ALLELOPHATIC SUBSTANCES AND THEIR ABILITY TO INFLUENCE THE GRASSES QUALITY

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Abstract*: The allelochemical compounds represent one of the factors that threaten the biodiversity and to which the specialized research should be conducted. Their structure and the action type are various and can be starting points for the manufacture of new herbicides. As a start point, it is essential to identify the classes of the involved allelophatic compounds and their concentrations, respectively the mechanisms by which they reach in the environment, in order to realize more precise impression of the function that these compounds perform. The similar chemical composition and the coexistence of different plants species can be strongly affected by the interactions between them. Due to the fact that generally the grasses present in grasslands grow as bushes separated by areas where the vegetation is missing, determined the researchers to consider that these plants have an allelophatic character. This study was realized to determine the allelophatic properties of Dactylis glomerata species and the capacity of the allelophatic substances to influence the perennial grasses quality (Festuca rubra, Lolium perenne and Poa pratensis). The research design consisted of three repetitions. The plants were treated with low alcoholic extracts of pirolizidinici alkaloids, respectively ergot alkaloids obtained from the air part of Dactylis glomerata species, except the blank. The extracts were applied in three different doses: D1 = 10 ml, D2 = 40 ml, D3 = 80 ml. The preliminary chemical analysis of the extracts revealed the presence of the alkaloids (NAL - N-acetyl loline and NFL-N-formyl loline). The identification of alkaloids was confirmed by HPLC-UV analysis. The HPLC (High Performance Liquid Chromatography) analyses of the extracts corresponding to Dactylis glomerata species have shown six compounds of phenolic acids class. Three of these compounds belong to the cinnamic acid derivatives (p-coumaric acid, caffeic acid, respectively ferulic acid), and three are benzoic acid derivatives (p-hydroxybenzoic acid, vanillic acid, syringes acid). The results showed that the alkaloids influence the quality index change at the treated plants. That was reflected by a decrease in crude protein content; the highest sensitivity was noticed at Poa pratensis species. This work was financially supported by the project "Postdoctoral school of agriculture and veterinary medicine Posdru/89/1.5/S/62371, co–financed by the European Social Fund through the Sectorial Operational Programme for the Human Resources Development 2007–2013.*

Key words: *lolinic alkaloids, chemical composition, quality, Dactylis glomerata*

INTRODUCTION

Any alive community or biocenosis can be constituted only on the interactions between the individuals of the cohabiting different species. The interactions can occur at least in three aspects: substantial, energetic and informational. The most important aspect seems to be the substantial one, for two reasons: first because the substances in the interactions development can convey both power and information, secondly, because in all cases, in a specific time of information transmission, any signal is converted into a change at the molecular organization of living (BORZA AND COSTE, 2002).

On the background of the biotope geochemical configuration, it can be made for each ecosystem an own biochemical structure produced by the biocenosis metabolism for each ecosystem (STUGREN, 1982). These are metabolites eliminate by the organisms in their near

environment, notably in soil. These metabolites cause an interaction between species, known as allelopathy. The allelopathic substances can be biosynthesised in any organ of the plant, but most frequently can be found in roots, leaves, seeds and vegetable scraps (BOUTON, 2005; IRANBAKHSH ET AL., 2010). The main target processes for the alellopathic substances are: cell division, membranes permeability and stability, production and achievement of the plant hormones balance, protein production, photosynthesis and respiration (RIZVI ET AL., 1992, FERGUSON ET AL., 2003). These effects slow and even stop the fundamental processes of the existence of plants, and also provide superiority and competitiveness for the alellopathic plant even in the conditions in which the access to the nutritional resources is limited.

There have been reported less informations about the control mechanisms that regulate alellochemical synthesis. These information gaps are significant barriers in understanding the allelopathy physiology (FARR.ET AL., 2008). The secondary metabolic compounds with alellopathic potential are virtually presents in all plant tissues and exert their effect by their chemical structure or are precursors of other toxic compounds, resulted from microbial decomposition (OWAR ET AL., 2007) and from the production of some physical and chemical changes (ASGHARIPOUR ET AL., 2010). The most common question in the allellopathic study is whether these compounds are released in sufficient quantities in the environment and thus cause a reaction in the surrounding organisms (HAQ ET AL., 2010).

The bioassays are an integral part in all alellopathic studies. The dosage is needed to assess the alellopathic potential of the species and the purification and identification of the active biocompounds considering the activity during the extraction. This bioassay, in their simplest form, and the alellochemicals isolation and identification, are techniques for providing the initial informations (OLIVEIRA, 2006).

The existence of the inhibition zones around the perennial grasses and the decreased of the diversity of the other plant species that form meadows and the changes of the soil composition in these habitats, have suggested the involvement of the chemical compounds. Due to the complexity of the mechanisms by which the perennial grasses interact, we approached in this study, the hypothesis of allelopathic properties in *Dactylis glomerata* in laboratory conditions, in terms of chemical compounds type, secondary metabolites and the capacity of the determined substances to influence the grass quality treated with *Dactylis* extract.

MATERIAL AND METHODS

Vegetal material: the biological material studied is represented by four species of perennial grasses: *Dactylis glomerata, Lolium perenne, Poa pratensis* and *Festuca rubra*, studied under laboratory conditions and in vegetation pots.

Bioassay of the plant growth: the growing plants was made in vegetation pots under uniform conditions for all samples. The vessels were kept in the growth chamber, under stable temperature and humidity (25 ° ± 27 ° C and 45%). The research design included three repetitions. All plants were treated with low alcoholic extracts of pyrrolizidine *alkaloids*, respectivly ergot alkaloids obtained from the air part of *Dactylis glomerata* species, except blank sample. The extracts were applied in three different doses: D1 = 10 ml / pot, D2 = 40 ml / pot, D3 = 80 ml / pot.

The obtaining and analysis of plant extract. Extraction of phenolic compounds [Djurdjević et al., 2005, Provan et al., 1994, Lowry et al., 1993]: both phenolic acids and total phenolic compounds were extracted from a mixture of 20 g of dry vegetal material (only the air part of each species) and 90 ml ethanol (80%), using a Soxlet equipment under reflux conditions. The extraction time was 4 hours. Polyphenols were determined because the work protocol involved in the determination of the allelophatic substances (NAL - N-acetyl loline,

NFL-N-formyl loline and EGV-ergovaline) requires using as extraction solvent a mixture of ethylic alcohol-water in a ratio of 20% or 1/5. This aspect determined the polyphenols presence in the raw plant extracts.

Analysis of total phenolic compounds by Folin Ciocalteu method. Qualitative and quantitative analysis of phenolic acids by HPLC technique.

The determination of the ergovaline and the lolinic alkaloids content in the vegetable extracts. The vegetal material obtained from *Dactylis glomerata* (whole plant) was cleaned of earth and dried at 60 ° C over for 24 hours. The dried material was ground with a mill until the grist passed by 1mm diameter sieve. Then, approximately 0.5g of ground material were weighed using the analytical balance (e = ± 0.1mg) in a graduated tube (16x125mm) fitted with Teflon stopper. In the graduated tube was introducing 10.0 ml chloroform, 1.0 ml of ergotamine tartrate (1mg/ml, as internal standard) and 1.0 ml of sodium hydroxide. After this, the graduated tube was closed and left in the dark for 24 h in an hematology stirrer. The amorphous mixture that was obtained was centrifuged at 1700 rot / min for 5 min. 4.0 ml of supernatant was taken and purified by the SPE technique (Solid Phase Extraction).

RESULTS AND DISCUSSIONS

The chemical studies have shown that the substances, which represent the basis of the allelopthy appearance, are vegetal secondary metabolites belonging to the classes of alkaloids, isoprenoides, flavonoids, phenols, terpenes and glucosinolates.

Taking as reference the specialized data, it was determined the chemical composition of the vegetable extract of *Dactylis glomerata.*

The HPLC (High Performance Liquid Chromatography) analyses of the extracts corresponding to *Dactylis glomerata* species have shown six compounds of phenolic acids class. Three of these compounds belong to the cinnamic acid derivatives (p-coumaric acid, caffeic acid, respectively ferulic acid), and three are benzoic acid derivatives (p-hydroxybenzoic acid, vanillic acid, syringes acid).

Table 1

Linked phenolic acids content in the vegetal alcoholic extracts [ppm]

Species	PCA	FA	CA	PHB	VA	SA
Dactylis glomerata	519.9 ± 29.1	915,2 ± 82.6	280.8 ± 36.2	54.1 ± 9.9	125.7 ± 23.3	102.8 ± 18.4

PCA- p-cumaric acid, FA-ferulic acid, , CA- caffeic acid, PHB- p-hydroxybenzoic acid, VA-vanilic acid and SA- siringic acid.

Table 2

Free phenolic acids content in the vegetal alcoholic extract [ppm]

Species	PCA	FA	CA	PHB	VA	SA
Dactylis glomerata	40.3 ± 9.7	70.4 ± 12.9	187.2 ± 35.7	10.4 ± 1.97	8,7 ± 2.9	11.2 ± 2.4

PCA- p-cumaric acid, FA-ferulic acid, , CA- caffeic acid, PHB- p-hydroxybenzoic acid, VA-vanilic acid and SA- siringic acid.

The concentrations of total polyphenols (free and bound) are presented in table 3.

Table 3

Polyphenols content in the plants' alcoholic extracts

Plant Species	Total free polyphenols [mg/g]	Total bound polyphenols [mg/g]	Total polyphenols [mg/g]
Dactylis glomerata	1.26 ± 0.15	7.82 ± 2.04	9.08 ± 0.98

Lolinic alkaloids are also present in extracts of *Dactylis glomerata* , the concentration of N-formyl loline (NFL) exceded the corresponding concentration of N-acetyl loline (NAL); ergovaline (EGV) was not detected in *Dactylis* extract.

Table 4

Alkaloids content in the low-alcohol vegetal extracts *Dactylis glomerata*

Species	NFL [µg/g]		NAL [µg/g]		EGV [µg/g]	
	$\overline{x} \pm s_{\overline{x}}$	s %	$\overline{x} \pm s_{\overline{x}}$	s %	$\overline{x} \pm s_{\overline{x}}$	s %
Dactylis glomerata	42,9 ± 3,9	15,73	10,67± 1,2	19,45	ned	

The action of *Dactylis glomerata* plant extracts on the chemical composition was analyzed in three perennial grasses species of *Lolium perene*, *Festuca rubra* and *Poa pratensis.*

The main objective of our research was to determine the ability of the studied substances to influence the quality of the studied grasses.

Table 5

Festuca rubra chemical composition after spraying with *Dactylis glomerata* extract

	Ash %			CP %		
Version	**Average**	**%**	**Dif./Semnif**	**Average**	**%**	**Dif./Semnif**
Blank F.r.	11,37	100,00	-	17,98	100,00	-
D1	11,45	100,70	0,08	17,64	98,11	-0,34
D2	11,40	100,26	0,03	17,48	97,22	-0,50
D3	11,30	99,38	-0,07	17,02	94,66	-0,96
	$DL_{5\%}$=0,69(%) $DL_{1\%}$=1,04(%) $DL_{0,1\%}$=1,67(%)			$DL_{5\%}$=1,05(%) $DL_{1\%}$=1,60(%) $DL_{0,1\%}$=2,56(%)		
	NDF %			**ADF %**		
Blank F.r.	70,78	100,00	-	28,45	100,00	-
D1	69,89	98,74	-0,89	28,45	100,00	0,00
D2	69,08	97,60	-1,70	28,35	99,65	-0,10
D3	68,87	97,30	-1,91	28,00	98,42	-0,45
	$DL_{5\%}$=3,55(%) $DL_{1\%}$=5,38(%) $DL_{0,1\%}$=8.64(%)			$DL_{5\%}$=4,70(%) $DL_{1\%}$=7,12(%) $DL_{0,1\%}$=11,44(%)		
	NFL µg/g			**NAL µg/g**		
Blank F.r.	1550,00	100,00	-	771,00	100,00	-
D1	1550,00	100,00	0,00	771,00	100,00	0,00
D2	1550,00	100,00	0,00	771,00	100,00	0,00
D3	1550,00	100,00	0,00	771,00	102,46	0,00
	$DL_{5\%}$=24,62(µg/g) $DL_{1\%}$=37,29(µg/g) $DL_{0,1\%}$=59,90(µg/g)			$DL_{5\%}$=15,44(µg/g) $DL_{1\%}$=23,37(µg/g) $DL_{0,1\%}$=37,55(µg/g)		
	EGV µg/g					
Blank F.r.	0,129	100,00	-			
D1	0,128	99,22	-0,001			
D2	0,129	100,00	0,000			
D3	0,128	99,22	-0,001			
	$DL_{5\%}$=0,006(µg/g) $DL_{1\%}$=0,009(µg/g) $DL_{0,1\%}$=0,014(µg/g)					

Festuca rubra plants treated with the extract obtained from *Dactylis glomerata L.* does not present significantly differences from the blank, regardless of the applied dose. However, the application of *Dactylis lolinci* extracts containing lolinic alkaloids cause a small decrease in crude protein content in treated plants compared to blank, but these differences are not segnificant from statistical point of view (table 5).

Table 6

Lolium perenne chemical composition after spraying with *Dactylis glomerata* extract

	Ash %			CP %		
Version	**Average**	**%**	**Dif./Semnif**	**Average**	**%**	**Dif./Semnif**
Blank L.p.	11,10	100,00	-	17,78	100,00	-
D1	10,98	98,92	-0,12	17,55	98,71	-0,23
D2	10,48	94,41	-0,62	17,00	95,61	-0,78
D3	10,38	93,51	-0,72	16,54	93,03	-1,24
	$DL_{5\%}$=0,83(%) $DL_{1\%}$=1,26(%) $DL_{0,1\%}$=2,03(%)			$DL_{5\%}$=1,52(%) $DL_{1\%}$=2,30(%) $DL_{0,1\%}$=3,70(%)		
	NDF %			**ADF %**		
Blank L.p.	69,35	100,00	-	29,75	100,00	-
D1	69,01	99,51	-0,34	29,48	99,09	-0,27
D2	68,75	99,13	-0,60	29,00	97,48	-0,75
D3	68,01	98,07	-1,34	28,97	97,38	-0,78
	$DL_{5\%}$=4,21(%) $DL_{1\%}$=6,38(%) $DL_{0,1\%}$=10,25(%)			$DL_{5\%}$=2,89(%) $DL_{1\%}$=4,38(%) $DL_{0,1\%}$=7,03(%)		
	NFL µg/g			**NAL µg/g**		
Blank L.p.	541,00	100,00	-	127,00	100,00	-
D1	541,00	100,00	0,00	127,00	100,00	0,00
D2	541,00	100,00	0,00	127,00	100,00	0,00
D3	541,00	100,00	0,00	127,00	100,00	0,00
	$DL_{5\%}$= 8,82(µg/g) $DL_{1\%}$= 13,36(µg/g) $DL_{0,1\%}$=21,46(µg/g)			$DL_{5\%}$= 4,04(µg/g) $DL_{1\%}$= 6,12(µg/g) $DL_{0,1\%}$= 9,83(µg/g)		
	EGV µg/g					
Blank L.p.	0,071	100,00	-			
D1	0,070	98,59	-0,001			
D2	0,071	100,00	0,000			
D3	0,070	98,59	-0,001			
	$DL_{5\%}$=0,005(µg/g) $DL_{1\%}$=0,007(µg/g) $DL_{0,1\%}$=0,012(µg/g)					

Dactylis glomerata L. extract does not have any significant effect on *Lolium perenne L.*plants, although the crude protein, also in this case, presents a small decrease.

The blank plants of *Lolium perenne* have a crude protein content of 17.78%. The crude protein content decreases by 0.23% when *Lolium perenne* is treated with the extract of *Dactylis glomerata* in D1 (10 ml). The crude protein content in treated plants is 17.00%. when it is applyed a higher dose, namely D2 (40 ml) The biggest difference between crude protein content in plants treated with the extract, was recorded when applied D3, the difference being 1.24% (table 6).

Poa pratensis L. is influenced, from the point of view of chemical composition, by the low alcoholic extract obtained from *Dactylis glomerata L.*

The results presented in table 7 show that N-formyl loline (NFL) has a significantly higher increase in plants treated with *Dactylis glomerata L.* extract, when it is applying a dose of 80 ml (D3). Regarding the other alkaloid, N-acetyl loline (NAL), it can be observed that it has a pronounced tendency to accumulate in plants treated with *Dactylis glomerata L.*extracts, the increase being significantly distinct at *Poa pratensis L.*, when was applied D3 (80 ml).

Dactylis glomerata L. extract causes a significant decrease in crude protein content in *Poa pratensis L.* plants, when these were treated with extract in dose D3 (80 ml) , resulting a sensitivity of *Poa pratensis* species at the *Dactylis glomerata* extract. The previous studies conducted by us, showed that the extract of *Dactylis glomerata* has a strong influence on the seeds germination of *Poa pratensis*, which are totally inhibited.

Table 7

Poa pratensis chemical composition after spraying with *Dactylis glomerata* extract

	Ash %			CP %		
Version	**Average**	**%**	**Dif./Semnif**	**Average**	**%**	**Dif./Semnif**
Blank P.p.	10,12	100,00	-	16,45	100,00	-
D1	10,00	98,81	-0,12	16,01	97,33	-0,44
D2	9,80	96,84	-0,32	15,47	94,04	-0,98
D3	9,85	97,33	-0,27	15,02	91,31	-1,43 ^{0}
	$DL_{5\%}$=0,64(%) $DL_{1\%}$=0,96(%) $DL_{0,1\%}$=1,55(%)			$DL_{5\%}$=1,38(%) $DL_{1\%}$=2,10(%) $DL_{0,1\%}$=3,37(%)		
	NDF %			**ADF %**		
Blank P.p.	73,09	100,00	-	31,56	100,00	-
D1	72,48	99,17	-0,61	31,00	98,23	-0,56
D2	71,98	98,48	-1,11	30,48	96,58	-1,08
D3	71,38	97,66	-1,71	30,70	97,28	-0,86
	$DL_{5\%}$=4,53(%) $DL_{1\%}$=6,86(%)$DL_{0,1\%}$=11,03(%)			$DL_{5\%}$=2,96(%) $DL_{1\%}$=4,49(%) $DL_{0,1\%}$=7,21(%)		
	NFL µg/g			**NAL µg/g**		
Blank P.p.	0,045	100,00	-	0,015	100,00	-
D1	0,054	120,00	0,009	0,015	100,00	0,000
D2	0,054	120,00	0,009	0,015	100,00	0,000
D3	0,065	144,44	0,020 *	0,020	133,33	0,005 **
	$DL_{5\%}$=0,015(µg/g) $DL_{1\%}$=0,022(µg/g) $DL_{0,1\%}$=0,036(µg/g)			$DL_{5\%}$=0,002(µg/g) $DL_{1\%}$=0,004 (µg/g) $DL_{0,1\%}$=0,006(µg/g)		

In order to realize a better characterization of the inhibitory effect of the lolinic alkaloids, it was calculated the regression curves between dose and response.

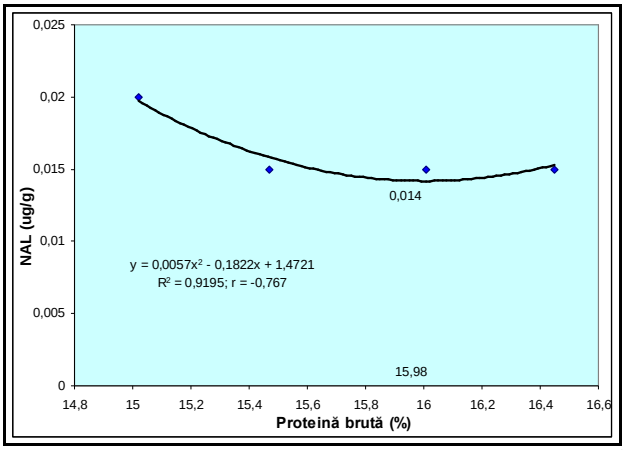

Figure 1. Regression curve between NAL and crude protein at *Poa pratensis* plants after spraying with *Dactylis glomerata* extract

The regression curve between NAL and crude protein is negative when *Poa pratensis* plants are treated with extracts of *Dactylis glomerata* (figure 1).

Thus, the increasing of N-acetyl loline amount causes a decrease of crude protein in *Poa pratensis* plants treated with extracts of *Dactylis glomerata*.

CONCLUSIONS

In the vegetable plants were found allelophatic compounds which corresponds with other studies carried out in the world;

Regarding the determination of allelophatic substances in the composition of *Dactylis glomerata* species, it can be identified the following alkaloids: NFL-N-formyl loline and NAL-N-acetyl loline, from the multitude of alkaloids that are known in the literature;

The application of *Dactylis glomerata* extracts lead to changes in chemical composition of treated plants, changes that vary with the applied dose;

Poa pratensis showed the highest sensitivity to the action of the allelophatic compounds that are present in *Dactylis* extract, compared with the other three species of perennial grasses that were studied;

The influence of the alkaloids on the change of the quality index at the treated plants was observed by a decrease in crude protein content.

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