

Comparative characteristics of *Lupinus perennis* L. under allelochemical sorgoleone stress

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Abstract

The aim of the present work was evaluation of the allelopathic potential of sorgoleones on *Lupinus perennis*. The compounds were isolated by column chromatography C_8 from *Sorghum halepense*. Were prepared different concentrations extracts from *Sorghum Halepense*, and then was assessed the tolerance of *Lupinus perennis* L. (Fabaceae) after treatment with these extracts. The effects were studied by the seeds germination biotest, plantlets growth and photosynthetic pigments dosage. The preliminary chemical analysis of *S. halepense* extract indicated the presence of the sorgoleone and dihydrosorgoleone, the identification of these compounds being confirmed by specific reactions. The germination was not affected by any of the tested extracts, but the growth was inhibited by the allelopathic compounds present in the composition of *S. halepense* extracts. Based on these results, it can be stated that the tested species showed sensitivity in the presence of the allelochemicals in the medium growth. The aqueous extracts of *S. halepense* are appropriate for use in situations when certain plants species are harmful.

Keywords: sorgoleone, dihydrosorgoleone, germination, growth of seedlings, *S. halepense*

Introduction

Allelopathy represents the influence that the plants exert upon one another through the chemicals substances released. Most of the time, the influence is inhibitory, which may explain why some plants do not grow in the presence of other selected plants. [Sexton *et al.*, 2002, Butnariu *et al.*, 2011]. Plants can develop competition and destroy one another in order to occupy a certain area. [Cramer *et al.*, 2007]. Thus, it can be affected the seeds germination, the roots development and the absorption of the nutrients. *Sorghum* (Grass Family: Poaceae), includes plants whose roots eliminate sorgoleone [Hejli *et al.*, 2004], a toxic substance that blocks the respiration and the photosynthesis of other plants [Butnariu, *et al.*, 2005].

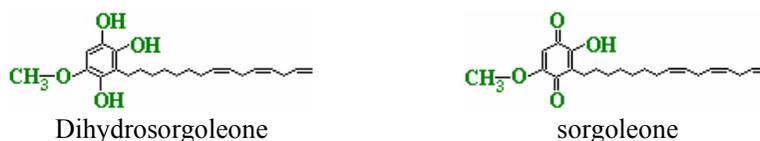


Figure 1. The chemical structure of sorgoleone and dihydrosorgoleone

S. halepense, a herbaceous plant of grass family, is one of the most harmful weeds for agricultural crops, especially in the dry regions. It reproduces by seeds and rhizomes and its roots are strongly developed to support the plants that sometimes grow higher than some cereals [Mihovsky, *et al.*, 2012].

The root system typically extends to a depth higher than 1 m. The strain grows up to three meters [Uddin *et al.*, 2010; Yang *et al.*, 2004]. From a chemical point of view, the plants contain major constituents (Figure 1) as sorgoleone and dihydrosorgoleone. *Sorghum* is considered to be one of the plant species with an important allelopathic potential [Dayan 2006; Butnariu *et al.*, 2012]. Sorgoleone, 2-hydroxy-5-methoxy-3-[(8'Z,11'Z)-8',11',14'-pentadecatriene]-p-benzoquinone, and the corresponding hydroquinone are the major components of *S. halepense* root exudate. Sorgoleone includes different similar compounds as length or unsaturation degree of 3-alkyl side chain [Dayan *et al.*, 2010].

These compounds possess a phytotoxic character emphasised by the inhibition of the photosystem II (PSII), process that is mediated by the oxygen. The isolation of these compounds was achieved using a C8 chromatography column and thin layer chromatography [Kagan *et al.*, 2003]. *Lupinus perennis* is a perennial species of Fabaceae family, being also a medicinal and melliferous plant with a relatively short flowering period [Halpern, 2005]. *Lupinus perennis* is known as forage crop and is used as green fertilizer. *L. perennis* presents therapeutic properties, also being a plant largely consumed by the vegetarians [Awad *et al.*, 2010; Zhekova, 2012].

Allelopathy is a field of the ecological biochemistry studying the reciprocal relations between plants through chemicals messages (signals). Proofs regarding the allelopathic interactions were also found in the agricultural systems [Nikolova, *et al.*, 2012; Butu *et al.*, 2012].

Besides competition, a large part of the production decrease for many crops was due to the allelopathy. There are plants that release in the environment (air, soil) toxic substances that inhibit the growth of the other selected plants, this mechanism providing them a competitive advantage [Hobbs *et al.*, 2006; Butnariu *et al.*, 2005; Nikolova, *et al.*, 2012].

The aim of this study is to evaluate the allelopathic potential of *S. halepense* plants on the quality characteristics necessary for germination and seedling growth of *L. perennis*. Using column chromatography, sorgoleones were isolated, structurally characterized, and evaluated for in vitro allelopathic potential.

Materials and methods

The experiment was carried out under greenhouse conditions.

Preparation of treatment solution. Were prepared three different concentration solutions of *Sorghum halepense* L. extracts as follows: domain (I)–standard substances (initial extract); domain (II)–obtained by dilution 1:1; and domain (III)–obtained by dilution 1:2

Seed germination biotest. The seeds selected to be used in the experiment, were initially verified to be highly qualitative, to have a good purity, not presenting any traces of dirt, damaged seeds or any other inert matter. The seeds of *Lupinus perennis* used for study had a 98% germination rate. In order to eliminate the risk of any infection, the seeds were soaked in sodium hypochlorite 5% for 10 minutes, and then washed with ionized water before planting. For the study of germination were used thirty petri plates which were kept under cold light emitted by fluorescent tubes with an intensity of $190\mu\text{mol/s/m}^2$, during a photoperiod of 16h, for 7 days, until the cotyledons developed completely. Each Petri plate contained 20 seeds of *Lupinus perennis*, carefully arranged on filter paper sprayed with 20 mL treatment solution.

The biometry of plant growth

The experiments protocol was carried according to the randomised block design containing three blocks. Each block was represented by one replicate, therefore containing all possible variants. Subsequently to germination, the seeds were cultured on a solid medium Murashige and Skoog (MS) ½, without growth hormones, with saccharose 10g/L, agar (Sigma) 8g/L, pH 5.9, in cylinders containing 100mL solution of extracts of *S. halepense*, in different concentrations. Each cylinder containing 20 mL Medium and 32 germinated seeds was transferred in the growth chamber providing constant temperature and humidity conditions (25±2°C and 45%). 12 Days old plants were used for the biometry of the plant growth.

Estimation of photosynthetic pigments. Leaf extraction was done in 80% acetone. In a Erlenmeyer flask we added 5mL of assimilating pigment alcohol extract and 2–3mL of petroleum ether and a few drops of water. The phases were subsequently separated as follows: the green coloured top phase contained chlorophyll a and b, and the lower part contained yellow pigments (carotene and xanthophyll). Chlorophyll and carotenoid content was assessed spectrophotometrically based on specific absorption coefficients. The absorbances were determined at wave lengths of 440.5nm, 662nm, and 646nm, with a PG Instruments UV–VIS spectrophotometer using UV WIN 5.05 software. The amount of these Pigments was calculated according to the equation: pigments (mg)=4.69 A_{440.5}–0.2679(chlorophyll a+ chlorophyll b). Synthesis pigment values were expressed in mg/g dry mass [Butnariu *et al.*, 2006].

Table 1. Overall mean values (± S.E.) of *L. perennis* seedlings grown under different concentrations extract

Characters	Control	Concentration extract (µg/g)		
		(III)	(II)	(I)
Germination (%)	99.2±0.01	98.1±0.02	97.5±0.07	97.3±0.03
Root length (cm)	3.35±0.02	3.23±0.07	2.31±0.05	1.89±0.03
Shoot length (cm)	6.22±0.01	5.32±0.03	5.23±0.02	4.53±0.02
Shoot/root ratio	1.86±0.5	1.60±0.42	2.26±0.04	2.39±0.66
Mean values (± S.E.)				

The presence of the sorgoleone and dihydrosorgoleone extract in the culture medium indicates a negative effect on roots growth. This suggests a prevention mechanism in order to reduce the alkaloids translocation in the root. The analysis of the photosynthetic pigments content at three concentrations of extract with allelopathic potential, showed similar values between photosynthetic pigments chlorophyll a (662–663) and chlorophyll b (616–617) and higher values for receptors from AIV at the seedlings treated with *S. halepense* extracts in the ratio 2:1 and near UV at seedlings treated with *S. halepense* extracts in the ratio 1:1 (Table 2).

Table 2. The variation of the average content of photosynthetic pigments in *L. perennis* seedlings

No.	Concentration extract (µg/g)	Wavelengths (nm)				
		335	431–432	453–455	616–617	662–663
1	Control	3.01	2.32	1.50	0.24	1.30
2	(I)	5.82	2.53	1.72	0.31	1.39
3	(II)	4.18	3.47	1.70	0.28	1.32
4	(III)	3.87	2.61	1.63	0.25	1.25

It can be noticed a relatively similar content of chlorophyll photosynthetic pigments (chlorophyll a and b), but a high content of photoreceptors photosynthetic pigments in the AIV at the seedlings treated with *S. halepense* extracts in the ratio 2:1 compared with the seedlings treated with *S. halepense* extracts in the ratio 1:1. By extrapolation, it can be considered that the genetic resistance of the *L. perennis* seedlings at *S. halepense* extract, which is more diluted, is manifested phenotypically by balanced photosynthetic pigments, which ensure a maximum photosynthetic efficiency and good resistance at the attack of the compounds with allelopathic potential. At *L. perennis* seedlings, it can be noticed a strong biosynthesis of the photoreceptors pigments in AIV for *S. halepense* extract in the ratio 1:1 and near UV for the extract where the ratio was 2:1. These pigments are known in literature for their physiological role of protection against the physical agents such as blue and ultraviolet radiations, and biological agents as fitoalexines. [Acciaresi *et al.*, 2010; Butnariu *et al.*, 2011, 2012.]. This behaviour can be considered as a compensation reaction in the metabolic, biochemical and physiological activity of *L. perennis* species, which ensures an optimal photosynthesis that is necessary to the plant development and the cold resistance increase [McDonald *et al.*, 2004; Bostan *et al.*, 2012, Butnariu *et al.*, 2011].

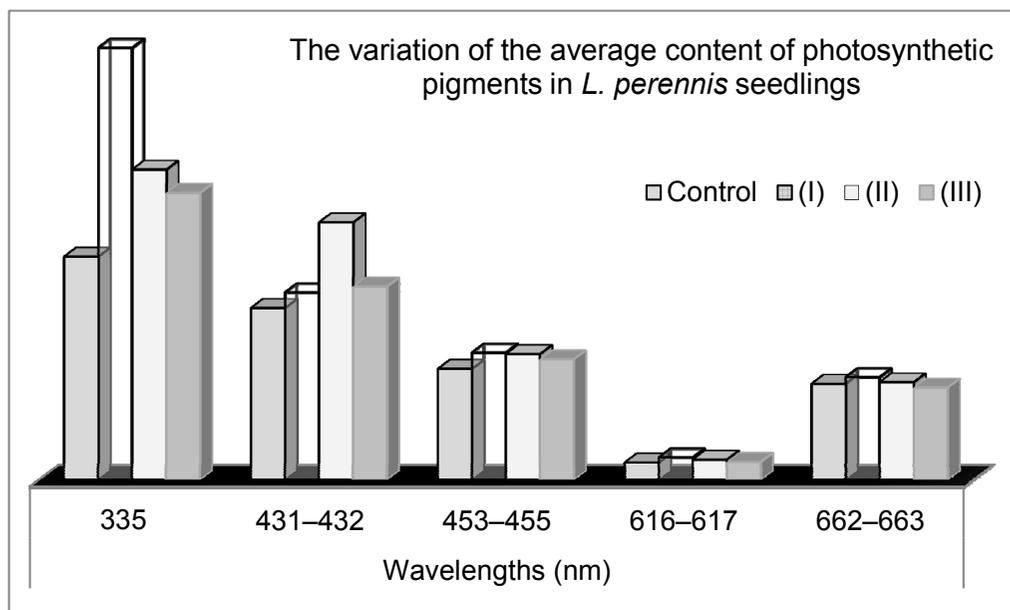


Figure 1. The variation of the average content of photosynthetic pigments in *L. perennis* seedlings

The method allowed the optical absorbance reading in the visible range characteristic for chlorophylls and carotenoids photosynthetic pigments, and other photoreceptors with maximum absorption in the near UV, characteristic of flavonoids and isoflavonoids pigments, which are important in the fitoalexine biosynthesis, endogenous substances with protective role against the biological stress.

Conclusion

The insignificant toxicity effects of the allelochemicals from *S. halepense* for *L. perennis* seed germination, suggested that seeds use their own reserves during the germination process.

On the other hand, the treatment with *S halepense* extract had a significant negative influence on the seedlings growth. The average content of photosynthetic pigments from *L. perennis* and the content pigments from the control samples are characterized by an intense biosynthesis of photoreceptors in the AIV the near UV, similar at *S. halepense* seedlings. The effect of sorgoleone and dihydrosorgoleone resulted in a decrease of the photosynthetic pigments content at *L. perennis* seedlings, treated with sorgoleone and dihydrosorgoleone initially extract and an increase of the photoreceptors content in the near UV in the other two concentrations extracts used in the bioassay. The foliar pigment content is higher at *L. perennis* seedlings considered as control, but lower at *L. perennis* seedlings treated with the extract with allelopathic potential; these can become susceptible to the attack of mildew, influencing the photosynthesis during the development. Understanding and controlling of such phenomena will create the possibility to use the allelopathic compounds as growth regulators or natural pesticides.

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