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Antiviral activity of plant extract from *Tanacetum vulgare* against Cucumber Mosaic Virus and Potato Virus Y

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ABSTRACT
Cucumber mosaic virus (CMV) and Potato virus Y (PVY) have been described among the top five important viruses infecting vegetable species worldwide. They cause severe damages in fruits and cultivated plants. There is currently no available effective pesticide to control these viral diseases. Higher plants contain a wide spectrum of secondary metabolites such as phenolics, flavonoids, quinones, tannins, essential oils, alkaloids, saponins, sterols and others. Extracts prepared from different plants have been reported to have a variety of properties including antifungal, antiviral and antibacterial properties against pathogens. *Tanacetum vulgare* (Tansy) is native to Europe, Asia, and North Africa. It has many horticultural and pharmacological qualities. *T. vulgare* is principally used in traditional Asian and North African medicine as an antihelminthic, antispasmodic, stimulant to abdominal viscera, tonic, antidiabetic and diuretic, and it is antihypertensive. In our research we established antiviral effect of methanol extract from *T. vulgare* against CMV and PVY in tomato plants.

Key words: *Tanacetum vulgare*, PVY, CMV

Introduction

PVY is one of the first described Potiviruses. It was first reported in potatoes in England and is the type species of the genus Potyvirus (Smith, 1931). Typical symptoms of PVY in tomato include mosaic, vein chlorosis, mild mottling, dark brown necrosis on leaflets, severe necrosis, leaf crinkling, and drooping (Jones et al., 1991). First report of PVY in Bulgaria was done by Kovachevski, who found symptoms on pepper (Kovachevski, 1942). Hristova and Kaytazova detect PVY on tomato and red pepper in Bulgaria (Hristova & Kaytazova, 1996). In 2001 PVY was found in mixed infection with CMV in some regions in Bulgaria, causing lethal necrosis and death of tomato plants (Hristova, 2002). Six different PVY strains are differentiated in potatoes in Bulgaria. These are PVYN, PVYNTN, PVYN/NTN, PVUN:O, PVYO and PVYC (Petrov, 2012; Petrov & Gaur, 2015). PVY symptoms in tomato leaves are mainly mosaic, chlorosis and distortion (Petrov, 2014a). In tomato fruits mild necrosis are observed (Petrov, 2014a).

Serious CMV infections have also occurred in tomato, causing significant yield losses by reducing fruit production and quality (Jorda et al., 1992). In Bulgaria, CMV was first reported by Kovachevski (Kovachevski, 1965). CMV epidemics have also been reported in tomato growing regions of Italy (Kaper et al., 1990), Spain (Jorda et al., 1992) and China (Kearney et al., 1990). CMV symptoms in infected tomato include stunting of vegetative growth, distortion and mottling of new growth, and a characteristic shoestring-like leaf appearance (Zitter, 1991). Strains from both subgroups have been observed to infect tomatoes grown in the same field and sometimes mixed infection in the same plant have occurred (Ilardi et al., 1995). Cucumber mosaic virus (CMV) is one of the five most important viruses which affect production and cause significant economic loss in many vegetable and horticultural crops (Palukaitis et al., 1992). CMV is difficult to control because of its extremely broad natural host range and there are no sources of genetic resistance to CMV available in commercial fresh-market tomato cultivars (Sikora et al., 1998). Different approaches are currently under way to find suitable control measures for CMV. Efforts using traditional breeding for CMV resistance have mostly not been successful (Watterson, 1993).

CMV symptoms in tomato leaves are mosaic, necrosis and leaf deformation (Petrov, 2015). A good perspective is to screen natural substances with antiviral effect in different plant cultivars. Such substances are produced by medicinal plants. One of these plants is *Tanacetum vulgare*.

T. vulgare L. (*Asteraceae*, common name tansy) is native to Europe, Asia, and North Africa (Figure 1). It has been introduced to other parts of the world, and in some areas it has become invasive. Tansy has many horticultural and

pharmacological qualities. It can be cultivated and it is used in companion planting, for biological pest control and sustainable agriculture. Tansy is planted alongside potatoes to repel the Colorado potato beetle. According to findings of Schearer (1984), tansy reduced beetle populations by 60-100%.

Figure 1. *T. vulgare.*

T. vulgare is principally used in traditional Asian and North African medicine as an antihelminthic, carminative, antispasmodic, stimulant to abdominal viscera, tonic, emmenagogue, antidiabetic, diuretic and it is antihypertensive (Stevovic et al., 2009). Raw or cooked young leaflets and the button-like flowers are used as garnish and in flavoring (Facciola, 1990). The bitter tea made from the leaves and flowering stems (Facciola, 1990) has been effectively used for centuries as a vermifuge. An infusion of the leaves or of the whole plant is used as an emmenagogue and for treating menstrual irregularities (Weiner, 1980). The leaves and flowering tops are said to have anthelmintic, antispasmodic, carminative, stimulant and tonic properties (Weiner, 1980). It is also used in treating hysteria, migraine, neuralgia, rheumatism, kidney weakness, stomach problems, and fever (Abad et al., 1995; PDR-HM, 2004). In addition, tansy extract has been reported to exhibit antitumor (Konopa et al., 1967), anti-inflammatory (Schinella et al., 1998) and antioxidant (Bandoniene et al., 2000; Mantle et al., 2000) properties. In regard to the antitumor properties of the herb, some studies on human cancer cell lines from breast, colon, lung and acute T leukemia indicate in vitro cytotoxic activity of *T. vulgare* extracts (Ramirez-Erosa et al., 2007; Wegiera et al., 2012). Extract of *T. vulgare* also inhibited mouse leukemia L1210 cells (Goun et al., 2002).

A large number of constituents have been identified in tansy, including thujone, camphor, alpha-pinene, borneol, tanacetin, sesquiterpenes, flavonoids, hydroxycoumarins, polyynes, sterols, tannic acid and resins (Chandler et al., 1982; PDR-HM, 2004).

In Bulgaria, the plant grows in all phytogeographical areas from 0 to 2000 m asl. For many years the herb has been widely used in traditional medicine for treating rheumatism, fever, gout, epilepsy, digestive disorders, helminthes and others. *T. vulgare* is toxic and its consumption may cause convulsions and even death. The intake of this plant should be done only on a medical doctor's prescription. At present, some pharmacopoeias have described the use of tansy in some medicines for treatment of colds and fever (LeCain & Sheley, 2006).

The present investigation was carried out to evaluate the safety of a methanol extract of *T. vulgare* by determining its potential antiviral effect after virus inoculation in tomato plants.

Materials and Methods

Extractions:

Fresh plant flowers from *T. vulgare* were collected and oven-dried (45°C) to absolute dry weight. Methanol was used as a solvent. Extractions were prepared in Soxhlet extractor, at water bath (80°C), for 4-5 hours. Methanol extracts were concentrated in vacuum evaporator at 55°C, 300 mbar. After the evaporation of the solvent, the concentrates were divided into liquid and soft fractions at 70°C, 72 mbar. The liquid fraction was diluted in water (%, v/v) up to 24 h before the assay. The soft fraction was diluted in water (%, w/v) just before the assay.

Treatment of plants and inoculation with CMV and PVY:

Tobacco plants were divided into four groups: 1) treated plants with the extracts before 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). Tomato plants cv. Ideal 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 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. Tomato plants were inoculated with the relevant virus according to Noordam (1973).

DAS - ELISA:

We used the method of Clark and Adams (Clark & Adams, 1977), according to DAS-ELISA kit for PVY (LOEWE, Germany) for estimation antiviral activity of the extracts in vivo in tobacco plants cv. Samsun.

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 (LOEWE Biochemica GmbH, Sauerlach, Germany) 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 was run at room temperature and after coloring it was 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. The results that exceeded three times optical density of the negative control were considered positive (positive result > 3x 0.305 OD (CMV) = 0.915; positive result > 3x 0.228 OD (PVY) = 0.684). Therefore, the tested samples with OD value more than 0.915 (for CMV) and 0.684 (for PVY) were considered positive for virus infection.

Results

Tomato plants were treated with % water concentrations of methanol extracts from 0.1% to 30% soft fraction and 0.5 to 30 % liquid fraction.

Tomato control plants cv. Ideal (inoculated with PVY only and not treated) needed at least 14 days after inoculation with PVY to develop symptoms. Before the 14th day they remained symptomless (Figure 2). The DAS-ELISA values of these plants remained under the cut off, about 0.228 (Table 1). Visible symptoms of PVY infection were observed after the 15th day as typical venous necrosis starting from the basis of the leaf and spreading by vascular tissues all over the plant (Figure 4). Mixed infection from PVY and CMV induced severe necrosis leading to drying of the tomato leaves (Figure 5).

Concentration from 0.5 to 5 % of Liquid fraction of methanol extracts did not reduce enough DAS-ELISA values of PVY and they remained above the cut off, ranging from 3.000 to 0.75 (Figure 7, Table 1). Treatment with 5% Liquid fraction reduced development of virus symptoms. 3% had no effect on the virus infection (Figure 7). 20 % of Soft fraction reduced the DAS-ELISA values from 3 to 0.365 (Figure 7, Table 1), but 10 % reduced development of symptoms.

Unlike PVY, CMV virus control tomato plants (inoculated with CMV only and not treated) remained symptomless and healthy until the 21st day (Figure 2). The DAS-ELISA values of these plants remained under the cut off, with Mean value 0.305 (Table 1). Visible symptoms of CMV infection were observed after the 21th day such as leaf mosaic and chlorosis (Figure 3).

Figure 1. *Healthy tomato plant cv. Ideal.*

Figure 3. *Necrotic symptoms of tomato plant cv. Ideal inoculated with CMV.*

Figure 4. *Necrotic symptoms of tomato plant cv. Ideal inoculated with PVY.*

Figure 5. *Necrotic symptoms of tomato plant cv. Ideal inoculated with PVY and CMV.*

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Table 1. *DAS-ELISA Mean values with ±SD of inoculated tomato plants with CMV and PVY: Mean – average value of three repeats; SD – standard deviation.*

Variants		Mean CMV inoculated	±SD	Mean PVY inoculated	±SD
% Methanol liquid fraction	30	0.271	± 0.018	0.219	± 0.009
	20	0.299	± 0.031	0.305	± 0.017
	10	0.312	± 0.023	0.285	± 0.052
	5	0.309	± 0.042	0.875	± 0.116
	3	0.716	± 0.118	1.528	± 0.111
	1	1.412	± 0.219	3	± 0.138
	0.5	2.875	± 0.145	3	± 0.281
% Methanol soft fraction	30	0.319	± 0.026	0.311	± 0.015
	20	0.361	± 0.038	0.365	± 0.028
	10	0.354	± 0.112	0.724	± 0.118
	5	0.718	± 0.109	1.252	± 0.174
	3	1.697	± 0.124	2.345	± 0.129
	1	1.318	± 0.165	2.867	± 0.184
	0.5	1.908	± 0.213	3	± 0.277
	0.1	2.589	± 0.274	3	± 0.318
K healthy		0.305	± 0.003	0.228	± 0.005
K infected		2.856	± 0.098	3	± 0.07
K water treated		0.291	± 0.005	0.213	± 0.007

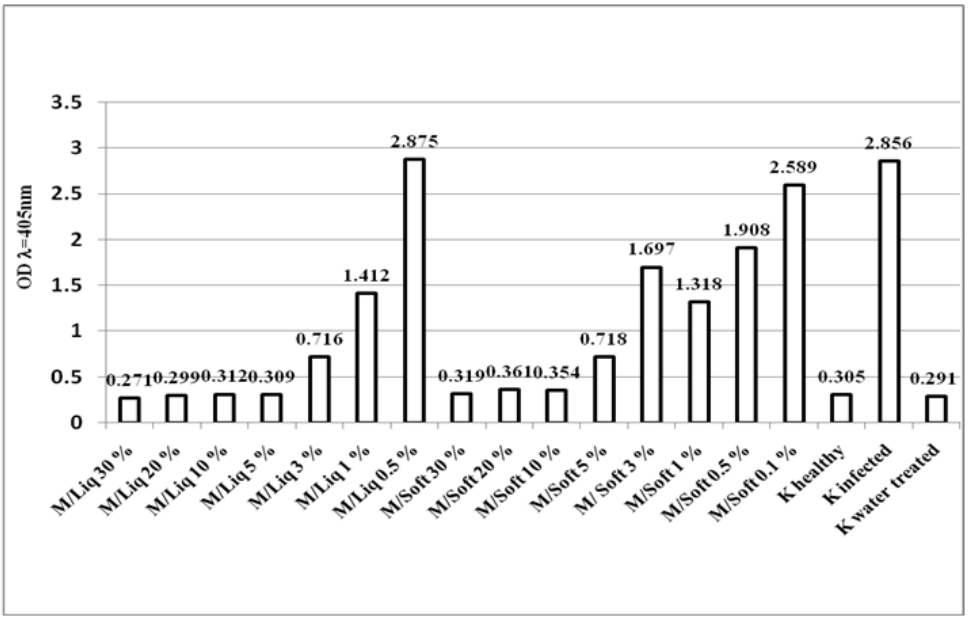

Figure 6. *DAS-ELISA results for CMV infection of the treated tomatoplants with liquid and soft fractions (water dilution, %) of methanol extract from T. vulgare: M/Liq- Liquid methanol fraction; M/Soft – Soft methanol fraction.*

Concentrations from 3 % of Liquid fraction and 5 % Soft fraction of methanol extracts from *T. vulgare* reduced development of virus symptoms, but DAS-ELISA values of CMV remained above the cut off, ranging from 2.875 to 0.716 (Figure 6, Table 1). 5 % of Liquid fraction and 10% soft fraction reduced the DAS-ELISA values to 0.309 (Figure 6) which is under the cut off.

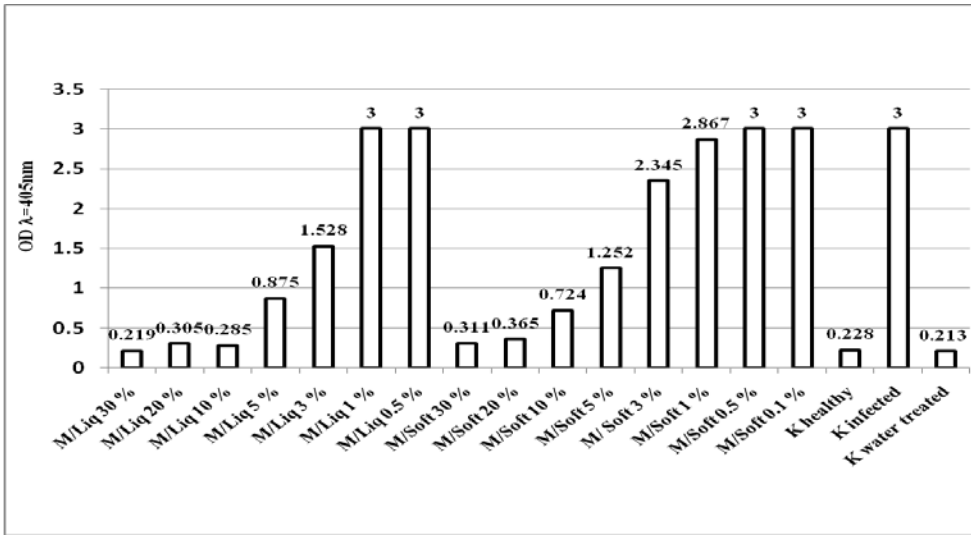

Figure 7. *DAS-ELISA results for PVY infection of the treated tomatoplants with liquid and soft fractions (water dilution, %) of methanol extract from T. vulgare: M/Liq- Liquid methanol fraction; M/Soft – Soft methanol fraction.*

DISCUSSION

In our research we demonstrated the antiviral effect of methanol extract from *T. vulgare* against economically important tomato viruses - CMV and PVY. We prepared different % water concentration from these methanol extracts and sprayed the tomato plants. Concentration of more than 20 % of *T. vulgare* reduced DAS-ELISA values of CMV and PVY inoculated plants under the Cut off straight line (Figure 6; Figure 7; Table 1). Concentration of 5 % for CMV and 10% for PVY were enough to stop development of virus infection. Concentrations lower than 5% was not sufficient to control the viral infections. In all tested plants phytotoxic effect of 30% of the extract was not observed.

Using extracts from medicinal plants would reduce damages caused by the virus infections. Other natural products that induced Systemic acquired resistance (SAR) against plant viruses were BION and EXIN. Treatment of plants with this combination resulted in 92% protection against PVY in tomato (Petrov & Andonova, 2012). Application of these elicitors resulted with 85 % reduction of the CMV infection in tomatoes by Inducing SAR (Petrov, 2014b), 78% against CMV in pepper (Petrov, 2014c), against ToMV and CMV in tomato (Petrov, 2014d) and against PVY in pepper (Petrov, 2014e).

Blocking the virus replication of PVY in another natural way was achieved by induction of posttranscriptional gene silencing with siRNAs against HC-Pro gene of PVY in potatoes cv. Agria (Petrov et al., 2015a) and cv. Arinda (Petrov et al., 2015b).

Another medicinal plant *Hypericum perforatum* also expressed antiviral activity. 10% water concentration of liquid fraction and 5% of soft fraction of methanol extracts from *H. perforatum* reduced significantly DAS-ELISA values of PVY in virus inoculated tobacco plants (Petrov et al., 2015c).

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References

Abad MJ, Bermejo P, Villar A. 1995. An approach to the genus *Tanacetum* (L.) (Compositae): Phytochemical and pharmacological review. Phytotherapy Research 9: 79–92.

Bandoniene D, Pukalskas A, Venskutonis P, Gruzdiene D. 2000. Preliminary screening of antioxidant activity of some plant extracts in rapeseed oil. Food Research International 33: 785–791.

Chandler RF, Hooper S, Hooper D, Jamieson W, Lewis E. 1982. Herbal remedies of the Maritime Indians: sterols and triterpenes of *Tanacetum vulgare* L. (Tansy). Lipids 17: 102–106.

Clark MF and Adams A. 1977. Characteristics of the micro plate method of enzyme inked immunosorbent assay for the detection of plant viruses. J. Gen. Virol, 34: 475-483.

Facciola S. 1990. Cornucopia—A Source Book of Edible Plants. Kampong Publications, Vista, CA, USA.

Goun EA, Petrichenko VM, Solodnikov SU. 2002. Anticancer and antithrombin activity of Russian plants. J Ethnopharmacol. 81(3): 337–342.

Hristova D, Kaytazova P. 1996.Viral diseases on tomato in Bulgaria during the period 1986-1994. Plant sciences (BG), 33 (3): 57-59.

Hristova D. 2002. Monitoring of lethal necrosis on tomato in Bulgaria in 2001. Proceedings of the tenth congress of the Bulgarian microbiologists, Plovdiv, Bulgaria, 358 – 361.

Ilardi V, Mazzei M, Loreti S, Tomassoli L and Barba M. 1995. Biomolecular and Serological Methods to Iden- tify Strains of Cucumber Mosaic Cucumovirus on Tomato. OEPP Bulletin, 25: 321-327.

Jones JB, Stall R and Zitter T. 1991. Compendium of Tomato Diseases. American Phytopathological Society, 73 pp.

Jorda C and Alfaro A. 1992. Epidemic of Cucumber Mosaic Virus plus Satellite RNA in Tomatoes in Eastern Spain. Plant Disease, 76: 363-366.

Kaper JM, Gallitelli D and Tousignant M. 1990. Identification of a 334-Ribonucleotide Viral Satellite as Principal Etiological Agent in Tomato Necrosis Epidemic. Research in Virology, 141: 81-95.

Kearney CM, Gonsalves D and Provvidenti R. 1990. A Severe Strain of Cucumber Mosaic Virus from China and Its Associated Satellite RNA. Plant Disease, 74: 819-823.

Konopa J, Jereczek E, Matuszkiewicz A. 1967. Screening of antitumor substances from plants. Arch Immunol Ther Exp, 15(1):129–132.

Kovachevski I. 1942. Viruses on pepper. Archive of the Bulgarian Agricultural Society (BG), 25-102.

Kovachevski I., 1965. Cucumber Mosaic Virus (CMV) in Bulgaria. Bulgarian Academy of Science, Sofia, Bulgaria, 79 pp. (in Bulgarian).

LeCain R and Sheley R. 2006. Common tansy (*Tanacetum vulgare*). Montana State University Extension MontGuide

Mantle D, Eddeb F, Pickering A. 2000. Comparison of relative antioxidant activities of British medicinal plant species in vitro. Journal of Ethnopharmacology 72: 47–51.

Noordam D. 1973. Identification of plant viruses: methods and experiments. Wageningen: Centre for Agricultural Publishing and Documentation; p. 207

Palukaitis P, Roossinck M, Dietzgen R and Francki R. 1992. Cucumber Mosaic Virus. Advances in Virus Research, 41: 281-347.

PDR-HM, 2004. Physicians' Desk Reference for Herbal Medicine. Joerg Gruenwald ed Medical Economics, Montvale, NJ.

Petrov N. 2012. Potato virus Y (PVY) in crop species from the family Solanaceae (PhD thesis). Sofia: ISSAPP "N. Pushkarov"; Bulgarian.

Petrov NM, Andonova R. 2012. Bion and Exin as SAR elicitors against Potato virus Y infection in tomato. Science & Technologies, 2(6): 46-49.

Petrov NM. 2014a. Damaging effects of Tomato mosaic virus and Potato virus Y on tomato plants. Science & Technologies, 4(6): 56-60.

Petrov NM. 2014b. Induction of systemic acquired resistance against Cucumber mosaic virus in tomato agro ecosystems. J. Bal. Ecol., 17(4): 385-390.

Petrov NM. 2014c. Environmental friendly methods of inducing resistance against Cucumber mosaic virus in pepper. Agri. Sci. & Tech., 6(4): 471-474.

Petrov NM. 2014d. Reduction of the infection with Tomato mosaic virus and Cucumber mosaic virus in tomatoes trough inducers of resistance. Proceedings of "Seminar of Ecology", IBER, Sofia, Bulgaria, 129-133.

Petrov NM. 2014e. Induction of resistance in pepper to Potato virus Y by activation of defense mechanisms of the host plant. In Proceedings of "Seminar of Ecology", IBER, Sofia, Bulgaria,134-139.

Petrov NM. 2015. Mixed viral infections in tomato as a precondition for economic loss. Agri. Sci. & Tech., 7(1): 124-128.

Petrov NM, Stoyanova M, Andonova R, Teneva A. 2015a. Induction of resistance to potato virus Y strain NTN in potato plants through RNAi. Biotechnology & Biotechnological Equipment, 29(1): 21-26.

Petrov NM, Teneva A, Stoyanova M, Andonova R, Denev I, Tomlekova N. 2015b Blocking the systemic spread of potato virus Y in the tissues of potatoes by posttranscriptional gene silencing. Bul.J.Agri.Sci, 21(2): 288-294.

Petrov NM, Stoyanova M, Valkova M. 2015c. The antiviral activity of extract from St. John's wort against Potato virus Y. Proceedings of the Union of scientists - Ruse, 7(3): 229-232

Petrov NM, Gaur R. 2015. Characterization of potato PVY isolates. Science & Technologies, 5(6): 17-21

Ramirez-Erosa I, Huang Y, Hickie R. 2007. Xanthatin and xanthinosin from the burs of Xanthium strumarium L. as potential anticancer agents. Can J Physiol Pharmacol. 85(11):1160-72.

Schearer WR. 1984. Components of oil of tansy (*Tanacetum vulgare*) that repel Colorado potato beetle (Leptinotarsa decemlineata). J. Nat. Prod. 47: 964–969.

Schinella GR, Giner R, Recio M, Mordujovich de Buschiazzo P, Rios J, Manez S. 1998. Anti-inflammatory effects of South American *Tanacetum vulgare*. Journal of Pharmacy and Pharmacology 50: 1069–1074.

Sikora EJ, Gudauskas R, Murphy J, Porch D, Andrianifahanana M, Zehnder G, Bauske E, Kemble J. and Lester D. 1998. A Multivirus Epidemic of Tomatoes in Alabama. Plant Disease, 82: 117-120.

Smith KM. 1931. On the composite nature of certain potato virus diseases of the mosaic group. Nature: 127:702.

Stevovic S, Surcinski M and Calic D. 2009. Environmental adaptability of tansy (*Tanacetum vulgare* L.). Afr. J. Biotechnol., 8: 6290–6294.

Watterson JC. 1993. Development and Breeding of Resistance to Pepper and Tomato Viruses. In: Kyle, M.M., Ed., Resistance to Viral Disease of Vegetables, Timber Press, Portland, 80-110.

Wegiera M, Smolarz H, Druch M. 2012. Cytotoxic effect of some medicinal plants from Asteraceae family on J45.01 leukemic cell line - pilot study. Acta Pol Pharm. 69(2):263-268.

Weiner MA. 1980. Earth Medicine—Earth Food: Plant Remedies, Drugs, and Natural Foods of the North American Indians. Fawcett Columbine, New York.

Zitter TA. 1991. Diseases Caused by Viruses. In: Jones JB, Jones J, Stall R and Zitter T. Eds., Compendium of Tomato Diseases, The American Phytopathological Society, St. Paul, 31-42.