EXAMINING PHYSIOLOGICAL PARAMETERS OF POLLUTION TOLERANCE ON
THE GERMINATION AND SEEDLING GROWTH OF AMARANTHUS
RETROFELEXUS

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ABSTRACT

Oil pollution is one of environmental stresses which impact the germination, emergence, and
growth of plants. Determining the threshold of plant tolerance in the gasoline contaminated
environment can be useful for selection of soil and irrigation water when yielding new plants.
Hence, to evaluate the response of Amaranthus Retrofelexus to gasoline and also to determine
the threshold of this plant at gasoline condition an experiment in the form of randomized
complete block design with five treatments and four replications was conducted in two stages in
growth chamber. In the first stage the zero, 2, 4, 6, and 8 percents of contamination were studied.
At this stage, the seeds were able to emerge at the contamination levels of zero and 6 percents,
and the emergence rate were 93 and 25 percents, respectively. By increasing the density of
gasoline, the emergence of seedlings stopped. In general, an increase in the amount of gasoline
led to reduction of emergence percentage, seedling length, shoot dry and fresh weights and the
percentage of dry mater. According to the results, the emergence threshold of Amaranthus
Retrofelexus was determined as the concentration of 6 percent of gasoline.

Keywords: Amaranthus Retrofelexus, emergence percentage, gasoline, threshold value,

INTRODUCTION

Green plants are the first link of the ecosystem chain and any adverse effect on the plants affects the health and life of other creatures. With the increasing environmental
pollution due to the development of industry and technology, it is clear that doing more research can provide solutions to prevent the current problems which become more serious in the future. Use of green plant-based systems to eliminate or reduce pollution includes a major part of research and scientific studies. In this context, the term phytoremediation refers to the concept of using green plants to eliminate or reduce the pollutions of soil, water and sediments. However, to use this process for cleaning up the water polluted by organic and inorganic materials is not new and dates back hundreds of years; while employing this method for soil contamination is a new and growing method and the scientific research in this area is ongoing (2). Since environmental pollutions have a feeding effect on each other, escalation of the harmful radiation from ozone depletion caused by air pollutants also intensifies hydrocarbon contamination of soil and plants. Gasoline is a bio-based material available at the natural resources with very poor solubility in water; and its presence in the soil causes pollution and toxicity. In a normal condition, the downward movement of gasoline is prevented due to its absorption by the organic matters in the soil surface; hence, the contamination remains in the surface and the root system remains safe in more plant species. This feature makes this pollutant as a good alternative in phytoremediation processes (2).

Amaranthus retroflexus is a pseudo cereal crop. It has wide leaves, deep root system, a main stem, short-day flowering (the flowering occurs at the end and the inflorescence develops at the top of plant by the end of vegetative growth); it is mainly self-pollinated (with a percentage of cross-pollination). The flowers are monoiclines in red, orange, and yellow colors which are crowded on the axis of inflorescence cluster. It is an annual tall plant (180 -240 cm) grown in summer, its C4 photosynthetic system is highly adaptable to the tropics with plenty of sunny days (18). Amaranthus forage produces a lot of fodder crop product in a short period of time which can be used in the form of hay and silage for ruminant and non-ruminant animals (23). In terms of quality, the protein content of amaranth forage is higher compared to other non-legume forage plants and even some forms of forage legumes (22). The primary emergence of plants in oil-polluted habitats depends on the thermal and salinity systems and their severity; as a result the number of plants per area is affected by these factors. Good seed germination and seedlings establishment is
among the other effective factors in the production of corps in the field.
In spite of the fact that an increase in the gasoline reduces or delays germination, most four-carbon plants have good germination in the non-contaminated condition (13), however their resistance to oil contamination may indicate their ability to compete with other plants in contaminated conditions (12). Amaranthus ability in coping with conditions such as nutrient-poor soils, wide range of temperature, high radiation, and drought tolerance make it a new forgotten product to the semi-arid regions. One of the effects of gasoline on plant is reduction of photosynthetic activity which in turn reduces the amount of chlorophyll (8), Co2 absorption, and photosynthetic capacity (3). Many studies report varying amount of sugars under gasoline effect. The total protein content which is a physiologic index also decreases under gasoline effect (2, 18).
One strategy of plant to resist gasoline is the accumulation of solutions including essential metal ions (e.g. K+) and basically organic solvents. The most important organic osmotic solutions include soluble sugars such as sucrose, glucose, Trehalose, and Raffinose (17).
Since determining the threshold of plant survival in gasoline condition is very useful for selection of soil and irrigation water, the present experiment was designed and conducted to evaluate the tolerance of Amaranthus Retrofelexus to salinity in the emergence and establishment of this plant in gasoline condition; the environmental factors were controlled.

**METHOD AND MATERIALS**
This experiment was done in randomized complete block design with five treatments and four replications. Five levels of gasoline contamination were studied: zero (witness), 2, 4, 6, and 8 percents. First, twenty Amaranthus Retrofelexus seeds (modified seeds purchased from Pakan-Bazr Isfahan with 99% viability and purity) were planted in the plastic pots with the dimensions 10 × 20 × 30 cm filled with soft sand. Each level of contamination with gasoline was obtained by applying a specific amount of gasoline with respect to the intended percentage in the solution. The percentages of gasoline in the treatments were determined based on the weight of soil and to achieve the desired amount of gasoline of the treatments and a uniform concentration of gasoline in all parts of the soil, the soil was mixed well manually. After sowing the seeds, the contamination treatment was done by adding 50 cc on a daily basis. Hoagland solution was used for irrigation. During treatments, the emerged
seedlings were counted and their length was measured; also measuring the average length and number of grown seedlings was done on a daily basis. When the witness plant was 10 cm long, the seedlings were harvested and their fresh weights were measured. The seedlings were exposed to 70 °C temperature for 48 hours in oven condition and their dry weights were measured at the end. The percentage of dry matter was measured to investigate the variations of produced dry matter in different treatments. In this stage, the salinity treatment was performed similar to the first method and after the germination and emergence of seedlings; the seedlings and their length were counted on a daily basis until their length were fixed and growing stopped. The seedlings were harvested and their dry weight was measured. Data analysis was done using SAS software, the comparison of means was done using LSD test, and the graphs were illustrated using Excel.

RESULTS AND DISCUSSION
The results of this study showed the experiment, there is a significant difference (P≤0.0001) between different levels of gasoline contamination in terms of seedling length, dry weight, fresh weight, percentage of dry matter, and the germination of Amaranthus seeds (table 1).

The results of experiments showed that by increasing the level of gasoline, the emergence percentage of Amaranthus seedlings decreased; where the witness treatment had the highest percentage of emergence and in the 8% gasoline treatment none of the seeds were able to produce seedling (table 2). Also, with the increase of gasoline from zero to 7%, the percentage of germination decreased by 47%. The slope of emergence under the effect of gasoline with the concentration ranging from zero to 8 was 4.7% on average per every two percents of increased gasoline (table 2).

<table>
<thead>
<tr>
<th>Features</th>
<th>Gasoline level</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>emergence percentage</td>
<td>0</td>
<td>2%</td>
</tr>
</tbody>
</table>

Table 1: Variance analysis (degree of freedom, and level of significance) the emergence percentage, seedling length, dry and fresh weights and the dry matter percentage in Amaranthus

<table>
<thead>
<tr>
<th>Sov</th>
<th>Df</th>
<th>emergence percentage</th>
<th>Seedling length</th>
<th>Dry weight</th>
<th>fresh weights</th>
<th>dry matter percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (r)</td>
<td>3</td>
<td>0.5789</td>
<td>0.3201</td>
<td>0.8218</td>
<td>0.8138</td>
<td>0.4618</td>
</tr>
<tr>
<td>Gasoline (t)</td>
<td>4</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error (e)</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: comparing the emergence percentage, seedling length (cm), dry and fresh weights (mg/plant) and the dry matter percentage in Amaranthus under the effect of gasoline
The first effect on the plant growth is lack of uniformity in germination and seedling emergence (6). In this regard, the researchers found that high levels of gasoline significantly inhibit seed germination and growth, this increase is due the increased osmotic potential and toxicity of ions (4). The results of this study are consistent with Kafi et al that reported reduced emergence of Kochia due to increased stresses. Moreover, the researchers reported that in gasoline condition, increased absorption of minerals and salts slows down the absorption of water by Atriplex leading to reduced germination percentage (11). Studies show that in the first stage of water absorption by seeds, the water moves in the intercellular space which is not dependent on the osmotic potential; and at the second stage, the water absorption is linear and slow. The movement of water is done across cell membranes determined by osmotic potential difference between seed and its surrounding solution (5 & 6). On the other hand, if gasoline can easily cross the cell membrane and into the cytoplasm, an active metabolic pump can prevent the accumulation and toxicity of a particular ion or reduce the availability of some nutrients (3, 9 and 10).

In a study conducted on Kochia it was observed that increased gasoline leads to reduced emergence rate and ultimately the number of emerged seedlings. The maximum salinity that Kochia plant survived was 6%. In the present study, the increased gasoline contamination lead to reduction in the number of emerged seedlings; and the maximum gasoline contamination that Amaranthus Retrofleexus tolerated to emerge about 20% was 25%. The results of mean comparison in the first stage showed that the length of Amaranthus seedlings reduced with the increased gasoline (table 2).

Since the harvest was done when the height of witness seedlings reached 10 cm, the highest seedlings were observed in the witness treatment and the shortest seedlings were observed in the 6% gasoline treatment; so that an increase in gasoline from zero to 6 percent decreased the length of seedlings by 49%. The reduction of Amaranthus seedlings under the effect of gasoline in the concentrations between zero and 6% was

<table>
<thead>
<tr>
<th>seedling length (cm)</th>
<th>10</th>
<th>5.1</th>
<th>2.8</th>
<th>2.1</th>
<th>0</th>
<th>0.81</th>
</tr>
</thead>
<tbody>
<tr>
<td>fresh weight</td>
<td>68.36</td>
<td>198.8</td>
<td>38.01</td>
<td>27</td>
<td>0</td>
<td>119.3</td>
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<tr>
<td>Dry weight</td>
<td>83.7</td>
<td>22.2</td>
<td>7.3</td>
<td>5.3</td>
<td>0</td>
<td>17.3</td>
</tr>
<tr>
<td>dry matter percentage</td>
<td>12</td>
<td>147.7</td>
<td>18.0</td>
<td>18.0</td>
<td>0</td>
<td>8.2</td>
</tr>
</tbody>
</table>

The reduction of Amaranthus seedlings under the effect of gasoline in the concentrations between zero and 6% was
49.0 cm on average per 1% increase of gasoline (table 2).

In gasoline condition, in addition to the reduced percentage and speed of germination, the seedling growth might be slow due to delay in the germination (5, 13). Also, in this context the seedlings consume some of their photosynthesis productions to mitigate the effects of gasoline, as a result the seedlings growth becomes slower compared to non-gasoline condition.

The reduced length of seedling due to increased concentration of gasoline was also reported in some of Atriplex and safflower species (23, 25). Studying the growth process of Amaranthus seedlings length showed that in non-salinity treatment the seedlings continued to growth in length slowly and reached 10 cm during 15 days.

In addition to delay of emergence of seedlings in 6% treatment, their growth completely stopped after 12 days. When plants are exposed to gasoline, an amount of carbon enters the vascular system of plant. Because the cytoplasm is filled, they will gradually and inevitably increase in the cytoplasm which causes a severe disruption in the cytoplasm and ion toxicity begins in this phase, followed by reduction of plant growth (15).

With the increased concentration of gasoline, the dry and fresh weights of shoot decreased. The cell is very sensitive to increasing concentrations of gasoline and the plant necessarily stores gasoline in the vacuole. With the increasing gasoline from zero to 8% in the first stage, the vacuoles were filled with minerals and the fresh weight of shoot decreased by 71 % (484.8 mg/plant).

At this stage, the dry weight of shoot also decreased significantly with the increasing concentration of gasoline; where at the 6% treatment of gasoline the dry weight was about 26.5% (61.5 mg/plant) of its similar witness treatment (table 2).

With increasing gasoline concentration, the percent of dry matter was significantly increased (P≤0.05). At this stage, with increasing gasoline concentration from zero to 6%, the dry matter increased 22.5% (table 2).

**CONCLUSION**

In general, the results of this study indicate that gasoline reduces the emergence percentage and length of seedlings in Amaranthus Retrofelexus, and the threshold of survival of this plant under the effect of gasoline were determined about 6%. Regarding that the tolerance of plants to salinity at the germination and emergence stages in the controlled condition does not
necessarily correlate with their response in field condition (17), it is suggested that to determine the exact response of Amaranthus Retrofelexus to gasoline, similar studies are conducted in the field condition.

REFERENCES


[8] Irma T., Jolan C. Gabriella S; Ferenc H; Attila P; Gabriella K; Agnes S; Margit s; Laszlo. E. 2002; Acclimation of tomato plants to salinity stress after a salicylic acid pre-treatment. Proceeding of the 7th Hungarian congress on plant physiol. S2-02.


