PAPER • **OPEN ACCESS**

Environmentally friendly technology for the destruction of tobacco mosaic viruses (TMV) from selected species of plants

To cite this article: N Toshpulatov *et al* 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **614** 012133

Environmentally friendly technology for the destruction of tobacco mosaic viruses (TMV) from selected species of plants

N Toshpulatov[1], O Tursunov[1,2,3], D Kodirov[1], and G Kholmuratova[4]

[1]Department of Power Supply and Renewable Energy Sources, Tashkent Institute of Irrigation and Agricultural Mechanization Engineers, 100000 Tashkent, Uzbekistan
[2]School of Mechanical and Power Engineering, Shanghai Jiao Tong University, 200240 Shanghai, China
[3]Research Institute of Forestry, 111104 Tashkent, Uzbekistan
[4]Department of Management, Tashkent Institute of Irrigation and Agricultural Mechanization Engineers, 100000 Tashkent, Uzbekistan

*E-mail: nusratillo59@mail.ru

Abstract. The article provides information on diseases of plants of melons and gourds of tomato, and cucumber. The analysis was carried out to identify the current state of the problem of infection and morbidity of tomato and cucumber plants with tobacco mosaic viruses (TMV). The positive and negative aspects of existing methods and methods of combating pathogens are described. The structure of the pathogen, the structure of cells in a spiral for the purpose of their destruction and elimination of harmful effects were studied. The description of the use of electric current discharges on the possibility of using TMV for breaking the cell spiral with the aim of destroying them is given.

1. Introduction

The yield and quality of crops grown largely depend on the variety, vegetation and how we treat them. However, a good variety, proper vegetation, timely care are not a guarantee of good yield. In addition, there are factors that negatively affect the receipt of good agricultural products.

These factors can be divided into two types:

objective - the correct selection of highly viable varieties and seeds, the influence of the structure and bonitet of the soil, the influence of weather and climatic conditions, vegetation, watering and plant care;

subjective - plant disease, infection with various viruses, bacteria, fungal infections, diseases.

As a rule, the influence of the first factor can only be prevented.

The influence of the second factor can be eliminated by its timely identification and the application of appropriate measures by the quarantine services. However, some diseases caused by infectious viruses lead to irreversible consequences in plant tissues and it is impossible to exclude them with the efforts of quarantine services [1]. The harmful effects of the tobacco mosaic virus can be counted among them [2].

Usually melons grown both in greenhouses and in open ground will suffer the most from TMV. The disease can cause great damage to plants of tomato, cucumber, cabbage, etc. It can wilt more in

greenhouses or nurseries for growing seedlings of plants. High humidity is favorable for the rapid multiplication and spread of the virus on plants. The infected plant withers quickly and changes the color of foliage and stems (Figure 1) [3-6].
The small tobacco mosaic is capable of rapid multiplication under appropriate conditions. Under the influence of external factors, on the plant organism (leaves, roots, stem), it penetrates into the plant's cellular structure in 3-4 hours and completely inhibits the entire organism of the plant-host within 25-30 days [7, 8]. In the leaves infected with TMV, a mosaic color appears, sometimes with mixed infection with other viruses, the leaves turn into threads, and the stem acquires a dark color, as in frost.
The first symptoms of tobacco mosaic virus infection can be seen on the leaves of the plant. The leaf plates are covered with a kind of mottling, which later takes the form of a mosaic of light or dark green color (Figure 2) [3, 4].

Figure 1. Tomato plants infected with tobacco mosaic viruses

Figure 2. Change in the shape and color of tomato leaves under the influence of TMV

The tobacco mosaic virus is one of the typical representatives of a large class of viruses with helical symmetry [9, 10]. Its particles are in the form of rigid hollow rods 15-18 × 300 nm in size and consist of protomeric proteins and RNA [7, 8]. The proteins are arranged in a spiral arrangement, forming a

total of 130 turns with a step of 23 angstroms. In total, the capsid contains 2,130 protomers, each 158 amino acid residues long. The main function of the capsid is to protect the genetic material of the virus - a single-stranded RNA molecule, immersed in the protein closer to the inner surface of the "stick" and repeats the steps of the protein helix [2-5].
The model of the tobacco mosaic virus consists of a protein molecule laid in a spiral, the capsid (shell) of which hides the RNA strand (Figure 3) [2-4, 11].

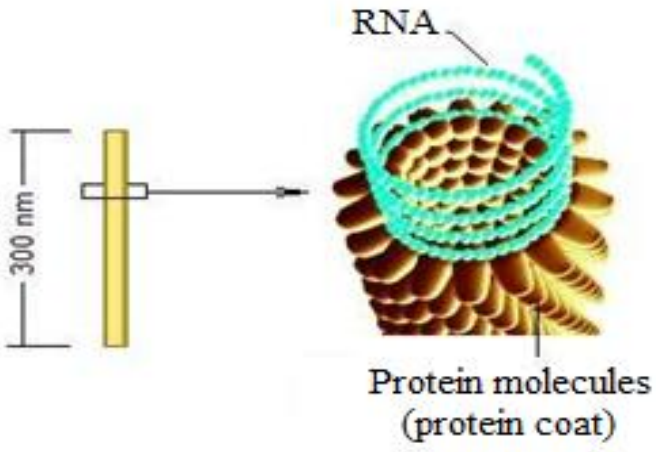

Figure 3. Spiral enveloped tobacco mosaic virus model

After entering the cellular structure, the helical TMV is exposed, i.e. releases its RNA. This is due to the peculiarities of the capsid proteins; they contain clusters of acidic amino acids that are stable outside the cell in the presence of a sufficient amount of calcium ions [12, 13]. However, in the cytoplasm of a low calcium concentration, they repel each other, which is why the first few protomers leave the capsid. RNA stripping is completed by the cellular ribosomes when they perform the first act of translation. Then the process is repeated several times and viral RNA and protein synthesis, after which new viruses are collected [5, 14, 15].

2. Methods

2.1. The main sources of TMV infection and methods of control to eliminate it

To eliminate the harmful effects of TMV, knowledge is required of the main causes and nuances of the occurrence and spread of infection on the cultivated areas of melons.
The results of our research have established that the main sources of infection with TMV infections are seeds, rhizomes, plant residues of perennial and annual weeds, remains of plants of the previous harvest, seed material infected with infections, new seedlings of tomato, cucumbers and other agricultural plants, spores of a pathogenic fungus found in the soil and in fertilizers for livestock and birds.
The spread of TMV occurs when caring for plants, watering, removing weeds, cultivating the soil, applying fertilizer, collecting fruits and crops with shoes, clothes, tools, etc. In large areas at the time of processing with mechanical tools attached to a tractor or other vehicles [2, 3].
The disease spreads especially quickly when the outer shell of bushes, leaves and fruits is damaged, where the outer protective layer of the skin is disturbed. In this case, insects such as aphids, thrips, cicadas and other pests become carriers of the infection. The disease spreads especially strongly with dense sowing of seedlings, poor lighting, high humidity and non-compliance with the norms and requirements of agricultural technology.

One of the features of tobacco mosaic is, depending on the variety, age, type and condition of growing plants, changes in the degree of harm. Therefore, the harmful effects of TMV on different varieties of tomato or cucumber in the same area are exposed in different ways [16-18].
If we take into account that TMV pathogens remain viable for up to 20 months or more [5], then to protect the next year's harvest, it is necessary to plant crops alternating with other crops using crop rotations.
To eliminate the harmful effects of the virus mosaic, in addition to quarantine measures, various methods and methods are recommended. These include agrotechnical - the introduction of crop rotations and tillage with various mechanical tools; chemical treatment - the use of various chemicals and preparations; heat treatment - heating, freezing and thawing of infected plants and their seeds; physical - treatment with various rays, ultraviolet radiation; electric - sonication, heating by electric current, etc.
Based on the analysis of scientific and technical information, the positive and negative aspects of the above methods and methods have been established, which are as follows.
The basis of the agrotechnical method is the use of land crop rotations, i.e. alternation of crops of plants and disease-resistant varieties, replacement of one crop with another, as well as the use of various methods and methods of mechanical soil cultivation.
Considering that TMV pathogens can remain viable for 20 or more months, then the use of crop rotation based on crop rotation and sowing disease-resistant varieties does not justify the ends and means due to the expectant ability of pathogens to more favorable conditions for spreading [4].
The use of mechanical treatment of the soil, such as deep plowing, hoeing, cultivation and others, in which various implements are used in a wide range are not very effective and sometimes they are contraindicated due to clogging by sown weeds. In most cases, mechanical soil cultivation leads to an extensive spread of seeds, stems and rhizomes of perennial weeds, which are foci for the spread of TMV infection and other pathogens of fungal diseases.
Chemical treatment of plants infected with tobacco mosaic viruses is carried out using weak and potent drugs [2, 3].
Weak-acting drugs (serums and chemical suspensions with a weak dose). are recommended to be used together with adaptogens and biostimulants.
For example, starting from the age of 14 days, tomato plants are recommended to be sprayed with adaptogens and biostimulants at intervals of two weeks throughout the season with "Heteroauxin", "Gibbersib", "Epin", "Epin extra".
If the seeds of a plant are infected with tobacco mosaic pathogens, then to strengthen them, prophylaxis is carried out before planting seedlings. In this case, it is recommended to soak the seeds of the plant in a 1% solution of potassium permanganate or two hours in the "Fitosporin" preparation.
When using potent drugs like boric acid, etc., it is recommended to carry out the first treatment before picking, i.e. the day before the pick, the last one - the day before planting seedlings in the ground with an interval of 8 days. It is recommended to spray with a 0.05% boric acid solution 5-7 days before planting seedlings. Such processing during the season for tomato must be repeated at least 4 times in the following preparations with a dosage: 0.05% boric acid; 0.01% cobalt nitrate; 0.01% copper sulfate [3].
As you can see, it is very laborious to carry out chemical treatment of contaminated areas of agriculture and plants, and it will take difficult work and a lot of time to conduct it. Therefore, usually on the part of farms, at the first sign of a disease, measures are taken to remove infected plants and burn it. To cleanse the lesion, the soil and equipment are disinfected with a 5% solution of potassium manganese.

2.2. *The method of temperature treatment*

With a gradual increase in temperature above the optimum in tobacco mosaic viruses, protein denaturation occurs, which contributes to a change in the shape of the viral particle, which allows it to

maintain its viability. It is impossible to sharply increase the temperature of the plant organism using conventional methods. For this reason, this technique is ineffective [2, 3].

2.3. Radiation treatment

a) in TMV, which received a large dose of X-rays, the damaging activity is not reduced;
b) irradiation with fast electrons: exposing TMV or infectious RNA TMV to the action of a beam of fast electrons with an energy of 5 MeV, we found that free RNA was inactivated approximately three times faster than the whole virus. But these results do not coincide with the results of other researchers, which mean the need for further research in this direction [3].
c) treatment with gamma rays: TMV was exposed to gamma rays with an intensity of 0 - 100 R. / h for several months. The research results showed insignificant changes in the amount of the virus contained in the cells. Only on the surface of plant leaves there was a slight decrease in infected areas;
d) ultraviolet treatment - TMV, which received an ultraviolet irradiation of about 15,000 - 30,000 quanta of energy, lose their activity, i.e. small changes occur in the protein coat of the virus. Also, using this method requires a long processing time and a large amount of energy, i.e. to inactivate one virus requires at least one quantum of energy [2, 3].

2.4. Treatment of TMV with ultrasound

For this it is necessary to carry out TMV treatment in a special solution, which, after processing, loses its ability to be refracted. Therefore, after each treatment, it is necessary to change the solution to a new one, which complicates the treatment process and reduces efficiency.

3. Results and Discussions

All of the above methods of processing plants infected with TMV viruses require significant resources, time, and money are not very effective.
For this reason, we have developed an electric pulse installation and conducted research to identify the negative impact of electrical discharges on tobacco mosaic viruses. To study the effect of high-voltage impulse discharges on the virus, the tomato strain TMV, propagated on the leaves of the Primoga tomato variety, was used. Before processing, about 100 g of tomato leaves 0.5-1 cm in size were cut and placed in a special vessel. Then these samples were divided into four equal groups. The first option is marked as a control, and the other three are experimental. Experimental versions of the samples before processing were in turn poured onto the surface of the processing area, connected to the negative side of the power source [2, 3, 6, 19].
Using the brushed positive electrode for 3s high voltage was used to treat the leaves of tomato infected with TMV. To increase the reliability of the experiments, after each treatment, the surface of the negative processing area was thoroughly cleaned.
The processing samples were subjected to electric pulse treatment at voltages of 3, 4, and 5 kV [2, 3, 19].
Then the control and experimental samples were separately placed in special vessels. Each sample was homogenized with 5 ml of distilled water in a mortar and the infectious juice was isolated. The virus-containing juice was filtered through 2 layers of gauze. Then, for additional purification, it was centrifuged at 5000 rpm for 20 min. During the rotation of the juice, the infected juice separated from the dense plant matter. The resulting juice was poured into a labeled tube, and the thick mass was discarded. To identify the degree of TMV damage on the treated samples of plant juice in comparison with the control, the following method was used [1, 3, 19].
From the tobacco plant Nicotinasilvestris, 12 leaves of the same size were treated, dividing them into 4 groups of 3 leaves; leaf surfaces were pollinated with 200-300 mesh corundum. And then, using a capillary pipette, samples of sap infected with TMV were evenly covered the surface of the plant leaves. The sediment is thrown away and the leaves of Nicotinasilvestr are contaminated above the sedimentary liquid.

We used corundum powder as a substance that creates microcracks on the leaf surface for TMV penetration into the cellular structure of plant material.
This technique is repeated for each experimental variant and for control in the same sequence.
For further observation of the development of TMV on infected tobacco leaves, they were placed in a moist desiccator chamber. After 4 days, the degree of leaf infestation was determined by the number of necrosis. The results obtained are presented in Table 1 [3, 19].

Table 1. Number of necrosis on infected leaves of Nicotinasilvestris

No.	Option	Voltage level, kV	Number of necrosis on leaves
1.	Control	-	40
2.	1	3.0	nine
3.	2	4.0	6
4.	3	5.0	five

Note: processing time 1 sec.

The results shown in Table 1 indicate that after processing plant materials with high-voltage electrical impulse discharges, their activity sharply decreases in comparison with the control. This allows us to assume that high-voltage electrical impulse discharges give positive results in the fight against HTM.
On the basis of the research carried out, a method for treating plants infected with diseases during the growing season was proposed.
This technique, with the correct selection of the voltage level in relation to the plant, can allow treatment to destroy diseases in the growth, development and vegetation phases.

4. Conclusions

The research has proven that plants are much more resistant to high-voltage discharges and impulse currents than tobacco mosaic viruses.
1. There are results confirming the improvement of plant growth and development, even an increase in yield compared to control.
2. Analysis of scientific and technical information shows that the technology of electro-pulse treatment of plants for the destruction of tobacco mosaic viruses is new.
3. The use of such technology in agriculture excludes pollution of the environment, soil, food and ecology. It allows you to save resources, extraordinary treatments, labor time and effort, technology, as well as various chemicals with all the ensuing consequences.

References

[1] Dobrowolski JW, Bedla D, Czech T, Gambus F, Gorecka K, Kiszcak W, Kuzniar T, Mazur R, Nowak A, Sliwka M, Tursunov O, Wagner A, Wieczorek J, Swiatek M 2017 Integrated Innovative Biotechnology for Optimization of Environmental Bioprocesses and a Green Economy *Optimization and Applicability of Bioprocesses* eds Purohit H, Kalia V, Vaidya A, Khardenavis A (Singapore: Springer) chapter 3 pp 27-71.
[2] Tashpulatov NT et al. 2001 *Problems of Informatics and Energy* **1** 22-26
[3] Tashpulatov N 2005 Application of energy-saving technologies for electro-treatment of plants, *International Conference on Sustainable Development, Safe Functioning of the Energy Sector in Uzbekistan: Prospects and Problems*, Institute of Energy and Automation of the Academy of Sciences of the Republic of Uzbekistan and the Academy of Education Development , USA-Tashkent.
[4] Dobrowolski JW, Kobylarczyk J, Tursunov O, Toh SQ 2015 Integration of Local Eco-Innovation with Global Problems of Protection of the Natural Environment and Bio-Based Green Economy, *In Proceedings : AASRI International Conference on Circuits and Systems (CAS)*, Atlantis Press, **9** 25-28.

[5] Muzafarov Sh, Tursunov O, Balitskiy V, Babayev A, Batirova L, Kodirov D 2020 *Int J Energy Clean Environ* **21**(2) 125-144.
[6] Tashpulatov N, Baizakov T, Khaliyarov M 1996 Method of harvesting plants, A.S. No. 3456 certificate. No. 504 registered in the state register of inventions of industrial designs and utility models of the Republic of Uzbekistan.
[7] Bhat S, Folimonova SY, Cole AB, et al 2013 *Plant Physiol*. **161**(1) 134-147.
[8] Liu C, Nelson RS 2013 *Front Plant Sci*. **2013** 4-12.
[9] Gelderblom HR 1996 Structure and Classification of Viruses *Medical Microbiology. 4th edition*. ed Baron S (Galveston (TX): University of Texas Medical Branch at Galveston) Chapter 41.
[10] Louten J 2016 *Essential Human Virology*. **19** 29.
[11] Taylor MW 2014 *Viruses and Man: A History of Interactions*. **2014** 23-40.
[12] Ge P, Zhou ZH 2011 *Proc Natl Acad Sci U S A*. **108**(23) 9637-9642.
[13] Chen MH, Sheng J, Hind G, Handa AK, Citovsky V 2000 *EMBO J*. **19**(5) 913-920.
[14] Hull R 2014 *Plant Virology*. **2014** 341-421.
[15] Phillips MB, Stuart JD, Simon EJ, Boehme KW 2018 *J Virol*. **92**(7) e02259-17.
[16] Sacristán S, Díaz M, Fraile A, García-Arenal F 2911 *J Virol*. **85**(10) 4974-4981.
[17] Alishiri A, Rakhshandehroo F, Zamanizadeh HR, Palukaitis P 2013 *Plant Pathol J*. **29**(3):260-273.
[18] Pazarlar S, Gümüş M, Oztekin GB 2013 *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **41**(2) 427-433.
[19] Tashpulatov N, Mirdzhalilov H, Bozorov E, Tashpulatov J 2005 Method of electrical impulse treatment of plants, Patent No. IAP 02758 Registered in the State Register of Inventions of the Republic of Uzbekistan.