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First Detection of Major Viruses Causing Mixed Infections in Melon (*Cucumis melo* L.) in Bengkulu Province, Indonesia

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Abstract

Melon cultivation has increased rapidly in recent years and is frequently associated with widespread virus-like symptoms in Bengkulu Province, Indonesia; however, thereis no formal documentation of the mixed viral infections has been available. Therefore, this study aimed to detect major viruses infecting melon crops in Bengkulu Province and to evaluate their occurrence based on field symptoms and serological detection. Field surveys were conducted at three melon cultivation sites: Kandang Limun Village (Bengkulu City), Air Sebakul Village (Central Bengkulu Regency), and Tawang Rejo Village (Seluma Regency). Symptomatic leaf samples were collected purposively. Virus detection was performed using the Dot Immunobinding Assay (DIBA) with antisera specific to *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV), and *Papaya ringspot virus* (PRSV). The results showed that the dominant symptoms of viral infection in melon plants included mosaic, vein banding, chlorosis, leaf curling, and stunted growth in both Snow & Rock and Merlin F1 varieties. Disease incidence varied among varieties and locations, with the highest incidence recorded in Snow & Rock (73.3%). Serological detection revealed that 100% of symptomatic samples tested positive for CMV, TMV, and PRSV, confirming that all infections occurred as mixed infections. This study represents the first report of mixed viral infections in melon crops in Bengkulu Province. These findings demonstrate that overlapping and variable field symptoms cannot be attributed to a single virus and cannot be reliably distinguished through visual diagnosis alone, highlighting the importance of laboratory-based diagnostic approaches for accurate virus identification.

Keywords: Cucumber mosaic virus, papaya ringspot virus, serological detection, tobacco mosaic virus, variety

Introduction

The Cucurbitaceae family comprises approximately 975 species distributed across 98 genera of flowering plants worldwide (Xu and Chang, 2017). Many members of this family, commonly referred to as gourds, are widely cultivated in tropical and subtropical regions due to their high economic and nutritional value. In Indonesia, several

cucurbit species are commercially important, including cucumber (*Cucumis sativus*), ridge gourd (*Luffa acutangula*), bitter gourd (*Momordica charantia*), chayote (*Sechium edule*), pumpkin (*Cucurbita moschata*), kabocha (*Cucurbita maxima*), watermelon (*Citrullus lanatus*), and melon (*Cucumis melo*). Melon is a horticultural crop native to Africa and Southwest Asia that has been domesticated and widely cultivated in tropical and subtropical regions due to its adaptability and high economic value (Endl et al., 2021; Grumet et al., 2021; Lija and Beevy, 2025).

Melon fruit is rich in essential nutrients, including carbohydrates, dietary fiber, vitamins A and C, potassium, magnesium, and bioactive compounds such as flavonoids and carotenoids, which contribute to its antioxidant, anti-inflammatory, and hydration-supporting properties. Regular melon consumption has been associated with various health benefits, including improved immune function, cardiovascular health, digestive regulation, and reduced oxidative stress (Manchali et al., 2021: Khalid et al., 2021).

In Indonesia, melon is an important commercial fruit crop intensively cultivated for the domestic market, particularly in major production centers, and several emerging production areas in Sumatra, including Bengkulu (Karimil et al., 2021). The increasing demand for high-quality melons has driven increased protection against pest and disease attacks, particularly viral diseases. They are particular concern because they cause not only direct damage to melon plants but also efficiently are transmitted by insect vectors, resulting in rapid disease spread and significant yield and quality losses (Desbiez, 2020). Nowadays, there are no effective curative methods to manage viral diseases in melon crops, making early detection and preventive management essential (Trisno et al., 2021; Riska et al., 2023).

A wide diversity of viruses has been reported to naturally infect cucurbit crops worldwide, with more than 90 virus species known to infect Cucurbitaceae under field conditions. Numerous studies have documented virus infections on cucurbit crops across different regions. Septariani et al. (2014) and Supyani et al. (2017) reported symptoms such as yellowing, leaf curling, and mosaic on cucumber plants in West Java, Central Java, and the Special Region of Yogyakarta caused by *Squash mosaic virus* (SMV), *Tomato leaf curl New Delhi virus* (ToLCNDV), *Zucchini yellow mosaic virus* (ZYMV), and *Cucumber mosaic virus* (CMV). Wiratama et al. (2015) further reported *Begomovirus* infection on cucumber in Bali, and Selangga et al. (2022) reported *Papaya ringspot virus*. Subsequent studies identified additional viruses infecting cucumbers on Java Island, including *Tobacco Mosaic Virus* (TMV) (Listihani et al., 2018), *Cucurbit Aphid-Borne Yellow Mosaic Virus* (CaBYV) (Listihani et al., 2020), and *Squash Mosaic Virus* (SqMV) (Listihani et al., 2022). Moreover, Subiastuti et al. (2019) reported *Squash leaf curl China virus* (SLCCV), a begomovirus, infecting watermelon in Purworejo, Central Java. In Bengkulu Province, *Tomato leaf curl New Delhi virus* (ToLCNDV) infection on cucumber was documented by Sutrawati et al. (2022). Cucurbit crops in Indonesia are exposed to a wide range of viruses with overlapping symptoms, which often complicates visual diagnosis and increases the likelihood of mixed infections in the field.

Viruses infecting one cucurbit species also have the potential to infect other cucurbit crops due to shared vectors, host susceptibility, and overlapping cultivation areas. In Indonesia, several viruses have been reported to infect melon, including *Cucumber*

mosaic virus (CMV) (Monica et al., 2022), *Squash leaf curl China virus* (SLCCNV) (Adnyani et al., 2018), and *Squash mosaic virus* (SqMV) (Lestari and Nurhayati, 2014). Currently, melon cultivation in Bengkulu Province has expanded rapidly, both under protected cultivation systems, such as net houses, and open-field conditions. During field observations, melon plants frequently exhibited virus-like symptoms such as mosaic, yellowing, leaf deformation, and stunting, often resulting in small, malformed fruits that are unmarketable and cause substantial economic losses to farmers.

Therefore, the identity of viruses associated with virus-like symptoms in melon fields of Bengkulu Province, as well as the occurrence of possible mixed infections, remains unclear. This lack of information represents a critical knowledge gap that may hinder the development of effective and region-specific virus management strategies. Accordingly, this study aimed to detect major viruses infecting melon (*Cucumis melo* L.) crops in Bengkulu Province using a Dot Immunobinding Assays. The results are expected to provide essential baseline data on the occurrence and infection patterns of viruses in melon fields of Bengkulu Province, thereby supporting the development of effective, location-specific viral disease management strategies.

Methods

Sample Collection

Sample collection was conducted at three melon cultivation sites in Bengkulu Province, Indonesia: Kandang Limun Village, Bengkulu City (3°47'15" LS, 102°19'40" BT, 80 m asl), Air Sebakul Village, Central Bengkulu Regency (3°29'50" LS, 102°24'25" BT, 15 m asl), and Tawang Rejo Village, Seluma Regency (3°48'00" LS, 102°29'30" BT, 50 m asl) (Figure 1).

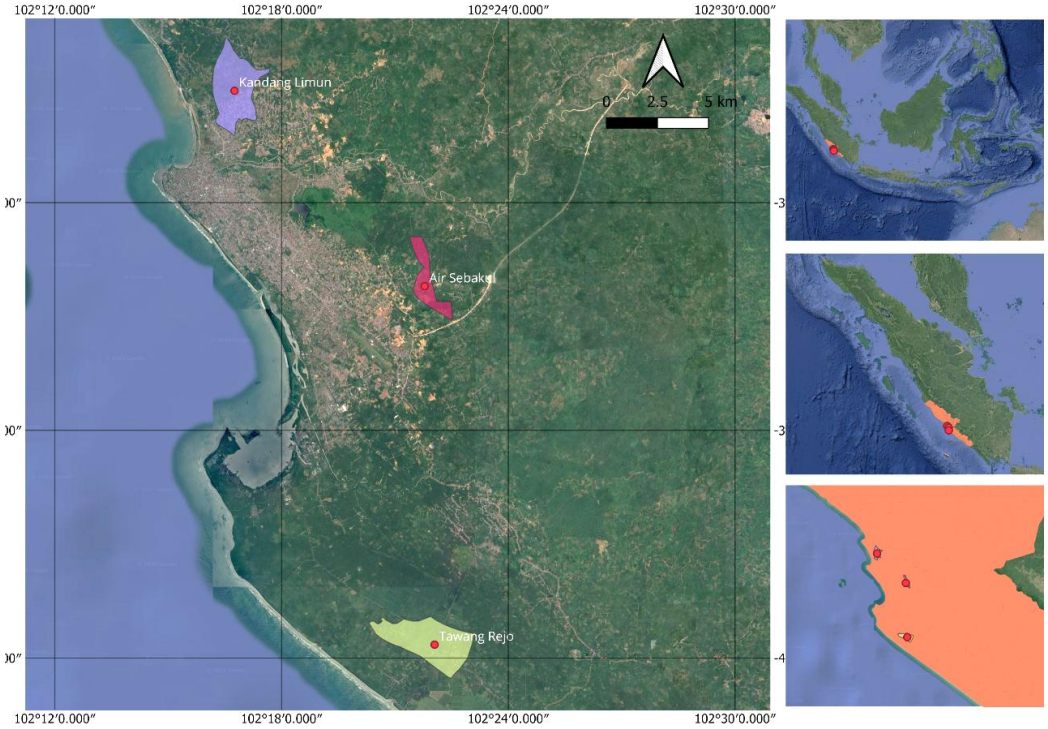

Figure 1. Study sites for the first detection of major viruses causing mixed infections in melon (*Cucumis melo* L.) in Bengkulu Province, Indonesia, specifically located in Kandang Limun, Air Sebakul, and Tawang Rejo.

A total of 20 symptomatic leaf samples were collected from each site, resulting in 60 samples overall. All samples were subsequently subjected to serological analysis using the Dot Immunobinding Assay (DIBA). Sampling was performed using a purposive sampling approach based on the presence of visible virus-like symptoms on melon plants, including mosaic, vein banding, vein clearing, leaf malformation, and stunting. The collected leaf samples were transported to the Plant Protection Laboratory, Department of Plant Protection, Faculty of Agriculture, University of Bengkulu, Indonesia, for further analysis.

Disease Incidence

Disease incidence was calculated based on the number of samples showing virus-like symptoms (mosaic, vein banding, vein clearing, leaf malformation, and stunting) compared with the total number of samples observed in the field. The disease incidence was determined using the following formula:

$$I = \frac{n}{N} x100\% \quad \text{..... (1)}$$

I = Incidence of viral disease (%)
n = Number of symptomatic samples
N = Total number of samples observed

Virus Detection

Dot Immunobinding Assay (DIBA)

Virus detection was performed using a modified Dot Immunobinding Assay (DIBA) based on Mahmood et al. (2007). Leaf samples were homogenized in Tris-buffered saline (TBS) containing 0.02 M Tris–HCl and 0.15 M NaCl (pH 7.5) at a ratio of 1:10 (w/v). Approximately 5 µL of each plant extract was spotted onto a nitrocellulose membrane (Hybond™-P, Amersham Biosciences, UK) and air-dried at room temperature. The membrane was then incubated for 2 h at 50 rpm in 10 mL of blocking solution consisting of 2% (w/v) non-fat milk and 2% (v/v) Triton X-100, followed by five washes with deionized water (dH_2O), each for 5 min with shaking at 100 rpm.

Detection of P*apaya ringspot virus (PRSV)*

For PRSV detection, the membrane was incubated in TBS containing 2% (w/v) non-fat milk and PRSV enzyme conjugate diluted at 1:1000 (v/v), and maintained at 4°C for 24 h. After incubation, the membrane was washed five times with TBS supplemented with 0.05% (v/v) Tween-20 (TBST), followed by rinsing with distilled water.

Detection of *Cucumber mosaic virus (CMV)* and *Tobacco mosaic virus (TMV)*

For CMV and TMV detection, the membrane was incubated with CMV or TMV primary antibody diluted at 1:1000 (v/v) in TBS containing 2% (w/v) non-fat milk for 2 h at 50 rpm. The membrane was then washed five times with TBST and re-incubated for 2 h at 50 rpm with the corresponding CMV or TMV enzyme conjugate diluted at 1:1000 (v/v) in TBS containing 2% (w/v) non-fat milk.

Color Interpretation

After the immunoreaction steps, all membranes were washed five times with TBST and incubated for 10–30 min in 10 mL of substrate buffer composed of 0.1 M Tris–HCl, 0.1 M NaCl, and 5 mM $MgCl_2$, supplemented with 33 µL nitro blue tetrazolium (NBT) and 16.5 µL 5-bromo-4-chloro-3-indolyl phosphate (BCIP). The color development reaction was terminated by immersing the membrane in distilled water. A positive reaction was indicated by a visible purple coloration at the site of sample application on the nitrocellulose membrane.

RESULT

Symptoms and Disease Incidence

Disease incidence in melon plants was estimated in the field based on the presence of visible virus-like symptoms. Observed symptoms at the three sampling locations—

Kandang Limun Village (Bengkulu City), Air Sebakul Village (Central Bengkulu Regency), and Tawang Rejo Village (Seluma Regency)—included mosaic patterns, vein banding, leaf curling, and stunted growth (Figure 2).

Figure 2. Variations of virus infection symptoms in melon plants: (A) Mosaic and vein banding, (B) Chlorosis and leaf margin curling, (C) Curled and thickened leaf lamina, (D) Mosaic with small leaves and vein chlorosis.

In addition, Figure 2 shows considerable variation in symptom severity among infected melon plants. Mild to moderate symptoms such as mosaic patterns, vein banding, and chlorosis were observed in some leaves, whereas more severe symptoms, including thickened and strongly curled leaf lamina, reduced leaf size, and pronounced growth suppression, were evident in others. This variation suggests differences in infection intensity and possibly the involvement of multiple viruses. The occurrence of complex symptom combinations, such as mosaic accompanied by vein chlorosis, leaf deformation, and stunting, is often associated with mixed viral infections rather than single-virus infection. Moreover, the deformation and reduction in leaf area observed in severely affected plants indicate substantial physiological disruption, which may impair photosynthetic capacity and ultimately reduce plant productivity.

Virus Incidence

The incidence of viral disease in melon plants was determined based on the proportion of plants exhibiting virus-like symptoms relative to the total number of plants observed. Disease incidence varied among the surveyed locations (Table 1). The highest incidence was recorded in the Snow & Rock variety at 63 days after planting in Kandang Limun, Bengkulu City, reaching 73.3%. In contrast, the lowest incidence was observed in the Merlin F1 variety at 40 days after planting in Air Sebakul, Central Bengkulu Regency, with an incidence of 29% (Table 1). Overall, disease incidence tended to increase with plant age in the field.

Table 1**.** Virus incidence in melon leaves planting in Kandang Limun, Air Sebakul and Tawang Rejo in Bengkulu Province, Indonesia

Location	Variety	Age (Days After Planting)	Visual Disease Incidence (%)	Virus Detection Using DIBA (%)		
				PRSV	CMV	TMV
Kandang Limun	Snow & Rock	63	73,3	100	100	100
Air Sebakul	Merlin F1	40	29,0	100	100	100
Tawang Rejo	Merlin F1	57	64,0	100	100	100

Serological detection using DIBA revealed that all symptomatic samples from the three locations were positive for PRSV, CMV, and TMV. This result indicates that all tested melon plants were simultaneously infected by the three viruses, confirming the occurrence of mixed infections across all surveyed sites. The presence of mixed infections may explain the diversity and severity of symptoms observed in the field, as well as the relatively high disease incidence recorded at later plant growth stages.

Although visual disease incidence differed among locations and plant ages, virus detection results were uniform, suggesting that symptom expression may depend on plant age, infection intensity, and host response rather than virus presence alone. The lower incidence observed at earlier growth stages may indicate latent infections or delayed symptom development, which became more apparent as plants aged in the field.

Mixed Infection of CMV, TMV, and PRSV

Based on the *DIBA* detection results shown in Table 1, 60 melon samples from the surveyed locations exhibited mixed infections involving three viruses: *Cucumber mosaic virus (CMV), Papaya ringspot virus (PRSV),* and *Tobacco mosaic virus (TMV).* Mixed infections can intensify disease symptoms due to synergistic interactions among the viruses, leading to more severe symptoms than those caused by single-virus infections (Figure 3).

The Figure 3 shows a clear and consistent color development in all melon samples when reacted with antisera against CMV, PRSV, and TMV. The uniform appearance of purple coloration across multiple sample spots indicates a strong and specific antigen–antibody reaction, confirming the presence of the three viruses in the tested samples.

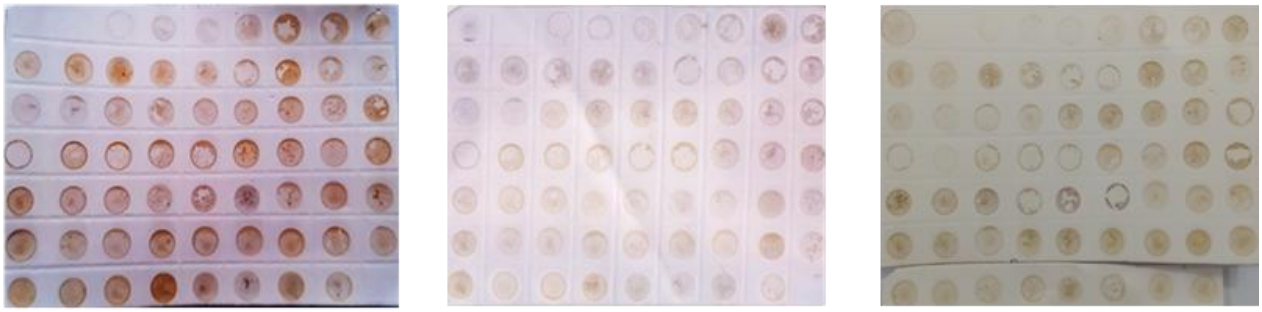

Figure 3**.** A color change to purple indicates a positive reaction to *CMV* (A), *PRSV* (B), and *TMV* antisera; no color change indicates a negative reaction (healthy plant samples used as negative controls).

In contrast, no color development was observed in healthy control samples, demonstrating the specificity and reliability of the assay. The simultaneous positive reactions observed for CMV, PRSV, and TMV provide visual evidence of mixed viral infections in melon plants from all surveyed locations. Although DIBA is a qualitative method and does not quantify virus concentration, the strong and consistent reactions suggest widespread virus infection, which is in agreement with the severe and diverse symptoms observed in the field.

Discussion

Field observations revealed that mosaic symptoms were the most frequently encountered in melon plants across all locations (Figure 2). Additional symptoms included vein thickening, vein banding, chlorosis, leaf curling, and abnormal or stunted plant growth. Such symptom diversity is commonly associated with viral infections in cucurbit crops. Infections caused by viruses belonging to the genus *Potyvirus*, such as PRSV, are typically characterized by mosaic patterns, leaf and fruit deformation, tissue thickening, and growth inhibition (Morteza et al., 2021). Likewise, infection by CMV is known to induce mosaic, chlorosis, and leaf malformation in melon plants (Monica et al., 2022).

In cucurbit plants infected by TMV, mosaic symptoms often appear as early indicators of infection (Damayanti et al., 2019). TMV infection in pumpkin (*Cucurbita* spp.) produces symptoms comparable to those observed in cucumber, although symptom severity is generally lower than that reported in plants of the Solanaceae family, which typically exhibit more pronounced mosaic patterns (Listihani et al., 2018). The overlap of visual symptoms observed in this study indicates that symptom expression alone cannot reliably distinguish between PRSV, CMV, and TMV infections, particularly under field conditions.

At the physiological level, viral infection induces various alterations in host plants, including changes in chloroplast structure, disruption of protein synthesis, hormonal imbalance, accumulation of reactive oxygen species (ROS), and interference with cell cycle regulation (Jiang and Zhou, 2023). The occurrence of stunted growth in infected melon plants suggests that viral infection likely began during the early vegetative growth stage, allowing sufficient time for systemic infection and symptom development.

Disease incidence varied among locations, plant ages, and melon varieties (Table 1). The results suggest that varietal differences and plant age influence symptom severity and expression rather than absolute resistance to viral infection. In general, disease incidence increased with plant age, suggesting cumulative infection pressure and progressive symptom expression over time. Despite variation in visual disease incidence, DIBA detection results were uniform, with 100% of symptomatic samples testing positive for PRSV, CMV, and TMV across all locations and varieties. This discrepancy indicates that visual disease incidence reflects the proportion of plants expressing symptoms rather than the actual presence of viruses. Plants with lower incidence values may harbor latent infections or exhibit delayed symptom development, which become more apparent as plants mature. Previous studies have shown that disease development is influenced by both host plant variety and plant age, where varietal differences affect the intensity of pathogen attack (Kone et al., 2017), while increasing plant age significantly influences disease incidence and severity (Cohen et al., 2022).

In the present study, DIBA detection using antisera specific to PRSV, CMV, and TMV yielded positive reactions for all three viruses in every symptomatic sample tested (Figure 3). The consistent development of purple coloration on the nitrocellulose membrane, accompanied by the absence of color change in healthy plant controls, confirms the specificity and reliability of the assay. These results provide clear serological evidence that all symptomatic melon samples were simultaneously infected by PRSV, CMV, and TMV, demonstrating the occurrence of mixed

viral infections across all surveyed locations and varieties.

Serological virus detection using the DIBA relies on substrate color development resulting from specific antigen–antibody reactions between plant extracts, virus-specific antisera, and enzyme substrates (Anggraini and Hidayat, 2014). This method has been widely applied for detecting cucurbit-infecting viruses, including CMV, PRSV, *Cucurbit aphid-borne yellow virus* (CABYV), and *Cucurbit green mottle mosaic virus* (CGMMV) in cucumber plants in West Java (Laili and Damayanti, 2019).

The consistent detection of PRSV, CMV, and TMV in all symptomatic samples indicates that mixed viral infections are a dominant epidemiological condition in melon cultivation areas in Bengkulu. Mixed infections are known to induce synergistic interactions among viruses, leading to more severe and complex symptoms than those caused by single-virus infections (Jiang and Zhou, 2023, Redinbaugh and Stewart, 2018). Such synergism may arise from the suppression of host defense mechanisms or enhanced viral replication facilitated by co-infecting viruses.

The complex symptom combinations observed in this study—including intense mosaic, vein banding, chlorosis, leaf deformation, tissue thickening, and stunted growth—are consistent with the effects of mixed infections reported in previous studies. Romay et al. (2014) reported that CMV, PRSV-W, WMV, ZYMV, and ZLCV commonly infect cucurbit crops through aphid- and thrips-mediated transmission, often resulting in mixed infections under field conditions. Similarly, a multiplex RT-PCR study by Han et al. (2024) demonstrated that dual and triple viral infections are more prevalent than single infections in melon fields in Xinjiang, China.

The findings of the multiplex RT-PCR study are highly relevant to the present research, as both studies highlight the limitations of symptom-based diagnosis and the epidemiological importance of mixed infections in open-field melon cultivation. In Bengkulu, the simultaneous presence of PRSV, CMV, and TMV detected by DIBA supports the conclusion that visual symptoms alone are insufficient for accurate virus identification, particularly when multiple viruses co-circulate.

Collectively, the visual symptoms observed in melon plants in Bengkulu, Indonesia, cannot be attributed to a single virus but rather arise from complex interactions among PRSV, CMV, and TMV. The universal detection of these viruses across all samples suggests that melon cultivation systems in the surveyed areas are in an epidemiological state that favors virus persistence, circulation, and co-infection. Therefore, reliance solely on visual symptom observation may lead to misdiagnosis and ineffective management strategies.

Conclusion

The dominant symptoms of viral infection in melon plants included mosaic, vein banding, chlorosis, leaf curling, and stunted growth in both Snow & Rock and Merlin F1 varieties. Disease incidence varied among varieties and locations, with the highest incidence recorded in Snow & Rock (73.3%). Serological detection revealed that 100% of symptomatic samples tested positive for CMV, TMV, and PRSV, confirming that all infections occurred as mixed infections. This study represents the first report of mixed viral infections in melon crops in Bengkulu Province. These findings demonstrate that overlapping and variable field symptoms cannot be attributed to a single virus and cannot be reliably distinguished through visual diagnosis alone, highlighting the importance

of laboratory-based diagnostic approaches for accurate virus identification.

Declaration

Author Contributions

Mimi Sutrawati as team leader, prepared the research plan, assisted with the work in the lab, and wrote the manuscript; Hendri Bustamam and Sempurna Br. Ginting corrected the manuscript, Ratna Nabila Pradita and Ella Mustika conducted field surveys and virus detection, and Ewa Aulia assisted with technical activities in the laboratory.

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Conflict of Interest

The authors declare that we have no financial or personal conflicts of interest that could have influenced the work reported in this article.

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