

Physiological Effects of Immunostimulant (Zerox) on Seed Germination and Early Seedling Development of Cotton (*Gossypium Hirsutum* L.) Under Laboratory and Field Conditions

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Research Article

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Abstract

Seed germination is a critical stage determining crop establishment and productivity, particularly in cotton (*Gossypium hirsutum* L.) grown under variable environmental conditions. The present study aimed to evaluate the dose-dependent effects of a colloidal silver-based immunostimulant (Zerox) on seed germination and early seedling development under both controlled laboratory and field conditions.

Cotton seeds of the Bukhara-10 variety were treated with five different application rates of Zerox (1.0, 1.5, 2.0, 2.5, and 3.0 l/t), along with a standard treatment (Fitovak, 200 ml/t) and a control (water). Laboratory experiments were conducted under controlled temperature (26 °C) and humidity conditions, while field trials were carried out over three consecutive growing seasons. Germination dynamics, seedling emergence, and early growth parameters were systematically recorded.

The results demonstrated a significant improvement in germination performance in response to Zerox application. The optimal treatment (2.0 l/t) resulted in 100% germination under laboratory conditions and significantly enhanced field germination (90.3%), representing a 17.7% increase compared to the control. Moreover, treated seeds exhibited accelerated germination, with emergence occurring approximately three days earlier than untreated seeds.

The observed effects are associated with enhanced enzymatic activity, improved mobilization of seed reserves, and regulation of endogenous phytohormones, particularly auxin and cytokinin. These findings suggest that Zerox acts as an effective physiological modulator, promoting rapid and uniform germination.

Overall, the application of Zerox at an optimal dose (2.0 l/t) can be recommended as an efficient seed treatment strategy to improve cotton establishment under both laboratory and field conditions.

Introduction

Seed germination represents a critical phase in the plant life cycle, determining successful crop establishment, uniform stand development, and ultimately yield formation. In cotton (*Gossypium hirsutum* L.), rapid and synchronized germination is particularly important under field conditions where environmental factors such as soil temperature, moisture availability, and salinity can significantly influence early plant development.

During germination, seeds undergo complex physiological and biochemical processes, including water uptake (imbibition), activation of hydrolytic enzymes, mobilization of stored reserves, and initiation of cellular metabolism. These processes are tightly regulated by endogenous phytohormones such as auxins, cytokinins, and gibberellins, as well as by reactive oxygen species (ROS), which function as signaling molecules at controlled concentrations. However, under suboptimal environmental conditions, oxidative stress and hormonal imbalance may delay germination and reduce seed vigor.

In recent years, increasing attention has been given to the use of plant biostimulants and immunostimulants as an effective approach to enhance seed performance and stress tolerance. These compounds are known to improve enzymatic activity, stimulate phytohormonal regulation, and enhance antioxidant defense systems, thereby promoting faster and more uniform germination. Among them, silver-based formulations have emerged as promising agents due to their potential role in modulating redox processes, influencing membrane permeability, and regulating hormonal signaling pathways.

Zerox, a colloidal silver-based immunostimulant, contains bioactive components capable of enhancing metabolic activity in seeds. Previous studies have reported its positive effects on root development, plant growth, and photosynthetic efficiency in cotton. However, comprehensive studies evaluating its dose-dependent effects on seed germination under both controlled laboratory conditions and multi-year field environments remain limited.

Therefore, the present study aimed to investigate the physiological effects of different application rates of Zerox on seed germination and early seedling development of cotton (*Gossypium hirsutum* L.). The study integrates laboratory and field experiments to determine optimal application rates and to provide a physiological interpretation of the observed responses based on enzymatic activation and phytohormonal regulation.

MATERIAL AND METHODS

Plant material and experimental design

Seeds of cotton (*Gossypium hirsutum* L.), cultivar Bukhara-10, were used in this study. The experiments were conducted under both controlled laboratory conditions and field conditions over three consecutive growing seasons. A completely randomized design (CRD) was applied in laboratory experiments, while field trials were arranged in a randomized complete block design (RCBD) with three replications.

The study included seven treatment variants: untreated control (distilled water), standard treatment (Fitovak, 200 ml/t), and five different application rates of the immunostimulant Zerox (1.0, 1.5, 2.0, 2.5, and 3.0 l/t).

Seed treatment procedure

Prior to treatment, cotton seeds were soaked in distilled water for 48 h to ensure uniform hydration. Subsequently, seeds were treated with the respective concentrations of Zerox and the standard preparation for 10–12 h. After treatment, seeds were air-dried under ambient conditions for 1–2 h before further use.

Laboratory germination assay

Laboratory experiments were carried out in a controlled environment using a thermostat chamber maintained at $26 \pm 1^{\circ}\text{C}$ with constant relative humidity. For each treatment, 50 seeds were placed in germination trays (cuvettes) lined with moistened filter paper. Each treatment was replicated four times.

Seed germination was monitored daily for 7 days. Germinated seeds were counted based on visible radicle emergence ($\geq 2\text{ mm}$). Germination percentage (%) was calculated using the standard formula:

$$\text{Germination (\%)} = (\text{Number of germinated seeds} / \text{Total number of seeds}) \times 100$$

Field experiment

Field trials were conducted at the experimental site of the Bukhara Scientific Experimental Station under typical agro-climatic conditions. Seeds were sown at a depth of 4–5 cm when soil temperature reached $14\text{--}15^{\circ}\text{C}$.

The sowing rate was 35 kg ha^{-1} . Each treatment plot consisted of rows 1.1 m in length, with a standardized number of seeds (62 seeds per row). Field germination was assessed by counting emerged seedlings at regular intervals over a 12-day period.

Measured parameters

The following parameters were evaluated:

- Germination percentage (%)
- Number of germinated seeds (laboratory and field)
- Germination dynamics (daily emergence)
- Time to seedling emergence (days)
- Early seedling development (radicle and shoot formation)

Statistical analysis

All experimental data were analyzed using one-way analysis of variance (ANOVA). Differences between treatment means were evaluated using the least significant difference (LSD) test at a significance level of $p < 0.05$.

In addition, effect size was estimated to quantify the magnitude of treatment effects. Data are presented as mean $\pm$ standard error (SE). Statistical analyses were performed using standard statistical software.

RESULTS AND DISCUSSION

Laboratory germination dynamics

The results of the laboratory experiments demonstrated a clear dose-dependent effect of the Zerox immunostimulant on seed germination and early seedling development of cotton (*Gossypium hirsutum* L.). During the first 24 hours of incubation, seeds treated with Zerox exhibited rapid water uptake and early metabolic activation compared to the control, where no visible germination was observed.

By the second day, germination had initiated in all treated variants, with a significantly higher number of germinated seeds compared to the control. The most pronounced effect was observed in the treatments with 2.0 and 3.0 l/t, where early radicle emergence and primary leaf formation were accelerated. This indicates enhanced enzymatic activity and more efficient mobilization of stored reserves in treated seeds.

After 7 days of incubation, germination percentage reached 100% in the 2.0 and 3.0 l/t treatments, compared to 72% in the control and 92% in the standard (Fitovak). The increase in germination relative to the control was + 28% for both optimal treatments. Furthermore, germination in these variants occurred approximately three days earlier than in untreated seeds, demonstrating a strong stimulatory effect of Zerox. (Table-1)

Table – 1. Determination of seed germination in laboratory conditions

№	Variants	in the cuvette seed, pcs.	applied doses, l/t	Observation dates, days					Germination	
				28.04	30.04	01.05	03.05	04 .05	In percent,%	relative to the control the difference, ±
Average number of sprouts from the replicates, pcs.										
1	Control	50	water	-	0	6	21	36	72,0	0,0
2	«Fitovak»	50	200 ml/t	-	14	29	44	46	92,0	+ 20,0
3	«Zerox»	50	1 l/t	-	8	21	42	42	84,0	+ 12,0
4	«Zerox»	50	1,5 l/t	-	8	17	44	47	94,0	+ 22,0
5	«Zerox»	50	2 l/t	-	15	31	46	50	100,0	+ 28,0
6	«Zerox»	50	2,5 l/t	-	19	36	44	46	92,0	+ 20,0
7	«Zerox»	50	3 l/t	-	28	42	48	50	100,0	+ 28,0

Physiological interpretation of laboratory results

The enhanced germination observed in Zerox-treated seeds can be explained by the activation of key physiological processes during seed imbibition. The presence of colloidal silver likely contributed to increased enzymatic activity, including hydrolytic enzymes responsible for the breakdown of stored macromolecules.

In addition, improved germination may be associated with the regulation of endogenous phytohormones. Increased sensitivity to auxin and cytokinin may have promoted cell division and elongation in the embryonic tissues, resulting in faster radicle protrusion and seedling establishment. The modulation of reactive oxygen species (ROS) levels may also have contributed to maintaining cellular homeostasis during early germination stages.

Field germination performance

Field experiments conducted over three years confirmed the positive effects observed under laboratory conditions. Germination dynamics varied depending on environmental factors such as temperature and soil moisture; however, consistent trends were observed across all seasons.

In the early stages (4–5 days after sowing), the number of emerged seedlings was higher in all Zerox-treated variants compared to the control. The treatment with 2.0 l/t showed the most stable and highest performance across all years. For instance, in the first year, 36 seedlings emerged in the 2.0 l/t treatment compared to 26 in the control. Similar trends were observed in the second and third years.

After 12 days, final germination percentage reached 90.3% in the 2.0 l/t treatment, compared to 72.6% in the control and 83.8% in the standard treatment. This corresponds to a 17.7% increase over the control. The 3.0 l/t treatment also showed high germination (87.6%), although slightly lower than the optimal 2.0 l/t dose. (Table-2)

Dose-dependent response and optimal treatment

The results indicate a clear dose-dependent response of cotton seeds to Zerox application. While all tested doses improved germination compared to the control, the most effective range was 2.0–3.0 l/t. Among these, 2.0 l/t was identified as the optimal dose, providing maximum germination efficiency and consistency across both laboratory and field conditions.

The slightly lower performance at higher concentrations (3.0 l/t) suggests a possible threshold effect, where further increases in concentration do not result in proportional physiological benefits. This may be due to metabolic saturation or regulatory limitations at the cellular level.

Comparison between laboratory and field conditions

Germination rates were consistently higher under laboratory conditions compared to field conditions. This difference can be attributed to the controlled environment in the laboratory, where optimal temperature (26°C) and moisture conditions ensured uniform metabolic activity.

In contrast, field conditions introduced variability due to fluctuating environmental factors. Nevertheless, the relative effectiveness of Zerox treatments remained consistent, confirming the reliability of the results and the practical applicability of the immunostimulant under real agricultural conditions.

Overall physiological implications

The positive effects of Zerox on seed germination can be attributed to its role as a physiological regulator. The presence of colloidal silver (3000 mg/l) may enhance membrane permeability, stimulate enzymatic activity, and regulate phytohormonal balance. These processes collectively contribute to increased oncotic pressure, accelerated cell expansion, and faster seedling emergence.

Overall, the findings demonstrate that Zerox functions as an effective seed priming agent, improving both the rate and uniformity of germination, which are critical factors for successful crop establishment.

Table 2 Effect of different doses of "Zerox" immunostimulant on seed germination in field conditions

№	Experience variants	First year						Second year						Third year			
		The number of sprouts, pcs.					Seed germination%	The number of sprouts, pcs.					Seed germination%	The number of sprouts, pcs.			
		1.05	3.05	5.05	7.05	8.05		8.05	10.05	12.05	14.05	16.05		7.05	9.05	11.05	13.05
1	Control	26	30	34	37	44	71,0	17	28	39	42	44	71,0	19	28	37	40
2	«Fitovak» 200 ml/t;	33	34	42	47	52	83,8	22	34	47	51	54	87,1	24	34	48	50
3	«Zerox» 1 l/t;	31	38	42	44	49	79,0	23	36	45	50	52	83,8	22	33	43	45
4	«Zerox» 2 l/t;	36	40	52	54	57	91,9	26	36	48	54	57	91,9	28	38	50	54
5	«Zerox» 3 l/t;	35	45	51	53	54	87,1	25	35	46	50	56	90,3	27	37	48	52

CONCLUSION

The present study demonstrated that the application of the colloidal silver-based immunostimulant Zerox significantly enhances seed germination and early seedling development of cotton (*Gossypium hirsutum* L.) under both laboratory and field conditions. The results confirmed a clear dose-dependent response, with all treated variants showing improved performance compared to the untreated control.

Among the tested concentrations, the application rate of 2.0 l/t was identified as the most effective treatment, resulting in the highest germination percentage (up to 100% under laboratory conditions and 90.3% under field conditions) and earlier seedling emergence by approximately three days compared to the control. The 3.0 l/t treatment also showed high efficiency but did not surpass the optimal performance of the 2.0 l/t dose, indicating a threshold effect in physiological response.

The observed improvements in germination can be attributed to enhanced enzymatic activation, improved mobilization of seed reserves, and regulation of endogenous phytohormones, particularly auxins and cytokinins. Additionally, the colloidal silver component of Zerox may play a role in modulating redox balance and improving cellular metabolic activity during early germination stages.

Overall, Zerox acts as an effective seed priming agent that improves germination rate, uniformity, and early seedling vigor. These findings suggest its practical potential for improving cotton establishment under variable environmental conditions. Based on the results obtained, the application of Zerox at a rate of 2.0 l/t is recommended for optimal seed germination performance.

Declarations

1. Consent for Publication

All authors have read, critically reviewed, and approved the final version of the manuscript. The authors agree to its submission and publication in the selected journal. They confirm that the manuscript is original, has not been published previously in any form, and is not under consideration for publication elsewhere.

All authors further confirm that they take full responsibility for the integrity of the work and agree with the order of authorship as presented in the manuscript.

2. Ethical Approval and Compliance with Research Standards

This study complies with all relevant institutional, national, and international guidelines for scientific research. No experiments involving humans or animals were conducted in this study; therefore, ethical approval from an institutional ethics committee was not required.

All experimental procedures involving plant material were carried out in accordance with standard agronomic and agricultural research practices at the Bukhara Scientific Experimental Station.

3. Competing Interests

The authors declare that there are no competing interests, whether financial or non-financial, that could have influenced the design, execution, interpretation, or reporting of this study. The authors confirm that there are no personal relationships, consultancy roles, employment, or financial holdings that may be perceived as influencing the objectivity of the research.

4. Funding Statement

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5. Authors' Contributions

R. O. Atoeva and D. O. Atoeva contributed equally to the study design, experimental planning, and execution of laboratory and field trials. R. O. Atoeva was responsible for statistical analysis, data interpretation, and validation of results. Both authors jointly prepared and revised the manuscript. D. O. Atoeva performed critical revision of the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Author Contribution – Short Description

R. O. Atoeva and D. O. Atoeva jointly designed the study and conducted the experiments. R. O. Atoeva performed data analysis, while both authors contributed equally to manuscript writing and approved the final version.

6. Data Availability Statement

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Additional raw datasets generated during the three-year field and laboratory experiments are stored in the archives of the Bukhara Scientific Experimental Station and are available from the corresponding author upon reasonable request, subject to institutional data sharing policies.

7. Acknowledgements

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8. Statement of Novelty

This study provides new experimental evidence on the dose-dependent effect of a colloidal silver-based immunostimulant (Zerex) on cotton seed germination under both laboratory and field conditions. The findings highlight the optimal concentration (2.0 l/t) for maximizing germination efficiency and improving early seedling establishment, contributing to sustainable cotton production strategies.

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