Influence of electromagnetic fields and microbial pesticide Vitaplan on stability of apples during storage

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Abstract. The article presents the results of studying the influence of treatments with electromagnetic fields of extremely low and super low frequency and the microbial pesticide Vitaplan containing bacteria *Bacillus subtilis* on the resistance of apples cv. Idared to microbial decay during storage, on the peroxidase activity change and the total polyphenolic content. It was found that the degree of damage to apples artificially infected with *Aspergillus niger* depends on the treatment parameters before storage. The highest degree of inhibition of the development of lesions caused by the phytopathogen was revealed in samples treated with SLF EMF with parameters 35 Hz, 12 mT and 30 min. It was also found that all the considered types of treatments increase the activity of peroxidase during storage during the first two weeks, as well as the content of polyphenolic substances. The data obtained suggest that the recorded increase in the resistance of apples to microbial decay may be the result of the induction of resistance under the influence of ELF/SLF electromagnetic fields and the microbial pesticide Vitaplan. The results of the study can be used to develop new methods for storing apples.

1. Introduction

The control and prevention of plant diseases are of the main tasks in agriculture. This is due to the fact that diseases can lead to significant economic losses. For example, according to the UN Food and Agriculture Organization (FAO), about a third of the fruit and vegetable harvest is lost in the field and in the post-harvest period due to pathogenic infections, primarily of fungal nature [1].

One of the most promising strategies to combat plant diseases at present is the induction of natural resistance to phytopathogens in plants (including fruits and vegetables in the post-harvest period) by means of biological, chemical or physical treatments.

This strategy is based on the ability of certain biological agents (such as fungi or bacteria), chemical substances and types of physical treatment (e.g., heat treatment, hypobaric treatment, ultraviolet irradiation) to activate defensive mechanisms in plants. That is, the induced resistance is a defensive response of a plant to external influences of one kind or another.

The defensive responses that occur in plants include various elements, such as the accumulation of antimicrobial polyphenolic substances and pathogenesis-related proteins, which include a variety of enzymes involved in plant control against phytopathogens. From research literature it is known that
the induction of resistance may be accompanied by an increase in the activity of enzymes such as peroxidase, chitinase, β-1,3-glucanase, phenylalanine ammonia lyase and others [2, 3].

Thus, when studying various methods of plant materials treatment, designed to increase its stability during storage, it is also of interest to study the change in the activity of defensive enzymes and the content of polyphenolic substances.

The aim of this work was to identify patterns of influence of pretreatment of apples with electromagnetic fields of extremely low and super low frequency (ELF/SLF EMF) and the microbial pesticide Vitaplan on the resistance of apples to microbial decay, on the peroxidase activity and the total polyphenolic content during storage.

The choosing of ELF and SLF EMF as the type of treatment for the induction of resistance is due to a number of factors: firstly, modern science considers the effect of EMF (including ELF and SLF) on biological objects to be proven [4]; secondly, EMF of low frequencies do not cause heating of the tissues of the treated material (unlike high-frequency EMF); thirdly, ELF and SLF EMF, like other types of physical treatment, do not pollute the surface of the objects of treatment.

An analysis of the research literature shows that there are a significant number of works devoted to studying the influence of various types of physical treatment on the stability of fruits and vegetables during storage. The most commonly studied types of treatment are ultraviolet irradiation [5], hypobaric treatment [6], heat treatment [7], high-frequency electromagnetic irradiation: radio frequencies (30 kHz – 300 MHz) and microwaves (300 MHz – 300 GHz) [8 – 11]. But we did not find any work on the influence of ELF/SLF EMF on the resistance to microbial decay and on the activity of defensive enzymes in fruits and vegetables during storage.

As for the choosing of a microbial pesticide, it should be noted that a large number of such products are present on the market today, and the development of new ones is actively continuing, since the effectiveness of their use has been proven in practice. However, manufacturers do not always indicate in detail the mechanism of action of manufactured products: it is often said that various options or a combination of them are possible. But research on this topic is ongoing, and more and more scientific work is appearing that evidence that the induction of resistance in plants is one such option, and, at least in some cases, this is the main mechanism of action of microbial pesticides.

One of the actively studied biological agents is the bacterium *Bacillus subtilis* [12, 13]. The commercially available microbial pesticide Vitaplan (manufactured by “AgroBioTehnologiya” group of companies, http://bioprotection.ru) contains strains of *Bacillus subtilis* VKM В-2604D and VKM В-2605D. The manufacturer claims that Vitaplan effectively inhibits the development of pathogens of many fungal and bacterial diseases of various crops. It was of interest to study the ability of these strains to influence on the resistance of apples to microbial decay, on the peroxidase activity and the total polyphenolic content during storage.

2. Materials and methods

2.1 Objects of research

Apples of the cultivar “Idared” removed from long-term storage (+2…+3 °C, relative humidity of 90±3%, 5 months) were used as objects of research. Before treatment, apples were stored for 5 days at a temperature of 16±1 °C.

Treatment with ELF/SLF EMF was performed in a laboratory experimental setup.

2.2 Microbiological research

A study of the influence of apple treatment modes on the development of diseases caused by the *Aspergillus niger* phytopathogen during storage was carried out with a suspension of a prepared test culture of a phytopathogenic microorganism. The suspension of a test culture was isolated from infected apples and cultured at +27±1 °C in Saburo medium for 7 days.

For the study, apples were selected in an amount of 15 pieces for each type of treatment, washed with water, dried and treated with 70% ethyl alcohol. On apples, two cuts were made with a sterile
scalpel, 3 by 3 mm in size, and a suspension of the phytopathogen was inoculated into each of the cuts in an amount of 10 μL. Then the apples with the suspension were treated according to the selected modes in the experimental setup.

Control samples were not subjected to treatment.

The degree of apple damage by the phytopathogen *Aspergillus niger* was determined by the diameter of the affected tissue.

### 2.3 Biochemical research

To determine the activity of the enzyme peroxidase, apples were selected without physical injuries and signs of microbiological decay. Each sample consisted of 3 apples, which were used to obtain a sample weighing 3 g. The obtained sample was homogenized with 5 mL of sodium phosphate buffer (100 mM, pH 6.0) with addition of 0.1 g of polyvinylpyrrolidone PVP K-90. After centrifugation for 20 min at 12000 rpm, the supernatant was used to determine the activity of the enzyme.

The determination of peroxidase activity was carried out by the photometric method using guaiacol as a substrate [14]. The increase in absorbance was determined at 460 nm for 120 s after adding hydrogen peroxide to the reaction mixture. Enzyme activity was expressed in relative units per gram of fresh weight per minute.

The total polyphenolic content was determined by the colorimetric method using the Folin–Denis reagent and was expressed as mg of gallic acid equivalent per 100 g of the fresh sample [15].

### 2.4 Statistical analysis

Experimental studies were carried out in triplicate. Mathematical processing of experimental data was performed by the method of descriptive statistics and analysis of variance using Statistica (StatSoft Inc.) and Office Excel (Microsoft) for Windows.

### 3. Results and discussion

#### 3.1 Influence of treatment with ELF/SLF electromagnetic fields and the microbial pesticide Vitaplan on the resistance of apples to microbial decay during storage

In order to establish the patterns of influence of treatment with ELF/SLF EMF and the microbial pesticide Vitaplan on the resistance of apples cv. Idared to microbial decay caused by *Aspergillus niger*, the treatment of objects of research was performed in three modes:

- mode 1 (E1) – treatment with ELF EMF: frequency – 20 Hz, magnetic induction – 8 mT, time of exposure – 40 min;
- mode 2 (E2) – treatment with SLF EMF: frequency – 35 Hz, magnetic induction – 12 mT, time of exposure – 30 min;
- mode 3 (V3) – treatment with Vitaplan, aqueous solution 10⁶ CFU/mL, 10 μL were inoculated into the same cuts as the phytopathogen.

In addition, two control samples were laid for storage:
- control 1 (C1) – without phytopathogen inoculation and without treatment;
- control 2 (C2) – phytopathogen inoculation, without treatment.

After the treatment, the samples were stored at temperatures of +25±1 °C for 48 hours and +2±1 °C for 28 days.

The results of the research are presented in table 1.

In samples not infected with the phytopathogen (C1), after 48 hours of storage at a temperature of +25±1 °C, no signs of damage were observed.

In samples of apples inoculated with the phytopathogen and stored at a temperature of +25±1 °C signs of tissue damage were observed already after 24 hours of storage. After 48 hours of storage, the diameter of the lesion was: C2 – 4.6±0.4 cm, E1 – 4±0.3 cm (13% lower than C2), E2 – 2.7±0.2 cm (41.3% lower than C2), V3 – 3.6±0.3 cm (21.7% lower than C2).
In all samples stored at a temperature of +2±1 °C, no visible tissue damage was observed after 48 hours.

In samples not infected with the phytopathogen (C1), after 28 days of storage at a temperature of +2±1 °C, no signs of damage were observed.

In samples of apples inoculated with the phytopathogen and stored at a temperature of +2±1 °C, after 28 days of storage, the diameter of the lesion was: C2 – 2.2±0.2 cm, E1 – 1.1±0.1 cm (50% lower than C2), E2 – 0.4±0.1 cm (81.8% lower than C2), V3 – 0.8±0.1 cm (63.6% lower than C2).

Table 1. Influence of the mode of treatment on the degree of apple damage by the phytopathogen Aspergillus niger during storage at +25±1 °C and +2±1 °C.

| Storage time | Diameter of the lesion, cm |
|--------------|----------------------------|
|              | Storage temperature +25±1 °C | Storage temperature +2±1 °C |
|              | C1  | C2  | E1  | E2  | V3  | C1  | C2  | E1  | E2  | V3  |
| 48 hrs       | -   | 4.6±0.4 | 4±0.3 | 2.7±0.2 | 3.6±0.3 | -   | -   | -   | -   | -   |
| 28 days      | -   | 2.2±0.2 | 1.1±0.1 | 0.4±0.1 | 0.8±0.1 |

The data obtained suggest that the degree of damage to apples artificially infected with Aspergillus niger depends on the treatment parameters before storage. The highest degree of inhibition of the development of lesions caused by the phytopathogen was revealed in samples treated according to mode 2 (E2): SLF EMF (35 Hz, 12 mT and 30 min).

3.2 Influence of treatment with ELF/SLF electromagnetic fields and the microbial pesticide Vitaplan on the peroxidase activity of apples during storage

We studied the activity of peroxidase, an enzyme that catalyzes H$_2$O$_2$-dependent oxidation and, thus, provides the occurrence of redox reactions, such as the oxidation of phenols to quinones. A number of studies are known that suggest a connection between an increase in peroxidase activity and plant resistance to diseases [2].

The treatment of objects of research was performed in 5 modes:
- mode 1 (E1) – ELF EMF: frequency – 20 Hz, magnetic induction – 8 mT, time of exposure – 40 min;
- mode 2 (E2) – SLF EMF: frequency – 35 Hz, magnetic induction – 12 mT, time of exposure – 30 min;
- mode 3 (V1) –Vitaplan, aqueous solution 10$^{10}$ CFU/mL, complete immersion followed by air drying;
- mode 4 (V2) –Vitaplan, aqueous solution 10$^{10}$ CFU/mL, partial immersion (1/2) followed by air drying;
- mode 5 (V3) –Vitaplan, aqueous solution 10$^6$ CFU/mL, complete immersion followed by air drying.

Control samples were not subjected to treatment.

After the treatment, the apples were stored for 5 weeks in one refrigerator at a temperature of +2±1 °C and a relative humidity of 75±3%. Sampling was carried out once a week.

Influence of different modes of treatment of apples cv. Idared on the peroxidase activity during storage is shown in Figure 1.
Figure 1. Change in the activity of peroxidase of Idared apples during storage, depending on the mode of treatment.

As follows from the data presented in Figure 1, during storage of untreated apples (control), the activity of peroxidase at the beginning, in the 1st week, decreases by 27.7%. After that, peroxidase activity gradually increases, appearing at the 4th week 124.1% higher than in apples before treatment and putting into storage. Further, peroxidase activity begins to decrease again: at the 5th week it is 56.7% higher than the initial value.

When storing apples treated according to modes E2 and V1, the activity of peroxidase at the beginning, in the 1st week, increases sharply: 126.4% and 121.5% higher than in apples before treatment and putting into storage, respectively. But already in the 2nd week, peroxidase activity is significantly reduced. Further, in the case of apples treated according to the mode E2, the decrease gradually continues, and at the 5th week the activity is 14.8% higher than the initial value. In the case of apples treated according to the mode V1, peroxidase activity in the period from the 2nd to the 5th week remains in a relatively narrow range, and at the 5th week it is 47.4% higher than in apples before treatment and putting into storage.

When storing apples treated according to modes V2 and V3, peroxidase activity initially increases, reaching a maximum in the 2nd week: 122.5% and 116.1% higher than in apples before treatment and putting into storage, respectively. Further, activity gradually decreases, exceeding the initial value by 38.6% and 3.1% at the 5th week, respectively.

When storing apples treated according to the mode E1, peroxidase activity gradually increases, reaching a maximum in the 3rd week: 67.6% higher than in apples before treatment and putting into storage. After that, peroxidase activity decreases, being at the 5th week 14.5% higher than the initial value.

Analyzing the data presented in Figure 1, we can conclude that the change in peroxidase activity in objects of research subjected to different modes of treatment has both similarities and differences.

The similarity lies in the nature of the change in the studied indicator: at the first stage - an increase with the achievement of a local maximum, at the second stage - a decrease. The only exception is the control (apples without treatment), where at the beginning of storage a decrease in peroxidase activity is observed, which is quite quickly replaced by growth.

The difference is that the achievement of a local maximum occurs at different times. So, when storing apples subjected to the most stringent treatment (E2, V1), the maximum of peroxidase activity is observed already in the 1st week. When storing apples treated according to modes V2 and V3, the
maximum is observed in the 2nd week. When storing apples treated according to the mode E1, the maximum is observed in the 3rd week. When storing apples without treatment (control), the maximum is observed in the 4th week.

3.3 Influence of treatment with ELF/SLF electromagnetic fields and the microbial pesticide Vitaplan on the total polyphenolic content of apples during storage

While studying the ability of ELF/SLF EMF and the microbial pesticide Vitaplan to increase the stability of apples during storage, it was also of interest to study the change in the content of polyphenolic substances (total polyphenolic content).

Polyphenolic compounds are widely present in plants, including apples. These compounds include a large group of substances: catechins, anthocyanins, flavones, flavonols, flavonones, chalcones, aurons and others. Polyphenolic substances fulfill many functions in plants, including defensive (have a toxic effect on pathogenic microflora, and also have antioxidant properties).

The objects of research were treated and stored as described above. Sampling was carried out at the 1st, 3rd and 5th weeks.

Influence of different modes of treatment of apples cv. Idared on the total polyphenolic content during storage is shown in Figure 2.

![Graph showing the total polyphenolic content of Idared apples during storage, depending on the mode of treatment.](image)

**Figure 2.** Change in the total polyphenolic content of Idared apples during storage, depending on the mode of treatment.

From the data presented in Figure 2, it follows that all the considered modes of treatment led to an increase in the content of total polyphenols compared to the initial content. However, the content of total polyphenols also increased in apples without treatment (control).

It should be noted that modes of treatment E1, E2, and V1 provided a higher content of total polyphenols compared to the control throughout the experiment. While modes of treatment V2 and V3 provided a higher content of total polyphenols compared to the control only at the 1st week.

4. Conclusion

Influence of pretreatment of apples cv. Idared with electromagnetic fields of extremely low and super low frequency (ELF/SLF EMF) and the microbial pesticide Vitaplan (contains strains of *Bacillus subtilis* VKM B-2604D and VKM B-2605D) on the resistance to microbial decay, the peroxidase activity and the total polyphenolic content during storage was studied.

It was found that the degree of damage to apples artificially infected with *Aspergillus niger* depends on the treatment parameters before storage. The highest degree of inhibition of the
development of lesions caused by the phytopathogen was revealed in samples treated with SLF EMF with parameters 35 Hz, 12 mT and 30 min.

It was also found that all the considered modes of treatment increase the activity of peroxidase during storage during the first two weeks, as well as the content of polyphenolic substances. The modes of treatment that led to the greatest changes are SLF EMF (35 Hz, 12 mT and 30 min) and Vitaplan, aqueous solution of 10^{10} CFU/mL (complete immersion).

The data obtained during the research suggest that the recorded increase in the resistance of apples to microbial decay may be the result of the induction of resistance under the influence of ELF/SLF electromagnetic fields and the microbial pesticide Vitaplan.

Further studies in this area are of interest for applied and fundamental research on the mechanisms of resistance induction in fruits and vegetables.

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