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MOLECULAR GENETIC IDENTIFICATION OF THE PRUNE DWARF VIRUS AND ITS INFLUENCE ON THE COMPOSITION OF SWEET CHERRY (*PRUNUS AVIUM* L.) FRUITS

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SUMMARY

The Prune dwarf virus (PDV), belonging to the family *Bromoviridae*, represents a significant pathogen affecting sweet cherry (*Prunus avium* L.) production globally. This study aimed to genetically identify PDV in Uzbekistan and determine its influence on sweet cherry fruit's biochemical composition. Leaf and fruit samples collected from symptomatic cherry trees occurred in the Piskent and Chirchik districts, Tashkent Region. Molecular identification employed the use of reverse transcription polymerase chain reaction (RT-PCR) with coat protein gene-specific primers. Biochemical analysis of water-soluble vitamins and flavonoids proceeded by high-performance liquid chromatography (HPLC). RT-PCR successfully detected PDV in infected samples, amplifying a 381 bp specific fragment. Chlorosis, leaf deformation, and fruit shrinkage were predominant symptoms. HPLC analysis revealed substantial reductions in biologically active compounds: vitamin B2 decreased by 64%, vitamin B12 by 57%, rutin by 82.3%, and gallic acid by 47%. Overall, water-soluble vitamins and flavonoids declined by 64%, respectively. These results demonstrate that PDV severely compromises sweet cherry fruits' nutritional quality and antioxidant properties. The findings underscore the necessity for implementation of virus diagnostic measures and certification of virus-free planting material to sustain cherry production in Uzbekistan.

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Keywords: Sweet cherries (*P. avium* L.), Prune dwarf virus, vitamins, flavonoid, productivity, biologically active substances, *Ilarvirus*, *Bromoviridae*

Key findings: The diagnostic results showed a considerable spread of prune dwarf virus (PDV) symptoms in the sweet cherry (*P. avium* L.) fruit fields of Uzbekistan. The specific prune dwarf virus (PDV) serum was successful in obtaining and identifying it by molecular-genetic methods

INTRODUCTION

Sweet cherry (*Prunus avium* L.) production exceeds 2.3 million tons globally, of which its main cultivation prevails in Asia (43%), Europe (37%), and America (15%). In cherry cultivation, Uzbekistan ranks fifth after Turkey (30%), the USA (15%), Iran (8%), and Chile (18%) (FAOSTAT, 2018). Cherries are very valuable, being rich in vitamins and having antimicrobial and antioxidant properties (Kamenova *et al.,* 2020) and anti-inflammatory activity (Diekmann and Putter, 1996) that help the human body remove free radicals. Moreover, the cherry contains sugar (7%–15%), acids (0.36%–1.1%), vitamins (0.2%), and pectin (0.7%). Cherry fruits are rich in various vitamins, folic acid, beta-carotene, calcium, potassium, magnesium, phosphorus, and flavonoids. With these valuable biochemical properties, the cherry strengthens the immune system and helps fight against free radicals as an antioxidant; it slows down the aging process.

Sweet cherries are one of the most important fruit trees in various countries, and the scope of studying viruses that infect this plant is expanding worldwide. In past studies, the recognition of about 30 viruses infecting this plant has been successful. PDV transmission is mechanical by grafting and through pollens, seeds, and rhizomes (Simkovich *et al.,* 2021). Gilmer and Way (1960) reported that pollens transmit PDV to cherry trees. The said virus has a geographical distribution and is also notable for having a wide range of experimental hosts, including more than nine families worldwide. The PDV has been proven to cause 10%–65% damage to plants' fertility (Diekmann and Putter, 1996).

Prune dwarf virus belongs to the genus *Ilarvirus* and the family *Bromoviridae*. The virus diameter and length are 18–26 and 30–85 nm, respectively, with a shape like a rod (Pallas *et al.,* 2013). PDV has a tripartite, positively charged, single-stranded RNA genome (Pallas *et al.,* 2012). It is approximately 1055 amino acids long and has a molecular mass of 118.9 kDa (Codoner and Elena, 2008). The PDV has become one of the first viruses identified in pome fruit trees. For the first time in 1928, Gloyer and Glasgow identified its symptoms of infection; however, that time, they assumed these symptoms may have resulted from some physical injuries. In 1936, Thomas and Hildebrand identified the PDV on Italian plums in New York, USA, and Ontario, Canada (Gilmer *et al.,* 1976).

As a result of PDV infection, morphophysiological variations occur within plant cells, including plastid deformation, disruption of chloroplast structure, cellular organelles' damage, and decline in metabolic processes (Kozieł *et al.,* 2020). PDV infection reveals various clinical symptoms, such as leaf yellowing, chlorosis, mosaic, ring spots, necrosis, leaf deformation, and the production of poor-quality fruits in plants (Parakh *et al*., 1995; Soltani *et al.,* 2013). However, in some cherry trees, reports of growth retardation, shoot shortening, and 35% to 65% fruit yield reduction have emerged (Nemeth, 1986; Diekmann and Putter*,* 1996). Furthermore, the virus naturally infects various *Prunus* species and exhibits diverse symptoms in peach, plum, and cherry fruits (Rampitsch and Eastwell*,* 1997a, 1997b; Kamenova *et al.,* 2020).

The PDV infection severity depends upon the host species and plant's genotype, climatic conditions, and the virulence of the viral isolate. For viral disease diagnosis, various molecular methods, such as DAS-ELISA, RT-PCR, and real-time RT-PCR, were widely used techniques (Jarošová and Kundu, 2010; Soltani *et al.,* 2013). The one-step RT-

PCR method exhibited the highest sensitivity for detecting PDV RNA segments, enabling early detection of infection in PDV-infected plants (Jarošová and Kundu, 2010). Additionally, Song *et al.* (2020) identified the complete genome sequence of PDV isolates in China, with RNA1, RNA2, and RNA3 segments measuring 3376, 2594, and 2129 NT in length, respectively. These genomic analyses revealed the genetic variability among the PDV isolates and provided insight into phylogenetic grouping criteria.

PDV infection adversely affects photosynthetic activity, cell wall biosynthesis, carbohydrate metabolism, and the antioxidant defense system in plants. As a result, key biochemical components of the fruits, such as flavonoids, phenolic compounds, and pectin substances, incurred significant reduction in various host species (Kozieł *et al.,* 2020; Kamenova *et al.,* 2020). Consequently, the PDV infection not only lowers the plant's productivity but also alters the biochemical composition of fruits.

Studies investigating the molecular structure, infection mechanism, transmission pathways, and physiological impacts of PDV were critically scientifically important in developing strategies for effective virus control. From this perspective, research on the influence of PDV based on major biochemical components of sweet cherry fruits, particularly flavonoids and antioxidant-active compounds, remains relevant and of significant scientific importance (Hadidi *et al.,* 2011; Pallas *et al.,* 2013; Song *et al.,* 2020).

PDV is one of the most common harmful viruses widely distributed in nature in several plants (Hadidi *et al.,* 2011; Simkovich *et al.,* 2021). In particular, using plant species, such as *Cucumis sativus, Cucurbita maxima, Crotalaria spectabilis, Momordica balsamina, Tithonia speciosa, Phlox drummondii, Thunbergia alata,* and *Melilotus officinalis* ensued for virus propagation and diagnosis (Honjo *et al*., 2020). According to environmental conditions, it widely varies based on virus isolates, host types, and cultivars. In PDV, important symptoms observed are leaf yellowing, chlorosis, mosaic, ring spots, necrosis, leaf deformation, and fruit reduction (Parakh *et al*., 1995). The said viral disease naturally occurs in plants such as peaches and plums. The PDV causes symptoms, such as necrotic and chlorotic spots, on cherry leaves and stunting in peach and plum trees (Rampitsch and Eastwell, 1997a, 1997b), with a 35%–40% reduction in plant height visible in these fruit trees (Nemeth, 1986; Simkovich *et al.,* 2021). Hence, it is vital to study the damage caused by this virus to productivity and the degree of reduction of essential structural elements in the fruits. Therefore, the presented study aimed to know the effect of the prune dwarf virus on the biochemical composition of sweet cherry (*P. avium* L.) fruits.

MATERIALS AND METHODS

The following research on sweet cherry (*P. avium* L.) fruits commenced in the Molecular Biology and Bioinformatics Laboratory, Department of Biology, Chirchik State Pedagogical University, Chirchik, Uzbekistan; the IFT and PCR laboratory of the State Center for the Diagnosis of Animal Diseases and Food Safety; and the Biochemical Analysis Laboratory of the Institute of Bioorganic, Uzbekistan. The selected cherry plants and their collected fruits took place in different cherry orchards of the districts of Piskent and Chirchik, Tashkent Region, Uzbekistan. The PCR performed used primer sequences synthesized at the Integrated DNA Technologies (IDT), Belgium. The PCR transferred to the laboratory was in a thermos bag (+4 °C) with special ice packs for analysis. The disease level of the cherry plants entailed determination using PCR based on the collected samples.

Total RNA extraction from plant tissues

Total RNA isolation from plant tissues followed a standard extraction protocol because RNA is highly susceptible to degradation; particular care occurred during sample handling. Immediately after collection, plant samples (leaves, stems, and roots) underwent immersion in liquid nitrogen and storage in 15

mL tubes under frozen conditions. Rapid freezing effectively inactivated RNases and preserved the integrity of RNA molecules. This procedure ensured high-quality RNA suitable for subsequent molecular analyses.

In the subsequent step, the coat protein (CP) gene of the viral RNA reached amplification by PCR. The primer pairs required for RT-PCR entailed selection based on conserved regions of the PDV genome. For the initial detection of PDV in the samples, the use of diagnostic primers ensued, developed by Nourolah Soltani *et al*. (2013) (Table 1).

Complementary DNA (cDNA) synthesis and reverse transcription polymerase chain reaction (RT-PCR) proceeded according to standard protocols. The reverse transcription reaction continued in a total volume of 20 µL. The conducted reaction has two stages. In the first stage, a 9 µL reaction mixture, as prepared for each sample, contained 5 µL of RNA, 2 µL of reverse primer, and 2 µL of ddH_2O. The mixture underwent incubation in a thermal cycler at 65 °C for 5 min for one cycle. In the second stage, the reaction volume gained adjustment to 20 µL by adding 9 µL of the first-stage product, 8 µL of 2.5× reaction buffer, 2 µL of dNTPs (10 mM), and 1 µL of Superscript IV reverse transcriptase. The reaction took place in a thermal cycler (HEAL FORCE-T960, China) under the following conditions: one cycle at 50 °C for 10 min and 85 °C for 10 min. The synthesized cDNA obtained subsequent storage at –20 °C until further use. The PCR reaction continued in a total volume of 25 µL for each sample.

A total of 10 µL of the PCR product succeeded in mixing with 3 µL of gel loading buffer (Invitrogen, USA) before being separated by electrophoresis on a 1% agarose gel prepared in 1× TBE buffer. The gel staining used 3 µL of ethidium bromide solution and proceeded with electrophoresis in 1× TBE buffer at 80 V for 120 min.

Sample collection for determination of vitamins and flavonoids

For the determination of vitamin and flavonoid contents in the sweet cherry fruits, the collection of samples progressed. As a research object, primary visual monitoring resulted in the field of Rivershon sweet cherry in the Chirchik District, Tashkent Region, studying the ways and conditions of disease transmission. Determining the vitamins and flavonoids contained in the cherry fruits continued with the fruits and leaves collected diagonally from different points of the orchard in separate polythene bags. In conducting the analysis of water-soluble vitamins, the algorithm used was as follows:

For this purpose, the study used sweet cherry plants prepared earlier and samples of fruits. For the analysis of water-soluble vitamins, running the high-performance liquid chromatography (HPLC) used a gradient elution mode and a diode array detector (DAD). Acetonitrile and buffer solution served as mobile phases. Spectral data studied and processed were in the spectral range from 200 to 400 nm.

Chromatographic conditions

The mobile phase (gradient mode) comprised acetonitrile-buffer solution pH = 2.92 (4%:96%, 0–6 min, 10%:90%) 6–9 min; (20%:80%) 9–15 min; (4%:96%) 15–20 min, and the injection volume was 10 µl. The speed of the mobile phase was 0.75 ml/min, Column Eclipse XDB-CC18. 5.0 micron, 4.6×250 mm. The diode-matrix detector has wavelengths of 272, 292, 254, 297, and 360 nm.

The determination of flavonoids used the following algorithm. Performing the said analysis was by high-performance liquid chromatography (HPLC) using an isocratic elution mode and a diode array detector

Table 1. Primers used for the detection of PDV by RT-PCR.

Primer name	Primer Nucleotide Sequence (5′–3′)	PCR product size (bp)	Reference
PDV1_F	GGAAAGCCTACTGCCCGATCAC	381 bp	Nourolah Soltani *et al*., 2013
PDV1_R	CCTACGTTGTAGGGGATTAGG		

(DAD). Acetonitrile and buffer solution served as mobile phases. Spectral data studies had the spectral range from 200 to 400 nm. Chromatograph Agilent Technologies 1260, mobile phase (isocratic mode), acetonitrile buffer solution (35:75), pH = 2.92, 15–20 min, and the injection volume was 5 ml. The speed of the mobile phase was 0.75 ml/min, with column Eclipse XDB C18. 5.0 micron and 4.6×250 mm. The detector was a diode-matrix detector with wavelengths of 254, 320, and 385 nm, with the processes sequenced.

RESULTS AND DISCUSSION

In the diagnostic reaction, the PDV1_F/PDV1_R primer pair used resulted in the amplification of a 381 bp product (Figure 1). During the sampling process, collecting leaf samples occurred from sweet cherry trees (*Prunus avium*) exhibiting characteristic disease symptoms, including chlorosis, mosaic patterns, leaf deformation, and growth retardation. Each sample sustained an individual label and storage under conditions preventing RNA degradation. RT-PCR products' analysis was by electrophoresis on a 1% agarose gel, with visualization under a UV transilluminator.

The analysis of the obtained results confirmed the presence of PDV in sweet cherry plants at the molecular level. The amplification products generated by RT-PCR appeared to be fully consistent with the specific genomic fragments of the virus. These findings demonstrate the high diagnostic accuracy, reproducibility, and sensitivity of the RT-PCR method for PDV detection.

In healthy sweet cherries (*P. avium* L.) and infected fruits with the prune dwarf virus, the water-soluble vitamins' quantification through HPLC utilized gradient elution mode and a diode array detector (DAD). The wavelength ensured checking at 265, 254, and 285 nm, with the results recorded. The results enunciated that 100 g of healthy cherry fruits contain water-soluble vitamins (B2=52.94 mg and B12=6.91 mg), while in the 100 g of infected cherry fruits, the vitamins observed were B2=19.11 mg and B12=3.38 mg (Table 2). However, the vitamins B1, B6, B9, RR, and C were undetected in these cherry samples (Figure 2).

The amount of flavonoids in 100 g of healthy and virus-infected cherry fruits also attained scrutiny, with the results illustrated in Table 1 and Figure 2. The wavelength, as analyzed, was at 254, 265, and 381 nm. According to the flavonoid analysis, the rutin and gallic acid were evident in both healthy and diseased cherry fruit samples. According to the results, the rutin (8.465 mg) and gallic acid (7.254 mg) resulted in the healthy cherry fruits, while rutin (1.521 mg) and gallic acid (4.587 mg) were evident in the infected cherry fruits (Table 2).

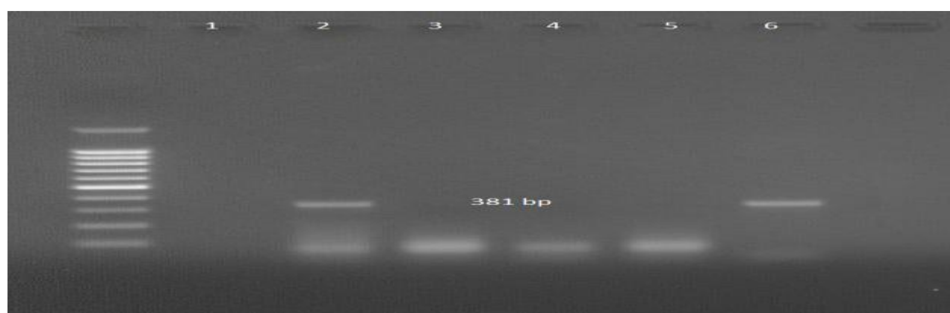

Figure 1. Agarose gel electrophoresis of RT-PCR products obtained for the detection of PDV. Electrophoresis performed on a 1% agarose gel resulted in M – 100 bp DNA Ladder Plus; Lane 1 – asymptomatic plant leaf; Lane 2 – sample obtained from sweet cherry plants exhibiting yellow chlorotic spots; Lane 3 – ring mosaic symptoms; Lane 4 – leaf curling; Lane 5 – vein shortening; and Lane 6 – marbled mosaic symptoms.

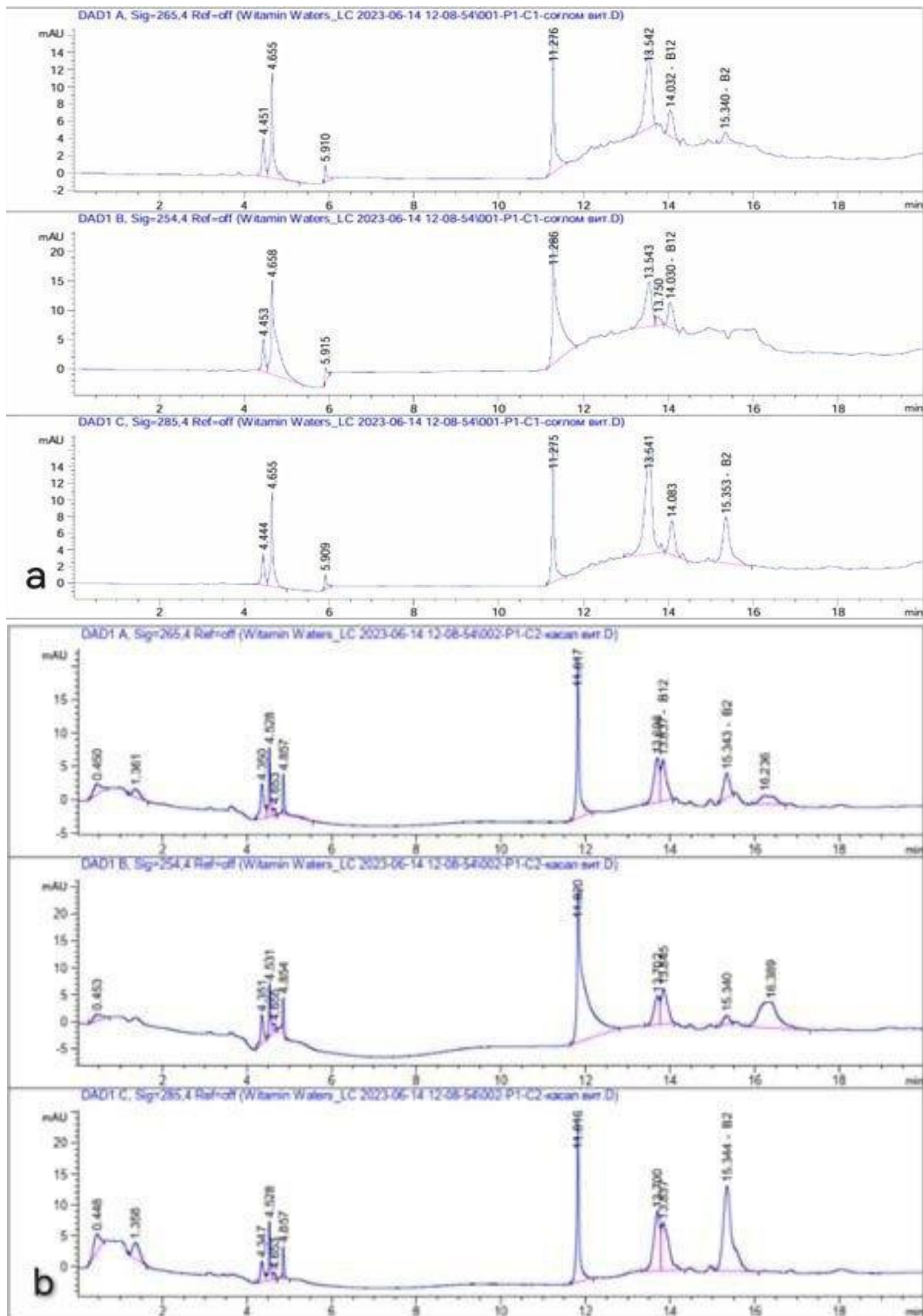

Figure 2. Chromatogram of water-soluble vitamin amines in the healthy (a) and PDV-infected (b) cherry fruits.

Table 2. Quantitative index of water-soluble vitamins and flavonoids in the cherry's healthy and PDV-infected fruits.

Cherry fruit samples	Water-soluble vitamins in 100 g of cherry fruits (mg)		Flavonoids in 100 g of cherry fruits (mg)	
	B2	B12	Rutin	Gallic acid
Healthy fruit (Rivershon)	52.94	6.91	8.465	7.254
Fruit infected with PDV (Rivershon)	19.11	3.38	1.521	4.587
Reduction rate (%)	64	57	82.3	47

With the increasing population in Uzbekistan, special attention is focusing on environmentally friendly food products, especially on crop plants, such as wheat, potatoes, tomatoes, corn, sunflowers, and cotton, which are the most in-demand crops. In previous years, the export and import of fruit tree seedlings have widely existed among different countries. As a result of that, the penetration and spread of various phytopathogenic viruses was notable in various regions of Uzbekistan. In past years, effective research has progressed on phytopathogenic viruses affecting important fruit trees (Sattarov *et al.,* 2020) and other crop plants (Akhmadaliev *et al*., 2024; Abduvaliev *et al.,* 2024; Kholmatova *et al.,* 2024; Sobirova *et al.,* 2024, 2025a, 2025b) in Uzbekistan.

Physiological characteristics of viruses and their effect on crop plants (Fayziev *et al.,* 2020) and molecular characterization of phytopathogenic viruses (Akhmadaliev *et al*., 2025; Yeginbay *et al.,* 2024) took place, and in obtaining special serum for viral immunodiagnostics, the conduct of its practical application succeeded (Jovlieva *et al.,* 2024). Similarly, the preliminary information about the distribution of phytopathogenic viruses is becoming available under the climatic conditions of Uzbekistan (Sattarov *et al.,* 2020).

Thus, in the presented investigations, the biological properties of the virus succeeded their studies, with the effects of the PDV on the biochemical composition of the cherry fruits analyzed. Phytopathogenic viruses have had studies in different countries regarding their influence on the biochemical composition of fruits (Homoki *et al.,* 2016; Paduch-Cichal *et al.,* 2024). In the course of this present study, the effect of PDV on the amount of water-soluble vitamins and flavonoids in cherry fruits received analysis (Figure 3). As we know, cherries are popular for their antioxidant properties. These bioactive compounds improve the nutritional value and positively affect the quality of cherry fruits. Among biologically active substances, the flavonoids are the phenolic compounds, providing health-promoting properties to cherry fruits (Wojdylo *et al.,* 2014; Borowy *et al.,* 2018; Paduch-Cichal *et al.,* 2024).

Prune dwarf virus causes a considerable decrease in the biologically active substances in cherry fruits. By comparing 100 g of healthy and virus-diseased cherry fruits, the vitamin B2 decreased by 64% (52.94 ± 19.11), and vitamin B12 decreased by 57% (6.91 ± 3.38). The results further revealed that flavonoids showed a significant decline in infected cherry fruits compared with healthy ones (Figure 2). In 100 g of healthy and diseased cherry fruits, the rutin decreased by 82.3% (8.465 ± 1.521), and gallic acid lowered by 47% (7.254 ± 4.587). The results authenticated that the prune dwarf virus reduced the content of biologically active substances in cherry fruits, which may also alter the quality and the nutritional values of cherry fruits.

The timely detection of viral disease infection, correct and quick diagnosis, and proper protection of plants from diseases will ensure the high-quality harvest from the world's rich fruit plants. Therefore, it is vital to have information about the identification of phytopathogenic viruses that cause infection, their development stages, distribution range, and how their preservation occurs from one season to another.

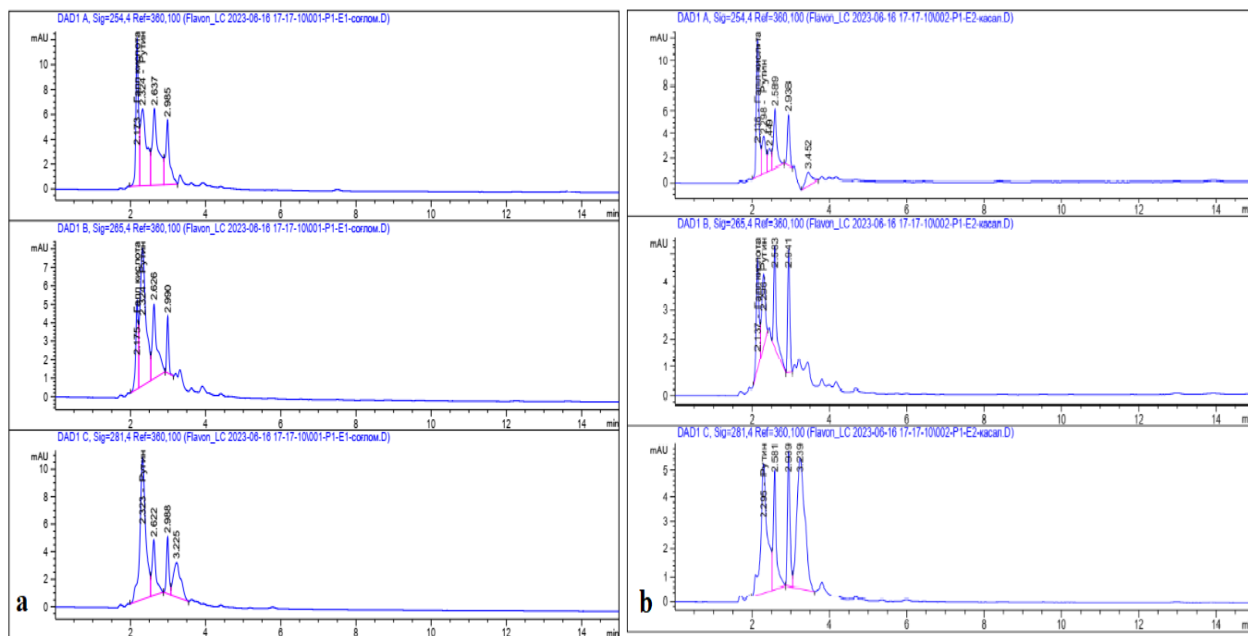

Figure 3. Flavonoids' analysis through chromatography in the cherry's healthy (a) and PDV-infected (b) fruits.

CONCLUSIONS

This study confirmed the presence of prune dwarf virus (PDV) in orchards in Uzbekistan using RT-PCR, identifying a 381-bp fragment of the coat protein gene. The virus caused significant deterioration in the biochemical composition of sweet cherries, reducing vitamin B2 by 64%, vitamin B12 by 57%, rutin by 82.3%, and gallic acid by 47%. These losses reduce the nutritional value and antioxidant capacity of infected fruits. The implementation of molecular diagnostics, virus-free certification programs, and the elimination of natural reservoirs of the virus are essential for protecting the quality of sweet cherries in Uzbekistan.

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