

## Allelopathic tolerance in broad bean (*Vicia faba* L.) accessions to *Sorghum halepense* extracts

Natalia Georgieva

*Institute of Forage Crops, Department of Forage Production and Livestocks, 5800 Pleven, Bulgaria*  
E-mail: [imnatalia@abv.bg](mailto:imnatalia@abv.bg)

### Abstract

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Due to the growing importance of organic farming and environmental protection, more and more attention should be paid to ecological interactions between species, and less to the use of pesticide products. The present study aimed to determine the allelopathic tolerance of ten *Vicia faba* accessions (Fb 1896, Fb 1903, Fb1929, Fb 2481, Fb 2486, Fb 3270, BGE 002106, BGE 029055, BGE 032012, BGE 046721) to extracts of *Sorghum halepense*. Aqueous extracts (in concentrations of 1.0, 5.0 and 10.0%) from shoot and root weed biomasses were compared with distilled water used as control. Germination and initial growth parameters, including the percentage of seed germination, length and weight of the germ, percentage of inhibition, tolerance index and seedling vigour index were reported. Analysis of variance showed significant influence of the factors “accession” and “concentration of the extracts” of *S. halepense* on the seed germination and initial growth parameters, and the influence of the type of weed extract was significant only for seed germination. The inhibitory effect of the extracts ranged from 7.7 to 100% in terms of seed germination, and it was in limits  $3.6 \div 100\%$  and  $7.1 \div 100\%$  regarding germ length and weight, respectively. As a whole, as the extract concentration increased, the depressive effect on the studied parameters enhanced. The summary effect of the allelopathic action of *Sorghum halepense* on all parameters studied (represented by GGE-biplot analysis) determined accessions Fb 3270, Fb 2481 and Fb 2486 as exhibiting higher tolerance, unlike Fb 1929, BGE 032012, BGE 002106, Fb 1903 and Fb 1896 which were more sensitive.

*Keywords:* tolerance; *Vicia faba*; allelopathy; *Sorghum halepense*

### Introduction

Although allelopathy has been known and used in farming since ancient times, its usage in modern farming is very limited. Allelopathy plays an important role in the study of suitable agricultural systems as well as in weed control (Cheng & Cheng, 2015). The weeds seriously compete with cultivated species because of their high adaptiveness to the environment. It is considered that they are one of the main factors reducing the yield. In addition to the competition, weeds decrease crop growth and yield by releasing allelopathic substances (Khanh et al., 2006). *Sorghum halepense* (L.) Pers. is one of the most harmful weeds in the world and its allelopathic potential is well known (Thahir & Ghafoor, 2011). The major allelochemicals it contains are phenolic compounds, chlorogenic acid, *p*-hydroxybenzaldehyde, *p*-coumaric acid (Zohaib et al., 2016), “sorgoleone” and “dihy-

drosorgoleone” (Movahedpour et al., 2010; Butnariu, 2012).

Allelopathy also has the potential to be applied in breeding programs for biological control of weeds by identifying or developing varieties with less sensitivity to allelopathic effects (Ebana et al., 2001). In this respect, differences between genotypes and varieties have found in crops such as wheat (Cheema et al., 2002), maize (Baličević et al., 2014), sunflower (Alsaadawi et al., 2012). In our previous studies, cultivars and accessions with a higher tolerance to sorghum water extracts were determined in pea (Georgieva & Nikolova, 2016), vetch (Georgieva et al., 2018) and lupine (Georgieva, 2019). According to some researchers (An et al. 1998; Kruse et al. 2000), allelopathic tolerance of crops and varieties is a promising complement to the weed control strategy. As the importance of organic farming and environmental protection increases, more and more attention will be paid to allelopathic researches. Obviously, allelopathy re-

quires further studies for wider application in modern agricultural production (Cheng & Cheng, 2015).

The purpose of this study was to determine the effect of *Sorghum halepense* extracts on the initial growth of broad bean accessions and to identify those that are tolerant to allelopathic compounds released by the weed.

## Material and Methods

### Treatments

The lab experiment was conducted at the Institute of Forage Crops (Pleven, Bulgaria) as three factorial study with four replicates in 2019. The first factor (A) included ten broad bean (*Vicia faba* L.) accessions (Fb 1896, Fb 1903, Fb1929, Fb 2481, Fb 2486, Fb 3270, BGE 002106, BGE 029055, BGE 032012, BGE 046721). The second factor (B) was root and shoot biomass of *S. halepense*, and the third factor (C) was *S. halepense* extract in three concentrations (1.0, 5.0 and 10.0%) and distilled water used as control.

### Sampling and plant extract preparation

Root and shoot biomass of *S. halepense* was collected at the flowering stage, dried to a constant dry weight at 60°C and grounded (Chon & Nelson, 2001). For extract preparation, 100 g of grounded plant material was suspended in 1 L of distilled water at 24 ± 1°C for 24 hours. The obtained extracts were decanted, filtered and brought to final concentrations of 1.0, 5.0 and 10.0%, respectively. To each of the extracts was added 1 g/L thymol as a preservative.

### Bioassay tests

Twenty broad bean seeds of each accession were placed in Petri dishes (14 cm diameter) with filter paper inside, and 8 ml extract was pipetted into each Petri dish. The seeds were incubated at 22°C ± 1°C. Seed germination percentage, length of primary germ (root + stem) (cm), fresh weight of primary germ (root + stem) (g), inhibition (%) were recorded after 7 days incubation. The percentage of germination was calculated using the formula: % germination = (germinated seeds/total number of seeds) × 100. The inhibition percentage (I, %) 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. Tolerance index (TI) was determined by the adapted formula of Tahseen and Jagannath (2015):

$$TI = \frac{L_{Set}}{L_{Sct}} \times 100,$$

where Lset – length of seedlings in each experimental treatment, mm

LSet – length of seedlings in the control treatment, mm.

### Data analysis

The data were analyzed using the GGEbiplot method by PB-STAT 1.2 software, and the software product Statgraphics Plus for Windows Ver. 2.1.

## Results and Discussion

### Analysis of variance

The results revealed that the evaluated traits of seeds are related to *V. faba* accessions, the concentrations applied and the type of extract tested (Table 1). The factor “accession” had significant and determining importance for seed germination and germ growth, 46.8 and 59.2% of the total variation, respectively, while regarding germ weight, the influence of the weed extract concentration was the strongest – 36.5% of the total variation. For all the parameters studied, the effect of the type of extract was the weakest, and its action was significant only in seed germination. The interaction of the factors (A × B, A × C, B × C, A × B × C) was statistically significant for all the parameters considered, with the highest effect being the interaction of the accessions with the concentration of *S. halepense* extract (A × C).

### Seed germination

Germination is undoubtedly one of the most important stages in plant development and is strongly influenced by the action of allelochemical substances (Bogatek et al., 2006). Plant reactions to allelochemicals are species-specific and concentration dependent (An et al., 2008). Usually, high concentrations of allelochemicals are inhibitory, and low concentrations are stimulating, a phenomenon known as hormesis (Hadacek et al., 2011). Aqueous extracts of *S. halepense* biomass showed an inhibitory effect ranging from 7.7 to 100% on the seed germination of *V. faba* accessions (Figure 1). Exception were found for Fb 3270 (for all concentrations), Fb 2481, BGE 002106 and BGE 029055 (at 1% concentration of extracts), in which such negative effect was not observed. In general, with increasing weed biomass concentration from 1% to 5 and 10%, the percentage of germinated seeds decreased disproportionately by 25.1, 39.1, and 57.9% compared to the control variant. According to Chaniago et al. (2006), increasing weed concentrations result in a strong reduction in plant respiration at the germination stage, which in turn causes a decrease in the germination percentage. Previous studies have shown that the key enzyme λ-phosphorylase, which participates in seed germination, can be inhibited by chlorogenic acid (Rice, 1984; Einhellig, 1995) contained in *S. halepense*.

Depending on the average value of the inhibition percentage for the different concentrations, the tested accessions can divide into the following four groups (Valcheva et al., 2018): (1) I<25% = high tolerant – Fb 3270, Fb 2481 and BGE 029055; (2) I<50% = tolerant – Fb 1896, Fb 2486, BGE 046721; (3) I<75% = sensitive – Fb 1903, BGE 002106, BGE 032012 and

**Table 1. Analysis of variance for seed germination and germ growth in *Vicia faba* accessions**

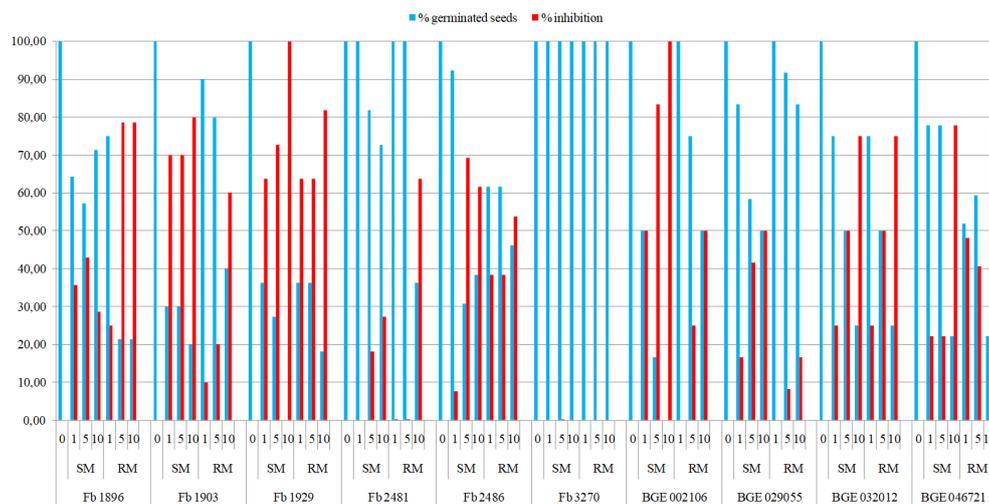
Causes of variation	Degrees of freedom	Sum of squares	Mean square	Influence of factors	Sum of squares	Mean square	Influence of factors	Sum of squares	Mean square	Influence of factors
Parameters	Germination, %			Germ length, cm			Germ weight, cm			
Total	319	269787.0		100.0	5830.32		100.0	10.3164		100.0*
Factor A- accession	9	126167.0	14018.5	46.8*	3449.05	383.23	59.2*	3.6831	0.4092	35.7*
Factor B – type of extract	1	3082.0	3082.0	1.1*	7.41	7.41	0.1 <sup>ns</sup>	0.0072	0.0073	0.1 <sup>ns</sup>
Factor C – concentration of the extracts	4	61394.2	20464.7	22.8*	1576.82	525.61	27.0*	3.7697	1.2566	36.5*
A×B	9	13367.3	1485.3	5.0*	100.16	11.13	1.7*	0.3034	0.0337	2.9*
A×C	27	34504.9	1278.0	12.8*	292.42	10.83	5.0*	0.6652	0.0246	6.4*
B×C	3	2028.4	6762.2	0.8*	44.38	14.79	0.8*	0.1288	0.0429	1.2*
A×B×C	27	16321.2	604.5	6.0*	173.02	6.41	3.0*	0.3459	0.0128	3.4*
Error	240	12922.5	53.8	4.8	187.05	0.78	3.2	1.4131	0.0059	13.7*

LSD at 0.05 probability level

(4) I>75%) = high sensitive – Fb 1929. In accordance with the results obtained, a number of researchers reported an inhibitory effect of allelochemicals on plant seed germination (Chon & Nelson, 2010; Aryakia et al., 2015; Cheng & Cheng, 2015). Bagheri et al. (2014) established that leaf extracts (at concentrations of 0, 25, 50, 75 and 100%) of *Chenopodium album* reduced germination (on average by 44%) in five sorghum varieties (Khaleseh, Galboos, Yengabad, Kimia and Spidfid). Among sorghum varieties, Galboos and Spidfid were the most tolerant ones to allelopathic substances of *Ch. album* at the germination stage. Similarly, Balicevic et al. (2014) reported a different sensitivity of maize hybrids to the effects of water extracts (1, 5 and 10% of plant biomass) of *Convolvulus arvensis* L. Compared to the control variant, the suppressive effect on germination in Bc 574 hybrid reached 24.9%, while in OSSK hybrid was 50.7%, which defined the first hybrid as more tolerant.

### Germ elongation

Unfavorable environmental conditions have a strong impact not only on the germination process but also on the overall growth and development of the plants. The plant roots first come into contact with the environment and the growth inhibition is a consequence of that of the root, as it limits the intake of water and minerals, and also because of the direct action of the inhibitors on the cellular metabolism (Vince & Zoltan, 2011). The length of the primary germ (root + stem) of *V. faba* accessions was considerably and negatively affected by the action of the weed extracts, with the suppression effect varying from 3.6 to 100% (Table 2). Compared to the controls, the effect was statistically significant for all tested variants (except for 1% extract of root and shoot mass in Fb 2481) and had mean values of decrease of 25.1, 43.4 and 60.3%, respectively for 1, 5 and 10%. Significantly stimulating effect on germ growth, as well as on



**Fig. 1. Influence of water extracts of *Sorghum halepense* on seed germination in *Vicia faba* accessions**  
SM – shoot mass, RM – root mass

biomass accumulation, manifested 1% extract of shoot biomass in accession BGE 046721. According to Hallak et al. (1999), the reduced growth is due partly to the allelochemical sorgoleone. It reduced the cell numbers in each cell division period, causing damages to the tubulins and resulting in polyploid nuclei. It is also found that sorgoleone, as well as *p*-coumaric acid, contained in *S. halepense*, inhibit H-ATPase activity, which is associated with water and nutrient uptake – a fact found in peas, soybeans (Hejl & Koster, 2004) and maize (Abenavoli et al. 2010). The results of a research of Cheng & Cheng (2015) showed that phenolic acids, also included in the composition of *S. halepense*, can increase the activity of phenylalanine ammonia-lyase and  $\beta$ -glucosidase, while reducing the activity of phenol- $\beta$ -glucose transferase. In this way, they inhibit root growth. All phenolic acids can impair DNA and RNA integrity, which, in turn, negatively affected protein biosynthesis and cell growth (Li et al., 2010).

Comparing the two types of extracts, from the root and shoot mass of *S. halepense*, it could note that there are no essential differences in their action, and the inhibition had average values of 44.7 and 43.1%, respectively.

According to tolerance index values, *V. faba* accessions can classify as high-tolerant (TI > 75%) – Fb 2481, tolerant (TI < 75%) – BGE 046721, Fb 3270, Fb 2486, BGE 029055 and sensitive (TI < 50%) – Fb 1896, Fb 1903, Fb 1929, BGE 002106, BGE 032012. In a similar study with genotypes of *Medicago sativa* and *Lotus corniculatus*, Valcheva et al. (2018) determined alfalfa variety Multifoliolate and birdsfoot trefoil populations LP1 and LP2 as characterized by an increased tolerance to the allelopathic action of *Cuscuta epithimum* aqueous extracts. The initial development of these forage crops was significantly inhibited, with the reduction of growth of 72.9 and 48.1%, and biomass accumulation of 88.9 and 80%, respectively, in alfalfa and birdsfoot trefoil. In a comparative evaluation of five rice genotypes, Mahmood et al. (2013) found a significant variation in their sensitivity to coumarin allelochemical. Although overall coumarin reduced the root length, the number of lateral roots, and the total number of root hairs, inhibition was more pronounced in BS-2000 (described as “coumarin susceptible”) than in BR-41 (“less susceptible”).

### Germ weight

The ten accessions of *V. faba* showed different sensitivity to *S. halepense* extracts regarding biomass accumulation (Table 2). Fb 1929, Fb 1903 and BGE 032012 exhibited greater sensitivity, with reductions in germ weight ranging from 55.4 to 60.0%. A less sensitivity was demonstrated by Fb 3270, Fb 2486 and BGE 046721, with reductions of 17.5 to 26.4%, while the intermediate position was occupied by BGE 029055, BGE 002106, Fb 2481, Fb 1896. The obtained results are in line with those of Siddiqui et al. (2010), who found a differential response of two wheat varie-

ties to the influence of six weed species. The weight reduction of aboveground mass, reported 120 days after field sowing, was 20-75% for Inqalab 91, and 14-32% for Punjab 96.

Similar to the results obtained in germ length, increasing concentrations of weed extract enhanced the inhibitory effect on germ weight by values of 21.9, 38.8 and 58.5%, respectively, while the type of extract (shoot and root mass) had no substantial influence on the parameter considered, with reduction values of 41.6 and 39.7%. In our previous studies, carried out at the same methodological scheme, extracts of aboveground mass (Georgieva, 2019) showed a stronger inhibitory effect in lupine accessions, whereas in pea varieties, the opposite was observed – stronger effect of the root mass extracts (Georgieva & Nikolova, 2016). In a comprehensive laboratory study, Thahir & Ghafoor (2011) found the following relationships: 1) stronger influence of *S. halepense* aboveground mass on germ development in *Avena fatua* L. 2) a stronger action of the root mass of the same weed on the germ development in *Lolium temulentum* Gaud., 3) and an equivalent effect of both extracts in *Lathyrus sativa* L. Therefore, the effect of both types of the extract is also determined by the recipient plant.

### GGE-biplot analysis

Figure 2 presents the summary result of the effect of weed extracts on seed germination and initial development of plants in *V. faba* accessions. The GGE-biplot method and the software product PBSTAT 1.2. were used for data evaluation. The center of the coordinate system indicates the position of the ideal genotype, which is least affected by the depressive effect of Env 1 (1.0% concentration of *S. halepense*), Env 2 (5.0% concentration), and Env 3 (10.0% concentration) (Figure 2). In the present experiment, the GGE-biplot clearly demonstrated genotypic variation in the sensitivity of the different accessions to allelopathic stress of *S. halepense*. Among the ten accessions, a relatively higher tolerance was shown by Fb 3270, Fb 2481 and Fb 2486, which were located closest to the center, on the right side of the graph. On the other hand, in Fb 1929, BGE 032012, BGE 002106, Fb 1903 and Fb 1896 was observed a stronger negative effect of weed extracts, and respectively, a lower tolerance. They were positioned on the left side of the coordinate system, at the farthest points from the center. Accessions BGE 029055 and BGE 046721 occupied an intermediate position. In confirmation, the results of a study of Shahrokhi et al. (2011) demonstrated different tolerance of barley varieties to allelopathic stress of *Amaranthus retroflexus*. The authors noted that Reyhan and Kavir varieties were more tolerant to the weed extracts action and that their cultivation could reduce the weed damage.

In earlier studies, Javaid et al. (2007) reported genotypic variation in rice varieties to allelopathic stress of *Cyperus rotundus* L. They found that “IRRI-8” and “IRRI-Fine” varieties were more tolerant to the phytotoxicity of *C. rotundus* than

**Table 2. Influence of *Sorghum halepense* extracts on germ length and fresh biomass accumulation in *Vicia faba* accessions**

Accessions	Type of <i>S. halepense</i> extract	Concentration, %	Germ length cm	Inhibition, % of control	Tolerance index, %	Germ weight g	Inhibition, % of control
Fb 1896	Shoot mass	control	6.77			0.359	
		1.0	4.92	-51.5	72.7	0.257	-48.4
		5.0	2.85		42.1	0.162	
	10.0	1.78	26.2		0.099		
	Root mass	1.0	4.30		63.6	0.228	
		5.0	3.47		51.2	0.207	
10.0		2.40		35.4	0.160		
Fb 1903	Shoot mass	control	6.33			0.332	
		1.0	4.50	-53.8	71.1	0.253	-57.5
		5.0	2.50		39.5	0.108	
	10.0	1.77	27.9		0.090		
	Root mass	1.0	3.59		56.8	0.161	
		5.0	2.83		44.7	0.147	
10.0		2.33		36.9	0.087		
Fb 1929	Shoot mass	control	9.52			0.587	
		1.0	5.47	-59.9	57.4	0.403	-55.4
		5.0	2.40		25.2	0.213	
	10.0	0.00	0.0		0.000		
	Root mass	1.0	6.88		72.3	0.468	
		5.0	5.02		52.7	0.342	
10.0		3.15		33.1	0.145		
Fb 2481	Shoot mass	control	6.84			0.482	
		1.0	6.84	-19.9	99.9	0.405	-34.7
		5.0	5.38		78.7	0.297	
	10.0	5.01	73.2		0.258		
	Root mass	1.0	6.59		96.4	0.357	
		5.0	5.54		81.0	0.314	
10.0		3.52		51.4	0.258		
Fb 2486	Shoot mass	control	11.18			0.632	
		1.0	9.55	-31.6	85.4	0.553	-25.0
		5.0	8.08		72.3	0.513	
	10.0	7.78	69.6		0.438		
	Root mass	1.0	7.78		69.6	0.548	
		5.0	7.57		67.7	0.517	
10.0		5.13		45.9	0.272		
Fb 3270	Shoot mass	control	19.23			0.547	
		1.0	16.73	-30.2	87.0	0.563	-17.5
		5.0	12.90		67.1	0.473	
	10.0	6.54	34.0		0.166		
	Root mass	1.0	15.07		78.4	0.496	
		5.0	15.43		80.2	0.508	
10.0		13.87		72.1	0.501		
BGE 002106	Shoot mass	control	8.08			0.383	
		1.0	6.00	-60.1	74.2	0.320	-47.9
		5.0	2.80		34.6	0.167	
	10.0	0.00	0.0		0.000		
	Root mass	1.0	6.26		77.5	0.314	
		5.0	2.48		30.6	0.228	
10.0		1.80		22.3	0.170		
BGE 029055	Shoot mass	control	13.97			0.663	
		1.0	10.74	-37.2	76.8	0.531	-33.9
		5.0	9.01		64.5	0.403	
	10.0	5.08	36.3		0.351		
	Root mass	1.0	10.11		72.3	0.553	
		5.0	7.48		53.6	0.427	
10.0		10.28		73.5	0.367		
BGE 032012	Shoot mass	control	11.30			0.612	
		1.0	4.73	-68.1	41.9	0.363	-60.0
		5.0	1.90		16.8	0.165	
	10.0	1.50	13.3		0.120		
	Root mass	1.0	6.84		60.5	0.304	
		5.0	4.56		40.3	0.264	
10.0		2.13		18.8	0.253		

Table 2. Continued

BGE 046721	Shoot mass	control	6.19			0.439	
		1.0	7.38	-26.8	119.2	0.522	-26.4
		5.0	6.99		111.3	0.468	
	10.0	2.50		40.4	0.250		
Root mass	1.0	4.73		76.3	0.264		
	5.0	3.38		54.6	0.241		
	10.0	2.30		37.1	0.193		
LSD at the 0.05 probability level							
A×B×C	0.79			0.047			

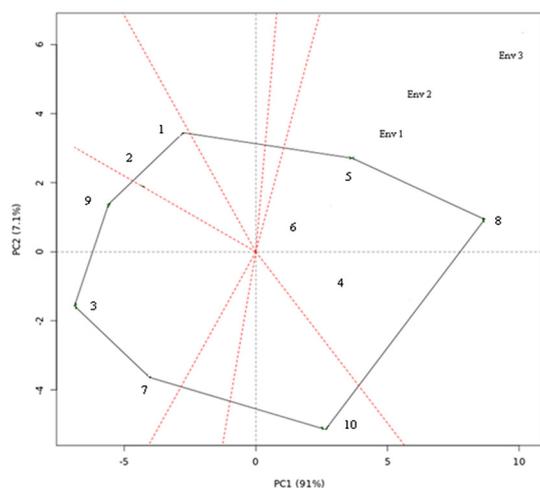


Fig. 2. GGE-biplot of allelopathic tolerance in *Vicia faba* accessions

1 – Fb 1896, 2 – Fb 1903, 3 – Fb 1929, 4 – Fb 2481, 5 – Fb 2486, 6 – Fb 3270, 7 – BGE 002106, 8 – BGE 029055, 9 – BGE 032012, 10 – BGE 046721 Env1 (1.0% concentration of water extract of *S. halepense*), Env2 (5.0%), Env3 (10.0%)

“Pak Basmati”, “Basmati Super” and “Basmati 385”. This unequal susceptibility could be due to inherent differences in the physiological and morphological characteristics of the different genotypes (Macias et al., 1992). Correlation analysis of the data showed a negative dependence of the seedling vigor index (SVI) with the inhibitory effect on seed germination ( $r = -0.985$ ), length and weight of the germs ( $-0.519$  and  $-0.668$ , respectively), as well as of the protein seed content with the inhibitory effect on germ length and weight ( $-0.435$  and  $-0.441$ , respectively). The last dependence was due to the property of proteins to adsorb molecules of organic compounds on their surface (Filipovich, 1985). A negative correlation was also observed between germ length and weight with the inhibitory effect of *S. halepense* regarding the same parameters ( $r = -0.577$  and  $-0.840$ , respectively). Thus confirms the dependence found in our previous studies (Georgieva & Nikolova, 2016; Georgieva, 2019), namely that accessions whose seeds are characterized by higher values of protein content and initial growth parameters (germ length and weight, seedling vigor index) are

less affected by the inhibitory effect of *S. halepense* extracts, respectively, exhibit a higher tolerance.

## Conclusions

Analysis of variance showed significant influence of the factors “accession” and “concentration of the extracts” of *Sorghum halepense* on the seed germination and initial growth parameters in ten *Vicia faba* accessions. The influence of the weed extract type, from root or shoot biomass, was significant only for seed germination.

The inhibitory effect of the extracts ranged from 7.7 to 100% in terms of seed germination. It was in limits from  $3.6 \div 100\%$  and  $7.1 \div 100\%$  regarding germ length and weight, respectively. In general, as the extract concentration increased, the depressive effect on the studied parameters enhanced. Significantly stimulating effect on germ growth, as well as on biomass accumulation, demonstrated 1% extract of shoot biomass in accession Fb 10.

The summary effect of the allelopathic action of *Sorghum halepense* on all parameters studied, represented by GGE-biplot analysis, determined accessions Fb 3270, Fb 2481 and Fb 2486 as exhibiting higher tolerance, unlike Fb 1929, BGE 032012, BGE 002106, Fb 1903 and Fb 1896 which were more sensitive. A dependence found in previous studies was confirmed, namely that accessions whose seeds are characterized by higher values of protein content and initial growth parameters, are less affected by the inhibitory effect of sorghum extracts. Respectively, they exhibit a higher tolerance.

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