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Investigation of the Influence of an Extremely Low-Frequency Electromagnetic Field on Carrot Phytopathogens *In- vivo* and *in-vitro*

T. V. Pershakova[1], G. A. Kupin[1], L. V. Mihaylyuta[1], M. V. Babakina[1], S. M. Gorlov[1], V. V. Lisovoy[1]
[1]*Krasnodar Research Institute of Agricultural Products Storage and Processing (branch of FSBSO "North-Caucasian Federal Scientific Center of Horticulture & Viniculture)*
Topolinaya alleya, 2, Krasnodar, 350072, Russia

Abstract

Pathogenic microorganisms that cause microbiological damage are a potential threat to the production and provision of post-harvest storage of vegetables. This problem is relevant for the cultivation and storage of carrots in the southern regions of Russia due to the special climatic conditions and physiological features of this root crop. Investigation of the influence of electromagnetic fields of extremely low frequencies on the viability of phytopathogens serves as a basis for developing methods for their control.

Keywords: carrots, electromagnetic field, inactivation of microorganisms, phytopathogens, storage.

INTRODUCTION

Ensuring the safety of plant raw materials is largely associated with the inhibition of the development of phytopathogens, causing diseases and, as a result, quality and quantity losses. Losses depend on the type of plant products, growing conditions, climatic and meteorological conditions, soil chemical composition, agrotechnical measures and the composition of pathogenic soil microorganisms [1].

Root vegetables are one of the types of plant products that are subject to long-term storage, but due to the low price of their sale, the use of methods that are acceptable for fruit - refrigerated storage, storage in regulated gas environments, becomes profitable only if there is a significant reduction in losses with maximum shelf life. In this regard, studies on the development of new technologies for reducing root vegetable losses during storage, in particular, due to the reduction of losses from microbial damage, are relevant.

The fact of the influence of the electromagnetic field on biological objects does not cause doubts in the scientific world [2].

Processing by electromagnetic fields has minimal effect on consumer properties of food products. In this case, the development or inactivation of microorganisms is inhibited [3, 4]. Electromagnetic fields of extremely low frequencies, unlike the high-frequency ones, do not cause heating of processed objects, their application is more environmentally friendly since the influence of chemical preparations is excluded, and more economically friendly since the expensive equipment is not required.

According to the International Commission on Protection from Non-Ionizing Radiation (ICZNI), the mechanisms of the action of alternating electromagnetic fields (EMF) on bio-objects are different depending on the frequency of the field. The ICZN distinguishes two frequency ranges of EMF variables: low-frequency fields (up to 100 kHz) and high-frequency fields (from 100 kHz to 300 GHz). In the low-frequency range, biological and medical effects are separately considered when exposed to alternating electric and magnetic fields in the frequency range below 300 Hz. According to the international classification, this range covers extremely low frequencies of ELF (3-30 Hz) and ultra-low frequencies of SNF (30-300 Hz).

The action of an external alternating electric field on bio-objects causes the transfer of electric charges (electric current), the polarization of the bound charge (the appearance of electric dipoles), and the reorientation of the dipoles present in the tissue. The action of an external variable magnetic field on bio-objects induces an electric field and a circulating electric current inside the body [2].

The impact of electric fields on biological objects is accompanied by various effects depending on its strength. Researchers studying the effect of low-frequency EMF on biological objects have established that induction electric current can directly drive the nervous and muscular systems at a current density exceeding a certain threshold value [5].

At current densities, at which stimulation of excitable tissues does not occur, neurons are also susceptible to excitability, as well as a direct effect on the electrical processes occurring in the body. As a result, the properties of cell membranes (ion transport, including the transport of Ca2 + ions through cell membranes and the intracellular concentration of this ion and the interaction of mitogens with receptors located on the surface of cells) and cellular functions (for example, increased proliferation and changes in metabolism, gene expression, protein biosynthesis and enzyme activity) [5-10].

The effectiveness of inactivation of microorganisms based on electromagnetic fields is high. Gayán et al. (2012) illustrate that the combination of UV light and soft magnetic field treatment can achieve 99.99% (4D) reduction in Salmonella enterica.

The mechanism of action is most often associated with changes in the morphology and structure of cells, such as endogenous key enzymatic inhibition of cellular metabolism, damage to cellular and genetic DNA, and changes in gene expression [11, 12].

Despite the fact that studies of the effect of low-frequency EMF on biological objects have been ongoing for many years, they are far from complete. Impact mechanisms are not fully understood, and the resulting effects are diverse and difficult to predict.

Scientists who studied changes in enzyme activity under the influence of low-frequency EMF showed that under the same conditions, the activity of certain enzymes can increase, while others may decrease or remain unchanged [13, 14].

In works devoted to the influence of low-frequency EMF on microorganisms, it is established that it depends on the type of microorganisms, the intensity of radiation and the processing time.

Strasak et al. (1998) [15] found that treatment of E. coli bacteria with EMF at a frequency of 50 Hz leads to a decrease in their viability and growth rate.

Fojt et al (2004) [16] also reported a decrease in the viability of E. coli, S. aureus and L. adecarboxylata bacteria when treated with a 50 Hz field and 10 mT induction. It was found that the effect manifests itself more in the case of E. coli and to a lesser extent in the case of S. aureus.

El-Sayed et al. (2006) [17] showed that the sensitivity of E. coli to EMF ELF varies with time: it is maximum in the first hours, but then decreases, which, apparently, is due to the adaptation of bacteria to stress.

However, not all researchers believe that the EMF variables always suppress the development of microorganisms. So, in the work of Tessaro et al. (2015) [18] showed the effect of static and variable magnetic fields of extremely low frequency on four types of bacteria. While static fields had no noticeable effect, the alternating field in one of the modes led to an increase in the growth rate of three species (Staphylococcus epidermidis, Staphylococcus aureus, and Escherichia coli) and a decrease in the growth rate of one species (Serratiama rcescens).

Walkling-Ribeiro et al. (2008) [19] found a decrease in the concentration of S. aureus in apple juice treated with an electromagnetic field (40 kV cm-1, 100 μs).

Altuntas et al. (2010) [20] showed the dependence of the degree of inactivation of Escherichia coli, Staphylococcus aureus and Listeria monocytogenes on the intensity of the electric field and the time of treatment.

The effectiveness of inactivation of microorganisms depends on many factors. Kuldiloke and Eshtiaghi (2008) [21] found that the degree of inactivation of pathogenic microorganisms under the influence of the electromagnetic field depends on the treatment time.

Amiali, Ngadi (2012) [22], Morris et al. (2007) [23] found the influence of such factors as electrical conductivity, pH; types, growth stage, concentration, size and shape of microorganisms.

After conducting experiments on the viability of microbial cells after treatment at a frequency of 18 GHz microwave and at a temperature below 40 °C, Shamis et al. (2011) [24] found morphological changes taking place in E. coli cells.

Due to the fact that the mechanisms of influence and possible effects of EMF treatment on biological objects, in particular on pathogenic microflora on the surface of root crops, have not been fully studied, studies of the effect of the electromagnetic field of extremely low frequencies on pathogenic microorganisms causing microbiological damage are relevant.

The magnitude of the magnetic induction is an important parameter of the EMF ELF. The values of the magnetic induction vary depending on the current strength, the length of the solenoid (source of irradiation) and the resistance of the solenoid (the number of turns of the winding on the solenoid) at the same frequency of the EMF ELF. This allows us to expand the range of EMF processing parameters. Under the influence of an electromagnetic field with different parameters of magnetic induction at the same frequency resonance absorption of the field, energy occurs by atoms of alkaline and alkaline-earth elements and a change in the spin orientation of the valence electrons of these atoms. As a result, there is a change in the rates of chemical reactions occurring on the membranes of microorganism cells.

In previous studies, EMF ELF parameters (frequency 16-32 Hz, current intensity 15 A, processing time 15 minutes) were established, which allow maximum inhibition of the development of pathogenic microorganisms [25].

Investigations on the effect of induction change on the development of microorganisms were of interest.

Microbiological damage during storage can be done by more than 20 types of microorganisms. The greatest damage is caused by Byssochlamys, Aspergillus, Penicillium, Rhizopus stolonifer, Sclerotinia sclerotiorum, Sclerotium rolfsii and Erwinia carotovora [26]. In this connection, the influence of electromagnetic fields of extremely low frequencies on such microorganisms as, Alternaria radicina and Erwinia carotovora, which are the most frequent causes of microbiological spoilage during storage in the Southern regions of Russia, were investigated.

The aim of the research was to study the influence of very low-frequency electromagnetic fields on the phytopathogens of carrot Sclerotinia sclerotiorum, Alternaria radicina and Erwinia carotovora in experiments in vivo and in vitro.

For this purpose, the degree of inactivation of the selected pathogens was studied as a function of the induction quantity of the EMF ELF. Based on the conducted studies, the optimal parameters for processing carrot roots were established, the effect of processing carrots with the EMF ELF with established parameters on the degree of development of diseases caused by the phytopathogens under study at different temperatures was studied.

MATERIALS AND METHODS

For research, root crops of carrots of Cordoba variety without mechanical damages and signs of infection with infectious and physiological diseases were selected.

Fungal pathogens Sclerotinia sclerotiorum and Alternaria radicina (causing white and black rot) were isolated from affected carrot roots and cultivated on Saburo medium at (+ 27 ± 1) °C for 14 days.

Spores of pathogenic fungi were obtained by washing cultivated mold cultures with sterile distilled water containing 0.05% Tween-80. The suspensions were filtered through three layers of sterilized gauze and adjusted to a concentration of 10^5 spores/ml. Accounting was performed with the use of the Garyaev chamber.

The causative agent of wet bacterial rot, Erwinia carotovora, was isolated from the affected carrot roots and cultivated on medium: DNA (dry nutrient agar) at a temperature of (+ 37 ± 1) °C for 48 hours. At the end of the thermostating, the population was flushed with a sterile saline solution, centrifuged and the concentration was adjusted to 10^5 cells/ml. The number of cells was counted by seeding the prepared dilutions with a deep inoculation into solid nutrient media.

To study the effect of electromagnetic fields of extremely low frequencies (EMF ELF) on microbial phytopathogens, we used an experimental laboratory for processing plant material. The processing was carried out by EMF ELF (frequency 16-32 Hz, current intensity 15 A, processing time 15 minutes), varying the magnitude of electromagnetic induction in the range from 3 to 15 mT.

In vitro studies were performed with suspensions of prepared test cultures of phytopathogenic microorganisms Sclerotinia sclerotiorum, Alternaria radicina and Erwinia carotovora. Suspensions of prepared test cultures were treated with EMF ELF and cultured at + (27 ± 1) °C on Saburo medium for 7 days – for fungi, at + (37 ± 1) °C on dry nutrient agar for 48 hours – for a bacterial pathogen. Control samples of suspensions were not subjected to treatment.

At the end of the cultivation period, the number of microorganisms was counted.

The dynamics of in vivo populations of Sclerotinia sclerotiorum, Alterna riaradicina and Erwinia carotovora were studied in sections of carrot roots and determined by the diameter of damaged root tissue when stored under different temperature conditions: +25 °C for 10 days and at +2 °C for 20 days.

For the study, 15 carrots were selected for each type of pathogenic microorganism, washed with water, dried and processed with 70% ethyl alcohol. Three root incisions were made with a sterile scalpel, 3 by 3 mm in size, and 10 μl of a suspension of one of the three microorganisms individually were added to each of the incisions in equal amounts.

Table 1 – Influence of the EMF ELF treatment regimes on the test cultures of microorganisms

Phytopathogen	**Processing Modes**					
	value of magnetic induction, mT					
	Initial concentration, CFU / g	**3**	**6**	**9**	**12**	**15**
		the number of microorganisms after treatment, CFU / cultivation conditions				
Saburo medium, 168 hours, temperature (27 ± 1) °C						
Sclerotinia sclerotiorum	(50±2.5) ×10^3	(50±2.5) ×10^3	(45±2.2) ×10^3	(40±2) ×10^3	**(30±1.5) ×10^3**	(40±2) ×10^3
Alternaria radicina	(40±2) ×10^3	(40±2) ×10^3	(38±1.9) ×10^3	(38±1.9) ×10^3	**(32±1.6) ×10^3**	(37±1.8) ×10^3
DNA medium, 48 hours, temperature + (37 ± 1) °C						
Erwinia carotovora	(45±2.2) ×10^3	(43±2.1) ×10^3	(35±1.7) ×10^3	**(27±1.3) ×10^3**	(28±1.4) ×10^3	(28±1.4) ×10^3

Then the root crops with the introduced suspensions were processed according to the selected regimes in the experimental setup. Control samples of carrot roots were not subjected to treatment. The experiment was carried out twice.

All root crops were placed in closed transparent plastic containers, a container with liquid was also placed there to provide high humidity. The containers were stored at temperatures of +2 °C and +25 °C.

Diagnosis of the incidence and size of characteristic lesions caused by Sclerotinia sclerotiorum, Alterna riaradicina and Erwinia carotovora was carried out every 48 hours for 10 days at +25 °C and every 4 days for 20 days at +2 °C.

RESULTS AND DISCUSSION

Table 1 presents data characterizing the effect of the EMF ELF treatment regimes on the test microorganism cultures.

From the data presented in Table 1, it can be concluded that the selected parameters for EMF ELF treatment have different effects on the development of the investigated phytopathogens. The highest growth inhibition efficiency against Sclerotinia sclerotiorum and Alternaria radicina was detected in *in vitro* experiments with a magnetic induction of 12 mT. For Erwinia carotovora, the most effective was the treatment option with a magnetic induction of 9 mT.

In connection with this, the following treatment regimens were used in further studies: for fungal pathogens, a frequency of 28 Hz, a current strength of 15 A, a treatment time of 15 minutes, a magnetic induction value of 12 mT; for pathogens of a bacterial nature, a frequency of 28 Hz, a current strength of 15 A, a treatment time of 15 minutes, a magnitude of magnetic induction of 9 mT.

At the next stage of the study, the hypothesis of inhibiting EMF ELF diseases caused by the phytopathogens Sclerotinia sclerotiorum, radicina and Erwinia carotovora, in the treatment of root crops of carrots by electromagnetic fields of extremely low frequencies, was tested.

The effect of EMF ELF treatment regimes on the diameter of the lesion caused by Sclerotinia sclerotiorum depending on the storage temperature is shown in Figure 1.

After storage at +25 °C for 10 days, the average diameter of the lesion in the reference samples (not treated with EMF ELF) infected with Sclerotinia sclerotiorum was 5 mm.

In samples treated with EMF ELF and infected with Sclerotinia sclerotiorum, the average diameter of the lesion was 3.2 mm.

After storage at +2 °C for 20 days, the median diameter of the lesion in reference samples infected with Sclerotinia sclerotiorum was 3 mm.

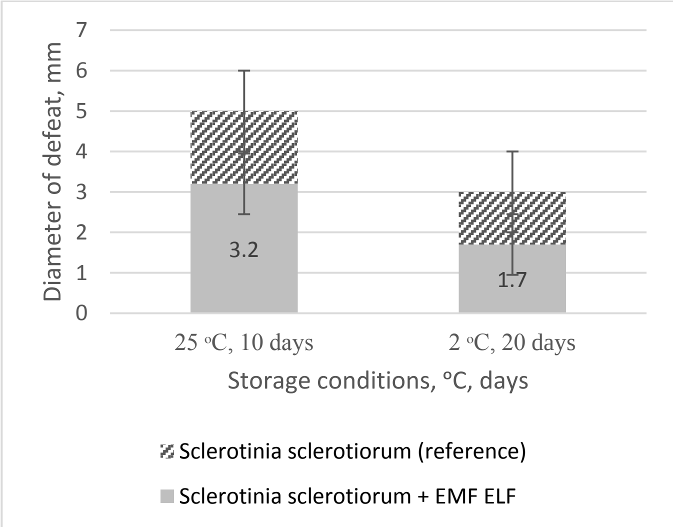

Figure 1 – Effect of EMF ELF treatment on the diameter of the lesion caused by Sclerotinia sclerotiorum, depending on the storage temperature. Error bars are the standard deviation of three repetitions

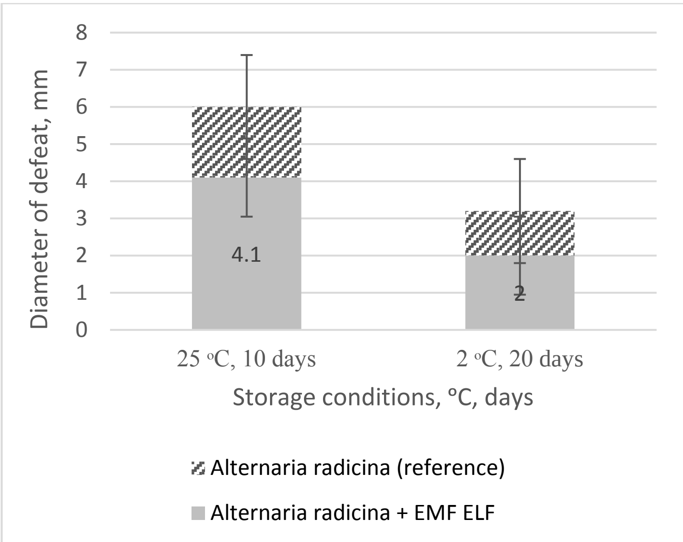

Figure 2 – The effect of EMF EMF treatment regimes on the diameter of the lesion caused by Alternaria radicina, depending on the storage temperature. Error bars are the standard deviation of three repetitions.

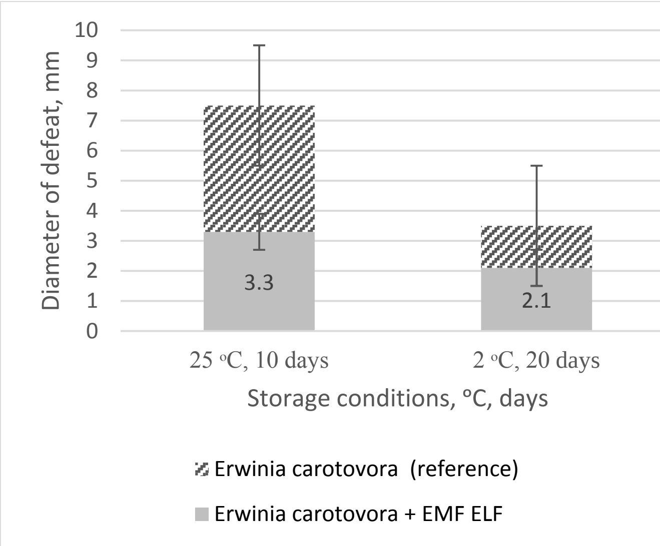

Figure 3 – Effect of EMF ELF treatment on the diameter of the lesion caused by Erwinia carotovora, depending on the storage temperature. Error bars are the standard deviation of three repetitions

In samples treated with EMF ELF with a magnitude of magnetic induction of 12 mT and infected with Sclerotinia sclerotiorum, the average diameter of the lesion was 1.7 mm.

The effect of EMF ELF treatment regimes on the diameter of the lesion caused by Alternaria radicina depending on the storage temperature is shown in Figure 2.

After storage at +25 °C for 10 days, the median diameter of the lesion in the reference samples (not treated with EMF ELF) infected with Alternaria radicina was 6 mm.

In samples treated with EMF ELF and infected with Alternaria radicina, the diameter of the lesion was 4.1 mm.

After storage at +2 °C for 20 days, the median diameter of the lesion in reference samples infected with Alternaria radicina was 3.2 mm.

In samples treated with EMF ELF and infected with Alternaria radicina, the average diameter of the lesion was 2 mm.

The effect of EMF ELF treatment regimes on the diameter of the damage caused by Erwinia carotovora depending on the storage temperature is shown in Figure 3.

Figure 3 – Effect of EMF ELF treatment on the diameter of the lesion caused by Erwinia carotovora, depending on the storage temperature. Error bars are the standard deviation of three repetitions.

After storage at +25 °C for 10 days, the average diameter of the lesion in the reference samples (not EMF-treated ELF) infected with Erwinia carotovora was 7.5 mm.

In the samples treated with EMF ELF with a magnitude of magnetic induction of 9 mT and infected with Erwinia carotovora, the average diameter of the lesion was 4.2 mm.

After storage at +2 °C for 20 days, the average diameter of the lesions in reference samples infected with Erwinia carotovora was 3.5 mm.

In samples treated with EMF ELF with a magnitude of magnetic induction of 9mT and infected with Erwinia carotovora, the average diameter of the lesion was 1.4 mm.

CONCLUSIONS

1. Based on the conducted studies, it has been established that the treatment of EMF ELF has an inhibitory effect on the development of the pathogens Sclerotinia sclerotiorum, Alternaria radicina and Erwinia carotovora.

2. The greatest efficiency of growth inhibition in *in vitro* experiments was established by EMF ELF treatment with the following parameters: for Sclerotinia sclerotiorum and Alternaria radicina, the frequency was 28 Hz, the current strength was 15 A, the processing time was 15 minutes, and the magnetic induction was 12 mT. For Erwinia carotovora: the frequency is 28 Hz, the current strength was 15 A, the processing time was 15 minutes, the magnetic induction was 9 mT.

3. It has been established that the treatment of the EMF ELF of carrots root crops, inoculated with the phytopathogens Sclerotinia sclerotiorum, Alternaria radicina and Erwinia carotovora, reduces the intensity of the development of the disease. The diameter of lesion caused by the investigated pathogens in samples treated with EMF ELF compared to untreated samples at storage at 25 °C after 10 days of storage is less by 36% for Sclerotinia sclerotiorum, 31.6% for Alternaria radicina and 44% for Erwinia carotovora. When stored at +2 °C, within 20 days the diameter of the lesion is 43.3% less for Sclerotinia sclerotiorum, 37.5% for Alternaria radicina and 60% for Erwinia carotovora.

4. The conducted studies justify the use of EMF ELF to reduce losses from diseases caused by phytopathogenic microorganisms during storage of carrot roots both during long-term storage at low temperatures and in short-term storage without artificial cooling.

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