

PATHOLOGICAL STUDIES ON TOMATO EARLY BLIGHT

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ABSTRACT: Tomato (*Lycopersicon esculentum* Mill.) is one of the most important and economic vegetable crops in Egypt. It is subjected to infect by numerous fungi that causing considerable losses in yield and quality. Early blight disease caused by *Alternaria solani* was wide spread in El-Sharkia governorate during 2004-2005 and 2005-2006 growing seasons. The highest percentage of disease incidence was recorded in El-Ebrahimia district while the lowest one was recorded in Abo- Hammad district during two growing seasons.

The causal organism was isolated from infected leaf and fruit samples (exhibited typical symptoms of early blight) collected from different localities mentioned before, and identified as *A. solani*. Growth of *A. solani* on leaves and fruits of tomato agar media, then exposed to Ultra-Violet irradiation (UV) was the best method to obtain *A. solani* spores. All tested isolates of *A. solani* were found to be pathogenic at different degrees against tomato plants Gs cv. Hynez tomato cv. was the most resistant tested cvs., while, Gs cv. was the most susceptible one. On the other hand, the other tested cultivars were moderately susceptible to be infect with *A. solani*. Old tomato leaves were more susceptible to be infect by *A. solani* than young one. Also, direct seed cultivation method enhanced tomato plants to be infect with *A. solani* than the transplanting method.

The fungicide Mmancozeb reduced both mycelial growth and /or spore germination of *A. solani in vitro* as well as disease severity and percentage of infection *in vivo* followed by Cure-plus and Vacomil-plus, respectively.

Key words: Tomato, early blight disease, *Alternaria solani*, pathological studies, chemical control, sporulation.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) early blight caused by *Alternaria solani* is one of the most important diseases of tomato world wide as well as in Egypt (Ozcelik and Ozcelik, 1997, Vloutoglou *et al.*, 2000). Different isolates of *A. solani* lost their ability to produce spores during the cultivation *in vitro*. The pathogen produces chlamydospores on hyphal, conidial cells on basal salts agar medium and infected tissues of several tomato cultivars. Conidia required desiccation. In contrast, chlamydospore production on hayphal cells was inhibited by desiccation (Patterson, 1991). Choulwar and Datar (1992) found that, the optimum temperature for *A. solani* growth on PDA medium was 28°C. Exposure the fungal growth to sunlight produced maximum growth and distinct zonation but not spores, they also stated that other light treatments (electric UV and fluorescent) increased growth over controls but did not induce zonation. Brij (1967) mentioned that, the intensity of light (sunlight and fluorescent), duration of exposure, temperature at the time of exposure and scraping the cultures have considerable effects on spores yield of *A. solani*. Also, sporulation can be induced when

fully grown culture were given dip or sprayed with distilled water (cold or hot) and there after, kept partially covered at different temperatures (Bhawani *et al.* 1973). On the other hand, Benlioglu and Delen (1996), reported that, tomato juice medium was found to be the most suitable (with 6 days dark at 23C°, 12h light at 26 C° 12h dark at 18 C° incubation) for sporulation of different isolates of *A. solani*. Also, Moretto and Barreto (1997) used two culture media with or without CaCO₃ with respect to mycelial growth of *A. solani*. The fungus produces different pigments in its culture media. Tong *et al.* (1997) found grey-brown and yellow pigments were secreted by some isolates.

Breeding tomato cultivar for resistance to early blight pathogen plays an important role in controlling the disease (Banerjee *et al.*, 1999, Suryavanshi *et al.*, 2000). Kashlendra *et al.* (2003) mentioned that, screening tomato cvs. against *A. solani* under artificial conditions was more informative than that under natural epidemic conditions.

Different fungicides (Copper oxychloride, Zineb, Mancozeb, Carbendazim, [Mancozeb], Topsin M-70 [thiophanate- methyl] and

Ridomil 5G [Metalaxy] Dithianon, Iprodione, Captafol and Difenconazole) have been tested *in vitro* and *in vivo* against *A. solani*, the tomato early blight pathogen (Singh *et al.*, 2000 and Mate *et al.*, 2005). Fungicides can be used alone or in combination with others (Choulwar and Datar, 1992 and Tomescu, 2002).

MATERIALS AND METHODS

Survey Studies

An extensive survey of tomato early blight disease incidence and severity were carried out at El-Sharkia governorate (Diarb negm, Abo-Hammad, El-Ebrahimia and El-Hossania districts) during two successive growing seasons (2004-2005 and 2005-2006). Two villages were randomly selected for each district and three fields were taken into consideration for each one. Three samples were collected from the selected fields where each one contains 100 plants. Percentage of infection was recorded for each sample and number of infected tomato fruits was calculated in each one. Disease severity was determined according to (Christ, 1992) which ranged between 0 and 7, where 0 = no symptoms, 1 = trace to 1%, 2 =

2 to 5%, 3 = 6 to 10%, 4 = 11 to 25%, 5 = 26 to 50%, 6 = 51 to 75 and 7 = 76 to 100% of total foliage on infected Canopy. Percentage of early blight index (PEBI) was calculated using the following formula:

Percentage of disease index =

$$\frac{\text{Sum of all ratings}}{\text{Number of plants} \times \text{maximum rating grade}} \times 100$$

Isolation, Purification and Identification of the Causal Organism

Leaf and fruits of diseased tomato plants showing typical early blight symptoms were collected from different locations of El-Sharkia governorate. Samples were washed several times using tap-water, surface sterilized with 0.01% mercuric chloride solution for two minutes, then washed three times in sterilized distilled water to get rid of the remained poisonous. Samples were then dried between two layers of sterilized filter paper to remove the excess water. The sterilized samples were then cut with adjacent healthy tissues using a sterile scalpel and placed on plain agar medium in sterilized Petri-dishes. The inoculated dishes were then incubated at 28°C for 72 hours. Hyphal tips from the outer

ends of the growing colonies were transferred into plates of potato dextrose agar (PDA) medium and incubated at 28°C. Pure cultures were obtained for each of the isolated fungus using single spore technique according to Hansen (1926) and or hyphal tip technique according to Brown (1924). The purified fungi were identified according to their morphological characters using the description of Ozcelik and Ozcelik (1997) and Perez and Martine (1997). Stock cultures were maintained on PDA slants and stored in a refrigerator at 5-10°C for further studies.

Fungal Sporulation

Different media and methods were used for inducing sporulation by *A. solani*. Cultivation of *A. solani* on S-medium (Shahin and Shepard (1979), water agar medium, V8 agar medium at 21 to 23°C (Miller, 1955), on Czapek-Dox agar medium (Salam *et al.*, 2006) and tomato medium (200g leaves or tomato fruits, 20g agar and 12g dextrose) at 21 to 23°C. Fungal growth was then exposed to ultra-violet irradiation for 2 min. (Benlioglu and Delen, 1996). *A. solani* was also grown on PDA medium (Rotem, 1994) after that

the fungal growth was wounded in cross fashion.

Pathogenicity Tests

Detached leaf technique

This experiment was carried out to study the pathogenicity of *A. solani* isolates using detached leaf technique described by (Atia, 2000, Atia *et al.* 2005 and Reni *et al.* 2007). Tomato plants with 5-6 full true leaves were used during these studies. Detached healthy leaflets from leaf No. 3 were taken. Tomato Gs cv. was grown in plastic pots (30 cm in diameter) was filled with field soil (9 kg/pot). Two plants were transplanted per pot under greenhouse conditions of Agric. Bot. and Plant Path. Dept. Fac. Agric., Zagazig Univ.

Inoculum preparation

Inoculum of *A. solani* isolates was prepared from cultures grown on PDA medium for 7 days at 28±2°C. Mycelial mats were washed several times with sterilized distilled water, then blended with water for 3 min. The fungal suspension concentration was adjusted microscopically with aid of the hemocytometer technique to 10⁵ cfu/ml Brame and Flood (1983).

Inoculation of detached leaves

Healthy looking tomato leaflets were gathered and placed in 15 cm Petri dishes with moistened filter papers. The lower surface of the leaflets was inoculated with 6 drops from the previously prepared inoculum (30 µl /leaflet) of each isolate. Other leaflets were inoculated with the same volume of distilled water and served as control. The inoculated leaflets were incubated in a growth chamber at 23±2°C. Inoculated leaflets were observed daily for 3-5 days after inoculation, for the appearance of lesions. Number and diameter of necrotic lesions (mm) as well as blighted area/leaflet (mm²) were determined and calculated (Atia *et al.* 2005). The fungus was re-isolated from the aforementioned leaflets.

Intact leaves

Pathogenicity tests of the different *A. solani* isolates was carried out, under greenhouse conditions on Gs tomato cv. When the plants were 7-8 weeks old, inoculum (10⁵ cfu/ml) was sprayed on each plant. On the other hand, plants sprayed with distilled water were used as control. The inoculated plants were kept under polyethylene bags for 48 h then transplanted to greenhouse. Disease reaction was determined

after 7 days from inoculation, based on the size and number of lesions on the inoculated and adjacent leaves. Disease severity was rated on 1-9 scale: 1 = symptom less; 2 = few small lesions; 3 = several small lesions; 4 = < 10% of leaf area with infection; 5 = 10-20%; 6 = 21-50%; 7 = 51-80%; 8 = 81-99% of leaf area with infection; 9 = dead plant. Leaves that were not completely unfurled during the inoculation were not assessed. The disease scales were converted into percentage of early blight index (PEBI) for each plant using the following formula (Pandey *et al.* 2003):

$$PEBI = \frac{\text{Sum of all ratings}}{\text{Number of plants} \times \text{maximum rating grade}} \times 100$$

Varietals Reaction

These experiments were designed to test the susceptibility of different tomato cultivars. i.e Super marmand, Hyenz, Gs and Castel rock to early blight disease, using detached and /or intact leaf techniques. Tomato plants with 5-6 full true leaves of different tomato cultivars were used for each particular cultivar separately.

Reaction of Tomato Leaf Age to *A. solani* Infection

This experiment was carried out to study the effect of tomato leaf

age to infection with *A. solani*, using leaflets from leaves No. 2, 3, 4 and 5 of tomato Gs cv. separately.

Effect of Cultivation Methods

This experiment aimed to study the effect of transplanting and direct cultivation with seeds methods on the infection with *A. solani*. Seeds of tomato Gs cultivar were directly sown in pots for 50-60 days. On the other hand, in case of transplanting seedlings of tomato were transplanted in pots 30 cm containing soil (9kg/pot). Both detached and/or intact leaves, were used.

Chemical Control

In vitro experiments

The inhibitory effect of different concentrations (0, 25, 50, 100, 125, 200, 250, 500 and 1000 ppm of active ingredients) of six tested fungicides (Kocide 101, Cure-plus, Vacomil-plus, Ridomil gold plus, Mancozeb and Copperikh) on linear growth of *A. solani* was investigated *in vitro* and *in vivo* conditions.

Different concentrations of each fungicide were suspended and added separately to PDA medium before solidification. Medium containing fungicides was poured

in Petri dishes (9 cm in diameter) where three. Petri-dishes were used for each concentration. The dishes were inoculated in the center with an equal disc (5 mm in diameter) of *A. solani* of 7 days old culture and incubated at 28°C. Linear growth was measured when the mycelial growth completely covered the medium surface in control treatment, then reduction percentage was calculated as mentioned in the following formula:

$$A = B - C/B \times 100$$

Where:

A= the percentage of growth reduction.

B= the mean diameter of growth of the pathogenic fungus in control treatments.

C= the mean diameter of growth of pathogenic fungus in different treatments.

Effect of fungicide concentrations on spore germination was studied as described by Nair and Ellingboe (1962). A drop of each fungicide concentration was deposited on dried clean glass slide as a film. A drop of *A. solani* spore suspension (10^5 cfu/ml) was spread over this film. Control treatment was prepared as a film of sterilized

distilled water. Three slides were used as replicates for each particular concentration. Each slide was placed on glass rod in Petri-dish in a moistened condition and incubated for 24h at 28°C. Four microscopic fields ($\times = 10 \times 40$) for each replicate (slide), were tested.

The percentage of spore germination was calculated from the following formula: $A/B \times 100$. Where:

A= the mean number of germination in the treatment.

B= the mean number of spores.

***In vivo* experiments**

To study the effect of the tested fungicides on the disease severity, tomato leaves (leaf No.2 and 3) were separately sprayed with different concentrations of the four tested fungicides 0.75, 1.5, 3 g/liter of Mancozep, Cure-plus and Vacomil-plus as well as 1.25, 2.5, 5g/liter, Kocide 101. Two days after spraying treated leaves of tomato plants were detached and placed in Petri-dishes (15cm in diameter) containing saturated filter paper with 10 ml. of sterilized water. Detached leaves were inoculated with 6 drops (10 μ l)/leaflet of *A. solani* D₁ spore suspension (10⁵ cfu/ml). Healthy

detached leaves of Gs tomato were also inoculated with 6 drops (10 μ l) from the mixture of the same amount of fungicides and *A. solani* D₁ spore suspension to test its direct effect on the pathogen. Three Petri-dishes were used as replicates for each concentration. For control treatment, detached leaves were inoculated with 6 drops (10 μ l)/leaflet of *A. solani* spore suspension only. Inoculated leaflets were incubated at 23°C and the disease incidence was calculated after 7-10 days. Number and diameter of necrotic lesions (mm) and blighted area/ leaflet were determined. Percentage of protection was calculated as follows:

Percentage of protection = $100 - A/B$

A= Percentage of disease incidence in treated [100 X blighted area in treated/blighted area in the untreated (control)]

B= percentage of disease incidence in untreated (control) mentioned before.

RESULTS AND DISCUSSION

Early blight disease of tomato, caused by the necrotrophic fungus

Alternaria solani is one of the most common foliar diseases. The disease can occur over a wide range of climatic conditions, but is most prominent in areas with dew, rainfall and high relative humidity. On tomato it causes damping-off of seedlings, later collar rot, leaf spots, stem lesions and fruit rot (Chaerani *et al.*, 2006).

Survey studies have been carried out during two successive growing seasons (2004/2005 and 2005/2006) of different localities of El-Sharkia governorate. The percentage of disease incidence and severity were higher in 2005-2006 growing season than that of 2004-2005 season Table 1. Also, the highest disease severity, percentage of disease incidence and number of infected tomato fruits were recorded in El-Ebrahimia locality. While, the lowest disease severity, percentage of disease incidence and number of infected tomato fruits was recorded in Abo-Hammad locality. Difference between localities in percentage of disease incidence and disease severity might be due to differences in relative humidity rainfall, temperature and time of sowing (Rotem and Reichert 1964).

The causal organism was isolated from diseased samples of tomato leaves and fruits collected from different localities of El-Sharkia governorate. The isolated fungi were identified as *Alternaria solani* according to Perez and Martinez (1997) and Ozcelik and Ozcelik (1997).

A. solani and other *Alternaria* spp. could be isolated from infected plant tissues but it was difficult to maintain these isolates on agar media and produce conidia. Often sporulation capacity declines or is lost after a few serial transfers on these media and the colonies might become completely mycelial. But mostly an induction of sporulation is required when high amounts of spores are needed.

Results in Table 2 show that tomato leaves and fruits agar media +UV was the most effective medium in inducing sporulation of *A. solani* which gave 6.2 spores /field followed by tomato leaves agar media + UV 4.53 number of spores/field. While fruits tomato agar +UV medium was the least effective one in inducing sporulation of *A. solani*. Data also indicate that the other media don't exhibit very slight of or were nell sporulation.

Table 1. Percentage of tomato early blight disease incidence, disease severity and number of infected fruits at different localities of El- Sharkia governorate during two successive growing seasons (2004-2005 and 2005-2006)

Localities	Percentage of disease incidence			Number of fruit infection			Disease severity		
	2004-2005	2005-2006	Mean	2004-2005	2005-2006	Mean	2004-2005	2005-2006	Mean
EL-Hossania	32.56	48.09	40.32	4.66	5.66	5.16	10.10	16.13	13.11
Diarb negm	37.14	50.03	43.37	5.33	8	6.66	12.78	19.51	16.14
El-Ebrahimia	47.28	58.09	52.68	9	8.33	10.66	17.52	25.55	21.53
Abo -Hammad	22.04	40.61	31.32	3.66	5	3.33	7.98	14.13	11.05
Average	34.75	49.20		5.66	6.74		12.09	18.83	
L.S.D at5%	5.21	4.91	2.13				3.72	2.35	1.56

Table 2. Effect of different media on *A. solani* sporulation

Media	Number of spores/field
Potato dextrose agar (PDA)	0.00
Czpex dox agar	0.00
V ₈ juice agar	0.2
S-medium	0.06
Cutting of PDA media	0.00
Water agar (WA)	0.00
Fruits tomato agar media	0.20
Fruits tomato agar media + UV	1.46
Leaves tomato agar media	0.20
Leaves tomato agar media +UV	4.53
Leaves and fruits tomato agar media	0.20
Leaves and fruits tomato agar media +UV	6.2
L.S.D at 5%	0.43

Similar results were obtained by Benlioglu and Delen, (1996) and Brji, (1967) who mentioned that, commonly used methods for sporulation induction include growing the fungus on tomato media, then expose to Ultra Violet light (UV) was the best way to encourage the sporulation while tomato media without exposure Ultra Violet light, V₈ juice agar (Miller (1955), S-medium (Shahin and Shepard, 1979) gave few numbers of spores.

Pathogenicity tests of the isolated fungi on Gs cv. (using detached leaf technique Table 3 show variation in their reactions. However, the highest pathogenic ability of the disease incidence (mean number of lesion, mean diameter of lesion, infected area and total infected area) was obtained in case of Diarb Negm isolate (D₁), while the lowest pathogenic ability of the disease incidence was obtained in case of isolate E₂. Other isolates were moderately effective. In case of intact leaves Table 4, D₁ was the most pathogenic (mean disease severity and percentage of infection). While, the lowest percentage of disease incidence and disease severity was obtained with El-Ebrahimia (E₂ isolate).

Other isolates were moderately effective. Differences in the pathogenic capabilities of the tested isolates might be due to one or more of several factors related to genetic make up of host variety and pathogen as well as their interaction, or might be due to the rate of mycelial growth as well as their ability to sporulate *in vitro* (Henning and Alexander, 1959 and Stancheva and Stamova, 1990). Also, a high genetic diversity was detected among the *A. solani* isolates (VCG, Van der Waals *et al.*, 2004). In addition, pathogenic isolates of *A. solani* might produce a non-specific and/or host-specific toxins (Thomma, 2003). Furthermore, germination fluids of *A. solani* contain alternaric acid and a nontoxic substance that acts as susceptible-inducing factor (Langsdorf *et al.*, 1991). At the same time, the disease caused by *A. solani* progressively weakens the plant and increases susceptibility to infection by reducing the photosynthetic leaf area and increasing the imbalance between nutrient in the fruits and nutrient supply from the leaves (Rowell, 1953).

Susceptibility of different tomato cultivars to infection with *A. solani* was studied Tables 5 and 6. Data

Table 3. Pathogenicity test of different *A. solani* isolates on tomato Gs cv. using detached leaf technique *in vitro*

Isolates	Mean number of lesions	Mean diameter of lesions (mm)	Infected area (mm ²)	Total Infected area mm ²
EL-Ebrahimia (E ₁)	4.25	4.31	14.68	63.39
EL-Ebrahimia (E ₂)	4.08	3.70	10.85	45.75
EL-Hossania (H ₁)	5.66	5.88	27.38	154.86
EL-Hossania (H ₂)	4.41	3.81	11.56	53.19
Diarb nigm (D ₁)	6	7.73	47.09	282.58
Diarb nigm (D ₂)	5.91	6.22	30.62	181.62
Abo-Hammad (A ₁)	4.33	5.09	20.42	87.47
Abo-Hammad (A ₂)	5.66	5.13	20.71	115.87
Mean	5.00	4.62	17.38	99.61
L.S.D at 5%	0.91	0.91	7.54	44.63

Table 4. Pathogenicity test of different *A. solani* isolates on tomato Gs cv. plants using intact leaves, under greenhouse conditions

Isolates	Disease severity	Percentage of infection
EL-Ebrahimia (E ₁)	38.04	66.7
EL-Ebrahimia (E ₂)	26.28	59.06
EL-Hossania (H ₁)	44.59	74.49
EL-Hossania (H ₂)	51.90	82.00
Diarb nigm (D ₁)	67.58	90.48
Diarb nigm (D ₂)	61.27	87.51
Abo-Hammad (A ₁)	40.46	69.98
Abo-Hammad (A ₂)	52.26	84.72
Mean	74.79	76.86
L.S.D at 5%	2.57	1.94

Table 5. Susceptibility of different tomato cultivars to infection with *A. solani* using detached leaf technique *in vitro*

Cultivars	Mean number of lesions	Mean diameter of lesions (mm)	Infected area (mm ²)	Total infected area (mm ²)
Super marmand	4.91	5.20	21.34	105.08
Hyenz	2.83	3.02	7.18	20.38
Gs	6	7.00	38.46	230.76
Kcastl rock	3.33	3.90	11.96	39.98
Mean	4.26	4.78	19.73	99.05
L.S.D at 5%	1.03	0.35	2.88	24.08

Table 6. Susceptibility of different tomato cultivars against infection with *A. solani* on intact leaves, under greenhouse conditions

Cultivars	Disease severity	Percentage of infection
Super marmand	47.99	84.47
Hyenz	30.39	66.63
Gs	67.13	91.21
Kcastel rock	37.28	73.85
Mean	45.69	79.04
L.S.D at 5%	2.74	2.23

obtained indicated that, Hyenz cv. was the most resistant cultivar showing the lowest mean number of lesions 2.83, mean diameter of lesions 3.02 mm, infected area 7.18 mm² and total infected area 20.38 mm²/leaflet by detached leaf technique. The lowest disease severity 30.39% and percentage of infection 66.63% were also obtained when cultivar Hyenz was examined. However, Gs cv. was the highest susceptible one resulting the highest mean number of lesions, mean diameter of lesion, infected area and total infected area (230.76 mm² using detached leaf and the highest disease severity 67.13% and percentage of infection 91.21% under intact leaves experiment. Similar results were obtained by Chaerani *et al.* (2007a) who found that of 81 tested genotypes, 11 were highly resistant, 23 were resistant, 22 were moderately resistant and 10 were highly susceptible.

Differences between cultivars in their susceptibility might be due to differences in genetic make up, (Banerjee *et al.*, 1998 and Perez and Martine 2000), quantitative expression and polygenic inheritance of the resistance

(Thirthamalappa and Lohithaswa 2000 and Chaerani *et al.*, 2007b).

They also reported that higher chitinase and peroxidase activity was observed in infected leaves compared with uninfected ones. As well as the increase of chitinase activity being greater in resistant than in susceptible plants (Fernandez *et al.*, 1995 and Garcia *et al.*, 1998). Chemical components of the tested varieties might play a role in cultivars resistant and/or susceptibility (Fernandez *et al.*, 1999). In addition, cultivar resistance depended on the presence of proper environmental conditions such as humidity, temperature, and the pathogen inoculum (Foolad and Ntahimpera 2000).

Effect of different cultivation methods on tomato early blight disease was studied using detached leaf technique and intact leaves Table 7 and 8. Obtained results proved that transplanting was the best method in reducing total infection area (mm²) being 122.28, when detached technique was applied. In consequence the least infection percentage 82.29% and also least disease severity was obtained compare with the direct method of cultivation.

Table 7. Effect of different cultivation methods in controlling tomato early blight disease incidence using detached leaf technique (*in vitro*)

Cultivation method	Mean number of lesions	Mean diameter. of lesions (mm)	Infection area(mm ²)	Total infection area(mm ²)
Transplanting	5.25	5.44	23.27	122.28
Direct seed	6	7.60	45.55	273.34
Mean	5.62	6.52	34.41	197.81
L.S.D at 5%	n.s	0.72	11.12	76.85

Table 8. Effect of different cultivation methods in controlling tomato early blight disease incidence using intact leaves, under greenhouse conditions (*in vivo*)

Cultivation method	Disease severity	Percentage of infection
Transplanting	43.72	82.29
Direct seed	62.14	91.73
L.S.D at 5%	13.80	9.36

With transplant method the farmers choose the healthy and strong plants, with a thick stems, healthy plants, while in case of direct seed method, the plant were thin and highly susceptible (Johen and Gerber 1979).

On the other hand, direct seed method of cultivation exhibit the highest total infection area (mm) being 273.34. Detached leaves reveal highest disease severity 62.14 and in consequence the highest infection percentage 91.73 as it could be shown from both tables.

Effect of tomato leaf age on tomato early blight disease incidence was studied using detached leaf technique. Data reveal that older tomato leaf is more susceptible to be infect with *A. solani* than the younger one. Leaf No 1 and 2 were the most susceptible, showing the highest number (5.91, 5) and largest diameter of lesions (6.97, 5.64mm). In addition, the highest total blighted area (226.19, 125.92 mm²) was also obtained. Leaf No 3 and 4, exhibit (4 and 3.16), (4.43 and 3.05mm) of lesion No. and diameter and (62.01, 22.59 mm²), of the total infection area respectively. On the other hand, the youngest leaf (No.5) was the

least affected one (1.58), (1.44) and 2.70 (mm²). This was expected as the susceptibility of tomato to early blight increases with senescence. Similar results were obtained by Rotem, (1994) and Vloutoglou and Kalogerakis (2000). Several reasons reported to increase susceptibility of senescent tissues to necrotrophic pathogen, among these reasons are higher sensitivity of senescent plant tissues to pathogen toxins, to cell wall degrading enzymes and autolysis of membrane lipids (Barna and Györgyi, 1992). The differences in resistance or susceptibility of different tomato leaves might be due to correlation between carbohydrate (total soluble sugar and starch in potato leaves) and resistance to early blight infection which higher in susceptible cultivars than the resistance one (Phukan, 1995). Also might be due to anti-fungal substances (steroidal, glycoalkloid saponin) which is higher in old tomato leaves and enhanced tomato plants to be attack with phytopathogenic fungi (Phukan, 1995).

Six fungicides (Mancozeb, Copperikh, Kocide, Ridomil gold, Cure-plus and Vacomil-plus) were tested for their effect on mycelial

growth and spore germination of *A. solani* under laboratory conditions and also on disease incidence using detached leaf technique and intact leaves under greenhouse conditions.

Obtained data as it could be shown from Tables 10 and 11 indicate that increasing concentration of tested fungicides resulted in a decrease of mycelial growth and number of germinated spores of *A. solani*. All tested

fungicides reduced *A. solani* growth and number of spore germination. Mancozeb was the most effective one, while Copperikh was the least effective fungicide in reducing mycelial growth and number of germinated spores of *A. solani*. Other fungicides were moderately effective. These results were in agreement with those obtained by (Prasad and Naik 2003 and Holm *et al.*, 2003).

Table 9. Effect of tomato leaf age on tomato early blight disease incidence using detached leaf technique

Leaf stage	Number of lesion.	Diameter of lesion (mm)	Infected area (mm ²)	Total infected area (mm ²)
Leaf No. 1	5.91	6.97	38.21	226.19
Leaf No. 2	5	5.64	25.15	125.92
Leaf No. 3	4	4.43	15.48	62.01
Leaf No. 4	3.16	3.05	7.40	22.59
Leaf No. 5	1.58	1.44	1.74	2.70
L.S.D at 5%	0.76	0.76	5.25	2.3

Table 10. Effect of different fungicide concentrations on *A. solani* linear mycelial growth *in vitro*

Fungicides	Linear area	Concentration (ppm.)									Mean
		0	25	50	100	125	200	250	500	1000	
Mancozeb	Dim.	90.00	68.33	59.33	47.66	30.00	29.00	26.66	22.00	0.00	35.37
	Red.	0.00	22.40	34.04	47.00	66.60	67.77	70.37	75.55	100	60.46
Copperikh	Dim.	90.00	90.00	90.00	89.33	87.00	84.00	74.66	53.66	47.33	88.24
	Red.	0.00	0.00	0.00	0.74	3.33	6.66	17.01	40.37	46.98	14.38
Kocid	Dim.	90.00	81.66	73.00	62.00	51.66	41.66	32.33	22.66	19.33	59.28
	Red.	0.00	9.25	18.86	31.11	42.56	53.7	64.07	74.81	78.51	46.60
Redomil gold	Dim.	90.00	90.00	86.66	81.66	78.00	64.33	57.66	37.33	15.33	75.12
	Red.	0.00	0.00	3.7	9.25	13.33	28.49	35.88	58.51	82.96	29.01
Cure-plus	Dim.	90.00	90.00	73.00	54.00	52.00	23.00	15.00	8.0	0.00	50.62
	Red.	0.00	0.00	18.80	40.00	42.22	74.44	83.33	91.11	100	56.23
Vacomil-plus	Dim.	90.00	90.00	87.33	75.33	48.66	30.66	26.66	19.00	2.33	58.74
	Red.	0.00	0.00	2.96	16.25	45.92	65.99	70.36	78.88	97.40	47.25
L.S.D at 5%			0.05	1.84	3.72	2.44	5.54	2.52	3.55	2.95	3.43

Dim = mycelial growth dimeter percentage

Red = reduction percentage of mycelial growth

Table 11. Effect of different fungicide concentrations on *A. solani* spore germination

Fungicides	Con.	% of spore germination									Mean
		0	25	50	100	125	200	250	500	1000	
Vacomil- plus	G.	90.95	60.28	56.88	46.73	39.55	35.37	31.77	25.18	16.36	44.78
	Red.	0.00	33.66	37.42	48.69	56.50	61.03	65.05	72.48	81.95	50.75
Cure-plus	G.	90.95	70.27	65.14	53.41	44.18	39.36	32.47	25.55	18.30	43.93
	Red.	0.00	22.75	28.23	41.29	51.43	56.65	64.19	71.88	79.91	46.25
Kocide	G.	90.95	82.65	75.98	68.35	56.66	50.76	45.73	38.38	33.48	60.32
	Red.	0.00	9.07	20.08	24.77	37.63	44.20	49.61	57.18	63.17	33.96
Mancozeb	G.	90.95	52.75	45.15	37.62	31.86	23.4	18.67	13.46	0.00	34.87
	Red.	0.00	41.94	50.38	57.66	64.94	74.26	79.39	85.22	100	61.53
L.S.D at 5%			3.61	2.86	3.80	4.87	3.24	3.40	4.24	1.91	2.92

Ger = germinated spores percentage

Red = reduction percentage of spore germination

Obtained results of detached leaf technique showed that all tested fungicides decreased disease incidence, also disease incidence was reduced with increasing the fungicide doses Table, 12. One day later of application Mancozeb was the most effective fungicide in reducing mean number of lesions (1.70), diameter of lesion (1.55mm), infected area (41.70 mm²) and percentage of disease protection (74.99%). However, Kocide was the least effective one. Other fungicides were moderately effective on tomato early blight disease. Generally, the tested fungicides were more effective when applied at the same time of *A. solani* inoculation.

Results obtained from intact leaves showed that all tested fungicides decreased disease incidence, also disease incidence decreased with increasing the fungicide concentrations Table 13. Mancozeb was the most effective one in decreasing disease severity and infection percentage (25.94% and 61.55%, respectively) However, Kocide was the least effective one (48.42% and 87.82% respectively). Similar results were obtained by (Keinath, *et al.*, 1996;

Patil, *et al.*, 2001 and Aslam khan *et al.*, 2003).

The chemical class of the ethylene bisdithiocarbamates (EBDCs) includes the preventative fungicides Mancozeb, Maneb, and Metiram. This class has broad-spectrum activity and a multi-site mode of action, is generally effective in controlling early blight, and its constituent chemicals break down to cyanide, which reacts with thiol compounds in the cell and interferes with sulfhydryl groups Eckert, (1988). Because of their multi-site mode of action the risk of plants becoming resistant to these fungicides is generally considered low Georgoplus, (1997). Mancothane (manganese ethylene bisdithiocarbamate complex with zinc salt) also possesses a multi-site mode-of-action, and inhibit enzyme activity in fungi by forming a chemical complex with metal-containing enzymes, including those involved in the production of ATP. Mancothane, which is also known as Mancozeb, Tatodust, Dithane M-45, and Manzate, is rapidly degraded in the environment by hydrolysis, oxidation, photolysis, and plant metabolism (Tomlin, 2000).

Table 12. Effect of different concentrations of selected fungicides on tomato early blight disease incidence using detached leaf technique

Fungicides	Concentration g/100L water	Number of lesions.		Diameter of lesion (mm)		Infected area (mm)		% of protection	
		At the same time	One day later	At the same time	One day later	At the same time	One day later	At the same time	One day later
Vacomil-plus	0	6	6	5.96	5.96	166.79	166.79	0	0
	75	0	1.91	0	1.08	0	1.77	100	98.93
	150	0	1.16	0	0.70	0	0.45	100	99.72
	300	0	0.25	0	0.32	0	0.02	100	99.98
	Mean	1.5	2.33	1.49	2.01	41.69	42.25	75	74.65
Cure-plus	0	6	6	5.96	5.96	166.79	166.79	0	0
	75	0	2.41	0	2.16	0	8.97	100	94.58
	150	0	1.91	0	1.75	0	4.40	100	97.35
	300	0	0.83	0	1.19	0	1.7	100	98.97
	Mean	1.5	2.78	1.49	2.76	41.69	45.46	75	72.72
Kocide	0	6	6	5.96	5.96	166.79	166.79	0	0
	75	2.08	4.33	0.89	3.97	1.41	53.08	99.15	68.06
	150	0.83	3.25	0.38	2.97	0.10	22.7	99.93	86.33
	300	0	2.83	0	2.31	0	11.86	100	92.86
	Mean	2.22	4.10	1.80	3.80	42.07	63.60	74.77	61.81
Mancozeb	0	6	6	5.96	5.96	166.79	166.79	0	0
	125	0	0.66	0	0.20	0	0.03	100	99.99
	250	0	0.16	0	0.05	0	0.0006	100	99.99
	500	0	0	0	0	0	0	100	100
	Mean	1.5	1.70	1.49	1.55	41.69	41.70	75	74.99
L.S.D at 5%		0.24	0.41	0.16	0.41	5.43	6.47	0.05	2.13

Table 13. Effect of different concentrations of selected fungicides on tomato early blight disease incidence under greenhouse conditions

Fungicides	Concentration g/100L water	Disease severity	Reduction of disease severity.	Percentage infection	Reduction of Percentage infection.
Vacomil-plus	0	57.6	0.00	94.7	0.00
	75	33.4	42.00	67.48	28.14
	150	26.76	53.54	61.19	35.37
	300	18.46	67.87	56.15	40.68
	Mean	34.05	40.85	69.88	26.04
Cure-plus	0	57.6	0.00	94.7	0.00
	75	39.26	31.76	80.07	15.41
	150	32.41	43.73	72.28	23.65
	300	25.89	54.98	67.19	29.02
	Mean	38.79	32.61	78.56	17.02
Kocide	0	57.6	0.00	94.7	0.00
	75	53.14	7.75	89.94	5.07
	150	44.06	23.54	86.58	8.55
	300	38.9	32.44	80.09	15.39
	Mean	48.42	15.93	87.82	7.25
Mancozeb	0	57.6	0.00	94.7	0.00
	125	19.4	66.29	55.93	40.95
	250	15.78	72.57	50.56	45.63
	500	10.99	80.91	45.04	53.4
	Mean	25.94	73.25	61.55	34.99
L.S.D at 5%		2.66	2.74	14.31	1.86

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دراسات مرضيه على الندوة المبكرة في الطماطم

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تعتبر الطماطم أحد أهم المحاصيل التى تتبع العائلة الباذنجانية، وهى واحدة من أهم محاصيل الخضراوات الاقتصادية فى جمهورية مصر العربية. تصاب الطماطم بالعديد من الفطريات الممرضة و التى تسبب فقدا فى الإنتاج و الجودة. ومن أهم تلك الأمراض، مرض الندوة المبكرة والذى يتسبب عن الفطر أترناريا سولانى. وقد أظهرت نتائج حصر المرض أنتشاره فى نطاق واسع من محافظة الشرقية وذلك أثناء موسمى الزراعة ٢٠٠٤-٢٠٠٥ و ٢٠٠٥-٢٠٠٦ حيث كانت أعلى نسبة أصابه بالمرض فى مركز الإبراهيمية بينما أقل نسبة للإصابة كانت فى مركز أبو حماد وذلك خلال فترة الحصر المذكورة سابقا. تم عزل المسبب المرضى من عينات أوراق و ثمار طماطم التى تظهر عليها أعراض الندوة المبكرة و التى جمعت من مناطق الحصر و تم تعريفه على أنه فطر أترناريا سولانى. وفى اختبارات المعمل تبين أن بيئة أوراق و ثمار الطماطم بعد تعريضها للأشعة فوق البنفسجية أفضل بيئة ينتج الفطر عليها جراثيمه. أظهرت اختبارات العدوى أن العزلات المختلفة لفطر أترناريا سولانى اختلفت فى مدى قدرتها المرضية على إصابة نباتات الطماطم من صنف جى اس. وفى اختبار مقاومة أصناف الطماطم المختلفة للإصابة كان صنف هاينز أكثر الأصناف المختبرة مقاومة للإصابة بينما الصنف جى اس كان أكثر الأصناف المختبرة قابلية للإصابة بالمرض بينما الأصناف الأخرى كانت متوسطة القابلية للإصابة. و كانت أوراق الطماطم الأكبر عمرا أكثر حساسية للإصابة بالفطر المسبب من الأوراق الحديثة. أما طريقة الزراعة بالشتل فكانت أفضل من زراعة البذور مباشرة حيث قل معها حدوث الإصابة وتبين من اختبار المبيدات الفطرية على المسبب المرضى أن مبيد الماتكوزيب أكثر المبيدات قدرة على تثبيط النمو الميسليومى و إنبات جراثيم الفطر و كذلك شدة الإصابة سواء فى المعمل أو الصوبه ينيه مبيد الكيوروبلاس والفاكوميل بلاس.