Allelopathic Effect of Aqueous Extract from Root Systems of Johnson Grass on Seed Germination and Initial Development of Soybean, Pea and Vetch

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Introduction

Studies of plant communities are often closely connected with the problems of interspecific relations between crops and weeds. The relatively low competitive ability of soybean, peas and vetch at the early stages of growth determine the weeds as a factor inhibiting the yield per grain (Marinov-Serafimov & Dimitrova, 2007).

The studies conducted so far (Hoque et al., 2003, Marchin-Silva & Aquila, 2006, Stoimenova et al., 2008) have demonstrated that many of the weed species accumulated both in their roots and shoots water soluble inhibitory substances that, after washing, evaporation and decomposition of the plant remain, spread in the environment and thus inhibit the seed germination of a large number of crops. Other studies present different rates of inhibitory or stimulating effect of the studied weed species on the seed germination of crops (Kadioglu et al., 2005, Marinov-Serafimov, 2005, Marinov-Serafimov, 2010).

The interaction between weeds and cultivated plants is simultaneous and/or subsequent with direct or indirect impact of one plant species on another, through synthesis of different chemical compounds (allelochemicals) that are released in the environment and have an inhibitory and/or stimulatory effect on the seed germination and development of crops (Verma & Rao, 2006, Aleksieva & Serafimov, 2008). Number of studies (Vasilakoglou et al., 2006, Ashrafi et al., 2007, Koloren, 2007) determined allelopathic interaction between weeds and crops using plant material extracted from the dry weed biomass. The lack of similar studies in Bulgaria motivated our interest to determine the allelopathic effect of root systems of Johnson grass on some legume plants.

Summary: This paper presents the results of the research on allelopathic effect of cold aqueous extracts from root systems of Johnson grass (Sorghum halepense (L.) Pers.) on the seed germination and the initial development of soybean (Glycine max (L.)), pea (Pisum sativum (L.)) and vetch (Vicia sativa (L.)) under laboratory conditions. It was established that the inhibitory effect of aqueous root extracts on the seed germination of soybean, peas and vetch varied between 28.8 and 86.3%. Furthermore, the inhibitory effect of aqueous root extracts of Johnson grass varied between 17.1 and 86.1% on the development of the initial germ and between 8.3% and 97.9% on the accumulation of fresh biomass in the initial germ of soybean, peas and vetch. The values of LC$_{50}$ (P=0.05) were established to vary between 46.40 and 98.28 g l$^{-1}$ dry biomass from root systems of Johnson grass and can be arranged in the following order: soybean → peas → vetch.

Keywords: allelopathy, extract, Johnson grass, peas, roots, seeds, Sorghum halepense (L.) Pers., soybeans, vetches
The purpose of the study was to determine the allelopathic effect of root systems of Johnson grass on seed germination and initial plant development in agrophytocoenoses of soybean, peas and vetch. The interdependences in the allelopathic relations between the Johnson grass and the crops in the studied plant communities would be an important element of the theoretic base for sustainable plant production. Furthermore, the results of such study would provide additional information about the indirect harmful effect of Johnson grass on the normal growth and development of the plants in beans agrophytocoenoses.

Material and Methods

The study was conducted in 2010 under laboratory conditions at the Institute of Forage Crops in Pleven, Republic of Bulgaria. Seeds of the following legumes were tested: soybean (Glycine max (L.) Merr., Srebrina variety; pea (Pisum sativum (L.)), Pleven variety and vetch (Vicia sativa (L.), Obrazets 666 variety.

Collection of the plant material: The root systems of Johnson grass were taken from depth of 0 to 30 cm at weed phenophase 71 (Weber & Bleiholder, 1990) in experimental areas sown with medium early potatoes from the Sante and Fontane varieties, with natural weeding background, at the experimental field of the General agriculture and Herbology Department at the Agricultural University of Plovdiv, Republic of Bulgaria.

Preparation of weed extracts: The root systems of Johnson grass were chopped in pieces between 0.5 and 3.0 cm long, dried to obtain constant dry mass at 55 ± 3°C and then ground in a Retsch SM-1 grinder with 1.0 mm sieve size. Portions of 100 g dry biomass from the Johnson grass roots were soaked in 1 l of distilled water. The obtained samples were then cold extracted at 24 ± 2°C for 8 h in a Shuttle decanter at 120/60 s⁻¹. The obtained extract was then taken out from the decanter, filtered and centrifuged in a K24 centrifuge at 5000/60 s⁻¹. The aqueous extracts were brought to 12.5; 25.0; 50.0 and 100.0 g dry biomass content per 1 l of distilled water (hereinafter designated as g l⁻¹). Thymol (C_{10}H_{14}O) as preservative was added to each of the extracts (Marinov-Serafimov et al., 2007a).

Bioassays technique: Batches of 100 seeds of soybean, peas and vetch were placed between layers of filter paper in plastic containers in four replications. The surface of the seeds was sterilized following the method of Voll et al. (2005). All of the available extracts ranging by root biomass content were pipetted in a proportion of 1:6 to the seed mass (Marinov-Serafimov et al., 2007b), and distilled water was used as control. Each variant was laid out in six replications. The samples were placed in a thermostatic device at 22 ± 2°C for 7 days. A biochemical analysis of the roots was performed for content of: cyanogenic glucosides (Ermakov et al., 1987), total phenols (Swain & Hillis, 1959), trypsin inhibitory activity (Page et al., 2000), condensed tannins (Terril et al., 1992) and water soluble sugars (Ermakov et al., 1987).

The following characteristics were determined: Percentage of germinated seeds (%); Germ length at phenophase 08 of the development of soybean, peas and vetch (cm) according to Weber & Bleiholder (1990); Inhibition Rate (IR) at seed germination and at germ growth calculated according to the formula of Ahn & Chung (2000).

SPEARMAN estimates software (Hamilton et al., 1978) was used to determine the LC_{50} of the extract in the test samples. The index of initial growth (GI) of the plants was determined using the formula of Gariglio et al. (2002). The growth rate (K_{cm}) and the biomass accumulation rate (K_{g}) of the germ were calculated using the formula of Mamonov & Kim (1978). The acidity of the solutions was measured using Gallenkamp pH stick.

The experimental data were statistically processed after transformation of the percentage of germinated seed by the formula

\[ Y = \arcsin\left(\frac{100}{\sqrt{x_0/100}}\right) \]

(Hinckelmann & Kempthorne, 1994). All experimental data were statistically processed using the Statgraphics Plus for Windows Version 2.1 software.

Results and Discussion

The aqueous extracts from the roots of Johnson grass inhibited the germination of the seeds of soybean, peas and vetch at rates between 28.8% and 86.3%, the differences being statistically proven at P=0.01. The rate of inhibition (IR) of the germination of seeds of soybean, peas and vetch at different concentrations of the Johnson grass roots extract varied in a wide range between 15.8% and 62.8% compared to the control sample (Table 1).
Concerning the impact of the Johnson grass root content in the aqueous solutions (g l\(^{-1}\)), it was found that with increasing concentrations of weed biomass the percentage of germinated seeds decreased non-proportionally (from 84.2% to 37.2%) on average in all test plants, the differences being statistically proven at \(P=0.01\) (Table 1).

This interdependence could be explained by the presence of cyanogenic glycosides and general phenols, as well as the complex impact of water soluble sugars contained in the tested extracts (Table 2). It is well known that cyanogenic glycosides and phenols are highly toxic and have pronounced protoplasmic effect (Iqbal et al., 2003). In higher concentrations, they may have a lethal impact on the seed germination whereas in lower concentrations they inhibit their germination to different degrees, which is probably due to the lower glycoalkaloid and phenol content.

The determination of the rate of inhibition (IR) of the seed germination in the tested beans gave the same results. In accordance with these results, the tested crops could be divided into two groups. The first group comprises of the soybean the seed germination of which is inhibited up to 25%. To the second group belong the peas and the vetch the seed germination of which is inhibited between 35% and 40% (Table 1). Obviously, the tested beans react in different ways to the inhibitory effect of the aqueous extracts from roots of Johnson grass. The seeds of pea and of the vetch are much more sensitive than the seeds of soybean (Table 1).

The different inhibitory effects of the extracts from roots of Johnson grass on the seed germination in the test plants can be explained on the one hand by the diffusion of the soluble allelochemicals during extraction of the biomass (Jiménez-Osornio et al., 1996, Cheema, 1998) and on the other hand by the different raw protein content in the seeds of soybean, pea and vetch (Kertikov, 2005, Marinov-Serafimov, 2005, Stoimenova et al., 2008). Similar results were reported by Marinov-Serafimov et al. (2007b) who determined the inhibitory effect of aqueous extracts from weeds typical of the biocoenoses of soybean, pea and vetch.

### Table 1. Effect of aqueous extracts from roots of Johnson grass on seed germination in test plants

| Weed biomass in the water extracts, g l\(^{-1}\) | Species | Glycine max | Pisum sativum | Vicia sativa | Average |
|---|---|---|---|---|---|
| | Characteristics | N | % | IR | N | % | IR | N | % | IR | N | % | IR |
| 0 | 63.4 | 100 | 0.0 | 65.9 | 100 | 0.0 | 61.1 | 100 | 0.0 | 63.5 | 100 | 0.0 |
| 12.5 | 54.7 | 86.3* | 13.7 | 52.7 | 80.0** | 20.0 | 52.7 | 86.3** | 13.7 | 53.4 | 84.2 | 15.8 |
| 25 | 54.7 | 86.3** | 3.7 | 50.8 | 77.0*** | 23.0 | 37.3 | 61.0*** | 9.0 | 47.6 | 74.8 | 11.9 |
| 50 | 50.8 | 80.0*** | 0.0 | 33.2 | 50.4*** | 49.6 | 32.2 | 52.6*** | 47.4 | 38.7 | 61.0 | 32.3 |
| 100 | 31.0 | 49.0*** | 51.0 | 21.4 | 32.5*** | 67.5 | 18.4 | 30.2*** | 69.8 | 23.6 | 37.2 | 62.8 |

Legend: \(N\)–number of germinated seeds; \%–percentage compared to control; \(IR\)–rate of inhibition of seed germination, \%; * statistically proven differences at \(t_{\text{critical}}\) \(P=0.5=2.0; P=0.1=2.7; P=0.01=3.6\)

### Table 2. Biochemical analysis of roots of Johnson grass

| Parameters | Cyanogenic glycosides (mg HCH/100g\(^{-1}\)) | Total phenols (relative units extinction) | Condensed tannins (%) | Tripsin inhibitor activity (TIU/mg\(^{-1}\)) | Water soluble sugars content (%) |
|---|---|---|---|---|---|
| | 16.48 | 0.674 | 0.0 | 1.43 | 15.6 |
Furthermore, analogous results were obtained while determining LC$_{50}$(P=0.5) on the germination of seeds of soybean, pea and vetch depending on the inhibitory effect of the extracts from roots of Johnson grass. The values of LC$_{50}$(P=0.5) varied from 46.40 g l$^{-1}$ to 98.28 g l$^{-1}$ and could be conventionally grouped in the following order: soybean [98.28 (-79.44 +121.60)] → pea [52.97 (-42.39 +66.19)] → vetch [46.40 (-36.86 +58.42)]. The different values of LC$_{50}$ could be explained by the different sensitivity of the test plants seeds, which is mainly due to their morphologic particularities.

The aqueous extracts from root systems of Johnson grass had different suppressive effects on the growth of the germ (from 17.1% to 86.1%) and on the accumulation of fresh biomass in the primary seedling (from 8.3% to 97.9%) of soybean, peas and vetch seeds (Table 3). With increase of the concentration of the extracts from root systems of Johnson grass, the growth and the accumulation of fresh biomass in the primary seedling of all test plants decreased disproportionately compared to the control, the differences being statistically proven at P=0.05.

Similar were the results about the growth rate and the rate of accumulation of fresh biomass in the primary seedlings of soybean, pea and vetch seeds depending on the concentration of the water root extracts (Table 3).

The growth rate of the seedling (K/cm) and the rate of accumulation of fresh biomass in the seedling (K/g) of the studied crops also seemed to depend on the concentration of the extract from Johnson grass. In all three crops relatively smaller inhibition of the growth rate of the seedling (K/cm) (r varied in the range from -0.676 to -0.788) and greater inhibition of the rate of accumulation of fresh biomass (K/g), where r varied from -0.896 to -0.938 - were found (Figures 1 and 2).

According to Iman et al. (2006), the mechanism of inhibition of the growth and of the accumulation of fresh biomass in the primary seedling of the seeds results from the effect of allelochemicals and could be explained by reduced cell division rate and/or by the elongation of the cells. Therefore, the stage of germination of the seeds can be considered as a less sensitive period in the individual development of the plants while the growth of the primary seedling could be used as a potential test to determine the allelopathic effect of the studied extract under laboratory conditions due to the direct contact of the extracts during the bioassays.

Table 3. Effect of aqueous extracts from Johnson grass roots on the growth rate and the accumulation of fresh biomass in the seedlings of test plants

| Weed biomass in the water extracts (g l$^{-1}$) | Species                   | Glycine max | Pisum sativum | Vicia sativa | Average |
|-----------------------------------------------|---------------------------|-------------|---------------|--------------|---------|
| Length of seedlings (cm plant$^{-1}$)         |                           |             |               |              |         |
| 0                                             |                           | 11.0d (0.0)*| 9.6d (0.0)    | 10.5c (0.0)  | 10.4d (0.0) |
| 12.5                                          |                           | 9.1c (17.1) | 6.6cd (31.4)  | 6.4b (39.2)  | 7.4cd (29.2) |
| 25                                            |                           | 8.7c (21.3) | 5.1bc (46.7)  | 3.7a (65.0)  | 5.8bc (44.3) |
| 50                                            |                           | 6.3b (42.9) | 3.3ab (65.9)  | 2.8a (72.9)  | 4.1ab (60.6) |
| 100                                           |                           | 2.9a (74.1) | 1.3a (86.1)   | 1.8a (83.1)  | 2.0a (81.1)  |
| Fresh biomass of seedlings (g plant$^{-1}$)   |                           |             |               |              |         |
| 0                                             |                           | 0.33c (0.0) | 0.24c (0.0)   | 0.10d (0.0)  | 0.22b (0.0) |
| 12.5                                          |                           | 0.29b (11.2)| 0.22c (8.3)   | 0.06c (40.4) | 0.19ab (20.0) |
| 25                                            |                           | 0.23b (28.6)| 0.18b (26.4)  | 0.06c (40.4) | 0.16ab (31.8) |
| 50                                            |                           | 0.23b (29.6)| 0.10ab (59.0) | 0.03b (64.5) | 0.12ab (51.0) |
| 100                                           |                           | 0.04a (87.1)| 0.005a (97.9) | 0.009a (91.0)| 0.02a (92.0) |

Legend: a, b, c, d, are statistically proven differences in P=0.05; * Degree of inhibition in the germination of respective control, %
by the morphologic and biological differences between them since the comparisons were made in identical conditions and with the same concentrations of the extracts applied.

The germination of the seeds of soybean, pea and vetch also depended on the acidity of the extracts, which varied between 5.5 and 6.9. A strong positive correlation was discovered between the germination of the seeds of soybean, pea and vetch and pH of the extracts where \( r \) varied between 0.932 and 0.974.

Furthermore, the effect of the extracts on the germination of seeds of soybean, pea and vetch can be expressed as function of the oxidation processes for extracts made from dry biomass of roots of Johnson grass in the following order: soybean – \[ \text{Germination} = \frac{1}{0.222129 - 0.001327463 \times \text{pH}}; R^2 = 0.944; \text{St. error} 0.0062 \]; pea \[ \text{Germination} = 1/(0.19742-0.000897213 \times \text{pH}); R^2 = 0.930; \text{St. error} 0.0050 \] and vetch – \[ \text{Germination} = 1/(0.19322-0.000892291 \times \text{pH}); R^2 = 0.856; \text{St. error} 0.0049 \].

The experimental data confirm the findings of other authors (Cheema, 1998, Turk & Tawaha, 2002, Ashrafi et al., 2007), namely the effect of allelochemicals is manifested in the germination inhibition, but it is more pronounced on the growth of the seedlings.

The index of initial growth of the plants (GI) depends on the same factors and follows the observed interdependences as concerns the seed germination and the growth of the primary seedling in the test plants under laboratory conditions (Table 4).

The results from the laboratory analyses show that the studied extracts from roots of Johnson grass have a delaying and/or inhibitory effect on the initial growth of soybean, pea and vetch. With increase of the dry biomass from roots of Johnson grass in the aqueous extracts the initial plant growth index (GI) decreased by 40.4% and 92.2%. Hence, the differences observed in the studied bean crops in respect of the allelopathic effect of the extracts could also be explained by the morphologic and biological differences between them since the comparisons were made in identical conditions and with the same concentrations of the extracts applied.

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Table 4. Effect of aqueous extracts from roots of Johnson grass on the initial growth (GI) of the test plants

| Species          | Weed biomass in the water extracts (g l⁻¹) |
|------------------|-----------------------------------------|
|                  | 0           | 12.5      | 25           | 50         | 100          |
| Glycine max      | 100         | 71.5      | 67.9         | 45.7       | 12.7         |
| Pisum sativum    | 100         | 54.9      | 41.0         | 17.2       | 4.5          |
| Vicia sativa     | 100         | 52.5      | 21.4         | 14.3       | 6.1          |
| Average          | 100         | 59.6      | 43.4         | 25.7       | 7.8          |
Conclusions

Aqueous extracts from the roots of Johnson grass inhibited the germination of soybean, pea and vetch. Inhibitory effect varied between 28.8 and 86.3%, the differences being statistically proven at P = 0.01.

Aqueous extracts from the roots of Johnson grass have suppressive effect on the seedling growth (from 17.1% to 86.1%) and the fresh biomass of seedlings (from 8.3% to 97.9%) of soybean, pea and vetch. The values of L_{C50}, which vary from 46.40 g l^{-1} to 98.28 g l^{-1} of fresh biomass from roots of Johnson grass, can be arranged in the following order: soybean → pea → vetch.

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Alelopatski efekat vodenog ekstrakta iz korenorskog sistema
divljeg sirka na klijavost semena i početni razvoj soje, graška i grahorice

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Izvod: U ovom radu se predstavljeni rezultati istraživanja alelopatskog efekta hladnog vodenog ekstrakta iz korenovog sistema divljeg sirka (Sorghum halepense (L.) Pers.) na klijavost semena i početni razvoj soje (Glycine max (L.)), graška (Pisum sativum (L.)) i grahorice (Vicia sativa (L.)) pod laboratorijskim uslovima. Utvrđen je raspon inhibitornog efekta vodenog korenskog ekstrakta na klijavost semena soje, graška i grahorice od 28,8% do 86,3%. Takođe, inhibitorni efekat vodenog korenovog ekstrakta divljeg sirka na razvoj inicijalne klice ovih vrsta varirao je u rasponu 17,1-86,1% a na akumulaciju sveže biomase u inicijalnoj klici u rasponu 8,3-97,9%. Utvrđene su vrednosti LC_{50}(P=0,05) koje variraju između 46,40 i 98,28 g l^{-1} suve biomase iz korenovog sistema divljeg sirka i mogu se postaviti u sledećem redosledu: soja → grašak → grahorica.

Kljucne reci: alelopatija, divlji sirak, ekstrakt. grahorica, grašak, koren, seme, soja, Sorghum halepense (L.) Pers.