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VIRAL DISEASES OF TOMATOES AND CUCUMBERS IN GREENHOUSES OF BELARUS

В статті наведено інформацію про випадки вірусних захворювань томатів та огірків, що вирощуються в умовах закритого ґрунту. Показано, що при інтенсивному розвитку вірусних захворювань огірків, їх шкодочинність становить 14,7-46,2 %, в залежності від гібриду. На основі біологічного тестування та ІФА ідентифіковані віруси ВТМ, ВОМ, ВМТ, ВЗКМО. Доведена можливість використання біологічно активних речовин для обмеження експресії ВЗКМО на ранніх стадіях розвитку огірків.

In the article the information on virus diseases incidence in tomato and cucumber cultivated under protected ground conditions is presented. It is shown that at the intensive virus diseases development in cucumber plants their harmfulness makes 14,7-46,2% depending on hybrid. Based on biological test and ELISA-test TMV, CMV, ToMV and CGMMV viruses are identified. A possibility of biologically active substances application for restriction the CGMMV expression and development at the early stage of cucumber plant development is determined.

Virus diseases are widely spread in vegetables and are one of the reasons of yield and production quality decrease. According to literary data more than 100 viruses of different taxonomic belonging differing by biological, serological, ecological peculiarities and ways of its transference are registered [6]. The most spread and harmful in tomato are: *Tobacco mosaic virus* (*TMV), Cucumber mosaic virus* (*CMV*), *Tomato mosaic virus* (*ToMV*), *Tomato aspermy virus* (*TAV*), *Tomato spotted wilt virus* (*TSWV*); in cucumber – *Cucumber green mottle mosaic virus* (*CGMMV*) and *Cucumber mosaic virus* (*CMV*) [3, 4, 9, 10].

The most actual problem of virus diseases concerns tomato and cucumber which are the most important protected ground crops. At present in greenhouses small-volume technologies allowing to decrease the material expenses essentially are widely used. However, the characteristic feature of tomato and cucumber cultivation using such technologies is the dominance of seed-born pathogens and the ones transmitted by contact and aerogenic means.

The objective of our researches was to study the phytoviral situation of greenhouses tomato and cucumber crops. The virus diseases monitoring was carried out in 2009-2010 by way of itinerary inspections of vegetable farms of different regions of the Republic. During budding-blossoming and fruit-bearing 100 plants from every tomato and cucumber hybrid were under visual inspection and leaf samples for laboratory studies were selected.

Plants with chlorosis, mosaic spot and leaf deformation symptoms were the object of studies. To determine virus etiology of diseases and their agents identification the selected tomato and cucumber plant samples were analyzed by plant-indicator and enzyme-linked immunosorbent assay (ELISA-test). *Nicotiana tabacum* L. (cv. Samsun), *N. glutinosa* L., *N. debneyi* Domin., *Datura stramonium* L., *Capsicum annum* L.*, Lycopersicon esculentum* Mill., *Tetragonia expansa* L., *Cucumis sativus* L. were used as test plants. ELISA delivery was accomplished with the test set use the Sediag (France) and ADGEN (Scotland).

By inspecting the greenhouse tomato and cucumber plantings it was determined that some hybrids are rather heavy infected by virus diseases, the incidence of which varied depending on hybrid and cultivation conditions. So, 40-90% of plants of tomato hybrids Alkasar, Barselona, Admira, Bomax have got the symptoms of mosaic and leaf deformation and the plants of cucumber hybrids Raiss, Kurazh, Evergreen, Tristan, Mirabel, Sharzh, Ceroz have a got – y 45-100% (Table 1).

Table 1. **Distribution of virus diseases in greenhouses (2009-2010)**

Tomato		**Cucumber**	
Hybrids (F1)	Distribution,%	Hybrids (F1)	Distribution,%
Admira	50-90,0	Ceros	65-75,0
Alkasar	45,0	Estafeta	32,0
Bomax	30-35,0	Evergreen	26-80,0
Barselona	40,0	German	25-35,0
Bolduin	30,0	Gladiator	21,0
Emoushen	15,0	Kolanell	75,0
Gayana	18,0	Kurazh	20-80,0
Greis	15-35,0	Malkum	20,0
Grodena	20,0	Marsel	25,0
Makarena	21,0	Meva	0
Pablo	0	Mirabell	20-70,0
Persei	0	Raiss	40-100,0
Raissa	25-30,0	Rafael	12-21,0
Siluet	10,0	Pacto	15-55,0
Starbuck	20,0	Pikas	24,0
Zhyronimo	15-30,0	Tristan	21-100,0
Zuko	30,0	Sharzh	85,5

The virus diseases damage brings vegetable crop yield decrease and trade production quality worsening. The evaluation of viral diseases influence on cucumber plant productivity has shown that they decrease yield and fruit trade qualities. So, in cucumber Kurazh F1 the intensive infection symptoms were developed during blossoming and active plant fruit-bearing. There was a delay in growth and bright mosaic accompanied by rugosity was discovered. The fruits have got unevenness and mosaic spot, as a result, 30% turned out to be non-standard. As a result, the disease harmfulness in the hybride Kurazh F1 has made 46,2% (Table 2).

Table 2. **Virus infection influence on cucumber productivity**

Hybrids (F1)	Plants	R, %	Ovaries number, pcs/plant	Fruit yield, kg/m^2	P, %
Rafael	with symptoms	15,6	13	1,0	14,7
	without symptoms	-	17	1,5	-
LSD05			1,5	0,11	
Kurazh	with symptoms	42,5	15,0	0,6	46,2
	without symptoms	-	19,3	1,1	-
LSD05			2,31	0,14	
Sharzh	with symptoms	33,1	17,3	1,1	26,1
	without symptoms	-	21,0	1,4	-
LSD05			1,76	0,23	

Note – R – disease development, %; P – harmfulness, %.

The analysis of the infected tomato plant samples by ELISA method and plant –indicator methods has shown that the main disease agents are *Tomato aspermy virus* (*TAV*) и *Tomato mosaic virus* (*ToMV*), cucumber – *Cucumber green mottle mosaic virus* (*CGMMV*) (Figure 1).

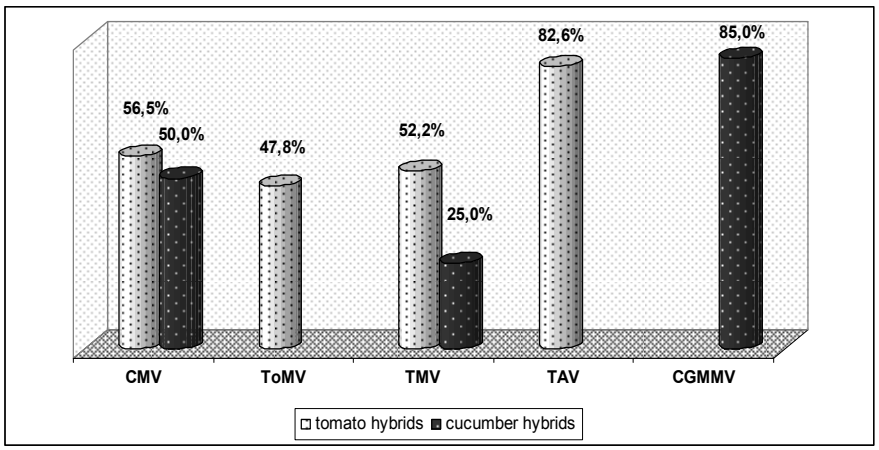

Figure 1. Frequency of virus incidence in studied tomato and cucumber hybrids (2009-2010 ELISA-data)

According to literary data the most effective antiviral measure against *CGMMV* is seed dressing in 15% trisodium phosphate solution [7]. At present in the technologies of vegetable crops growing the biological active substances (BAS) are more frequently used. It is determined that these combinations are able to raise both agricultural crop yield and essentially decrease the negative influence of abiotic and biotic factors through the hormonal exchange and plant immunity stimulation raising their disease resistance. Brassinosteroids showing high growth-stimulating are of interest and can be a basis for the effective and ecologically safe application [8]. The active biological stimulators are the preparations containing a complex of amino acids , micro and macro elements. The essential role in raising plant resistance to phytopathogens play the preparations the main active ingredient of which are the proteins- the proteolytic ferment inhibitors rendering the protective action [2, 5].

We studied a possibility of *CGMMV* inhibition by way treatment of cucumber see phytoregulators epine, s. and tubelak, WSP which are included into the "State register of plant protection products pesticides and fertilizers allowed for application on the territory of Belarus" A range of active concentrations of preparations and their influence on *CGMMV* expression and development at pre-sowing seed dressing is determined.

The results of researches have shown that pre-sowing cucumber seed treatment (Raiss F1, Evergreen F1) by growth regulators promoted seedlings emergence, cucumber plant growth and development of studied hybrids. At the same time seed retting in growth-regulating substances solutions held in cucumber plant symptoms appearance and development induced by *CGMMV* at an early stage of plant ontogenesis. Based on ELISA-test it is determined that the virus concentration in cucumber plants grown from seeds treated by the above-indicated preparations was 1,5-2 times less in comparison with the control (Figure 2).

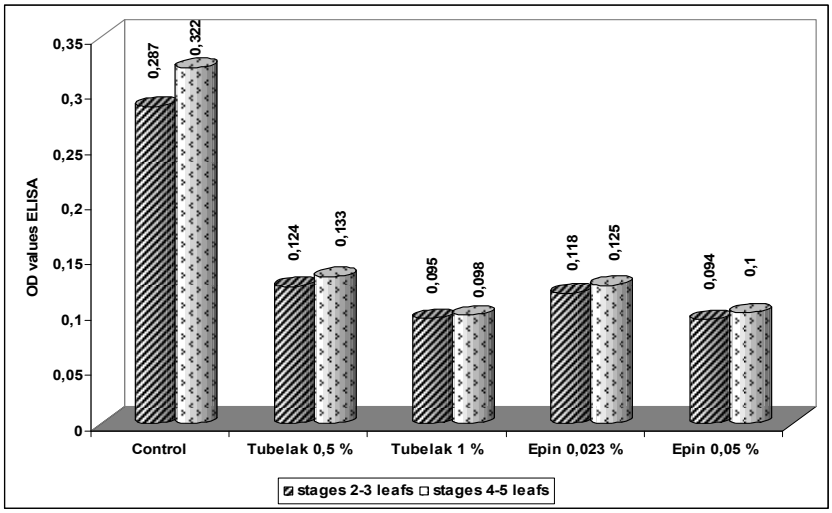

Figure 2. The inhibition of *CGMMV* by the treatment of seeds of cucumber (Evergreen F1) with plant growth regulators tubelak, WSP and epin, s (laboratory experiments 2009-2010)

So, the inspection of vegetable farms of the republic carried out in 2009-2010 has shown a wide spread of viral diseases in greenhouses tomato and cucumber plantings. It is determined that at the intensive viral diseases development in cucumber crop their productivity depending on hybrid is decreased for 21,0-45,4% and harmfulness index makes 14,7-46,2%. Based on biological test and ELISA-test *TMV, CMV, ToMV* and *CGMMV* viral diseases agents are identified. The prevalent ones are *Tomato aspermy virus* (*TAV*) и *Cucumber green mottle mosaic virus* (*CGMMV*). A possibility of biologically active substances application (epin, s., tubelak, WSP) to reduce *CGMMV* development at early cucumber plant ontogenesis stage is established.

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CHARACTERISTICS OF THE INTERACTION BETWEEN VIRUS AND SPECIFIC ANTIBODIES IN CELL HOMOGENATES

Розглянуто підхід до детекції методом поверхневого плазмонного резонансу з використанням формування комплексу антиген-антитіло під час преінкубації замість послідовного нанесення. На прикладі Nicotiana tabacum за допомогою запропонованого підходу досліджено вірусну інфекцію. Досягнуто узгодження між методами ППР та ІФА. Розглянуто також кінетику взаємодії.

Surface plasmon resonance approach using a detection of complex formed by viral antigen and antiviral antibody during the preincubation step instead of their consecutive application is discussed. With the example of Nicotiana tabacum the development of the viral infection in the plant was studied by the mentioned approach. The agreement between SPR and ELISA methods was achieved. Kinetics of the interaction is discussed.

Detection of the specific interactions against the background of the unspecific components of the sample is the one of the important tasks of the modern analytical biochemistry. The new ways to overcome the matrix effect are especially essential for the methods based on surface plasmon resonance (SPR) technique, particularly those which could be used for the express-diagnostics of viruses.The main advantage of the SPR technique is possibility of direct registration of surface interactions without any additional procedures or labels. Real time response registration enables the investigation of interaction kinetics and, thereby, tracking of a system's evolution.

There exist many approaches for detection of virus specific proteins and nucleic acids, but only few of them can detect intact virus particles. Enzyme-linked immunosorbent assay (ELISA), the traditional technique for detection of virus specific antigens, enables one to obtain exact and reliable information concerning presence of virus infection using interaction between the virus particle elements and corresponding antibodies. This technique, however, has certain constraints; most essential of them is the lack of kinetic information regarding the reaction under consideration.

Among the optoelectronic methods the surface plasmon resonance technique is widely used when studying virus specific macromolecules. However most works in this field deal with individual components of viruses rather than entire virus particles [8]. Although virus peptides, recombinant and refined virus proteins can serve as convenient model to investigate antibody affinity, their structure, conformation and microsurroundings of subunits, both separate and incorporated into virus particle, may essentially differ. That is why investigations of interaction between