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Allelopathic Effect of *Artemisia herba alba* on Germination and Seedling Growth of *Anabasis setifera*

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Abstract: Mature and immature fruit aqueous extract of *Artemisia herba alba* ASSO (Asteraceae) were evaluated for their effect on germination and seedling growth of *Anabasis setifera*. Germination percentage and seedling growth of *Anabasis setifera* decreased significantly as the concentration of the aqueous fruit extracts of *A. herba alba* increased. The percentage of reduction in germination and seedling length was significantly higher in immature fruit extract compared to mature. Mature and immature aqueous fruit extract significantly inhibited root length more than shoot. A significant linear correlation was found between aqueous fruit extracts concentration and total phenolic content in the extract. This relationship was coupled with the results of germination and seedling growth which indicated that phenolics were the responsible agent for the phytotoxic effect of *A. herba alba* residue on germination and seedling growth of *Anabasis setifera*.

Key words: *Artemisia herba alba*, *Anabasis setifera*, germination, seedling growth, phenolics, aqueous extract

INTRODUCTION

Artemisia herba alba is a common perennial shrub in semi-deserts of the Middle East (Zohary, 1973) and it is one of the most common medicinal species of *Artemisia*. It is widely used in folk medicine by Bedouins (Al-Shamaony *et al.*, 1994) as hot or cold drinks.

Medicinal plants may contain bioactive compounds that possess inhibitory activity. The seed of *A. herba alba* is a single fruit with a transparent envelope, that has mucilaginous cells which contain phytotoxic chemicals (Al-Charchafchi *et al.*, 1987). These chemicals were toxic to the germination and seedling growth of other plants (Alam *et al.*, 2001) or even autotoxic to the same plant species (Al-Charchafchi *et al.*, 1987). The effects of one plant on other plant through the release of chemical compounds in the environment can be defined as allelopathy (Hussain and Khan, 1988). The overwhelming evidence suggests that plant phenolics play a major role in allelopathy (Inderjit, 1996; An *et al.*, 2000). *Anabasis setifera* is a perennial shrub that grows wild in the Campus of Hashemite University -Zarqa/Jordan, but it can not grow around and between *Artemisia herba alba* (personal communication). The present investigation was therefore, conducted to envisage the allelopathic effect of aqueous fruit extract of *A. herba alba* on germination and seedling growth of *Anabasis setifera* and also determine the correlation of allelopathic activity with total phenolic contents.

MATERIALS AND METHODS

Plant material: Fruits of *A. herba alba* were harvested before and after maturity from the medicinal and aromatic plant garden at Hashemite University in Jordan. Plant material was shade dried then ground and kept in glass jar at 5°C until use for extraction. Seeds of *Anabasis setifera* were harvested at maturity stage from Hashemite University Campus and husked seeds were used in germination and seedling growth tests.

Preparation of extracts from *A. herba alba* fruits: Ten grams of dried powder from each mature and immature fruits of *A. herba alba* were extracted separately with 100 mL of boiled distilled water in a glass jar for 24 h at 20°C. The mixture has been shaken every 1 h. The mixture was placed in boiled water bath for 1 h, centrifuged at 6000 rpm for 15 min and filtered through micro-filter Millipore (0.2 µm). The filtrate designated as full strength (100%) and stored in a freezer (-50°C) prior to use for bioassay.

Determination of total phenolics: Total phenolic contents of mature and immature fruit aqueous extracts of *A. herba alba* were determined by the Folin-Ciocalteu method with vanillic acid as standard (Lowe, 1993). Total phenolic were then calculated from the calibration curve.

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Effect of extract on seed germination and seedling growth:

Fifty seeds of *Anabasis setifera* were germinated in petri dishes on Whatman filter paper with 5 mL of the aqueous extracts or distilled water as control. Three replicates were incubated in a randomized complete block design at 20°C in an incubator with fluorescent light. Germination criteria were the emergence of the radical through the pericarp. Germination percentages were recorded and total seedling length was measured after 5 days of incubation. Total seedling length was measured using five seedlings taken randomly from each dish after five days of incubation.

Statistical analysis: ANOVA test was used to determine the level of significance within the *Anabasis setifera* regarding the effect of *Artemisia herba alba* fruits aqueous extract on germination and seedling growth of *Anabasis setifera*. Significance of differences was accepted when $p < 0.05$.

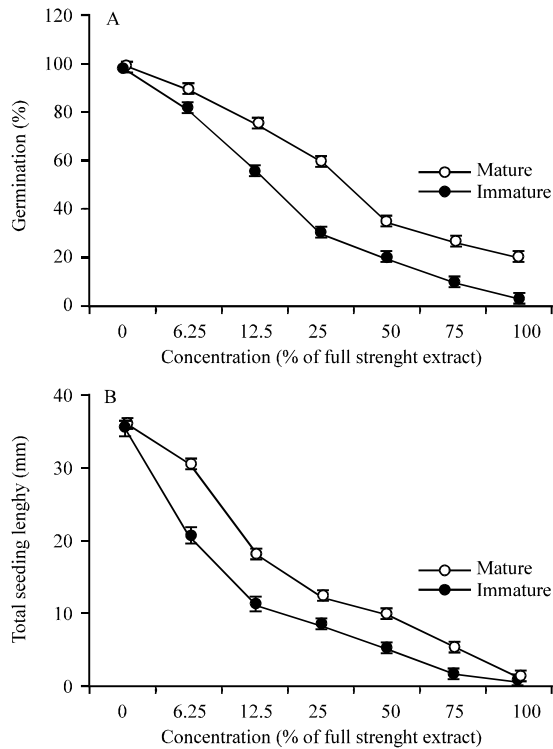


Fig. 1: Effect of different concentrations of *Artemisia herba alba* (maure and immature) fruit aqueous extract on germination percentages and subsequent seedling growth of *Anabasis setifera*. A: Germination, B: Seedling growth

RESULTS

Effect of aqueous *A. herba alba* fruit extract on germination and seedling growth:

Anabasis setifera seed germination percentages were significantly decreased ($p < 0.05$) as the concentration of the aqueous fruits extract increased (Fig. 1A). Immature fruit extract showed significantly ($p < 0.05$) higher reduction in germination as compared to mature fruit extract in all concentrations used (Fig. 2A).

On the other hand, total seedling growth of *Anabasis setifera* decreased gradually with increasing fruit extract

Table 1: Correlation between aqueous fruit extracts concentration *Artemisia herba alba* and total phenolic content in the extract

Extract concentrations (% of full strength extract)	Total phenolic content in the extracts (μg)	
	Mature	Immature
6.25	30	50
12.5	44	65
25	65	87
50	104	126
75	155	177
100	196	218

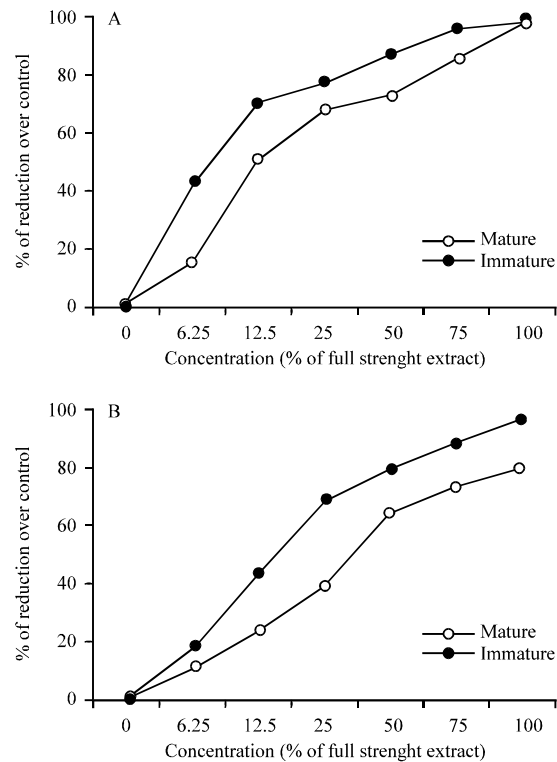


Fig. 2: Average percent of reduction over control in germination and total seedling length of *Anabasis setifera* A: Germination B: Total seedling length

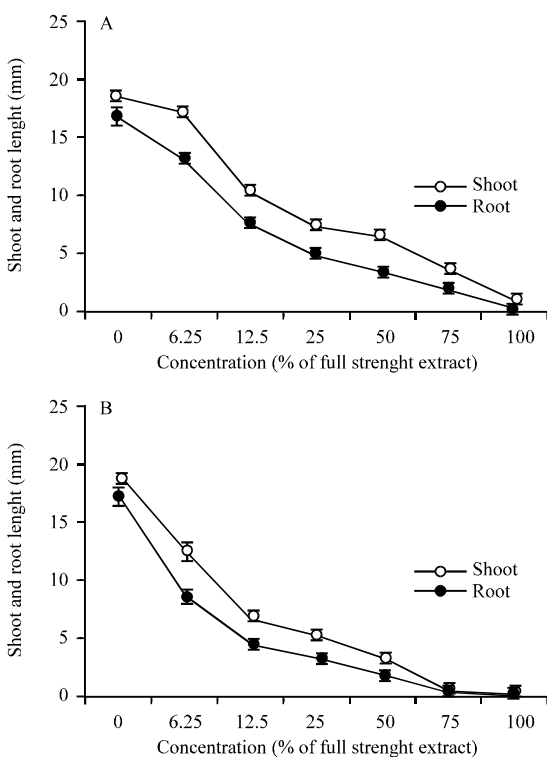


Fig. 3: Effect of different concentrations of *Artemisia herba alba* (mature and immature) fruit aqueous extract on shoot and root lengths of *Anabasis setifera* A: mature, B: immature

concentration (Fig. 1B). A significant differences ($p < 0.05$) were shown between mature and immature fruit extract on the inhibition of total seedling length. Immature fruit extract showed higher percentage of reduction in total seedling length ($p < 0.05$) as compared to mature fruit extract in all concentrations used (Fig. 2B). Therefore its worthwhile to investigate which part of seedling length (root or shoot) is mainly inhibited by crude aqueous fruit extract; inhibition experiment was administrated that determined the effect of mature fruit aqueous extract on shoot and root length (Fig. 3A) and immature fruit aqueous extract on the same seedling parts (Fig. 3B). Both extracts has significant allelopathic inhibitors on root and shoot length, but the toxicity was higher in the immature fruit extract (Fig. 4B) as compared to the mature fruit extract (Fig. 4A). On the other hand, the inhibitory effect on root length was higher than shoot length ($p < 0.05$).

The result of the present investigation indicated also that the total phenolic content in the aqueous extract of immature fruit was higher than mature as shown in Table 1.

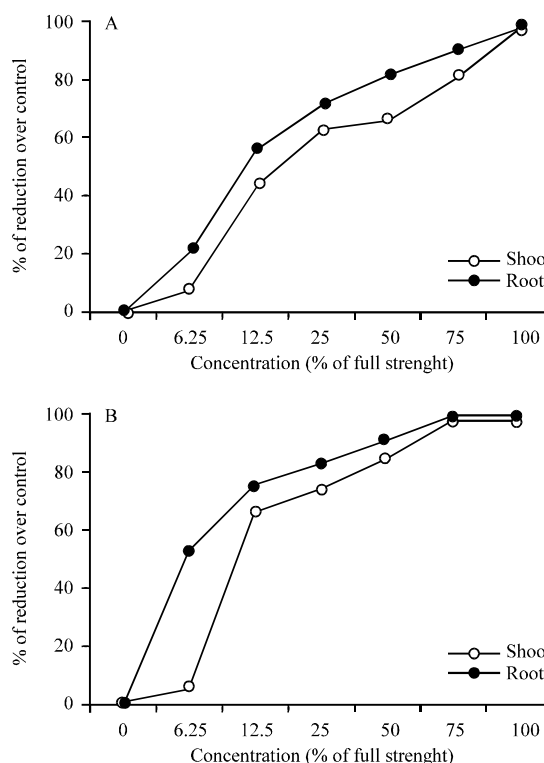


Fig. 4: Average percent of reduction over control in shoot and root length of *Anabasis setifera*. A: Mature fruit aqueous extract of *Artemisia herba alba* B: Immature fruit aqueous extract of *Artemisia herba alba*

DISCUSSION

Fruit aqueous extracts of the *A. herba alba* (mature and immature) exerted a typical allelopathic effects on germination and seedling growth of *Anabasis setifera*; severe toxicity at high extract concentration and moderate toxicity at low concentration. There by *A. herba alba* fruit extract (mature and immature) may contain some phytotoxic substance(s) that inhibits germination and growth of *Anabasis setifera*. Aqueous extract of plants may contain phenolics such as ferulic acid, P-coumaric, vanillic, caffeic, chlorogenic and others (Hussain and Khan, 1988; Habib and Abdul Rehman, 1988). These phenolics inhibit the germination process (Williams and Hoagland, 1982; Al- Charchafchi *et al.*, 1987), which was due to their interference with indol acetic acid metabolism, or synthesis of protein and ion uptake by the plants (Hussain and Khan, 1988). Therefore, *A. herba alba* might release some soluble phenolic allochemicals to the environment (Xu *et al.*, 2003), which has a growth

inhibitory effect on new seeding of *Anabasis setifera* or other plant species.

Interestingly, present results reveal that fruit aqueous extracts (mature and immature) capable of inhibiting root length more than shoot. This may be due to the direct contact between the root and phytotoxic compounds of the aqueous extracts which in turn may inhibit cell division (Rietjens and Alink, 2003) which is highly active in meristematic tissue in the growing root.

There was a significant correlation between aqueous fruit extract concentration and total phenolic content in the fruit extract. Such a highly significant relationship, coupled with the results of germination and seedling growth inhibition, indicated that the phenolics were the responsible agents for the allelopathic effects of *A. herba alba* extracts on *Anabasis setifera*. This interpretation was in agreement with that found by Ben-Hammouda *et al.*, (1995). Phenolic compounds are well known potential phytotoxins (Al-Charchafchi *et al.*, 1987) and exist as free forms, esters, or as glycosides when combined with sugars and are water soluble that may be indirectly related to chemicals that is finally responsible for the observed allelopathic effect (Seal *et al.*, 2004).

Although phenolic extract concentration was higher in immature fruit aqueous extract and it showed higher inhibition in germination percentages and seedling growth compared to mature fruit aqueous extract. This may be due to differences in quality and quantity of phenolic compounds that were isolated from fruits (Bewley and Black, 1994).

The present study indicated that the phenolic compounds inside fruits of the common medicinal plant *A. herba alba* exhibit some potential inhibitory activity of germination and seedling growth of some plant species.

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