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INFLUENCE OF THE BIOLOGICAL MEDIUM "ZAMIN-M" ON THE ACTIVITY OF OXIDATION-REDUCTION FERMENTS IN POTATO TUBERS DURING THE STORAGE PERIOD.

Khojanazarova Mo'tabar Koshakovna

Doctoral student (DSc) at Tashkent State Agrarian University

https://orcid.org/0000-0003-4400-5818, tel: +998(99) 760-79-07

Sayyora Sobirovna Murodova

Professor of the Department of "Biotechnology" of the Jizzakh branch of the National University of Uzbekistan.

https://0000-0001-7106-3234. tel: : +998(97) 462-83-03
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Abstract. This article examines the effect of the Rhizobacterium-based biological agent "Zamin-M" on the antioxidant defense system and enzymatic activity of potato tubers during storage. During the study, the dynamics of activity in the main oxidative-reducing enzymes—peroxidase, catalase, and polyphenol oxidase—were analyzed within the tuber cells. Laboratory results indicate that biological treatment regulates oxidative stress in tubers and ensures stable high activity of antioxidant enzymes. This allows for the slowing down of cell membrane degradation and the formation of induced resistance to phytopathogens. The article scientifically substantiates the fundamental importance of the biological agent in regulating potato tuber metabolism.

Keywords: Potato, "Zamin-M," storage, biological agent, antioxidant system, peroxidase, catalase, polyphenol oxidase, oxidative stress, rhizobacteria, enzymatic activity, metabolism.

Introduction

Potato (Solanum tuberosum L.) is one of the strategic crops ensuring food security in global agriculture and is rightfully called "second bread" due to its nutritional value and high calorie content. Under the conditions of Uzbekistan, potato production volumes are increasing from year to year, but the long-term preservation of the harvested crop remains one of the most pressing problems in the agricultural sector. According to data, the amount of post-harvest losses during the storage period can reach 20% to 40% of the total harvest.

Even after potato tubers are harvested, they continue their vital activity as a living organism. The natural loss of mass during storage occurs under the influence of two main factors: physiological processes (respiration, transpiration) and phytopathogenic microorganisms (fungal and bacterial decomposition). Oxidation processes occurring in tubers lead to the degradation of cell membranes and a decrease in antioxidant resources, which reduces not only the weight of the product but also its nutritional value and marketable appearance.

Currently, chemical fungicides widely used to reduce storage losses are effective, but their negative impact on the environment and human health, the accumulation of residual toxins in the product, and the formation of resistance to pathogens necessitate a transition to biological methods.

In recent years, the use of rhizabacteria-based biopreparations in agricultural biotechnology has become a promising direction. Such preparations not only exert a direct antagonistic effect

against phytopathogens but also possess the ability to activate the plant's own defense system, triggering a mechanism of induced systemic resistance.

In particular, the biological agent "Zamin-M," developed based on strains of beneficial bacteria and a polymer carrier (Gipan), has shown high efficiency in potato storage. However, the effect of this agent on the oxidative-reducing enzyme system and antioxidant protection mechanisms within the tubers has not been sufficiently studied at a fundamental level.

The primary objective of this study is to scientifically substantiate the role of the biological agent "Zamin-M" in forming an antioxidant defense system and stabilizing cellular metabolism by studying the dynamics of the enzymatic activity of potato tubers during the storage period.

Research materials and methods. Potato varieties "Gala" (early ripening) and "Picasso" (mid-ripening) were used for the research. Healthy, uniform-sized (60–80 g) tubers without mechanical damage were selected as samples. The complex biological agent "Zamin-M" was used in the treatment of tubers. The composition of the agent consists of a microbial component, i.e., a mixture of strains Pseudomonas stutzeri SKB 308, Bacillus megaterium SKB 310, and Bacillus subtilis SKB 30), as well as the reagent Hypan, which possesses flocculant properties as a polymer carrier.

Before storage, the tubers were treated by spraying with a working solution of the biological agent in a ratio of 1:500 (consumption rate - 10 liters of solution per 1 ton of product). The control variant consisted of tubers treated with clean water. The tubers were placed in mesh bags of 10 kg each and stored in a warehouse at a temperature of 4±1 °C and a relative humidity of 85-90%.

Samples for biochemical analysis were taken on the first day of storage and at the end of each month (for 4 months). Core tissues from the middle part of the tuber were used for the analysis. Peroxidase activity was determined by the Boyarkin method based on the oxidation rate of benzidine in the presence of hydrogen peroxide. The results were measured using the photometric method (wavelength 490 nm).

Catalase activity was determined using the Chance and Maehly method based on a decrease in the concentration of H_2O_2 used as a substrate. Measurements were carried out on a spectrophotometer at 240 nm. Polyphenol oxidase activity was determined at a wavelength of 420 nm based on the intensity of the colored compounds formed during the oxidation of pyrogallol.

All experiments were performed in three repetitions. The reliability of the obtained results was analyzed using the Microsoft Excel and OriginPro 2026 software packages according to Student's t-test. Mean values and standard errors ($P < 0.05$) were calculated.

Results and discussion. The natural weight loss and microbiological stability of potato tubers during storage are directly dependent on their internal biochemical state and metabolic intensity. During storage, tubers undergo varying degrees of oxidative stress, which leads to the degradation of cell membranes and tissue aging. In this regard, when studying the effect of the biological agent "Zamin-M" on potato preservation, the main strategic goal of our study was to analyze not only external indicators but also the activity of enzymes in the antioxidant protection system of the tubers.

The primary objective of the conducted laboratory experiments was to determine the ability of a complex consisting of rhizobacteria and the Hypan polymer to balance oxidation-reduction processes within the tuber. At the same time, we focused on the dynamics of catalysis, which breaks down hydrogen peroxide, peroxidase, which forms an immune response to phytopathogens, and polyphenol oxidase, which is an indicator of tissue necrosis.

Analysis of monthly samples obtained during the experiment showed that biological treatment produced an "induced resistance" effect on the tubers, ensuring the stable functioning

of the enzymatic apparatus. Particularly during critical storage periods (3–4 months), sharp differences in enzyme activity were observed between the control and experimental variants.

The results of the obtained long-term and repeated laboratory analyses are summarized in Table 1:

Table-1.

Dynamics of antioxidant enzyme activity in potato tubers during the storage period

Varieties and Treatments	Initial	1 month	2 month	3 month	4 month
"Gala" variety					
Catalase, mg H_2O_2/g·min					
Control (untreated)	2,42	2,15	1,80	1,35	0,92
"Zamin-M"	2,42	2,38	2,25	2,10	1,85
Polyphenol oxidase, U/g·min					
Control (untreated)	1,20	1,35	1,42	1,15	0,85
"Zamin-M"	1,20	1,65	1,82	1,78	1,72
Polyphenol oxidase, U/g·min					
Control (untreated)	0,85	1,12	1,45	1,78	2,10
"Zamin-M"	0,85	0,92	1,05	1,18	1,25
"Pikasso" variety					
Catalase, mg H_2O_2/g·min					
Control (untreated)	2,65	2,40	2,10	1,75	1,20
"Zamin-M"	2,65	2,62	2,55	2,42	2,20
Polyphenol oxidase, U/g·min					
Control (untreated)	1,45	1,58	1,65	1,40	1,10
"Zamin-M"	1,45	1,95	2,15	2,12	2,05
Polyphenol oxidase, U/g·min					
Control (untreated)	0,72	0,95	1,25	1,55	1,85
"Zamin-M"	0,72	0,78	0,85	0,95	1,02

The enzyme catalase serves as the primary "shield" ensuring cellular viability by decomposing hydrogen peroxide (H2O2), one of the most toxic compounds formed as a result of oxidative stress in tuber cells, into water and molecular oxygen. The results of Table 1 show that at the beginning of the storage period, catalase activity was relatively high in both varieties (Gala - 2.42; Picasso — 2.65 mg H2O2/g·min).

However, as the shelf life increased, a sharp decrease in the activity of this enzyme was observed in the control variant. By the 4th month of storage, the enzyme activity in the "Gala" variety decreased by 2.6 times compared to the baseline, and in the "Picasso" variety by 2.2 times. This condition is explained by the natural aging process of plant tissues and the depletion of antioxidant resources. Such a decrease in enzyme activity accelerates the process of lipid peroxidation in cell membranes, resulting in impaired membrane permeability, making the tubers extremely vulnerable to pathogenic infections.

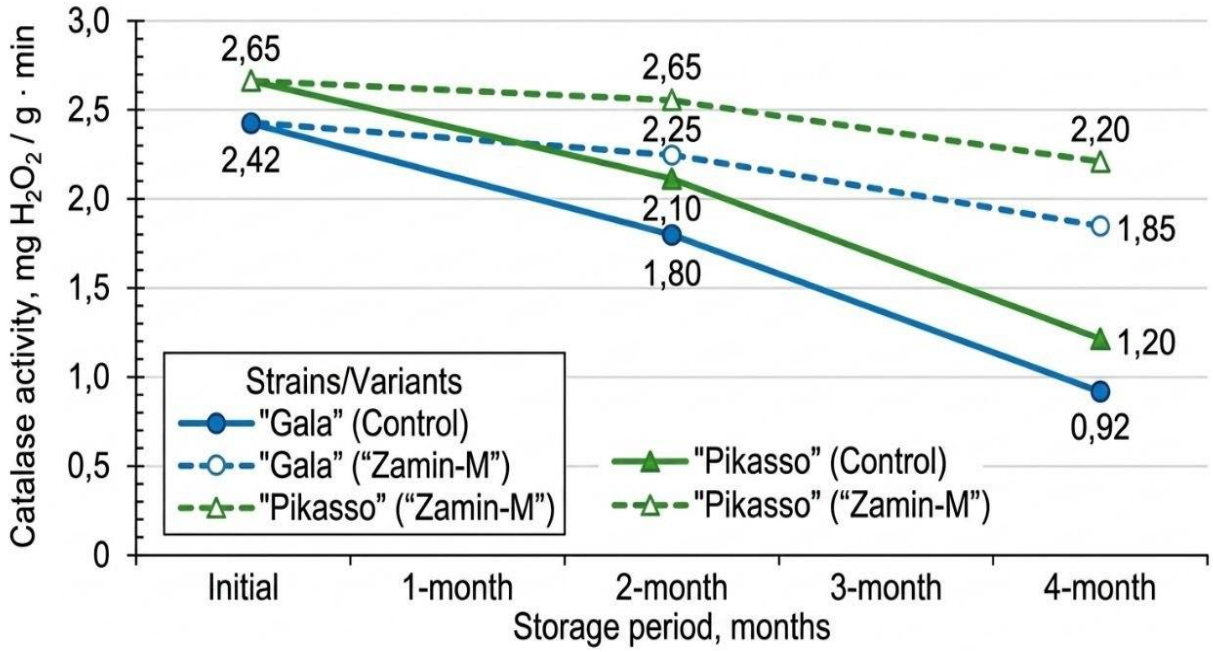

Figure 1. Dynamics of "Catalase" enzyme activity in potato tubers during storage, mg H2O2/g·min.

In contrast, in the variants treated with the "Zamin-M" biological product, catalase activity remained stable and high throughout the entire storage period. By the end of the 4th month, the enzyme activity of the "Picasso" variety was 2.20 mg H2O2/g·min, which is 83.3% higher than the control. This biochemical phenomenon can be consideUred the result of the mutual exchange of signals between bacteria of the genus Bacillus and Pseudomonas in the biopreparation and the plant. Bacterial metabolites activate the expression of genes that regulate redox homeostasis (balance) in tuber cells, allowing for the preservation of cellular integrity even during critical storage periods.

The logical relationship between the analyses in Table 1 and Figure 1 shows that "Zamin-M" immobilized with the flocculant not only reduces weight loss but also achieves this result by maintaining stable expression of fundamental antioxidant genes within the tubers. This dynamics of catalase activity fully reveals the biochemical mechanism of the high preservation efficiency noted in our first article.

The dynamics of the peroxidase enzyme reveal not only the preservative but also the immunomodulatory properties of the biological agent "Zamin-M." According to the table data, a sharp increase in peroxidase activity was observed in the treated variants within the first month of storage (Gala - 1.65; Picasso — 1.95 U/g · min). In the control variant, this indicator was much weaker, and the growth dynamics were unstable.

From a scientific point of view, such early and high manifestations of peroxidase activity are associated with the mechanism of "Induced systemic resistance." The mechanism of induced resistance is the process by which a plant activates its natural defense mechanisms under the influence of external stimuli and brings this system to a state of readiness. Simply put, it can be called a "vaccine" for plants. When a plant comes into contact with an agent (a beneficial bacterium, a weak pathogen, or a specific chemical), it perceives this as a "danger signal" and prepares for defense before the actual damage to the plant begins.

When the rhizabacteria in "Zamin-M," immobilized in the flocculant, come into contact with the tuber crust, the plant perceives it as "positive stress." As a result, the peroxidase enzyme is activated, accelerating the synthesis of high-molecular-weight compounds such as lignin and suberin in the cell walls. This process creates an additional mechanical and chemical barrier on

the surface of the tubers, preventing phytopathogenic fungi (especially the genus Fusarium) from entering the tubers.

In the 3rd and 4th months of storage, a decrease in peroxidase enzyme activity (0.85–1.10 U/g·min) in the control variant indicates a weakening of tuber immunity, while a stable maintenance of activity (1.72–2.05 U/g·min) in the variant using immobilized "Zamin-M" proves the long-term protective effect of the biological agent. This is the main biochemical factor for reducing weight loss in potatoes.

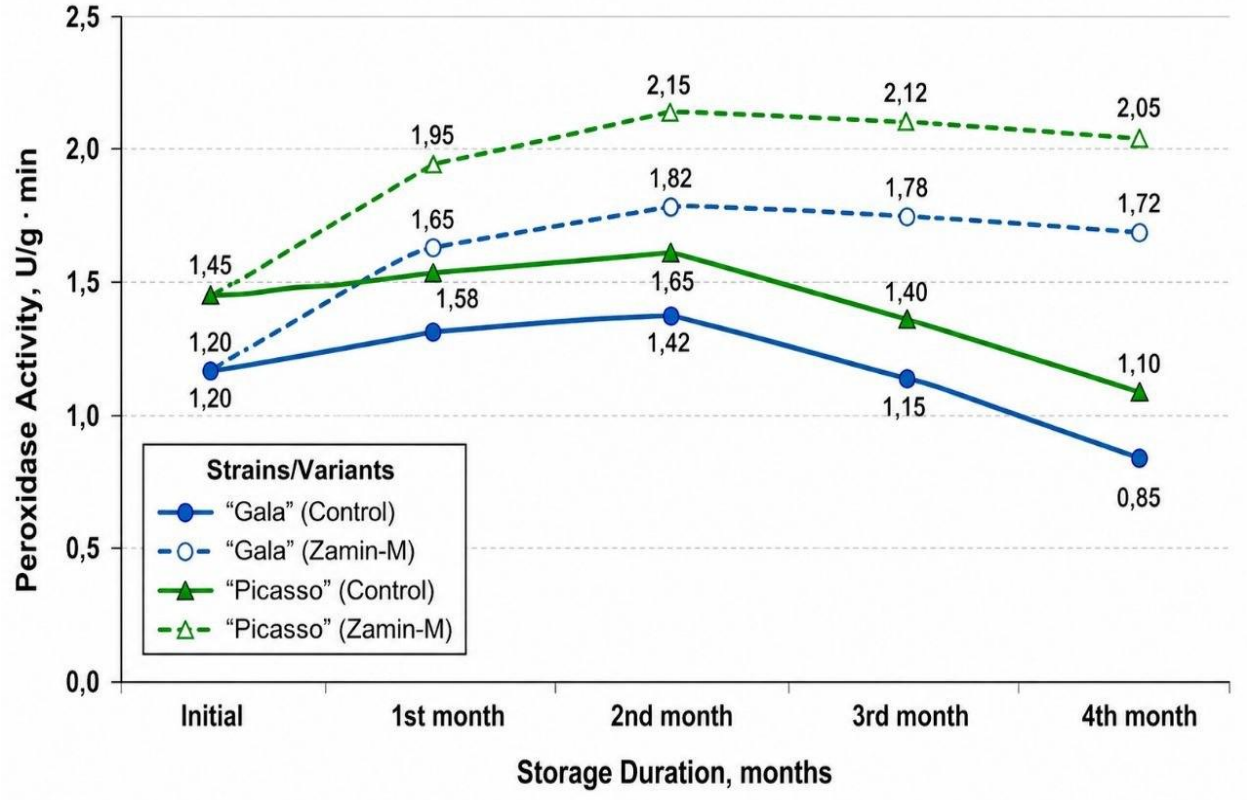

Figure 2. Dynamics of "Peroxidase" enzyme activity in potato tubers during the storage period, U/g·min.

The studied dynamics of peroxidase activity (Fig. 2) provide a fundamental explanation for the mechanism by which the biological agent forms an immune barrier against phytopathogens in potato tubers. Following the application of the immobilized "Zamin-M" variant on the flocculant, the rapid induction and maintenance of a stable high level of enzyme activity contributed to the acceleration of lignin synthesis and the strengthening of cell walls, which led to a sharp reduction in the microbiological losses identified in our first study.

The enzyme polyphenol oxidase is typically activated when tubers are damaged or diseased, converting phenolic compounds into toxic quinones, leading to potato blackening and tissue death (necrosis). In our study, the activity of the polyphenol oxidase enzyme was studied as a "negative indicator"; that is, the higher its activity, the faster the tuber undergoes degradation.

The table results show that in the control variant, polyphenol oxidase activity increased steadily during storage and reached its maximum point (2.10 U/g·min) by the 4th month. This indicates the intensification of internal necrotic processes and hydrolytic decomposition in the tubers.

In contrast, in the immobilized "Zamin-M" variant, the activity of the polyphenol oxidase enzyme was almost 2 times lower than in the control. For example, in the "Picasso" variety, the activity of the polyphenol oxidase enzyme at the 4th month was only 1.02 U/g·min. This result is

directly related to the role of the Hypan polymer in the biological agent. Hypane forms a microbial membrane on the surface of the tuber, limiting excessive oxygen diffusion. The enzyme polyphenol oxidase requires molecular oxygen to function. Thus, by optimizing the substrate environment (oxygen content) necessary for the enzyme, the biological agent prevents the darkening and internal deterioration of the tubers.

Intervarietal differences and a genotypic reaction were also observed in this experiment. The data obtained during the study indicate that the effectiveness of the "Zamin-M" biological agent also depends on the genetic characteristics of the potato variety.

The "Picasso" variety demonstrated higher indicators in terms of antioxidant system stability compared to the "Gala" variety. This condition is explained by the genetic tendency of this variety for long-term storage and its high degree of complementarity (adaptability) to the bacteria contained in the biological product.

Although the enzymatic activity of the "Gala" variety was slightly lower, its preservation indicators under the influence of the biological agent improved significantly (by an average of 35-40%) compared to the control.

The most significant scientific novelty of this study is the synergistic (mutually reinforcing) effect of the components within the immobilized "Zamin-M." While rhizabacteria "stimulate" the synthesis of enzymes in the tubers, the Hypan polymer protects these bacteria from unfavorable factors and retains moisture in the tubers. The stability of moisture provides an aquatic environment necessary for enzymes (especially catalase).

CONCLUSION

In conclusion, the studied dynamics of enzyme activity prove that the "Zamin-M" immobilized in the flocculant is not merely a superficial protective agent, but an environmentally safe regulator that regulates biochemical metabolism within the tubers. This regulation is carried out by controlling oxidative stress, enhancing the immune system response, and halting the premature aging of tissues.

As a result of the conducted comprehensive biochemical studies, the following scientific conclusions were formulated regarding the effectiveness of the biological agent "Zamin-M" in regulating the antioxidant defense system during the storage period of potato tubers:

- Treating flocculans with the immobilized biological agent "Zamin-M" plays a crucial role in managing oxidative stress in tubers. In the control variant, catalase activity decreased by 2.2–2.6 times by the 4th month of storage, whereas in the variant using the biological agent, this indicator remained at a steadily high level (1.85–2.20 mg H_2O_2/g·min). This proves that the cell membranes of the tuber are protected from peroxide oxidation and the lifespan is extended.

- Studies have shown that rhizobacteria in the biopreparation activate the mechanism of "induced systemic resistance" in the plant. The increase in peroxidase enzyme activity in the experimental variant by 35-45% compared to the control indicates an increase in immune barriers (synthesis of lignin and suberin) against phytopathogens in the tubers.

- The activity of the polyphenol oxidase enzyme in the experimental variant was 1.5–2.0 times lower than the control, indicating that the biopreparation effectively controls tissue necrosis and "darkening" processes in tubers. This indicator is important not only for preserving the nutritional properties of potatoes but also for preventing their microbiological degradation.

- As a result of the synergistic interaction between the hypan polymer and bacterial metabolites, an optimal balance of oxidation-reduction processes in the tubers is ensured. This serves as the fundamental basis for the results obtained in our first study regarding the reduction of natural mass loss and the extension of shelf life.

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