

SHORT COMMUNICATION

Allelopathic potential of fodder galega at different growth stages

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ABSTRACT

Fodder galega is a forage legume which effectively competes with weeds, perhaps allelopathy contributes to this interference with neighbours. Aqueous extracts of shoots and roots of fodder galega were prepared from different growth stages and assayed on rape germination. Concentration of total phenolic compounds in aqueous extracts was also measured. Extracts of shoots of fodder galega were more suppressive to germination than root extracts. Maximum allelopathic potential of shoots was found at flowering stage. Concentration of phenolic compounds in extracts of shoots was higher than in root extracts. Maximum concentrations of phenolic compounds were found in shoots at budding and flowering stages. Our results suggested that shoots of fodder galega are the main source of its allelochemicals, specially at flowering stage. These allelochemicals may be phenolic compounds but other metabolites may also participate.

Keywords: Allelopathy, *Galega orientalis*, germination, growth stages, phenolic compounds

INTRODUCTION

Forage crops suppress the weeds by promoting conditions unfavorable for the germination and establishment of weeds and thereafter competing for available resources (28). Allelopathy, the inhibitory or stimulatory effect of chemicals released by a donor plant on a receptor one, may contribute to such interference (24). Many phytochemicals including phenolic compounds are allelopathic to plants and related organisms (6,15,19,23). Phenolics are allelopathic compounds present in forage legumes and influences the availability of soil nutrients, seed germination and plant growth (10,13,14,18).

Fodder galega is highly productive forage legume with good nutritive value (1,3,4,5,21,22). In Lithuania, this perennial legume reduces the weed stands and growth. Allelopathy may participate in this interference process, as observed in other forage

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legumes (2). The objectives of this research were (i) to evaluate the allelopathic potential of parts of fodder galega (*Galega orientalis* Lam.) (ii) at different growth stages and (iii) to determine the relationship between the phenolic content and allelopathic potential of fodder galega plant parts.

MATERIALS AND METHODS

The study consisted of 3 factors: (i). Plant parts: 2 (Shoots, roots), (ii). Growth stages: 5 (shooting, budding, flowering, maturity, senescence) and (iii). Aqueous extract concentrations: 5 (0, 0.02, 0.05, 0.1 and 0.2%). Shoots and roots of fodder galega were collected at different growth stages from a perennial stand of fodder galega (*Galega orientalis*) at Research Farm of Lithuanian University of Agriculture. Shoots and roots of fodder galega were collected at (i). shooting (May 10th to 20th), (ii). budding (May 21th to 30th), (iii). flowering (June 1th to 10th), (iv). maturity (July 1th to 10th) and (v). senescence (September 1th to 10th). Soil of the perennial stand was *Endocalcari-Epihypogleyic Cambisol*. Plant parts (shoots and roots) were separately chopped into 0.5 cm long pieces and 50 g biomass of each plant part was immersed in 250 ml distilled water for 12 h and kept at 5 °C in Incubator. Then, aqueous extracts were filtered through Whatman No 1 filter paper. These aqueous extracts were diluted to concentrations of 0.02, 0.05, 0.1 and 0.2 % (w/v) and used for germination assays.

Germination assay

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 was added per Petri dish as per treatment. Treatments were replicated four times. Petri dishes were kept at 20°C for 16 h. Seeds sown in distilled water served as control. Seed germination was used to calculate the allelopathic potential of aqueous extracts in conventional coumarine units (CCU) as per Grodzinsky's method (11).

Phenolic compounds content

Sixty ml of each plant extract was acidified with sulphuric acid to pH 3. The acidified aliquot was shaken with 10 ml ethyl acetate for 30 s in a separating funnel and the organic phase was carefully collected. Then, the remaining aqueous phase was extracted thrice using 4 ml ethyl acetate. Fractions of organic phase were mixed and evaporated to dryness under reduced pressure. The dry residue was dissolved in a known volume of distilled water and total concentration of phenolic compounds was estimated using the Folin-Denis reagent (17). Concentrations of phenolic compounds in the aqueous plant extracts were expressed as parts per million (ppm) equivalent to chlorogenic acid. This phenolic acid was selected because it provides reliable results in colorimetric reaction with Folin Denis reagent (17).

Statistical analysis

The least significant differences (LSD₀₅), standard errors (SE) and standard deviation (SD) were calculated at p<0.05. Data were analyzed using the statistical package STATISTICA of StatSoft for Windows. Results for germination, phenols concentration and CCU are presented as mean ± SD of 4 independent analyses at the significance level of 0.05.

RESULTS AND DISCUSSION

Shoot aqueous extracts of fodder galega collected at different growth stages significantly reduced the rape germination (Fig. 1). However, the inhibitory activity of shoots depended on its growth stage and the extract concentration. The maximum inhibition in germination was observed at flowering (38% to 76%) followed by maturity (18% to 78%) and budding (21% to 59%), while the lowest was at shooting (7% to 34%) and senescence (6% to 29%). These results suggest that fodder galega shoot have the lowest concentrations of allelochemicals in the early as well as in the last growth stages. A similar response has been observed in other forage and crop species (12,16,22).

Root extracts of fodder galega were less inhibitory to rape germination than shoot extracts (Fig. 2). Similar observations were made on other forage legumes such as alfalfa (*Medicago sativa* L.) (19). However, this response can not be generalized for all legume crops. For example, root extracts of winged bean (*Phaseolus acutifolius* A. Gray) and tepary bean (*Psophocarpus tetragonolobus* L.) are more phytotoxic than shoot extracts (26). Differences in shoot and root extract effects may indicate the presence of different allelochemicals or concentrations of allelochemicals in them. On the other hand, the inhibitory activity of root extracts also depended on growth stage and extract concentration. The maximum inhibition was observed at flowering (41 to 48%) and maturity (0% to 48%). Thus allelopathic potential of fodder galega depends on plant age and shoots are the main source of allelochemicals (20,25). In agreement with these results, shoot extracts from reproductive stage of alfalfa were more inhibitory than from the vegetative stage (7,8,19,23,27,30). Nevertheless, further research is needed in this regard, to determine if the observed response is general one forage legumes.

Phenolic compounds content

Phenolic compounds play a significant role in allelopathy (13). They were implicated in allelopathic interactions among different groups of plants such as algae, fungi, lichens, bryophytes, pteridophytes, gymnosperms, and angiosperms (9,26). For this reason, we determined the levels of phenolic compounds in extracts of fodder galega at different growth stages and related their presence with the observed phytotoxicity on the receptor plant. The fodder galega shoots were rich in phenolic compounds at budding (0.57 to 1 ppm) and flowering (0.41 to 1.4 ppm) stages (Figure 3). However, the biological activity of extracts at budding stage was lower than at flowering. These results suggested that other metabolites are also participating in phytotoxicity of shoot extracts from the fodder galegas or the composition of phenolic compounds is responsible for the observed phytotoxicity more than their concentrations (5,25,27).

Content of total phenolic compounds was significantly lower (0.058 - 0.512 ppm) in root extracts than in shoot extracts (0.140 - 1.450 ppm) (Figs. 3 and 4). These results suggested that phenolic concentration is responsible for the phytotoxicity of extracts from plants parts of fodder galega.

Content of conventional coumarine units (CCU)

The toxicity of aqueous extract from fodder galega were expressed as CCU (10,11,30). The content of CCU ranged between 8.1 and 275, depending on the growth

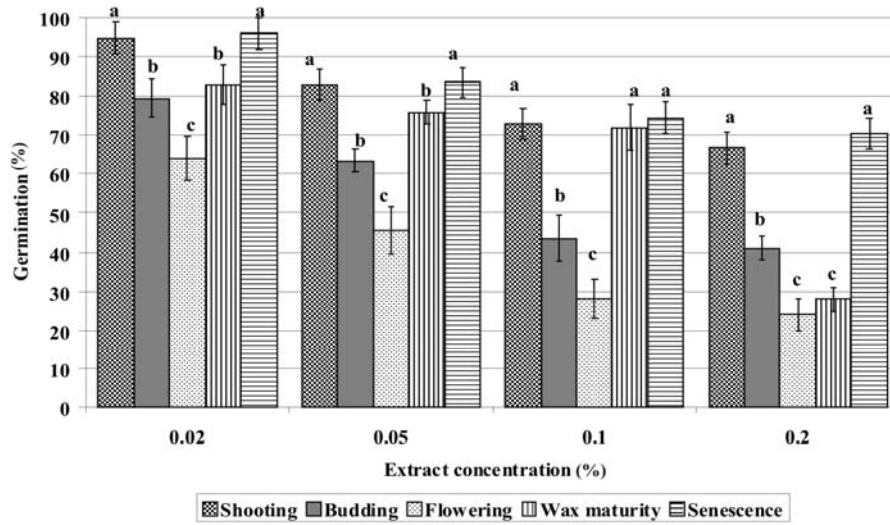


Figure 1. Allelopathic potential of shoot extracts from fodder galega at different growth stages on rape germination (mean±sd). Different letters indicate significant differences among means at $p < 0.05$.

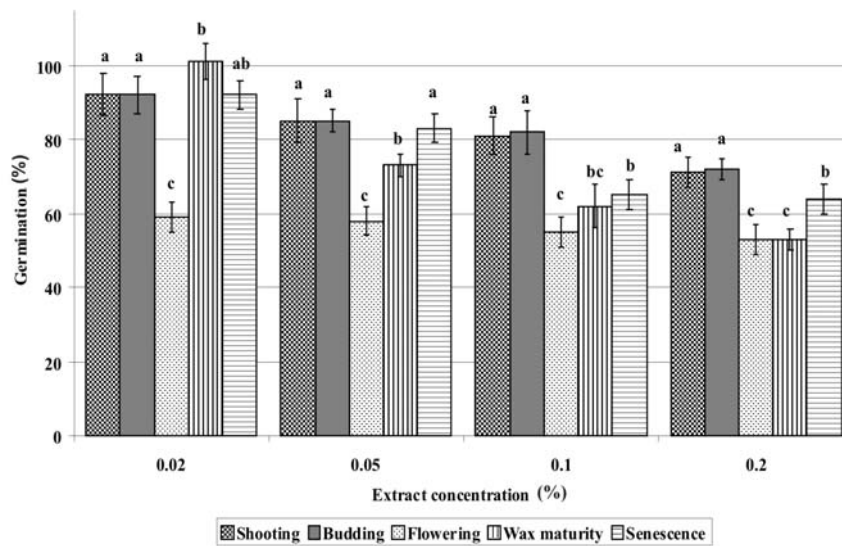


Figure 2. Allelopathic potential of root extracts of fodder galega at different growth stages on rape germination (mean±sd). Different letters indicate significant differences among means at $p < 0.05$.

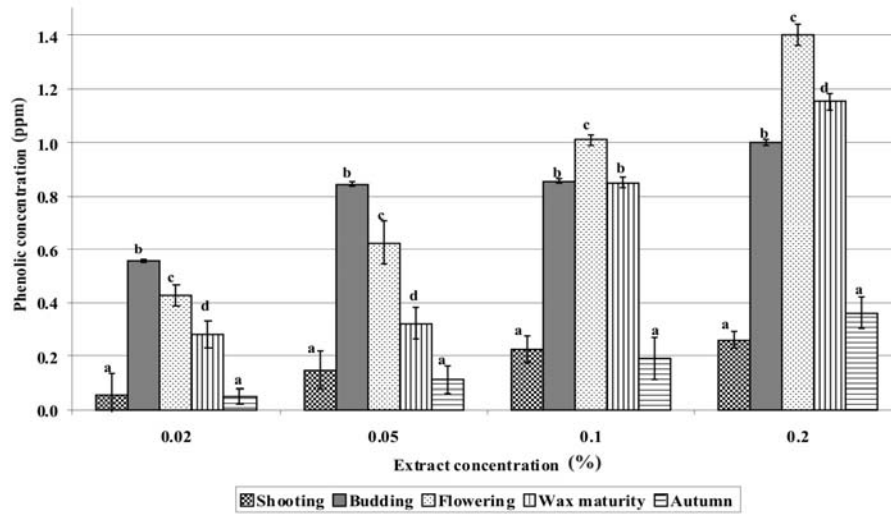


Figure 3. Total phenolic compounds contents in shoot extracts of fodder galega at different growth stages (mean \pm SD). Different letters indicate significant differences among means at $p < 0.05$.

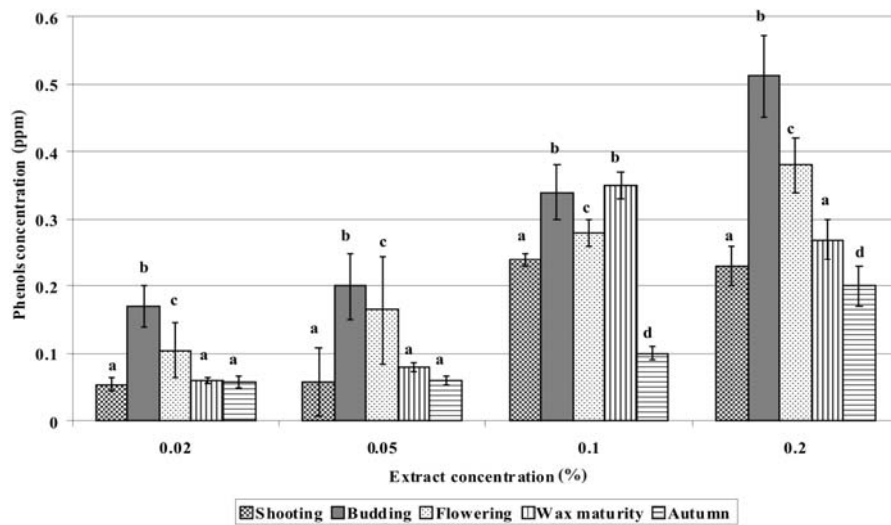


Figure 4. Total phenolic compounds contents in root extracts of fodder galega at different growth stages (mean \pm SD). Different letters indicate significant differences among means at $p < 0.05$.

stages and extract concentrations. These values are similar to other forage grasses such as *Lolium perenne* (18). This index suggested that allelopathic potential of fodder galega is not as higher as that of other forage crops (Table 1). The concentration of CCU in shoots of fodder galega sequentially increased from shooting to flowering and then decreased until senescence stage. Stronger phytotoxicity and higher CCU content were found in fodder galega shoots and roots, except at senescence in roots.

Table 1. Effects of aqueous extract concentrations on Conventional Coumarine Units (CCU) content in shoots and roots of fodder galega

Concentration of aqueous extracts (% w/v)	Growth stages				
	Shooting	Budding	Flowering	Maturity	Senescence
	Shoot				
0.0	8.4	8.4	8.4	8.4	8.4
0.02	12.5	19.4	89.0	17.2	9.4
0.05	18.2	43.0	192.0	23.0	16.4
0.1	28.0	106.0	241.0	27.3	23.9
0.2	35.0	113.0	275.0	241.0	30.0
LSD ₀₅	2.41	3.61	4.98	4.03	2.51
	Root				
0.0	8.4	8.4	8.4	8.4	8.4
0.02	11.5	11.5	48.9	8.1	11.5
0.05	15.7	15.7	51.0	26.1	16.7
0.1	18.9	21.2	59.0	43.0	37.5
0.2	30.5	27.3	64.9	64.9	39.1
LSD ₀₅	2.37	1.82	1.78	2.04	2.01

CONCLUSIONS

The allelochemicals of fodder galega shoots were more phytotoxic than those of roots. Maximum allelopathic potential of shoots was observed at flowering stage, perhaps due to phenolic compounds. Nevertheless, participation of other bioactive compounds cannot be excluded. More research is needed to determine the real effects of fodder galega allelochemicals in natural conditions on representative receptor plants.

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