OPEN ACCESS Plant Protection, 10 (01) 2026. 27-33 DOI:10.33804/pp.010.01.5934

Check for updates

Available Online at EScience Press

Plant Protection

ISSN: 2617-1287 (Online), 2617-1279 (Print)

http://esciencepress.net/journals/PP

Research Article

Comparative Performance of ELISA and Lateral Flow Immunoassay for Distinguishing *Cucumovirus CMV* Strains in Iraqi Field Samples

[a]Ammar Aish, [b]Marwa Ahmed

[a] *Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.*
[b] *Department of Forensic Evidence, College of Sciences, Al-Karkh University of Sciences, Iraq.*

ARTICLE INFO

Article history
Received: 25th October, 2025
Revised: 16th January, 2026
Accepted: 21st January, 2026

Keywords
CMV
Diagnostic sensitivity
Serological assays
Virus detection
Strain identification

ABSTRACT

Pandemic and epidemic plant infections with *Cucumovirus* (CMV) poses a continuing threat to agricultural stability. Therefore, diagnostic tools that are both accurate and expedient have become an urgent need. This study compared the performance of Enzyme-Linked Immunosorbent Assay (ELISA) and Lateral Flow Immunoassay (LFIA) for detecting CMV in symptomatic plant hosts in the middle of Iraq. A total of 200 field samples were collected from tomato, pepper, eggplant, datura, cucumber, and zucchini squash across Baghdad Governorate and neighboring territories during the 2024 growing season. Comparative analysis demonstrated a clear difference in diagnostic sensitivity. ELISA outperformed LFIA in successfully detecting the virus in samples that tested negative by LFIA. This high-level sensitivity is likely due to enzymatic amplification in the ELISA, which enables detection of lower viral titers that might not be captured by the direct antigen-antibody binding mechanism of standard LFIA. Geographical distribution data showed that CMV infection was common in Al-Jadiriya but not in Khan Bani Sa'ad. Despite the apparent CMV-like symptoms observed in cucumber samples collected from Khan Bani Sa'ad, the samples tested negative by both diagnostic methods. This might be due to varietal resistance to CMV or the presence of divergent CMV strains not detected by commercial immunodiagnostic kits. Generally, the better sensitivity and quantitative competency of ELISA makes it suitable for comprehensive CMV surveillance, whereas LFIA remains a valuable rapid, on-site screening tool. Further molecular characterization of local CMV isolates is recommended to improve understanding of genetic diversity and regional detection strategies.

Corresponding Author: Ammar Aish
Email: ammar.amjad@coagri.uobaghdad.edu.iq

Introduction

Recently, plant viral diseases have become a persistent and escalating challenge to global food security, resulting in economic setbacks by reducing crop yields and compromising quality (Adhab and Alkuwaiti, 2022; Kanapiya et al., 2024). Therefore, the need for a rapid, high-precision detection and identification method for the causal agents is key to the successful management of agricultural threats (Adhab et al., 2021). Effective control measures depend mainly on early and accurate diagnosis. Most quarantine protocols, certification of virus-free plant materials, and virus-vector management work effectively at the early stages of infection (Rubio et al., 2020; Obaid and Adhab, 2025a).

Different diagnostic techniques were developed to address this need. Some are classical, such as biological indexing and types of electron microscopy, and others are advanced, such as molecular and serological assays. Despite high sensitivity and specificity of molecular methods, their requirement for expensive instruments and a sterile environment with highly skilled personnel may limit their use. These factors often make molecular methods impractical for immediate on-site decision-making (Mohammad et al., 2021; Adhab and Schoelz, 2024).

Serological methods exploit the specific affinity between viral antigens and antibodies. They have become the best choice as a vital, cost-effective alternative for routine diagnostics in the field (Al-Ani et al., 2011). Among these serological choices, the Enzyme-Linked Immunosorbent Assay (ELISA) has been considered the standard-bearer for plant virology. It is adaptable and has high-throughput potential. The Double-Antibody Sandwich (DAS-ELISA) format is widely considered the benchmark for laboratory-based screening (Clark and Adams, 1977; Kanapiya et al., 2024). In Iraq, several studies have validated the efficacy of various ELISA assays and recommended them for the reliable on-site detection of diverse pathogen species (Al-Ani, 2010; Al-Neami et al., 2011; Adhab et al., 2019; 2023; Khalaf et al., 2023a; 2023b; Banra et al., 2025; Timm et al., 2025).

In Iraqi fields, diagnostic methods that function effectively at the point-of-need, specifically under field conditions, are in high demand. Therefore, farmers and specialists pushed interest in alternative serological assays that require no laboratory infrastructure. The virus-specific Lateral Flow Immunoassays (LFIA), also known as immunostrips or dipsticks, have gained significant interest recently (Selvarajan et al., 2020). Virus-specific LFIA allows the visualization of viruses in a short time through the interaction of virus with antibodies over a porous membrane. It has been confirmed in various studies that this method is practically useful in identifying plant viruses in Iraqi field conditions (Adhab et al., 2021; Obaid and Adhab, 2025b).

Both the CMV-specific ELISA and the CMV-specific LFIA commercial kits are based on same immunological principles, but their distinct operational methodologies offer different advantages. Therefore, the objectives of this study include evaluating the diagnostic performance of both the CMV-specific ELISA and the CMV-specific LFIA under Iraqi field conditions and determining whether either method has superior capacity to detect distinct CMV variants, thereby improving CMV surveillance and management strategies in the middle of Iraq.

Materials and Methods

Collection of samples

A total of 200 symptomatic leaf samples were collected from various Cucurbitaceae and Solanaceae species from fields and nurseries in the middle of Iraq. Only *Cucumovirus CMV*-like symptoms were targeted in this sampling activity. Different fields were sampled during the 2024 growing season at locations near Baghdad. These locations include the University of Baghdad's experimental stations (33.2791°N, 44.3848°E), various commercial nurseries in Baghdad Governorate (33.3152° N, 44.3661° E) and surrounding agricultural areas, including Khan Bani Sa'ad (33.5701° N, 44.5412° E). The process of sampling was planned in the following way:

Cucurbitaceae

Cucumber (*Cucumis sativus*) leaf samples exhibiting CMV symptoms viz., pale mosaic, vein clearing and chlorotic spotting along the veins were taken. The pathogenicity was confirmed by mechanically inoculating cucumber and cowpea (*Vigna unguiculata*) indicator plants with the inoculum taken from the infected leave samples (Nasir and Adhab, 2021).

Squash (*Cucurbita pepo*) plants showing characteristic symptoms of reduced size of lamina blade, linear constriction of foliage, interchangeably dark- and light-green mosaic patterns and deformation of leaves were also sampled.

Luffa acutangula specimens displaying mosaic-like spots and small, distorted leaves were also collected to assess the presence of the virus.

Solanaceae

Solanaceous samples, including tomato (*Solanum lycopersicum*), bell pepper (*Capsicum annuum*), eggplant (*Solanum melongena*), and datura (*Datura stramonium*) exhibiting CMV-like symptoms were collected. The common symptoms include mosaic, mottling, leaf malformation, and plant stunting.

Extraction procedures

Newly, fully expanded indicator leaves were harvested and ground in liquid nitrogen using a pestle and mortar. A 0.1 g aliquot of the finely ground leaf tissue was homogenized in 3 ml of 0.1M phosphate buffer, pH 7.0, supplemented with 0.1% Tween-20. The resultant homogenate was purified by centrifugation at 10,000 × g for 10 min, and the supernatant was decanted and stored on ice until further use.

Inoculation of indicator plants

Virus inoculation was performed mechanically by applying an aliquot of the purified leaf extract of experimental plants to a set of indicator plant species for host range studies. *Chenopodium amaranticolor* and *Nicotiana benthamiana* were selected as indicators, along with *V. unguiculata* and *C. sativus* (Adhab et al., 2025).

Serological assays

Immunostrip (LFIA)

For immunodiagnosis, crude plant sap (50 µl) was extracted for a test spot, and the LFIA strip was used for rapid detection. *Cucumis CMV* ImmunoStrip (Agdia, Elkhart, IN) with a test band was analyzed after about 15 min according to the manufacturer's manual.

Enzyme-Linked Immunosorbent Assay (ELISA)

DAS (double-antibody sandwich) ELISA was performed to assess the presence of *Cucumovirus CMV*. The 96-well polystyrene plates were initially coated with approximately 100 µL of anti-CMV coat protein. Subsequently, the plates were blocked with PBS-Tween (0.1% Tween) to prevent any nonspecific interaction. Afterward, 100 µL of each sample, previously ground in the extraction buffer, was added, along with a reference sample, to the sample buffer used to dilute the samples. After overnight incubation, the plates were washed, and then added to a 100 µL (1:1000 v/v) dilution of the anti-CMV, which was conjugated to the ELISA-based reagent HRP (horseradish peroxidase).

After the reaction was complete (30 min), the plates were rewashed, and 100 µL of ELISA buffer containing PNP substrates was added. The reaction was determined after 30 min at 405 nm in the spectrophotometer (Reader ELISA, Biotek, USA).

Results and Discussion

Biological assay

Following mechanical inoculation with purified leaf extracts of experimental plants, indicator plant species were observed for symptom development. *C. sativus* consistently exhibited mosaic-like lesions, characteristic of CMV infection, while *V. unguiculata* displayed localized necrotic or chlorotic lesions on inoculated leaves. This differential host response served as a preliminary confirmation of viral pathogenicity and as a bioassay for assessing CMV host range.

Serological assays comparison and strain

The data provided a snapshot of the presence of *Cucumovirus CMV* in various plant species across Baghdad and nearby regions of Iraq. Two detection methods, ELISA and LFIA, were used to detect the virus. The results presented in Table 1 showed that there was an obvious discrepancy in the performance of the CMV-specific ELISA and CMV-specific LFIA kits in this study; the ELISA showed better sensitivity to CMV epidemiological surveillance.

Samples collected from Al-Jadiriya and Al-Yusufiya had strong unidirectional discordance between two techniques. The samples of pepper, tomato, and eggplant were positive for CMV-specific ELISA and negative for CMV-specific LFIA strips. This trend supports the fact that ELISA is more sensitive to the presence of CMV in the solanaceous hosts.

The method of amplification of the enzymatic activity in ELISA probably contributes to the better sensitivity of this test that enables it to detect low CMV titers in the host plant. These titers have a chance to be below the visual detection limit of LFIA strips. There were no CMV field samples (no samples with CMV +/+, excluding controls) indicating that the viral loads in these symptomatic solanaceous plants were always low to moderate, which usually invalidates LFIA results.

The findings in Table 1 also suggest that the Al-Jadiriya region is an important location of CMV. The Solanaceous host plants found at this site such as bell pepper, eggplant, tomato and *Dataura* spp. were always positive for CMV-specific ELISA. This observation demonstrates that CMV persistence and transmission in this place might be associated with localized agricultural practices, environmental factors or vectors. On the other hand, different trend was recorded in the agricultural fields of the Khan Bani Sa'ad.

All cucumber samples exhibiting apparent CMV-like symptoms, including leaf mosaic, were negative for both CMV-specific-ELISA and the CMV-specific-LFIA serological assays. However, the presence of CMV at this particular site in cucurbits has been reported in previous surveys (Adhab et al., 2021).

The current uniform lack of detection with CMV commercial kits might indicate the presence of potential cucumber resistance mechanisms in the commonly grown cultivars. Moreover, more concerning, there might be a resurgence of novel CMV strains or other viral pathogens that escape detection by standard commercial antibodies. Alternatively, the observed symptoms in the samples might be caused by environmental stress and mimic viral infection.

As far as sensitivity and specificity are concerned, the results in Table 1 show consistent outperformance of CMV-specific-ELISA over CMV-specific-LFIA, which can be attributed to fundamental methodological differences.

One of the major benefits of the CMV-specific DAS-ELISA kit is the enzymatic amplification, which allows the catalytic antibody-virus interaction and the production of an observable signal, which reduces the detection threshold to a considerable extent. This attribute plays a crucial role in the detection of CMV in low viral titers in samples, in early phases of infection, or in cucumber tolerant lines. On the other hand, LFIA is based on direct antibody-virus interaction and lacks any signal amplification, which, in turn, reduces the sensitivity of the given technique (Selvarajan et al., 2020).

The CMV-ELISA's spectrophotometric optical density readings provide estimated CMV concentration and objective data, which enables high-throughput processing and precise comparative analysis. Critical agricultural applications can be built on this, such as monitoring CMV spread or evaluation of CMV-antiviral activity. On the other hand, LFIA provides only presence/absence of results that are based on visual interpretation and lacks the capacity to estimate virus titer (Kanapiya et al., 2024).

Table 1. Comparative identification of *Cucumovirus* CMV in symptomatic solanaceous and cucurbitaceous host tissues taken in the 2024 growing season in central Iraq.

Plant Species	Location of Collection	Symptoms	ELISA / LFIA test Result
Pepper	Al-Jadiriya	Necrosis on Leaves	+ / -
Pepper	Al-Jadiriya	Mosaic on Leaves	+ / -
Eggplant	Al-Jadiriya	Mosaic on Leaves, Aphids Present	+ / -
Tomato	Al-Jadiriya	Leaves Striped and Twisted	+ / -
Tomato	Al-Jadiriya	Mosaic on Leaves	+ / -
Datura	Al-Jadiriya	Spots on Leaves	+ / -
Eggplant	Al-Jadiriya	Mosaic and Crinkling, Aphids Present	+ / -
Eggplant	Al-Jadiriya	Mosaic on Leaves	-/ -
Eggplant	Al-Jadiriya	Mosaic on Leaves	-/ -
Cucumber	Khan Bani Sa'ad	Mosaic on Leaves	-/ -
Cucumber	Khan Bani Sa'ad	Mosaic on Leaves	-/ -
Cucumber	Khan Bani Sa'ad	Mosaic on Leaves	-/ -
Cucumber	Khan Bani Sa'ad	Mosaic on Leaves	-/ -
Cucumber	Khan Bani Sa'ad	Mosaic on Leaves	-/ -
Cucumber	Khan Bani Sa'ad	Mosaic on Leaves	-/ -
Cucumber	Khan Bani Sa'ad	Mosaic on Leaves	-/ -
Cucumber	Khan Bani Sa'ad	Mosaic on Leaves	-/ -
Cucumber	Al-Jadiriya	Mosaic and Crinkling	+ / -
Cucumber	Al-Yusufiya	Mosaic and Crinkling	+ / -
Cucumber	Al-Jadiriya	Mosaic and Stunting	+ / -
Cucumber Gethaa	Al-Jadiriya	Mosaic on Leaves	-/ -
Zucchini	Al-Yusufiya	Mosaic on Leaves	-/ -
Cucumber	Al-Yusufiya	Mosaic on Leaves	-/ -
Cucumber	Al-Yusufiya	Mosaic on Leaves	-/ -
Positive Control	----	----	+ / +
Positive Control	----	----	+ / +
Negative Control	----	----	- / -
Negative Control	----	----	- / -

Agdia, USA kits were used to perform the assays. + means a positive result; and, -, a negative result.

Strain diversity and diagnostic implications

Based on the manufacturer's validation data (Agdia, USA), both the ELISA and LFIA kits are engineered to detect a comprehensive range of CMV isolates from Subgroups I and II. It also covers various geographic origins from Asia, Europe, and the Americas. The provided target lists largely overlap with minor variances; one example is that the ELISA kit is validated for CMV-CNU-1 from South Korea, while the LFIA includes specific detection for CMV-94 and CMV-93, not explicitly listed for the ELISA.

The reported LFIA's high diagnostic sensitivity is approximately 1:21,600 dilution (limit of detection). The present field results suggest that the strain diversity in Iraq may challenge these limits. Having LFIA fail to confirm ELISA-positive samples in our survey supports the hypothesis that local CMV variants may evolve to gain different epitopes, which eventually affect binding affinity on the LFIA assay. This result is in line with the previous findings of Nasir and Adhab (2021), who reported a novel CMV isolate infecting cucurbits in Baghdad and the adjacent areas. The observed difference between CMV immunostrips and CMV-ELISA in the current study shows that there is a dire need to use molecular diagnostic methodologies to explain the genetic makeup of the circulating CMV strains in Iraq, and whether they are divergent viral strains.

Strategic selection of diagnostic tools

CMV-specific ELISA and CMV-specific LFIA are both based on the choice of the required operational setting. CMV-specific LFIA gives fast results in 5-15 min, and therefore is invaluable for decentralized, point-of-need uses. Also, it is portable and does not require electricity or specialized equipment, making it the optimal tool for border quarantine inspections and immediate field surveying of CMV. This rapid screening method is affordable, time-saving, and sustainable in the Iraqi environment which is perfectly aligned with the United Nations Sustainable Development Goals (Anaz et al., 2023).

However, CMV-specific ELISA remains the optimal method in epidemiological studies of CMV, in certification programs and when maximum diagnostic sensitivity is needed. The fact that it is able to handle high volumes of solanaceous and cucurbitaceous samples with great precision is enough to justify the required infrastructure, which exists in the laboratory.

Conclusion and future directions

This study highlights that although commercial CMV-specific serological detection kits are robust; the local CMV evolution might affect their functioning. To determine the sensitivity difference between CMV-specific ELISA and CMV-specific LFIA quantitatively and improve future diagnostic plans, it is recommended that side-by-side limit-of-detection assays on serial dilutions of locally isolated CMV strains be conducted.

The current data support the use of CMV-specific ELISA as the more reliable tool for confirming CMV in this surveillance context. CMV-specific-LFIA is not suitable for declaring plants "CMV-free" in the Baghdad region without confirmatory testing. Next-generation sequencing may accurately identify the causal agents of the observed symptoms on solanaceous and cucurbitaceous plants in Khan Bani Saad.

Authors' Contributions

AA and MA conceived and designed the study, conducted the data analysis, and prepared the original draft of the manuscript. MA provided critical supervision, constructive feedback, and substantive revisions to enhance the clarity and scientific quality of the manuscript. All authors reviewed and approved the final version and accept responsibility for the accuracy and integrity of the work.

Research Funding

This research did not receive any grant from funding agencies.

Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 3: Good Health and Well-Being
SDG 9: Industry, Innovation, and Infrastructure

References

Adhab, M., Al-Ani, R., 2021. Biodiversity and occurrence of plant viruses over four decades: Case study for Iraq. In: 2021 Third International Sustainability and Resilience Conference: Climate Change. IEEE, pp. 159-163.

Adhab, M., Al-Kuwaiti, N., Al-Ani, R., 2021. Biodiversity and occurrence of plant viruses over four decades:

Case study for Iraq. In: 2021 Third International Sustainability and Resilience Conference: Climate Change. IEEE, pp. 159-163.

Adhab, M., Alkuwaiti, N.A., 2022. Geminiviruses occurrence in the Middle East and their impact on agriculture in Iraq. In: Geminivirus: Detection, Diagnosis and Management. Academic Press, pp. 171-185.

Adhab, M., Angel, C., Rodriguez, A., Fereidouni, M., Király, L., Scheets, K., Schoelz, J.E., 2019. Tracing the lineage of two traits associated with the coat protein of the Tombusviridae: silencing suppression and HR elicitation in *Nicotiana* species. Viruses 11, 588.

Adhab, M., Ezzulddin, D.L., Ahmed, A.S., 2025. Cultivating Resilience: Sustainable Plant Virus Control in Iraq's Agricultural Landscape. BIO Web of Conferences 192.

Adhab, M., Schoelz, J.E., 2024. Influence of the P6 effector protein of *Cauliflower mosaic virus* (CaMV) on the sustained expression and subcellular localization of the CaMV movement protein. Virology 600, 110240.

Adhab, M., Zhang, Y., Schoelz, J., 2023. Transient expression of *cauliflower mosaic virus* (CaMV) P6-GFP complements a defective CaMV replicon to facilitate viral gene expression, replication and virion formation. Virology 587, 109854.

Al-Ani, L.K., 2010. Susceptibility of watermelon cultivars to infestation by the two spotted spider mites. Iraqi Journal of Agricultural Sciences 41(4), 86-91.

Al-Ani, R.A., Adhab, M.A., Diwan, S.N., 2011. Systemic resistance induced in potato plants against *Potato virus Y* common strain (PVYO) by plant extracts in Iraq. Advances in Environmental Biology 5, 209-215.

Al-Neami, K.T., Al-Dorri, O.K., Kahtan, L.K., 2011. Effect of water extracts of some plants on two-spotted spider mites *Tetranychus urticae* Koch (Acariformes: Tetranychidae). Iraqi Journal of Agricultural Sciences 42(1), 111-117.

Anaz, A., Kadhim, N., Sadoon, O., Alwan, G., Adhab, M., 2023. Sustainable utilization of machine-vision-technique-based algorithm in objective evaluation of confocal microscope images. Sustainability 15, 3726.

Banra, S., Choudhary, J.S., Khalaf, L.K., Paray, B.A., Al-Sadoon, M.K., Thakur, A.K., Naaz, N., 2025. Comparative Assessment of different rearing diets on the developmental and reproductive behaviour of fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Neotropical Entomology 54, 41-52.

Clark, M.F., Adams, A.N., 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology 34, 475-483.

Kanapiya, A., Amanbayeva, U., Tulegenova, Z., Abash, A., Zhangazin, S., Dyussembayev, K., Mukiyanova, G., 2024. Recent advances and challenges in plant viral diagnostics. Frontiers in Plant Science 15, 1451790.

Khalaf, L.K., Adhab, M., Aguirre-Rojas, L.M., Timm, A.E., 2023a. Occurrences of wheat curl mite *Aceria tosichella* Keifer 1969 (Eriophyidae) and the associated viruses, (WSMV, HPWMoV, TriMV) in Iraq. Iraqi Journal of Agricultural Sciences 54, 837-849.

Khalaf, L.K., Zhang, Y., Adhab, M., 2023b. *Grapevine vein-clearing virus* is mealybug-borne but not mealybug-transmitted. Iraqi Journal of Agricultural Sciences 54, 1469-1477.

Mohammed, D., Adhab, M., Al-Kuwaiti, N., 2021. Molecular characterization of viruses associated to leaf curl disease complex on zucchini squash in Iraq reveals Deng primer set could distinguish between New and Old World Begomoviruses. Anais da Academia Brasileira de Ciências 93, e20210050.

Nasir, A.A., Adhab, M., 2021. A Biologically Distinct Isolate of *Cucumber mosaic virus* from Iraq. In: 2021 Third International Sustainability and Resilience Conference: Climate Change. IEEE, pp. 148-150.

Obaid, H., Adhab, M., 2025b. Decoding Viral Threats: Understanding ToBRFV and PepMV impact on tomato production in Iraq. BIO Web of Conferences 192.

Obaid, H.K., Adhab, M., 2025a. Outbreak of tobamoviruses and potexviruses associated with disease epidemics in tomato production area of Iraq. Iraqi Journal of Agricultural Sciences 56(Special), 237-246.

Rubio, L., Galipienso, L., Ferriol, I., 2020. Detection of plant viruses and disease management: relevance of genetic diversity and evolution. Frontiers in Plant Science 11, 1092.

Selvarajan, R., Prasanya Selvam, K., Balasubramanian, V., Sundaram, S.S., 2020. A rapid and sensitive lateral flow immunoassay (LFIA) test for the on-site detection of *banana bract mosaic virus* in banana plants. Journal of Virological Methods 284, 113929.

Timm, A.E., Khalaf, L.K., Smith, C.M., 2025. Genetic differentiation in populations of the corn leaf aphid *Rhopalosiphum maidis* (Fitch) Hemiptera: Aphididae. Iraqi Journal of Agricultural Sciences 56, 531–545.