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ORIGINAL PAPERS

ANTIOXIDANT ENZYMES INVOLVED IN SEEDS' GERMINATION MODULATED BY ACTIVE PRINCIPLES FROM PLANT EXTRACTS AND HYDROLYZED PROTEINS

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Abstract. *Transition to organic farming requires the development of new methods to protect seeds from adverse factors. The germinative process and the post-germinative phase are strictly controlled by the oxidative balance involving lipid peroxidation, reactive oxygen species and enzymes activity, directing the plant viability and its further development. We focus our studies mainly on the seeds protection and fortification through natural solutions based on marigold and fenugreek extracts, steroid alkaloids from tomatoes and hydrolyzed proteins derived from leather waste industry. The structural configuration of the plant protection products proves significant effects fighting on different mechanisms of seeds' oxidative stress. As well as the experimental design highlighted a tandem correlative mechanism between germination, decrease of lipid peroxidase and activation of catalase and superoxide-dismutase. SEM-PROTECT II was the most active biopesticide involved in these processes. Our findings directed complex research oriented to technological and experimental optimizations for the development of an innovative, efficient and competitive plant protection product to meet modern agriculture requirement.*

Keywords: germination, antioxidant, peroxidase, catalase, germinative energy

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INTRODUCTION

Pollution and biological and physical stress are important and vulnerable factors for seed growth and plant development. The rise in agriculture production is imposed by a larger world's population and also by the by the population's demand for good-quality food and a better life. Pollution and biological and physical stress are important and vulnerable factors for seed growth and plant development. The rise in agriculture production is imposed by a larger world's population and also by the by the population's demand for good-quality food and a better life.

The ratio between crop yields must lead to a better understanding of the fine mechanisms of plant growth and the discovery of solutions to improve their development as well as their resilience to aggressors. This is usually achieved through the use of chemical fertilizers and/or pesticides, ameliorating the afore-mentioned factors and those of biotic and abiotic stresses [1]. Research conducted in this field highlighted a variety of different plants' extracts and natural protein complexes having the capacity to improve plant growth and development, increase nutrient uptake, yield, and water content, as well as the nutritional value and quality of the harvest [2].

After the generalization of the use of chemical fertilizers and pesticide products, which allowed a considerable increase in yield in the twentieth century, the rise of biotechnologies and new cultivation techniques is underway. The concept of biocontrol has caused an important technological, economic, and political debate aiming to develop sustainable agriculture at a lower ecological cost [3].

In the development cycle of plants and agricultural production, there are certain critical parameters that determine the yield of production at harvest. Among them, the limitation of fungal attack and pests, as well as the stimulation of germinative capacity and energy, are essential. Although the quality of the seeds is mainly determined by the genetic endowment, it can deteriorate in the post-harvest stage during the processing and storage period. The vigor of the seeds, respectively, the cell viability, directs the performance in the field and the quality of the crops, being necessary treatments that improve the germination and growth of the seedlings or facilitate the delivery of the seeds. Among the techniques for improving the quality of the seeds can be listed: hydration (pre-hydration), fortification, osmotic conditioning, and bio-/osmo-priming [4]. We focus our studies mainly on seed protection and fortification through natural solutions based on plant extracts and hydrolyzed proteins derived from the leather waste industry. We chose the marigold and fenugreek extracts, as well as the steroidic alkaloids from tomatoes.

The germinative process and the post-germinative phase are strictly controlled by the oxidative balance involving lipid peroxidation, reactive oxygen species, and enzyme activity, directing plant viability and further development [5–7].

Polynesaturated fatty acids from cellular lipids are a very sensitive target for reactive oxygen species, affecting the germination capacity and longevity of seeds [8]. Hydrogen peroxyde induces cytotoxic effects, and early seed imbibition is associated with cellular membrane damage and malondialdehyde (MDA) production. The concentration of malondialdehyde (MDA) could reflect the degree of deterioration of seeds resulting from adverse conditions. Under water stress conditions, a gradual decrease in germination capacity was concomitant with an increase in MDA concentration. The water stress-induced decrease in membrane stability indicates the degree of lipid peroxidation caused by reactive oxygen species, and the changes in MDA levels recorded by seed pretreatment could be the result of treatment-induced changes in the activities of free radical scavenging enzymes and peroxide. Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) are the main protective enzymes, as they are involved in the elimination of free radicals and reactive oxygen species [9]. Reactive oxygen species (ROS) accumulate during the imbibition phase of seed germination. Seed germination essentially requires low levels of ROS that could be maintained by CAT and POD involved in the removal of oxygenated water [10].

In order to point out the capitalization of natural compounds and leather industry waste in the circular bio-economy, especially in healthy food production, we conducted our research in the following directions:

- Modulation of antioxidant enzymes and lipid peroxidation by a natural complex with fungicide and stimulation properties [11].
- Marigold and Fenugreek extract capitalization
- Hydrolyzed protein waste from leather industry valorisation
- Highlighting the importance of multifunctional biopesticides in the circular bio-economy.

MATERIALS AND METHODS

Preparation of the planting material

The transition to organic farming requires the development of new methods to protect seeds from adverse factors. A contemporary and effective method is the pre-sowing treatment of seeds with natural bioregulators, as it is known that growth regulators of an exogenous nature manifest their action by changing the endogenous level of natural hormones already present in the plant, thus altering the directed growth and development of plants. The study of the biological actions of these natural compounds is based on knowledge of their physiological activities, depending on their chemical structure, concentration, and application methods.

The study was performed on the following seeds: LYCOPERSICON ESCULENTUM, tomato (semi-early variety SAINT PIERRE), and CAPSICUM ANNUM, peppers (semi-early variety SOROKSARY).

The effect of selected bioactive substance combinations was examined:

- GLYCAM-SSTIM combo I-GGLY T (tomato glycoalkaloids) 0.5% + camelina oil 6% + Ska fenugreek butylene glycol extract 10% + CHC3B protein hydrolysate 1.5% (biopesticide with ambivalent, antifungal, and fertilizing activity);

- GLYCAM-SSTIM combo II: GLY T (tomato glycoalkaloids) 0.5% + camelina oil 6% + marigold butylene glycol extract 10% + protein hydrolysate CHC2B 1.5% (biopesticide with ambivalent, antifungal, and fertilizing activity);

- SEM-PROTECT I: marigold butylene glycol extract 10% + protein hydrolysate CHC3B 1.5% (biostimulator and phytoprotector);

- SEM-PROTECT II: butylene glycol extract of fenugreek Ska 10% + protein hydrolysate CHC2 B 1.5% (biostimulator and phytoprotector);

on germination capacity, germination dynamics and biometric parameters of seedlings.

Two methods were used to sterilize the seeds of bell peppers and tomatoes:

1. incubation of seeds for 30 minutes at 500 °C in distilled water
2. surface sterilization by immersion in a 2% potassium permanganate solution for 30 minutes, followed by repeated washing with distilled water.

The seeds thus conditioned were evenly distributed in Petri dishes of 50 mm diameter on a soapy bed of filter paper that had been previously sterilized by UV irradiation and moistened with distilled water. In the case of samples where it was desired to simulate moderate drought conditions, the saddling material was moistened with PEG 6000 to achieve an osmotic potential of -0.6 MPa [12]. Tweezers were used to manipulate the saddling material because the seeds are quite small. They were covered with new filter paper, and then the test solution was added by pipette (about 10 mL). Three replicates

species. The control variant was moistened with distilled water. The test solution was dripped in as many places as possible to wet the paper evenly. The Petri dish was covered with the upper half, where the test variant and the repetition were marked with a marker.

Plates were covered with aluminum foil and randomly placed under temperature-controlled conditions (25 °C). Seed checks were carried out daily. Germination was monitored, but it was also ensured that the filter paper was not dry to the touch. If necessary, a few drops of test solution were added to maintain moisture. Seeds were considered germinated when the root was visible. The germination criterion was the emergence of the root through the seed coat. The germination index (Gi) was calculated based on the germination percentage as follows: Gi = PGt/Dt, where Gt is the percentage of germinated seeds at 1-day intervals and Dt is the number of days of germination (Huang et al. 2003). Seeds treated for 6 days were sampled for the determination of biochemical parameters (catalase, superoxide dismutase, peroxidase, and lipid peroxidation).

Catalase, superoxide dismutase, peroxidase, and lipid peroxidation assessment

Catalase activity was investigated according to the method of Aebi. The estimation was done spectrophotometrically, measuring the decrease in absorbance at 240 nm. The reaction mixture contained 0.01 M phosphate buffer (pH 7.0), 2 M H2O2, and cell lysates. The specific activity of catalase is expressed in terms of units per mg of protein. A unit is defined as the velocity constant per second.

Superoxide dismutase, is a metalloenzyme that catalyzes the dismutation of the superoxide anion into oxygen and hydrogen peroxide. We have used the spectrometric procedures described by Sigma Aldrich to determine the SOD activity in samples. The method is based on the spectrophotometric evaluation (550 nm absorbance spectra) of the inhibition rate of cytochrome C reduction by competing for the superoxide radical with superoxide dismutase.

Peroxidase - was analyzed according to Prochazkova [14]. The 3 ml reaction mixture contained 28 µl guaiacol, 2 mM H2O2, 0.1 M phosphate buffer (pH 6.0), and 0.1 ml enzyme. Absorbance was recorded at 470 nm.

Lipid peroxidation was evaluated using the method with thiobarbituric acid (TBA). The MDA-TBA adducts formed subsequent to the reaction of TBA and MDA in the biological sample were measured using a colorimetric method (l = 532 nm).

RESULTS AND DISSCUTION

In this study, in order to determine the optimal forms of association between plant extracts and protein hydrolysates, the impact of these solutions on the germination process of tomato and pepper seeds of different varieties was evaluated.

Under laboratory conditions, the germination capacities (germination energy, germination faculty, and root length) of tomato and pepper seeds treated with solutions of different combinations of biologically active substances were determined. The most effective combination for tomatoes was found to be GLYCAM-STIM combo II because it increased germination energy by 34% and germination capacity of tomato seeds by 15.8% compared to the control (see Fig. 1), and for pepper seeds, a more effective stimulating effect was shown by

SEM-PROTECT II, which increased germination energy by 18% compared to the control and germination capacity by 14.3%. In both tomato and pepper seeds, it was found that spraying the seed material with the test solution for 24 hours improved the germination capacity of the seeds. But keeping into account that not only the final germination percentage obtained is an important aspect, but also other germination parameters such as mean germination time, germination index, and germination

velocity coefficient, germination timing index, and germination uncertainty index, which are often used to assess the agronomic relevance of treatments, it can be said that seed soaking with GLYCAM-STIM combo II gave the best results according to the indicators mean germination time (MGT), germination index (GI), germination velocity coefficient (CVG), germination rate index (GRI), and germination synchronization index (Z). The data obtained showed that the studied solutions stimulate the primary physiological processes of tomato and pepper seeds, namely growth energy and germination. At the same time, they influence root development. The results obtained allow us to conclude that treating seeds before sowing with GLYCAM-STIM combo II bioregulators significantly increases seed growth energy and root development, an important factor when using tomato and pepper seeds after a long storage period and reduced germination. Application of these seed treatments further ensures simultaneous and uniform sprouting, which will subsequently lead to increased yield and quality of production.

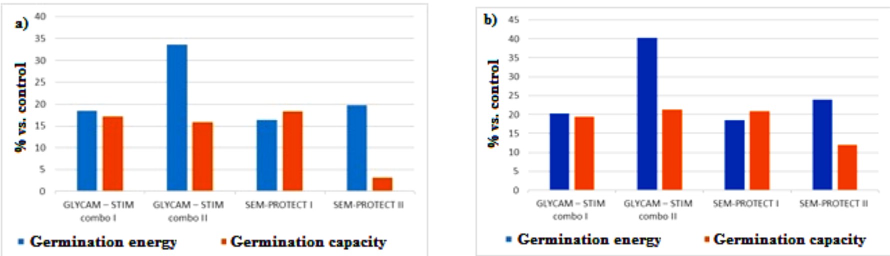


Fig. 1. Evaluation of the germination capacity of tomato seeds in a) the seed material was pre-treated with the test solutions for 24 hours, and after soaking was sprayed with distilled water; b) the seed material after sterilization was soaked and sprayed with the test solution every 24 hours.

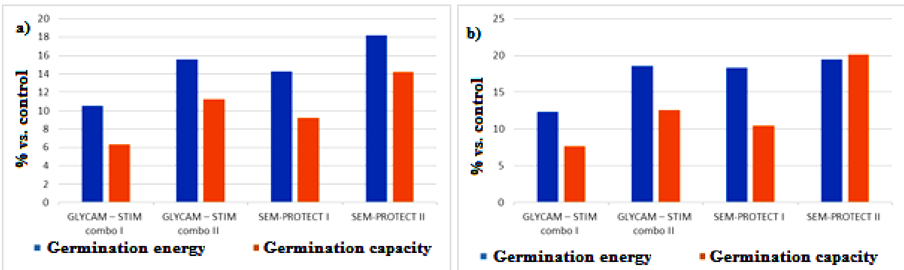
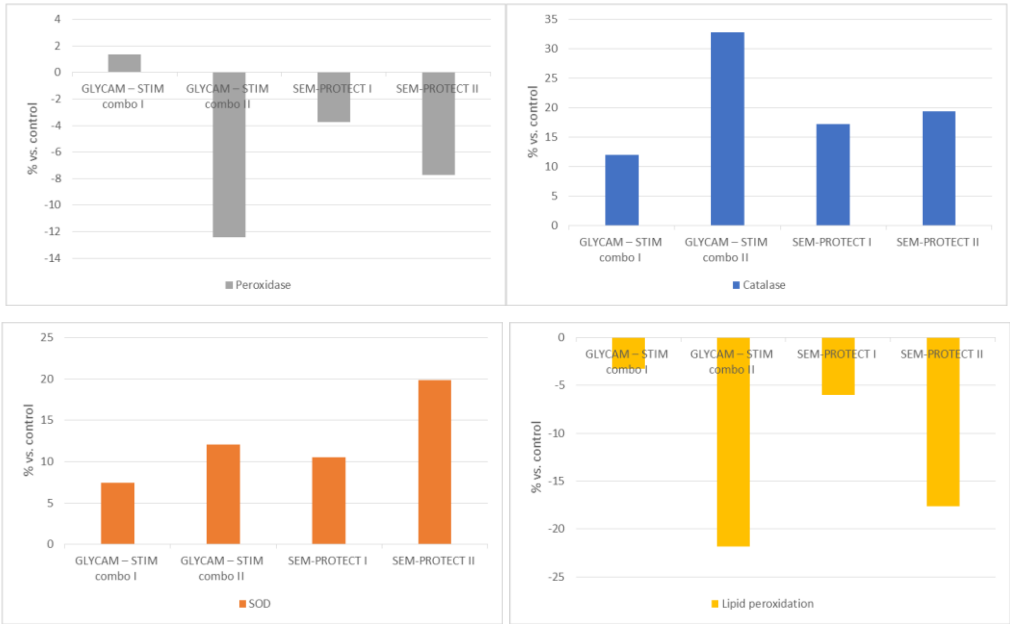


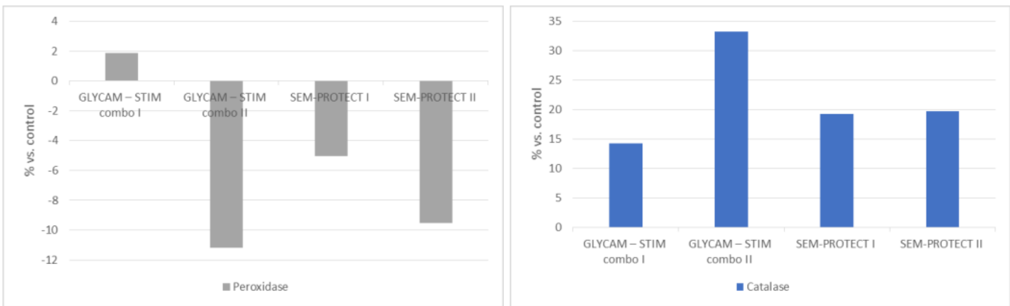
Fig. 2. Evaluation of the germination capacity of pepper seeds in a) the seed material was pre-treated with the test solutions for 24 hours, and after soaking was sprayed with distilled water; b) the seed material after sterilization was soaked and sprayed with the test solution every 24 hours.

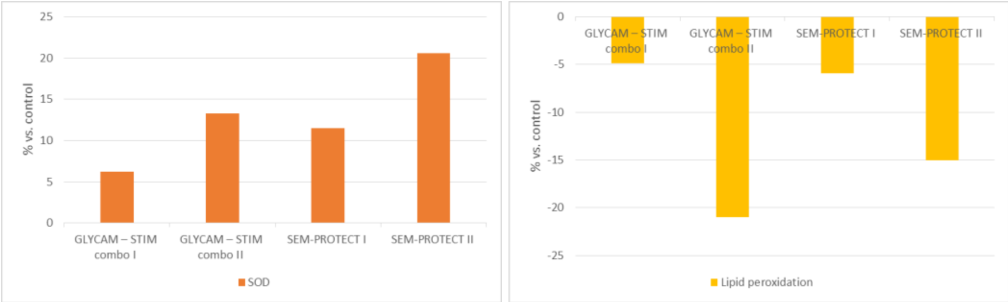
In the germination process, and especially in the one that takes place under water stress conditions, an essential role is played by the level of antioxidant enzymes, especially catalase and peroxidase. In the following, the results obtained in the tests to quantify the level of malondialdehyde present as a consequence of the degree of lipid peroxidation and the enzymatic activity of the protein enzymes involved in counteracting oxidative stress are presented. Statistical analysis of the experimental data revealed that, in the case of tomato seeds, between the two methods of treatment application (pretreatment for 24 hours or periodic spraying with the test solution), there are no statistically significant differences, i.e.

Either of the two methods can be applied with a similar result, but in the case of pepper seeds, periodic spraying with a biostimulator increases the enzymatic activity for catalase and superoxide dismutase and decreases the peroxidase activity and the degree of lipid peroxidation..



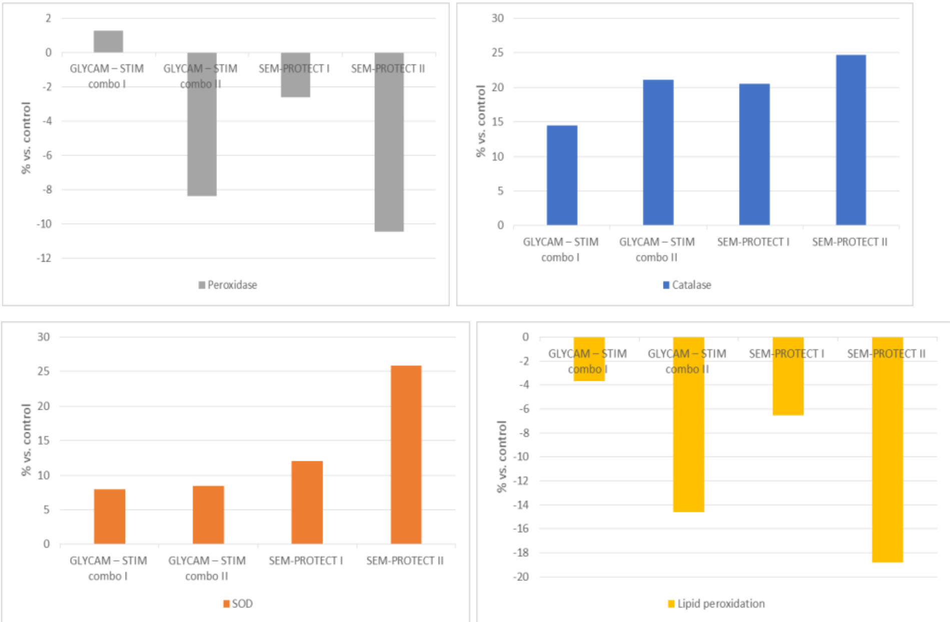
a)the saddling material was pretreated with the test solutions for 24 hours, and after sterilization it was sprayed with distilled water



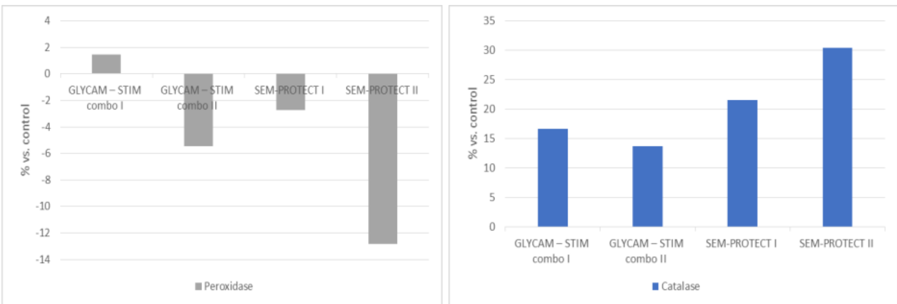


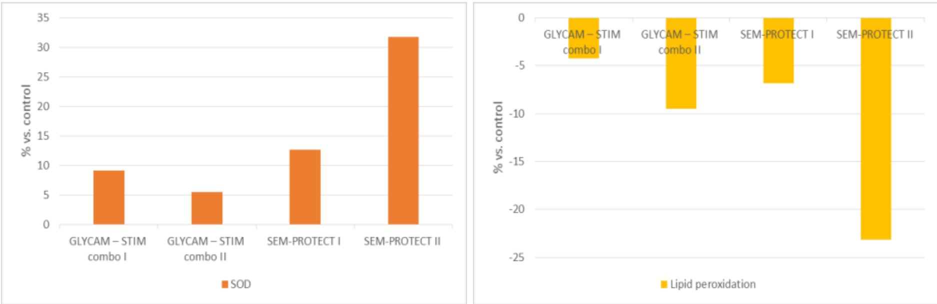
b) the saddling material after sterilization was seeded and sprayed with the test solution every 24 hours

Fig. 3. Evaluation of enzyme activity of enzymes involved in counteracting oxidative stress and the degree of lipid peroxidation in tomato seeds



a) the saddling material was pretreated with the test solutions for 24 hours, and after sterilization it was sprayed with distilled water





b) the saddling material after sterilization was seeded and sprayed with the test solution every 24 hours

Fig. 4. Evaluation of enzyme activity of enzymes involved in counteracting oxidative stress and the degree of lipid peroxidation in pepper seeds

CONCLUSIONS

The structural configuration of the plant protection products proves significant effects on different mechanisms of the seeds' oxidative stress. The experimental design highlighted a tandem correlative mechanism between germination, a decrease in lipid peroxidase, and the activation of catalase and superoxide dismutase. SEM-PROTECT II was the most active biopesticide involved in these processes. Our findings directed complex research oriented to technological and experimental optimizations for the development of an efficient and competitive plant protection product to meet modern agriculture requirements. Obtaining natural compounds from marigold and fenugreek and protein extracts from tannery waste, testing their capacity to modulate the antioxidant balance in seeds, and combining them in multifunctional prototypes are the main directions for defining an efficient active complex, formulation variants, and treatment schemes. The modern farming solution must be organized to avoid any damage and to preserve the germinative processes; therefore, antioxidant protection is an important option.

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