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Effect of macro and micro elements on the formation of secondary metabolites in *Ornithogalum sintenisii*

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Abstract. The ecological environment created by the influence of anthropogenic factors has made the living conditions of living creatures difficult. The development of industry, the creation of new cities, and new workplaces created to meet the needs of the increasing world population disrupt the ecological balance. One of the plant species exposed to these effects is the *Ornithogalum sintenisii* Freyn species. Although this species is widely distributed in the northern region of Azerbaijan, its population has decreased much compared to the 1980s. In this study, the soil-plant relationship of *O. sintenisii* species and the effects of this relationship on phenolic and flavonoid formation were learned. As a result of the research, the amounts of phenolic and flavonoids in the bulb and leaf parts of the species are normal and it is thought that this is due to the effect of some micro and macro elements found in the soil and plant structure. *O. sintenisii* species, distributed in Mumlu village of Şabran district, grows in gray-brown soils, and the normal amounts of Cu (7.09 ppm) and Mn (24.83 ppm) in these soils positively affected the formation of flavonoids. Finally, it is possible to say that the extracts of this species are a rich source of secondary metabolites and have pharmacological value..

1 Introduction

In the face of the scientific world, the development of life on earth and the plants that are a part of it have become one of the most important subjects to be learned. The development of plant organs and the addition of new chemical elements to their structures as a result of this development have brought them to the highest level of living things over time. Each of the chemical elements definitely has a role in the life of the plant [1].

Plant organisms constantly produce primary and secondary metabolites to maintain their own life. Secondary metabolites are chemical substances produced by plants that are not directly related to the basic vital functions of the plant, but are at least as important as primary metabolites (protein, fat, carbohydrate) [2]. By producing secondary metabolites, plants can have the ability to create self-defense mechanisms against adverse environmental conditions, diseases, bacteria, insects and animals and have an allelopathic effect [3, 4]. Secondary metabolites are also used in industrial areas in paint, fiber, glue, oil, flavor, perfume and medicines. Recognition of the biological properties of numerous secondary metabolites has triggered the search for new drugs, antibiotics, insecticides and herbicides [5, 6]. Recently, studies have been carried out on many secondary metabolites that are thought to be effective in inhibiting the coronavirus protease enzyme. All these studies reveal the importance of secondary metabolites [7].

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Just as soil has an important place in the life of plants, plants also have an extraordinary role in the renewal and functioning of the soil [8]. To learn about this interaction, scientists are constantly conducting research on soil-plant relationships. Shen *et al.* [9] investigated the role of P in plant life and the needs for this element in agriculture in the coming years. Wyttenbach et al. [10] investigated the role of the element Ca in soil formation and its effect on plants. Cataldo and Wildung [11] investigated the effect of heavy metal pollution in the terrestrial environment on plants and the current transport mechanisms of nutrient ions Cu, Zn and K. They also investigated the absorption mechanisms of some non-nutrient ions (Cd, Tl and Ni) and performed detailed kinetic studies [2, 11].

Our aim in this study was to investigate the soil-plant relationship and its effect on secondary metabolites on the *Ornithogalum sintenisii* Freyn species. For this purpose, the amount of macro and micro elements in the structure of the soil and the plant was determined. At the same time, the amounts of phenolic and flavonoid substances in the bulb and leaf extracts of the plant were determined and a connection was established between them and the amounts of macro and micro elements in the structure of the plant.

2 Material and Metod

2.1 Plant material

The *O. sintenisii* was collected in the autumn of 2021 from the land of Mumlu village in Shabran district of Azerbaijan. Species identification was made. After the bulbs and leaves of the plants were dried in the dark, they were crushed into small pieces and made ready for extraction.

2.2 Preparation of the extracts

After drying, plant parts (bulb and leaves) were extracted in methanol and acetone in a shaking water bath at 55°C. The extraction process was repeated twice for 12 hours. Then, the obtained solution was purified from solvent (at 45-48°C) with the help of a rotary evaporator (Rotavapor IKA VB 10, Germany) and dried by removing the water in a lyophilizator (Thermosavant Modulyo D, USA). The extracts were stored in a deep freezer at -20°C for use in future studies [12].
Plant extracts: Ornithogalum (O), Bulb - Methanol (OBM), Bulb - Acetone (OBA), Leaf-Methanol (OLM), Leaf-Acetone (OLA).

2.3 Total phenolic and flavonoid content

The total phenolic content of extracts were determined by colorimetric Folin- Ciocalteau method [13], and expressed as gallic acid equivalents (GAE) in mg/mL plant extract. The total flavonoid content of extracts was detected by the aluminum chloride method [14] and expressed as mg of quercetin equivalents (QE) per g of extract (mgQE/g). Total phenolic and flavonoid amount determination experiments were repeated three times.

2.4 Soil and plant analysis methods

Different methods are used to carry out soil and plant mineral matter analysis. The most important of these methods are AAS (Atomic Absorption Spectrometer) and ICP-OES (Inductively Coupled Argon Plasma-Optical Emission Spectrometer). The analysis of bulb and leaf mineral elements of the Ornithogalum sintenisii plant was carried out using the ICP-OES (Inductively Coupled Argon Plasma-Optical Emission Spectrometer) method. In order to determine the general amount of nitrogen, the sample was prepared by wet combustion and the data obtained on the Kjeldahl device was recorded [15]. For other macro and micro elements, samples were prepared by aging in a microwave device and scanned in the ICP-OES device.

2.5 Statistical analysis

The experimental data are presented as mean ± standard error. All of the statistical analyses were performed by SPSS Statiscal program using one way analysis of variance (ANOVA) adn Duncan ($p < 0.05$).

3 Results and Discussion

Plants in contact with potentially hazardous external environmental factors have developed a wide variety of complex defense strategies. Plants, which are a part of the natural environment, are around abiotic and biotic factors. It is an indisputable fact that secondary metabolites are produced by the plant in order to overcome all kinds of stress conditions that arise. [16, 17]. Most researchers have adopted that the formation of secondary metabolites is linked to soil and other environmental factors. Especially the macro and micro elements found in the structure of the soil and their role in plant life come to the fore. It is known that plants producing saponins collect Mn, Mo, W and Cr, and plants synthesizing terpenoids collect Mn [18]. Research has proven that alkaloid-producing plants accumulate Co, Mn, Zn and Cu [19]. Fe, Cr, Cu, Co and Mn are cofactors and activators of ferments that catalyze the main stages of phenol exchange [20]. Skórzynska-Polit *et al.* [21] They determined that flavonoid synthesis increased in *Phaseolus coccineus* L. under the influence of Cd^{2+} and Cu^{2+} ions.

Table 1. Mineral substance analysis results of *O. syntenisi*

Test name	Methods	Mineral content	Position
Body (Sand)	Hydrometer	36 %	Sandy Clay Loam
Body (Clay)	Hydrometer	33 %	Sandy Clay Loam
Body (Silt)	Hydrometer	24 %	Sandy Clay Loam
pH(25 °C)	1:2,5	7,016	Medium Acid
Lime	Calcimetric	7,34 %	Less Chalky
Organic Matter	Walkey-Black	5,38 %	Middle
Nitrogen (N)	Burning method	0,67 %	Middle
Phosphorus (P)	Spectrophotometric	94,13 ppm	Very little
Potassium (K)	A.A/ICP-OES	288 ppm	Morek
Calcium (Ca)	A.A/ICP-OES	3,415 ppm	More
Magnesium (Mg)	A.A/ICP-OES	173 ppm	More
Sodium (Na)	A.A/ICP-OES	59,46 ppm	Middle
Iron (Fe)	DTPA/ICP-OES	12,78 ppm	More
Manganese (Mn)	DTPA/ICP-OES	10,58 ppm	More
Zinc (Zn)	DTPA/ICP-OES	14,71 ppm	Too much
Copper (Cu)	DTPA/ICP-OES	2,46 ppm	Normal
Boron (B)	ICP-OES	2,38 ppm	Sufficient

The soils where *O. sintenisi* species is distributed in the Şabran district are generally humus and fertile soils [22]. As a result of the analysis of soil samples taken from the Şabran region, it was determined that these soils are slightly calcareous, partially clayey, slightly acidic and unsalted. These soils, which are at medium levels in terms of organic matter and nitrogen, are poor in phosphorus, less than normal in potassium and calcium, and sufficient in terms of magnesium (Table1). The current chemical structure of the soil also affects the structure of plants. As a result of the research, it has been determined that nitrogen, phosphorus, magnesium, iron and boron are sufficient, while potassium, calcium, copper, manganese and zinc elements are less than normal in the structure of the plant (Table 2).

Table 2. Mineral matter analysis results of the leaf part of *O. syntenisi*

Element	Methods	Mineral content	Situation
Total Nitrogen (N)	Kjeldahl	0,32%	Sufficient
Phosphorus (P)	ICP-OES	3,51%	Sufficient
Potassium (K)	ICP-OES	2,93 %	less than normal
Calcium (Ca)	ICP-OES	0,48 %	less than normal
Magnesium (Mg)	ICP-OES	0,30 %	Sufficient
Iron (Fe)	ICP-OES	135.03 ppm	Sufficient
Copper (Cu)	ICP-OES	7.09 ppm	Normal
Manganese (Mn)	ICP-OES	24.83 ppm	Normal
Zinc (Zn)	ICP-OES	18.88 ppm	less than normal
Boron (B)	ICP-OES	35, 17 ppm	Sufficient

Phenolic compounds and flavonoids constitute an important group of secondary metabolites. Flavonoids regulate the movement of auxin at the intracellular and intercellular level and in this way ensure the development of individual organs of the plant [23]. Since the amount of potassium in Mumlu village soils is low, the formation of flavonoids, tannins and glycosides may be reduced. The normal amount of Cu (7.09 ppm) and Mn (24.83 ppm) positively affected the formation of flavonoids in the plants in these soils. The amounts of phenolic and flavonoid substances in the extracts of the *O. sintenisi* plant collected from Mumlu village were examined. The highest amount of phenolic substances (5.11±2.08 mg GAE/mL extract) was in bulb-methanol (OBM) extracts, and the highest amount of flavonoids (77.06±2.46 mgQE/g) was in leaf methanol (OLM) extracts. was seen. It is possible to associate the fact that these amounts are higher compared to other plants with the normal amount of Cu and Mn chemical elements in the soil and plant structure (Table 3).

Table 3. Total phenolic and total flavonoid contents of the extracts of *O.sintenisii*

Plant extracts	Total phenolic content (mg GAE/mL extract)*	Total flavonoid content (mgQE/g)*
OBM	5.11±2.08	67.61±1.71
OLM	3.85±1.61	77.06±2.46
OBA	3.70±2.33	28.91±2.36
OLA	4.26±3.19	49.08±3.16

*There is no statistical difference if lowercase letters are the same in a column ($p \leq 0.05$).\

What has been explained above reveals that there is a relationship between the amount of phenolic components and flavonoid substances and the chemical structure of the soil and the plant.

Conclusion

The effect of soil-plant relationships on secondary metabolite formation has been revealed by research. In this context, our studies on O. syntenisi gave us some results. The results show a link between soil-plant relationships and the formation of secondary metabolites. The abundance of metals such as K, Ca, Mg, Fe and Mn and the sufficient amount of Cu and B elements in the soil and plant structure of Mumlu village caused the normal formation of secondary metabolites (phenolics and flavonoids) in the plant structure. Secondary metabolites depend on plant structure not only from the presence of the mentioned elements in the soil or plant structure, but also from other ecological and edaphic factors. Therefore, it has been understood that the formation of secondary metabolites depends on the soil-plant relationship.

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