

## ABSTRACT

of the dissertation for the degree doctor of philosophy (PhD)  
on specialty «6D061300 – Geobotany»

**Izbastina Klara Serzhankyzy**

**«Assessment of cenopopulations rare species *Anthemis trotzkiana* Claus ex Bunge in the Aktobe region with use of botanical and molecular genetic methods»**

**General characteristics of the work.** The thesis assesses the state of cenopopulations of a rare plant *Anthemis trotzkiana* Claus ex Bunge in the Aktobe region using botanical and molecular genetic methods.

**The relevance of research topic.** The extinction of some species on Earth leads to disturbance within ecosystems and destruction of the biosphere's integrity. These days, it is one of the most dangerous and rapidly occurring changes in nature. Anthropogenic factors are often the cause of the decline and extinction of plant populations in ecosystems. According to the latest edition of the Red Book of the Republic of Kazakhstan (2014), 387 plant species are under state protection. To preserve these species, monitoring and, if possible, conservation and restoration work is carried out on their populations. Nevertheless, the list of rare plant species is constantly updated, since today the methods of accurately determining the number and range of certain species still require in-depth study. In view of this, the issue of improving various methods for assessing and preserving rare species at the population level in different regions of the country is relevant.

Mesozoic chalk mountain ranges are formed in the Aktobe region, located at the intersection of Europe and Asia. *Anthemis trotzkiana* Claus is one of the most ancient species preserved in areas with a separate landscape and climate. A rare species that needs special protection, listed in the Red Book of Kazakhstan, endemic of the Volga region - Western Kazakhstan. Populations of species harvested due to their medicinal properties have declined in number due to livestock grazing and chalk production. To date, the relevance of the work is determined by the fact that the condition of the species population in the region, soil, chemical composition and genetic diversity have not been studied. Work in this direction requires the use of a wide range of botanical, phytochemical, molecular genetic methods. Peculiarities and genetic features of cenopopulations of *A. trotzkiana*, studied by scientifically reliable methods, allow us to develop protection principles and consider ways to preserve this rare species.

**The aim of the work** is a comprehensive study of the current state of the cenopopulations of the plant *Anthemis trotzkiana* Claus in the Aktobe region using botanical and molecular genetic methods, as a result to give recommendations on the conservation and protection of rare species.

**The tasks of the work:**

1. Study of the floristic and ecobiomorphological composition of plant composition of plant communities in which the cenopopulations of *Anthemis trotzkiana* are found;

2. To determine the age structure and ontogenetic state of cenopopulations of rare species *A. trotzkiana*;
3. To describe the physicochemical properties of the chalky soil cover on which the plant grows;
4. To study the effect of soil on germination and seed growth rate of the studied plants;
5. To identify the morphological and anatomical features of the plant *A. trotzkiana*;
6. To conduct a molecular genetic analysis of populations of rare species *A. trotzkiana* in the Aktobe region;
7. To study the phytochemical composition;
8. To evaluate the current state of cenopopulations of the studied plant *A. trotzkiana*.

**Objects of the research.** Natural populations of *Anthemis trotzkiana* in the Aktobe region (Akshatau, Bestau, Ishkaragantau).

**Scientific novelty of research.**

- For the first time, a comprehensive study of 3 populations and 9 cenopopulations of a rare species *Anthemis trotzkiana* in the Aktobe region has been carried out;
- A species of *Pimpinella titanophila* has been discovered in Ishkaragantau population, which had not previously been included in the list of plants of the Aktobe region, and its geographical location has been first clarified;
- For the first time, the floristic composition, age structure, ontogenetic status and the main spectrum of plant communities in which *A. trotzkiana* is found have been determined;
- X-ray diffractometry and X-ray spectral analysis have been carried out for the first time to determine the moisture content, pH, salinity, humus of chalky soils on which *A. trotzkiana* grows;
- The germination of seeds of the endangered species *A. trotzkiana* has been studied in laboratory conditions, a positive effect of nano-sulfur solutions on seed germination in the greenhouse has been noted;
- Morphological and anatomical features of the vegetative and reproductive organs of the species have been clarified;
- For the first time on the basis of ISSR markers, a molecular genetic analysis of the structural features of *A. trotzkiana* populations and the interpopulation and intrapopulation diversity was carried out;
- For the first time, the composition of biologically active substances, chemical elements and essential oils in the structure of *A. trotzkiana* was determined.

**Scientific and practical significance of the work.** The scientific results obtained and concepts make it possible to evaluate the current state of rare *A. trotzkiana* cenopopulations, to supplement the base of the Department of Ecology and Bioresources of the Aktobe region with new data. Herbarium samples of plant species belonging to three populations of the rare *A. trotzkiana* species in the Aktobe region were transferred to the herbarium fund of the Institute of Botany

and Phytoproducts of the Ministry of Education and Science of the Republic of Kazakhstan (Appendix Θ). In order to preserve the gene pool of the endangered *A. trotzkiana* species, an act was received on the introduction of seeds collected from three populations in 2017 and 2018 into the seed bank of the Institute of Botany and Phytointroduction of the Ministry of Education and Science of the Republic of Kazakhstan (Appendix Б). The technology, qualitative and quantitative results of the isolation of the main groups of biologically active substances from *A. trotzkiana* (Appendix B) are presented. New information on the composition of plant essential oils has been obtained.

**The main statements for defense:**

1. Floral description of plant communities in which *A. trotzkiana* is present;
2. Age structure and demographic indicators of rare cenopopulations of *A. trotzkiana*;
3. Physico-chemical parameters of the soil's chalky layers of *A. trotzkiana* populations, qualitative and quantitative content of calcite and chemical elements;
4. Seed germination and growth potential in the soil of a rare plant in laboratory conditions;
5. Morphological and anatomical features of the vegetative and reproductive organs of *A. trotzkiana*;
6. Phylogenetic position of the species *A. trotzkiana* in the tribe Anthemideae according to the phylogenetic tree of Neighbor Joining based on ITS sequences;
7. Results of the analysis of the structure and diversity of *A. trotzkiana* populations based on ISSR markers;
8. Qualitative and quantitative results of the phytochemical composition, chemical elements, and essential oils of *A. trotzkiana*;
9. Therapeutic and prophylactic properties of the main biologically active substances and essential oils in the plants.

**The main results of research and conclusions:**

1. The location and coordinates of 3 populations, 9 cenopopulations of a rare species *A. trotzkiana* have been found in the mountains of the Aktobe region: Akshatau, Bestau, Ishkaragantau. The number of species in the floristic composition of plant communities where cenopopulations are found is small: 40 species belonging to 36 genera and 18 families. *Crambe tatarica*, *Linaria cretacea*, and *Tulipa biebersteiniana* were found in the population, which are listed in the Red Book of Kazakhstan along with the rare species *A. trotzkiana*. It has been noted that 8 species: *Allium globosum*, *Gypsophila diffusa*, *Zygophyllum macropterum*, *Trinia hispida*, *Linaria cretacea*, *Anthemis trotzkiana*, *Artemisia salsoloides*, *Centaurea sibirica* have been all found in 3 populations. The species *Pimpinella titanophila*, belonging to the genus Apiaceae, which had not previously included to the flora of the Aktobe region before our studies of the Ishkaragantau, was first discovered by us; this mountain has been marked as the new geographical location of this species. Perennial grasses predominate in the vegetation of populations, 11 species in Akshatau 55%, 12 species in Bestau 63.1% and 14 species in Ishkaragantau 66.6%. In second place are shrubs adapted to the conditions of local chalk massifs. The number of annual herbaceous plants is

small, trees and shrubs are not identified. There are hemicryptophytic, chamaephytic, cryptophytic and teraphyte types according to the K. Raunkier system. All populations are characterized by overgrown hemicryptophytes, while annual theophytes are rare. More than 50% of mesoxerophytes have been found in population regions with a temporary lack of moisture. *Ephedra distachya*, *Anabasis cretacea*, *Camphorosma monspeliaca*, *Kochia prostrata*, *Nanophyton erinaceum*, *Zygophyllum pinnatum*, *Linaria cretacea*, *Artemisia salsoloides* have been found in carbonate soil along with *A. trotziana*.

2. *A. trotziana* plant has relatively average indices in the population 1 – Akshatau (1 – CP 11.8 samples / m<sup>2</sup>, 2 – CP 11 samples / m<sup>2</sup>, 3 – CP 12.1 samples / m<sup>2</sup>). The plant grew densely in population 2 – Bestau (4 – CP 13 samples / m<sup>2</sup>, 5 – CP 13.3 samples / m<sup>2</sup>, 6 – CP 12.7 samples / m<sup>2</sup>) and rarely in population 3 – Ishkaragantau (7 – CP 8.9% samples / m<sup>2</sup>, 8 – CP 9% samples / m<sup>2</sup>, 9 – CP 8.4% samples / m<sup>2</sup>). Nevertheless, juvenile and immature individuals have been not found in any coenopopulation. The age and energy efficiency index of cenopopulations are similar. By the predominance of the number of generative trees, the base spectra are concentrated and belong to the mature type. The low displacement index and the aging rate of 7, 8, and 9 CP are due to the predominance of trees in the sub-senile and senile states and soil cover indicators.

3. The humus content in the chalky soils of the three populations on which the plant grows is 3.15%-4.90%. The high content of organic substances (4.90%) in the soil of Mount Ishkaragantau is associated with a large amount of rainfall in the area. The moisture content of the cuttings at a depth of 0 - 100 cm is stable, moisture is well preserved in the solid carbonate bottom layer. The salt concentration at the levels of populations and horizons is 0.14% - 0.63%. Despite the fact that in nature the high alkalinity of the soil of plants is one of the indicators restraining the growth of plants, the growth of *A. trotziana* is not affected by highly alkaline soil (pH > 7.49 - 8.41); it has been found that this is a strongly basifilic plant, characteristic of chalky deposits with an alkalinity of more than 8 indicators. Elements C, O, Mg, Al, Si, K, Ca, Fe, Cl have been determined from the soil. The main phase of the mineral composition is calcite (93-98%), the mixture is quartz (1-6%). The mass fraction of the mineral in soil samples found in *A. trotziana* is pure natural boron Ca (CO<sub>3</sub>). This species is a reliable indicator of chalky soils, and its distribution area depends on soils with a high calcium content (more than 95%).

4. Seed germination and growth rate of *A. trotziana* in a Petri dish is 90% higher under laboratory conditions (Akshatau 93.3%, Bestau 96.2% and Ishkaragantau 92.3%), and germination of seeds grown in natural soil is 50-70%. Chalky soil is an important factor for the stable existence of the species and proves that the species is an obligate calcephite. A 0.2% calcium polysulfide solution had a positive effect on the growth and productivity of the species when applied to other soils.

5. The plant *A. trotziana*, found in the Aktobe region, is a shrub. Signs of xeromorphism have been observed in the morphological and anatomical structure of the plant, which arise as a result of adaptation to drought. The anatomical

features of the xeromorphic structure of the roots, stems and leaves of virginal and generative trees collected from populations No. 1 and No. 2 are similar. The various anatomical structure and relatively low biometric parameters of the virginal and medium generative trees collected from population No. 3 are related to the level of isolation between populations.

6. Neighbor - Joining phylogenetic genealogy based on internal transcribed spacers (ITS) of nuclear ribosomal DNA showed that *A. fruticulosa*, *A. calcarea*, *A. marschalliana* are closely related and genetically close to *A. trotziana*. Based on ISSR markers, the highest value of genetic polymorphism among *A. trotziana* populations was observed in the Bestau population (63.41%). The Bestau population has the highest values of the Shannon index – 0.283, and the diversity of genes – 0.181. The paired genetic distance matrix PhiPT and Nei showed that the 1-Akshatau and 2-Bestau populations were close in the genetic space, 0.172 and 0.181, respectively, and it has been noted that the 3-Ishkaragantau population was isolated (0.129). As a result of the AMOVA analysis, the difference between the populations and within the populations of *A. trotziana* has been determined: the value  $\Phi_{PT} = 0.283$ ,  $P = 0.001$ . It is clear that *A. trotziana* in the Aktobe region, compared with interpopulation genetic polymorphism, has a higher intrapopulation genetic polymorphism. Genetic diversity within populations is 72% and between populations is 28%.

7. Essential oils, alcohols, phenols, carbohydrates, aldehydes and ketones were found in the roots, leaves and flowers of *A. trotziana*. Leaves contain 36.02% of essential oils, 26.89% of ketones and 11.51% of phenolic compounds. Healing properties, considered in traditional and indigenous medicine, of 14 phytochemical compounds found in the leaves have been determined. 51 monoterpene and 35 sesquiterpenoid compounds have been found in essential oils from flowers and leaves. The content of essential oils in the leaves is higher than in the flowers, 90.2% in the 1st population, 72.2% in the 2nd population and 55.6% in the 3rd population. The predominance of natural compounds of camphor, 1,8-zinol, camphene, borneol, which are widely used in medicine, can serve as a basis for use as a medicine. Only the removal of leaves during the isolation of natural compounds for treatment, without damaging the roots and flowers of a rare species, will not only help to rationally use this endangered species, but also conserve it.

8. Due to the proximity of the natural populations of *A. trotziana* to the settlements in the region, we can conclude that grazing, chalk production and trampling are the main threats to the species, which affect its habitat and lead to a decrease in its population. The research results showed that the morphological characteristics and genetic diversity of *A. trotziana* trees in the Bestau population are higher than in other populations. In this regard, we recommend protecting the Bestau population as a specially protected area.

**The author's contribution.** In achieving the aim and objectives of the study, the author of the work fully analyzed the literature, studied the research object in the field and laboratory conditions, summarized and processed the results, wrote and designed the thesis.

### **The connection of work with the plan of state programs.**

The dissertation work was carried out within the framework of the projects of the RSE on REM "Institute of Botany and Phytointroduction" O.0860 «Implementation by the State Botanical Gardens of Kazakhstan's priority scientific and practical tasks of the Global Strategy for Plant Conservation as a sustainable system for maintaining biodiversity» (2018-2020) and the RSE on REM "Al-Farabi Kazakh National University" O.0898 «Development and approbation of technologies for obtaining new sulfur-containing nanocomposites and drugs» (2018-2020).

**Approbation of the work.** The basic principles and results of the thesis were discussed at a scientific meeting of the Department of Biodiversity and Bioresources of al-Farabi KazNU. The main ideas and results of the work have been presented and discussed at the following conferences:

“Biodiversity Conservation and Rational Use of Bioresources” Republican Scientific Conference (Almaty, Kazakhstan, 2016); "Problems of biodiversity conservation study and sustainable use of biological " International Scientific Conference (Almaty, Kazakhstan, 2016); PlantGen 2017, IV International Conference (Almaty, Kazakhstan, 2017); International scientific-practical conference “Actual problems of environmental genetics and experimental biology” (Almaty, Kazakhstan, 2018); IV (XII) International Botanical Conference of Young Scientists in St. Petersburg (St. Petersburg, Russia, 2018); 2nd International Conference “Smart Bio” 2018 (Kaunas, Lithuania, 2018); International scientific conference of students and young scientists “Farabi Alemi” (Almaty, Kazakhstan, 2018, 2019).

**Publications.** The main content of the thesis is presented in 13 published works, including 1 article in a scientific journal included in the Scopus database, 4 articles in national scientific journals included in the list of journals approved by the Committee for Control in Education and Science of the Republic of Kazakhstan, 2 articles and 6 abstracts at international scientific conferences.

**The volume and the structure of dissertation.** The thesis includes definitions, designations and abbreviations, introduction, literature review, object and research methods, discussion of results, conclusion, list of 330 literature sources and 4 appendices. The research work of 148 pages includes 30 tables, 11 formulas and 57 figures.