Allelopathic potential of soybean (*Glycine max* L.) on the germination and root growth of weed species

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Abstract: Water extracts that were obtained from the shoots and roots of *Glycine max* were used to determine their allelopathic potential in relation to the germination and seedling growth of the weed species *(Sorghum halepens L.)* and *(Secale cereale L.)*, in laboratory bioassays. The shoots and roots of *G. max* were soaked separately in distilled water in a ratio of 1:1 (w/v) for 24 h in order to prepare the aqueous extracts. Distilled water was used as the control. The seeds of the target species were germinated in Petri dishes and counted daily for up to 7 days. The *G. max* shoot extract (100%, 75%, and 50%) decreased the seed germination of *S. halepense* and *S. cereale*. The shoot extract caused the most reduction in the germination index and germination speed in *S. halepense*. The mean LC_{50} value of *G. max* shoot and root extracts in relation to the germination inhibition of *S. cereale* was 40% and 38%, respectively, and 43% and 41%, respectively, in *S. halepense.* All four concentrations of the shoot extract proved to be more phytotoxic than the root extract, reducing the root length of all four species, while the root extract decreased the root length of *S. cereale* at the 100% concentration. The *S. halepense* seeds were more sensitive regarding germination, as compared to *S. cereale.* The shoot aqueous extract of *G. max* was more phytotoxic, as compared to the root aqueous extract, even at the lowest concentration (25%).
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Key words: *Glycine max* L., *Sorghum halepens* L., *Secale cereale L.,* allelopathy, seedling growth

Introduction

Allelopathy offers potential for weed control through the production and release of allelochemicals from leaves, flowers, seeds, stems and roots of living or decomposing plant materials. Allelopathy is a challenge at present and allelochemicals a resource [16]. Allelopathy is regarded as a natural strategy in plants protecting them against environmental enemies and competing plants. This process involve plant secondary metabolites that suppress the growth and development of surrounding biological systems and named as allelochemicals. Thus, allelopathy interactions between plants and other organisms may become an alternative to synthetic herbicides and other pesticides [21]. Cover crop may help decrease herbicide use by providing a non-chemical means of reducing the impact of weed interference. Weed suppression by cover crop systems attributed to competition by the live cover crop, physical obstruction of weed emergence and allelopathic potential[19]. Soybean *(Glycine max)* has been reported to contain some allelochemicals and therefore suggested as being capable of posing a serious threat of phytotoxicity to weeds [10,11,12]. Organic compounds extracted with ethanol from soybean rhizospheic soil on continuous and alternate cropping at pod-string were identified by gas chromatography-mass spectrometry (GC-MS). The results showed the compounds mainly include organic acids, alcohol, acetone, aldehyde, naphthalene phenyl and furan hydrocarbon, many of which were reported as allelochemcals[13]. Detrimental effects of allelochemicals on plant germination and growth have been reported [22,7,5,17,29,24,27,4]. Mays et al. (1998) conducted an experiment to compare N from soybeans with fertilizer N resulted in a 46% reduction in wheat yield following soybeans where wheat was not fertilized with N. Subsequent research showed that when soybeans were allowed to grow to bloom stage or later preceding a wheat or triticale crop a significant reduction in grain yield resulted from the previous soybean crop. When the grain crop was fertilized with 80 lbs/acre of N this response did not occur. The experimental results suggest that soybean root exudates are responsible for the decrease in wheat and triticale growth and yield. A research was initiated to determine how soybean [*Glycine max* (L.) Merr] cultivars daer in competitive ability and also whether allelopathy functions to inhibit surrounding weed growth. Exudates from roots of soybean cultivars grown in sand reduced the dry weight of 4-week-old velvetleaf plants an average of 15%, but foxtail millet was not inhibited. Allelopathy appears to be one mechanism for competition among soybeans and weeds. Soybean cultivars possessing the ability to chemically inhibit competing weed growth would be a great benefit to the soybean producer [22]. Yan and Yang (2008) investigated on the problems caused by continuous cropping in soybean and especial focused on origin and activity of allelochemicals under biotic

stress. Interaction between phytotoxins and pernicious microorganisms in the soils of soybean under continuous cropping practice was suggested as a possible mechanism. The allelopathy of different densities of soybean root exudates on 4 main crops was studied. The results showed that the increasing of soybean root exudates density promoted the germination of wheat seeds, but inhibited the germination of soybean, corn and Chinese cabbage seeds; with the increasing of soybean root exudates density, the gross activity of SOD and the root activity of wheat and corn were enhanced significantly, but those of soybean were inhibited. However, the role of MDA content was different, with the increasing of soybean root exudates density, the MDA content of wheat decreased significantly, the MDA content of soybean increased significantly, the MDA content of corn changed little. The low density of soybean root exudates promoted plant height and dry weight, radial length and root dry weight of wheat and soybean, while the high density inhibited them;and for those of corn, the alleopathy was enhanced [24]. The aim of the present investigation was to assess the allelopathic potential of the shoot and root extracts of *G. max* on the germination, emergence, root elongation, and seedling growth of three weed species. This can provide basic information about the management of these weeds.

Material and Methods

Target species

The experiment was carried out at the Department of Biology, Islamic Azad University, Mashhad Branch, Iran (2012). The seeds of *Glycine max*, *Secale cereal* and *Sorghum halepens* were supplied by the Agricultural Research Center of Khorasan province, Iran.

Water extraction of Glycine max

The fresh shoots and roots of *G. max* were collected from six weeks old plants. Each part was soaked separately in distilled water in the ratio of 1:1 (w/v). Similar extraction techniques were used by Molina et al. (1991) in their studies of *Eucalyptus* spp. The extracts were prepared at room temperature and left in the laboratory for 24 h. The extracts were collected, filtered through filter paper, and described as 100%. Distilled water was added to the solutions to make different dilutions (75, 50, and 25%).

Germination bioassays

Glass Petri dishes (9 cm diameter) were used and contained blotting paper (3MM; Whatman). Twenty-five seeds of each species were placed in the Petri dishes to which 3 mL of solution were added at the start, while the control received 3 mL of distilled water. An additional 1 mL of each solution was added every 48 h thereafter. Three replicates of each treatment were incubated in a germination chamber. The light was provided by cool, white fluorescent tubes with an irradiance of 35 $\mu mol\ m^{-2}\ s^{-1}$. The germination was assessed after every 24 h by counting the number of germinated seeds for up to 7 days. Germination was considered as the rupture of the seed coat and radicle emergence of ≥1 mm. Seedling growth bioassays. The Petri dishes were placed in a cold chamber at 4°C after 1 week in order to stop seedling growth and the radicle length was measured with a measuring tape.

Statistical analysis

The germination rate index (G_T) was determined, as described by Jäderlund et al. (1996), and the speed of germination (S) was calculated, as proposed by Ahmed and Wardle (1994). The collected data were statistically analyzed by using a one-way ANOVA and the Dunnett test was used to determine the differences between the treatment means at the 5% probability level. The mean LC_{50} value (the dose for 50% inhibition of seedling growth) was calculated by using a probit analysis, as described by Finney (1971). A logistic equation was fitted to the germination data as a function of the logarithm of the oncentrations of the *G. max* shoot and root extracts by using SPSS. where Y= the probit value, a= the intercept, b= the slope of the line, and X= the $\log_{10}$ concentration. The value of X was obtained in order to calculate the LC_{50} values of the concentrations of the *G. max* shoot and root extracts.

Results

Effect of the Glycine max extracts on germination at each exposure time

The laboratory test showed that the *G. max* shoot aqueous extract (100, 75, and 50%) significantly inhibited the germination of *S. halepense* and *S. cereal* (Tables 1–2). The *G. max* root extract (100% and 75%) completely inhibited the germination process of *S. cereale* during the second, third, and sixth day. However, there was a tendency for a stimulation of the germination of *S. cereale* by different concentrations of the root extract.

Effect of the Glycine max *extracts on the germination indices*

As shown in Table 5, the inhibitory effect of the water extracts on the G_T and S depended on the extract concentration and the plant species. For *S. halepense*, the G_T and S were completely inhibited by the *G. max* shoot extract at all four concentrations. Both indices were less sensitive to the root extract at the lowest concentration, which did not significantly affect *S. halepense*. For *S. cereale*, the aqueous extracts of the *G. max* shoots and roots did not significantly inhibit the G_T and S at any of the tested concentrations (Table 3).

Effect of the* Glycine max *extracts on germination

The effect of the *G. max* shoot and root extracts on the germination of the tested species after the probit analysis is presented in Tables 4-5. The total number of seeds, ungerminated seeds, expected response, and probability were determined against the four different concentrations (100, 75, 50, and 25%) of the *G. max* shoot and root extracts. The data of the ungerminated seeds were fitted to the probit model. The data regarding the ungerminated seeds were best fitted to the probit model after log transformation of the data. The result of the χ^2-tests for goodness-of-fit was d= 10 (at the 95% confidence limit) for the ungerminated seeds. The regression equation was Y=−0.091 + 0.675X in relation to the *S. halepense* germination data after exposure to the root extract of *G. max*. No regression equation was computed in relation to *S. halepense* after exposure to the shoot aqueous extract of *G. max* because there was no seed germination. The concentration of 46% of the root of *G. max* was a diagnosed concentration that inhibited 50% of the seed germination of *S. halepense* (Table 4). The result of the χ^2-tests for goodness-of-fit was d= 10 (at the 95% confidence limit) for the ungerminated seeds of *S. cereale* and the regression equation was Y= 0.447 + 0.204X following exposure to the shoot aqueous extract of *G. max*. The concentration of 40% of the *G. max* shoot inhibited 50% of the seed germination of *S. cereale*. The regression equation was Y= 0.180 + 0.079X following exposure to the *G. max* root extract. The concentration of 38% of the roots of *G. max* inhibited 50% of the seed germination of *S. cereale* (Table 5).

Table 1. Effects of the *Glycine max* shoot and root water extracts on the germination of *Sorghum halepens* at each exposure time during 1 week

Treatment	**Exposure time (days after sowing)**						
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Shoot aqueous extract Control	0.00±0.00	0.42±0.33	4.92±0.90	4.75±0.65	3.50±0.89	3.09±0.80	2.33±0.63
100%	0.00±0.00	0.00±0.00	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
75%	0.00±0.00	0.00±0.00	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
50%	0.00±0.00	0.00±0.00	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
25%	0.00±0.00	0.00±0.00	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
root aqueous extract Control	0.00±0.00	0.42±0.33	4.92±0.90	4.75±0.65	3.50±0.89	3.09±0.80	2.33±0.63
100%	0.00±0.00	0.00±0.00	2.00±1.15	4.00±2.00	3.00±0.57	0.00±0.00	1.67±0.88
75%	0.00±0.00	0.00±0.00	4.00±1.15	4.00±1.15	2.67±0.66	2.67±1.76	3.33±2.02
50%	0.00±0.00	0.00±0.00	3.33±0.41	2.00±2.00	2.22±0.83	3.33±2.02	2.00±0.57
25%	0.00±0.00	0.00±0.00	3.67±0.88	4.33±1.20	2.13±0.76	1.33±0.66	1.67±0.88

*Significant differences, compared to the control, at $P < 0.05$, according to the Dunnett test. The results represent the mean (±SE) of three replicates

Table 2. Effects of the *Glycine max* shoot and root water extracts on the germination of *Secale cereal* at each exposure time during 1 week

Treatments	**Exposure time (days after sowing)**						
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Shoot aqueous extract Control	0.00±0.00	2.08±0.417	2.25±0.55	2.17±0.405	1.25±0.37	0.27±0.19	0.00±0.00
100%	0.00±0.00	0.00±0.000*	1.67±0.33	1.67±0.330	2.00±0.57	1.33±0.33	0.33±0.33
75%	0.00±0.00	0.00±0.000*	1.33±0.33	4.33±0.880*	2.00±1.52	0.67±0.33	0.00±0.00
50%	0.00±0.00	0.00±0.000*	1.33±0.34	2.33±0.330	2.67±0.33	0.67±0.33	0.67±0.45
25%	0.00±0.00	0.00±0.000*	2.67±1.66	3.33±0.880	2.33±0.88	0.33±0.33	0.00±0.00
root aqueous extract Control	0.00±0.00	2.08±0.417	2.25±0.55	2.17±0.405	1.25±0.37	0.27±0.19	0.00±0.00
100%	0.00±0.00	0.00±0.000*	3.33±0.88	2.00±1.000	1.67±0.66	1.67±0.88*	0.33±0.33
75%	0.00±0.00	0.00±0.000*	5.00±0.57*	3.67±0.330	2.33±0.33	2.33±1.20*	0.67±0.66
50%	0.00±0.00	0.00±0.000*	5.00±0.15*	1.33±0.330	2.00±1.15	1.00±1.00*	0.00±0.00
25%	0.00±0.00	0.00±0.000*	5.33±0.88*	2.67±0.330	1.67±0.88	1.67±0.33*	0.33±0.33

Table 3. Effect of the different concentrations of the *Glycine max* shoot and root aqueous extracts on the germination indices of *Secale cereal* and *Sorghum halepens*

Treatments	*Sorghum halepens*		*Secale cereal*	
	G_T	**S**	**G_T**	**S**
Shoot aqueous extract Control	82.67±7.05	1.24±0.207	40.00±8.00	0.94±0.20
100%	0.00±0.00*	0.00±0.000*	28.00±4.00	0.66±0.52
75%	0.00±0.00*	0.00±0.000*	37.33±5.33	0.55±0.68
50%	0.00±0.00*	0.00±0.000*	38.67±6.67	0.71±0.19
25%	0.00±0.00*	0.00±0.000*	34.67±3.52	0.67±0.08
root aqueous extract Control	82.67±7.05	1.24±0.200	40.00±8.00	0.94±0.20
100%	42.67±8.11*	0.70±0.110*	36.00±10.06	0.52±0.13
75%	66.67±2.66*	1.16±0.100	37.33±13.92	0.83±0.11
50%	70.67±11.85	1.22±0.210	35.33±2.66	0.57±0.18
25%	70.67±1.85	0.93±0.170	46.00±6.11	0.72±0.46

*Significant differences, compared to the control, at $P < 0.05$, according to the Dunnett test. The results represent the mean (±SE) of three replicates. G_T, germination rate index; S, speed of germination.

Table 4. Probit analysis for the seed germination of *Sorghum halepense*, exposed to four concentrations of *Glycine max* root aqueous extract

***E*xtract concentration (%)**	**Total no. of seeds**	**No. of ungerminated seeds**	**Expected response**	**Probability**
100	25	15	11. 59	0. 464
75	25	9	10. 75	0. 430
50	25	8	9. 60	0. 384
25	25	7	7. 76	0. 309

Regression line parameters (*G. max* shoot): $Y=a+bX$; $Y= 0.447 + 0.204X$; LC_{50} value = 2.23; diagnostic concentration = 40%.
Regression line parameters (*G.max* root): $Y=a+bX$; $Y= 0.180 + 0.079X$; LC_{50} value = 6.10; diagnostic concentration = 38%.

Table 5. Probit analysis for the seed germination of *Secale cereale*, exposed to four concentrations of *Glycine max* aqueous extract

Extract concentration (%)	**Total no. of seeds**	**No. of ungerminated seeds**	**Expected response**	**Probability**
Shoot				
100	25	18	16.81	0.673
75	25	16	16.58	0.663
50	25	16	16.25	0.650
25	25	15	15.67	0.627
Root				
100	25	16	14.28	0.571
75	25	16	14.18	0.567
50	25	14	14.04	0.562
25	25	11	13.81	0.553

Effect of the* Glycine max *extracts on root growth

The effect of the *G. max* shoot and root extracts on the root length of the plant species was concentration- and species-dependent. The root length of *S. cereale* was significantly reduced as a result of the *G. max* shoot extract at all concentrations (Figure 1A). The aqueous extract of the *G. max* root significantly reduced the root length of *S. cereale* at all the tested concentrations. The *G. max* shoot extract significantly reduced the root length of *S. halepense* at all concentrations. No significant variation was detected due to the *G. max* root extract on the root length of *S. halepense* at any of the tested concentrations (Figure 1B).

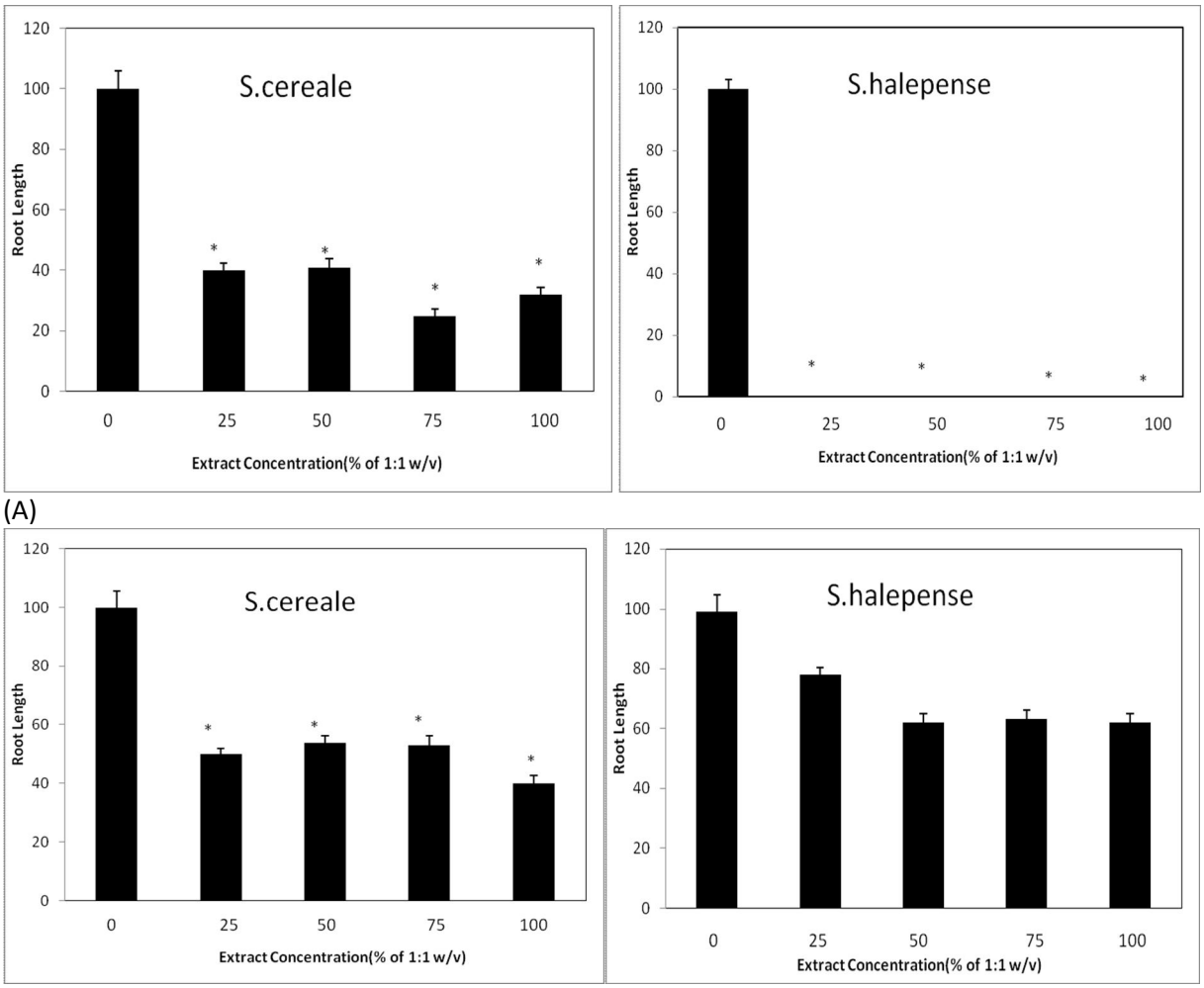

Fig 1. Effect of the (a) shoot and (b) root aqueous extracts of *Glycine max* on the root length of *Secale cereale* and *Sorghum halepense*. Every column in each graph represents the mean value ± standard error from three replicates. *Significant difference at the 0.05 probability level, compared to the control, according to the Dunnett test.

Discussion

The allelopathic phenomenon has received much attention, as shown by numerous reports on the subject (26)

The present study revealed that *G. max* significantly reduced the germination and seedling growth of two weed species. Compared to the distilled water, the continuous application of the *G. max* shoot or root extracts for up to 7 days significantly reduced the seed germination of the weed species. This finding is supported by the results of Iman et al. (2006), who identified several phenolic compounds in the *G. max* shoot and root extract. These compounds that are present in the shoot and root extract might be responsible for the retardation of germination and other growth parameters of *S. cereale* and *S. halepense* in the present study. Phenolics are widely recognized for their allelopathic potential in plants and can be found in a variety of plant tissues [6]. The effects of allelochemicals have been studied mostly on seed germination and the suggested mechanisms for its inhibition are the disruption of mitochondrial respiration[1] through the influence of allelochemicals on glycolysis, the Krebs cycle, electron transport and oxidative phosphorylation[20], and the mitochondrial membrane. For *S. cereale,* the LC_{50} values of the *G. max* shoot and root extracts were 40 and 38%, respectively. The mean LC_{50} value of the *G. max* root extract in the germination inhibition of *S. halepense* was 46%. Generally, the rate of seed germination decreased with an increasing concentration of the extracts, indicating that seed germination was quantitatively related to the extracts' concentration. The increasing inhibitory rate with the increasing concentration was in accordance with previous reports [8,25,3] for other allelopathic species. Among the

shoot and root extracts, the shoot extract was observed to be the most inhibitory. The delayed seed germination with the shoot extract of *G. max*, compared with the root extract, could be related to the more inhibitory effect of the allelochemicals that are present in the shoot parts of the plant. These results are supported by the findings of Iman et al. (2006), who reported that the effect of the aerial parts of *G. max* on the germination and growth of different plant species was greater than the effect of the subaerial parts. The maximum decrease in the germination percentage of the seeds of the weed species, when treated with the shoot extract of *G. max*, indicated the presence of water-soluble allelochemicals in maximum concentration. The lower concentration of the root extract actually promoted seed germination in some bioassay species, but the higher extract concentrations significantly reduced the germination, which suggests that the stimulatory or inhibitory effect is a function of the concentration[23]. The inhibition of seed germination was recorded in both test species, although the extent of inhibition varied across the species and treatments. The aqueous extracts of the shoot and root of *G. max* suppressed the germination and seedling growth of *S. halepense*, and *S. cereale* and the inhibitory effect increased with increasing concentrations of the extracts. A marginal improvement in the germination at low concentrations of the root extract could be the result of the detoxification of the allelochemical(s) through the conjugation, sequestration, or secretion of carbohydrates and the oxidation of phytotoxic compounds[14]. Iman et al. (2006) reported that the lower doses of *G. max* shoot and root extract stimulated the growth and germination of corn varities, although the shoot extract of *G. max* appeared to have a more negative effect on seed germination than the root extract. This could be related to a greater concentration of diffusible active compounds in the shoot than in the root or to a variation in the chemical composition between the tissues. As a result of the more dramatic effects of allelochemicals on seed germination than on the growth and viability of adult plants[28], the seed germination bioassay commonly has been used to establish the allelopathic activity of plant extracts. In the present study, two indices were used to assess the allelopathic effects on the germination of the test species. It is evident from the data that the G_T, commonly used in allelopathic bioassays, was not sensitive enough at the lowest concentration of the root extract to conclusively confirm allelopathic activity. It is because this index gives a global interpretation of germination [15] and does not take into account the speed of germination. In view of the failure of the G_T to convincingly demonstrate allelopathic activity of the shoot and root extracts of *G. max*, the S was used to monitor the germination behavior of the seeds of the target species because of its sensitivity. The limitations of any index to adequately reflect the effect of allelochemicals on germination behavior is evident from present studies and the use of multiple indices seems to be necessary in order to validate the allelochemical activity of plant extracts. The root length of both target species was significantly suppressed by the different concentrations of the *G. max* shoot extract, as compared to the root extract. Shunjie et al. (2008) concluded that the percentage of germination and plumule and radicle length of corn and Chinese cabbage decreased with an increasing concentration of *G. max* root extract.

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