RESEARCH ARTICLE

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Allelopathic tolerance in white lupine (*Lupinus albus* L.) accessions to *Sorghum halepense* extracts

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ABSTRACT

Modern farming requires a better understanding of the response of species, and in the particular experiment, of accessions, to the influence of weeds in order to develop agricultural systems that are more dependent on ecological interactions and less dependent on the use of pesticides. The present lab study aimed to estimate the tolerance of white lupine accessions to *Sorghum halepense* extracts on the seed germination and initial growth of the crop. Studied factors were: white lupine accessions – ten levels (Lp01, Lp04, Lp06, Lp10, Lp21, Lp23, Lp25, Lp27, Lp28 Lp29), aqueous extracts – two levels (shoot and root biomass of S. halepense) and concentrations of the extracts – four levels (1.0%, 5.0%, 10.0% and distilled water as control). The results showed a different tolerance of the ten accessions to allelopathic action of the aqueous extracts. Increasing concentrations of the extracts inhibited seed germination (by 5.0 to 10% in the different accessions), elongation and accumulation of primary germ (2.4 to 71.2% and 3.0 to 56.2%, respectively. The GGEbiplot analysis, representing the summary effect of the action of weed extracts on the germination and growth parameters in different concentrations, determined Lp 01, Lp 04 and Lp 10 as tolerant. Sensitivity exhibited Lp 21, Lp 25 and Lp 23. The correlation analysis of data showed that accessions whose seeds had a higher 1000 seeds mass, higher seed vigor index and protein content were less affected by the inhibitory effect of *S. halepense* extracts, respectively, they exhibited a higher tolerance.

Key words: allelopathy, lupine, tolerance, *Sorghum halepense*

Introduction

One of the main problems in modern agriculture is weed control. Only soil cultivation cannot control weeds effectively, and in some cases could increase their density as it increases the number of weed rhizomes. It is even more complicated to manage weeds in natural and urban areas where environmental and human health risks can completely hinder the use of chemical herbicides (Thahir & Ghafoor, 2011).

One of the most widespread and harmful weeds is *Sorghum halepense* (L.) Pers. (Holm et al., 1991; Vasilakoglou et al., 2005; Hristoskov, 2013). A number of researchers report competition regarding water and nutrients between *S. halepense* and crop species when they have grown together. In some cases, the reduction in growth and yields of crops is higher than expected as a result of competition for water and nutrients. This fact is explained by the presence of allopathic compounds that have a considerable additional effect on the growth and yields of agronomic crops such as maize (Kalinova et al., 2012), cotton (Uludag et al., 2007), potatoes (Hristoskov, 2013) and others. Several allelopathic phenolic compounds and terpenoids have been identified in different species of genus *Sorghum* (Einhellig & Souza, 1992; Duke et al., 2000; Vasilakoglou et al., 2005). Czarnota et al. (2001), working with *Sorghum* species, established the presence of an allelopathic compound, known as sorgoleone {2-hydroxy-5-methoxy-3-[(8'*Z*,11'*Z*)- tadecatriene]-*p*-benzoquinone}, in extracts from the roots. Also, *Sorghum* species, including *S. halepense*, release cyanogenic conglicosides (dhurrin and taxiphyllin) and phenolic acids (*p*-hydroxybenzoic acid and *p*-coumaric acid) that suppress plant growth (Sene et al., 2001).

According to a number of researchers (Rice, 1984; Cheema et al., 1997; Liebman & David, 2006), allelopathy is a natural and ecological approach which is useful and applicable in the weed manage. Studies by some authors (Liebman & David, 2006; Asghari & Tewari, 2007) show that the sensitivity of crops to the allelopathic compounds released from weeds differs between species and genotypes within the species. Cultivars (accessions, populations, genotypes) showing allelopathic tolerance to different weeds were found in crops such as wheat (Cheema et al., 2002), barley (Shahrokhi et al., 2011), maize (Baličević et al., 2014) etc. Considerable variation in the allelopathic potential of crops has been reported in rice (Sitthinoi et al., 2017) and vetch (Georgieva et al., 2018). Knowing the variation in the allelopathic tolerance of cultivars is important in the crop management and could lead to lower production costs as the need of application of herbicides is reduced (Benckiser & Schnell, 2006). Such investigations are particularly important for organic production conditions where the weed control is extremely difficult (Olofsdotter, 1998). Alternatively, the inclusion of allelopathic traits from weeds or wild plants in crop plants by methods of traditional selection or genetic engineering may also improve the biosynthesis and release of allelochemicals. Genetic basis of allelopathy has been demonstrated in rice and wheat, where cultivars with increased allelopathic potential are developed (Ferguson et al., 2003).

The present study aimed to establish the allelopathic effect of *S. halepense* on germination and initial growth parameters of white lupine accessions and to identify such ones which are tolerant to allelopathic substances released by the weed.

Material and methods

The lab experiment was conducted at the Institute of Forage Crops (Pleven, Bulgaria) during 2018 as a multifactorial study based on a completely randomized design.

Collecting and preparing plant material

Seeds of 10 accessions (factor A) belonging to white lupine (*Lupinus albus* L.) species were used: Lp01, Lp04, Lp06, Lp10, Lp21, Lp23, Lp25, Lp27, Lp28 Lp29. Shoot and root biomass (factor B) of *S. halepense* were collected at the phenological stage of flowering of the weed. The plant material was dried to a constant dry weight at 60°C and grounded into fine powder (Chon and Nelson, 2001).

Preparing weed extracts

One hundred grams of the grounded shoot and root biomass of *S. halepense* were cold-extracted in 1 L of distilled water at 24 ± 2^{0}C for 24 hours. The obtained extracts were decanted and filtered with filter paper. The aqueous extracts were brought to final concentrations of respectively 1.0, 5.0 and 10.0% (factor C). Distilled water was used as a control. To each of the extracts was added 1 g/L thymol as a preservative.

Biological techniques

Twenty seeds of each accession were placed in Petri dishes (9 cm diameter) on filter paper. Six ml of the aqueous extracts were pipetted into each Petri dish. Each variant was in four replications. Petri dishes were placed in a thermostat at 22 ° C ± 2 ° C for seven days. The following parameters were recorded: seed germination (%), length of primary germ (root + stem) (cm), fresh weight of primary germ (root + stem) (g), inhibition (%). The percentage of germination was calculating using the formula: % germination = (germinated seeds/total number of seeds) × 100. The inhibition percentage was determined using the formula of Chung et al. (2003): % inhibition = [(control-extracts)/control] × 100, and the seedling vigour index (SVI, %) – according to Abdul–Baki & Anderson (1973): *SVI* = germ length (cm) × germination.

Results and discussion

Germination bioassay

Seed germination process, as well as growth processes in the plant organism, is influenced by the presence of allelochemicals in the environment due to the fact that these substances are related to basic physiological processes such as photosynthesis, cell division, protein synthesis, enzymatic and metabolic activities (Lin et al., 2004). The results in the present experiment showed that in four (Lp 06, Lp23, Lp 25, Lp 29) of the lupine accessions, the allelopathic effect of S. halepense extracts on seed germination was not found, and in six of them (Lp 01, Lp 04, Lp 10, Lp 21, Lp 27, Lp 28), it was relatively low, with a variation in the inhibition percentage of 5.0 to 10.0 (Figure 1).

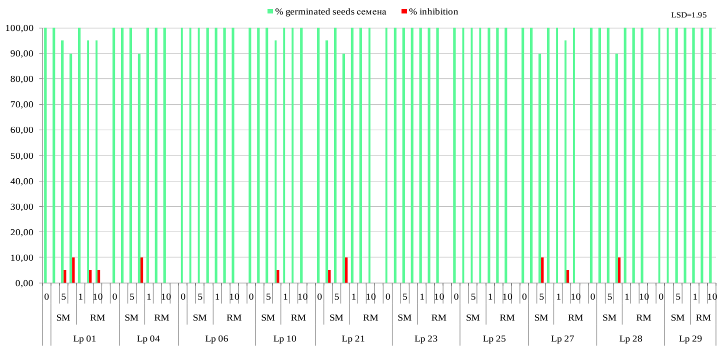

Figure 1. *Effect of water extracts of Sorghum halepense on seed germination in white lupine accessions.* ***Legend*:** SM – shoot mass, RM – root mass.

The effect was observed at the higher concentrations (5.0 и 10.0%) of extracts of root and shoot weed biomass. These data are in agreement with results obtained in our previous studies in other leguminous crops conducted under the same methodological framework. For example, in various cultivars of *Pisum sativum*, the suppressive action of water extracts was in the limits of 8.0 ÷ 31.2% (Georgieva et al., 2016), and in *Vicia sativa* and *Vicia villosa* species – between 7.3 ÷

21.9% (Georgieva et al., 2018). A similar dependence was observed by Treber et al. (2015) in seeds in soybean cultivars showing different susceptibility in the germination process and inhibition values of *Polygonum lapathifolium* L. extracts (at concentrations of 1, 5 and 10%) between 18 and 38%.

Interest in the present experiment represented almost half of the white lupine accessions, which exhibited tolerance and were not affected by the action of weed extracts. Stef et al. (2013) reported similar results and lack of inhibitory effect on seed germination in other lupine species (*Lupinus perennis* L.) after testing with extracts of *S. halepense*. The authors suggested that in this case, the seeds use their own reserves in the germination process. A possible reason could be their different protein content. The analysis of data showed a correlation with an average negative value (r = -0.395) between the parameters. According to Filipovich (1985), one of the characteristic physical properties of proteins was their ability to adsorb molecules of organic compounds and ions on their surface. Therefore, under the experimental conditions, accessions with higher protein content in the grain were less affected by the depressing effect of the weed extracts at germination. The last dependence was established in our previous researches (Georgieva et al., 2016; Georgieva et al., 2018).

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Germ elongation

It has been found that allelopathic substances isolated from different plant parts have a stimulating or inhibitory effect on the acceptor plants and their intensity depends on the concentration (Sikora & Berenji, 2008; Hossain & Alam, 2010). In general, the data presented in Table 1 showed that germ elongation in the majority of the studied accessions was negatively influenced by the increase in the concentration of *S. halepens* aqueous extracts, with mean values of 6.5, 32.8 and 51.1% for respectively 1, 5 and 10%. The suppressing action of the concentrations was statistically significant with the exception of the 1% concentration of the shoot weed biomass in accessions Lp 10, Lp 27 and Lp 28. A stimulating effect (statistically significant) was observed at the lowest concentrations of root mass (in Lp 04, Lp 06, Lp 10, Lp 21, Lp 23 and Lp 28) and shoot mass (in Lp 04). This effect was particularly pronounced in Lp 28, where the germ growth under the influence of the 1% extract of shoot weed mass was 26.5% compared to the control. For the other accessions, the stimulation averaged 13.0%.

On the other hand, the germ length of the lupine plants was influenced to a greater extent by the aqueous extracts of shoot mass (decrease by 36.8% on average, compared to the control) than by the root mass (29% on average).

Table 1. *Influence of Sorghum halepense extracts on length and fresh biomass accumulation in germs of Lupinus albus accessions.*

Accessions	Plant part of *S. halepense*	Concentration %	Germ length, cm	%, K	Germ weight g	%, K
Lp 01		0 (Control)	22.71	(-11.7)	0.185	(-17.8)
	Shoot mass	1.0	21.43		0.173	
		5.0	20.21		0.156	
		10.0	18.16		0.149	
	Root mass	1.0	20.99		0.138	
		5.0	20.53		0.130	
		10.0	19.04		0.167	
Lp 04		0	8.21	(-25.7)	0.206	(-10.3)
	Shoot mass	1.0	8.83		0.209	
		5.0	4.77		0.164	
		10.0	2.91		0.138	
	Root mass	1.0	9.69		0.214	
		5.0	6.01		0.206	
		10.0	4.40		0.176	
Lp 06		0	8.55	(-30.4)	0.230	(-20.6)
	Shoot mass	1.0	7.26		0.197	
		5.0	5.34		0.192	
		10.0	3.58		0.154	
	Root mass	1.0	9.39		0.212	
		5.0	6.00		0.185	
		10.0	4.12		0.154	
Lp 10		0	7.63	(-34.0)	0.181	(-12.7)
	Shoot mass	1.0	7.44		0.158	
		5.0	5.07		0.187	
		10.0	2.24		0.103	
	Root mass	1.0	8.40		0.220	
		5.0	3.46		0.111	
		10.0	3.62		0.170	
Lp 21		0	9.86	(-48.7)	0.307	(-44.1)
	Shoot mass	1.0	6.24		0.189	
		5.0	5.01		0.190	
		10.0	2.95		0.134	
	Root mass	1.0	6.54		0.179	
		5.0	5.58		0.169	
		10.0	4.05		0.169	
Lp 23		0	8.91	(-38.8)	0.246	(-27.5)
	Shoot mass	1.0	5.32		0.188	
		5.0	5.34		0.175	
		10.0	3.19		0.139	
	Root mass	1.0	9.95		0.251	
		5.0	4.78		0.160	
		10.0	4.13		0.156	
Lp 25		0	11.06	(-46.8)	0.23	(-23.4)
	Shoot mass	1.0	8.04		0.22	
		5.0	6.40		0.19	
		10.0	3.19		0.12	
	Root mass	1.0	8.58		0.20	
		5.0	4.95		0.17	
		10.0	4.19		0.17	
Lp 27		0	8.28	(-31.0)	0.214	(-14.5)
	Shoot mass	1.0	8.02		0.214	
		5.0	6.33		0.196	
		10.0	3.19		0.162	
	Root mass	1.0	7.73		0.195	
		5.0	5.18		0.167	
		10.0	3.86		0.165	

		0	9.14		0.257	
		1.0	9.10		0.209	
	Shoot mass	5.0	5.83		0.169	
Lp 28		10.0	2.83	(-26.8)	0.121	(-26.7)
		1.0	11.57		0.269	
	Root mass	5.0	6.49		0.189	
		10.0	4.32		0.175	
		0	10.26		0.252	
		1.0	9.53		0.244	
	Shoot mass	5.0	5.72		0.233	
Lp 29		10.0	3.74	(-35.4)	0.157	(-17.3)
		1.0	8.71		0.223	
	Root mass	5.0	7.54		0.215	
		10.0	4.56		0.177	
LSD at the 0.05 probability level A×B×C			0.38		0.015	

When comparing extracts from different plant parts, many researchers have found that leaves and stems usually have the highest allelopathic potential between the plant parts, which is due to a higher content of allelochemicals (Tawana & Turk, 2003; Ravlić et al., 2012, Šćepanović et al., 2007). Fractionation-based biological analyzes in *S. halepense* (Thahir & Ghafoor, 2011) have proved that leaves are more effective source of allelochemicals and can be used as a bioherbicide. Variable results, however, were observed by the authors subsequently under laboratory conditions. Extracts of shoot parts of the weed have reduced the germ length in *Avena fatua* L., in *Lolium temulentum* Gaud. the results have shown the opposite - a stronger effect of root mass extracts, and in *Lathyrus sativa* L. - the action of the two extracts has been absolutely equivalent. Obviously, the inhibiting effect is also determined by the recipient plant.

The ten accessions, subjects of the study, differed in their response to S. halepense extracts. The averaged values in different accessions regarding germ elongation showed the lowest depressing effect (on average by 11.76 and 25.7%) of the extracts, respectively a high tolerance, in Lp 01 and Lp04. In contrast, Lp 21, Lp 25 and Lp 23 exhibited susceptibility with values of the inhibitory effect of 48.7, 46.8 and 38.8%. In a similar experiment, Treber et al. (2015) found a different tolerance in soybean varieties to *Polygonum lapathifolium* L. aqueous extracts as the suppressive influence on the germ elongation varied from 2.8 to 31.5%. Shahrokhi et al. (2011) reported a different level of susceptibility to *Amaranthus retroflexus* extracts in five barley cultivars in relation to all tested parameters (rate of germination, germ length, fresh and dry weight of the germs). The authors indicated cultivars Reyhan and Kavir as more tolerant than others to *A. retroflexus* allelochemicals, and that the planting of these cultivars could reduce the weed damage. In another experiment, Baličević et al. (2014) studied the reaction of maize hybrids to Convolvulus arvensis. The results showed a strong variation in the inhibitory effect regarding the growth of shoot (from 3.8 to 50.0%) and root (from 1.7 to 92.0%), as hybrid Bc 574 was distinguished with a higher tolerance to the high concentrations of the extracts. Genotypic variation in allelopathic tolerance was also observed in other crops as wheat and rice (Bashir et al., 2012).

Germ weight

As a whole, the trends, established in the germ elongation in white lupine accessions under the influence of *S. halepense* extracts, were also observed in terms of biomass accumulation (Table 1). With the concentration increase of 1 to 5 and 10%, the negative effect on the germ weight also increased: the decrease was by 15.1, 23.4, 33.8% respectively, and was mathematically significant, except for the lowest concentration of the shoot weed biomass in Lp 01, Lp 25, Lp 27 and Lp 29. Some stimulation (on average by 13.0%), which, however, was not statistically significant, was observed under the influence of the lowest concentration of root (in Lp 04, Lp 10, Lp 23, Lp 28) and shoot biomass (in Lp 04).

Similar to the dependence in germ elongation, the averaged values regarding germ weight determined a relatively stronger inhibitory effect of the concentrations of shoot weed biomass (23.2%) compared to the root mass (19.7%).

Accessions, whose capacity to accumulate fresh mass was less affected by the suppressive action of the extracts were Lp 04 and Lp 10 (a decrease of 10.3 and 12.7%, respectively, compared to the control variants), unlike Lp 21 and Lp 23, in which the decrease was 44.1 and 27.5%, respectively.

By comparing the germ quantitative parameters after treatment with aqueous extracts of *S. halepense*, it can be concluded that the changes in germ length in white lupine were more pronounced than those in the biomass accumulation. This relationship was confirmed by other authors (Sitthinoi et al., 2017) who reported that the primary germ lenght is a more sensitive variable than its weight.

Reduced growth was determined by the inhibitory effect of the extract on cell division in plant tissues by the impediment of the microtubule arrangement in cell division process (Singh et al., 2002) or by inhibiting the expansion of the root and shoot cells (Zimdahl, 1999). Rice (1974) established that the allelopathic effects on the germination and plant growth were could occur by different mechanisms, including reduction in mitotic activity in the hypocotyl and root, reduction of the ion exchange rate, suppression of hormonal activity, , inhibition of photosynthesis, respiration and protein synthesis, reduced permeability of cell membranes etc.

The variance analysis showed a dominant impact of the factor "accession" on seed germination and germ elongation in the white lupine (62.5 and 75.9% of the total variation, respectively) (Table 2). It was determined by the different reaction of the accessions to the changing environmental conditions. Regarding germ weight, the influence of weed extract concentration was most pronounced - 35.9% of the total variation. The impact of extract type in all studied parameters was the weakest (from 0.3 to 2.1%).

The interactions of the accession with extract concentration (A × C) and the type of extract with its concentration (B × C) were statistically significant. There was no significant interaction between accession and extract type (A × B) for all parameters considered, and among the three experimental factors (A × B × C) in terms of seed germination and germ weight.

Figure 2 presents the summary effect of the action of aqueous weed extracts on the seed germination and initial plant development in white lupine accessions. GGEbiplot method and GenStat software product are used to evaluate the data. The inhibitory effect of the various concentrations is ranked as Env 1 (1.0% concentration of *S. halepense*) < Env 2 (5.0% concentration) < Env 3 (10.0% concentration). As an "ideal" accession, is determined this one, which is least affected by the depressing influence of aqueous extracts on the parameters studied (germination, growth and accumulation of biomass). The center of the coordinate system shows the location of such an ideal genotype and, respectively, how much closer to the center is a given genotype, the higher are the values of observed parameters. In the experimental conditions, Lp01, Lp04, and Lp10 that were positioned closest to the center, on the right side of the graph, were characterized by maximum values of germination and growth. Therefore, *S. halepense* extracts had a minimal allelopathic effect on them, i. e. the accessions exhibited a high tolerance. In contrast, accessions Lp21, Lp28, Lp25 and Lp23 were located at the most distant points of the center, on the left side of the coordinate system, suggesting a high inhibitory effect and sensitivity exhibition. Lp06, Lp29 and Lp27 occupied an intermediate position.

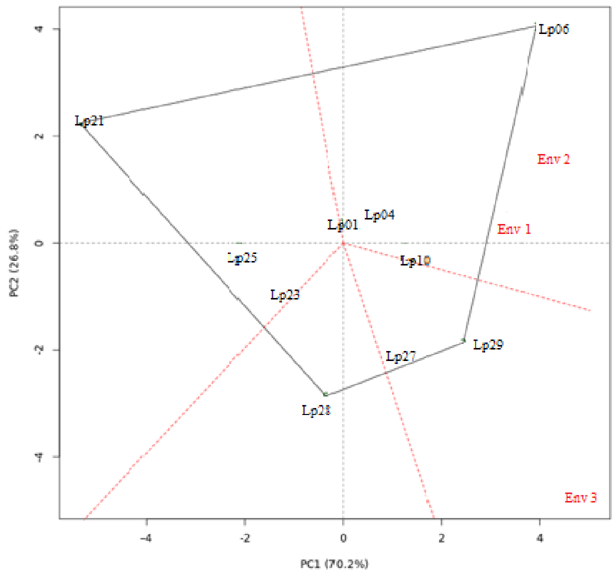

Figure 2. *GGEbiplot of allelopatic tolerance of white lupine accessions.* ***Legend:*** Env1 (1.0% concentration of water extract of *S. halepense*), Env2 (5.0%), Env1 (10.0%).

There is still a lack of sufficient researches in the scientific literature to explain the causes of tolerance or sensitivity of genotypes to the allopathic effect of weed species. In general, the germination of white lupine was less inhibited (from 5.0 to 10.0% in the various accessions) compared to the growth parameters: elongation and accumulation of biomass the primary germ, with 2.4 to 71.2% and 3.0 to 56.2%, respectively. As in our previous studies, dependencies with different characteristics (morphological, biochemical) of seeds were sought. The analysis of data showed a negative correlation between the seed vigor index (SVI) of tested accessions and the inhibitory effect of *S. halepense* on seed germination (r = - 0.412), germ length and weight (-0.626 and -0.590, respectively). There was also a negative relationship between the 1000 seeds mass and the stated parameters with values of the correlation coefficient of 0.705, -0.706 and - 0.616, respectively. This dependence confirmed the relationship between seed mass and stress tolerance observed by Liebman & David (2006), namely that small seeds were more susceptible to the action of phytotoxins. The authors reached to this conclusion by comparing the germination and growth of the shoots in 44 crops and 18 weed species (whose mass per 100 seeds ranged from 20 to 26.25 mg) after treatment with extracts of red clover biomass. The variation among species with respect to seed mass, according to Liebman & David (2006), was related to different sensitivity to phytotoxins for several reasons.

Table 2. Analysis of variance for seed germination and germ growth in white lupine accessions.

Causes of variation	Degrees of freedom	Sum of squares	Mean square	Influence of factors	Sum of squares	Mean square	Influence of factors	Sum of squares	Mean square	Influence of factors
Indicators	Germination, %				Germ length, cm			Germ weight, cm		
Total	319	6080.0		100.0	7628.67		100.0	0.768954		100.0
Factor A-accession	9	3800.0	15.8333	62.5*	5793.65	643.739	75.9*	0.0920574	0.0102286	12.0*
Factor B - type of extract	1	125.0	125.0	2.1*	21.6008	21.6008	0.3*	0.00273195	0.00273195	0.4^{ns}
Factor C - concentration of the extracts	3	280.0	93.3333	4.6*	1444.01	481.337	18.9*	0.275997	0.0919992	35.9*
Interaction										
A×B	9	125.0	13.8889	2.1^{ns}	18.0444	2.00494	0.2^{ns}	0.0138164	0.00153515	1.8^{ns}
A×C	27	770.0	28.5185	12.7*	128.89	4.77371	1.7*	0.0943814	0.00349561	12.3*
B×C	3	215.0	71.6667	3.5*	20.8124	6.93748	0.3*	0.0227035	0.00756784	3.0*
A×B×C	27	435.0	16.1111	7.2^{ns}	59.642	2.20896	0.8*	0.03681	0.00136333	4.8^{ns}
Error	240	330.0	36.6667	5.4	142.02	0.59175	1.9	0.230456	0.000960232	30.0

Legend: LSD at 0.05 probability level.

First, in comparison with large seeds, small seeds had a "greater quantity of roots per unit of root mass" (Seibert & Pearce, 1993). In this way, they had a larger quantity of absorbent surface through which phytotoxins can penetrate. Second, small seeds usually had fewer reserves to maintain respiration during stress periods. Thus, they could undergo a disproportionate reduction in growth in early stages of development, which limited their access to resources and their competitiveness subsequently (Westoby et al., 2002). Finally, it may be assumed that differences in seed reserves may also contribute to differences among species in the ability to detoxify allelochemicals, although this hypothesis has not been tested (Liebman & David, 2006). Regardless of the mechanisms that determine tolerance, a better understanding of the response of species, and in the present experiment - of accessions, to the influence of weeds is needed in order to develop farming systems which are more dependent on ecological interactions and less dependent on the use of pesticides.

Conclusions

The results of the conducted experiment showed a different tolerance of ten accessions of white lupine to the allelopathic action of aqueous extracts of root and shoot mass of *Sorghum halepense*. Increasing concentrations (1, 5 and 10%) of the extracts inhibited seed germination (by 5.0 to 10% in the different accessions), elongation and accumulation of primary germ (2.4 to 71.2% and 3.0 to 56.2%, respectively). A significant stimulating effect was found after testing with the lowest concentration in some of the accessions, especially strong pronounced in Lp 28.

The GGEbiplot analysis, representing the summary effect of the action of weed extracts on the germination and growth parameters of the accessions in different concentrations, determined Lp 01, Lp 04 and Lp 10 as tolerant. Sensitivity exhibited Lp 21, Lp 25 and Lp 23.

The correlation analysis of data showed that accessions whose seeds had a higher 1000 seeds mass, higher seed vigor index and protein content were less affected by the inhibitory effect of *S. halepense* extracts, respectively, they exhibited a higher tolerance.

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