Vietnam Journal of Food Control

A Scientific Journal of National Institute for Food Control
Journal homepage: https://vjfc.nifc.gov.vn

Research article

Development of a real-time PCR method for detection and enumeration of *Plutella xylostella granulovirus* in plant protection product

Pham Ngoc Ha[1*], Nguyen Tuan Thanh[1], Ha Anh Thu[1], Tran Hong Ba[1], Vu Thi Quy[2]
[1]*National Institute for Food Control, Hanoi, Vietnam*
[2]*Hanoi Medical University, Hanoi, Vietnam*

(Received: 06 Aug 2025; Revised: 01 Dec 2025; Accepted: 21 Dec 2025)

Abstract

Plutella xylostella granulovirus (PlxyGV) is a baculovirus that specifically infects diamondback moths (*Plutella xylostella*) and is widely used in commercial biopesticide formulations. To ensure the quality and efficacy of PlxyGV-based biopesticides, this study developed a quantitative real-time PCR (qPCR) method for viral DNA detection and quantification. A 222 bp fragment of the *ORF30* gene encoding the envelope protein ODV-E66 was cloned into the pGEM-T Easy vector, transformed into *E. coli DH5α*, and verified by Sanger sequencing, showing ≥ 99% identity with the published *ORF30* sequence of *PlxyGV* (GenBank accession No. MN099286.1). The real-time PCR method demonstrates high sensitivity, with both the limit of detection and limit of quantification reaching 10^1 copies/µL. The assay exhibits specificity and accuracy are 100%. A standard curve was established showing a strong linear correlation between cycle values and the log of viral DNA concentration (R^2 = 0.9957), with an amplification efficiency of 90.1%. Repeatability (RSDr = 0.20%) and reproducibility (RSDR = 0.28%) were within acceptable ranges based on ISO 22118:2011, confirming the method's reliability and applicability for quantifying PlxyGV in plant protection products.

Keywords: *real-time PCR, Plutella xylostella granulovirus, cloning.*

1. INTRODUCTION

In modern agriculture, plant protection products play a crucial role in pest control and crop yield preservation [1]. However, the extensive use of chemical pesticides over recent decades has highlighted significant limitations, including environmental pollution, food residues, disruption of ecological balance, and adverse effects on human health [2]. It is estimated that millions of tons of chemical pesticides are used worldwide every year, of which only 2% reach the target organisms, the rest affect non-target organisms, soil, water and air [3]. The overuse of chemicals in farming has also contributed to soil degradation and increased the risk of pesticide resistance in pests [4].

In response to these challenges, biopesticides have become a sustainable alternative trend [5]. Biopesticides are derived from microorganisms, animals, plants or minerals, with advantages such as: less harmful to the health of users, less residue in agricultural products, environmentally friendly and highly biodegradable [6, 7, 8]. Among these, biopesticides, particularly insect-specific viruses of the *Baculoviridae* family, have gained prominence [9]. Baculoviruses infect hosts through midgut epithelial cells, where viral DNA is released from occlusion bodies (OBs). Subsequent secretion of chitinase disrupts cellular structures, leading to host mortality [10], while maintaining high target specificity that preserves beneficial organisms [11]. *Plutella xylostella granulovirus* (PlxyGV), a member of the *Betabaculovirus* genus, demonstrates exceptional efficacy against the diamondback moth (*Plutella xylostella*), a devastating pest of cruciferous crops [12].

*Corresponding author: Pham Ngoc Ha (E-mail: hapn0411@gmail.com)
https://doi.org/10.47866/2615-9252/vjfc.4594

The diamondback moth is a serious pest on cruciferous vegetables such as cabbage, broccoli, Chinese cabbage, and cauliflower, with strong reproductive capacity, short life cycle, and increasing resistance to chemical pesticides [13, 14]. Damage caused by diamondback moth is estimated to be as high as US$4–5 billion per year globally [4]. In that context, PlxyGV, a member of the genus *Betabaculovirus*, has been researched and developed as a specific biopesticide for diamondback moth. This virus has an occlusion body (OB) form in the first infection stage and a budded virus form in the secondary infection stage. Each OB contains a complete genome, capable of infecting and killing larvae after only 3 to 5 days. PlxyGV can spread effectively in pest populations, causing widespread epidemics under favorable conditions. In addition, this virus also has high biological specificity, does not affect beneficial organisms or the environment, thereby meeting the requirements for safe and sustainable pest control in agriculture [15]. As a result, PlxyGV is increasingly incorporated into biopesticide formulations as a viable alternative to chemical pesticides for managing diamondback moth infestations.

Despite these advantages, a critical bottleneck in biopesticide commercialization remains the absence of robust quality control protocols [16]. Current OB quantification relies on error-prone hemocytometry, suffering from operator dependency and inability to distinguish viable from defective particles [17]. In this context, real-time PCR presents a promising solution, providing sensitivity, specificity, and accurate quantification of viral content in biological products. Unlike conventional PCR, which provides only end-point detection, qPCR allows precise determination of the cycle threshold (Ct) that correlates with the logarithm of target DNA concentration. The method combines high sensitivity with a broad dynamic range, often exceeding 6–7 orders of magnitude, and can discriminate single-copy targets within complex biological matrices. In recent years, qPCR has been applied successfully to the quantification of baculoviruses such as *Helicoverpa armigera* nucleopolyhedrovirus and *Spodoptera exigua nucleopolyhedrovirus* [18, 19]. For *Plutella xylostella granulovirus*, Zhang *et al.* (2023) established a qualitative qPCR detection assay targeting the *ORF30* gene, which encodes the envelope protein ODV-E66, a major structural component of the occlusion-derived virion responsible for mediating host cell entry. However, to date, a fully validated quantitative qPCR method for PlxyGV has not been reported in Vietnam, particularly one conforming to ISO 22118:2011 standards for molecular quantification of biological agents.

In Vietnam, the urgency to develop effective testing methods for biopesticides is increasingly critical, particularly as the agricultural sector embraces a strategy of sustainable, organic, and biosafe development. This urgency is underscored by Decision No. 5415/QD-BNN-BVTV, issued on December 18, 2023, which outlines the Ministry of Agriculture and Rural Development's "Project on the Development of Production and Use of Biopesticides by 2030, with a Vision to 2050." This initiative aims to boost the usage of biopesticides to 30% and elevate the percentage of qualified production facilities to 90% nationwide [20]. Consequently, establishing effective quality control measures for biological plant protection products, including those containing PlxyGV, is essential to ensure pest control efficacy and consumer safety. In this study, we present a real-time PCR method specifically designed to detect and quantify PlxyGV, addressing this urgent need in the industry.

2. MATERIALS AND METHODS

2.1. Testing samples

The study was conducted using plant protection products containing PlxyGV provided by Henan Jiyuan Baiyun Industry Co., Ltd. through the distributor Nong Sinh Co., Ltd.

2.2. Reagents and reference materials

2.2.1. Reagents

Luminaris HiGreen qPCR master mix 2X (Thermo Fisher Scientific, USA), Gene JET genomic DNA purification kit (Thermo Fisher Scientific, USA), Primer (IDT, USA), real time PCR tube strips (Thermo Fisher Scientific, USA), BHI broth (Merck, Germany), DNA ladder from 50 bp to 1,000 bp (Thermo Fisher Scientific, USA), Agarose (Thermo Fisher Scientific, USA), DNA dye (Intron), 1X TAE buffer (Thermo Fisher Scientific, USA), pGEM-T Easy cloning vector (Promega, USA). The remaining chemicals meet the requirements for molecular biology research.

2.2.2. Reference materials

The study used a purified PlxyGV strain provided by Henan Jiyuan Baiyun Industry Co., Ltd. through distributor Nong Sinh Co., Ltd.

2.3. Equipment

Machines and equipments used in the study include: CFX96 Touch Real-time PCR machine (Bio-Rad, USA), Hettich MIRKO 220R refrigerated centrifuge (Hettich, Germany), horizontal electrophoresis device (Bio-Rad, USA), Nanodrop spectrophotometer (Thermo Fisher Scientific, USA), Geldoc gel imaging system (Bio-Rad, USA), vortex shaker (IKA, Germany), spindown (GeneReach, Taiwan) and other ancillary equipment.

2.4. Methods

2.4.1. Genomic DNA extraction

Total DNA from *Plutella xylostella granulovirus* (PlxyGV) was extracted using two different methods to compare extraction efficiency and DNA purity. The first method employed the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions, which relies on silica membrane binding and ethanol-based washing to obtain purified genomic DNA. The second method followed a modified alkaline lysis protocol based on Zhang *et al.* (2023) [17], designed to improve the disruption of occlusion bodies (OBs) and release encapsulated viral DNA. In this procedure, 500 µL of the virus suspension was treated with alkaline buffer containing 100 mM NaCl, 100 mM Na_2CO_3, and 5 mM EDTA, and incubated at room temperature for 1 h. After lysis, the mixture was diluted 1:1000 in nuclease-free water to obtain a working DNA solution suitable for amplification. The concentration of PlxyGV DNA was quantified using a Nanodrop at 260 nm, and the DNA solution was stored at -20°C until further use.

2.4.2. Real-time PCR method to amplify target genes

For amplifying the *ORF30* gene sequence (222 bp), which encodes the ODV-E66 protein crucial for forming the coat of PlxyGV inclusion bodies, we utilized primer sequences from Zhang *et al.* [17]: the forward primer (5'-CCTGCCCGGTCTGTCTACG-3') and the reverse primer (5'-ACCGCCAAGTGAGGGAAATC-3'). Each PCR reaction consisted of Luminaris HiGreen qPCR master mix (2X), 10 µM of each primer, template DNA (100-200 ng), and water, reaching a total volume of 25 µL. The thermal cycling conditions included an initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 30 s and annealing at 62°C for 1 min, with the final product held at 4°C until analysis.

2.4.3. Cloning of the ORF30 gene sequence encoding the ODV-E66 protein

The amplified *ORF30* gene sequence was ligated directly into the pGEM-T Easy cloning vector (Promega). The ligation reaction, totaling 10 µL, included 2 µL of PCR product, 1 µL of T4 DNA ligase, 5 µL of T4 DNA ligase buffer (2X), 1 µL of the pGEM-T Easy vector (50 ng), and 1 µL of sterile deionized water [21]. This mixture was incubated overnight at 4°C and subsequently stored at -20°C. The pGEM-T Easy vector containing the insert was transformed into *E. coli* DH5α cells via heat shock. Transformants were selected using the blue-white colony method on TSA medium supplemented with 100 µg/mL ampicillin, 100 mM IPTG, and 20 µL of X-Gal (50 mg/mL), and incubated at 37°C for 18-20 h.

2.4.4. PCR and sequencing

The white colonies observed on TSA agar plates indicated successful transformation of *E. coli* with the plasmid containing the *ORF30* gene sequence. Colonies were picked with an inoculating loop and cultured in 10 mL of liquid BHI solution containing 100 µg/mL ampicillin, incubated overnight at 37°C. Plasmid isolation was conducted using the GeneJET Plasmid Miniprep Kit. The plasmid DNA was PCR tested for the presence of the target DNA and the pUC19 DNA (300 bp) of the pGEM-T Easy vector using specific primers for the PlxyGV *ORF30* gene and pUC19: forward primer (5'-GCTGCAAGGCGATTAAGTTG-3') and reverse primer (5'-GTTGTGTGGAATTGTGAGCG-3') [21]. The expected band size was 522 bp. Each PCR reaction included 2X PCR master mix, 10 µM of each primer, template DNA (100-200 ng), and water, totaling 25 µL. The thermal cycling protocol involved an initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 30 s. A final extension step at 72°C for 10 min concluded the reaction, which was held at 4°C until analysis. PCR products were analyzed

using electrophoresis on a 1.5% agarose gel containing Redsafe 20,000X, and those amplified with the pUC19 primer pair were sent to Genlab for Sanger sequencing and comparison with data from US GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.4.5. Method validation

Validation parameters were established according to ISO 22118:2011 and Guidelines on performance criteria and validation of methods for detection, identification and quantification of specific DNA sequences and specific protiens in foods, including limit of detection (LOD 95), accuracy (AC, ≥ 90%), specificity (SP, ≥ 90%), sensitivity (SE, ≥ 90%), limit of quantification (LOQ), repeatability (≤ 25), and reproducibility (≤ 35).

3. RESULTS AND DISCUSSION

3.1. Amplification of *ORF30* primer pairs

The comparison of DNA concentrations revealed that the method proposed by *Zhang et al.* achieved significantly higher extraction efficiency than the GeneJET kit (**Table 1**).

Table 1. *Comparison of total DNA concentration extracted from PlxyGV by two different methods*

Sample	GeneJET (ng/µL)	Zhang *et al.* (ng/µL)	Difference (Zhang – Kit)
L1	42	138	96
L2	46	150	104
L3	40	147	107
Mean ± SD	42.7 ± 3.1	145.0 ± 6.2	102.3 ± 5.5

*Statistical analysis: Paired t-test (df = 2, *t* = 24.92, *p* < 0.001)*

The alkaline lysis method achieved an average DNA concentration of 145.0 ± 6.2 ng/µL and an A260/A280 ratio of 2.2 ± 0.04, whereas the GeneJET kit yielded 42.7 ± 3.1 ng/µL with an A260/A280 ratio of 1.86 ± 0.05. The average difference between the two methods was 102.3 ± 5.5 ng/µL. Statistical analysis using a paired *t*-test showed that this difference was highly statistically significant ($t(2) = 24.92$; $p < 0.001$). These results indicate that the alkaline lysis buffer method described by Zhang was more effective at disrupting the structure of viral inclusion bodies, leading to higher DNA recovery. The total DNA extracted using Zhang's method was then utilized as a template for checking the amplification of the *ORF30* primer pairs.

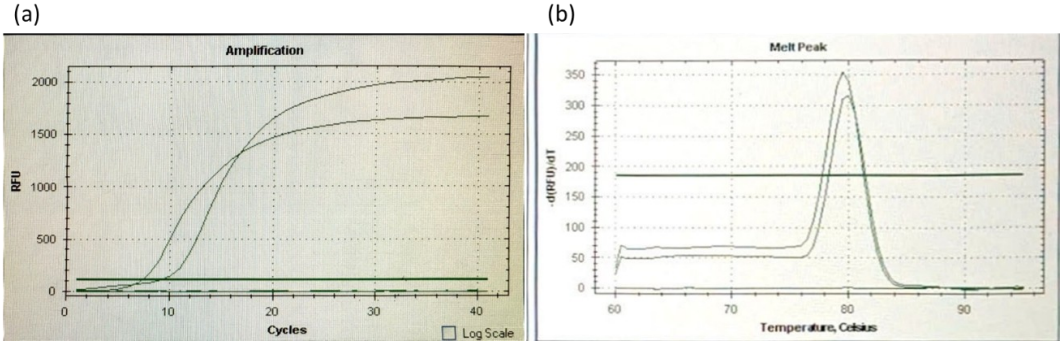


Figure 1*. (A) Typical amplification curve: positive control (Ct~7), negative control (under threshold); and (B) melting curve of ORF30 gene*

Real-time PCR with primers specific to the *ORF30* gene generated clear amplification signals, as evidenced by stable fluorescence curves (**Figure 1A**). The sample produced amplification values of 10 cycles. Melting curve analysis indicated that the real-time PCR products exhibited a characteristic melting temperature of approximately 80°C, confirming the amplification of a single specific product (**Figure 1B**). The resulting real-time PCR products were subsequently used to generate recombinant vectors, pGEM-PlxyGV, and transformed into *E. coli* DH5α cells.

3.2. Results of gene cloning for *ORF30* coding the ODV-E66 protein

To assess cloning efficiency, plasmid DNA was extracted from eight white colonies and analyzed for the presence of the vector and the *ORF30* gene sequence using PCR with specific pUC19 primers (300 bp).

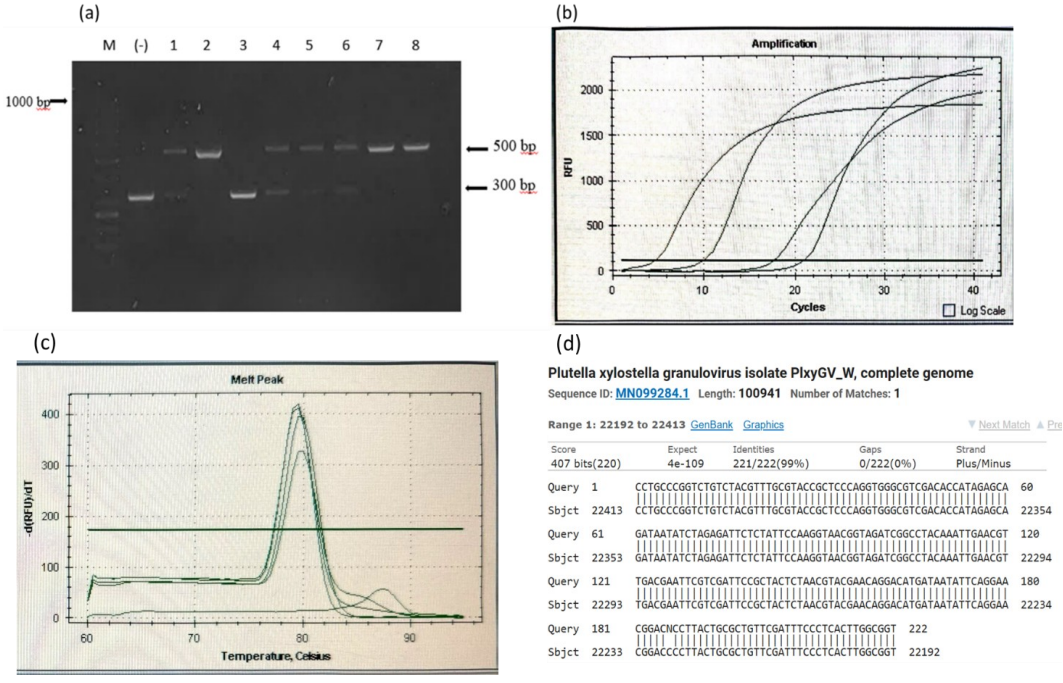


Figure 2. *(A) Results of testing the recombinant plasmid carrying the ORF30 gene sequence on agarose gel; (B) Fluorescence amplification curve of the real-time PCR product: positive control (Ct~10), colony 8 (Ct~4), colony 7 (Ct~20), colony 8 (Ct~19), negative control (under threshold); (C) Melting curve of the real-time PCR product; (d) Sequence comparison of the ORF30 gene with the reference sequence from NCBI (GenBank code: MN099284.1)*

Agarose gel electrophoresis results (**Figure 2A**) revealed DNA bands of the theoretical size (~522 bp) in white colonies 2, 7, and 8, confirming the successful insertion of the *ORF30* gene into the pGEM-T Easy vector. Colony 3 exhibited a DNA band of approximately 300 bp, indicating unsuccessful recombination. Colonies 1, 4, 5, and 6 displayed two bands (~522 bp and ~300 bp), suggesting the presence of both the recombinant plasmid and the vector without the gene insertion. Plasmids from colonies 2, 7, and 8 were chosen as templates for the subsequent real-time PCR reaction. The amplification results (**Figure 2B**) demonstrated consistent fluorescence curves, with the positive control showing a Ct value of approximately 10, and colonies 2, 7, and 8 exhibiting Ct values of around 4, 20, and 19, respectively, indicating efficient amplification of the ORF30 gene in the recombinant plasmids. The melting curve analysis (**Figure 2C**) showed that the melting temperatures of colonies 2, 7, and 8 were 80.0°C, 79.5°C, and 79.5°C, respectively, which were identical to that of the positive control (79.5°C), confirming the specificity of the amplification products. To verify the sequence of the cloned gene, Sanger sequencing of these recombinant plasmids was performed, revealing a 99% similarity with the reference sequence (GenBank: MN099284.1) (**Figure 2D**), thereby confirming that the cloned gene was indeed *ORF30* of PlxyGV. The recombinant plasmids carrying the *ORF30* gene will serve as standard plasmids for future studies.

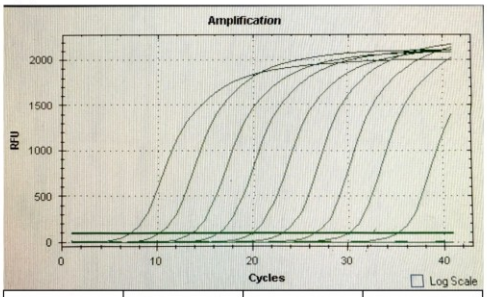
3.3. Validation parameters

The parameters to be evaluated include: limit of detection (LOD 95), accuracy (AC, ≥ 90%), specificity (SP, ≥ 90%), sensitivity (SE, ≥ 90%), limit of quantification (LOQ), repeatability (≤ 25%), and reproducibility (≤ 35%).

3.3.1. Determination of detection limit

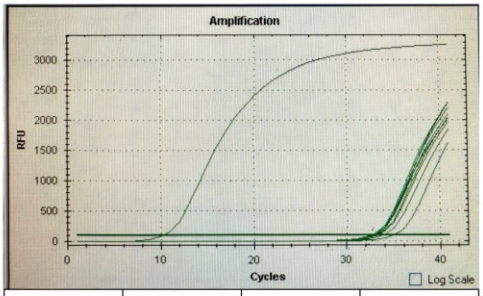
The limit of detection (LOD) was calculated based on the lowest DNA concentration in which at least 95% of samples yielded positive results, in accordance with ISO 22118:2011. To determine the LOD, the standard plasmid carrying the PlxyGV *ORF30* gene was serially diluted in a log base 10 scale from 10^9 to 10^1 copies/µL, resulting in concentrations of 10^8, 10^7, 10^6,10^5, 10^4, 10^3, 10^2, 10^1 copies/µL, which were then used as templates in a real-time PCR reaction with specific primers for *ORF30* gene.

(a)



Log	Ct	Log	Ct
9	7.01	4	23.1
8	10.14	3	26.45
7	13.53	2	29.89
6	16.35	1	35.06
5	19.99	0	N/A

(b)



Log	Ct	Log	Ct
(+)	12.28	1	37.1
1	34.97	1	34.61
1	35.92	1	34.91
1	34.79	1	34.78
1	35.3	1	34.88
1	35.16		

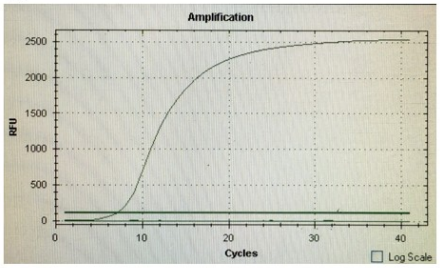
Figure 3. *Results of the detection limit survey with standard plasmid concentrations (10^9 to 10^1 copies/µL).*

At a plasmid concentration of 10^1 copies/µL, the Ct cycle threshold was approximately 35.06 (**Figure 3A**). This concentration was identified as the lowest at which the assay could reliably detect the *ORF30* gene sequence. To confirm the LOD, the experiment was repeated 10 times at this plasmid concentration, resulting in a 100% positive detection rate **(Figure 3B)**. Therefore, the limit of detection (LOD) of the method was established at 10^1 copies/µL.

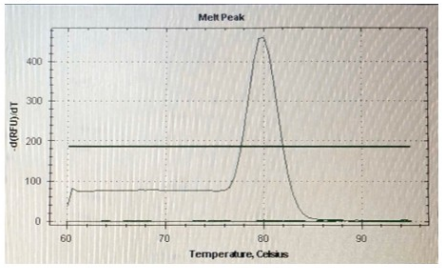
3.3.2. Accuracy (AC), specificity (SP) and sensitivity (SE)

Real-time PCR reactions were conducted using a primer pair amplifying *ORF30* gene across two sample groups: Group 1 included PlxyGV strains provided by Henan Jiyuan Baiyun Industry Co., Ltd., while Group 2 consisted of non-PlxyGV virus strains used as biopesticides, such as *Helicoverpa armigera nucleopolyhedrovirus (HearNPV), Spodoptera exigua nucleopolyhedrovirus (SeNPV), and Spodoptera litura nucleopolyhedrovirus (SpltNPV)*.

(a)



(b)



(c)

Sample	Ct value	Melting point
PlxyGV	7.26	80°C
HearNPV	N/A	N/A
SeNPV	N/A	N/A
SpltNPV	N/A	N/A

Figure 4. *(A) Typical amplification curves; (B) melting temperatures for the two sample groups; and (C) Ct value and melting point of the two sample groups*

The PlxyGV strain group exhibited a typical amplification curve with a melting temperature (Tm) of 80°C (**Figure 4A**). In contrast, the heterologous group demonstrated no typical amplification or melting curves across all samples (**Figure 4B**). These results confirm that the real-time PCR method, utilizing specific primers for the *ORF30* gene, can effectively differentiate between virus species used as biopesticides.

3.4. Construction of standard lines

The quantitative method was established based on the linear correlation between the base 10 logarithm of the standard plasmid copy number (log_{10} copies) and the threshold cycle value (Ct) obtained in the real-time PCR reactions.

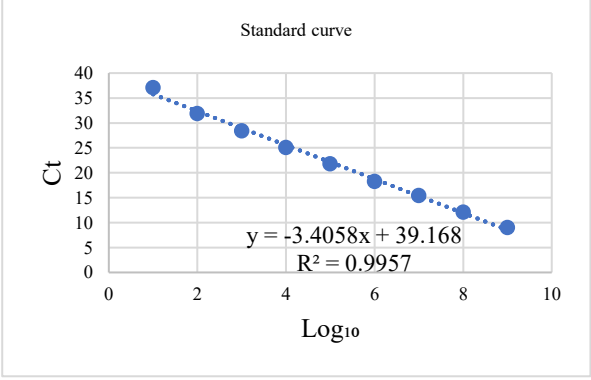


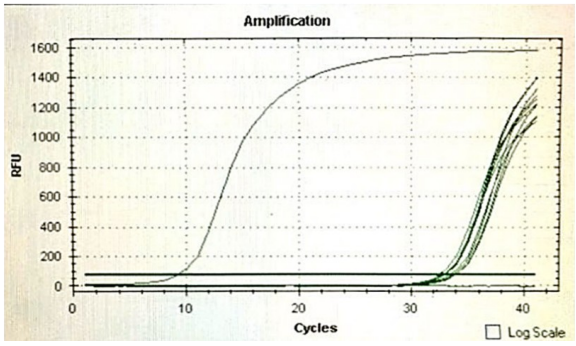
Figure 5. *PlxyGV standard curve from dilution concentrations of 10^1 to 10^9*

The calibration curve indicated a slope of the linear equation of -3.4058, with a correlation coefficient of $R^2 = 0.9957$, signifying a strong relationship between DNA concentration and amplification signal (**Figure 5**). The amplification efficiency (E), calculated using the formula E = 10^(-1/slope), reached 90.1%, which is within the acceptable limits per ISO 22118:2011 standards. Thus, the standard curve established in this study demonstrates high reliability for accurate quantification of PlxyGV. The equation representing the linear correlation between the threshold cycle (Ct) and the log_{10} of initial DNA copy number (log_{10} Sq) is as follows: $Ct=-3.4058\times\log10(Sq)+39.168$. From this equation, the initial target DNA quantity (Sq) can be derived using: $Sq=10[(Ct-39.168)/-3.4058]$.

3.5. Limit of quantification (LOQ)

The limit of quantification (LOQ) was established based on the lowest concentration detectable. This was determined by repeating the experiment 10 times at a plasmid concentration of 10^1 copies/µL (**Figure 6**).

(a)



(b)

Well	Fluor	Content	Sample	C(t)
A01	SYBR	Pos Ctrl		10.93
A02	SYBR	Unkn	5-8	34.93
A03	SYBR	Unkn	5-8	34.28
A04	SYBR	Unkn	5-8	34.43
A06	SYBR	Unkn	5-8	35.13
A07	SYBR	Unkn	5-8	33.93
B01	SYBR	Unkn	5-8	34.39
B02	SYBR	Unkn	5-8	34.33
B03	SYBR	Unkn	5-8	35.33
B04	SYBR	Unkn	5-8	34.29
B05	SYBR	Unkn	5-8	35.39
B07	SYBR	Neg Ctrl		N/A

Figure 6. *Amplification curve (a) and Ct threshold cycle value (b) of 10 real-time PCR reaction replicates at a plasmid concentration of 10^1 copies/µL*

The results indicated a 100% positive reaction across all 10 replicates. Thus, it can be concluded that the limit of quantification of the method is also 10^1 copies/µL.

3.6. Repeatability (RSDr) and reproducibility (RSDR) of the method

To evaluate repeatability, real-time PCR reactions were conducted in duplicate, each consisting of five replicates at a standard plasmid concentration of 10^1 copies/µL. For reproducibility (RSDR), two independent analysts performed 10 replicates on the same sample concentration. The results are summarized in **Table 2**.

Table 2. *Ct value of 10 replicates at plasmid concentration of 10^1 copies/µL*

Inspector 1		Inspector 2	
1st time	2nd time	1st time	2nd time
34.65	34.89	34.57	34.9
34.51	34.93	34.63	34.96
34.74	35.02	34.66	34.91
34.59	34.85	34.7	35.06
34.68	34.98	34.6	34.94

The mean Ct values were 34.63 (KNV1) and 34.94 (KNV2), with standard deviations of 0.068 and 0.067, respectively. The relative coefficients of variation (RSDr) were calculated as 0.20% (KNV1) and 0.19% (KNV2), both within the acceptable limit per ISO 22118:2011 (RSDr ≤ 25%). The reproducibility (RSDR) was determined by evaluating Ct values from two different analysts conducting 10 independent replicates on the same sample concentration. The mean Ct value was 34.78, with a standard deviation of 0.098, resulting in an RSDR coefficient of variation of 0.28%, which is below the specified limit (RSDR ≤ 35%).

3.7. Experimental results of quantifying PlxyGV in plant protection products

To assess the applicability of the developed quantitative method, the research team analyzed PlxyGV content in plant protection product provided by Henan Jiyuan Baiyun Industry Co., Ltd. The samples were extracted using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, USA) and analyzed via real-time PCR with specific primers for the *ORF30* gene. The experiment was conducted in three independent trials.

The quantification results of PlxyGV in plant protection product indicated that Ct values ranged from 9.73 to 10.05, corresponding to viral DNA copy numbers in the reaction from 3.11×10^8 to 3.89×10^8 copies/µL (**Table 3**). The average value was approximately 3.51×10^8 copies/µL, exceeding the manufacturer's published threshold of ≥ 3.0×10^8 copies/µL (equivalent to 3.0×10^{10} OB/mL).

***Table 3**. Ct value obtained from real-time PCR reaction when quantifying PlxyGV in plant protection product*

Experimental times	*Ct*	*Copies/µL in reaction (Sq)*
1	9.88	3.52×10^8
2	10.05	3.11×10^8
3	9.73	3.89×10^8

4. CONCLUSION

The study successfully established a procedure for detecting and quantifying PlxyGV using the real-time PCR technique with specific primers targeting the ORF30 gene. The method demonstrated high sensitivity, with detection and quantification limits both established at 101 copies/µL. Accuracy, specificity, and sensitivity were all achieved at 100%. The constructed standard curve, correlating the threshold cycle (Ct) with log virus density, displayed a strong linear relationship (R2 = 0.9957) and an amplification efficiency of 90.1%, well within acceptable limits. Additionally, repeatability (RSDr = 0.20%) and reproducibility (RSDR = 0.28%) were both found to be within established thresholds (RSDr ≤ 25%%, RSDR ≤ 35%%), aligning with ISO 22118:2011 standards.

REFERENCES

[1]. M. Tudi *et al.,* "Agriculture development, pesticide application and its impact on the environment," *International Journal of Environmental Research and Public Health*, vol. 18, no. 3, p. 1112, 2021.

[2]. V. M. Pathak *et al*., "Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review," *Frontiers in Microbiology*, vol. 13, p. 962619, 2022.

[3]. C. Shekhar *et al.,* "A systematic review of pesticide exposure, associated risks, and long-term human health impacts," *Toxicology Reports*, vol. 13, pp. 101840–101855, 2024.

[4]. R. Jayaraj, P. Megha, and P. Sreedev, "Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment," *Interdisciplinary Toxicology*, vol. 9, no. 3–4, pp. 90–100, 2016.

[5]. M. S. Ayilara *et al.,* "Biopesticides as a promising alternative to synthetic pesticides: A case for microbial pesticides, phytopesticides, and nanobiopesticides," Frontiers in Microbiology, vol. 14, p. 1040901, 2023.

[6]. G. M. Daraban, R.-M. Hlihor, and D. Suteu, "Pesticides vs. Biopesticides: From pest management to toxicity and impacts on the environment and human health," *Toxics*, vol. 11, no. 12, p. 983, Dec. 2023

[7]. P. Cai and G. Dimopoulos, "Microbial biopesticides: A one health perspective on benefits and risks," *One Health*, vol. 20, p. 100962, 2025.

[8]. A. K. Priya, A. Alagumalai, D. Balaji, and H. Song, "Bio-based agricultural products: a sustainable alternative to agrochemicals for promoting a circular economy," *RSC Sustainability*, vol. 1, no. 4, pp. 746–762, 2023.

[9]. M. Martínez-Balerdi, J. Caballero, E. Aguirre, P. Caballero, and I. Beperet, "Baculoviruses as microbial pesticides: Potential, challenges, and market overview," *Viruses*, vol. 17, no. 7, p. 917, 2025.

[10]. H.-R. Lee, J. Jung, M. Riu, and C.-M. Ryu, "A new frontier for biological control against plant pathogenic nematodes and insect pests I: By microbes," *Research in Plant Disease*, vol. 23, no. 2, pp. 114–149, 2017.

[11]. Y. Gelaye and B. Negash, "The role of baculoviruses in controlling insect pests: A review," *Cogent Food & Agriculture*, vol. 9, no. 1, p. 2254139, 2023.

[12]. S. Bhattacharyya and D. K. Bhattacharya, "Pest control through viral disease: Mathematical modeling and analysis," *Journal of Theoretical Biology*, vol. 238, no. 1, pp. 177–197, 2006.

[13]. P. Bhadoria, M. Nagar, V. Bharihoke, and A. S. Bhadoria, "Ethephon, an organophosphorous, a fruit and vegetable ripener: Has potential hepatotoxic effects?," *Journal of Family Medicine and Primary Care*, vol. 7, no. 1, pp. 179–183, 2018.

[14]. C. A. Damalas and S. D. Koutroubas, "Farmers' exposure to pesticides: Toxicity types and ways of prevention," *Toxics*, vol. 4, no. 1, p. 1, 2016.

[15]. J. H. Chang *et al*., "An improved baculovirus insecticide producing occlusion bodies that contain Bacillus thuringiensis insect toxin," *Journal of Invertebrate Pathology*, vol. 84, no. 1, pp. 30–37, 2003.

[16]. R. Chaudhary *et al*., "Microbial bio-control agents: A comprehensive analysis on sustainable pest management in agriculture," *Journal of Agriculture and Food Research*, vol. 18, p. 101421, 2024.
[17]. H. Zhang et al., "Establishment of a rapid detection method for Plutella xylostella granulovirus based on qPCR," *Heliyon,* vol. 9, no. 4, p. e15170, 2023.
[18]. R. I. Graham *et al*., "Development of a real-time qPCR assay for quantification of covert baculovirus infections in a major African crop pest," *Insects*, vol. 6, no. 3, pp. 746–759, 2015.
[19]. L. A. da Silva *et al*., "Identification and detection of known and new viruses in larvae of laboratory-reared fall armyworm, Spodoptera frugiperda," *Journal of Invertebrate Pathology*, vol. 210, p. 108290, 2025.
[20]. "Vietnam to increase the use of biological plant protection products by an additional 30%," Tuoi Tre Online, Dec. 28, 2023. [Online]. Available: https://tuoitre.vn/viet-nam-se-su-dung-thuoc-bao-ve-thuc-vat-sinh-hoc-tang-them-30-20231228155953896.htm
[21]. N. T. Hanh, L. V. Hoa, T. H. Ba, P. V. Quan, L. T. Long, and P. T. Loan, "Producing positive control materials for template in PCR testing to detect botulinum neurotoxin types A and B genes," *Vietnam Journal of Food Control*, vol. 7, no. 1, pp. 53–67, 2024.