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PREVALENCE OF INFECTIONS WITH ONION YELLOW DWARF VIRUS, LEEK YELLOW STRIPE VIRUS AND GARLIC COMMON LATENT VIRUS IN PLANTS FROM THE GENUS *Allium*

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Abstract. The aim of the present work was to identify GCL, OYD and LYS and to assess the degree of their distribution in crop and ornamental plants from the genus *Allium*. Two groups of plants from the genus *Allium* were used. The first group included 10 botanical species, while the second group was composed of seven commercial *A. sativum* cultivars and two genotypes. Identification of GCL, OYD and LYS in leaves, inflorescences, and bulbs was performed with the use of the ELISA test. All plants in the first group consisting of botanical species of the genus *Allium* were free from the viruses studied, whereas in commercial *A. sativum* cultivars a high prevalence of GCLV, OYDV and LYSV infection reaching 88.2%, 75% and 32.1%, respectively, was reported. Varying severity of infection in the particular plant organs was found.

Key words: *Allium*, garlic varieties, GCLV, OYDV, LYSV, ELISA test

INTRODUCTION

Garlic (*Allium sativum*) is one of the oldest vegetables cultivated worldwide for over 6 thousand years. It is an important plant due to its usability and medicinal properties provided by bioactive components present in underground bulbs and young leaves. The most common is the Asian *A. sativum* ecotype characterized by a diploid karyotype 2n = 16. Plants with this genotype are sterile and do not produce seeds in natural conditions; they reproduce exclusively in a vegetative mode via aerial bulbils or cloves from underground bulbs [Simon and Jenderek 2003]. Some researchers investigating sterility of garlic hypothesize secondary loss of sexual reproduction caused by a variety of developmental disorders [Etoh and Simon 2002]. In turn, Konwicka [1973] suggested that

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A. sativum sterility is caused by infections of plants with mycoplasmas, rickettsiae, and viruses. Vegetative reproduction is associated with a possibility of transmission of pathogens, including viruses, which directly reduces yield quality and disinfection of reproductive material increases cultivation costs substantially [Salomon 2002].

A majority of known plant viruses are not or to a limited degree transmitted via seeds. Therefore, plants with generative reproduction, e.g. onion and leek, begin their life cycle as virus-free plants. Since garlic does not have such ability and reproduces in the vegetative mode exclusively, it always transmits viruses to successive generations. This phenomenon is very common due to high virus survival rates in infected plant tissues; therefore, a plant that does not exhibit signs of infection may be a virus carrier [Salomon 2002].

The most prevalent viral pathogens infecting garlic are viruses from the genera Potyvirus – Onion yellow dwarf virus OYDV and Leek yellow stripe virus LYSV, Carlavirus – Garlic common latent virus GCLV, and Shallot latent virus [Hillman and Lawrence 1995, Dovas et al. 2001a, b, Chen et al. 2004], and Allexivirus [Sumi et al. 1993, Barg et al. 1994, Yamashita et al. 1996, Chen et al. 2004]. Heterogeneous garlic infections caused by several viruses have been reported most frequently [Klukáčková et al. 2007].

Potyviruses are currently the best – described garlic viruses [Klukáčková et al. 2007]. With its 143 species, it is the most abundant genus in the family *Potyviridae* and among all plant viruses [King et al. 2011]. Its genetic material is composed of ca. 9,7 kb-long ssRNA. Potyvirus molecules have a length ranging from 680 to 900 nm and a diameter of 11–13 nm. The size of the capsid protein varies from 30 to 47 kDa between individual species. Viruses from this genus are characterized by a limited host range and are most frequently carried by aphids on their proboscises. Only a few species can be transmitted via seeds [King et al. 2011].

The Onion yellow dwarf virus infects plants from the genus *Allium* worldwide [Diekmann 1997]. It is transmitted mechanically by aphids as a non-persistent virus. It is not transmitted via seeds [Brunt et al. 1996], but by vectors and vegetative propagation material. The host range of this virus is limited to the genus *Allium*; the infection is particularly prevalent in onions and shallots. Reduction in bulb weight caused by strains of this virus in different garlic varieties ranges from 25 to 60% [Barg et al. 1997, Lot et al. 1998]. Co-infection with other viruses results in even greater yield losses [Diekmann 1997].

The Leek yellow stripe virus has been found in Europe, North America, and Australia [Diekmann 1997]. It is transmitted by aphids on their proboscises, but not via seeds [Brunt et al. 1996]. Vegetative propagation material is regarded as the primary means of transmission of the virus. The host range of LYSV is limited to species from the genus *Allium*: common garlic, broadleaf leek (*Allium ampeloprasum* var. *ampeloprasum*), pearl onion (*A. ampeloprasum* var. *sectivum*), and several wild type and ornamental *Allium* species [Diekmann 1997]. Onions and shallots exhibit minimum susceptibility to this virus [Bos 1981].

Carlavirus is the most abundant genus from the family *Betaflexiviridae* comprising 43 species, including the Garlic common latent virus, which is distributed worldwide [King et al. 2011]. Both the host range and the mode of transmission differ between

particular virus species. A majority of them can be transmitted on aphid proboscises as non-persistent viruses; moreover, they can relatively easily be transmitted mechanically .Similar to other garlic viruses, they are not transmitted via seeds. Vegetative propagation material is the primary infection source. The virus is characterized by a wide range of hosts from the family *Alliaceae*. It is prevalent mainly in the common garlic, although it was identified in more than 50 other species from the genus *Allium* [Diekmann 1997].

The genus Allexivirus belongs to the family *Alphaflexiviridae* and comprises eight species [King et al. 2011]. The main host for this genus are plants from the genus *Allium*, and the viruses are transmitted by mites feeding on plants (*Eriophyodea*) [King 2011]. Occurrence of light green stripes on garlic leaves is a sign of infection with allexiviruses [Barg et al. 1997, Takaichi et al. 1998, Daniels 1999]. A recent study conducted by Perotto et al. [2010] has shown that the two most prevalent allexiviruses GarV-A and GarV-C may reduce the size of *A. sativum* bulbs by even 96%.

Currently, thermotherapy is the most efficient method for elimination of garlic viral infections. Unfortunately, as shown by the investigations conducted by Fajardo et al. [2001], after elimination of viruses from the propagation material, re-infection of *A. sativum* occurs as early as after the second period of cultivation. In the case of allexiviruses, re-infection was observed after the first yield [Perreto et al. 2010]. Nevertheless, cultivation of virus-free material is characterized by a lower number of infected plants; therefore, yield losses are considerably lower [Takaichi 1998, Melo-Filho et al. 2006]. The aim of the work was to identify the GCL, OYD and LYS and assessment of their prevalence rate in crop and ornamental plants from the genus *Allium*.

MATERIAL AND METHODS

Study material. Two groups of plants from the genus *Allium* were used for the study. The first group consisted of 10 botanical species originating from the UMCS Botanical Garden in Lublin (*A. angulosum*, *A. carinatum*, *A. cernuum*, *A. fistulosum*, *A. ledebourianum*, *A. senescens*, *A. tuberosum*, *A. turkestanikum*, *A. victorialis* and *A. vineale*). The other group comprised seven commercially available *A. sativum* varieties (Arkus, Botanik, Ceves, Harnaś, Huzar, Kna, Mega, Ornak) and two genotypes growing in the UMCS Botanical Garden. Genotype L originated from eastern Poland, and genotype L13 from the Botanical Garden in Marburg (Germany). The experiment was set up in two plots P1 and P2 located at a 5-km distance, on which no *Allium* plants had been cultivated previously. In each plot, 20 plants of each species, variety, and genotype were planted. The propagation material, i.e. cloves from underground bulbs, was planted in the soil in November 2011.

From each species, variety, and genotype, 12 samples of leaves, inflorescences, and underground bulbs were randomly collected. Aqueous extracts of sampled plant organs were used for virusological analyses. Samples were pooled then submitted for testing: 1 g of sampled plant organs were weighted, placed in a mortar, and ground in 20 ml of extraction buffer (20 mM buffer Tris,pH 7.4 containing 137 mM NaCl, 3 mM KCl, 2% PVP 24 kD, 0.05% Tween 20 and 0.02% NaN3). Extraction of the bulb material was

performed in the same buffer supplemented with albumin. 2 ml aliquots of the aqueous extracts obtained were placed in Eppendorf tubes.

Detection of viruses in thus prepared plant extract samples was carried out using a commercial ELISA kit (BIOREBA AG, Switzerland) in accordance with the manufacturer's instructions. To this end, 200 μl of each sample in three replicates were placed into wells, which had previously been coated with specific antibodies against the GCL, OYD and LYS s. The plates were incubated in a humid chamber at 4ºC overnight. Virus-antibody complexes were detected using specific enzyme-conjugated anti-viral antibodies. To accomplish this, 200 μl of conjugate were added, incubated at 30ºC for 5 hours, and supplemented with the substrate. The intensity of the colour reaction was assessed with Sunrise^{TM} (Tecan) reader at wavelength λ = 405 nm and reference wavelength λ = 495 nm. Absorbance values three times or more than those given by healthy controls were considered to indicate infection. Absorbance was analysed with the Magellan^{TM} software.

RESULTS

All plants from the first group, comprising botanical species of the genus *Allium*, were free from GCLV, OYDV and LYSV, whereas high prevalence of viral infections was found in the second *A. sativum* group. The distribution of the infection among the different organs is shown in Table 1. The absorbacne was showed as an average value.

Infection of garlic with Garlic common latent virus. A majority, i.e. 88.2% of the *A. sativum* varieties and genotypes examined, were infected by the Garlic common latent virus (GCLV) (fig. 1). Only plants of the Kna 210 variety growing in plot P1 and P2 as well as the Ceves variety from plot P1 were free from the Garlic common latent virus. Among the *A. sativum* varieties from plot P1 infected with the GCLV the Mega variety exhibited a significantly lower degree of infection; the mean absorbance for the leaves was 0.724, inflorescence 0.579, and for bulbs 0.498. In turn, a significantly more severe infection was detected in the Arcus variety, where the absorbance values were 1.328 for the leaves, 1.234 for the inflorescence, and 0.746 for the bulb.

Among the *A. sativum* varieties growing in plot P2 and infected with the GCLV, the Harnaś and Mega varieties were characterized by the lowest level of infection. In turn, compared with the other varieties, the Botanik variety was characterized by a significantly higher infection degree with mean absorbance values of 1.176 for the leaves, 1.052 for the inflorescence, and 1.099 for the bulb. The genotype L plants were severely infected by the virus as well and were characterized by absorbance values of 1.489 for the leaves, 0.498 for the inflorescence, and 0.708 for the bulb.

The GCLV leaf infection in both plots was comparable. The highest level of infection was found in inflorescences of garlic varieties growing in plot P1, whereas the level of infection in inflorescences of varieties growing in P2 was the lowest. The level of GCLV infection of the bulbs of *A. sativum* varieties was comparable in both plots.

Infection of garlic with Onion yellow dwarf virus. Infection with OYDV was diagnose in 75% of *A. sativum* varieties studied (fig. 1). The virus was detected in the bulbs, with the exception of the Harnaś variety from plot P1, in which it was detected

only in the inflorescence (absorbance value 0.411). The Harnaś variety from plot P1 as well as the Huzar, Ornak, and Mega varieties and genotype L13 from plot P2 were OYDV-free.

Table 1. Identification GCLV, OYDV and LYSV in leaves, inflorescences and garlic bulbs

	Varieties	Plot 1			Plot 2		
		leaf	inflorescence	bulb	leaf	inflorescence	bulb
GCLV	Arkus	P	P	P	P	P	P
	Botanik	P	P	P	P	P	P
	Ceves	N	N	N	P	P	P
	Harnaś	P	P	P	P	P	P
	Huzar	P	P	P	P	P	P
	Kna 210	N	N	N	N	N	N
	Mega	P	P	P	P	P	P
	Ornak	P	P	P	P	P	P
	Genotype L	P	P	P	P	P	P
	Genotype L13	P	P	P	P	P	P
OYDV	Arkus	N	N	P	N	N	N
	Botanik	P	N	N	N	N	P
	Ceves	N	N	P	N	N	P
	Harnaś	N	N	N	N	P	N
	Huzar	N	N	P	N	N	N
	Kna 210	N	N	P	N	N	P
	Mega	N	N	P	N	N	N
	Ornak	N	N	P	N	N	N
	Genotype L	N	N	P	N	N	P
	Genotype L13	N	P	P	N	N	N
LYSV	Arkus	N	N	P	N	N	N
	Botanik	P	N	N	N	N	P
	Ceves	N	N	P	N	N	P
	Harnaś	N	N	N	N	P	N
	Huzar	N	N	P	N	N	N
	Kna 210	N	N	P	N	N	P
	Mega	N	N	P	N	N	N
	Ornak	N	N	P	N	N	N
	Genotype L	N	N	P	N	N	P
	Genotype L13	N	P	P	N	N	N

P – positive result of ELISA test for the presence of virus; N – negative result of ELISA test for the presence of virus

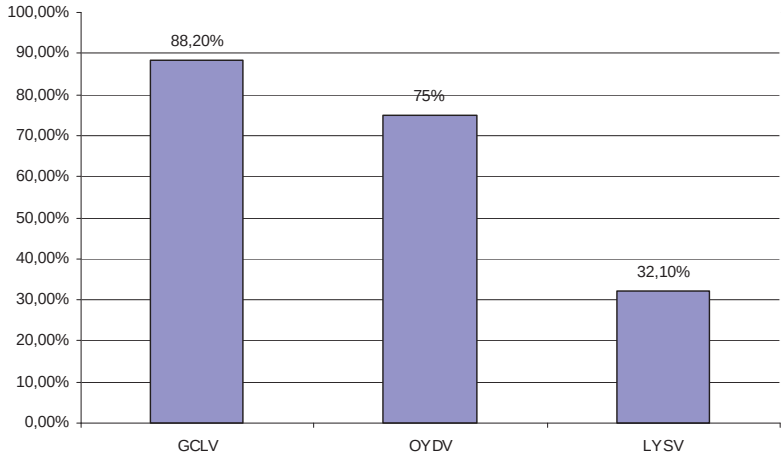

Fig. 1. Percentage of *A. sativum* infection with GCLV, OYDV and LYSV

With the exception of the Kna 210 variety, infection of garlic bulbs with the Onion yellow dwarf virus (OYDV) in the varieties growing in plot P2 was more severe than in the varieties from plot P1. Among the *A. sativum* varieties from plot P1 infected by the Onion yellow dwarf virus, the Arcus variety was characterized by a significantly lower level of infection, whereas the highest level was detected in the Mega variety.

In plot P2, the lowest severity of infection was found in the Kna 210 variety, but the other varieties were characterized by a high level of infection.

Infection of garlic with Leek yellow stripe virus. More than half of the *A. sativum* varieties examined were LYSV-free. The frequency of LYSV infection was at the level of 32.1% (fig.1). In plot P1, only the Arkus (absorbance value 0.634), Huzar (absorbance value 1.485) and Ornak (absorbance value 0.456) varieties were infected. Similarly, in plot P2, bulb infection was only detected in the Ceves (absorbance value 0.387), Huzar (absorbance value 1.298), and Ornak (absorbance value 0.834) varieties and in genotype L13 (absorbance value 0.370).

DISCUSSION

It is generally agreed that carlaviruses, e.g. the Garlic common latent virus and Shallot latent virus, are transmitted by aphids less effectively than potyviruses; yet they may pose a considerable problem locally. In the present study, the Garlic common latent virus (GCLV) was detected in 88.2% of the commercial plant varieties. The Kna 210 cultivar was GCLV-free. A high prevalence of the virus was also reported from garlic cultivation in the Czech Republic, where the virus infected over 98% of garlic plants out of five examined varieties: Anton, Anin, Benátčan, Bjetin, and Vekan [Klukáčková et al. 2007]. In Italy, differences in the geographical distribution of the GCL viral infection

of garlic were described; in the south, the infection rate reached 23%, while in the north of the country it was 98% [Dovas and Vovlas 2003]. Differences in GCLV prevalence in garlic related to the location of cultivation was also reported from Greece [Dovas et al. 2001b]. Material collected from five regions of Greece displayed a 20% level of infection; however, in three regions of northern and central Greece, infection of garlic with the virus did not exceed 3% of plants. The highest level of infection, i.e. 97.6%, was reported from the southern part of the country. Use of virus-free propagation material in Brazil [Fayad-André et al. 2011] and Iran [Shahraeen et al. 2008] resulted in a reduction of the level of GCLV infection.

In the present study, the second most prevalent of garlic infection was caused by the Onion yellow dwarf virus (OYDV), which was identified primarily in the bulbs of 78.6% of the examined plants. The pathogen was detected in all the plant varieties. Similar results were obtained in the Czech Republic, where 75% of plants from five local varieties were infected with this virus; the lowest proportion (6.9%) of infected plants was reported from the Vekan variety, indicating that it is less susceptible to infection caused by this pathogen [Klukáčková et al. 2007]. A high prevalence of OYDV garlic infections was reported in Greece and Iran; in Greece, the virus infects almost 100% of plants, which renders it the most prevalent garlic viral pathogen. In comparison to GCLV, whose garlic infection rates differed in the particular regions of the country, OYDV infected over 95% of plants in every region [Dovas et al. 2001b]. In Iran [Shahraeen et al. 2008] and Italy [Dovas and Vovlas 2003], OYDV was identified in 91.5% and 98% of garlic plants, respectively. As in the case of GCLV, OYDV was rarely detected in garlic cultivated in Brazil. Depending on the region of the country, the infection rate was between 3 and 21.5% of plants. In plants propagated from non-virus-free material, the rate of this viral infection was 4-fold higher than in virus-free plant material [Fayad-André et al. 2011].

In our study, the prevalence of the Leek yellow stipe virus (LYSV) was the lowest, as it was detected in 32.1% of the plants. Harnaś, Kna 210, Mega, Botanik and genotype L13 were not infected by this virus. A similar LYSV prevalence rate in garlic was reported from the Czech varieties of this species [Klukáčková et al. 2007]. The percentage of infected plants reached 31.2; however, the Vekan variety was free from this virus and exhibited a low level of infection with the Onion yellow dwarf virus, what indicates considerable resistance of this variety to potyviruses. In Iran, the LYSV prevalence was 40.6%; material sampled from four of the nine regions was virus-free, whereas the proportion of infected garlic plants in the other regions ranged from 60 to 86.2% [Shahraeen et al. 2008]. In Brazil, LYSV was detected the most frequently of all the viruses prevalent in Garlic cultivations throughout the country, and the infection rate in the plants examined reached 30–46%. Use of virus-free material for propagation of garlic reduced the LYSV occurrence rates by even 45% [Fayad-André et al. 2011]. The prevalence of the LYSV in the Polish garlic varieties was low. Only one virus, i.e. GCLV, infected the whole plant, while the other two of the viruses examined, OYDV and LYSV, were detected primarily in bulbs. Dovas et al. [2001b] have demonstrated that all the three viruses infected garlic leaves, which may imply that the strains of the viruses recognized in the investigations of garlic infections in Poland differ in tissue specificity from those detected in Greece.

In Poland and the Czech Republic, infection of *A. sativum* with the Potyviruses (OYDV and LYSV) was less prevalent that the infection with the carlavirus (GCLV), which may suggest higher resistance of varieties cultivated in these countries to potyviruses or lower occurrence of vectors of these pathogens. Potyviruses are more economically important, as they produce distinctive disease symptoms, whereas infections with the carlavirus GCLV are asymptomatic [Takaichi et al. 1998, Klukáčková et al. 2007].

Latent asymptomatic viral infections cause lower yield losses than overt infections that lead to manifestation of disease symptoms. Therefore, the highest garlic yield losses have been reported from Greece and Iran, where the rate of plant infection with potyviruses is higher than that with carlaviruses [Dovas et al. 2001b, Shahraeen et al. 2008]. The most efficient mode of garlic virus control is to eliminate these pathogenic agents from plants by approval of pathogen-free propagation material for trade. This approach has substantially improved the health of garlic crops in Brazil. It should be emphasized that the rates of garlic infection prevalence are related the size of aphid populations in a particular region. The greater the number of pathogen-transmitting vectors is, the more plants may be potentially infected [Takaichi et al. 1998, Fayad-André et al. 2011].

A comparison of our study results with literature data indicates a great impact of environmental conditions on the biology of garlic development. This refers not only to susceptibility to various pathogenic infections but also to the capability of seed production. Shemesh Mayer et al. [2013] have reported that some *A. sativum* genotypes that produced seeds in the Asian climate gradually lost this capability in cultivations located in Israel.

The present study shows that all the viruses analysed have been detected in the commercial garlic cultivars, while none of the viruses have been reported to infect the botanical species from the genus *Allium*. The viruses were identified in various organs, most frequently in the bulbs, and less frequently the inflorescences and leaves. The differences in the degree of infection of the plants between the two experimental plots were insignificant. In the light of our study and other authors' reports, it is difficult to support the hypothesis proposed by Konwicka [1973], who claims that failure to produce seeds in garlic results from degenerative diseases caused by mycoplasmas, rickettsiae, and viruses. Viral infections can not be considered as the only cause of garlic sterility. The mode of propagation seems to be the mechanism that enhances or reduces susceptibility to infection with these viruses. Propagation via seeds may interrupt the infection chain, whereas vegetative reproduction promotes transmission of these pathogens due to tissue continuity and sustenance of latent and asymptomatic infections, which does not allow selection of appropriate propagation material. Evidently, infected plants produce reduced yield; yet, presence of viruses in plant tissues is not a direct cause for the sterility of this species. Therefore, research aimed at obtaining garlic seeds is one of the ways to produce virus-free crops.

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ROZPOWSZECHNIENIE ZAKAŻEŃ ROŚLIN Z RODZAJU *Allium* PRZEZ WIRUSY ŻÓŁTEJ KARŁOWATOŚCI CEBULI (OYD), ŻÓŁTEJ SMUGOWATOŚCI PORA (LYS) ORAZ WIRUS LATENTNY CZOSNKU (GLV)

Streszczenie. Czosnek jest rośliną ważną ze względu na swoje użytkowe i lecznicze właściwości, które zawdzięcza bioaktywnym komponentom występującym w podziemnej cebuli oraz w młodych liściach. Najbardziej rozpowszechniony jest azjatycki ekotyp *A. sativum* posiadający diploidalny kariotyp wynoszący 2n = 16. Rośliny o takim genotypie są niepłodne i nie zawiązują nasion w warunkach naturalnych, dlatego są rozmnażane wyłącznie w sposób wegetatywny poprzez cebulki powietrzne lub ząbki z podziemnych cebul. Rozmnażanie wegetatywne wiąże się z możliwością przenoszeniem patogenów, w tym wirusów, co bezpośrednio wpływa na obniżenie jakości plonu, a zabiegi odkażające materiał reprodukcyjny istotnie zwiększają koszty uprawy. Większość znanych wirusów roślinnych nie jest przenoszona przez nasiona lub są przenoszone za ich pośrednictwem tylko w ograniczonym stopniu. Z tego względu rośliny rozmnażane generatywnie, np. cebula i por, rozpoczynają swój cykl życiowy jako wolne od wirusów. Czosnek, który nie posiada takiej możliwości i rozmnaża się tylko drogą wegetatywną zawsze przenosi wirusy na kolejne pokolenia. Zjawisko to jest bardzo rozpowszechnione, ponieważ przeżywalność wielu wirusów w tkankach roślinnych jest wysoka i nawet jeżeli roślina nie wykazuje objawów porażenia, może być nosicielem wirusa. Celem niniejszej pracy była weryfikacja tezy, że przyczyną sterylności u *A. sativum* są infekcje patogeniczne. W tym celu przeprowadzono badania mające na celu wykrywanie wirusów GCL, OYD i LYS oraz określenie stopnia ich rozprzestrzenienia w uprawianych i ozdobnych gatunkach roślin z rodzaju *Allium*. Do badań użyto dwu grup roślin z rodzaju *Allium*. Pierwsza grupa obejmowała 10 gatunków botanicznych, drugą grupę stanowiło 7 handlowych odmian

A. sativum oraz 2 genotypy. GCL, OYD i LYS w liściach, kwiatostanach i cebulach wykrywano za pomocą testu ELISA. Wszystkie rośliny z pierwszej grupy obejmującej gatunki botaniczne rodzaju *Allium* były wolne od badanych wirusów. Natomiast w drugiej grupie *A. sativum* stwierdzono dużą częstotliwość występowania infekcji GCLV, OYDV oraz LYSV odpowiednio 88,2, 75 i 32,1%. Stopień porażenia poszczególnych części roślin był zróżnicowany. Analiza wyników badań własnych w świetle danych literaturowych wskazuje na znaczny wpływ warunków środowiskowych na biologię rozwoju czosnku – dotyczy to głównie podatności na infekcje różnymi patogenami. Można postawić więc wniosek, że sterylność czosnku jest spowodowana wieloma czynnikami, w tym również infekcjami wirusowymi.

Słowa kluczowe: *Allium*, odmiany czosnku, GCLV, OYDV, LYSV, test ELISA

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