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Phytotoxic Effects of *Chenopodium album* L. Water Extract on Higher Plants

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Abstract: In a laboratory study phytotoxic effects of aqueous leaf extract prepared from *Chenopodium album* L. on seed germination of several weeds (*Echinochloa crus-galli*, *Amaranthus retroflexus* and *Cuscuta campestris*), mitotic cell division and coleoptile elongation were studied. Also, the effects of GA and IAA on counteracting the inhibitory effects of leaf extract were investigated. At a relatively high leaf extract concentrations (30 and 50% v/v prepared from 10% w/v of original stock solution), only *Cuscuta* seed germination was reduced. At 50% leaf extract, seedling growth in *Echinochloa* and *Amaranthus* was reduced significantly. In the presence of GA and IAA applied separately, the leaf extract had less inhibitory effects on seed germination and coleoptile elongation respectively. In the presence of leaf extract, the number of mitotic cells in *Allium cepa* root tips reduced significantly.

Key words: Phytotoxic, *Chenopodium album* L., water extract

INTRODUCTION

In Iran *Chenopodium album* L. is a common weed on cash crop farms and also in cereal and sugar beet fields. Due to the production and release of allelochemicals including phenolic acids, this weed has an allelopathic potential which affects growth of economically important plants. Earlier Le-Tourneau *et al.* (1956) and coworkers reported that aqueous extract (2 g dry tops in 100 mL H₂O) prepared from *C. album* reduced seed germination, coleoptile elongation and root growth of Mida wheat significantly. The inhibitory effects of *C. album* and its residues on the growth and development of other economical plants such as cucumber (Williams, 1964) and radish (Malik *et al.*, 1994) have been reported. Malik *et al.* (1994) have identified seven phenolic acids in *C. album* shoot. The residual materials of this weed in soil have been reported to reduce growth and development of tomato and sunflower (Reinhardt *et al.*, 1997). Allelopathic effects of *C. album* leaf extracts on nitrification have also been reported. In the presence of leaf extracts, complete nitrite oxidation to nitrate was delayed significantly in culture solutions with soil. Also, in the presence of leaf extract the amount of both nitrite and nitrate produced were significantly higher than those in control solutions (Jafari and Kholdebarin, 2002). In the present study, we report on the potential of *C. album* leaf extract in controlling the growth of other weeds and the effects it has on the development of economical plants (cell division and cell elongation). Consequently, we also report on the possible interaction of leaf extract with growth hormones, GA and IAA.

MATERIALS AND METHODS

All the experiments were conducted at the Biology Department of Shiraz University. *C. album* plants were collected after anthesis from a tomato field located in Research Center For Agricultural

and Natural Resources Of Fars Province, Iran. Leaves were separated, put in plastic bags and stored at -9°C for later use. Leaf extract was prepared by homogenizing 10 g frozen leaf material in 100 mL distilled water in a blender. The homogenate was stirred on a magnet stirrer plate overnight and filtered through Whatman filter paper. The filtrate was centrifuged at 3000 g for 20 min and the resulting supernatant was used as stock solution for biological test. The inhibitory effects of leaf extract on biological activities such as cell division, cell elongation and seed germination observed after 12 h of homogenization, points to the stability of leaf extract active ingredients.

Seed Germination

Cuscuta seeds were scarified in concentrated (98%) sulfuric acid for 4 h and then washed several times with distilled water. *Echinochloa* seeds were washed in running water for 48 h followed by surface sterilization in 10% sodium hypochlorite for 10 min and rinsed with distilled water thoroughly. *Amaranthus* seeds were surface sterilized with 10% sodium hypochlorite for 10 min and then rinsed throughly with distilled water. Batches of 10 seeds in each group were placed on Whatman filter papers inside Petri dishes and were treated with 5 mL of various concentrations of leaf extract (0, 1, 5, 10, 20, 30 and 50%). *Cuscuta* and *Echinochloa* seeds were incubated at 20°C±1 and *Amaranthus* seeds were kept at 31°C±1. After 3 days, seed germination rate and early seedling growth were recorded.

Interaction with GA and IAA

Hordeum vulgare seeds (CV. Doradifeh Aras) were surface sterilized in 10% sodium hypochlorite for 10 min and then rinsed several times with distilled water. For each treatment, seeds were placed on Whatman filter papers inside sterilized Petri dishes. To each Petri dish, 5 mL leaf extract at different concentrations (0, 1, 5, 10, 20, 30 and 50%) plus 3 mg L⁻¹ GA3 were added. Petri dishes were kept in incubator at 20°C±1 for 4 days. At the end of this period, the rate of seed germination for each treatment was determined.

To study the leaf extract-IAA interaction, 10 detipped 6 mm long barley coleoptile sections were put in vials containing different concentrations of leaf extract plus 3 mg L⁻¹ IAA and 0.1% sucrose. In some treatments, 2, 4 -dinitrophenol (DNP) as uncoupler of oxidative phosphorylation at concentrations of 0.1 and 0.01 mM was added to incubation media. All vials were shaken at 100 rpm on a gyratory shaker for 24 h.

Effects on Mitotic Division

Allium cepa bulbs were allowed to root by contacting their bases with the surface of distilled water in small beakers. When roots were 0.5-1 cm long, bulbs were transferred individually to other small beakers and kept there for 24 h with their roots suspended in solutions containing different concentrations of *C. album* leaf extract. The solutions pH was adjusted to 6.5. Feulgen staining method was used to detect cells undergoing mitotic division in the meristematic (1-1.5 mm from root tips) regions of the roots under microscope.

Statistical Analysis

In all experiments three replicates were used for each treatment and the experiments were designed as completely randomized blocks and Duncan test was used to compare means.

RESULTS AND DISCUSSION

Of the various weed seeds tested, *Cuscuta* seeds were the most susceptible as the 30 and 50% leaf aqueous extracts inhibited seed germination by 30 and 50% respectively (Duncan test, $p \leq 0.05$). With the increase in extract concentrations both radicle and plumule growth in *Amaranthus* (Fig. 1) and

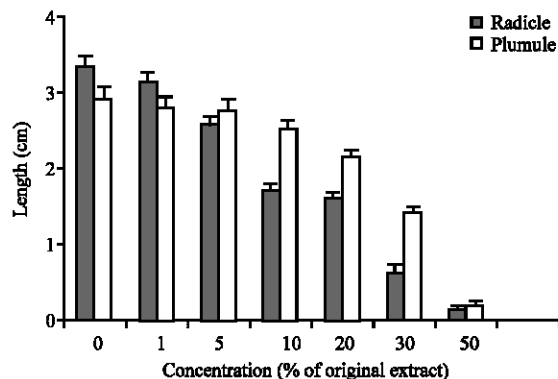


Fig. 1: Effect of *C. album* L. leaf aqueous extract on plumule and radicle length of *Amaranthus retroflexus*

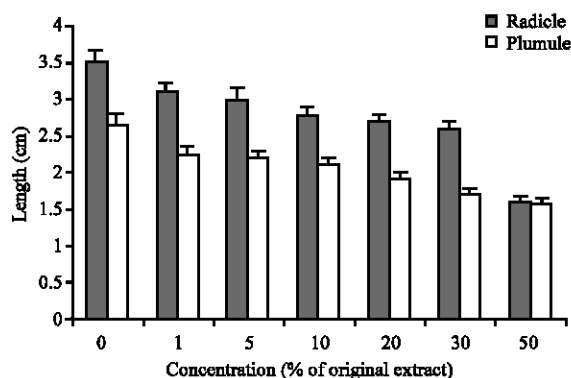


Fig. 2: Effect of *C. album* L. leaf aqueous extract on plumule and radicle length of *Echinochloa crusgalli*

Echinochloa (Fig. 2) were reduced. However, the inhibitory effect on plumule growth was more drastic at 30 and 50% leaf extract ($p \leq 0.05$).

Leaf extract at all concentrations reduced barley seed germination rate. The inhibition was complete at a concentration of 50% leaf extract ($p \leq 0.05$). In the presence of GA, the leaf extract had less inhibitory effect on germination (Fig. 3).

The presence of phenolic compounds in leaf extract may have interfered with either GA synthesis or α -amylase activity required for barley seed germination. In the same way, the inhibitory effects of leaf extract on *Echinochloa* and *Amaranthus* radicles and plumules elongation and on onion root cells mitosis may be attributed to interference of phenolic compounds which GA and IAA synthesis and their actions. Mitochondrial ATP synthesis may have been affected too. In agreement with this hypothesis, Singh *et al.* (1989) reported that aqueous *Lanata camara* leaf extract inhibited both seed germination and seedling growth in *Lolium multiflorum*. They found 13 phenolic compounds in *Lanata* leaf extract. Using paper chromatography and HPLC, Malik *et al.* (1994) reported the presence of 7 phenolic acids in *C. album* shoots.

Except at 1% level, all other concentrations of *C. album* leaf extract reduced the elongation of barley coleoptile segments (Fig. 4). When mixtures of both auxin and leaf extract were used, the stimulatory effects of auxin on barley coleoptile segments elongation were reduced ($p \leq 0.05$). 2, 4-dinitrophenol (DNP) as an uncoupler of oxidative phosphorylation, at concentrations of 0.1 and

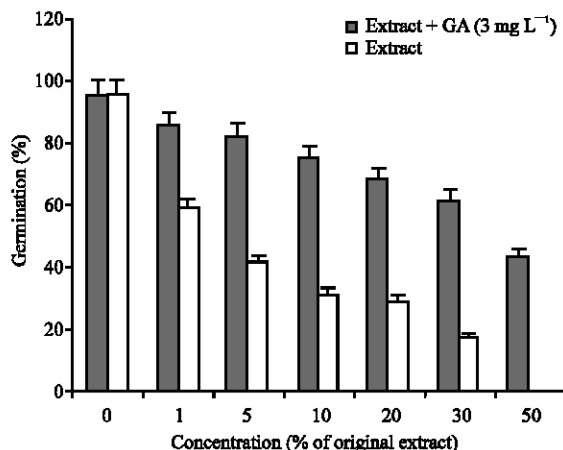


Fig. 3: Effect of different concentration of *C. album* L. leaf aqueous extract and GA on *Hordeum vulgare* seed germination

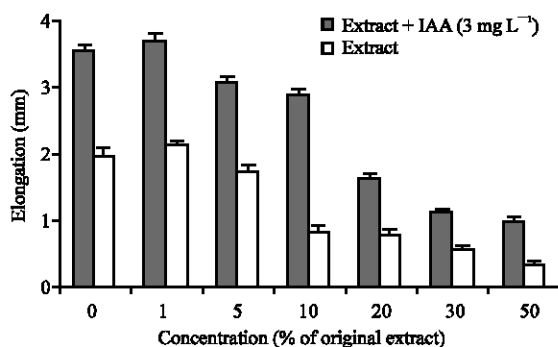


Fig. 4: Effect of different concentrations of *C. album* L. leaf aqueous extract and IAA on *Hordeum vulgare* coleoptile segments elongation

0.01 mM had similar inhibitory effects on barley coleoptile segments elongation in solutions containing 5 and 10% aqueous leaf extract (Fig. 5). It seems that *C. album* leaf extract effect on cell elongation is similar to the effects of DNP. However, they might have similar or different targets in their actions.

Allelochemicals such as phenolic compounds present in *C. album* leaf extract may have either interfered with membrane ATPase activity required for auxin-induced cell elongation or have directly influenced mitochondrial ATP production. In support of this contention, a number of phenolic compounds such as cinamic acid and coumarin have been reported to antagonise gibberellin inducer growth of light grown dwarf pea seedlings. The inhibition was reversed by increasing GA concentration (Corcoran *et al.*, 1972). In other studies, allelopathic phenolic compound, diacetyl-piquerol has been shown to inhibit H-ATPase activity in microsomal fraction from *Impomea purpuree* radicles (Ortega *et al.*, 1990). The activity of mitochondrial and tonoplast ATPases prepared from pea stems have been inhibited by several phenolic compounds (Macri *et al.*, 1986).

With the increase in concentration of *C. album* leaf extract the onion root cells mitotic index decreased significantly ($p \leq 0.05$) (Fig. 6). How the extract affects mitotic cell division remains to be investigated. It is possible that phenolic compounds present in leaf extract have interfered with cell division. The reduction in the rate of mitotic cell division by allelochemicals present

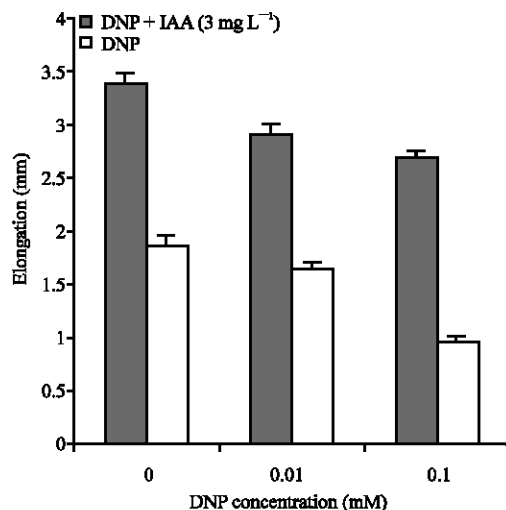


Fig. 5: Effect of DNP and IAA on *Hordeum vulgre* coleoptile segments elongation

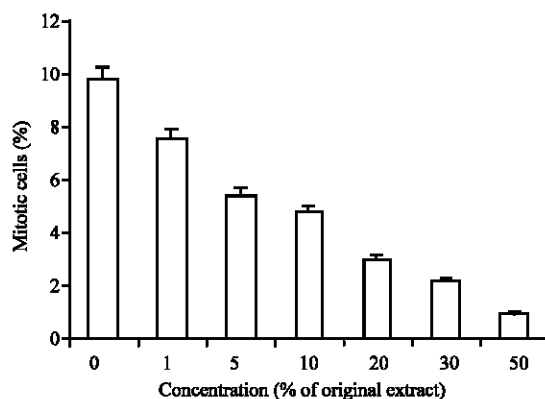


Fig. 6: Effect of different concentrations of *C. album* L. leaf aqueous extract on mitosis in *Allium cepa* root tips

in other plants has been reported by other workers. Thus, Jimenez-Estrada *et al.* (1996), reported that aqueous extracts prepared from *Juglans nigra* fruit skin prevented pea root cells from entering cell division stage.

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