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Isolation and identification of *Fusarium oxysporum*, the causal fungus of wilt disease of tomato (*Solanum lycopersicum*)

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Abstract :*F. oxysporum* is a fungal plant disease that causes tomato plant losses and severe wilting. This pathogen causes widespread diseases in host plants such as crown rot and wilting. The goal of this study was to isolate *F. oxysporum*, a fungus that causes wilt disease. Plant and soil samples were obtained from several Mansoura University sites. Isolates of *F. oxysporum* were obtained from soil, tomato roots, tomato seeds, and potato tubers. The pathogen isolates were identified using microscopic and morphological analysis. *F. oxysporum* morphological identification on growth media was accomplished by describing the oval to kidney/ellipsoid shaped oval tapering, white cottony mycelium with dark-purple undersurface, and 3 septate spores. According to their morphological and microscopic characterization, the isolates were confirmed as *F. oxysporum*. The purpose of this study was to isolate and determine the morphological characteristics of fungal isolates from *F. oxysporum* f. sp. lycopersici, the causative agent of tomato wilt disease. Pathogenicity testing is used to select the most aggressive isolate.

keywords: Tomato; Morphological appearance; Microscopic identification; *Fusarium oxysporum*; Pathogenicity test

Introduction

Worldwide, the tomato (*Lycopersicon esculentum*) is one of the widely cultivated vegetables that is universally touted as a popular and commercially important garden crop [1; 2]. Owing to rich amount of vitamins C and A, it is an essential daily nutritional component and is consumed in various types of processed fruit products and also as fresh unprocessed [3]. *Fusarium* species are known to make a wide range of disorders on an unusual variety of host plants. *Fusarium oxysporum* is filamentous fungus of the class Ascomycetes and the family Hypocreaceae [4]. It is a soil-inhabiting fungus that is widespread throughout the world. Some species of *Fusarium* are confined to the tropics, some prevail in temperate regions, while others are present in arctic, alpine and desert regions, where harsh climatic conditions prevail [5].

Indeed, the near spread of *F. oxysporum* to soils worldwide led to its inclusion in what has been termed the cosmopolitan mycorrhizal fungus. *Fusarium* species are usually identified based on their micro and macroscopic features [6]. However, phytopathogenic strains cause devastating vascular diseases and often limit production of economically important crops [7; 8]. Characteristic of soil-borne pathogens, *F. oxysporum* can live extensively in the soil as dormant chlamydospores. The presence of the host root leads to germination of chlamydial spores. Threads of infection adhere to the surface of the root and then penetrate it. The fungus invades the intercellular root cortical cells and enters the vascular system by excavating the xylem. Thereafter, the pathogen shows a specific route of infection as it be prone to exclusively colonize within xylem vessels, resulting in more rapid colonization of the host. Inside vessels, the pathogen begins to generate microconidia, which, based on detachment, are carried upward by the sap stream. Moreover into the upper vessels, microconidia germination causes fungal penetration [9].

The wilt characteristic symptoms are caused by vascular occlusion caused by aggregation of

hyphae and host-pathogen interactions combination of like toxins release, gingiva, telose formation and gels. Disease typical markers, such as defoliation and wilting appear, vein removal, leaf epilation and eventually precede the death of the host plant. At this stage, fungus vascular wilt, which remains limited to the xylem vessels, spreads among the parenchymal tissue and starts to abundantly multiply on the plant surface as leaves, steams, etc. or other means [10]. This study aimed to isolate the fungal phytopathogenic *F. oxysporum* from various sources in different localities in Mansoura district. Purification and identification of *F. oxysporum* were performed by morphological and microscopic identification. To detect the most aggressive isolate, different isolates were subjected to pathogenicity test.

2. Materials and methods

2.1. Pathogen isolation

Isolation of fungal phytopathogenic *F. oxysporum* from different resources potato tuber, soil, infected tomato seeds, wilt tomato roots and infected tomato plant with wilt and damping off symptoms collected from Mansoura University, Mansoura, Egypt.

Isolation from soil: Dilution plate method was performed by adding the collected soil (one g) to sterile distilled water. Using sterile distilled water, the soil sample serial dilution of up to 6-10 dilutions was done. For pathogen isolation enhancing, 1mL of each dilution was remove using sterile pipette and subjected to PDA medium surface. For fungal growth at room temperature, plates were incubated (72 hours).

Isolation from tomato seeds: the seeds were treated for five minutes with sodium hypochlorite (1%). After that using sterile distilled water, they were washed many times to remove any residuals, then dried on sterile filter paper, inserted into the surface PDA medium and incubated at room temperature, for 72 hours.

Isolation from tomato roots: Isolation was performed and showing characteristic wilt indices. Root samples were cut into small pieces and these pieces were then sterilized by immersing into sodium hypochlorite (1%) for five minutes. After that, these pieces were washed many times in sterile distilled water to remove any residues and then dried on sterilized filter paper. Then, they were subjected to PDA medium surface that amended with 0.01% streptomycin sulphate. PDA poured plates under Laminar flow chamber. Then for fungal growth, plates were incubated at room temperature for 4-7 days [11].

2.2. Pathogenicity test of wilt pathogen

2.2.1. Isolation of inocula

F. oxysporum were isolated from infected tomato roots and seeds, potato tuber and soil. tested for their Pathogenicity test was undertaken under greenhouse conditions, where pathogenic potential of 4 isolates of *F. oxysporum* were tested on *Solanum lycopersicum* susceptible cultivar. Using soil infested with *F. oxysporum* isolates into plastic pots, three 35 days-old tomato plantlets were transplanted.

2.2.2. Pot and soil sterilization

All pots were sterilized by immersing them, for 15 minutes, in formalin (5%), left overnight in plastic sheets cover, air dried and filled with autoclaved soil.

2.2.3. Inocula preparation and soil infestation

This was carried out in sterilized sand barley medium (barley grains, 75 g); clean sand, 25 g; and 10 ml distilled water to cover the mixture), packages inoculated with pathogen isolates, after incubation for two years at 28°C. Mushrooms were well mixed with sterile light sandy soil (1: 2) at the rate of 1g inoculum/100 g soil. Before planting, pots were watered for one week regularly. The control pots were filled with the same soil and without fungus medium and treated in the same way [12].

2.2.4. Transferring of transplants and cultivation:

Sterilized infested soil, were planted with 35 days-old transplants at the 4th of March 2022 into. In sterilized non-inoculated soil, other transplants were planted as control. After 14 days of cultivation of inoculated tomato plantlets the disease incidence and disease severity of wilt symptoms were assessed.

2.2.5. Disease assessment

The disease severity was estimated as the degree of plant damage according to the scale of wilt disease according to Cal et al. [13] [all leaves green (0); lower leaves yellow (1); lower leaves dead (2); upper leaves wilted and lower leaves dead (3) and dead plant (4)].

Disease incidence was identified as:

$$\frac{\text{Number of wilting plants}}{\text{Total number of plants}} \times 100 \text{ [14]}$$

The number of browning spots, yellowing number and percentage of branches and leaves of the inoculated tomato plants with *F. oxysporum* was also measured**.**

2.3. Purification and identification

Individually, growing fungi were transferred to PDA medium. Pure cultures were detected using single spore/filamentous tip method proposed by Dhingra [15]. Pure cultures of isolated fungi were identified according to the colonial and microscopic features (mycelial development and spore formation) [16; 17].

2.4. Statistical analysis

For each specific treatment, 3 pots (replicates) were used. In completely randomized block design, inoculation treatments were arranged in the glasshouse.

3. Results and discussion

Several disorders are affect tomato involving *Fusarium* wilt resulted from the host-specific pathogen *F. oxysporum* [2]. The seed-borne and soil *Fusarium* nature makes it not easy to manage and, in host absence, quiescent chlamydospore can survive, in soil, for long times [18]. Infected plants are weak and have necrotic, yellow leaves. Disease transmission from infected seedlings or pathogenic strains, pathogen persistence in soil, farming and irrigation tools, contaminated soil and airborne spread of spores make disease management difficult [2].

3.1.Pathogenicity test and isolates selection

From infected tomato (roots, soil, seeds) and infected potato tuber *F. oxysporum* isolates were collected and tested for their pathogenicity to determine the most aggressive one (**Figure 1**). The disease severity of *Fusarium* wilt on tomato plants inoculated with *F. oxysporum* in plant. At each observation, disease severity was obtained by leaves showed wilting number (percentage from each plant total leaves number).

Pathogenicity test of isolates collected from different infected sources revealing wilt symptoms and, on artificial inoculated root, were able to cause wilt symptoms and damping-off. The highest wilt and damping-off were seen by isolate of infected tomato roots followed by isolate from infected potato tuber. The least harmful isolate was from infected tomato seeds. The pathogenic potential of the four isolates demonstrated that all isolates had the ability to infect tomato plants, where disease incidence was ranged between 50-73% and the severity was ranged between 1 and 2. *F. oxysporum* isolated from tomato roots showed the highest disease incidence (73%) and severity (2) and thus this isolate are considered as the most pathogenic one so far it was selected for the present study (**Table 1**). Alike, Sagitov et al. [19] were observed, after 2 months from inoculation, wilt symptoms specifically stem brown vascular discoloration of seedlings of the Tomato Carolina Gold cultivar. They revealed that, *F. oxysporum* f. sp. *lycopersici* isolates G and A gives the highest disease incidence (52.78%) and dead and diseased tomato percentage (77.78%). In addition, Mohammed et al. [17] found that the strains IB19503 identified as *F. redolens* and IB19521, IB19506 and IB19505 identified as *F. solani* have generated slight tap roots browning and showed between 22.22- 33.33% of disease incidence.

Figure 1. Pathogenicity test of tomato plants

infected with four *F. oxysporum* isolates to determine the most aggressive one. A) at the beginning of the test (35 days- aged tomato plants), B) at the end of the test (after 14 days of cultivation).

Table 1. Pathogenicity test, disease incidence and severity percentages of *F. oxysporum* isolates from different sources.

Isolate	Source of isolates	Disease incidenc %	Disease severity
Control	without isolate	0.00	0.00
A	Soil	60%	2
B	Potato tuber	66.6%	2
C	Tomato roots	73%	2
D	Tomato seeds	50%	1

One of the chief tomato disorders is tomato *F. oxysporum* wilt [20]. Vascular system brown discoloration is a disease characteristic and can be used to identify fungal isolates such as *F. oxysporum* [21]. *F. oxysporum* enters the root epidermis, and later inhabits the xylem vessels of the plant after spreading through the vascular tissue, causing severe water stress and vascular occlusion, as a result of which wilt-like symptoms appear [22]. Morphologically, the disease was identified by wilted plants with scant/absent yield and bearing yellow-colored leaves. In infected soil, the dormant *F. oxysporum* chlamydospore, in host absence, can survive indefinitely [23]. Development of vascular infection of *F. oxysporum* is a complex phenomenon, and occurs in sequential steps include: pathogen-host root signals recognition, root hairs surface attachment and hyphae propagation, and differentiation within xylem vessels after invasion of vascular tissue and root cortex and finally exudation of virulence factors and toxins. Vessels colonization results in host plant wilting and the disease development [1]. Other studies in Egypt reported a similar findings of disease incidence and severity percentages [24].

3.2. Colonies cultural, morphological, and microscopic identification

In this investigation, after isolates selection according to the highest disease incidence and severity, selected isolates from tomato roots were cultured. The isolates cultural characteristics varied and in general they presented mycelia covering with vigorous growth after 4 days. Colonies color in PDA medium varied from white, light pink and pink (**Figure 2**). Moreover, morphological analysis of microscopic structures showed variation among the isolates to produce micro- and macro-conidia (spores) (**Figure 3**). Additionally, their microscopic structures morphological analysis showed variation among different isolates for micro-and macro-conidia production **[**25**]**.

In the different culture media, findings reported a marked variability in conidia production compared to the other media, PDA medium presents a higher amount of complex carbohydrates and a greater nutrient richness. These features can induce the proliferation of many microscopic fungi [25], as has been observed in other studies reporting increased *F. solani* formation in PDA medium [26].

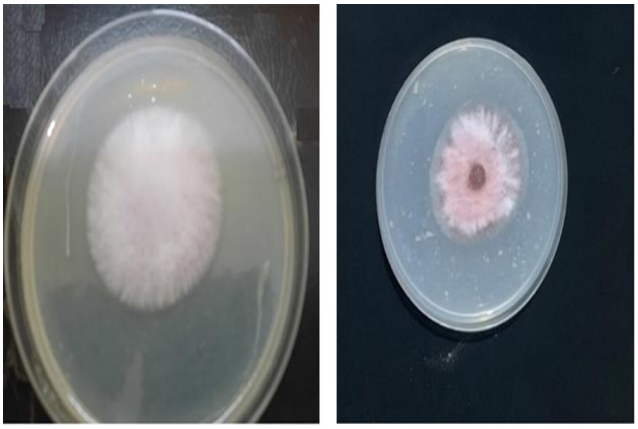

Figure 2. *F. oxysporum* isolates cultural characteristics. Colony was aspect on potato dextrose agar.

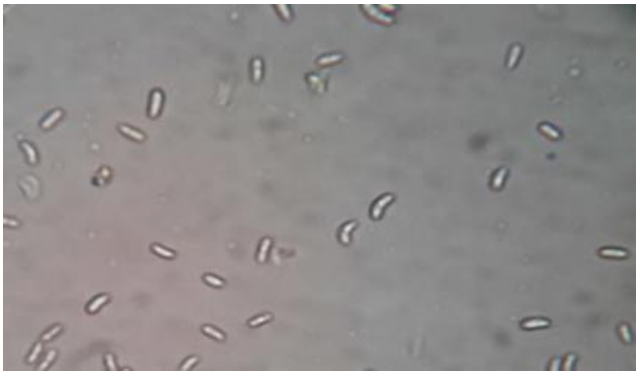

Figure 3. *F. oxysporum* microscopic characteristics with randomly spread spores under light microscope at 40X.

Conclusion

Fusarium wilt remains a major problem worldwide causing severe losses in economic yields. *Fusarium* wilt causes tomato crop losses. It is very important to study the

morphology and microscopic structure of *F. oxysporum* to ascertain the plant disorders causes. Based on observations of microscopic and microscopic structures, this study could be the basis for a future study to identify the most aggressive isolate.

References

1 Srinivas C, Nirmala Devi D, Narasimha Murthy K, Mohan CD, Lakshmeesha TR, Singh B, Kalagatur NK, Niranjana SR, Hashem A, Alqarawi AA, Tabassum B, Abd_Allah EF, Chandra Nayaka S, Srivastava RK. (2019). Fusarium oxysporum f. sp. lycopersici causal agent of vascular wilt disease of tomato: Biology to diversity– A review. *Saudi J Biol Sci*; **26**:1315-1324.

2 Jamil A, Musheer N, Kumar M. (2021). Evaluation of biocontrol agents for management of wilt disease of tomato incited by F. oxysporum. Archives of Phytopathology and Plant Protection; **54:**1722-1737.

3 Brookie KL, Best GI, Conner TS. (2018). Intake of Raw Fruits and Vegetables Is Associated With Better Mental Health Than Intake of Processed Fruits and Vegetables. Frontiers in psychology; **9**:487-487.

4 Pitt, J. I., Hocking A.D., Bhudhasamai K., Miscamble B.F., Wheeler, K.A., and Tanboon-Ek, P. (1994). The normal mycoflora of commodities from Thailand. 2. Beans, rice, small grains and other commodities. *International Journal of Food Microbiology* **23**:35-43.

5 Francis, R.G. and Burgess, L.W. (1975). Surveys of fusaria and other fungi associated with stalk rot of maize in eastern Australia. Australian *Journal of Agricultural* Research **26**:801-807.

6 Singha, I. M., Kakoty, Y., Unni, B. G., Das, J., & Kalita, M. C. (2016). Identification and characterization of Fusarium sp. using ITS and RAPD causing fusarium wilt of tomato isolated from Assam, Northeast India. *Journal of Genetic Engineering and Biotechnology*, **14(1),** 99-105

7. Servin A, Elmer W, Mukherjee A, la Torre-Roche D, Hamdi H, White JC, Bindraban P, Dimkpa C. (2015). A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. *Journal of Nanoparticle* Research; **17**:1-21.

8 Shahzad, R., Abdul Latif Khan., Saqib Bilal., SajjadAsaf., In-Jung Lee., (2017). Plant growth- promoting endophytic bacteria versus pathogenic infections: an example of Bacillus amyloliquefaciens RWL-1 and Fusarium oxysporum f. sp. *lycopersici in tomato. Peer J*. **5**, e3107.

9 McGovern, R.J., (2015). Management of tomato diseases caused by Fusarium oxysporum. Crop Prot. **73**, 78–92.

10 Joshi RJJMPS. (2018). A review of Fusarium oxysporum on its plant interaction and industrial use. **6:**112-115.

11 Darmadi, A. A. K., Suriani, N. L., Sudirga, S. K., & Khalimi, K. (2019). First Study on Fusarium equiseti: Causes Fusarium Wilt in Tomato Crop in Bali, Indonesia. *SABRAO Journal of Breeding & Genetics*, ***51*(4),** 442-450.

12. Shehata, M.M, (2001). Studies on tomato root-rot disease and their control. M. Sc., thesis Fac. of Agriculture El-Azhar University, Cairo, Egypt.

13 Cal, A., Pascual, S., Larena,I. & Melgarejo, P. (1995). Biological control of Fusarium oxysporum f. sp. Lycopersici. Plant pathology,**44**,909-917

14 Song W., Zhou L., Yang C., Cao X., Zhang L., Liu X. (2004). Tomato Fusarium Wilt its chemical control strategies in a hydroponic system Crop Protection, **23** 243-247.

15 Dhingra OD, Sinclair JB, (1995). Basic plant pathology methods. second edition, Lewis Publishers, CRC Press, USA, 400-450pp.

16. Domsch KH, Gams W, Anderson TH, (1980). Compendium of soil fungi. Academic Press. A Subbsidiary of Harcout Brace Jovanovich, Bublishers, London, 1: 859 pp.

17 Mahmoud A. F. (2016). Evaluation of certain antagonistic fungal species for biological control of faba bean wilt disease incited by Fusarium oxysporum. *Journal of Phytopathology and Pest Management* **3(2):** 1-14.

18 Khan MI, Fatma M, Per TS, Anjum NA,

Khan NA. (2015). Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Front Plant Sci; 6:462.

19 Sagitov AO, El-Habbaa GM, El-Fiki IA (2010). Occurrence of tomato Fusarium wilt disease in Kazakhstan. Research Results (Kaznau Univ.), Almaty, Kazakhstan, **2:(046),** 212-215.

20 Borisade, O. A., Uwaidem, Y. I., & Salami, A. E. (2017). Preliminary Report on Fusarium oxysporum f. sp. lycopersici (Sensu lato) From Some Tomato Producing Agroecological Areas in Southwestern Nigeria and Susceptibility of F1-Resistant Tomato Hybrid (F1-Lindo) to Infection. Annual Research & Review in Biology, **18(2),** 1-9. https://doi.org/10.9734/ARRB/2017/34626

21 Reis, A.; Costa, H.; Boiteux, L. S. and Lopes, C. A. (2005): First Report of Fusarium oxysporum f. sp. lycopersici Race 3 on Tomato in Brazil. Fitopatol. Bras, **30(4):** 426-428.

22 Singh VK, Singh HB, Upadhyay RS. (2017). Role of fusaric acid in the development of 'Fusarium wilt' symptoms in tomato: Physiological, biochemical and proteomic perspectives. Plant Physiol Biochem; **118:**320-332.

23 Khan N, Maymon M, Hirsch AM. (2017). Combating Fusarium infection using Bacillus-based antimicrobials. Microorganisms.;**5(4):**75.

24 Nofal AM, El-Rahman MA, Abdelghany TM, Abd El-Mongy M. (2021). Mycoparasitic nature of Egyptian Trichoderma isolates and their impact on suppression Fusarium wilt of tomato. Egyptian *Journal of Biological Pest Control*; 31:103.

25 Teixeira L, Coelho L, Tebaldi ND. (2017). Characterization of Fusarium oxysporum isolates and resistance of passion fruit genotypes to fusariosis. Revista brasileira de fruticultura; 39.

26 Silva JL, Teixeira RNV. (2012). Sporulation and mycelial growth of Fusarium solani in different culture media and steady bright. RevistaAgro@mbiente On-line; **6**:47-52.