Induction and resistance against *Fusarium* wilt disease of tomato by using sweet basil (*Ocimum basilicum* L) extract

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Received 28 March 2014, accepted 10 November 2014. Published on the web 28 November 2014.

Akladious, S. A., Isaac, G. S. and Abu-Tahon, M. A. 2015. **Induction and resistance against *Fusarium* wilt disease of tomato by using sweet basil (*Ocimum basilicum* L) extract**. Can. J. Plant Sci. **95**: 689–701. The antifungal activity of *Ocimum basilicum* (sweet basil) extract against *Fusarium oxysporum* f. sp. *lycopersici* race 3 the causal agent of tomato wilt and its ability in inducing disease resistance were studied in vivo using seed-soaking treatment before sowing. Plants were harvested at 45 and 105 d (vegetative and flowering stages) after sowing. Treatment with *O. basilicum* extract decreased the disease incidence from 94.70 to 18.00%. Results revealed that growth parameters and photosynthetic pigments were markedly inhibited in tomato plants in response to *Fusarium* wilt disease, whereas the contents of non-enzymatic and enzymatic antioxidants were increased as compared with healthy control plants. Moreover, presoaking in basil extract enhanced all the mentioned parameters in both healthy and infected plants. SDS-PAGE analysis of tomato leaves revealed that seed treated with basil extract resulted in an induction of novel protein bands during the vegetative stage. These new proteins were not detected in untreated healthy or infected control plants. Electrophoretic studies of polyphenol oxidase, esterase and malate dehydrogenase isoenzymes showed wide variations in their intensities and densities among all treatments. It seems that *O. basilicum* extract was able to enhance the biological control of *Fusarium* wilt disease of tomato.

Key words: Sweet basil, *Fusarium oxysporum* f. sp. *lycopersici*, disease incidence, antioxidants, SDS-PAGE electrophoretic, isoenzymes

Akladious, S. A., Isaac, G. S. et Abu-Tahon, M. A. 2015. **Résistance à la flétrissure due à *Fusarium* et induction de celle-ci par l'extrait de basilic (*Ocimum basilicum* L) chez la tomate**. Can. J. Plant Sci. **95**: 689–701. Les auteurs ont étudié le pouvoir antifongique de l'extrait de basilic (*Ocimum basilicum*) contre la race 3 de *Fusarium oxysporum* f. sp. *lycopersici* responsable de la flétrissure de la tomate; ils se sont également intéressés à la capacité de l'extrait à induire la résistance à cette maladie *in vivo* par trempage des semences avant leur mise en terre. Les plants ont été récoltés 45 et 105 jours après les semis (stade végétatif et floraison). Le traitement avec l'extrait d'*O. basilicum* abaisse l'incidence de la maladie de 94,70 à 18,00 %. Les résultats indiquent que la flétrissure causée par *Fusarium* inhibe nettement les paramètres de croissance et les pigments de la photosynthèse tout en augmentant la concentration d'antioxydants enzymatiques et non enzymatiques, comparativement à ce qui se produit chez les plants témoins, non attaqués par la maladie. Par ailleurs, le trempage préalable dans l'extrait de basilic rehausse les paramètres précités chez les plants sains et malades. L'analyse des feuilles de tomate par électrophorèse sur gel de polyacrylamide en présence de SDS (SDS-SAGE) révèle que le traitement des semences avec l'extrait de basilic entraîne l'induction de nouvelles bandes protéiques pendant le stade végétatif. Ces nouvelles protéines n'ont pas été décelées chez les plants témoins infectés ou sains non traités. L'analyse par électrophorèse des isoenzymes de la polyphénol oxydase, de l'estérase et de la malate déshydrogénase indique d'importantes variations au niveau de l'intensité et de la densité pour tous les traitements. Apparemment, l'extrait d'*O. basilicum* accroît le contrôle biologique de la flétrissure de la tomate attribuable à *Fusarium*.

Mots clés: Basilic, *Fusarium oxysporum* f. sp. *lycopersici*, incidence de la maladie, antioxydants, analyse SDS-PAGE, isoenzymes

Tomato (*Lycopersicon esculentum* Mill.) is considered one of the most important and solanaceous vegetable crops in Egypt for local consumption and export. Tomato plants are subjected to attack by numerous diseases, wherever the crop is planted, and fungal pathogens, like *Fusarium oxysporum* f. sp. *lycopersici*, the causal agent of wilt disease (Stone et al. 2000). *Fusarium* wilt disease by *F. oxysporum* f. sp. *lycopersici* is one of the most devastating diseases of tomato (Amini and Sidovich 2010). The effects of the pathogen on tomato plantations may either cause their death in severe cases or at least a considerable reduction of the crop yield. Three races of *F. oxysporum* f. sp. *lycopersici* have been reported. They are distinguished by their virulence to tomato cultivars that contain single resistance genes. Race 1 was initially described in 1886 (Booth 1971), and race 2 was first

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Abbreviations: **APX**, ascorbate peroxidase; **CAT**, catalase; **EST**, esterase; **GR**, glutathione reductase; **Mdh**, malate dehydrogenase; **POX**, peroxidase; **PPO**, polyphenol oxidase; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase

reported in 1945 in Ohio (Alexander and Tucker 1945). Race 3 was observed in Australia in 1978 (Grattidge and O'Brien 1982) and was reported in several US states. Due to its relatively recent discovery, the majority of commercial tomato cultivars are still susceptible race 3 (Reis and Bioteux 2007). Studies on race 3 are very limited. In recent years, much attention has been given to non-chemical systems for seed treatment to protect them against many plant pathogens. Plant extracts have played significant roles in the inhibition of the seed-borne pathogen *F. oxysporum* and in the improvement of seed quality and emergence of plant seeds. Many studies showed that the application of some plant extracts can induce systemic resistance in many plants through the accumulation of pathogenesis-related proteins (PR-proteins) (Satheesh and Pari 2004). Treatment of some plants with plant extracts provided control of many fungal diseases through metabolic changes in plants, including induction of phenol biosynthesis enzymes, antioxidant defensive enzymes and phenol accumulation (Kamalakannan et al. 2004).

The genus *Ocimum* (basil) belongs to the family Lamiaceae, subfamily Ocimoideae and includes over 150 different species and varieties distributed in tropical regions of Asia, Africa, Central and South America (Darrah 1980). Members of the family to which *Ocimum* belongs are strongly aromatic due to essential oils, which consist of monoterpenes, sesquiterpenes and phenylpropanoids. Several essential oils of aromatic plants including *Ocimum basilicum* (basil) have totally inhibited fungal development on maize kernels (Montes-Belmont and Carvajal 1998). Many studies have showed that basil contains antioxidants such as rosmarinic acid, β-carotene and tocopherol, which have anti-fungal, antimicrobial and anti-apoptotic properties (Leung and Foster 1996; Kaya et al. 2008; Isaac and Abu-Tahon 2014).

The aim of the current study is to determine the efficacy of aqueous extract of *O. basilicum* for controlling the important tomato pathogenic fungi, *Fusarium oxysporum* f. sp. *lycopersici* race 3, by studying some of the physiological and biochemical attributes of infected and healthy tomato plants.

MATERIALS AND METHODS

Plant Pathogen

The causal pathogen of tomato wilt disease *F. oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen NRRL 26037 race 3 was obtained from NRRL (Agricultural Research Service Culture Collection) through United State Department of Agriculture (USDA), New Orleans, LA 70179. The cultures were grown on Czapek's medium (Tuite 1969) at 28°C for 7 d before being used. Cell suspensions of pathogenic fungus were prepared by filtration of cultures through cheese cloth to remove mycelial fragments and used as a source of inoculum.

Preparation of Aqueous Extract of *Ocimum basilicum* (Sweet Basil)

The leaves and flowers of sweet basil (*Ocimum basilicum* L.) plants were collected at the flowering stage of growth from different plant gardens in Ain Shams University (the botanical garden of the Faculty of Education, botanical garden of the Faculty of Science, botanical garden of the Faculty of Agriculture) during the period 2013 Jun. 1–7. The selected parts were cut into small pieces and washed several times with running tap water, then surface sterilized with 0.1% mercuric chloride solution for 30 s and repeatedly washed with sterilized water. Plant materials were dried at 50°C for 24 h; the dried plant parts were ground to fine powder and then sieved with 80 mesh sieve. Two hundred grams of the powdered plant materials were blended in 1000 mL of sterilized cold distilled water for 48 h. The macerated materials were squeezing through double cheese cloth sheets, and then filtered through a Whatman no. 1 filter paper. The aqueous extract was set as the original concentration (20%). The extracts were utilized for the experiments according to the method described by Isaac and Abu-Tahon (2014).

Preparation of Cultivated Soil

Clay pots 30 cm in diameter containing loam-based garden soil were sterilized with a 5% formalin solution for 15 min. The soil was covered with a polyethylene sheet for 7 d to retain the gas and left to dry for 2 wk until all traces of formaldehyde disappeared. Soil sterilization was confirmed by weighting 1.0 g of sterilized soil then spraying it on the surface of a 9.0-cm sterilized petri dish containing 20 mL of Czapek's medium under sterilized conditions. The plates were incubated at 30°C for 7 d. At the end of the incubation period, no fungal growth was noticed on the surface of the dish. The experiment was carried out with five replicates. Pathogen inocula were added to the potted soil at a rate of 3% (vol/wt) and mixed thoroughly with the soil 1 wk before planting (Abdel-Monaim et al. 2011).

Plant Cultivation and Treatments

Tomato super strain B (*Lycopersicon esculantum* Mill., Castle rook cultivar) seeds were obtained by the Vegetables Research Center, El-Dokki, Giza, Egypt. Tomato seeds were surface sterilized by immersing them in 70% ethanol for 2 min, then in 0.2% sodium hypochlorite (NaOCl) for 3 min. They were washed several times with sterile distilled water and then divided into four groups (100 seeds per group) and treated as follows:

- Seeds of the first group were soaked in distilled water for 10 h, then sowed in sterilized soil free from pathogens and irrigated with tap water to serve as a control (untreated healthy plants).
- Seeds of the second group were soaked in distilled water for 10 h, then sowed in sterilized soil infested

with *F. oxysporum* and irrigated with tap water (untreated infected plants).

- Seeds of the third group were soaked in sweet basil extract for 10 h then sowed in sterilized soil infested with *F. oxysporum* and irrigated with tap water (treated infected plants).
- Seeds of the fourth group were soaked in sweet basil extract for 10 h, then sowed in sterilized soil free from pathogens and irrigated with tap water (treated healthy plants).

Each group of seeds was placed into 30-cm diameter pots, which were filled with about 3 kg soil. The seeds were sown to a depth of 3 cm in each pot. Ten pots (each containing 10 seeds) were used for each group. The pots were then placed in a nursery protected with wire, which received adequate direct sunshine from all directions; the freely draining clay pots were watered every 48 h with equal amount of water. This experiment was conducted under favorable environmental conditions (day length 12–14 h, temperature 28–30°C and humidity 65%). When emergence was complete (~7 d) the percentage of seed germination was assessed and the seedling density was reduced to seven seedlings per pot. The growing tomato plants were checked for foliar symptoms of *Fusarium* wilt disease after sowing.

Pathogenicity Test of *Fusarium oxysporum*

This experiment was carried out under field conditions in nursery protected with wire to evaluate the potential capabilities of the tested fungus according to the method described by Hilaal (1992). The percentage of infection was determined at 45 and 105 d after sowing. To determine the incidence of *Fusarium* infection in host plants, root pieces (1 cm long) were surface sterilized with 1% $Ca(OCl)_2$ after washing with tap water, and transferred to Czapek's Agar plates supplemented with penicillin (100 000 units L^{-1}) and streptomycin sulfate (200 mg L^{-1}) (five root pieces per plate). Plates were incubated at room temperature (28°C) and after 1 wk, infection of roots by *Fusarium* was recorded.

Parameters Used

Measurement of Disease Incidence

Disease incidence was measured after 45 d (vegetative stage) and 105 d (flowering stage) of sowing as described by Weitang et al. (2004). Disease incidence was recorded on a 0–4 scale, where 0 represents no infection and 4 denotes complete infection. Eight replications were maintained for each treatment in two separate experiments.

$$\text{Disease incidence (\%)} = [(\Sigma \text{ scale} \times \text{number of plants infected})/(\text{highest scale} \times \text{number of plants})] \times 100.$$

$$\text{Percentage of wilt disease control of tomato} = 100 - \text{disease incidence} \times 100$$

Growth Parameters

When the developed plants reached 45 d (vegetative stage) and 105 d (flowering stage) they were carefully uprooted from the soil of each treatment to assess morphological characteristics (shoot and root lengths, number of leaves and flowers as well as fresh and dry weights of shoots and roots) in addition to some physiological and biochemical criteria as follows.

Determination of Photosynthetic Pigment

The total photosynthetic pigments (chlorophyll $a+b$, carotenoid) were quantified with acetone (Vernon and Seely 1966). Photosynthetic pigments (chlorophyll $a+b$, carotenoid) were measured in each leaf pair. Leaf samples (500 mg) were homogenized with acetone (90% vol/vol), filtered and made up to a final volume of 50 mL. Pigment concentrations were calculated from the absorbance of extract at 663, 644 and 452.5 nm using the formula of the method of Lichtenthaler (1987) with some modification, given as below:

$$\text{Chlorophyll } a \text{ (mg g}^{-1} \text{ FW)} = (10.3 \times A663 - 0.92 \times A644) \times 50/500$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1} \text{ FW)} = (19.7 \times A644 - 3.87 \times A663) \times 50/500$$

$$\text{Carotenoids (mg g}^{-1} \text{ FW)} = (4.2 \times A452.5) - [(0.026 \times \text{Chl } a) + (0.426 \times \text{Chl } b)] \times 50/500.$$

Ascorbic Acid

Ascorbic acid was determined as described by Mukherjee and Choudhuri (1983). Four milliliters of the extract was mixed with 2 mL of 2% dinitrophenyl-hydrazine (in acidic medium) followed by the addition of 1 drop of 10% thiourea (in 70% ethanol). The mixture was boiled for 15 min in a water bath and after cooling to room temperature, 5 mL of 80% (vol/vol) H_2SO_4 was added to the mixture at 0°C (in an ice bath). The absorbance was recorded at 525 nm by spectrophotometer.

Determination of Total Phenol Content

The total phenol was determined using the Folins–Ciocalteau method. Leaf extracts (0.5 mL) were placed in test tubes with 2 mL of Folins–Ciocalteau reagent. After 3 min, 2 mL of 35% sodium carbonate was added and the test tubes were shaken. The mixture was allowed to stand for 1 h and the absorbance was measured at 745 nm using a spectrophotometer (Arash et al. 2010). Total phenolic contents

were determined as a gallic acid eguivalent (GAE) based on Folins–Ciocalteau calibration curve using gallic acid (ranging from 50 to 500 mg L^{-1}) as the standard and expressed as milligrams gallic acid per 100 g of fresh weight of tomato leaves.

Determination of Total Flavonoids Content

The amount of flavonoids was determined by the method of (Zhishen et al. 1999) using Quercetin as the standard. A known volume of the extracts was placed in a 10-mL volumetric flask. Distilled water was added to make the volume to 5 mL and then mixed with 0.3 mL $NaNO_2$ (1:20 wt/vol). Three mL of 10% aluminum trichloride (1:10 wt/vol) was added 5 min later. Six minutes later, 2 mL of 1 N NaOH was added. The total absorbance was measured at 510 nm. The flavonoid content was determined from the calibration curve, and expressed as mg quercetin 100 g^{-1} of fresh weight of tomato leaves.

Assay of Enzymatic Antioxidants

Catalase (CAT) activity was determined by measuring the initial rate of disappearance of hydrogen peroxide as described by Velikova et al. (2000). The reaction mixture (3 mL) contained 10 mM potassium phosphate buffer (pH 7.0) and 0.1 mL enzyme extract and the reaction was started by adding 0.035 mL of 3% hydrogen peroxide. A decrease in hydrogen peroxide concentration was followed by a decline in optical density at the wavelength of 240 nm. The non-enzyme extract mixture served as a blank. The CAT activity was calculated using the extinction coefficient of 40 mM^{-1} cm^{-1} and the activity was expressed as μmol H_2O_2 reduced mg $protein^{-1}$ min^{-1}.

Peroxidase (POX) activity was determined as an increase in optical density due to the formation of guaiacol dehydrogenation product according to Velikova et al. (2000). The reaction mixture (3 mL) contained 10 mM potassium phosphate buffer (pH 7.0), 0.04 mL enzyme extract, 0.6 mL guaiacol, and 1% (wt/vol) aqueous solution, and the reaction was started by adding 0.15 mL of 100 mM hydrogen peroxide. The absorbance was recorded at the 470 nm wavelength in a spectrophotometer. The non-enzyme extract mixture served as a blank. The POX activity was determined using the extinction coefficient of 26.6 mM^{-1} cm^{-1} and the activity was expressed as μmol GDHP mg $protein^{-1}min^{-1}$.

Ascorbate peroxidase (APX) activity was determined by measuring a decrease in optical density at the 290 nm wavelength as ascorbate was oxidized, as described by Prochazhava et al. (2001). The reaction mixture (3 mL) contained 50 mM ascorbic acid, 0.1 mM EDTA, and 0.1 mL enzyme extract and the reaction was started by adding hydrogen peroxide to 1.5 mM. The non-enzyme extract mixture served as a blank. The APX activity was calculated using the extinction coefficient of 2.8 mM^{-1} cm^{-1} (Nakano and Asada 1981) and the activity was expressed as mmol ascorbate oxidized mg $protein^{-1}$ min^{-1}.

Superoxide dismutase (SOD) activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium chloride, using a reaction mixture consisting of 1 M Na_2CO_3, 200 mM methionine, 2.25 mM nitroblue tetrazolium chloride, 3 mM EDTA, 60 mM riboflavin and 0.1 M phosphate buffer (pH 7.8). Absorbance was read at 560 nm (Beauchamp and Fridovich 1971).

Glutathione reductase (GR) activity was determined based on the decrease in absorbance at 340 nm due to the oxidation of NADPH to NADP according to the method of Foyer and Halliwell (1976), with minor modifications. The reaction mixture (3 mL) consisted of 50 mM Tris-HCl (pH 7.6), 5 mM $MgCl_2$, 0.5 mM GSSG, 0.2 mM NADPH and 0.1 mL enzyme extract. The reaction was started by the addition of GSSG and the NADPH oxidation rate was monitored at 340 nm for 3 min.

Electrophoretic of Protein Patterns

The electrophoretic protein profile of tomato leaves was analyzed according to SDS-PAGE technique (Laemmli 1970), which was performed using 12% running gel and 3% stacking gel. Running gel contained 30% wt/vol acrylamide, 0.8% wt/vol methyl bis acrylamide, 10% wt/vol SDS, 1.5 M Tris-HCl (PH = 8.3), 9.3 mL H_2O, 10% wt/vol ammonium persulfate solution and 23 μL of tetramethylethylenediamine. Stacking gel contained 30% wt/vol acrylamide, 0.8% wt/vol methyl bis-acrylamide, 10% wt/vol SDS, 0.5 M Tris-HCl (PH = 6.8), 10.10 mL of H_2O, 10% wt/vol ammonium persulfate and 23 μL of tetramethylethylenediamine. Running buffer of the tank contained 0.125 M Tris-HCl (PH = 8.5), 0.96 M glycine and 0.5% wt/vol SDS. The sample buffer was used with an equal volume of protein extraction supernatant.

Enzyme Extraction and Isozyme Analysis

Fresh and young leaves from each accession were homogenized in cold extraction buffer [0.05M Tris-HCl pH: 8.3 (1.5 wt/vol)] using a mortar. The extract was centrifuged (10 000 rpm) for 10 min at 4°C and the resultant supernatant was used as enzyme source.

Isozyme polyphenol oxidase (PPO) was stained according to the procedure described by Sato and Hasegawa (1976) (0.1 M Na-phosphate buffer pH 6.8, 15 mg catechol and 50 mg sulfonic acid); Isozyme esterase (EST) was stained according to Tanksley and Orten (1983) (0.1 M Na-phosphate buffer pH 6.2, 0.1% Fast blue RR salt, 0.05% α-naphthyl propionate or β-naphthyl acetate), whereas isozyme malate dehydrogenase (Mdh) was stained according to Brown et al. (1978) (0.1 M Tris-HCl buffer pH 7.5, 0.12 M DL-Malate, 0.015% NAD, 20 Mm MTT and 4 mM PMS).

Isozymes were separated on 10% PAGE vertical slab gels (16 × 18 × 0.2 cm) according to Wendel and Weeden (1989). The gel buffer was composed of 45 mM Tris-HCl, 25 mM boric acid and 1 mM EDTA-Na_2 pH 8.6, and the electrode buffer was composed of 0.18 M Tris-HCl, 0.1 M boric acid and 4 mM EDTA-Na_2. The gels were stained by shaking in the dark at 37°C in the appropriate staining solution as previously described for each isozyme. After staining, the reaction was stopped by washing the gel two to three times with tap water. The gel was then kept in the fixing solution (glycerol and water 1: 1 vol/vol) for 24 h and rinsed twice in tap water, then photographed. Interpretation of banding patterns followed standard principles (Wendel and Weeden 1989).

Statistical Analysis

The experiment was designed as a completely randomized design with three replicates ($n = 3$). The results were subjected to two-way analysis of variance (ANOVA) and the mean differences were compared by the Duncan test at 5% significance level.

RESULTS

Disease Incidence

The induced resistance in tomato plants treated with sweet basil extract was measured by the reduction of wilt disease severity in tomato plants infected with *F. oxysporum*. Data in Table 1 show that the highest percentage of disease incidence in tomato plants was recorded in the untreated infected plants 45 and 105 d after sowing (Group 2). The wilt disease started to appear 4 wk after sowing; the morphological symptoms appeared in the form of chlorosis, dropping and epinasty of leaves followed by yellowing, stunting and death of plant. The results obtained clearly demonstrate that the percentage of disease incidence was decreased in infected plants treated with sweet basil extract from 52% in infected plants to 18% in infected treated plants 45 d after sowing, from 94% to 18% 105 after days of sowing. The plant extract of sweet basil seems to be more effective at flowering stage (105 d). The observed disease control by sweet basil may be due to the presence of biologically active constituents, which have either direct antimicrobial activities or induce a host plants defense response resulting in a reduction of *Fusarium* wilt development.

Changes in Growth Parameters

Data presented in Table 2 show that growth parameters of tomato plants in terms of shoot and root lengths, numbers of leaves and flowers as well as fresh and dry weights of shoots and roots during vegetative and flowering stages were markedly inhibited due to the infection with *F. oxysporum* as compared with healthy plants (control). On the other hand, the infected and healthy plants produced from seeds presoaked in *O. basilicum* extract (Group 3 and 4) showed significant increases in their growth parameters as compared with untreated healthy plants (control). The magnitude of such increases was much more pronounced in healthy plants produced from seeds treated with *O. basilicum* extract (Group 4).

Changes in Photosynthetic Pigments

Data in Table 3 reveal that infection of tomato plants with *F. oxysporum* f. sp. *lycopersici* resulted in a significant decrease in chlorophyll *a* and *b* and total photosynthetic pigments at vegetative and flowering stages in the leaves as compared with the healthy control. Application of *O. basilicum* extract caused increases in total photosynthetic pigments and carotenoid contents in the leaves of both infected and healthy plants. The maximum increase in total pigment content was observed in healthy plants treated with *O. basilicum* extract during the flowering stage; this may be due to the increase in leaf number and area observed at the flowering stage.

Changes in Ascorbic Acid, Total Phenols and Flavonoids Contents

The present investigation showed that ascorbic acid, total phenols and flavonoids contents were significantly increased in all treatments as compared with the control plants during both vegetative and flowering stages. All these contents were recorded to be higher when *F. oxysporum* infected plants were treated with *O. basilicum* extract at the vegetative stage. Untreated infected tomato plants (Group 2) showed no noticeable increases in these contents (Table 4).

Changes in Enzymatic Antioxidants

Data presented in Table 5 show that infection with *F. oxysporum* f. sp. *lycopersici* significantly increased the activities of CAT, POX, APX, SOD and GR at

Table 1. Effect of aqueous extract of *Ocimum basilicum* (basil) on percentage of seed germination, percentage of wilt disease incidence and percentage of wilt disease control

Treatment	Seed germination (%)	Disease incidence (%)		Disease control (%)	
		45 d	105 d	45 d	105 d
Healthy plants (control)	100	0.00	0.00	0.00	0.00
Infected plants with *F. oxysporum*	74	52.1	94.7	47.9	5.3
Infected plants produced from seeds soaked in basil extract	87	18.0	18.0	82.0	82.0
Healthy plants produced from seeds soaked in basil extract	100	0.00	0.00	0.00	0.00

Table 2. Effect of *Ocimum basilicum* (basil) extract on growth parameters of healthy and infected tomato plants with *Fusarium oxysporum* f. sp. *lycopersici*

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves/ plant	No. of flower	Fresh weight (g)		Dry weight (g)	
					Shoot	Root	Shoot	Root
			Vegetative stage					
Healthy plants (control)	21.8*de*	20.8*bc*	6.0*bc*	–	5.8*d*	0.4*e*	0.6*c*	0.07*c*
Infected plants with *F. oxysporum*	15.2*e*	18.0*c*	5.0*c*	–	2.6*e*	0.2*e*	0.2*d*	0.05*c*
Infected plants produced from seeds soaked in basil extract	24.6*cd*	21.7*bc*	6.6*bc*	–	6.4*cd*	0.6*de*	0.6*c*	0.09*c*
Healthy plants produced from seeds soaked in basil extract	29.0*bcd*	22.3*bc*	7.3*b*	–	9.0*c*	1.2*cde*	0.9*c*	0.11*c*
			Flowering stage					
Healthy plants (control)	31.0*abc*	23.0*bc*	7.3*b*	4.33*a*	13.0*b*	2.1*abc*	1.3*b*	0.31*ab*
Infected plants with *F. oxysporum*	29.6*bcd*	21.3*bc*	6.6*bc*	2.33*a*	8.8*cd*	1.7*bcd*	0.9*c*	0.29*b*
Infected plants produced from seeds soaked in basil extract	36.0*ab*	26.3*ab*	9.3*a*	5.33*a*	15.8*b*	2.5*ab*	1.6*ab*	0.36*ab*
Healthy plants produced from seeds soaked in basil extract	38.6*a*	30.6*a*	10.6*a*	7.33*a*	18.8*a*	3.2*a*	1.8*a*	0.40*a*

a–e Data presented as means of three replicates. In a column, means followed by different letters indicate a significant difference at $P \leq 0.05$ according to Duncan's multiple range test.

vegetative and flowering stages as compared with the healthy control. The APX and SOD activities were enhanced to a higher extent at all treatments than CAT and POX activities. Moreover, presoaking the seeds in *O. basilicum* extract before sowing markedly increased the CAT, POX, APX, SOD and GR activities in both infected and non-infected plants (Groups 3 and 4) as compared with the control. The maximum values in such activities were recorded in infected plants treated with *O. basilicum* extract (Group 3).

Changes in Protein Pattern

Table 6 and Fig. 1a, b reveal the changes in the pattern of protein electrophoresis (SDS-PAGE) extracted from the leaves of tomato plants infected with *F. oxysporum* f. sp. *lycopersici* or treated with *O. basilicum* extract either as separate treatments or as a combined treatment. At the vegetative stage (Fig. 1a), the results obtained reveal that 17 protein bands with molecular weights ranging between 139.56 and 23.12 kDa were observed in tomato leaves of healthy plants (control). The protein patterns of tomato leaves under all treatments comprise 15 major bands (common bands) having molecular weights of 139.5, 134.7, 129.5, 88.6, 77.0, 69.5, 61.4, 56.4, 44.1, 42.6, 40.4, 28.7, 27.3, 23.8 and 22.1 kDa, respectively. Two protein bands of molecular weights 84.28 and 64.23 kDa disappeared in tomato plants infected with *F. oxysporum* f. sp. *lycopersici* as compared with control plants. The total number of protein bands was decreased from 17 bands in the control plants to 15 in the infected plants.

Treatment of infected and healthy plants with *O. basilicum* extract resulted in the appearance of two newly synthesized protein bands, which have molecular weights of 31.87 and 24.95 kDa. These new proteins were not detected in untreated healthy (control) or untreated infected plants. Healthy plants treated with *O. basilicum* extract were characterized by the disappearance of only one protein band having the molecular weight of 64.23 kDa as compared with untreated healthy plants (control). A more or less similar pattern of change was observed in the protein banding pattern of tomato leaves during the flowering stage, but this stage was distinguished by the presence of three higher molecular weight protein bands having molecular weights of 193.83, 183.3 and 179.63 kDa.

Isozymes Expression

In the present investigation, PPO, EST and Mdh isozymes were studied. Photographs of the banding pattern produced by the application of isozymes technique under all treatments at both vegetative and flowering stages are shown in Fig. 2. Their scored bands are given in Table 7, whereas the number and types of bands and percentage of the total polymorphism are given in Table 8.

Table 3. Effect of *Ocimum basilicum* (basil) extract on photosynthetic pigments (mg g^{-1} fresh weight (FW)) of leaves of healthy and/or infected tomato plants with *Fusarium oxysporum* f. sp. *lycopersici*

Treatments	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)	Chlorophyll (*a*+*b*)	Carotenoids (mg g^{-1} FW)	Total pigments
			Vegetative stage		
Healthy plants (control)	31.5*d*	10.4*cd*	41.9*ef*	9.6*de*	51.6*ef*
Infected plants with *F. oxysporum*	24.6*e*	8.6*d*	33.2*f*	7.1*e*	40.4*f*
Infected plants produced from seeds soaked in basil extract	37.1*cd*	10.6*cd*	47.7*de*	9.9*de*	57.6*de*
Healthy plants produced from seeds soaked in basil extract	38.1*cd*	15.3*cb*	53.4*d*	10.3*de*	63.7*de*
			Flowering stage		
Healthy plants (control)	52.7*b*	20.3*b*	73.1*c*	16.3*bc*	89.4*c*
Infected plants with *F. oxysporum*	43.9*c*	13.9*cd*	57.8*d*	12.5*cd*	70.3*d*
Infected plants produced from seeds soaked in basil extract	56.2*b*	29.3*a*	85.6*b*	20.4*ab*	106.0*b*
Healthy plants produced from seeds soaked in basil extract	67.6*a*	32.6*a*	100.2*a*	24.3*a*	124.5*a*

a–f Data presented as means of three replicates. In a column, means followed by different letters indicate a significant difference at $P \leq 0.05$ according to Duncan's multiple range test.

Polyphenol Oxidase

Electrophoretic patterns of PPO isozyme illustrate the appearance of four bands. Bands nos. 1 and 2 were present in all treatments (common bands), whereas, Band no. 3 was present in some treatments and absent from the others (polymorphic). The fourth band was a unique band which appeared only in tomato plants infected with *F. oxysporum* f. sp. *lycopersici* at flowering stage. This band may be stress-related, as a response to infection with *F. oxysporum* (Fig. 2).

Esterase Isozyme

Expression of the EST isozyme demonstrated that the total number of bands was increased not only in the infected plants, but also in infected and healthy plants treated with *O. basilicum* extract. There is only one monomorphic band, which was present in all treatments. Another band appeared in some treatments but was absent from others (polymorphic band). The treatment of healthy tomato plants with *O. basilicum* extract resulted in the appearance of one unique strong band at the vegetative stage, which disappeared in all other treatments. Thus, the presence of such a band may be due to a stimulation effect of basil extract for the synthesis of this enzyme.

Malate Dehydrogenase

Isozyme profiles of Mdh in tomato leaves demonstrate that the total number of bands increased under all treatments as compared with control. Bands nos. 1 and 2 were present in all treatments (common bands). The other two bands were present in some treatments and absent from the others (polymorphic).

DISCUSSION

Biological control had attained importance in modern agriculture in order to curtail the hazards of the intensive use of chemicals for pest and disease control (Tuber and Baker 1988). Accordingly, the efficacy of plant extract from leaves and flowers of sweet basil against *F. oxysporum* f. sp. *lycopersici* race 3, the causal agent of wilt diseases of tomato, was studied. The initial interest in studying race 3 is that the majority of commercial tomato cultivars are still susceptible to race 3. This new *Fusarium* wilt might become an

Table 4. Effect of *Ocimum basilicum* (basil) extract on ascorbic acid, total phenol and total flavonoids contents of leaves of healthy and/or infected tomato plants with *Fusarium oxysporum* f. sp. *lycopersici*

Treatments	Ascorbic acid (µg g^{-1} FW)	Total phenol (mg gallic acid 100 g^{-1} FW)	Total flavonoids (mg quercetin 100 g^{-1} FW)
		Vegetative stage	
Healthy plants (control)	0.9*e*	45.2*d*	22.8*d*
Infected plants with *F. oxysporum*	1.1*e*	51.7*cd*	27.3*cd*
Infected plants produced from seeds soaked in basil extract	1.9*cd*	98.0*ab*	41.5*bc*
Healthy plants produced from seeds soaked in basil extract	1.3*de*	76.8*bc*	35.5*cd*
		Flowering stage	
Healthy plants (control)	0.3*c*	30.9*cd*	17.0*cd*
Infected plants with *F. oxysporum*	0.4*b*	36.2*bcd*	19.5*bc*
Infected plants produced from seeds soaked in basil extract	0.7*a*	78.9*a*	28.1*a*
Healthy plants produced from seeds soaked in basil extract	0.5*b*	62.4*bcd*	22.9*a*

a–e Data presented as means of three replicates. In a column, means followed by different letters indicate a significant difference at $P \leq 0.05$ according to Duncan's multiple range test.

Table 5. Effect of *Ocimum basilicum* (basil) extract on enzymatic antioxidants of leaves of healthy and/or infected tomato plants with *Fusarium oxysporum* f. sp. *lycopersici*

Treatments	Catalase (unit. min^{-1} g^{-1} FW)	Peroxidase (unit. min^{-1} g^{-1} FW)	Ascorbate peroxidase (unit. min^{-1} g^{-1} FW)	Superoxide dismutase (unit. min^{-1} g^{-1} FW)	Glutathione reductase (unit. min^{-1} g^{-1} FW)
			Vegetative stage		
Healthy plants (control)	225*de*	17.4*d*	94.2*e*	105.5*e*	166.7*e*
Infected plants with *F. oxysporum*	315*bc*	28.3*c*	201.2*d*	124.9*cd*	209.4*c*
Infected plants produced from seeds soaked in basil extract	390*a*	44.5*a*	314.8*a*	161.4*a*	268.6*a*
Healthy plants produced from seeds soaked in basil extract	340*b*	37.1*b*	251.6*b*	140.3*b*	241.9*b*
			Flowering stage		
Healthy plants (control)	190*e*	13.6*e*	89.4*e*	83.8*f*	125.0*f*
Infected plants with *F. oxysporum*	210*de*	17.5*d*	136.0*e*	113.3*de*	184.3*d*
Infected plants produced from seeds soaked in basil extract	320*bc*	34.7*b*	290.8*b*	156.1*a*	255.6*ab*
Healthy plants produced from seeds soaked in basil extract	270*cd*	28.6*c*	230.14*c*	133.5*bc*	223.0*c*

a–f Data presented as means of three replicates. In a column, means followed by different letters indicate a significant difference at $P \leq 0.05$ according to Duncan's multiple range test.

economically important disease since race 3-resistant cultivars are generally not yet available (Reis and Bioteux 2007).

The present study revealed that tomato plants infected with *F. oxysporum* showed a reduction in all growth parameters. This reduction may be due to the toxins produced by the fungus, which affect the stomatal function leading to uncontrolled transpiration and excessive loss of water, thus leading to wilted plants. In addition, the decrease in shoot dry weight might be related to increased respiration rate, decompartmentalization due to membrane degradation (Ahmed et al. 2009). Our results are in agreement with those of Hassanein et al. (2008), who found that the reduction in growth may also be related to the accumulation and action of phenolics produced from the degradation of cell wall lignin mainly via depolymerization resulting from fungal elicitors. On the other hand, treatment of plants with *O. basilicum* extract was highly effective on growth promotion and protection of tomato against *Fusarium*, and this might be carried out in a number of ways including antibiotic production, induced resistance and reducing the production of the mycotoxins fumonisin secreted by the pathogen (El-Khallal 2007). Similar results were obtained by Hassanein et al. (2008), who found that the fresh weight of tomato plant was increased when plants were treated with leaf extracts of neem and chinaberry, and these treatments have the potential for use in the control of *Fusarium* in tomato.

This may represent an indirect contribution to biocontrol through the conservation of root system function, both by fungal hypha growing out into the soil and increasing the absorbing surface of the roots, and by the maintenance of root cell activity (El-Khallal 2007). Also, the extracts from many different plant parts contain common growth-promoting substances, such as the essential oils extracted from the leaves and flowers of *O. basilicum* (Makri and Kintzios 2007). In addition, vitamins A and C, rosmarinic acid, phenols, alkaloids, steroids, sterols and saponins that were isolated from the leaves and flower of *O. basilicum* in relatively high concentrations have antimicrobial, antioxidant, antifungal and anti-inflammatory activities (Leung and Foster 1996).

The decrease in photosynthetic pigments of plants infected with *F. oxysporum* recorded in this study could be attributed to the consequence of the fungal effect on the release of transported toxins, which leads to the liberation of reactive oxygen species (ROS) (El-Khallal 2007), or it might have resulted from the high level of lipid peroxidation mediating cell damage in tomato tissues (Haberer and Kierber 2002). Meanwhile, soaking seeds in *O. basilicum* extract had a positive effect on increasing the amount of all photosynthetic pigments in the plants produced. This effect may be due to its role in activation of enzymes that regulate photosynthetic carbon reduction and protect chloroplast from oxidative damage. In all photosynthetic organisms, β-carotene, flavone, phenol and vitamins, all known to be present in *O. basilicum* extract, serve important photoprotective roles by scavenging ROS. Carotenoid pigments protect chlorophylls from photo-oxidative destruction and reduce chlorophyll degradation, where they function as energy carriers and photo-oxidation

Table 6. Effect of *Ocimum basilicum* (basil) extract on electrophoretic pattern of leaves of healthy and/or infected tomato plants with *Fusarium oxysporum* f. sp. *lycopersici* at vegetative and flowering stages of plant growth

	Vegetative stage[z]			
Molecular weight (kDa)	Lane 1	Lane 2	Lane 3	Lane 4
139.56	+	+	+	+
134.71	+	+	+	+
129.54	+	+	+	+
88.62	+	+	+	+
84.28	+	−	+	+
77.08	+	+	+	+
69.58	+	+	+	+
64.23	+	−	+	−
61.42	+	+	+	+
56.49	+	+	+	+
44.18	+	+	+	+
42.65	+	+	+	+
40.41	+	+	+	+
31.87	−	−	+	+
28.74	+	+	+	+
27.39	+	+	+	+
24.95	−	−	+	+
23.81	+	+	+	+
22.12	+	+	+	+
Total number of bands	17	15	19	18
	Flowering stage[y]			
Molecular weight (kDa)	Lane 5	Lane 6	Lane 7	Lane 8
193.83	+	+	+	+
183.32	+	+	+	+
179.63	+	+	+	+
132.81	+	+	+	+
126.91	+	+	+	+
120.75	+	+	+	+
107.81	+	+	+	+
93.14	+	+	+	+
82.16	+	+	+	+
73.95	+	+	+	+
63.8	+	−	+	+
43.9	+	+	+	+
36.76	+	+	+	+
23.54	+	+	+	+
19.66	+	−	−	+
Total number of bands	15	13	14	15

[z]Vegetative stage: Lane 1: Healthy plants (Control). Lane 2: Plants infected with *F. oxysporum*. Lane 3: Plants infected with *F. oxysporum* and treated with *O. basilicum*. Lane 4: Healthy plants treated with *O. basilicum* extract.
[y]Flowering stage: Lane 5: Healthy plants (Control). Lane 6: Plants infected with *F. oxysporum*. Lane 7: Infected plants with *F. oxysporum* and treated with *O. basilicum*. Lane 8: Healthy plants treated with *O. basilicum* extract.

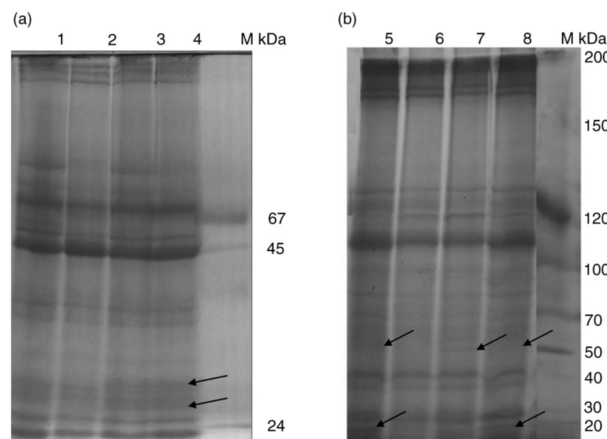

Fig. 1. Electrophoretic banding profiles of protein extracted from the leaves of tomato plants and the effects of various treatments. (a) Vegetative stage. M, Marker protein. Lane 1: Healthy plants (Control). Lane 2: Plants infected with *F. oxysporum*. Lane 3: Plants infected with *F. oxysporum* and treated with *O. basilicum*. Lane 4: Healthy plants treated with *O. basilicum* extract. (b) Flowering stage. Lane 5: Healthy plants (Control). Lane 6: Plants infected with *F. oxysporum*. Lane 7: Plants infected with *F. oxysporum* and treated with *O. basilicum*. Lane 8: Healthy plants treated with *O. basilicum* extract.

protectors because carotenoids are free radical scavengers (Abbas and Akladious 2013). Moreover, Sen and Mukherjee (2009) reported that in tomato plants carotenoids (β-carotene and xanthophylls) play an important role in protecting cells against stress and have the ability to quench ROS. The antioxidant effectiveness of *O. basilicum* is very important in the regulation of photosynthesis due to the presence of many substances in basil extract, such as tocopherol, phenols, flavonoids and high levels of important carotenoids, which are responsible for its antioxidant properties (Dean et al. 2005).

Development of an antioxidant defense system in plants protects them against oxidative stress damage by either partial suppression of ROS production or the scavenging of ROS which are generated during plant pathogen interactions (Cavalcanti et al. 2007). In the present study *O. basilicum* extract significantly increased total phenolic content in infected and non-infected tomato plants with a higher magnitude in infected plants as compared with healthy control. Accumulation of phenolic compounds at the infection site has been correlated with the restriction of pathogen development, since such compounds are toxic to pathogens. Also, phenolic compounds may impede pathogen infection by increasing the mechanical strength of the host cell wall. These results agree with the general speculation, that when plant cells are recruited into infection, there is a switch from the normal primary metabolism to a multitude of the secondary defense pathways, and activation of novel defense enzymes and genes takes place (Tan et al. 2004). Baâtour et al. (2012) noticed an increase in secondary metabolism, as shown by the enhancement of high phenolic contents and antioxidant activities at vegetative stage as compared with flowering stage in sweet marjoram plants under stress conditions. Ascorbic acid (vitamin C), the most abundant, powerful and water-soluble non-enzymatic antioxidant, acts to

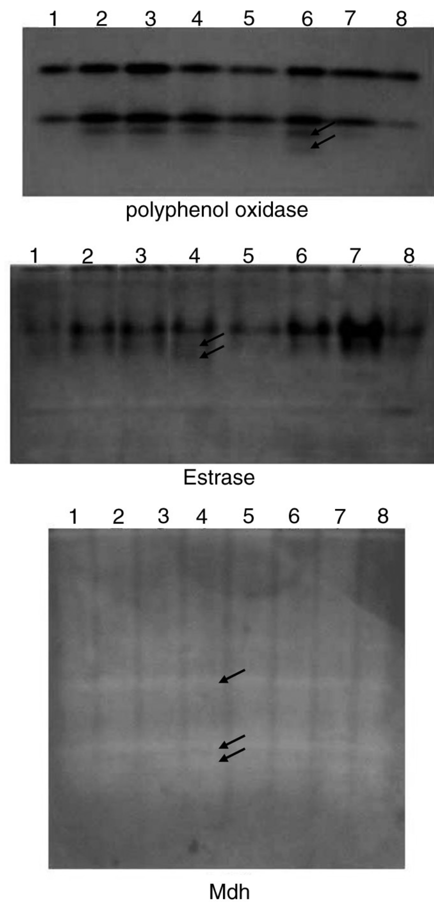

Fig. 2. Electrophoretic patterns of polyphenol oxidase (PPO), esterase (EST) and malate dehydrogenase (Mdh) isoenzymes of tomato leaves in response to various treatments. Lanes 1–4: Vegetative stage. Lanes 5–8: Flowering stage.

prevent or minimize the damage caused by ROS. It can directly scavenge superoxide, hydroxyl radicals and singlet oxygen and reduce H_2O_2 to water via APX reaction (Abbas and Akladious 2013). The chemical evolution and significance of flavonoids have been assumed to play an important role in overcoming the oxidative stress in cells (Dean et al. 2005). Presently, flavonoid contents showed enhanced synthesis in *F. oxysporum* infected plants treated with *O. basilicum* in comparison with either untreated infected plants or control plants. Seed soaking in *O. basilicum* extract before sowing might have caused an increase in the level of vitamins and antioxidants, which all are known to be present in *O. basilicum* extract.

Various antioxidant enzymes, such as POX and CAT, can participate in reactive oxygen metabolism of the species during infection. Catalase can protect the cell from H_2O_2 by catalyzing its decomposition into O_2 and H_2O. The higher APX activity enhances H_2O_2-scavenging capacity and protects the host cells from lipid peroxidation (Sato et al. 2011). It is apparent from the obtained results that in tomato leaves the activities of APX and SOD were enhanced to a higher extent at all treatments than CAT and POX activities. SOD is the first and the most important defense line among the antioxidant systems. Morkunas and Gmerek (2007) stated that POX may be one of the elements in the defense system in response to pathogens like *F. oxysporum*. Garcia-Limones et al. (2012) reported that the increase in the antioxidant enzyme system (CAT, APX and GR) was associated with resistance to *Fusarium* wilt in chickpea. Generally, the plants defend against ROS by induction antioxidative enzymes, which scavenge ROS.

Table 7. The presence (+) and absence (−) of bands in three isozymes, polyphenol oxidase, esterase and malate dehydrogenase at the effect of various treatments

	Vegetative stage[z]				Flowering stage[y]			
PPO	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8
				Polyphenol oxidase				
1	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+
3	−	+	+	+	−	+	+	−
4	−	−	−	−	−	+	−	−
Total	2	3	3	3	2	4	3	2
				Esterase				
1	+	+	+	+	+	+	+	+
2	−	+	+	+	−	+	+	+
3	−	−	−	+	−	−	−	−
Total	1	2	2	3	1	2	2	2
				Malate dehydrogenase				
1	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+
3	−	−	−	+	−	+	+	−
4	−	+	+	−	−	−	−	+
Total	2	3	3	3	2	3	3	3

[z]Vegetative stage: Lane 1: Healthy plants (Control). Lane 2: Plants infected with *F. oxysporum*. Lane 3: Plants infected with *F. oxysporum* and treated with *O. basilicum* Lane 4: Healthy plants treated with *O. basilicum* extract.
[y]Flowering stage: Lane 5: Healthy plants (Control). Lane 6: Plants infected with *F. oxysporum*. Lane 7: Plants infected with *F. oxysporum* and treated with *O. basilicum* Lane 8: Healthy plants treated with *O. basilicum* extract.

Table 8. Number and types of bands as well as the percentage of the total polymorphism generated by three isozymes

		Polymorphic			
Isozymes[z]	Monomorphic Bands	Unique	Non Unique	Total bands	Polymorphic (%)
PPO	2	1	1	4	50
EST	1	1	1	3	67
Mdh	2	–	2	4	50

[z]PPO, polyphenol oxidase; EST, esterase; Mdh, malate dehydrogenase.

The high levels of enzymatic antioxidant activities observed in infected plants treated with *O. basilicum* extract may be due to the fact that when tomato plants are challenged with the pathogen and the antagonist, the host plant secretes more phenol oxidase enzyme for defense (Ojha and Chatterjee 2012). Kamalakannan et al. (2004) demonstrated that in addition to direct antagonism, the biocontrol agents also increase the activity of various defense-related enzymes and chemicals in response to pathogen infection. The essential oil of *O. basilicum* was effective against damping-off disease causing fungi (Nahak et al. 2011). Investigations of the mechanisms of disease suppression by plant products have suggested that the active principles present in plant extracts may either act on the pathogen directly or induce systemic resistance in host plants resulting in a reduction of disease development (Kagale et al. 2004). This may explain in part the promotive effect of *O. basilicum* extract on increasing the activities of antioxidant enzymes in infected tomato plants. These data clearly suggest that *O. basilicum* extract prevents *F. oxysporum* wilt disease development through a mechanism involved in activation of antioxidant defensive enzymes (Farag Hanaa et al. 2011).

The SDS-PAGE protein profile of diseased leaves of tomato plants showed differences in band patterns when compared with healthy plants. The fungal infection caused the disappearance of protein bands at both stages of plant growth which were present in the healthy control plants. The absence of these protein bands in the infected plants can be explained as a result of retarding the trigger of resistance gene transcription, which leads to pathogen-related proteins, as previously reported by Satheesh and Pari (2004) and El-Khallal (2007). On the other hand, some new protein bands were observed in both diseased and healthy plants treated with *O. basilicum* extract (lanes 3 and 4), so that these bands act as a protein marker in resistance mechanisms; such a response allows plants to become more tolerant to the pathogens. This finding has previously been recorded by several authors (El-Askary et al. 2003). Woloshuk et al. (1991) reported that protein of 24 KDa is related to osmtin, a member of the pathogen related protein (PR-5) and have antifungal effect. Thus, it could be suggested that in resistance-induced plants the accumulation of PR-proteins forms the first line of defense to a challenging pathogen and they are implicated in plant defense because of their antifungal activity. In this respect, Nafie and Mazen (2008) consider that such protein expression may enhance plant responses to overcome further pathogen invasion. Plants have flexible detection systems and probably employ several recognition and signal transduction pathways to activate their defense.

The induction and change in the isozyme profile are considered to play an important role in the cellular defense against oxidative stress, caused by pathogen infection. The present investigation showed that application of *O. basilicum* extract was associated with remarkable changes in PPO isozymes patterns of tomato plants upon infection with *F. oxysporum*. The treatment induced new PPO bands that are not detected in the healthy control. Furthermore, new PPO isozymes were increased upon infection with the pathogen during flowering stage. Higher levels of PPO isozymes were associated with greater resistance. Induction of new PPO isozymes in response to infection with the pathogen indicated that the induced isozyme has a definite role in suppressing disease development in plant tissue. In this respect, a number of studies have suggested that PPO may participate in defense reactions and confer hypersensitivity to plants resistant to diseases, for example, potato, cotton and vicia. The new generation of PPO isozymes and the performance of disease resistance for organization are positively correlated. The increased activity of PPO was reported to be due to either solubilization of polyphenolases from cellular compartments or activation of latent PPO (Jyosthna et al. 2004). The disappearance of the fourth band in infected plants treated with basil extract, which was observed in the present study, can be due to the suppression of *Fusarium* growth in host plant tissue and so the stress proteins ceased to be formed. In addition, new EST isozyme bands were increased in response to fungal infection and/or application of basil extract. Our results are in agreement with Zhao et al. (2012), who reported that new EST isozyme bands increased in response to different fungal infections, and induce resistance of pear against pathogens and maintain the health of the plant. Moreover, the presence of a clear extra band (EST-3) in the isozyme profile of EST at the vegetative stage as a result of treating the healthy plants with basil may be due to a stimulation effect of basil extract for the synthesis of this enzyme. The Mdh expression pattern of tomato plants infected with *F. oxysporum* and/or treated with basil extract showed an increase in its isozyme pattern at both stages of plant growth as compared with the control. Correlation between the increase in Mdh expression and the resistance to stress conditions has been established in many plant species (Hassanein 2004). Malate dehydrogenase has been proposed as a component of an apoplast system that may generate H_2O_2 required for POX-mediated lignification (Fry 1986); the lignification of the cell wall has important functions in response to fungus infection. The increased activity of Mdh that was observed in this study may be due to the activation of enzyme precursor or the synthesis of new enzyme protein. These changes may also be due to interaction of enzyme subunits of both host leaf tissue and the fungus to form hybrid enzyme with an increased activity. Such change in isozyme expression suggests that the gene involved in the synthesis of this isozymes form is differentially activated under specific conditions (El-Tayeb and Hassanein 2000).

CONCLUSION

The present study concludes that *O. basilicum* has a good antioxidant potential and can be used to produce novel natural antioxidants to control the devastating *Fusarium* wilt of tomato plants.

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