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IDENTIFICATION AND CONTROL OF THE FUNGUS *ALTERNARIA SOLANI* (CAUSAL AGENT OF EARLY BLIGHT ON TOMATOES) IN THE CITY OF MOSUL

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Abstract: Early blight caused by the fungus *Alternaria solani* , is one of the most devastating foliar , foliar diseases affecting tomato production worldwide... This study investigated the pathogenicity characteristics, phytotoxin production and fungicide treatment of A. solani isolates from infected tomato plants in northern Iraq. Like, Morphological and pathogen evaluations confirmed the identity and virulence of the pathogen under greenhouse conditions. The fungus can qualitatively and quantitatively produce cell wall-degrading enzymes, including cellulase and pectinase, that suggests that it plays , plays a role in host tissue maceration. You know what? The culture , culture shows necrotic changes and wilting symptoms, that confirms the toxic activity of the plant. And oh yeah, Chromatographic and spectroscopic analyzes revealed the presence of a phytotoxic compound associated with the development of the disease. Like, The in vitro evaluation of three , three systemic fungicides at different concentrations resulted in a significant reduction in the actinic growth of the pathogen, complete inhibition was achieved at the highest , highest concentrations of the selected treatments. Other greenhouse experiments have shown that fungicide use reduces , reduces disease incidence and severity while increasing defense-related enzyme activities, including peroxidases, polyphenol oxidase, and catalase. Like, These results emphasize the importance of toxin production in the pathogenicity of A. Seriously, solani and demonstrate the efficacy of selected fungicides in controlling early rot in protected cropping systems.
Keywords: *Alternaria solani*, systemic fungicides, early blight disease, mycotoxins.

Introduction:

The fungus *Alternaria solani* is the causative agent of early blight disease in tomatoes; it was first identified as a pathogen in New Jersey in 1882 by researchers Ellis and Martin (Agrios, 2005). Tomatoes have been known by various names; some Europeans called them the "poor man's apple," while the French dubbed them the "love apple" a term from which the Arabic words bandoura or tomatim* were derived in certain Arab countries (Arunakumora, 2006). Global annual tomato production is estimated at 1.1 million tonnes, cultivated over a total area of 500,620 hectares—an average yield of 17.6 tonnes per hectare—with Iraq contributing 1.3% of the total global production (Statistic/Factfish, 2013; FAO, 2012).

The disease affects the crop under various conditions—whether grown in open fields or protected structures—and also causes post-harvest damage during transport or storage, particularly under poor storage conditions. The severity of disease fluctuates with the environment conducive to the pathogen and is related to the relationship between the pathogenic fungus and the rainy season and changes in relative humidity. Infection also takes place in the desert areas, due to the alternate dew formation at night and dry conditions during the day (Pandey, 2008). The fungus is primarily dispersed and infected by fungal spores and fungal spore type is used as the basis for fungal taxonomy. Persistence of the disease is ensured by the conidia that can survive for long periods either on the plant or in the soil (Sallam et al., 2012). The classification of the fungus is given below (Simmons, 2007):

Kingdom:	**Fungi**
Phylnu:	Ascomycota
Class:	Dothideomycetes
Order:	Pleosporales
Family:	Pleosporaceae
Genus:	*Alternania*
Species:	*solani*

Alternaria solani is a polycyclic pathogen of plants as it can cause numerous infection cycles during the season using its wintering inoculum. The growth of early blight of Alternaria solani (Simb, 2009) are influenced by environmental factors. This research will accomplish the following goals:

1. Isolation and identification of the fungus which causes early blight disease.
2. Quantitative and Qualitative testing for the pathogen's cellulase and pectinase enzyme production.
3. Identification of pathogenicity (virulence) of the pathogen.
4. Testing fungicides in a greenhouse.

Materials and Methods

- Sample collection and fungal isolation of diseased plants:

Leaves with early blight spotting symptoms were taken from areas and at different times. Samples were then stored in polyethylene bags and sent to the lab for the isolation and identification of the pathogenic fungus (Amita, 2016). The leaves were washed, and pieces of tissue with and without the lesions were cut out. These sections were surface sterilized for 3 minutes in 1% sodium hypochlorite solution and then placed on filter paper. Five pieces of infected tissue were placed in each Petri dish containing Potato Dextrose Agar (PDA) medium (autoclaved). The dishes were then incubated at 27°C for 5 – 7 days and checked for 5 days during the incubation

period.

- Purification & identification of fungus *Alternaria solani*:

Single spore purification was carried out according to Pryor and Michailides (2002). One colony of fungus was taken with the help of an inoculation loop and placed in a tightly closed glass vial containing 10ml of distilled water with a drop of the surfactant Tween 80. The vial was shaken and then tenfold serial dilutions (10^{-1} and 10^{-2}) were prepared. Then 1 ml of the spore suspension was evenly spread over the surface of a Potato Dextrose Agar (PDA) plate, before it was solidified, to cultivate the fungus and isolate a pure culture, based on a single spore (Pevalta, 2005).

- Identification of the Pathogen:

Taxonomic keys of Ellis (1971), Bose and Som (1987), Shahid (2002) and Naik et al. (2010) were used for identification. Various characters such as spore morphology, spore arrangement, conidiophores and spore size were used. The size of the spores was measured by measuring 25 spores on each objective lens (4x, 10x and 40x) at 40x using a calibrated eyepiece micrometer (0.01 mm graduation) and the appropriate changes were made to ensure accurate calibration under each objective lens. The isolates were then grown on Potato Dextrose Agar (PDA) slants and kept at 4°C until further use (Reda, 2008).

- Pathogenicity Test:

The study was carried out in greenhouse employing soil-peat moss mixture (1:2) treated with 1% formalin. This mixture was put into the pots of 1 kg capacity, and 60-day-old seedlings were transplanted into the pots. The leaves were sprayed with 5ml of the pathogenic fungal suspension (three replicate pots), and then covered with plastic bags and kept under high relative humidity for three days for the fungus to grow. The control treatment was spray with only sterile distilled water. Plants were thereafter grown until disease symptoms were observed. Once symptoms of early blight appeared the pathogen was re-isolated from infected plants to support Koch's postulates and to confirm pathogenicity (Pezet, 1999).

- **Tomato Leaf Necrosis Assay:**

To make tomato leaves permeable, they were put in glass Petri dishes on filter paper moistened with sterile distilled water. Two types of media (Czapek-Dox and vegetable juice) yielded three replicates of filtrates each. Using sterile needle, several punctures were made in each leaf and the fungal culture filtrate was applied to the punctures. Dishes were incubated at 25 ± 2°C and symptoms of yellowing and necrosis of tissues observed after 24–72 hours, with sterile distilled water used as a control (Yoder et al., 1977).

- Tomato shoot wilting test:

Freshly cut tomato shoots were used, and the ends of the shoots were dipped into test tubes containing the toxin filtrates of Czapek-dox and vegetable juice media at different concentrations (25%, 50% and 100%). Shoots were grown for 24-48 hours at a moderate temperature of 25 ± 2°C. There were three replicates for each treatment, and one shoot of each replicate for each treatment, and the control treatment included shoots only in sterile distilled water (Zhang, 2003).

In laboratory tests, fungicide effect on the growth inhibition of Alternaria solani at different concentrations.

Fungicides Fibrosat, Topsin-M and Alsa were used in this study at concentrations of 50, 100, 150 and 250 mg active ingredient per liter. The fungicides were applied to 250 ml of Potato Dextrose Agar (PDA) media and the glass flasks shaken to ensure uniform distribution of the suspension of the fungicides in the media. Then a fungicide

amended media was poured in to the sterilized Petri dishes in three replications of each treatment according to Completely Randomized Design (CRD). For the control treatment, the growth medium was used without the addition of the pesticide suspension. The pathogenic fungus was transferred to the center of all the plates as a 0.5 cm diameter disk cut from the edge of a fresh, 5-day-old fungal colony with a cork borer that had been sterilized with alcohol and alcohol flame, to the centre of the plate, which marks the intersection of two lines drawn on the outer surface of the plate. After inoculation the plates were incubated at 25 ± 2°C. At the end of 10 days when the fungal colonies were grown up on the control plates, the average diameter of each fungal colony was measured on all the pesticide plates and the inhibition percentage for each pesticide was estimated by the following equations:

% Inhibition= (Diameter Colony Treatment Mean – Qatar Colony Comparison/ Diameter Colony Treatment Mean) X 100

The results were statistically analyzed, and the means were tested using Duncan's Multiple Range Test (Rotem, 1994).

Results and Discussion

- Isolation and Identification:

The fungus *Alternaria solani* was characterized by the formation of green colonies during the initial growth stage (at 48 hours); the secretion of a yellow pigment onto the PDA medium was observed, followed by a color transition to grey, while the colony margin appeared white. Microscopic examination revealed the formation of large, spindle-shaped macroconidia featuring long beaks and a pale golden or brown coloration. It was also observed that the conidia formed singly at the apex of the conidiophore. These characteristics—including colony shape and color; conidial dimensions (length, beak length, and width); and the presence of transverse and oblique septa—served as the basis for identification. The fungus formed transverse septa numbering between 5 and 11, while oblique and longitudinal septa ranged from 0 to 5; the fungus was identified based on the characteristics described by Shi (2002), Ellis (1971), and Bose and Som (1986).

- Pathogenicity Test:

A pathogenicity test (Table,1) conducted on tomato plants in a greenhouse ten days after inoculation with the fungus *Alternaria solani* revealed symptoms characterized by small, dark spots appearing on the lower leaves. These spots expanded to form concentric, overlapping rings surrounded by a yellow halo a hallmark symptom of the disease. As the infection intensified and the plant aged, the spots coalesced, leading to total leaf death and defoliation. This progression is attributed to the fungus's ability to secrete cellulase and pectinase enzymes, as well as phytotoxins. The fungus was subsequently re-isolated from the artificially infected tomato leaves and cultured in pure form on Potato Dextrose Agar (PDA) medium. Results from the re-isolation of the fungus from tomato leaves (fulfilling Koch's postulates) in this test demonstrated that its phenotypic characteristics matched those of the pathogenic fungus originally sprayed onto the leaves. The results also indicated that the general characteristics of *Alternaria solani* vary depending on the culture medium; the following table illustrates the effect of the culture medium type on the characteristics of *Alternaria solani.*

Table 1: Pathogenicity test results.

Medium	Colony color	Pigment production	Colony diameter	Average number

			(cm)	of spores
PDA	Dark grey	Yellow-colored	7.5	**$12.11x10^6$**
V-8j	Light green	Was not	7.3	**$8.75x10^6$**
TMCA	Light grey	Orange-colored	5.9	**0**
SSA	white	Yellow-colored	4.7	**0**

- Local Necrosis Test on Tomato Leaves:

Local necrosis tests using filtrates from *Alternaria solani* cultures—grown on Czapek-Dox and vegetable juice media—resulted in localized necrosis at the puncture sites on tomato leaves. This was seen as black puncture area surrounded with yellow area, which was not seen in leaves that were only punctured and treated with distilled water. Necrosis was seen as a dark central area surrounded by a yellow-coloured zone, indicating the dead area surrounded by a zone of yellow color. The spots found are the result of the death and degradation of chlorophyll at the spot with the yellow halo which indicates the area of toxin diffusion after partial chlorophyll breakdown in the leaf tissue, as described by Wood (1967), Lou et al. (2013) and Siciliano et al. (2015).

- Tomato Shoot Wilting Test:

Results showed that tomato plant cuttings immersed in "Tashabuk Dox" and vegetable juice filtrates at three different concentrations (25%, 50% and 100%) had an effect from *Alternaria solani* filtrates. After 12 hours, wilting symptoms were observed in the 100% concentrations of both the filtrates. Symptoms occurred after 24 hours at 50% concentration, and after 48 hours at the 25% concentration for both filtrates. Remarkably, at 100% concentration, shoots completely perished after 48 hours from both filtrates.

Branches placed in distilled water (no pectin) however, did not wilt or exhibit any changes; this suggests that the filtrate (100% pectin) moved rapidly to all parts of the branches and leaves through the conducting vessels, in particular the water-conducting xylem. For both filtrates the 50% concentration was the second earliest concentration to show wilting symptoms, followed by the 25% concentration. The toxicity of the filtrate caused by *Alternaria solani* to the conducting vessels led to the wilting of the branches, which caused the water to be less absorbed and moved from the roots. The results obtained in this study are similar to the results of Soylu (2003) for treatment of citrus branches with Alternaria solani filtrate, and Tally (2000) for treatment of broad bean cuttings with *Alternaria solani* filtrate.

- Laboratory evaluation of the fungicide inhibition of Alternaria solani fungus at different concentrations:

The results of the research showed that all fungicides tested (Fibrosat, Elsa and Topsin-M) at all concentration rates were effective in inhibiting the growth of Alternaria solani colonies. The fungicide Elsa produced the highest level of inhibition at 100 mg, 150 mg and 200 mg of the active ingredient per litre of water with 100% compared to the fungicide Fibrosat which achieved the same inhibition rate (100%) at 200 mg of the active ingredient per litre of water. Pesticides Elisa (50 mg/L of active ingredient) and Fibrosat (150 mg/L of active ingredient) were the second most effective with inhibition rates of 80.26% and 83.20%, respectively, not significantly

different from each other. The pesticide in third rank was Fibrosat (50 mg active ingredient/L and 100 mg active ingredient/L) with inhibition rates of 75.86% and 77.20% respectively as indicated in Table, 2.

Table 2: Effect of pesticide concentrations on the inhibition of the fungus *Alternaria solani* under laboratory conditions.

Concentration (mg active ingredient/L)	fungicides						Concentration impact rate	
	Fbrosate		Alsaa		Topsin-M			
	Colony diameter (cm)	Inhibition rate (%)	Colony diameter (cm)	Inhibition rate (%)	Colony diameter (cm)	Inhibition rate (%)	Colony diameter (cm)	Inhibition rate (%)
50	1.81 e	75.86 c	1.48 ef	80.26 b	4.58 b	38.93 f	2.62 b	65.01 d
100	1.71 ef	77.2 c	0.0 c	100 a	4.30 c	42.66 e	2.00 c	73.33 c
150	1.26 f	83.20 b	0.0 c	100 a	3.96 d	47.20 d	1.74 d	76.80 b
200	0.0 c	100 a	0.0 c	100 a	3.80 d	94.33 d	1.26ه	83.20 a
Comparison without pesticide	7.5 a	0.0 f	7.5 a	0.0 f	7.5 a	0.0 f	7.5 a	0.0 e
Pesticide application rate	2.45 b	67.25 b	1.79 c	76.05 a	4.82 a	35.26 c		

The values in the table represent the mean of three replicates.

Values followed by the same letter indicate no significant differences according to Duncan's Multiple Range Test at the 5% probability level.

The fungicide Topsin-M had the lowest inhibition rates than fungicides Sa and Vibrosat under all concentrations. The inhibition rates were 47.20% and 49.33% for the active ingredient concentrations of 150 and 200 mg/L respectively (not significantly different) and 42.66% for the 100 mg/L active ingredient concentration. The 50 mg active ingredient/L concentration exhibited the lowest inhibition rate of 38.33% for Topsin-M. As far as overall effect of the concentrations employed in the study is concerned, the results indicated that the rate of inhibition was highest in the 200 mg active ingredient/L followed by the concentration of 150, 100, and 50 mg active ingredient/L, with 76.80%, 73.28% and 65.01%, respectively. As far as the effect of the concentrations used in the study, it is revealed that the highest inhibition rate was achieved at 200 mg active ingredient/L (83.20%), followed by the 150, 100 and 50 mg active ingredient/L concentrations (76.80%, 73.28% and 65.01%, respectively). Regarding the effect of the pesticides, it was found that the pesticide Alsaa had the highest effect with an inhibition rate of 76.05%, followed by the pesticide Fbrosate with 67.25% while "Topsin-M" recorded the lowest inhibition rate of 35.26% compared to Alsaa and Fbrosate.

Conclusions and Recommendations

Conclusions:

1. The cultural and morphological characteristics of the fungus *Alternaria solani* varied depending on the type of growth medium and temperature.
2. The fungus *Alternaria solani* demonstrated significant qualitative and quantitative efficiency in secreting cellulase and pectinase enzymes.
3. The fungus *Alternaria solani* exhibited a high capacity for toxin production.
4. Tests on the effects of various pesticide concentrations showed that the fungicide Alsaa at concentrations of 100, 150, and 200 mg active ingredient/L and the fungicide Fbrosate at a concentration of 200 mg active ingredient/L completely inhibited fungal growth and sporulation.
5. Both Alsaa and Fbrosate yielded positive results in reducing the incidence and severity of infection and improved plant characteristics; the fungicides were applied via two methods: foliar spraying and soil drenching.

Recommendations:

1. Expand research into the enzymatic and toxicological efficiency of the fungus *Alternaria solani* to determine its specific capacity to secrete other enzymes and toxins.
2. Identify tomato cultivars and hybrids that exhibit high resistance to the fungus *Alternaria solani*.
3. Investigate the effects of various fungicides in agricultural fields to identify the most effective types for controlling early blight disease.
4. Expand future agricultural research to determine the optimal method for eradicating the disease, and utilize genetic engineering and molecular techniques to detect the causative agents.

References:

Agrios, G. N. (2005). Plant pathology, 5th edition, Elsevier Academic Press, London. Pp 454-455.

Amita, P.; I. Sharma.; R. Sasode and R. N. Sharma (2016). Collection of Different Isolated of *Alternoria solani* in Bhind, Morena and microbiology, International J. Appli, Research: 2(6)-2017-21.

Arunakumara, K. T (2006). Studies on *Alternaria solani* (Ellis and Martin) Jones and Grout causing Early Blight of tomato, M. Sci. Thesis, Dharwad: 1-70.

Bose, T. K. and M. G. Som (1986). Vegetable Grops un India, Nanya Prakash Publishing, Caleutta, p. 443.

Ellis, M. M (1971). Dematiaceous hyphomycetes. Commonwealth mycological institute, Kew, surry, England, Pp. 608.

Factfish/ statistic. (2013). http//www.factfish.com/statistics/country Iraq/tomatoes+ area+harvested.

FAO (2012). Research And Technology paper7. Food and Agriculture Organization Of The United Nations. Rome Italy, M. 99.

Lou.; Fu, K.; Peng, Y and Zhou, L (2013). Metabolitos from *Alternaria* Fungi and their Bioactivities. Journal/ Molecules, 18: 5891-5935.

Pandey, K. K.; R. P. Singh.; A. K. Singh and P. C. Singh (2008). Cultural Morphological, Pathogenic and Molecular Variability of *Alternaria solani* in India, World journal microbiology, Biotechnology, 24:1003-1009.

Pevalta, I. E; knapp, S. and spoonar, D. W. (2005). New species of wild tomatoes (*Solanum lycopersicom* solanaceae) from Northern peru, Sys Bot, 30: 424-434.

Pezet, R.; V. Pont and R. Tabacchi (1999). Simple analysis of 6-penty-1- xpyrone a major anti-fungal metabolite of *Trichoderma* spp. useful for testing the antagonistics activity of these Fungi, Phytopath. Anal. 10 (5): 285-288.

Pryor, B. M and R. L. Gilbertson (2002). Relationships and taxonomic status of *Alternavia radicina* A. *carotiincultae* A. *pertroselini* based upon morphological, biochemical and molecular characteristic, Mycologia, 94: 49-61.

Reda, A. M.; A. Mohamoud and Z. Ebtsam (2008). Production of bacterial pectinase from agro-industrial wastes under solid state fermentation conditions, Journal of Applied Science Research, 4(12): 1708-1721.

Rotem, J. (1994). The genus *Alternaria*: Biology, Epidemiology and Pathogenicity, The American psychopathological society, APS Press. St. Paul, Minna, pp. 326.

Sallam, M. A, Hwa Kamal, A. M. and Abo- Elyousr (2012). Evaluation of various plant Extracts against the Early Blight Disease and field conditions, Plant proctect, Sci. 48 (2): 74-79.

Shi, C.; Y. Dai.; X. Xu.; X. Xie and Q. Liu (2002). The purification of polyphenol oxidase from tobacco protein Experiment and purification, 24: 51-55**.**

Siciliano, L.; G, Ortu.; G, Gilardi.; M. L, Gullino and A, Garibaldi (2015). my cotoxin production in Liquid culture and on plants infected with *Alternaria* spp. Isolated from Rocket and cabbage, J**.** Toxins, 7:743-154.

Simb, A. (2009). The role of pectinase enzyme in the development of soft rot caused by *Pseudomonas Fluorecens* in the Purple variety of on ions (Allium Cepa). African Journal of Microbiology Research, 3(4): 163-167.

Simmons, E. G. (2007). Alternaria: an Ideentification manual CBS Biodirrensityseries, 6: 1-77 spp.

Soylu, S.; O. Basel and E. M. Soylu. (2003). Induction of disease resistance by the plant activator, acibenzolar-s-Methyl (ASM) against bacterial canker (*Clavibacter Michiganensis* subsp. *Michiganensis*) in tomato seedling plant, Sci. 165: 1069-1075.

Tally, A.; M, Oostendrop.; K, Lawton.; T, Staub and B, Bassi (2000). Commercial development of elicitors of induced resistance to pathogens. In: A. A. Agrawal, S. Tuzun and E. Bent (Eds). Induced plant defenses against pathogens, Aps press. st. paul). pp. 357-369.

Wood, RK. S. (1967). Physiological plant pathology, Blackwell scientific publication Oxford and Edinburg, 57 opp.

Yoder, O. C.; G. A. Gregory and V. E Grescen (1977). Bioassay for detection and quantification of *Helminthosporium* Maydis race T- toxin a Comparison, Physiological Plant Pathology, 10: 232-245.

Zhang, LP.; Gy. Lin.; Nino-Lin and MR. Foolad. (2003). Mapping QTLs conferring early blight (*Alternaria solani*) resistance in tomato using backcross population of *lycopersicom esulentum* X *lycopersicom hirsutum cross*. By selective genotypeing mol breed, 12: 3-19.