ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Impact of greenhouse environmental factors and fungicide Trinol (triadimend) treatment on berry leaves infected with powdery mildew (*Uncinula necator* (Schwein.) Burrill: Role of host antioxidant systems against pathogen infection.

Khalaf A Fayez[1, 2], Yassin M Al-Sodany[1, 3]*, and Alaa Abouzaid[4]

[1]Biology Department, Faculty of Science, Taif University, Taif, KSA
[2]Botany Department, Faculty of Science, South Valley University, Sohag, Egypt
[3]Botany Department, Faculty of Science, Kafr El-Sheikh University, Kafr El-Sheikh, Egypt
[4]Botany Department, Faculty of Science, Tanta University, Tanta, Egypt

ABSTRACT

Unfavorable environmental conditions such as temperature and humidity within greenhouse enhanced powdery mildew (*Uncinula necator*) fungal plant disease on black mulberry (*Morus nigra*). The initial symptoms of powdery mildew were appeared on leaves as light green to yellow spots associated with leaf chlorosis. As the disease progresses, the spots get larger due to formation of large numbers of spores. With late fungus infection, the chlorosis and necrosis of leaves were observed. Ultramicroscopic examination (SEM) of infected leaves showed a colonization of fungus on leaves. In response to Trinol (triadimend) fungicide, the fungus growth on plant leaves was strongly inhibited. Physiological activities of healthy plants, fungal infected plants as well as that infected with fungus and sprayed with fungicide were investigated. Photosynthetic pigments, that affect the efficiency of photosynthesis in higher plants, showed a high sensitivity with powdery mildew infection. Fungus infected leaves that sprayed with Trinol fungicide had more contents of photosynthetic pigments compared to those of infected and healthy plants. Soluble carbohydrate contents of infected leaves and that sprayed with the fungicide were significantly increased compared with that of healthy one. Soluble protein contents of infected leaves significantly increased in response to fungicide spraying. The response of infected plants and fungicide treated plants stimulated own defense systems for against pathogen infection. The contents of phenolic compounds and proline increased in fungus infected and fungicide sprayed leaves. Malondialdehyde (indicate lipid peroxidation) was increased in fungus infected leaves and that sprayed with the fungicide. Total antioxidant activities of infected leaves, and fungus infected leaves that sprayed with fungicide were slightly high than that of healthy one. The results confirmed a reduction in *U. necator* infected berry due to application of Trinolfungicide.

Keywords: Black mulberry (*Morus nigra*); environmental factors, powdery mildew; Antioxidants; Fungicide.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Black mulberry (*Morus nigra*) is a species of mulberry. It is native to southwestern Asia, where it has been cultivated for so long that its precise natural range is unknown. Black mulberry cultivated for its edible fruit and is planted and often naturalized west across much of Europe, including Ukraine, and east into China. Multiple fruits of black mulberry become purplish-black at maturity. Black, red and white mulberry are widespread in Pakistan, Iran, North India and Afghanistan, where the tree and the fruit are known by the Persian-derived names *toot* (mulberry) [1].

Phytopathogenic fungi can cause local or general symptoms on their hosts. According to Agrios [2] the most common symptoms are leaf-spots, blight, canker and die-back. Powdery mildew fungi are obligate, biotrophic parasites of the phylum Ascomycota of Kingdom Fungi. Powdery mildew is a fungal disease that affects a wide range of plants [3, 4]. Powdery mildew diseases are caused by many different species of fungi in the order Erysiphales. Infected plants display white powdery spots on the leaves and stems. The first symptoms occur on lower leaves as bright yellow spots that range from 1/8 to 1/2 inch in diameter. The spots enlarge and eventually turn brown. As infections progress, the leaf dies but remains attached to the stem. However, with extensive loss of foliage, many exposed fruit will sunburn. The yield losses may exceed 50% in heavily infected fields. The extent of loss depends on environmental conditions, date of disease, and effectiveness of fungicide control. Worm days with an occasional rainstorm are conducive to disease development [5-9].

Biotic and abiotic environmental stressors produced characteristic changes in physiological processes of higher plants [10-16]. Oxidative metabolism of normal cells and different stress situations generate highly reactive oxygen species (ROS). The ROS, such as superoxide radical ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), and singlet oxygen ($^1O_2$), have been implicated in a number of physiological disturbances in plants [18,19]. Biotic and abiotic stresses were reported to cause oxidative stress, by resulting in accumulation of reactive oxygen species such as H_2O_2 and $O_2^{\bullet-}$ [20,21]. Plants have a set of enzymes and redox metabolites that carry out ROS detoxification and known as antioxidant systems [22,23].

Disease is also strongly dependent on environmental conditions. The association of reactive oxygen species (ROS) formation and increased activity of enzymes participating in their metabolism with the induction of defense responses has been demonstrated in many plant–pathogen interactions [20,24,25]. ROS have direct antimicrobial activity inhibiting germination of spores of many fungal pathogens [26]. The pathogen itself may also generate H_2O_2 as a pathogenicity factor [27].H_2O_2 generation affects gene expression and causes inhibition or retardation of fungal development [28].

It has been demonstrated that proline inhibits ROS-mediated apoptosis in fungal pathogenesis [29]. Proline decreases amounts of free radicals [30] and reactive oxygen species (ROS) [29,31]. Protein synthesis is essential for normal cell differentiation and growth. A variety of environmental stressors have been reported to influence the synthesis of plant proteins [32].

Phenolic compounds are plant secondary metabolites that constitute one of the most common and widespread groups of substances in plants. Plants need phenolic compounds for pigmentation, growth, reproduction, and resistance to pathogens and for many other functions [33]. In a survey of 22 different fruits and vegetables, blueberries had the highest antioxidant capacity when measured with the oxygen radical absorbing capacity (ORAC) assay [34,35]. Phenolic compounds are secondary metabolites that are quite widespread in plants and can act as free radical scavengers and protect cells from oxidative toxicity [36,37]. The fungus *Gymnospora ngiumsabinae* infected pear leaves caused an increase in the total content of phenolic compopunds [38].

Malondialdehyde (MDA) content is widely used to measure the extent of lipid peroxidation as an indicator of oxidative stress and membrane damage [39].The present project evaluated the following: (1) the effect of fungicide triadimend application on control of downy mildew and on the maintenance of leaf photosynthetic pigments; (2) the antioxidant metabolites contents of fungal infected leaves as well as interaction of fungicide and fungal pathogen compared to healthy one; and (3) the total antioxidant activity of leaves in response to pathogen and fungicide application. Moreover, the present study aims toincrease the fundamental understanding of appropriate environment factors affecting health, growth and productivity of

berry plants; to detect the pathogen affected plants and the ability of fungicide for host plant protection and to know if the open field or greenhouse conditions were appropriated for breeding berry plants. This will generate useful information to improve plant protection against fungi pathogen and physiological activities of berry plant cultivated within greenhouse and open field.

MATERIALS AND METHODS

Plant materials and treatments

Black mulberry (*Morus nigra*) plants have two - three years age was grown in greenhouse and open field of Taif University will be used for the experiments. The air temperature and relative humidity will be assessed by using data logger inside and outside the greenhouse.

Isolation, identification and control of powdery mildew pathogenic fungi

Pathogenic powdery mildew fungi (*Uncinula necator* (Schwein.) Burrill, *Syn. Erysiphe necator*) were isolated from the infected plant and identify with modern techniques and electron microscope [40,41]. Then, we will try to control the disease by fungicide Triadimend [42,43,44].

Photosynthetic pigments determination

Contents of Chlorophyll a (*Chl a*), chlorophyll b (*Chl b*) and total carotenoids were spectrophotometrically determined according to Metzner et al. [45]. The pigment contents of treated plants were then compared to the control. The photosynthetic pigment contents were extracted from a known fresh weight of leaves in 85% (v/v) aqueous acetone. The extract was centrifuged at 4000g for 10 min. The supernatant was then taken and diluted by 85% aqueous acetone to the suitable concentration for spectrophotometer measurements. The extinction was measured against a blank of a pure 85% aqueous acetone at three wavelengths of 452.5, 644 and 663 nm. Using the following equations:

$$Chlorophyll\ a = 10.3 \times E_{663} - 0.918 \times E_{644} = \mu g / ml$$
$$Chlorophyll\ b = 19.7 \times E_{644} - 3.87 \times E_{663} = \mu g / ml$$
$$Total\ carotenoids = 4.2 \times_{E452.5} - \{(0.0264 \times Chl\ a) + (0.426 \times Chl\ b)\} = \mu g / ml.$$

The pigment contents(*Chl a*, *chl b* and carotenoids) were calculated as mg/g fresh weight.

Soluble protein determination

Soluble protein content of leaves will determine according to Lowry et al. [46] using Bovine serum albumin as a standard. Leaf samples (0.1 g dry weight) were extracted in 10 mL distilled water for 2 h at 90°C. The extracts were centrifuged and the supernatants were collected. One mL of extract was added to 5 mL of alkaline reagent (50 mL 2% Na_2CO_3 prepared in 0.1 N NaOH and 1 mL 0.5% $CuSO_4.5H_2O$ prepared in 1% sodium potassium tartarate) and mixed thoroughly then allowed to stand for 10 min. A total of 0.5 mL of Folin phenol reagent diluted 1:2 (v/v) was then added and mixed immediately. After 30 min, the extinction against appropriate blank was measured at 700 nm. Protein contents were expressed as mg g^{-1} DW.

Soluble carbohydrate determination

Soluble carbohydrate content was determined in aqueous solution with anthrone sulfuric acid reagent according to Fales [47] and Schlegel [48], using glucose as a standard. To extract water-soluble carbohydrates, a known weight (0.1 g dry weight) of leaf tissue powder was boiled in distilled water in a water bath for 1 h. The extracts were then cooled and filtrated through a centered glass funnel. A total of 0.5 mL of the extract was mixed with 4.5 mL of anthrone reagent (0.2 g anthrone, 8 mL absolute ethyl alcohol, 30 mL distilled water and 100 mL sulfuric acid (D= 1.84). The mixture was then boiled in a water bath for 7 min. After cooling, the developed blue green color was measured at 620 nm against blank. Soluble carbohydrate contents were expressed as mg g^{-1} DW.

Lipid peroxidation determination

Malondialdehyde (MDA) content was determined as an indication of leaf lipid peroxidation according to Hernández and Almansa [49]. Fresh leaf samples (500 mg) were homogenized in 5 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000g for 20 min at 4°C. A one mL aliquot of the supernatant was mixed with 3 mL of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA, and incubated at 90 ºC for 20 min. After stopping the reaction in an ice bath, samples were centrifuged at 10,000g for 5 min. The supernatant absorbance at 532 nm was then measured. After subtracting the non-specific absorbance at 600 nm, MDA concentration was determined using the extinction coefficient (E = 155 $mM^{-1}cm^{-1}$).

Proline determination

Proline content of leaves was determined according to Bates et al [50]. A known dry weight (0.1 g) of leaves was extracted in 10 mL of aqueous 3% sulfosalicylic acid over-night. The extract was centrifuged at 3000g for 10 min. Two mL of the supernatant was mixed with 2 mL of fresh acid ninhydrin solution and 2 mL of glacial acetic acid for reaction in a test tube for 1 h at 100 ºC. The reaction was terminated in an ice bath, and the mixture was extracted with 4 mL toluene. The extract was vigorously stirred for 20 s using a test tube stirrer. The chromophore-containing toluene was aspirated from the aqueous phase, and its absorbance was measured at 520 nm. Proline content was determined from a standard curve and calculated as mg g^{-1} DW.

Phenolic determination

Total phenolic content of leaves were determined using Folin-Ciocalteau reagents [51]. Gallic acid standard solution (2.0 mg/mL) was prepared by accurately weighing 0.01 g and dissolving 50 mL of distilled water. Forty µL of extract (in 80% methanol) or gallic acid standard was mixed with 1.8 mL of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min, and then 1.2 mL of sodium bicarbonate (7.5% w/v) was added to the mixture. After standing 60 min at room temperature, absorbance was measured at 765 nm. Results are expressed as mg/g gallic acid equivalents.

DPPH free radical scavenging assay

Leaf extract in methanol were subjected to the free radical-scavenging activity assay using the method described by Shimada et al. [52]. Each extract (0.2–10 mg /mL) in methanol (2 mL) was mixed with 2 mL of freshly prepared methanolic solution containing 80 ppm of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. The mixture was shaken vigorously and left to stand for 30 min in the dark. The absorbance was then measured at 517 nm. The percentage of DPPH scavenging activity was calculated as follows:

$$\text{DPPH scavenging ability} = [1- (A_i-A_j)/Ac] \times 100.$$

Ai is absorbance of extract + DPPH, Aj is absorbance of extract + methanol, and Ac is absorbance of DPPH + methanol. A lower absorbance indicates a higher scavenging effect.

Data analyses

All data were subjected to ANOVA test and means were compared by two conventional methods of analysis. The LSD values for significant mean differences at levels P <0.05 and P <0.01 were separated. All statistical tests were carried out using SPSS software.

RESULTS AND DISCUSSION

The results indicated that relative humidity increased inside the greenhouse than that of outside it during the day and night times. Generally, the relative humidity increased in the night time as compared with that of day time and it reaches to the minimum values at 10 am to 1 pm. inside the greenhouse, its values ranges from 22.07±10.03% at 12 pm to 52.48±12.24% at 7 pm (Table 1). Regarding the growing months, the relative humidity increased during April and May months compared with that of June and July. Inside the greenhouse, the relative humidity fluctuated during the four months, but it increased during April and May (Fig. 1a). On the other hand, the relative humidity is approximately stable during June and July outside the greenhouse and retains the lowest values during these months (Fig. 1b).

ISSN: 0975-8585

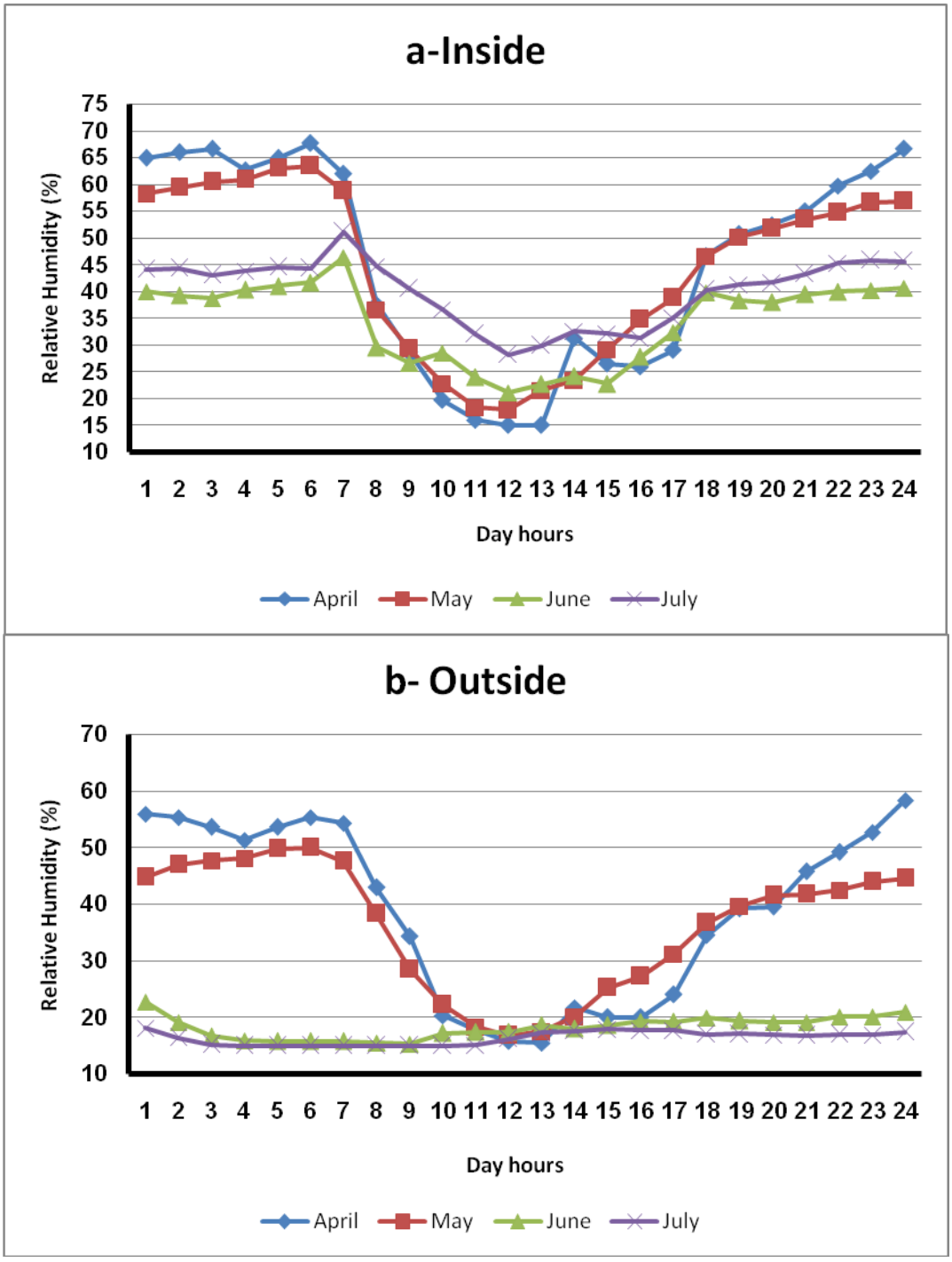


Fig 1: The relative humidity (%) inside and outside the green house at Taif University in relation to growing months of berry tree.

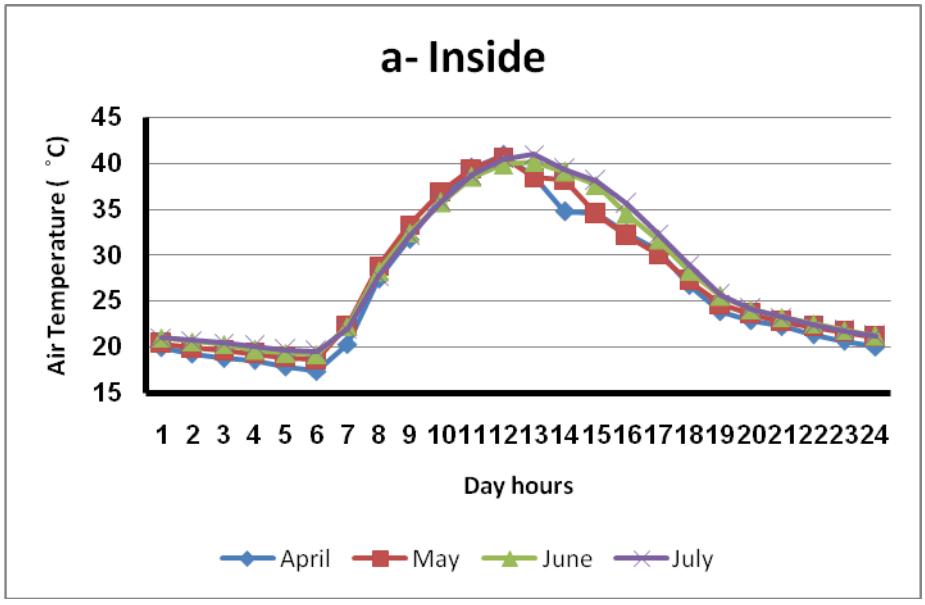
Table 1: Means ± SD of temperature (˚C) and relative humidity (%) inside and outside the green house at Taif University

Time hrs.	Temperature (˚C)		Humidity (%)	
	inside	outside	inside	outside
1	20.77±2.33	23.30±2.32	48.11±13.75	29.48±17.53
2	20.37±2.35	24.75±3.17	48.29±14.38	28.51±18.59
3	20.08±2.29	26.12±4.25	48.12±15.19	27.48±19.30
4	19.64±2.20	27.95±5.86	48.87±15.29	27.24±20.01

ISSN: 0975-8585

5	19.24±2.21	29.32±7.27	50.09±15.77	27.82±20.66
6	19.04±2.17	30.66±8.49	50.45±16.04	27.96±20.67
7	22.15±1.49	31.65±7.85	52.48±12.24	27.11±18.53
8	28.23±1.58	33.11±6.37	37.05±11.30	23.65±13.55
9	32.61±1.91	34.98±5.25	32.08±10.95	20.08±8.92
10	36.20±2.06	35.33±3.73	28.99±11.09	18.21±8.37
11	38.94±2.15	34.55±1.98	24.52±11.24	16.97±7.89
12	40.37±2.53	33.49±2.71	22.07±10.03	16.75±6.00
13	39.81±3.75	31.59±4.39	24.27±10.82	17.71±7.33
14	38.72±3.82	30.79±4.74	26.94±12.40	18.73±7.69
15	36.65±4.09	29.50±4.84	28.00±13.60	20.60±12.71
16	33.98±3.67	28.77±4.80	31.10±15.36	21.39±14.18
17	31.33±2.79	27.86±4.15	35.17±14.30	22.83±15.40
18	28.09±2.26	26.42±3.29	42.44±11.06	25.05±16.18
19	25.24±2.14	25.67±2.59	43.61±12.38	26.04±16.81
20	23.89±2.05	25.11±2.52	44.24±12.69	26.49±17.29
21	23.06±2.00	24.60±2.37	45.86±12.61	26.80±17.56
22	22.32±2.04	24.03±2.44	47.29±12.88	27.53±18.03
23	21.70±2.20	23.48±2.46	48.25±13.40	28.15±18.47
24	21.12±2.30	23.08±2.36	48.54±13.48	28.95±18.39
Total	**27.66±7.88**	**28.58±5.98**	**39.85±16.36**	**24.23±16.18**

The results indicated that the mean temperature increased inside the greenhouse than that of outside it during the day time, but decreased during the night time. As contrast to relative humidity, the mean temperature increased in the day time as compared with that of night time and it reaches to the minimum values inside the greenhouse at early morning of 6 am (19.04±2.17 ˚C) and to the maximum value at noon of 12 pm (40.37 ± 2.53 ˚C), while outside the greenhouse, it ranges from 23.08 ± 2.36 ˚C at midnight to 35.33 ± 3.73 ˚C at 10 am (Table 1). Regarding the growing months, the mean temperature inside the greenhouse is approximately stable during the four months (April, May, June and July months) (Fig. 2a). It fluctuated outside the greenhouse during the four months, it increased during June and July as compared with that of April and May (Fig. 2b).



ISSN: 0975-8585

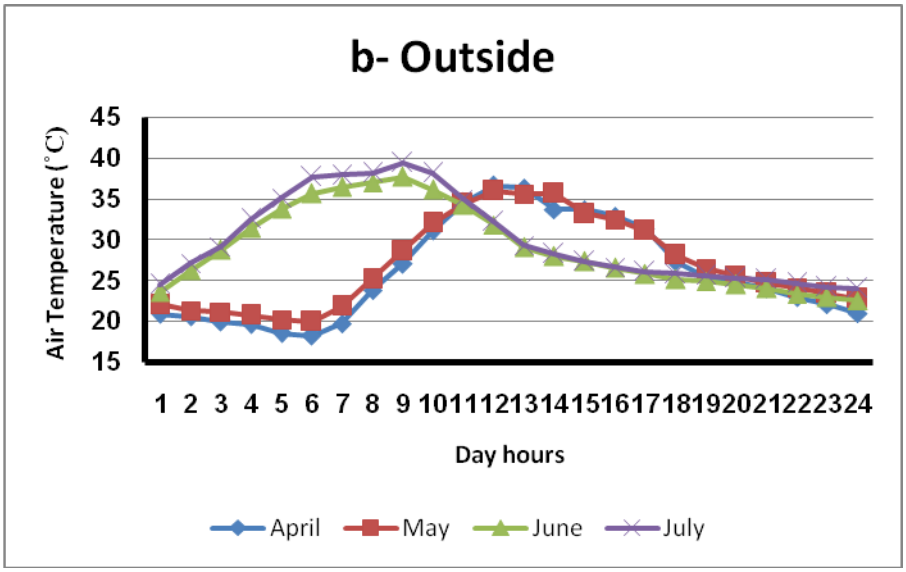
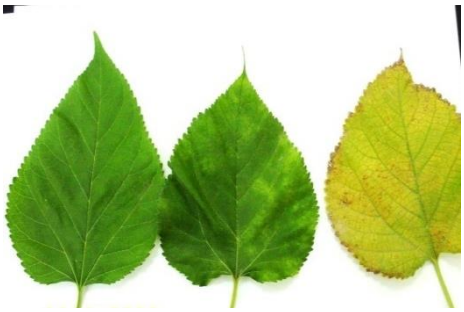


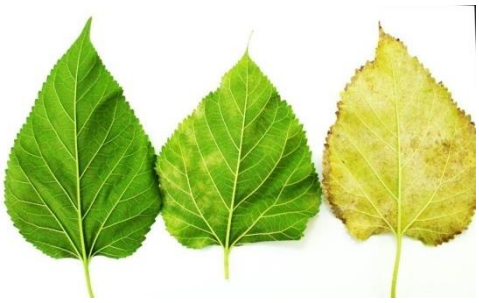
Fig 2: The mean temperature (˚C) inside and outside the green house at Taif University in relation to growing months of berry tree.

Powdery mildew is a fungal disease that affects many plants. Environments with high humidity and moderate temperatures enhanced powdery mildew deseases. The greenhouse environment is very conducive to several diseases, among which grey mould and powdery mildews arc the most important ones [53].Powdery mildew is a common problem in greenhouses where relative humidity is kept low to create environmental conditions unfavorable for other foliar pathogens [54,55].The initial symptoms appeared on berry leaves are white patches of mycelium on the lower or upper leaf surfaces. As the disease progresses, leaf edges may roll upward and reddish asymmetrical spots appear on the upper surface of the leaves (Figs. 3A-D). Moreover, powdery mildew appear on leaves as chlorotic spots on the upper and lower leaf surfaces. Powdery mildew produces a white powdery coating on the surfaces of plant leaves and resulted plants to become distorted. As shown in Figures 3 A-B, the powdery mildew produces a white powdery coating on the lower surface of plant leaves of black berry. Dark brown spots on leaves displayed in affected plants. webby mycelium on the lower leaf surface are are detected. As spores are produced, the infected areas take on a white, powdery or dusty appearance. Due to fungicide treated pinfected plants, powdery mildew symptoms were controlled. The desesaes of powdery mildew can be controlled using fingicides.In the present study, powdery mildew infected black berry was significantly reduced by foliar application of fungicide Trinol (triadimend). The effects of fungicidal activity on leaves applied with trinol were detected through restriction of progress of colonies and disease severity compared with control plants. The leaf symptoms due to infection of powdery mildew on leaf lower surfacewas more obivous than upper surfaces (Fig. 3C and D).Because powdery mildew grows primarily on the outside of leaves, interaction fungicides are effective for managing powdery mildew on the upper leaf surfaces [56].

A



B



ISSN: 0975-8585

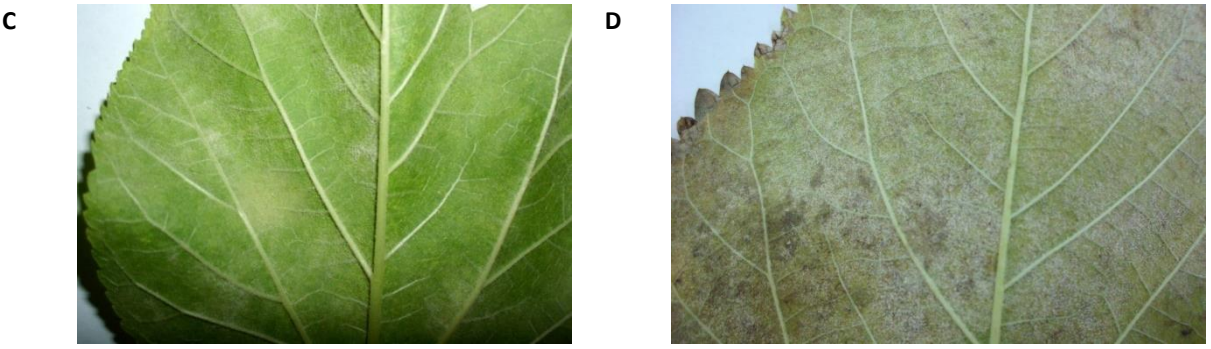
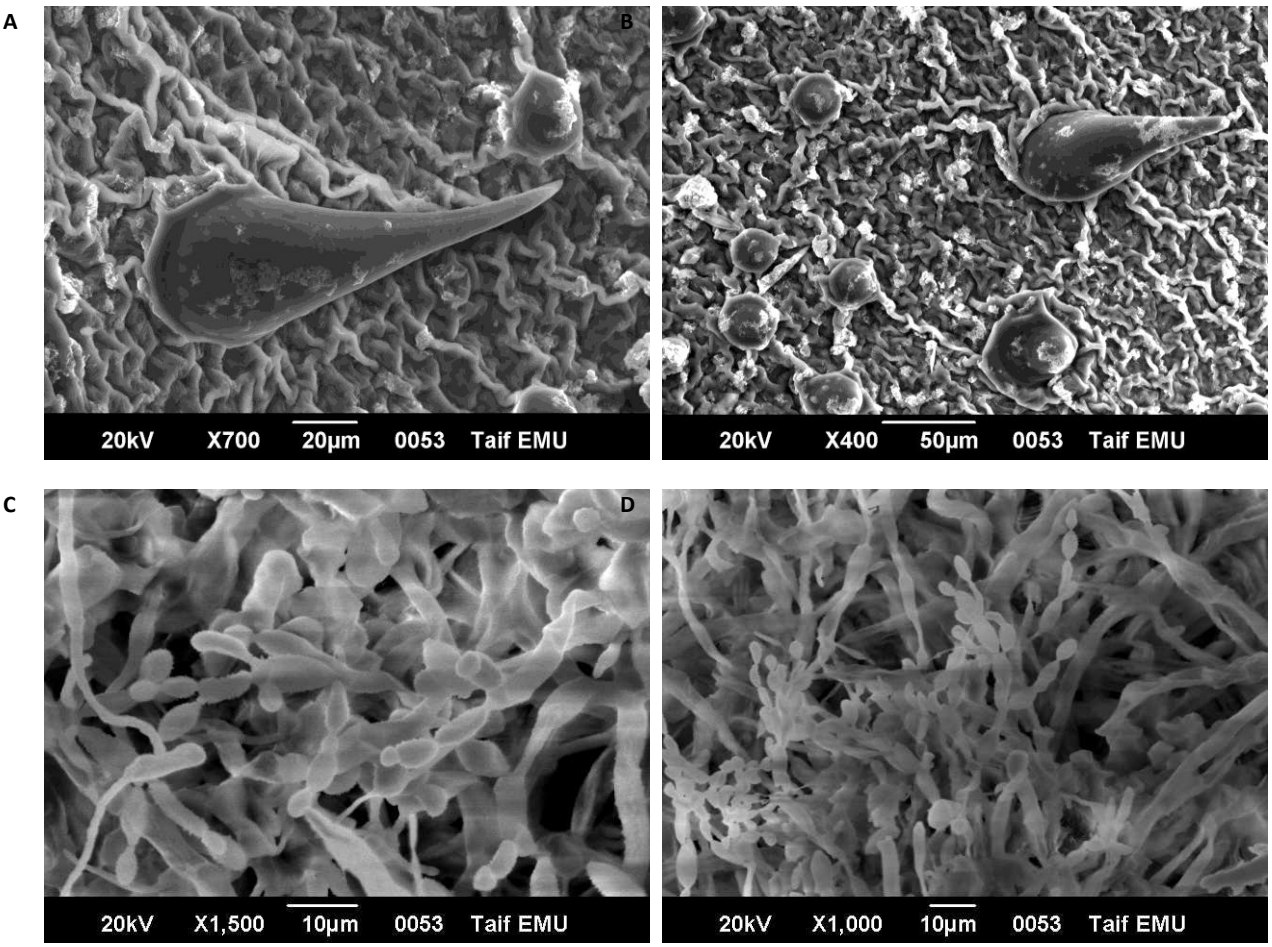


Fig 3 (A): The upper surface of berry leaves; helthay (left side), fungus infected+ fungicide treated (middle) and fungus infeted (right). (B): the lower surface of berry leaves; helthay (left side), fungus infected + fungicide treated (middle) and fungus infected (right). (C): the early infecton of leaf with fungus of powdery mildew. (D): the late infecton of leaf with fungus. Powdery mildew were covering lower berry leaf surface. Leaf infected with powdery mildew has a white-to light gray dusting.

SEM images of health and powdery mildew infected leaves are presented in figures 6A-6E. SEM image of a healthy leaf surface showing blackberry idioblasts located in epidermal layer of leaf surface like hooked dome (Figures 4A and 4B). The hyphae and conidia of powdery mildew are covering the surface of berry leaves (Figs. 4C-4E).



ISSN: 0975-8585

E

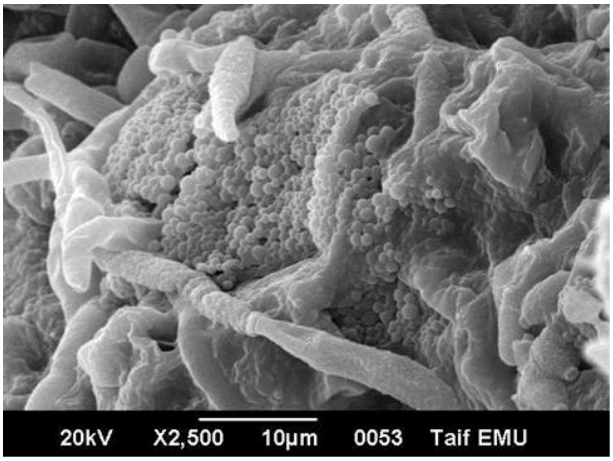


Fig 4 (A): SEM image of a healthy leaf surface showing blackberry idioblasts located in epidermal layer of leaf surface SEM image of healthy leaf surface showing a hooked dome-like protrusion; bar: 20 µm. (B): Mulberry idioblasts located in epidermal layer, SEM image of leaf surface infected with powdery mildew + fungicide treated of infected leaves showing a hooked dome-like protrusion; bar: 50 µm. (C-E): SEM images of leaf surfaces infected with powdery mildew .Hyphae and conidia of powdery mildew on the surface of berry leaves; bars: 10 µm.

It has been well documented that an important group of plant pathogenic fungi, such as rusts and powdery mildews, reduces the rate of photosynthesis by leaves and isolated chloroplasts. The lower rate is accompanied by reduced activity of certain enzymes and by aberrations in chloroplast ultrastructure [57-60].

Table 2: Levels of photosynthetic pigments (mg g^{-1} FW)and of total antioxidant activities (%) in healthyleaves, powdery mildew infected leaves and powdery mildew infected leaves that sprayed with fungicide of berry (*Morusnigra)*. Values are means (M) of three replicates ± SD. For each data, statistically significant of differences compared to the value of healthy leaves was conducted. *, significant *at P*<0.05; **, significant at *P*<0.01.

Treatment	*Chl A*		*Chl B*		*Carotenoids*		*A/B* ratio	Total	
	M±SD	%	M±SD	%	M±SD	%		M	%
Healthy	1.18±0.07	100	0.39±0.03	100	0.39±0.02	100	3.02	1.96	100
Infected	0.62±0.06**	52	0.20±0.02**	51	0.23±0.02**	59	3.10	1.05	53
Infected + Fungicide	1.21±0.02	102	0.35±0.01	88	0.41±0.01	105	3.45	1.97	101

Fungal infection decreased the photosynthetic pigment contents of black mulberry *(Morus nigra)* leaves (Table 2**).** Contents of *chla*, *chlb* and carotenoids of black mulberry leaves decreased by 48, 49, and 41 %, respectively, due to powdery mildew infections. These data indicate that the *chl* a, and *chlb* contents were declined affecting fungal infection to a greater extent than that of carotenoids. Interestingly, contents of *chla,* and carotenoids of black mulberry leaves increased by 2 and 5%, respectively, while *chlb* decreased by 12% in response to fungicide treatments. The most commonly observed symptoms characteristic to specific host–virus interactions, were often accompanied by a decrease in total chlorophylls contents, photosynthetic rate, delayed chlorophyll biosynthesis and inhibition of the electron transport on the donor side of PSII[61-64].In this respect ,chloroplasts isolated from powdery mildew-infected (*Erysiphe polygoni* DC) sugar beet leaves (Beta vulgaris L) showed a reduction in the rate of electron transport , ATP formation, decreased rate of photosynthetic CO_2 assimilation ,alterations in chloroplast ultrastructure and a reduction in the activity of enzymes necessary for the formation of organic acids [65].

The levels of soluble carbohydrate significantly increased due to powdery mildew infections. This increase was more response to fungicide treatment. In details, the increases in soluble carbohydrate due to fungal infection and that treated with the fungicide were about 96 and 113% in comparison with that of the control (Fig. 5).

The soluble protein contents of infected leaves significantly increased with fungicide treatment. In contrast, the protein content of leaves infected with powdery mildew approximately had content of that the

ISSN: 0975-8585

control. The soluble protein of fungicide- treated leaves increased about 41% compared with that of the control (Fig 5).

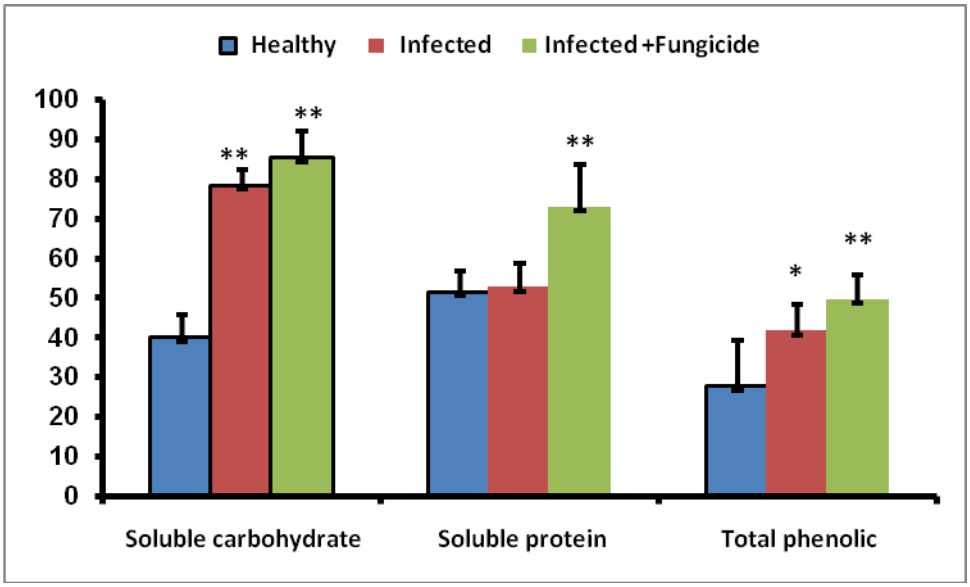


Fig 5: Levels of soluble carbohydrate (mg g^{-1} DW), soluble protein (mg g^{-1} DW) and total phenolic compounds (µM Gallic acid g^{-1}DW) in healthy, powdery mildew infected and powdery mildew infected leaves that sprayed with fungicide of berry (*Morus nigra)*. Values are means (M) of three replicates ± SD. For a given data, statistically significant of differences compared to the value of healthy leaves was conducted. *, significant *at P*<0.05; **, significant at *P*<0.01.

The levels of total phenolic contents of infected leaves as well as that treated with fungicide significantly increased. The increases in leaves of total phenolic contents due to fungal infection and that treated with the fungicide were about 50 and 79% in comparison with that of the control (Fig 5). Plants need phenolic compounds for pigmentation, growth, reproduction, resistance to pathogens and for many other functions [66].

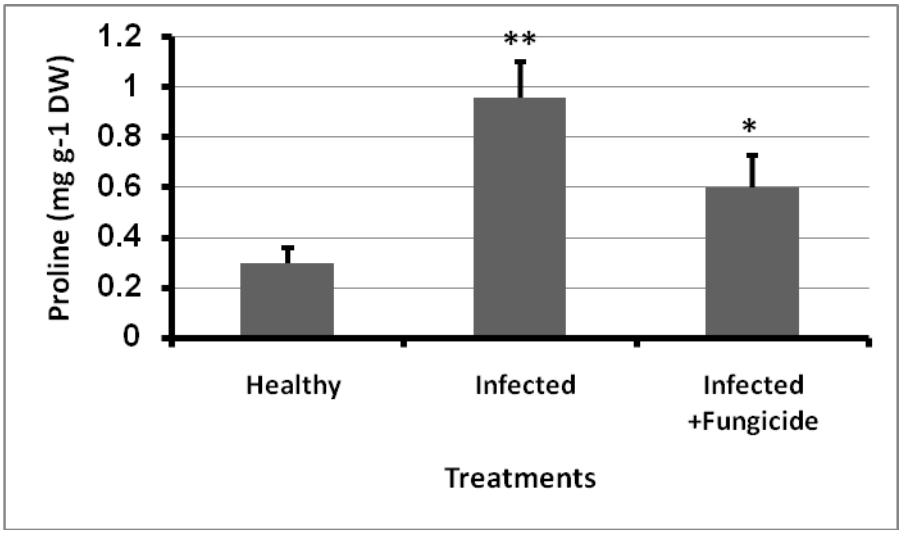


Fig 6: Levels of proline (mg g^{-1} DW) in healthy leaves, powdery mildew infected leaves and powdery mildew infected leaves that sprayed with fungicide of berry (*Morus nigra*). Values are means (M) of three replicates ± SD. For each date, statistically significant of differences compared to the value of healthy leaves was conducted. *, significant at P<0.05; **, significant at P<0.01.

Proline content of powdery mildew infected leaves increased three fold compared to that of the control. In response to fungicide treatment, the proline content of powdery mildew infected leaves increased tow fold compared to the control (Fig. 6). Proline accumulates during several stresses and acts as a compatible

solute that buffers cellular redox potential [67]. Proline is suggested to protect plants by acting as a cellular osmotic regulating, ROS detoxifying molecule, thereby maintaining membrane integrity and stabilizing antioxidant enzymes [68]. The proline concentration in leaves was significantly higher in fungus –infected leaves and in that of treated- fungicide than in healthy leaves, suggesting that infection with powdery mildew induced more proline accumulation in leaves, thus enhancing resistance of the host plant to the powdery mildew. Probably as suggested by Chen and Dickman [29], proline as non-enzymatic antioxidants could counteract the inhibitory effects of ROS in microbes, animals, and plants.

MDA leaf content of both infected plants and that infected and traded with fungicide significant increased. In details, the increases in MDA due to fungal infection and that treated with the fungicide were about 99 and 273%, respectively, in comparison with that of the control (Fig. 7).

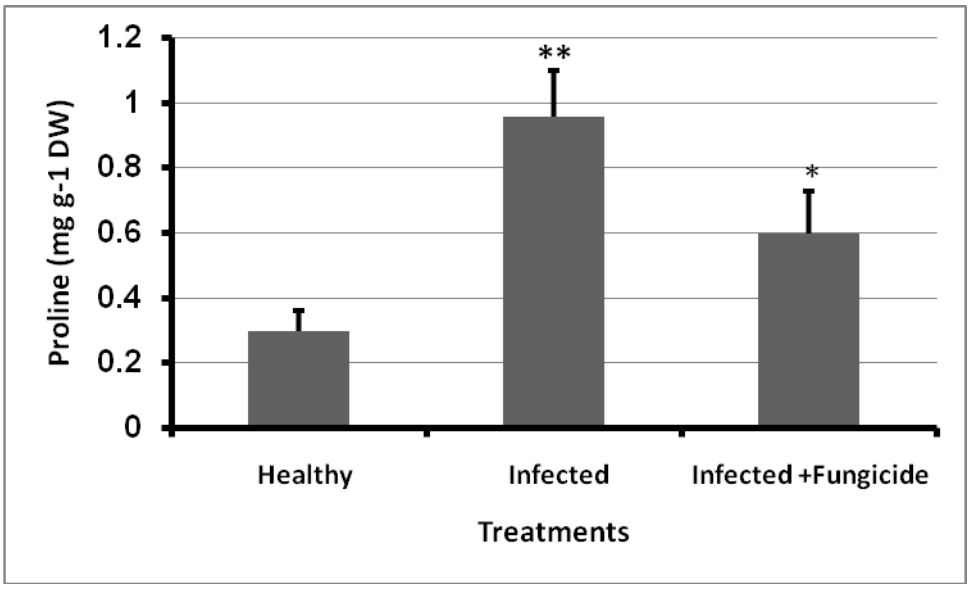


Fig 7: Levels of MDA (µmol g^{-1} FM) and total antioxidant activities (%) in healthy leaves, powdery mildew infected leaves and powdery mildew infected leaves that sprayed with fungicide of berry (*Morus nigra*). Values are means (M) of three replicates ± SD. For each date, statistically significant of differences compared to the value of healthy leaves was conducted. *, significant at P<0.05; **, significant at P<0.01.

Table 3: Levels of total antioxidant activities (%) in healthy leaves, powdery mildew infected leaves and powdery mildew infected leaves that sprayed with fungicide of berry (*Morus nigra)*. Values are means (M) of three replicates ± SD.

Treatment	*Total antioxidant activities*	
	M±SD	%
Healthy	87±1.7	100
Infected	88.63±2.29	102
Infected +Fungicide	89.21±2.58	103

Total antioxidant activities of fungus infected and treated with fungicide slightly increased compared with that of health one (Table 3) Antioxidant systems are produced during interactions between pathogens and plant hosts [69,70]. ROS inhibit chloroplast development, decrease seed viability and root growth, stimulate leaf abscission and desiccation, cause peroxidation of essential membrane lipids in the plasma lemma and intracellular organelles (lipid peroxidation) and damage proteins, carbohydrates, nucleic acids and pigments such as chloroplasts or carotenoids [71].The highly susceptible one of three *Lycopersicon* spp. infected with *Oidium neolycopersiciL.* showed correlation between production of reactive oxygen species (ROS) and activity of enzymes participating in their metabolism and hypersensitive response was evident during plant defence response [72]. Lipid peroxidation occurred in the *Lycopersicon* accessions in response to *Oidium neolycopersici* L. Kiss [73].

ISSN: 0975-8585

CONCLUSIONS AND RECOMMENDATIONS

Agriculture is the world's largest economic sector. Agricultural productivity suffers a heavy loss due to plant pathogens. Plant protection from diseases has been necessary for the higher yields in agriculture. Late infection with Powdery mildew affected most leaves of berry. This disease is most easily noticed by the dusty appearance or white powdery growth occurring in patches on leaves. Infection and disease development are favored by moderate temperatures and high humidity. Early control of the infections with fungicides is important to managing this disease. The results of these studies have been reported with the aim to provide further insight into connections between plants, fungal pathogen and fungicides.

Based on the results of the present study, we having wider area of opportunities in agricultural productivity in future research studies. The results showed chlorosis of powdery mildew infected berry leaves as a result of chlorophylls decreasing. Physiological activities were altered and oxidative stress occurred. The greenhouse environments enhanced powdery mildew infection for berry plants. On this, from our findings we recommend to avoid planting berry plants in environments contain fungal spores of powdery mildew. The effects of fungicidal activity of Trinol (triadimend) on leaves applied were detected through restriction of progress of colonies and disease severity compared with control plants. Early control of primary infections is important to managing this disease. Because powdery mildew grows primarily on the leaf surface where contact fungicides are effective.

REFERENCES

[1] Boulos L. Flora of Egypt. Volume one (Azollaceae – Oxalidaceae). Al-Hadara Publ., Cairo, Egypt1999; pp. 1-419.

[2] Agrios. GN. Plant Pathology, Fourth Edition. London: Academic Press 1997; pp. 1-635.

[3] Cao X, Luo Y, Zhou Y, Duan X, Cheng D. Detection of powdery mildew in two winter wheat cultivars using canopy hyperspectral reflectance. Crop Prot. 2013; 45: 124-131.

[4] Sylla J, Beatrix W, Alsanius BW, Krüger E, Becker D, Wohanka W,. In vitro compatibility of microbial agents for simultaneous application to control strawberry powdery mildew (*Podosphaera aphanis*).Crop Prot. 2013; 51: 40-47.

[5] Johnson DA, Rowe RC,. Powdery mildew. Pages 34-35 in: Compendium of Potato Diseases, 2nd ed. WR Stevenson, R Loria, GD Franc, DP Weingarter, eds. American Phytopatholog. Society, St. Paul, MN.2001.

[6] Darriet P, Pons M, Henry R. Impact odorants contributing to the fungus type aroma from grape berries contaminated by powdery mildew (*Uncinula necator*); incidence of enzymatic activities of the yeast *Saccharomyces cerevisiae*. J. Agric. Food Chem.2002; 50: 3277-3282.

[7] Glawe DA, du Toit LJ, Pelter GQ,. First report of powdery mildew on potato caused by *Leveillula taurica* in North America Online. Plant Health Progress 2004; doi:10.1094/PHP-2004-1214-01-HN.

[8] Romberg MK, Nuñez JJ, Farrar JJ. First report of powdery mildew on potato caused by *Golovinomyces cichoracearum* in California. Plant Dis. 2004; 88: 309.

[9] Hartney S, Glawe DA, Dugan F, Ammirati J. First report of powdery mildew on *Corylusa vellana* caused by *Phyllactinia guttata* in Washington state. Online. Plant Health Progress 2005; doi: 10.1094\PHP-2005-1121-01-BR.

[10] Shaddad MA, Ahmed AM, Fayez KA. Alleviation of the adverse effects of salinity by nitrogen fertilization. Biol. Plantarum. 1988; 30: 343-350.

[11] Fayez KA, Kristen U. The influence of herbicides on the growth and proline content of primary roots and on the ultrastructure of root caps. Environ. Exp. Bot. 1996; 36: 71-81.

[12] Fayez KA. Action of photosynthetic diuron herbicide on cell organelles and biochemical constituents of the leaves of two soybean cultivars. Pest. Biochem. Physiol. 2000; 66: 105-115.

[13] Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 2002; 7: 405-410.

[14] Miteva E, Hristova D, Nenova V, ManevaS.. Arsenic as a factor affecting virus infection in tomato plants: changes in plant growth, peroxidase activity and chloroplast pigments, Sci. Hortic2005; 105: 343-358.

[15] Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 2011; 48: 909-930.

[16] Fayez KA, Mahmoud SY. Detection and partial characterization of a putative closterovirus affecting *Ficus carica*: molecular, ultrastructural and physiological aspects of infected leaves. Acta Physiol. Plant.2011; 33: 2187-2198.

[17] Scandalios JG. Oxygen stress and superoxide dismutases. Plant Physiol. 1993; 101: 7-12.

[18] Blokhina O, Virolainen E, Fagerstedt, E. Antioxidants, oxidative damage and oxygen deprivation stress: a review, Ann. Bot, 2003; 91: 179-194.
[19] Baker CJ, Orlandi EW. Reactive oxygen in plant pathogenesis. Annu. Rev. Phytopathol. 1995; 33: 299-321.
[20] Turkan I, Demiral T. Recent developments in understanding salinity tolerance. Environ. Exp. Bot. 2009; 67: 2–9.
[21] Mittova V, Tal M, Volokita M, Guy M. Up-regulation of the leaf mitochondrial and per oxisomal antioxidative systems in response to salt-induced oxidative stress in the wild salt-tolerant tomato species *Lycopersicon pennellii*. Plant Cell Physiol. 2003; 41: 845-56.
[22] Foyer CH, Noctor G. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 2005; 17: 1866-1875.
[23] Bolwell GP, Wojtaszek P. Mechanisms for the generation of reactive oxygen species in plant defence-a broad perspective. Physiol. Mol. Plant Pathol.1997; 51: 347-66.
[24] Wojtaszek P. Oxidative burst: an early plant response to pathogen infection. Biochem. J. 1997; 322: 681-92.
[25] Peng M, Kuc J. Peroxidase-generated hydrogen peroxide as a source of antifungal activity in vitro and on tobacco leaf disks. Phytopathology 1992; 82: 696-699.
[26] Schouten S, Hopmans EC, Schefuß E, Sinninghe Damste´ JS. Distributional variations in marine crenarchaeotal membrane lipids: a new organic proxy for reconstructing ancient sea water temperatures? Earth and Planetary Science Letters 2002; 204: 265–274.
[27] Mellersh DG, Foulds IV, Higgins VJ, Heath MC. H_2O_2 plays different roles in determining penetration failure in three diverse plant–fungal interactions. Plant J. 2002; 29: 257-268.
[28] Chen C, Dickman M.B. Proline suppresses apoptosis in the fungal pathogen *Colletotrichum trifolii*, PNAS 2005; 102: 3459-3464.
[29] Okuma E, Soeda K, Tada M, Murata Y. Exogenous proline mitigates the inhibition of growth of *Nicotiana tabacum* cultured cell under saline conditions. Soil Sci. and Plant Nutri.2000; 46: 257-263.
[30] ChenC, Wanduragala S, Becke DF, Dickman MB. Tomato QM-like protein protects *Saccharomyces cerevisiae* cells against oxidative stress by regulating intracellular proline levels. Appl. Environ. Microbiol.2006; 72: 4001–4006.
[31] Willium A. The effect of cell cycle regulators on protein profiles in cultured root meristems of *Pesium* sativum. Enviro. Exp. Bot. 1989; 29: 317–322.
[32] Lattanzio V, Lattanzio VMT, Cardinali A. Role of phenolics in the resistance mechanisms of plant against fungal pathogens and insects. Phytochemistry: Advances in Research, 2006; 23-67 ISBN: 81-308-0034-9.
[33] Cao G, Sofic E, Prior RL. Antioxidant capacity of tea and common vegetables.J. Agric. Food Chem. 1996; 44: 3426-3431.
[34] Wang H, Cao G, Prior RL.Total antioxidant capacity of fruits. J. Agric. FoodChem. 1996; 44: 701–705.
[35] Costa RM, Magalhãs AS, Pereira JA, Andrade PB, Valentão P, Carvalho M, Silva BM. Evaluation of free radical scavenging and antihemolytic activities of quince (*Cydonia oblonga*) leaf: a comparative study with green tea (*Cammelia sinensis*). Food Chem. Toxicol.2009; 47: 860-865.
[36] Oliveira AP, Valenao P, Pereira JA, Silva BM, Tavares F, Andrade PB. *Ficus carica* L.: Metabolic and biological screening. Food Chem. Toxicol.2009; 47: 2841-2846.
[37] Kalisz S, Oszmiański J, Wojdyło A. Increased content of phenolic compounds in pear leaves after infection by the pear rust pathogen. Physiol. Molecular Plant Pathol2015; 91: 113-119.
[38] Lin CC, Kao CH. Effect of NaCl stress on H_2O_2 metabolism in rice leaves. Plant Growth Regul.2000; 30: 151-155.
[39] Braun U, Cook RTA, In man AJ, Shin HD. The Taxonomy of the Powdery Mildew Fungi2002. in: The Powdery Mildews: a Comprehensive Treatise, Berlanger RR, WR Bushnell, AJ Dik, TLW Carver (eds.). American Phytopathological Society, St Paul. pp. 13-55
[40] Heffer V, Johnson KB, Powelson ML, Shishkoff N. Identification of powdery mildew fungi. The Plant Health Instructor 2006. DOI: 10.1094/PHI-I-2006-0706-01
[41] Reuveni R, Dor G, Reuveni M. Local and systemic control of powdery mildew (*Leveillula taurica*) on pepper plants by foliar spray of mono-potassium phosphate. Crop Prot. 1998; 17: 703-709.
[42] Kasselaki AM, Shaw M, Malathrakis N, Haralambous J. Control of *Leveillula taurica* in Tomato by *Acremonium alternatum* is by Induction of Resistance, not Hyperparasitism. Euro J. Plant Pathol.2006; 115: 263-267.

[43] Manoj KA, Kalpana NR, Sundaresha S, Ramachandra YL. Compatibility of fungal antagonists filtrates against germination of powdery mildew spores, *Leveillula taurica* (Lev.) Arn. of Bell pepper. Plant pathology J. 2006; 5: 248-252.
[44] Metzner H, Rau H, Senger H. UntersuchungenzurSynchronisierbakeiteinzelnerPigmentmangel-Mutanten von Chlorella. Planta.1965; 65: 186-194.
[45] Lowry OH, Rosebrough NJ, Farr AI, Randall RJ. Protein measurement with the folin phenol reagent. J Biol. Chem. 1951; 193: 291-297.
[46] Fales FW. The assimilation and degradation of carbohydrates by yeast cells. J. Biol. Chem. 1951; 193: 113-124.
[47] Schlegel HG. Die verwertungorgani schersaurendurch Chlorella irlichet, Planta1956; 47, 510.
[48] Hernández JA, Almansa MS. Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. Physiol. Plant 2002; 115: 251-257.
[49] Bates LS, Waldern RP, Teare ID. Rapid determination of free proline for water stress studies. Plant Soil 1973; 39: 205-207.
[50] Dai GH, Andary C, Cosson-Monodol L, Boubals D. Polyphenols and resistance of grapevines to downy mildew. ActaHorticult.1994; 381: 763-766.
[51] Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food. Chem. 1992; 40: 945-948.
[52] Elad Y, Malathrakis NE, Dik AJ. Biological control of Botrytis-incited diseases and powdery mildews in greenhouse crops. Crop Protection 1996; 15: 229-240.
[53] Jarvis WR. Managing Diseases in Greenhouse Crops. APS Press, St. Paul, MN 1992.
[54] Keinath AP, DuBose VB. Controlling powdery mildew on cucurbit rootstock seedlings in the greenhouse with fungicides and biofungicides. Crop Protec. 2012; 42: 338-344.
[55] McGrath SP, Zhao FJ, Lombi E. Phytoremediation of metals, metalloids and radionuclides. Advances in Agronomy 2002; 75: 1–56.
[56] Allen PJ. Changes in the metabolism of wheat leaves induced by infection with powdery mildew. American Journal of Botany 1942; 29: 425-435.
[57] Allen PJ. Physiological aspects of fungus disease of plants. Annual Review of Plant Physiology 1954; 5: 225-248.
[58] Magyarosy AC, Schurmann P, Buchanan BB. Effect of powdery mildew infection on photosynthesis by leaves and chloroplasts of sugar beets. Plant Physiol. 1976; 57: 486-489.
[59] Montalbini P, Buchanan BB. Effect of a rust infection on photophosphorylation by isolated chloroplasts. Physiol Plant Pathol.1974; 4: 191-196.
[60] Almasi M, Kiani S, Louimi N. Principles of Agricultural Mechanization. Ma'soumeh (PBUH) Publication. Ghom, Iran2000; pp. 19-40.
[61] Arias CA, Brix H, Johansen NH. Phosphorus removal from municipal wastewater in an experimental two-stage vertical flow constructed wetland system equipped with a calcite filter. Water Sci. Technol. 2003; 48 (5): 51–58.
[62] Synková H, Semorádová Š, Schnablová R, Müller K, Pospíšilová J, Ryšlavá H, Malbeck J, Čeřovská N. Effects of biotic stress caused by Potato virus Y on photosynthesis in ipt transgenic and control *Nicotiana tabacum* L. Plant Sci2006; 171: 607–616.
[63] Radwan J, Kawalko A, Wojcik JM, Babik W. MHC-DRB3 variation in a free-living population of the European bison, Bison bonasus. Molecular Ecology 2007; 16: 531-540.
[64] Magyarosy AC, Malkin R. Effect of powdery mildew infection of sugar beet on the content of electron carriers in chloroplasts. Physiological Plant Pathology 1978; 13: 183-188.
[65] Lattanzio V, Lattanzio VMT, Cardinali A. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In: Imperato F, editor. Phytochemistry advances in research. India: Research Signpost2006; pp. 23-67.
[66] Wahid A, Gelani S, Ashraf M, Foolad MR. Heat tolerance in plants: an overview. Environ. Exp. Bot. 2007; 61: 199-223.
[67] Joshi R, Ramanarao VM, Baisakh N. Arabidopsis plants constitutively over expressing cordgrass exhibits enhanced level of tolerance to salt stress. Plant Physiol. Biochem. 2013; 65: 61-66
[68] Kwon SI, Anderson AJ. Differential production of superoxide dismutase and catalase isozymes during infection of wheat by a *Fusarium proliferatum*-like fungal isolate. Physiol. Mol. Plant Pathol.2001; 58: 73-81.
[69] Malencic DJ, Vasic D, Popovic M, Devic, D. Antioxidant Systems in Sunflower as Affected by Oxalic Acid. Biol. Plantarum, 2004; 48: 243-247.

[70] Philosoph-Hadas S, Meir S, Akiri B., Kanner J. Oxidative defense systems in leaves of three edible herb species in relation to their senescence rates, J. Agr. Food Chem. 1994; 42: 2376-2381.
[71] Mlíčková K, Luhová L, Lebeda A, Mieslerová B, Peč P. Reactive oxygen species generation and peroxidase activity during *Oidium neolycopersici* infection on *Lycopersicon* species. Plant Physiology and Biochemistry 2004; 42: 753-761.
[72] Tománková K, Luhová L, Petřivalský M, Peč P, Lebeda A. Biochemical aspects of reactive oxygen species formation in the interaction between *Lycopersicon* spp. and *Oidium neolycopersici*. Physiol. Mol. Plant Pathol.2006; 68: 22-32.