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BIOASSAY OF PHENOLICS ACCUMULATION AND ACTIVITY IN FODDER GALEGA AT DIFFERENT GROWTH STAGES

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Abstract

Alleochemical interactions have multifaceted influences on receptor species and ecosystems in total. Phenolic compounds are allelochemicals the most common in plants. Plants may concentrate 1–3% of phenols in fresh mass. Multifunctional activity of allelochemicals stimulates increasing of their employment spectrum in order to stabilize balance of biological processes in chains of agro–ecosystems "soil–plants–atmosphere".

The objectives of this research were to establish and compare the total amount of phenolic compounds and allelopathic activity of the aqueous extracts produced from the different shoot parts (leaves, stems, blossoms and seed) and root of the new crop fodder galega (*Galega orientalis* Lam.) at different growth stages. Allelopathic impact of fodder galega was examined during 2004–2005 at the Lithuanian University of Agriculture.

The character of biochemical impact of shoot and root aqueous extracts on rape germination significantly depended on galega growth stage and extract concentration. The biochemical effect of all extracts and concentrations tested exhibited the same trend towards inhibiting rape seed germination. The shoot extracts were more toxic than root extracts and had a stronger suppressive effect on germination.

Phenols concentration and conventional coumarine unit (CCU) content increased evenly depending on the total phenols concentration at all plant growth stages and according to extracts concentration gradient. Phenols concentration and activity in shoot and root increased from shooting to flowering stage.

Key words: allelopathy, fodder galega, phenols, germination, allelochemicals.

Introduction

Biochemical or alleochemical interactions are considered as effects of chemical compounds (allelochemicals) released from plants, including other existing types of organisms in their vicinity /Broekaert et al., 1997; Blanco, 2007; Louis et al., 2007/. Allelochemicals as biological active compounds released from growing donor plants, their secretions and residue have multifaceted influences on receptor species and

ecosystems in total /Alford et al., 2007; Macías et al., 2007; Weih et al., 2008/. They influence soil microbial ecology, soil nutrients also physical, chemical and biological soil features. Effects of any one of these abiotic and biotic constituents of soil and other components of ecosystems may influence growth, distribution, productivity and total survival of plant species /Inderjit, Dakshini, 2000; Carlini, Grossi, 2002/. Allelopathy is one of many in ecosystems existing interactions such as organisms interference, microbial nutrient immobilization, mycorrhizal activity which separating is difficult if not impossible /Anaya, 1999; Wu et al., 2001/. Involvement of eco-physiologists and microbiologists widened the scope of allelopathic research and identification, isolation, characterization of allelopathic compounds and determining of their role importance as stress factors in interference were demonstrated as well /Inderjit, 2006; Ninkovic et al., 2007/. These studies emphasized the significance of understanding multifunctional aspects of allelopathy in structuring of trophical levels, affecting predators and pests, forming symbiotic relations, mediating competitive circumstances. Allelopathic interactions include both inhibitory and stimulatory effects of allelochemicals released or incorporated in plant debris. Nowadays a great attention is paid to identification of plant physiological active compounds, their production and application as components for bio-preparations: phytoherbicides, phytopesticides etc. /Pistelli et al., 2002; Hoagland et al., 2008/.

The main allelochemicals are phenolic compounds which are common for plant cell. Plant may concentrate 1–3% of phenols in fresh mass /Popa et al., 2008; Аллелопатия…, 1990/. Plant phenolics have the potential to influence soil nutrients and implement the growth pattern /Qu, Wang, 2008/.

The objectives of this research were to establish and compare the total amount of phenolic compounds and allelopathic activity of the aqueous extracts produced from the different shoot parts (leaves, stems, blossoms, and seed) and root of the new crop fodder galega at different growth stages.

Materials and methods

Perennial stand of fodder galega (*Galega orientalis* Lam.) grown on the Training Farm of the Lithuanian University of Agriculture was used for investigation of allelopathic potential of this crop. Allelopathic impact (phenols content, total concentration and dynamics, influence on seed germination) of fodder galega was examined during 2004–2005 at the Lithuanian University of Agriculture.

The soil was characterized as sandy moraine loam humic horizon of *Calcari-Epihypogleyic Luvisol* (*LVg-p-w-cc*). The soil pH varied from 7.1 to 7.0, humus content was medium 2.3–2.5%, available P_2O_5 180 mg kg^{-1} and K_2O 209 mg kg^{-1}. The agrochemical analyses were carried out at the Agrochemical Research Centre of the Lithuanian Institute of Agriculture. Soil pH_{KCl} was analyzed by the potentiometric method, mobile P_2O_5 and K_2O by acetone-lactate (A-L) analysis method, and humus percentage by the Tyurin methods.

Allelopathic activity of fodder galega and its separate parts was estimated on the basis of seed germination bio–screening and recalculating to conventional coumarine units (CCU) according to A. Grodzinsky's method /Краткий…, 1973/. The plants of fodder galega were sampled in spring, summer and autumn for producing aqueous extracts which were used for bio–screening (Table 1). The biochemical (allelopathic)

characteristics of fodder galega aqueous extracts at different growth stages: shooting (2nd ten-day period of May), budding (3rd ten-day period of May), flowering (1st ten-day period of June), seed wax maturity (1st ten-day period of July) and in autumn (1st ten-day period of September) were examined. The plant samples were taken at the periods when 50% of plants in the stand had reached the same growht stage.

Table 1. Experimental design
1 lentelė. *Bandymo schema*

Stage, part *Tarpsnis, dalis*	Aqueous extract concentration % *Vandeninių ekstraktų koncentracija %*	
	shoot / *ūgliai*	root / *šaknys*
Shooting / *Krūmijimasis*	0.2, 0.1, 0.05, 0.02	0.2, 0.1, 0.05, 0.02
Budding / *Butonizacija*	0.2, 0.1, 0.05, 0.02	0.2, 0.1, 0.05, 0.02
Flowering / *Žydėjimas:*	0.2, 0.1, 0.05, 0.02	0.2, 0.1, 0.05, 0.02
leaves / *lapai*	0.2	–
stems / *stiebai*	0.2	–
blossoms / *žiedai*	0.2	–
Wax maturity / *Vaškinė branda:*	0.2, 0.1, 0.05, 0.02	0.2, 0.1, 0.05, 0.02
seeds / *sėklos*	0.2	–
Senescence / *Senėjimas*	0.2, 0.1, 0.05, 0.02	0.2, 0.1, 0.05, 0.02

The 0.2, 0.1, 0.05 and 0.02% concentrations of fodder galega whole shoot and root aqueous extracts were produced and examined at every growth stage. The extracts of 0.2% concentration produced from separated plant parts (leaves, stems, blossoms and roots) were estimated at the flowering stage as well as from seeds (0.2%) at wax maturity stage.

The total content of phenolic compounds as the essential allelopathic characteristic for plant cells was estimated according to A. Jermakov's et al. photo-calorimetric method /Методические…, 1976/ using Folin-Ciocalteau reagent /Inderjit, 2006/. Chlorogenic acid was used as a standard phenolic compound. 1 ml of extract solution was mixed with 45 ml of distilled water. One millilitre of Folin-Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 3 min 3 ml of Na_2CO_3 was added, then the mixture was allowed to stand for 2 h. The absorbance was measured at 760 nm. The concentrations of phenolic compounds in the plant aqueous extracts were expressed as parts per million (ppm) equivalents to chlorogenic acid.

According to the bio-method of A. and D. Grodzinsky /Краткий …, 1973/, the seed germination screening was applied and used for recalculating of biochemical activity into conventional coumarine units (CCU). Quickly germinated rape (*Brassica napus* L.) cv. 'Valesca' was chosen as the receptor plant. One hundred seeds of rape were placed on filter paper in each 6-cm diameter Petri dish. Five ml aqueous plant extract (0, 0.2, 0.1, 0.05 and 0.02% concentration) was added per Petri dish as per treatment. Treatments were replicated four times. Petri dishes were kept at +25 °C for 16 h. Seeds sown in distilled water served as control.

The weather conditions that influenced growth, growth and biochemical qualities of the fodder galega were adverse due to lasting droughts.

The confidence limits of the data were based on Student's theoretical criterion. Standard error (*SE*) and standard deviation (SD) were calculated at level of statistical significance $P < 0.05$. The results of allelopathic effects were statistically processed using the statistical package *Statistica* of *StatSoft* for *Windows* standards.

Results and discussion

Allelopathic effect of shoot. The character of biochemical impact of the aqueous extracts produced from fodder galega shoot on the germination data significantly depended on different growth stage and extract concentration (Fig. 1).

The biochemical effect of all extracts and concentrations tested exhibited the same trend of inhibiting germination. As many authors reported /Wu et al., 2003; Chon et al., 2005; Orr et al., 2005/, the extracts of the fodder galega shoot were more toxic than those of root and had a stronger suppressive effect on the receptor plant germination. 0.2% extracts showed the strongest phytoinhibitory effect on seed germination (24%) at flowering stage. Allelopathic activity of shoot extracts decreased sequentially in accordance with descending concentration at all growth stages. Germination at 0.05–0.02% concentrations ranged from 46–64% at flowering stage to 84–96% in the autumn. The allelopathic effect on seed germination of fodder galega shoot increased with plant age from shooting to budding–flowering stages and ranged between 96–24%. At the seed wax maturity the allelopathic effect stopped to decline. At this stage the strongest inhibition of seed germination (28%) was determined at the maximal 0.2% extract concentration. The germination significantly increased from 72 to 83% with the decreasing of extract concentration from 0.1 to 0.02%. The minimal level of allelopathic impact was recorded at senescence stage and seed germination ranged between 70–96% depending on extract concentration.

Allelopathic effect of root. Root extracts of fodder galega were less inhibitory to germination than shoot extracts and showed not only inhibitory effect (Fig. 2). Seed germination ranged between 53–101% in root extracts. Only the least concentration of root extract tested exhibited exceptionally week stimulatory effect (101%) at seed wax maturity in contradistinction to inhibitory effect of other root and shoot treatments.

Alelopathic effect of root extracts had no such evident impact on seed germination during plant vegetation as shoot ones. The strongest inhibitory effect of extracts on germination was recorded only at the flowering and seed wax maturity stages. A noteworthy fact is that during flowering the seed germination fluctuated within a rather narrow range 53–59%. The tested extracts were less active in spring and autumn. Under the effect of these extracts the germination ranged between 71–92 and 64–92%, respectively.

The descending concentration of root extracts showed the tendency to reduce inhibitory effect on seed germination. The extracts of the greatest concentration (0.2%) in all stages were more toxic and showed the strongest suppressive effect on the germination of receptor plant.

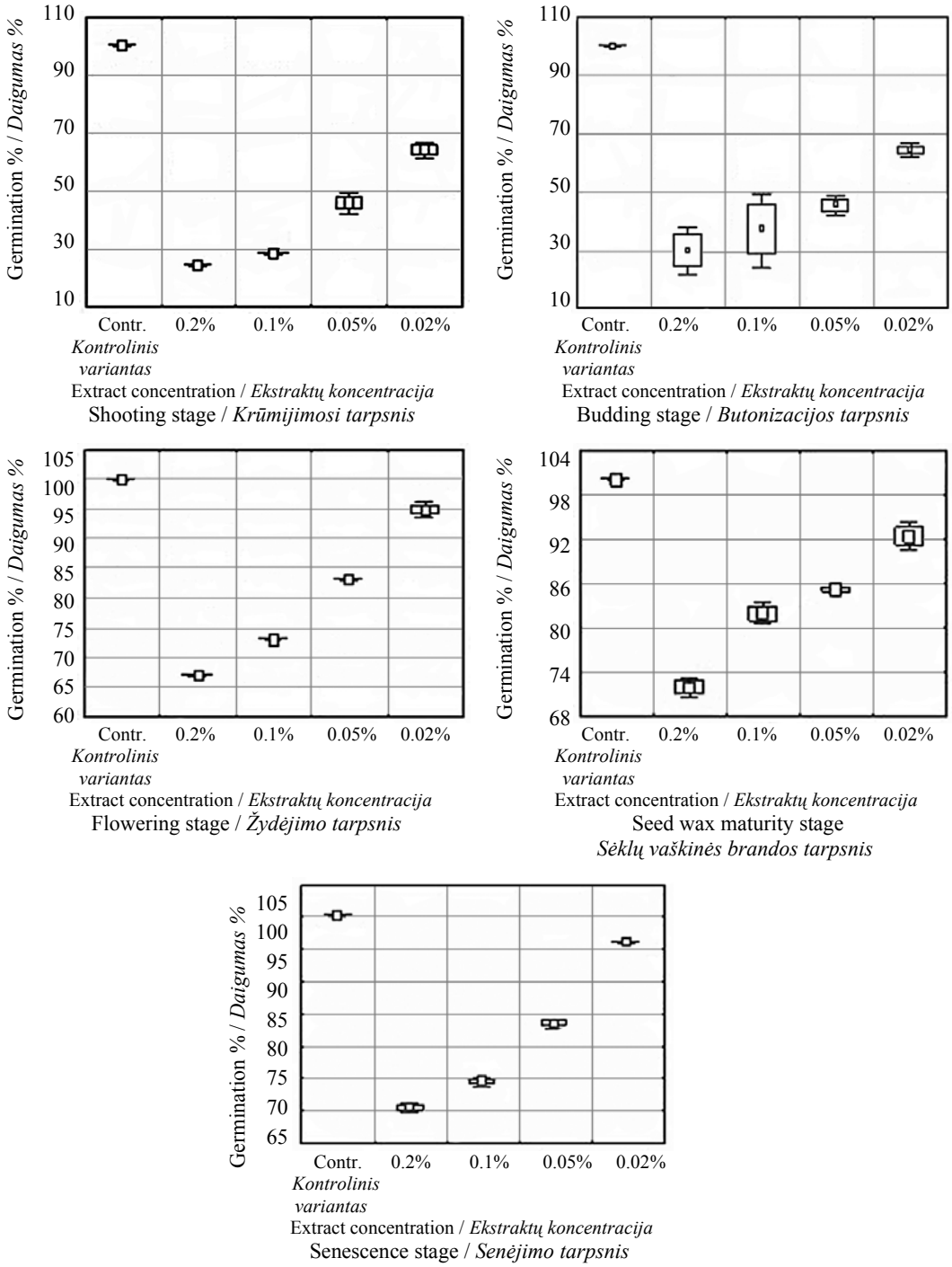

Figure 1. Allelopathic impact of fodder galega shoot on germination at different stages ($P < 0.05$; square – mean, rectangle – SE, whiskers – SD)

1 paveikslas. *Rytinio ožiarūčio ūglių alelopatinis poveikis daigumui įvairiais tarpsniais (P < 0,05; kvadratai – vidurkis, stačiakampiai – SE, ūseliai – SD)*

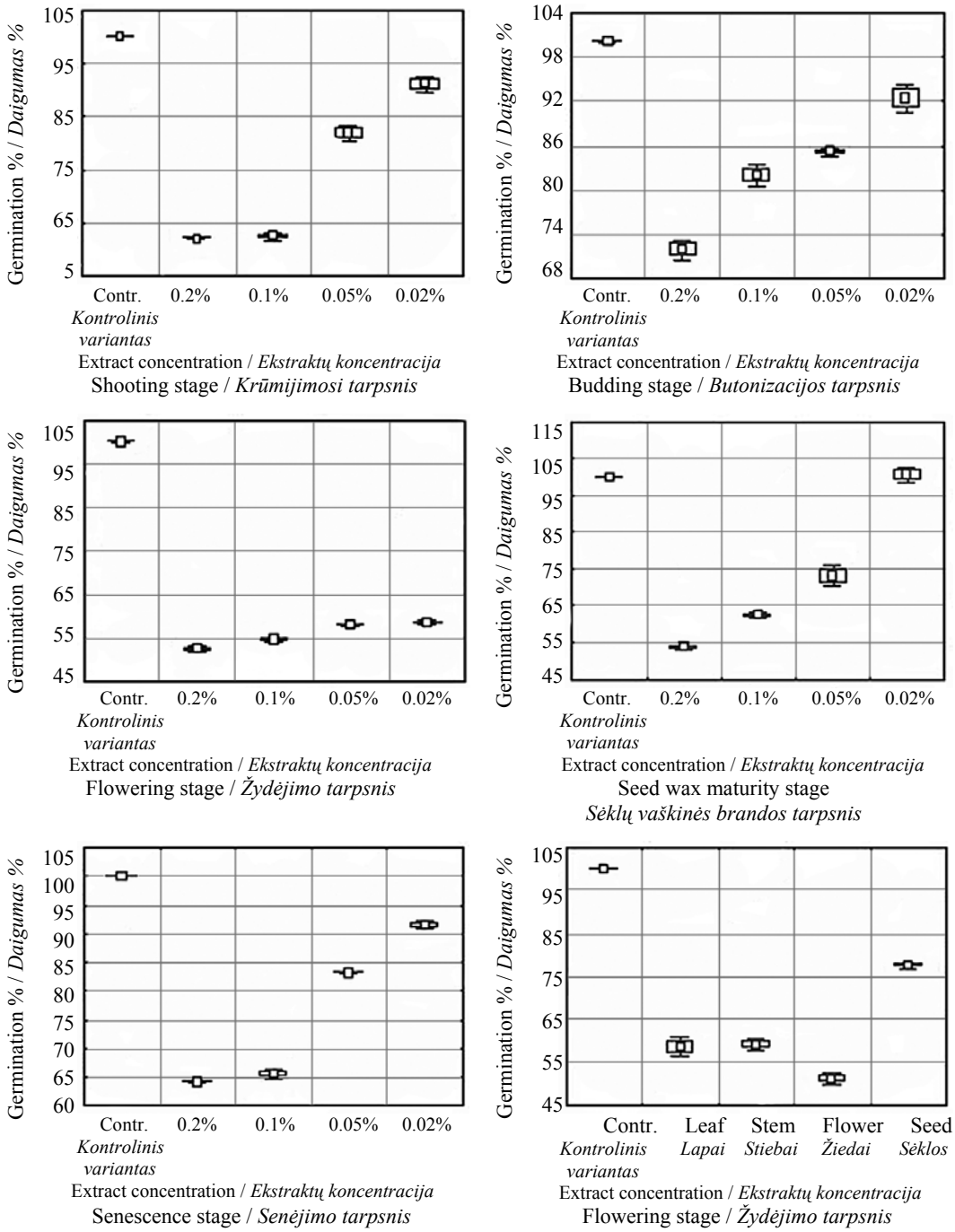

Figure 2. Allelopathic effect of root and different fodder galega parts on germination at different stages ($P < 0.05$; square – mean, rectangle – SE, whiskers – SD)

2 paveikslas. *Rytinio ožiarūčio šaknų ir įvairių dalių alelopatinis poveikis daigumui įvairiais tarpsniais (P < 0,05; kvadratai – vidurkis, stačiakampiai – SE, ūseliai – SD)*

Allelopathic effect of the shoot parts. The strongest suppressive effect of extracts was determined at flowering stage. 0.05% aqueous extracts of shoot vegetative (leaves and stem) and generative (florets and seed) parts were produced and processed at flowering stage, also of seed at wax maturity stage. All of them suppressed seed germination (Fig. 2).

While ground and macerated materials are thought to be less ecologically meaningful /Inderjit, Callaway, 2003/, the fodder galega inflorescence extract indicated the highest toxicity. The seed germination was only 55% under the effect of inflorescence extract. The inhibitory effect of the stem and root extracts decreased, under which the germination amounted to 57 and 58% or was by 43 and 42% lower compared with the control. The germination determined by 39% less in leaf extract than that in control treatment.

Among the tested extracts the seed extract had the minimal toxic and suppressive effect on germination. Therefore germination was significantly the highest (73%) in this extract than in the other treatments.

Phenols concentration at different stages. Phenolics concentration measurement model was chosen to estimate their content at different growth stages of galega. The highest total content of phenols was determined at budding stage which was characterized as the most intensive growth period of galega shoot of (Fig. 3).

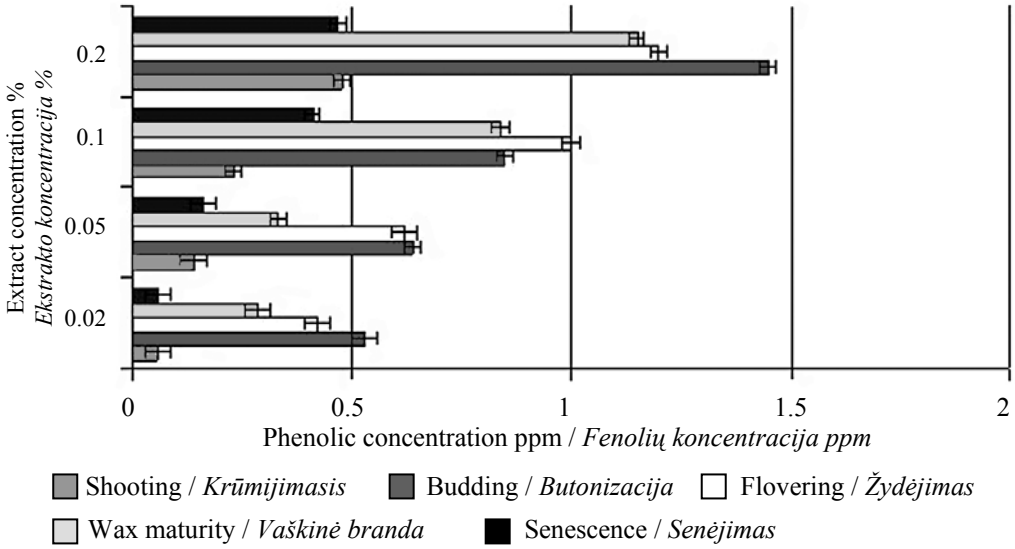

Figure 3. The variation of phenolic compounds concentration in fodder galega shoot extracts at different growth stages ($P < 0.05$; mean ± SD)

3 paveikslas. *Fenolių koncentracijos kitimas rytinio ožiarūčio ūglių ekstraktuose įvairiais augimo tarpsniais (P < 0,05; vidurkis ± SD)*

With the exception of 0.05% concentration, all shoot extracts at budding stage had the highest phenolics concentration (0.555–1.445 ppm), but their biological activity was less in comparison with extracts at flowering stage. Different activity of higher concentration extracts may indicate the presence of different phenolics composition /Varnaitė, 1994/. As Varnaitė (1994) reported, the phenols composition fluctuated during fodder plants vegetation. More precise estimation of biochemical activity is required with identification of phenols composition in galega vegetative extracts. Biochemical activity was recorded less of shoot extracts at flowering stage than that of budding stage due to decrease of phenols concentration (0.425–1.205). These extracts indicated the strongest suppressive activity on seed germination.

Many authors noted the multifaceted importance of allelopathy in soil nutrient dynamics, microbial and rhizosphere ecology of natural and agricultural soils /Dakshini, 2000; Inderjit, 2006; Blanco, 2007/. Phenolics concentration in galega root extracts ranged between 0.058–0.512 ppm and was significantly lower in comparison with shoot (Fig. 4).

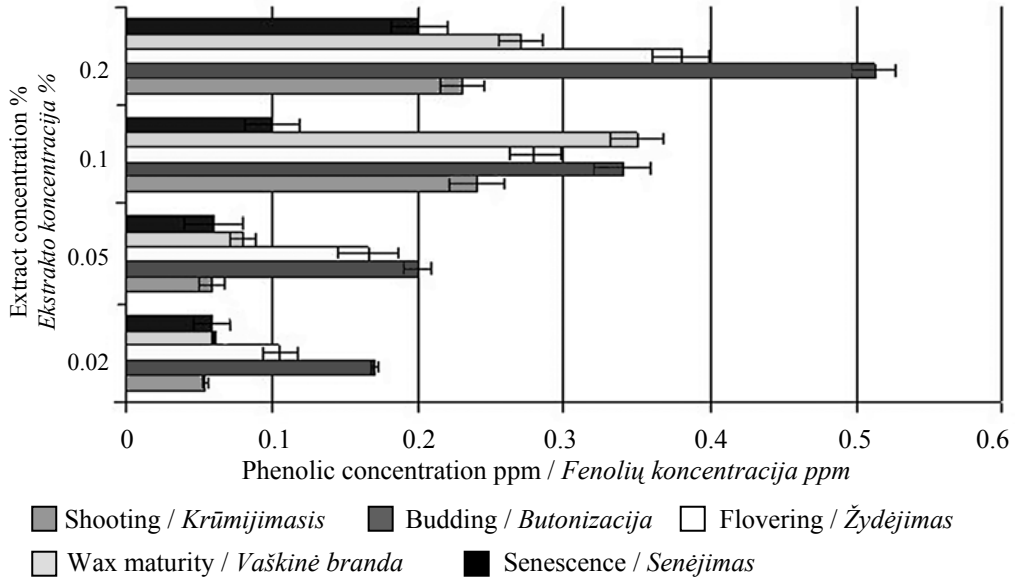

Figure 4. The variation of phenolic compounds concentration in fodder galega root extracts at different growth stages ($P < 0.05$; mean ± SD)

4 paveikslas. *Fenolių koncentracijos kitimas rytinio ožiarūčio šaknų ekstraktuose įvairiais augimo tarspniais (P < 0,05; vidurkis ± SD)*

The highest phenols concentration was determined at budding8 and flowering stages with the exception of 0.1% extract at seed wax maturity stage. The least phenolics concentration was recorded at seed wax maturity (0.06–0.27 ppm) and in the autumn (0.058–0.2 ppm). Variations of galega total phenols concentration during vegetation affirm the influence of environment and climate factors on production and release of

these allelochemicals /Anaya, 1999/. Negligible concentrations of phenols indicate the low level toxicity of root exudates. These results suggest that galega is suitable for long-term growing in the same place without causing soil chemical exhaustion.

Content of conventional coumarine units (CCU). As Inderjit et al. (2000), Inderjit 2006), Wu et al. (2003) and Broeckling and Vivanco (2008) have reported that phenols have very diverse agro-ecological impact. They are the basic phytotoxins causing soil chemical degradation, inhibiting oxidation of NH_4^+ to NO_3^-, reducing the rate of decomposition and N mineralization, influencing soil inorganic ions etc. Therefore it is important to determine phenols content in cultivated crops.

The toxicity and biochemical activity of galega aqueous extracts were expressed by conventional coumarine units /Краткий..., 1973/. In accordance with Kryževičienė, Paplauskienė (2002) and Grodzinskyj /Аллелопатия…, 1990/ reports, the total content of CCU ranged between 8.1–275 depending on the growth stage and the extract concentration and was recorded to be similar to that of other forage grasses, especially to *Lolium perenne* /Kryževičienė, Paplauskienė, 2002/. CCU index suggests that phytoinhibition and allelopathic activity of galega is not higher as that of other forage grasses in agrophytocenoses. Variation of CCU content and germination had the same tendencies in galega extracts. Shoot and root 0.2% extracts tested exhibited the highest CCU content (Table 2).

The concentration of CCU content in galega shoot increased from shooting to flowering and ranged between 35–12.5 and 275–89 at respective stage and extract concentration. The content of CCU decreased and ranged between 241–17.2 and 30–9.4 at the latest galega growth stages: seed wax maturity and in autumn (2nd cut), respectively.

Table 2. The content of conventional coumarine units in fodder galega shoot and root
2 lentelė. *Sutartinių kumarino vienetų kiekis rytinio ožiarūčio ūgliuose ir šaknyse*

Extract concentration % *Ekstraktų koncentracija %*	Shooting *Krūmijimasis*	Budding *Butonizacija*	Flowering *Žydėjimas*	Wax maturity *Vaškinė branda*	Senescence *Senėjimas*
		Shoot / *Ūgliai*			
Control *Kontrolinis variantas*	8.4	8.4	8.4	8.4	8.4
0.2%	35	113	275	241	30
0.1%	28	106	241	27.3	23.9
0.05%	18.2	43	192	23	16.4
0.02%	12.5	19.4	89	17.2	9.4
LSD_{05} / R_{05}	2.41	3.61	4.98	4.03	2.51
		Root / *Šaknys*			
Control *Kontrolinis variantas*	8.4	8.4	8.4	8.4	8.4
0.2%	30.5	27.3	64.9	64.9	39.1
0.1%	18.9	21.2	59	43	37.5
0.05%	15.7	15.7	51	26.1	16.7
0.02%	11.5	11.5	48.9	8.1	11.5
LSD_{05} / R_{05}	2.37	1.82	1.78	2.04	2.01

Stronger phytotoxicity and higher CCU content were recorded of galega shoot extracts than those of root with the exception of root extracts in autumn. Low total content of phenols in root extracts indicated extensive galega impact on soil chemical characteristics, but more detailed investigations are needed to study the composition and impact of galega phenols.

Conclusions

1. Shoot and root aqueous extracts of fodder galega (*Galega orientalis* Lam.) were prepared at different growth stages and assayed on rape germination. The extracts with different inhibitory impact on germination and CCU rates depended on galega growth stage, plant parts/organs and concentration gradient.

2. Shoot extracts exerted a stronger suppressive effect on rape germination than those of root due to the higher phenols content. Galega extracts of the least concentrations (0.05% and 0.02%) indicated the minimal biochemical activity at shooting stage and in autumn compared to those of the highest concentrations (0.1 and 0.2%) at flowering stage.

3. Maximal concentration of phenolic compounds was found in shoot extracts at budding and flowering stages. Our experimental evidence suggests that shoots of fodder galega are the main source of its allelochemicals, especially at flowering stage. Nevertheless, the presence of other bioactive metabolites cannot be excluded.

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RYTINIO OŽIARŪČIO FENOLIŲ KAUPIMASIS IR AKTYVUMAS ĮVAIRIAIS AUGIMO TARPSNIAIS

L. Baležentienė

Santrauka

Alelopatinė sąveika pasireiškia įvairiapusiu poveikiu receptorinėms augalų rūšims ir apskritai ekosistemai. Pagrindiniai alelochemikalai yra fenoliniai junginiai, būdingi augalų ląstelėms, ir jose susikaupia 1–3 %. Dėl daugiafunkcio aktyvumo alelochemikalai naudojami siekiant subalansuoti biologinius agroekosistemos grandies dirva–augalai–atmosfera procesus.

Tyrimo tikslas – nustatyti ir palyginti bendrą fenolių kiekį ir alelopatinį aktyvumą vandeniniuose ekstraktuose, paruoštuose iš šiuo atžvilgiu dar netirto pašarinio augalo rytinio ožiarūčio (*Galega orientalis* Lam.) įvairių antžeminių dalių (lapų, stiebų, žiedų bei sėklų) ir šaknų įvairiais augimo tarpsniais. Rytinio ožiarūčio alelopatinis poveikis daigumui 2004–2005 m. buvo tirtas Lietuvos žemės ūkio universitete.

Rytinio ožiarūčio antžeminės dalies ir šaknų vandeninių ekstraktų biocheminis poveikis augalo daigumui iš esmės priklausė nuo augimo tarpsnio ir ekstrakto koncentracijos. Visų tirtų ekstraktų ir koncentracijų biocheminis poveikis buvo inhibuojantis sėklų daigumą. Antžeminės dalies ekstraktai buvo toksiškesni nei šaknų ir labiau slopino augalo daigumą.

Fenolių koncentracija ir sutartinių kumarino vienetų (SKV) kiekis tolygiai didėjo didėjant ekstraktų koncentracijos gradientui. Antžeminės dalies bei šaknų fenolių koncentracija ir jų aktyvumas ekstrakte didėjo augalų krūmijimosi ir žydėjimo tarpsniais.

Reikšminiai žodžiai: alelopatija, rytinis ožiarūtis, fenoliai, daigumas, alelochemikalai.