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# Evaluating Allelopathic Effects of Some Plant Species in Tissue Culture Media as an Accurate Method for Selection of Tolerant Plant and Screening of Bioherbicides

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## ABSTRACT

**Plant tissue culture technique could provide sterile and controllable condition in order to assay direct effect of different compounds on plant growth accurately. In this study, the effects of aqueous extracts prepared from roots and shoots of goosefoot (*Chenopodium album* L.), redroot pigweed (*Amaranthus retroflexus* L.), fennel (*Foeniculum vulgare*), and wormwood (*Artemisia absinthium* L.) were evaluated on the seed germination and growth criteria in tissue culture media. The fennel root extract, nearly without phenolic content and with low antioxidant activity, showed the most drastic allelopathic effect on goosefoot, especially at 100 mg $mL^{-1}$ concentration, which might be due to the presence of some substance potentially useful for biological control of goosefoot, an invasive weed. Goosefoot was resistant to extract of fennel shoot, wormwood root, and shoot, while fennel and radish (*Raphanus sativus* L.), at high concentration (100 mg $mL^{-1}$), were not resistant to the root and shoot extracts of both goosefoot and redroot pigweed. In response to allelopathic components, shoot:root ratio was increased, and more peroxidase and superoxide dismutase activity were detected in roots. There was no direct relationship between allelopathic effects with total phenolic content and antioxidant activity. In conclusion, our results showed that allelopathic effects of extracts on growth and biochemical criteria depended on both the concentration levels and the plant parts from which the aqueous extract was derived. Therefore, tissue culture media is an accurate and suitable tool to screen plants resistant to allelopathic components of weeds, and to identify high allelopathic plants as potential bioherbicide and invasive plant controller.**

**Keywords**: Aqueous extract, Growth criteria, Invasive plant, Total phenolic content, Antioxidant activity.

## INTRODUCTION

The phenomenon of allelopathy encompasses all types of direct and indirect chemical interactions among plants and microorganisms (Einhellig, 1995). Almost all kinds of plant species could produce allelochemicals that vary among plant species, plant part, and growing stages and could be harmful and poisonous for one or more species (Sodaeizadeh *et al.,* 2009; Oueslati, 2003; Cheel *et al.,* 2012). Evaluating allelopathic effect of plant species could be important in different environmental conditions such as producing agricultural crops (Xuan *et al.,* 2012; Kato-Noguchi *et al.,* 2012), bioherbicides in weeds controlling strategies (Teerarak *et al.,*

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2012; Tigrea *et al.,* 2012) and the effect of allelochemicals on biodiversity and establishing of invasive species (Valera-Burgos *et al.,* 2012; Inderjit, 1996; Tognetti and Chaneton, 2012). Research has shown that many factors including nutrition, humidity, radiation, temperature, pest and disease influence the allelopathy process (Einhellig, 1996). For example, microorganisms positively or negatively interfere with plant-plant allelopathy (Mishra and Nautiyal, 2011; Anaya *et al.,* 2013; Cipollini *et al.,* 2012) or could directly have negative (Goodal *et al.,* 2012; Elliott and Cheng, 1987) or positive (Mallik and Williams, 2008; Sturz and Christie, 2003) allelopathic effects on plant (Barazani and Friedman, 2001).

It seems that by control stressful environmental conditions, real effect of plant would be emerged. Plant tissue culture media can provide sterile and controllable conditions in which accurate and direct effect of different components such as organic materials (Aryakia and Hamidoghli, 2010; Siril and Joseph, 2013) or mineral elements (Bouman and Tiekstra, 2001) on growth could be assayed. The aims of this study were: 1) to evaluate allelopathic effects of some plant species on germination and growth criteria using tissue culture media, as a new method for identifying plant with high allelopathic effect (probably as bioherbicides and invasive plants controllers); and 2) to identify resistant plants to allelopathic components.

## MATERIALS AND METHODS

### Plant Material and Preparation of Aqueous Extracts

Seeds, shoots, and roots of goosefoot (*Chenopodium album* L.), redroot pigweed (*Amaranthus retroflexus* L.), fennel (*Foeniculum vulgare*), wormwood (*Artemisia absinthium* L.) and radish (*Raphanus sativus* L.) were separately collected at reproductive stage in the glasshouse of Iranian Biological Resource Center, Alborz province, Iran. The surfaces of roots were washed with distilled water and were desiccated between the sheets of paper for one minute. Then, 250 g fresh matter of each part was separately cut into one-centimeter pieces, and shaken in 1000 mL double distilled water at 125 rpm at a temperature of 25 ℃ for 48 hours. Extracts were separated from the residue using two layer Whatman filter paper No. 42 (125 mm) and centrifuged at 5,000 rpm for 30 minutes. Supernatant was used as 100% stock solution (250 mg $mL^{-1}$) and stored at -20℃.

### Preparation of Tissue Culture Medium

To evaluate allelopathic effects of plant extracts on tissue culture media, modified MS medium (Murashige and Skoog, 1962) containing macronutrients: ammonium nitrate ($NH_4NO_3$) 1.650 mg $L^{-1}$, calcium chloride ($CaCl_2{\cdot}2H_2O$) 440 mg $L^{-1}$, magnesium sulfate ($MgSO_4{\cdot}7H_2O$) 370 mg $L^{-1}$, potassium phosphate ($KH_2PO_4$) 170 mg $L^{-1}$, potassium nitrate ($KNO_3$) 1.900 mg $L^{-1}$, and micronutrients: boric acid ($H_3BO_3$) 6.2 mg $L^{-1}$, cobalt chloride ($CoCl_2{\cdot}6H_2O$) 0.025 mg $L^{-1}$, cupric sulfate ($CuSO_4{\cdot}5H_2O$) 0.025 mg $L^{-1}$, ferrous sulfate ($FeSO_4{\cdot}7H_2O$) 27.8 mg $L^{-1}$, manganese sulfate ($MnSO_4{\cdot}4H_2O$) 22.3 mg $L^{-1}$, potassium iodide (KI) 0.83 mg $L^{-1}$, sodium molybdate ($Na_2MoO_4{\cdot}2H_2O$) 0.25 mg $L^{-1}$, zinc sulfate (ZnSO4·$7H_2O$) 8.6 mg $L^{-1}$, $Na_2EDTA{\cdot}2H_2O$ 37.2 mg $L^{-1}$ were used. This medium was supplemented with vitamins including inositol 100 mg $L^{-1}$, niacin 0.5 mg $L^{-1}$, pyridoxine-HCl 0.5 mg $L^{-1}$, thiamine-HCl 0.1 mg $L^{-1}$ and sucrose 30 g $L^{-1}$. The pH of the medium was adjusted to 5.8 before heat sterilization (at 121°C for 15 minutes) by 0.1N KOH and then 8 gr $L^{-1}$ agar was added. With the aim of conserving heat-sensitive allelochemicals, extracts were added after autoclave. The pH of the extracts was adjusted to 5.8 and was sterilized by 0.2 micron-rated filter membrane. Later, 30 mL of the media was added to each 300 mL flask.





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### Disinfection and Seed Culture

Small and damaged seeds were removed before starting disinfection. Seeds were washed with tap water, rinsed with 70% (v/v) ethanol for 30 seconds and then were disinfected by sodium hypochlorite (NaClO) 1.5% (w/v) for 20 minutes. Seeds were washed with sterile distilled water several times under laminar flow and cultured in the media. The culture condition (temperature 24±2°C, 16 hours photoperiod using cool white fluorescent light, and 3,000–4,000 lux light intensity) were maintained throughout the study. Germination, growth, and biochemical criteria were measured during 60 days of culture initiation.

### Methanolic Extract Preparation

To determine total phenolic content and antioxidant activity, plants root and shoot were collected from the fields at the reproductive stage and dried at 40°C for 48 hours. Five hundred mg of dry weight for each sample was powdered and shaken for 24 hours in 10 mL of 80% (v/v) methanol. Extracts were then separated from the residue using Whatman No.1 filter paper and then centrifuged at 4,000 rpm for 20 minutes. The supernatant was used to determine the total phenolic content and antioxidant activity.

### Determination of Total Phenolic Content

The amount of total phenolic was determined according to the method of Singleton and Orthofer (1999), which used Folin-Ciocalteu reagent and gallic acid as standard. Thirty µL of methanolic extract of root or shoot was diluted to the volume of 500 µL by distillated water, and then 500 µL of undiluted Folin-Ciocalteu reagent were added and mixed. The mixture was allowed to stand for a 1 min period, followed by the addition of 500 µL of 20% (w/v) sodium carbonate $(Na_2CO)_3$. After standing in the dark and room temperature for 120 minutes, the absorbance was read at 730 nm using a UV–Vis spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram dry weight sample.

### Determination of Total Antioxidant Activity

Antioxidant activity of methanolic extract of each part was assessed according to the method described by Brand *et al.* (1995) using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging capacity with some modifications. Afterwards, 30 µL of methanolic extract of each part was mixed with 350 µL of DPPH (1mM in methanol) and methanol 100% was added to bring to the final volume of 2 mL. Following incubation of the mixture in the dark and room temperature for 20 min, the absorbance of the reaction mixture was read at 517 nm using a UV–Vis spectrophotometer. The volume of the sample extract required to cause a 50% decrease in the absorbance at 517 nm relative to the control (100%) was calculated. Methanol and ascorbic acid were used as the control and positive control, respectively. The inhibition ratio was calculated from the following equation:

Inhibition (%)= [(Absorbance of the control-Absorbance of test sample)/Absorbance of the control]×100

### Extraction

The fresh tissues of treated plants were thoroughly ground with a cold mortar and pestle in an ice bath, until no fibrous residue could be seen. The grinding medium (500 mg) were completely extracted in 1,000 µL cool buffer phosphate (containing $K_2HPO_4$+$KH_2PO_4$ 50 mM ،EDTA 0.5 mM and pH= 7) and centrifuged at 15,000 rpm at 5°C for 15 minutes. The supernatant was





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used for determination of the superoxide dismutase (SOD) and peroxidase (POD) activity in the tissue.

### Total Peroxidase

Peroxidase activity was assessed according to the method of Kalir *et al.,* (1984) with minor modifications. The reaction solution (1 mL) contained phosphate buffer (475 µl, 50 mM; pH 7.0), guaiacol (475 µL; 45 mM), $H_2O_2$ (475 µL; 100 mM), and crude enzyme extract (50 µL). Absorbance due to the formation of tetra-guaiacol was recorded at 470 nm using a UV–Vis spectrophotometer and enzyme activity was calculated as per its coefficient of extinction( 26.6 $mM^{-1}$ $cm^{-1}$). One unit of enzymatic activity was defined as the amount of the enzyme that causes a change of 0.01 in absorbance per minute.

### Total Superoxide Dismutase Activity

The activity of Superoxide dismutase was determined as the inhibitation of the *p* - nitroblue tetrazolium chloride (NBT) photoreduction (Giannopolitis and Ries, 1977). The assay was conducted at 25°C in a total volume of one mL of 50 mM potassium phosphate buffer (pH 7.8), containing 0.1 mM EDTA, 1.3 µM riboflavine, 13 mM methionine, 75 µM NBT, and 50 µL of enzymatic extract. Absorbance was monitored at 560 nm after 15 minutes of illumination, using a UV–Vis spectrophotometer. One unit of SOD is defined as the amount of enzyme that inhibits 50% of NBT photoreduction to blue formazan that monitored at 560 nm. SOD activity of the extracts was expressed as units of SOD per milligram of protein.

### Analytical Methods and Statistical Analysis

The effect of three concentrations (1, 10 and 100 mg $mL^{-1}$) of each aqueous extract was evaluated in the treated plants and growth criteria (germination, root length, shoot length, root fresh weight, shoot fresh weight, leaf number, shoot: root ratio) and biochemical parameters (POX and SOD) were assessed. The shoot:root ratio was calculated based on shoot and root fresh weight measurements. This experiment was carried out in factorial completely randomized design with four replicates and ten samples per replicate for all evaluated characters, except for POX and SOD which were done in one replicate. The treatment means were separated by Duncan's multiple range tests using SPSS 10.0.

## RESULTS AND DISCUSSION

### Germination and Growth Criteria

The results of Duncan's multiple range test showed that by increasing the concentration of root and shoot extract from 1 to 100 mg $mL^{-1}$ in the culture media, the growth criteria of treated plant including germination, root length, shoot length, root fresh weight, shoot fresh weight and leaf number were decreased, but the ratio of shoot to root was enhanced (Table 1). The most significant effect on germination and growth criteria of treated plants was obtained in 100 mg $mL^{-1}$ concentration that prevented germination and subsequent growth in most cases (Table 1). Goosefoot was highly resistant in the cases of fennel shoot, wormwood root and wormwood shoot extracts, even in the high concentration of 100 mg $mL^{-1}$ (Table 1). In other words, goosefoot was germinated and continued to grow in all extracts, except fennel root extract, in 100 mg $mL^{-1}$ concentration compared with lower concentrations (1 and 10 mg $mL^{-1}$) as the highest level of POX and SOD were observed in this concentration (Table 2).

At the same concentration (100 mg $mL^{-1}$), fennel and radish were not resistant to the goosefoot and redroot pigweed extracts, as in most treatments, germination and growth





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**Table 1**. Duncan's multiple range tests comparing the mean of the effect of root and shoot extracts on growth criteria. [a]

| Treated plant | Growth criteria | Root fresh weight (mg) | | | Root length (mm) | | | Shoot fresh weight (mg) | | | Shoot length (mm) | | | Leaf number | | | Shoot: root ratio | | | Number Germination | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Concentration [mg.mL$^{-1}$] | 1 | 10 | 100 | 1 | 10 | 100 | 1 | 10 | 100 | 1 | 10 | 100 | 1 | 10 | 100 | 1 | 10 | 100 | 1 | 10 | 100 |
| | Extract | | | | | | | | | | | | | | | | | | | | | |
| Goosefoot | Fennel root | $8.21^{a}$ | $3.46^{b}$ | $0^{c}$ | $34.38^{a}$ | $15.65^{b}$ | $0^{c}$ | $110.27^{a}$ | $56.7^{b}$ | $0^{c}$ | $49.8^{a}$ | $34.6^{b}$ | $0^{c}$ | $7.3^{a}$ | $3.6^{b}$ | $0^{c}$ | $14.11^{a}$ | $16.58^{a}$ | $0^{b}$ | $8.5^{a}$ | $8^{a}$ | $4^{b}$ |
| | Fennel shoot | $9.28^{a}$ | $7.05^{b}$ | $4^{c}$ | $35.52^{a}$ | $27.8^{b}$ | $15.8^{c}$ | $115.2^{a}$ | $94.78^{b}$ | $60.02^{c}$ | $51^{a}$ | $45.4^{b}$ | $29.6^{c}$ | $7.65^{a}$ | $7.1^{ab}$ | $4.15^{b}$ | $13.11^{b}$ | $13.51^{b}$ | $15.34^{a}$ | $8.5^{a}$ | $8.5^{a}$ | $5.75^{b}$ |
| | Wormwood shoot | $9.09^{a}$ | $5.26^{b}$ | $2.99^{c}$ | $35.1^{a}$ | $20.7^{b}$ | $14.2^{c}$ | $113.15^{a}$ | $76.97^{b}$ | $51.53^{c}$ | $50.7^{a}$ | $41.15^{b}$ | $20.3^{c}$ | $7.35^{a}$ | $6.6^{ab}$ | $4^{b}$ | $12.61^{c}$ | $15.02^{b}$ | $17.49^{a}$ | $8.5^{a}$ | $8.25^{a}$ | $6^{b}$ |
| | Wormwood root | $8.81^{a}$ | $5.03^{b}$ | $3.01^{c}$ | $34.65^{a}$ | $22.45^{b}$ | $14.5^{c}$ | $114.8^{a}$ | $77.31^{b}$ | $54.06^{c}$ | $52.05^{a}$ | $40.64^{b}$ | $22.2^{c}$ | $7.5^{a}$ | $6.2^{b}$ | $3.95^{c}$ | $13.18^{c}$ | $15.53^{b}$ | $18.07^{a}$ | $8.5^{a}$ | $8.5^{a}$ | $5.75^{b}$ |
| Fennel | Pigweed root | $8.7^{a}$ | $5.94^{b}$ | $0^{c}$ | $48.6^{a}$ | $29.34^{b}$ | $0^{c}$ | $63.96^{a}$ | $44.33^{ab}$ | $0^{b}$ | $74.21^{a}$ | $49.63^{b}$ | $0^{c}$ | $3.86^{a}$ | $2.98^{b}$ | $0^{c}$ | $7.36^{a}$ | $7.51^{a}$ | $0^{b}$ | $9.33^{a}$ | $8^{b}$ | $0^{c}$ |
| | Pigweed shoot | $8.71^{a}$ | $6.06^{b}$ | $0^{c}$ | $49.45^{a}$ | $31.46^{b}$ | $0^{c}$ | $64.65^{a}$ | $44.03^{ab}$ | $0^{b}$ | $74.2^{a}$ | $50.96^{b}$ | $0^{c}$ | $3.65^{a}$ | $3.27^{a}$ | $0^{b}$ | $7.45^{a}$ | $7.35^{a}$ | $0^{b}$ | $9.25^{a}$ | $8^{a}$ | $0^{b}$ |
| | Goosefoot root | $9.11^{a}$ | $6.17^{b}$ | $0^{c}$ | $50.8^{a}$ | $30.1^{a}$ | $0^{c}$ | $62.96^{a}$ | $45.03^{ab}$ | $0^{b}$ | $72.27^{a}$ | $51.33^{b}$ | $0^{c}$ | $3.53^{a}$ | $3.2^{ab}$ | $0^{b}$ | $6.93^{a}$ | $7.36^{a}$ | $0^{b}$ | $9.33^{a}$ | $8^{a}$ | $0^{b}$ |
| | Goosefoot shoot | $9.27^{a}$ | $7.52^{b}$ | $3.1^{c}$ | $50.13^{a}$ | $40.8^{b}$ | $9.85^{c}$ | $65.36^{a}$ | $49.8^{b}$ | $25^{c}$ | $73^{b}$ | $60.9^{b}$ | $20.25^{c}$ | $3.66^{a}$ | $3.7^{ab}$ | $2^{b}$ | $7.06^{b}$ | $6.66^{b}$ | $8.32^{a}$ | $8.66^{a}$ | $8.25^{a}$ | $6^{b}$ |
| Radish | Goosefoot shoot | $15.71^{a}$ | $10.24^{b}$ | $0^{c}$ | $27.2^{a}$ | $18.07^{b}$ | $0^{c}$ | $184.27^{a}$ | $126.39^{b}$ | $0^{c}$ | $45.25^{a}$ | $38^{b}$ | $0^{c}$ | $3.75^{a}$ | $3.10^{b}$ | $0^{c}$ | $12.51^{a}$ | $12.17^{a}$ | $0^{b}$ | $5.5^{a}$ | $3.75^{b}$ | $0^{c}$ |
| | Pigweed root | $14.72^{a}$ | $6.72^{b}$ | $0^{c}$ | $28.06^{a}$ | $11^{b}$ | $0^{c}$ | $185.91^{a}$ | $103.98^{b}$ | $0^{c}$ | $45.51^{a}$ | $28.12^{b}$ | $0^{c}$ | $2.96^{a}$ | $2.6^{ab}$ | $0^{b}$ | $12.62^{b}$ | $16.23^{a}$ | $0^{c}$ | $5^{a}$ | $3.5^{b}$ | $0^{c}$ |
| | Goosefoot root | $15.35^{a}$ | $10.38^{b}$ | $0^{c}$ | $27.75^{a}$ | $19.28^{b}$ | $0^{c}$ | $183.91^{a}$ | $127.5^{b}$ | $0^{c}$ | $48.05^{a}$ | $38.41^{b}$ | $0^{c}$ | $3.39^{a}$ | $3^{ab}$ | $0^{b}$ | $12.45^{a}$ | $12.3^{a}$ | $0^{b}$ | $5^{a}$ | $3.5^{b}$ | $0^{c}$ |
| | Pigweed shoot | $14.98^{a}$ | $6.42^{b}$ | $0^{c}$ | $28.15^{a}$ | $10.72^{b}$ | $0^{c}$ | $185.01^{a}$ | $104.15^{b}$ | $0^{c}$ | $46.65^{a}$ | $27.87^{b}$ | $0^{c}$ | $3.6^{a}$ | $2.65^{b}$ | $0^{c}$ | $11.79^{b}$ | $17.98^{a}$ | $0^{c}$ | $5.75^{a}$ | $3.25^{b}$ | $0^{c}$ |

[a] Different letters (a-c) indicate significant differences (Duncan's test at $P< 0.05$ level).

**Table 2**. Peroxidase and superoxide dismutase activity of plant parts treated with different extracts.

| Extract | Concentration (mg $mL^{-1}$) | Treated plant | SOD (µm $min^{-1}$ $cc^{-1}$) | | POX (U $gr^{-1}$ FW) | |
|---|---|---|---|---|---|---|
| | | | Root | Shoot | Root | Shoot |
| Control | 0 | Goosefoot | 165.48 | 41.73 | 28.62 | 2.36 |
| Wormwood root | 1 | Goosefoot | 283.19 | 87.58 | 56.73 | 4.34 |
| Wormwood root | 10 | Goosefoot | 909.57 | 116.04 | 131.78 | 5.01 |
| Wormwood root | 100 | Goosefoot | 1543.42 | 146.70 | 209.45 | 5.86 |
| Wormwood shoot | 1 | Goosefoot | 261.07 | 93.81 | 69.99 | 3.61 |
| Wormwood shoot | 10 | Goosefoot | 912.12 | ۱۳۱.16 | 118.60 | 3.86 |
| Wormwood shoot | 100 | Goosefoot | 1484.62 | 159.37 | 187.52 | 4.93 |
| Fennel root | 1 | Goosefoot | 376.61 | 127.77 | 89.25 | 3.19 |
| Fennel root | 10 | Goosefoot | 1472.13 | 257.24 | 118.78 | 4.32 |
| Fennel shoot | 1 | Goosefoot | 301.08 | 68.34 | 27.43 | 2.97 |
| Fennel shoot | 10 | Goosefoot | 638.74 | 94.84 | 41.80 | 3.14 |
| Fennel shoot | 100 | Goosefoot | 763.59 | 189.99 | 72.02 | 4.84 |
| Control | 0 | Fennel | 285.20 | 99.51 | 7.18 | 1.48 |
| Goosefoot root | 1 | Fennel | 321.00 | 161.52 | 14.70 | 0.95 |
| Goosefoot root | 10 | Fennel | 412.93 | 182.27 | 18.86 | 2.51 |
| Goosefoot shoot | 1 | Fennel | 316.71 | 125.15 | 8.22 | 1.04 |
| Goosefoot shoot | 10 | Fennel | 374.02 | 163.77 | 10.43 | 1.90 |
| Goosefoot shoot | 100 | Fennel | 752.16 | 506.76 | 39.90 | 3.45 |
| Redroot pigweed root | 1 | Fennel | 336.82 | 154.85 | 16.73 | 1.63 |
| Redroot pigweed root | 10 | Fennel | 419.35 | 244.15 | 25.38 | 1.43 |
| Redroot pigweed shoot | 1 | Fennel | 323.43 | 160.55 | 29.08 | 0.79 |
| Redroot pigweed shoot | 10 | Fennel | 394.35 | 217.24 | 34.04 | 2.31 |
| Control | 0 | Radish | 194.71 | 114.91 | 92.60 | 9.07 |
| Goosefoot shoot | 1 | Radish | 437.82 | 168.12 | 174.59 | 17.83 |
| Goosefoot shoot | 10 | Radish | 726.55 | 206.32 | 387.93 | 26.37 |
| Goosefoot root | 1 | Radish | 393.55 | 129.58 | 226.70 | 17.12 |
| Goosefoot root | 10 | Radish | 712.37 | 220.22 | 417.38 | 23.24 |
| Redroot pigweed root | 1 | Radish | 272.90 | 128.57 | 280.61 | 14.47 |
| Redroot pigweed root | 10 | Radish | 637.15 | 264.187 | 461.43 | 16.61 |
| Redroot pigweed shoot | 1 | Radish | 793.96 | 176.24 | 368.66 | 109.69 |
| Redroot pigweed shoot | 10 | Radish | 921.02 | 327.20 | 593.97 | 101.02 |

of fennel and radish were prevented. In the present study, for all growth criteria, no significant difference was detected between lower concentrations of 1 mg $mL^{-1}$ and the control condition, while 100 mg $mL^{-1}$ almost inhibited the germination and subsequent growth (Table 1). Therefore, we focused on 10 mg $mL^{-1}$ concentration in order to determine their responses to the allelopathic effects.

Undoubtedly, germination is one of the most important plant growing stages and is severely affected by allelochemical components (Bogatek *et al.,* 2006). No significant difference was observed between the effect of plants extracts and control conditions on germination at 10 mg.$mL^{-1}$ concentration (Figure 1a-c), but at 100 mg $mL^{-1}$ concentration, root and shoot extracts severely decreased the seed germination (Table 1). In agreement with our results, many studies have reported the inhibitory effect of allelochemicals on germination. The effect of plant extract of *Nepeta meyeri* on germination and growth of seven plant species showed that with increasing the extract concentration, germination was reduced (Mutlu *et al.,* 2010). Moreover, the allelopathic effects of two cultivars of sunflower (*Helianthus annuus* L.) at 2.5, 5, and 10% (m/v) concentration on germination of mustard (*Sinapis alba*) revealed that with increasing the extract concentration, seed germination was inhibited as decease in





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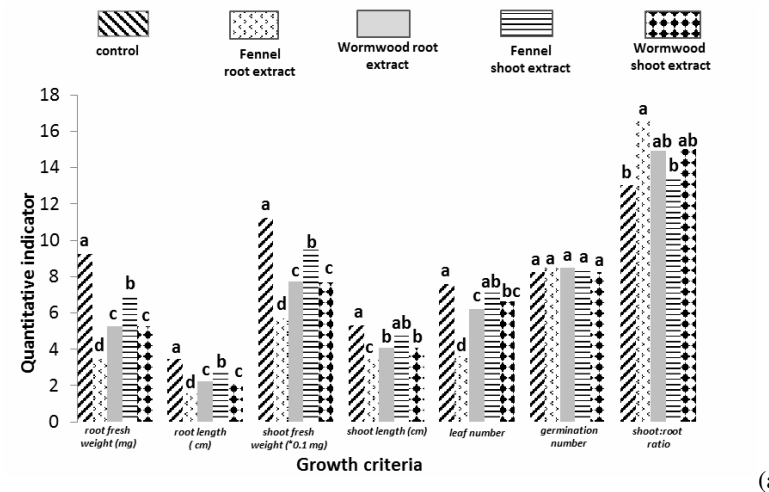
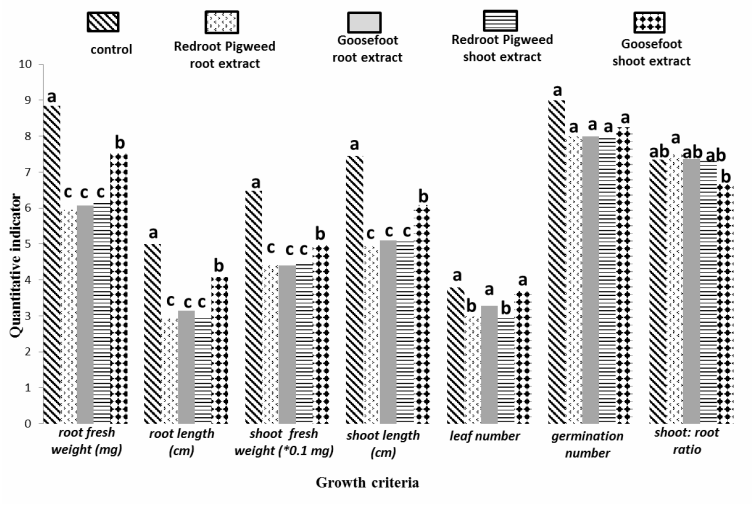



(a)

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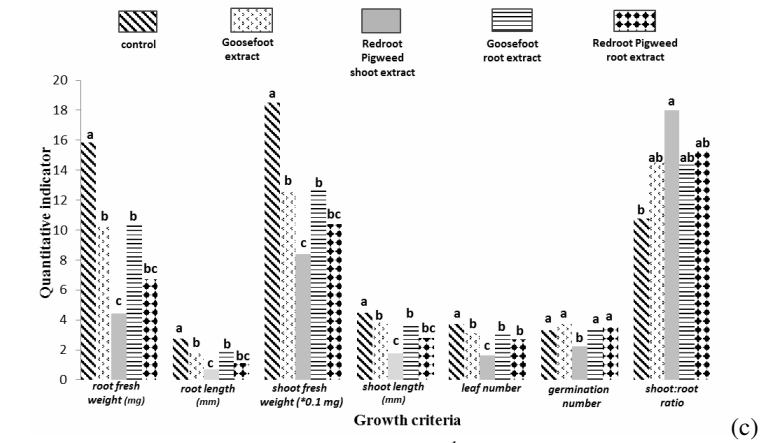


(c)

**Figure 1**. Comparison of the mean effect of 10 mg $mL^{-1}$ concentration of root and shoot extracts of (a) fennel and wormwood on goosefoot growth criteria, (b) redroot pigweed and goosefoot on fennel growth criteria and (c) redroot pigweed and goosefoot on radish growth criteria.. Different letters (a-d) indicate significant differences (Duncan's test at $P< 0.05$ level).





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germination was significantly correlated with increased membrane deterioration (Bogatek *et al.,* 2006). Aqueous extract of plant parts at low concentrations had no effect on seed germination, but by increasing the concentration of the extract, seed germination displayed more reduction (Valera-Burgos *et al.,* 2012; Lin *et al.,* 2004).

Growth criteria of fennel, goosefoot, and radish at 10 mg $mL^{-1}$ concentration revealed that extracts severely reduced shoot length, fresh weight, root length and fresh weight, while shoot: root ratio was greatly increased compared to the control conditions (Figure 1a-c). Results showed that the highest effect on growth criteria were related to the fennel root extract (in the case of goosefoot), goosefoot root extract (in the case of fennel), and redroot pigweed shoot extract (in the case of radish). The highest amount of POX and SOD in both root and shoot of fennel, goosefoot, and radish at 10 mg $mL^{-1}$ concentration, were related to goosefoot root, fennel root, and redroot pigweed shoot extract, respectively. The least effect on growth criteria was related to the fennel shoot extract (in the case of goosefoot) and goosefoot shoot extract (in the case of fennel) (Table 2). The least amount of POX and SOD in both root and shoot of fennel and goosefoot at 10 mg $mL^{-1}$ concentration, were related to goosefoot and fennel shoot extract, respectively. Therefore, the goosefoot (in the case of fennel shoot extract) and fennel (in the case of goosefoot shoot extract) germinated and continued to growth at 100 mg $mL^{-1}$ concentration (Table 3). The effects of root and shoot extracts of wormwood (in the case of goosefoot) and goosefoot (in the case of radish) on growth were similar and no significant was observed between them.

In view of biochemistry, our results showed that root system was influenced more than shoot system (Table 2), as in all treated concentrations the increase in activities of POX and SOD of root were more than that of shoot. Many studies have reported the inhibitory effect of allelochemicals on growth (Soltys *et al.,* 2011; Chowhan *et al.,* 2013; Mishra and Nautiyal, 2011). The effect of humic substance and its fractions were studied on morphology of *Arabidopsis thaliana* and results showed that humic fractions, especially at high concentrations, reduced root and shoot fresh weight and enhanced shoot: root ratio (Muscolo *et al.,* 2010). It is reported that shoot: root ratio of *Quercus rubra* was enhanced in the presence of *Dennstaedtia punctilobula* (Lyon and Sharpe, 1996).The effect of leaf extract of *Acacia pennatula* on germinability of three plant species revealed that general growth and especially the development of root in seedlings was reduced, reflecting shifting their biomass allocation model to a reduced root: shoot ratio which interferes in the development of the root system (Peguero *et al.,* 2012). Impairment of various metabolic activities due to plant leachate resulted in decreased root and shoot length (Singh *et al.,* 2009). Extract of *Pinus pinea* decreased root length of *Cistus* (Valera-Burgos *et al.,* 2012), which may influence seedling establishment in the field due to the differential access to water sources, hence, poor development of root could affect the ability of plant to cope with water deficiency condition, especially during summer.

**Biochemical Parameters**

The control treatments showed the maximum growth and minimum POX and SOD activity in both root and shoot compared to the other concentrations (Table 2). It was reported that the controls had the least POX activity and when plants were treated with different extract concentrations, POX and SOD were increased (Singh *et al.,* 2009; Bai *et al.,* 2009). Similarly, another study showed that by increasing the concentration of plant extract (*Eupatorium adenophorum*), POX and SOD activity of goosefoot and redroot pigweed leaves were





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enhanced (Jinhu *et al.,* 2012). Allelochemicals cause oxidative damage, as evidenced by enhanced activity of ROS-scavenging enzymes and increased membrane lipid peroxidation (Lara-Nunez *et al.,* 2006). Moreover, antioxidative enzymes are the most important components in the scavenging system of ROS; and SOD is a major scavenger of superoxide, and its enzymatic action results in the formation of $H_2O_2$. The $H_2O_2$ produced is then scavenged by catalase (CAT) and a variety of POX (Noctor and Foyer, 1998). Therefore, according to our results, POX and SOD systems of root and shoot of the plants encountered allelochemicals of different concentrations (even low concentration) and these systems were activated as a resistance mechanism in stressful condition in tissue culture media. Plants that germinated and continued to grow at high concentration of 100 mg $mL^{-1}$ also had the highest POX and SOD activities (Table 2) Indicating further resistance.

According to the results, the plant parts containing more phenol have more antioxidant activity as wormwood shoot has the highest phenolic content and antioxidant activity (Total phenol= 57 µg $mg^{-1}$ DW and DPPH IC50= 0.4 mg) (Table 3). In accordance to our results, previous researches (Caia *et al.,* 2004; Wojdylo *et al.,* 2007; Lu *et al.,* 2011) showed that there was a positive correlation between antioxidant activity and total phenolic content. Our data shows that different plant parts have different phenolic and antioxidative activity and subsequently different allelopathic effects. For example, the root of fennel (in the case of goosefoot) and goosefoot (in the case of fennel) showed low phenolic content and, consequently, low antioxidant activity, revealing more allelopathic effects on growth criteria (Figure 1a and b) compared to fennel and goosefoot shoot with high amount of phenolic content and high antioxidant activity (Table 3). In addition, redroot pigweed shoot extract (in the case of radish) revealed low phenolic content and, consequently, low antioxidant activity (Table 3) and, therefore, more allelopathic effect on growth criteria than the other extracts (Figure 1c). More POX and SOD activity of parts (Table 2) accompanied by reducing growth criteria (Table 1). In accordance to our results, many researches have indicated that different plant parts contained different allelopathic effects (Sodaeizadeh *et al.,* 2009; Oueslati, 2003; Fernandez *et al.,* 2009). Some previous researchers have reported that phenolic components are the main cause of allelopathic effects (Garcia-Sanchez *et al.,* 2012; Li *et al.,* 2010; Jarchow and Cook, 2009), as more total phenolic content have more allelopathic effect on germination and subsequent growth (Ben-Hammouda *et al.,* 1995; Balezentiene and Seziene, 2010; Chon and Nelson, 2010). However, it is not the fact that all phenolic components have allelopathic effects (Inderjit 1996) and there is no simple relationship between total phenolic content and allelopathic effect (Yang *et al.,* 2009). These statements are in

**Table 3**. Total phenolic content and antioxidant activity of root and shoot.

| Plant material | DPPH | Total phenol [µg.$mg^{-1}$ DW] |
|---|---|---|
| Wormwood shoot | IC50= 0.4 mg | 57 |
| Wormwood root | IC50= 0.53 mg | 50 |
| Fennel root | IC50= 12.53 mg | ND |
| Fennel shoot | IC50= 0.73 mg | 38 |
| Redroot pigweed shoot | IC50= 11.87 mg | ND[a] |
| Redroot pigweed root | IC50= 23.04 mg | ND |
| Goosefoot shoot | IC50= 0.8 mg | 27 |
| Goosefoot root | IC50= 23.64 mg | ND |

[a] No Detection.





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agreements with our finding that plants containing more phenolic and antioxidative activity reveal less allelopathic effects.

## CONCLUSIONS

Our finding explains that different plant parts have different allelopathic effects on plant species. In addition, more allelopathic effects due to increasing extract concentration lead to reducing growth criteria and increasing biochemical (POX and SOD) activity of each plant part. Germination inhibition depends on the extract concentration and confirms the importance of allelopathic effects of extract (especially high concentrations) as a mechanism for controlling seed germination. Therefore, tissue culture media could be an accurate and suitable tool for: (1) screening resistant plant and alien (non-endemic) plants that are not yet cultivated and no data are available about their resistance to prevalent weeds of the region, and (2) identifying high allelopathic plants as potential bioherbicide and invasive plant controller.

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# ارزیابی اثرات آللوپاتیک برخی گونه های گیاهی در محیط کشت بافت به عنوان یک روش دقیق انتخاب گیاه مقاوم و غربال گری علف کشهای زیستی

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## چکیده

تکنیک کشت بافت گیاهی می‌تواند شرایط استریل و قابل کنترلی را فراهم کند که در آن اثر دقیق و مستقیم ترکیبات مختلف را بر شاخص‌های رشد و نمو گیاهان مورد بررسی قرار داد. در این پژوهش اثر عصاره آبی (۱، ۱۰ و ۱۰۰ mg.mL-1) ریشه و شاخه گیاهان سلمه (Chenopodium album L.)، تاج خروس (Amaranthus retroflexus L.) ، رازیانه (Foeniculum vulgare) و درمنه (Artemisia absinthium L.) بر جوانه زنی و پارامترهای بیوفیزیکی گیاه در بستر کشت بافت مورد بررسی قرار گرفت. نتایج نشان داد عصاره ریشه رازیانه که تقریبا فاقد ترکیبات فنولی و متعاقبا توان آنتی اکسیدانی کم بود، بیشترین و شدید ترین تاثیر آللوپاتیک را به ویژه در غلظت ۱۰۰ mg.mL-1روی علف هرز سلمه دارد که ممکن است ناشی از ترکیباتی با پتانسیل کنترل بیولوژیک علف هرز و مهاجم سلمه باشند. گیاه سلمه نسبت به عصاره شاخه رازیانه، ریشه و شاخه آرتمیزیا مقاوم است ولی رازیانه و تربچه، در غلظت زیاد و مشابه ۱۰۰ mg.mL-1 ، نسبت به عصاره ریشه و شاخه تاج خروس و سلمه مقاوم نیستند. در پاسخ به ترکیبات آللوپاتیک نسبت شاخه: ریشه افزایش یافت و بیشترین میزان پراکسیداز و سوپراکسید دیسموتاز نیز در ریشه وجود داشت. بین پتانسیل اللوپاتیک گیاهان و محتوای فنول و توان آنتی اکسیدانتی رابطه مستقیم وجود نداشت. نتایج نشان داد تاثیرات آللوپاتیک عصاره روی شاخص‌های بیوفیزیکی و بیوشیمیایی به سطح غلظت عصاره و نوع اندام گیاهی بستگی دارد. بنابراین بستر کشت بافت ابزار دقیق و مناسبی برای غربال‌گری گیاه مقاوم به ترکیبات آللوپاتیک علف‌های هرز، شناسایی گیاهان با پتانسیل آللوپاتیک زیاد؛ احتمالا به عنوان علف‌کش بیولوژیک و کنترل کننده گیاهان مهاجم می‌باشد.





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