The influence of plant growth-promoting rhizobacteria (PGPR) on the cultivation of *Cynara Scolymus L.* under salinity stress

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Abstract. This article highlights laboratory and field experiments based on the results of treatment of *Cynara scolymus L.* with biologically active products. According to the obtained results, compared to the control variant in the field experiments, the highest result was 3.4% to 4.8% in 1:100 and 1:1000 ratios of "Elisitor". Similar results were observed to increase 0.3%, 3.0% respectively in 1:1000 and 1:100 ratios of "Zamin-M" biopreparation. According to the analyzes carried out in laboratory conditions, the fertility indicator was found to be 95.0% in plants treated with the biopreparation "Elicitor". This result was 6.0% higher than the control and 3.4% higher than the standard. Fertilization in plants inoculated with "Zamin-M" biopreparation was found to be 93.85%, it was increased by 4.85% compared to the control variant and by 2.25% compared to standard variant.

1. Introduction

Microorganisms present in the rhizosphere and soil organic matter are estimated to occupy less than 5% of the total area. Microorganisms are a tool that transforms the environment into a habitat necessary for plants, and play a key dynamic role in the process of decomposition and circulation of nutrients as a result of the mineralization process. Microorganisms used in agroecological and organic systems affect soil quality and microbial activity. The soil structure is diverse and consists of plant growth stimulants, fungi and bacteria [1, 2,3]. In ancient times, Theophrastus (372-287 BC) suggested mixing different soil samples in order to increase plant viability and protect against diseases [4]. Since the number of some natural medicinal plants is decreasing, it is important to grow them on farms [5, 6]. However, differences can be observed between the phytochemical composition and productivity of medicinal plants grown on farms and those produced under the influence of the natural environment [7].

Medicinal plants, according to their natural origin, require different climatic conditions for their growth. Most medicinal plants require sunny, ventilated areas protected from strong winds and late winter frosts. The soil should be fertile and have the right amount of Na, P, Cu, minerals, organic and other elements required for crop growth in an optimal combination. It is well known that intensive tillage, in addition to affecting the physical properties of the soil, leads to a rapid decrease in the level of organic matter and nutrients. Conversely, management practices using organic materials affect agricultural sustainability by improving the physical, chemical, and biological properties of soils. The use of organic additives has long been recognized as an effective means of improving soil structure and fertility, increasing the diversity, activity and population of microbes, as well as maintaining moisture in the soil and increasing productivity [8, 9, 10, 11].

Currently, the use of biological fertilizers as an additive to improve the growth and productivity of agricultural, horticultural and medicinal plants plays an important role [12, 13].

Medicinal plants are exposed to a number of biotic and abiotic environmental stresses that adversely affect their growth and development. Among various environmental stresses, salinity, floods, heavy metals, drought, cold climate, soil compaction, mechanical resistance and lack of nutrients are some of the main factors of abiotic stress [14, 15]. Under stress conditions, some physiological imbalances in plants, such as increased production of ethylene, as well as regulation of nutritional and hormonal balance, can affect the growth and therapeutic properties of plants. The use of conventional approaches to mitigate abiotic stresses associated with medicinal plants has had little success. The role of PGPR in relation to medicinal plants and their effect on the growth and synthesis of therapeutic metabolites under stress is still unclear [16, 17, 18].

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Plants have a complex innate immune system to prevent pathogenic microorganisms from entering and colonizing their internal structures. The first signal of the induction response is controlled by the plant, in which it accepts modified molecules from the microbe or plant. To counteract the initial plant defense response, successful microbes produce special effectors that disrupt the recognition of defense mechanisms or plant defense mechanisms to stimulate ETS- effector-triggered susceptibility. However, if these pathogenic effectors in turn are recognized by plant resistance proteins, the second layer of the inductive response, i.e. immunity to the ETI- effector-triggered immunity begins [19, 20]

Thus, it is important to assess the extent of the threat to the plant and adopt appropriate and proportionate responses. These can lead to incompatibility, from responses to attack, expression of PTI (PAMP- or pattern-triggered immunity)-based defense mechanisms, if the microbe/pathogen is unable to suppress these responses. The use of elicitors in agriculture reduces the need for pesticides by using the plant's own defense system [21]. From this point of view, the purpose of this study was to use rhizosphere microorganisms in the cultivation of medicinal plants under salinity stress conditions.

2. Methods

The prickly artichoke plant (*Cynara scolymus L.*), "Zamin-M" (composed of *Bacillus subtilis* SKB-309, *Bacillus megaterium* SKB-310 and *Pseudomonas stutzeri* SKB-308 strains) and its autolysate Elicitor were used as the research material for the inoculation with rhizobacteria.

Steps to extract "Elicitor". The most important active stage of the formation of a biotechnological system is the development of the mode of growth (cultivation) of producer cells. In the microbiological industry, the principle of moving liquids using compressed air is widely used, including in the transfer of sterile nutrient media from containers and from inoculators to sterile fermenters producing pure cultures. In this case, a montage was used, provided with containers and inoculators for the nutrient medium, as well as mixers, nozzles, steam and water jackets, and other structures.

2.1 Technology of preparation of cultivation material from pure culture

Preparation of cultivation material, depending on the type of producer and its physiological and biochemical characteristics, consists of several main stages: initial culture (in a test tube) → growing in agar medium (in a test tube) → growing in flasks in a liquid nutrient medium in microbiological shakers (one or two stages) → in special equipment cultivation (in one or more inoculators) → accumulation of microorganism cultures in small fermenters → cultivation material [13].

Primary cultures stored in laboratories are used to obtain cultivation material. It is necessary to have a document about the name of the culture used in each production (species, series and generations), collection series, number, storage period, average levels of activity, date of study. This document provides a description of the temperate nutrient medium for culture growth and culture maintenance methods. Usually, it is necessary to use optimal storage methods so that the beneficial properties of microorganisms do not change. It is known that the physiological characteristics of cultures stored for a long time and replanted several times change easily and quickly [22].

Preparation of nutrient environment is one of the important stages in the production of microbiological synthesis. Depending on the physico-chemical properties of the components of the nutrient media, they were dissolved or suspended in certain proportions in water at a specified temperature and pH. According to technological requirements, they are enriched in the process of preparation of nutrient media, which includes neutralization, tempering, filtering, cooling, removal of components inhibiting the activity of microorganisms, enrichment of media with biologically active substances and other steps. Various equipment were used for preparation of air and nutrient media: neutralizers, hydrolysis devices, quenchers, devices with mixers, sterilizers, heat exchangers, filters, etc.

2.2 Analyzes of vegetative experiments

These experiments were carried out in a comparative manner in relation to the control option on seed germination [23].

To carry out the research in field conditions, working solutions of "Elicitor" in the ratio of 1:1000, 1:100 were prepared. Artichoke seeds were soaked in the prepared working solution for 1 hour, and after 1 hour, the seeds were sown in the field at a distance of 40 cm. Untreated seeds were used as a control, biopreparation "Zamin-M" at a ratio of 1:100 was used as a standard. The seeds were dried in a cool, direct sunlight place and then planted. During the artichoke vegetation, the soil was treated by spraying the working solution in the amount of 300-350 liters/ha.

The analysis of vegetative experiments was carried out during the period of seed germination, leaf formation, budding, flowering and ripening stages. Biometric indicators were compared to the control option in terms of length of the main stem, number of sympodial branches, buds, flowers.

In the experiments, agrotechnics of the thorny artichoke was established according to generally accepted methods.

During the vegetation period, "Zamin-M" preparation was used as follows:
1) in the amount of 1liters/ha before planting seeds;
2) before budding;
3) in the amount of 2.5 liters/ha during flowering and ripening.

3. Results and Discussion

3.1 "Elicitor" extraction technology based on association of rhizobacteria

In the process of processing primary raw materials, microbiological synthesis includes a complex of several complex technological processes that enable the production of finished products necessary for human activity. The microbiological approach to plant protection is more important, and the use of microorganisms allows to solve the tasks of biologizing agriculture and increasing soil fertility. However, exposure to microorganisms with chemical factors affects the natural microflora and causes their death. This has a negative effect on the effectiveness of their use. In the modern form of microbiological production, each of the production of various biopreparations is produced by means of specific, separate technologies. Also, the microorganisms used in all the processes carried out go through almost the same life cycle stages. Accordingly, a model drawing of these technological processes suitable for the microbiological synthesis process was adopted [20].

When developing the composition of the nutrient medium used in the cultivation of microorganisms during scientific research, it was considered necessary to use Peptone -10 g/l; $MgSO_4$x $7H_2O$ -0.3 g/l; glucose - 20 g/l; K_2HPO_4 -0.4 g/l; NaCl -3.0 g/l; $CaCO_3$-3.0 g/l; pH-6.8; 1000 ml. distilled water.

In production, the preparation of cultivation material is carried out in the laboratory. At the first stage, cultivation material is grown in a microbiological laboratory. Initially, the culture was propagated in test tubes (1) on agar slants in a sterile condition, in a moderate nutrient medium, according to a specific regime (pH 6.8-7.0; temperature 28±2°C, storage for 72 hours).

Grown cultures are washed over the agar medium (1) in a test tube with sterile clean water, and were transferred to 50 or 100 ml liquid nutrient medium (1) in 250 ml Erlenmeyer flasks, and the growth temperature was 28 ± 2^0C, the duration of the growth period was 72 hours.. The flasks were placed on shakers in a temperate room (28°C). The growth rate of the cultures was varied depending on the agitation speed of the shaker. Moderate mixing speed was carried out at 120-240 rpm. The duration of growing culture in flasks on shakers was continued for 72 hours based on its physiological characteristics.

Morphological indicators of microorganisms were observed at the initial stage. The best result was shown in the logarithmic phase of cultures. In the second step, the optimal sterile feed for the development of microorganisms was cooled to a temperature of 28 ± 2^0C, and 5-8% of the cultivation material in the flask was put in the cultivation equipment (inoculator) (3) (temperature 28 ± 2°C, 72 hours, pH 6.8-7.0). Maintaining a moderate growth regime in the equipment during the preparation of the cultivation material is the main factor. To control this process, it is necessary to take samples for biochemical and microbiological analysis and identify them. Cultivation was continued until the amount of rhizobacteria in the nutrient was 1.5%/l (on a dry weight basis). This process usually takes 12 hours.

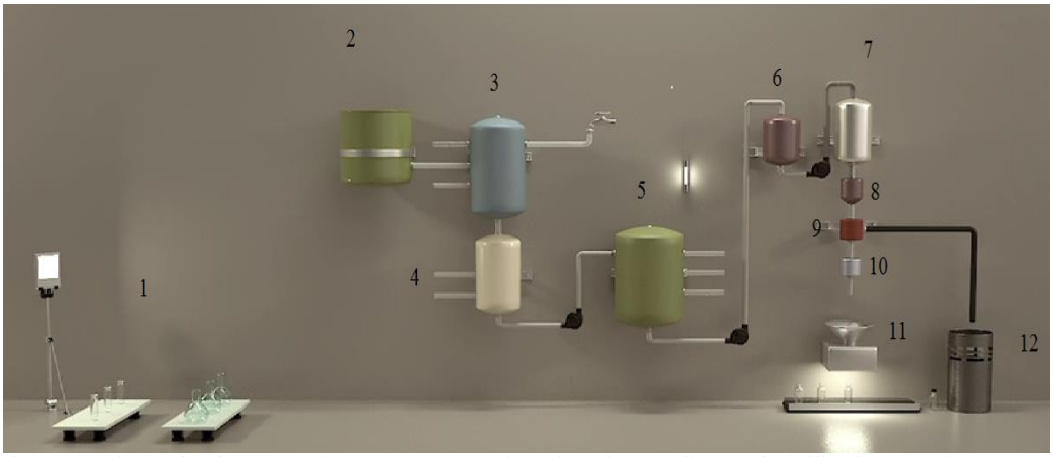


Fig. 1. Elicitor extraction technology: 1. Culture. 2-3. Preparation of nutrient medium and sterilization container. 4. Fermenter for growing the primary cultivation material (Barbatyor). 5. Basic growth fermenter. 6. Small-scale growth fermenter. 7. Extractor. 8. Biomass storage tank. 9. Filter. 10. Dispenser. 11. Packaging equipment. 12. Waste collection container

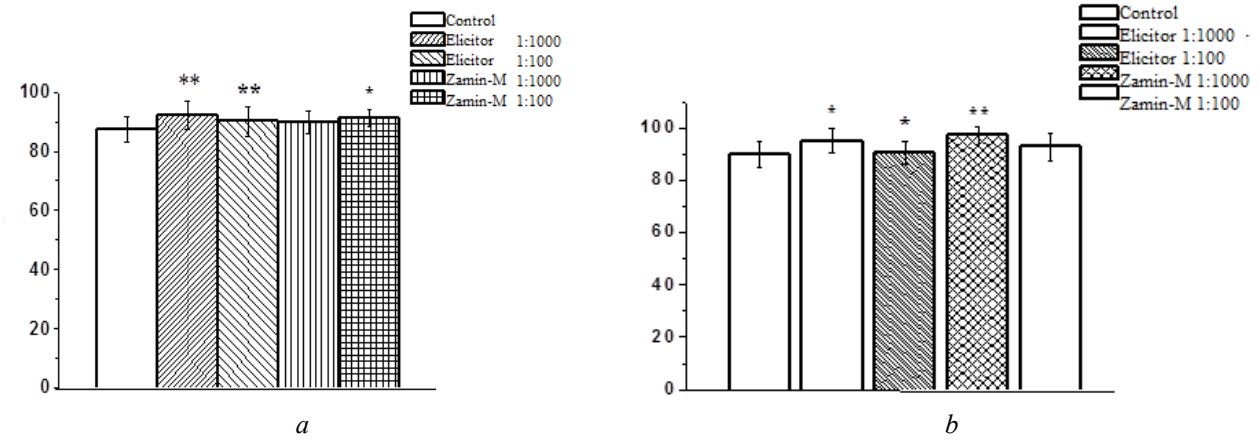
The third stage was continued in 50 l equipment. In this case, all the culture fluid is transferred from the small volume inoculator (4) to the large volume equipment (5) with pre-sterilized nutrient medium. The duration of cultivation is 12 hours. Stage 4 of the process was continued in a 5 l apparatus (6). The suspension was transferred from the fermenter to the extractor (7), the process was continued at 120^0 C for 20 minutes and the extract was obtained. The obtained extract was directed to the biomass storage tank (8), through which it was passed through a filter (9) and the filtrate was obtained. The filtrate is transferred to a dispenser (10) of a certain size, and the product is packaged in 1 liter (11). As a result of filtering, the remaining waste is disposed of or can be collected in a special container (12) and drained as juice. In our future studies, a model drawing of technological processes suitable for microbiological synthesis of "Elicitor" (see Fig. 1) based on rhizobacterial association was developed based on generally accepted methods for biotechnological processes.

3.2 Effect of biopreparations on the seed germination of thorny artichoke plant

Experiments conducted on plants require the study of the laws of their individual development (ontogeny), including the characteristics of seeds and the level of fertilization. The shape of the seed of *Cynara scolymus L.* is inverted ovoid, 5-6 mm long, 2-3 mm wide, hard, hairless, smooth, four-sided, brown, oozing or slightly gray, sometimes with dark spots, the length of the pod is 2-3 cm, yellow in color, the fruit is a grain. Considering the importance of rhizobacterial association autolysate- "elicitors" in the life of plants, laboratory and field experiments were conducted. Field experiments were conducted in Jizzakh State Forestry plots. For this, the research area was thoroughly plowed and rows were taken. The study was carried out on the basis of seeds planted by inoculation of 5 different options:

1- Control;
2- "Elicitor" solution in 1: 1000 ratio;
3- "Elicitor" solution in 1: 100 ratio;
4- "Zamin-M" biopreparation in 1: 1000 ratio;
5- "Zamin-M" biopreparation in 1:100 ratio.

As a control, seeds that were not treated at all were used. The results of the study on seed germination are shown in Fig. 2 below.



a *b*

Fig. 2. Indicators of plant seed germination on days 7 (a) and 14 (b)under field conditions

As can be seen from Fig. 2, the treatment with biologically active products has a positive effect on the fertility indicators of the plant, based on the obtained results. According to the obtained results, compared to the control variant in the field experiments, the highest result was 3,4% to 4.8% in 1:100 and 1:1000 ratios of "Elisitor". Similar results were observed to increase 0,3%, 3,0% respectively in 1:1000 and 1:100 ratios of "Zamin-M" biopreparation.

Table 1. Effect of bioproducts on seed germination of *Cynara scolymus L.* under laboratory conditions

No	Variants	n	Germination %	
			Day 7	Day 14
1	Control	5	85.4	89.0
2	Organic –C(standart)	5	88.2	91.6
3	Zamin-M	7	91.8	93.85
4	Elisitor	7	92.0	95.0

Experiments to study the effect of Elisitor on plants in laboratory conditions were conducted in the biotechnology laboratory of Tashkent State Agrarian University. Plant seeds inoculated with the complex biopreparation "Zamin-M"

based on local strains of rhizobacteria and "Elicitor" based on it were planted in special cassettes in four variants. Fertilization of plants in each option was determined on 7 and 14 days and the results were recorded. The results on plant seed germination are shown in Table 1 below.

As can be seen from the above table 1, in laboratory experiments, According to the analyzes carried out in laboratory conditions, the fertility indicator was found to be 95.0% in plants treated with the biopreparation "Elicitor". This result was 6.0% higher than the control and 3.4% higher than the standard. Fertilization in plants inoculated with "Zamin-M" biopreparation was found to be 93.85%, it was increased by 4.85% compared to the control variant and by 2.25% compared to standard variant (Fig. 3).

Fig. 3. Fertilization of artichoke plant grown in phytotron

4. Conclusions

The pharmaceutical properties of the prickly artichoke, the raw material of which is imported for the pharmaceutical industry of our country, i.e., the rich content of leaf extract and the presence of high-level antioxidants, the synthesis of biologically active substances, are theoretically and practically important in the development of the technology of growing this plant in plantations in our country. The conclusions drawn from the results show that inoculation with the minimum concentration of "Elicitor" can achieve higher efficiency than other options. At the same time, it was analyzed based on the above sources that replacing chemical fertilizers with PGPR in plantations will increase their demand and make it an important component in the management of a sustainable agricultural system, and the use of "Elicitor" will reduce the need for pesticides by using the plant's own defense system.

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