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ASMA EL AYEB

HICHEM BEN JANNET

FETHIA HARZALLAH SKHIRI

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Effects of *Acacia cyanophylla* Lindl. extracts on seed germination and seedling growth of four crop and weed plants

Asma EL AYEB[1], Hichem BEN JANNET[2], Fethia HARZALLAH-SKHIRI[1,*]

[1]Laboratory of Genetics, Biodiversity and Valorisation of Bioresources (LR11ES41), Higher Institute of Biotechnology of Monastir, University of Monastir, Monastir, Tunisia

[2]Laboratory of Heterocyclic Chemistry and Reactivity: Bio-organic Chemistry and Natural Products, Faculty of Sciences, University of Monastir, Monastir, Tunisia

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Abstract: The aqueous and organic extracts of the roots, stems, phyllodes, flowers, legumes, and seeds of *Acacia cyanophylla* Lindl. were assayed at different concentrations to assess their allelopathic potential. The extracts were tested on the seeds of 2 crops (*Triticum aestivum* L. and *Lactuca sativa* L.) and 2 weeds (*Peganum harmala* L. and *Silybum marianum* L.) species. The final germination percentages and the seedling shoot and root lengths were significantly reduced by the *A. cyanophylla* extracts as compared to the control. Aqueous extract from seeds was the strongest inhibitor of germination, shoot length, and root length for *L. sativa* and *P. harmala. Triticum aestivum* was tolerant to the aqueous extracts tested; however, *S. marianum* was moderately inhibited by those extracts. Organic extracts have reduced the germination of weed and crop seeds and seedling growth in high percentages. This study indicates that the biomass of *A. cyanophylla* contains allelochemicals and could be used as a postemergence herbicide.

Key words: *Acacia cyanophylla* Lindl., crop, weed, seeds, extracts

1. Introduction

Damages to human health and the environment caused by herbicides are regarded as a real problem today. In fact, herbicide residues have been shown to have a direct impact on human and animal health and may cause a loss of crops and already endangered plant species (1,2). This has resulted in an increased interest in alternative strategies leading to the development of biodegradable compounds (1). Many plants synthesize toxic substances for defense against other plants and microorganisms including viruses, bacteria, and fungi (3,4). Allelopathy is a biological phenomenon by which an organism produces one or more biochemicals that influence the growth, survival, and reproduction of other organisms (5). It is a natural and environmentally friendly technique that might prove useful in controlling weeds, increasing crop yields, and decreasing the use of synthetic pesticides (6). These biochemicals are known as allelochemicals and can have beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on the target organisms (5). The possible application of allelopathy in agriculture has been the subject of much research (7). Biologically active natural products extracted from plants have the potential to replace herbicides (8). Among plants, trees often produce an important biomass that should be exploited. *Acacia* Mill. is the second largest genus belonging to the subfamily Mimosoideae of the family Fabaceae (9), with about 1350 species (10). *Acacia* species range in size from small shrubs to large trees and are ecologically important as "pioneer" species because they rapidly establish cover following major natural disturbances (11). Researchers showed that *Acacia* trees are known as a source of components with bioactive properties, suggesting that there is a large inhibitory potential in this genus. The main use of *Acacia* species is as a fodder source, but they are also used in traditional medicine for their hypoglycemic (12), antibacterial (13), and antiinflammatory (14) activities. Other authors reported the genus' spasmogenic and vasoconstrictor actions (15) as well as its cytotoxic (16) and antioxidant (17) activities.

Acacia species affect crop growth by competing for various environmental resources as their litter interferes with the establishment and growth of the adjoining crop plants (18) by releasing numerous chemical substances, including phenolic compounds (19). Some of these substances act as allelochemicals (20) and influence

* Correspondence: fethiaprosopis@yahoo.fr

germination and seedling growth. The toxicity of *Acacia dealbata* Link on the germination and growth of some meadow species (21) and lettuce (22) during flowering has been studied. Leachates of this species showed allelopathic interference with the tested species *Zea mays*, *Dicranum* sp., *Hedera hibernica*, *Leucobryum* sp., and *Dactylis glomerata* (23). Furthermore, Jadhar and Gayanar (24) showed that *Acacia auriculiformis* A.Cunn. ex Benth. leaf extracts significantly inhibit the germination and growth of rice and the radicle growth of cowpeas. This same material decreased the germination and growth of aster (*Callistephus chinensis*) (25), wheat (26), and some agricultural crops (27,28). Oyun (29) showed that leaf leachates of *Acacia auriculiformis* significantly decreased the germination percentage of maize seeds and all the seedling growth parameters compared to the control. Similarly, the allelopathic potential of *Acacia nilotica* (L.) Willd. ex Delile was estimated, and it was determined that its leaf extracts are highly toxic for wheat (26) and for the growth of several crop and weed species (30).

Acacia cyanophylla Lindl. (syn. *Acacia saligna* (Labill.) H.L.Wendl.) is an Australian tree species introduced in Tunisia for the first time in 1930 for range land rehabilitation, particularly in the semiarid zones, and it is now extensively grown there (31). *Acacia cyanophylla* plantations are located in the subhumid and the semiarid bioclimatic regions as the species is highly resistant to drought and salinity (32). These plantations were created under various development programs of agrosilvopastoral rangeland and were used as fodder reserves particularly during periods of drought (leaves and pods) as well as for fixing coastal dunes, because they improve the soil quickly and have a high production of humus (33). *Acacia* tree trunks were used as firewood, stakes, wooden packaging, and for making paper pulp (34). Works concerning the allelopathic potential of *A. cyanophylla* are very few. This species showed an antinematode activity against gastrointestinal nematode parasitism in sheep (33). The growth of herbaceous species beneath and around *Acacia cyanophylla* trees is completely lacking. This lack of ground vegetation under its canopy indicates that it has some allelopathic potential possibly caused by fallen leaves (through decomposition of litter), plant leachates, or root exudates. Consequently, the release of allelochemicals (organic substances) into the soil inhibited seed germination and the establishment of agricultural crops and vegetation (35,36).

In the present study, we investigated the allelopathic effects of the different parts of *Acacia cyanophylla* (roots, stems, phyllodes, flowers, legumes, and seeds) in aqueous and organic extracts on 2 crops, lettuce (*Lactuca sativa* L.) and wheat (*Triticum aestivum* L.), and 2 weeds, thistle (*Silybum marianum* L.) and peganum (*Peganum harmala* L.). The work consisted of screening the effect of each of organic and aqueous extract and identifying the most effective in the aim to use its biomass as a postemergence herbicide or a template for new herbicide classes.

2. Materials and methods

2.1. Plant material

Acacia cyanophylla vegetative and reproductive parts were collected in September 2008 and February 2009 (the period for flower collection) from the area of Monastir (35°46′0″N, 10°59′0″E, in the coastal region of eastern Tunisia with a subhumid climate). After separating the different plant parts (roots, stems, phyllodes, flowers, legumes, and seeds) and cleaning them with tap water, the fresh material was oven-dried at 45 °C for 3 days, ground into a powder using a Wiley mill, and stored at 4 °C until use.

2.2. Aqueous and organic crude extract preparation

To create the extract preparations, 40 g of powder from each dried plant part was separately extracted by soaking in 1 L of distilled water at ambient temperature for 24 h to give a concentration of 40 g dry tissue L^{-1}. The 6 crude aqueous extracts were filtered through Whatman filter paper No. 1 to remove excess debris. The clear extracts were used freshly in bioassay tests.

The powdered material of the different parts of *A. cyanophylla* (75 g) was successively extracted at ambient temperature using 3 solvents with increasing polarity: petroleum ether (PE), ethyl acetate (EA), and methanol (M). Other methanolic extracts were directly (dM) prepared from 75 g of powder of the different plant parts. The 24 organic extracts were filtered and evaporated to dryness in a vacuum at 40 °C with a rotary evaporator. After determination of the yield, the extracts were stored at 4 °C until use.

2.3. Bioassays with aqueous extracts

Four target species, including 2 crops, lettuce (*Lactuca sativa*) and wheat (*Triticum aestivum*), and 2 weeds, thistle (*Silybum marianum*) and peganum (*Peganum harmala*), were used to test germination and early growth responses. Lettuce was used as a test plant because it is very sensitive to chemicals at low concentrations (37). Wheat was used because it is one of the most important agricultural foods and feed crops worldwide (38). Each crude aqueous extract was diluted with sterile distilled water to give final concentrations of 10, 20, 30, and 40 g L^{-1}. Five milliliters of each solution was added onto 2 layers of Whatman No. 1 sterilized filter paper at the bottom of a sterile petri dish (90 mm) and allowed to dry under reduced pressure. All target seeds were surface sterilized by immersion in 0.525 g L^{-1} of sodium hypochlorite for 5 min, rinsed in sterile deionized water 4 times, and soaked in a final water bath at

22 °C for 4 h. Preliminary assays proved that the bleach did not inhibit germination. For each target species, 30 swollen seeds were sown in a petri dish where the filter paper was moistened with 5 mL of distilled water. The petri dishes were sealed with Parafilm to prevent the loss of moisture and to avoid contamination; thereafter, they were kept in a growth chamber to germinate in the dark with an average temperature of 23 ± 2 °C for 7 days. Distilled water was the control. The experimental design was a randomized block in 3 replicates for each treatment. A seed was considered germinated when the radicle protruded ≥2 mm.

2.4. Bioassays with organic extracts

The 24 dried organic extracts were dissolved in methanol to compare their phytotoxic effects. Five milliliters of each extract dissolved at 6000 ppm (6 mg mL^{-1}) was put on a sheet of filter paper in a petri dish and evaporated to dryness for 24 h at 24 °C. The filter paper was moistened with 5 mL of sterile distilled water, and then 30 imbibed seeds from each target species were arranged in each petri dish and allowed to grow in a growth chamber in the dark at 23 ± 2 °C for 7 days. The treatments were arranged in a completely randomized design with 3 replications. The test conditions and parameters that were measured were the same as in the previous bioassay. The control petri dishes contained only methanol and distilled water.

2.5. Parameters measured and statistical analysis

Percentage germination and shoot and root length of each target species were measured for all seedlings in each petri dish on day 7 after placing the seeds on the medium. The data were transformed to percent of control for analysis. The inhibitory or stimulatory percent was calculated using the following equation given by Chung et al. (39):

inhibition (–) / stimulation (+) % = [(extract – control) / control] × 100,

where 'extract' is the parameter measured in the presence of the *A. cyanophylla* extract and 'control' is the parameter measured in the presence of distilled water.

The laboratory bioassays were conducted with 3 replications. The data from the experiments were subjected to analysis of variance (ANOVA) using SPSS 13.0 for Windows. The percentage data were transformed using arcsine-square root (arcsine √x) before ANOVA analysis. The means were separated at the 5% significance level by the least significant difference test (Student's test).

3. Results

The allelopathic effects of organic and aqueous *Acacia cyanophylla* extracts on seed germination and early growth in 4 weed and crop seeds are presented in Table 1. The results indicated that extracts have a varying degree of inhibitory and stimulatory effect on germination and seedling growth.

3.1. Effects of aqueous extracts

The variance analysis showed that the *A. cyanophylla* aqueous extracts had a significant effect on germination of the target seeds and on the shoot/root length of their seedlings ($P < 0.05$), except for the *Peganum harmala* seeds (Table 1).

Seed germination and growth of *Lactuca sativa* seedlings were completely inhibited by the *A. cyanophylla* seed aqueous extract at 40 g L^{-1}. All the other aqueous extracts at all the concentrations tested significantly reduced the seed germination (except the aqueous extracts from flowers) and root length of lettuce. Inhibition varied from 1.7% to 40% for seed germination and from 12.9% to 90.1% for root length. Root aqueous extracts at concentrations of 10–40 g L^{-1} were the greatest inhibitors of germination, while extracts from seeds at the same concentrations were more toxic to root growth. However, shoot length was improved. This improvement was more significant when the seedlings were exposed to the extracts from legumes and flowers and the stimulation percentages varied between 112.6% (legume aqueous extract at 20 g L^{-1}) and 46.0% (legume aqueous extract at 40 g L^{-1}). Aqueous extracts from roots (10–40 g L^{-1}) and those from seeds (30 g L^{-1}) both moderately inhibited the shoot length.

Triticum aestivum seeds were less sensitive to aqueous extracts. In fact, the highest inhibition of germination (13.4%) was by the aqueous extract from flowers at 10 g L^{-1}. The percentage inhibition of seedling growth varied based on concentration of the aqueous extract. The wheat seedlings were remarkably inhibited when treated with root aqueous extracts (25.7%–46.9% for shoot length and 36.2%–51.9% for root length) and by all the extracts tested at the highest concentration (40 g L^{-1}).

Peganum harmala seeds exhibited the least sensitivity to aqueous extracts. In fact, a nonsignificant difference in their percentage of germination was reported when compared to the control, except for those treated with aqueous extracts from phyllodes and seeds at 30 and 40 g L^{-1}. The shoot length of peganum was strongly stimulated in the presence of root aqueous extracts at 10–30 g L^{-1}, stem aqueous extracts at 10–40 g L^{-1}, and the phyllode aqueous extract at 30 g L^{-1}. All other extracts inhibited shoot seedling growth; the highest inhibition was reported in the presence of seed aqueous extracts at concentrations of 30 (67.7%) and 40 g L^{-1} (90.5%) and the phyllode aqueous extract at 40 g L^{-1} (66.0%). The roots of the peganum were more sensitive to the root aqueous extracts.

All of the aqueous extracts moderately inhibited the percentage germination of *Silybum marianum* seeds except those from roots and stems at 40 g L^{-1} and from flowers at 20 g L^{-1}. The percent inhibition reached 38.3% with the phyllode aqueous extracts at 40 g L^{-1}. Root and shoot lengths were significantly reduced in the presence of

Table 1. Percentage inhibition or stimulation of seed germination and root and shoot lengths of target species *Lactuca sativa* L., *Triticum aestivum* L., *Peganum harmala* L., and *Silybum marianum* L. in the presence of aqueous extracts (at 10, 20, 30, and 40 g L^{-1}) of *Acacia cyanophylla* plant parts.

Target species		Percentage inhibition or stimulation											
		Lactuca sativa			*Triticum aestivum*			*Peganum harmala*			*Silybum marianum*		
Plant part AE	g L^{-1}	Seed germ	Shoot length	Root length	Seed germ	Shoot length	Root length	Seed germ	Shoot length	Root length	Seed germ	Shoot length	Root length
Roots	10	−30.0bc	−28.8b	−62.1f–i	−5.5a–c	−35.0b–e	−36.2 a–d	−1.7ef	+15.8e–g	−26.1hi	−3.3e–g	−35.9c–e	−26.4f–i
	20	−40.0b	−27.9b	−78.1c–e	−5.5a–c	−33.3a–c	−46.6ab	+3.4f	+14.0e–g	−36.4e–h	−6.7d–g	−23.6e–i	−14.8jk
	30	−40.0b	−5.4c	−82.4b–d	−12.2a	−25.7b–d	−40.0a–c	+1.6ef	+25.9fg	−38.9efg	−6.7d–g	−19.1g–i	−10.2kl
	40	−36.7b	−8.6c	−88.8b	−6.7ab	−46.9a	−51.9a	+1.6ef	−12.0d	−57.4d	0.0g	−12.7ij	−2.8l
Stems	10	−6.6d–g	+28.7d–f	−39.2lm	−3.3bc	−1.3h	−0.4j	0.0ef	+21.2fg	−23.5ij	−3,3e–g	−18.6g–i	−26.8f–i
	20	−5.0d–g	+39.7e–h	−47.2j–l	−6.7ab	−1.4h	−3.4ij	−1.7ef	+17.5e–g	−40.6 e–g	−6.7d–g	−23.7e–h	−29.5e–i
	30	−3.3d–g	+47.5g–i	−51.2i–l	−7.8ab	−11.8d–g	−11.4g–i	+1.6ef	+30.8fg	−41.9e–g	−6.7d–g	−31.4c–g	−45.2de
	40	−8.3d–f	+51.2hi	−62.1f–i	−8.9ab	−12.7d–g	−17.7f–h	−1.7ef	+37.7g	−45.7d–f	−0.0g	−32.6c–g	−47.1c–e
Phyllodes	10	−5.0d–g	+32.6e–g	−12.9n	−8.9ab	−9.3f–h	−11.5g–i	−27.7bc	−3.9de	−26.4hi	−16.7a–e	−35.6c–f	−45.9de
	20	−5.0d–g	+39.5e–h	−28.4m	−7.8ab	−26.3b–d	−33.0b–e	−38.0ab	−55.4c	−89.4b	−28.3a–c	−43.1b–d	−56.3a–d
	30	−5.0d–g	+38.6e–h	−43.2kl	−6.7ab	−12.6e–g	−18.2e–h	−38.0ab	+7.4ef	−47.0de	−30.0ab	−48.9a–c	−65.0abc
	40	−10.0d–f	+25.2de	−50.3i–l	−11.1ab	−24.9b–e	−32.5b–f	−34.6bc	−66.0bc	−75.7c	−38.3a	−60.5a	−72.2ab
Flowers	10	0.0g	+91.3jk	−64.9f–h	−13.4a	−20.6c–f	−12.7gh	−1.7ef	−33.8g	−22.1ij	−1.7fg	−19.7f–i	−19.1i–k
	20	0.0g	+83.3j	−59.1f–j	−3.3abc	−19.8c–f	−19.0e–g	+1.6ef	−19.9fg	−39.1e–g	0.0g	−22.5e–i	−22.1h–j
	30	−1.7fg	+95.7jk	−66.7f–h	−8.9ab	−31.8a–c	−40.5a–c	0.0ef	−15.3e–g	−55.8d	−11.7c–g	−32.6c–g	−37.8c–g
	40	0.0g	+86.2j	−69.1e–g	−13.3a	−31.9a–c	−43.2a–c	0.0ef	−32.0g	−56.6d	−13.3b–f	−34.7c–f	−47.7c–e
Legumes	10	−5.0defg	+58.9i	−59.5f–j	−4.5a–c	−12.7fg	−8.0hi	−5.2de	−15.0e–g	−14.6j	−11.7b–g	−26.1d–g	−39.8d–g
	20	−1.7fg	+112.6k	−56.2g–k	−5.6a–c	−19.0c–f	−27.7c–f	−5.2de	−17.3e–g	−33.6f–i	−11.7b–g	−35.9c–e	−54.4cd
	30	−1.7fg	+93.2jk	−55.2h–k	−3.3a–c	−20.4c–e	−46.8ab	0.0ef	−34.0g	−29.3gh	−21.7a–d	−38.8cde	−55.6b–d
	40	−6.7d–g	+46.0f–i	−66.6f–h	−10.0ab	−39.9ab	−53.2a	−1.7ef	−35.4g	−35.9e–h	−33.3ab	−58.7ab	−73.0a
Seeds	10	−3.3e–g	+42.6f–i	−71.9d–f	−4.5a–c	−5.1gh	−2.9ij	−7.0de	−12.8d	−58.7d	−1.7fg	−18.7g–i	−21.5h–j
	20	−13.3c–e	+14.8d	−85.4bc	−7.8ab	−10.4f	−12.4gh	−7.0de	−18.3d	−75.6c	−5.0d–g	−12.0h–j	−23.1g–j
	30	−13.3c–e	−17.4bc	−90.1b	−12.3a	−3.7gh	−21.4d–g	−17.3cd	−67.7b	−88.0b	−8.3c–g	−20.3f–i	−37.9d–h
	40	−100.0a	−100a	−100a	−5.6ab	−9.3fg	−36.1a–d	−58.6a	−90.5a	−97.6	−1.7fg	−8.2j	−41.5d–f

Values in a column followed by the same letter are not significantly different at $P < 0.05$. AE = aqueous extract; germ = germination.

aqueous extracts. Shoots were more sensitive to phyllode extracts (35.6%–60.5%) and legume extracts (26.1%–58.7%), while legume extracts were more toxic to roots (39.8%–73.0%).

3.2. Yield in organic extracts

In order to determine the chemical group to which the active molecules of the *A. cyanophylla* plant parts might belong, 4 organic extracts were prepared with PE, EA, and M and directly with dM extract. The yields for each extract are reported in Table 2. Yield increased with an increase in the polarity of the solvents from hexane to direct methanol. The highest yields were obtained with dM extracts from stems, seeds, phyllodes, and flowers (12.60%, 11.12%, 10.54%, and 10.26%, respectively).

3.3. Effects of organic extracts

Table 3 illustrates the percent inhibition of seed germination and root and shoot lengths of the target plants in the presence of the *A. cyanophylla* organic extracts. The organic residues were dissolved in methanol. The results showed that methanol did not affect germination and the effects could be attributed to allelochemicals present in the organic extracts.

All the extracts significantly inhibited germination and seedling growth of the tested seeds. This inhibition varied based on the nature of the organic extract. The crop seeds tested were less sensitive to the organic extracts, especially the wheat seeds. In fact, for this crop, percentage inhibition of seed germination did not exceed 36.7% (PE phyllodes extract). PE, EA, and dM extracts from roots; PE, EA, and M extracts from phyllodes; and M extracts from stems and flowers were the most effective (20.0%–25.0%, 18.3%–36.7%, and 25.0% and 23.3%, respectively). Wheat shoot seedling growth was highly inhibited by the organic extracts (35.1%–96.6%), except for the PE extract from flowers and the dM extract from phyllodes, whose percentages of inhibition did not exceed 16.9%. Roots were more sensitive than shoots and the root length inhibition percentages were 42.5%–99.6%.

For lettuce, the inhibition percentages of germination were more important (8.9%–88.9%), and in contrast to the other tests, most of the M extracts were less inhibitory. Lettuce root growth was significantly reduced when roots were treated with *A. cyanophylla* organic extracts, especially the PE extract from phyllodes (88.1%); the M extracts from phyllodes (86.7%), flowers (88.2%), and seeds (99.4%); and the dM extracts from roots (98.4%) and stems (81.1%). Those 3 last organic extracts, along with the EA extract from stems, also inhibited shoot growth (50.1%–95.4%). For the rest of the organic extracts, the inhibition percentages for shoot length were less important, though a weak stimulation was observed with the EA extract from roots (12.2%) and the dM extract from phyllodes (6.2%).

For weed seeds, a significant toxicity was recorded in the presence of vegetative and reproductive organs. PE extracts from stems and legumes, EA extract from flowers, and M extracts from stems and seeds all showed a low inhibition of peganum seed germination, which did not exceed 6.7%. A percent inhibition greater than 50% was reported with the PE extracts from phyllodes (80.0%), roots (65.0%), and flowers (76.7)%; the EA extract from legumes (76.7%); the M extracts from roots (65.0%), phyllodes (76.7%), and flowers (76.7%); and the dM extracts from phyllodes (80.0%), legumes (55.0%), and seeds (90.0%).

Peganum shoot growth was completely inhibited by the dM extracts from stems, phyllodes, legumes, and seeds and by the M extract from flowers. Most of the other extracts inhibited the growth of shoots more than 50%, except the PE extracts from roots and seeds and the EA extract from flowers. The roots of peganum seedlings were more sensitive to organic extracts as the lowest percent inhibition was 42.5% (EA extract from flowers).

Table 2. Yield, in percent of dry matter, of organic extracts of the different parts of *Acacia cyanophylla*. The petroleum ether, ethyl acetate, and methanol extracts were obtained by fractional extraction. The direct methanol extract was obtained by a direct powder extraction in methanol.

Acacia cyanophylla plant parts	Petroleum ether	Ethyl acetate	Methanol	Direct methanol
	Yield (%)			
Roots	1.12	1.80	4.11	8.01
Stems	0.90	6.41	5.13	12.60
Phyllodes	0.42	6.91	3.92	10.54
Flowers	0.37	5.75	4.68	10.26
Legumes	0.34	0.95	3.49	6.95
Seeds	5.14	3.74	4.87	11.12

Table 3. Percentage inhibition or stimulation of seed germination and root and shoot lengths of target species *Lactuca sativa* L., *Triticum aestivum* L., *Peganum harmala* L., and *Silybum marianum* L. in the presence of organic extracts of *Acacia cyanophylla* plant parts.

Target species		Percentage inhibition or stimulation											
		Lactuca sativa			*Triticum aestivum*			*Peganum harmala*			*Silybum marianum*		
Solvent extract	Plant parts	Seed germ	Shoot length	Root length	Seed germ	Shoot length	Root length	Seed germ	Shoot length	Root length	Seed germ	Shoot length	Root length
PE	Roots	−28.9b–e	−2.0g–i	−17.4h–j	−20.0a–c	−47.3d–f	−51.5n	−65.0bcd	−39.9h	−51.5n	−70.0b–f	−79.3bc	−63.2gh
	Stems	−27.8b–e	−3.8g–i	−35.3e–g	−10.0b–e	−52.8de	−69.5lm	−5.0ij	−59.8fg	−69.5lm	−90.0a	−96.4a	−93.4ab
	Phyllodes	−32.2b–e	−27.5d–f	−88.1b	−36.7a	−87.2b	−95c–f	−80.0ab	−90.0c	−95c–f	−60.0c–f	−85.2a–c	−87.8b–d
	Flowers	−40.0b–d	−9.3e–i	−67.1c	−1.7fg	−7.5h	−80j–l	−76.7a–c	−70.0ef	−80.0j–l	−63.3b–f	−76.1b–d	−73.4d–g
	Legumes	−28.9b–e	−1.2g–i	−24.1f–i	−5.0 e–g	−49.9de	−83.2h–k	−3.3ij	−76.1de	−83.2h–k	−60.0c–g	−68.9cd	−36.8i
	Seeds	−41.1b–d	−28.4de	−62.4c	−13.3b–e	−68.5c	−48.7n	0.0j	−30.2h	−48.7n	−76.7a–e	−78.6bc	−80.5c–g
EA	Roots	−27.8b–e	+12.2i	−48.5de	−18.3a–d	−72.1c	−65.7m	−20.0fgh	−83.7cd	−65.7m	−66.7b–f	−57.5d	−54.2hi
	Stems	−23.3c–f	−53.2c	−55.2cd	−1.7fg	−53.2de	−66.9m	−33.3ef	−83.7cd	−66.9m	−36.7gh	−0.0e	−40.7i
	Phyllodes	−10.0e–g	−4.4g–i	−33.1fg	−21.7a–c	−43.6def	−82.0i–k	−10.0g–i	−80.7d	−82.0ijk	−73.3a–f	−77.6bc	−77.5d–g
	Flowers	−32.2b–e	−11.5e–h	−65.0c	−16.7b–e	−40.5ef	−42.5n	−6.7h–j	−5.1g	−42.5n	−80.0a–d	−78.5bc	−74.5d–g
	Legumes	−24.5c–f	−19.7d–g	−60cd	−8.3c–f	−74.7c	−94.2	−76.7abc	−98.3ab	−94.2c–g	−83.3ab	−80.3bc	−81.2b–f
	Seeds	−20.0d–f	0.6g–i	−67c	−11.7b–e	−55.3d	−79.0	−30.0^{e}–g	−84.6cd	−79.0j–l	−56.7e–g	−75.6b–d	−65.3f–h
M	Roots	−11.1fg	−7.7f–i	−10.2j	−5.0 e–g	−36.1f	−97.1a–d	−65.0bcd	−97.1b	−97.1a–d	−73.3a–f	−86.4a–c	−75.9d–g
	Stems	−11.1fg	−14.4e–h	−47.5de	−25.0ab	−71.4c	– 89.6e–i	−5.0ij	−96.1b	−89.6e–i	−75.0a–e	−93.2a	−89.9a–c
	Phyllodes	−8.9fg	−34.3d	−86.7b	−18.3a–d	−91.4ab	−93.6d–g	−76.7a–c	−97.1b	−93.6d–g	−81.7abc	−84.7a–c	−83.6b–e
	Flowers	−11.1fg	−29.9de	−88.2b	−23.3a–c	−95.0a	−99ab	−76.7a–c	100a	−99ab	−50.0fg	−69.9cd	−73.8d–g
	Legumes	−17.8d–f	−9.9e–i	−14.3ij	−5.0e–g	−37.2f	−73.9k–m	−46.7de	−77.3de	−73.9k–m	−80.0a–d	−88.9ab	−86.8b–e
	Seeds	−88.9a	−95.4a	−99.4a	−8.3b–f	−54.7d	−89.7f–i	−3.3ij	−95.6b	−89.7f–i	−76.7a–e	−88.7ab	−84.1bc–e
dM	Roots	−48.9bc	−88.4b	−98.4a	−25.0ab	−96.6a	−91.4e–h	−50.0cde	−97.6ab	−91.4e–h	−90.7a	−96.7a	−98.7a
	Stems	−56.7b	−50.1c	−81.1b	−15.0b–e	−91.9ab	−94.2c–g	−43.3d–f	100a	−94.2c–g	−76.7a–e	−94.9a	−98.2a
	Phyllodes	−30.0b–e	+6.2hi	−26.8f–h	−5.0 e–g	−16.9g	−98.6a–c	−80.0ab	100a	−98.6a–c	−53.3e–g	−69.7cd	−74.3d–g
	Flowers	−31.2b–e	−7.5f–i	−32.6fg	0.0	−35.1f	−87.5g–j	−10.0g–i	−68.9ef	−87.5g–j	−56.7d–g	−72.4b–d	−70.7e–h
	Legumes	−18.9d–f	−1.2g–i	−22.8g–i	−5.0 e–g	−50.4de	−96.4b–e	−55.0b–e	100a	−96.4b–e	−23.3h	−33.0e	−20.4j
	Seeds	−34.4bce	−6.7g–i	−36.3ef	−13.3b–e	−54.7d	−99.6a	−90.0a	100.0a	−99.6a	−10.0i	−19.3e	−19.7j

Values in a column followed by the same letter are not significantly different at $P < 0.05$. PE = petroleum ether; EA = ethyl acetate; M = methanol; dM = direct methanol; germ = germination.

The thistle seeds were sensitive to all organic extracts. A low percent inhibition of germination was recorded for only the dM extract from seeds (10.0%). All the organic extracts inhibited germination in the range of 36.7% (EA extract from stems) to 90.0% (PE extract from stems) and 90.7% (dM extract from roots). Along with this inhibition, there was a net deceleration of seedling growth.

4. Discussion

The results revealed that seedling elongation was more reduced with the organic extracts than the aqueous extracts and roots were more sensitive than shoots. In all cases, the target plants were greatly inhibited by the different extracts and this inhibition varied based on organ, organic fraction, and target species. The weed seeds were more sensitive than the crop seeds, and the inhibitory effect was much more pronounced on growth than on germination. In fact, Prati and Bossdorf (40) reported that the degree of allelopathic interference is species-specific and can even vary within a species. Table 4 summarizes which aqueous and organic extracts of *Acacia cyanophylla* organs give the best allelopathic activity and are probably rich in allelochemicals compounds, reducing or stimulating germination and growth. For vegetative organs, the most toxic extracts were the aqueous extracts from phyllodes at 20 and 40 g L^{-1} and the dM organic extracts from roots and phyllodes. For reproductive extracts, the aqueous extract from seeds (40 g L^{-1}), the methanolic extracts (dM and M) from seeds, and the M extract from flowers were the most effective. Those extracts were rich in polar compounds.

The present findings corroborate the earlier report obtained by Kamal et al. (26), who found that *Acacia auriculiformis* significantly inhibited the germination and growth of rice and cowpea. Reports revealed that the inhibition of roots and shoots was more pronounced than that of germination and the inhibitory effect on root length was greater than on shoot length. The inhibitory effect of *A. auriculiformis* leaf extracts on germination of some agricultural crops was proportional to the extract concentration (27). The same result was found by El-Khawas and Shehata (41) when they tested the effect of *A. nilotica* on monocot (*Zea mays* L.) and dicot (*Phaseolus vulgaris* L.) plants. Based on the overall findings, it can be concluded that allelopathy is a concentration-dependent phenomenon. Carballeira and Reigosa (22) found that leachates of blossoming *A. dealbata* were allelopathic to *Lactuca sativa* germination and growth. We confirmed this in our results with aqueous extracts from *A. cyanophylla* flowers, which reduced shoot length by 83.3%–91.3%. Kohli et al. (18) and Seigler (19) found that some *Acacia* spp. affect crops through allelopathy, as their litters interfere with the establishment and growth of adjoining crop plants due to presence of numerous substances including phenolic compounds in the litter. Some of these substances act as allelochemicals (20) and influence germination and seedling growth (42). An indirect relation between a lower germination rate and allelopathic inhibition may be the consequence of inhibition of water uptake (43) and alteration in the synthesis or activity of gibberellic acid (37), which regulates de novo amylase production during seed germination (44). Allelochemicals decrease the elongation, expansion, and division of cells, all of which are growth prerequisites (45), and they inhibit absorption of ions (46) and therefore arrest growth (47) and repress protein synthesis and/or stimulate its degradation (48). Putnam (49) and Seigler (19) reported that such allelochemicals are frequently secondary plant products such as phenolics, terpenoids, organic acids, or alkaloids.

We also noticed that the roots of plants exposed to allelochemicals (aqueous and organic extracts) became brownish, stunted, and void of root hairs. This might be due to a rapid inhibiting effect on respiration of the root tips, which ultimately reduced elongation. Identical results were reported by Shahid et al. (50) when they tested the aqueous extract of *Acacia nilotica* on wheat and its weeds.

The bioassays indicated that in some cases we registered a high stimulatory effect on shoots with aqueous extracts from legumes (20 and 30 g L^{-1}) and from flowers (30 g L^{-1}). The ability of plant part exudates to stimulate germination is of wide occurrence in plants. It has been demonstrated that a number of natural compounds have the ability to stimulate weeds. *Striga* species are stimulated by lactone-forming acids found in the genus *Euphorbia* (51). Chang and Lynn (52) demonstrated the role of para-benzoquinones as natural seed germination stimulants for *Striga*. Root exudates of *Pisum arvense* and *Vicia villosa* produce substances that apparently stimulate both photosynthesis and the absorption of phosphorus by barley and oat plants. A great number of compounds stimulatory to the growth of seedlings have been found, such as agrostemin, allantoin, and strigols (53).

The varying degree of inhibition obtained in this work highlights the differential responses and the need to evaluate the allelopathic compatibility of crops with *Acacia* trees before their introduction into the agroforestry system. Allelochemicals escaping into the litter from *A. cyanophylla* plant parts (seeds, legumes, phyllodes, and flowers) should be identified and characterized, and those substances with strong allelopathic activity have potential as possible alternatives for achieving sustainable weed

Table 4. Aqueous and organic extracts of *Acacia cyanophylla* organs selected for giving the best allelopathic activity by testing seed germination (percent inhibition of seed germination) and early seedling growth (percent inhibition of roots and shoots) of the target species. The aqueous extracts were at 20, 30, or 40 g L^{-1} and the organic extracts were at 6000 ppm.

Target species	Seed germination/ seedling growth	Aqueous Extracts (g L^{-1})								Methanol extracts		Direct methanol extracts		
		Seeds	Legumes		Flowers	Stems	Phyllodes		Roots	Flowers	Seeds	Roots	Phyllodes	Seeds
		40	20	30	30	40	20	40	40	6000 ppm				
Lactuca sativa	Seed germination	–100	–	–	–	–	–	–	–	–	–88.9	–	–	–
	Shoot length	–100	+112.6	+93.2	+95.7	–	–	–	–	–	–95.4	–	–	–
	Root length	–100	–	–	–	–	–	–	–	–	–99.4	–98.4	–	–
Triticum aestivum	Seed germination	–	–	–	–	–	–	–			–	–	–	–
	Shoot length	–	–	–	–	–	–	–	–46.9	–95.0	–	–96.6	–	–
	Root length	–	–	–	–	–	–	–		–99.0	–	–	–	–99.6
Peganum harmala	Seed germination	–58.6	–	–	–			–	–		–	–	–80.0	–90.0
	Shoot length	–90.5	–	–	–	+37.7		–	–	–100	–	–	–100	–100
	Root length	–97.6	–	–	–		–89.4	–	–	–99.0	–	–		–99.6
Silybum marianum	Seed germination	–	–	–	–	–		–38.3	–	–	–	–90.7	–	–
	Shoot length	–	–	–	–	–		–60.5	–	–	–	–96.7	–	–
	Root length	–	–	–	–	–		–72.2	–	–	–	–98.7	–	–

(–): percentages of inhibition were not selected; they are reported in Tables 1 and 3.

management. On the other hand, the results suggest that *A. cyanophylla*, a multipurpose tree used for rehabilitation of dry lands and soil amelioration, could be a feasible source of natural herbicides; its biomass could be used as a postemergence herbicide or as a template for new herbicide classes.

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