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Investigation of the Allelopathic Potential of *Alhagi graecorum* Boiss.

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ABSTRACT

The current study evaluated the allelopathic potential of *Alhagi graecorum* on germination and seedling growth of two common crop plants; bean (*Vicia faba*) and corn (*Zea mays*). Water soluble allelochemicals were extracted from the air dried-powdered shoots of *A. graecorum* at three different concentrations (2.0, 4.0 and 6.0%, w/v). The germination experiment revealed that seeds of both bean and corn have tolerance to the aqueous extract of *A. graecorum*, where concentrations up to 6.0% had no significant effect on percent of germination as compared with the untreated seeds. The results showed that the lowest concentration (2.0%) of the aqueous extract stimulated elongation of radicle and plumule as well as seedling biomass of both bean and corn, while the highest concentration (6.0%) was inhibitory. In addition, the growth of corn seedlings was retarded at the modest dose (4%) of the aqueous extract, while that for bean seedlings was promoted at the same concentration. Similarly, water soluble allelochemicals extracted from *A. gaecorum* shoots influenced accumulation of soluble sugars and proteins in a concentration and species dependent manner.

Key words: Allelopathy, *Alhagi graecorum,* germination, growth, sugars, proteins

INTRODUCTION

Plants produce vast array of secondary metabolites that escape in to the environment and affect the growth and development of neighboring plants and other organisms, a phenomenon known as "allelopathy" (Rice, 1984; Chou, 2006). These metabolites are called allelochemicals and are belonging to several chemical classes such as flavonoids, phenolics, alkaloids, terpenoids and cyanogenic glycosides (Einhellig, 1999; Chou, 2006). The impact of allelochemicals on germination, growth and development of plants is governed by their complexity, interaction and concentration (Inderjit *et al*., 2002; Mallik and Williams, 2005; Li *et al*., 2011; Saleh, 2013). They cause alteration in plant metabolism owing to their interactions with vital growth processes and activities of many enzymes. (Einhellig, 2002; Weir *et al*., 2004; D'Abrosca *et al*., 2013).

Alhagi graecorum Boiss. is a shrubby evergreen perennial herb, woody at the base, erect to ascending up to 60-100 cm high, very much branched with rigid spiny twigs about 1 inch long. The plant is belongs to family Leguminasae and native to North Africa, the Middle East and Southeast Europe (Awmack and Lock, 2002). In Egypt, *A. graecorum* is widely distributed and seems to have wide ecological amplitude, it recorded from Nile region, oasis, Mediterranean region, Eastern and Western Desert, Red Sea coast and Sinai (Boulos, 2009). It grows naturally in xeric, halic and mesic habitats (Hassanein and Mazen, 2001). The species is sometimes confused with *A. maurorum* and the two may be distinct ecotypes or even subspecies (Awmack and Lock, 2002).

Different categories of secondary metabolites are extracted from *A. graecorum* including: flavonoids, alkaloids, phenolics, steroids, terpenoids, resins and tannins (El-Demerdash *et al*., 1991; Kamil *et al*., 2001; Laghari *et al*., 2010, 2011). Pharmacological studies on purified phytochemicals or crude extracts of *A. graecorum* and its related species revealed their hepatoprotective, antimicrobial, cytotoxic, antioxidant and antiproliferative activities (Batanouny, 1999; Alqasoumi *et al*., 2008; Sulaiman, 2013). However, the allelopathic potential of *A. graecorum* is poorly undertaken. Thus, the current work aims *in vitro* assess the effect of water soluble allelochemicals extracted from *A. graecorum* on the germination and early seedling growth of two common crop plants; *Vicia faba* and *Zea mays*.

MATERIALS AND METHODS

Preparation of aqueous extract from *A. graecorum* shoots: *A. graecorum* plants were collected from the western desert of Egypt. The aerial part of the plant was air dried for few days and ground to pass through 1 mm mesh. Ten grams of the air dried and powdered shoots mixed with 100 mL of distilled water and shake overnight at 4°C, in order to avoid fermentation or microbial growth. The mixture was filtered through multi-layered cheesecloth and then centrifuged at 3000 g for 30 min. The supernatant was served as a stock solution of concentration 10% (w/v) and used to prepare concentrations of 2, 4 and 6% by subsequent dilutions with distilled water.

Germination experiment: The seeds of *Vicia faba* and *Zea mays* were surface sterilized using 0.1% (w/v) $HgCl_2$, washed several times under running water and finally washed in distilled water. Seeds of each species divided in to 4 groups, the first group soaked in distilled water for 4 h, to serve as the control, while the remaining groups soaked in 2.0, 4.0 or 6.0%, w/v aqueous extract. After that, 10 uniform seeds placed in each of 5 clean, oven-dried Petri dishes which have been lined with 2 layers of filter paper and moistened with 10 mL distilled water or with 10 mL of the appropriate concentration of the aqueous extract. The Petri dishes were incubated at 25°C for ten days. Emergence of 1 mm radicle was used as the criterion for germination. At the end of the incubation period, the length of plumule and radicle was measured in 5 seedlings picked up randomly. Thereafter, embryonic axes detached, their fresh weight determined and then oven-dried for dry weight measurements.

Extraction and determination of soluble reducing sugars: Water-soluble carbohydrates were extracted by boiling a known weight of dry powdered tissues in distilled water for 1 h in a water bath. The extract was cooled and centrifuged at 5000 g for 10 min then the supernatant was completed up to known volume.

Reducing value of each sugar extract was determined according to the method adopted by Clark and Switzer (1977). One milliliter of each sugar extract was mixed with 1 mL of freshly prepared Nelson's alkaline copper reagent. (Nelson's A:B; 25:1) and heated in a boiling water bath for 20 min, then rapidly cooled under running water. Nelson's A; 12.5 g of anhydrous Na_2CO_3, 12.5 g K, Na tartrate, 10 g $NaHCO_3$ and 100 g anhydrous Na_2SO_4 in 500 mL distilled water, Nelson's B; 7.5 g $CuSO_4$ in 50 mL distilled water. Thereafter, 1 mL of arsenomolybdate reagent (25 g ammonium molybdate in 450 mL distilled water mixed with 21 mL concentrated sulphuric acid and 3 g sodium arsenate in 25 mL distilled water) was added with several shaking to dissolve Cu_2O.

When effervescence stopped, the mixture was made up to 10 mL with distilled water and its color intensity was measured at wavelength 540 nm against water-reagent blank. The content of reducing sugar was determined from glucose standard curve and then calculated as mg sugar g^{-1} dry weight.

Extraction and determination of soluble proteins: Extraction of water soluble proteins was carried out according to the method described by El-Tayeb *et al.* (2006). Soluble protein was extracted by incubating 100 mg of dry powdered tissues in 10 mL distilled water for 2 h at 90°C. After cooling, the mixture was centrifuged at 5000 g for 10 min and the clear supernatant was completed upto known volume with distilled water.

Protein determination was carried out according to the modified Folin-Lowry method adopted by Hartree (1972). One mL of the clear protein extract was mixed with 0.9 mL of alkaline sodium carbonate solution and heated in a water-bath at 50°C for 10 min. After cooling, 0.1 mL copper sulphate-potassium sodium tartrate solution was added to the mixture and allowed to stand for 10 min at room temperature, followed by addition of 3 mL of 10% Folin-phenol reagent with immediate mixing. After 30 min, the absorbance of the blue colour was recorded at 750 nm against water reagent blank. The concentration of protein was determined using bovine serum albumin standard curve, then expressed as mg g^{-1} dry weight.

Statistical analysis: Data analyzed using the computer program SPSS (version 12). All the data were subjected to one-way Analysis of Variance (ANOVA) following a randomized complete block design. The treatment means were compared using Duncan's Multiple Range Test at $p = 0.05$. Where needed, data were transformed by log (x+1) before statistical analysis.

RESULTS

Effect of aqueous extract of *A. graecorum* on seed germination: Data illustrated in Fig. 1a and b showed that the used concentrations of aqueous extract (2.0, 4.0 and 6.0%, w/v) of *A. graecorum* did not significantly affect the germination percentage of bean and corn seeds. Where, the percent of germination in both species was approximately 100% even in seeds treated with the highest concentration of the aqueous extract.

Effect of aqueous extract of *A. graecorum* on elongation of plumule and radicle: The highest concentration (6.0%) of the aqueous extract significantly inhibited the elongation of both radicle and plumule of *Vicia faba* seedling (Fig. 2a). On the other hand the lower concentrations of the aqueous extract caused significant increment in the length of radicle and plumule. The most stimulatory concentration was the 2.0% which increased the length of radicle and plumule by about 31, 18%, respectively, over the untreated seedlings.

As shown in Fig. 2b the distinct doses of *A. graecorum* aqueous extract differently affect the elongation of *Zea mays* seedlings. The lowest concentration (2.0%) of the aqueous extract significantly improved the elongation of both radicle and plumule, while the higher doses had adverse effect. The inhibition in elongation was directly proportional with the concentration of aqueous extract.

Effect of aqueous extract of *A. graecorum* on fresh and dry masses: The effect of different doses of aqueous extract from *A. graecorum* on the biomass of *Vicia faba* and *Zea mays* seedlings is shown in Fig. 3a and b. The fresh and dry masses of bean seedling were significantly affected

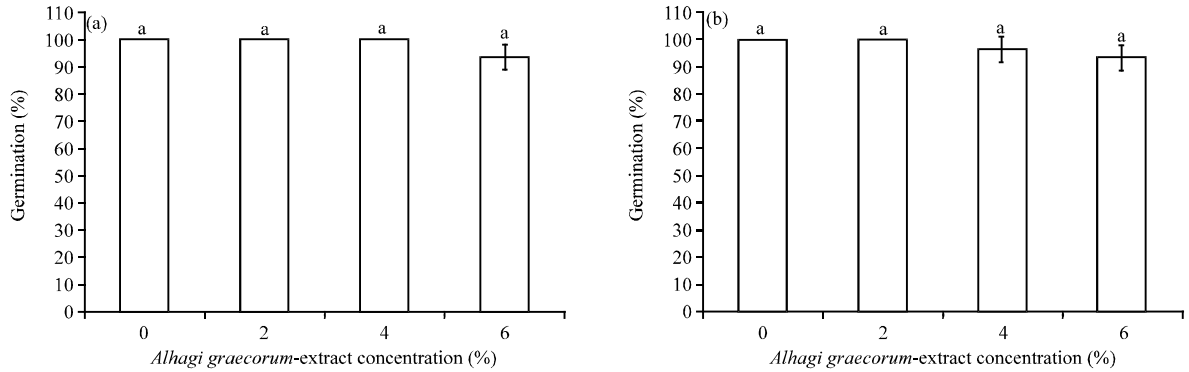

Fig. 1(a-b): Effect of aqueous extract of *A. graecorum* on germination of (a) Bean and (b) Corn seeds

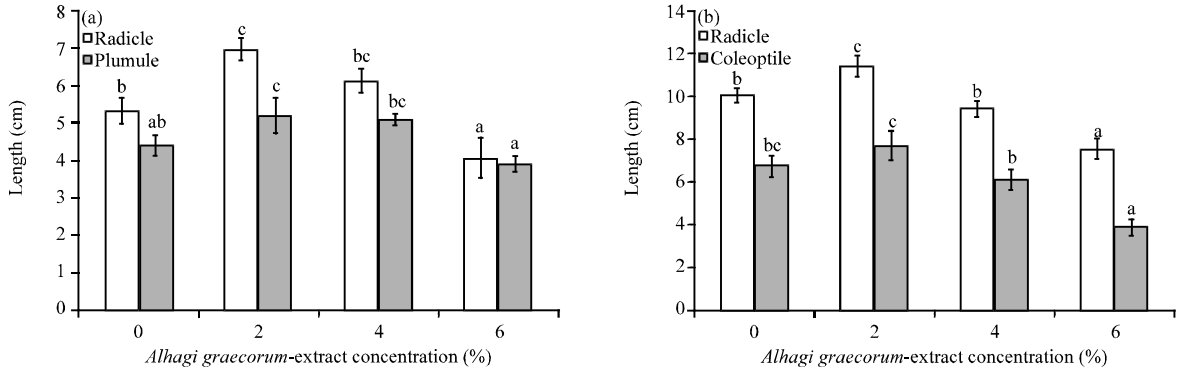

Fig. 2(a-b): Effect of aqueous extract of *A. graecorum* on radicle and plumule length of (a) Bean and (b) Corn seedlings

by the aqueous extract. The lower concentrations of the aqueous extract caused significant increase in biomass production, where the 2.0% treatment resulted in the maximum enhancement which estimated at 50 and 34% in the fresh and dry weights, respectively, as compare with the untreated seedlings. On contrast, the highest concentration of the aqueous extract was inhibitory (Fig. 3a). Regarding corn seedlings, the last two concentrations (4.0, 6.0%) had adverse effect on the biomass production, where they caused about 9, 40 and 13, 32% reduction in fresh and dry weight, respectively. While, the lowest concentration (2.0%) resulted in significant increase in the fresh and dry masses (Fig. 3b).

Effect of aqueous extract of *A. graecorum* on accumulation of soluble sugars and proteins: It is obvious from data presented in Fig. 4a that treatments of bean seeds with different doses of aqueous extract of *A. graecorum* had significant impact on the accumulation of soluble sugars and proteins in seedling tissues. Similar patterns obtained for both metabolites, where the highest dose of the aqueous extract significantly lowered their levels by 14 and 12%, respectively. On the other hand, the lower concentrations caused significant accumulation in soluble sugars and proteins.

Figure 4b showing that aqueous extract of *A. graecorum* at concentration of 2.0% caused a significant increase in the level of soluble sugars in tissues of corn seedlings. After that, the

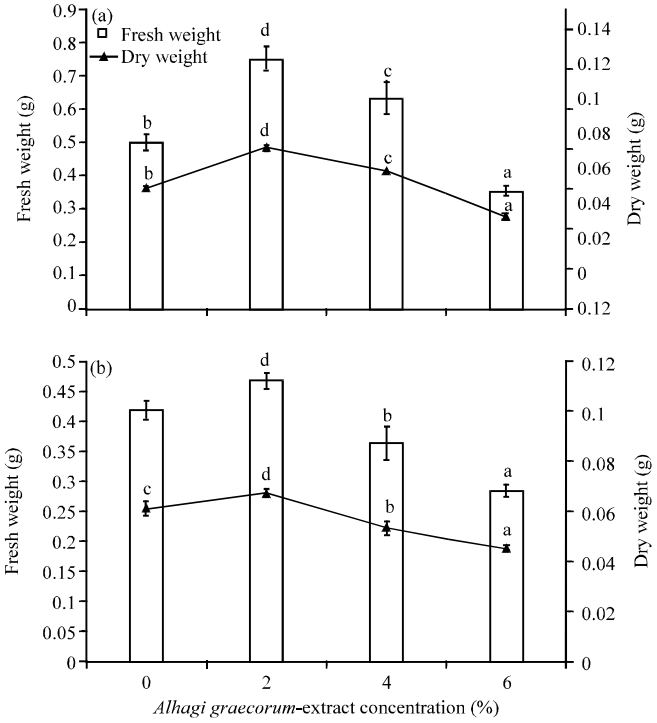

Fig. 3(a-b): Effect of aqueous extract of *A. graecorum* on fresh and dry masses of (a) Bean and (b) Corn seedlings

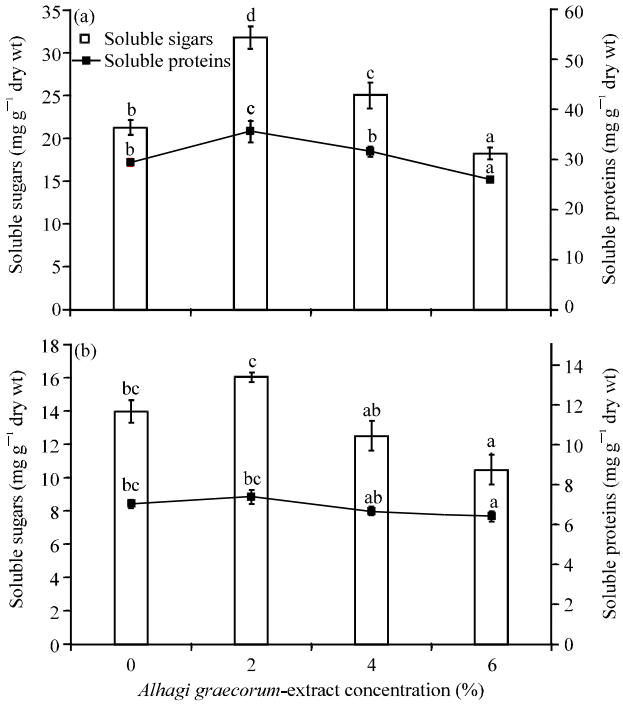

Fig. 4(a-b): Effect of aqueous extract of *A. graecorum* on contents of soluble sugars and proteins in tissues of (a) Bean and (b) Corn seedlings

accumulation of soluble sugars was inhibited. A less pronounced effect observed in case of soluble proteins, where the treatments were ineffective except for the highest concentration which was significantly inhibitive.

DISCUSSION

Most of investigation in the field of allelopathy focused on the growth inhibitory action of allelochemicals, while neglecting their stimulatory effects. However, the stimulation of plant growth by residues or extracts of other plants is also proved (Mallik and Williams, 2005; Saleh, 2013). In addition, some plant growth promotive allelochemicals were isolated and identified (Yokotani-Tomita *et al.*, 1998; Higashinakasu *et al.*, 2005). In this context, the experimental results of the present study revealed that water soluble allelochemicals extracted from the aerial part of *A. graecorum* affected the growth of bean and corn seedlings in a concentration dependent manner. The germination experiment demonstrated that seeds of both bean and corn have tolerance to the aqueous extract of *A. graecorum*, where concentrations up to 6.0%, w/v had no significant effect on percent of germination as compared with the untreated seeds. Generally, seed germination is less sensitive to allelochemicals than seedlings growth (Einhellig, 2004). In this connection, El-Khatib (2000) reported a significant inhibition in seed germination of *Chenopodium murale*, *Glinus lotoides* and *Mulva parviflora* treated with aqueous extract of *A. gaecorum*. Similarly, Sadaqa *et al.* (2010) reported that allelochemicals released from shoot residue of *A. maurorum* reduced the percent of germination of onion seeds. Moreover, aqueous extracts of leaf and root of *Pluchea dioscoridis* significantly inhibited the seed germination of *Corchorus olitorius*, *Lepidium sativum* and *Cynodon dactylon* (Fahmy *et al.*, 2012). On the other hand, Al-Watban and Salama (2012) reported that aqueous extracts of aerial parts of *Artemisia monosperma* (1.0 and 2.0%, w/v) stimulated the germination percentage of common bean seeds. In addition, Saleh (2013) demonstrated that the lower concentration (1.0 and 3.0%, w/v) of Olive Processing Wastes (OPW) aqueous extract did not significantly affect germination of corn grains, while the higher concentrations (6.0, 9.0%) were inhibitory.

The present results indicated that the lowest concentration of *A. graecorum*-extract (2.0%) significantly stimulated elongation of radicle and plumule as well as seedling biomass of bean and corn. On the other hand, the highest concentration (6.0%) was inhibitory. In addition, the growth of corn seedlings was retarded at the modest dose (4%) of the aqueous extract, while that for bean seedlings was promoted at the same concentration. In accordance with these results, Einhellig (1986) reported that the biological activity of allelochemicals is concentration dependent with a response threshold below which growth is stimulated in some instances.

The inhibitory effect of aqueous extract of root and shoot of *A. gaecorum* on seedling growth of some weed species was reported El-Khatib (2000). Also, El-Darier (2002) demonstrated that treatment of maize and bean with either *Eucalyptus rostrata* leaf powder or its aqueous extract decreased the elongation of root and shoot as well as their dry masses. Moreover, incorporation of shoot residue of *A. maurorum* in the soil at rate of 100 g kg^{-1} drastically reduced the length and dry weight of onion shoot and root Sadaqa *et al.* (2010). In addition, Al-Watban and Salama (2012) reported reduction in the early seedling growth of common bean treated with aqueous extracts of aerial parts of *Artemisia monosperma* at concentrations of 3.0 and 4.0%, w/v. In a previous study, I demonstrated that the effect of water soluble allelochemicals extracted from OPW on elongation and biomass of corn radicles and coleoptiles might be promotive or suppressive depending on the used concentration (Saleh, 2013).

The present results revealed that water soluble allelochemicals extracted from *A. gaecorum* shoot influenced the level of soluble sugars and proteins in a concentration dependent manner. This impact may ascribe to the effect of allelochemicals on the activities of amylases and proteases (Devi and Prasad, 1992; Kato-Naguchi and Macias, 2005; Batish *et al*., 2008). In this context, results obtained by El-Darier (2002) revealed that treatment of maize and bean seedlings with *Eucalyptus rostrata* leaf powder leads to accumulation of mono and polysaccharides. Also, leaf leachates of *Eucalyptus rostrata* and *Acacia nilotica* differentially affect the accumulation of soluble sugars and proteins in tissues of corn and common bean seedlings (El-Khawas and Shehata, 2005). Moreover, Al-Watban and Salama (2012) reported that aqueous extracts of *Artemisia monosperma* aerial parts at concentrations 2.0 and 4.0%, w/v decreased the content of soluble sugars, while increased the content of proteins in tissues of common bean seedlings. They observed that the depletion in the level of soluble sugars was accompanied with inhibition in amylase activity. In addition, Saleh (2013) demonstrated that treatment of corn seeds with OPW extract at concentration of 3.0%, w/v significantly increased the soluble sugars and proteins content in seedling tissues, while the higher concentrations were inhibitory.

In summary, there is apparent disparity in response of bean and corn seedlings to the different concentrations of the aqueous extract of *A. graecorum*. Thus, the present results supports the hypothesis that type and extent of impact of allelochemicals on plant growth is concentration and species dependent (Einhellig, 1986; Mallik and Williams, 2005; Saleh, 2013).

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