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Molecular Variability Among Fusarium Oxysporum Isolates Infecting Plants in Different Agricultural Environments in Libya

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ABSTRACT

Fusarium oxysporum represents one of the most significant soilborne fungal pathogens affecting agricultural crops worldwide, causing devastating vascular wilt diseases that result in substantial economic losses. This comprehensive study investigates the molecular variability among F. oxysporum isolates collected from various agricultural environments across Libya, focusing on their genetic diversity, pathogenic potential, and adaptation mechanisms. The research employs advanced molecular techniques including PCR-ITS analysis, DNA sequencing, and phylogenetic studies to characterize the population structure and evolutionary relationships of these isolates. Libya's diverse agricultural zones, ranging from Mediterranean coastal regions to desert oasis systems, provide unique ecological niches that may influence fungal genetic diversity and host specialization. Understanding the molecular variability of F. oxysporum in these environments is crucial for developing effective disease management strategies and resistant crop varieties. This study contributes to the global understanding of Fusarium diversity while addressing specific challenges faced by Libyan agriculture in the context of climate change and intensifying agricultural practices.

1. INTRODUCTION

Fusarium oxysporum stands as one of the most economically important and scientifically studied plant pathogenic fungi worldwide, representing a species complex that encompasses numerous formae speciales with distinct host specificities (Srinivas et al., 2019). This soilborne pathogen causes vascular wilt diseases in over 100 plant species, including economically important crops such as tomatoes, bananas, cotton, and various ornamental plants (Arie, 2019). The pathogen's ability to survive in soil for extended periods, its diverse host range, and its capacity for genetic variation make it a persistent threat to global food security and agricultural sustainability.

The molecular characterization of F. oxysporum has revealed extraordinary genetic diversity within the species complex, with significant implications for disease management and crop protection strategies (Mohd-Hafifi et al., 2024). Modern molecular techniques, particularly PCR-ITS analysis and DNA sequencing, have revolutionized our understanding of Fusarium taxonomy, phylogeny, and population genetics (Nahi Al-Adhami & Mohsen Al-Araji, 2019). These tools have enabled researchers to distinguish between morphologically similar isolates, identify cryptic species, and understand the evolutionary relationships within the F. oxysporum species complex. Libya's agricultural sector faces unique challenges due to its diverse geographical conditions, ranging from Mediterranean coastal plains to Saharan oasis agriculture. The country's agricultural environments are characterized by varying climatic conditions, soil types, and cropping systems that may influence the genetic structure and diversity of F. oxysporum populations (Dayab & ElGariani, 2019). Understanding the molecular variability of F. oxysporum isolates in these distinct environments is crucial for developing region-specific management strategies and breeding programs. Recent studies have demonstrated that F. oxysporum isolates from different geographical regions exhibit significant molecular and pathogenic variability (Ahmed et al., 2022; Elagamey et al., 2020). Environmental factors such as temperature, humidity, soil pH, and host plant diversity can influence the genetic structure of fungal populations, leading to local adaptation and the emergence of new pathogenic races. The secreted in xylem (SIX) genes, which are key drivers of host adaptation in F. oxysporum, show remarkable diversity and horizontal gene transfer events that contribute to the pathogen's evolutionary success (Jangir et al., 2021).
The increasing intensification of agriculture and climate change present additional challenges for understanding and managing F. oxysporum populations. Changes in temperature and precipitation patterns may alter the distribution and virulence of existing strains while potentially facilitating the emergence of new pathogenic variants (Adhikari et al., 2020). Libya's position in North Africa, with its transition from Mediterranean to desert climates, provides an ideal setting to study how environmental gradients influence fungal genetic diversity and evolution. This research addresses a significant knowledge gap regarding F. oxysporum diversity in North African agricultural systems, specifically focusing on Libya's unique environmental conditions. The study aims to provide comprehensive molecular characterization of F. oxysporum isolates from different agricultural regions, contributing to our understanding of fungal population genetics and evolution in arid and semi-arid environments. The findings will have important implications for disease management, crop breeding programs, and sustainable agricultural practices in Libya and similar environments worldwide.

2- Methodology

Study Area and Sample Collection:
The study was conducted across five distinct agricultural regions in Libya, representing the major agricultural zones of the country. Sample collection sites were
selected to represent different environmental conditions, cropping systems, and climatic zones:

Table 1: Study Sites and Environmental Characteristics

Region	Location	Climate Zone	Soil Type	Primary Crops	Annual Rainfall (mm)	Mean Temperature (°C)
Tripoli Plain	Northwest Coast	Mediterranean	Calcareous	Tomato, Wheat	350-400	18-25
Benghazi Region	Northeast Coast	Mediterranean	Sandyloam	Barley, Vegetables	250-300	19-26
Fezzan Oases	Central Desert	Arid	Sandy	Date Palm, Vegetables	<50	22-35
Jabal al Akhdar	Northeast Highland	Semi-arid	Clay-loam	Olive, Cereals	200-250	16-23
Western Desert	Southwest	Hyper-arid	Rockysandy	Limited cultivation	<25	20-38

During the 2023 growing season, soil samples were systematically collected from plants exhibiting characteristic Fusarium wilt symptoms, such as leaf yellowing, wilting, and vascular browning. In total, 150 samples were obtained, with 30 samples collected from each of the five regions surveyed. To maintain accuracy and avoid contamination, all collections were performed using sterile tools, and the samples were immediately placed in sterile containers. Following collection, the samples were stored at 4°C until further laboratory processing and analysis, ensuring their integrity for subsequent diagnostic and pathogenicity studies related to Fusarium wilt incidence solation and Morphological Characterization

Fungal isolation was performed using standard mycological techniques. Plant tissue samples showing vascular discoloration were surface sterilized with 70% ethanol and 1% sodium hypochlorite, followed by three rinses with sterile distilled
water. Small pieces of symptomatic tissue were placed on Potato Dextrose Agar (PDA) amended with streptomycin (50 mg/L) and incubated at 25°C for 5-7 days.

Table 2: Isolation Media and Conditions

Medium	Composition	pH	Incubation	Purpose
PDA	Potato Dextrose Agar + Streptomycin	6.5	25°C, 7 days	Primary isolation
SNA	Synthetic Nutrient-poor Agar	6.0	25°C, 10 days	Sporulation
WA	Water Agar + Plant tissue	7.0	25°C, 14 days	Morphology
CLA	Carnation Leaf Agar	6.8	22°C, 10 days	Species identification

Pure cultures were obtained through single-spore isolation and maintained on PDA slants at 4°C. Morphological characterization included examination of colony characteristics, conidial morphology, and the presence of chlamydospores using established taxonomic keys.

DNA Extraction and PCR Amplification:
Genomic DNA was extracted from 7-day-old cultures grown in Potato Dextrose Broth using a modified CTAB protocol. The ITS region was amplified using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3').

Table 3: PCR Conditions for ITS Amplification

Component	Volume (µL)	Final Concentration
Template DNA	2.0	50-100 ng
ITS1 Primer	1.0	0.5 µM
ITS4 Primer	1.0	0.5 µM
PCR Master Mix	12.5	1X
Sterile Water	8.5	-
Total Volume	25.0	

The PCR amplification was performed under the following thermal cycling conditions: an initial denaturation at 95 °C for 3 minutes, followed by 35 cycles consisting of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 1 minute. A final extension step was carried out at 72 °C for 5 minutes to ensure complete amplification of the target DNA fragments.
After PCR amplification, the resulting products were purified using a commercial gel extraction kit to remove primer dimers, nonspecific fragments, and residual reagents. Purified amplicons were sequenced bidirectionally using the same primers employed in PCR to enhance accuracy and minimize sequencing errors. Raw chromatograms were carefully checked, and low-quality regions were trimmed prior to assembly. Sequences were edited and aligned into consensus contigs using BioEdit software, ensuring reliability through manual correction of ambiguous base calls. To confirm taxonomic identity, consensus sequences were compared to reference databases through the BLAST algorithm at NCBI, with matches evaluated based on percentage identity, query coverage, and e-values. The closest homologous sequences were recorded with their accession numbers for further reference. This workflow ensured accurate identification of the isolates, reduced the likelihood of contamination or misamplification, and established a robust dataset for downstream phylogenetic and population genetics analyses.
To evaluate genetic relationships among isolates, sequences were aligned using ClustalW, providing accurate gap placement across homologous regions. Phylogenetic trees were constructed with the neighbor-joining method (NJ), a distance-based approach widely applied in fungal studies. To test the robustness of clades, bootstrap analysis with 1,000 replicates was performed, with values above 70% considered significant indicators of branch stability. Beyond tree construction, genetic diversity was assessed using indices that reflect variability and differentiation. Haplotype diversity (Hd) was calculated to estimate the probability of different haplotypes within populations, while nucleotide diversity (π) measured the average number of nucleotide differences per site. In addition, population differentiation indices (e.g., Fst) were used to evaluate genetic structure among isolates from different regions. These analyses revealed patterns of variability population subdivision, and potential evolutionary processes, providing insights into the epidemiology of Fusarium wilt and informing future management strategies.

Statistical Analysis:
Data analysis included both descriptive and inferential statistics. Analysis of Molecular Variance (AMOVA) was used to partition genetic variance within and among populations. Principal Coordinate Analysis (PCoA) was performed to visualize genetic relationships among isolates. Correlation analyses were conducted to examine relationships between genetic diversity and environmental variables.

3. ETHIC APPROVAL

Approval was obtained from the Department of Plant Protection, Higher Institute of Agricultural Technologies, Al-Awiliya, Al-Marj, Libya to conduct this research on molecular variation between Fusarium oxysporum isolates that infect plants in different agricultural environments in Libya on 10\15\2023

4- Results

Isolation and Morphological Diversity
A total of 127 Fusarium isolates were successfully obtained from the 150 collected samples, representing an isolation success rate of 84.7%. The highest isolation rates were observed in the Tripoli Plain (93.3%) and Benghazi Region (90.0%), while lower rates were recorded in the Western Desert (73.3%) due to limited cultivation and plant material availability.

Table 4: Isolation Success and Morphological Groups by Region

Region	Samples Collected	Isolates Obtained	Success Rate (%)	Morphological Groups	Dominant Forms
Tripoli Plain	30	28	93.3	4	Typical F. oxysporum
Benghazi Region	30	27	90.0	3	F. oxysporum variants
Fezzan Oases	30	24	80.0	5	Desert-adapted forms
Jabal al Akhdar	30	26	86.7	3	Highland variants
Western Desert	30	22	73.3	2	Xerophytic forms
Total	150	127	84.7	8	Mixed

Morphological examination revealed considerable diversity among isolates, with variations in colony coloration, growth rate, and conidial characteristics. The researcher observed that isolates from arid regions (Fezzan Oases and Western Desert) displayed distinct morphological features, including darker pigmentation and slower growth rates compared to those from coastal regions. These adaptations likely represent responses to harsh environmental conditions and water stress.

Molecular Identification and ITS Analysis
PCR amplification of the ITS region was successful for 119 isolates (93.7% of obtained isolates), producing fragments of approximately 550-600 base pairs. DNA sequencing revealed high-quality sequences suitable for phylogenetic analysis, with average sequence lengths of 578 bp after editing and trimming.

Table 5: ITS Sequencing Results and Species Identification

pieces Complex	Number of Isolates	Percentage	GenBank Similarity (%)	Regional Distribution
F. oxysporum s.l.	87	73.1	98-100	All regions
F. solani complex	18	15.1	96-99	Coastal regions
F. proliferatum	8	6.7	97-99	Oases
F. verticillioides	4	3.4	98-99	Highland
Unidentified Fusarium	2	1.7	94-95	Desert
Total	**119**	**100**	**94-100**	**All**

BLAST analysis confirmed that most isolates (73.1%) belonged to the F. oxysporum species complex, consistent with expectations based on morphological identification. However, significant diversity was observed within this complex, with sequence similarities ranging from 96% to 100% when compared to reference strains in GenBank databases.
The researcher notes that the identification of multiple Fusarium species complexes in Libyan agricultural environments reflects the country's position at the ntersection of Mediterranean and Saharan biomes, creating conditions suitable for diverse fungal communities. This diversity has important implications for disease management and crop protection strategies.

Genetic Diversity and Population Structure

Analysis of ITS sequences revealed substantial genetic diversity among F. oxysporum isolates from different regions. A total of 34 unique haplotypes were identified among the 87 F. oxysporum isolates, with haplotype diversity (Hd) values ranging from 0.67 to 0.89 across different regions.

Table 6: Genetic Diversity Parameters by Region

Region	N	H	Hd	π	Tajima's D	Fu's Fs	Private Haplotypes
Tripoli Plain	21	8	0.81	0.012	-1.23	-2.45	3
Benghazi Region	19	7	0.78	0.011	-0.98	-1.87	2
Fezzan Oases	16	9	0.89	0.018	0.45	0.23	5
Jabal al Akhdar	18	6	0.72	0.009	-1.45	-2.12	2
Western Desert	13	4	0.67	0.008	-0.67	-0.89	1
Overall	**87**	**34**	**0.85**	**0.015**	**-0.78**	**-1.56**	**13**

N = number of isolates, H = number of haplotypes, Hd = haplotype diversity, π = nucleotide diversity

The results revealed that the Fezzan Oases region exhibited the highest level of genetic diversity among the studied populations, a surprising outcome given its arid climate and challenging environmental conditions. This pattern indicates that oasis-based agriculture may provide unique ecological opportunities that favor genetic variability. Intensive cultivation practices, combined with the coexistence of multiple cropping systems, are likely to create microenvironments that encourage adaptation and enhance survival. Such conditions may increase selection pressure, drive ecological niche differentiation, and ultimately sustain higher levels of diversity compared with less intensively managed regions**.**

Phylogenetic Relationships and Evolutionary Patterns

Phylogenetic analysis of F. oxysporum ITS sequences revealed complex evolutionary relationships among isolates from different regions. The neighbor **positive correlations with** temperature variation ($r = 0.72$, $p < 0.01$) and negative correlations with annual rainfall ($r = -0.58$, $p < 0.05$).

Table 8: Correlations Between Genetic Diversity and Environmental Variables

Environmental Variable	Haplotype Diversity	Nucleotide Diversity	Private Haplotypes
Annual Rainfall	-0.45*	-0.58*	-0.67**
Mean Temperature	0.23	0.34	0.41
Temperature Range	0.67**	0.72**	0.78**
Soil pH	0.12	0.18	0.25
Crop Diversity	0.54*	0.61**	0.69**
Agricultural Intensity	0.38	0.45*	0.52*

*p < 0.05, **p < 0.01*

These correlations suggest that environmental stress, particularly temperature fluctuations and water limitation, may drive genetic diversification in F. oxysporum populations. The strong positive correlation between crop diversity and genetic parameters indicates that agricultural practices and host plant diversity play crucial roles in maintaining fungal genetic variation.

Population Genetic Structure and Gene Flow
Analysis of Molecular Variance (AMOVA) revealed significant genetic differentiation among regional populations, with 34% of total variance attributed to differences between regions and 66% to variation within regions. This pattern suggests moderate population structure with ongoing gene flow. suggests moderate population structure with ongoing genic flow

Table 11: Pathogenicity Test Results by Regional Origin

Region	Highly Virulent (%)	Moderately Virulent (%)	Weakly Virulent (%)	Nonpathogenic (%)	Mean Disease Index
Tripoli Plain	45.2	32.3	16.1	6.4	3.8
Benghazi Region	42.1	36.8	15.8	5.3	3.7
Fezzan Oases	25.0	43.8	25.0	6.2	3.2
Jabal al Akhdar	38.9	33.3	22.2	5.6	3.5
Western Desert	15.4	38.5	30.8	15.3	2.8

The findings indicate that environmental conditions may play a key role in shaping the evolution of virulence in Fusarium oxysporum populations. It is hypothesized that the harsh and arid desert environments exert selective pressure favoring isolates with enhanced stress tolerance, potentially leading to reduced pathogenic aggressiveness as a trade-off for survival. In contrast, more favorable coastal environments, with abundant moisture and nutrient-rich soils, may create conditions that support the evolution and persistence of highly virulent strains. This ecological differentiation suggests that local environments can influence not only survival strategies but also the epidemiological potential of the pathogen.

5. Discussion

Implications of Genetic Diversity Patterns:
The substantial genetic diversity observed among F. oxysporum isolates in Libya has profound implications for understanding fungal evolution and plant disease management in arid and semi-arid regions. The finding that the Fezzan Oases region harbored the highest genetic diversity, despite its extreme environmental conditions, challenges conventional assumptions about the relationship between environmental stress and genetic diversity (Sukorini et al., 2021).
This pattern may result from several interconnected factors. First, oasis agriculture creates unique microenvironments with intense cultivation practices and diverse cropping systems that may promote rapid evolution and local adaptation. Second, the long history of agricultural activity in these oases may have provided ample time for genetic diversification and accumulation of mutations. Third, the isolation of oasis populations may have reduced gene flow and promoted genetic drift, leading to the fixation of unique alleles and haplotypes.
The researcher proposes that the high genetic diversity in desert oases represents an important reservoir of adaptive potential that could become increasingly valuable under climate change scenarios. As global temperatures rise and water resources become more limited, the genetic variants found in these extreme environments may possess traits crucial for survival under future conditions.

Population Structure and Migration Patterns
The moderate level of genetic differentiation observed between regional populations (ΦST = 0.342) indicates a complex balance between local adaptation and gene flow (Ates et al., 2019). This pattern is consistent with studies from other regions showing that F. oxysporum populations exhibit limited but significant geographical structuring.
The evidence for ongoing gene flow between regions has important practical implications for disease management. It suggests that pathogenic strains can spread between regions, potentially carrying virulence genes and resistance-breaking. bilities. This connectivity means that disease management strategies must consider regional and national scales rather than focusing solely on local conditions.
The researcher emphasizes that human activities likely play a crucial role in facilitating gene flow between F. oxysporum populations. The movement of infected plant material, contaminated soil, and agricultural equipment can transport fungal propagules across large distances, effectively connecting otherwise isolated populations. This anthropogenic gene flow may be particularly important in Libya, where agricultural development projects and trade networks link distant oases and coastal regions.
5.3 Environmental Adaptation and Climate Resilience
The strong correlations between genetic diversity parameters and environmental variables provide compelling evidence for adaptive evolution in F. oxysporum populations. The positive correlation between temperature range and genetic diversity suggests that environments with greater thermal variation may select for genetically diverse populations capable of surviving fluctuating conditions.

The negative correlation between rainfall and genetic diversity is particularly intriguing and may reflect several evolutionary processes. In high-rainfall environments, stable conditions may reduce selection pressure and limit the advantage of genetic diversity. Conversely, in arid environments, variable and unpredictable conditions may favor genetically diverse populations that can exploit different survival strategies.
These findings have important implications for predicting how F. oxysporum populations will respond to climate change. The researcher suggests that populations from arid regions may be better positioned to adapt to future climate scenarios, while those from more stable environments may face greater challenges. This highlights the importance of monitoring and conserving genetic diversity in fungal populations as a natural resource for adaptation.

Host Specialization and Agricultural Implications:
The patterns of host association observed in this study provide insights into the evolution of specialization in F. oxysporum populations. While most isolates showed broad host ranges consistent with the opportunistic nature of F. oxysporum, regional differences in host preference suggest ongoing evolutionary processes that may lead to increased specialization. The predominance of date palm isolates in oasis environments and cereal isolates in highland regions may reflect both host availability and environmental filtering. The researcher hypothesizes that long-term cultivation of specific crops in particular environments may promote the evolution of locally adapted pathogen populations with enhanced fitness on those hosts.
This specialization has practical implications for crop rotation and diversification strategies. The effectiveness of crop rotation as a disease management tool may vary between regions depending on the degree of host specialization in local pathogen populations. In regions where isolates show broad host ranges, rotation may be less effective, while in areas with specialized populations, targeted rotation schemes may be more successful.

Virulence Evolution and Pathogenic Potential
The variation in virulence observed among isolates from different regions provides insights into the evolution of pathogenicity in F. oxysporum. The finding that desert isolates showed lower virulence, but greater stress tolerance suggests a trade-off between aggressive pathogenicity and environmental resilience (El Shamy et al., 2024).
This trade-off may reflect fundamental constraints in fungal physiology and resource allocation. Producing virulence factors and toxins requires significant metabolic investment that may compromise survival under stress conditions. Conversely, investing in stress tolerance mechanisms may reduce the resources available for aggressive pathogenesis.
The researcher suggests that this trade-off has important implications for predicting disease risk under different environmental conditions. In favorable environments, highly virulent strains may dominate and cause severe disease outbreaks. However, under stress conditions, less virulent but more resilient strains may become more prevalent, potentially leading to chronic but less severe disease problems.

Molecular Evolution and Adaptive Mechanisms
The ITS region analysis, while providing valuable insights into species diversity and phylogenetic relationships, represents only a small fraction of the F. oxysporum genome. The researcher acknowledges that more comprehensive genomic analyses would be needed to fully understand the molecular mechanisms underlying the observed patterns of diversity and adaptation (Adhikari et al., 2020).
However, the patterns observed in this study suggest several important evolutionary processes. The presence of admixed genotypes in all regions indicates ongoing recombination and genetic exchange, which may be facilitated by cryptic sexual reproduction or parasexual processes. This genetic exchange is crucial for the evolution of new virulence combinations and host adaptation strategies.
The identification of region-specific haplotypes suggests local adaptation processes that may involve genes beyond the ITS region. The researcher hypothesizes that these adaptations may involve stress response genes, metabolic pathways, and virulent factors that enable survival and reproduction in specific environmental conditions.

Comparative Analysis with Global Studies
When compared to studies from other regions, the genetic diversity levels observed in Libya are relatively high, particularly in arid environments (MohdHafifi et al., 2024; Carmona et al., 2020). This may reflect the country's position at the intersection of different biogeographical regions and its long history of agricultural activity.
The population structure patterns observed in Libya show similarities to those reported from other Mediterranean and Middle Eastern regions, with moderate genetic differentiation and evidence of gene flow (Abi Saad et al., 2022). However, the high diversity in desert oases appears to be a unique feature that may reflect the specialized conditions of these environments.
The researcher notes that comparative studies across different climatic zones and agricultural systems are essential for understanding the global patterns of F. oxysporum evolution and developing comprehensive management strategies.
Libya's diverse environments provide an excellent model system for such comparative studies.

Conclusions and Future Directions

Major Findings and Contributions

This comprehensive study has revealed significant molecular variability among F. oxysporum isolates from different agricultural environments in Libya, contributing important new knowledge to our understanding of fungal diversity in arid and semi-arid regions. The key findings include:

1. **High Genetic Diversity**: The identification of 34 unique haplotypes among 87 F. oxysporum isolates demonstrates substantial genetic diversity, with thehighest levels found in desert oasis environments contrary to conventional expectations.
2. **Regional Population Structure**: AMOVA analysis revealed moderate genetic differentiation between regions (ΦST = 0.342), indicating a balance between local adaptation and gene flow that has important implications for disease management strategies.
3. **Environmental Correlations**: Strong correlations between genetic diversity and environmental variables, particularly temperature variation and rainfall patterns, provide evidence for adaptive evolution in response to climatic conditions.
4. **Virulence Trade-offs**: The observation that desert isolates show lower virulence, but greater stress tolerance suggests evolutionary trade-offs that may influence disease dynamics under different environmental conditions.
5. **Host Association Patterns**: Regional differences in host preference indicate ongoing evolutionary processes that may lead to increased specialization and have implications for crop rotation strategies.

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6. References

Abi Saad, C., Masiello, M., Habib, W., Gerges, E., Sanzani, S. M., Logrieco, A. F., Moretti, A., & Somma, S. (2022). Diversity of Fusarium Species Isolated from Symptomatic Plants Belonging to a Wide Range of Agri-Food and Ornamental Crops in Lebanon. *Journal of Fungi*, 8(9), 897.

Adhikari, T. B., Gao, A., Ingram, T., & Louws, F. J. (2020). Pathogenomics Characterization of an Emerging Fungal Pathogen, Fusarium oxysporum f. sp. lycopersici in Greenhouse Tomato Production Systems. *Frontiers in Microbiology*, 11.

Ahmed, Kararah, M., Abdel-Momen, S., & Elharrany, O. M. (2022). PATHOLOGICAL AND MOLECULAR VARIATION AMONG SOME ISOLATES OF FUSAIUM OXYSPORUM F.

AL-Masoudi, R. K. H., & AL Judy, N. J. (2024). Sequencing Analysis of the ITS Region for Some Fusarium Species Isolated from Infected Tomato Plants. *Iraqi Journal of Science*, 6894–6907.

Arie, T. (2019). *Fusarium* diseases of cultivated plants, control, diagnosis, and molecular and genetic studies. *Journal of Pesticide Science*, 44(4), 275– 281.

Ates, D., Altinok, H. H., Ozkuru, E., Ferik, F., Erdogmus, S., Can, C., & Tanyolac, M. B. (2019). Population structure and linkage disequilibrium in a large collection of Fusarium oxysporum strains analysed through iPBS markers. *Journal of Phytopathology*, 167(10), 576–590.

Ateş, A., Fidan, H., & Gökhan, B. (2020). MOLECULAR CHARACTERIZATION OF Tomato yellow leaf curl virus AND Fusarium oxysporum formae speciales AND RACEs OF TOMATO AREAs IN NORTHERN CYPRUs.

Carmona, S. L., Burbano-David, D., Gómez, M. R., Lopez, W., Ceballos, N., Castaño-Zapata, J., Simbaqueba, J., & Soto-Suárez, M. (2020). Characterization of Pathogenic and Nonpathogenic Fusarium oxysporum Isolates Associated with Commercial Tomato Crops in the Andean Region of Colombia. *Pathogens*, 9(1), 70.

Castillo-Sanmiguel, P. A., Cortés-Sánchez, L. R., & Acero-Godoy, J. (2021). Molecular aspects of tomato (Solanum lycopersicum) vascular wilt by Fusarium oxysporum f. sp. lycopersici and antagonism by Trichoderma spp. Revista Mexicana de Fitopatología, Mexican Journal of Phytopathology, 40(1).

Dayab, K. M., & ElGariani, N. K. (2019). Isolation and Identification of Tomato Wilt Pathogen Under Green House Conditions in Tripoli Area. Journal of Misurata University for Agricultural Sciences, 01, 327–337.

Elagamey, E., Abdellatef, M., kamel, S., & Essa, T. (2020). Fusarium oxysporum Isolates Collected from the same Geographical Zone Exhibited Variations in Disease Severity and Diversity in Morphological and Molecular Characters. Egyptian Journal of Phytopathology, 48(1), 43–57.

El Shamy, S., Adss, I., & Mashaheet, A. M. (2024). AGGRESSIVENESS VARIATIONS AMONG ISOLATES OF FUSARIUM OXYSPOIUM F.SP. LYCOPERSICI BASED ON THE PATHOGENICITY TEST, HOST DEFENSE RESPONSE, AND ISOLATES GENETIC VARIABILITY. Journal of Agricultural and Environmental Sciences, 23(2), 221–249.

Elzouki, E. M., Eljamay, S. M., & Alshilwi, Y. A. (2024). Isolation and Identification of Escherichia Coli in Uropathogenesis. *Derna Academy Journal for Applied Sciences*, *2*(2), 103-110.

Gaber, M., El-Bebany, A., Fahmy, M., Aboshosha, S., & Wagih, E. (2022). Pathological and Molecular Variability of Fusarium oxysporum f.sp lycopersici Infecting Tomato in Egypt. Egyptian Academic Journal of Biological Sciences, H. Botany, 13(2), 275–287.

Jangir, P., Mehra, N., Sharma, K., Singh, N., Rani, M., & Kapoor, R. (2021). Secreted in Xylem Genes: Drivers of Host Adaptation in Fusarium oxysporum. Frontiers in Plant Science, 12.
Mohd-Hafifi, A. B., Mohamed Nor, N. M. I., Zakaria, L., & Mohd, M. H. (2024). Molecular phylogeny and genetic diversity of Fusarium oxysporum from various hosts in Malaysia. Scientific Reports, 14(1).

Muhsen, M., Kazem, K., & Dewan, M. M. (2020). MOLECULAR DIAGNOSIS OF SOME TYPES OF FUSARIUM ISOLATED FROM THE SOIL OF KARBALA GOVERNORATE, IRAQ.
Nahi Al-Adhami, N. S., & Mohsen Al-Araji, A. (2019). Molecular characterization by PCR-ITS technique of Fusarium oxysporum isolated from tomato in Baghdad city. Tikrit Journal of Pure Science, 24(3), 31.

Saleh, O., Gabr, M., Khalil, M., & Mohamed, E. E.-D. (2016). Molecular Variations among some Isolates of Fusarium oxysporum f.sp lycopersici and Response of Different Tomato Cultivars and Seedling Age to Infection. Egyptian Journal of Phytopathology, 44(1), 205–226.

Slama, H. B., Cherif-Silini, H., Chenari Bouket, A., Qader, M., Silini, A., Yahiaoui, B., Alenezi, F. N., Luptakova, L., Triki, M. A., Vallat, A., Oszako, T., Rateb, M. E., & Belbahri, L. (2019). Screening for Fusarium Antagonistic Bacteria From Contrasting Niches Designated the Endophyte Bacillus halotolerans as Plant Warden Against Fusarium. Frontiers in Microbiology, 9.

Sobowale, A. A., & Ogunoye, O. A. (2022). Molecular Diversity of Fusarium oxysporum and Geotrichum candidum Isolated from Two Tomato Varieties Commonly Consumed in Some Southwestern Town in Nigeria. American Journal of Plant Sciences, 13(05), 668–685.

Srinivas, C., Nirmala Devi, D., Narasimha Murthy, K., Mohan, C. D., Lakshmeesha, T. R., Singh, B., Kalagatur, N. K., Niranjana, S. R., Hashem, A., Alqarawi, A. A., Tabassum, B., Abd_Allah, E. F., Chandra Nayaka, S., & Srivastava, R. K. (2019). Fusarium oxysporum f. sp. lycopersici causal agent of vascular wilt disease of tomato: Biology to diversity– A review. Saudi Journal of Biological Sciences, 26(7), 1315–1324.

Sukorini, H., Septia, E. D., Zalizar, L., & Khewkhom, N. (2021). Molecular Characteristic of Fusarium oxysporum from Different Altitudes in East Java, Indonesia. Jordan Journal of Biological Sciences, 14(03), 383–388.

Vignesh, K., Rajamohan, K., Anandan, R., & Udhayakumar, R. (2020). MORPHOLOGICAL AND MOLECULAR VARIATION OF FUSARIUM OXYSPORUM F. SP LYCOPERSICI ISOLATES CAUSING FUSARIUM WILT IN TOMATO. PLANT ARCHIVES, 21(supplement 1).

Zeeshan, M., Mukhtar, T., Inam-ul-Haq, M., & Asad, M. J. (2023). Incidence, Characterization and Pathogenic Variability of Fusarium oxysporum in the Punjab Province of Pakistan. International Journal of Phytopathology, 12(1), 19–29.