Plant Archives Volume 20 No. 2, 2020 pp. 7995-7999 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

MOLECULAR DIAGNOSIS AND PATHOGENICITY OF A NOVEL ISOLATE OF *FUSARIUM ANTHOPHILUM* FROM EGG PLANT SEEDLINGS

Leith Hassan Kriedy[1*], Jamal Hussein Kahdim[2] and Nazar Rashid Merzah[3]

[1*]Plant Protection Department, Karbala Agriculture Directorate, Karbala, Iraq.
[2]Department of Plant Protection, Faculty of Agriculture, University of Kufa, Najaf, Iraq.
[3]Ministry of Agriculture, Plant Protection Directorate, Baghdad, Iraq.

Abstract

Samples were collected from dead or infected with wilted and stunted eggplant seedlings from the gardening station in Karbala governorate. The causes were isolated and diagnosed in the laboratory of plant diseases at the Faculty of Agriculture, University of Kufa. The phenotypic diagnosis showed that the pathogenic isolates were *Fusarium* species. Polymerase chain reaction (PCR) technique was used to diagnose the isolates and a nucleotide sequence of the double DNA product was determined. The results, analyzed by PCR products using BLAST program, showed that the pathogen isolates that belong to *Fusarium anthophilum* differed from the isolates belonging to the same type recorded in NCBI. Therefore, it was registered in the NCBI as Leith and entry number MT548905. The results of the pathogenicity test of the diagnosed isolate showed that it reduced the germination of eggplant seeds by 30% compared to the germination rate of 93% in the control treatment. The isolation of the same fungus under the nursery conditions led to a germination rate of about 40% and the death rate of seedlings to 50%, compared to the control treatment. Also, the treatment of seeds with the fungal filtrate led to a significant reduction in the germination percentage of eggplant seeds to 40% compared to 93.33% in the control treatment. The fungal filtrate also resulted in a significant reduction in seedlings length and the fresh and dry weight of seedlings compared to untreated seedlings of the control.

Key words : *Fusarium*, molecular diagnosis, eggplant, BLAST.

Introduction

Solanumm melongena L. is an important vegetable crop spread all over the world. Eggplant is affected by many fungal diseases such as root rot diseases, seedling death and wilting, which are among the most common widespread soil diseases (Agrios, 2005 and Dar and others, 2018). Root rot and seedling death are among the most dangerous diseases affecting the nightshade crops and economic plants worldwide (Hadwan & Khara, 1992). *Fusarium* species are among the most common causes of root rot disease and eggplant seedling death under greenhouse and field conditions (Elbehadly, 1996). The accurate diagnosis of pathogenic fungi is very important in controlling various plant diseases. Several studies have indicated that relying on phenotypic characteristics in the diagnosis alone may give inaccurate results in addition to requiring considerable experience in the field of diagnosis, especially for *Fusarium* species (O'Donnell *et al.,* 2008).

Relying on phenotypes only may be incorrect in 50% of diagnosed cases of *Fusarium* Healy (*et al.,* 2005). Therefore, to avoid cases of inaccurate diagnosis, it is preferable to adopt molecular diagnostic methods, especially with economically important fungi species (Schroeder *et al.,* 2013). Therefore, this study aimed at isolation and molecular diagnosis of *Fusarium*, and to confirm the pathogenic ability of the diagnosed isolate to cause seed rot disease and eggplant seedling death.

***Author for correspondence :** E-mail: leithhassan447@gmail.com

Materials and Methods

Fusarium isolate: Infected eggplant seedlings that showed symptoms of wilting and stunting were collected from the horticulture station of the city of Karbala - Iraq. The samples were brought to the Plant Pathology Laboratory in the Faculty of Agriculture - University of Kufa in order to isolate fungi accompanying the infection. Seedlings roots of each sample were thoroughly washed with running water for 20 min, cut into 2 cm small pieces and sterilized with NaOCL solution for 3 minutes. They were then washed with sterile distilled water several times to remove NaOCl residues, dried on sterile filter paper and planted in 9 cm Petri dishes containing PDA treated with antibiotic Chloramphenicol at 200 mg/L. The dishes were incubated at 25 ± 2 ± for four days, after which the fungal isolates were purified on the same nutritional medium (PDA) by the haifa tip method. The isolates were diagnosed morphologically depending on the colony's characteristics and conidia types according to the taxonomic keys (Leslie and Summerell, 2006).

Molecular diagnoses of *Fusarium* isolate using PCR technique

A molecular diagnosis was performed to confirm the phenotypic and microscopic diagnosis of *Fusarium* isolate from eggplant seedling under study in the Plant Virus Laboratory of the Faculty of Agriculture - University of Karbala. DNA was extracted from the *Fusarium* isolate in this study according to the method provided with the extract kit obtained from Favorgen Company, Taiwan - China. The polymerase chain reaction was performed using the (Maxime PCR PreMix, Cat. No. K-2012) kit that obtained from the South Korean company Bioneer. A serial polymerase reaction was carried out with a total volume of 20µl consisting 1µl of forward initiator (TCCGTAGGTGAACCTGCGG: ITS1) and revers TCCTCCGCTTATTGATATGC: TS4, as well as 1µl of extracted DNA (DNA). All of the materials were placed in the tube supplied by the manufacturer and the volume was completed to 20µl by Nuclease-free water.

The DNA of the *Fusarium* isolate was amplified in PCR reaction in conditions and steps involved Initial denaturation of DNA for 5 min at 94 C° followed by 35 cycles of Final denaturation for 30s at 94 C°, Primer annealing for 30s at 55 C°, then Initial elongation of the PCR-amplified product for 1 minute at 72 C° and completion of the reaction with a Final elongation step at 72 C° (Zhang *et al.,* 2012). Gel electrophoresis was performed and the result was photographed using under the UV trans-illumination.

For the diagnosis, PCR amplicons for *Fusarium* isolate with ITS1 and ITS4 were sent to the Korean company Macrogen to determine the Nucleotide sequence of amplified forward-revers DNA products. The Basic Local Alignment Search Tool (BLAST) was used to analyze sequences Nitrogenous bases and the results were compared with the globally diagnosed fungal data available in American National Center for Biotechnology Information, NCBI.

Pathogenicity of *Fusarium anthophilum* on eggplant seeds in PDA medium

Local eggplant (black head) seeds were washed for two minutes with running water and 10% sodium hypochlorate solution to sterilize the seeds surface, after which they were washed with sterile distilled water and dried on sterile filter papers. The seeds were planted in Petri dishes containing the prepared PDA at a rate of 10 seeds per plate. The center of each plate, for three replicates, was inoculated with a 0.5 cm diameter disc from the tip of the *Fusarium* colony isolated from eggplant seedlings. Three plates were planted with eggplant seeds without fungal inoculation as a control treatment. The plates were incubated for 7 at 25 ± 2C°, after which the seed germination rate was calculated.

Pathogenicity of *F. anthophilum* on eggplant seeds in a nursery conditions

Sterilized soil was used to fill the holes of a cork planting tray. Three tablets of 0.5 cm diameter of *Fusarium* culture media were added to each hole and left for 3 days with irrigation. Three seeds and three replicates were sown inside each hole and holes planted with seeds without *Fusarium* inoculum served as control. The dishes were placed under the canopy and irrigation was done when needed for a period of 21 days after which seed germination rate and seedlings fresh and dry weight were measured and compared among treatments.

Effect of *F. anthophilum* filtrate on eggplant seeds and growth indicators

Broth PDA medium was prepared in 250 mL flasks, by placing in each flask 100 mL of liquid medium and inoculated with a 0.5 cm diameter disc of 7days old *F. anthophilum* culture and the flasks were shaken to distribute the spore suspension evenly. Fungal filtrate was taken with a sterile syringe and passed through a fine filter Millipore of 0.22µm. Eggplant seeds were used, with 10 seeds for each petri dish containing filter paper moisturized with the fungal filtrate with 3 replicates, in addition to the control treatment containing filter paper treated with distilled water only. The plates were incubated at 25 ± 2C° for 10 days and growth indicators

were measured.

Results and Discussion

Isolation and diagnosis of fungi of infected eggplant seedlings.

The isolation was initially identified as *Fusarium* sp. through phenotypic dependence. The sporodochia appeared in orange to red color with the presence of small conidiospores Microconidia which were identified to be divided by 3 septa usually or 4 septa sometimes. The large conidiospores Macroconidia are mostly not divided or divided by one septum were also found while the chlamydospores were not present (Leslie and Summerell, 2006).

Molecular diagnosis of *Fusarium* isolate

The results of extracting DNA from *Fusarium* isolate from eggplant seedlings that, using PCR showed the possibility of multiplying the DNA product (PCR-amplified product) at the expected size about 500-800 bp when the forward and revers primers ITS1 and ITS4 were used (Fig. 1). Results of amplification PCR were subjected to Nucleotide Sequence Analysis using the BLAS program (Fig. 2) and the findings were compared to the available data for the same fungus in the NCBI. The isolate was found to be *Fusarium anthophilum* which was 98.28% similar to the closest *Fusarium* isolate recorded in NCBI (Fig. 3). Thus, the *Fusarium* isolate under study was recorded in the NCBI as new isolate with an entry number MT548905 with name Leith.

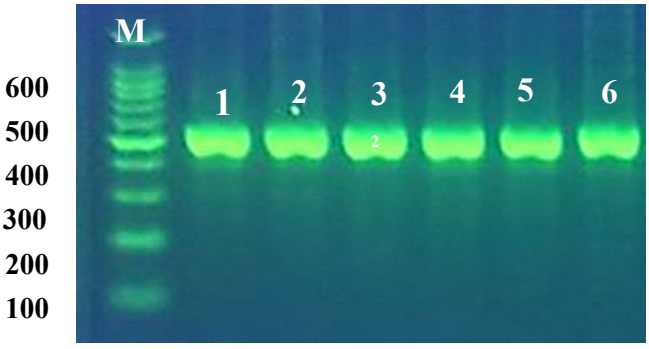

Fig. 1: DNA amplification products by PCR technique for *Fusarium solani* (1, 2 and 3), *F. fujikuroi* (4), *F. oxysporium* (5) and *F. anthophilum* (6) isolated in this study using the primer pair ITS1 and ITS4. M= 1Kbp DNA ladder marker.

Many previous studies have shown that diagnosing fungi based on (Morphological characters only) is insufficient and sometimes gives imprecise results. Emphasis has been placed on finding advanced classification systems or universal codes such as DNA barcoding, which are considered one of the quick and easy classification methods by using short genetic marker in the genome of organisms (Chu *et al.,* 2006). Iwen *et al*., (2002) mentioned that the current taxonomic studies used ITS Intragenic transcriptional space for the sequential nitrogen bases on the ribosomal DNA strand (rDNA) within the organism's gene, where the difference in ITS regions of fungi was adopted in the diagnosis and classification of many species and strains. Fungal. And the possibility of adopting the ITS region in the diagnosis of many types of Penecillium (Seifert *et al.,* 2007).

The study demonstrated the possibility of relying on the (PCR) technique in diagnosing the fungi under study, as it is characterized by high accuracy in diagnosing many organisms and reduces errors that accompany the phenotypic diagnosis (Huang *et al.,* 2006). In one study to diagnose *Fusarium* species based on phenotypic characteristics, the results indicated that about 50% of cases were inaccurate (Healy *et al.,* 2005). It was found that the phenotypic diagnosis was incorrect in some *Fusarium* species such as *Fusarium* verticillioides and Fuarium subglutinans after being re-diagnosed using PCR technique (Giantsis *et al.,* 2017). The differences in DNA sequences in the ITS region (Internal transcribed spacer showed significant efficiency in the ability to diagnose many fungi of the genera *Fusarium* and Cladosporium (Arif *et al.,* 2012; Alhussaini *et al.,* 2016; Al-Fadhal *et al.,* 2018).

Pathogenicity of *Fusarium anthophilum* on eggplant seeds in PDA medium

The results showed that *F.anthophilum* significantly reduced the percentage of eggplant seed germination after 7 days of inoculation and incubation (Table 1). The germination rate in the fungus treatment was 30% with seedling death rate of 91.66% compared to the germination rate of 93.33% and no seedling death recorded in the control treatment.

Table 1: Pathogenicity of *Fusarium anthophilum* on eggplant seeds in PDA medium.

Treatments	Seed germination %	Seedling death %
F. anthophilum	30	91.66
Control	93.33	0
L.S.D. $_{(P\leq 0.05)}$	18.51	23.14

Pathogenicity of *Fusarium anthophilum* in eggplants grown in planting trays

The results table 2 indicated that *F.anthophilum* had a significant decrease in seed germination rate and a significant increase in seedling death percentage compared with the control treatment. *Fusarium*

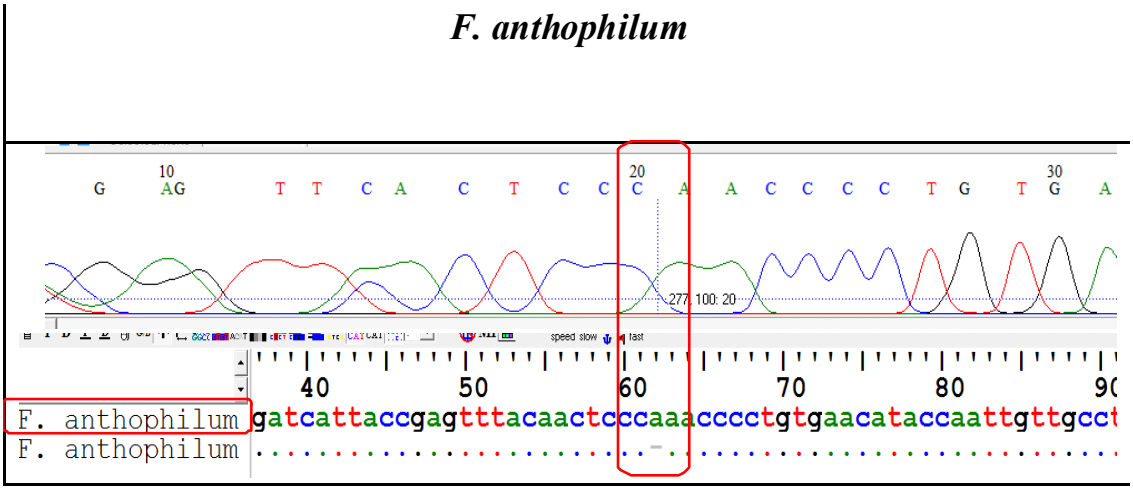

Fig. 2: Sequence variation of some nitrogenous bases of the DNA amplified by PCR from *F. anthophilum* isolated in this study and the closest isolate of same fungus recorded in NCBI.

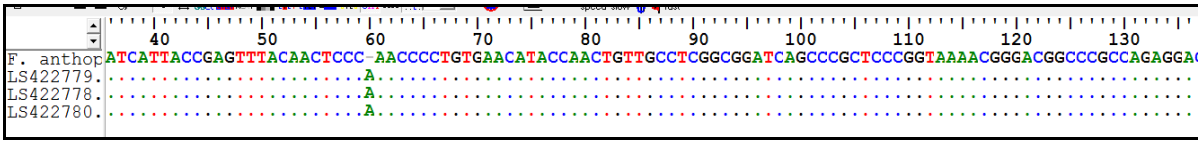

Fig. 3: Nitrogenous base sequences of the PCR-amplified products from *F. anthophilum* isolated in this study which differed in some site locations from the other isolates of the same fungus recorded in NCBI.

anthophilum presence in the soil of eggplant plants reduced plant's shoot and root length and fresh and dry weight of the 21 days old seedlings, with a significant difference from the control treatment in the absence of the pathogenic fungus. These findings are in agreement with previous studies reporting that soil pathogenic fungi associated with eggplant crop were the main cause of death and seed rot when *Fusarium* spp. That caused the disease were isolated (Mwaniki and others 2016).

Effect of *Fusarium anthophilum* on eggplant seeds and seedlings

Evaluating the effect of *F.anthophilum* filtrate on eggplant plants showed that the fungal filtrate of led to a significant reduction in seed germination and the lengths of epicotyl and rootlet of young eggplant seedlings cultured in Petri dishes after 10 days of incubation compared to the control treatment. Many researchers have indicated that filtrates of some plant pathogens have the ability to reduce the germination rate of many plant seeds. This is due to the negative effect of the pathogene filtrate, which is due to the ability of the pathogenic fungi to secrete enzymes and some other compounds that break down plant pectin and cellulose (Weinhold and Sinsclar, 1996).

Table 2: Pathogenicity of *Fusarium anthophilum* on eggplants grown in planting trays.

Treatments	Seed germination %	Seedlings death %	Shoot length	Root length	Fresh weight	Dry weight
F. *anthophilum*	40	50	2.067	1.167	0.0133	0.0004
Control	100	0	4.800	1.833	0.0667	0.0036
L.S.D. $_{(P\leq 0.05)}$	2.267	1.603	0.434	0.381	0.0130	0.00124

Table 3: Effect of *F. anthophilum* filtrate on germination and growth of eggplant seedlings in petri dishes after ten days under laboratory conditions.

Treatments	Seed germination %	Epicotyl length Cm	Rootlet length Cm	Seedling's fresh weight g	Seedling's dry weight g
F. *anthophilum*	40	1.43	1.306	0.015	0.000021
Control	93.33	3	2.6	0.038	0.000463
L.S.D. $_{(P\leq 0.05)}$	20.69	2.276	7.79	0.0641	0.000807

References

Agrios, G.N. (2005). Plant Pathology. 5th Ed. Elsevier Inc. USA, 998.

Al-Fadhal, F.A., A.N. AL-Abedy and M.M. Al-Janabi (2018). Molecular identification of novel isolates of *Rhizoctonia solani* Kuhn and *Fusarium* spp.(Matsushima) isolated from petunia plants (*Petunia hybridal*.). *Plant Archives*, **18(1):** 703-711.

Alhussaini, M.S., M.A. Moslem, M.I. Alghonaim, A.A. Al-Ghanayem, A.A. AL-Yahya, H.M. Hefny and A.M. Saadabi (2016). Characterization of *Cladosporium* species by internal transcribed spacer-PCR and microsatellites-PCR. *Pakistan Journal of Biological Sciences*, **7:** 143-157.

Arif, M., S. Chawla, M.W. Zaidi, J.K. Rayar, M. Variar and U.S. Singh (2012). Development of specific primers for genus *Fusarium* and *F. solani* using rDNA sub-unit and transcription elongation factor (TEF-1α) gene. *African Journal of Biotechnology*, **11(2):** 444-447.

Dar, W.A., M.G. Hassan, P.A. Sheikh, B. Summuna and S.A. Ganaie (2018). Integrated Disease Management Capsule for Wilt/Root Rot Complex of Chili. *Int. J. Curr. Microbiol. App. Sci.*, **7(1):** 1253-1261.

Elbehadly, A.H. (1996). Pathogenic fungi in peat moss and soil and their impaction on covered farm vegetable crops. Bulleten of Iraq natural History Museum, **8(4):** 47-53.

Giantsis, I.A., A. Chaskopoulou and M. Claude (2017). Direct multiplex PCR (dmPCR) for the identification of six phlebotomine sand fly species (Diptera: Psychodidae), including major Leishmania vectors of the Mediterranean. *Journal of Economic Entomology*, **110(1):** 245-249.

Hadwan, H.A. and H.S. Khara (1992). Effect of inoculum level and temperature on the incidence of damping off and root rot of tomato by *Rhizoctonia solani*. *Plant Disease Research*, **7:** 242-244.

Healy, M., K. Reece, D.Walton, J. Huong, S. Frye, I.I. Raad and D.P. Kontoyiannis (2005). Use of the DiversiLab System for species and strain differentiation of *Fusarium* species isolates. *Journal of clinical microbiology*, **43(10):** 5278-5280.

Huang, A., J.W. Li, Z.Q. Shen, X.W. Wang and M. Jin (2006). High-throughput identification of clinical pathogenic fungi by hybridization to an oligonucleotide microarray. *Journal Clinic Microbiology*, **44(9):** 3299-3305.

Leslie, J.F. and B.A. Summerell (2006). The *Fusarium* Laboratory Manual. Blackwell Publishing Professional, Ames, IA, USAlipoeptides by *Pseudomanas fluorescens* Strains in bulk soil and Sugar beet Rhizosphere. *Appl. Environment. Microbial.*, **69(2):** 861 -868.

Mwaniki, P.K., M.M. Abang, I.N. Wagara, J.N. Wolukau and S. Hans-Josef (2016). Response of African eggplants to *Fusarium* spp. and identification of sources of resistance. *African Journal of Biotechnology*, **15(11):** 392-400.

O'Donnell, K., D.A. Sutton, A. Fothergill, D. McCarthy, M.G. Rinaldi, M.E. Brandt and D.M. Geiser (2008). Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the *Fusarium solani* species complex. *Journal of Clinical Microbiology*, **46(8):** 2477-2490.

Pathak, V.N. (1974). Diseases of fruit crops. Oxford & IBH Publishing Co., New Delhi, 940.

Schroeder, K.L., F.N. Martin, A.W. de Cock, C.A. Lévesque, C.F. Spies, P.A. Okubara and T.C. Paulitz (2013). Molecular detection and quantification of *Pythium* species: evolving taxonomy, new tools, and challenges. *Plant Disease*, **97(1):** 4-20.

Weinhold, A.S. and J. Sinsclair (1996). *Rhizoctonia solani*: Penetration, colonization and Host response, 163-174.

Zhang, S., X. Zhao, Y. Wang, J. Li, X. Chen, A. Wang and J. Li (2012). Molecular detection of *Fusarium oxysporum* in the infected cucumber plants and soil. *Pakistan Journal of Botany*, **44(4):** 1445-1451.