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Influence of melafen on redox enzymes in sprouting sugar beet

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ABSTRACT

The article contains the study results of the melafen influence, a new generation phytoregulator, on the activity of redox enzymes at the initial stages of ontogenesis. The catalase activity under the influence of melafen increased throughout the entire experiment. The greatest activity was observed on the 6th day then it decreased. The maximum value in the experimental variant was already reached on the 3d day. The activity increase of peroxidase and polyfenoloxidase also was seen the results of which were the increase of ascorbic acid, glutathione, the reducing activity of tissues and respiration intensity of sprouts.

Keywords: melafen, catalase, peroxidase, redox enzymes, polyfenoloxidase, polyfenols.

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INTRODUCTION

One of the most important processes of a living plant is the catalytic action of biochemical processes which is carried out by various enzyme systems. Redoxreactions underlie the fundamental processes such as cell respiration and photosynthesis. A physiological and biochemical response of the plant body depends on the aggregate action of the life environment, physiological state of the organism and development stage. While acting on the seeds with various factors and also with growth regulatorsa response reaction of the seeds is observed in the form of a level change (fallor elevation) of metabolismat the initial stage of ontogenesis.

Melamine salt bis (oxymethyl) of phosphinic acid (melafen, MF) is a highly efficient growth and development plant regulatorthat is of significant interest for practical use in agriculture and biotechnology [1, 2]. Studying the influence of the MF solutions on growth and energy processes of a plant cell [3, 4], on a physical and chemical state of biological membranes of various origin [5], functional characteristics of mitochondria [6, 7], also in studying the action mechanism of preparation solutions in the plant cell metabolism regulation it has been found that MF solutionspossess a high physiologic activity in low and ultra low concentrations. The obtained experimental data on chlorella culture give us a possibility to make a conclusion that melafen has a wide range of action and possesses a high physiologic activity comparable withnatural growth regulators and with the action of ATPin low concentrations [8, 9, 10].

The initiating role belongs to an active phosphinic group of the melafen preparation that in contact with the external membrane of the cell can have an influence on the functional state of membranes. The change of the membranes' functional state can trigger physiological and genetic programs.

Melafen has an impact on the expressiveness of the gene Elip2 participating in regulating chlorophyll and biogenesis of chloroplasts [11]. The obtained data [4] imply the regulatory role of melafen in the process of collecting the active enzyme ribulose biphosphate carboxylase/oxygenase– a supramolecularproteincomplexthat participates in reactions of the dark stage of photosynthesis.

We have established in our studies [12, 13, 14] thatunder the influence of melafen in a concentration 10^{-6}-10^{-7}%, a more intensive flow of redox processes takes place in spring wheat seeds' sprouting– the activity increase of amylase demonstrates this. The activity rise of this enzyme leads to a higher mobilization of nutrients, facilitates the intensive growth of sprouts and increases the respiration intensity up to 15,6%.

The purpose of the investigation was to study the influence of stimulating melafen concentrations on redox processes progressing in the sugar beet sprouting which underlie metabolic processes in photosynthesis and respiration.

In this connection the following objectives were set:

- to study the the character of a change of redox catalase enzymes, peroxidase and polyfenoloxidase in sprouting treated and control seeds;
- to determine the content of ascorbic acid, glutathione and total reducing activity of tissues for the forecasting of the ascorbic acid and ascorbatoxidase systems which is connected with oxidation and restoration of glutathione.

OBJECTS AND INVESTIGATION METHODS

To study the objectives set we conducted multiple lab tests for the purpose of investigating the action of melafen inconcentrations $1 \cdot 10^{-6}$and $1 \cdot 10^{-7}$%.

The properties of this compound have not been described in literature. The compounds which are close to melafen in structure and possess the same kind of activity are unknown. Salts of ortho-phosphoric acid and dialkyl-phosphoric acid with melamine were studied as flame retardants or their useful properties were not studied at all.
It is known that bis (oxymethyl)–phosphinic acidis a multifunctional compound having in its structure acidic, phosphoryl and oxymethyl groupscapable of interacting with various biotargets. The preparation is completely

dissoluble in waterand its water solutions are stable; melafen is less toxic for warm blooded, itsLD_{50}=2000 mg/kgfor mice.

As an outcome of the studies conducted at the laboratory of genotoxicity of Kazan State University named after O.N. Ilyinskayawas found:
-The preparation does not show toxic effects on the strain Salmonella typhimurium TA 100 in studied concentrations from 0,4 mM to 0,46 mM;

- The DNA-damagedactivity was not detected in any of the investigated melafen concentrations;

- In Ames test it did not show mutagenic properties in experiment variants with metabolic activation and without it (did not induce point mutations in cells of Salmonella typhimurium, microcosmic fraction of the liver in rats practically did not modifythe mutagenic potential of melafen).

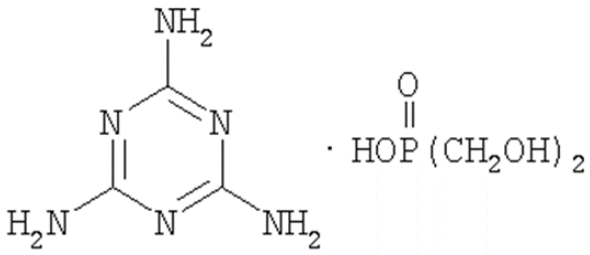

Figure 1: Melafen formula

In conformity with the Federal Law of July 19 1997 №109-FL «Ofsafe pecticide and agrochemicals handling» melafen – is melamine salt bis (oxymethyl)phosphinic acidwas officially registered with authorization number №2222-11-11-167-0-0-3-0 for a term up to 15.11.2021 and was permitted to be used in the territory of the Russian Federation.

The determination of the activity of catalase, peroxidase and polyfenoloxidasewas carried out – by Mikhlin and Bronovitskaya's method, the content of ascorbic acid, glutathione, reducing activity – by Pett's method modified by Prokoshev as expounded by TretyakovN.N., KarnaukhovaT.V., PanichkinaL.A. and others. [16], respiration – by Varburg in thermostatic conditions.

RESEARCH RESULTS

Knowing the mechanisms of enzyme activity will help to reveal deeper biochemical and physiological transformations that take place in crops including also in sugar beet. We studied the dynamics of the activity change of catalase, peroxidaseand polyfenoloxidase, the ascorbic acid content, glutathione and reducing activity of sugar beet tissues. Similar researches on melafen and other growth regulators in literature are not present.

Catalase : H_2O_2 - oxidoreductase (Enzyme classifier 1.11.1.6). This enzyme in a cell carries out in general the function of protecting from hydrogen peroxide which is formed in the respiration process. It is known that hydrogen peroxide is a strong poison for a cell decomposes under the action of the enzyme of catalase:

$$2H_2O_2 \xrightarrow{catalase} 2H_2O + O_2$$

Catalase is referred to oxidoreductase because it performs the transfer of two hydrogen atoms from one molecule to another molecule:

$$H_2O_2 + H_2O_2 \rightarrow O_2 + 2H_2O$$

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Considering the dynamic pattern of the catalase enzyme activity it should be pointed out that it plays a leading role in redox processes regulating the oxidative regime in a plant body. It also can be considered as a measure of intensity and productivity of total metabolism that ultimately can have a great influence on the course of the production process not only of sugar beet but also other crops.

The analysis of the catalase activity makes possible to trace physiologic and biochemical processes of sprouting seeds which treated with melafen in concentrations$10^{-6}$and 10^{-7}%; first of all – the state of redox reactions intrinsic to respiration. In table 1 and in figure 2the catalase activity is presented.

Table 1: The catalase activity change in sugar beet sprouts (mg H_2O_2, decomposed during the incubation time for 1 g of the plant material)

Experiment variant	Germination term, hour/day							
	12 hours	24 hours	2	3	6	9	12	15
Control	8,7±0,2	13,1±0,4	13,8±0,2	14,8±0,5	16,6±0,3	16,9±0,5	12,1±0,7	8,2±0,3
Melafen $1\cdot10^{-6}$	9,4±0,4	16,9±0,3	17,8±0,2	18,8±0,4	18,1±0,5	15,6±0,3	11,0±0,3	8,1±0,4
Melafen $1\cdot10^{-7}$	9,4±0,3	16,2±0,4	17,8±0,3	19,7±0,5	19,7±0,4	15,7±0,3	12,0±0,3	8,1±0,1

The data analysis (table 1) shows that the catalase activity under the action of melafen increases throughout 6 days by 0,6-3,8 mg H_2O_2depending on time. It should be pointed that in the experimental variant the maximum value is reached on day 3 whereas in the control variant it is reached on day 9. Further, the activity decreases in the control variant on day 12 and in the experiment variant on day 9. On day 15 the catalase enzyme inactivation takes place in all the variants.

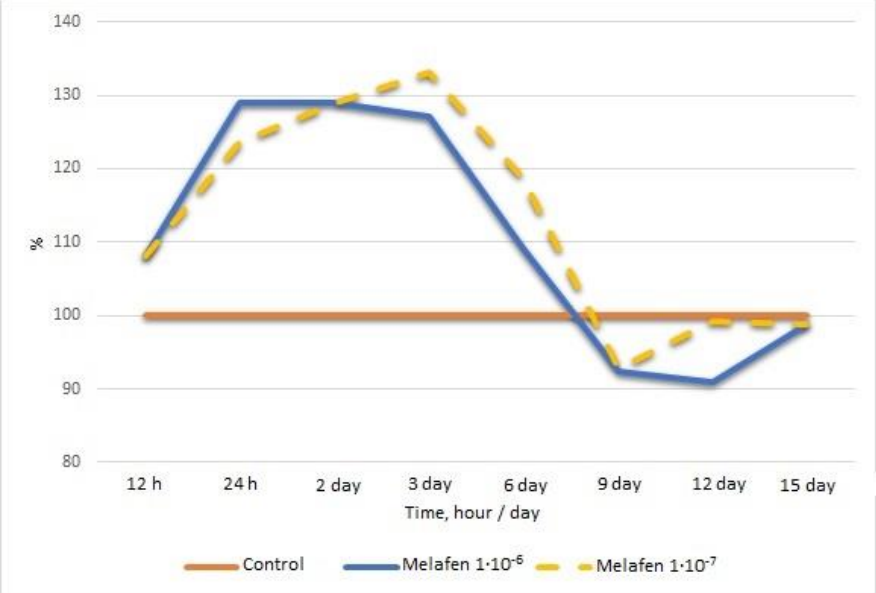

Figure 2: Catalase activity in % in relation to control

From fig.2we can see that on the first 6 days the catalase activity is more than 20%, in comparison with the control group, later the tendency to a fall is observed. The similar character of the catalase change we observe in other plants, in spring wheat in particular.

A slightly lowered level of catalase beginning from day 9 of the studies in experimental plants in comparison with the control group can be explained by the fact that the chain of biochemical reactions connected with respiration in seeds and sprouts is not completely formed, since its final formation takes place at complete sprouting and sprout development. In connection with the metabolic processes mobilization in

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experimental plants (seeds treated with melafen) it is quite clear from the data of figure 2 that the respiration system has been formed more intensively than in the control group.

Peroxidase (Enzyme classifier1.11.17, a donor: H_2O_2 - oxireductase) in the presence of hydrogen dioxidecatalizes oxidation of a wide range of fenols, whereas polyfenooxidase (Enzyme classifier1.10.3.1, o-diphenol: oxygen-oxireductase)oxidizes polyfenols. These two enzymes are referred to oxidoreductase. At present in literature there is no information about the activity change of peroxidase and polyfenoloxidase under the influence of any factors in plants with exception of soil studies. We think that melafen as a regulator of energy processes and having a high physiologic activity is likely to lead to the «enzyme release». They are transferred from a latent state to an active one as a result,oxidoreductases and hydrolases become active in the first place. The activity of peroxidase in sprouting sugar beet seeds increases reaching its maximum on day 3 in both concentrations, whereas in control plants it reaches on day 7-9 (fig. 3, 4). In experimental variants the peroxidase acitivity rise is observed throughout the 7th day.

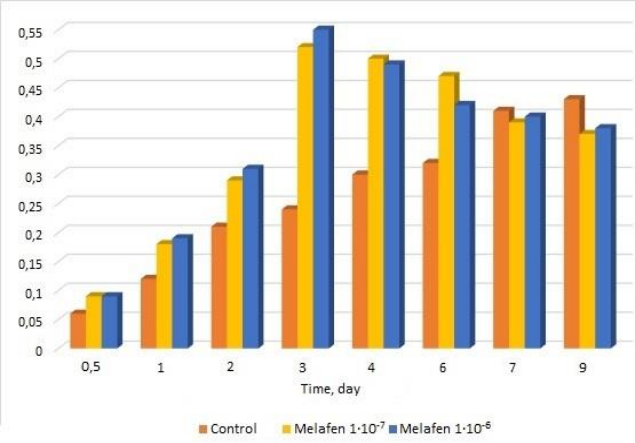

Figure 3: Bar chart of peroxidase activity in sprouting sugar beet seeds (ml $I_2$for 1 g of dry matter)

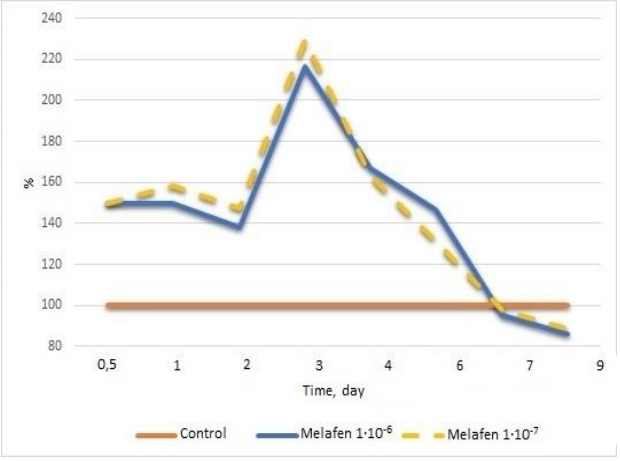

Figure 4: Activity of peroxidase in % in contrast to control

It follows thence that in experimental variants redox processes flow more intensively.
The same picture is observed in polyfenoloxidase but at a lower level. It is likely connected with the fact few polyfenol compounds are contained and synthesized in the seeds (fig. 5, 6).

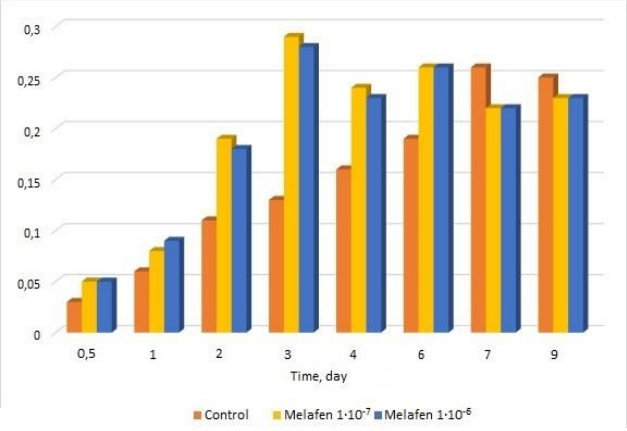

Figure 5: Bar chart of polyfenoloxidase activity in sprouting sugar beet seeds (ml $I_2$for 1 g of dry matter)

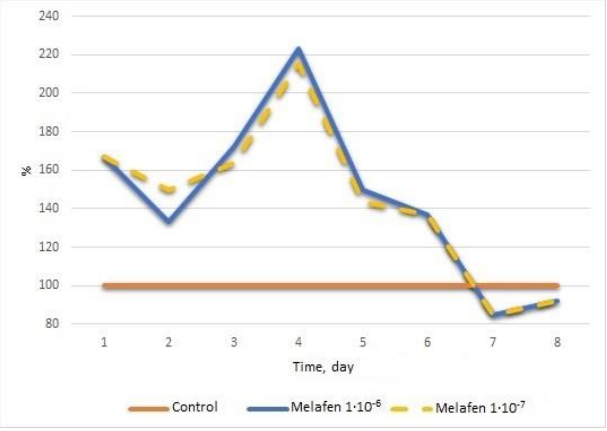

Figure 6: Activity of polyfenoloxidase in % in contrast to control

Under the action of melafen up to six days the activity remained steady approximately at the same level and then was decreasing. The observed fall of activity we explain by the increase of the ascorbic acid

content (fig. 7). At the expense of the ascorbic acid rise the inactivation of peroxidase and polyfenooxidase takes place. From the data we see that both concentrations of melafen has an effect in the same way on the character of the peroxidase and polyfenoloxidase activity change.

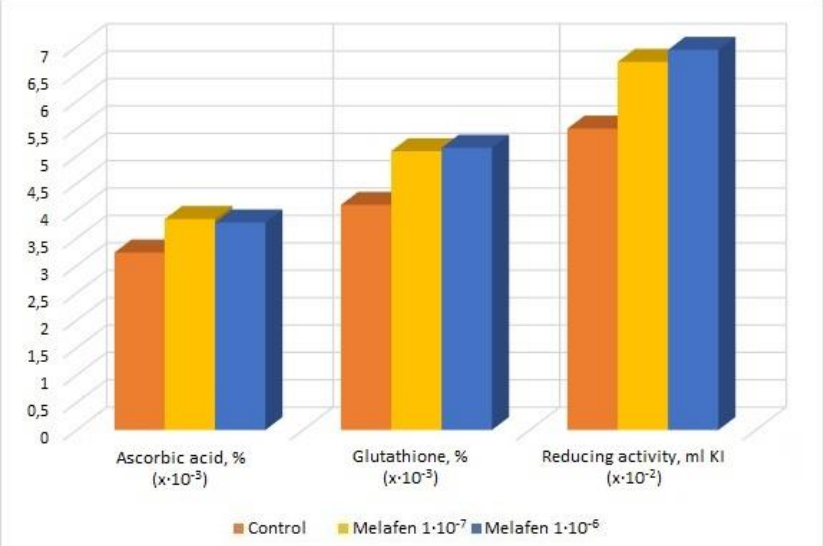

Figure 7: Influence of «Melafen» on the ascorbic acid content, glutathione content and reducing activity of sugar beet tissues

The results of the studies show that under the influence of melafenthe increase of the ascorbic acid content takes place by 16,9-18,8%, glutathione – 23,8-25,3%. Total reducing activity of tissues increases by 22,1-26,2% respectively. It should be noted that the increase of total reducing ability of sprout tissues demonstrates their high ecologic flexibility [15].

Glutathione is known to work for maintaining the redox potential in the cell.

A moderate rise of the oxidoreductases activity is an integral part of metabolism processes at the initial stages of ontogenesis and by the end of the vegetation periodwill have manifested itself as a delayed effect because the yielding capacity of crops is an integral factor of all physiologic and biochemical processes taking place in a plant during its individual development.

The results of the studies (fig. 8)point to positive shifts in metabolic processes at the initial stages of ontogenesis and stimulation.

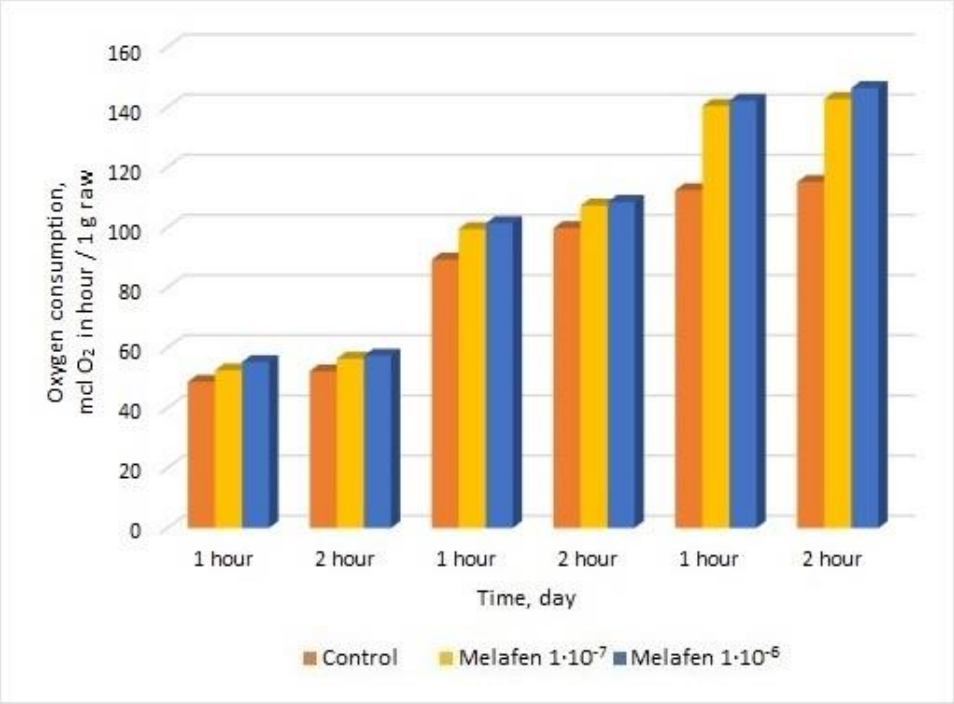

Figure 8: Oxygen consumption by sugar beet sprouts

The second and third days are characterized by an increase of water-bearing nature up to 37% and more that facilitates more active progress of metabolic processes. In this period respiration increases almost in 2 times.

The increase of the respiration rate even in cells of chlorella has been shown in other authors' studies [17]. Consequently, the obtained data on the respiration intensity demonstrate that melafen regulates the energy exchangein the process of sprouting sugar beet seeds. Thus a favorable energy balance is created which gives a possibility to raise biologic full-value of seeds.

CONCLUSION

Thus, the results of the presented data show thatmelafen demonstrates a high efficiency as a primary metabolism regulator because it takes a wide effect on redox cell processes resembling various manifestations of the phytohormones effect.

It was found in sugar beet sprouts that under the influence of melafen in a concentration of $1 \cdot 10^{-6}$-$1 \cdot 10^{-7}$% the catalase activity increased reaching its maximum on days 2-3, in control on day 9. The activity of peroxidase and polyfenoloxidase reached its maximum on days 3-4. Then a fall is observed associated with the content of ascorbic acid, glutathione and reducing activity of tissues.

Due to these changes we should forecast the intensification of metabolic and growth processes of sugar beet plants.

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