Effect of Chlorogenic Acid on Germination and Seedling Growth, and on the Enzymes Activity Extracted from *Artemisia herba alba* ASSO. Part I: Germination and Seedling Growth

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**ABSTRACT**

Studies were carried out on the effects of chlorogenic acid on *Artemisia herba alba* seed germination and on the subsequent growth development at concentrations of 0.05, 0.1, 0.2, 0.3, and 0.4 mM, and under controlled conditions (20 °C, continuous illumination and continuous darkness). The compound showed inhibition of seed germination and subsequent seedling growth in dark treated seeds more than light treated seeds in most concentrations. Moreover, the percent of reduction in root length over control was higher than shoot length in both light and dark conditions. Possible mechanisms of action are discussed.

**KEYWORDS**: Phenolics, chlorogenic acid, *Artemisia herba alba*, germination, root growth, shoot growth.

1. **INTRODUCTION**

*Artemisia herba alba* ASSO (Asteraceae) is a common perennial shrub in the semi-deserts of the Middle East (Zohary, 1973) and the dominant plant in the Mediterranean area. *Artemisia herba alba* is one of the most important medicinal species of *Artemisia*. It is widely used in Iraqi folk medicine (Al-Shamaony et al., 1994) and used by Bedouins as a hot or cold drink in all the Middle East.

The seeds of *A. herba alba*, in botanical terms, are achenes surrounded by a loosely receptacular bracts (Rechinger, 1964; Al-Charchafchi and Jawad, 1982A). These bracts had an important role in germination and seedling growth; germination percentages of the husked seeds (bracts removed) and the unhusked seeds (with bracts) were about 100 and 15 percent, respectively (Al-Charchafchi et al., 1987).

Many researchers reported that phenolic compounds might be involved in germination inhibition of *A. herba alba* (Al-Charchafchi et al., 1987). Clor et al. (1974), and Al-Charchafchi and Jawad (1982A) observed that the seed of *A. herba alba* is a single fruit with a transparent gelatinous envelope which develops around the seed after a few minutes contact with water. Bewley and Black (1994) reported that coats may contain mucilaginous cells which burst upon contact with water and they may contain phenolics.

Previous work found that many plant species possess a strong phytotoxic potential and its residues contain water-soluble materials which were toxic to germination and seedling growth (An et al., 1997; Alam et al., 2001). This phytotoxicity is concentration dependent. It was found that phenolics were the most likely phytotoxic constituents of the extract of many plant species (Hussain and Khan, 1988; Habib and Rehman, 1988; An et al., 2000; Atoum et al., 2005). Al-Charchafchi et al. (1987), found that *A. herba alba* contains phenolic compounds that inhibited the germination and growth of its own and other seeds. At the medicinal and aromatic plant garden of the Hashemite University in Jordan, it was noticed that *A. herba alba* cannot be replaced by new seedlings of *A. herba alba*, which might be related to autotoxicity or other factors. Many researchers have suggested a possible role of the Pentose Phosphate Pathway (PPP) in phenolic compound biosynthesis (Ashihara and Komamine, 1964; Bewley and Black, 1995). Glucose-6-phosphate dehydrogenase (G6PDH) and 6 phosphogluconate
dehydrogenase (6PGDH) have been considered as controlling enzymes of PPP (Schnarrenberger et al., 1973). The activities of these dehydrogenases were inhibited by phenolic compounds (Farkas and Kiraly, 1960; Al-Quadan et al., 1981; Bewley and Black, 1995). Phytochemicals have been present in virtually all plant tissues, including leaves, flowers, fruits, stems, roots, seeds, and pollen. Several compounds isolated from different plant species have been identified to exhibit phytotoxic effects; among these compounds were phenolics, flavonoids and alkaloids (Lodhi, 1979). It was indicated previously that the seeds' dormancy of *A. herba alba* were due to the presence of phenolic compounds in their bracts and seed coat (Al-Charchafchi et al., 1987); moreover, the percentage of phenolic compounds were reduced in nondormant seeds of *A. herba alba*.

In the present investigation, the nondormant seeds of *A. herba alba* (germination percentage was higher than 90 %) were imbibed under different concentrations of chlorogenic acid phenolic compound for one to five days in order to evaluate its effect on germination percentage and growth of *A. herba alba*; moreover, chlorogenic acid effect on glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activity extracted from *A. herba alba* were investigated, the results will be the subject of a separate paper.

### 2. MATERIALS AND METHODS

**Plant Material**

Achenes of *Artemisia herba alba* (husked) were harvested during December 2001, 2002 and 2003 from the medicinal and aromatic plant garden of the Hashemite University / Zarka, Jordan. The harvested seeds were shade dried and stored in brown paper bags at 5 °C until use.

**Germination Test Method**

Germination was performed in 12 cm petri dishes lined with one layer of Whatman filter paper No.30 and moistened with 5 ml of distilled water or the experimental solutions (0.05, 0.1, 0.2, 0.3 and 0.4 mM chlorogenic acid). 100 husked seeds per dish were included (three replicates). All the dishes were incubated under controlled conditions (20 °C, continuous light illumination and continuous darkness) for many days. The germination percentages were calculated after 1, 2, 3, 4, and 5 days of seeds imbibition. In case of continuous darkness, germination counting was performed only at the end of the experimental period. Dark treatment was conducted by wrapping the dishes with aluminum foil immediately after seed imbibition. The seeds were considered as germinated when radicles were less than 1 mm in length.

**Seedling Development Test Method**

The average roots and shoots length of seedling were calculated by measuring 10 seedlings, taken randomly from each dish at the end of each period of imbibition as mentioned above.

**Statistical Analysis**

Data were statistically analyzed and means were tested separately. Paired T-test was used to test the significance between means at P ≤ 0.05.

### 3. RESULTS AND DISCUSSION

**Effect of Light and Dark Conditions on Germination Percentages of *A. herba alba* After Different Periods of Imbibition**

The germination percentage of *A. herba alba* increased gradually with time and reached more than 80 % after five days of imbibition at 20 °C either under continuous light or darkness (Figure-1). The germination percentage of *A. herba alba* under light condition was higher than that under dark condition; however, the reduction in germination percentage was not significant (P ≤ 0.05). This result was in agreement with that observed by Al-Charchafchi and Jawad 1982B, who concluded that *A. herba alba* was a positive photoplastic seed, but the sensitivity of these seeds to light decreased gradually with seed age.

**Effect of Chlorogenic Acid on Germination Percentages of *A. herba alba***

After five days of imbibition and under both light and dark conditions, germination percentage of *A. herba alba* was decreased significantly (P ≤ 0.05) as the concentration of chlorogenic acid was increased; this was compared to controls under the same condition (Figure-2); moreover, the reduction in germination percentage in dark treated seeds was higher than that of light treated seed (P ≤ 0.05). These results showed that chlorogenic acid acts as a phytotoxic compound because this reduction in germination may be due to inhibitory effect...
of chlorogenic acid on metabolic processes during germination. Chlorogenic acid was reported earlier as germination inhibitor (Williams and Hoagland, 1982; Blum et al., 1984). Moreover, phenolics were responsible agents for the phytotoxic effects of Vulpia residue (An et al., 1997). Phenolic compounds cause inhibition in germination due to indol acetic acid metabolism interference, protein synthesis inhibition, and effects on ion uptake (Castro et al., 1984; Hussain and Khan, 1988).

Chilogenic acid at 0.4 mM causes a significant decrease in germination of A. herbal alba (P ≤ 0.05) compared to water control after all periods of imbibition in both light and dark (Figures 3 and 4); moreover, the percentage of reduction in dark treated seeds was significantly higher (P ≤ 0.05) than that of light treated ones (Table-1). This may be related to both the effect of chlorogenic acid and darkness on germination of A. herba alba.

Effect of Chlorogenic Acid on Seedling Growth of A. herba alba

The total seedling length of A. herba alba was significantly decreased (P ≤ 0.05) as chlorogenic acid concentration was increased both under light and dark conditions (Figure-5), however, reduction in total seedling length in dark treated seeds were higher than that of light treated seeds (P ≤ 0.05) compared to water controls. These results were consistent with the results obtained in germination. The reduced seedling length of A. herba alba may be related to the toxic effect of chlorogenic acid on seedling growth. Ben-Hammouda et al. (1990) found that the phytotoxic potential of sorghum plant parts were positively correlated with phenolic content.

The effect of different concentrations of chlorogenic acid on shoot and root length of A. herba alba incubated at 20 °C under continuous light and dark conditions was investigated. Figures - 6 (a and b) showed that shoot and root length of A. herba alba decreased gradually with the increasing concentration of chlorogenic acid. The reduction in root length over control was higher than that of shoot length (P ≤ 0.05) in both light and dark conditions as shown in Table (2). Our results indicated that chlorogenic acid inhibited root length more than shoot length.

Table 1: Average % of reduction over control in germination percentages of A. herba alba achene in both light and dark.

<table>
<thead>
<tr>
<th>Period of imbibition in 0.4 mM of chlorogenic acid (day)</th>
<th>Light</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>43</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>36</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>48</td>
</tr>
</tbody>
</table>

Table 2. Average % of reduction over control in root and shoot length of A. herba alba achene imbibed in different concentrations of chlorogenic acid.

<table>
<thead>
<tr>
<th>Chlorogenic acid (mM)</th>
<th>Length (mm) in Light</th>
<th>Length (mm) in Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>0.05</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>0.1</td>
<td>39</td>
<td>47</td>
</tr>
<tr>
<td>0.2</td>
<td>56</td>
<td>63</td>
</tr>
<tr>
<td>0.3</td>
<td>74</td>
<td>83</td>
</tr>
<tr>
<td>0.4</td>
<td>75</td>
<td>92</td>
</tr>
</tbody>
</table>
Figure 1. Effect of light and dark conditions on germination percentages of *A. herba alba* incubated at 20 °C after different periods of imbibition.

Figure 2. Effect of chlorogenic acid on germination percentages of *A. herba alba* after five days of imbibition at 20°C.
Figure 3. Effect of chlorogenic (0.4mM) on germination (%) of *A. herba alba* at 20 °C under continuous light during different periods of imbibition.

Figure 4. Effect of chlorogenic (0.4mM) on germination (%) of *A. herba alba* at 20 °C and under continuous darkness during different periods of imbibition.
Figure 5. Effect of chlorogenic acid on total seedling length of *A. herba alba* at 20 °C under continuous light and darkness.

Figure 6a. Effect of chlorogenic acid on root and shoot length of *A. herba alba* incubation at 20 °C under continuous light.
Figure 6b. Effect of chlorogenic acid on root and shoot length of *A. herba alba* incubation at 20 °C under continuous darkness.

REFERENCES


