

вестник ЗАЩИТЫ РАСТЕНИЙ

PLANT PROTECTION NEWS

2025 том 108 выпуск 3





Санкт-Петербург St. Petersburg, Russia OECD+WoS: 1.06+QU (Microbiology); 4.01+AM (Agronomy)

https://doi.org/10.31993/2308-6459-2025-108-3-17058

Full-text article

MONITORING OF OKRA YELLOW VEIN MOSAIC VIRUS IN THE SARGODHA DIVISION, PAKISTAN

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Okra Yellow Vein Mosaic Virus (OYVMV) is an important and significant threat to okra production, causing great yield losses in tropical and subtropical regions. The distribution and prevalence of OYVMV infection were estimated in the okra fields of Punjab, Pakistan, during 2020 and 2021. Surveys were conducted across Sargodha division (Sardogha, Khushab, Mianwali, and Bhakkar districts) to collect symptomatic and asymptomatic leaf samples. The presence of OYVMV was confirmed through PCR using specific primers. Disease incidence and severity were calculated using established formulas and rating scales. Significant differences were found in the incidence and severity of OYVMV infection among the districts and crop varieties. In 2020, Sargodha had the highest incidence and severity (80% and 52%), followed by Mianwali and Khushab, while in Bhakkar, the lowest incidence and severity were recorded (70% and 47%). In 2021, Mianwali showed the highest incidence and severity (73% and 47%), followed by Bhakkar and Khushab with Sargodha showing the least values for both parameters (69% and 44%). Among the varieties, Sultan-121 displayed the highest incidence and severity of the disease, while Sabz Pari had the lowest indices. Heat maps were utilized to visualize spatial variations in the incidence and severity of the virus infection. These findings highlight the widespread presence of OYVMV in Sargodha division and determine a baseline for future control and management strategies intended to mitigate the OYVMV-incurred losses in okra production.

Keywords: OYVMV, plant disease, incidence, severity, Sargodha, Khushab, Mianwali, Bhakkar

Submitted: 17.05.2025 Accepted: 10.07.2025

Introduction

Okra crop is threatened by many fungal, bacterial and viral diseases, such as damping off (Matny 2013), leaf spot disease (Arain et al., 2012), powdery mildew (Gogoi et al., 2013) and Okra Yellow Vein Mosaic Virus (OYVMV) (Kumar and Vashisth 2024). The latter is the major disease in okra fields, being one of the main yield hindrances in okra production. OYVMV is a systemic pathogen, broadly transmitted by the whitefly Bemisia tabaci vector as a considerable threat to okra cultivation in the tropical and subtropical regions (Davis, Thompson 2024). OYVMV infects plants belonging to several species in the genus Abelmoschus and brings severe havoc into okra fields (Akinyele, Osekita 2006). Viral diseases like OYVMV have become a serious challenge in the agricultural scenario of South Asia and Africa, contributing to huge losses (Jamir et al., 2020). It causes symptoms like vein yellowing, stunted growth and a reduction in fruit yield, which may lead to total crop failure if management practices

are not employed in a timely manner. Unlike many other plant viruses, the molecular diagnostics for OYVMV are more developed and PCR-based techniques have been effective in detecting the virus even in asymptomatic plants (Shabbir et al., 2025). Virus-induced damage reported in okra plants of several divisions in Pakistan and India was associated with the whitefly transmission (Mubeen et al., 2017). Other means of disease dispersal is grafting. The infection finally turns symptomatic with observations like yellowing of veins, leaf curling, and stunting. It severely affects okra pod quality and quantity (Shwetha et al., 2024). Research focused on OYVMV infection in major okra-growing regions, but it remained noticeably deficient in Pakistan (Ali et al., 2012). Hence, this study targets monitoring the incidence and spread of OYVMV in okra fields within important growing divisions of Pakistan, and as such, aims at developing a baseline for future control and management strategies.

Materials and Methods

A survey was carried out to collect leaf samples in okra growing areas of division Sargodha, viz. Sargodha, Bhakkar, Mianwali and Khushab between 2020 and 2021. Leaf samples were collected from infected okra fields in all areas. Areas selected for monitoring and sample collection were Musakhel, Rokhri, Chasma and Harnoli in Mianwali, Duellewala, Goharwala, Jhok Mehar Shah and Kotla Jam in Bhakkar, Jauharabad, Mitha Tiwana, Padhrar and Naushehra in

Khushab along with Chak 104 SB, Chak 128 SB, Chak 136 SB and Sial Sharif in Sargodha district. Samples were randomly collected from symptomatic and asymptomatic plants from okra fields (Fig. 1). Infected samples were diagnosed based on OYVMV symptoms (Fig. 2A, B, C, D): yellowing of veins, leaf curling, stunted growth and reduced fruit yield. Infected fruits are yellow, fibrous, small and tough (Chaudhary et al., 2017). These symptoms can be associated with significant

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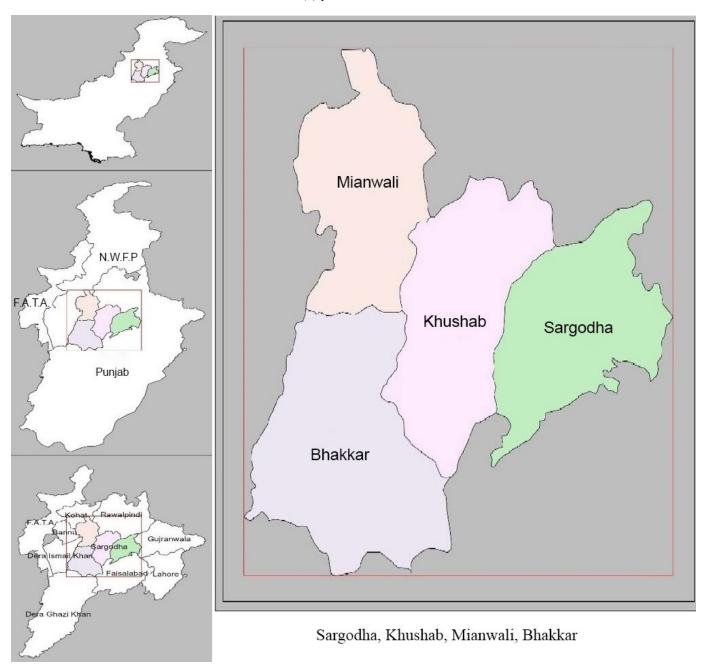


Figure 1. Map of the districts under study **Рисунок 1.** Карта районов в исследований

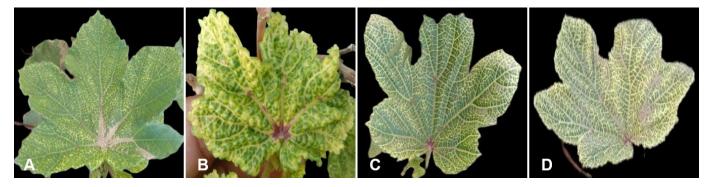


Figure 2. Okra Yellow Vein Mosaic Virus infection symptoms on okra plants **Рисунок 2.** Симптомы заражения растений бамии вирусом жёлтой жилковой мозаики бамии

yield losses if not managed effectively (Mubeen et al., 2021). The severity of virus infection (Mubeen et al., 2017) was based on a visible manifestation of symptoms by the plants

during the sample collection. Hot spots were marked for future investigation. The incidence of viral infection in okra plants was calculated based on positive samples confirmed through

polymerase chain reaction (PCR). Five leaf samples for each variety were subjected to PCR and the formula is mentioned as follows:

Disease Incidence (%) =
$$\frac{Number\ of\ Diseased\ Plants}{Total\ number\ of\ Plants\ Examined} \times 100$$
(Mubeen et al., 2017)

The severity of OYVMV infection was recorded as described by Mubeen et al. (2017) and disease rating scale is given in Table 1. The DSI was calculated according to the formula given by McKinney (1923).

Disease Severity Index =
$$\frac{\text{Severity Scale} \times \text{Number of Diseased Plants}}{6 \times \text{Total number of Plants Examined}} \times 100$$

Disease incidence and severity data were collected for the years 2020 and 2021 and analyzed using R programming language (version 4.4.3). Data were imported from Microsoft Excel files using the readxl package and pre-processed using

Table 1. Severity rating scale of Okra Yellow Vein Mosaic Virus infection

Rating scale	Severity,	Symptoms	Remarks
0	0	No symptoms	Immune
1	Up to 10	Few scattered lesions only	Highly resist- ant
2	11–25	Visible lesions	Moderately resistant
3	26–50	Visible lesions without stem girdling	Tolerant
4	51–60	Lesions with stem girdling	Moderately susceptible
5	61–70	Early vein yellowing with mosaic pattern	Susceptible
6	71–100	Vein yellowing with mosaic pattern and stem girdling	Highly suscep- tible

dplyr. The mean incidence or severity was calculated as the arithmetic average while the standard error was determined as the standard deviation divided by the square root of the sample size. Visualizations were generated using the ggplot2 and cowplot packages. Bubble maps were created for each variety in both years, where circle sizes represented the magnitude of disease incidence or severity and color gradients were used to denote intensity levels. The heat map technique was utilized to illustrate the combined effect of different varieties. A heat map is defined as the 2-dimensional data visualization technique which is used to represent the intensity of individual values within a dataset as a color. The variation of value is as the color intensity. Blue, grey, green, yellow and red colors of different intensityshow the values of severity which are being exploited for comparison in different locations over two years, as well as for different locations within a year.

Таблица 1. Шкала оценки пораженности вирусом желтой жилковой мозаики бамии

Балльная шкала	Степень поражения, %	Симптомы	Примечания
0	0	Нет симптомов	Невоспри- имчивый
1	до 10	Одиночные разроз- ненные поражения	Высоко- устойчивый
2	11–25	Заметные поражения	Средне- устойчивый
3	26–50	Поражения без опоя- сывания черешка	Толерант- ный
4	51–60	Поражения с опоясы- ванием стебля	Среднечув- ствительный
5	61–70	Начало пожелтения жилок с мозаичным распределением	Чувстви- тельный
6	71–100	Пожелтение жилок с мозаичным распределением и опоясыванием черешка	Высокочув-

Results

The data was transformed to analyze the incidence and disease severity statistically. PCR showed that the incidence was significantly different in all the districts of Sargodha division. Among the four varieties, disease incidence (Fig. 3A, B) and severity (Fig. 4A, B) significantly differed. The disease incidence and severity scores were high in Sultan-121 with mean values of 83.94% and 55.23%, respectively, followed by BS728 (78.88%, 51.62%) and Ujala (76.56%, 51.43%). The least disease incidence and severity were recorded in Sabz Pari with mean values of 66.56% and 41.52%, respectively in 2020. In 2021, the highest values were found in Sultan-121 with mean values of 81.81% and 41.52% respectively, followed by BS728 (72.18%, 47.95%) and Ujala (67.00%, 42.54%). The disease incidence and severity were found lowest in Sabz Pari, with mean values of 61.18% and 36.72%, respectively. During 2020, the highest incidence was recorded in Sargodha, with a value of 80.06%, followed by Mianwali (79.69%) and Khushab (75.75%). The lowest incidence was recorded in Bhakkar (70.44%) (Fig. 4), While in 2021, the highest incidence was recorded in Mianwali, with a value of 72.88%, followed by Bhakkar (71.00%). The least was recorded in Khushab

(69.19%), at par with Sargodha (69.06%). The same trend was found in disease severity. During 2020, the highest severity (DS) was recorded in Sargodha with the value of 52.02% followed by Mianwali (51.07%) and Khushab (49.85%) and the least severity was recorded in Bhakkar (46.85%) while in 2021 the highest Severity was detected in Mianwali with the value of 47.26% followed by Bhakkar (47.02%), Khushab (44.93%) and least was observed in Sargodha (44.04%). There was a difference in disease incidence and severity among the same areas and varieties for two years which might be due to environmental conditions, agronomic practices and insect vectors. For example, Duellewala displayed red- and yellow-colored severity bubbles for Sultan-121 in 2020 and 2021, respectively; i.e. the severity was reduced. Heat map analysis for same year showed that the severity was higher in Duellewala than in Harnoli. Jhok Mehar Shah exhibited high severity (over 90%) for the Sultan-121 in both years, being slightly less intense red in 2020, which became dark red in the next year. Heat maps of the incidence (Fig. 5) of different varieties and heat maps of varying severity (Fig. 6) of varieties are given in this chapter for a clear picture of the situation.

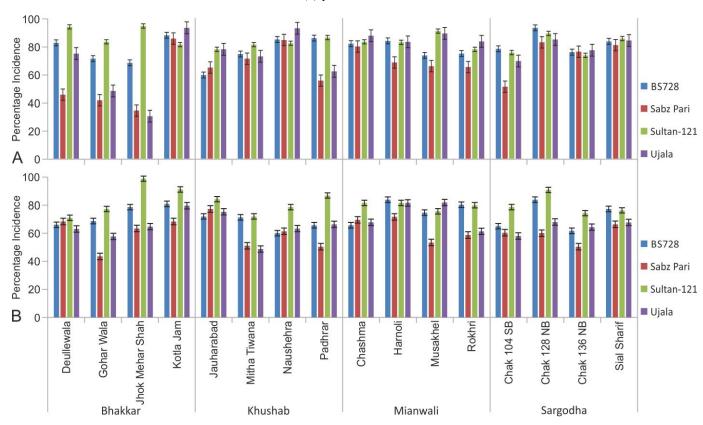


Figure 3. Okra yellow vein mosaic virus incidence in different varieties across districts of Sardogha division in 2020 (A) and 2021 (B)

Рисунок 3. Распространенность вируса жёлтой жилковой мозаики бамии на разных сортах среди районов округа Сардогха в 2020 (A) и 2021 гг. (B)

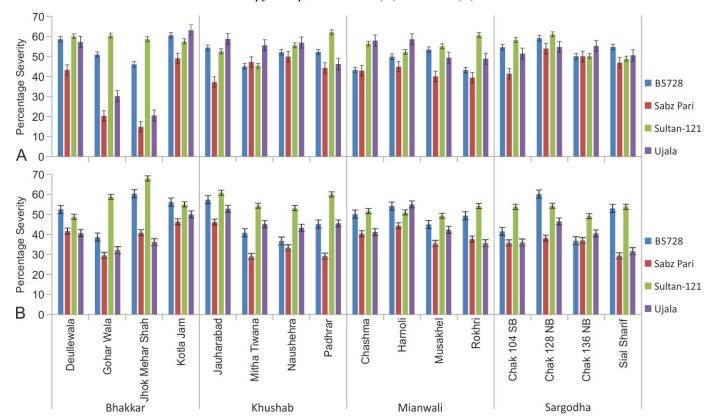


Figure 4. Okra yellow vein mosaic virus-induced disease severity of different varieties grouped by districts of Sardogha division in 2020 (A) and 2021 (B)

Рисунок 4. Степень поражения разных сортов, сгруппированных по районам округа Сардогха в 2020 (A) и 2021 гг. (B), вирусом жёлтой жилковой мозаики бамии

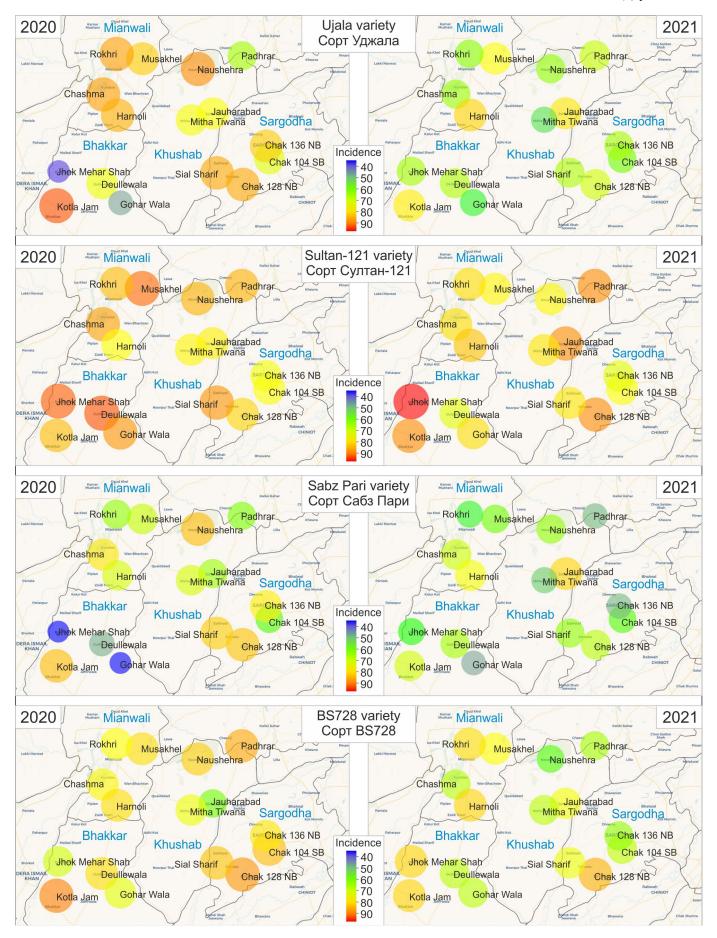


Figure 5. Okra yellow vein mosaic virus incidence heat maps of different varieties in 2020 and 2021 **Рисунок 5.** Тепловые карты распространенности вируса жёлтой жилковой мозаики бамии на разных сортах в 2020 и 2021 гг.

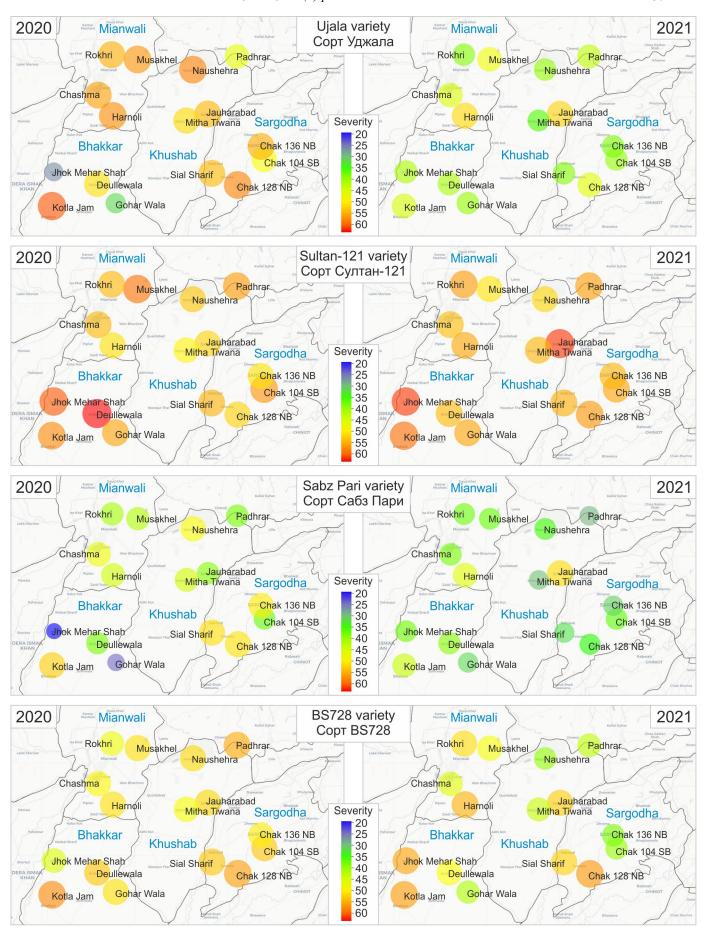


Figure 6. Okra yellow vein mosaic virus-induced disease severity heat map of different varieties in 2020 and 2021 **Рисунок 6.** Тепловая карта степени поражения разных сортов вирусом жёлтой жилковой мозаики бамии в 2020 и 2021 гг.

Discussion

The data analysis revealed the prevalence of infection across all okra-growing regions of the Sargodha division in Punjab, Pakistan. While virus detection based on symptomology is often unreliable and time-consuming (Ali et al., 2005), as it depends on the visible manifestation of symptoms in indicator plants, PCR offers a more practical, sensitive and effective detection method using specific primer pairs (Senevirathna et al., 2016). This study was the continuation of a previous study by the authors where OYVMV-infected samples showed the desired amplification levels when subjected to PCR. Our results confirmed the presence of virus in the infected samples in the previous study by Shabbir et al. (2025). The present study on OYVMV's distribution across okra-growing areas agreed with the already published data. Similar survey had also been carried out in Bangladesh and PCR was applied to show the presence of the virus OYVMV (Hossain et al., 2023). Subsequently, OYVMV was discovered globally as an important pathogen of the okra crop. It was first discovered

in the USA in 2010 (Hernandez-Zepeda et al., 2010), though Venkataravanappa et al. (2015) detailed its genomic features, with DNA A encoding 5-7 proteins and DNA B encoding two proteins, providing a total survey of virus sizes ranging from 2.5 to 2.8 kbp (Shuja et al., 2022). It was recorded for the first time in Sri Lanka by Tharmila et al. (2017) and later reported in Thailand by Tsai et al. (2013). OYVMV was detected in 17 of 18 okra genotypes in PCR analysis and resistance was shown by one genotype (Shabbir 2025). Recent research also covers the study conducted by Tharmila et al. (2017), which described a qPCR methodology used in the detection and quantification of beta satellites linked to OYVMV. Furthermore, several studies considered PCR as a significant method to indicate the presence of OYVMV in okra plants. Mondal et al. (2015) also studied image processing techniques to detect and classify the presence of yellow vein mosaic virus in okra leaves, thereby solidifying PCR's reliability as a successful detection method.

Conclusions

Our study concludes that OYVMV is widely distributed across all the okra-growing areas in the Sargodha Division, Punjab. The virus has a significant impact on various okra varieties with notable differences in incidence and severity between districts and cultivars. Sultan-121 showed the highest disease incidence and severity and highlighted its vulnerability to the virus. The successful detection of OYVMV through PCR-based methods proved to be a reliable and sensitive approach which enabled the identification of infected plants including those with no visible symptoms. This underscores the importance of early and precise detection in managing the

disease. Regular monitoring coupled with PCR diagnostics is essential for effective disease management and early intervention especially in the areas where virus prevalence is high. The development of resistant varieties, integrated pest management strategies targeting whitefly vectors and timely interventions could help mitigate the impact of OYVMV on okra crops. Future research should focus on assessing the long-term impact of OYVMV on okra productivity and exploring potential control measures. Overall, this study provides a foundation for future efforts aimed at controlling OYVMV and ensuring the sustainability of okra cultivation in Pakistan.

Acknowledgements

Checking English grammar and style was the courtesy of Ken Smith, Master in TESOL (Santo Domingo, Dominican Republic).

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Вестник защиты растений, 2025, 108(3), с. 182-189

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https://doi.org/10.31993/2308-6459-2025-108-3-17058

Полнотекстовая статья

МОНИТОРИНГ ВИРУСА ЖЁЛТОЙ ЖИЛКОВОЙ МОЗАИКИ БАМИИ В ОКРУГЕ САРДОГХА, ПАКИСТАН

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Вирус жёлтой жилковой мозаики бамии (OYVMV) – важная и значительная угроза производства бамии, вызывающая огромные потери урожая в тропических и субтропических регионах. Распространённость OYVMV оценивалась на полях бамии в Пунджабе, Пакистан, в 2020 и 2021 гг. Обследования проведены в районах Сардогха, Хушаб, Мианвали и Бхаккар округа Сардогха для сбора образцов листьев с симптомами и без них. Присутствие ОУУМУ подтверждено с помощью ПЦР с видоспецифичными праймерами. Распространенность болезни и степень поражения растений рассчитывалась с помощью общепринятых формул и балльных оценок. Выявлены существенные различия этих показателей при сравнении разных районов и сортов. В 2020 году наибольшие значения распространенности болезни и степени поражения растений (80 и 52%) наблюдались в районе Сардогха; затем следовали Мианвали и Хушаб, а в районе Бхаккар были наименьшие показатели (70 и 47%). В 2021 году максимальные значения отмечены в Мианвали (73 и 47%), минимальные - в Сардогхе (69 и 44%). Среди проанализированных сортов, Султан-121 продемонстрировал наиболее высокие значения распространенности болезни и степени поражения растений, а Сабз Пари – наиболее низкие. «Тепловые карты» использованы для визуализации пространственного варьирования распространенности вирусной инфекции и степени поражения растений. Эти результаты подчеркивают широкую встречаемость OYVMV в округе Сардогха и задают отправную точку для последующей разработки стратегий борьбы, направленных на снижение потерь производства бамии, связанных с заражением OYVMV.

Ключевые слова: OYVMV, болезни растений, встречаемость, степень поражения растений, Саргодха, Хушаб, Мианвали, Бхаккар

Поступила в редакцию: 17.05.2025 Принята к печати: 10.07.2025

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