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Dramatic biochemical and anatomical changes in eggplant due to infection with *Alternaria solani* causing early blight disease

Haifa Abdulaziz S Alhaithloul[1], Mohamed S Attia[2], Mohamed A Abdein[3]
[1] Biology Department, College of Science, Jouf University, Sakaka, Kingdom of Saudi Arabia
[2] Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt
[3] Biology Department, Faculty of Arts and Science, Northern Border University, Rafha, Kingdom of Saudi Arabia

Abstract
The purpose of the present study is to investigate the biochemical and anatomical responses in eggplant infected with *Alternaria solani* isolate responsible for early blight disease. Disease symptoms, physiological, antioxidant enzyme activities as well as anatomical changes as due to *Alternaria solani* infection in eggplant plants were determined. All growth characters, photosynthetic pigments, total soluble carbohydrate as well as total indole in shoots was shown to be significantly decreased due to *A. solani* infection after (20- and 40-days following inoculation. Total soluble protein on the other hand, was slightly decreased, during first stage of growth, with increases in total soluble protein contents as compared with uninfected plants during second stage of growth. Total phenol contents, oxidative enzyme activities and endogenous hormonal contents (IAA, GA_3, ABA and SA) of eggplant were increased compared to uninfected plants. The mesophyll cells of challenged plants appeared relatively small, compact or without intercellular spaces.

Keywords: biochemical reaction, histopathological studies, endogenous hormonal contents, defense enzymes

Introduction
Eggplant (*Solanum melongena*) is a warm season vegetable cultivated worldwide for its fleshy fruits and it is a second important vegetable crop next to potato in Egypt. The total world production of eggplant is about 32 million tons. The fruits rich in 1.4 g proteins, 4.0 g carbohydrates, 0.3 g fat, 18 mg calcium, 2.0 mg potassium and 0.9 mg iron per 100 g of edible portion. It also provides vitamins like A, B and C. (Choudhary and Gaur, 2009). Early blight disease reported on vegetable crops are being the most destructive to plant and cause considerable inhibition in yield. (Abd-El-Khair and Haggag, 2007, and Waqas *et al*., 2016) [1, 39]. Among all the fungal diseases, Alternaria leaf spot, Alternaria leaf blight and fruit rot diseases are of regular incidence at relative occurrence causing extensive damage to the quality of fruits (Ghosh *et al*., 2002). It was reported that *A. solani* caused a destructive in photosynthesis, making to inhibit mass growth. The pathogen decreases the rate of photosynthesis in infected leaves through inhibition of photosystem II activity and decrease in chlorophyll content (Hossain *et al.*, 2002) [14]. Fungi have Promotes a defense of tools, such as pathogens related proteins, to block plant resistance and/or to establish Appropriate conditions for their invasion of plant cells (Kamoun, 2007) [15]. Pathogen produce compounds that are like endogenous hormones. These compounds act as control plant development and to trigger important plant signaling events during biotic and a biotic stress (Pozo *et al*., 2015) [25].

Materials and Methods
For the present work, uniform four weeks eggplant seedlings (*Solanum melongena*) got it from Agricultural Research Center (ARC), Ministry of Agriculture, Giza, Egypt.

Isolation and maintenance of the pathogen
Infected plant organs such as leaves, fruits and stems with typical blight disease symptoms were collected from different localities 2017 cropping season. Macroscopic and microscopic examinations were made according to Ellis, 1976. Keeping fungus was maintained on PDA medium. To produce sporulation, cultures were transferred on 23-25˚C for 6 days on PDA medium. Conidial suspensions were prepared as described in Boedo *et al*., (2012) [4]. Spore density was counted by a hemocyto meter and density was adjusted to 10^6 spores per ml.

Pot experiment
The pots experiment was performed in the botanical garden of Faculty of Science, Al Azhar University Egypt in 2017. Four-week old eggplant seedlings were planted in (21 cm diameter) pots filled with autoclaved sandy loam soil (1:5, V: V) each pot contained one eggplant seedling. Complete block design was used with two controls (Healthy & infected).

Disease symptoms and disease index
Disease symptoms were assessed 15 days after inoculation and the disease index was determined according to leath *et al*., 1989 [18].

Morphological characters
Plant samples were taken from healthy and infected plants with *A. solani* at the vegetative stages (20 and 40 days after inoculation) to measure phenotypic traits stem and root lengths as well as number of leaves per plant.

Histological influences
Anatomical changes of leaves were studied according to

(Corgan and Widmoyer, 1971) [9].

Determination of metabolic changes

The Quantitative estimation of plant pigments according to Vernon and Selly, 1966 [37]. The contents of total soluble carbohydrates were estimated using another one technique according to Umbriet *et al*., 1969 [35]. Estimation of total soluble proteins according to the method of Lowery *et al.*, 1951 [21]. Estimation of phenolic compounds according to the method of Daniel and George, 1972 [10]. Peroxidase enzyme activity assayed according to that method described by Srivastava, 1987 [33]. On the other hand, the activity of polyphenol oxidase enzyme was estimated according to the method of Matta and Dimond, 1963 [23]. Determination of endogenous hormones (IAA, GA_3, ABA and SA) in the terminal buds of the infected plants as well as the control implemented as described by (Lee *et al*., 1989) [19].

Statistical analyses

Experimental results were exposed to one-way analysis of variance (ANOVA). The difference between the means was separated by using (L.S.D) at 5% level of probability using Co-state software (Snedecor and Cochran, 1982) [31].

Results

Identification of causal pathogen

Isolation of fungi was obtained from eggplant leaves and fruits, blight symptoms appeared and identified *A. solani*, (Table 1 & Figure 1) based on the morphological characteristics according to (Simmons 2007) [29].

Table 1: Culture and microscopic examination of *A. solani*.

Culture examination	
Surface color (Top)	Grey to black colonies brown mycelium
Reverse color (Bottom)	Grey brown to black bottom of colony
Microscopic examination	
Conidiophore	Short irregularly branched
Conidia	Spectating in both laterally and longitudinally, with up to six transversals and two to three longitudinal or oblique barriers, usually of clavate or pyriform shape overall, tapering towards the apices, forming a short beak, in culture usually 20–40 µm, with walls smooth to conspicuously rough

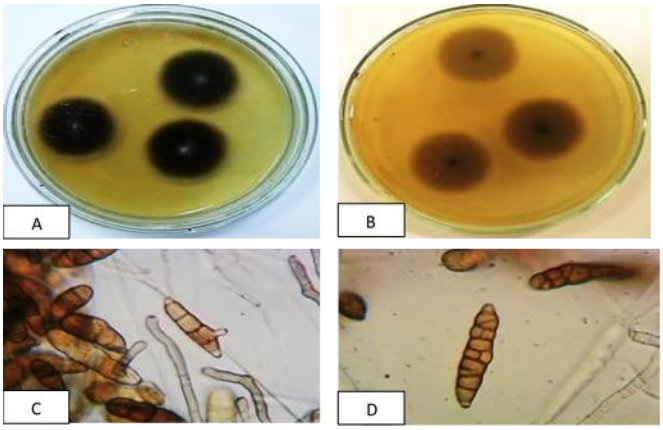


Fig 1: Light micrograph showing conidia of *A. solani* spectating in both laterally and longitudinally, with up to six transverse and two to three longitudinal or oblique septa; (Mag. power 20×40x).

Effect of *A. solani* infection on growth characters

Results in Table (2) revealed that all plant parameters as shoot lengths, root lengths and number of leaves of infected plants were significantly decreased throughout the duration of the experiment. The percentages of decrease in plants subjected to *A. solani* were 46.7 and 37.6 % after 20 and 40 days, in shoot length. Also, (51.5 and 46) % in root length at the same period and (42.8 and 48.1) % in leaf number per plant at the vegetative stages.

Changes in photosynthetic pigments

Results in (Table 3) Cleary show that, chlorophyll a, b was greatest decrease in challenged plants, during the two stages of growth. This reduction recorded in chlorophyll a as (49.6 and 55.6) % and chlorophyll b as (45.9 and 38.1) % at the vegetative stages (20 and 40 days after inoculation). Results also show that, carotenoids contents were significantly increased in plants in response to *A. solani* infection.

Changes in total soluble carbohydrate and protein contents

Results listed in Table (3) reveled that, total soluble carbohydrate contents in eggplant shoots were significantly decreased due to *A. solani* infection at the vegetative stages (20 and 40 days after inoculation with *A. solani*). The decrease in total carbohydrate contents in the affected plants was estimated by (36.6 and 36.7) % during the two vegetative stages of growth. Table (3) shows, total soluble protein in eggplant shoots was slightly decreased due to *A. solani* infection. The decrease in total dissolved protein contents in the affected plants was estimated (2.6) % during the two vegetative stages of growth.

Total phenols

Data generated in Table (3) showed that, *A. solani* showed marked significant increase in total phenols of shoots. The increases in total phenols in infected plants was estimated by (51.3 and 40.4) % respectively.

Table 2: Effect of *A. solani* infection on shoot, root lengths and number of leaves in eggplant plants.

Treatments	After 20 days				After 40 days			
	Healthy	Infected	decrease %	LSD at 0.05%	Healthy	Infected	decrease %	LSD at 0.05%
Shoot length (cm)	25.33	13.66	46.7	7.4	47.56	29.66	37.6	8.8
Root length (cm)	13	4.3	51.5	2.9	17.3	9.33	46	3.4
Leaves number/plant	9.33	5.33	42.8	3.4	45.66	23.67	48.1	6.5

Table 3: Effect of *A. solani* infection on photosynthetic pigments, total soluble carbohydrate contents, total soluble protein contents and total phenols in eggplant plants.

Treatments	After 20 days			After 40 days		
	Healthy	Infected	LSD at 0.05%	Healthy	Infected	LSD at 0.05%
Chlorophyll a (mg/g fresh weight)	8.52	4.29	1.80	11.33	5.03	3.2
Chlorophyll b (mg/g fresh weight)	5.22	2.82	1.52	6.84	4.23	2.33
Carotenoids (mg/g fresh weight)	1.20	2.26	0.7	1.56	2.93	1.01
Total soluble carbohydrate contents (mg/g dry weight)	34.53	21.86	8.01	55.25	34.95	12
Total soluble protein contents (mg/g dry weight)	45.33	44.13	14	86.15	61.80	22
Total phenol(mg/100g dry weight)	0.71	1.46	0.56	1.4	2.35	1.02

Oxidative enzymes activity

The resulting data in the Table. (4) Appeared that the highest enzymatic activity of superoxide dismutase, peroxidase and polyphenol oxidase (PPO) were observed in eggplants challenged with *A. solani* related to healthy (uninfected) plants. This was throughout different growth intervals.

Table 4: Effect of *A. solani* infection on superoxide dismutase, peroxidase enzyme activity and polyphenol oxidase enzyme activity in eggplant plants.

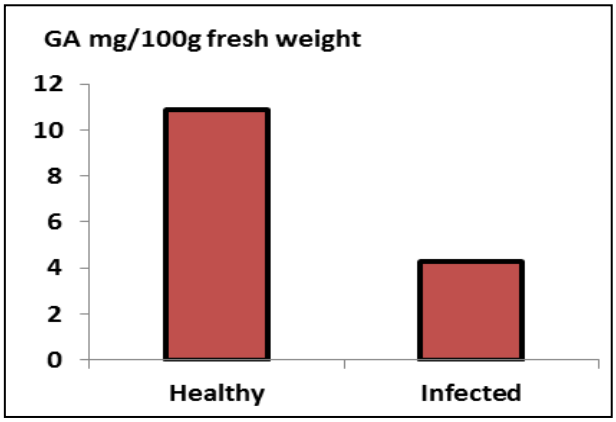
Test	After 20 days			After 40 days		
	Healthy	Infected	LSD at 0.05%	Healthy	Infected	LSD at 0.05%
superoxide dismutase enzyme activity(ug/g fresh weight)	1.03	1.72	0.6	2.27	3.07	0.9
Peroxidase enzyme activity (ug/g fresh weight)	0.82	1.62	0.63	1.03	2.67	0.8
polyphenol oxidase enzyme activity(ug/g fresh weight)	3.64	5.45	1.01	4.04	6.00	1.54

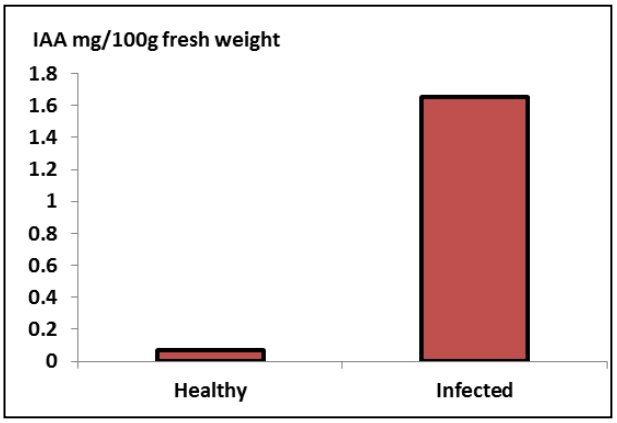
Endogenous hormones.

Results shown in Table (5) showed that amounts of IAA, ABA and SA were markedly increased in infected plants than that of healthy ones. Meanwhile, marked decreases in the contents of GA_3 were observed in infected plants as being compared with healthy ones. These results can be demonstrated as follows: The decrease in GA3 contents was estimated by 46.75 %. While detected increases in IAA, ABA and SA contents as compared with uninfected plants were estimated by (95.7, 91.7 and 141.1) % respectively. That case was at the second stage of growth (40 days after inoculated with *A. solani*).

Table 5: Effect of *A. solani* infection on endogenous hormones in eggplant.

	GA mg/100g fresh weight	IAA mg/100g fresh weight	ABA mg/100g fresh weight	SA mg/100g fresh weight
Healthy	10.90273	0.070692	0.273002	0.060988
Infected	4.284634	1.649719	3.296517	1.559029





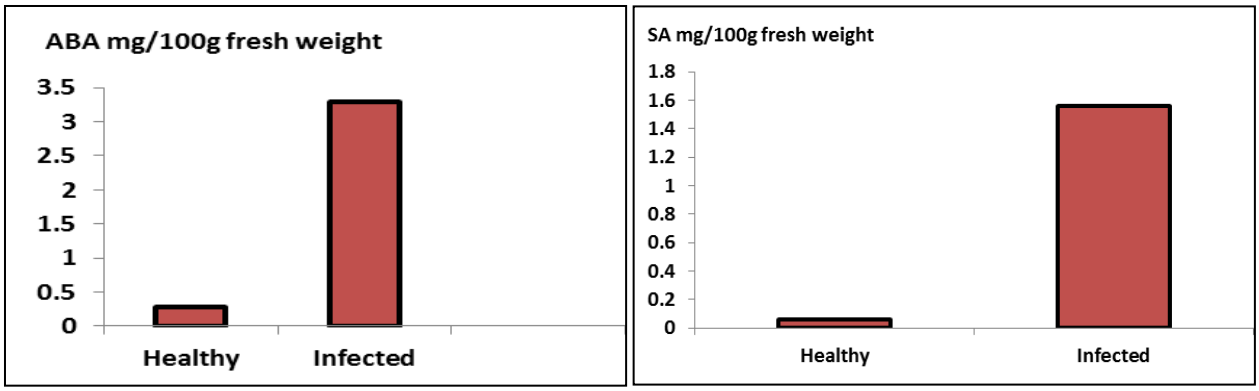


Fig 2: Effect of *A. solani* infection on endogenous hormones in eggplant.

Histopathological changes:

Light microscopic examination of uninfected (control) leaf of eggplant showed the normal structure (Figure 3, A and B), where the upper epidermis is consists of tubular parenchyma cells. Cortex layer was located next to the upper epidermis. Cells of cortex were separated by intercellular spaces. The mesophyll cells (palisade parenchyma) were cylindrical. Spongy parenchymal cells contain large interfacial spaces (Figure 3, A and B).

A. solani infected leaf revealed destroyed epidermis, cell of cortex, mesophyll, parenchyma and vascular bundles (Fig 4-B). The upper and lower epidermis were very compact and smaller cells as well as abnormal growth in the lower epidermal cells. A light microscopic examination of the infected leaves showed that *A. solani* penetrates into the tissues that are hardened by the breakdown of the inner cell walls. Also, complete decomposition of plant tissue, includes dissolution of lignin. The decomposition of both lignin occurs as a result of the action of one or more groups of enzymes secreted by the pathogen causing a highly sensitive response. (HR). The mesophyll and palisade tissues are large and contain a few cells and lacking chlorenchyma with thin-walled cells (Fig. 4, A and B). Excessive growth (hyperplasia) or enlargement (hypertrophy) demonstrated as enlarged parts of lower epidermis, compared with healthy ones.

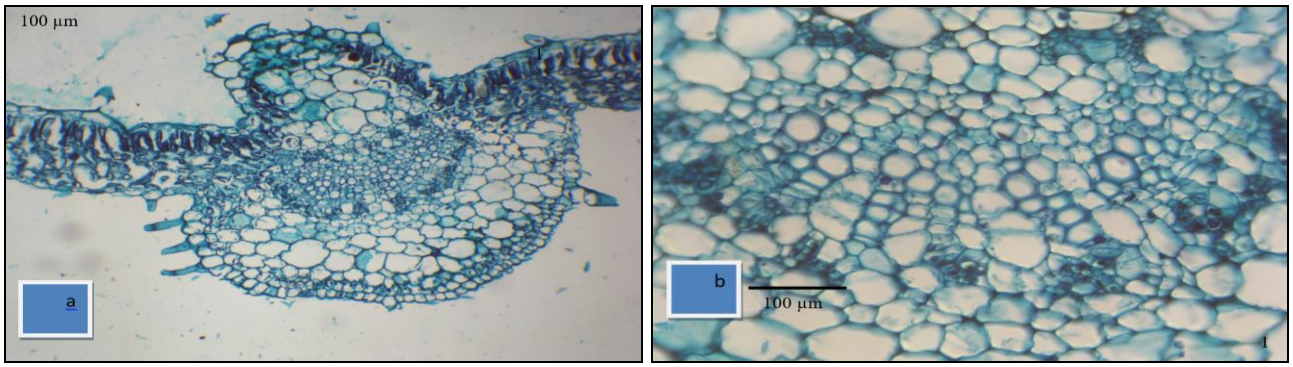


Fig 3: Light micrograph of eggplant leaves cross section, (A) and (B) healthy leaf showing normal cells and tissues of 20 days post infection.

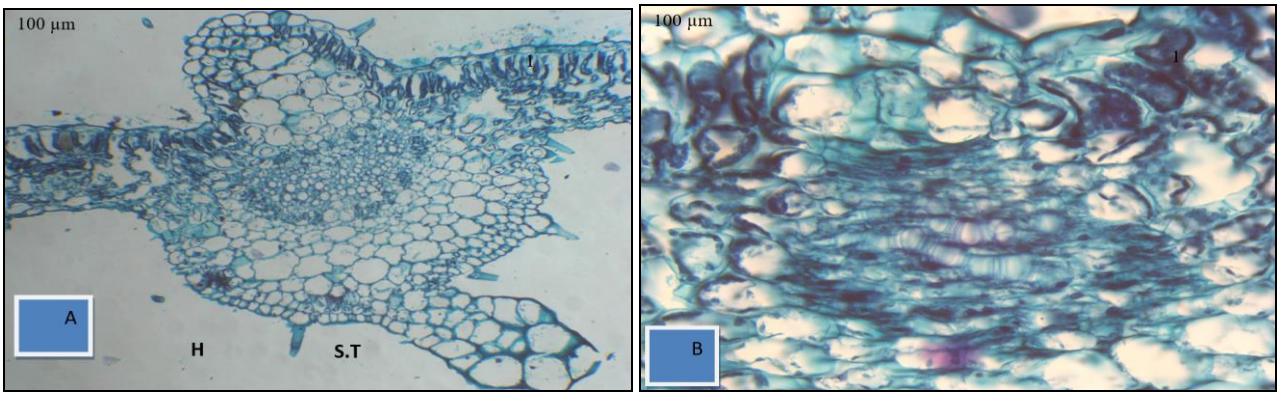


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Fig 4: Light micrograph of eggplant leaves cross section, (A) and (B) infected leaf showing different changes in cells and tissues of 20 days post infection

Discussion

In the present work *A. solani* significantly decreased vegetative characters and metabolic responses of eggplants. In this regard, all vegetative characters were significantly reduced with proceeding *A. solani* infection. Plant height and the leaf number /plants were significantly decreased as a result *A. solani* infection. In this regards the reduction in all growth parameters development may be correlated with the disturbances in the supply or distribution of the growth regulating hormones (Bos, 1978, Orcutt and Nilsen, 2000, Faheed *et al*., 2005, and Mohamed *et al*., 2017) [5, 25, 11, 24].

The process of photosynthesis is the main function through which the plants can convert light energy into chemical energy, which can be used in all the activities of the cell is strongly affected by the infection pathogenic infection (Bassanezi *et al*., 2001 and Arfan *et al*., 2007) [3, 2].

In this research, the incidence of *A. solani* resulted in a significant decrease in both photosynthesis and inhibition of overall growth. The reduction in chlorophyll is explained by Kyselakova *et al.,* (2011), This deficiency after infection may be due to oxidative stress that causes damage to chlorophyll. This means that the plant fails to capture light so photosynthesis will decrease or stop. However, plant pathogens inhibit photosynthesis, especially in the late stages of disease (Goodman *et al*., 1986 and Hossain *et al*., 2002) [13, 14]. Total soluble carbohydrate contents in eggplants shoots were significantly decreased due to *A. solani* infection during different stages of growth. The decrease in total soluble carbohydrate contents is explained by Schweizer (2000) [28] who mentioned that when plants become infected with pathogens, the respiratory rate generally increases. This means that infected tissues consume spare carbohydrates faster than healthy tissues. Increased respiratory rate appears soon after infection by the time the symptoms appear obvious. Thereafter, breathing decreases to normal rates or to lower levels than those found in healthy plants.

In the present work total soluble protein contents in eggplant shoots was slightly decreased due to *A. solani* infection. The decrease in total proteins in the affected plants was estimated by (2.6) % during first stage of growth (20 days after inoculation). A decrease of total soluble proteins due to pathogen infection has been reported (Taiwo and Akinjogunla, 2006) [34]. It was found that *A. solani* caused significant reductions in total soluble protein levels in infected leaves of tomato plants as compared to uninfected controls (Mohamed *et al*., 2017) [24]. The significant decrease in protein content as a result of the disease is likely due to some interactions related to a highly sensitive response and increased soluble protein. This was case at the second stage of growth (40 days after inoculation with *A. solani*). Several studies have been devoted to illustrating the numerous metabolic changes associated with a highly sensitive response, such as the local defensive reaction of a pathogen resistant plant. In a highly sensitive response, many enzymes are activated, known as proteins (PR). The continuous accumulations of newly induced proteins may help in the localization of fungal infection; the reverse is not true. (Walter *et al.*, 2007) [38]. It has been shown that the production of various toxins by necrotrophic fungus *A. alternate* is required for its pathogenesis (Chung, 2012) [8] and after successful infection the host plants induces cell membrane, production of ROS molecules and increased H_2O_2 accumulation followed by cell death. Our results showed that, *A. solani*, cause a significant decrease of total indoles in shoots of the infected plants. On the other hand, eggplant infected with *A. solani* showed significant increase in total phenols of shoots. Phenols play an important role in regulating plant metabolism and lignin formation. (Lewis and Yamamoto, 1990) [20]. Also, phenols act as pickers for free electrolytes as well as substrates for many antioxidant enzymes In addition, phenols act as free radical scavengers as well as substrates for many antioxidant enzymes (Martin-Tanguy, 2001) [22]. Many defense enzymes that have been associated with disease infection include, peroxidase (PO), polyphenol oxidase (PPO), superoxide dismutase (SOD), and proteinase inhibitors (Van Loon, 1997) [36]. In the present work the results showed that, antioxidant enzymes activity increased significantly in eggplants infected with *A. solani*.

Our results indicated that contents of both IAA and gibberellic acids ($GA_{3)}$ were decreased in infected plants than that of healthy ones. At the same time, an increase in content of abscisic acid (ABA) and jasmonic acid (JA) were observed in infected plants as being compared with healthy ones**.** Fungi produce compounds that are like plant hormones, such as auxins, cytokinins (CKs), (GAs), ethylene (ET), (ABA), (JA) and salicylic acid (SA). These hormones play an important role in plant development and to induce significant plant signal events during biotic and abiotic stress (Pozo *et al*., 2015) [25].

Histopathological studies in the present work showed that, the upper and lower epidermis of infected leaves were very compressed as well as appearance abnormal growth in lower epidermis cells, than these in healthy ones. Upper epidermal cells were completely pressed. These results agree with (Gómez-Rodríguez *et al*., 2003; Khalil *et al*., 2014 and Mohamed *et al*., 2017) [12, 16, 24]. The mesophyll and Palisade tissues are large and have a lower number of cells and lacking chlorenchyma with thin cell walls compared with healthy ones. Successful injuries leads to the onset of symptoms, such as distorted or distorted or necrotic areas of the host plant. These results may be explained by Riedle-Bauer (2000) [27] which reported that, the thickness and rigidity of the outer wall of epidermal cells appear to be important factors in the resistance of some plants to certain pathogens.

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