Research Article

Enhanced Resistance in Okra (*Abelmoschus esculentus* L. Moench) Against Okra Yellow Vein Mosaic Virus Through the Application of *Trichoderma* spp.

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Abstract | This study investigates the efficacy of *Trichoderma atroviride* and *Trichoderma asperellum* in Induced Systemic Resistance (ISR) in okra (*Abelmoschus esculentus*) against *Okra Yellow Vein Mosaic Virus* (OYVMV). The experiment was conducted using a controlled randomized design with eight treatments, including single and combined applications of the two fungal species. Results revealed a significant reduction in disease severity in treated plants, with the combination treatment (A7) achieving the lowest severity at 27.67%. Enzymatic assays indicated enhanced peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) activities in fungal-treated plants, particularly in the A7 group, Showed higher total phenolic content, that improved biochemical defenses. our results demonstrate the potential of *T. atroviride* and *T. asperellum* as a sustainable biocontrol elicitation resistance agents in Okra plant against OYVMV.

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Keywords | Okra, Okra yellow vein mosaic virus, Induced systemic resistance (ISR), *T. atroviride*, *T. asperellum*, Plant enzymes

Introduction

Okra (*Abelmoschus esculentus*), a significant agricultural crop of the Malvaceae family, which is grown in warm temperate, tropical, and subtropical climates (Benchasri, 2012). But insects, nematodes, mites, and viruses pose a serious threat to its production, resulting in yields that are substantially reduced (Singh *et al*., 2014; Ounis *et al*., 2024). Virus infections represent a significant challenge, with field infection rates reported to reach as high as 88% (Appiah *et al*., 2020), frequently culminating in substantial or near-total yield losses (Jamir *et al*., 2020). Okra yellow vein mosaic disease (OYVMD), okra enation leaf curl disease, and okra mosaic disease are the three main viral illnesses that impact okra (Mishra *et al*., 2017). Okra Yellow Vein Mosaic Disease (OYVMD) is caused by begomoviruses classified under the family Geminiviridae (Hossain *et al*., 2023). Globally, several begomoviruses are linked to OYVMD, including Okra Yellow Vein Mosaic Virus (OYVMV), Bhendi Yellow Vein Mosaic Virus (BYVMV), Okra Enation

Leaf Curl Virus (OELCuV), Cotton Leaf Curl Multan Virus (CLCuMuV), Okra Yellow Crinkle Virus (OYCrV), Radish Leaf Curl Virus (RaLCV), and Okra Mosaic Virus (OkMV). Among these, OYVMV is one of the most extensively studied begomoviruses. It possesses a monopartite genome approximately 2.7 kb in size (Davis and Thompson, 2024) and encodes six proteins distributed between the virion-sense and complementary-sense strands. Notably, the arrangement of open reading frames (ORFs) may vary among the begomoviruses responsible for OYVMD (Venkataravanappa et al., 2015; Ghosh *et al*., 2009). The virus is transmitted by the whitefly vector (*Bemisia tabaci*), which acquires virions through its stylets and foregut while feeding on infected plants (Barman *et al*., 2022). Infected plants display characteristic symptoms, including chlorosis, dwarfing, and vein yellowing. Severe infections can result in stunted growth, defoliation, and malformed fruits, potentially leading to complete yield loss under conditions favorable for viral spread (Appiah *et al*., 2020).

To manage OYVMD, several strategies have been proposed, such as developing resistant okra cultivars, controlling *B. tabaci* populations, and employing plant defense activators (Mubeen *et al*., 2021). Biological control agents, particularly *Trichoderma* spp., have shown promise in inducing systemic resistance (ISR) in plants against viral pathogens (Kannojia *et al*., 2019; Al-Kaeath *et al*., 2024). Various *Trichoderma* spp. have successfully elicited ISR in different plants against a broad spectrum of pathogens, including viruses like tomato yellow leaf curl virus (TYLCV) (Kream *et al*., 2023a, b). This study aims to evaluate the efficacy of two *Trichoderma* spp. in combating OYVMD and integrating them into sustainable pest management programs for okra.

Materials and Methods

Fungal growth conditions

Trichoderma atroviride and *Trichoderma asperellum* (Laboratories of the College of Agriculture, University of Karbala, Iraq) were cultivated for 10 days at 28°C in the dark on potato dextrose agar (Sigma, St. Louis, Mo, USA) plates. Using a hemocytometer, 10^{-7} spores/ml, were counted and utilized as the inoculum for fungal pre-cultures in 250 ml Erlenmeyer flasks that contained 100 ml of liquid minimum media (Penttilä *et al*., 1987) with 2% glucose added as a carbon source. After that, the flasks were kept at 28°C and 150 rpm for 48 hours. The fungal biomass was then collected by filtration, administered several sterile distilled water washes, and then moved to the final cultures.

Plant growth conditions

Experimental procedure: Assessing resistance in okra against okra yellow vein mosaic virus (OYVMV): Plant material and soil preparation: The study utilized the local Hussainawya variety of okra. Seeds were sown in plastic trays with planting holes 8–10 cm deep. The growth medium consisted of a sterilized soil mixture of one part peat moss to two parts sand. After germination, seedlings were maintained under optimal growth conditions until they developed three true leaves, Use three plants (three replicates) for each experimental unit.

Virus inoculation

Having previously fed on an okra plant infected with the virus, whiteflies were transferred to the seedlings after they reached the three-true-leaf stage (10 insects per plant). The insects were then left on the healthy plants for virus transmission over one week. Once it was confirmed that they had fed on all seedlings, the insecticide Mospilan® (acetamiprid 20%) was used to eliminate them, at a rate of two sprays, one week apart.

Fungal treatments and transplantation

Following inoculation, the seedlings were transplanted into sterilized plastic pots (22 cm × 24 cm) filled with soil pre-inoculated with Trichoderma spores based on the experimental treatments listed in Table 1. The fungal spore suspensions were prepared at a concentration of 1×10^{7} spores/mL.

Table 1: *Treatments applied and details.*

Treatment	Details
A0	Plant healthy (control)
A1	OYVMV -inoculated plant
A2	*T. atroviride* + Plant Healthy
A3	*T. asperellum* + Plant Healthy
A4	*T. atroviride* + *T. asperellum*+ Plant Healthy
A5	*T. atroviride* + OYVMV-inoculated plant
A6	*T. asperellum* + OYVMV-inoculated plant
A7	*T. atroviride* + *T. asperellum* + OYVMV inoculated plant

Controlled growing conditions

To minimize external interference, the pots were transferred to a controlled area covered with insect-

proof netting. Environmental conditions such as temperature (35 ± 2°C) and relative humidity (60 ± 5%) were monitored daily. The plants were watered daily according to their requirements to maintain consistent soil moisture levels.

Evaluation of symptoms and virus confirmation

The plants were monitored for symptom development at 7, 14, and 21 days post-inoculation. Observations included symptoms such as vein yellowing, leaf curling, and stunting. Young symptomatic leaves were collected from each treatment group and analyzed using the Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) method (Clark and Adams, 1977) to confirm the presence of OYVMV as the causal agent.

Experimental treatments

The experiment was designed with eight treatments (Table 1):

Screening for resistance under natural conditions

In addition to the controlled experiment, a field screening was conducted to evaluate the performance of the okra plants under natural conditions. Plants were grown in an open field where they were naturally exposed to OYVMV. Disease severity was assessed using a standardized scoring system adapted from Karem and Haidery (2022).

Assays of antioxidant enzymes

Enzyme extraction: The Assay activities of peroxidase (POD) (U/mg) Pitotti *et al.* (1994), superoxide dismutase (SOD) (U/mg) Magnani *et al.* (2000), catalase (CAT) (U/mg) Hadwan and Kadhum (2018), and total phenolic (µg/ml) by Cl and Indira (2016) were measured in okra leaf samples following a standardized procedure. Fresh leaf samples (0.2 g each) were collected and immediately ground to a fine powder in liquid nitrogen using a sterilized pestle and mortar to prevent enzyme degradation. The powdered samples were homogenized with 2 mL of ice-cold 50 mM phosphate buffer (pH 7.7) containing 1 mM ethylenediaminetetraacetic acid (EDTA) to stabilize the enzymes. The homogenate was centrifuged at 4°C for 15 minutes at 15,000 rpm (Figure 1). The resulting supernatant, containing the crude enzyme extract, was carefully collected and stored on ice until used for enzymatic assays. This method ensured the extraction of active antioxidant enzymes while minimizing potential degradation during sample preparation.

Statistical analysis

The experiment was designed following a Completely Randomized Design (CRD). The data were analyzed statistically using GenStat version 18, employing the Least Significant Difference (LSD) test at a 0.05 significance level.

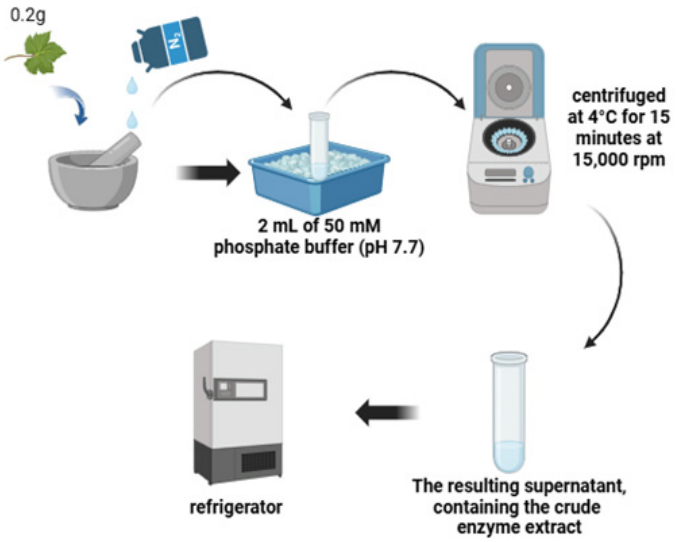

Figure 1: *Representation of the procedure for extracting antioxidant enzymes (POD, SOD, and CAT) from okra leaf samples.*

Results

Disease severity of OYVMV

The results presented in Figure 2 demonstrate that treating okra plants with *Trichoderma* spp. significantly influenced the appearance of okra yellow vein mosaic virus (OYVMV) symptoms after 21 days of inoculation. The lowest symptom severity was observed in plants treated with a combination of *T. atroviride* and *T. asperellum*, with a severity percentage of 27.67% (Karem and Haidery, 2022). This was followed by plants treated with *T. asperellum*, which showed a slightly higher severity rate of 28.33%. Statistical analysis indicated no significant difference between these two treatments. In contrast, untreated and virus-inoculated plants showed the highest symptom severity of 65%, confirming the effectiveness of Trichoderma treatments in mitigating the impact of the disease.

Peroxidase (POD) (U/mg)

The data shown in Figure 3 demonstrate the effect of different treatments on the peroxidase (POD) enzymatic activity within okra leaves. Enzymatic analysis revealed a significant increase in peroxidase (POD) activity in plants treated with *T. atroviride* and *T. asperellum* simultaneously with okra yellow

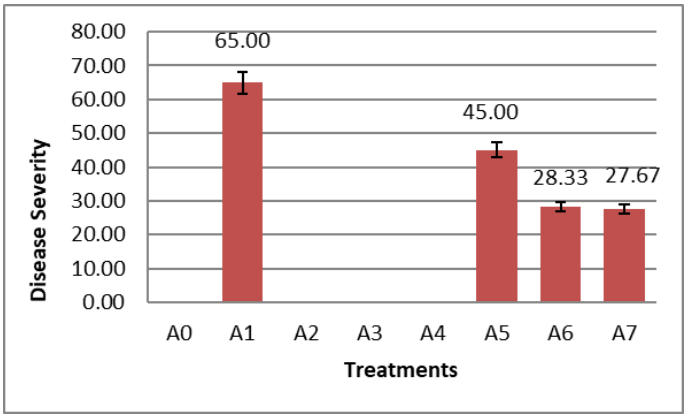

Figure 2: *Shows the different symptoms (OYVMV) of infection and their severity on treated okra plants, A0=Plant Healthy (control), A1= OYVMV -inoculated plant, A2= T. atroviride+ Plant Healthy, A3= T. asperellum+ Plant Healthy, A4= T. atroviride+ T. asperellum+ Plant Healthy, A5= T. atroviride + OYVMV-inoculated plant, A6= T. asperellum+ OYVMV-inoculated plant, A7= T. atroviride + T. asperellum+ OYVMV-inoculated plant.*

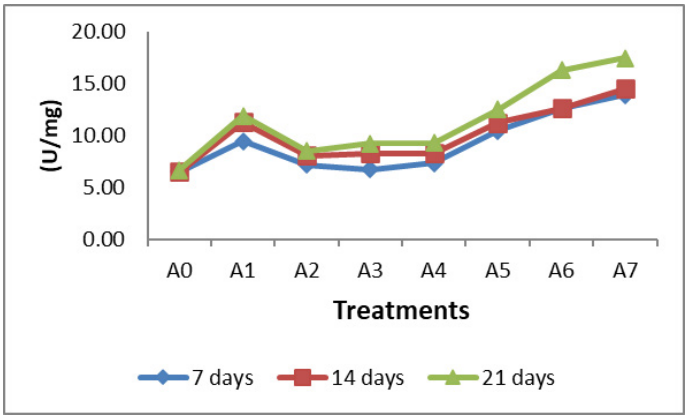

Figure 3: *Okra leaf content of peroxidase (POD) (U/mg), A0=Plant Healthy (control), A1= OYVMV -inoculated plant, A2= T. atroviride+ Plant Healthy, A3= T. asperellum+ Plant Healthy, A4= T. atroviride+ T. asperellum+ Plant Healthy, A5= T. atroviride+ OYVMV-inoculated plant, A6= T. asperellum+ OYVMV-inoculated plant, A7= T. atroviride+ T. asperellum+ OYVMV-inoculated plant. LSD $_{(0.05)}$ Treat. = 0.38, Times= 0.23 and for Intersection= 0.67*

vein mosaic virus (OYVMV) inoculation (A7). More specifically, the observed POD activity in treatment A7 significantly exceeded all other treatments, especially after 21 days, reaching 17.5 U/mg, reflecting the enhanced systemic resistance induced by the synergistic action of these fungal agents. Compared to the control group (A0, untreated healthy plants), which reached 6.67 U/mg, plants inoculated with OYVMV (A1) showed a significant decrease in enzymatic activity, reaching 11.9 U/mg, confirming the detrimental effect of viral infection on the plant's inherent defense capabilities. In contrast, treatments that included single fungal inoculations (A2, A3, A5, A6) showed mean levels of POD activity of 16.33, 12.5, 9.23 and 8.53 U/mg, respectively, indicating partial recovery of defense responses.

Superoxide dismutase (SOD) (U/mg)

Figure 4 shows the changes in superoxide dismutase (SOD) activity across the various treatments applied to okra plants. Close examination reveals a significant increase in SOD activity within plants exposed to specific fungicides, particularly in the context of viral inoculation with Okra yellow vein mosaic virus (OYVMV). On day 7 post-treatment, the highest SOD activity was observed in plants treated with a mixture of *T. atroviride* and *T. asperellum* along with viral inoculation (A7), recording a value of 5.30 U/mg. This value was significantly higher than in the control group (A0), which maintained baseline levels of 2.27 U/mg. The trend of increasing enzyme activity continued through periods 14 and 21, culminating in a maximum SOD activity of 7.23 U/mg for A7 at day 21. Treatments that included single fungal applications, either in conjunction with virus inoculation or administered to healthy plants (A2, A3, A5, and A6), showed average SOD activity over the 21-day period of 6.63, 6.13, 5.77, and 5.2 U/mg, respectively. Notably, the application of *T. asperellum* to infected plants (A6) resulted in 6.63 units/mg of SOD activity on the 21st day, demonstrating its significant contribution to the antioxidant defense system. In contrast, plants inoculated with OYVMV without fungal treatment (A1) showed elevated SOD levels compared to the control group, reaching 4.77 U/mg, but they remained lower than the fungicide-treated groups, highlighting the virus-suppressive effects and the mitigating capacity of *Trichoderma* spp.

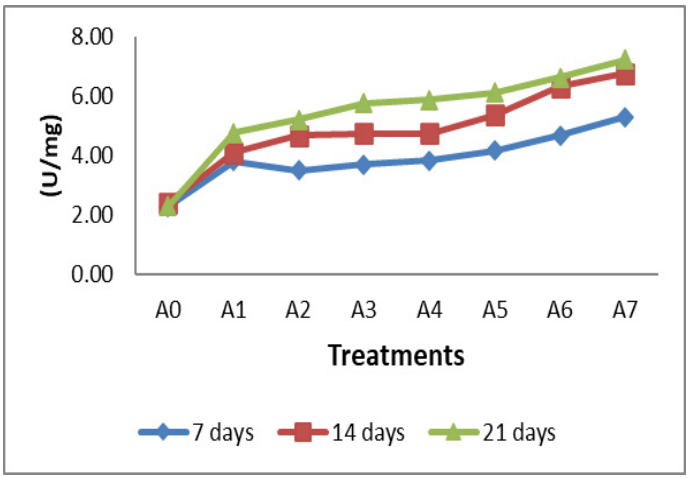

Figure 4: *Okra leaf content of Superoxide dismutase (SOD) (U/mg), A0=Plant Healthy (control), A1= OYVMV -inoculated plant, A2= T. atroviride+ Plant Healthy, A3= T. asperellum+ Plant Healthy, A4= T. atroviride+ T. asperellum+ Plant Healthy, A5= T. atroviride+ OYVMV-inoculated plant, A6= T. asperellum+ OYVMV-inoculated plant, A7= T. atroviride+ T. asperellum+ OYVMV-inoculated plant. LSD $_{(0.05)}$ Treat. = 0.174, Times= 0.106 and for Intersection= 0.302.*

Catalase (CAT) (U/mg)

Figure 5 revealed that there were changes in the content of catalase enzyme in okra leaves during different periods of inoculation with the virus and also with different treatments. At 7 days, plants show to dual treatment with *T. atroviride* and *T. asperellum* in conjunction with OYVMV inoculation (A7) demonstrated the highest CAT activity, registering 2.77 U/mg. This value significantly surpassed the baseline activity recorded in the healthy control group (A0), which was 1.29 U/mg. Similarly, treatments involving individual fungal applications (A2, A3, A4) exhibited moderate enhancements in CAT activity, with values ranging from 2.33 to 2.55 U/mg.

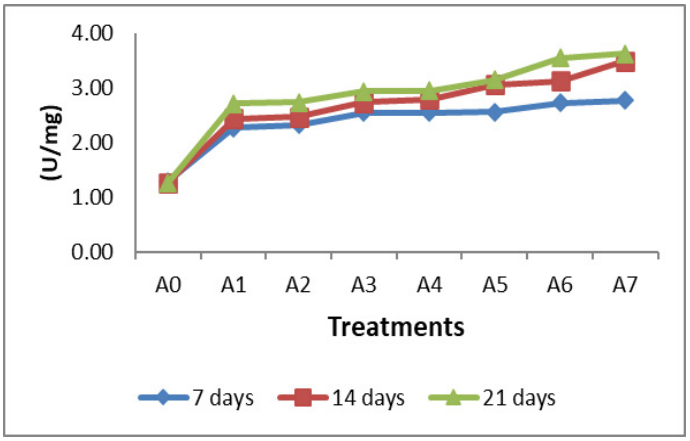

Figure 5: *Okra leaf content of Catalase (CAT) (U/mg), A0=Plant Healthy (control), A1= OYVMV -inoculated plant, A2= T. atroviride+ Plant Healthy, A3= T. asperellum+ Plant Healthy , A4= T. atroviride+ T. asperellum+ Plant Healthy , A5= T. atroviride+ OYVMV-inoculated plant, A6= T. asperellum+ OYVMV-inoculated plant, A7= T. atroviride+ T. asperellum+ OYVMV-inoculated plant. LSD $_{(0.05)}$ Treat. = 0.038, Times= 0.023 and for Intersection= 0.066*

By the 14th day, CAT activity exhibited a marked increase across all treatments, with A7 achieving a peak value of 3.50 U/mg. Also, plants treated with *T. asperellum* and OYVMV inoculation (A6) closely followed, reaching 3.13 U/mg. The OYVMV-inoculated plants without fungal treatment (A1) recorded a CAT activity of 2.44 U/mg, which, although elevated compared to the control group (1.27 U/mg), remained lower than fungal-treated groups. At the 21-day, CAT activity reached its zenith in A7, with a recorded value of 3.63 U/mg, followed by A6 at 3.55 U/mg. Plants treated with *T. atroviride* and OYVMV inoculation (A5) recorded a CAT activity of 3.15 U/mg. In contrast, untreated OYVMV-infected plants (A1) displayed a CAT activity of 2.72 U/mg, significantly exceeding the control group's value of 1.26 U/mg but remaining inferior to all fungal-treated groups.

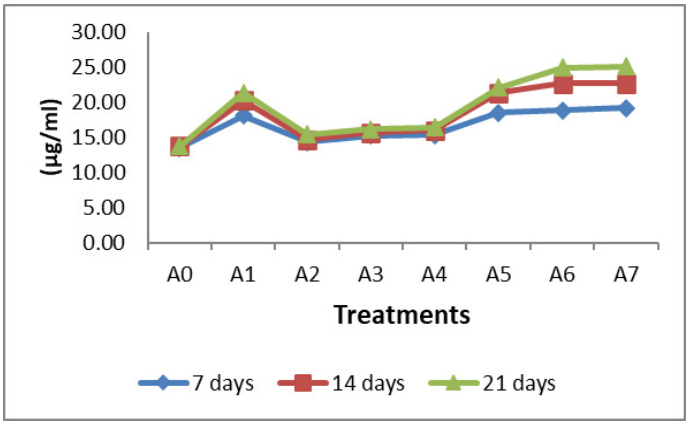

Figure 6: *Okra leaf content of Total phenolic (µg/ml), A0=Plant Healthy (control), A1= OYVMV -inoculated plant, A2= T. atroviride+ Plant Healthy, A3= T. asperellum+ Plant Healthy, A4= T. atroviride+ T. asperellum+ Plant Healthy, A5= T. atroviride+ OYVMV-inoculated plant, A6= T. asperellum+ OYVMV-inoculated plant, A7= T. atroviride+ T. asperellum+ OYVMV-inoculated plant. LSD $_{(0.05)}$ Treat. = 0.25, Times= 0.15 and for Intersection= 0.44*

Total phenolic (µg/ml)

Figure 6 shows changes of total phenolic content in okra leaves after 7, 14, and 21 days under different experimental treatments. The results show the considerable effect of fungal treatments and viral inoculation on phenolic accumulation. The period 7-day, okra plants subjected to combined treatment with *Trichoderma atroviride* and *T. asperellum* alongside okra yellow vein mosaic virus (OYVMV) inoculation (A7) exhibited the highest phenolic content, measured at 19.23 µg/ml. This remarkable increase notably exceeded the baseline phenolic level recorded in the healthy control group (A0), which stood at 13.50 µg/ml. Plants treated with individual fungal applications (A2, A3, A4) displayed moderate increases, with phenolic values ranging from 14.37 to 15.37 µg/ml. By the 14th day, phenolic content had significantly amplified, with treatment A7 maintaining a peak value of 22.70 µg/ml. interestingly, plants treated with *T. asperellum* and OYVMV inoculation (A6) mirrored this concentration, demonstrating comparable efficacy. Conversely, OYVMV-inoculated plants without fungal treatment (A1) recorded a phenolic level of 20.30 µg/ml, which, while elevated relative to the control group, remained lower than that observed in fungal-treated plants. At the final period after 21 days, the phenolic content reached its highest value in A7, with a recorded concentration of 25.13 µg/ml, followed closely by A6 at 24.93 µg/ml. Plants treated with *T. atroviride* and OYVMV inoculation (A5) exhibited a phenolic level of 22.13 µg/ml. In comparison, untreated OYVMV-infected plants (A1) displayed a phenolic content of 21.33 µg/

ml, significantly exceeding the control group's value of 13.80 µg/ml, but remaining inferior to all fungal-treated groups.

Discussion

The results of this study highlight the important role of Trichoderma fungi in inducing systemic resistance in okra plants against yellow vein mosaic virus (OYVMV). Treatments with *T. atroviride* and *T. asperellum* effectively reduced the severity of OYVMV symptoms, with the lowest symptom severity in plants treated with the combined fungicide (A7), resulting in a 27.67% reduction in disease severity. This finding suggests a synergistic interaction between these fungi, enhancing their ability to induce systemic resistance (ISR). These results are consistent with those achieved by Karen and Haidery (2022) when they used amino acids and algae extracts to induce systemic resistance in okra plants against the same virus. Plants infected with the virus and treated with algae extracts and amino acids showed a 13% lower disease severity compared to untreated infected plants, which reached 41%. This decrease in the severity of symptoms resulted from the healthy growth of plants due to the presence of the Trichoderma fungus, which stimulated good plant growth and helped it induce systemic resistance.

Enzymatic assays revealed compelling evidence of enhanced plant defense mechanisms. Peroxidase (POD) activity, a key indicator of plant resistance, increased significantly in the A7 treatment group. This enhanced activity highlights the role of Trichoderma fungi in stimulating oxidative stress, which is crucial for defense against pathogens. These results also match those of Karem and Haidery (2022), when okra plants were treated with algae extracts and amino acids. Peroxidase levels increased to 17.32 U/mg after 21 days of treatment. Similarly, superoxide dismutase (SOD) and catalase (CAT) activities increased significantly in plants treated with A7, reaching 7.23 and 3.63 U/mg, respectively. The results also showed an increase in total phenolics after 21 days, reaching 75.4 U/mg, confirming their role in eliminating reactive oxygen species (ROS) and maintaining cellular homeostasis under viral stress. Abdelkhalek *et al.* (2022) also reported the ability of the *T. hamatum* Th23 strain to promote plant growth, induce systemic resistance, and enhance innate immunity against *Tobacco mosaic virus* TMV infection. Th23 has been identified as a potential biocontrol agent for managing plant viral infections, by observing its effect on increasing the activity of oxidative enzymes in plants infected with the virus and treated with fungi.

This study's results contribute to the growing body of evidence supporting the use of biological control agents to manage viral diseases in economically important crops like okra. The combined use of *T. atroviride* and *T. asperellum* represents a promising approach to reducing disease severity, enhancing plant resilience, and improving yield potential in the face of OYVMV challenges. Future studies could explore the molecular mechanisms underlying the observed effects and the long-term field applicability of these treatments in diverse environmental conditions.

Conclusions and Recommendations

This study demonstrates the significant potential of *Trichoderma atroviride* and *Trichoderma asperellum* in Induced Systemic Resistance in okra (*Abelmoschus esculentus*) against okra yellow vein mosaic virus (OYVMV). The combined treatment of these two fungal species (A7) resulted in the lowest disease severity and the highest levels of defense-related enzymatic activities, including peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT). Additionally, the total phenolic content was significantly elevated, indicating enhanced structural and biochemical defenses.

The findings highlight the synergistic effects of dual fungal treatments in activating multiple plant defense pathways, thereby providing superior protection against OYVMV compared to individual applications. This biocontrol strategy not only reduces disease severity but also enhances the overall resilience of okra plants, making it a promising component of sustainable agricultural practices.

The study emphasizes the importance of integrating *T. atroviride* and *T. asperellum* into pest management programs for okra as a cost-effective and environmentally friendly alternative to chemical controls. Further research is recommended to explore the molecular mechanisms underlying these effects and to assess the long-term field applicability of these treatments under diverse environmental and agronomic conditions.

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Novelty Statement

This research explores, for the first time, how Trichoderma species might enhance okra's innate defenses against Okra Yellow Vein Mosaic Virus, offering a sustainable and environmentally friendly alternative to conventional management methods.

Author's Contribution

Zina Abdul-Hussein Jawad: Contributed to the statistical analysis and data interpretation, including the ranking methodology.
Nabeel Al-Kaeath: Was responsible for drafting and refining the manuscript.
Malik H. Karem: Conceived the original research idea and conducted the laboratory experiments.

Conflict of interest
The authors have declared no conflict of interest.

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