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The Allelopathic Effect of the Exotic Tree Acacia saligna on the Germination of Wheat and Canola

Mohamed Kamel, Sabah A. Hammad*

Botany Department, Faculty of Science, South Valley University, Qena 83523, EGYPT
* Corresponding author: sa_ham2ad@yahoo.com

Abstract. This study was carried out to investigate the allelopathic effect of aqueous extracts derived from leaves and stems of *Acacia saligna* (Labill.) H.L.Wendl. upon two agricultural crops, wheat and canola. Seed germination (%), shoot and root elongation, fresh and dry weight, vigor index and phytotoxicity parameters were estimated. Leaf extract exhibits higher inhibitory effect than stem extract. Wheat seeds were more tolerant to the allelopathic action of *A. saligna* extracts than canola. Canola germination minimized to 8.33% at concentration 10% of leaf extract but the percent of germination was 60% in the case of stem extract. At 10% leaf extract, 76.67% of wheat seeds germinated; but at 10% stem extract, 93.33% of the seeds were germinated. The other growth parameters as shoot and root length, fresh and dry weight and vigor index also showed continued decrease with the increasing of allelopathic extract concentration. Leaf extract exhibits the stronger allelopathic effect. The phytotoxic effect was stronger on the germination of canola compared with wheat. It reached up to 91.76% inhibition at concentration 10%, but reached up only 23.33% in the case of wheat, respectively.

Key words: *Acacia saligna*, Allelopathy, Allelochemical, Phytotoxicity, Canola, Wheat.

Introduction

Allelopathy is one of the important phenomena used by plant to defense against competition with others. Many species use allelopathy as effective strategy to invade, stablish and increase their area of occupancy. MAY & ASH (1990) defined allelopathy as the interference mechanism, in which live or dead plant materials release chemical substances, which inhibit or stimulate the associated plant growth. These chemical substances are called allelochemicals (intermediary metabolic products) and include: phenols, terpenes, glycosides, alkaloids, amino acids and sugars as preferably defense compounds (HARBORNE, 1989).

Acacia saligna (Labill.) H.L.Wendl. (subfamily Mimosoideae, family Fabaceae) is a very adaptable and fast growing tree native to Western Australia (MIDGELY & TURNBULL, 2003). In some areas, *A. saligna* has gone on to become an invasive species with a wide range of impacts. In areas where it has become invasive, *Acacia saligna* is known to form dense monospecific stands, excluding native species and preventing their regeneration (HOLMES & COWLING, 1997; HADJIKYRIAKOU & HADJISTERKOTIS, 2002). *Acacia saligna* has been planted extensively in Africa and the Mediterranean region including Egypt (MIDGELY & TURNBULL, 2003).

According to SCHWARTZ *et al.* (1996) and SHINWARI *et al.* (2012); the exotic invasive species are considered to be the second largest cause of biodiversity loss after habitat destruction. Most of the exotic plant

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effects reported have been identified as caused by allelopathic interaction which resulted in interference with physiological and biochemical processes in plants, due to chemicals released by exotic plants (ALHAMMADI, 2008). RAFIQUL HOQUE *et al.* (2003) showed that trees of genus *Acacia* are known as a versatile source of components with bioactive properties. BARNES *et al.* (1996) suggested a large inhibitory potential in *Acacia* genus which dominates the dry south Saharan regions of Africa.

Many workers (BITENDE & LEDIN, 1996; CODER KIM, 1999; SHAYO & UDEN, 1999; ABDULRAZAK *et al.*, 2000; DUBE *et al.*, 2001; RUBANZA *et al.*, 2005 and NAKAFEERO *et al.*, 2007) found that tannins, phenols and phenolic compounds are the most common compounds in different *Acacia* organs (leaves, stem, pods, fruits, petiole, and root). DUKE (1983) reported that natal-grown bark of *Acacia saligna* contains up to 30.3% tannin.

The aim of the current work was to test the allelopathic effect of *Acacia saligna* leaf and stem extracts on the seed germination of canola and wheat.

Material and Methods

Preparation of extracts. Aerial parts (stems and leaves) of *Acacia saligna* plants at the vegetative stage were collected separately, air dried and ground to obtain fine powder. For extract preparation: 10 g powder (leaves or stem) was added to 100 ml distilled water and left at room temperature for 24 h to obtain 10% (w/v) extract. The extract was filtered through filter paper. This was served as original stock solution and stored at 4ºC for further use (EL-KHATIB & ABD-ELAAH, 1998; EL-KHATIB & HEGAZY, 1999; EL-KHATIB, 2000). Different concentrations (2, 4, 6, 8 and 10%) of aqueous leaf and stem extracts were prepared.

Germination and seedling growth bioassay. Seeds of wheat (*Triticum aestivum* variety Giza 168) and canola (*Brassica napus* variety sero 4) were obtained from the Faculty of Agriculture, South Valley University. The healthy seeds were surface sterilized with 3% ethanol and thoroughly rinsed with sterilized distilled water. The comparative assessments of the influence of various concentrations of aqueous extracts on seed germination and early growth of seeds were carried out in 9 cm Petri dishes.

Five ml of the various concentrations of leaf and stem extracts (control (distilled water), 2, 4, 6, 8 and 10 %) was added to the dishes (4 replicates per treatment) containing 10 uniformly seeds of the tested species on two pieces of Whatman no. 1 filter papers. Dishes were incubated under the growth chamber conditions with 25/18˚C day and night temperature respectively, 13/11 hours light/dark period (WU *et al.*, 2007). Germination of seeds and early seedling growth were recorded after 7 days of germination. Germination percentage, shoot and root length, fresh and dry weight were estimated. Also vigor index (VI) and phytotoxicity percentage (reduction) was computed using the following equations:

% Germination = (no of germinated seeds / total no of seeds) × 100 (after SCOTT *et al.*, 1984).

VI = [seedling length (cm) × germination percentage] (after ABDUL-BAKI & ANDERSON, 1973).

% Phytotoxicity = [(Control - Extracts) ÷ Control] × 100 (after VOKOU, 1992).

Data were statistically analyzed using ANOVA and difference between means under different treatments were calculated by Least Significant Differences (LSD) test at $P<0.05$ and $P<0.001$using SPSS program v. 20 (IBM Corp., 2011).

Results

Data of the test species demonstrates significant degrees of suppression and a negative response to the increasing concentration of different extracts. There are significant differences between the different concentrations and control especially at higher concentrations.

Germination percentages of both canola and wheat seeds decreased under the effect of different concentrations of *A. saligna* extracts (Table 1). The treated seeds with leaf extract inhibited significantly (up to 92% inhibition in canola and 23% in wheat) compared with those treated with stem extract (40% inhibition in canola 20% in wheat). The allelopathic effect was stronger upon canola seeds compared with wheat.

Recorded germination percentage of canola was 100% under the control treatment. The application of different concentrations of *A. saligna* leaf extract reduced germination significantly from 98.33% at concentration of 2% to 8.33% at concentration 10%. On the other hand, stem extracts showed lower effect on germination rate. At concentration 10%, the germinated seeds reached 60%. In case of wheat, seeds were more tolerant to the allelopathic effect of *A. saligna* extracts, whether extracted from leaves or stems. At concentration 10%; 76.67% of the seeds germinated under leaves extract. With the application of stem extracts, the percentage varied between increase and decrease with different concentrations. The lowest percentage was recorded at concentration 2% (80%) while other percntages increased with concentration (Table 1).

Table 2 explains the allelopathic effect of *A. saligna* extracts on canola fresh and dry weight. Canola exhibits a significant decrease in both fresh and dry weight under the different concentrations of leaf extract. The estimated fresh weight was 380 mg at control. It decreased down to 16 mg at concentration 10%. On the other hand, stem extract didn't exhibit any significant effect on the fresh or dry weight. On contrast, the lower concentrations of stem extract (2% and 4%) showed a simulating effect on the fresh and dry weight, but at concentrations 6% - 10% a slight decrease was noticed.

Table 1. Germination percentage (%) ± standard deviation of canola and wheat under the different concentrations of *Acacia saligna* leaf and stem extracts.

Treatment	**Canola**		**Wheat**	
	Leaf extract	**Stem extract**	**Leaf extract**	**Stem extract**
Control	100.0±0.00	100.00±0.00	100.0±0.00	100.00±0.00
2%	98.33±2.89	98.33±2.89	100.00±0.00	80.00±17.32
4%	83.33*±17.56	98.33±2.89	80.00**±17.32	93.33±11.54
6%	46.67**±7.63	93.33±2.88	76.67**±5.78	90.00±17.32
8%	15.00**±8.66	85.00*±5.24	73.33**±11.54	86.67±5.77
10%	8.33**±7.64	60.00**±6.55	76.67**±11.55	93.33±5.77

* at P<0.05 and ** P<0.01.

Table 2. Fresh and dry weight (mg/plant) ± standard deviation of canola seedlings under the different concentrations of *Acacia saligna* leaf and stem extracts.

Treatment	**Leaf extract**		**Stem extract**	
	Fresh wt., mg	**Dry wt., mg**	**Fresh wt., mg**	**Dry wt., mg**
Control	380 ±12	51±8	429±59	38±3
2%	310* ±53	38*±6	489±52	41±3
4%	129** ±26	34**±11	493±48	40±2
6%	46.0**±22	15**±4	406±123	35±9
8%	23.0**±14	7.0**±3	273±89	11±11
10%	16.0**±15	6.0**±5	143±47	5.0±8

* at P<0.05 and ** P<0.01.

Table 3. Fresh and dry weight (mg/plant) ± standard deviation of wheat seedlings under the different concentrations of *A. saligna* leaf and stem extracts.

Treatment	Leaf extract		Stem extract	
	Fresh wt., mg	Dry wt., mg	Fresh wt., mg	Dry wt., mg
Control	1204±15	302±17	1144±31	265±1
2%	1373±14	367±13	1223±25	280±65
4%	1019±28	308±6	1346±14	227±17
6%	745.0**±5	309±15	1278±35	291±87
8%	633.0**±9	266±52	1290±13	307±4
10%	596.0**±16	307±71	1344±16	331±41

* at P<0.05 and ** P<0.01.

The results of wheat seedlings were illustrated in Table 3. Significant differences appeared at concentrations 6%, 8% and 10% of leaf extract on the fresh weight only. Under the effect of stem extract there was a slight increase in both fresh and dry weight with increasing concentration compared with control.

Leaf extract caused a significant decrease in both shoot and root lengths of canola at concentrations 4% - 10% (Table 4). At concentration 2% there was a significant increase in the shoot length (3.66 cm) compared with control (2.92 cm). At concentrations 4% - 10%, the recorded shoot lengths were 1.21, 1.03, 1.58 and 0.42 cm respectively, while the recorded root lengths were 3.55, 1.74, 0.53, 0.57 and 0.25 cm respectively.

Canola shoot lengths exhibit different reactions toward stem extract. A stimulating effect appeared at concentrations 2%, 4% and 6% with significant values at the first two concentrations. At the higher concentrations, shoot lengths decreased compared with control. Root length decreased with the increasing concentration showing significant values starting from 6% to 10%. The lowest length was detected at 10% concentration. The reductions in both shoot and root length (2.92 and 1.90 cm) reach to about 60% at 10% concentration.

In case of wheat, the extracts of *A. saligna* leaves and stems caused a significant decrease in the shoot and root length (Table 5). The decrease in shoot and root length was significant at 4%, 6%, 8% and 10% concentrations of leaves extract. The highest decrease was recorded at concentration 10%. Stem extract decreased the length of wheat seedlings shoots significantly at concentration 10%, while the roots length decreased significantly at 8% and 10%. The stems extract at concentration 2% showed a stimulating effect where the length of both shoots and roots increased comparing with control.

Table 4. Shoot and root length (cm) ± standard deviation of canola under the different concentrations of *Acacia saligna* leaf and stem extracts.

Treatment	Leaf extract		Stem extract	
	Shoot length, cm	Root length, cm	Shoot length, cm	Root length, cm
Control	2.92±0.20	6.20±1.63	3.41±0.29	4.76±2.40
2 %	3.66**±0.21	3.55**±0.76	4.45*±0.50	4.35±0.85
4 %	1.21**±0.19	1.74**±0.42	4.47*±0.47	3.79±0.40
6 %	1.03**±0.27	0.53**±0.15	3.92±1.01	2.72*±0.47
8 %	1.58**±0.37	0.57**±0.08	2.80±0.56	2.49*±0.33
10 %	0.42**±0.38	0.25**±0.22	2.92±0.77	1.90**±0.54

* at P<0.05 and ** P<0.01.

Table 5. Shoot and root length (cm) ±standard deviation of wheat seedlings under the different concentrations of *Acacia saligna* leaf and stem extracts.

Treatment	Leaf extract		Stem extract	
	Shoot length, cm	**Root length, cm**	**Shoot length, cm**	**Root length, cm**
Control	8.58±0.33	11.12±0.71	8.87±0.63	11.30±0.40
2 %	7.56±0.33	10.53±0.69	9.73±0.44	11.89±1.27
4 %	6.08**±0.42	7.16**±1.05	8.80±0.53	10.69±0.56
6 %	3.39**±1.01	3.71**±0.62	9.33±0.44	10.96±1.21
8 %	3.00**±0.26	3.21**±0.13	8.56±0.31	8.54**±0.23
10 %	0.78**±0.59	2.25**±0.11	7.54**±0.60	7.43**±0.76

* at P<0.05 and ** P<0.01.

Subjecting canola and wheat seeds to different concentrations of *A. saligna* extracts reduced the vigor index of both species seedlings (Fig. 1A and B). Canola seedlings were affected more than wheat ones. The effect of leaves extract was stronger than stems extract. Vigor index of canola decreased quickly, under the effect of allelo-compounds extracted from *A. saligna* leaves, from 912 to 2.89 at concentration 10%. On the other hand, vigor index of wheat was 2016 and dropped gradually to 236.9.

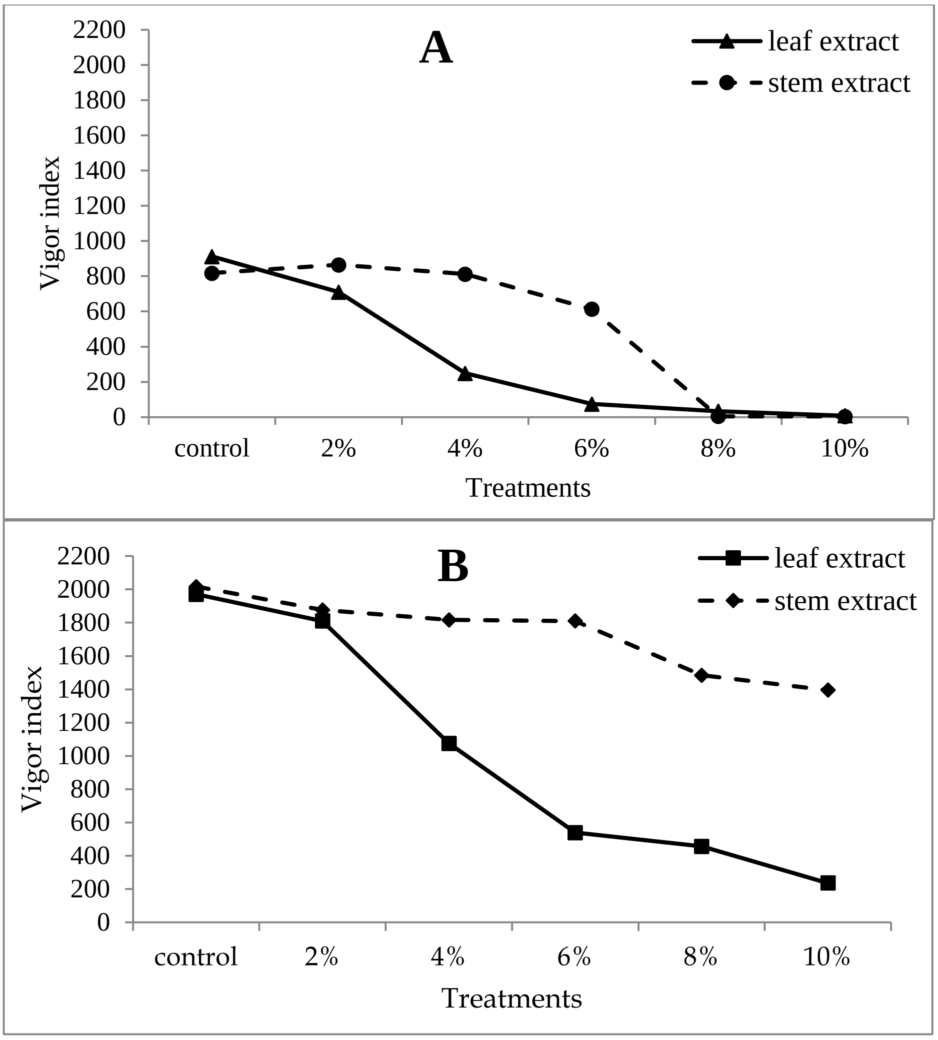

Fig. 1. Vigor index (VI) for canola (A) and wheat (B) seeds under different concentrations of *A. saligna* leaf and stem extracts.

Results represented in Fig. (2A and B) indicate the phytotoxic effect of *A. saligna* extracts on canola and wheat species. There was a remarkable reduction in the germination percentage for the two examined species comparing with control. The phytotoxic effect was stronger on canola compared with wheat. It reached up to 91.76% inhibition at concentration 10%, but reached up only 23.33% in the case of wheat. At lower concentration (2%), leaf extract exhibit neglected phytotoxic effect on canola. It cleared that the phytotoxicity increased paralleled with the increasing of extract concentration in the case of canola. In case of wheat, toxicity action was smaller and did not exceed 26% (leaf extract) and 13% (stem extract).

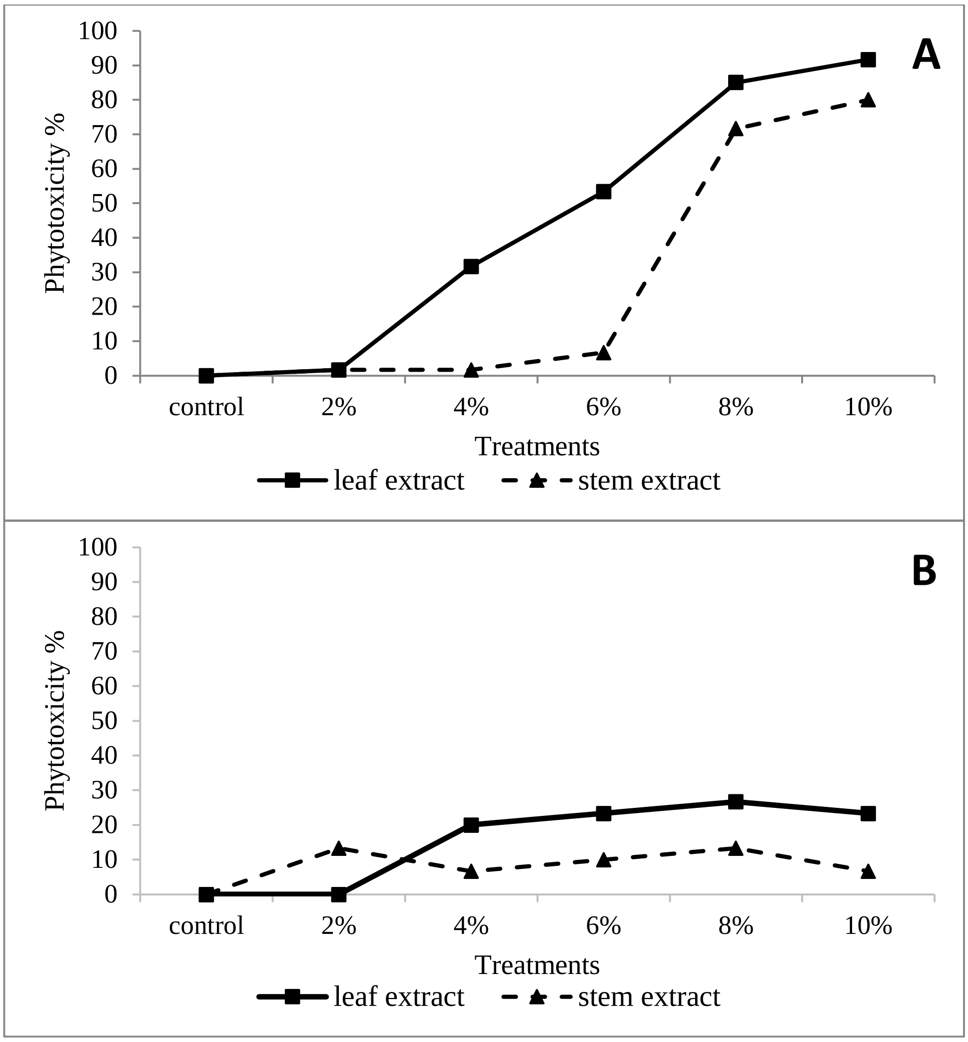

Fig. 2. Phytotoxic effects of the different concentrations of *A. saligna* leaf and stem extracts on the germination of canola (A) and wheat (B) seeds.

Discussion

Data presented in this study demonstrates the allelopathic effect of *A. saligna* leaf and stem extract on seed germination and seedling growth of two different seeds (canola and wheat). Data revealed that the inhibition in the germination percentage occurred in both canola and wheat may be owing to the alteration of enzyme activity, which affects the mobilization of storage compounds during germination (EINHELLIG, 1995) or inhibition of mitosis (ROSHCHINA, 2001; IRSHAD & CHEEMA, 2004; MOHAMADI & RAJAIE, 2009). Delaying or cessation of storage compounds transmission can reduce respiration substrates and metabolic energy in allelochemicals exposed seeds which decrease germination and seedling growth (YARNIA *et al.*, 2009).

The inhibition caused by leaf extract of *A. saligna* was more effective than by stem extract. SUMAN *et al*. (2002), TEFERA (2002), DEVI & DUTTA (2012), VERMA *et al*. (2012) reported that leaf extract of plants showed most prominent allelopathicity than root, stem and seed extract. PALANI & DASTHAGIR (1998) observed a significant yield reduction in cowpea, sesame, horse gram and sorghum by aqueous leaf extracts of *Acacia holosericea*.

Data also revealed that at lower concentrations of the extract it might have a positive effect or non-inhibitory effect on the germination or growth of the test seeds. These results were also obtained by Cheema in 1988 (after RANDHAWA *et al*., 2002), who reported that lower concentration show promoting effect, while higher concentration had inhibitory effect.

The reduction in the fresh and dry weight of the studied seedlings under leaf extract was higher than stem extract, especially in canola. The same results were obtained by El-KHATIB *et al*. (2004) who suggests that the effect of allelopathic extracted substances on cell division causes a reduction in root growth. The root growth reduction affects the root efficiency. Consequently, this decreases mineral uptake, nutrient absorption and the transport of nutrients from the root to other plant parts. In summation, the dry matters become reduced.

Both shoot and root lengths of tested canola and wheat were more sensitive to the leaves extract of *A. saligna* compared with extract of stems. The inhibitory effect on root length was obvious than shoot length. The same results were obtained by AL HAMDI *et al*. (2001), INDERJIT & STREIBIG (2001) and BELZ & HURLE (2004) who reported that wheat root length had the highest sensitivity to allelochemicals and root length is more affected than shoot growth. Root length may be a key parameter to verify allelopathic strength. The higher inhibition of roots compared with shoots may be due to their more intimate contact with the treated filter paper.

The drop in germination percentage and growth parameters indicates the negative effect of the allelopathic action of different concentrations of the *A. saligna* extracts on the vigor status and viability of seed embryo and seedling growth. The decrease in the vigor of canola or wheat with the increase of extract concentration or vice versa is expected result that explains the allelopathic effect of *A. saligna*. Vigor index reflects the ability of seeds to produce normal seedlings under less than optimum or adverse growing conditions similar to those which may occur in the field. Reduced seed germination and seed vigor index of test crops under laboratory conditions indicated the accumulation of toxic allelopathic substances of the donor plant, which was harmful to the growth of seedlings of receptor plants (CHOU, 1992 and MANDAL *et al*., 2012).

The inhibitory action on the test species reveals the phytotoxic effect of allelochemicals in *A. saligna* extracts. Wheat tended to prevent the toxicity may by increasing its tolerance or by produce substances which can play antagonistic effect against allelopathic substances. Inhibition does not affect seed germination, seedling growth and dry matter production only but can cover all the stages of plant growth. El-KHATIB *et al*. (2004) point out that allelopathic compounds do not cause an inhibition of plant development and growth for the individual plant only but can influence an ecosystem by changing the pattern of vegetation.

Conclusion

Wheat seeds are more tolerant than canola seeds to the allelopathic effect resulted from the treatment with different concentrations of *A. saligna* extracts. The extracts of the exotic tree *Acacia saligna* showed an inhibitory effect on the germination of seeds. The unique allelopathic effect of some exotic species on native, 'inexperienced' communities also contribute to invasive success. Allelopathy is expected to be an important mechanism in the plant invasion process.

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