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DETECTION OF POTATO VIRUS Y INFECTION USING IMMUNOCHROMATOGRAPHIC ASSAY

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Abstract. It is well known that in the modern era, the continuous expansion of the distribution range of phytopathogenic viruses necessitates the use of rapid diagnostic methods with high sensitivity for their detection. This, in turn, requires the development of reliable and efficient tools for early diagnosis. Therefore, sensitive approaches such as polymerase chain reaction (PCR) and immunological methods (ELISA, immunochromatographic tests) have been widely applied. In the present study, we report the results of detecting *Potato virus Y* (PVY) using the immunochromatographic assay, which is considered a rapid and convenient method. Its effectiveness and applicability were scientifically evaluated and discussed. During the study, samples were collected from artificially inoculated indicator plants exhibiting disease symptoms, as well as from naturally growing reservoir plants found in virus-infected potato fields and along field margins. These samples were tested using the ImmunoStrip lateral flow assay system. The analysis revealed that the presence of two red signals (lines) on the test strips clearly indicated virus infection in the examined plants. The application of the ImmunoStrip method under field conditions demonstrated that *Potato virus Y* can be rapidly detected, making this approach an important diagnostic tool for monitoring and preventing the spread of the virus. According to the research results, Potato Virus Y (PVY) was detected in 64% of the plants tested using the ImmunoStrip test. Additionally, a weak viral reaction was observed in 18% of the plants, while the virus was not detected in the remaining 18% of the samples. These results confirm the widespread prevalence of PVY.
Keywords: *Potato virus Y* (PVY), indicator plants, reservoir plants, polymerase chain reaction (PCR), immunochromatographic assay, ImmunoStrip, rapid diagnostics.

Introduction

In recent years, due to various climate changes, the expansion of transportation networks, and the steady increase in interregional trade of agricultural products, the geographical distribution of plant viruses has been widening. This, in turn, has led to a higher incidence of plant infections and a rise in the number of newly emerging virus strains and isolates. Such circumstances highlight the urgent need to enhance the efficiency of monitoring and management measures in the field of plant virology [7].

Based on numerous research findings, it can be stated that almost all plant species are susceptible to viral infections, which disrupt physiological processes and may result in yield losses of up to 50–80% [2].

Potato virus Y (PVY) is currently among the most widespread and economically important phytopathogens, causing significant reductions in both yield and crop quality. At a

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time when the risk of phytopathogenic virus spread continues to increase, timely and accurate diagnosis has become crucial for sustainable agricultural production [3, 11].

Potato virus Y (PVY) is recognized as a major threat in agriculture, particularly in potato production. PVY belongs to the family *Potyviridae* and typically induces mosaic patterns, leaf deformation, and necrotic spots on infected plants. These symptoms disrupt the photosynthetic process and significantly reduce crop yield (Gray et al., 2010) [10, 12].

According to the literature, Potato virus Y (PVY) can reduce potato yield by 3.8–80%. Experimental studies conducted by A.L. Ambrosov scientifically confirmed that PVY infection may decrease yield by up to 60.4% and reduce starch content in potato tubers by 1.8% (*Crosslin et al.,* 2005) [5].

PVY is mainly transmitted mechanically through aphids, and early detection is therefore a critical factor in its management (Salazar, 2003) [6, 10, 11, 13]. For this reason, controlling and preventing the spread of viral diseases, as well as mitigating their negative impacts, requires the use of highly accurate, rapid, and reliable diagnostic methods [2, 9].

The role of diagnostics in preventing the spread of plant viral diseases is of paramount importance. With the increasing emergence of new plant viruses driven by climate change and the intensification of agricultural trade, early detection through reliable diagnostic tools is essential to limit their dissemination.

Commonly used approaches for the identification of phytopathogenic viruses include enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). These methods rely on virus-specific antibodies or genetic sequences, thereby allowing the detection of only the targeted virus. However, both ELISA and PCR are relatively expensive, time-consuming, and require sophisticated equipment and reagents for successful implementation [8, 13].

In recent years, low-cost methods that do not require specialized equipment and allow rapid analysis within 10–15 minutes have been developed. One such approach is the immunochromatographic assay (ICA). ICA can be performed in several formats, and highly practical variants are now available [2].

Immunochromatographic assay is an immunochemical diagnostic method based on the principle of thin-layer chromatography and represents a rapid immunodiagnostic tool for the easy detection of biological samples. The method relies on the specific interaction between an antigen and its corresponding antibody and is commonly implemented in the form of rapid test systems, using specialized test strips, dipsticks, or cassettes [2, 4, 14].

The principle of the immunochromatographic assay is based on the movement of liquid samples along membranes, resulting in the formation of specific visible lines [1, 14].

Immunochromatographic tests have been successfully applied not only for potato but also for other members of the *Solanaceae* family, including tomato, pepper, eggplant, and tobacco, in the detection of PVY. In the development of these technologies, researchers from China, the Netherlands, the United States, and Japan have played a leading role, and their contributions have been crucial in establishing effective diagnostic systems against Potato virus Y (*Mumford et al.*, 2016) [10]. In recent years, immunochromatographic assays have become widely used for the rapid field diagnosis of plant viruses.

Therefore, the present study aimed to evaluate the detection of *Potato virus Y* using the ImmunoStrip method.

Materials and Methods

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In this study, a set of indicator plants mechanically inoculated with PVY, several naturally growing reservoir plants located near PVY-infected crops, and an immunochromatographic test kit manufactured by LOEWE Biochemica (Germany) (catalog number: LFS07038/50) were used.

For the purposes of this study, visual monitoring of potato fields was conducted in several districts of the Tashkent region. During field surveys, samples were collected from plants exhibiting disease symptoms, as identified based on scientific references, and transported to the laboratory in polyethylene bags. In the subsequent stage of the research, symptomatic leaf samples were homogenized in phosphate buffer (1:1), followed by centrifugation at 6000 rpm for 15 minutes. The resulting supernatant, representing the viral sap, was separated into individual tubes and used for further experiments.

Viral sap was mechanically inoculated onto *Nicotiana samsun* plants grown under laboratory and greenhouse conditions and the plants were subsequently observed for symptom development. Symptomatic tissues were then homogenized and reinoculated onto a series of indicator plants. For diagnostic purposes, an **immunochromatographic test kit (catalogue No. LFS07038/50) manufactured by LOEWE Biochemica, Germany** was employed. From the symptomatic parts of the plants primarily leaf tissue fragments of approximately 1.0–1.5 cm^2 were excised using sterile, disinfected cutting tools. Each fragment was transferred into a sterile plastic bag, followed by the addition of 3 ml of phosphate buffer provided in the kit. The plant tissue was thoroughly homogenized with a pestle until completely disrupted.

From the resulting homogenate, 1.0–1.5 ml was carefully transferred into a sterile Eppendorf tube using a clean pipette. The sensitive end of an ImmunoStrip was then immersed into the prepared extract, and the reaction was monitored within the prescribed time. A positive result was indicated by the appearance of a disti nct test line in addition to the control line, whereas the presence of only the control line confirmed a negative reaction (Figure 1).

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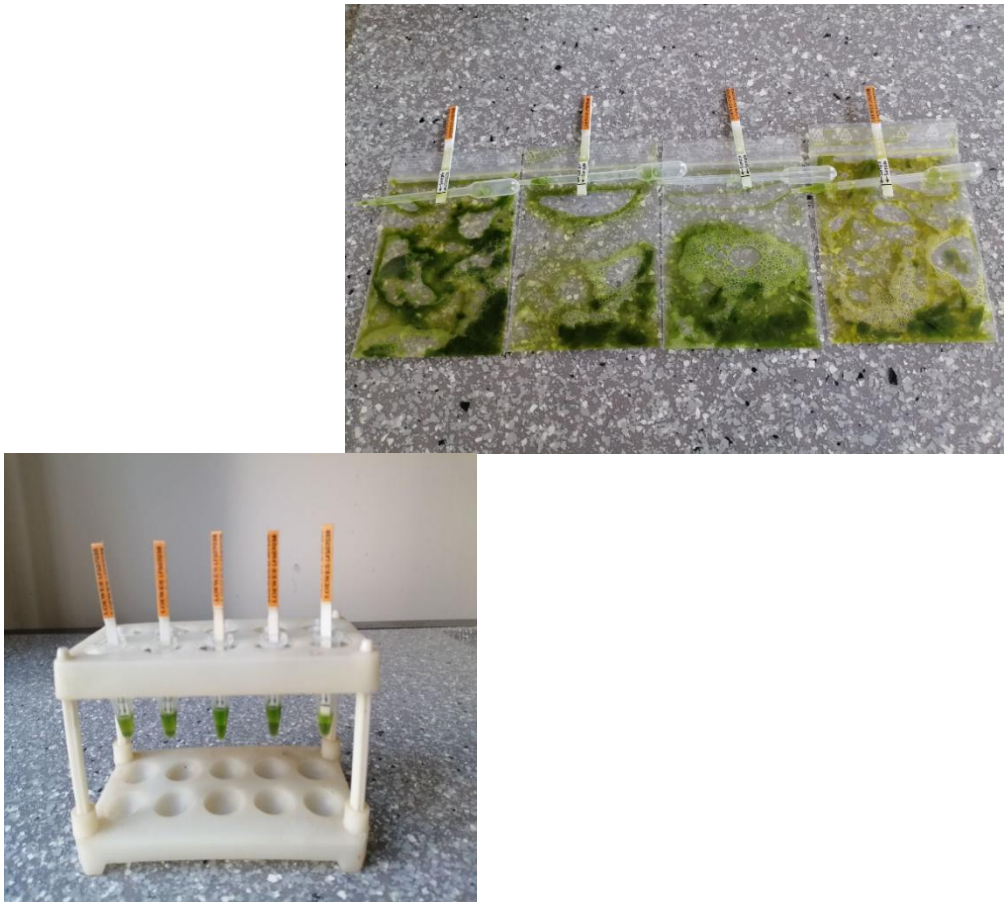

Figure 1. Testing procedure on PVY-infected indicator plants using the ImmunoStrip assay.

Within 5–15 minutes of incubation, the ImmunoStrip assay revealed the appearance of two distinct red lines. The line formed near the sample application zone corresponded to the **test line**, indicating the presence of the pathogen. The second line, which consistently appeared regardless of the infection status, served as the **control line**, confirming that the assay had been performed correctly.

Results and Discussion

The experiment was conducted under greenhouse and laboratory conditions using indicator plants. Samples were collected both from indicator plants that developed characteristic symptoms and from naturally growing reservoir plants located near potato fields infected with Potato virus Y (PVY). Each sample was placed in polyethylene bags, transported to the laboratory, and subjected to further analysis.

Below, representative examples of diagnostic results obtained from several indicator plants using the ImmunoStrip lateral flow test system are presented (Figure 2).

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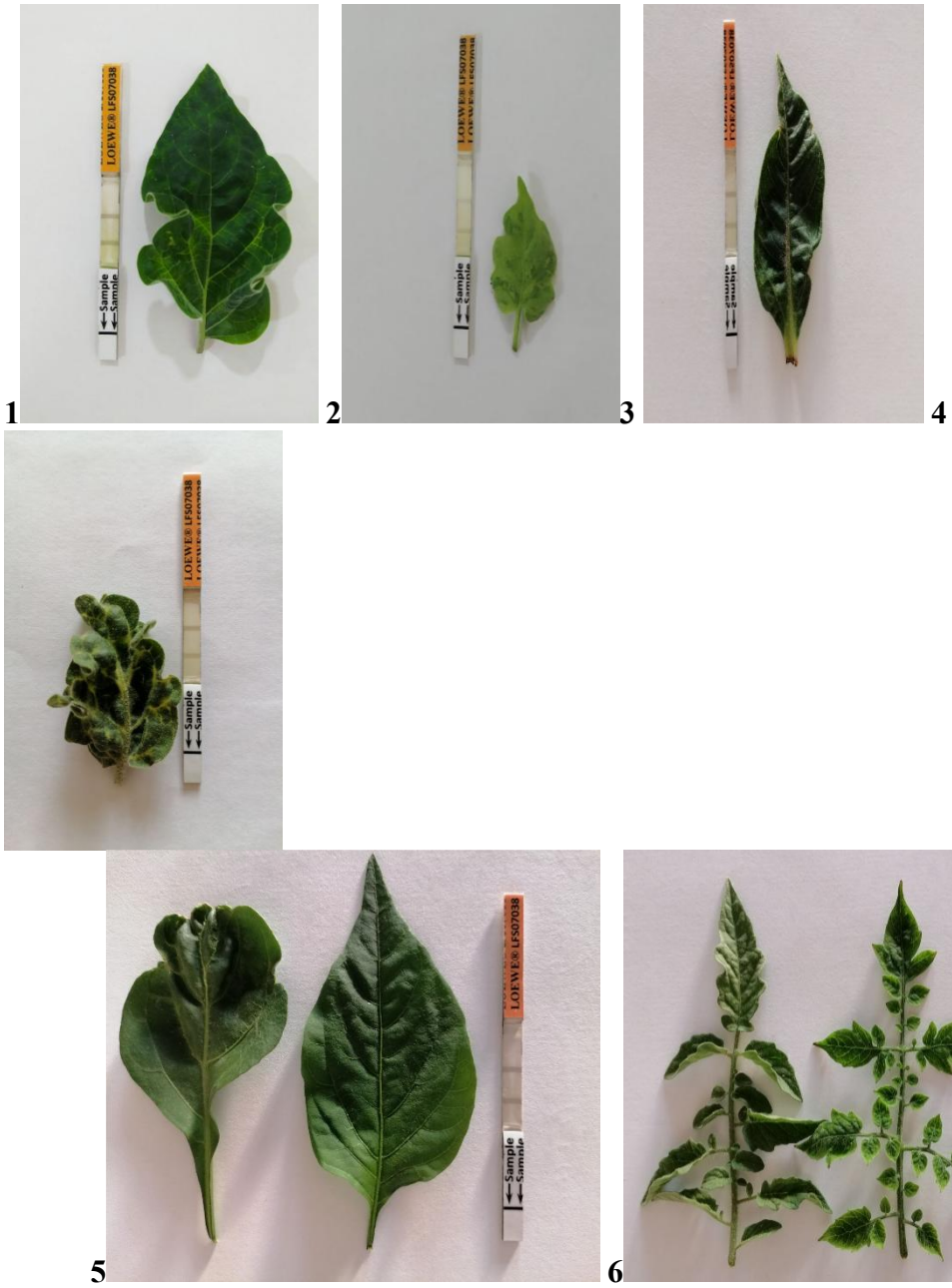

Figure 2. ImmunoStrip assay results for *Datura metel* (1), *Nicotiana glutinosa* (2), *Dahlia* (3), *Solanum melongena* (4) *Capsicum annuum* (5) and *Solanum lycopersicum* (6).

The results of the immunochromatographic assays conducted for the detection of Potato virus Y (PVY) are presented in the table below (Table 1).

Table 1.

Detection and diagnosis of Potato virus Y (PVY) in cultivated and wild plant species using the immunochromatographic assay.

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	Plant species	Sample type	Symptom characteristics	ICA result
1	*Solanum lycopersicum*	**Leaf**	**Deformation, mosaic, necrosis**	+
2	*Solanum melongena*	**Leaf**	Severe mosaic, deformation, chlorosis	+
3	*Capsicum annuum*	**Leaf**	Wavy deformation, mosaic	+
4	*Nicotiana tabacum cv. Samsun*	**Leaf**	Deformation, mosaic, vein darkening, necrosis	+
5	*Nicotiana glutinosa*	**Leaf**	Deformation, severe systemic mosaic	+
6	*Nicotiana tabacum cv. Dyubek*	**Leaf**	Deformation, mosaic, necrotic streaks	+
7	*Nicotiana tabacum cv. Vergenya*	**Leaf**	Deformation, systemic mosaic, necrosis	+
8	*Nicotiana rustica*	**Leaf**	Deformation, systemic mosaic	+
9	*Datura metel*	**Leaf**	Systemic chlorotic spots, mosaic, deformation	+
10	*Dahlia spp*	**Leaf**	Mosaic, leaf chlorosis	+
11	*Astrameriya*	**Leaf**	Systemic mosaic	±
12	*Petunia hybrida*	**Leaf**	Mild mosaic, chlorosis	+
13	*Physalis floridana*	**Leaf**	Severe mosaic, deformation, necrosis	+
14	*Solanum nigrum*	**Leaf**	Leaf distortion, mosaic	+
15	*Juglans regia L*	**Leaf**	Deformation, mosaic	±
16	*Prunus domestica L*	**Leaf**	Systemic chlorotic spots, mosaic	±
17	*Lactuca serriola*	**Leaf**	Deformation, systemic mosaic	+
18	*Artemisia vulgaris*	**Leaf**	Systemic mosaic	±
19	*Chenopodium album*	**Leaf**	Mosaic	-
20	*Chenopodium amaranticolor*	**Leaf**	Mosaic, chlorotic spots	+
21	*Chenopodium quiona*	**Leaf**	Mosaic, chlorotic spots	+
22	*Alhagi*	**Leaf**	Mild mosaic	+
23	*Amaranthus retroflexus*	**Leaf**	Leaf distortion, mosaic	±
24	*Capsella bursa-pastoris (L.) Medik.*	**Leaf**	Mosaic	+
25	*Lactuca sativa L*	**Leaf**	Deformation, systemic mosaic	-
26	*Datura stramonium*	**Leaf**	Mosaic, leaf chlorosis	-
27	*Portulaca oleracea*	**Leaf**	Mosaic, chlorosis	-
28	*Rumex*	**Leaf**	Leaf mosaic	-

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Note. In the table, the "+" symbol indicates that the presence of Potato virus Y (PVY) in indicator plants was clearly confirmed by the ImmunoStrip test. The "±" symbol indicates weak detection of the virus, suggesting that in such cases additional verification using more sensitive diagnostic methods is required. The "-" sign indicates a negative test result, meaning the virus was not detected.

Within the scope of the study, 28 plant samples (indicator and reservoir plants) suspected of being infected with Potato virus Y (PVY) were analyzed using the Immunochromatographic Assay (ICA) via the ImmunoStrip lateral flow test system. The results confirmed the extent of the virus's spread and the effectiveness of the ImmunoStrip method under field conditions. The analytical distribution of the total samples is as follows:

- Positive result (+): the PVY virus was clearly detected in 64% of the tested plants (18 species), which was confirmed by the distinct appearance of both the test line and the control line.

- Weak reaction (±): the presence of the virus was recorded as a weak reaction in 18% of the samples (5 species). These cases suggest a low virus concentration or a slow antibody reaction depending on the plant species, requiring further verification using more sensitive methods (e.g., PCR).

- Negative result (−): the virus was not detected in the remaining 18% of the samples (5 species); only the control line was visible, indicating that these plants were not infected with PVY.

The analysis results demonstrated that PVY is widespread not only in potato crops but also among important cultivated species such as *Solanum lycopersicum*, *Solanum melongena* and *Capsicum annuum*, as well as reservoir plants like *Datura metel* and *Lactuca serriola*. Specifically, the virus was found or a weak reaction was recorded in 23 out of 28 species (82%) tested in the experiment. This confirms the high risk of PVY persistence in the environment and among weeds.

Phytopathogenic viruses can be rapidly detected in the symptomatic tissues of infected plants using ImmunoStrip assays, as reported in the literature [Eshboev, 2015; Akhmadaliev, 2023].

Conclusion

Immunochromatographic assay is an effective tool for the rapid diagnosis of Potato virus Y (PVY), enabling the prompt detection of infected plants. It represents a convenient method for field-based identification of potato viral diseases. The simplicity and accessibility of this approach make it highly suitable for monitoring potato viral infections directly under field conditions. Due to its rapid results and minimal equipment requirements, the method can be applied not only in laboratory settings but also directly at agricultural sites. This facilitates timely implementation of control measures against virus spread, thereby minimizing yield losses and improving the quality of harvested potatoes.

Further research aimed at developing new test systems with higher sensitivity and specificity may lead to the creation of universal diagnostic tools for the early detection of various pathogens. Such advancements would have a significant impact on controlling viral diseases in plants and ensuring the overall sustainability of agricultural production.

According to the results of this study, when a number of indicator and reservoir plants infected with *Potato virus Y (PVY)* were diagnosed using the immunochromatographic assay, the appearance of two red lines on the ImmunoStrip confirmed the presence of the virus. The

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use of this method provides opportunities for implementing rapid control measures to prevent the spread of viral diseases in plants.

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