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Viruses Infecting Garlic in Indonesia: Incidence and its Transmission to Shallot and Spring Onion

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ABSTRACT

Garlic (*Allium sativum*) is an important horticultural crop globally and in Indonesia. Its production faces significant challenges due to diseases caused by viral infections. Therefore, this study aimed to determine the distribution of garlic viruses across cultivation areas in Indonesia and analyze the potential for transmission to shallot (*Allium cepa var. agregatum*) and spring onion (*Allium fistulosum* L.). In the process, garlic leaves and bulbs samples were collected from several growing areas in West Nusa Tenggara, Central Java, West Java, and East Java. Onion yellow dwarf virus (OYDV), leek yellow stripe virus (LYSV), garlic common latent virus (GCLV), and shallot latent virus (SLV) were detected by reverse transcription-polymerase chain reaction method using specific primers. The results showed that mixed infections of several viruses were more common than the single form. The average frequency of viral infection in order from the highest was LYSV (75.15%), OYDV (64.95%), SLV (36.47%), and GCLV (26.66%). Single isolates of LYSV, OYDV, GCLV, and SLV were selected for the transmission experiment in the screen house, and each was inoculated mechanically to shallot (cultivars Sanren F1 and Lokananta) and spring onion (cultivar Blaze F1). All viruses were successfully transmitted to shallot, but only LYSV, OYDV, and SLV could be mechanically transmitted to spring onion. The infection of shallot and spring onion significantly affected the number of bulbs but had no considerable effect on plant height, leaf number, and bulb diameter.

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INTRODUCTION

Garlic (*Allium sativum*) is a horticultural crop cultivated in the highlands. Its main producing areas in Indonesia are Central Java, West Nusa Tenggara, and East Java.

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In the country, garlic production has been unable to meet domestic market demand. Hence, the consumption relies on imports. A limitation in the cultivation of this crop is the availability of good seed bulbs free from pathogens, particularly viruses. The main viruses infecting garlic belong to the genera *Potyvirus, Carlavirus,* and *Allexivirus* (Katis et al., 2012). Symptoms of the infection include yellow mosaic, green mosaic, yellow-striped, green-striped, curly, and notched upper surface of leaves. Viruses in garlic can be transmitted through insect vectors (aphids), contaminated plant material, and mechanical transmission (Mang et al., 2022).

Virus infection is systemic, affecting the entire plant body, including the bulbs used as planting material (Bhusal et al., 2021). Bulbs from infected plants in the previous planting season, reused as seed materials tend to be a source of disease. Furthermore, Bagi et al. (2012) explained that virus infection caused a 21.5% decrease in bulb weight. Hidayat et al. (2023) and Nurulita et al. (2024) reported four main viruses infecting garlic plants in Indonesia, namely onion yellow dwarf virus (OYDV), leek yellow stripe virus (LYSV), garlic common latent virus (GCLV), and shallot latent virus (SLV). These infections are referred to as "Garlic viral complex" because single infections are rare (Cremer et al., 2021; Majumder & Baranwal, 2014).

Wulandari et al. (2016) detected OYDV, SLV, and GCLV in shallots, signifying virus transmission among Allium species. Plants in the same genus often share genetic similarities, facilitating susceptibility to the same viruses. Additionally, only specific genetic variants (genotypes) can infect certain host genotypes in virus species. PRSV-P infects papaya and cucurbits, while PRSV-W exclusively infects cucurbits (Tripathi et al., 2008). These cases explain the complex interactions between viruses and the hosts, driven by genetic factors (McLeish et al., 2019).

In Indonesia, garlic is typically cultivated in an intercropping system with spring onion (*Allium fistulosum* L.) or shallot (*Allium cepa var. agregatum*), as observed in Karanganyar Central Java, and Bandung West Java (Rahmawati & Jamhari, 2019). The cropping pattern increases the risk of virus transmission due to the overlapping host range (Kadwati & Hidayat, 2015; Taglienti et al., 2018). This study determines the distribution of garlic viruses across cultivation areas in Indonesia and analyzes the potential for transmission to shallot and spring onion.

METHODS

Sampling Methods

Samples were collected from the main garlic-producing areas in Indonesia to determine the spread of the virus. The study locations consisted of 3 fields in West Nusa Tenggara (Sembalun: 8°23'53.16"S, 116°32'49.2"E), 1 in West Java (Ciwidey: 7°06'51.6"S, 107°26'24.1"E), 2 in Central Java (Tawangmangu: 7°39'40.8"S, 111°06'28.6"E), and 4 in

East Java (Ngantang: 7°52'34.8"S, 112°22'40.5"E; Junrejo: 7°53'07.7"S, 112°33'16.3"E; Pacet: 7°40'55.9"S, 112°32'28.4"E).

This study determined five sampling blocks diagonally in fields, each with a minimum area of 800 m^2. The total area of the sampling blocks is 5/9 of the field area. Leaf samples were collected from symptomatic plants showing symptoms such as twisted or wrinkled, yellow mosaic, green mosaic, yellow stripes, green stripes, and curly (Bhusal et al., 2021; Cremer et al., 2021). Furthermore, ten symptomatic leaves from each block were combined into a composite sample for virus detection. Additional seed bulb samples sourced from the previous planting season were collected from village storage warehouses during visits to the production sites. A total of 35 bulbs were sampled from each warehouse.

Virus Detection and Identification

Leaf samples from the field were used directly for detection, while the bulbs were grown in water media until leaves appeared. The detection stage was preceded by total ribonucleic acid (RNA) xtraction using the CTAB method (Doyle, 1991). Subsequently, RNA was used as a template in the amplification of the target viruses (LYSV, OYDV, GCLV, and SLV), applying a one-step reverse transcriptase-polymerase chain reaction (RT-PCR) method described by Nurulita et al. (2024). The process started with cDNA synthesis at 45°C for 60 min and pre-denaturation at 94°C for 1 min. It was followed by 35 cycles consisting of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, extension at 72°C for 1 min, and a final elongation at 72°C for 10 min.

Specific primers for LYSV and SLV (Haque & Hattori, 2017), OYDV (Sumi et al., 2001), and GCLV (Parrano et al., 2012) were adopted. RT-PCR premix comprised of 12.5 µl Dreamtaq Green master mix (2×) (Thermo Scientific), 1 µl 0.1 M DTT (Smobio, Taiwan), 0.5 µl RNAse inhibitor (Bioline London, UK), 0.25 µl RT enzyme (200 U/µl) (Smobio, Taiwan), 2 µl template RNA, 2 µl each of 10 µM forward and reverse primers, and 4.75 µl nuclease-free water. Amplification results were visualized using gel electrophoresis. Selected amplified DNA fragments were sent to 1st BASE Malaysia to obtain virus sequences using the Sanger sequencing method. Finally, sequence analysis was performed using BioEdit 7.7 (Hall, 1999), MEGA 11 (Tamura et al., 2021), and SDTV1.3 (Muhire et al., 2014).

Transmission of Garlic-origin Viruses

The LYSV, OYDV, GCLV, and SLV isolates used for the mechanical transmission experiment were obtained from a single infection and confirmed by sequencing to ensure identity. The plant materials used in the study were true seeds of shallot cv. Lokanata and cv. Sanren F1 (East-West Seed Indonesia), as well as spring onion cv. Blaze F1 (East-West Seed Indonesia). Initial virus detection was conducted 21 days after sowing (DAS) to

ensure the test plants were virus-free before transplantation into polybags at 35 DAS. The garlic-origin virus was transmitted by mechanical inoculation, where sap (exudate) from the inoculum source plants was applied to the test plants at 2 weeks post-transplanting (WPT). The sap was made by grinding the infected leaf in a mortar with 10,000 µl of cold 0.01 M phosphate buffer at pH 7 containing 1% mercaptoethanol, following the method of (Nurviani et al., 2016). Carborundum 600 mesh was sprinkled on the leaves of the test plants before the application. Subsequently, it was allowed to sit for 3 min until the sap dried, and the leaves were rinsed with distilled water to remove any residual carborundum. The inoculation was conducted simultaneously for all test plants.

The experiment was arranged using a two-factor, completely randomized design. The first and second factors were plant cultivar (TSS cv. Lokanata, TSS cv. Sanren F1, and spring onion cv. Blaze F1) and virus type (LYSV, OYDV, GCLV, SLV, and without virus inoculation as healthy control), respectively. This study experimented with control conditions in a screen house. All test plants were grown in polybags using a 1:1 mixture of soil and compost as a planting medium. The experimental design consisted of 3 replications for treatments. It was important to acknowledge that each replication had ten plants/treatments. The parameters observed were disease incidence, incubation period, plant height, number of leaves, and number and diameter of bulbs. Data were then analyzed using ANOVA followed by Tukey's test of 5%.

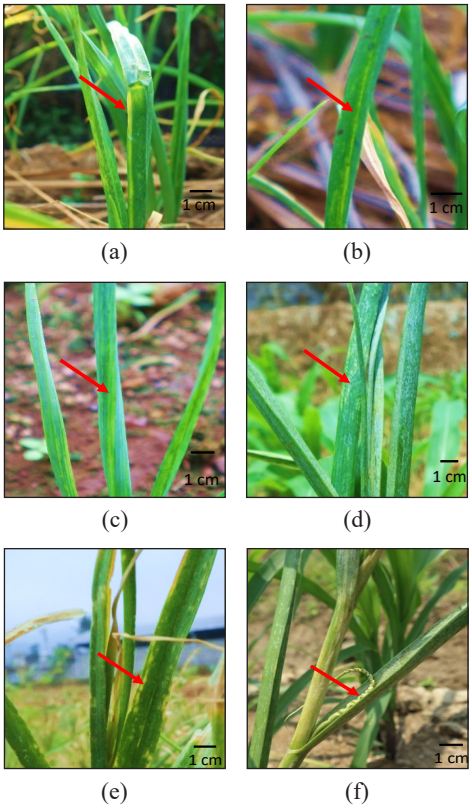


Figure 1. Symptoms of virus infection discovered in the field include: (a) yellow stripes and wrinkles; (b) yellow stripes; (c) green stripes; (d) green mosaic; (e) yellow mosaic; and (f) a curly leaf
Note. The red arrows signify the intended symptoms

RESULTS

Disease Symptoms and Virus Incidence

Virus infections were identified by symptoms in the fields, such as twisted or wrinkled leaves, yellow stripes, green stripes, green mosaic, yellow mosaic, and curly leaves, as shown in Figure 1. The dominant type in each field varied, as detailed in Table 1. Symptoms were also observed in young leaves growing out of the bulb samples but were less varied than those from the field (Figure 2).

Table 1
Symptoms of virus infection found on garlic plants in the field

Symptoms	Site					
	Tawangmangu	Ngantang	Pacet	Junrejo	Ciwidey	Sembalun
Twisted leaf	+	-	-	-	-	-
Striped, green on the leaf	-	-	-	+	+	+
Striped, yellow on the leaf	-	-	-	+	-	-
Leaf curling	+	-	-	-	-	-
Green mosaic on leaf	+	+	-	-	+	+
Yellow mosaic on leaf	-	-	+	-	-	-

(a) (b) (c)

Figure 2. Abnormal growth of garlic bulbs: (a) green stripe; (b) leaflet malformation; and (c) leaf curl
Note. The red arrows signify the intended symptoms

Virus infections were detected in all field samples collected from several garlic-growing areas (Figure 3). The average frequency of infection in order from the highest was LYSV (75.15%), OYDV (64.95%), SLV (36.47%), and GCLV (26.66%). All four target viruses from Tawangmangu, Ngantang, Pacet, Junrejo, and Ciwidey were present in each garlic cultivar. In samples from Sembalun, only LYSV and OYDV were consistently detected, while GCLV and SLV were exclusive to cv. Sangga Sembalun and cv. Lumbu Kuning, respectively. Based on the planting location, virus distribution varied in the same cultivar, as shown in Figure 3. For instance, all four viruses were detected on cv. Lumbu Putih from Tawangmangu, while only two were detected on cv Lumbu Putih from Sembalun. A similar pattern was observed for cv. Lumbu Hijau, with four viruses identified in Pacet and 2 in Sembalun.

The results showed that mixed infection was more common than the single-virus counterpart. As detailed in Figure 4, mixed infection of three viruses, particularly LYSV + OYDV + SLV, was mostly observed in leaf samples. In contrast, single and double infections, with the highest frequencies by LYSV and LYSV + SLV, respectively, were observed from bulb samples. It was important to acknowledge that the number and diversity

of viruses detected from leaf samples were more complex. Mixed infection of four viruses in leaf and bulb samples was low, while double infection of GCLV + SLV was absent in both samples.

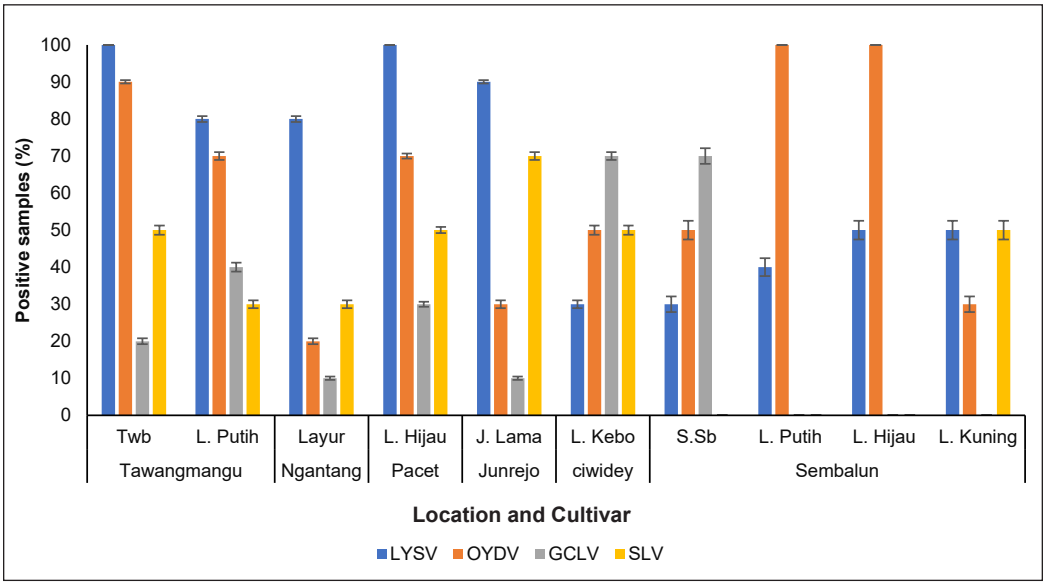
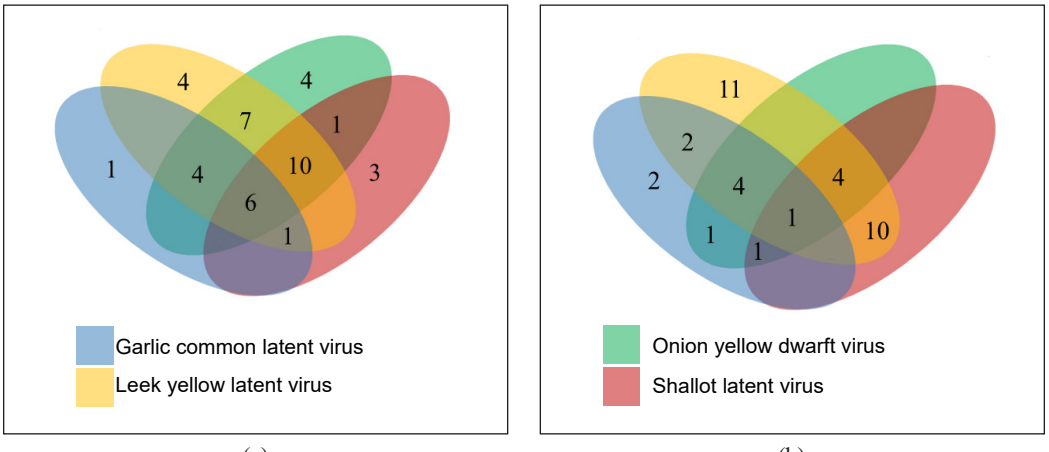


Figure 3. Frequency of virus infection on garlic cultivars planted in West Java (Ciwidey), Central Java (Tawangmangu), East Java (Ngantang, Pacet, and Junrejo) and West Nusa Tenggara (Sembalun)
Note. Garlic cultivars were Tawangmangu Baru (Twb), Lumbu Putih (L. Putih), Lumbu Hijau (L. Hijau), Jawa Lama (J. Lama), Lumbu Kebo (L. Kebo), Sangga Sembalun (S.Sb), Lumbu Kuning (L. Kuning). The viruses identified were Leek yellow stripe virus (LYSV), Onion yellow dwarf virus (OYDV), Garlic common latent virus (GCLV), Shallot latent virus (SLV). The error bars in the figures represent the standard deviation



(a) (b)

Figure 4. Venn diagram of garlic virus mixed infection on: (a) leaf : and (b) bulb samples
Note. The numbers in the figure represent the positive samples infected with the virus based on RT-PCR detection

Identity of Garlic Virus Isolates

Single LYSV, OYDV, and SLV infection were detected in shoots of bulbs from cv. Layur (East Java), cv. Sangga Sembalun (West Nusa Tenggara), and cv. Layur (East Java), respectively. GCLV infection was discovered in field samples of cv. Lumbu Kebo (West Java). The isolates of LYSV, OYDV, GCLV, and SLV shared the highest identity with the isolates from China (homology 98.43%; GenBank MN059534.1), China (homology 93.06%; GenBank MN059637.1), Argentina (homology 95.91%; GenBank KJ124847.1), and Australia (homology 90.90%; GenBank JF320811.1), respectively, as shown in Figure 5. The sequences of these isolates have been deposited in the GenBank with accession numbers LC831819.1, LC831799.1, LC831600.1, and LC831613.1, respectively.

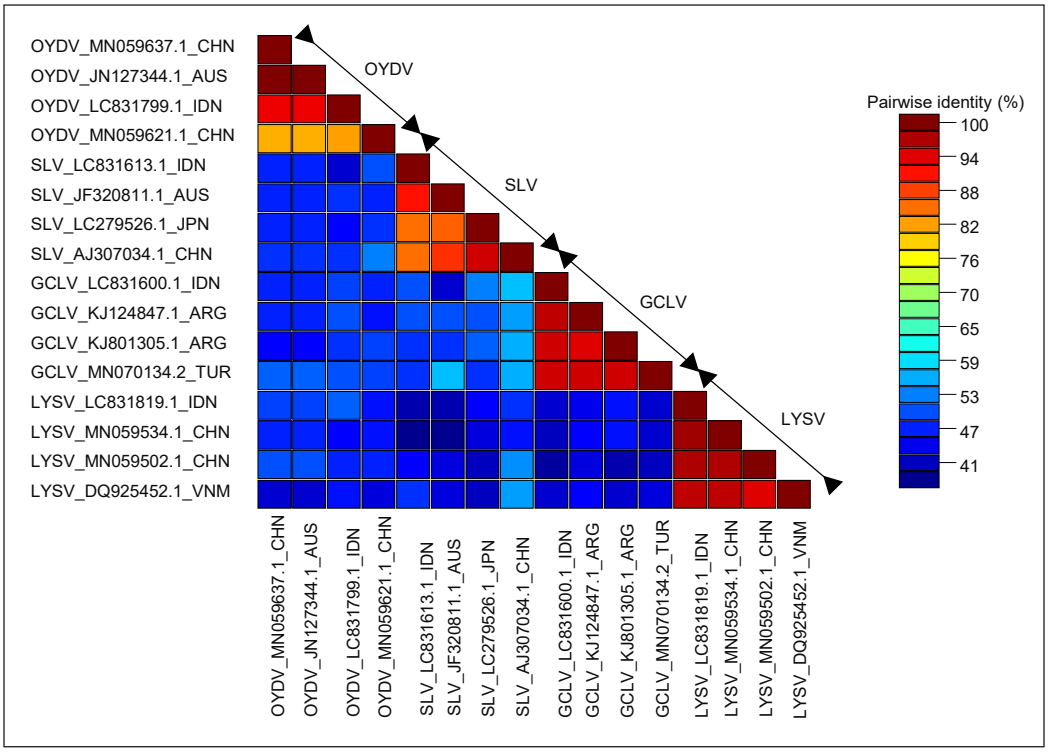


Figure 5. Pairwise alignment of garlic virus isolates with the Genbank database
Note. The isolates from this study are the blue-coded accession numbers (LC831799.1, LC831613.1, LC831600.1, and LC831819.1). The black-coded accession numbers are isolated from Genbank

Incidence of Virus Transmission from Garlic to Shallot and Spring Onion

Transmission studies of viruses originating from garlic successfully confirmed that shallot and spring onion are included in the host range. Shallot and spring onion showed symptoms after being inoculated with target viruses, except for GCLV. However, confirmation by

RT-PCR method proved that all plants were positively infected. Infection of GCLV was confirmed on cv. Lokananta and cv. Sanren F1 but not on cv. Blaze F1.

The highest disease incidence was 42 days after inoculation (DAI), discovered on cv. Blaze F1 and infected by LYSV (76.66%). SLV, OYDV, and LYSV followed it on 'Lokananta' (76.66%), cv. Lokananta (70%), and cv. Lokananta (63.33%). There was no significant difference in virus incidence among the 4 treatments, as shown in Figure 6. The incidence increased from the beginning of inoculation until 35 DAI and remained constant. However, the exception was observed for SLV and OYDV inoculation on cv. Blaze F1, which experienced a decrease starting from 27 and 33 DAI, respectively. The reduction in disease incidence in both treatments was 42.68% and 49.96%. It was important to acknowledge that spring onion cv. Blaze F1 inoculated with SLV or OYDV experienced a decrease in disease severity.

The experiment shows that OYDV and SLV originating from garlic only caused light green stripes and wrinkles on the leaves until 42 DAI, as detailed in Figure 7. Furthermore, twisted leaves and yellow mosaic appeared at 72 DAI on cv. Sanren F1 inoculated with OYDV, as presented in Figure 8. It signified differences in the symptoms that arise from the response of shallot and garlic plants.

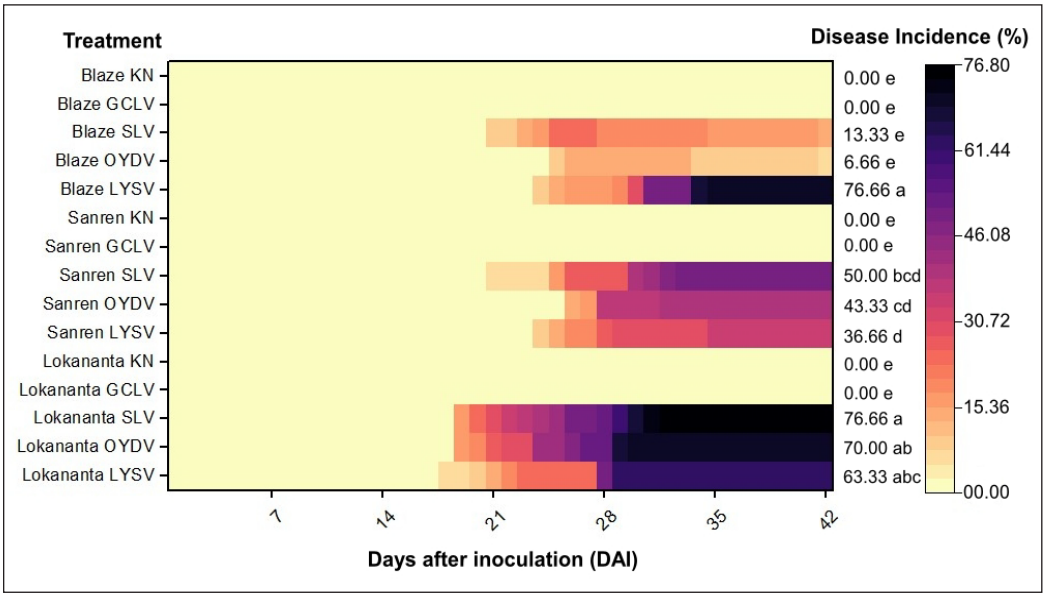
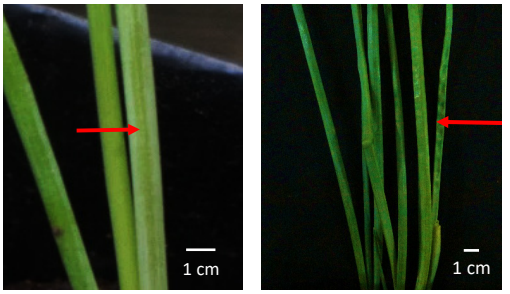


Figure 6. Heat map of the incubation period (days after inoculation) and incidence of virus infection based on symptoms appearance in test plants (%)

Note. The numbers on the right side of the diagram signify the incidence of symptoms at 42 DAI (days after inoculation). Meanwhile, the letter after the number showed that there was no significant difference between treatments based on Tukey's 5% test. The treatments on the left side of the diagram signify a combination of cultivar (cv. Blaze F1, cv. Sanren F1, cv. Lokananta) and virus (GCLV, SLV, OYDV, LYSV), KN: treatment without virus inoculation



(a) (b)

Figure 7. Symptoms of OYDV and SLV on cv. Lokananta and cv. Blaze F1: (a) green stripes at 18 DAI; and (b) wrinkled leaves at 42 DAI
Note. The red arrows represent the intended symptoms

(a) (b)

Figure 8. Symptoms of OYDV on cv. Sanren F1 at 72 DAI: (a) twisted leaves; and (b) yellow mosaic
Note. The red arrows show the intended symptoms

Effect of Virus Transmission from Garlic on Agronomic Characters

Garlic virus infection did not affect plant height and number of leaves, except for SLV on cv. Blaze F1 and OYDV on cv. Sanren F1, as shown in Figures 9 and 10. However, genetic factors influence this condition more, as cv. Blaze F1 is a spring onion and cv. Sanren F1 is a shallot. In contrast to plant height and number of leaves, a significant effect was observed on the number of bulbs, as detailed in Figure 11. It was particularly

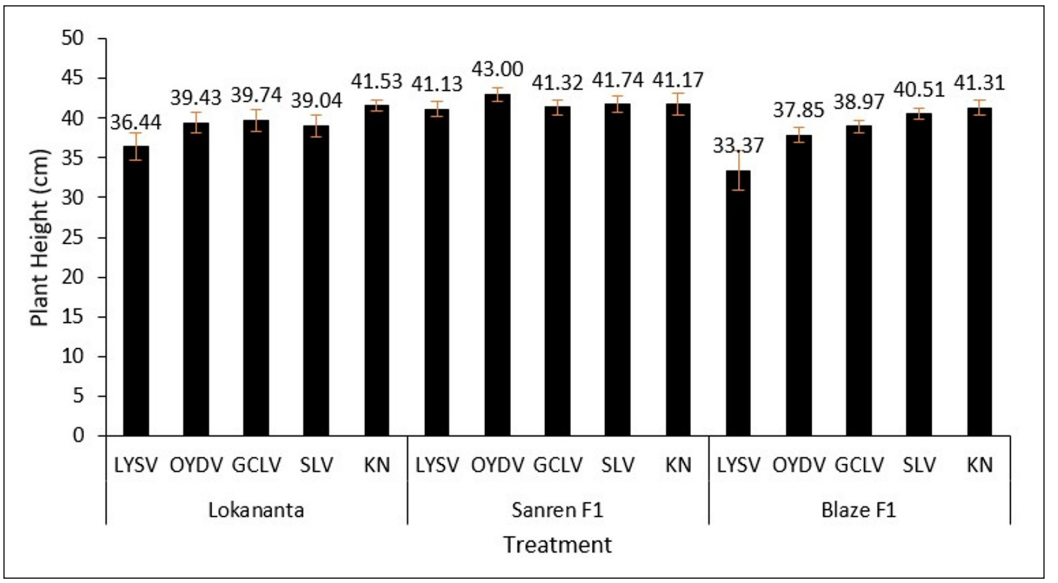


Figure 9. Average plant height of shallot (cv. Lokananta and cv. Sanren F1) and spring onion (cv. Blaze F1) given the inoculation of LYSV, OYDV, GCLV, and SLV
Note. KN = Treatment without virus inoculation. The numbers at the top of the diagram represent the average treatment values, while the error bars in the figures represent the standard deviation

for SLV, LYSV, and OYDV infections on cv. Sanren F1, as well as LYSV infection on cv. Lokananta. Despite the difference in the number of bulbs, the diameter was not affected. The study also shows that the effect of virus infection on yield reduction was different between the cultivars. For example, cv. Sanren F1 appeared to be more affected by the number of bulbs than cv. Lokananta.

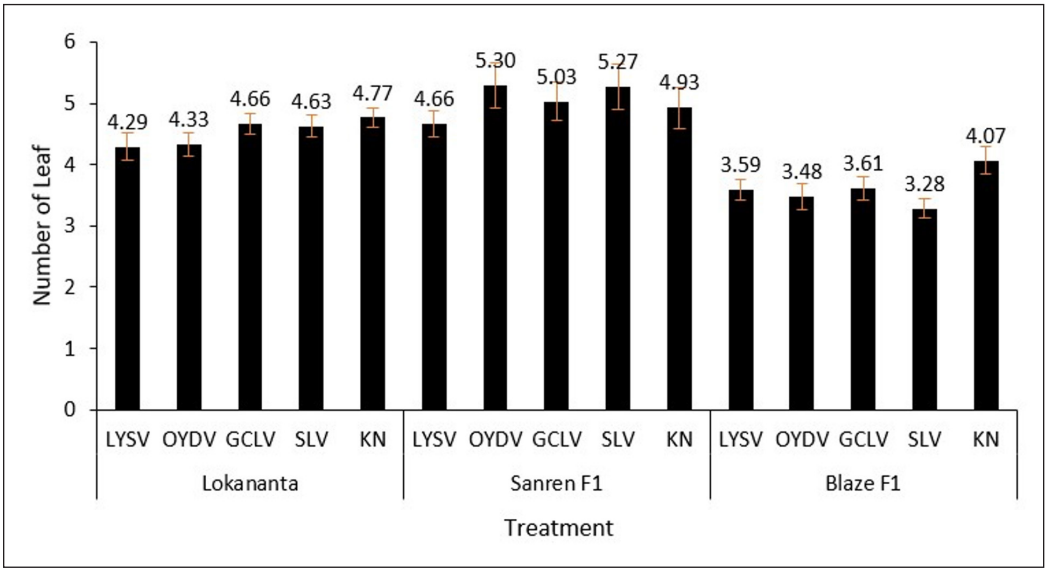
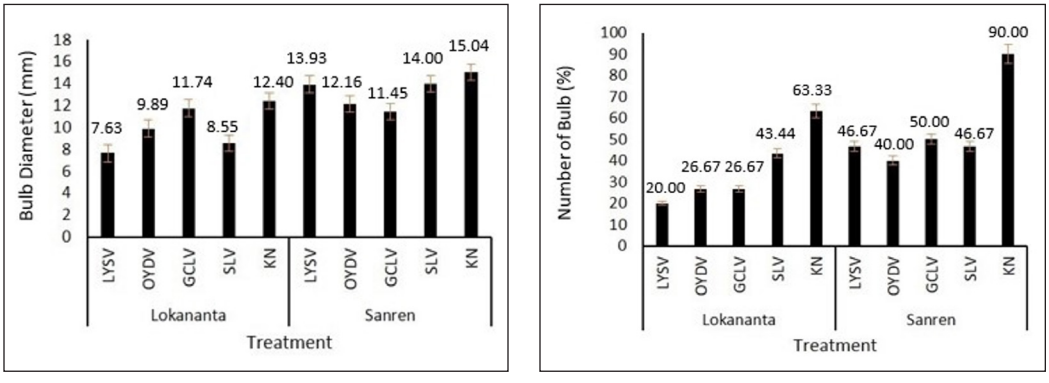


Figure 10. Average number of leaf of shallots (cv. Lokananta and cv. Sanren F1) and spring onion (cv. Blaze F1) given the inoculation of LYSV, OYDV, GCLV, and SLV
Note. KN = Treatment without virus inoculation. The numbers at the top of the diagram represent the average treatment values, while the error bars represent the standard deviation



(a) (b)

Figure 11. Average of bulb diameter (mm) (a) and the number of bulb (%) (b) of shallot (cv. Lokananta and cv. Sanren F1) given the inoculation of LYSV, OYDV, GCLV, and SLV
Note. KN = treatment without virus inoculation. The numbers at the top of the diagram represent the average treatment values, while the error bars represent the standard deviation

DISCUSSION

Identifying 50 symptomatic plants in an 800 m^2 field presented no challenge during sampling. It signified the high prevalence of garlic virus infection in Indonesia. Variations in symptoms observed across locations were influenced by interaction between the host plant, virus, and environment. A single virus can present different symptoms in the same plant, while distinct viruses can produce similar symptoms in the same plant (Kumar et al., 2012). Mosaic symptoms in garlic are often associated with mixed infections of several viruses, specifically members of the genera *Potyvirus, Carlavirus,* and *Allexivirus* (Chen et al., 2001; Tsuneyoshi et al., 1998).

Symptom variations are evident across field locations, leaf samples, and young shoots grown from bulbs. This variation may relate to the infection process and disease development. Viruses detected from bulbs are carried over from a previous harvest, while those in leaves arise from various sources, including primary infections on seed bulbs (Bhusal et al., 2021) and transmission through contact between plants (Coutts & Jones, 2015) or insect vectors (Mang et al., 2020). The detection of viruses in bulbs confirmed the ability to infect all plant parts, including planting material (Bhusal et al., 2021). Potyvirus and carlavirus can spread through the help of aphid vectors in a non-persistent manner (Mang et al., 2022). Another reason for the complexity of the symptoms observed in the field is that the plants sampled were in the final vegetative phase. Meanwhile, the bulb samples were obtained from plants two weeks after planting. Paudel and Sanfacon (2018) explained that the virus accumulated to higher levels later in life. This factor leads to the more pronounced symptoms observed in the late stages of plant development.

Virus detection is essential to verify that the symptoms observed are caused by viral infection. According to this study, viruses infecting garlic have spread across the cultivation areas in Indonesia. The detection results do not clearly indicate whether garlic viruses are specific to certain locations or cultivars. Inconsistency originated when comparing viruses infecting with garlic from the same area and those infecting specific cultivars. For example, a cultivar may harbor different virus combinations, while a single location may yield varying results across cultivars. The current results suggest that viruses are not associated with specific locations or cultivars.

The data obtained explains that LYSV and OYDV are the two most dominant viruses compared to others. Using seed bulbs from previous planting seasons is a key factor in the extensive spread of infections. This practice increases the tendency of virus-carrying bulbs to serve as primary inoculum, spreading diseases in fields (Khan et al., 2017). Reducing sources of inoculum in the field can be achieved by producing pathogen-free seed bulbs and implementing a controlled seed certification system.

Based on RT-PCR detection, viruses infecting garlic are more frequently detected in the form of mixed infections. Kadwati and Hidayat (2015) previously reported mixed infections

of two or three viruses in samples from Indonesia. Abraham et al. (2019) stated that mixed infections caused most viral infections in Ethiopia (65.7%). In this study, a combination of GCLV and SLV was not observed. The same results were reported by Kadwati and Hidayat (2015) in that the viruses were not discovered in local garlic samples from Bandung.

Infection of a virus may cause detrimental effects to the presence of another. The most common interference is attributed to the activation of the host cell defense response by a primary virus infection. It facilitates the prevention of subsequent infection by a secondary virus (Alves-Junior et al., 2009; Singhal et al., 2021). Furthermore, the reduced rate of viral multiplication in mixed infections may be due to competition for host cell resources. Generally, closely related virus strains do not invade the same cells in their hosts. This condition is known as the phenomenon of spatial exclusion or spatial separation between viruses (Singhal et al., 2021). GCLV, SLV, and other carlaviruses often cause latent infections without symptoms. Acknowledging that co-infections can worsen symptoms and greatly impact crop yield and quality (Katis et al., 2012; Santosa & Ertunc, 2021).

Studies confirmed that shallots cv. Lokananta and cv. Sanren F1 are susceptible to LYSV, OYDV, GCLV, and SLV, while cv. Blaze F1 was only infected by LYSV, OYDV, and SLV. The virus inoculation procedure conducted in this study was performed only once. It implied that the lack of positive detection of GCLV could be attributed to the need for multiple inoculations. There have been no studies addressing cv. Blaze F1's resistance to the virus.

Based on the incidence analysis, not all viruses that were inoculated displayed symptoms. For example, the results of GCLV inoculation in 'cv. Lokananta and cv. Sanren F1 showed no symptoms despite being detected as positive. Asymptomatic virus infection, as evidenced by GCLV, may result from the tolerance response of the host plant. Tolerant plants are generally able to suppress virus replication. It ensures that the virus titer decreases and inhibits cytopathic or harmful effects on the host (Takahashi et al., 2019; Zhang et al., 2018).

Spring onion cv. Blaze F1 inoculated with SLV or OYDV was subjected to a recovery process after several days, signifying a decrease in disease severity. A factor contributing to reducing disease incidence is the recovery process from the plant's resistance response and its interaction with the environment. Trebicki (2020) explained that the compatibility of the virus and the host plant and its interaction with the environment had positive, negative, or neutral effects on disease development and severity. Given the differences between the host isolates being transmitted from garlic to spring onion, this mechanism can possibly occur. The transmission also showed mild symptoms, unlike those observed in garlic infected with the virus. The symptoms observed from OYDV and SLV inoculations were primarily green stripes and wrinkling on the leaf. It is important to acknowledge that severe viral infections can lead to stunting or leaf malformation (Nurulita et al., 2024).

Garlic virus infection in shallot and spring onion did not affect plant height and number of leaves. It is with the exception of SLV on cv. Blaze F1 and OYDV on cv. Sanren F1, which influenced leaf number due to genetic factors. In contrast to plant height and number of leaves, a significant effect was observed on the number of bulbs. The need for proper development of the material caused the reduction in bulbs. The equatorial diameter should reach twice the diameter of the bulb neck to meet the criteria of good quality (Sullivan et al., 2021). In this study, many bulbs did not meet this criterion and had to be eliminated. The condition signified that the virus did not reduce the bulb size during the initial infection. As explained by Cafrune et al. (2006), the infection of Allexivirus in the first season did not lead to a significant yield reduction but was significantly decreased in the next planting season. This study also shows that the effects of virus infection are different among the different cultivars. Cultivar Sanren F1 appeared to be more affected by the number of bulbs than cv. Lokananta.

CONCLUSION

The incidence of garlic virus infection was widely spread in Indonesia. Infection of LYSV, OYDV, SLV, and GCLV was detected from leaf and bulb samples. This signified that seed bulbs played an important role as the primary source of disease inoculum. All four viruses were transmitted to shallot, but only LYSV, OYDV, and SLV were mechanically transmitted to spring onion. Therefore, virus-free seed bulbs were recommended to suppress the spread of the virus in the field.

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