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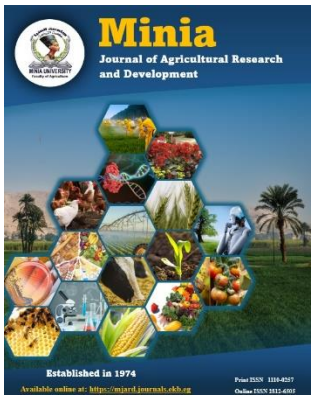

Occurrence of tomato leaf spot caused by Alternaria alstroemeriae In El-Minya governorate, - Egypt

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ABSTRACT

Isolation process carried out from tomato plants in different locations shown a typical leaf spot grown in commercial open fields grown in El-Minya Governorate. This process revealed 10 isolates designated from Als1 to Als 10. In general, the tested isolates are able to infect tomato plant genotype Syria 084 and the highest virulence one is Als 4. All tested *Alternaria* isolates produced conidiospore contained about 3.9-5.2 cells (mean 4.6), length 120-151 µm (mean 131 µm) and 42.2-55.1 µm width (mean 51.0. µm). The length/width ratio was ranged from 2.6 to 3.1 with mean 2.8. The measurements referred that the isolated pathogen is typically *A. alstroemeriae*. This identification was confirmed by PCR as a molecular identification.

Out of 7 tomato genotypes, Syria 084 shows high susceptible one followed by F1 023. The highest resistant genotype was F1 K-186 followed by Sajda. The isolated *A. alstroemeriae* can infect 3 plants belong to Family Solanaceae i.e. *Solanum tuberosum (*potato cv. Pinto*), Capsicum annuum (*pepper cv Ropy King*)* and *Solanum melongena (*eggplant cv Baldy Black*).* On the other hands, *Datura stramonium* was resistance toward the fungus infection. Actymal 70% shows the highest inhibitory effect on the fungal growth compared to Cure M 45 and Metrodex 80%. Also under field condition, it shows the highest protection effect for tomato plant against *A. alstroemeriae.*

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L., syn. *Lycopersicon esculentum* Mill.) belong to the important vegetables for human nutrition. It is cultivated across all continents in open fields or in protected cultures. According to data collected and updated in December 2022 by the Food and Agriculture Organization (FAO), total world tomato production for both processing and fresh consumption in 2022 amounted to just over 186.8 million metric tons of tomato fruits cultivated across 5.1 million hectares achieving an average yield of 37.1 metric tons per hectare. In Egypt, the total

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cultivated area under tomato was half million hectares produce about 6.2 million metric tons. Egypt is fifth in total global tomato production after China, India, the United States of America and Turkey.

Simmons (2008) summarized and described about 275 *Alternaria* species. This genus is recorded as a pathogen to more than 4000 host plants. These hosts include very important crops such as cereals, ornamentals, vegetables and fruits, caused direct economic losses (Thomma, 2003). Additionally, several *Alternaria* species produce toxic and hazardous compounds as secondary metabolites, including AAL toxin, tenuazonic acid, alternariol, and alternariol monomethyl ether (Meena *et al.* 2016 and Boyce *et al.* 2010). According to Jambhulkar *et al.* (2016) and Gherbawy *et al.* (2018) a number of *Alternaria* species, including *A. alternata, A. solani,* and *A. tenuissima*, have been identified as tomato plant pathogens that cause symptoms such as stem canker, early blight, leaf spot, and leaf blight.

Traditionally, the disease management of these diseases in commercial farms mainly depends on disease monitoring, cultural control, planting resistant cultivars, and the application of synthetic agrochemicals such as fungicides. However, frequent application of fungicides that fall within the same chemical families causes strains of the organism to develop a resistant to the detrimental effects of those fungicides and become insensitive to specific active ingredients. (Nehela *et al.,* 2023). Moreover, several fungicides have been deregistered as a result of growing worries about the effects of fungicides on the environment and residues in food. The need to replace these has raised interest in more environmentally friendly, sustainable, and successful disease-management options, such as resistant varieties, biological control, microbial fungicides, botanical fungicides, agro-nanobiology techniques, and inducing local or systemic resistance (O'Brien, 2017).

This work was planned to 1) isolate the causal agent(s) of tomato leaf spots, 2) identify this causal agent(s), 3) study the reaction of some tomato genotype toward the infection in intact plants or detached leaves and 4) study the infectivity of the fungus toward some Solanacaous plants.

MATERIALS AND METHODS

1- Isolation of the causal pathogens:

Naturally diseased tomato plants showing leaf spots in various localities in Minya s governorates were used for isolation. Isolation process was carried out as described by Roopa (2012). The leaf disinfected leaf parts were moved to sterile Petri plates containing PDA media and incubated at 27±1 °C for 4 days. After incubation period, developed colony were purified by hyphal tip technique and held at 4 °C on a PDA medium until used.

2- Pathogenicity test:

The isolated pathogen from diseased leaves was purification and tested for its pathogenicity for proving Koch's postulates. Pathogenicity tests were carried out under greenhouse conditions at the Department of Plant Pathology, Faculty of Agriculture, Minia University. Two tomato seedlings (genotype Syria 084) were planted in sterilized pots (30 cm diameter) containing autoclaved sand clay soil (3 kg/ pot). Artificial inoculation was achieved as described by Kumar *et al.,* (2017) using propagules suspension ($4x10^3$ CFU/ml). Healthy-apparent tomato leaves were detached and placed in petri Dishes 15-cm in diameter containing filter paper saturated with sterilized water and inoculated by spraying 5 ml of the fungal suspension. Each treatment contained three replicates (three

pots or dishes/ replicate). After 4 and 8 days from inoculated tomato leaves were evaluated to leaf spots development.

Disease severity (DS) and disease incidence (DI) percentage was recorded using the numerical rate of infected leaves which included scale from 0 to 4 that suggested by Zheng *et al*. (2015). The formulation was then modified as follows: Disease severity (%) $=\{\Sigma (n \times r)/NR\} \times 100$,

Where, n= Number of infected leaves on the plant, r= Numerical rate of infected leaves, N= Total number of leaves on the plant and R= Maximum numeric rate.

3- Identification of the causal pathogens

The identification was carried out at Dept. Pl. Pathol., Fac. Agric., Minia Univ. according to morphological and cultural character described by Simmons (2008). The developed fungal isolates were subjected to microscopic examination (Carl Zeiss with eyepiece screw micrometer micrometer). The colour, size, no. of cell/spore and number of septum/ spore were recorded.

Molecular identification of fungal isolates:

The fungal isolates were maintained on potato broth medium and incubated at 28°C for 5 days (Pitt and Hocking, 2009). Fungal DNA was extracted at the Molecular Biology Research Unit, Assiut University using Patho-gene-spin DNA/RNA extraction kit (Intron Biotechnology Company, Korea). Polymerase chain reaction (PCR) and sequencing were performed at SolGent Company, Daejeon, South Korea. The ITS region of rRNA gene was amplified using the universal primers ITS1 (forward) and ITS4 (reverse). The 18S rDNA and ITS regions from the fungal strain *Alternaria* sp. were amplified using PCR with a final reaction mixture volume of 10 µl. Amplified PCR products were analyzed using 1% agarose gel in TAE buffer solution at 80 V for 40 minutes at 25° C. Sequencing of PCR amplified products was carried out using the commercial service (Eurofins Genomics India Pvt Ltd).

Primers have the following composition: ITS1 (5'-TCCGTAGGTGAA CCTGCGG-3'), and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). Purified PCR products were sequenced using the same primers with the incorporation of ddNTPs in the reaction mixture (White *et al*., 1990). To further characterize the *Alternaria* sp. at species level. the The species specificspecies-specific primers AaF (5’ GTGCCTTCCCCCAAGGTCTCCG 3’) and AaR (5’ CGGAAACGAGGTGGTTCAG GTC 3’) 9 were used to identify the leaf blight pathogen using PCR.

The obtained sequences were analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Analysis of sequences and establishment of phylogenetic trees were done using MegAlign (DNA Star) software version 5.05.

4-Respose of tomato genotypes to inoculation

Response of 7 genotypes of tomato seedlings i.e. Sajda, Azura, Syria 084, El-Kods 448, F1k-186, F1 023 and Salymia 65010 (30 days old) were used in this study to clarify their response to *A. alstroemeriae* (isolate Als 4) infection. This experiment was carried out as explained above. Disease severity and incidence were calculated 4 and 8 days after inoculation. Each treatment was presented by 10 pots. Detached leaves of tomato varieties were tested towered the infection with the fungus as mention before.

5-Respose of some Solanious plant to inoculation

Four plants belong to Family Solaneceae i.e. *Solanum tuberosum (*Potato*), Capsicum annuum (*Pepper*)* and *Solanum melongena (*Eggplant*)* and, *Datura stramonium (*Datoura*)* were used to study their response toward inoculation with *A. alstroemeria* isolate Als 4. Inoculation was carried out as mention in pathogenicity tests.

6. Disease control using fungicide

6-1. Effect of some fungicides on growth of *A. alstroemeriae.*

Three fungicides i.e. Actamyl® 70% (systemic fungicide-70% Thiophanate-methyl), Cure M® 80% (systemic fungicide-25% Tebuconazole) and Metrodox® (local fungicide-80% Dithiocatbamate) at different concentrations (5.0, 2.5, 1.75, 0.7 and 0.35mg/ml PD solid or liquid media) were prepared individually from commercial formulation. A disc of 7-day old pathogen mycelial culture (4-mm diameters) was transferred to the center of Petri dishes containing solidified PDA medium with fungicides in a desired concentration. The plates were incubated at 27±2°C 12h light and 12h dark. After 7 days, linear growth was calculated as described by Prasad *et al*. (2012).

Five disks of 1 cm-diameter each were taken at a certain distance from the original inoculum. The disks were placed in a test tube containing 5 ml sterilized distilled water. Conidiospores of fungus under each treatment were counted using heamocytometer slide and light microscope (Tzeng and DeVay, 1989). Four replicates were used for each treatment

Another experiment was prepared at 100 ml conical flasks containing 50 ml PD broth. The flasks containing broth with different concentrations of certain fungicides were inoculated with A disc of 7-day old pathogen mycelial culture (4-mm diameters). Fungal dry weight was determined after 10 days of incubation at 27±2°C by methods of Carolina *et al*. (2019)

6-2. Effect of some fungicides on disease occurrence caused by of *A. alstroemeriae.*

Management trails were conducted out in the Farm of Pl. Pathol. Dept., Fac. of Agric., Minia Univ. to control tomato leaf spots caused by *A. alstroemeriae*. The susceptible tomato plant cv Syria 084 and fungal the virulent isolate Al s 4 were used in these experiments. Seedlings of tomato 30 days, Seedlings of tomato 30 days were inoculated by sprayed fungal suspension containing 5×10^4 propagules till raining off. After another 1 days, inoculated plants were sprayed by the tested fungicides i.e. Actamyl 70%, Cure M 45 and Metrodex 80% at different concentrations (5.0, 2.5, 1.75, 0.7 and 0.35 mg/ml distilled water). Three rows, 10 plant in each considered as a replicate, 3 replicates were represented each treatment. Disease incidence and severity were recorded after 6 days of inoculation. Data were subjected to statistical analysis using analysis of variance and means were compared using the LSD test according to Durner (2021)

RESULTS

1. Pathogenicity tests on:

1.2. Intact plants under greenhouse condition

Isolation process carried out from tomato plants in different location shown a typical leaf spot grown in commercial open fields in El-Minya governorate. Data in Table 1 and fig 1. show the pathogenicity of the isolated fungi toward tomato plant grown in greenhouse. In general, all the tested isolates are able to infect tomato plant

genotype Syria 084. Also, both disease incidence (DI) and disease severity (DS) increased after 8 days after inoculation comparing after 4 days. The highest DI and DS was resulted after inoculation with Als 4. It caused DI and DS 80.9 and 45.4 respectively after 4 days and 100 and 55.0 after 8 days. The lowest virulence was detected in isolate Als 9. It caused DI and DS 33.9 and 20.8 after 4 days 45.8 and 30.7 respectively after 8 days after inoculation.

Table 1: Ability of *A. alstroemeriae* isolates to cause spots in tomato leaves cv Syria 084 in greenhouse.

Isolates	Evaluation 4 days after inoculation		Evaluation 8 days after inoculation		Mean	
	DI%	DS%	DI%	DS%	DI%	DS%
Al s. 1	70.2	30.2	83.4	41.1	76.8	35.6
Al s. 2	63.3	27.5	73.6	41.6	68. 5	34.6
Al s. 3	56.6	19.6	66.9	33.8	61.7	26.7
Al s. 4	80.9	45.4	100	55.8	90. 5	50.6
Al s. 5	70.4	34.6	80.8	44.4	75.6	39.5
Al s. 6	56.5	16.6	60.4	16.5	583	16.5
Al s.7	56.6	35.8	60.5	54.1	58.6	44.9
Al s.8	60.6	34.4	80.9	54.6	70.76	44.5
Al s. 9	33.9	20.8	45.8	30.7	39.8	25.7
Al s. 10	73.4	29.2	83.4	29.5	78.4	29.3
LSD $_{5\%}$ Isolates (A)				5.6	2.6	
Time (B)				3.5	2.1	
AB				10.5	6.4	

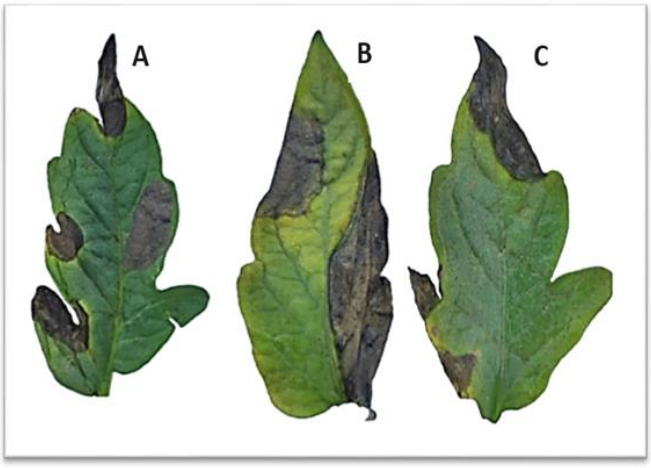

Fig 1. Tomato leaf spots caused by *A. alstroemeriae*. A) naturel infection B and C) artificial inoculation

1.2. Detached leaves under laboratory condition

The response of tomato detached leaves placed under laboratory condition toward the inoculation by A. *alstroemeriae* propagules were presented in Table 3. In general disease incidence and disease severity relatively increased after 8 days comparing with 4 days. The most virulent isolate is Als 4. It causes DI 30.2 and DS 16.8%. This isolated was followed by Als 5 which caused DI and DS 26.2 and 14.7% respectively. The lowest virulence was detected in Al s 9 that produced DI and DS 13.3 and 6.1% respectively.

Table 2: Ability of *A. alstroemeriae* isolates to cause spots in tomato detached leaves cv Syria 084 in laboratory condition.

Isolates	Evaluation 4 days after inoculation		Evaluation 8 days after inoculation		Mean	
	DI%	DS%	DI%	DS%	DI%	DS%
Al s. 1	23.4	10.1	27.8	13.7	25.6	11.9
Al s. 2	21.1	9.2	24.5	13.8	22.8	11.5
Al s. 3	18.9	6.5	22.3	11.2	20.6	8.9
Al s. 4	26.9	15.1	33.3	18.6	30.2	16.8
Al s. 5	23.5	11.5	28.9	14.8	26.2	13.2
Al s. 6	18.9	7.1	20.1	7.1	19.5	7.1
Al s.7	18.9	11.9	20.2	18.0	19.5	14.9
Al s.8	20.2	11.5	26.7	18.2	23.6	14.8
Al s. 9	11.3	6.0	10.3	6.2	13.3	6.1
Al s. 10	20.4	10.7	23.8	14.8	22.1	14.7
LSD $_{5\%}$ Isolates (A)			2.8	1.5		
Time (B)			3.1	1.7		
AB			5.4	4.3		

2-Identification of the causal pathogen:

2.1. By light microscope examination

All tested *Alternaria* isolates produced long chain, dark brown, multicellular, melanized, relatively large conidiospores, which can possess both longitudinal and transverse septae. Spores are typically broadest at the base and taper towards the end. Conidiospore contained about 3.9-5.2 cells (mean 4.6), length 120-151 µm (mean 131 µm) and 42.2-55.1 µm width (mean 51.0. µm). The length/width ratio was ranged from 2.6 to 3.1 with mean 2.8.

Table 3: Morphological character for conidiospore of different isolates of A. *alstroemeriae*.

Measurements*		Fungal isolates			Mean
		Al.s 1	Al.s 2	Al.s 3	
No of cell/spore		3.9 ±0.5	4.7±0.5	5.2±0.8	4.6±0.76
Long (nm)	rang	141-151	120-150	133-129	120-151
	Mean	128.0±8.0	135.0±11.0	130.0±13.0	131±15.6
Width (nm)	rang	42.2-50.5	43.5-52.0	51.2-55.1	42.2-55.1
	Mean	46.5±2.0	56.3±1.7	50.4±2.4	51.0±7.5
No. of septa	Horizontal	3.2±0.9	4.0±0.8	3.9±0.8	3.7±0.4
	Vertical	1.6±0.2	1.2±0.3	2.1±0.5	1.6±0.5
length/width ratio		2.7	2.4	2.6	2.5

* Data are the mean of 50 spores ± SEM or SD

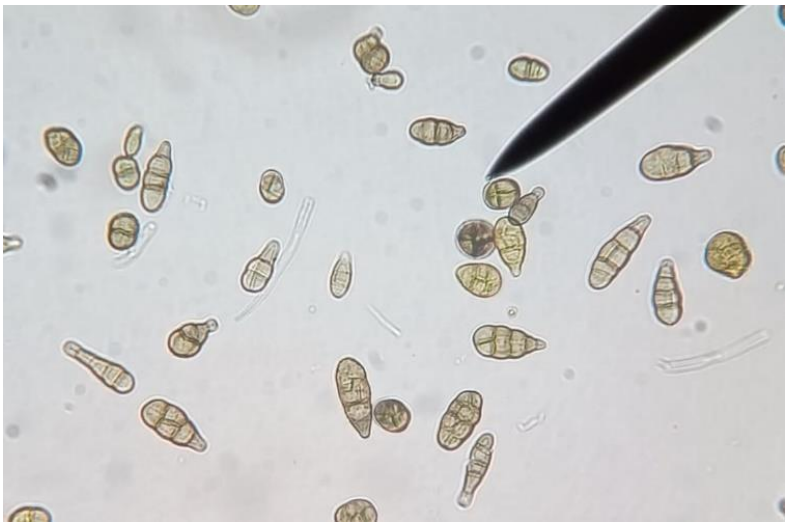

Fig 2: Dark brown, multicellular, melanized, relatively large conidiospores of *A. alstroemeriae*.

2.2. Molecular identification

Identification of A. *alstroemeriae* at molecular level was done by using universal ITS primer pairs ITS1 and ITS4. Agarose gel electrophoresis of PCR amplified products resulted in amplification of 528bp in tested isolate. The rDNA-ITS analyses performed on genomic DNA of *A. alstroemeriae* isolate revealed the presence of high level of genetic diversity with other *Alternaria* isolates infecting another host. The rDNA-ITS has the unique potential for providing information across an entire genome. The isolates showed strong genetic similarity within a range of 99.82% to 100%, indicating high level of identity among them irrespective of hosts. In the phylogenetic tree, the present isolate grouped separately from the other isolates from different hosts indicating *A. alstroemeriae* infecting marigold is distinct from other *Alternaria* sp. reported from different host.

Sample MF-3: Alternaria alstroemeriae AUMC15941 (541 letters)

GCGGAGGGATCATTACACAAATATGAAG GCGGGCTGGAACCTCTCGGGGTTACAG

CCTTGCTGAATTATTCACCCTTGTCTTTT
GCGTACTTCTTGTTTCCTTGGTGGGTTC
GCCCACCACTAGGACAAACATAAACCTT
TTGTAATTGCAATCAGCGTCAGTAACAAA
TTAATAATTACAACTTTCAACAACGGATC
TCTTGGTTCTGGCATCGATGAAGAACGC
AGCGAAATGCGATAAGTAGTGTGAATTG
CAGAATTCAGTGAATCATCGAATCTTTGA
ACGCACATTGCGCCCTTTGGTATTCCAA
AGGGCATGCCTGTTCGAGCGTCATTTGT
ACCCTCAAGCTTTGCTTGGTGTTGGGC
GTCTTGTCTCTAGCTTTGCTGGAGACTC
GCCTTAAAGTAATTGGCAGCCGGCCTAC
TGGTTTCGGAGCGCAGCACAAGTCGCA
CTCTCTATCAGCAAAGGTCTAGCATCCAT
TAAGCCTTTTTTTCAACTTTTGACCTCGG
ATCAGGTAGGGATACCCGCTGAACTTAA
GCATAT

The rDNA-ITS analyses performed on genomic DNA of *A. alstroemeriae* isolate revealed the presence of high level of genetic diversity with other Alternaria isolates infecting another host. The rDNA-ITS has the unique potential for providing information across an entire genome. The isolates showed strong genetic similarity within a range of 99.82% to 100%, indicating high level of identity among them irrespective of hosts. In the phylogenetic tree, the present isolate grouped separately from the other isolates from different hosts indicating *A. alstroemeriae* infecting marigold is distinct from other *Alternaria* sp. reported from different host

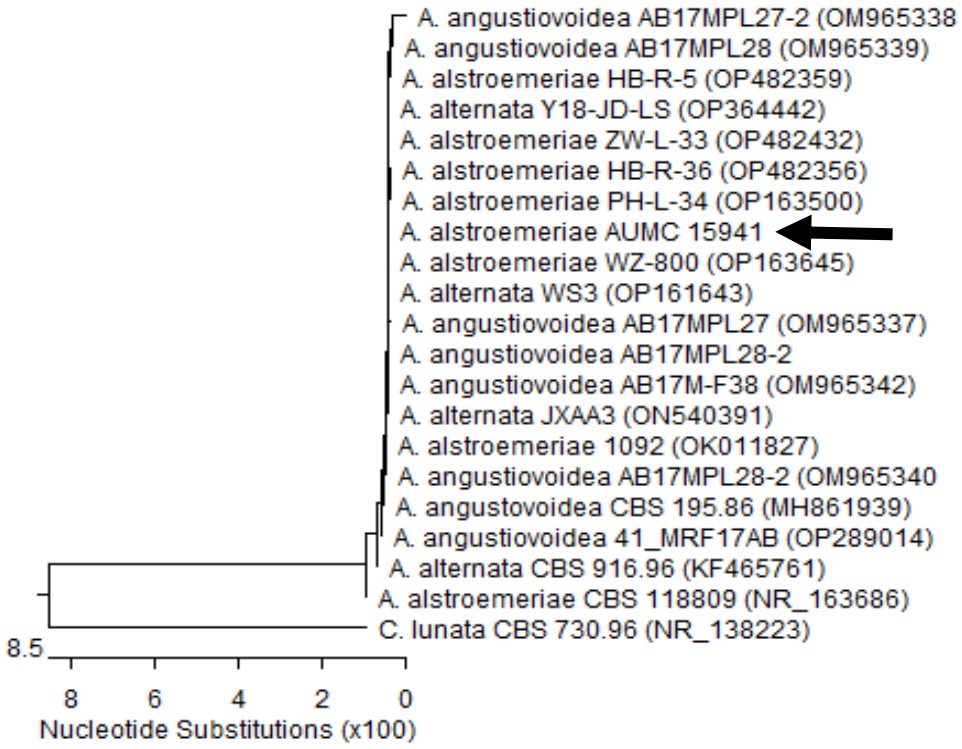

Figure (3): Phylogenetic tree based on ITS sequencing of rDNA of *Alternaria alstroemeriae* AUMC15941 isolated in the present study (arrowed) aligned with closely related sequences of fungal strains accessed from the GenBank. *A. alstroemeriae* (OK-011825.1) showed 99.82% -100% identity and 100% coverage with several strains of the same species. *Curvularia lunata* represents an outgroup strain. A.= *Alternaria*, C. =*Curvularia*

3-Respose of tomato genotypes to inoculation

3.1. Intact plants under greenhouse condition

Data in Table 4 demonstrate the response of 7 tested tomato genotypes to *A. alstroemeriae* isolate Als 4 infection. Out of 7 tomato genotypes, Syria 084 shows high value of DI and DS even after 4 days from inoculation. It is 100 and 59.4 followed by F1 023 that shows 87.6 and 28.2 respectively. The highest resistant genotype was F1 K-186 which shows DI 53.1 and DS 15.6 followed by Sajda which pronounced DI 36.3 and DS 27.9%.

Table 4: Response of different genotypes of tomato towered inoculation with *A. alstroemeriae* in greenhouse condition

Tomato Genotypes	Evaluation 4 days after inoculation		Evaluation 8 days after inoculation		Mean	
	DI%	DS%	DI%	DS%	DI%	DS%
Sajda	50.2	12.5	76.4	43.7	63.3	27.95
Azura	75.5	43.7	75.7	50.5	75.6	47.1
Syria 084	100	56.2	100	62.5	100	59.35
EL-KODS 448	65.4	31.8	75.5	31.5	70.45	31.65
F1 K-186	50.8	12.5	55.4	18.7	53.1	15.6
F1 023	75.2	25.2	100	31.2	87.6	28.2
Salymia 65010	65.4	18.7	75.4	18.7	70.4	18.7
$LSD_{5\%}$ Genotype (A)			4.9	3.1		
Time (B)			3.6	2.2		
AB			7.3	5.6		

3.2. In detached leaves of under laboratory condition.

Data in Table 5 show the response of detached leaves of tomato toward inoculation of *A. alstroemeriae* isolate Als 4. DI and DS slightly increased after 8 days more than 4 days. Disease incidence ranged from 13.4 to 30.9% after 4 days and from 19.9 to 35.9% after 8 days. Meanwhile, DS ranged from 5.5 to 12.2 after 4 days and from 10.5 to 21.5% after 8 days. The lowest DI was recorded in Syria 086 (16.7) and the highest in F1 023 leaves (26.6) while the lowest DS was recorded in Azura (8.6) and the highest in EL-KODS 448 leaves (16.1).

Table 5: Response of different genotypes of tomato detached leaves towered inoculation with *A. alstroemeriae.*

Tomato genotypes	Evaluation after 4 days of inoculation		Evaluation after 8 days of inoculation		Mean	
	DI%	DS%	DI%	DS%	DI%	DS%
Sajda	18.5	12.2	23.5	14.5	21.0	13.3
Azura	19.9	5.5	23.6	11.7	21.8	8.6
Syria 084	13.4	11.8	19.9	14.2	16.7	13.0
EL-KODS 448	20.6	10.7	31.8	21.5	26.2	16.1
F1 K-186	24.4	11.9	28.7	13.5	26.6	12.7
F1 023	30.5	10.8	35.9	11.7	33.2	11.3
Salymia 65010	20.7	10.7	20.9	10.5	20.8	10.6
$LSD_{5\%}$ Genotype (A)			4.2	3.4		
Time (B)			1.9	1.7		
AB			6.3	5.4		

4-Respose of some Solanacaous plant to inoculation

Data in Table 7 show that *A. alstroemeria* isolate Als 4 can infect 3 plants belong to Family solanceceae i.e. *Solanum tuberosum (*potato cv pinto*), Capsicum annuum (*papper cv Ropy King*)* and *Solanum melongena (*eggplant cv Balay Black*).* On the other hands, *Datura stramonium* was resistance toward the fungus infection. The highest DI and DS% was recorded on *Solanum tuberosum* leaves. It was 48.5 and 16.9 after 4 days and 100 and 386 after 8 days respectively. The lowest DI and DS were recorded on *Solanum melongena* leaves. It was 16.6 and 4.5 after 4 days and 16.6 and 8.3% after 8 days respectively.

Table 7: Response of some solanacaous plant toward inoculation by *A. alstroemeriae.*

Plants	Disease occurrence after					
	4 days of inoculation		8 days of inoculation		Means	
	DI	DS	DI	DS	DI	DS
Solanum tuberosum	48.5	16.9	100	38.6	74.25	27.8
Capsicum annuum	50.2	12.5	83.3	33.3	66.75	22.9
Solanum melongena	16.6	4.5	16.6	8.3	16.6	6.4
Datura stramonium	0.0	0.0	0.0	0.0	0.0	0.0
$LSD_{5\%}$ Plants(A)				3.4	2.9	
Time (B)				4.5	3.5	
AB				13.8	14.3	

5. Disease control using fungicide

5-1. Effect of some fungicide on growth of *A. alstroemeriae*

Data in Table 8 illustrate the effect of some fungicides i.e. Actamyl 70%®, Cure M 45® and Metrodex 80%® on growth of *A. alstroemeriae*. All tested fungicides caused complete inhibition on the fungal growth at concentrations of 5.0 and 2.5 mg/ml media. Meanwhile, only Actamyl 70% caused complete inhibition at concentration of 1.25 mg/ml media. At concentrations of 0.75 and 0.37 mg/ml media the linear growth is 41.3 and 64.6, 41.3 and 41.3,72.8 and 31.4 and 42.2, 81.2 in case of Actamyl 70%, Cure M 45 and Metrodex 80% respectively compared to 90.0 in control treatment.

Regarding to dry weight, no growth was detected at concentrations 5.0 and 2.5 mg/ml media. The highest inhibitory effect was recorded in case of Actymyl 70% at concentration of 0.75 and 0.37 gm/ml. It was 90.2 and 152.5 mg compared to 101.2 and 186.1 mg and 103.2 and 202.2 in case of Cure M45% and Metrodex 80% respectively. Regarding to sporulation, it is clear that all tested fungicides caused complete inhibition for the fungal growth and sporulation at concentration of 5.0 and 2.5 mg/ ml. Also, at concentration of 1.25 mg/ml Actymal 70% caused the same effect. At concentration of 0.75 mg/ml, sporulation was 18.5×10^4, 28.5×10^4 and 38.3×10^4 in case of Actamyl 70%, Cure M 45 and Metrodex 80% respectively. At concentration of 0.37 mg/ml, sporulation was 25.5×10^4, 31.3×10^4 and 42.5×10^4 respectively. Sporulation rate in control treatment was 50.5×10^4.

Table 8: Growth parameters* of *A. alstroemeriae* i.e. linear growth (mm), dry weight (mg/50 ml media) and sporulation as effected by different concentrations of Actamyl 70%, Cure M 45 and Metrodex 80% fungicides.

Fungicides	Conc. (mg/ml)	Fungal growth		
		Linear growth (mm)	Dry weight (mg)	Sporulation ($X\times10^4$)
Actamyl 70%	5.0	0.0±0.0	0.0±0.0	0.0±0.0
	2.5	0.0±0.0	0.0±0.0	0.0±0.0
	1.25	0.0±0.0	0.0±0.0	0.0±0.0
	0.62	31.4±3.2	90.2±6.8	$18.5\pm2.2\times10^4$
	0.30	64.6±6.5	152.5±9.2	$25.5\pm3.9\times10^4$
Cure M 45	5.0	0.0±0.0	0.0±.0.	0.0±0.0
	2.5	0.0±0.0	0.0±0.0	0.0±0.0
	1.25	15.2±3.2	41.2±4.2	$20.2\pm2.5\times10^4$
	0.62	41.3±3.5	101.2±5.3	$28.9\pm3.0\times10^4$
	0.30	72.8±4.9	168.1±6.8	$31.3\pm3.9\times10^4$
Metrodex 80%	5.0	0.0±0.0	0.0±0.0	0.0±0.0
	2.5	0.0±0.0	0.0±0.0	0.0±0.0
	1.25	13±1.2	51.2±6.5	$23.2\pm2.8\times10^4$
	0.62	42.2±4.5	103.2±8.5	$38.3\pm4.6\times10^4$
	0.30	81.2±5.1	202.2±13.4	$42.5\pm5.6\times10^4$
control		90.4±6.7	215.2±10.2	$50.5\pm4.8\times10^4$

- Values are means of three replicates ± SD.

5-2. Effect of some fungicide on disease caused by *A. alstroemeriae*

Data in Table 9 summarized the effect of spraying different concentrations of some fungicides, 2 days before inoculation, and evaluated after 8 days of inoculation. All tested fungicides caused complete protection for tomato plants at concentrations of 5.5 and 2.5 mg/ ml. At concentration of 1.25 mg/ml DI and DS were 14.1 and 6.4, 19.2 and 10.2 and 10.5 and 26.2 and 13.2 in case of Actamyl 70%, Cure M 45 and Metrodex 80% fungicides respectively. At concentration of 0.75 mg/ml DI and DS were 31.2 and 14.5, 30.1 and 20.3 and 43.3 and 25.2 and 13.2 in case of using the same fungicides respectively. At concentration of 0.37 mg/ml DI and DS were 55.1 and 6.4, 24.2 and 64.4 and 30.2 and 64.2 and 42.5 respectively. Actamyle 70% shows the highest protection activity under field condition comparing the same concentration of the other tested fungicides.

Table 9: Occurrence of tomato leaf spots caused by *A. alstroemeriae* as affected by some fungicide i.e. Actamyl 70%, Cure M 45 and Metrodex 80% after 8 days from inoculation.

Fungicides	Conc. (mg/ml)	Disease Occurrence	
		Disease incidence%	Disease severity%
Actamyl 70%	5.0	0.0	0.0
	2.5	0.0	0.0
	1.25	14.1	6.4
	0.75	31.2	14.5
	0.37	55.1	24.2
Cure M 45	5.0	0.0	0.0
	2.5	0.0	0.0
	1.25	19.2	10.5
	0.75	30.1	20.3
	0.37	64.4	30.2
Metrodex 80%	5.0	0.0	0.0
	2.5	0.0	0.0
	1.25	26.2	13.2
	0.75	43.3	25.2
	0.37	74.2	42.5
Control		100	46.2
$LSD_{5\%}$ fungicide (A)		3.4	4.9
Conc. (B)		4.5	3.7
(AB)		10.8	11.3

DISSCUSION

Isolation process carried out from tomato plants in different location show leaf spot grown in commercial open fields in El-Minya governorate. This process revealed 10 isolates. All isolates are able to infect tomato plant genotype Syria 084. The highest DI and DS was resulted after inoculation with Als 4 in both intact plants and detached leaves.

More than fifty morphologically identical pathogenic and nonpathogenic fungi belong to *Alternaria sp.* With latest advances in molecular biology, researchers have stated to use of DNA-primarily based whole molecular methods and sequencing of genetic regions for the quick and precise identification of fungi on the species level (Mohammadi and Bahramikia 2019). This fungal genus includes saprophytic, endophytic and pathogenic species. It is associated with a wide variety of substrates including seeds, plants, agricultural products, animals, soil and the atmosphere. Species of *Alternaria* are known as serious plant pathogens, causing major losses on a wide range of crops.

Microscopic examination and molecular identification indicate that the isolates belong to *Alternaria sp.* The spore contained about 3.9-5.2 cells (mean 4.6), length 120-151 µm (mean 131 µm) and 42.2-55.1 µm width (mean 51.0. µm). The length/width ratio was ranged from 2.6 to 3.1 with mean 2.8. These measurements are in a good line with that mentioned before (Yamagishi *et al.,* 2009; Valdes *et al.,* 2014 and Zhou *et al.,* 2023) described *A. alsrtoameriae.* According available literature, this is the first report about *A. alsrtoameriae* in upper Egypt. Deokar and Raghuwanshi (2002) studied the morphological characters in six isolates of *Alternaria carthami* and reported variability with regards to conidial size, number of septa, beak size and color (Patriarca and Fernández-Pinto, 2018). Alternaria has north of 50 pathogenic and nonpathogenic species which are morphologically very much like one another. With most recent advances in sub-atomic science, scientists have started out the utilization of DNA-basically based absolutely sub-atomic procedures and sequencing of hereditary regions for the quick and precise identification of parasites on the species level (Mohammadi and Bahramikia 2019).

The pathogenicity of the isolated fungi toward tomato plant grown in greenhouse or on detached leaves were varied. In general, the highest DI and DS was resulted after inoculation with Als 4. It caused DI and DS 80.9 and 45.4% respectively. Hubballi *et al.* (2010) reported that the pathogenicity of *Alternaria* is known to be variable. This variability arises because the mycelium may become heterokaryon because of the nature of the pathogen and this consequently led to variability in pathogenicity, sporulation, growth rate and other environmental requirements.

Out of 7 tomato genotypes, Syria 084 shows high value of DI and DS after four and seven days of inoculation in both intact or detached leaves test. These are 100 and 59.4 and 100 and 62.5%, respectively. Weihmann *et al.* (2016) reported that the detached leaf method is a useful rapid and unexpansive tool to evaluating large numbers of genotypes for determining their resistance to foliar pathogens. Also, it proved to be a reliable method to differentiate the pathogenic and non-pathogenic isolates in many pathogens on their hosts. Griffiths *et al.* (2011) found that most of cultivated tomato-genotypes in Egypt are susceptible to *Alternaria* infection. So, the use of resistant cultivars, varieties or genotypes is one of the most effective and sustainable controls of

Alternaria diseases in tomato or other crops. Eid *et al.* (2017) evaluated pathogenicity of 14 isolates of *Alternaria sp.* with five tomato hybrids for their response. They found that the tested 14 isolates of *Alternaria* react differently on the five tested hybrids. Moreover, the tested isolates show different pathogenicity in field condition or under greenhouse condition.

Obtained data indicate that *A. alsrtoameriae* can be control using many fungicides. The fungal growth completely inhibited by using 1.25 mg/ml media of Actamyle 70% or 0.62 mg ml^{-1} Cure-M 45 and Metrodex 80%. Verma and Verma (2010) tested efficacy of seven compounds viz., Chlorothalonil, Copper oxychloride, Azoxystrobin, Propineb, Copper hydroxide, Mancozeb at concentrations ranged from 250 to 2500 ppm against *A. alternata*. They reported that all the fungicides significantly reduced the fungal growth. However, hexaconazole was very effective as it caused 100% growth inhibition). Haviland *et al.* (2021) found that the systemic fungicides were highly effective in inhibiting mycelial growth. It showed inhibition percentages significantly higher than the rest of the fungicides. Several chemical classes are recommended in spray guides for Alternaria control on several crops in the United States. A few fungicide products can provide partial control of Alternaria rot when used at postharvest according to the California citrus pest management guidelines (Grafton-Cardwell *et al.,* 2021 and Boris *et al.,* 2022).

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الملخص العربى

تواجد مرض تبقع أوراق الطماطم الناتج عن *Alternaria alstroemeriae* في محافظة المنيا-مصر

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تمت عملية العزل من نباتات الطماطم التى تظهر علي اوراقها بقعة سوداء وتنمو في الحقول المفتوحة بمواقع مختلفة من محافظة المنيا. وتكشفت عن هذه العملية ١٠ عزلات. بشكل عام، كانت جميع العزلات قادرة على إصابة نبات الطماطم من الصنف سوريا ٠٨٤. والجراثيم الكونيديه للفطر المعزول كانت تتكون من ٣.٩-٥.٢ خلية (بمتوسط ٤.٦) وطولها ١٢٠-١٥١ ميكرومتر (بمتوسط ١٣١ ميكرومتر) وعرضها ٤٢.٢-٥٥.١ ميكرومتر (بمتوسط ٥١.٠ ميكرومتر) .تراوحت نسبة الطول إلى العرض من ٢.٦ إلى ٣.١ (بمتوسط ٢.٨) . وأشارت القياسات إلى أن الفطر الممرض المعزول هو *A. alstroemeriae* . تم تأكيد هذا التعريف بواسطة تفاعل البوليميراز المتسلسل كتعريف جزيئي .ومن بين ٧ أنماط طرز وراثية مختبره للطماطم ضد الفطر ، أظهر الطراز سوريا ٠٨٤ حساسية عالية تلاه الطراز النمط F1 023 وكان الطرازالوراثى جيني الأكثر مقاومة هو F1 K-186تلاها .Sajda. كما اصاب الفطر *A. alstroemeriae* ثلاث نباتات تنتمي إلى العائله البازنجانيه solanceceae وهي البطاطس *Solanum tuberosum* والفلفل*Capsicum annuum* والباذنجان *Solanum melongena* ومن ناحية أخرى، كان نبات الداتورا *Datura stramonium* مقاومًا للإصابة بالفطر. هذا واظهر المبيد اكتيمال ٧٠% اعلى ثاثير مثبط لنمو الفطر وكذلك اعلى حمايه حماية للطماطم في الحقل مقارنة بالمبيدين كيور م ٤٥ وميترودكس ٨٠%