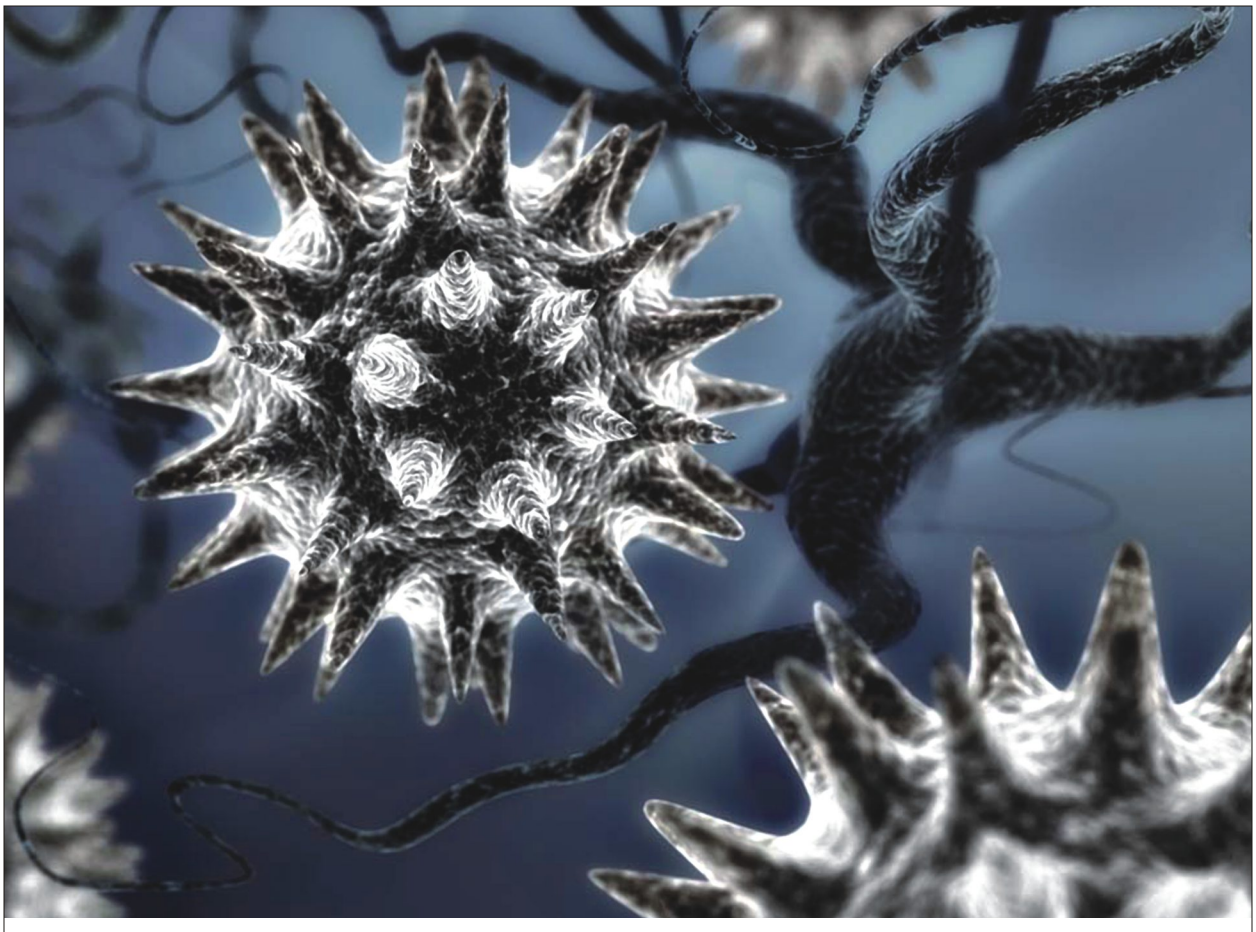

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Research Article
Chemotherapy of Potato Virus Y Infecting Potato Plants Using Antiviral Drugs

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Abstract

Background and Objective: Potato Virus Y (PVY) is classified as the most dangerous viral pathogen that infects potato plants and lowers the yield quantity and quality of tubers. Thus, the present study aimed at evaluating three antiviral compounds (ribavirin, acyclovir and oseltamivir) at different concentrations for the management of potato virus Y and potato growth parameters. **Materials and Methods:** DAS-ELISA and RT-PCR were used for the identification of PVY isolates. Virus concentration was measured by DAS-ELISA 1 and 2 weeks post-inoculation. The plant growth parameters (plant height, leaf number/plant, chlorophyll content and leaf area) were measured 40 and 80 days post-planting. Fresh and dry weights of plants and tubers weight/plant were measured 95 days after planting. **Results:** Oseltamivir and ribavirin were the most effective compounds in reducing virus concentration (0.442 and 0.447, respectively) when compared to PVY-infected control. Interestingly, among all plant growth parameters measured, only the leaf area was affected with no significant differences between preventive and curative applications. The highest fresh weight per plant was obtained by ribavirin (19.72 g) followed by acyclovir (19.50 g) and oseltamivir (17.55 g) compared to the PVY-infected control treatment (12 g) and the healthy, untreated control (24.66 g) with significant differences between preventive and curative applications. **Conclusion:** All antiviral compounds used reduced the virus concentration in the preventive and curative applications. Fresh, dry weights of potato plants and tuber weight were significantly increased by the application of all antiviral compounds. While, all compounds did not affect the chlorophyll content, leaf number/plant and plant height.

Key words: Potato, PVY, ribavirin, acyclovir, oseltamivir

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a member of the *Solanaceae* family, which is considered one of the most important crops worldwide. In Egypt, it comes in second place among vegetables after tomato. In 2018, Egypt ranked sixteen in the world and the first in Africa for potato production[1]. International Potato Center (ICP) stated that the potato crop has a double role in food security; being a cash crop and important food with high nutritive value[2]. Potato Virus Y (PVY) is classified as the most dangerous pathogen of potato plants, causing yield losses to range from 10-100%[3]. *Potato virus Y* belongs to the *Potyvirus* genus, the family Potyviridae. Its genome consists of a monopartite, single-stranded, positive-sense RNA with approximately 9.7 kb in length[4]. In nature, PVY exists within mixed three groups (PVY^N, PVY^O and PVY^C) with a genetic variation between them[5]. The PVY has a wide host range, including many plant species that belong to different families, particularly the family *Solanaceae*. The previous studies reported that PVY is the most prevalent virus on potato worldwide, due to the genetic variation among its strains[6]. The symptoms of PVY on potato plants appear as mild to severe mosaic, vein necrosis, mottling and malformations[7]. For the elimination of plant viruses, many trials of thermotherapy, tissue culture and chemotherapy were reported. The most effective antiviral compounds, which have been used with a significant elimination of plant viruses, belong to inhibitors of neuraminidase (NA), inosine monophosphate dehydrogenase (IMPDH) and inhibitors of S-adenosylhomocysteine hydrogenase (SAH), mentioned by Panattoni *et al*.[8]. Studies of Panattoni *et al*.[8] and Hu *et al*.[9] mentioned that many antiviral drugs, which used in clinical chemotherapy were applied against some plant viruses such as apple and grapevine viruses. The chemotherapy of Phyto-viral is limited due to lack of enough knowledge concerning molecular properties of many plant viruses and deficit resources in this field compared to clinical viruses[10]. *In vitro*, ribavirin and amantadine are common antiviral compounds, which have been used in previous studies for the elimination of Plum Pox Virus (PPV) virus from the infected shoots of plum plants[11]. Elimination of Bean yellow mosaic virus, *Potato spindle tuber viroid* and *Potato virus Y* from the infected gladiolus and potato explants, respectively by ribavirin *in vitro* was successfully done by Nascimento *et al*.[12], Mahfouze *et al*.[13], Nasir *et al*.[14] and Kaur *et al*.[15]. Studies of Ram *et al*.[16] on amantadine and acyclovir for the elimination of infected *Chrysanthemum morifolium* cv. Regol from *Chrysanthemum B carlavirus* were conducted. Elimination of *Grapevine fleck virus* was done through treatment of grapevine plants with a mix of ribavirin and oseltamivir[17]. In general, previous studies of Yang *et al*.[18], Sastry and Zitter[19] and Gong *et al*.[20] demonstrated that chemotherapy using ribavirin substances considered the most promising antiviral compounds against potato viruses such as PVY, PVA, PVS, PVM and PVX. In tissue culture technique, potato plantlets free of PVY were obtained when ribavirin and oseltamivir were mixed in the culture media[17]. While Singh[21] mentioned that acyclovir did not have a high eradication effect on potato viruses to produce free-virus potato plantlets as was anticipated. Oseltamivir has been succeeded in the elimination of the *Grapevine leafroll virus in vitro* chemotherapy[22]. To overcome ribavirin toxicity on regeneration of potato meristems *in vitro*, oseltamivir was mixed with a low concentration of ribavirin[17]. The eradication of plant viruses using antiviral drugs become an important tool for the production of virus-free plantlets[23]. The ribavirin has shown some adverse effects on humans when tested *in vitro* and *in vivo* based upon the dose[24]. The oseltamivir did not affect electrocardiogram at overdose, but there are some post-marketing studies, which proclaimed that it has some adverse effects[25]. The acyclovir did not have adverse effects on the genetics of humans and animals such as (mutagenicity, carcinogenicity and teratogenicity) when tested *in vitro* and *in vivo*, but it may affect animal fertility under the overdose[26].

The present work aimed at evaluation of some clinical antiviral compounds such as ribavirin, acyclovir and oseltamivir against plant viruses and study their efficacy at various concentrations through the foliar application on leaves of potato cv. Spounta infected with PVY, under controlled conditions in the insect-proof growth room.

MATERIALS AND METHODS

Source of potato tubers: Potato (*Solanum tuberosum* cv. Spounta) tubers used in this study were obtained from the Department of Potato Research and Vegetatively Propagated Vegetable Crops, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.

Source of the virus isolate: During spring 2019, potato plants naturally infected and showing symptoms typical to those of PVY were collected from different locations in Dakahlia governorate, Egypt. Polyclonal antibodies specific for PVY detection were obtained from the German Collection

of Microorganisms and Cell Cultures GmbH DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH). The double antibodies sandwich enzyme-linked immunosorbent assay (DAS-ELISA) technique was employed to confirm the PVY existence in the collected samples according to methods of Clark and Adams[27] in the laboratory of Seed Pathology and Tissue Culture, Faculty of Agriculture, Mansoura University, Egypt. ELISA-reader was used for measuring virus concentration (absorbance value was measured at 405 nm). The infected potato plants that gave a positive result with PVY-specific antibody were used for isolation and identification of the virus. For virus isolation, the sap of infected potato leaves [prepared using 0.01 M phosphate buffer (1.362 g KH_2PO_4 dissolved in 1 L of deionized water and 1.781 g $Na_2HPO_4.2H_2O$ dissolved in 1 L of deionized water, then mix 49 mL of KH_2PO_4 with 51 mL of $Na_2HPO_4.2H_2O$ to adjust the pH to 7.0)] was used to inoculate *Datura metel* L. (20 plants) with the aid of carborundum (400 mesh size), in insect-proof greenhouse. For virus propagation, the sap of PVY-inoculated datura leaves was taken after the development of symptoms and used for mechanical inoculation to *Nicotiana tabacum* cv. White burley (15 plants), in insect-proof greenhouse. Back mechanical inoculation from *N. tabacum* cv. White burley to healthy potato plants was done.

Protocol of Schubert *et al.*[28] was used to identify the virus strain; all Reverse Transcriptase (RT)-PCR reactions were used as follows: Based on the instructions of total RNA mini extraction Kit (Spin Column) from Applied Biotechnology ABT, the total RNA was extracted from potato leaves infected with PVY-isolate. A one hundred mg leaf tissue was ground in 700 µL of RNA lysis buffer and then incubated for 2 min at room temperature to complete the dissociation of the nucleoprotein complex. Chloroform in the amount of 200 µL was added to homogenized samples and vortex vigorously and then incubated for 2-3 min at room temperature. The homogenized samples were centrifuged at 12000 rpm for 5 min at room temperature. The aqueous phase was transferred to fresh tubes; 700 µL of 70% ethanol was added and gently mixed for 3 min. The mixture and precipitate were transferred to a Spin-column AC and centrifuged at 12000 rpm for 30 sec at room temperature, then discarded the flow through. The Spin-column and collection tubes were re-used and 500 µL of washing buffer was added to each one from them, centrifuged for the 30 sec at 12000 rpm at room temperature and the flow-through was discarded. The washing step was repeated once again. The empty columns were centrifuged at 12000 rpm for 1 min to get rid of any remaining washing buffer. Finally, the Spin-columns were placed in a 1.5 mL RNase-free centrifuge tube, then added 100 µL of elution buffer to the centre of the columns and incubated for 2 min at room temperature and then centrifuged at 12000 rpm for 1 min. The spin was repeated. The eluted RNA was stored at -80°C for later analysis. The RNA extraction was confirmed by agarose gel electrophoresis. Viral cDNA was synthesized and amplified according to the instructions of Applied Biotechnology (ABT H-minus cDNA synthesis Kit) as follows: for each reaction, 2 µg of templet RNA, 1 µL of PVY-specific oligonucleotides and up to 13.5 µL with nuclease-free water, incubated for 5 min at 65°C and chilled on ice. The mixture of 4 µL of 5X first strand buffer, 0.5 µL of H minus MMLV (200 unit μL^{-1}) and 2 µL of the dNTPs mixture (10 mM) was added to each reaction and incubated at 42°C for 60 min. Finally, the reactions were terminated by heating at 70°C for 5 min. The cDNA was stored at -20°C for later analysis. The PCR reaction of 25 µL volume contained 2 µL cDNA of each isolate, 1×buffer (supplied by the manufacturer), 0.4 µL dNTPs, 0.2 µL of specific primers for PVY^N from Invitrogen (YN5-F-1780 TCCGAATGGGACAAGAAAACTTG and YN3-R-2438 TGGTTCATCCAGTAGCAATTGCT), 1 enzyme unit and ddH_2O was prepared. Cycling protocol according to Schubert *et al.*[28] was: 2 min incubation at 96°C, followed by 35 cycles of 96°C for 30 sec, 62°C for 30 sec, 72°C for 2.5 min and a final extension at 72°C for 10 min. Five microliters of RT-PCR product were analyzed on 1.2% agarose gel in 1X TBE buffer (10X continuing Tris base 108 g, boric acid 55 g and EDTA 7.4 g, then dissolved in 1 l water) at 100 volts. 10000 bp sharp DNA ladder marker (100 bp DNA Ladder RTU Ready-to-Use, GeneDirex) was used to compare the size of RT-PCR products. Gels were stained with ethidium bromide at 5 µg mL^{-1} and photographed using a gel documentation system. So, the virus identification process mainly based on symptomatology, DAS-ELISA and RT-PCR[29].

Cultivation and inoculation of potato plants: Tubers of potato cv. Spounta were sown (2 tubers/bag) in black plastic bags (25×30 cm) filled with sterilized soil (5 kg/bag of clay) and placed in an insect-proof greenhouse (28±2°C with 16 hrs daylight) at Plant Pathology Department Experimental Farm, Faculty of Agriculture, Mansoura University, Egypt. All potato plants (at 25 days old) were dusted with carborundum (400 mesh) and mechanically inoculated with PVY-infectious sap by forefinger rubbing. Uninfected control plants were treated with only the buffer solution.

Antiviral application: Three antiviral compounds at three concentrations (C1, C2 and C3) of each were applied as follows: ribavirin (trade name is Ribavirin, Memphis for Pharmacology and Chemical Industry) used at 100 mg L^{-1} (C1), 200 mg L^{-1} (C2) and 400 mg L^{-1} (C3), acyclovir (trade name is Acyclovir 400 Stada, Global Napi Pharmaceuticals, Stada, Germany for Germa Pharm Ltd.) used at 200 mg L^{-1} (C1), 400 mg L^{-1} (C2) and 800 mg L^{-1} (C3) and oseltamivir (trade name is Tamiflu, Switzerland by F. Hoffmann-la Roche Ltd.) used at 37.5 mg L^{-1} (C1), 75 mg L^{-1} (C2) and 150 mg L^{-1} (C3). Treated potato plants were divided into two groups: 1) plants treated once at 24 hrs pre-PVY-inoculation and 2) plants treated twice; 24 hrs post-PVY-inoculation and again 7 days post the first treatment. Potato plants were sprayed with antiviral compounds using a hand-held low-pressure sprayer until drop-off. Untreated healthy plants and PVY-infected plants sprayed solely with water were used as negative and positive controls, respectively. Three replicates were used for each treatment. Pots were arranged in a completely randomized design.

Determination of virus concentration: Virus concentration was measured by double-antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) technique[27] in the laboratory of Seed Pathology and Tissue Culture, Faculty of Agriculture, Mansoura University, Egypt, at 7 and 14 days after inoculation with PVY, using PVY-specific polyclonal antibodies obtained from German Collection of Microorganisms and Cell Cultures GmbH DSMZ. Three leaves from each plant were cut into small pieces and mixed, then 1 g from that mix thoroughly homogenized with PBS-Tween (with 2% PVP). P-nitrophenyl phosphate was used as the substrate for the linked enzyme. All samples were compared with the positive sample included with the provided kits. Samples were considered positive when the number obtained from the ELISA reader (optical density OD at 405 nm) is almost similar to that of the positive sample.

Growth parameters and yield components: Three samples (three plants) of each treatment were taken at 40 and 80 days post-planting for measurement of the plant height (cm), leaf number/plant, chlorophyll content (SPAD-unit) using Chlorophyll Meter SPAD-502, leaf area (cm^2)/leaf using Easy Leaf Area 1.2 (a free application software). Fresh and dry weight (g)/plant and tuber weight (g)/plant were measured at 95 days after planting.

Statistical analysis: Experiments were repeated twice and each experiment contained three replicates. The complete randomized design was used. The data were analyzed using the CoStat software package (CoStat 6.4.0.0, CoHort Software, Birmingham, UK). Means of the values of the two experiments were first subjected to analysis of variance (three-way ANOVA) and significant differences between treatment means of repeated experiments were determined using Duncan's Multiple Range Test at p 0.05.

RESULTS

Virus isolation: Potato plants grown under open field conditions and naturally infected with potato viruses were diagnosed. The naturally infected potato leaves exhibited certain systemic virus symptoms, i.e., mosaic, yellowing between veins, mottling, stunting, chlorosis and vein necrosis. Confirmation of the PVY existence in the collected potato plant samples naturally infected with potato viruses was done using the DAS-ELISA technique in Table 1. Data in Table 1 showed that potato plant samples collected from Talkha location (No. 1, 9 and 11), Aga (12 and 13) and Faculty of Agriculture's farm (7) were infected with PVY through ELISA-reading (OD at 405 nm) compared to negative and blank samples. Potato virus Y was biologically isolated and propagated on *D. metel* and *N. tabacum* cv. White burley plants, respectively, from the selected DAS-ELISA positive sample (No. 7) of naturally infected potato plants. Typical systemic symptoms of mosaic, mottling and leaf crinkle were observed after 15 days from inoculation on *D. metel* plants, while, symptoms of mosaic, mottling and leaf deformation were observed after 35 days from inoculation on tobacco white burley plants. Typical symptoms of PVY were observed when back inoculation was done from *N. tabacum* cv. White burley plants to healthy plants of potato cv. Spounta.

Table 1: Detection of PVY in potato plants naturally infected, using DAS-ELISA technique

Sample (Dakahlia governorate)	ELISA-reading (OD at 405 nm)
Negative sample	0.110
Talkha location (1)	0.915
Faculty of agriculture's farm (7)	1.095
Talkha location (9)	0.463
Talkha location (11)	0.386
Aga location (12)	0.883
Aga location (13)	0.403
Blank sample	0.112

OD: Optical density

Table 2: Effect of antiviral compounds on PVY concentration, one and two weeks after inoculation

	PVY concentration	
Factor	One-week post-inoculation	Two weeks post-inoculation
Main treatment		
Ribavirin	0.224[ba]	0.447[c]
Acyclovir	0.217[b]	0.493[b]
Oseltamivir	0.219[b]	0.442[c]
Control^{+} (PVY-infected)	0.264[a]	0.744[a]
Control^{-} (healthy)	0.189[c]	0.277[d]
p-value	0.000***	0.000***
Concentration (C)		
C1	0.227[a]	0.5[a]
C2	0.218[a]	0.477[b]
C3	0.222[a]	0.465[b]
p-value	0.1232[ns b]	0.0123*
Inoculation time		
Pre-inoculation	0.222[a]	0.447[b]
Post-inoculation	0.223[a]	0.515[a]
p-value	0.7796[ns]	0.0000***
Interactions (p-values)		
Time*treatment (T)	0.0482*	0.0000***
Time*concentration (C)	0.0033**	0.0000***
T*C	0.4103[ns]	0.0017**
Time*T*C	0.0003***	0.0000***

[a]Numbers in the same column (means) followed by the same letter are not significantly different according to Duncan's Multiple Range Test at p = 0.05. [b]Ns: Non-significant, C1: First concentration, C2: Second concentration, C3: Third concentration. *,**,***Indicate the different significance level

Virus identification: Identification was done through virus symptomatology, ELISA and RT-PCR. Identification was done through virus symptomatology on *D. metel* plants, which exhibited typical systemic symptoms of mosaic, mottling and leaf crinkle. Also, *N. tabacum* cv. White burley showed typical systemic symptoms of mosaic, mottling and leaf deformation. DAS-ELISA method was used and confirmed the presence of PVY in the tested plants. In addition, PVY was identified by RT-PCR through isolation of total RNA from leaves of PVY^{N}-infected potato cv. Spounta plants compared to healthy ones. After reverse transcription, amplification of the cDNA by PCR using primer sets was done. To estimate the size of the amplified PCR product, a standard DNA ladder of 10000 bp was used. The expected size of the amplified DNA was 658 bp.

Effect of antiviral compounds on virus concentration: Ribavirin, acyclovir and oseltamivir reduced the virus concentration in potato plants when used as preventive or curative applications compared to PVY-infected control (untreated) in Table 2. Two weeks after inoculation with PVY, oseltamivir and ribavirin were more effective in reducing the virus concentration (0.442 and 0.447, respectively) than acyclovir (0.493). The preventive application was more effective in reducing the virus concentration than the curative application two weeks after inoculation (0.447 and 0.515, respectively), while there were no significant differences between the two methods of application one week after inoculation.

Interactions between concentrations of all treatments in Fig. 1 showed that all compounds reduced the virus concentration (measured 1 week after inoculation) when applied preventively or curatively (no significant differences were occurred either between the used concentrations or the time of application) compared to the untreated control (PVY-infected). Data in Fig. 1 show that acyclovir C1, C2 and C3 pre-inoculation treatment reduced the virus concentration measured one week after inoculation (0.227, 0.201 and 0.243), post-inoculation (0.218, 0.212 and 0.204), respectively. Followed by oseltamivir in pre-inoculation treatment (0.215, 0.226 and 0.225) and post-inoculation (0.256, 0.192 and 0.204) and ribavirin in pre-inoculation (0.217, 0.219 and 0.233) and post-inoculation (0.237, 0.261 and 0.208), respectively. Significant differences occurred between the used concentrations and the untreated control of PVY-infected (0.264 and 0.264).

On the other hand, interactions between concentrations of all treatments in Fig. 2 showed that all compounds reduced the virus concentration (measured 2 weeks after inoculation) when applied preventively or curatively (significant differences were occurred either between the used concentrations or the time of application) compared to the untreated control (PVY-infected). The 2nd (C2) and 3rd (C3) concentrations of all compounds significantly reduced virus concentration when compared to 1st concentration (C1), untreated control (infected) and healthy control. Data in Fig. 2 show that ribavirin in C1, C2 and C3 pre-inoculation (0.565, 0.302 and 0.452) and post-inoculation (0.503, 0.467 and 0.398), respectively. Followed by oseltamivir in pre-inoculation treatment (0.333, 0.368 and 0.419) and post-inoculation (0.547, 0.492 and 0.495) and acyclovir in pre-inoculation treatment (0.433, 0.277 and 0.493), post-inoculation (0.578, 0.708 and 0.472), respectively. Significant differences occurred between the used concentrations and the untreated control of PVY-infected (0.744 and 0.744). Also, the interaction between the time of application (before or after PVY-inoculation) revealed that the most effective time of application was before virus inoculation (preventive application).

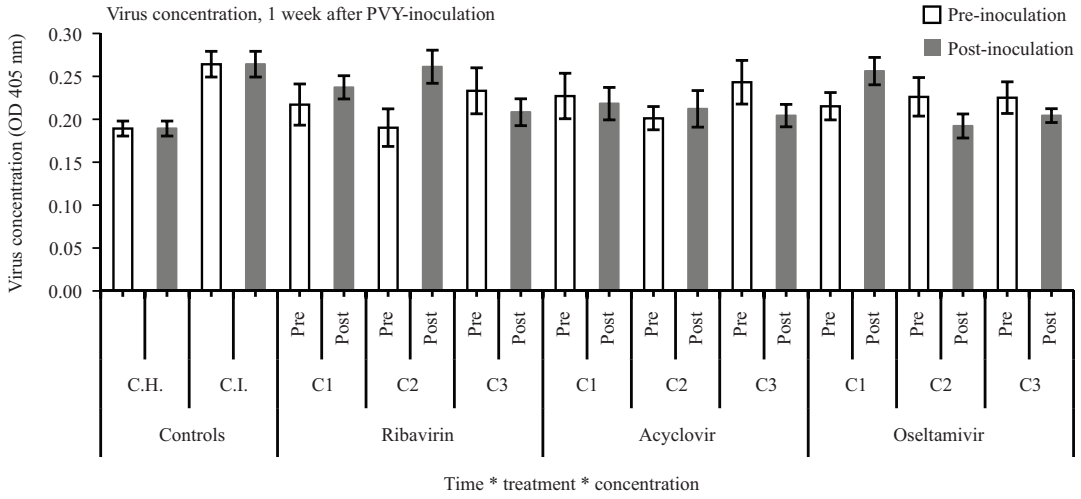

Fig. 1: PVY concentration affected by the interaction between different concentrations of the antiviral compounds and the time of application at one-week pre-and-post-inoculation

CH: Control^{-} (healthy), C.I: Control^{+} (PVY-infected). Columns represent means of OD values at 405 nm of PVY level using DAS-ELISA. Error bars represent the standard deviation (SD)

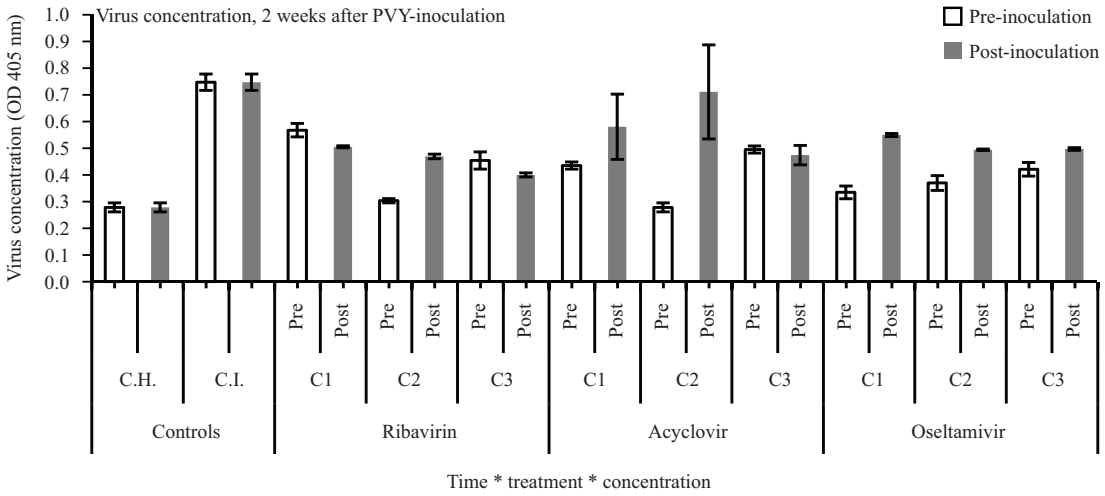

Fig. 2: PVY concentration affected by the interaction between different concentrations of the antiviral compounds and the time of application at two weeks pre-and-post-inoculation

C.H: Control^{-} (healthy), C.I: Control^{+} (PVY-infected). Columns represent means of OD values at 405 nm of PVY level using DAS-ELISA. Error bars represent the standard deviation (SD)

Effect of antiviral compounds on plant growth parameters and yield components: Ribavirin, acyclovir and oseltamivir did not affect the chlorophyll content (29.75, 30.55 and 30.35, respectively) after 40 days from planting compared to PVY-infected control treatment (30.85), in Table 3. However, they led to an increase in the chlorophyll content (43.03, 44.68 and 43.19, respectively) 80 days after planting when compared to the PVY-infected control treatment (39.16) in Table 4. They also increased the leaf area and leaf number/plant after 40 and 80 days from planting compared to PVY-infected control treatment. However, there was no constant trend as to their effect on plant height (Table 3 and 4).

Concentrations' interaction in Fig. 3 shows that acyclovir at the 2nd concentration (C2) and ribavirin at the 3rd concentration (C3) were the most effective treatments in

Table 3: Effect of antiviral compounds on growth parameters of potato plants, 40 days after planting

	Plant growth parameters, 40 days after planting			
Factor	Chlorophyll content (SPAD unit)	Leaf area (cm^2/leaf)	Leaf number/plant	Plant height (cm)
Main treatment				
Ribavirin	29.75[ba]	34.05[b]	8.33[bc]	43.5[c]
Acyclovir	30.55[b]	38.27[a]	9.55[a]	48.27[b]
Oseltamivir	30.35[b]	36.61[ab]	8.11[c]	41.22[d]
Control$^+$ (PVY-infected)	30.85[b]	29.33[c]	7.33[d]	43.33[cd]
Control$^-$ (healthy)	41.51[a]	39.33[a]	9[ab]	53.66[a]
p-value	0.0000***	0.0000***	0.0000***	0.0000***
Concentration (C)				
C1	32.31[a]	32.23[b]	8.5[a]	46.86[a]
C2	32.35[a]	37.73[a]	8.2[a]	46.16[ab]
C3	33.14[a]	36.6[a]	8.7[a]	44.96[b]
p-value	0.5891[ns b]	0.0000***	0.1834[ns]	0.0788[ns]
Inoculation time				
Pre-inoculation	31.95[a]	36.11[a]	8.57[a]	45.8[a]
Post-inoculation	33.26[a]	34.93[a]	8.35[a]	46.2[a]
p-value	0.0812[ns]	0.2096[ns]	0.3165[ns]	0.5593[ns]
Interactions (p-values)				
Time* treatment (T)	0.0132*	0.0001***	0.4042[ns]	0.1684[ns]
Time * concentration (C)	0.9360[ns]	0.2849[ns]	0.6090[ns]	0.0206*
T * C	0.8653[ns]	0.0001***	0.6583[ns]	0.0080**
Time * T * C	0.6147[ns]	0.0000***	0.6976[ns]	0.4192[ns]

[a]Values in the same column (means) followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test at p = 0.05. [b]Ns: Non-significant, C1: First concentration, C2: Second concentration, C3: Third concentration. *,**,***: Indicate the different significance level

Table 4: Effect of antiviral compounds treatments on growth parameters of potato plants, 80 days after planting

	Plant growth parameters, 80 days after planting			
Factor	Chlorophyll content (SPAD unit)	Leaf area (cm^2/leaf)	Leaf number/plant	Plant height (cm)
Main treatment				
Ribavirin	43.03[ba]	39.33[b]	8.55[ab]	49.22[c]
Acyclovir	44.68[b]	36.22[c]	8.66[ab]	54.72[b]
Oseltamivir	43.19[b]	31.66[d]	8.94[a]	49.61[c]
Control$^+$ (PVY-infected)	39.16[c]	37[c]	6.66[c]	56[b]
Control$^-$ (healthy)	54.23[a]	44.66[a]	8.05[b]	62.66[a]
p-value	0.0000***	0.0000***	0.000***	0.0000***
Concentration				
C1	44.83[a]	34.33[c]	8.26[a]	55.43[a]
C2	45.15[a]	37.53[b]	8.1[a]	54.1[a]
C3	44.6[a]	41.8[a]	8.16[a]	53.8[a]
p-value	0.7927[ns b]	0.0000***	0.7982[ns]	0.1749[ns]
Inoculation time				
Pre-inoculation	45.28[a]	39.42[a]	8.15[a]	56.08[a]
Post-inoculation	44.43[a]	36.35[b]	8.2[a]	52.8[b]
p-value	0.2008[ns]	0.0003***	0.8280[ns]	0.0000***
Interactions (p-values)				
Time* treatment (T)	0.7268[ns]	0.0000***	0.0181*	0.0000***
Time * concentration (C)	0.8691[ns]	0.1290[ns]	0.0124*	0.0097**
T * C	0.9993[ns]	0.0000***	0.9774[ns]	0.4225[ns]
Time * T * C	0.1974[ns]	0.0000***	0.1054[ns]	0.0484*

[a]Values in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test at p = 0.05. *,**,***: Indicate the different significance level. [b]ns: Non-significant, C1: First concentration, C2: Second concentration, C3: Third concentration

increasing the leaf area (cm^2) of potato plants infected with PVY at 40 days after planting in pre-inoculation treatment (55 and 49) and post-inoculation (39 and 28.33), respectively compared to other used concentrations and the untreated control of PVY-infected (29.33 and 29.33). The same trend had also occurred with the concentrations'

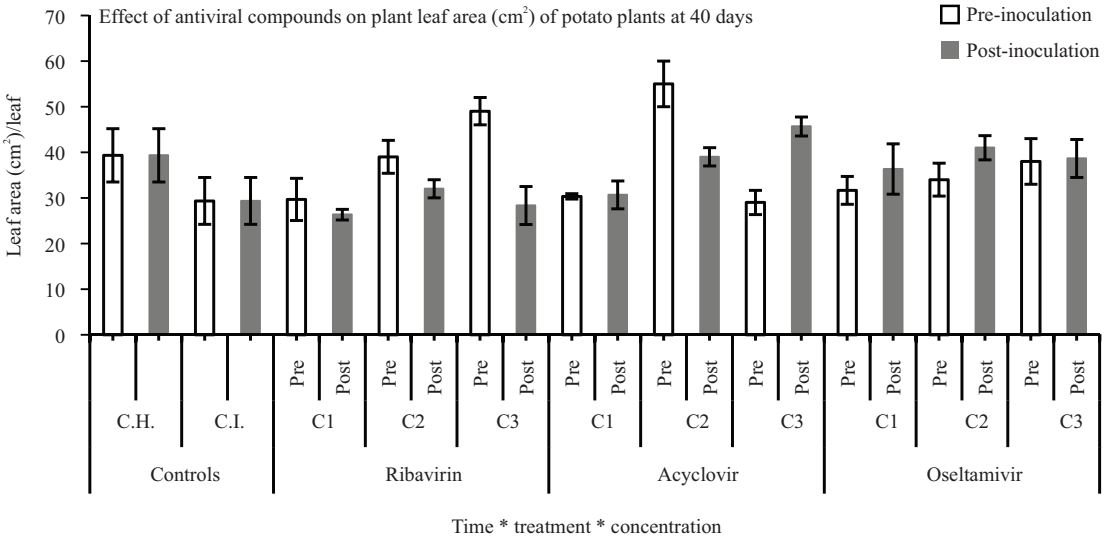

Fig. 3: Leaf area of PVY-infected potato plants affected by the interaction between different concentrations of antiviral compounds and time of application (before or after inoculation) at 40 days from planting

C.H: Control⁻ (healthy), C.I: Control⁺ (PVY-infected). Error bars represent the standard deviation (SD)

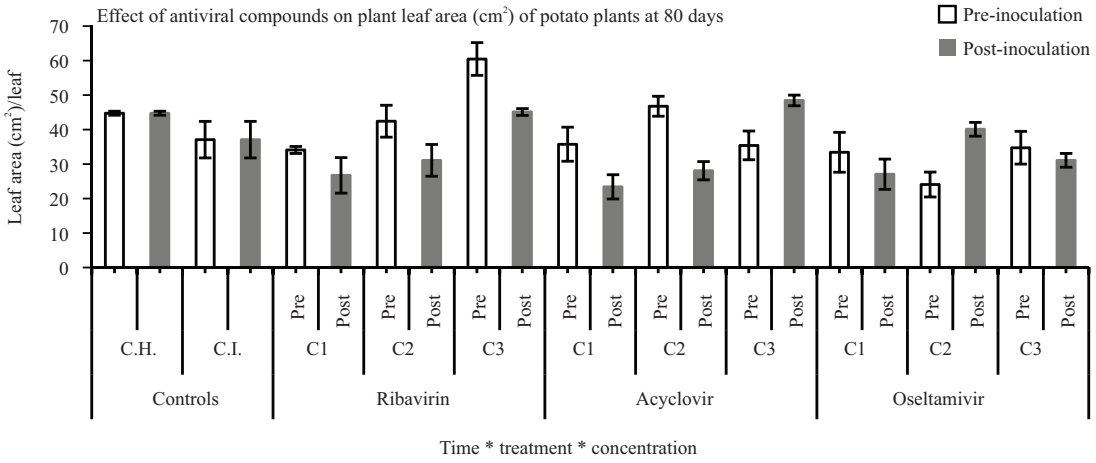

Fig. 4: Leaf area of PVY-infected potato plants affected by the interaction between different concentrations of antiviral compounds and time of application (before or after inoculation) at 80 days from planting

C.H: Control⁻ (healthy), C.I: Control⁺ (PVY-infected). Error bars represent the standard deviation (SD)

interaction in Fig. 4 where acyclovir at the 2nd concentration (C2) and ribavirin at the 3rd concentration (C3) were the most effective treatments in increasing the leaf area in pre-inoculation and post-inoculation, compared to other used concentrations and the untreated control of potato plants infected with PVY at 80 days after planting.

Fresh and dry weights of potato plants, as well as tuber weight estimated after 95 days from planting, were also positively affected by all antiviral compounds when compared to the PVY-infected control treatment. There were no significant differences between preventive and curative applications of antiviral compounds on all three yield parameters except for the fresh weight per plant whereas the post-inoculation (curative) treatment (20.46 g/plant) was more effective than pre-inoculation (preventive) treatment (16.91 g/plant) in Table 5.

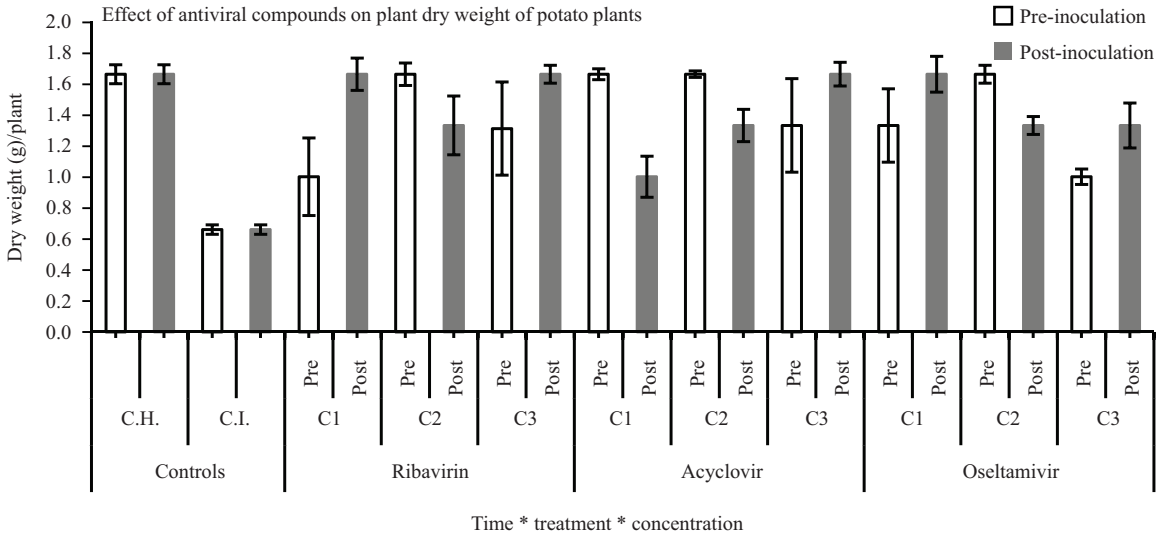

Fig. 5: Dry weight of PVY-infected potato plants affected by the interaction between the concentrations of antiviral compounds and the time of application (before or after inoculation) at 95 days from planting

C.H: Control^{-} (healthy), C.I: Control^{+} (PVY-infected). Error bars represent the standard deviation (SD)

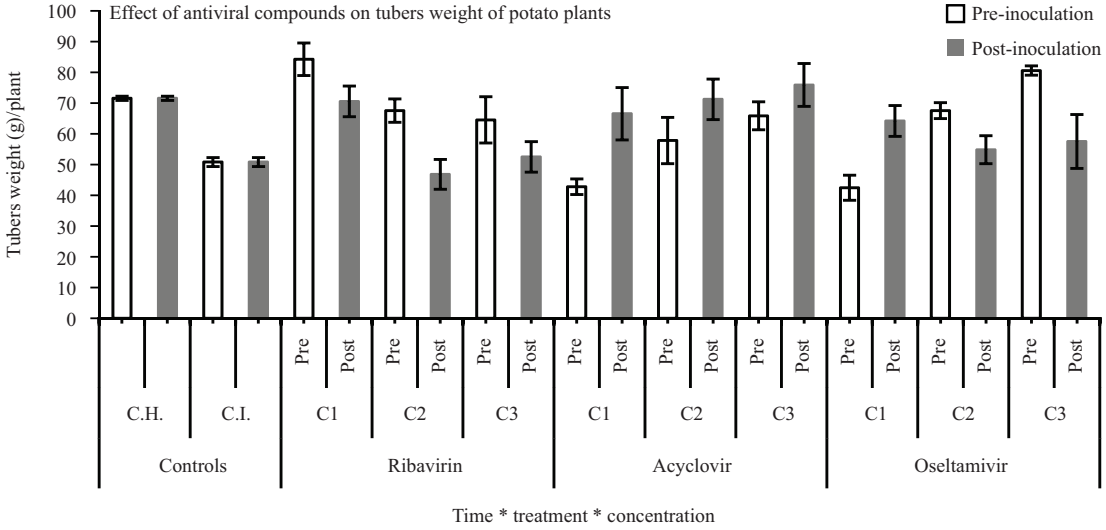

Fig. 6: Tuber weight of PVY-infected potato plants affected by the interaction between the concentrations of antiviral compounds and the time of application (before or after inoculation) at 95 days from planting

C.H: Control^{-} (healthy), C.I: Control^{+} (PVY-infected). Error bars represent the standard deviation (SD)

Data in Fig. 5 concludes that all compounds significantly increased the plant dry weight (95 days after planting) of potato plants infected with PVY in pre-inoculation treatment (1.66 g) compared to the untreated control of PVY-infected (0.66 g). There were no significant differences between the three antiviral compounds (preventive and curative applications under three concentrations) on plant dry weight. Also, the same trend was found through the data presented in Fig. 6 which concludes that all compounds significantly increased the tuber weight (95 days after planting) of potato plants infected with PVY compared to the untreated control of PVY-infected. There were no significant differences between the three antiviral compounds (preventive and curative applications under three concentrations) on tuber weight.

Table 5: Effect of antiviral compounds on fresh and dry weight of potato plants and tuber weight, 95 days after planting

	Parameter		
Factor	Fresh weight (g)/plant	Dry weight (g)/plant	Tuber weight (g)/plant
Main treatment			
Ribavirin	19.72[b a]	1.43[b]	64.16[b]
Acyclovir	19.5[b]	1.44[b]	63.16[bc]
Oseltamivir	17.55[c]	1.38[b]	61[c]
Control^{+} (PVY-infected)	12[d]	0.66[c]	50.66[d]
Control^{-} (healthy)	24.66[a]	1.66[a]	71.33[a]
p-value	0.0000***	0.0000***	0.0000***
Concentration			
C1	18.93[a]	1.3[b]	61.36[b]
C2	18.56[a]	1.36[a]	60.86[b]
C3	18.56[a]	1.29[b]	63.96[a]
p-value	0.7297[ns b]	0.0609[ns]	0.0181*
Inoculation time			
Pre-inoculation	16.91[b]	1.3[a]	62.51[a]
Post-inoculation	20.46[a]	1.33[a]	61.62[a]
p-value	0.0000***	0.3581[ns]	0.3417[ns]
Interactions (p-values)			
Time* treatment (T)	0.0000***	0.0000***	0.0000***
Time * concentration (C)	0.0316*	0.0000***	0.0000***
T * C	0.1004[ns]	0.0002***	0.0000***
Time * T * C	0.5619[ns]	0.0000***	0.0000***

[a]Values in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test at p = 0.05. [b]Ns: Non-significant, C1: First concentration, C2: Second concentration, C3: Third concentration. *,**,***: Indicate the different significance level

DISCUSSION

Chemotherapy, thermotherapy and tissue culture were reported as effective methods for the elimination of plant viruses. Our results show that all concentrations of ribavirin (100, 200 and 400 mg L^{-1}), acyclovir (200, 400 and 800 mg L^{-1}) and oseltamivir (37.5, 75 and 150 mg L^{-1}) reduced the PVY-concentration in treated potato plants in the preventive and curative applications compared to the PVY-infected, untreated control. These results are in agreement with reports of Panattoni *et al.*[8]. who mentioned that most of the antiviral compounds that eliminated plant viruses belong to inhibitors of neuraminidase (NA), inosine monophosphate dehydrogenase (IMPDH) and inhibitors of S-adenosylhomocysteine hydrogenase (SAH). The antiviral compounds used against plant viruses are not viricidal in action but considered as inhibitors of virus replication and this act play an essential role in stopping infection dispersal within the plant[8]. Chemotherapy has been used effectively against human and animal viruses and interestingly considerable analogous with plant viruses have occurred despite the absence of a normal immune system in plants[8]. Ribavirin (1,β-D-ribofuranosy1-1,2,4-triazole-3-carboxamide) is an artificial guanosine nucleoside, the antiviral agent which interferes with the viral mRNA synthesis, metabolized to nucleoside analogues that prevent the synthesis of RNA and capping of viral mRNA[18]. In the present study, ribavirin used under high concentrations of 200 and 400 mg L^{-1} exhibited a significant reduction of PVY-concentration in treated potato plants in the preventive and curative applications compared to 100 mg L^{-1} and the PVY-infected control treatment. Previous studies concerning PVY control, which reported that PVY was sensible to the concentration of 75 mg $L^{-1}$of ribavirin, while another study demonstrated that PVY was less sensible for the same concentration, as reported by Gong *et al.*[20] and Yang *et al.*[18] found that adding a high concentration ranged from 75-200 mg L^{-1} of ribavirin mixed into potato tissue culture media, succeeded in the elimination of potato viruses including PVY. Elimination of PVY and Potato Leaf Roll Virus (PLRV) through *in vitro* chemotherapy of infected potato stem cuttings due to ribavirin treatment (amended in 40 mL of solid Murashige and Skoog medium) has shown a decrease of virus concentration with the high concentration of the complex (50 mg L^{-1} ribavirin+100 mg L^{-1} of 2,4-dioxo-hexahydro-1-3-5 triazine) with both viruses[30]. To reduce the phytotoxic effect of ribavirin, Badarau *et al.*[17] found that the addition of oseltamivir with ribavirin may lead to the reduction of phytotoxicity. The highest elimination rate (100%) to PVY and PVX has been obtained when ribavirin mixed with oseltamivir (20 mg+80 mg L^{-1}, respectively) sprayed on the infected potato plantlets acclimatized in greenhouse[17]. Furthermore, in the present study, acyclovir used under high

concentrations of 400 and 800 mg L^{-1} exhibited a significant reduction of PVY-concentration in treated potato plants in the preventive and curative applications compared to 200 mg L^{-1} and the PVY-infected control treatment. Acyclovir or aciclovir {9-[(2-hydroxyethoxy) methyl] guanine} is a synthetic purine nucleoside analogue. This compound could be converted to acyclovir monophosphate by viral thymidine kinase (TK), then after that transformed by host cell kinase to diphosphate. After that diphosphate could change to triphosphate, which interferes with the viral DNA polymerase, thus inhibits or prevents virus replication without any effect on the normal cellular processes, because the uninfected cells do not use the acyclovir as a substance[26,31]. In a previous study of Singh[21], it has been reported that acyclovir did not show any significant effects on potato viruses' eradication when tested *in vitro* to produce virus-free potato plantlets. While acyclovir with 30 g dm^{-3} was used to produce chrysanthemums free-CVB with an elimination rate ranged from 20-30%[16]. According to reports of Panattoni *et al.*[8] acyclovir did not show any effect on *Potato virus S*, but inhibition effect was shown when splashed on lima bean plants before inoculation with *Bean golden mosaic virus*. Oseltamivir, {ethyl, (3R,4R,5S)-5-amino-4-acetamido-3-(pentan-3-yloxy)-cyclohex-1-ene-1-carboxylate}, is known as an inhibitor of neuraminidase enzyme. This enzyme is reported to be responsible for viral penetration into healthy cells and release viral particles from infected cells[32]. Badarau *et al.*[17] found that the highest eradication rate of PVY and PVX in infected potato plantlets was obtained when treated with 20 g ribavirin combined with 80 g oseltamivir. Results of the present study show that oseltamivir used at 75 and 150 mg L^{-1} was the most effective treatment causing a significant reduction of PVY-concentration in the treated potato plants in both preventive and curative applications compared to other antiviral compounds and the PVY-infected untreated control. The same trend was reported by Badarau *et al.*[17] when 40 mg L^{-1} of oseltamivir mixed with 40 mg L^{-1} of ribavirin was used as *in vitro* chemotherapy for the elimination of Grapevine fleck virus.

CONCLUSION

Using antiviral compounds (ribavirin, acyclovir and oseltamivir) at different concentrations reduced the PVY concentration in potato plants when used as preventive or curative applications compared to PVY-infected control treatment (untreated). All of the tested antiviral compounds increased the chlorophyll content after 80 days from planting when compared to the PVY-infected control treatment. All of the tested antiviral compounds increased the leaf area and leaf number/plant after 40 and 80 days from planting compared to PVY-infected control treatment. Fresh and dry weights of potato plants, as well as tuber weight estimated after 95 days from planting, were positively affected by all antiviral compounds when compared to the PVY-infected control treatment.

SIGNIFICANCE STATEMENT

This study evaluated the effect of some antiviral compounds in combating PVY infecting potato plants cv. Spounta. Results indicate that ribavirin and oseltamivir are promising compounds to manage potato viruses. However, further studies are needed to explain how these compounds act at the molecular level of viral pathogen and host plant.

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