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**To,**

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**Dear authors,**

I have immense pleasure to inform that your research manuscript BJAS/ 119-19, Entitled “**A study of eggplant leaf spot disease in greenhouses in Basrah Province**” has been accepted and the editorial board agreed to publish this paper in the forthcoming issue of the Basrah Journal of Agricultural Sciences (BJAS), Vol. 32[Special Issue], 2019. As per management policy you are required to pay the amount below in advance for scheduling your paper for online / print publication. Then your article will be freely available on our journal website.

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**Prof. Dr. Asaad R. S. Al-Hilphy**

**Editor in Chief**

# **A study of eggplant leaf spot disease in greenhouses at Basra Province**

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## **Abstract**

The present study has been conducted to isolate and identify the leaf fungal spot pathogens of eggplant at different regions of Basra city and their chemical control. The results showed that the disease was spread at all regions cultivated with eggplant at Basra province with infection percentages of 46.6-88.5%. The highest infection percent was recorded at Garmat Ali region (88.5%), while the lowest percent was recorded at the Agricultural station (46.6%). The highest percent of disease severity (50 %) was recorded at Tomato Cultivars Breeding Project, while the lowest percent was recorded at Agricultural station (20%). *Alternaria alternata*, *Cladosporium oxysporum* and *Curvularia lunata* were isolated from the leaves infected with spot disease, the pathogenicity test revealed that all isolated fungi were pathogenic. *C. oxysporum* and *C. lunata* were recorded for the first time in Iraq as causal agents of eggplant leaf spot. The results showed that the fungicide Ortiva inhibited the growth of the fungi with an average of 95.7%, followed by Difecor ( 94.9% ), additionally, it was noticed that all examined fungicides completely inhibited the growth of both *C. oxysporum* and *C. lunata* ( %100 for each one). The study revealed that the fungicide Ortiva significantly reduced the disease severity of eggplant leaves spot disease up to 11.6% compared with control treatment which was 25%. Finally the results explained that the fungicide Ortiva gave the best protection to eggplant which infected with leaves spot disease compared to the other fungicides, it achieved a best plant height, higher dry weight of shoot and root systems, higher leaf area and higher leaves number. Therefore it is recommended for controlling the spot disease.

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Key words: Eggplant, Fungi, Fungicides, Leaf spot, Ortiva.

## **Introduction:**

Eggplant (*Solanum melongena* L.) which belongs to the family Solanaceae is considered one of most important vegetable crops (Choudhary, 1976) containing vitamins A, B1, B2, B9 and C, besides minerals P, Mg, Fe, Zn, Na and Cu (Salunkhe and Kadam, 1998; Gopalan *et al.*, 2007). Eggplant is infected by several fungal diseases, and the leaf spot disease is one of most important diseases which caused by many fungal pathogens such as: *Alternaria alternata*, *A. tenuissima*, *Cercospora melongenae*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Phomopsis vexans*, *Septoria lycopersici* and *Stemphylium solani* (Sivanesan, 1987; Thomma, 2003; Jiang and Zhang, 2007; Kamaludeen and Lal, 2013; Chaudary *et al.*, 2016; Xie *et al.*, 2016;

Matrood, 2018). The diseases' symptoms represented by small, yellow, brown or black rounded to elongate spots on the leaves that differed according to the causal agents (Raja et al, 2005; Manamgoda *et al.*, 2011; Chaudary *et al.*, 2016).

Leaf spot disease can be controlled by chemical fungicides, so the chemical control was more effective for controlling the plant diseases (Marsh, 1977 and Siedle, 1995). Some fungicides were previously used for controlling the spot disease such as Iprodione, Mancozeb, Ortiva, Score and Topas (Babu, 2000; Prasad, 2002; Al-Zubaidy, 2005; Panwar *et al.*, 2014).

Nowadays it was found that the leaves spot disease was spread on eggplant which cultivated in greenhouses at Basra Province. Because of the crop importance and the little studies on this disease, therefore this study was carried out to study the following aims:

- 1 – Field survey of leaves spot disease on eggplant in plastic houses.
- 2 – Isolation and identification of the fungi associated with the disease.
- 3 – Testing the pathogenicity of the isolated fungi.
- 4 – Evaluation of some fungicides efficiency against spot disease.

## **Materials and methods**

### **Field survey and estimation of infection percentage:**

A field survey was conducted to estimate the infection percentage with leaf spot disease in greenhouses at Basra Province in the growing season of 2016-2017. The survey included the regions: Garimat Ali, Shatt Al-Arab, Zubair, Qurnah, Agricultural station, and Hybrid Tomato Breeding Project. Three standard greenhouses were randomly chosen for each region and the infection percentage was calculated as the following:

$$\text{Infection percentage} = \frac{\text{Number of infected plants}}{\text{Number of total plants}} \times 100$$

### **Evaluation of disease severity:**

Disease severity was evaluated by taking 10 eggplant leaves randomly from each greenhouse and location according to a scale based on 0 to 3 index, where 0 = symptomless leaves, 1 = 1-3 spots, 2 = 4-6 spots and 3 = more than seven spots. The percentage of disease severity was calculated according to McKinney (1923) cited from Al-Waily (2004) as the following:

$$\begin{aligned} \% \text{Disease severity} \\ = \frac{\text{Sum}(\text{leaves number in each degree} \times \text{degree number})}{\text{Total leaves} \times \text{higher degree}} \times 100 \end{aligned}$$

### **Isolation and identification of fungi causing leaves spot:**

Samples of infected eggplant leaves with spots were collected, carefully washed with tap water, cut to 1cm pieces, sterilized with a concentration of %10 NaOCl for 2 minutes, washed with sterile distilled water to remove NaOCl residues and carefully blotted by filter papers. Five pieces were put in each plate containing sterile medium (PDA) with 250 mg/L Chloramphenicol, with three replicates for each one and incubated at  $25\pm 2^{\circ}\text{C}$ . After 3-5 days, fungi purified by taking a 5 mm disk from the tip of seven days old colony by Cork borer, added to PDA and incubated for 3-5 days. The fungi identified according to their macro and microscopic features by preparing lactophenol slides and using compound microscope named Optika B-180 according to Ellis (1971 and 1976), Sivanesan (1987) and Bensch *et al.* (2012).

### **Preparation of fungal spore suspension:**

The fungi were grown on PDA in plastic plates for 10 days in an incubator at  $25\pm 2^{\circ}\text{C}$ . Ten ml of distilled water were added to each plate and well shaken to make a suspension. The suspension filtered by three layers of gauze. The spore number was estimated by using hemocytometer and adjusted to  $10^5$  spores/ml.

### **Testing the pathogenicity of the isolated fungi:**

The eggplants (Barcelona cultivar) were grown in plastic pots which were treated with fungal suspension ( $10^5$  spores/ml) of each isolated fungus. A 2L handle atomizer was used to spray the fungal suspension on the plant leaves, control treatment was sprayed with distilled water only. The plants were covered with polyethylene bags for two days to keep the moisture, then the covers were removed and the plants were dropped out in a plastic house for observing the symptoms.

### **Effect of some fungicides on fungal growth on PDA:**

The medium Potato Dextrose Agar (PDA) was prepared, poured in 250 ml conical flasks (150 ml for each flask) and sterilized in an autoclave at  $121^{\circ}\text{C}$  and 15 pounds/inch<sup>2</sup> for 30 minutes. Four fungicides named: Difecor (Difenconazole), Ortiva (Azoxystrobin), Score (Tebuconazole) and Topas (Benconazole %10) with recommended concentrations 0.4, 0.6, 0.4 and 0.4 ml/L respectively, were added to each flask, well homogenized and poured in plastic plates. A disk (0.5 cm diameter) of each fungus was taken from the seven days old colony margin and cultured in each plate with three replicates for each treatment, while the distilled water was added to control treatment. All plates were incubated at  $25\pm 2^{\circ}\text{C}$  in the incubator. When the fungal growth in the control treatment reached to the plate margin, the radial growth was measured by using an average of two vertical diameters passed throw the reverse of plate center. The inhibition percentage was estimated according to Abbott equation (1925) which cited from Shaaban and Al-Mallah (1993) as the following:

$$\%Inhibition = \frac{\text{Control radial growth} - \text{Treatment radial growth}}{\text{Control radial growth}} \times 100$$

## **Effect of some fungicides on disease-severity:**

Four selective fungicides named Difecor, Ortiva, Score, and Topas were used to study the growth inhibition of the isolated fungi *A. alternata*, *C. oxysporum* and *C. lunata*, the causal agents of leaf spot disease. Healthy eggplant plants were transplanted to plastic pots. Artificial infection with a spore suspension of the fungi mentioned-above which previously prepared was carried out and the leaves packed with polyethylene bags for two days. Then the bags removed. After three days the treated plants with fungal suspensions were divided into five groups, four groups were completely sprayed with 1 ml/L from each one of the four fungicides mentioned-above by using 2 L handle atomizer. The fifth group was sprayed with distilled water as a control treatment. After 10 days the disease severity was estimated as formerly described. The experiment was replicated three times for each treatment.

## **Field Experiment:**

### **Soil preparation and transplanting:**

A greenhouse experiment was carried out in Agricultural station of College of Agriculture. The greenhouse (16 X 4 m) was prepared and divided into three rows (1.5 m between one row and another and 40 cm between plant and another). The plants were transmitted to the greenhouse and planted with three plants in each pit. Dripping irrigation method was used. The plants were fertilized by Urea (1 gm/L water). NPK 20-20-20 (0.5 gm/L water) was also used after one week from planting. NPK 20-48-20 (1 gm/L water) also added after three weeks. The plants divided into two groups, the first group was treated with isolated fungi, while the other group was considered as control treatments.

### **Treatment of plants with fungal suspensions and fungicides:**

Two months old eggplant leaves were wounded by sterilized needle and treated with fungal spore suspension which prepared as formerly described, using 2L handle atomizer. After three days the plants divided into five groups, four groups were completely sprayed with 1 ml/L from each of the fungicides were performed as a foliar spray using 2L handle atomizer, the fifth group was sprayed with distilled water. All treatments were performed with three replicates.

The following treatments were applied:

*Alternaria alternata* + 1 ml/L Difecor, *A. alternata* + 1 ml/L Ortiva, *A. alternata* + 1 ml/L Score, *A. alternata* + 1 ml/L Topas, *Cladosporium oxysporum* + 1 ml/L Difecor, *C. oxysporum* + 1 ml/L Ortiva, *C. oxysporum* + 1 ml/L Score, *C. oxysporum* + 1 ml/L Topas, *Curvularia lunata* + 1 ml/L Difecor, *C. lunata* + 1 ml/L Ortiva, *C. lunata* + 1 ml/L Score, *C. lunata* + 1 ml/L Topas, *A. alternata* alone, *C. oxysporum* alone and *C. lunata* alone.

### **Parameters and features:**

The parameters, plant height (cm), shoot and root systems dry weight (gm), leaf area (cm<sup>2</sup>) and leaves number (leaf/plant) were measured at the end of the experiment to evaluate the effect of fungicides on eggplant leaf spot disease.

### **Statistical analyses:**

The laboratory experiments were analyzed by using Completely Randomized Design (CRD), while the field experiments were analyzed by using Randomized Completely Block Design (RCBD), a least significant difference (LSD) was used to compare means, all treatments were repeated for three times (Al-Rawi and Khalaf-Allah, 1980). All statistical analyses were conducted by using GenStat program.

### **The results and discussion:**

#### **Infection percentage and severity:**

The results of field survey of eggplant leaf spot disease in greenhouses at some regions Garmat Ali, Shatt Al-Arab, Zubair, Abu Al-Khaseeb, Agricultural station, Hybrid Tomato Cultivars Breeding Project, and Qurnah revealed that the higher percentage of infection (88.5%) was recorded at Garmat Ali, followed by Shatt Al-Arab with a percent of 85%, while the lowest percentage was recorded at Agriculture College Fields which was 46% (Table 1).

The infection severity in the same regions also estimated. It was found that the higher infection severity (50%) was noticed at Hybrid Tomato Cultivars Breeding Project, while the lowest one was noticed at Agriculture College Fields which reached 20% (Table 1). The high percent of infection at Garmat Ali could be attributed to the carelessness of agricultural processes and not application the chemical control against diseases, while the lowest infection percentage at Agriculture College Fields may be as a result of good agricultural processes, good fertilization, good disease control and the type of cultivated variety.

#### **Isolation and identification of fungi causing eggplant leaf spot disease:**

Three species of fungi *Alternaria alternata*, *Cladosporium oxysporum* and *Curvularia lunata* were isolated from eggplant leaves infecting with spot disease which planted in greenhouses (Table 2). *C. oxysporum* and *C. lunata* were recorded for the first time in Iraq as causal agents of eggplant leaf spot disease. The fungi features were in agreement with Eliss (1971 and 1976), Sivanesan (1987) and Bensch *et al.* (2012).

Table (1) Infection percentage and severity of eggplant leaf spot disease in greenhouses at Basra Province.

Region	% Infection	% disease severity
Garmat Ali	88.5	27
Shatt Al-Arab	85	37
Hybrid Tomato Cultivars Breeding Project	78.5	50
Abu Al-Khaseeb	64.2	45
Qurnah	57.8	30
Zubair	50	25
Agriculture College Fields	46.6	20
LSD 0.05	25.52	21.71

Table (2) fungi isolated from eggplant leaves infected with leaf spot disease

No.	Fungi
1	<i>Alternaria alternata</i>
2	<i>Cladosporium oxysporum</i>
3	<i>Curvularia lunata</i>

### Pathogenicity test:

The pathogenicity test results revealed that *Alternaria alternata*, *Cladosporium oxysporum* and *Curvularia lunata* have the ability for causing the eggplant leaf spot disease. The symptoms appeared as small angular brown spots surrounded or not surrounded with yellow rings, enlarged with time to cover most leaf area. This result was in agreement with many studies explained that the eggplant leaf spot can be caused by *A. alternata*, *C. lunata* and *C. cladospoides* (Raja *et al.*, 2005; Hassan *et al.*, 2013; Chaudary *et al.*, 2016; Matrood, 2018). Also, El-Meleigi *et al.* (1986), Abdul Qader and Mohammed (1997), Al-Zayat *et al.* (2002) and Al-Zubaidy (2005) mentioned that many fungi can cause the spots on date palm leaves and other plants in different regions, among them *A. alternata*, *C. herbarum* and *Cladosporium* spp.

## Effect of some fungicides on the growth of fungi caused eggplant leaf spot on PDA:

The study showed the ability of examined fungicides for inhibition the isolated fungi growth on PDA (Table 3). It was found fungicides Ortiva significantly inhibited the fungal growth with an average of 95.7%, followed by Difecor and Topas with an average of 94.9 and 94.8% respectively, while Score inhibited the growth with a percent of 89.3%. Also, the results indicated that all fungicides inhibited the growth of both fungi *C. oxysporum* and *C. lunata* (100%), while they inhibited the growth of *A. alternata* in a percent of 81.1% with significant differences between them. This study agreed with Chatidimopoulos and Pappas (2016) and Galves *et al.* (2016) who found that the fungicide Ortiva inhibited the growth of pear leaves spot disease caused by *Septoria pyricola* and garlic leaves spot disease caused by *Stemphylium* sp. This result was also in agreement with Al-Zubaidi (2005) who found that the fungicide Topas was one of the best fungicides using for inhibition the growth of date palm leaves spot pathogens. The high inhibition ability may be caused by oxidation and reduction processes or by the effect on enzymes, then they effect on energy and protein production and lead to negative effects on fungal growth (Shaaban and Al-Mallah, 1993; Al-Adel, 2006). So the resistance of some fungi against other fungicides may be attributed to their ability for degrading the fungicide particles (Bedan,1996).

Table (3) Effect of some fungicides on the growth of some fungi causing eggplant leaf spot disease

Fungicides Fungi	% Inhibition				Fungus means
	Ortiva	Score	Difecor	Topas	
<i>A.alternata</i>	87.3	68	84.8	84.5	81.1
<i>C.oxysporum</i>	100	100	100	100	100
<i>C.lunata</i>	100	100	100	100	100
Fungicide means	95.7	89.3	94.9	94.8	
LSD <sub>0.01</sub> Fungicides = 2.35 ; Fungi = 1.8 ; Interaction = 3.7					

\*Each number in the table represents an average of three replicates.

## Effect of some fungicides on disease severity of eggplant leaf spot disease:

The results elucidated that the fungicide Ortiva significantly reduced the disease severity of eggplant leaf spot up to 11.6%, followed by Topas with a percent of 13.6% compared with control treatment which was 25% (Table 4). The other two fungicides Difecor and Score have no significant effects compared with control. This



result was in agreement with Willis and Mavuso (2007) who mentioned that the fungicide Ortiva was very effective against avocado leaf spot caused by *Pseudocercospora purpurea*. Also, it was found that Ortiva can be used against garlic spot disease (Galves *et al.*, 2016). Rohini *et al.* (2015) found that the fungicide Carbendazim 50% gave the highest percentage of eggplant leaf spot disease protection. The effect of fungicides on the disease severity may be caused by their strong connection with pathogen hyphae and effect on cell mitosis and lead to reduce its division (Dane, 2005).

Table (4) Effect of some fungicides on % disease severity of eggplant leaf spot disease

Fungicides Fungi	% disease severity					Fungus means
	Control	Ortiva	Difecor	Score	Topas	
<i>A.alternata</i>	30	13	25	28	15	23.2
<i>C.oxysporum</i>	25	12	28	22	12	18.2
<i>C.lunata</i>	20	10	20	17	14	16
Fungicide means	25	11.6	24.3	22.3	13.6	
LSD <sub>0.01</sub> Fungicides = 3.35 ; Fungi = 5.43 ; Interaction = 7.32						

\*Each number in the table represents an average of three replicates.

### Effect of some fungicides on eggplant growth parameters in the presence of leaf spot disease

#### Plant height:

It was noticed from the Table (5) that the best height (23.89 cm) was recorded in the treatment of fungicide Ortiva, followed by Topas (23.28 cm) which were significantly differed from the control treatment (21.72 cm), while Score achieved lowest height reached 18.39 cm. The increase in plant height may belong to the fungicide ability for giving the plant enough protection against the pathogens which destroy the plant tissue and decreasing its ability for growth, therefore the plant growth was improved (Younis *et al.*, 2004; Rohini *et al.*, 2015).

Table (5) Effect of some fungicides on plant height (cm) in the presence of eggplant leaf spot pathogens

Fungicides Fungi	Plant height (cm)					Fungus means
	Control	Ortiva	Difecor	Score	Topas	
<i>A.alternata</i>	24.00	25.17	22.83	20.00	19.33	22.27
<i>C.oxysporum</i>	20.67	22.17	17.33	18.00	25.67	20.77
<i>C.lunata</i>	20.50	24.33	15.00	19.00	24.83	20.73
Fungicide means	21.72	23.89	18.39	19.00	23.28	
LSD <sub>0.05</sub> Fungicides = 1.28 ; Fungi = 1.11 ; Interaction = 3.32						

\*Each number in the table represents an average of three replicates.

### Dry weight of shoot and root systems:

The experiment results showed that the high shoot system dry weight (5.16 gm) was reported when the fungicide Ortiva was applied, it was significantly differed from other fungicides treatments, while it had no significant differences with control treatment (4.62 gm), whereas the fungicide Score gave the lowest dry weight reached 3.47 gm (Table 6). From another hand, Table (7) revealed that the high root system dry weight (0.81 gm) was achieved in the fungicide Ortiva treatment with significant differences with the fungicides Score and Difecor which were 0.60 and 0.58 gm respectively. The fungicide may inhibits the fungal enzymes such as cellulase, lipase, and proteinase which have negative effects on plant growth (Fawzi *et al.*, 2009), therefore the plant growth improved when the fungicide was applied.

### Leaf area:

The study explained that the best leaf area reached 86.7 cm<sup>2</sup> was recorded in the treatment of fungicide Ortiva with significant differences with all other treatments including control treatment which reached 76.4 cm<sup>2</sup>, while Difecor gave a less leaf area reached 63.2 cm<sup>2</sup> (Table 8). It was found from the studies that the fungicide Ortiva inhibits the leaf spot disease such as avocado leaf spot (Willis and Mavuso, 2007), therefore the leaves become more healthy when the fungicide be used.

### Leaves number:

It was showed (Table 9) that the fungicide Ortiva treatment achieved a higher leaves number reached to 25.56 leaf/plant with significant differences with other three fungi Topas, Score and Difecor which gave 18.11, 17.56 and 15.22 leaf/plant respectively. Some studies showed that the leaf spot disease can be inhibited when

Ortiva used (Galves *et al.*, 2016), as a result of that, the leaves number significantly increase.

Table (6) Effect of some fungicides on shoot system dry weight (gm) in presence of eggplant leaf spot pathogens

Fungicides Fungi	Shoot system dry weight (gm)					Fungus means
	Control	Ortiva	Difecor	Score	Topas	
<i>A.alternata</i>	4.86	5.39	3.99	3.91	5.07	4.64
<i>C.oxysporum</i>	5.24	4.78	2.79	4.10	3.20	4.02
<i>C.lunata</i>	3.77	5.31	3.63	3.48	3.90	4.02
Fungicide means	4.62	5.16	3.47	3.83	4.06	
LSD <sub>0.05</sub> Fungicides = 0.82 ; Fungi = 0.53 ; Interaction = 1.44						

\*Each number in the table represents an average of three replicates.

Table (7) Effect of some fungicides on root system dry weight (gm) in the presence of eggplant leaf spot pathogens

Fungicides Fungi	Root system dry weight (gm)					Fungus means
	Control	Ortiva	Difecor	Score	Topas	
<i>A.alternata</i>	0.86	0.85	0.61	0.48	0.81	0.72
<i>C.oxysporum</i>	0.99	0.81	0.42	0.67	0.59	0.69
<i>C.lunata</i>	1.07	0.76	0.77	0.60	0.79	0.80
Fungicide means	0.97	0.81	0.60	0.58	0.73	
LSD <sub>0.05</sub> Fungicides = 0.18 ; Fungi = 0.10 ; Interaction = 0.33						

\*Each number in the table represents an average of three replicates.

Table (8) Effect of some fungicides on leaf area (cm<sup>2</sup>) in the presence of eggplant leaf spot pathogens

Fungicides Fungi	Leaf area (cm <sup>2</sup> )					Fungus means
	Control	Ortiva	Difecor	Score	Topas	
<i>A.alternata</i>	67.2	86.1	76.3	67.0	74.9	74.0
<i>C.oxysporum</i>	86.7	89.0	54.9	65.8	88.7	74.5
<i>C.lunata</i>	76.4	84.9	70.7	56.8	67.2	74.9
Fungicide means	76.8	86.7	67.3	63.2	76.9	
LSD <sub>0.05</sub> Fungicides = 3.40 ; Fungi = 1.40 ; Interaction = 8.33						

\*Each number in the table represents an average of three replicates.

Table (9) Effect of some fungicides on leaves number (leaf/plant) in presence of eggplant leaf spot pathogens

Fungicides Fungi	Leaves number (Leaf/plant)					Fungus means
	Control	Ortiva	Difecor	Score	Topas	
<i>A.alternata</i>	26.00	28.33	18.67	17.00	20.33	22.7
<i>C.oxysporum</i>	23.67	22.67	14.67	12.00	15.00	17.60
<i>C.lunata</i>	25.33	25.67	19.33	16.67	19.00	21.20
Fungicide means	25.00	25.56	17.56	15.22	18.11	
LSD <sub>0.05</sub> Fungicides = 4.11 ; Fungi = 3.70 ; Interaction = 7.60						

\*Each number in the table represents an average of three replicates.

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