



Biochemical Impact of Fodder Galega (*Galega orientalis* Lam.) on Agro-ecosystems

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Multifunctional allelochemicals activities stimulate an increase in an employment spectrum of biologically active compounds in biological farming. The understanding of the allelochemical action mechanisms makes it possible to use these compounds to enhance crop production and develop a more sustainable agriculture, including weed and pest control through crop rotations, residue management and a variety of approaches in bio-control.

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

Biochemical impact of the aqueous extracts produced of fodder galega ground part and roots on the germination data of the test-object subjected significantly on the galega growth stage and extract concentration. The biochemical effect of all tested extracts and concentrations had the same tendency to inhibit the test-object seed germination. The extracts of the ground part were more toxic than those of roots and had a stronger suppressive effect on the test-plant germination. Phenols concentration and conventional coumarine unit (CCU) content increased evenly in dependence on total phenols concentration at all plant development stages accordingly to the extracts concentration gradient. Phenols concentration as well as their activity of ground part and roots increased from shooting to flowering stage.

Key words: *ecology, allelopathy, galega, phenols.*

1. Introduction

The phenomenon of allelopathy encompasses all types of chemical interactions among organisms in all ecosystems. Biochemical or allelochemical 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). Allelopathy is tightly coupled with competition for resources and stress caused by disease, temperature extremes, moisture deficit and herbicides. Such stresses often increase allelochemical production and accentuate their action. Allelopathic inhibition typically results from a combination of allelochemicals which interfere with several physiological processes in the receiving plant or other organisms. 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, Macias 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 1999; Carlini, Grossi 2002). Allelopathy is one of many in ecosystems existing interactions such as organisms interference, microbial nutrient immobilization, mycorrhizal activity whose 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, while 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 have emphasized the significance of understanding the multifunctional aspects of allelopathy in structuring trophic 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 to 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 (Ignat et al. 2011). Plant may concentrate 1–3% of phenols in fresh mass (Popa et al. 2008, Grodzinsky 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 of different shoot parts (leaves, stems, blossoms, and seed) and root of new crop fodder galega at their different growth stages.

2. Methods

Perennial stand of fodder galega (*Galega orientalis* Lam.) grown in the Training Farm of the Lithuanian University of Agriculture was used for investigation of allelopathic potential of that crop. Allelopathic impact (phenols content, total concentration and dynamics, influence on seed germination) of fodder galega was examined 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 P₂O₅ 180 mg kg⁻¹ and K₂O 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 potentiometric method, mobile P₂O₅ and K₂O by acetone–lactate (A-L) analysis method, and humus percentage by 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 (1990). The plants of fodder galega were sampled in spring, summer and autumn for producing aqueous extracts which were used for bio–screening. The biochemical (allelopathic) characteristics of fodder galega aqueous extracts at their 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 growth stage.

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 of separated plant parts (leaves, stems, blossoms and roots) were estimated at a flowering stage as well as from seeds (0.2%) at a wax maturity stage.

The total content of phenolic compounds as the essential allelopathic characteristic for plant cells was estimated according to Slinkard and Singleton (1977) photo-calorimetric method using Folin-Ciocalteu 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 Na₂CO₃ 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 (1990), 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 the student's theoretical criterion. The least significant difference (LSD₀₅) and standard deviation (SD) were calculated at the 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.

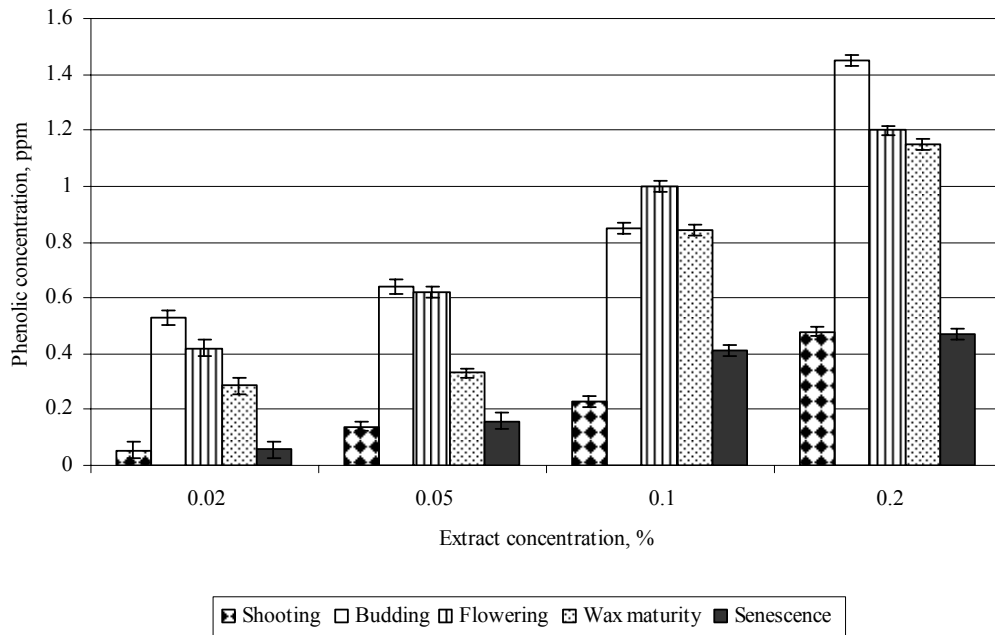


Fig. 1. Variation of phenolic compounds concentration in fodder galega shoot extracts at different growth stages ($p < 0.05$; mean \pm SD)

3. Results and Discussion

3.1. Phenolic concentration during growth stages

Phenolics concentration measurement model was chosen to estimate their content at different growth stages of galega (Ignat et al. 2011). The highest total content of phenols was determined at a budding stage which was characterized as the most intensive growth period of galega shoot of (Fig. 1).

With an exception of 0.05% concentration, all shoot extracts at a budding stage had the highest phenolics concentration (0.555–1.445 ppm), but their biological activity was less in comparison with extracts at a flowering stage. Different activity of higher concentration extracts may indicate the presence of different phenolics composition

(Baležentienė, Sėžienė 2010). 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 a flowering stage than that of a budding stage due to a decrease in 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. 2).

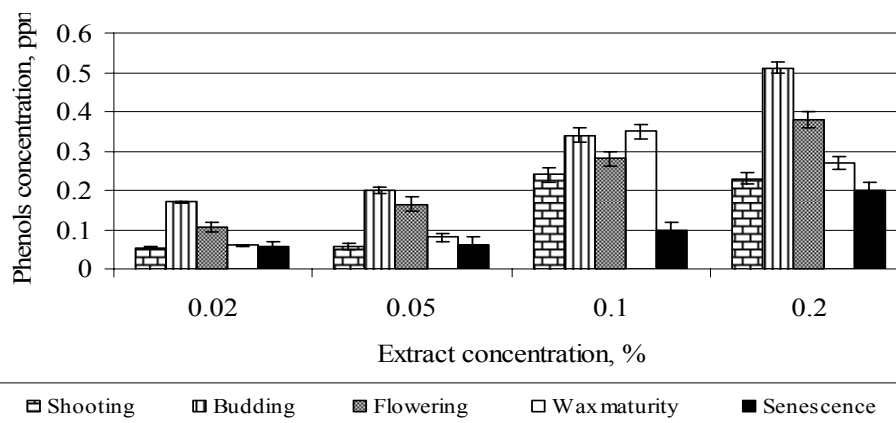


Fig. 2. Variation of phenolic compounds concentration in fodder galega root extracts at different growth stages ($p < 0.05$; mean \pm SD)

The highest phenols concentration was determined at budding and flowering stages with the exception of 0.1% extract at a seed wax maturity stage. The least phenolics concentration was recorded at seed wax maturity (0.06–0.27 ppm) and in 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.

3.2. Content of conventional coumarine units

Inderjit et al. (2000), Inderjit (2006), Wu et al. (2003) and Broeckling, Vivanco (2008) have reported that phenols have a 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 the phenols content in cultivated crops.

The toxicity and biochemical activity of galega aqueous extracts were expressed by conventional coumarine units (Grodzinsky 1990). In accordance

with Kryževičienė, Paplauskienė (2002) and Grodzinsky (1990) reports, the total content of CCU ranged between 8.1–275 depending on the growth stage and the extract concentration. This CCU rate of galega biomass 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 1).

The concentration of CCU content in galega shoot increased from shooting to flowering and ranged between 35–12.5 and 275–89 at a 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.

Stronger phyto-toxicity 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.

Table 1. Content of conventional coumarine units in fodder galega shoot and root

Extract concentration, %	Shooting	Budding	Flowering	Wax maturity	Senescence
Shoot					
Control	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	2.41	3.61	4.98	4.03	2.51
Root					
Control	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	2.37	1.82	1.78	2.04	2.01

3.3. Phyto-inhibitory effect of shoot

The character of biochemical impact of the aqueous extracts produced from fodder galega shoot on the germination data depended significantly on a different growth stage and extract concentration (Fig. 3).

The biochemical effect of all extracts and concentrations tested exhibited the same trend of

inhibiting germination. As many authors reported (Wu et al. 2003; Chun 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 phyto-inhibitory effect on seed germination (24%) at a 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 a flowering stage to 84–96% in 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 a decrease in extract concentration from 0.1 to 0.02%. The minimal level of allelopathic impact was recorded at a senescence stage and seed germination ranged between 70–96% depending on the extract concentration.

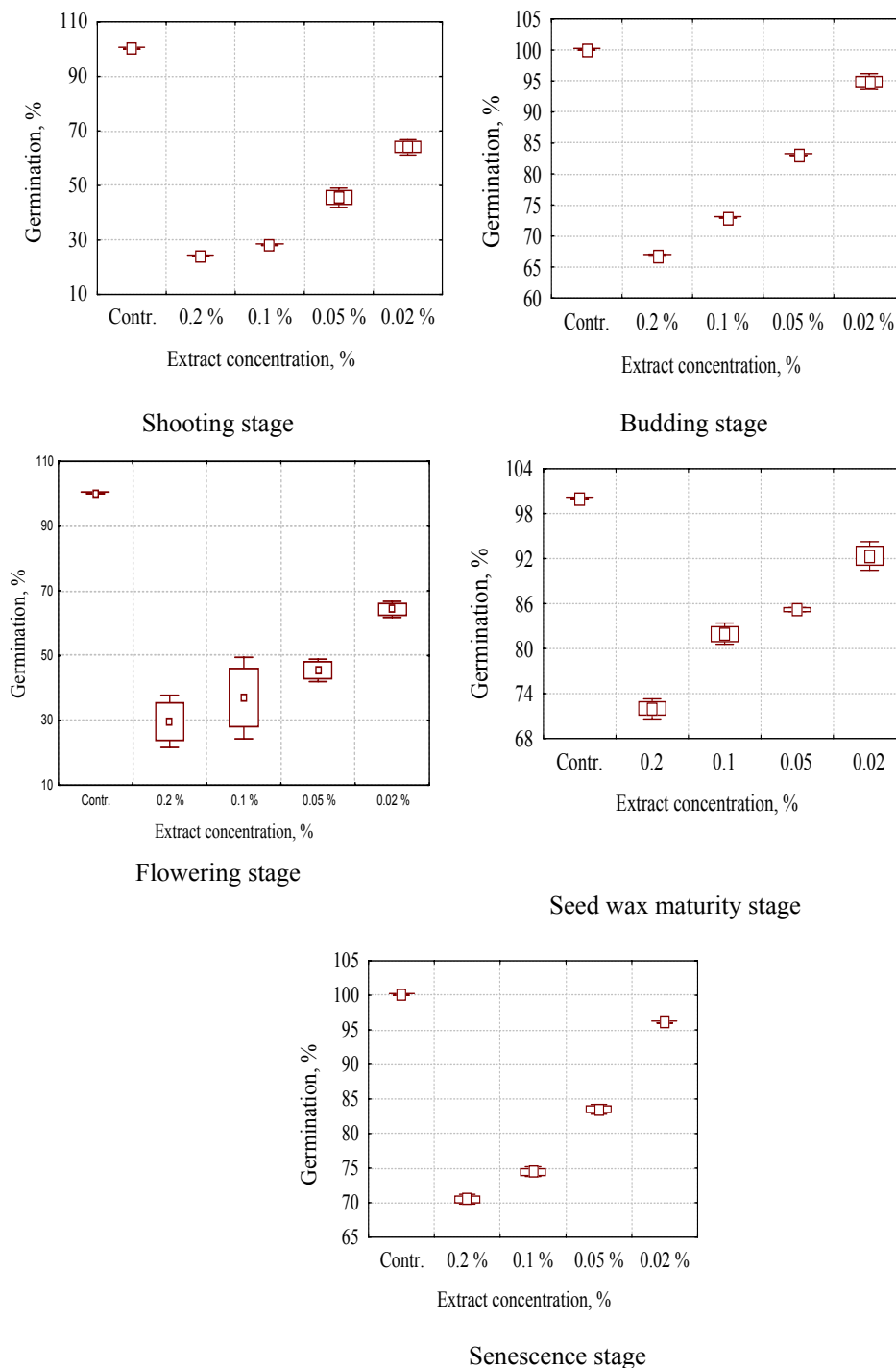


Fig.3. Phyto-inhibitory impact of fodder galega shoot on germination at different stages ($p < 0.05$; square – mean, rectangle – SE, whiskers – SD)

3.4. Phyto-inhibitory effect of root

Root extracts of fodder galega were less inhibitory to germination than shoot extracts and showed not only the inhibitory effect (Fig. 4). Seed germination ranged between 53–101% in root

extracts. Only the least concentration of the root extract tested exhibited an exceptionally weak stimulatory effect (101%) at seed wax maturity in contradistinction to the inhibitory effect of other root and shoot treatments.

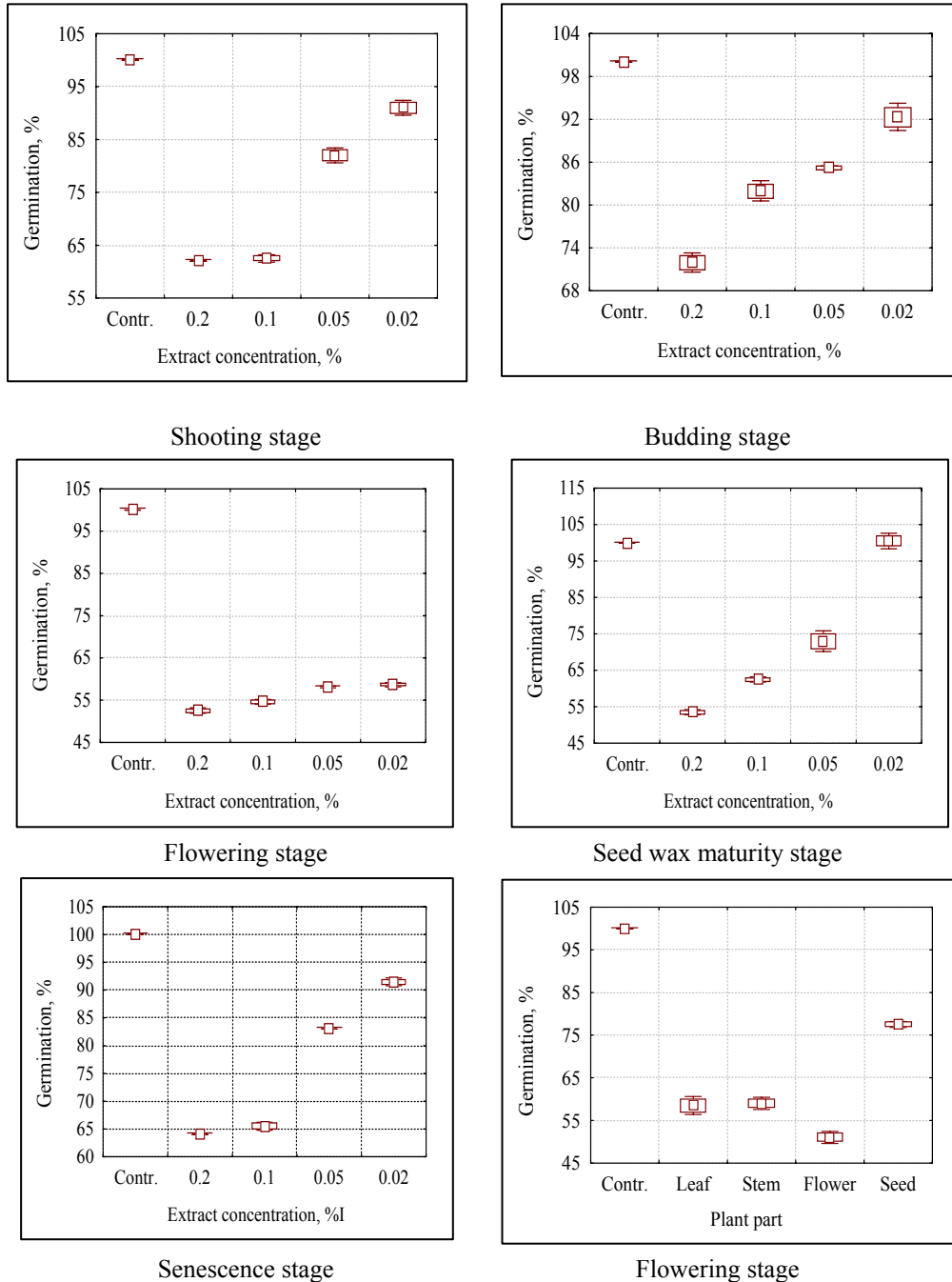


Fig.4. Phyto-inhibitory effect of root and different fodder galega parts on germination at different stages ($p < 0.05$; square – mean, rectangle – SE; whiskers – SD)

Alelopathic effect of root extracts had no such evident impact on seed germination during plant vegetation as the shoot ones. The strongest inhibitory effect of extracts on germination was recorded only at 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 the 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.

3.5. Phyto-inhibitory effect of shoot parts

The strongest suppressive effect of extracts was determined at a flowering stage. 0.05% aqueous extracts of shoot vegetative (leaves and stem) and generative (florets and seed) parts were produced and processed at a flowering stage, also of seed at a wax maturity stage. All of them suppressed seed germination (Fig. 4).

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 to the control. The germination was 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.

4. Conclusions

Our results suggest that allelochemicals of the aerial part of fodder galega (*Galega orientalis* Lam.) are more phytotoxic than those of its roots due to the higher phenols content. Nevertheless, participation of other bioactive compounds could not be excluded. The maximal allelopathic potential of aerial plant part was detected at a flowering stage and phenolic compounds could be involved. The extracts with different inhibitory impact on germination and CCU rates depended on a galega growth stage, plant parts and an extract concentration gradient. Galega extracts of the least concentrations (0.05% and 0.02%) indicated the minimal biochemical activity at a shooting stage and in autumn compared to those of the highest concentrations (0.1 and 0.2%) at a flowering stage. More research is needed to determine the real effect of fodder grass allelochemicals on representative receptor plants in natural conditions.

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Rytinio ožiarūčio (*Galega orientalis* Lam.) biocheminis poveikis agroekosistemoms

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Aleksandro Stulginskio universitetas, Lietuva

(gauta 2011 m. rugsėjo mėn.; atiduota spaudai 2011 m. gruodžio mėn.)

Daugiafunkcinis alelochemikalų aktyvumas skatina plėsti biologiškai aktyvių junginių panaudojimo galimybes biologiniame ūkininkavime. Alelochemikalų veikimo mechanizmo supratimas leidžia mums naudoti šiuos junginius skatina gerinti augalų produktyvumą ir plėtoti tvarų žemės ūkį, įskaitant piktžolių ir kenkėjų kontrolės per sėjomainą, likučių valdymą ir biologinės kontrolės metodų įvairovę.

Pagrindinis šio tyrimo tikslas buvo nustatyti ir palyginti bendrą fenolinių junginių kiekį ir alelopatinį aktyvumą vandeniniuose ekstraktuose, pagamintuose iš rytinio ožiarūčio įvairių ūglio dalių (lapų, stiebų, žiedų ir sėklų) ir šaknų skirtingose augimo stadijose.

Biocheminis poveikis vandeninių ekstraktų, pagamintų iš pašarų ožiarūčių antžeminės dalies ir šaknų daigumo rezultatai patikimai priklausė nuo augimo tarpsnio ir ekstrakto koncentracijos. Visų tirtų ekstraktų ir koncentracijų biocheminis poveikis rodė tą pačią tendenciją slopinti bandymo objekto sėklų daigumą. Antžeminės dalies ekstraktai buvo labiau toksiški ir stipriau slopino daigumą nei šaknų ekstraktai. Fenolių koncentracija ir sutartinių kumarino vienetų (CCU) kiekis didėjo tolygiai priklausomai nuo bendros fenolių koncentracijos visais augalų vystymosi tarpsniais pagal ekstraktų koncentracijos gradientą. Fenolių koncentracija, taip pat jų aktyvumas antžeminėje dalyje ir šaknyse didėjo nuo krūmijimosi iki žydėjimo tarpsnio.