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Full Length Research Paper

Phytotoxic effects of *Stellera chamaejasme* L. root extract

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***Stellera chamaejasme* is a traditional Chinese medicinal plant with a wide range of medicinal properties and biological activity. In order to evaluate the phytotoxity of *S. chamaejasme*, the effects of aqueous extracts (6.25, 12.5, 25, 50 and 100 g/L) prepared from roots was studied on the seed germination and seedling growth of rapeseed (*Brassica napus*), sesame (*Sesamum indicum*), wheat (*Triticum aestivum*) and maize (*Zea mays*). The treatments with high concentration extract (50 and 100 g/L) significantly reduced the germination rate of four crops compared with the control but the treatments with low concentration extract (from 6.25 to 25 g/L) had no obvious inhibitory effect. The mean germination time (MGT) of rapeseed and sesame increased with increasing extract concentration generally. The MGT of wheat and maize extended only at above 50 g/L concentration and at 100 g/L concentration respectively, whereas the treatments at 6.25, 25 and 50 g/L had a decreased effect on MGT of maize. The concentration-dependent effects were also found on shoot length, root length, number of lateral roots and dry weight of rapeseed, wheat and sesame seedling. Lower concentration extract treatments (6.25, 12.5 and 25 g/L) significantly promoted the seedling growth of maize, whereas the higher concentrations extracts had inhibitory effect. The results demonstrated that the high concentration aqueous extracts from *S. chamaejasme* had strong phytotoxity on seedlings growth of tested species. Dicotyledonous plants (rapeseed and sesame) were more sensitive to *S. chamaejasme* extract than monocotyledonous plants (wheat and maize). This result might be an important reference for further investigating the phytotoxic effect of *S. chamaejasme* for weed control.**

Key words: *Stellera chamaejasme*, phytotoxity, allelopathy, germination, seedling growth.

INTRODUCTION

The secondary metabolism products produced by plants have been reported to function as chemical agents against plant pathogenic microorganisms, insects, stored-grain pests and weeds. Many such natural compounds have the potential to be exploited as antibacterial agents, fungicides, insecticides and herbicides or as leads for discovery of new derivatives (Duke et al., 2000b; Jacob and Walker, 2005; Copping and Duke, 2007; Dayan et al., 2009). Many medicinal plants especially those native medicinal plants have been selected to assess their phytotoxity or allelopathic potential on seed germination and seedling growth of agricultural crops (Turker and Usta, 2008; Anjum et al., 2010; Gilani et al., 2010; Umer et al., 2010). Fujii et al. (2003) surveyed allelopathic potential of 239 Japanese medicinal plants by using sandwich method. Reigosa and Pazos-Malvido (2007) tested phytotoxic effects of 21 plant secondary metabolites on *Arabidopsis thaliana* germination and root growth. Razavi et al. (2010) evaluated phytotoxity of a medicinal plant of Iran, *Prangos uloptera* on growth of lettuce. In addition, several studies reported the phytotoxity of organic solvent extracts of plants such as: methanol, ethanol, hexane and dichloromethane extracts (Turker and Camper, 2002; Goncalves et al., 2009; Saeed et al., 2010) . Phytotoxity analysis has been the important approach for identifying plants that are likely sources of herbicidal compounds of interest (Duke et al., 2000a).

Some potential candidates with strong allelopathic

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properties have been found out and have shown promising prospect for natural herbicides development (Duke et al., 2002; Vyvyan, 2002; Batish et al., 2007).

Stellera chamaejasme, belongs to the family of Thymelaeaceae, is a perennial grassland poisonous plant widely distributed in north and southwest China. The root of *S. chamaejasme* is a well-recognized traditional Chinese medicine for treatment of hydrothorax, ascites, carbuncles, sores, scrofula, cough, chronic tracheitis and skin diseases (Jiangsu New Medical College, 1977). Recently, studies also showed that *S. chamaejasme* had antitumor (Yoshida et al., 1996), antiviral (Yang and Chen, 2008), free radical scavenging (Myagmar and Aniya, 2000), inhibition of leukemia cell growth (Tsolmon et al., 2010) and immunomodulatory activities (Xu et al., 2001a).

Different types of secondary metabolites of *S. chamaejasme* such as: flavonoids (Jin et al., 1999b), biflavanones (Xu et al., 2001a), coumarins (Modonova et al., 1985), bicoumarin (Xu et al., 2001b), diterpene (Jiang et al., 2002), phenolic compounds (Feng et al., 2008) and phenylpropanoid glycosides (Jin et al., 1999a) have been isolated and characterized. The insecticidal activity (Tang and Hou, 2008), acaricidal activity (Shi et al., 2004) and antimicrobial activity (Yang et al., 2005; Ma et al., 2009) of *S. chamaejasme* extracts have been reported.

S. chamaejasme has more competitive capability than surrounding plants in growth (Liu et al., 2004). The allelopathic effect of *S. chamaejasme* on some pasture grasses has been described, which showed that the aqueous extracts of root, stem, leaf and flower of *S. chamaejasme* had inhibitory effect on seed germination and seedling growth (Zhou et al., 1998; Cao et al., 2007; Wang et al., 2009). These results implicate that *S. chamaejasme* contains phytotoxin or allelochemicals impairing the growth of other plants, which may also act as a potential inhibitory agent on field weed. However, few works have been done for evaluating allelopathic effect of *S. chamaejasme* on crop plants and weeds. Bi and Liao (2010) investigated allelopathic effect of extract from mixture of root, stem and leaf of *S. chamaejasme* on eight crops, but they merely tested the influence of equeous extract on seed germination and vigor. The objective of this study was to assess the phytotoxicity of aqueoues extracts from *S. chamaejasme* root on seed germination and seedling growth of rapeseed (*Brassica napus*), sesame (*Sesamum indicum*), wheat (*Triticum aestivum*) and maize (*Zea mays*).

MATERIALS AND METHODS

Plant material and extracts preparation

Roots of *S. chamaejasme* were collected from Ruoergai grassland, Sichuan province (China). The plant material was authenticated by Prof. Taiping Hou from Sichuan University (Chengdu, China). Fresh roots were washed and air dried in shade for a week, the dried root was crushed into flocci powder.

The dry root powder (100 g) of *S. chamaejasme* was soaked in 1000 ml distilled water at room temperature for 24 h. The aqueous extracts were vacuum filtered through double layer filter paper to remove debris and the final volume was adjusted to 1000 ml which served as stock solution of 100 g/L aqueous extract. This stock solution was stored at 5°C and was later diluted with distilled water to obtain the different concentrations to be tested (50, 25, 12.5, and 6.25 g/L).

Phytotoxic effects analysis of the plant extracts

Seeds of rapeseed (*Brassica napus*), sesame (*Sesamum indicum*), wheat (*Triticum aestivum*) and maize (*Zea mays*) were obtained from the Xinglong seeds company (Mianyang, China). Before germination tests, the healthy crop seeds were surface- sterilized with 1% sodium hypochlorite (NaOCl) water solution for 5 min and then washed five times with distilled water.

To test the phytotoxicity, 10 ml of the extract at different concentrations were added separately on double layer sterile filter papers in 9 cm petri dishes, and 30 wheat seeds, 40 rapeseed and sesame seeds, 20 maize seeds of uniform size and weight were evenly placed into the treated filter papers. The same volume distilled water (10 ml) was added to serve as the control.

The petri -dishes were incubated in a dark germinating chamber for 10 days at 25 $\pm$ 2°C and 97% relative humidity, each treatment was replicated four times in a completely randomized experiment design. During this period the petri -dishes were observed daily and an equal amount of distilled water was added to each petri-dish as needed to prevent seeds or seedlings from drying out. Germination was defined as the point coleoptiles were visible and counted daily for 10 days. After 10 days, the germination percentage and speed were calculated. The mean germination time (MGT) has been chosen for measure the germination speed, it can be calculated by using the relation: MGT = ($n \times d$)/ N, where n is number of seeds germinated on day d and N is the total number of germinated seeds (Oyun, 2006).

Growth measurements of four crops seedlings were determined after 10 days of germination. The length of root and shoot of seedlings, and the number of lateral roots were measured. All seedlings were harvested and oven dried at 60ºC to a constant weight to determine the dry weight. The total dry weight of each replication was converted to dry weight per seedling.

Statistical analysis

The results of bioassay experiments were subjected to analysis of variance (ANOVA) using data processing system (DPS V7.05). Differences between means were determined using least significant difference (LSD) test (P<0.05).

RESULTS

Germination

The influence of aqueous extract of *S. chamaejasme* root on germination of four crops is presented in Table 1. The low concentration treatments (6.25, 12.5 and 25 g/L) showed no significant effect on seed germination as compared to the control. However, the obvious inhibitions on germination percentage were found at high concentration treatments (50 and 100 g/L) in four crops and the differences between these treatments and the

Table 1. Effect of aqueous extract of *S. chamaejasme* on germination percentages of four crops.

Concentration (g/L)	Germination rate (%)			
	Rapeseed	Sesame	Wheat	Maize
0	75.0 ± 9.4a	93.8 ± 6.0a	85.0 ± 8.8a	41.3 ± 4.8ab
6.25	75.6 ± 3.1a (-0.8)	92.5 ± 6.1a (1.4)	81.7 ± 4.3ab (3.9)	43.8 ± 4.8a (-5.9)
12.5	77.5 ± 5.4a (-3.3)	91.9 ± 6.6a (2.1)	78.4 ± 6.9ab (7.8)	40.0 ± 4.1abc (3.5)
25	76.3 ± 7.5a (-1.7)	92.5 ± 6.1a (1.4)	75.0 ± 7.9ab (11.8)	36.3 ± 2.5bc (12.2)
50	36.3 ± 3.2b (51.7)	70.6 ± 4.3b (24.7)	72.5 ± 9.6b (14.7)	33.8 ± 4.8c (18.3)
100	33.1 ± 5.2b (55.8)	50.0 ± 8.9c (46.7)	71.7 ± 4.3b (15.7)	23.8 ± 4.8d (42.5)

Each value is the mean ± S.D. of four repetitions. Means with different letters in the same column differ significantly from each other at the 5% level as determined by LSD test. Values in parenthesis indicate inhibitory percentage in germination as compared to control (0 g/L).

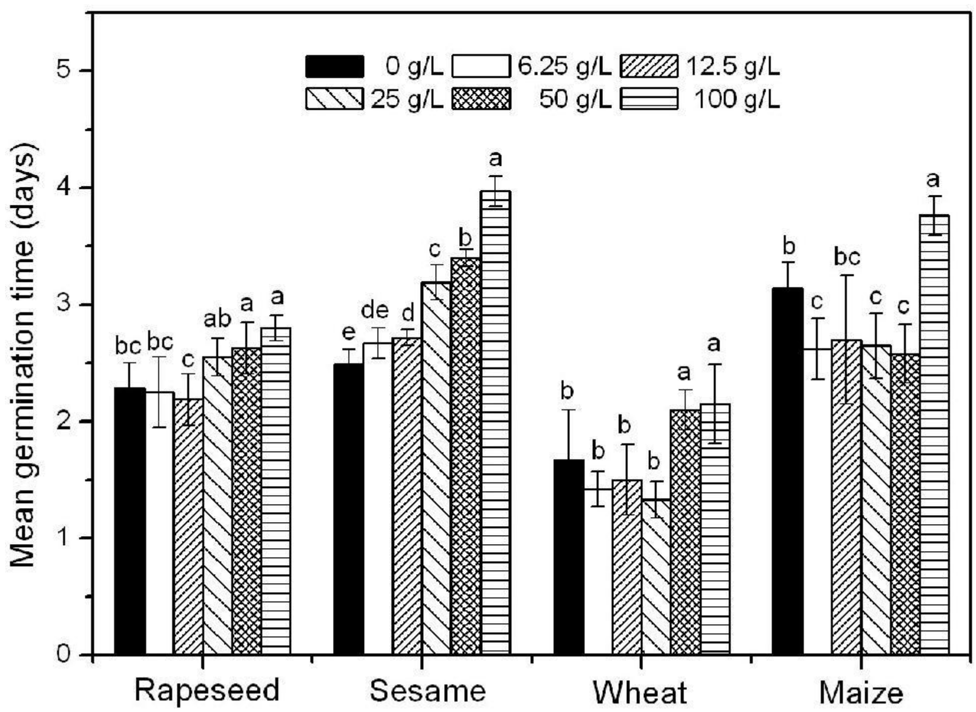

Figure 1. Effect of aqueous extract of *S. chamaejasme* on MGT of four crops. Vertical bars show standard deviation of means of four replicates (n=4). Values with different letters show significant difference as determined by LSD Test at $p<0.05$.

control reached significant level. The extent of inhibition increased with increasing extract concentration.

Mean germination time

Mean germination time (MGT) reflect the seed germination speed. Figure 1 shows different response among four crops for MGT. The aqueous extract delayed the germination of sesame most obviously, which displayed significant increase of MGT at 12.5 g/L and above concentration. However, a significant increase of MGT of rapeseed and and wheat was only found at 50 and 100 g/L concentration treatment. In maize, only the highest concentration treatment (100 g/L) showed significant increase effect on MGT whereas lower concentration treatments (6.25, 25 and 50 g/L) significantly decreased the germination time.

Shoot length

The aqueous extracts of five concentrations almost showed the negative action on shoot length of rapeseed,

Table 2. Effect of root aqueous extract of *S. chamaejasme* on shoot length of four crops.

Concentration (g/L)	Shoot length (cm)			
	Rapeseed	Sesame	Wheat	Maize
0	2.83 ± 0.26a	2.40 ± 0.26a	17.33 ± 0.89a	7.83 ± 0.46c
6.25	2.84 ± 0.36a (-0.4)	2.00 ± 0.18b (16.7)	17.07 ± 0.72a (1.5)	10.30 ± 0.47a (-31.6)
12.5	2.56 ± 0.09a (9.6)	1.93 ± 0.05b (19.8)	16.39 ± 1.21ab (5.4)	9.73 ± 0.93ab (-24.2)
25	2.46 ± 0.31a (13.0)	2.05 ± 0.13b (14.6)	14.98 ± 0.85b (13.6)	9.00± 0.24b (-14.9)
50	1.52 ± 0.33(46.3)	0.98 ± 0.10c (59.4)	12.55 ± 1.19c (27.6)	8.93± 0.75b (-14.0)
100	1.08 ± 1.02c (61.8)	0.33 ± 0.05d (86.5)	10.17 ± 1.12d (41.3)	6.23 ± 0.83d (20.5)

Each value is the mean ± S.E. of four repetitions. Means with different letters in the same column differ significantly from each other at the 5% level as determined by LSD test. Values in parenthesis indicate inhibitory percentage in shoot length as compared to control (0 g/L).

Table 3. Effect of root aqueous extract of *S. chamaejasme* on root length of four crops.

Concentration (g/L)	Root length (cm)			
	Rapeseed	Sesame	Wheat	Maize
0	5.08 ± 0.81a	3.38 ± 0.13a	10.30 ± 0.66a	8.25 ± 0.51d
6.25	4.90 ± 0.63a (3.5)	2.68 ± 0.25b (20.9)	10.13 ± 0.43a (1.7)	13.08 ± 0.82a (-58.5)
12.5	3.33 ± 0.83b (34.6)	2.13 ± 0.22c (37.1)	10.28 ± 0.39a (0.2)	11.50 ± 0.79b (-39.4)
25	1.40 ± 0.37c (72.4)	1.75 ± 0.33d (48.2)	9.73 ± 0.67a (5.6)	9.53 ± 0.76c (-15.5)
50	0.20 ± 0.00d (96.1)	0.13 ± 0.05e (96.3)	5.58 ± 0.62b (45.9)	7.43 ± 0.82d (10.0)
100	0.18 ± 0.05d (96.6)	0.10 ± 0.00e (97.0)	3.55 ± 0.48c (65.5)	4.30 ± 0.74e (47.9)

Each value is the mean ± S.E. of four repetitions. Means with different letters in the same column differ significantly from each other at the 5% level as determined by LSD test. Values in parenthesis indicate inhibitory percentage in root length as compared to control (0 g/L).

wheat and sesame (Table 2). Sesame was more sensitive to extracts than rapeseed and wheat. Compared with the control, significant decrease of shoot length was observed in sesame at all treatments with aqueous extract, but in wheat and rapeseed, the minimum concentration of extract significantly inhibited shoot growth was 25 and 50 g/L respectively. It was interesting to record that only the maximum concentration of extract (100 g/L) showed adverse effect on shoot growth of maize but the extracts of low concentration (from 6.25 to 50 g/L) displayed distinct stimulation effects.

Root length

Similar to the effect on shoot growth, all of the extract treatments decreased the root length of rapeseed, wheat and sesame and the minimum concentrations showed significant inhibition compared with the control were 12.5, 50 and 6.25 g/L respectively (Table 3). However, the root length of maize in the treatments with 6.25, 12.5 and 25 g/L extracts was significantly higher than in the control. Only in the treatments with 50 and 100 g/L extracts, the root length of maize was observed to be decreased.

Number of lateral roots

The effect of aqueous extracts on the number of lateral roots is shown in Table 4. A significant inhibitory effect on the number of lateral roots of sesame was observed at all extract concentrations. In rapeseed, significant inhibitory effect was found only at 12.5 g/L and above concentration extracts but slight stimulation effect at 6.25 g/L concentration. In wheat, a significant stimulation effect on growth of lateral roots was recorded at 50 g/L concentration extract and no inhibitory effect was observed at other concentration extracts compared with the control. The lateral roots of maize increased significantly when treated with the aqueous extracts from 6.25 to 50 g/L concentration. The maximum number of lateral roots was 10.0 at 25 g/L concentration treatment.

Dry weight of seedling

Different effect of aqueous extract on seedling biomass of four crops was obtained (Table 5) . The minimum extract concentrations in which the dry weight of rapeseed, wheat, maize and sesame seedling decreased significantly compared with control were 25, 6.25, 50 and

Table 4. Effect of root aqueous extract of *S. chamaejasme* on number of lateral roots.

Concentration (g/L)	Number of lateral roots			
	Rapeseed	Sesame	Wheat	Maize
0	9.0 ± 0.8a	4.7 ± 0.4a	5.8 ± 0.3b	7.5 ± 0.4c
6.25	9.3 ± 1.0a (-2.8)	3.5 ± 0.8b (25.5)	5.4 ± 0.3b (7.3)	9.3 ± 0.5ab (-24.3)
12.5	5.0 ± 0.8b (44.4)	3.4 ± 0.9b (28.7)	5.7 ± 0.2b (1.3)	8.6± 0.4b (-14.7)
25	2.8 ± 1.0c (69.4)	3.6 ± 0.6b (23.4)	5.8 ± 0.1b (0)	10.0 ± 0.9a (-33.0)
50	1.5 ± 0.6d (83.3)	3.4 ± 0.5b (27.1)	6.9 ± 0.6a (-18.1)	9.3 ± 0.9ab (-23.3)
100	1.0 ± 0.0d (88.9)	3.2 ± 0.3b (31.9)	5.8 ± 0.5b (0)	6.8 ± 0.6c(9.7)

Each value is the mean ± S.D. of four repetitions. Means with different letters in the same column differ significantly from each other at the 5% level as determined by LSD test. Values in parenthesis indicate inhibitory percentage in lateral roots as compared to control (0 g/L).

Table 5. Effect of root aqueous extract of *S. chamaejasme* on dry weight of seedling.

Concentration (g/L)	Dry weight per seedling (mg)			
	Rapeseed	Sesame	Wheat	Maize
0	4.40 ± 0.14a	2.60 ± 0.18a	51.15 ± 2.46a	58.50 ± 5.51b
6.25	4.58 ± 0.26a (-4.0)	2.30 ± 0.16b (11.5)	45.53 ± 3.08b (11.0)	71.25 ± 4.03a (-21.8)
12.5	4.30 ± 0.34a (2.3)	2.20 ± 0.18bc (15.4)	45.15 ± 2.34b (11.7)	58.25 ± 3.59b (0.4)
25	3.50 ± 0.42b (20.5)	2.10 ± 0.18bc (19.2)	45.55 ± 2.72b (11.0)	57.75 ± 6.02b (1.3)
50	3.00 ± 0.29c (31.8)	1.98 ± 0.22cd (24.0)	41.88 ± 2.85b (18.1)	41.75 ± 4.65c (28.6)
100	1.19 ± 0.02d (95.7)	1.70 ± 0.22d (34.6)	37.58 ± 2.76c (26.5)	36.00 ± 5.03c (38.5)

Each value is the mean ± S.D. of four repetitions. Means with different letters in the same column differ significantly from each other at the 5% level as determined by LSD test. Values in parenthesis indicate inhibitory percentage in dry weight as compared to control (0 g/L).

6.25 g/L respectively. At 6.25 g/L concentration treatment, the dry weight of maize seedling significantly increased with 21.8% as compared to the control.

From the inhibitory percentage of the test traits at concentration of 50 and 100 g/L (from Table 1 to 5, and Figure 1), it showed that seed germination and seedling growth of small-seeded crops (rapeseed and sesame) were more sensitive to *S. chamaejasme* aqueous extract than large-seeded crops (wheat and maize) except for the dry weight of maize.

DISCUSSION

This study has revealed that *S. chamaejasme* possesses high phytotoxity on tested crops. The aqueous extract of *S. chamaejasme* root is likely to contain flavonoids (Jin et al., 1999b), coumarins (Modonova et al., 1985; Jin et al., 1999a), diterpenes (Jiang et al., 2002) and phenolic compounds (Feng et al., 2008). One or some of these chemical components may play a significant role in the phytotoxic effect. These plant secondary metabolites have already been recognized as allelopathic chemicals in plants (Haig, 2008). Coumarin, as main and typical component of *S. chamaejasme*, might act as a plant growth inhibitor which can severely inhibit cell growth at low concentrations (Abenavoli et al., 2003). However, it is unknown to date what components of *S. chamaejasme* act as allelopathic chemicals.

The phytotoxicity effect of aqueous extract on seed germination showed a significant concentration-dependent effect. The treatments with 50 and 100 g/L significantly reduced the germination rate, whereas the treatments with low concentration extract (from 6.25 to 25 g/L) had no obvious inhibitory effect. This result is supported by the findings of Mutlu and Atici (2009) and Zahed et al. (2010), who observed a pronounced concentration-dependent effect in barley treated with root and leaf extract of *Nepeta meyeri* and in wheat treated with essential oil from *Schinus molle* fruit and leaf. Cao et al. (2007) have also reported that the germination of two pastures (*Galium verum* and *Lilium pumilum*) decreased with increasing aqueous extract concentration of *S. chamaejasme*. Bi and Liao (2010) researched the allelopathic effect of *S. chamaejasme* aqueous extract on eight crop plants, who reported the germination inhibitory of rapeseed and wheat at 50 g/L concentration was 17.8 and 3.3% respectively. In our study, the germination inhibitory of rapeseed and wheat at 50 g/L concentration was 51.7 and 14.7% respectively. This difference might possibly be attributed to different material and soakage time used. We applied the crushed dry root powder of *S. chamaejasme* to extract for 24 h whereas Bi and Liao (2010) used the uncrushed mixture materials of root, stem and leaf to extract for only 15 h. Different material and extract method may lead to different concentration of phytotoxic chemicals.

Response on the mean germination time (MGT) also demonstrated the different sensitivity of crops to *S. chamaejasme*. The MGT of dicotyledonous plants (rapeseed and sesame) increased with increasing extract concentration except at 6.25 and 12.5 g/L concentration in rapeseed. Interestingly, the MGT of monocotyledonous plants (wheat and maize) extended only at above 50 g/L concentration and at 100 g/L concentration respectively, while other treatments had a decreased effect on the MGT. Similar results were observed by Tanveer et al. (2008), who reported that the MGT of wheat and maize reduced when treated with the leaf aqueous leachate of *Xanthium strumarium*. However, Oyun (2006) reported the delay effect on the MGT of maize with the extracts of *Gliricidia sepium* and *Acacia auriculiformis* at three tested concentration. These inconsistent results might be due to the different allelopthic chemicals in different plants.

The reduction in shoot length, root length, number of lateral roots and dry weight of rapeseed and sesame further reflected its higher susceptibility to *S. chamaejasme* than wheat and maize. The root growth of rapeseed was effected more than shoot growth, but similar phenomenon were not observed in other three crops. This might be the reason for different action of same allelopathic chemicals from *S. chamaejasme* on different plant organs. We found that some concentration treatments of *S. chamaejasme* extract showed promotion effect on seedling growth of maize. This result is supported by the findings of Mubarak et al. (2009), who reported that the leaf water extract of some trees significantly increased hypocotyl and radicle length of maize. Wang et al. (2009) also reported that the decayed residue of *S. chamaejasme* in soil had evident stimulation effect on seeding growth of *Lolium perenne*.

From the inhibitory percentage of aqueous extracts on tested traits compared with control in our study, it is noteworthy that dicotyledonous and monocotyledonous plants had different sensitive response to aqueous extract of *S. chamaejasme*. Germination and growth of rapeseed and sesame (dicotyledon) were inhibited more strongly by aqueous extract of *S. chamaejasme* than wheat and maize (monocotyledon). Goncalves et al. (2009) also found aqueous and hexane extracts from *Drosophyllum lusitanicum* leaf significantly inhibited the seed germination of lettuce and wheat, but wheat was less sensitive. Similar phenomenon that wheat and maize were less sensitive than other dicotyledon was observed by Maharjan et al. (2007) and Umer et al. (2010) . This phenomenon may probably be due to the size of seed. Small seeded crops and weeds are more susceptible to allelochemicals than the large seeded plants, which is attributed to greater surface to volume ratio, resulting in more exposure of such seeds to allelochemicals (Cheema et al., 2008). Our bioassay results indicated low sensitivity of wheat and maize on *S. chamaejasme* that may be assumed a potential for controlling small-seeded weeds in large-seeded crops production such as wheat and maize. Further research works for evaluating phytotoxity of *S. chamaejasme* on other crops and weeds with different solvent extracts are underway.

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