Study of enzymatic activity of microbiological biopreparation in the cultivation of cotton plant (*Gossypium hirsutum* L.) under saline stress conditions

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Abstract. The changes in enzymic activity of the soils have been studied in the result of inoculation of cotton plants with microbiological preparation “Zamin-M” which was obtained on the base of association of strains *Pseudomonas stutzeri* SKB-308, *Bacillus subtilis* SKB-309 and *Bacillus megaterium* SKB-310 isolated from the rhizosphere of cotton plant grown in different saline soils. The results of the study showed that when the biopreparation “Zamin-M” was used, the enzymic activity of peroxidase was 1.76 ± 0.32 and in the control 1.5 ± 0.31 (mg purpurgalin/ g soil, 24 hours), as well as the enzymic activity of polyphenol oxidase was 2.1 ± 0.53 and in the control 1.64 ± 0.51 (mg purpurgalin / g soil, 24 h). In the control variants, the activity of the enzyme of catalase relative to 1 g of absolutely dry soil was 1.0 ± 0.55 according to the amount of oxygen released in 5 minutes, while using the preparation its increase was observed and made 1.8±0.27 ml O₂. Similar indicators were found in the study of invertase enzymic activity, when the preparation “Zamin-M” was applied, its amount constituted 271 ± 0.55 (mkg / absolutely dry soil), while in the control variant it made 111 ± 0.41 (mkg / absolutely dry soil). Urease enzymic activity was active under 24 ± 0.3 units when the preparation was used and in the control it was noted to be 14 ± 0.64 (mkg N/NH₄ /1 g soil) and phosphatase enzymic activity made 74±0.21 and in the control 53±0.32 (mkg P₂O₅/g soil, 24 hr) which led to the activation of biochemical processes in the soil.

1. Introduction

The study of application of rhizobacteria to accelerate the growth and development of plants, to protect them from various external affects, diseases and to improve the quality of the yield of growing plant, has been revealed in several research work of scientists, such as Alizadeh *et al.* [1], Yang *et al.* [2], Ahmad *et al.* [3], L. Peregr and M. McMillan [4], Vejan *et al.* [5], Pandove *et al.* [6], C.K. Odoh [7], T.M. Patel and F.P. Minocherhomji [8] and other. Also there were mentioned the names of microbiological means (Kodiak, Ascend/BuRIZE, PIX PLUS, Deny, Intercept, SoilGard, Contans WG, Afla-Guard, BioAg Soil and Seed) created basing on rhizobacteria that are intended for cotton growing. Tikhonovich *et al.* [9], Chebotar *et al.* [10], A.M. Boronin [11], O.N. Loginov [12] proved that it was possible to treat agricultural crops with biopreparations based on rhizobacteria, protect plants from phytopathogens, accelerate growth and development, adapt to soil conditions, to achieve
preservation of elements reserve such as nitrogen, phosphorus and potassium which were reducing as a result of the amount of nutrients extracted from the soil.

Similar research was carried out by Davranov K. [13], Khodijbaeva S.M. [14], Fedorova O.A. [15], Akhmedova Z.R. [16], Jumaniyozova G.I. [17] and other. However, in the production of complex biopreparations based on rhizobacteria, a number of factors, namely the selection of rhizobacteria that ensure the growth and development of the target plant, their resistance to salinity and phytopathogens, soil type, local microbial communities, environmental conditions, tillage, crop conformity to cultivation system, crop suitability to varietal characteristics, and the influence of biopreparations on soil’s enzymic activity haven’t been considered to conduct complete and further research yet.

In this regard, based on the beneficial properties of various rhizobacteria, the issue of testing and implementation of scientifically substantiated agrobiotechnologies of microbial preparations in saline soils is regarded relevant and of scientific and practical importance.

The object of research included “Zamin-M” microbiological preparation obtained on the base of the strains Pseudomonas stutzeri SKB-308, Bacillus subtilis SKB-309 and Bacillus megaterium SKB-310 and rhizosphere bacteria which include to the families Bacillaceae and Pseudomonadaceae isolated from the rhizosphere of cotton plant grown in the soils of different salinity, low saline, medium saline, strongly saline and non-saline meadow-gray, gray meadow and meadow soils, biological fertilizers, cotton plant (Gossypium hirsutum L.) cultivars C6524, An-Boyovut-2, Andijon 37, Buhoro 6, C4727.

2. Material and Methods

Enzymatic activity of microorganisms was identified by the methods under the authorship F.Kh. Khaziev [18]. In determining the activity of the catalase enzyme, the cultures were firstly grown in agar medium, then 1-2 drops of 10% hydrogen peroxide H$_2$O$_2$ solution were added, depending on the quality of the formation of oxygen bubbles [18] the conclusion was made.

In the agrochemical analysis of the soil of the experimental field, the amount of humus was determined by the Tyurin method, total nitrogen, phosphorus by K.E. Ginzburg, G.M. Sheglova, E.A. Woolfius [19] method, nitrate nitrogen, mobile phosphorus by Machigin (Machigin, 1993) and metabolic potassium was determined with photometer with flames under the method of P.V. Protasov. Agrochemical analysis of soil was carried out in collaboration with the staff of the Agrochemical Laboratory of the Institute of Soil Science of the Republic of Uzbekistan.

The study of the effect of biopreparation on the growth and development of cotton plant was performed under the method of Sh. Abdualimov that was recorded by him at UzCGRS [20]. Hairy cotton seeds of 45 kg/ha were treated before sowing. Number of replications was 4 times. The area of the furrows was 60 m$^2$, width was 2.4, length 25 m, and the calculated area was 30 m$^2$.

In the experiments conducted on the farms, agricultural practices of cotton plant were organized with the common methods (Methods for conducting field experiments, 2007) [22]. At the growing period “Zamin-M” preparation was applied:

- to the seeds before sowing in the amount 2.0-2.5 and 3 L/ha;
- before budding;
- flowering and yielding period in the amount 2.0 L/ha.

The preparation “Zamin-M” was tested at the Institute of Cotton Growing Agrotechnology and Selection by order of the State Chemical Commission. There, as a standard were used association of microorganisms, i.e. “Baykal-EM1” (registered in the list of pesticides and agro-chemicals approved for use in agriculture of the Republic of Uzbekistan from 1.12.2011. to 30.12. 2015) complex preparation made of lactic acid bacteria, yeasts, single-celled microscopic, photosynthetic bacteria and the control variants without any treatment.
Table 1 The scheme of field experiments conducted with “Zamin-M” microbial composition

| Experiment variants | The norm of preparation use, l/t | Pre-sowing treatment of seed, l/t | Treatment at vegetation period, l/ha |
|---------------------|---------------------------------|----------------------------------|-------------------------------------|
| Control without treatment |                                |                                  |                                     |
| Baykal EM1– standard | 3.0                             | 3.0                              | 3.0                                 |
| Zamin-M             | 2.0                             | 2.0                              | 2.0                                 |
| Zamin–M             | 2.5                             | 2.5                              | 2.5                                 |
| Zamin–M             | 3.0                             | 3.0                              | 3.0                                 |

Field experiments on the study of the salinity level of soil were carried out on the farms “Zamindor” in Jizzakh district, Jizzakh region, “Semurg” in Dustlik district (2012-2013), “Bozorobod” in Jondor district of Bukhara region, “Ergashev Alpomish Murtazo ugli”, “Bobomurod Roziya Ilyos Dilovar” (2013-2014) farms, “Seitimov Ulugbek” farm in Amudarya district of Karakalpakstan Republic (2012-2013).

The effect of the association of individual strains and microorganisms on plants and the main indicators of plants was analyzed with the method of Dospekhov [21].

The data obtained basing on the experiments was analyzed statistically under the program Stat View 5. The indicators of differences were calculated according to Fisher value and Student method under Anova program (packet) Anova Post Hoc Tests.

The result was achieved by the following formulae:

\[
\bar{X} = \frac{x_1 + x_2 + x_3}{n} = \frac{\Sigma x}{n}
\]

\[
\sigma = \sqrt{\frac{\Sigma(x_i - \bar{X})^2}{n - 1}}
\]

\[x\] - individual measurement importance,
\[\bar{X}\] - mean arithmetic value
\[n\] - number of observations
\[\Sigma(x_i - \bar{X})^2\] - the sum of square deviations

\[
S_X = \frac{\sigma}{\sqrt{n}}
\]

\[S_X\] - mean arithmetic standard deviations
\[n\] - number of measurements

Determining exact intervals

\((\bar{X} - t_pS_X) < \bar{X} < (\bar{X} + t_pS_X)\)

Check the accuracy of the two selected free mean values
(using t parameter criteria of Student)

\[
t_d = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{S^2\bar{X}_1 + S^2\bar{X}_2}}
\]

If it is \(t_d \geq t\), the differences are found to be exact. It should be particularly considered that when comparing two mean indicators, the amount of \(t\) coefficient is equal to \((n_1 + n_2 - 2)\).
3. Results and Discussions

Sulfate (SO$_4^{2-}$ mg/eq) and chlorine (Cl$-$ mg/eq) in the soils of the selected area to study the effect on soil enzymatic activity and the number of microbial populations are suitable for sulfate-type soils by its composition. Variation in the composition of the dry residue of chlorine and sulfate ions was observed according to soil-ground profile. It was determined that the soil had weak alkaline property (respective to $\leq 0.05\%$ dry residue) in which the experiment was conducted by HCO$_3$ ions content and its amount varied from 0.021 to 0.024% through soil profile. Salt reserve made 62.220 m/ha in 0-30 cm deep tilling layer, while in 100-150 cm depth this indicator constituted 118.9 mg/ha.

It is known that the catalase enzyme belongs to the class of oxidoreductases and catalyzes basic oxidation-reduction reactions and it plays a leading role in biochemical reactions in soil. The activity of catalase was studied by a specially modified gasometric method using a device recommended by O. Myachina, a senior researcher at the Institute of General Chemistry of the Academy of Sciences of Uzbekistan. In this case, it was taken into account depending on the amount of oxygen released as a result of the decomposition of hydrogen peroxide after its interaction with the soil.

Microorganisms are also sensitive to small changes in the environment. A slight increase in the osmotic pressure of the soil solution makes it difficult for water and nutrients to enter the cell, which reduces the vital activity of living plant cells or causes them to die. This phenomenon is clearly reflected in the example of the enzyme catalase. As shown in Figure 2, enzyme activity decreases sharply when soil salinity is high. The activity of the enzyme is kept within a certain limit in the tilling layer of soil due to salinity-resistant microorganisms.

In the studies it was observed that salinization of the soil leads to a depletion of the microflora, which in turn reduces the activity of the enzyme catalase. An increase in the amount of toxic salts from 0.203 to 1-1.06% under saline conditions led to a decrease in the amount of oxygen released in 5 minutes per 1 g of soil from 4.5 to 1.05 ml under the conditions of chloride-sulfate salinity (Figure 1). The high content of water-soluble salts affects the activity of microorganisms and enzymes [23, 24].

![Figure 1. Variations of catalase enzymic activity in the soils of cotton plant rhizosphere that was sown after treating with “Zamin-M” biopreparation](image)

It was found that an increase in the concentration of salts leads to a decrease in the number of microorganisms that feed on soil substances.

In the control variants, the activity of the catalase enzyme relative to 1 g of absolutely dry soil was 1.0 ± 0.55 according to the amount of oxygen released in 5 minutes, but after using preparation it increased and made 1.8±0.27 ml O$_2$.

Similar values were found in the study of invertase enzymic activity, when the preparation “Zamin-M” was applied, its amount constituted 271 ± 0.55 (mkg / abs dry soil), in the control variant it made 111 ± 0.41 (mkg / abs dry soil) (Figure 2).
Figure 2. Variations of invertase enzymic activity in the soils of cotton plant rhizosphere that was sown after treating with “Zamin-M” biopreparation

Urease enzyme activity was active in 24 ± 0.3 units while using the preparation and in the control 14 ± 0.64 units (mkg N // NH₄ / 1 g soil) . Phosphatase enzymic activity was 74±0.21 under the effect of preparation, while in the control 53±0.32 (m kg P₂O₅/g. soil, 24 hours) and this led to the activation of biochemical processes in the soil (Figure 3).

Figure 3. Variations of urease enzymic activity in the soils of cotton plant rhizosphere that was sown after treating with “Zamin-M” biopreparation

Peroxidase enzymic activity indicated 1.76 ± 0.32 when applied the biopreparation “Zamin-M” and 1.5 ± 0.31(mg purpurgalin / g soil, 24 hours) in control, as well as polyphenol oxidase enzyme activity made 2.1 ± 0.53 and in the control 1.64 ± 0.51 (mg purpurgalin / g soil, 24 h) units were noted (Figures 4 and 5).

The results of the study and the data in the scientific literature show that the reasons for the decrease in the activity of soil enzymes as a factor are salinity, lack of adequate moisture and organic matter, accumulation of antimicrobial compounds.
Figure 4. Variations of peroxidase enzyme activity in the soils of cotton plant rhizosphere that was sown after treating with “Zamin-M” biopreparation.

Figure 5. Variations of polyphenol oxidase enzymic activity in the soils of cotton plant rhizosphere that was sown after treating with “Zamin-M” biopreparation.

Figure 6. Variations of phosphatase enzymic activity in the soils of cotton plant rhizosphere that was sown after treating with “Zamin-M” biopreparation.
Soil enzymes are one of the main factors in the main biological processes that take place in soil, oxidation-reduction reactions and the synthesis of humus compounds in the soil. Considering all of this, the study of the activity of these enzymes concluded that the effectiveness of the preparation may serve as one of the main parameters of bioindication in saline soils.

4. Conclusions

The main share of the cotton grown in the world comes from arid and semi-arid regions. Under such conditions, additional problems arise in the production and use of bio inoculants. In such areas, lack of nutrients in the soil, a tendency to salinity, an abundance of insoluble compounds of R and its only 2-4% suitability for the plant were observed due to low precipitation and high temperatures. Having analyzed such studies based on the literature and the results obtained, it was confirmed that the microbial composition “Zamin-M” had a positive effect on soil enzymatic activity and the number of microbial populations under salinity stress. Consequently, it has been concluded that the use of “Zamin-M” biopreparation will serve for increasing biological activity of the soil.

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