ALLELOPATHIC INFLUENCE AMONG SOME GRASSLAND SPECIES

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Abstract

The purpose of this work is to evaluate the allelopathic effect of herbage cold water extracts of two species of perennial grasses, Dactylis glomerata and Festuca arundinacea and two species perennial legumes:Trifolium repens and Lotus corniculatus on seed germination (%) and seedling development (shoot and root lenght) on , Lolium perene and Phleum pratense and on themselves. The experiment was carried out in petri dishes and the measurements were performed after 14 days. Seed germination, growth and development of roots and shoots of the perennial grasses and legumes studied were influenced by the concentration of the substrate solution, by the donor species of vegetal extract and by the tested species. Regarding the roots and shoots development on the 4 solutions extract, we noticed that the reaction was very different depending on the species. The inhibitory effect of the tested extracts was the strongest on the species Dactylis glomerata and Trifolium repens regarding both seed germination and seedling development, and the least affected were Lolium perenne and Lotus corniculatus. Positive allelopathic effect was noted at the seedling development at Lolium perenne species.

Keywords: *allelopathy, grasses. legumes, germination, shoots and roots development*

1. INTRODUCTION

In the grassland ecosystems, interactions between plants are carried out through competition, for nutritional resources, water, light, based on some morpho-physiological characteristics (bush shape, plant height, root length, abundance of vegetative shoots, precocity, etc.) or through some secondary metabolites, released into the environment, called allelopathic substances, which can have beneficial (positive allelopathy) or harmful (negative allelopathy) effects on target organisms, the two mechanisms often interfering (Einhelling F.A., 1995)

Allelopathic substances are located in all plant organs; and can be eliminated in the environment by leaching from the leaves surface, by the decomposition of plant residues, by root exudates or by the decomposition of organic matter (Einhelling F.A., 1995).

In natural ecosystems, the allelopathic interaction is manifested initially on seed germination and later on seedling development (Mozden et al, 2020), with an effect on plant establishment, species abundance and distribution (Macfarlane et al, 1981).

A number of perennial grassland forage species have been studied for allelopathic interrelationships: *Festuca arundinacea,* (Cope 1982; Koo et al, 2022) *Dactylis glomerata* (Cope 1982; Mozdezn et al, 2020) *Poa pratensis* (Cope 1982; Lepinska et Wanda, 2009; Bostan et al, 2020), *Trifolium repens* (Cope 1982; Breazu 1998; Macfarlane 2012), *Festuca rubra* (Jankowska et al, 2014; Bostan et al 2013), *Lolium perenne* (McCarty et al), *Lotus corniculatus* (Breazu 1998).

The purpose of this paper is to evaluate and quantify the allelopathic potential of water extracts of plant material on seed germination and seedling development in several species of perennial grasses and legumes included in the breeding program of the institute, on seed germination and seedling development: *Dactylis glomerata, Lolium perenne*, *Trifolium repens, Lotus corniculatus, Festuca arundinacea* and *Phleum pratense.*

2. MATERIAL AND METHODS

To carry out this experiment, the aqueous vegetative extracts of four grasses: *Dactylis glomerata, Trifolium repens, Lotus corniculatus, Festuca arundinacea* were tested for potential allelopathic effects on 6 receiving species: *Dactylis glomerata* Intensiv variety, *Lolium perenne* Mara variety, *Trifolium repens* Mioriţa variety, *Lotus corniculatus* Doru variety, *Festuca arundinacea* Adela variety and *Phleum pratense* Carpatica variety. The vegetative materials were harvested from field plants after cutting regrowth. This material is made up of the vegetative part of the plant, leaves and stems, without inflorescences. It was harvested 35 days after the first mowing, and these no longer formed inflorescences. After a preliminary pre-drying, natural drying in the shade, the materials were dried at 60^0 C and then they were ground. Water solutions were made from the 4 donor species with two concentrations: 5% and 10%. These water solutions were left to macerate for 48 hours and then filtered. The extracts were used as a substrate for seed germination. Distilled water used for control was obtained by simple distillation and handled and stored under the same conditions as the other solutions.

To continue the experiment, seeds from the 6 studied species were germinated in Petri dishes on filter paper. 100 seeds from each species were used in 3 replicates for each concentration extracts and all Petri dishes were wetted with the same amount of liquid. Germination was counted and the seedlings measurements were carried out after 14 days, for each concentration and species.

The results were processed statistically by analysis of variance, for germination %, root length (cm) and shoots length (cm).

3. RESULTS AND DISCUSSIONS

Seed germination, growth and development of roots and shoots of the 6 perennial grasses and legumes studied were influenced by the concentration of the substrate solution (5%, 10%) by the the donor species of vegetal extract and by the tested species (fig.1). On the distilled water substrate, used in this case as a control, all species had normal germination, growth and development.

Dactylis glomerata extract influenced the monitored aspects depending on the concentration of the solution used. At the solution concentration of 5%, seed germination was not affected in the species *Lolium perenne* and *Lotus corniculatus* (even slightly stimulated) and was reduced in the species *Festuca arundinacea* and *Phleum* pratense. The strongest inhibitory effect was manifested in the species *Dactylis glomerata* and *Trifolium repens*. The application of the 10% dose had an inhibitory effect on all species, the most strongly affected being *Dactylis glomerata*, *Phleum pratense* and *Trifolium repens* (total inhibition) (fig. 1).

Festuca arundinacea extract had inhibitor effect on 5 % concentration at *Dactylis glomerata*, and *Trifolium repens,* and on 10 % concentration at *Dactylis glomerata*, *Trifolium repens* and *Lotus corniculatus* (fig. 1).

Regarding the influence of *Trifolium repens* extract on the germination of the other species, an increased sensitivity was noted for the *Dactylis glomerata* and *Trifolium repens* species, at both concentrations and for *Phleum pratense* at the 10% concentration, the other species being little affected. The germination at *Lotus corniculatus* it was not affected at none of the concentrations.(fig. 1)

Lotus corniculatus extract was inhibitor effect on 5% and 10% concentration at *Dactylis glomerata* and *Trifolium repens* (fig. 1).

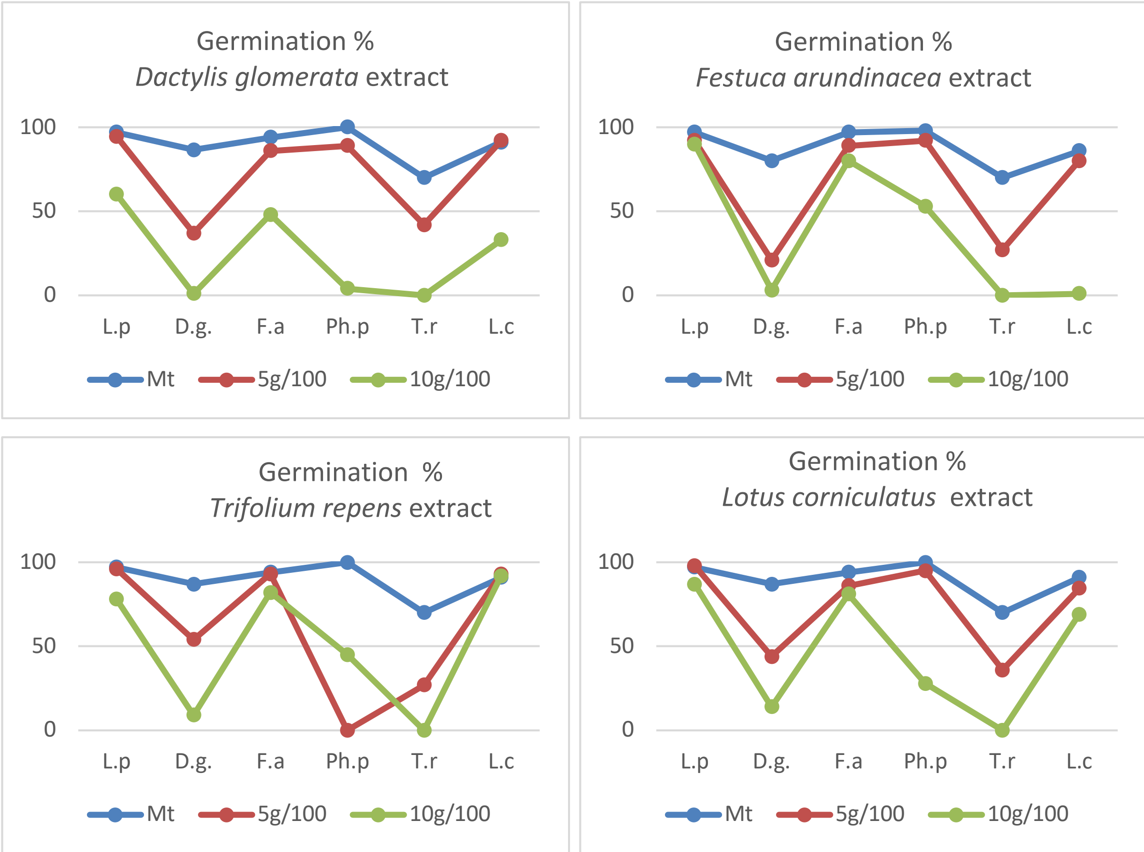

Fig. 1. Seed germination of some grassland species on different concentrations of *Dactylis glomerata, Festuca arundinacea, Trifolium repens* and *Lotus corniculatus* extracts

Regarding the roots and shoots development on the 4 solutions extract, we noticed that the reaction was very different depending on the species.

On *Dactylis glomerata* extract, the roots length was the most strongly affected at all the studied specie. At the concentration of 5%, the length was reduced to almost half of the values recorded on the control solution, with *Lotus corniculatus* exception, for which 126.2% higher values were recorded. The 10% solution had a strong inhibitory effect, drastically reducing the growth, at *Trifolium repens* being zero value. This species showed significant negative values at 10% concentration level.

At species *Dactylis glomerata, Lolium perenne* and *Phleum pratense*, there were obtained very significant negative values for roots length at both concentration levels.

At *Festuca arundinacea* a distinct significant negative result was obtained at 5% concentration level, 67.3% less than control level and very significant negative values at 10% concentration level with

The shoots length development was less affected at the concentration of 5%, being even stimulated at *Lolium perenne* (120%), distinct significant result, but at the concentration of 10% the reduction in the size of the shoots was more pronounced, especially at *Dactylis glomerata* and *Trifolium repens* (Table 1).

Table 1. Measurements of grassland species seedlings on different concentration of *Dactylis glomerata* extract

Species	Extract Concentration	Root length		Diff	Signif.	Shoot length		Diff.	Signif.
		[cm]	%	[cm]		[cm]	%	[cm]	
Dactylis glomerata	**Control**	**4.33**	**100**	**mt**		**3.71**	**100**	**mt**	
	5%	2.00	46.2	-2.33	000	1.72	46.4	-1.99	000
	10%	0.04	0.9	-4.29	000	0.07	2.0	-3.64	000
Lolium perenne	**Control**	**5.42**	**100**	**mt**		**4.80**	**100.0**	**mt**	
	5%	3.19	58.8	-2.23	000	5.80	120.8	1.00	**
	10%	0.44	8.0	-4.98	000	2.43	50.6	-2.38	000
Trifolium repens	**Control**	**0.90**	**100**	**mt**		**2.38**	**100.0**	**mt**	
	5%	0.29	32.4	-0.61		1.16	48.8	-1.22	00
	10%	0.00	0	-0.90	0	0.00	0.0	-2.39	000
Lotus corniculatus	**Control**	**1.13**	**100**	**mt**		**2.93**	**100.0**	**mt**	
	5%	1.42	126.2	0.30		2.53	86.5	-0.40	
	10%	0.20	17.3	-0.93	0	0.69	23.7	-2.24	000
Festuca arundinacea	**Control**	**4.35**	**100**	**mt**		**4.16**	**100.0**	**mt**	
	5%	2.93	67.3	-1.42	00	4.15	99.6	-0.02	
	10%	0.08	1.8	-4.27	000	1.14	27.4	-3.03	000
Phleum pratense	**Control**	**2.85**	**100**	**mt**		**3.17**	**100.0**	**mt**	
	5%	0.70	24.4	-2.15	000	2.92	92.1	-0.25	
	10%	0.03	0.9	-2.82	000	0.47	15.0	-2.70	000
	DL 5% = 0.896 cm; DL 1% = 1.227; DL 0.1% = 1.673					DL 5% = 0.665cm; DL 1% = 0.911; DL 0.1% = 1.241			

On *Festuca arundinacea* extract, the roots development was strongly inhibited at the 5% concentration in all species, the most affected being *Phleum pratense* and *Trifolium repens*, and the 10% concentration totally inhibited root growth, being the solution with the greatest negative impact.

The shoot length was influenced differently, depending on the concentration of the solutions. At the concentration of 5%, the negative impact was more obvious in the species *Dactylis glomerata* and *Trifolium repens*, but at the species *Lolium perenne* and *Phleum pratense* the growth of shoots was stimulated. The 10% concentration totally inhibited the growth of shoots (table 2).

Table 2. Measurements of grassland species seedlings on different concentration of *Festuca arundinacea* extract

	Extract Concentration	**Root length**		**Diff**	**Signif.**	**Shoot length**		**Diff.**	**Signif.**
		[cm]	**%**	**[cm]**		**cm**	**%**	**[cm]**	
Dactylis glomerata	**Control**	**3.17**	**100**	**mt**		**3.44**	**100.0**	**mt**	
	5%	0.49	15.5	-2.68	000	1.72	50.0	-1.72	000
	10%	0.00	0	-3.17	000	0.12	3.5	-3.32	000
Lolium perenne	**Control**	**4.46**	**100**	**mt**		**5.06**	**100.0**	**mt**	
	5%	1.98	44.4	-2.48	000	5.66	111.7	0.60	
	10%	0.06	1.5	-4.40	000	2.94	58.0	-2.13	000
Trifolium repens	**Control**	**0.97**	**100**	**mt**		**2.10**	**100.0**	**mt**	
	5%	0.07	7.2	-0.91	000	0.83	39.5	-1.27	00
	10%	0.00	0	-0.98	000	0.00	0.0	-2.10	000
Lotus corniculatus	**Control**	**1.14**	**100**	**mt**		**2.45**	**100.0**	**mt**	
	5%	0.57	50.4	-0.57	0	1.99	81.3	-0.46	
	10%	0.00	0	-1.14	000	0.010	0.4	-2.45	000
Festuca arundinacea	**Control**	**4.52**	**100**	**mt**		**4.57**	**100.0**	**mt**	
	5%	1.13	25.1	-3.39	000	4.18	91.6	-0.39	
	10%	0.16	3.5	-4.37	000	2.07	45.3	-2.50	000
Phleum pratense	**Control**	**1.90**	**100**	**mt**		**2.80**	**100.0**	**mt**	
	5%	0.12	6.3	-1.79	000	3.26	116.4	0.46	
	10%	0.05	2.6	-1.85	000	2.25	80.4	-0.55	
	DL 5% = 0.484 cm; DL 1% = 0.663; DL 0.1% = 0.903					DL 5% = 0.701 cm; DL 1% 0.961; DL 0.1% = 1.309			

On *Trifolium repens* extract, the root development was strongly inhibited by the 10% solution, in all species. the impact of the 5% solution was less, *Lolium perenne* and *Festuca arundinacea* species being less affected.The shoots development was differently influenced by the concentration of the solutions used. The 5% solutions had a positive influence on the growth of shoots at *Lolium perenne*, *Phleum pratense* and *Lotus corniculatus* species. At the concentration of 10% in the species *Dactylis glomerata* and *Trifolium repens* the development of stems was totally inhibited (table 3).

Table 3. Measurements of grassland species seedlings on different concentration of *Trifolium repens* extract

Species	Extract Concentration	Root length [cm]	Root length %	Diff [cm]	Signif.	Shoot length cm	Shoot length %	Diff. [cm]	Signif.
Dactylis glomerata	**Control**	**4.32**	**100**	**mt**		**3.71**	**100.0**	**mt**	
	5%	0.64	14.9	-3.68	000	2.46	66.4	-1.25	
	10%	0.01	0.2	-4.32	000	0.19	5.1	-3.52	000
Lolium perenne	**Control**	**5.41**	**100**	**mt**		**4.80**	**100.0**	**mt**	
	5%	3.77	69.6	-1.65	000	5.78	120.3	0.97	
	10%	0.08	1.6	-5.33	000	2.90	60.4	-1.91	00
Trifolium repens	**Control**	**0.89**	**100**	**mt**		**2.38**	**100.0**	**mt**	
	5%	0.23	26.3	-0.66	0	0.74	31.0	-1.65	0
	10%	0.00	0	-0.90	00	0.00	0.0	-2.39	00
Lotus corniculatus	**Control**	**1.12**	**100**	**mt**		**2.93**	**100.0**	**mt**	
	5%	0.44	39.1	-0.69	0	3.02	103.2	0.10	
	10%	0.50	44.9	-0.62	0	1.75	59.7	-1.18	
Festuca arundinacea	**Control**	**4.34**	**100**	**mt**		**4.16**	**100.0**	**mt**	
	5%	2.70	62.3	-1.64	000	3.94	94.6	-0.23	
	10%	0.03	0.7	-4.31	000	2.35	56.5	-1.81	00
Phleum pratense	**Control**	**2.84**	**100**	**mt**		**3.17**	**100.0**	**mt**	
	5%	0.42	14.8	-2.43	000	3.29	103.9	0.13	
	10%	0.04	1.4	-2.80	000	1.51	47.6	-1.66	0
	DL 5% = 0.613 cm DL 1% = 0.840; DL 0.1% = 1.145					DL 5% = 1.313 cm DL 1% = 1.799; DL 0.1% = 2.451			

On *Lotus corniculatus extract*, the root development was inhibited by both solutions, the 10% concentration having a strong negative effect. At the concentration of 5%, the *Lolium perenne* and *Festuca arundinacea* had a higher growth compared to the other species.

The shoots development was positively influenced by the 5% concentration in the *Lolium perenne* and *Festuca arundinacea* species, the impact being negative in the other species. At the concentration of 10%, the growth was totally inhibited at *Trifolium repens*, the other species having a reduction of 26.1% - 52.2% (table 4).

Table 4. Measurements of grassland species seedlings on different concentration of *Lotus corniculatus* extract

Species	Extract Concentration	Root length [cm]	Root length %	Diff [cm]	Signif.	Shoot length cm	Shoot length %	Diff. [cm]	Signif.
Dactylis glomerata	**Control**	**4.32**	**100**	**mt**		**3.71**	**100.0**	**0.00**	
	5%	0.10	2.3	-4.23	000	1.58	42.6	-2.13	000
	10%	0.02	0.5	-4.31	000	0.60	16.2	-3.11	000
Lolium perenne	**Control**	**5.41**	**100**	**mt**		**4.80**	**100.0**	**mt**	
	5%	3.42	63.2	-2.00	000	5.90	122.9	1.10	*
	10%	0.08	1.5	-5.34	000	3.08	64.2	-1.72	000
Trifolium repens	**Control**	**0.89**	**100**	**mt**		**2.38**	**100.0**	**mt**	
	5%	0.19	21.2	-0.71	0	1.21	50.9	-1.17	00
	10%	0.00	0	-0.90	0	0.00	0.0	-2.39	000
Lotus corniculatus	**Control**	**0.55**	**100**	**mt**		**2.93**	**100.0**	**mt**	
	5%	0.36	65.4	-0.19		2.24	76.5	-0.69	
	10%	0.39	70.9	-0.16		1.39	47.4	-1.54	000
Festuca arundinacea	**Control**	**4.34**	**100**	**mt**		**4.16**	**100.0**	**mt**	
	5%	2.39	55.0	-1.96	000	4.50	108.0	0.34	
	10%	4.50	103.6	0.16		1.91	45.9	-2.26	000
Phleum pratense	**Control**	**2.84**	**100**	**mt**		**3.17**	**100.0**	**mt**	
	5%	0.07	2.5	-2.78	000	2.64	83.3	-0.53	
	10%	0.00	0	-2.85	000	0.69	21.9	-2.48	000
	DL 5% = 0.697 cm; DL 1% = 0.955; DL 0.1% = 1.301					DL 5% = 0.820 cm; DL 1% = 1.124; DL 0.1% = 1.531			

4. CONCLUSIONS

The extract of *Dactylis glomerata* had an inhibitory effect on the germination, growth and development of seedlings, of the 6 studied species, except for *Lotus corniculatus* that was stimulated the development of roots, and for *Lolium perenne* the development of shoots.

Trifolium repens extract had the strongest inhibitory effect on all species.

For *Dactylis glomerata, Trifolium repens and Lotus corniculatus*, the *Festuca arundinacea* extract was strongly inhibitory.

In general, seed germination and root growth were inhibited on the studied extracts, while shoots length was the least affected.

Among the studied species, *Lolium perenne* stood out on all 4 extracts, being even stimulated on some of them.

REFERENCES

Alburquerque, M., Santos, R., Lima, L., *et al.*, 2011. Allelopathy, an alternative tool to improve cropping systems. A review, Agronomy for sustainable development, 31 (2), 379-395.

Bostan C., Moisuc A., Radu F.,*et al.*, 2010. *Effect of Poa pratensis* extracts on growing and development of perennial grasses seedlings. Research Journal of Agricultural Science, 42 (I), 372-377.

Breazu I.T. 1998.Grass/clover mixture merits, allelopathic influence among some grassland species and varieties. Breeding for multifunctional agriculture. Proc. of the 21st Meeting of the Fodder Crops and Amenity Grasses Section of EUCARPIA, Kartause Ittingen, Switzerland: 98-100.

Czeke, L., J., Kaufman P., B., 2006.Regulation of metabolite synthesis in plants. Natural products from plants, CRC Press, Boca Raton, Fl, 101-141.

Einhelling F.A., 1995.Allelopathy: Current Status and Future Goals, Red. Inderjit, K.M.M, Am. Chem. Soc., Washington, 1-241.

Favaretto A., Scheffer-Baso S.M., Perez N.B., 2020. Allelopathy in Poaceae species present in Brazil. A review. Agronomy for Sustainable Development, 38 22, 1-12.

Koo D., Goncalves C.G., Askew S.D. 2022. *Lolium arundinaceum* leaf and root developmental temperatures influence its allelopathic potency on *Poa annua International* Turfgrass Society Research Journal 14 (1), 787-790.

Kruse M., Strandberg, M. 2000. Ecological Effects of Allelopathic Plants – A review NERI Technical Report nr 315, 1-66.

Macfarlane M.J., Scott D., Jarvis P. 1982. Allelopathic effects of white clover. Germination and chemical bioassay, NZ Journal of Agricultural Research, 25:4, 503-510.

Macfarlane M.J., Scott D., Jarvis P., 1982. Allelopathic effects of white clover 2. Field investigations in tussock grasslands, NZ Journal of Agricultural Research, 25:4, 511-518.

McCarty, L.B., McCauley R.K., Liu H.,*et al*., 2010. Perennial Ryegrass Allelopathic Potential on Bermudagrass Germination and Seedling Growth, Hortscience, vol 45 (12).

Mozdzen. K., Barabasz – Krasny B., Stachurska-Swakon *et al*. 2020.Allelopathic interaction between two common meadow plants *Dactylis glomerata* L and *Trifolium pratense* L, Biologia vol 75, 653-663.

Narval S.S., 1998.Allelopathy in ecological agriculture, Proceedings of the III International Congress in Allelopathy in Ecological Agriculture and Forestry, Dharward, India, 11-32,

Jankowska, J, Ciepiela, G.A., Jankowski, et al., 2014.The allelopathic influence of Taraxacum officinale on the initial growth and development of Festuca rubra L. Journal of Ecological Engineering, vol 15, nr. 1, 38-44.

Peters, E.J., 1981. Allelopathic Effects of Tall Fescue Genotypes, Agronomy Journal, vol 73 (1), 56-58.

Sanchez-Moreiras A.M., Weiss O. A., Reigosa-Roger M., J., 2004, Allelopathic Evidence in the Poaceae, The botanical Review 69 (3), 300-319.