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Overview of *Fusarium Oxysporum* f. Sp. *Vasinfectum* Causing Okra Wilt and its Management

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Abstract

Okra (*Abelmoschus esculen*tus) is susceptible to various diseases caused by fungi, bacteria, nematodes, and viruses, with fungal pathogens being the most destructive. One of the most destructive pathogens is *Fusarium oxysporum*, a soil-borne fungus responsible for causing wilt in okra and other vegetables. In Pakistan, okra wilt is widely distributed and poses a significant threat to crop production. Both chemical and non-chemical control strategies have been explored to manage this disease. Morphological and microscopic examination of the isolated fungus revealed the colony characteristics of typical *F. oxysporum*, including white, cottony, circular colonies and the presence of macroconidia. *In vitro* experiments were conducted using three fungicides, e.g., Topsin-M, Mutagen, and Carbendazim, to assess their efficacy against F. oxysporum. Trichoderma spp. was employed as a biocontrol agent, offering a promising eco-friendly alternative for disease management and demonstrating the ability to suppress the pathogen effectively. It is further recommended that optimal concentrations of the tested fungicides be determined to enhance their practical application and minimize environmental impact.

Keywords: Bio-control, Fungicides, Morphological Traits, *Trichoderma* spp.,
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Introduction

Okra is believed to have originated in East Africa and is the most widely cultivated vegetable in South Asia and other parts of the world (Singh & Nigam, 2023). Okra is commonly referred to as lady's finger. Okra grows best in warm climates, as the plant does not tolerate frost and chilling, which limits its cultivation in cooler regions. Okra plant belongs to the *Malvaceae* family and the genus *Abelmoschus* (Mubeen et al., 2017). Scientific literature has documented its taxonomy and agricultural importance (Terell & Winters, 1974). The okra flower is characterized by a spoon-shaped calyx with five small teeth that merge with the crown and fall soon after flowering. Okra is cultivated in different geographical locations in the Middle East and North Africa, Turkey, Iran, Japan, West Africa, Yugoslavia, India, Pakistan, Bangladesh, Afghanistan, Malaysia, Thailand, Brazil, Ethiopia, Cyprus, and the south of the United States (Mubeen et al., 2021). Its global distribution emphasizes its culinary and nutritional value (Kotha et al., 2019) and the need to understand and effectively manage diseases for better production.

Fusarium oxysporum f. sp. vasinfectum is the causative agent of Fusarium wilt, a significant disease that predominantly affects okra crops (Keinath et al., 2023). The fungus infects the roots and colonizes the vascular system, limiting water transport within the plant (Agbaglo et al., 2020). Being a soil-borne pathogen, it can be spread through practices like cross-cropping and contaminated equipment (Fatima et al., 2023). Saleem et al. (2020) reported that Fusarium wilt in okra initially causes symptoms of wilting of seedling leaves and cotyledons. The cotyledons first develop chlorosis at the tips, which eventually turns necrotic. In mature plants, symptoms include wilting and yellowing of the leaves. The disease progresses gradually but may intensify after heavy rainfall during the summer season, while plants may become stunted or even die of severe infections. A key diagnostic feature is the discoloration of the vascular system, which is visible upon cutting the stem of the infected plant (Agrios, 2005). The heat encourages the growth of fungi, which can enter the area through infected seeds, contaminated equipment, or human activity (Ploetz, 2006). To control this disease, certified and disease-free seeds and resistant varieties to Fusarium wilt are recommended, particularly in areas with a history of repeated infection, which can effectively resist the disease incidence. Soil fumigation can also assist in reducing the occurrence of disease (Fravel et al., 2003).

The causal agent of Okra wilt

Fusarium oxysporum f. sp. *vasinfectum* is the pathogen responsible for okra wilt, which was identified in commercial okra cultivars in Pakistan. This pathogen was found in various Clemson spineless heirloom cultivars, which continue to be widely distributed. *F. oxysporum* f. sp. *vasinfectum* causes discoloration of the vascular cells within the stems of infected plants (Snyder et al., 1940). Two inoculation methods were compared: the injection of microconidia into plant stems and the soaking of microconidia at the base of the plant. Edel-Hermann et al. (2019) proposed that the six races of *F. oxysporum f. sp. vasinfectum* evolved based on their selective pathogenicity to different hosts (These races included 1, 2, 3, 4, 6, and 8. Previously identified races 5 and 7 were later reclassified as 3 and 4, respectively. Nelson et al. (1983) reported that *F. oxysporum* could reproduce asexually by producing chlamydospores, microconidia, and macroconidia, while Bell et al. (2016) observed that microconidia are uninucleate and exhibited low germination efficiency ranging from 1% to 20%. Researchers used RAPD markers to differentiate *F. oxysporum* isolates, and Forty-six isolates collected globally were grouped into three clusters based on the results of inoculation within different host plants. Three RAPD groups of isolates were identified in China; one resembled race 3, while the others were defined as races 7 and 8, which differed from previously known races. The identified most dominant race, 7, was grouped within a single RAPD cluster, while race 8 was grouped into two based on genetic diversity (Zhu et al., 2021). Davis et al. (2006) reported that the pathogenic response of each race varied in plants outside the *Gossypium* genus. Kim et al. (2005) indicated that some researchers have categorized races 1, 2, and 6 under a single genetic group called race A. Bell et al. (2016) identified two distinct types of Fusarium pathogens: one capable of causing root rot without colonizing the plant stem and another that effectively colonized the stem but did not induce root rot symptoms. Races 3, 4, and Australian biotypes are root rot pathotypes, while Races 1, 2, 6, and 8 are vascular colonization (or vasoactive) pathogens.

Morphological and Microscopic Characterization of F. oxysporum

Ayada et al. (2022) reported that *F. oxysporum* is a ubiquitous soil-borne fungus identifiable by its distinct morphological and microscopic features. Vavre et al. (2021) observed that its colonies typically proliferate. Farhaoui et al. (2023) further characterized the colonies as having a cottony appearance with a white or pinkish aerial mycelium, occasionally displaying a contrastingly colored reverse side. Ajmal et al. (2022) described that *F. oxysporum* produces three main types of asexual spores. (Patel et al., 2020 reported the macroconidia as slender, slightly curved to sickle-shaped structures measuring 20–55 µm in length and 3–5 µm in width containing 3–5 septa. Vavre et al. (2021) noted that these macroconidia possess tapered apical and characteristic foot-shaped basal cells. Pithiya & Kanzaria (2024) reported that the microconidia, the second type of spores produced by *F.oxyporum*, are much smaller, oval to ellipsoidal measuring 5–10 µm long, and either aseptate or have single septum which is produced abundantly from unbranched monophialides arranged in "false heads." (Hou et al., 2020) described the third type of spores, chlamydospores, as thick-walled globose structures present singly or in small clusters form. These spores allow the fungus to survive under adverse environmental conditions. These combined morphological traits are important for accurately distinguishing *F. oxysporum* from other *Fusarium* species.

Disease Cycle of Okra Wilt

Todorović et al. (2023) reported that Fusarium survived in the soil for long periods by producing chlamydospores, which persist in plant debris and soil. Kalbande and Yadav (2021) stated that these chlamydospores germinate under favorable warm and moist conditions, producing hyphae that penetrate okra root cells through natural openings or wounds. Srivastava et al. (2024) described that the fungus colonizes the plant's xylem vessels, which multiply and release conidia transported upward with the water flow. Fungal invasion disrupts the regular transport of water and nutrients, which causes characteristic yellowing and wilting of leaves. Hassan (2020) mentioned that the fungus infection gradually spreads from the lower parts of the plant upward until the entire plant collapses. *F. oxysporum* sporulates and releases additional chlamydospores from decaying tissues into the soil to continue the cycle (Figure 1).

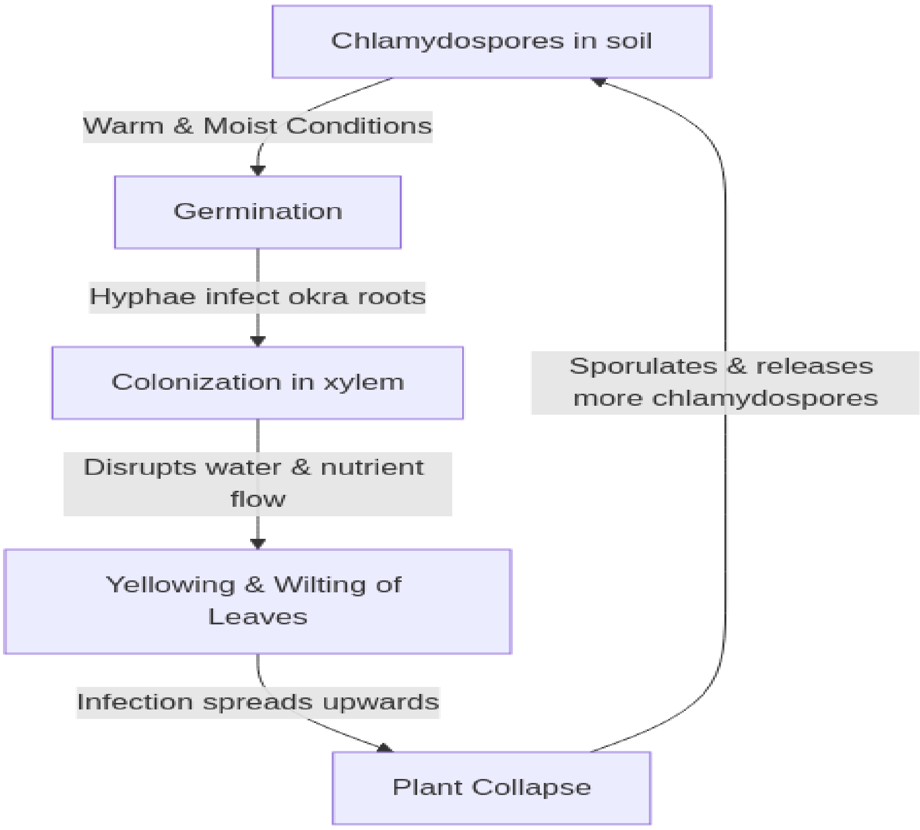

Figure 1. The life cycle of Fusarium Wilt of okra

Symptoms of Okra Wilt

Ounis et al. (2024) reported that Fusarium wilt in okra exhibits a progressive yellowing of older leaves that begin from the plant's lower part and advance upward. Keinath et al. (2023) also described that affected leaves exhibit wilting during the hottest part of the day, though they often showed temporary recovery in the evening hours. Plants show stunted growth, reduced vigor, and defoliation. According to Hossain et al. (2021), the symptoms include brown or dark vascular discoloration within the stem. The entire plant wilts permanently in severe form, and ultimately, leaf drop and death occur (Figure 2).

Disease Progression **Early Leaf Symptom** **Severe Infection**

Figure 2. Symptoms of Fusarium Wilt of okra

Integrated Disease Management

Jiménez-Díaz et al. (2015) proposed that managing Fusarium wilt is quite a challenge, and all plant species typically need an integrated approach to disease control. The primary objective of these approaches is to eliminate pathogens and reduce fungal inoculum size and persistence in soil. Hariharan et al. (2022) reported an increasing trend in crop losses due to various plant pathogens. They emphasize the urgency of current disease challenges and demand the development of eco-friendly and non-chemical control alternatives. Biocontrol, or biological control of plant diseases, is a promising and effective way to overcome many hazards from existing control systems. Biological control agents (BCAs) include the nontoxic, filamentous fungal parasite Trichoderma spp. It is known for its versatility in agriculture. The interaction between *Trichoderma*, the host plant, and the pathogen is important in disease suppression. Once activated, *Trichoderma* engages with the infected plant through mechanisms such as mycoparasitism, antibiosis, and competition while indirectly enhancing plant growth and yield by producing systemic resistance responses. This interaction promotes disease management and crop rehabilitation from different diseases, resulting in increased and protected crop production. Many species of Trichoderma are effective against many plant diseases by improving the general health of plants and increasing their yields. Biocontrol activity, plant-trichoderma interaction, and effectiveness vary depending on the species-specific and environmental factors. In addition, both commonly used and newly identified *Trichoderma* strains require thorough evaluation under diverse field conditions to ensure their efficacy and safety as biocontrol agents.

Cultural Control

Gordon et al. (1997) indicated that human activity is the primary factor contributing to new *Fusarium oxysporum* (Fo) infestations. Haware (1998) emphasized that using Fo-free seeds on non-infested soils is crucial for maximizing their usefulness. Avoiding cultivation on infected soils significantly reduces disease incidence. Agrios (2005) reported that crop rotation may lessen the inoculum in the soil for soil-borne pathogens such as Fo. However, it will be less effective because Fo-chlamydospores can survive inside the soil for extended periods (Pande et al., 2007). Adding traditional agronomic practices also helped reduce the impact and prevalence of Fo. Seeds that are tainted or sick can cause disease. A successful quarantine or certified pathogen-free seedlings are crucial strategies for controlling *Fusarium* wilt. Jiménez-Díaz et al. (2015) acknowledged that while these measures were effective still, they are often costly and need to be evaluated in the context of crop economics and disease forecasting. Rubiales et al. (2015) and Panth et al. (2020) proposed using resistant cultivars, which is generally acknowledged as the safest, most practical, and efficient crop protection technique to manage soil-borne disease aside from all the previously mentioned control measures. Cultural techniques such as crop rotation and soil solarization must be established to reduce the occurrence of Fo inoculum in these situations. Solarization can decrease soil inoculum, which is achieved by covering the soil with mulch to raise its temperature.

Chemical Control

Singh et al. (2022) reported that various fumigant fungicides such as carbendazim, dazomet, chloropicrin, and 1,3-dichloro propene had been used to manage Fusarium wilt as alternatives to methyl bromide chemicals. Historically, chloropicrin and dazomet were effectively employed to control pea wilt in soils with high levels of infection (Singh et al., 2022). Pea wilt in heavily infected soils was satisfactorily treated with chloropicrin and dazomet. Yadeta et al. (2013) reported many drawbacks to this strategy regarding the economy, the environment, and public health. Gullino et al. (2013) proposed that Methyl bromide was a commonly used fumigant until the Montreal Protocol, which was implemented in 1986 as part of an international agreement to protect the ozone layer because of its strong efficacy against diseases spread via soil. Panth et al. (2020) and Zhao et al. (2017) reported that it is crucial to emphasize that their excessive and careless usage can harm aquatic habitats, change the makeup of the soil microbial population, and even cause fungicide resistance to emerge. The public's intense concern about environmental issues has led to suggestions to investigate new environmentally friendly control methods. Ons et al. (2020) reported that chemical control is a management strategy for soil-borne diseases, and using fungicides to control *Fusarium* wilt was common practice in the past. However, in addition to the harmful effects of agricultural chemicals on human health and the environment, the development of fungicide resistance has led to the search for alternative applications (Table 1).

Table 1. Fungicides used, dosages, and chemical

Fungicides	Dose	Active Ingredient	Source	Reference
Dolomite	4 grams in 4L water	Metalyxl-Mancozeb	Target	Arain et al. (2012)
Ridomil MZ	4 grams in 4L water	Metalyxl-Mancozeb	Sygenta	
Topsin-M	8 grams in 4L water	Thiophanate-methyl	Agro Pakistan Pvt	
Tahfuz	4 grams in 4L water	Fostel Aluminum	Target	
Diesomil	4 grams in 4L water	Cymoxil- Mancozeb	Target	
Protest	6 grams in 4L water	Propinib	Target	

Biological control

Kapoor et al. (2006) and Chakraborty et al. (2009) investigated Trichoderma spp. as one of the most commonly used biological controls for *Fusarium* wilt. Biological control techniques can be used with other methods for efficient disease management in the field, or they can aid in creating alternative management plans. Fravel et al. (2003) and Fatima et al. (2015) reported that research on the biocontrol of plant diseases has dominated recent years. The results provide awareness of the effectiveness of biological control agents in treating okra wilt and their potential as environmental alternatives to other fungicides. Salih et al. (2019) investigated the effects of the biological agent *Trichoderma harzianum* Rifai and the fungicide topsin-m on okra root rot in the field. This study aimed to explore the outcomes of the interaction between *T. harzianum* and Topsin-M fungicide on root rot of okra disease in the field. 3 fungal species have been isolated from root rot disease: *F. solani*, *R. solani,* and *R. phaseolus*. Strains of these bacteria were tested and found to cause root rot with a prevalence of 41.7%, 6.7%, and 31.7%, respectively. Indoor tests show that *Trichoderma harzianum* has level 1 and 2 antagonistic ability against the *fungus Macrophemina phaseolina, Fusarium solani, and Rhizoctonia. solani* respectively. It was also found that Topsin-M fungicide prohibited the growth of all fungi by 100%, while the growth inhibition rate of toxic fungi was 50.4%, as recommended for discussion experiments. Field results are biological. When each fungus was compared individually, Harzia harzianum and the topsin-M decrease the disease percentage and severity of *F. solani, R. solani, and M. phaseolina* fungal diseases by 65.3%, 21.20%, 13.20%, and 46%, respectively. It decreased by .20. %, 25.70% and 18.20%, 71.00%, 60.20, 60.20, 66.80, 80.20 and 60.20%. Interaction of chemical *T. harzianum* and topsin-M enlarge plant height, dry and fresh weight of shoot and root system and fruit production in studied okra plant. Lahlali et al. (2022) proposed that biological control agents developed distinct approaches to inhibit the growth of pathogens, including producing antimicrobial substances, competing for resources, engaging in parasitism-inducing metabolizing germination stimulants, and plant defense responses.

Conclusion and Future Prospects

This review concludes that managing okra wilt caused by *Fusarium oxysporum* requires an integrated approach combining pathogen identification with integrated disease management strategies. Detailed morphological and microscopic analyses have confirmed the presence of *F. oxysporum*, emphasizing the need for targeted interventions. However, chemical fungicides have proven effective in controlling pathogens, environmental concerns, and pathogen resistance, which demands the exploration of eco-friendly alternatives like biological agents. Biocontrol agents such as Trichoderma spp. or resistant breeding emerge as promising tools to manage this disease. Future research should focus on refining integrated management practices that improve chemical and biological control. There is a need to determine optimal fungicide concentrations to minimize environmental. Further exploration of biocontrol efficacy and the development of resistant okra cultivars should be the primary focus in the future against pathogens and diseases of okra. Awareness about field sanitation is also important to avoid or manage future diseases.

Competing Of Interest

The authors declare that the research was carried out without commercial or financial relationships that could be construed as a potential conflict of interest.

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