RESEARCH ARTICLE

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Antiviral activity of sukomycin against Potato Virus Y and Tomato Mosaic Virus

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ABSTRACT
Potato Virus Y (PVY) and Tomato Mosaic Virus (ToMV) are one of the most important plant viruses that strongly influence the quality and quantity of vegetable production and cause substantial losses to farmers. The most conventional and common method of pest and disease control is through the use of pesticides. Unfortunately, most of them are synthetic compounds without antiviral activities and possess inherent toxicities that endanger the health of farm operators, consumers and the environment. In order to carry out a control of viral infections in plants and to reduce the loss of production it is necessary to search for alternative and environmentally friendly methods for control. Sukomycin is a complex of substances with antimicrobial and antiviral activities produced from *Streptomyces hygroscopicus* isolated from soil. This natural complex significantly reduces symptoms and DAS-ELISA values of Potato virus Y and Tomato mosaic virus in tobacco plants.

Key words: Sukomycin, PVY, ToMV

Introduction

PVY is the most common viral pathogen found in potato and tobacco, and it infects plants of a wide range of species, primarily within the family *Solanaceae* (Danci et al., 2009). PVY is distributed all over the world and causes losses in potato, tobacco, tomato and pepper production in the form of reduced yield or quality (Singh et al., 2008). The virus is transmitted non-persistently by aphids of more than 50 species (Radcliffe & Ragsdale, 2002). Three main distinct groups of strains of PVY have been described (Singh et al., 2008): the common group PVY^{O}, the stipple streak group PVY^{C} and the tobacco veinal necrosis group PVY^{N}. As a result of genomic recombination between viruses of the PVY strain groups, additional necrotic strains have emerged, including the recombinant PVY^{NTN} and PVY^{NW} (Glais et al., 2002).

Six different PVY strains are differentiated in potatoes in Bulgaria. These are PVY^{N}, PVY^{NTN}, $PVY^{N/NTN}$, $PVU^{N:O}$, PVY^{O} and PVY^{C} (Petrov, 2012; Petrov & Gaur, 2015).

Tobacco PVY strains are considered as "strong" and "mild" according to their ability to cause necrosis on some tobacco genotypes (Gooding, 1985). Three strain groups have been distinguished: MsMr, MsNr and NsNr, according to their reaction with tobacco cultivars which were resistant or sensitive to *Meloidogyne incognita*. A virus strain group was found - VAM-B in tobacco plants, breaking the resistance of the cultivars (Blancard, 1994). Latore & Flores (1985) suppose that tobacco genotype VAM can be used as additional host for genotyping PVY tobacco strains.

Other economically important viruses on tobacco are Tobacco mosaic virus (TMV) and Tomato mosaic virus from Tobamovirus genus. ToMV is distinguished from TMV by its ability to produce local necrotic lesions in *Nicotiana tabacum* var. White Burley and *N. sylvestris* (Green & Kim, 1991). ToMV strains include those, which cause corky ring, crusty fruit, yellow streak and aucuba symptoms (Kang et al., 1981; Jones et al., 1991).

In Bulgaria ToMV was first reported by Kovachevski (1977). Consequently, it is not easy to correctly identify ToMV by symptoms because it causes a variety of them. However, common ToMV symptoms known include mosaic, systemic chlorosis, local necrotic lesions, leaf abscission, as well as systemic leaf and stem necrosis, which ultimately cause death (Brunt et al., 1990; Green & Kim, 1991; Jones et al., 1991). The virus is transmitted by human activities, through seed, and from leaf and root debris (Green & Kim, 1991). It is also readily sap-transmissible and cosmopolitan (Brunt et al., 1990).

Up to now there is no effective antiviral drug against plant viruses in tobacco. Sukomycin is a complex of substances with antiviral activities extracted from *Streptomyces hygroscopicus* from soil. The active ingredient with antimicrobial activity is believed to be nigericin. This extract from Streptomyces has effect against different bacteria, fungi, *Trichomonas vaginalis*, Herpes viruses (Tishkov et al., 1989) and Tobacco mosaic virus. Its potential is not fully established.

The aim of this study is to test the effect of sukomycin against PVY and ToMV in test tobacco plants.

Materials and Methods

Substances: Sukomycin extracted from *Streptomyces hygroscopicus*.

S. hygroscopicus was grown in media at 28°C for 120 hours with aeration and agitation. The fermentation broth was filtered and wet mycelial cake was extracted twice with 80% ethanol (1:2 w/v) followed by centrifugation. The combined extracts were concentrated under reduced pressure at 45°C to 10 fold volume reducing. The residual aqueous suspension was extracted 3 times with petroleum ether (1:1 w/v) and the combined organic layers were vacuum evaporated to give an oily residue. The oil was suspended in NaOH (1:4 w/v) and cooled at 4°C over night. After centrifugation the clear solution was vacuum evaporated and dried. The residue was dissolved in diethyl ether (1:3 v/v). The ether solution was treated with 1M NaOH (2:1 v/v) and the aqueous layer was still extracted twice with ether. The combined ether extracts were dried with Na_2SO_4 and were vacuum evaporated and dried. The residue was treated with n-hexane and the precipitated fine crystals of Na-salt of the complex were filtered and washed 3 times with minimal amounts of n-hexane. After that they were dried under reduced pressure.

Treatment of plants and inoculation with viruses (ToMV and PVY):

Tobacco plants were divided into four groups: 1/ treated plants with the extracts before the relevant virus inoculation; 2/ Not treated plants, only inoculated with the relevant virus (K - infected); 3/ treated plants with the extracts only (K-healthy, for toxicity) and 4/ Not treated and not inoculated plants (K-water treated). Tobacco plants cv. Samsun was grown at 22-25°C, 75-85% relative humidity, constant photo-period of 16/8 hours, light intensity 3000 lux. The reporting of the symptoms was made 7-25 days after virus inoculation. Plants were treated one day before artificial infection with the relevant virus strain by water dilution of the extracts. Sprays were conducted in a greenhouse at a temperature of 21°C to 24°C and a relative humidity of 45% with a dose of 5-15 ml solution of extracts. Tobacco plants were inoculated with the relevant virus according to Noordam (Noordam, 1973).

DAS - ELISA: We used the method of Clark and Adams, according to DAS-ELISA kit for the relevant virus (LOEWE Biochemica GmbH, Germany) for estimation antiviral activity of the extracts in vivo in tobacco plants cv. Samsun (Clark & Adams, 1977). Plants were tested with DAS-ELISA for the relevant virus using sap from homogenized potato leaves. Micro titer ELISA plate wells were coated with the relevant virus IgG polyclonal antiserum diluted in 0.05 M carbonate buffer (pH 9.6) according to the supplier's specifications. Plates were incubated for 4 h at 37°C, followed by 3, 5-minute washing steps with PBS-T buffer and then loading with homogenized in coating buffer with 1% PVP and albumin (BSA) plant extracts. After that plates were incubated at 4°C overnight. After washing off the crude plant extract, virus was detected by the relevant virus antibodies conjugated with alkaline phosphatase and diluted in conjugate buffer according to the supplier's specifications in incubation step for 4h at 37°C. P-nitro phenyl phosphate diluted in diethanolamin buffer (1mg ml^{-1}, pH 9.8) is a substrate for the alkaline phosphatase enzyme reaction which runs at room temperature and after coloring is stopped with 3N NaON. Optical density at 405 nm was measured by Multifunctional detector DTX 880 (Beckman, USA). Tissue samples from healthy and infected plants were used as negative and positive controls. Positive results are these that exceed three times optical density of the negative control (positive result > 3x 0.297 OD (ToMV) = 0.891; positive result > 3x 0.277 OD (PVY) = 0.831). Therefore, tested samples, with OD value more than 0.9, were considered positive for virus infection.

Results

Until the 7th day after inoculation with PVY virus control tobacco plants cv.Samsun NN (inoculated with PVY only and not treated) remained symptomless and healthy (Figure 1). The DAS-ELISA values of these plants remained under the cut off, with values around 0.277 (Table 1). Visible symptoms of PVY infection were observed after the 14th day consisting of chlorotic and necrotic patterns and leaf deformation (Figure 2).

Figure 1. *Healthy tobacco plant cv. Samsun NN.*

Figure 2. *Mosaic and necrotic symptoms of tobacco plant cv. Samsun NN inoculated with PVY.*

Plants were treated with different % water dilution of sukomycin from 0.01% to 20%. Water dilutions from 0.01 to 6% did not reduce enough DAS-ELISA values of PVY and they remained above the cut off, ranging from 3.000 to 0.816 (Figure 5, Table 1). However, treatment with 1% water dilution of sukomycin reduced development of virus symptoms significantly. Treatment under 1% of sukomycin had no effect on the virus infection in the tested tobacco plants (Figure5). 3% of sukomycin reduced the DAS-ELISA values from 3 to 1 (Figure 5). Ten and twenty % sukomycin reduced the DAS-ELISA values of PVY under the off and plants were symptomless and considered virus free (Figure 5, Table 1).

ToMV was completely different plant virus from PVY and tobacco plants reacted differently. Until the 2nd day after inoculation with ToMV virus control tobacco plants (inoculated with ToMV only and not treated) remained symptomless and healthy (Figure 1). The DAS-ELISA values of these plants remained under the cut off, with values around 0.297 (Table 1). Visible symptoms of ToMV infection were observed after the 3rd day consisting of small necrotic spots on the leaf lamina (Figure 3).

Figure 3. *Necrotic symptoms of tobacco plant cv. Samsun NN inoculated with ToMV.*

Dilutions from 0.01 to 0.5 % of sukomycin reduceed development of virus symptoms to the stage of a healthy plant (without any necrotic spot), but DAS-ELISA values of ToMV remained above the cut off, ranging from 2.7 to 1.4 (Figure 4, Table 1). Treatment under 0.5% of sukomycin had no effect on the virus infection in the tested tobacco plants (Figure 4). 8 % of sukomycin reduceed the DAS-ELISA values from 2.7 to 0.4 (Figure 4) which was under the cut off. Using higher % from 8 of sukomycin reduced the DAS-ELISA values of ToMV significantly considering plants virus free (Figure 4, Table 1).

Discussion

The present research was based on the antiviral effect of polyether complex extracted from *S. hygroscopicus* called sukomycin with active antiviral ingredient Nigericine against economically important tobacco viruses ToMV and PVY. Different % water concentrations from this complex were used. Water concentration of 8 % Sukomycin was the lowest concentration that reduces DAS-ELISA values of ToMV and PVY inoculated plants under the Cut off straight line (Figure 4, Figure 5, Table 1). Concentration of 0.5 % for ToMV and 1% for PVY were enough to stop development of virus infection. Concentrations lower than 1% were not sufficient to control the viral infections. In all tested plants phytotoxic effect of 20% dilutions of the sukomycin was not observed.

These findings were of great importance given the lack of antiviral drugs with natural origin and no phytotoxicity. Using this substance will reduce damages and loss of plant production caused by the virus infections. Presented production technology was inexpensive and easy to apply. Similar results were achieved with 10% water concentration of liquid fraction and 5% of soft fraction of methanol extracts from *Hypericum perforatum.* They reduced significantly DAS-ELISA values of PVY in virus inoculated tobacco plants (Petrov et al., 2015). Extracts from natural products had a great potential for controlling virus diseases but there is a lot to be done for future research, considering testing different viruses and plant cultivars.

Table 1. *DAS-ELISA Mean values with ±SD of inoculated tobacco plants with ToMV and PVY.*

Variants		Mean ToMV inoculated	±SD	Mean PVY inoculated	±SD
	20	0.293	± 0.012	0.219	± 0.016
	10	0.279	± 0.016	0.334	± 0.038
	9	0.348	± 0. 022	0.417	± 0.101
	8	0.466	± 0. 017	0.481	± 0.126
	7	0.691	± 0.024	0.5	± 0.106
	6	0.723	± 0.018	0.816	± 0.09
% of Sukomycin	5	0.714	±0.03	0.882	± 0.083
	4	0.816	± 0.036	1.123	± 0.091
	3	0.773	± 0.032	1.072	± 0.112
	2	0.902	± 0.040	2	± 0.204
	1	0.849	± 0.036	1.925	± 0.166
	0.5	1.465	± 0.052	2.112	± 0.147
	0.1	1.312	± 0.084	3	± 0.219
	0.05	1.981	± 0.107	3	± 0.382
	0.01	2.718	± 0. 195	3	± 0.274
K healthy		0.297	± 0.012	0.277	± 0.003
K infected		2.872	± 0.098	3	± 0.108
K water treated		0.318	± 0.018	0.243	± 0.007

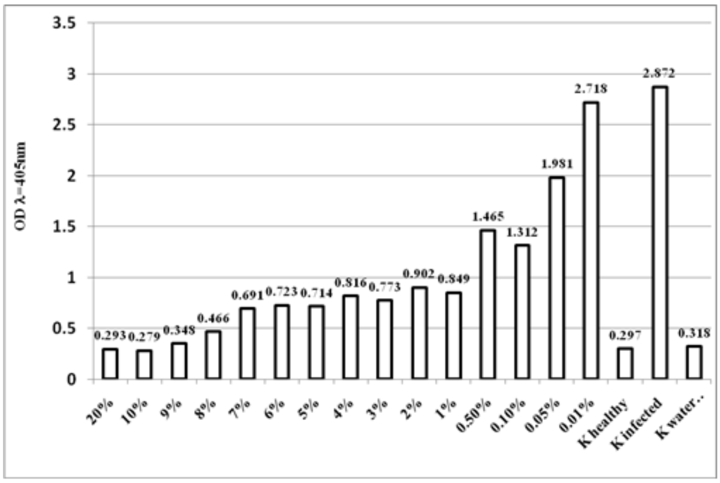

Figure 4. *DAS-ELISA results for ToMV infection of the treated tobacco plants with water dilution (%) of sukomycin.*

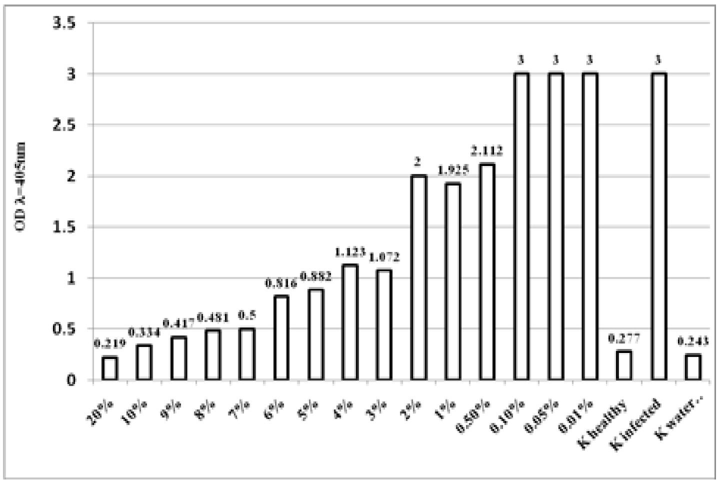

Figure 5. *DAS-ELISA results for PVY infection of the treated tobacco plants with water dilution (%) of sukomycin.*

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