44	0.067 *	0.124	248.00	0.074 ***
D3	0.320	355.56	0.230 ***	0.189	378.00	0.139 ***
$DL_{5\%}$=0,047(µg/g);$DL_{1\%}$=0.070(µg/g);$DL_{0.1\%}$=0.113(µg/g)				$DL_{5\%}$=0.028(µg/g);$DL_{1\%}$=0.043(µg/g);$DL_{0.1\%}$=0.069(µg/g)		

The regression curve between NFL and the crude protein of *D. glomerata*, plants after applying a treatment *F. rubra* extracts, is a negative curve [Schardl *et at*., 2007].

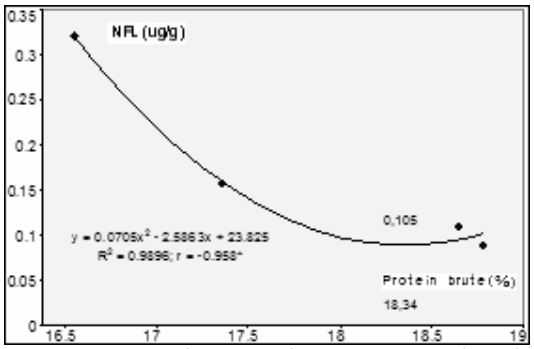

Figure 4: Regression curve between NFL and crude protein at *D. glomerata* plants after spraying with *F. rubra* extract.

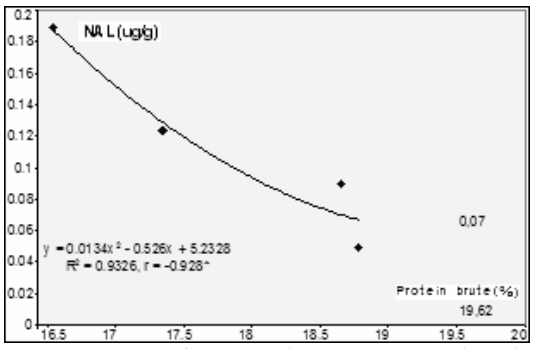

Figure 5: Regression curve between NAL and crude protein at *D. glomerata* plants after spraying with *F. rubra* extract.

As the NFL amount increases, the crude protein decreases, influencing in a negative way its amount. The same increasing trend of the allelopathic substances, respectively NFL and NAL, can be noticed at *P. pratensis L.* species.

At dose D1, the accumulation of N–formyl loline in *P. pratensis L*. plants is significantly higher compared to the blank sample and very significantly higher at the doses 2 and 3.

N–acetyl loline shows an accumulation in plants treated with extracts of *F. rubra L.*, very significantly higher, when *P. pratensis L.* was treated with extract doses of 40 mL and 80 mL. Crude protein shows a slight decrease as the applied extract dose of extract is higher (Table 4).

Tabel 4. *Poa pratensis* chemical composition after spraying with *Festuca rubra* extract.

	Ash %			**CP %**		
Version	Average	%	Dif./Semnif	Average	%	Dif./Semnif
Blank sample P.p.	10.12	100.00	–	16.45	100.00	–
D1	9.80	96.84	–0.32	16.31	99.15	–0.14
D2	10.71	105.83	0.59	16.01	97.33	–0.44
D3	10.22	100.99	0.10	15.82	96.17	–0.63
	$DL_{5\%}$=0.88(%);$DL_{1\%}$=1.34(%);$DL_{0.1\%}$=2.15(%)			$DL_{5\%}$=1.46(%);$DL_{1\%}$=2.21(%);$DL_{0.1\%}$=3.35(%)		
	NDF %			**ADF %**		
Blank sample P.p.	73.09	100.00	–	31.56	100.00	–
D1	72.80	99.60	–0.29	30.89	97.88	–0.67
D2	71.97	98.47	–1.12	29.75	94.26	–1.81
D3	68.28	93.42	–4.81	28.89	91.54	–2.67
$DL_{5\%}$=5.10(%);$DL_{1\%}$=7.72(%);$DL_{0.1\%}$=12.40(%)				$DL_{5\%}$=3.68(%);$DL_{1\%}$=5.57(%);$DL_{0.1\%}$=8.94(%)		
	NFL µg/g			**NAL µg/g**		
Blank sample P.p.	0.045	100.00	–	0.015	100.00	–
D1	0.085	188.89	0.040 *	0.035	233.33	0.020
D2	0.100	222.22	0.055 ***	0.650	4333.33	0.635 ***
D3	0.250	555.56	0.205 ***	0.200	1333.33	0.185 ***
$DL_{5\%}$=0.032(µg/g);$L_{1\%}$=0.048(µg/g); $DL_{0.1\%}$=0.077(µg/g)				$DL_{5\%}$=0.051(µg/g); $DL_{1\%}$=0.078(µg/g); $DL_{0.1\%}$=0.125(µg/g)		

It can be observed a negative regression between NFL and crude protein at *P. pratensis* plants treated with *F. rubra* extract (Figure 7).

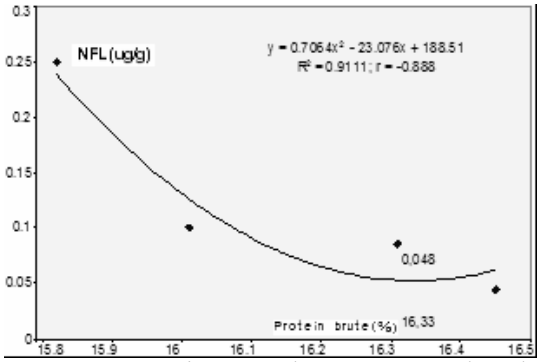

Figure 7: Regression curve between NFL and crude protein at *P. pratensis* plants after spraying with *F. rubra* extract

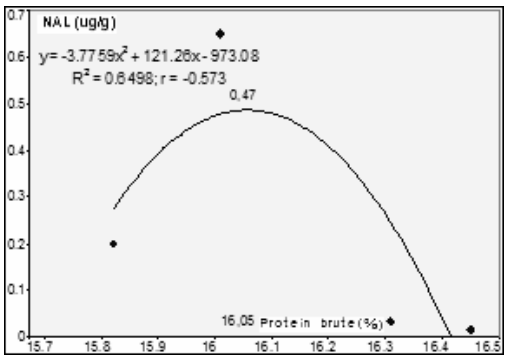

Figure 8: Regression curve between NAL and crude protein at *P. pratensis* plants after spraying with *F. rubra* extract

In Figure 8 it can be observed that the protein amount increases up to 16.05 as N–acetyl loline (NAL) increases up to a maximum level of 0.47 **µg/g**.

Conclusions

In this paper was highlighted the alelopathtic potential of alkaloids: N-formyl loline (NFL), N-acetyl loline (NAL) and ergovaline (EGV), identified by HPLC method. The treatment of herbaceous plants with extracts of *Festuca rubra* L. in different doses resulted in modifications of their chemical composition. Therefore, the quality index of the tested plants has changed, by a decrease in crude protein content. Simultaneously with the increasing concentration of lolinic alkaloids, the amount of crude protein decreased.

Acknowledgments

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