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Research Article

Fenton as advanced oxidation process for controlling downy mildew of cucumber under greenhouse conditions

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Abstract: In this research, the curative action of Fenton reagent (H_2O_2)/$Fe^{(2+)}$), Fenton like reagent (H_2O_2)/$Fe^{(3+)}$), Fenton complex (H_2O_2)/$Fe^{(3+)}$/oxalic acid) and famoxadone + cymoxanil as foliar applications were examined against downy mildew of cucumber caused by *Pseudoperonospora cubensis* (Berk. and Curtis) under greenhouse conditions during two successive growing seasons. Likewise, the impact of these treatments was also investigated on some biochemical and growth characters of cucumber plants. In addition the toxicity of Fenton solutions were assessed on rats as for biochemical and histological changes in liver and kidney of treated rats with respect to control. Results demonstrated that famoxadone + cymoxanil was the best treatment against downy mildew followed by Fenton like reagent, Fenton reagent and Fenton complex, in both growing seasons. There was marked increase in each biochemical parameter of cucumber plants (chlorophyll, peroxidase and polyphenoloxidase) and also in cucumber yield under all treatments compared to untreated control. No noticeable alterations were observed in liver and kidney of rats treated with the tested Fenton solutions compared to control. Fenton solutions could be utilized as efficient and safe means to control downy mildew of cucumber in greenhouse conditions.

Keywords: Downy mildew, fungicide, Fenton, disease, toxicity, histopathology

Introduction

Downy mildew caused by *Pseudoperonospora cubensis* Berk and Curt. Rostov. is a common disease of greenhouse and field-grown cucumber plants and closely related crop species (squash, pumpkin, melons) and it can command to a huge yield loss (Ko *et al*., 2008; Yang *et al*., 2008). The utilization of conventional fungicides is now a main problem in plant disease control and may result in environmental pollution, phytotoxicity and fungicide resistance (De Waard *et al.*, 1993). Resistance under field conditions has been accounted for several classes of fungicides including benzimidazoles, dicarboximides, phenylamides, and sterol biosynthesis inhibitors groups (Leroux *et al.,* 2000). However, agronomists prioritize environmentally safe materials for plant protection since generality of consumers need vegetables and traditional crops free of pesticide residues (Suzuki *et al.,* 2006). Therefore, the objective of reducing the risk of resistance evolution and rely on fungicides confirms the necessity for materials with new mechanism of action for disease control. These objectives can be accomplished by the constant general demand for minimizing

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pesticide levels in water and the inaccessibility of traditionally appropriate disease-resistant plants (Suzuki *et al.,* 2006).

Organic agriculture growers and others have regularly used hydrogen peroxide (H_2O_2) as a preventive measure for crop diseases (Yang *et al.*, 2008). While reports on the utilization of food and/or pharmacy-grade peroxide in controlling plant pathogens is still in preparatory phases of advancement, Bio safe systems has newly released a peroxygen formulation named OxiDate, which is classified as a broad-spectrum pesticide for fungi and bacteria. Mildews of cucurbits are one the diseases that can be managed by this formulation. Among the recorded advantages of this drug are its biodegradability, practically zero phytotoxicity, and its efficacy against parasitic spores (Ko *et al.,* 2008).

Generation of reactive oxygen species (ROS), for example, superoxide (O^{-2}), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2) is one of the primary reactions of plants against pathogens, ecological stimulants, and protection activators (Vanacker *et al.,* 2000). Hydroxyl radical (OH) is an intense oxidant in the vaporous and aqueous phases of atmospheric photochemical rotation. Acidic species such as nitric acid, nitrous acid, hydrogen peroxide and organic compounds are considered as origins of hydroxyl radical in the natural aqueous phase, and the interaction of H_2O_2, Fe and oxalic acid by photo Fenton reactions can be a main exporter of ·OH production in the aqueous phase (Arakaki *et al.*, 1998).

In contaminated water treatment, several photo Fenton solutions are utilized to degrade toxic organic materials (Chaudhuri and Elmollai, 2009; Zaror *et al.*, 2009). Conventional Fenton or photo Fenton solutions need low pH value (2–3) for high generation rate of hydroxyl radicals. On the other hand, the low pH value (2-3) may induce phytotoxicity to plant itself by affecting the cell wall and releasing of nutrient elements such as Ca and Mg. Ultra violet (UV) - light some of the time is utilized to enhance Fenton solution to obtain higher generation rate of OH, but UV light is harmful to plants. Therefore, Fenton solutions with high ·OH generation rates and low acidity (instead of high acidity) under natural sunlight (instead of UV light) are expected to successfully control plant pathogens and at the same time avoid plant damage (Sakugawa *et al.*, 2012).

The efficacy of Fenton solutions in sunlight against strawberry downy mildew (*P. cubensis* Berk and Curt. Rostov.) has been evaluated. Fenton solution protected entire plants before inoculation and significantly reduced the conidial germination within 20 min of Fenton solution exposure to sunlight (Sakugawa, 2008). In this work, the adequacy of foliar application of Fenton solutions containing distinctive hydroxyl radicals producing sources utilizing different mixtures of H_2O_2, Fe^{2+}or Fe^{3+}, and oxalic corrosive ($H_2C_2O_4$) against downy mildew of cucumber under greenhouse conditions in comparison with famoxadone + cymoxanil, the recommended fungicide of downy mildew was evaluated. Likewise, the impact of these medications on some biochemical's and yield characters of cucumber was additionally researched. Also, the mammalian toxicity of the tested treatments was assessed in rats as for biochemical and histological changes in the liver and kidney with respect to control.

Materials and Methods

Chemicals

Chemicals including, Fe_2SO_4, $FeCl_3$. $6H_2O$, H_2O_2, HCl and $H_2C_2O_4$ with high purity were obtained from El-Gomhoria Company for Chemical and Glasses, Cairo, Egypt. High purity Milli-Q water was used to prepare solutions of these chemicals. Equation Pro (famoxadone 16.6% + cymoxanil 22.1%) produced by E. I. DuPont India Pvt. Ltd India was the tested fungicide.

Treatments

A conventional Fenton reagent (50 mM H_2O_2 and 0.7 mM Fe^{2+}, pH 5.5), a Fenton-like reagent (50 mM H_2O_2 and 0.7mM$Fe^{3+,}$ pH 5.5),

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a new type of Fenton oxalate complex (50 mM H_2O_2, 0.7 mM Fe^{3+}, and 3 mM oxalic acid, pH 5.5) (Zuo and Hoignea, 1992; Faust and Zepp, 1993); a conventional fungicide, famoxadone + cymoxanil, (0.1 mg/L) and pure water (Milli-Q water pH 5.5)as a control were the assessed treatments in this study against downy mildew. Fenton solutions were adjusted to pH 5.5 by H_2SO_4. Since the microbial populations do not survive at pH below 5, more than 48 h, the Fenton solutions were prepared at pH of 5.5 in order to guarantee that fungus death was not because of the acidity of the solutions itself (Rodrıguez-Chueca *et al.,* 2011). Besides, this is nearly the natural pH of fresh water that was used for pesticides application. These solutions were prepared just before use.

Impact of Fenton solutions on cucumber plants

Before testing the impacts of Fenton solutions on downy mildew of cucumber, a trial was carried out to determine the suitable concentration levels of Fenton solutions and their influence on cucumber plants. To perform this, infected cucumber leaves were sprayed with various levels of H_2O_2 (10, 15, 20, 25, 50 and100 mM), while keeping the concentration of Fe^{3+} at 0.7 mM and oxalic acid at 3 mM (Sakugawa *et al.,* 2012).

Application of Fenton solutions on cucumber

This work was performed under greenhouse conditions at Sakha Research Station, Kafr Elshiekh Governorate in two growing seasons (2012-2013/2013-2014). Seedlings of cucumber (D.P 164 cultivar), 21 days-old, were transplanted at spacing of 50 cm using double row perridge. The plot was 3.5 m long and 1.5 m wide, each plot had 14 plants. Randomized complete block design was used to arrange the tested treatments with four replicates for each treatment and normal cultural practices. The goal of this experiment was to examine the curative effects of Fenton solutions and the recommended fungicide on cucumber plants infected by *P. cubensis*. The artificial inoculation of downy mildew fungus was achieved using inoculum from a local greenhouse. the fungus conidia were gently brushed into a small quantity of distilled water containing two drops of Tween 20 and fungus spores were counted using hemocytometer to make a spore suspension with a specific concentration. For inoculation of cucumber plants, the upper 10 leaves of each plant were consistently treated with a suspension of *P. cubensis* ($5x10^5$conidia/ml). Five treatments (famoxadone + cymoxanil, three Fenton-based solutions and Milli-Q water) were used after symptoms of downy mildew infection appeared on cucumber plants. The tested treatments were applied to plants as mist, using an electronic spray machine with a fine nozzle, in the early morning. Treatments were applied three times after the symptoms of downy mildew infection appeared on cucumber plants (0, 7 and 14 days after disease appearance). Disease severity was assessed at the following times: 0 and 4 d for the 1st spray, 7 and 11 d for the 2nd spray and 14 and 18 d for the 3rd spray). Greenhouse mean temperatures ranged between 23 ± 2 ºC and 19 ± 2 ºC during the day and night, respectively with14 h of light per day, and relative humidity of 76.2–80%.

Disease severity determination

The disease severity (DS) of downy mildew was determined according to the following equation developed by Descalzo *et al*. (1990)**.**

$R = \sum (a \times b) / N \times K \times 100$

Where R = disease severity index, a = number of leaves within infection grade, b = numerical value of each grade, N = total number of the investigated leaves and K = the highest degree of infection in category. Area of lesions in each replicate (three plants each with ten leaves) was measured. Plants were scored visually for pathogen presence on a scale of 0, 1, 3, 5, 10, 20, 90 and 100% of leaf area covered with symptoms of downy mildew using a standard area diagram according to Gaunt (1987).

Reduction percentage in downy mildew DS was determined according to the following equation

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Reduction(%) = ((DS control% - DS treatment %) / DS control %)×100.
Where DS = disease severity

Biochemical analysis

Determination of chlorophyll

Cucumber leaves were extracted using 5 ml N, N dimethyl formamid in the dark for 48 h at room temperature as described by Moran and Porath (1980). This experiment was repeated three times for obtaining accurate data. The density of color was measured in three replicates at 645 and 663 nm for chlorophyll a and b, respectively by utilizing spectrophotometer. Chlorophyll a and b were calculated by the following equation as described by Arnon (1949).
Ch. a = 12.64(A663) - 2.49(A645)
Ch. b = 5.6(A663) + 23.26(A645)

Extraction of defense enzymes from cucumber

Sodium phosphate buffer (0.1M) at pH of 7.1 (2 ml buffer/ g tissue) was used to ground leaves samples by blender. The squashed tissues were pressured using four layers of cheese cloth and the sap was centrifuged at 3000 rpm for 20 min at 6 °C. The supernatant was used for polyphenoloxidase and peroxidase assessment by spectrophotometer (Mohamed *et al.* 2016). Each treatment was presented by three replicates.

Polyphenoloxidase assay

The method described by Matta and Dimond (1963) was used to assess polyphenoloxidase activity. The method was carried out by mixing 0.1 ml of enzyme crude extract with 1.0 ml of sodium phosphate buffer (0.2 M) at pH of 7.0 as well as 1.0 ml of 10^{-3} M catechol and the final volume was brought to 6.0 ml with Milli Q water. The absorbance of the mixture was measured three times at 495 nm using spectrophotometer to determine the enzyme activity (Matta and Dimond, 1963).

Peroxidase assay

Peroxidase activity was assessed by technique described by Allam and Hollis (1972) by estimating the oxidation of pyrogallol at the presence of H_2O_2 at wavelength of 425 nm. The procedure was performed by mixing 0.5 ml of sodium phosphate buffer (1%) at pH 7.0, with 0.3 ml crude enzyme extract followed by 0.3 ml of pyrogallol (0.5 M) and finally 0.1ml of H_2O_2 (1%). After that the mixture was brought to a final volume of 3 ml with pure water. The activity of peroxidase was estimated by measuring the absorbance of the mixture at 425 nm three times using spectrophotometer.

Toxicity assessment

The utilized grown-up Wistar male rats (*Rattus norvegicus*) w 8 weeks old and 80-100 g in weight were obtained from Faculty of Medicine, Tanta University. Rats were housed in wire confines under suitable conditions with free access to drinking water and food. The rats were kept in temperature-controlled room and given a standard diet as described by Romestaing *et al.* (2007). Prior to treatments, rats were allowed to feed for two weeks for acclamation. The rats were divided into four groups of three individuals. Three groups were treated with the Fenton solutions (for 30 days) and the fourth was used as control. Oral administration was used to treat rats with Fenton solutions at dose level of 500 mg/kg body weight and control group received water only. After 30 days of treatment the rats were bled and blood samples as well as liver and kidney organs were taken for further investigation.

Biochemical parameters

Blood samples were centrifuged at 4500 rpm for 15 min and the supernatant blood serum was used to assess the glutamic-pyruvate transaminase (GPT) and creatinine using colorimetric methods as described by Reitman and Frankel (1957) and Barham and Trinder (1972), respectively.

Histological test

Kidney and liver samples of each treatment were kept in neutral buffered formalin 10% for histological tests that were carried out

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following the method described by Bancroft and Stevens (1996).

Statistical analysis

The analysis of variance test (ANOVA) using SPSS software (version19) and Duncan's multiple ranges test (Duncan, 1955) were used to analyze the data.

Results

Phytotoxicity of Fenton solutions on cucumber plants

Results of preliminary tests demonstrated that 50 mM H_2O_2 was successful against downy mildew (data not shown) and with no hazardous effect on cucumber plants (i.e. chlorophyll content, enzymes activity, leaf damage and plant height). Lower concentrations of H_2O_2 did not significantly reduce the downy mildew severity, while the higher concentration harmed cucumber leaf tissues.

Efficacy of Fenton solutions against downy mildew

Severity of downy mildew decreased markedly in treated cucumbers compared to untreated control (Tables 1 and 2). famoxadone + cymoxanil was the most efficient treatment toward downy mildew followed by Fenton like reagent, Fenton reagent and Fenton complex in both tested seasons (Tables 1, 2 and Figs. 1, 2). The decrease in disease severity was from 53 to 96%, 44 to 94%, 41 to 93% and 47 to 96%, for Famoxadone + cymoxanil, Fenton like reagent, Fenton reagent and Fenton complex, respectively.

Table 1 Effect of the tested treatments on the severity of downy mildew in cucumber in the first season (2014).

Treatments	Severity of downy mildew (%)[1]					
	For 1st spray		For 2nd spray		For 3rd spray	
	0	4	7	11	14	18
Fe SO_4+ H_2O_2	20.25 ± 1.83e	17.39 ± 1.05bc	15.52 ± 2.64 bc	12.89 ± 2.58 b	9.53 ± 0.94 b	4.00 ± 0.25 bc
$FeCl_3$+ H_2O_2	23.60 ± 1.68 bc	16.39 ± 2.17 bc	13.25 ± 1.02 cd	11.49 ± 1.02 bc	7.28 ± 0.85 c	2.53 ± 0.53 d
$FeCl_3$+H_2O_2+ oxalic	24.38 ± 2.10 ab	18.23 ± 0.99b	16.59 ± 0.78 b	14.33 ± 1.02 b	10.50 ± 1.16 b	4.51 ± 1.19 b
Famoxadone + Cymoxanil	22.24 ± 1.81cd	14.62 ± 0.29c	12.13 ± 0.58 d	8.38 ± 1.2 c	6.25 ± 0.93 c	2.46 ± 0.5 d
Control	25.09 ± 2.45 a	30.84 ± 2.79a	35.64 ± 2.31 a	39.63 ± 02.43 a	43.37 ± 1.78 a	65.12 ± 0.96 a

[1] Means in a column followed by the same letters are not significantly different using Duncan's multiple ranges test at $P < 0.05$.

Table 2 Effect of the tested treatments on the severity of downy mildew in cucumber in the second season (2015).

Treatments	Severity of downy mildew (%)[1]					
	For 1st spray		For 2nd spray		For 3rd spray	
	0	4	7	11	14	18
Fe SO_4+ H_2O_2	25.33 ± 2.01 cd	22.42 ± 1.23 c	21.27 ± 5.19 b	16.39 ± 0.74 b	12.43 ± 1.06 bc	6.00 ± 1.23 bc
$FeCl_3$+ H_2O_2	27.38 ± 2.07 b	21.16 ± 2.01 cd	17.26 ± 1.1 c	13.87 ± 1.42 c	9.25 ± 6.83 d	4.71 ± 0.69 cd
$FeCl_3$+ H_2O_2+ oxalic	30.41 ± 2.16 a	26.17 ± 1 b	21.13 ± 0.71 b	17.46 ± 1.04 b	13.29 ± 1.07 b	7.41 ± 0.98 b
Famoxadone + Cymoxanil	24.50 ± 1.95 de	18.36 ± 1.12 d	14.33 ± 0.86d	8.32 ± 1.13 d	6.15 ± 0.14 e	3.66 ± 0.32 de
Control	26.16 ± 2.66 bc	35.28 ± 2.15 a	40.57 ± 1.42 a	52.66 ± 1.96 a	66.16 ± 6.8 a	78.30 ± 1.99 a

[1] Means in a column followed by the same letters are not significantly different using Duncan's multiple ranges test at $P < 0.05$.

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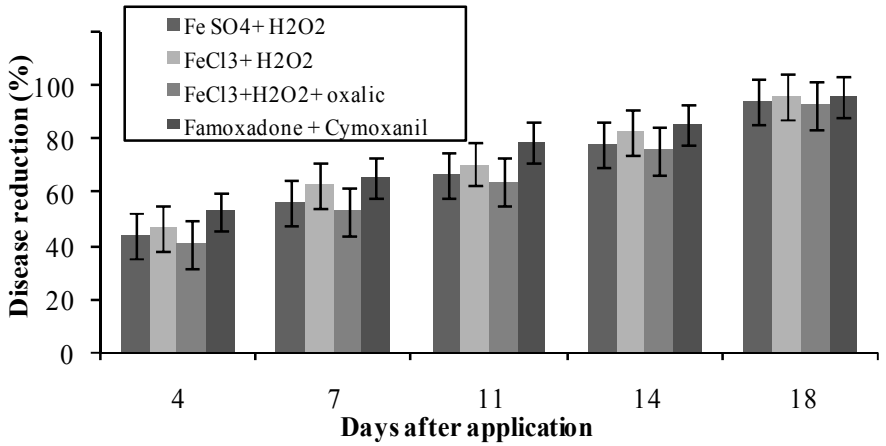

Figure 1 Efficacy (%disease reduction) of the tested treatments against *P. cubensis* at different days of application in the first season (2014). Bars indicate means of three observations with standard deviation (SD).

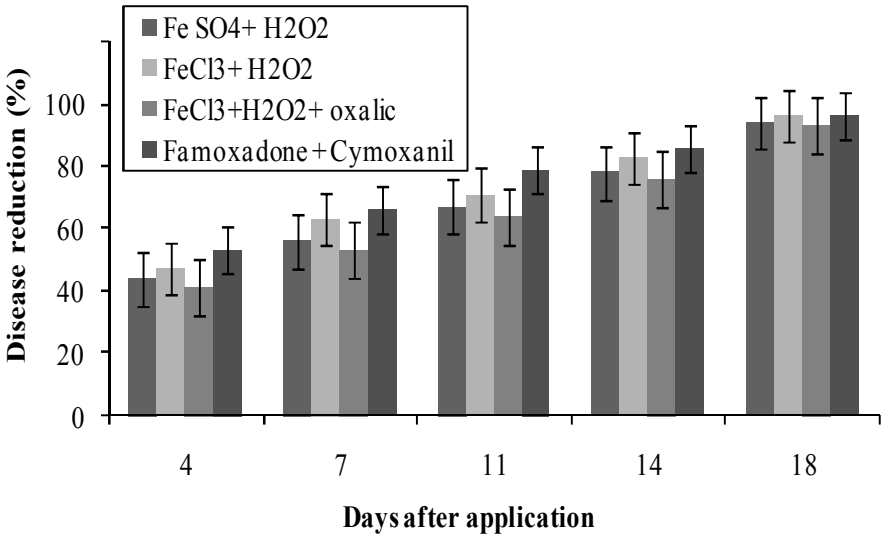

Figure 2 Efficacy (% disease reduction) of the tested treatments against *P. cubensis* at different days of application in the second season (2015). Bars indicate means of three observations with standard deviation (SD).

Impact of medicinal compounds on some biochemical parameters of cucumber plants

Results indicated that the assessed biochemical parameters (chlorophyll content, peroxidase and polyphenoloxidase activity) potentially raised in cucumber plants treated with Fenton solutions and fungicide compared to untreated control. The highest level of chlorophyll content (a and b) and defense enzymes activity (peroxidase and polyphenoloxidase) in treated cucumber plants was registered for famoxadone + cymoxanil followed by Fenton like reagent, Fenton reagent and Fenton complex, respectively as presented in Fig. (3) and Tables (3, 4). The enzymes activity increased gradually after application and finally decreased because of the reduction in disease severity by applied treatments.

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Effect of the treatments on some crop characters

The assessed growth and yield characters were considerably raised in cucumber treated with the examined medicinal treatments relative to untreated control.The best characters (plant height, fruit number/plant and fruit yield) were found in cucumber treated with famoxadone + cymoxanil took after by Fenton like reagent, Fenton reagent and Fenton complex, respectively (Table 5).

Safety of Fenton solutions

Biochemical factors

The data indicated there were no significant changes in glutamic–pyruvate tansaminase (GPT) and creatinine content of rats treated with the Fenton solutions compared to untreated control (Table 6).

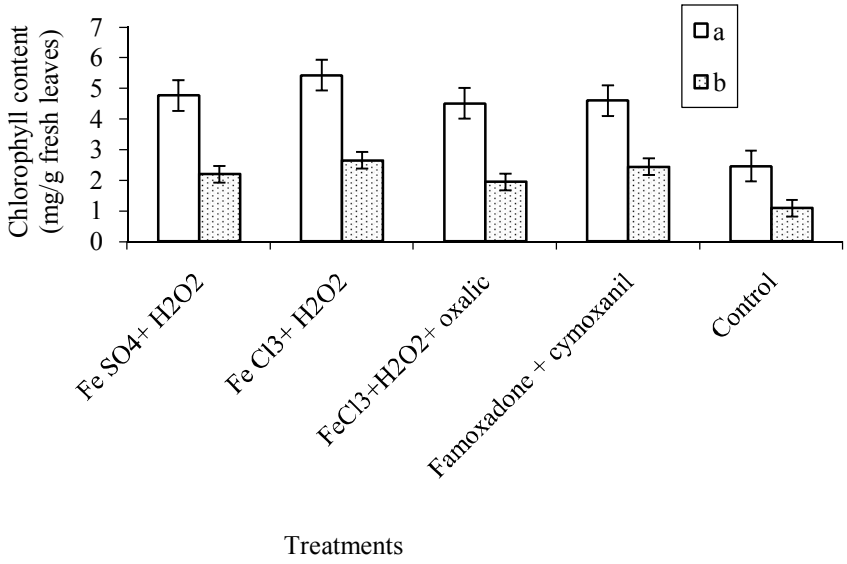

Figure 3 Effect of the tested treatments on chlorophyll content (a, b) in cucumber after 18 days from the third application. ∗Bars indicate means of three observations with standard deviation (SD).

Table 3 Effect of the tested treatments on peroxidase activity in cucumber.

Treatments	Activity of peroxidase (U/min/mg protein)[1]					
	For 1st spray		For 2nd spray		For 3rd spray	
	0	4	7	11	14	18
Fe SO_4+ H_2O_2	4.10 ± 0.03 ab	4.90 ± 0.03 b	5.80 ± 0.35 b	6.11 ± 0.01 b	6.73 ± 0.01 b	4.36 ± 0.01 bc
Fe Cl_3+ H_2O_2	4.20 ± 0.02 a	5.60 ± 0.02 a	6.53 ± 0.01 a	7.11 ± 0.01 a	7.33 ± 0.02 a	5.01 ± 0.01 a
$FeCl_3$+H_2O_2+ oxalic	3.90 ± 0.02 bc	4.20 ± 0.02 d	5.53 ± 0.01 b	5.91 ± 0.02 b	6.19 ± 0.01 c	4.33 ± 0.4 bc
Famoxadone + cymoxanil	4.12 ± 0.03 ab	4.63 ± 0.03 bc	4.69 ± 0.005 c	5.00 ± 0.02 d	5.20 ± 0.04 d	4.43 ± 0.01 b
Control	1.13 ± 0.05 d	1.23 ± 0.05 e	1.42 ± 0.01 d	1.93 ± 0.02 e	2.12 ± 0.03 e	1.35 ± 0.02 d

[1] Means in a column followed by the same letters are not significantly different using Duncan's multiple ranges test at $P < 0.05$.

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Table 4 Effect of the tested treatments on polyphenoloxidase activity in cucumber.

Treatments	Activity of polyphenoloxidase (U/min/mg protein) [1]					
	For 1st spray		For 2nd spray		For 3rd spray	
	0	4	7	11	14	18
Fe SO_4+ H_2O_2	1.12 ± 0.01 c	1.22 ± 0.01 c	1.45 ± 0.01 b	1.52 ± 0.03 b	1.63 ± 0.01 b	1.54 ± 0.005 b
Fe Cl_3+ H_2O_2	1.22 ± 0.005 b	1.32 ± 0.005 b	1.74 ± 0.02 a	1.91 ± 0.02 a	1.95 ± 0.01 a	1.87 ± 0.02 a
$FeCl_3$+H_2O_2+ oxalic	1.10 ± 0.01 c	1.19 ± 0.01 d	1.28 ± 0.01 c	1.36 ± 0.01 c	1.43 ± 0.01 c	1.22 ± 0.01c
famoxadone + cymoxanil	1.29 ± 0.01 a	1.39 ± 0.01 a	1.70 ± 0.01 a	1.90 ± 0.01 a	1.92 ± 0.02 a	1.81 ± 0.01a
Control	0.91 ± 0.01 d	0.96 ± 0.01 e	1.01 ± 0.01 d	1.10 ± 0.01 d	0.86 ± 0.02 d	0.86 ± 0.02d

[1] Means in a column followed by the same letters are not significantly different using Duncan's multiple ranges test at $P < 0.05$.

Table 5 Effect of the tested treatments on some growth and yield characters of cucumber.

Treatments	Plant height (m)[1]	Fruit number / Plant[1]	Fruit yield (kg/plant)[1]
Fe SO_4+ H_2O_2	3.00 ± 0.05 b	28.33 ± 0.57 c	3.60 ± 0.10 c
Fe Cl_3+ H_2O_2	3.21 ± 0.02 a	30.00 ± 1.0 b	3.80 ± 0.01 b
$FeCl_3$+H_2O_2+ oxalic	2.95 ± 0.05 b	26.00 ± 1.0 d	3.40 ± 0.10 d
famoxadone + cymoxanil	2.83 ± 0.07 c	33.66 ± 0.57 a	4.10 ± 0.10 a
Control	2. 50 ± 0.05 d	20.33 ± 0.57 e	2.70 ± 0.10 e

[1] Means in a column followed by the same letter are not significantly different using Duncan's multiple ranges test at $P < 0.05$.

Table 6 Effect of Fenton solutions on two biochemical parameters of treated rats.

Treatments	GPT (U/L)[1]	Creatinine (mg/dl)
Fe SO_4+ H_2O_2	49.00 a	0.369 a
Fe Cl_3+ H_2O_2	48.20 a	0.370 a
$FeCl_3$+H_2O_2+ oxalic	48.00 a	0.371 a
Control	48.10 a	0.372 a

[1] Glutamic-pyruvate transaminase (GPT).

The histological alterations in the kidney

Cross section of the normal kidney tissue is shown in Fig. (4 A). The tissues of rats treated with Fenton solutions were normal and without any histopathological changes (Fig. 4 B-D).

The histological alterations in the liver

The results showed that liver tissues of the rats treated with Fenton were normal similar to control (Fig. 5 A) but with mild histopathological changes such as a slight hydropic degeneration of hepatocytes (Fig. 5 B, D) and Kupffer cells activation (Fig. 5 C).

Discussion

The outcomes of the utilized Fenton solutions in this study demonstrated that Fenton like reagent, Fenton reagent and Fenton complex could control downy mildew of cucumber plants. The impacts of Fenton solutions likely happened because of its high •OH photoformation rate under sunlight and its acidity that is not so high as to harm the plant. The Fenton solutions are cost effective, biodegradable, utilizes ecologically safe reagents, such as H_2O_2, Fe and oxalic acid; therefore, may be utilized as alternatives to conventional fungicides to control downy mildew of cucumber (Sakugawa *et al.* 2012). The evolution of pathogens resistant to Fenton solutions will not happen since it is troublesome for them to stay away from the assault of •OH because of its high reactivity to different organic and inorganic compounds (Sakugawa *et al.,* 2012). Further and extensive studies to look at the feasible utilization of the Fenton solutions in rural fields are justified (Sakugawa *et al.,*

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2012). The results showed that the Fenton complex was more effective against downy mildew than Fenton and Fenton-like reagents. This may be due to the fact that Fe3+ organo complexes are able to absorb light from the solar spectrum and are stable at environmental pH; this avoids the pH dependency of traditional photo-Fenton systems (Sakuagwa *et al*., 2012). Strong and photoactive Fe3-complexes are formed by carboxylate or polycarboxylate groups (e.g. oxalate, malonate and citrate), Fe3-complexes are photoactive at neutral pH (Spuher *et al*., 2010).

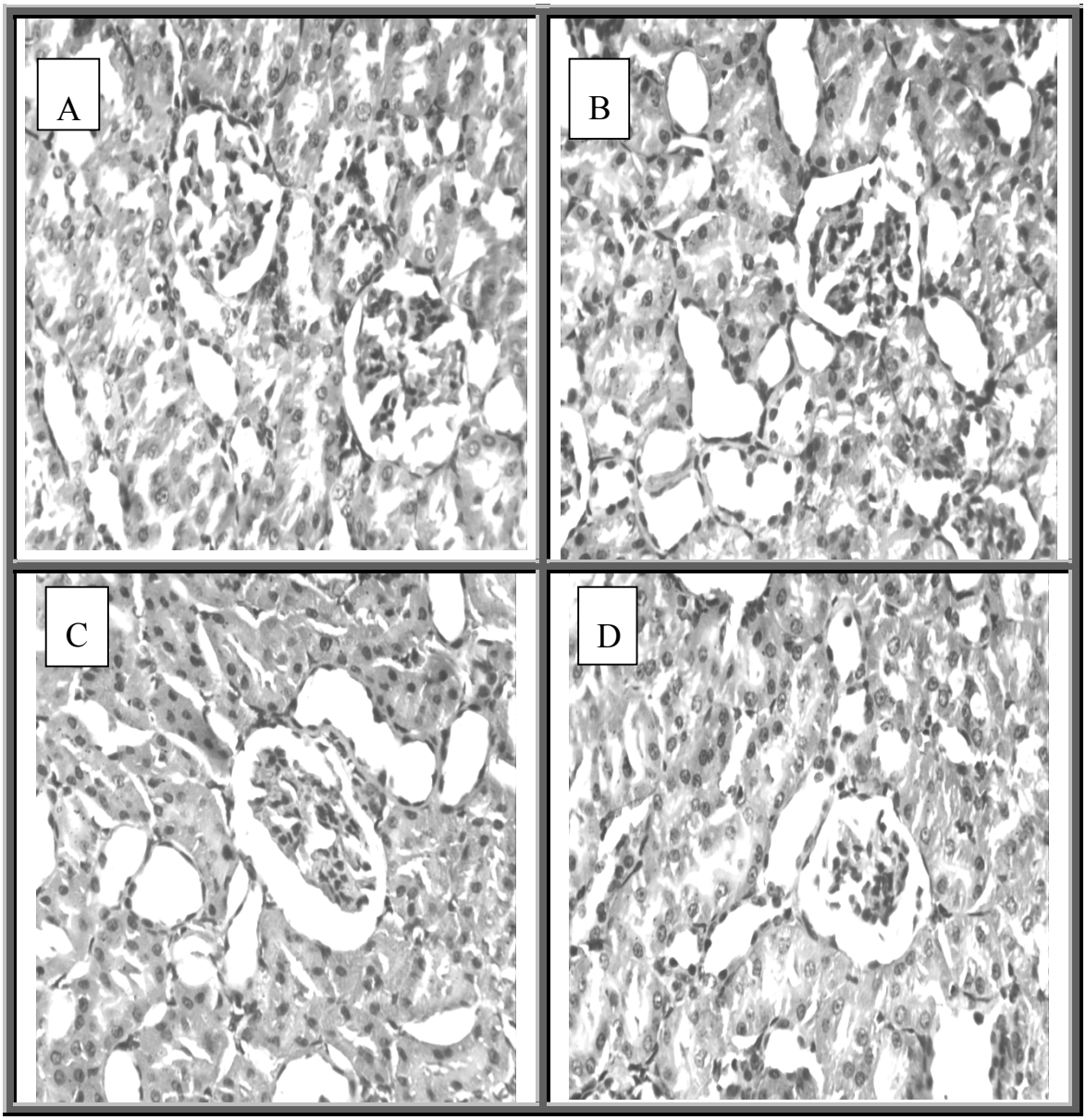

Figure 4 Sections in kidney tissue of rats treated with (H_2O_2)/ $Fe^{(2+)}$ (B), (H_2O_2)/ $Fe^{(3+)}$ (C), and a new type of solution, (H_2O_2) /$Fe^{(3+)}$/oxalic acid (D) compared to the control (A).

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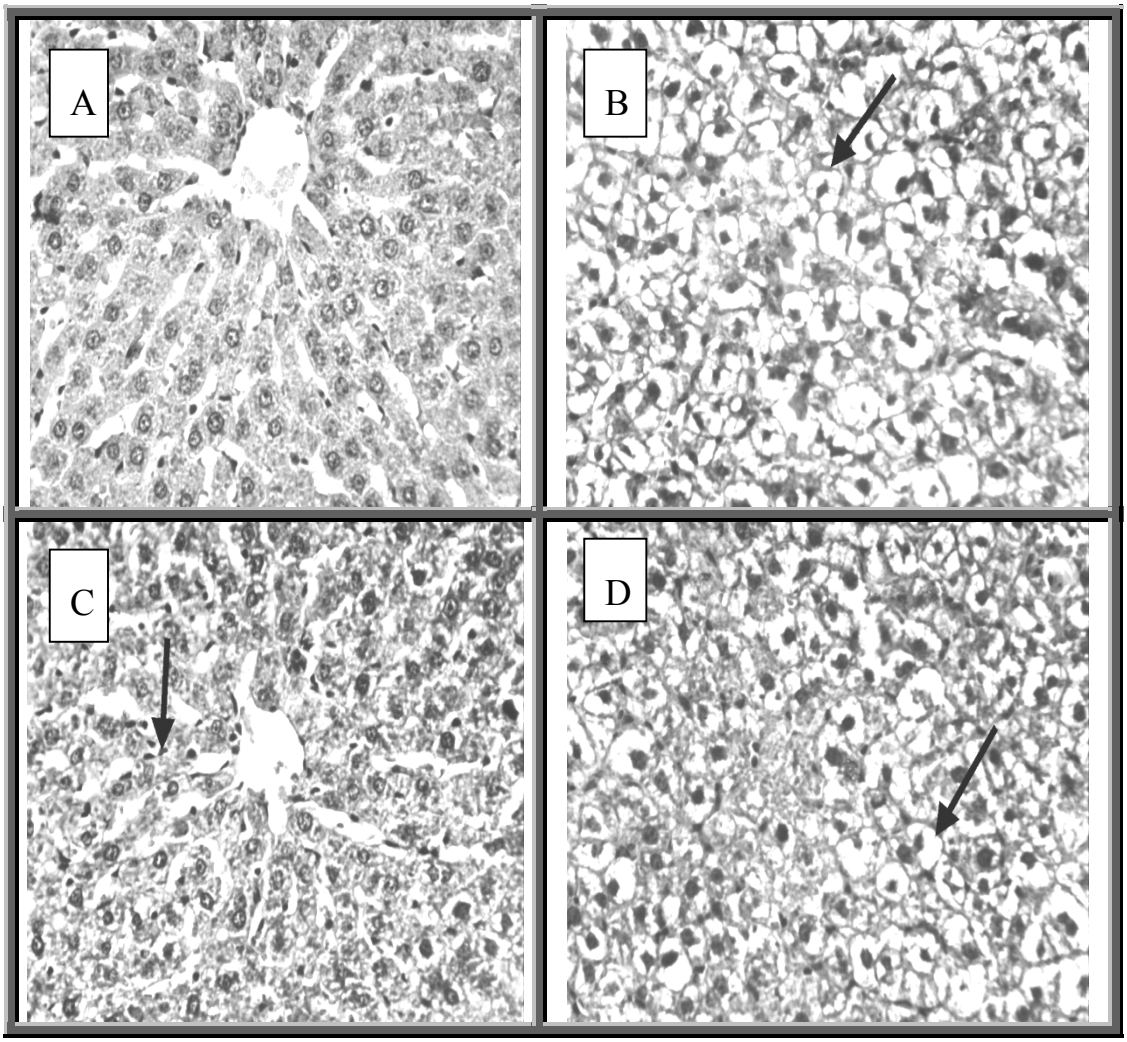

Figure 5 Sections of the liver tissue of rats treated with $(H_2O_2)/ Fe^{(2+)}$ (B), $(H_2O_2)/ Fe^{(3+)}$ (C), and a new type of solution, (H_2O_2) /$Fe^{(3+)}$/oxalic acid (D) compared to the control (A).

The mechanism by which foliar-applied Fenton solutions control this fungus on cucumbers has not been determined. However, it is clear that Fenton solutions are directly involved in the observed disease reductions and inhibition of the fungus, and that these effects occur through the plant tissue (Sakugawa *et al*., 2012). This study is a preliminary step in examination of the possible use of Fenton solutions to control downy mildew of cucumber. Treatment with Fenton solutions successfully suppressed the disease. Another mechanism is the antifungal activity is induced by the entrance of Fenton and photo-Fenton-type reactions into cells of the target microorganism (Polo-Lopez *et al*., 2011).This process is favored by the diffusion of H_2O_2 through membranes. Different oxidative reactions may occur inside the cell in the presence of extra hydrogen peroxide. Furthermore, the iron naturally present in cells can be released from the chelating molecules inside, which is trapped within cells under the radiation effect and may produce hydroxyl radicals via the Fenton/Habere Weiss Cycle (Imaly *et al*., 1988). It has also been reported that growing *E. coli* cells treated with Fenton contain approximately 20 mM of chelatable

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Fenton-active ferrous ions. The killing effect of Fenton reaction may be due to the degradation of polysaccharides by hydroxyl radicals. It has also recently been noted by Hammel *et al*. (2002) that the hydroxyl radicals pull hydrogen atoms from sugar subunits of polysaccharides, resulting in cleavage of the polysaccharide chain.

The results of this study showed increase of defense enzymes activity relative to control treatment and this is in agreement with findings of Stangarlin *et al*. (2011) who reported that the induction of resistance in plants involves the activation of defense enzymes in response to the treatment with elicitor agents, protecting against subsequent infection by pathogens. Therefore the control of downy mildew of cucumber by the tested treatments may be due to the induction of resistance in cucumber against downy mildew by the activation of defense enzymes (POX and PPO) which occurs through the oxidation of phenolic compounds to quinines that are known to be antimicrobial agents and toxic to the pathogen as well as synthesis of lignin which together with cellulose and other polysaccharides occurring in the cell wall of the plants, act as a physical barrier to the pathogen penetration. H_2O_2 in the Fenton solutions might be rapidly decomposed to low levels by peroxidase and a combination of antioxidant enzymes ubiquitously present in plant tissues (Foyer and Noctor, 2000; Lee, 2000). Therefore, the concentrations of H2O2 used in preparation of the Fenton solutions were defined in order to ensure that the plants could tolerate the oxidation capacities of the solutions and Fenton solutions would be safe to cucumber plants.

The results showed increase in cucumber yield and chlorophyll content of leaves under different treatments relative to control. This is because the tested treatments can suppress the leaf area affected by downy mildew fungus and serve to delay the loss of green leaf area due to disease, and thereby increase yield (Paveley *et al*., 1997). Application of different formulae of foliar fungicides as alternatives to synthetic fungicides resulted in reduction of foliar disease incidence and severity which reflected positively in plant stand, and its healthy growth and high yield (El-Mougy *et al*., 2013).

The biochemical and histological data for all tested Fenton solutions showed no significant changes in kidney or liver of treated rats relative to control treatment. This reflects the safety of these materials on mammalian health. Moreover, the observed changes in kidney and liver tissues were mostly uncorrelated with the dosages which may again indicate safety of the tested Fenton solutions on human health.

This study is considered a step toward more investigations on using these effective treatments as alternatives for control of plant pathogens. This will help to reduce the environmental pollution and the adverse effects on human health resulting from fungicide use. Since this Fenton solutions revealed non-significant toxicity in rats despite the high dosages administered orally therefore they will not be a hazard to humans as residue. The mechanisms of action of Fenton solutions against *P. cubensis* are not specific to it only and these compounds could also be used against other plant pathogens.

Conclusions

Fenton solutions had a significant effect on downy mildew of cucumber. Fenton solutions could be used as safe alternatives for conventional fungicides in downy mildew control. One of the major challenges for scientists using Fenton as a plant pathogen control agent is to find the conditions of greatest efficiency at neutral or near-neutral pH. *et al*.

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فنتون، فرآیند پیشرفته اکسیداسیون برای کنترل بیماری سفیدک دروغی خیار در شرایط گلخانه

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چکیده: در این پژوهش عمل درمان‌کننده معرف فنتون (H_2O_2)/$Fe^{(2+)}$)، ترکیب مشابه فنتون (H_2O_2)/$Fe^{(3+)}$) و کمپلکس فنتون (H_2O_2)/$Fe^{(3+)}$/oxalic acid) و هم‌چنین فاموکسادون + سیموکسانیل به‌صورت محلول‌پاشی علیه بیماری سفیدک دروغی خیار که توسط *Pseudoperonospora cubensis* (Berk. and Curtis) ایجاد می‌شود، در شرایط گلخانه در دو فصل زراعی مورد آزمایش قرار گرفت. هم-چنین، اثر این تیمارها بر برخی خصوصیات بیوشیمیایی و رشدی گیاه خیار بررسی شد. به‌علاوه، سمیت محلول‌های فنتون روی موش ارزیابی شد. به‌طوری‌که تغییرات بیوشیمیایی و بافت‌شناسی کبد و کلیه موش‌های تیمار شده در مقایسه با شاهد مورد ارزیابی قرار گرفت. نتایج نشان داد که در هر دو فصل زراعی، فاموکسادون + سیموکسانیل بهترین تیمار برعلیه سفیدک دروغی بود و بعد از آن معرف مشابه فنتون، معرف فنتون و کمپلکس فنتون قرار گرفتند. افزایش قابل‌توجهی در هر یک از شاخص‌های بیوشیمیایی گیاه خیار (کلروفیل، پراکسیداز و پلی‌فنل اکسیداز) و هم‌چنین در عملکرد خیارهای تیمار شده در مقایسه با شاهد تیمار نشده، مشاهده شد. تغییر قابل‌توجهی در کبد و کلیه موش‌های تیمار شده با محلول‌های فنتون مورد آزمایش در مقایسه با شاهد، مشاهده نشد. محلول‌های فنتون می‌تواند به‌عنوان ابزار مؤثر و بی‌خطر برای کنترل سفیدک دروغی خیار در شرایط گلخانه به‌کار گرفته شود.

واژگان کلیدی: سفیدک دروغی، قارچکش، فنتون، بیماری، سمیت، بافت‌شناسی بیماری

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