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## Isolation and Pathogenicity of the Fungus, *Fusarium Solani* A Causal of Dry Root Rot on Sour Orange in Baghdad Province, Iraq

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Al-Karboli, M. H. H. and Kuthair, W. M. (2016). Isolation and pathogenicity of the fungus, *Fusarium solani* a causal of dry root rot on sour orange in Baghdad province. International Journal of Agricultural Technology 12(5):927-938.

**Abstract** Laboratory and field experiment were conducted at the college of Agriculture, University of Baghdad to investigate the variability in cultural, Morphological characteristic and pathogenicity of the casual of dry root rot, *Fusarium solani* on sour orange from different regions in Baghdad province. Results indicated the presence of 16 *F. solani* isolates and the appearance of the fungus at high rates ranged between (93-100) percent in all regions. It might be the dominant fungus in the roots of the sour orange .There were a significant differences between these isolate, Pathogenicity tests of these isolates on cabbage and sour orange seeds indicated that the percentage of germination between (3.3-26.3) % and (30-70) % for cabbage seed and sour orange respectively compared with 96.66 % for the control treatment. Al-Jadriyah isolates considered the most effective and the high pathogenicity where sour orange seed germination did not exceed 38.3% , while it ranged from (48.3-66.6) % for the rest of other isolates .

**Keywords:** - *Fusarium solani* isolates, Pathogenicity, dry root rot, sour orange

### Introduction

*Fusarium solani* a ubiquitous fungus in the soil and affects a large number of crops , vegetables , trees , causing various symptoms which include: seed rotting , death and damping off seedlings , dry root rot (Kunta, *et al.*, 2015; Domsch, *et al.*, 1980; Farr, *et al.*, 1989; Brasileiro *et al.*, 2004 ) . *Fusarium solani* mainly infects citrus trees especially grape fruit, *citrus paradise*, orange *C. cinensis* and to a lesser extent lemon, *C. limon* and trifoliate orange. *Fusarium* spp. Were isolated for the first time from citrus roots by Sherbakoff (1953). Studies on the roles of *F. solani* on citrus trees indicated a

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significant reduction in seedlings growth and dry weight by 33%. Apparently, leaves of healthy citrus trees, suddenly wilt and turn yellow and die, roots are blackened and rotted with brown, vascular discoloration with the stem of the rootstock, fruits dry and stay on the tree (Olsen, *et al.*, 2000).

Tatum and Baker (1983) explain the ability of *F. solani* to produce 11 forms of toxins belonging to the naphthazarin group. And that some of these toxins can induce disease symptoms in citrus seedlings represented by vein coloration, leaves wilting and clogging of the transporting vessel. Also there is a relation between *Fusarium solani* races and their virulence and ability for toxin production and speed of disease symptom appearance (Kumar, *et al.*, 1995; Baker, *et al.*, 1981). In nature plant pathogens exist as different strains that exhibit variation in their morphological and cultural characters, pathogenicity and virulence (Ravi and Kumar, 2012). Morphological and pathogenic variations are known in many fungal pathogens (Kumar, *et al.*, 1995; Khosrow and Zakaria, 2015; Kunta, *et al.*, 2015).

Dry root rot is considered an important disease on citrus in Iraq and there are no detailed studies about it. The present study was conducted to investigate the variability in cultural and morphological characteristics and pathogenicity of *F. solani* isolates from different areas of sour orange orchards in Baghdad province.

## **Material and methods**

### ***Isolation and Identification of F. solani from sour orange roots***

Isolates of *F. solani* were collected from 8 sour orange orchards around Baghdad province (Table 1) during the period of September – December 2015. Five samples of roots were taken randomly from 5 trees showing symptoms of common dry root rot in each orchard, then roots were transported to the lab and put in the refrigerator until isolation, and rinsed in tap water for 30-60 min, cut the roots into small pieces (length of 0.5 – 1 cm) showing vascular discoloration. Surface sterilization for 3 minutes in sodium hypochlorite solution (1% free chlorine) and then washed with sterile water and dried with tissue paper. Four sterile root pieces were cultured in petri dishes (9 cm diameter) containing 15-20 ml of potato dextrose agar (PDA). All culture plates were incubated in darkness at  $25 \pm 1$  °C for 5 days. Fungal colonies resembling *F. solani* were identified depending on their cultural and characteristics using the method described by

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(Booth, 1977). *F. solani* were subcultured again and purified by using single spore technique (Salam,2009) isolates were also grown on PDA (Nelson,et al., 1983) for the development of color.

The percentage of the appearance of *F. solani* in the root pieces were calculated from the equation of (Booth, 1977) below:

$$\% \text{ of appearance } F. \text{ solani} = \frac{\text{No. of root pieces with } F. \text{ solani in petri dishes}}{\text{Total numbers of pieces used for each sample}}$$

#### ***Pathogenicity of F. solani isolates on cabbage seeds***

Pathogenicity of the 8 *F. solani* isolates Table 1 were tested first on cabbage seeds. Petri dishes (9 cm diameter) contained 20-15 ml of the cultured media (PDA) were inoculated with 5 mm of 6 days old of each. *F.solani* isolates taken from near the edge of each colony placed in the central of the petri dishes. 4 days later , cabbage seeds were superficially sterilized with sodium Hiboklorate (1% free chlorine) for two minutes and placed as a circle around each petri dishes, an average of 10 seeds were placed in each petri dishes and three replicates/treatment ( *F. solani* isolates ) in a total number of 48 petri dishes except the control treatment without *F. solani* all petri dishes were incubated at  $25 \pm 1 \text{ c}^\circ$  for 4 days then the percentage of seed germination was calculated for each isolates compared with the control treatment ( Bollkan and Butler ,1974 ).

#### ***Pathogenicity test of F. solani isolates on sour orange seeds***

Sour orange seed from ripened non-infected fruits were extracted and cleaned thoroughly under running tap water until they get rid from adhesive substance and sterilized superficially with sodium Hiboklorat ( 1% free chlorine) for two minutes , then washed with distilled water and dry in the air. Sour orange seeds planted in plastic pots (15 cm diameter) containing sandy soil sterilized twice in autoclave (for 121 m, under pressure of 1.5kg/cm for one hour), 8 seeds were planted in each pot and three replicates / treatment (*F. solani* isolate) in total of 51 pot including 3 pots for control. A 7.5 gm. of each *F. solani* isolate culture on millet seeds were added to each pot and mixed with

the sand soil, except for the control treatment (Kuthair, 2007). All pots was transferred to a suitable place in a greenhouse and watered regularly with distilled water, and checked for seed germination. After the germination of all seedling in the control treatment, the numbers of seedling was calculated in each treatment and compared with the control treatment to calculate the percentage of seed germination. Complete random design (CRD) and statistical analysis program –SAS (2012) were used to compare the differences between treatments (Least significant differences (L.S.D. P. 0.05).

## **Results and discussion**

### ***Isolation and Identification of F. solani from sour orange roots***

Results of the isolation of *F. solani* from sample of sour orange roots which showed symptoms of typical symptoms of dry root rot and brown coloration of infected roots is present in all roots samples collected from the orchard regions in Baghdad province, with a high appearance rates between (93.3-100) % (Table 1) *F. solani* isolates taken from root orchards of Jadriyah (JA1), Tarmiyah (TM1) and Hamdaniya (HA1) have reached 100%. Microscopic examination showed that *F. Solani* have formed the three usual types of spores: Microconidia, Macroconidia and Chlamedospores. Using the taxonomic key of Booth s (1977) show it belong to *F. solani*.

It is clear from these results and the appearance of the fungus, *F. solani* at high rates, it might be the dominant fungus in the roots of the sour orange that show the typical symptom of citrus root rot, especially citrus orchards in Iraq suffered from stress factors such as drought and lack of regular irrigation during hot summer months, which may help in the development of the disease (Kuthair, 2007; Olsen, *et al.*, 2000; O'Connell, 2006). These findings are consistent with previously results found by many Iraqi researchers who found *F. solani* in high rates in citrus roots samples collected from different governorates of Iraq (Abbas, *et al.*, 1990; Al-Hakim, 1975; Mohammad, *et al.*, 1981; Al-Heeti, *et al.*, 1995; Kuthair, 2007).

### ***Pathogenicity test of F. solani isolates on cabbage seeds***

Result in Table (1) show a significant difference on the effects of *F. solani* isolates that collected from the different Sour orange orchids around

Baghdad province on the germination of the cabbage seeds. Where percentage of seed germination ranged between (3.3 – 30) % Compared with 96.6% for the control treatment.

Result indicated a significant differences in effects of *F. solani* isolates on the seed germination taken from the same regions of Taji and Tarmiyah, where Taji isolates amounting to 30 and 10% for TG1 and TG2 respectively, while it was 23.3 and 6.66% for Tarmiyah isolates TM1 and TM2. There were no significant differences was observed between the rests of the isolates obtained from the same area of the other 6 regions within the province of Baghdad. All *F. solani* has caused a significant reduction in the percentage of seed germination where the mean germination rates ranged from (4.95 – 24.95) %, Compared with 96.64%, in the control treatment. The lowest percentage of seed germination of 4.95 % recorded for Jadriyah ( JA ) isolates (Fig. 1) , followed by isolates RS , TU , DO , TM which amounted to percentage of germination in 8.33 , 13.3 , 13.3 and 14.98 % respectively , and there in no significant differences between these isolates on their effect on seed germination . It was the highest percentage of germination of 20, 21.6, and 24.95 % for isolates TG, AC and HA respectively. Based on these Pathogenicity test , The 8 *F. solani* isolates can be divided into two groups , The first group which include isolates JA , RS , TU , DO and TM which germination rates ranged between ( 4.95 – 14.98 ) % on average of 10% , The second group includes isolates TG , AC and HA in which the proportions of germination high ( 20 , 21.6 and 24.95) % reached twice what in the first group.

**Table 1.** Percentage of *F. solani* isolate on sour orange roots and their effect on Cabbage seed germination.

Region	Isolates	% of <i>F. solani</i> appearance on sour orange roots	Percentage of cabbage seed germination	
			% seed germination / isolates	Mean
Al –Jadriyah (JA)	JA1	98.3	6.6	4.95
	JA2	100	3.3	
Al –Rashdiya (RS)	RS1	96.6	10	8.33
	RS2	98.3	6.6	
Al –Tuwaythah (TU)	TU1	95	10	13.3
	TU2	96.6	16.6	
Al-Dora (DO)	DO1	98.3	10	13.3
	DO2	95	16.6	
Al-Tarmiyah (TM)	TM1	100	23.3	

	TM2	96.6	6.66	14.98
<b>Al-Tajii (TG)</b>	TG1	93.3	30	
	TG2	98.3	10	20.0
<b>Agriculture College (AC)</b>	AC1	93.3	16.6	
	AC2	95	26.6	21.6
<b>Al-Hamdaniya (HA)</b>	HA1	100	26.6	
	HA2	96.6	23.3	24.95
	<b>Control</b>		96.6	96.66
	<b>L.S.D. 0.05</b>		<b>10.663 *</b>	<b>10.479 *</b>

The effects on germination of cabbage seeds have been used by many researchers to evaluate the pathogenicity of different isolates of many fungal plant diseases (Bollkan and Butler, 1974; Hasson, 2005). In a similar study Kuthair (2007) refers to the existence of significant differences between 4 *F. solani* isolates obtained from sour orange orchard collected from 4 provinces of between (4-53) % Several studies indicated that the different effects of *F. solani* isolates in the germination of cabbage and cauliflower seeds may be due to three reasons:

1-First is the genetic difference between fungus isolates of different regions (Nelson, *et al.*, 1983; Brasilerio, *et al.*, 2004; Juber and Hasson, 2006; Kuthair, 2007; O Connell, 2006).

2-Producing a toxic metabolite which inhibit seed germination ( Juber and Kuthair,2009; Jain and Thapliyal,1980) , and finally the ability of theses isolates to produce analyst enzymes (cellulolytic enzyme , protease ) , Mycotoxins (Fusarubin and Javanicin ) and other toxins (Baker *et al.*,1981;Nemec,1995; Nelson and Hansen,1997 ) . The cell wall leading to rotting and decomposition of seeds and lack of germination (Bateman and Lumsden, 1965; Pannecoucq and Hofte, 2009; Kuthair, 2007).

### ***Pathogenicity test of F. solani isolates on sour orange seeds***

Results in table (2) indicated a significant reduction in the percentage of the sour orange seeds germination grown in contaminated pots with 8 different *F. solani* isolates from different regions of Baghdad province, which ranged between (30 – 70)% compared with 96.66% in the control treatment. The lowest percentage of seed germination obtained for the two Jadriyah isolates amounted for 30, 46.6 % for JA1 and JA2 respectively and average mean of 38.3% which was significantly different from all other isolates obtained from other regions (Fig. 1). According to the results of the statistical analysis of the impacts of these isolates in the germination of the sour orange seeds we can

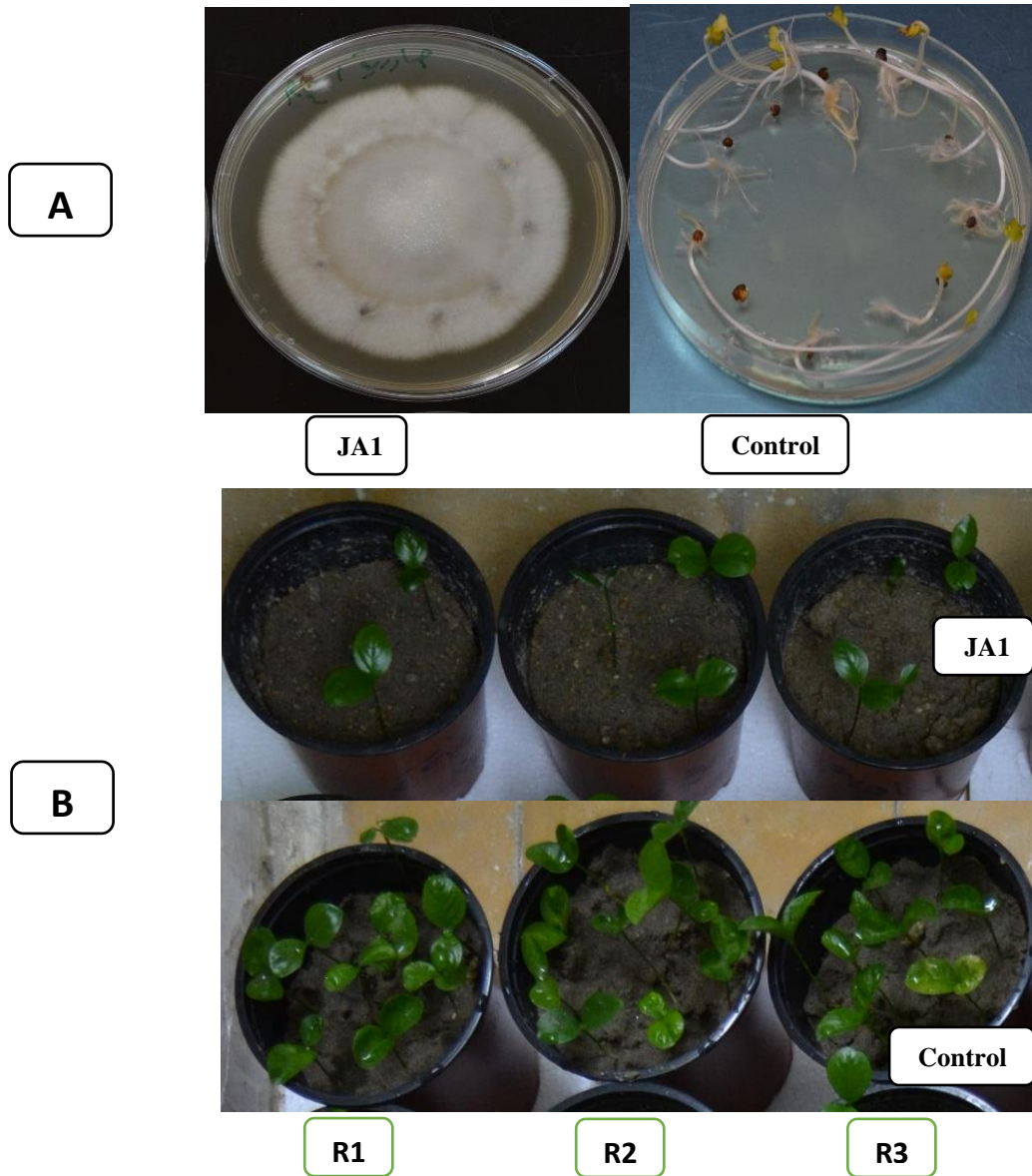
divided these isolates into 3 main groups , the first group include Al-Jadriyah isolates JA1 and JA2 which considered the most effective and the high pathogenicity where germination did not exceed 38.3% , while the second group where germination rates where decreased to 50% which include isolates taken from Rashdiya region ( RS ) , Tarmiyah ( TM ) , Tuwaythah (TU) and the Collage of Agriculture (AC) which the germination rates between ( 48.3 – 55.1 )%.

The third group which have the least effect where germination rate decreased ( 63.3-66.6)% for the isolates of Dora ( DO ) , Taji ( TG) and Hamdaniya ( HA) respectively. There were many reasons about the effects of *F. solani* on the germination of the sour orange seeds, the most important is the ability to produce analyst enzymes , (Cellulotic enzyme , Protease ) , Mycotoxins (Fusarubin , Javaicin) and other toxins ( Baker,et al.,1981;Obaid,2004;Nemec,et al.,1991 ).

Based on these two pathogenicity test, Al-Jadriyah (JA) isolates have been chosen to conduct the next subsequent experimentation due to their high effect (Pathogenicity) on germination of the cabbage and sour orange seeds.

**Table 2.** Pathogenicity test of *F.solani* isolates on sour orange seeds.

Region / isolates	Percentage of sour orang seed germination/ isolate	Mean
JA1	30	
JA2	46.6	38.30
RS1	46.6	
RS2	50	48.30
TM1	43.3	
TM2	60	51.65
TU1	56.6	
TU2	50	53.30
AC1	70	
AC2	43.3	55.15
DO1	60	
DO2	66.6	63.30
TG1	60	
TG2	70	65.55
HA1	66.6	
HA2	66.6	66.60
Control	96.6	96.66
L.S.D. 0.05	7.925 *	6.478 *



**Figure 1.** Pathogenicity test of *F. solani* Al- Jadriyah (JA1) isolate on Germination of: (A) Cabbage seeds , (B) Sour orange seeds



## Acknowledgement

The authors would like to offer particular thanks to Dr. Feras and Dr. Salam and their colleagues responsible for plant pathology Lab. , Plant Protection Research Station , Ministry of Agriculture for their help and cooperation in completing this research.

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(Received: 20 July 2016, accepted: 5 September 2016)