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Research Paper

Impact of Planting Bed and Culture Conditions on Phenolic Acid Production in Some *Lactuca undulata* Ledeb. Populations

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Introduction

Lactuca undulata Ledeb. is an annual herb belonging to the family of Asteraceae. In Iran, it has been reported from Golestan National Park, Tehran, Semnan, Isfahan, Hormozgan, Khorasan, Azerbaijan, Sistan and Baluchestan, Kerman, and Fars (Asadi *et al*., 2013). Phytochemical analyses have shown that *L. undulata* contains flavonoids, phenols, and caffeic acid derivatives such as chicoric acid (Ramezannejad *et al*., 2019). According to Ramezannejad results, the highest amount of chicoric acid (2.3 mg/g DW) was found in the stems during the reproductive phase.

Chicoric acid ($C_{22}H_{18}O_{12}$) was first isolated from the shoots of *Cichorium intybus* L. (Scarpati & Oriente, 1958) and has various pharmacological and biological properties. The amount of this compound depends on different factors such as plant species, plant organs, and growth conditions (Murch *et al.*, 2006).

The quality and quantity of secondary metabolites are dependent on the plant species, variety, and population (Holeski *et al.*, 2012). Also, genetic composition, environmental conditions, and the interaction between these two factors are the most important factors influencing the quality and quantity of secondary metabolites in plants (Nicolle *et al.*, 2004). Our previous study on five *L. undulata* populations collected from Qom, Biarjamand, Mirzabayloo in North Khorasan, Firoozkooh, and Cheshmeh Ali-Damghan showed that the highest amount of chicoric acid (3.5 mg/g dry weight) was obtained from Firoozkooh samples. In the present study we aimed to investigate the impact of planting bed and culture conditions on chicoric acid production in different populations of *L. undulata*.

Methods and Materials

Lactuca undulata seeds were collected from May to June from different regions (Cheshmeh Ali- Damghan, Qom, Mirzabailo, Biarjamand, and Firoozkooh) of Iran. Then seeds were planted in pots with two different soil types (potting mix and soil collected from natural habitats) and kept either outdoors or in a growth chamber. The pots were incubated at 25±2 °C and 16h light/ 8h dark photoperiod in growth chamber conditions. The other group of seeds was planted in 1×2 m plots in the field. This experiment was conducted using a completely randomized design. Three pots were selected for each culture medium and five seeds were planted in each pot. Pots and plots were irrigated every two days until the seeds were germinated. After germination, they were irrigated four days apart. Sampling was performed at the outset of the reproductive phase. Then the roots and shoots of the specimens were harvested, separated into batches and fresh weight of the roots and shoots were recorded. Samples were then air-dried in the shade with proper ventilation at 75°C for 3 days, and dry weight of the samples was measured. Dried powdered samples were used to quantify caffeic acid derivatives contents. Plant extraction was undertaken according to Luo *et al.*, (2003) method. Caffeic acid derivatives contents were determined by HPLC-UV system.

Along with seed collecting and plant harvesting from natural habits, plant rhizosphere soil and field soil were collected and various factors such as pH, electrical conductivity (EC), lime percentage, and soil texture were measured.

Statistical analysis was performed using SPSS software (version 22). ANOVA and Duncan's multiple range test ($p=0.05$) were used to compare the means.

Results & Discussion

The results showed that the texture of the soil samples collected from Cheshmeh Ali, Biarjamand, and Qom was sandy loam. But the texture of the soil samples from Mirzabayloo and Firoozkooh was loam and loamy-clay, respectively. The highest EC value was observed in the soil samples collected from Firoozkooh, Mirzabayloo, and Cheshmeh Ali regions. But the lowest EC value was observed in Qom and Biarjamand samples. Soil texture of the samples collected from the field plots was loamy-clay

The obtained data revealed that seeds collected from the Mirzabayloo region planted in the growth chamber had the highest growth rate compared to the other populations. Also, Cheshmeh Ali, Mirzabayloo, and Firoozkooh populations entered the reproductive phase with a delay of one month compared to Qom and Biarjamand populations.

Analysis of variance showed that there is a significant difference among the five investigated populations in root and stem length and fresh and dry weight, which grew in different conditions. The highest amount of root and stem fresh and dry weight in all populations was observed in the samples

which were grown in the field conditions. Among the samples grown in the field conditions, Mirzabailo (33.78±11.53) and Qom (26.11±4.78 gr) showed the highest amount of fresh weight. The present results also showed that plants that grew in the field had longer stems and roots compared to the plants grown in the pots. The highest stem length (52.66±4.23 cm) was observed in Mirzabayloo populations.

Analysis of variance also showed that there is a significant difference in the amount of chicoric acid and chlorogenic acid among different populations which grow in different conditions. The amount of chicoric acid in plants grown in pots and outdoors was higher than the samples grown in the field or growth chamber. The highest amount of chicoric acid (1.24 mg/kg DW) was observed in the Firoozkooh population which was grown in pots containing soil from the natural habitat. But the highest amount of chlorogenic acid (0. 98 mg/g DW) and caffeic acid (0.50 mg/g DW) were measured in the Cheshmeh Ali population which grew in the pots containing natural habitat soil. The highest amount of chicoric acid, chlorogenic acid, and caffeic acid was observed in plants that grew outdoors. It can be due to temperature fluctuations between day and night. This finding is in agreement with the results of Tudela *et al.,* (2017) who reported low temperature decreased phenolic acid metabolism in *Lactuca sativa* during the growing season. On the other hand, light intensity and wavelength are the most important factors which can stimulate phenolic acid biosynthesis in plants (Romani *et al.,* 2002). Yildirim and Turker (2014) showed that the production of phenolic acids increased in plants grown outdoors compared to plants that grew in the growth chamber.

Conclusion

The current results showed that there are high levels of diversity among *Lactuca undulata* populations in terms of phytochemical characteristics. Meanwhile, the obtained results revealed that the highest amount of chicoric acid (1.24 mg/kg DW) was observed in the Firoozkooh population which was grown in pots containing soil from the natural habitat.

Keywords*: Caffeic acid, Chlorogenic acid, Chicoric acid,* Lactuca undulata *Ledeb., Soil*

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