https://doi.org/10.17221/60/2020-HORTSCI

The effect of a magnetic field on the phenolic composition and virus sanitation of raspberry plants

Mikhail Upadyshev, Svetlana Motyleva, Ivan Kulikov, Vladislav Donetskih, Maria Mertvischeva, Klavdia Metlitskaya, Anna Petrova*

Federal Horticultural Research Center for Breeding, Agrotechnology and Nursery, Moscow, Russia

**Corresponding author: motyleva_svetlana@mail.ru*

Citation: Upadyshev M., Motyleva S., Kulikov I., Donetskih V., Mertvischeva M., Metlitskaya K., Petrova A. (2020): The effect of magnetic field on phenolic composition and virus sanitation of raspberry plants. Hort. Sci. (Prague), 48: 166–173.

Abstract: A magnetic pulse treatment led to an increase in the Raspberry bushy dwarf Idaeovirus-free microplants' output and their phenolic composition change. The greatest output of the virus-free raspberries microplants (80–82%) was marked after complex treatment with pulsed and rotating magnetic fields with a time-changing frequency from 3.2 to 51 Hz, as well as with a pulsed magnetic field with a frequency from 1 to 10 Hz. The pulsed and rotating magnetic fields' complex effect resulted in the gallic and salicylic acid content increase by 14 % and 71%, respectively, compared to the untreated variant. The chlorogenic, salicylic and gallic acids' active synthesis was observed 72 hours after the magnetic treatment with a frequency from 3.2 to 51 Hz. There was a tendency for the amount of the phenolcarbonic acid to decrease 14 days after the magnetic treatment, except for the variant with the pulsed and rotating field treatment.

Keywords: magnetic pulse treatment; phenolcarbonic acids; Raspberry bushy dwarf Idaeovirus; virus-free raspberries microplants; *in vitro*

A magnetic field is able to affect the plants cells, tissues and organs in different ways: to increase the enzymes activity, to activate the molecules electronic complex, to change the cell membranes permeability, and to change the direction and speed of the biochemical reactions (Weaver, Chizmadzhev 1996; Stange et al. 2002; Cakmak et al. 2012).

The research of various magnetobiology scientific schools showed the prospects of using magnetic fields in technologies based on plant organism bioregulation (Bingy 2002).

Numerous experiments indicate the plants high sensitivity to external low-frequency pulsed magnetic field (PMF) effects, which has a stimulating or inhibitory influence on the plants growth and development processes depending on the field characteristics: direction, frequency of electromagnetic radiation oscillations, intensity and duration (Baryshev 2002; Bingy, Savin 2003; Maffei 2014).

The magnetic field's positive effect on the seed and planting material during the propagation of various crops, as well as the crop output yield formation, the increase in the immunity to phytopathogens, including plant viruses was marked (Esitken, Turan 2004; Upadyshev, Donetskih 2008; Nadirov et al. 2009; Donetskikh et al. 2017). For example, in the experiments of Italian researchers, tobacco plants infected with the *Tobacco mosaic virus*, treatment with a permanent magnetic field (17 or 13 mTl) and with a permanent and sinusoidal variable magnetic field (10 Hz, 25.6 or 28.9 mTl) combination led to the reduction in the necrosis area and an increase in the ornithine decarboxylase and phenylalanine ammonia-lyase enzyme activity (Trebbi et al. 2007).

https://doi.org/10.17221/60/2020-HORTSCI

One of the most harmful viruses on raspberry plants is the *Raspberry bushy dwarf virus* (RBDV), which leads to an output yield decrease and a reduction in its product's qualities. A release from this virus with thermotherapy and with a meristem culture is difficult due to a high thermotolerance which leads to the need to search for alternative sanitation methods (Wang et al. 2008).

The majority of magnetic field activity research has been conducted on seeds, herbaceous and model plants (Maffei 2014). A magnetic field stimulated the lipid synthesis in radishes by 2.5–4 times (Novitskii et al. 2014), and increased the chlorophyll content and stimulated the photosynthesis in maize plants (Anand et al. 2014).

However, experimental data about a magnetic field effect on horticultural crops' phenol metabolism are not enough, although this kind of research will clarify the biochemical mechanisms of the magnetic treatment. Up to the present moment, resonance effect mechanisms, PMF thresholds, and action targets depending on certain frequencies and amplitude remain largely unclear. Solving these issues is of great application importance in addition to the fundamental importance.

The magnetic pulse treatment led to changes in the raspberry microplants' phenolic composition, increasing the chlorogenic acid content in the tissues by 33% in comparison with the control (Upadyshev et al. 2017). In addition to the quantitative changes, there were qualitative ones in the raspberry microplants' biochemical composition, as the number of characteristic peaks on the chromatographic profiles increased from 8 to 13. In addition to the chlorogenic acid, the study of other phenolic compounds (e.g., gallic and salicylic acids) that play an important role in the plants' metabolism processes and influence the growth and development processes as well as the induced immunity are also of a certain interest.

The purpose of this work was to study the effect of the magnetic induction pulses' periodic sequence with varying frequency on the microplants phenol composition cultivated on artificial nutrient environments and on a virus-free raspberry microplant's output.

MATERIAL AND METHODS

The research was conducted in 2015–2018. The objects of the research were the microplants of raspberry Gerakl variety infected with Raspberry bushy dwarf Idaeovirus. Explants (5–10 mm) were planted on a modified Murashige and Skoog medium (Murashige, Skoog 1962), containing 1 mg/L 6-benzyaminopurine and were cultivated in light room conditions (temperature – 25 °C, photoperiod – 16 hours, illumination – 5 000 lk). Repeat 5-fold, 6 explants in each repetition.

The explants were processed using the a ABIMP-4 device (a traveling pulsed magnetic field apparatus) of the original design of the Federal Horticultural Research Center for Breeding, Agrotechnology and Nursery (patent of the Russian Federation No. 2652818)) (Donetskikh et al. 2017, 2018) for the magnetic pulse treatment (MPT) (Figure 1) using magnetic induction pulses with the parameters in accordance with Table 1 and Figures 2 and 3. The control plants are without any treatment (experiment variant 5).

Figure 1. Explants' magnetic pulse treatment

https://doi.org/10.17221/60/2020-HORTSCI

Table 1. The magnetic field parameters in the different variants of the experiment (experiment variant – magnetic pulse treatment

Item	Frequency range (Hz)	Frequency change speed (Hz/s)	Magnetic field type	Magnetic induction pulses height (mTl)	Magnetic induction vector direction	Pulses polarity
1	3.2–51.2–3.2	0.1	pulsed and rotating in a clockwise order pulse	3.5 ± 0.3 and (8.8–7.2) ± 0.3	along the growth of and perpendicular to a microplant	single-polar
2	3.2–51.2–3.2	0.1	pulsed	(17.5–15.1) ± 0.3	perpendicular to a microplant	heteropolar
3	50–100	0.05	pulsed	(15.1-12.3) ± 0.3	perpendicular to a microplant	heteropolar
4	0.7–10.0	0.013	pulsed	17.5 ± 0.3	perpendicular to a microplant	heteropolar

After the MPT, the explants continued to be cultivated on a culture medium and two months later they were tested by enzyme-linked immunosorbent assay (ELISA) to indicate the presence of viruses according to the method by (Clark, Adams 1977) using Neogen diagnostic kits, followed by analysis of the results' registration on a "Stat Fax 2100" microplate reader at the 405 and 630 nm wavelengths and with the infection rate index definition (the ratio of the tested sample's optical density to the seronegative control optical density) and the virus-free plants output (in %).

After performing the MPT for a certain amount of time (in 3, 72, 168 and 336 hours), twenty explants of each variant were extracted from the vessels, a general sample consisting of four explants extracted in 70% alcohol in the ratio of 100 mg of the plant tissues per 1 mL of alcohol was formed. The quantitative definition of the phenolic compounds (chlorogenic, gallic, salicylic acids) was carried out by the High-Performance Liquid Chromatography (HPLC) method on a Knauer GmbH (Germany) chromatograph with the Eurochrom HPLC Software (Version 3/05 P4). Acetonitrile composition eluent – 0.03% TFC (trifluorouxic acid) in the ratio of 30:70 was used. The compounds' detection in the samples was carried out at a 325 nm wavelength. The results were calculated based on five replicate tests per mg/100 g of the raw mass. The experimental data's statistical processing was carried out by the method of dispersion analysis with the least significant difference (LSD_{05}) definition in the LSD_{05} figures and the standard error (M ± SEM) in the tables.

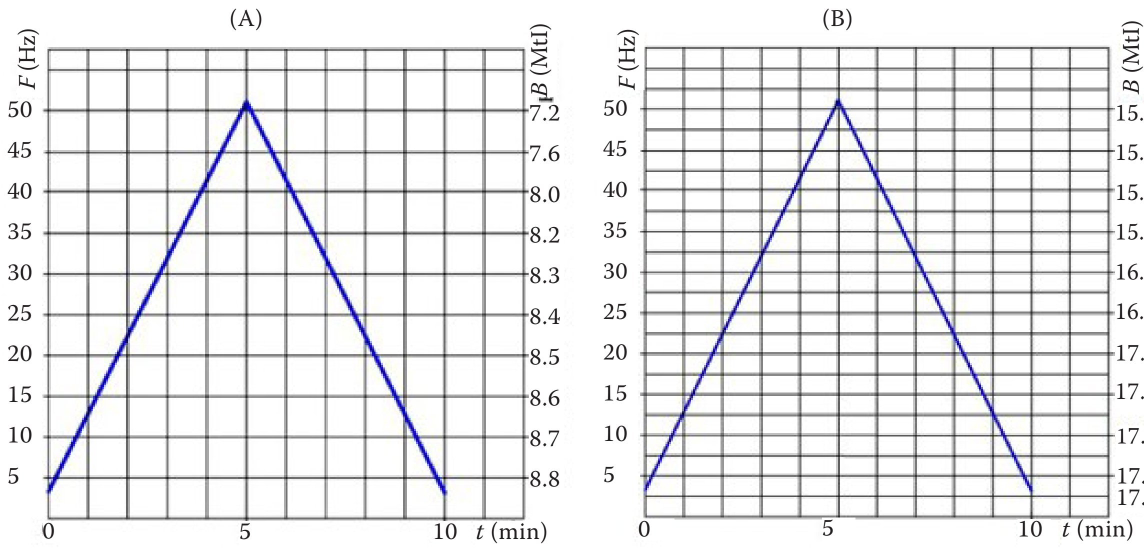

Figure 2. The magnetic pulse frequency and pulse height changes in the 1st variant (A) and in the 2nd variant (B) of the experiment

https://doi.org/10.17221/60/2020-HORTSCI

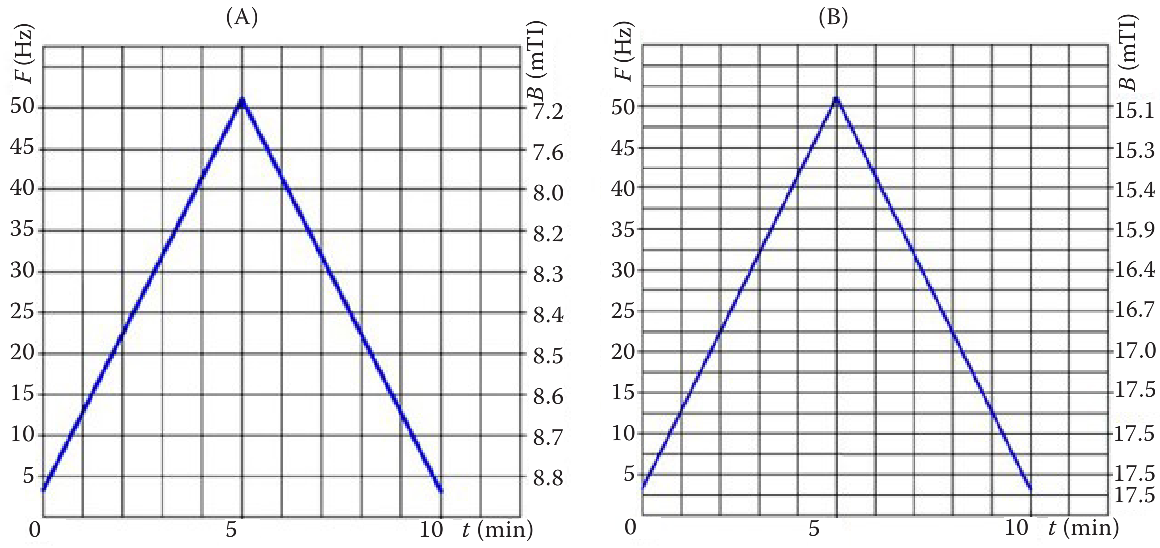

Figure 3. The magnetic pulse frequency and pulse height changes in the 3rd variant (A) and in the 4th variant (B) of the experiment

RESULTS AND DISCUSSION

The greatest output of the raspberry bushy dwarf virus-free plants was noted in variants 1 and 4 after the MPT (Table 2).

In addition to the RBDV-free plants' output increase in all the variants with the MPT, there was a significant (1.8–1.9 times) decrease in the infection rate index in comparison with the control.

The high elimination effect in the 1st variant is probably associated with the complex effect of two types of magnetic field (pulsed and rotating) that led to the gallic and salicylic acid content increasing by 14 and 71%, respectively, compared to the variant without the treatment. The amount of chlorogenic acid did not change. The use of the pulsed magnetic field only with the same frequency range (variant 2) had a smaller effect on the plants' sanitation from the virus in comparison with variant 1, although it increased the chlorogenic and salicylic acid content by 50 and 59%, respectively, compared to the control. The use of PMF in a low-frequency range (variant 4) provided the RBDV-free plants' high output with a simultaneous increase in the amount of chlorogenic acid by 43.8%.

The average positive correlation ($r = 0.46$) was established between the salicylic acid content in the plants' tissues and the RBDV-free raspberry explants' output. The correlation between the chlorogenic and gallic acid content and the virus-free

Table 2. The output of the RBDV-free plants and the content of the phenol compounds in Gerakl raspberry explant tissues ($M \pm$ SEM)

Variant No.	The output of RBDV free plants (%)	The content of phenol compounds** (mg/g of wet weight)		
		chlorogenic acid	salicylic acid	gallic acid
1	81.8^{c*}	0.16 ± 0.03	2.75 ± 0.15	0.70 ± 0.08
2	42.9^{b}	0.24 ± 0.05	1.35 ± 0.13	0.65 ± 0.06
3	53.8^{b}	0.13 ± 0.02	2.57 ± 0.20	0.59 ± 0.06
4	80.0^{c}	0.23 ± 0.04	2.29 ± 0.19	0.44 ± 0.05
5	0.0^{a}	0.16 ± 0.02	2.41 ± 0.18	0.41 ± 0.05

RBDV – *Raspberry bushy dwarf virus;* *different Latin letters indicate the statistically important various variants with a 5% significance level; **on average for 4 records

https://doi.org/10.17221/60/2020-HORTSCI

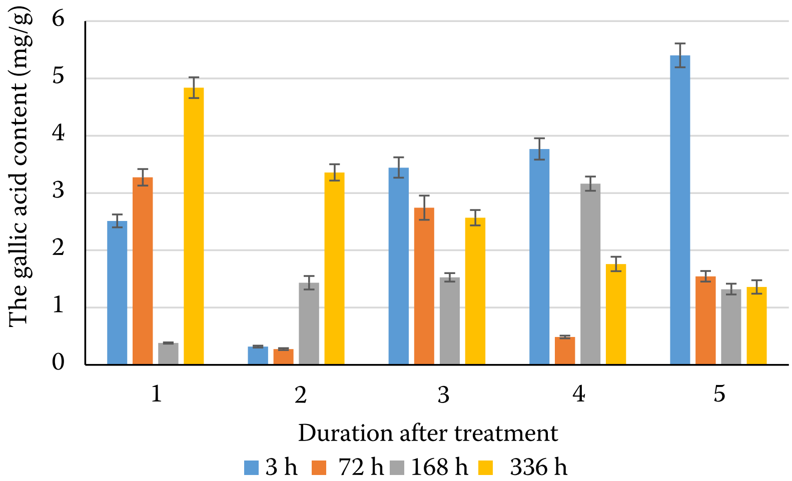

Figure 4. The chlorogenic acid content dynamics in the raspberry explants depending on the magnetic treatment regime and the exposition period (1, 2, 3, 4 – the experiment variant according to Table 1; 5 – without treatment)

explants' output was weak positive: the correlation ratio was 0.19 and 0.23, respectively. Consequently, it can be assumed that salicylic acid plays a more significant role in the raspberry plants' sanitation from the RBDV compared to chlorogenic and gallic acids.

The study of the phenolic compounds' content in the raspberry plants' tissues in the dynamics allowed one to identify certain dependences due to both the treatment regime and the period after the MPT.

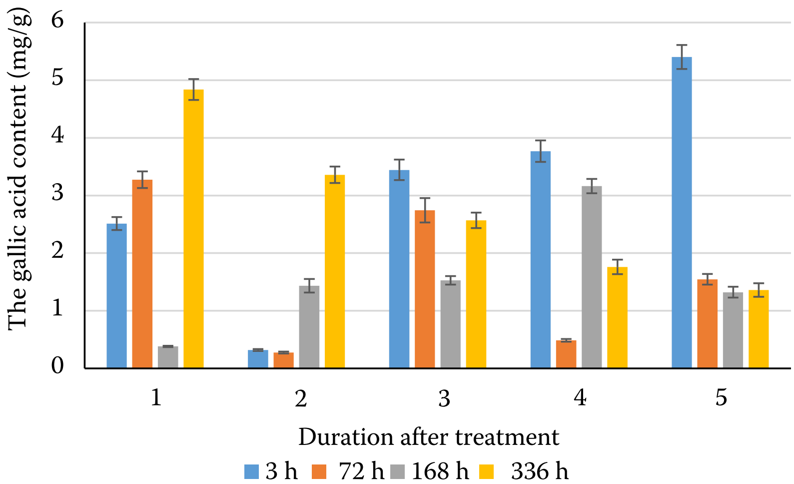

Figure 5. The gallic acid content dynamics in the raspberry explants depending on the magnetic treatment regime and the exposition period (1, 2, 3, 4 – the experiment variant according to Table 5 – without treatment)

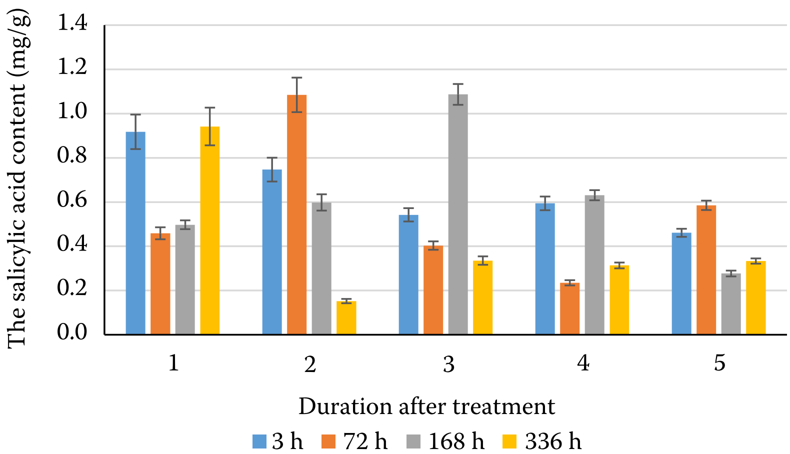

Figure 6. The salicylic acid content dynamics in the raspberry explants depending on the magnetic treatment regime and the exposition period (1, 2, 3, 4 – the experiment variant according to Table 1; 5 – without treatment)

https://doi.org/10.17221/60/2020-HORTSCI

In most cases, 3 hours after the MPT, there was a decrease in the phenolic compounds' content in the explants' tissues compared to the control, which is probably related to the plants' primary response to the magnetic treatment as a stress factor (Figures 4–6). The exception was the salicylic acid content, which increased by 18–99% in the raspberry explants depending on the treatment regime 3 hours after the MPT compared to the control. The most active salicylic acid synthesis is marked in variants 1 (0.92 mg/g) and 2 (0.75 mg/g).

In the raspberry explants' tissues, the chlorogenic acid content increased in the variant 2 (Figure 7) after 72 hours. After 72 hours, the salicylic acid content increased in the variant after a 3.2–51–3.2 Hz MPT (variant 2), the gallic acid content increased in the variant after a 3.2–51–3.2 Hz MPT (variant 2) and a 50-100 Hz (variant 3) MPT.

The phenolic compounds' active synthesis in the microplants' tissues is connected with non-specific resistance development, in our opinion, to a viral infection induced by the magnetic field.

At 168 hours, the amount of gallic and chlorogenic acids increased compared to the control after the MPT with a 1–10 Hz frequency, the amount of salicylic acid increased after a 50–100 Hz MPT.

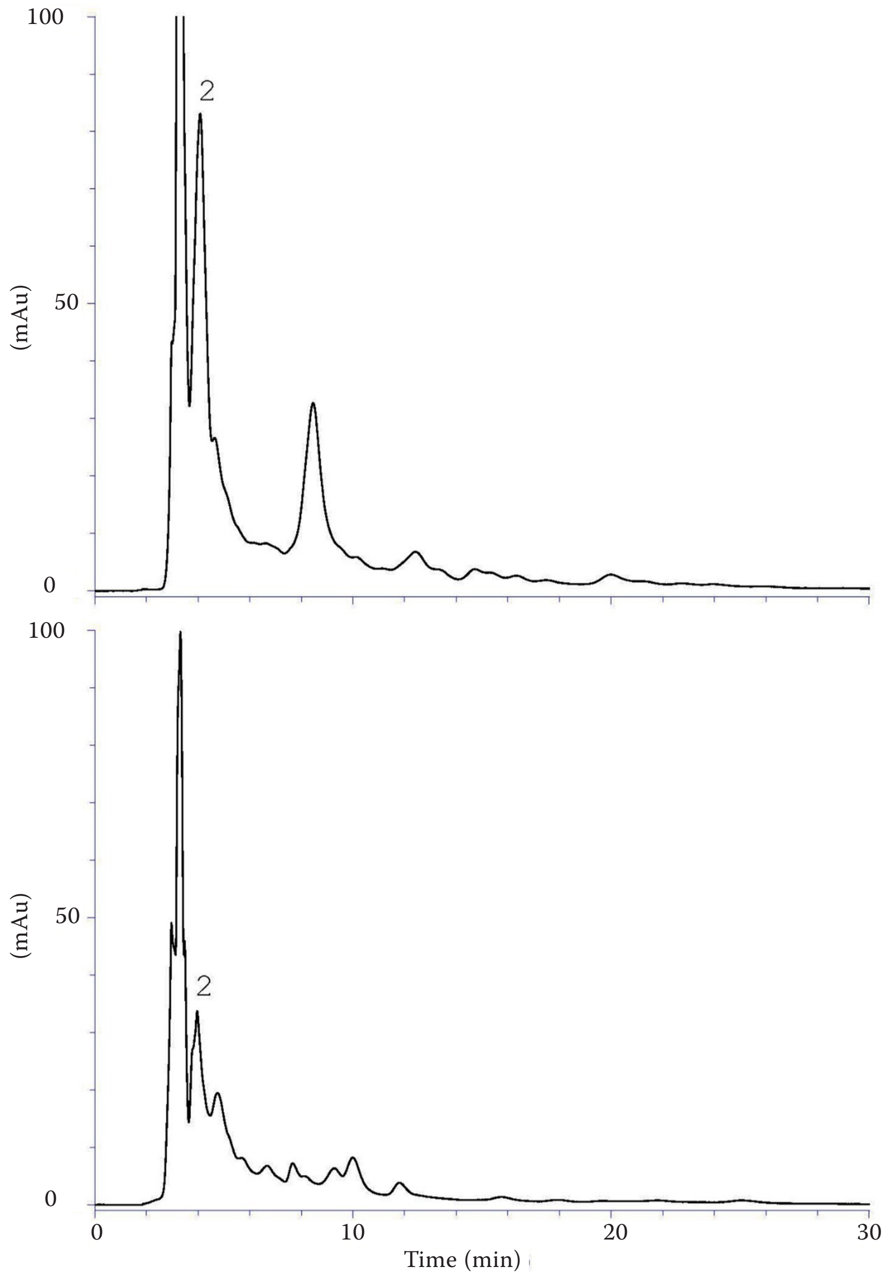

Figure 7. The chlorogenic acid content (2) chromatograms in the raspberry explant tissues 72 h after the MPT: at the top – experiment variant 2, at the bottom – control (experiment variant 5)

https://doi.org/10.17221/60/2020-HORTSCI

At 14 days (336 hours) after the MPT, there was usually a tendency to level the magnetic treatment effects in relation to the microplants' phenolic composition: in most cases, there was a decrease in the number of phenolic compounds except for the variants after the pulsed and rotating MPT fields with a 3.2–51–3.2 Hz frequency for all three acids under study. Consequently, the pulsed and rotating magnetic field treatment (variant 1) had the maximum after effect in relation to the studied phenolic compounds' high level in the microplants.

The pulsed magnetic field effects on the plants' sanitation from the viruses' processes, in our opinion, may be connected with the phenolic compounds' antioxidant activity, and their effect on the plants' enzyme and hormonal systems. The protective effect of some phenolic compounds in the plants' resistance to various pathogens forming is widely known. For example, in the experiments of Belarusian researchers, the oxycoric and oxybenzoic acids and flavonoid content increased in the plant's tissues under a barley mesh helminthosporiosis pathogen influence and, after an epibrassinolide treatment, the phenylpropanoid metabolism was activated, upon which the authors made a conclusion about the plant hormones and the phenolic compounds complex hormonal-inhibitory action (Manzhelesova 2019). The study by Shabrangi and Majd (2009) also shows suggested that pre-treated plants by magnetic fields are more resistant to harmful environmental factors.

The study by Belyavskaya (2004) determined that a plant's prolonged exposure to a weak magnetic field may cause different biological effects at the cellular, tissue and organ levels. In studies conducted by Lipiec et al. (2005), in laboratory experiments with oat seedlings after treatment with oscillating magnetic field, an increase in the level of polyphenols and antioxidant activity was shown and the number of microorganisms (bacteria, fungi) colonies was significantly reduced.

Salicylic acid can induce the acquired resistance to stimulate the pathogenesis-related (PR) proteins formation, to limit the spread of viruses on the plant tissues and organs, and to act as a signal causing the protective genes expression in the cells. Some stressors (ozone treatment, UV-C-light ray treatment) may stimulate the salicylic acid formation (Hara et al. 2012). Therefore, the salicylic and some other phenolcarbonic acid synthesis increase under the pulsed magnetic field effect is quite understandable in our experiments.

CONCLUSION

The magnetic pulse treatment led to the *Raspberry bushy dwarf virus*-free microplants' output increase and their phenolic composition change. The greatest output of the virus-free raspberry microplants was noted after the complex processing with the pulsed and rotating magnetic fields with the time-changing frequency from 3.2 to 51 Hz, as well as with the pulsed magnetic field with the frequency from 1 to 10 Hz. The pulsed and rotating magnetic field complex treatment resulted in the gallic and salicylic acid content increase by 14 and 71%, respectively, compared to the untreated variant. At 3 hours after the magnetic pulse treatment, the gallic and chlorogenic acid content decrease was observed with the simultaneous increase in the salicylic acid accumulation in the raspberry explants' tissues. At 72 hours after the magnetic treatment with a frequency from 3.2 to 51 Hz, the chlorogenic, salicylic and gallic acid active synthesis was observed. At 14 days after the magnetic treatment, there was a tendency that the number of phenolic compounds decreased except for the variants after the pulsed and rotating field treatment with the 3.2–51–3.2 Hz frequency for all three phenolcarbonic acids under study. The non-specific resistance induction is considered, by us, as one of the possible mechanisms of the magnetic pulse treatment during the microplants' sanitation from viruses.

REFERENCES

Anand A., Nagarajan S., Verma A., Joshi D., Pathak P., Bhardwaj J. (2012): Pre-treatment of seeds with static magnetic field ameliorates soil water stress in seedlings of maize (*Zea mays* L.). Indian Journal of Biochemistry and Biophysics, 49: 63–70.

Baryshev M.G. (2002): Electromagnetic processing of materials of plant and animal origin. Krasnodar, Kuban State University Publishing.

Belyavskaya N.A. (2004): Biological effects due to weak magnetic field on plants. Advances in Space Research, 34: 1566–1574.

Bingy V.N. (2002): Magnetobiology: Experiments and Models. Moscow, MILTA.

Bingy V.N., Savin A.V. (2003): Effects of weak magnetic fields on biological systems: physical aspects. Physics-Uspekhi (Advances in PhysicalSciences), 46: 259–291.

Cakmak T., Cakmak Z.E., Dumlupinar R., Tekinay T. (2012): Analysis of apoplastic and symplastic antioxidant system in shallot leaves: impacts of weak static electric and magnetic field. Journal of Plant Physiology, 169: 1066–1073.

Clark M.F., Adams A.N. (1977): Characteristics of microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. Journal of General Virology, 34: 475–483.

Donetskikh V.I., Upadyshev M.T., Selivanov V.G. (2018): An innovative device for exposing plants to a stationary, traveling and rotating pulsed magnetic field. Machinery and Equipment for Rural Areas, 7 (253): 32–37.

Donetskikh V.I., Upadyshev M.T., Petrova A.D., Metlitskaya K.V., Selivanov V.G. (2017): Application of AMIS-8 apparatus to combat viruses when preparing planting stock of fruit crops. Machinery and Equipment for Rural Areas, 1 (235): 16–23.

Esitken A., Turan M. (2004): Alternating magnetic field effects on yield and plant nutrient element composition of strawberry (*Fragaria* × *ananassa* cv. Camarosa). Acta Agriculturae Scandinavica, Section B – Soil and Plant Science, 54: 135–139.

Hara M., Furukawa J., Sato A., Mizoguchi T., Miura K. (2012): Abiotic stress and role of salicylic acid in plants. In: Ahmad P., Prasad M.N.V. (eds): Abiotic Stress Responses in Plants. New York, Dordrecht, Heidelberg, London, Springer: 235–251.

Lipiec J., Janas P., Barabasz W., Pysz M., Pisulewski P. (2005): Effects of oscillating magnetic field pulses on selected oat sprouts used for food purposes. Acta Agrophysica, 5: 357–365.

Maffei M.E. (2014): Magnetic field effects on plant growth, development and evolution. Plant Science, 5: 1–15.

Manzhelesova N., Bolynets N. (2015): Plant hormones and phenolic compounds in plants diseases control. Science and innovations: 62–65. Available at https://innosfera.by/files/2015/3.pdf

Murashige T., Skoog F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473–497.

Nadirov N.K., Solodova E.V., Ashirov A.M., Chirkin A.P., Polykhova S.M. (2009): Complex influence of the low-frequency electromagnetic field on qualitative composition of corn. Available at htpps://www.biophys.ru/archive/congress2009/abs-p153.pdf

Novitskiy Y.I., Novitskaya G.V., Serdyukov Y.A. (2014): Lipid utilization in radish seedlings as affected with weak horizontal extremely low frequency magnetic field. Bioelectromagnetics, 35: 91–99.

Stange D.C., Rowland R.E., Rapley B.J., Podd J.V. (2002): ELF magnetic fields increase amino acid uptake into *Vicia faba* L. roots and alter ion movement across the plasma membrane. Bioelectromagnetics, 23: 347–354.

Shabrangi A., Majd A. (2009): Effect of magnetic fields on growth and antioxidant systems in agricultural plants. In: Progress in Electromagnetic Research Symposium. Beijing, China, 2: 1142–1147.

Trebbi G., Borghini F., Lazzarato L., Torrigiani P., Calzoni G.L., Betti L. (2007): Extremely low frequency weak magnetic fields enhance resistance of NN tobacco plants to Tobacco Mosaic Virus and elicit stress-related biochemical activities. Bioelectromagnetics, 28: 214–223.

Upadyshev M.T., Donetskih V.I. (2008): New method of sanitizing berry and fruit crops from viruses by the magnetotherapy method. Russian Academy of Agricultural Sciences, 34: 223–226.

Upadyshev M.T., Motyleva S.M., Mertvischeva M.E., Donetskih V.I. (2017): About biochemical mechanism of magnetic treatment effect on raspberry plants sanitation from viruses processes. In: The Role of Physiology and Biochemistry in Vegetable, Fruit and Officinal Plants Introduction and Selection. Moscow: 315–317. Available at htpps://www.elibrary.ru/item.asp?id=29233478

Wang Q., Cuellar W.J., Raiamäki M. L., Hirata Y., Valkonen J.P.T. (2008): Combined thermotherapy and cryotherapy for efficient virus eradication: relation of virus distribution, subcellular changes, cell survival and viral RNA degradation in shoot tips. Molecular Plant Pathology, 9: 237–250.

Weaver J.C., Chizmadzhev Y. (1996): Theory of electroporation: a review. Bioelectrochemistry and Bioenergetics, 41: 135–160.

Received for publication: April 21, 2020
Accepted after correctiéon: April 7, 2021