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АНТИВИРУСНА АКТИВНОСТ НА РАСТИТЕЛЕН ЕКСТРАКТ ОТ *CHELIDONIUM MAJUS* СРЕЩУ КАРТОФЕН ВИРУС Y
ANTIVIRAL ACTIVITY OF PLANT EXTRACT FROM *CHELIDONIUM MAJUS* AGAINST POTATO VIRUS Y

Николай Петров*, Мария Стоянова, Мирослава Вълкова
Nikolay Petrov*, Mariya Stoyanova, Miroslava Valkova

Институт по почвознание, агротехнологии и защита на растенията
"Никола Пушкаров"
Institute of Soil Science, Agrotechnologies and Plant Protection *Nikola Pushkarov*

***E-mail: m_niki@abv.bg**

Abstract

Chelidonium majus is an important medical plant used in traditional and folk medicine throughout the world. In the folk medicine of the Balkan countries it is widely used for its anticholera, antispasmodic, and sedative properties. *C. majus* extracts exhibit antiviral, antitumour, antimicrobial and anti-inflammatory effects. In our research we demonstrate the antiviral activity of a methanol extract from *C. majus* against PVY in tobacco plants. To our knowledge, it is the first investigation of an extract from this plant against phytopathogenic viruses.

Key words: *Chelidonium majus*, PVY.

INTRODUCTION

Celandine (*Chelidonium majus* L.) (Papaveraceae) is an important medical herb used in traditional and folk medicine throughout the world. In China it is used as a remedy for whooping cough, chronic bronchitis, asthma, jaundice, gallstones and gallbladder pains (Chang and Chang, 1986). In folk medicine of the Balkan countries, it is widely used for its choleric, spasmolytic, and sedative properties. Extracts from celandine are supposed to have antibacterial, antiviral, antifungal and anti-inflammatory effects. Fresh latex is used to remove warts, which are a visible manifestation of papilloma viruses (Colombo and Tome, 1995). The main active constituents of celandine are the alkaloids chelidonine, chelerythrine, sanguinarine, isochelidonine, and isoquinoline alkaloids with protopine (Franz and Fritz, 1979; De Rosa and Di Vincenzo, 1992).

The methanolic extract of the roots of *C. majus* revealed a high resistance to *Fusarium* (Matos et al., 1999). Several flavonoids and phenolic acids were isolated from the aerial parts which exhibit interesting antiviral and antimicrobial

properties both *in vitro* and *in vivo* (Colombo and Bosisio, 1996). A glycoprotein isolated from *C. majus* exhibits good antibacterial activity against methicillin resistant staphylococci and multiresistant enterococci (Fik et al., 1997). *C. majus* is frequently prescribed to treat gastric and biliary disorders (Benninger et al., 1999).

Total alkaloidal extracts of *C. majus* showed antiviral activity against different types of viruses. The ethanol extract of *C. majus* inhibit the growth and development of herpes simplex virus type 1 (HSV-1) (Monavari et al., 2012). In addition, the crud extract of *C. majus* was found to inhibit HIV-1 and this action was related to its sulphated polyglycosaminoglycan content (Gerencer et al., 2006). *In vitro* study revealed that the benzophenanthridine alkaloidal fractions of different parts showed virucidal activity against HSV-1 and Adenovirus type 5 and 12 alkaloids such as chelidonine (IC 50 (Kery et al., 1987). The *C. majus* 200 µg/ml) and berberine (IC = 100 µg/ml) were found to have inhibitory action against HIV-I reverse transcriptase enzyme (Tan et al., 1991).Total alkaloidal extracts of *C. majus* showed antiviral activity against herpesvirus, poxvirus, grippevirus (Lozjuk et al., 1996).

CmGRP1 is a newly described glycine-rich RNA-binding protein found in *C. majus* milky sap. It can be classified as a class IVa plant GRP. It means that apart from the glycine-rich domain, it has one RNA recognition motif (RRM), and it is implicated to be involved in plant defense against different diseases. Additionally, study using Real-Time PCR showed increased CmGRP1 mRNA levels observed in samples from plants subjected to viral pathogen infection as well as high salt conditions. It might suggest the involvement of this protein in plant responses against different stress conditions (Nawrot et al., 2013).

Potato Virus Y (PVY) causes significant yield loss in variety of crops of *Solanaceae* family including potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), tomato (*Lycopersicon esculentum*) and pepper (*Capsicum* spp. L.), wherever they are cultivated (De Bokx and Huttinga, 1981; Petrov, 2012). Therefore it is very important to find out any substance that reduces PVY virus titer and symptoms in the plants. There are currently no established such a substance that directly affect the virus.

The aim of this study is to establish antiviral activity of methanol extracts from *C. majus* against PVY. In order to reduce virus induced symptoms on tobacco plants and the virus titer in inoculated plants we sprayed plants with different percent of water diluted methanol extracts of *C. majus*.

MATERIALS AND METHODS

Plant material: Fresh plant flowers and leaves from *Chelidonium majus* L. were collected and freezed in freezer at -20°C.

Extractions: Methanol was used as a solvent. Extractions were prepared in Soxhlet extractor from 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) using dimethylsulfoxide (DMSO) up to 24 h before the assay.

Treatment of plants and inoculation with PVY

Tobacco plants were divided into four groups: 1/ treated plants with the extracts before PVY virus inoculation; 2/ Not treated plants, only inoculated with PVY (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 strain PVY 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 PVY according to Noordam (1973).

DAS - ELISA: We used the method of Clark and 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 PVY using sap from homogenized potato leaves. Micro titer ELISA plate wells were coated with PVY 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.

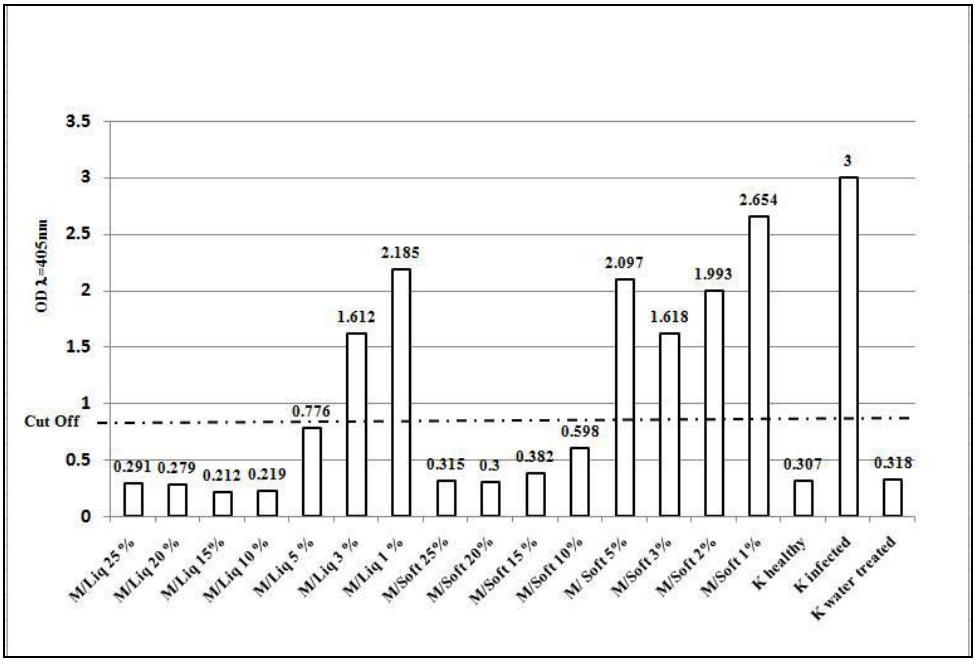
After that plates were incubated at 4°C overnight. After washing off the crude plant extract, virus was detected by PVY antibodies conjugated with alkaline phosphatase and diluted in conjugate buffer according to the supplier‘s specifications in incubation step for 4h at 37°C. P-nitro phenyl phosphate diluted in diethanolamin buffer (1mg ml-1, pH 9.8) is a substrate for the alkaline phosphatase enzyme reaction which run on 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 time optical density of the negative control (positive result >3x 0.307 OD = 0.914). Therefore, tested samples, with OD value more than 0.914, were considered positive for PVY infection.

RESULTS AND DISCUSSION

The liquid fraction of 5% of methanol water extract from *C. majus*was the highest dilution thatreducesDAS-ELISA values of PVY inoculated plants under the Cut off straight line (Fig. 1). Soft fractions of 10% was the highest dilution that reduced the virus titer to the DAS-ELISA values of healthy plants. Liquid fraction in concentration lower than 3% and soft fractions in concentrations 5 to1% were not

sufficient to control the viral infection. Higher % water concentrations expressed very good protection against PVY infection.

In all tested plants phytotoxic effect of 25% dilutions of the extracts was not observed.



Фиг. 1. *ДАС-ЕЛАЙЗА резултати на третираните тютюневи растения с течни и твърди фракции (процентни разреждания) на метанолни екстракти от C. majus*

Fig. 1*. DAS-ELISA results for PVY infection of the treated tobacco plants with liquid and soft fractions (dilution, %) of methanol extract from C. Majus*

Legend: (M/Liq) – liquid methanol fraction; (M/soft) – soft methanol fraction; (K) – control plants

Легенда: (M/Liq) – течна метанолна фракция; (M/soft) – твърда метанолна фракция; (K) – контролни растения

CONCLUSIONS

We established that spraying with at least 5% water dilution of liquid fraction and 10% water dilution of soft fraction of methanol extracts reduce significantly DAS-ELISA values of PVY in virus inoculated tobacco plants.

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