

## CONTROLLING FUSARIUM WILT DISEASE IN MELON (*CUCUMIS MELO* L.) USING TILLERED ONION BULB EXTRACT

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### Abstract

Melon wilt disease is a soil-borne disease caused by *Fusarium oxysporum* f. sp. *niveum*. This disease incur of the heavy economic loss in melon crops. To decrease damage to melons, many control methods have been developed. However, many of the current control methods have limitations and disadvantages. For example, fungicides may cause health concerns for both humans and the environment due to high toxin content and the presence of residues. Therefore, biological control methods that reduce or eliminate the risk of environmental contamination and threats to human health are urgently needed to solve these issues and to protect melon crops from wilt disease. In this research, we assessed the efficacy of tillered onion bulb extract (TOE) for biocontrol of melon wilt disease in melon. Different concentrations of the TOE have been shown to have inhibitory effects on *Fusarium* spore germination and growth, pathogenic bacterial biomass, and fungal sporulation, with increased inhibitory effects at higher TOE concentrations. In melon wilt disease, concentrations of TOE greater than 250 mg/mL produced the highest protective effects in both susceptible and resistant melon cultivars. The disease index in resistant varieties was 18%, and the disease control effect was 63.51%, while the disease index in susceptible varieties was 21.41%, and the disease control effect reached 65.96%. These values indicate stronger control effects than those achieved using 40% Ning WP melon blight. High concentrations (over 500 mg/mL) of TOE had strong inhibitory effects on melon seed germination and the activity of protective enzymes in melon cultivars.

**Key words:** *Fusarium oxysporum*; Tillered onion bulb extract; Biocontrol.

### Introduction

Melon is a typical economy crop grown in Heilongjiang Province, which produces crops known for unique flavor and quality both in China and abroad (Sun *et al.*, 2003; Abdul *et al.*, 2016). However, in recent years melon wilt disease has occurred to various degrees in the main producing area of this province, becoming one of the main restricting factors of melon industry development and having serious negative consequences on yield, quality, market competitiveness, and the incomes of farmers (Martyn & Amador, 1987; Champaco *et al.*, 1993). Comprehensive measures to control this disease are thus needed.

Wilt disease is a soil-borne disease that affects various production areas on a global scale (Louvet, 1986; Gonzalez *et al.*, 1994). This disease is also known as Fusarium wilt or stem rot (Hoitink & Fahy, 1986; Hoitink & Boehm, 1999; Hoitink *et al.*, 2001). During the onset of this disease in melon, all the vines or portions of the branches wilt during the day, become normal in appearance during the night, and then die after 5~6 d. Affected vines and roots rot, become water-logged, and then turn brown, sometimes with red clay content. At least seven types of melon *Fusarium* (e.g., *Fusarium oxysporum* f. sp. *niveum*) (Risser *et al.*, 1976) and several physiological races of melon *Fusarium* have been identified. For example, watermelon *Fusarium* wilt and melon *Fusarium* wilt have 3 physiological races, and cucumber *Fusarium* wilt is divided into physiological race No. 1 (USA), physiological race No. 2 (Israel), and physiological race No. 3 (Japan). Melon *Fusarium* wilt has occurred in most regions of China in recent years, especially in Heilongjiang, Jilin, Liaoning, Shandong, Hubei, and Peking as well as 18 other cities and

provinces, and annual losses are estimated between 10% and 30%. In serious cases, there is a total loss of melon crops (Chikh-Rouhou *et al.*, 2011; Herman & Perl-Treves, 2007; Chikh-Rouhou *et al.*, 2007).

Melon *Fusarium* wilt is a vascular disease, and to date, there are no effective pesticides for the prevention and control of the pathogen (Cebolla *et al.*, 2000; Fravel *et al.*, 2005; Brimmer & Boland, 2003; Boutler *et al.*, 2000). The existing disease-resistant varieties of melon have only relative resistance, and although rotation can reduce the incidence rate of blight, it cannot cure it (King *et al.*, 2008). (Soriano-Martin *et al.*, 2006). Because the current stock has an impact on the quality of the thin-skin melon, melon grafting is rarely used in production. The market demand for melon has increased in recent years, and to produce melon in all seasons, melon cultivation methods have become diverse. However, melon cultivation conditions are exacerbated by *Fusarium oxysporum* accumulation in soil and by the incidence of epidemic *Fusarium* wilt diseases. Therefore, the effective control of melon wilt disease has become a global concern.

Traditionally, crop rotation has been proven to be an effective strategy for controlling many soil-borne diseases, but because most of these pathogens (such as *Fusarium oxysporum* f. sp. *melonis*) can survive for a long period, the effectiveness of crop rotation is limited once a disease outbreak occurs (King *et al.*, 2008). Grafting is a promising practice in soil-borne disease control in vegetable farming, particularly for watermelon in Japan, Korea, and Taiwan. However, added cost still limits the feasibility of this method for Chinese farmers. Soil disinfection using various chemicals (Cebolla *et al.*, 2000) or pesticides (Fravel *et al.*, 2005) is also a traditional practice but must be phased out because of environmental and human health concerns (Brimmer & Boland, 2003; Boutler *et al.*, 2000).

Recently, increasing attention has been given to the prevention and cure of melon *Fusarium* wilt (Berrocal-Lobo and Molina 2008). The development and use of biocontrol agents (BCAs) for use against FOM is an appealing management strategy for both conventional and organic farming industries.

Using botanical pesticides as a potential biocontrol resource has become increasingly popular in recent years (De Cal, 1995; Larkin & Fravel, 1998; Habiba *et al.*, 2016; Garip *et al.*, 2017), especially for soil-borne diseases of plants. Many antagonistic strains have been proven to be effective biocontrol agents in controlled laboratory or greenhouse conditions. *Bacillus* spp. (Gong *et al.*, 2006; Hervas *et al.*, 1998), *Penicillium* spp. (Sabuquillo *et al.* 2006; Larena *et al.* 2003), *Aspergillus* spp. (Suárez-Estrella *et al.*, 2007), and *Trichoderma* spp. (Tawfic & Allam, 2004; Rojo *et al.*, 2007) are a few strains included in the long list of BCAs. However, few research reports have examined melon wilt disease prevention (Zhao *et al.*, 2011). This research uses fresh tillered onion bulb extract (TOE) in a preliminary study on the bacteriostasis of melon *Fusarium* wilt through several experiments, including an indoor bacteriostatic experiment and a prevention pot experiment. We also evaluated the effects of the TOE on the growth of melon and activity of related enzymes to explore the potential for using TOE in preventing and controlling melon wilt disease and to provide a basis for future guidance towards pollution-free melon production.

## Materials and Methods

**Test materials:** Melon wilt disease pathogen (*Fusarium oxysporum*) was acquired from the Northeast Agricultural University Horticulture College Melon and Watermelon Laboratory. Potato dextrose agar (PDA) was used to cultivate *Fusarium oxysporum* f. sp. *niveum*. Pathogenic spore suspension culture was cultivated by Czapek.

Selected fresh tillered onion (*A. cepa* var. *aggregatum* Don.) bulbs were purchased in a market in Harbin, Heilongjiang Province.

Tested resistant and susceptible strains of melon *Fusarium* wilt (MR-1 and M1-15) were provided by the Northeast Agricultural University College of Horticulture Melon and Watermelon Molecular Breeding Lab.

**Test drugs:** Glucose, anhydrous ethanol (analytical reagent), agar powder, beef extract, peptone, sodium chloride, sucrose, MgSO<sub>4</sub>, potassium dihydrogen phosphate, potassium hydrogen phosphate, sodium acetate, snail enzyme, borax, kelp polysaccharide (Sigma Company), and arsenic acid disodium hydrogen were used as test drugs.

**Instruments and equipment:** A DHP - 9082 type electric heating constant temperature incubator (Shanghai Yiheng Instruments Co., Ltd.), a DHG-9245 type thermostatic drum wind drying oven (Shanghai Yiheng Instruments Co., Ltd. production), a thermostat oscillator (Shanghai Zhicheng Analytical Instrument Manufacture Co., Ltd.), a -40°C low temperature preservation refrigerator (Haier Co., Ltd.), a super clean workbench (Beijing: Harbin Instrument Manufacturing Co., Ltd.), an H WS28 type thermostatic

water bath (Shanghai Yiheng Instruments Co., Ltd.), a high-speed centrifuge (TG-16B) (Shanghai Anheng Scientific Instrument Factory), an electron microscope, an automatic pressure sterilizer, and an ultraviolet spectrophotometer were used in the experiments.

**The separation and purification of melon *Fusarium*:** First, the rhizome junction was gathered on the onset of severe melon plant disease and rinsed with 70% alcohol. The skin was removed to reveal the brown part of the vascular bundle, and a blade was used to cut the bundle into a 0.5 cm piece, which was then cut into a 1/4 circular cylinder. The pieces were placed in a petri dish containing 70% alcohol for 2 ~ 3 s and then placed into a petri dish with mercuric chloride for 2 min. Samples were finally washed in sterile water, transferred to the isolation medium and cultivated for 2 d. New-growth white mycelium was then removed for purification and cultivation on PDA medium.

A purified colony was used to prepare a spore suspension and cultured in PSA medium. New single colonies were placed in sterile water, and single spore isolation was performed repeatedly for the colonies from a single spore. One portion was retained in PSA slant medium, and another was used in inoculation tests.

**The preparation of tillered onion bulb extracts:** Fresh onion bulbs were peeled, weighed to 200 g, chopped, and added to 200 mL of sterile water. The mixture was shaken on a shaking table at 150 r/min for 48 h, and then, four layers of cotton gauze were used to filter and remove the residue. The filtrate was then centrifuged at 8000 rpm for 15 min, and the supernatant was removed. The supernatant was filtered using a 0.22 µm bacteria filter, and a concentration of 1000 mg/mL tillered onion bulb crude extract of the liquid was made using the double dilution method (gradually diluting to solutions with concentrations of 500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL).

All instruments used in this process were sterilized by autoclaving, and the procedures were performed on a sterile bench.

**The Effects of tillered onion bulb extracts on melon wilt spore germination:** Fifty microliters of different mass concentrations of tillered fresh onion bulb was placed onto the groove of concave glass, and the same amount of melon wilt spore suspension was placed into the groove. The concave glass was then placed in a petri dish with three layers of filter paper (wet with distilled water) and covered after mixing. The mixture was cultivated at 25°C in a constant-temperature incubator, and spore germination was examined under a microscope after 12 h. There were three replicates per concentration, and 5 horizons were evaluated for each replicate. The average rate of spore germination and inhibition rate were calculated and compared with those of sterile water controls.

**The Effects of tillered onion bulb extracts on the growth of melon wilt mycelium:** The culture medium containing tillered onion bulb leaching liquid was prepared as follows: PDA medium was sterilized and cooled to approximately

50 °C. Next, 10 mL of various concentrations of tillered onion bulb leaching liquid was mixed with the same volume of liquid medium. The resulting culture media with different concentrations of tillered onion bulb leaching liquid were poured onto tablets.

**Preparation of flat colonies:** Activated melon *Fusarium* wilt was mixed with sterile water into a  $10^4 \sim 10^5$  spores/mL bacterial suspension, after which 100  $\mu$ L bacteria suspension was poured onto a PDA tablet, smoothed over with a sterile glass spatula, and cultivated in the dark at 28°C. The colonies were used after they reached confluence.

**Antibacterial activity assay:** Bacteriostatic activity was evaluated using the growth rate method. A perforator (diam. = 7 mm) was used to remove fungus cake with the same growth rate and the same medium thickness from the edge of tablet. Then, a vaccination needle was inserted in the center of the cake and different concentrations of tillered onion bulb leaching liquid were injected. One fungus cake was placed in each petri dish, and sterile water culture medium was used as a control. Each assay was repeated 3 times, and the cakes were cultivated at 28°C in a constant-temperature incubator. The extension diameter was measured after 3 d, and the bacteriostatic rate was calculated using the following formula:

$$\text{Bacteriostatic rate (\%)} = \frac{(\text{Contrast dish colony diameter} - \text{last colony diameter})}{\text{Contrast dish colony diameter}} \times 100$$

**The effects of tillered onion bulb leaching liquid on melon *Fusarium* biomass:** A perforator (diam. = 7 mm) was used to remove fungus cake with the same growth rate and the same medium thickness from the edge of tablet. Then, a vaccination needle was inserted into the middle of the cake, and different concentrations of tillered onion bulb leaching liquid were injected. One fungus cake was placed in each petri dish, and sterile water culture medium was used as a control. Each assay was repeated 3 times. The tablet was kept upside down and cultivated at a constant temperature of 28°C. After 72 h, the pathogenic bacteria biomass was determined.

Microbial biomass was calculated using the reference dry weight colorimetric method. The well-cultivated bacteria were washed with 2 M phosphate buffer and divided into two centrifuge tubes. The bacteria were centrifuged for 10 min at 3500 r/min. Then, the centrifugal tubes were placed in a 105°C oven and dried to a constant weight. One milliliter of distilled water and 2 mL of potassium dichromate solution were added to the tubes and heated to a boil for 30 min. Finally, 2 mL of water was added, and the OD value was measured at a wavelength of 620 nm

**The influence of fresh tillered onion bulb leaching liquid on the amount of melon wilt bacterial spores:** A perforator (diam. = 7 mm) was used to remove fungus cake with the same growth rate and the same medium thickness from the edge of tablet. Then, a vaccination needle was inserted into the middle of the cake, and different concentrations of tillered onion bulb leaching liquid were injected. One fungus cake was placed in each

petri dish, and sterile water culture medium was used as a control. Each assay was repeated 3 times. The tablet was kept upside down and cultivated at a constant temperature of 28°C. The pathogenic bacterial suspension (15 mL) was placed onto the culture dish. After 72 h, counting plates and a microscope were used to calculate the pathogen spore quantity ( $\times 10^3$  spores/mL) and inhibition rate.

**The influence of tillered onion bulb leaching liquid on seed germination in different melon varieties:** After melon seeds were soaked in lukewarm water, they were covered with three layers of filter paper in a petri dish, with 30 seeds per dish. Then, 10 mL of different concentrations of tillered onion bulb leaching liquid was added to each dish to cover the sprouts. Sterile water was used as the control. Seed germination was observed, and the germination and inhibition rates were calculated.

**The influence of tillered onion bulb leaching liquid on the growth of melon plants:** The melon seeds of resistant and susceptible varieties were placed in different concentrations of tillered onion bulb leaching liquid for 24 h. Seeds soaked with sterile water were used as controls. Seeds were then separately placed into bowls. The plant growth index was randomly surveyed using plant height, fresh weight, root length, leaf length, and leaf width. We calculated the averages and determined the inhibition rate after 15 d.

**Melon seedling preparation:** Seedlings were prepared in the greenhouse of the No. 1 garden station at Northeast Agricultural University. The soil was sterilized by high temperature and high pressure. The influence of tillered onion bulb leaching liquid on melon physiological and biochemical indices was evaluated at concentrations of 500 mg/mL, 250 mg/mL, 125 mg/mL (controls lacked tillered onion bulb leaching liquid but included direct inoculation of the pathogen). This experiment utilized a randomized block design, with three replicates per variety and 10 seedlings in each replicate. We chose 2 g of leaf tissue from plants 1 d before inoculation and 1, 3, 5, and 7 d after inoculation for the determination of physiological and biochemical indices.

#### The determination of physical and chemical indicators

**1. The determination of chitinase, referencing Boller (1983):** In total, 1.0 g of fresh weighed material was added to 5 mL of 0.1 mol/L acetate buffer (pH 5.0), and the mixture was ground in an ice bath. The mixture was then centrifuged at  $10\,000 \times g$  for 10 min, then the supernatant fluid was centrifuged for 10 min at  $10\,000 \times g$ . The supernatant fluid was then stored in a refrigerator (4°C) for later use. Next, 0.4 mL of enzyme and 40 mL of 1% snail enzyme were mixed and placed in a water bath at 37°C for 30 min, followed by the addition of 0.2 mL of saturated borax. The mixture was placed in a boiling water bath for 7 min, and after the mixture was cooled, 2 mL of 1% glacial acetic acid and 1 mL of dimethyl amino benzene formaldehyde (DMAB) were added. The mixture was then heated to 37°C for 15 min, and its optical density was measured at 585 nm. One unit of enzyme

activity is defined as the amount of enzyme required per gram of plant material under the above conditions to produce n-acetyl glucosamine.

**2. Determination of  $\beta$ -1,3-glucanase, referencing SIBS (Anon. 1999):** First, 0.5 g of fresh weighed material was pre-cooled in a mortar, and 3 mL of 0.05 mol/L sodium acetate buffer (pH 5.0) and 5.0 g of polyvinylpyrrolidone (PVP) were added. The mixture was fully ground in an ice bath and centrifuged at  $15\ 000 \times g$  at  $4^\circ\text{C}$  for 15 min. The supernatant was centrifuged at  $10\ 000 \times g$  for 10 min, resulting in crude enzyme liquid, which was stored in a refrigerator ( $4^\circ\text{C}$ ). Next, 0.4 mL of 1 mg/mL laminarin (Sigma, soluble in sodium acetate buffer) and 0.1 mL of enzyme were mixed. The mixture was heated to  $37^\circ\text{C}$  for 15 min, and 0.5 mL of copper reagent was immediately added. The mixture was blended, placed in a  $100^\circ\text{C}$  water bath for 10 min, and cooled with cold water. A total of 0.5 mL of arsenic molybdate reagent was added, and after the mixture turned blue, 3.5 mL distilled water was added. The solution was shaken well, and its color was examined at 660 nm. The light density was determined, and the amount of reducing sugar in the liquid sample was calculated using a standard curve. One unit of enzyme activity (U) was defined as 1 nmol  $\text{g}^{-1}$  FW  $\text{s}^{-1}$ .

**The effect of fresh tillered-onion bulb on melon *Fusarium* wilt inoculation:** This analysis was performed in the greenhouse of the No. 1 garden station of Northeast Agricultural University. The soil was sterilized by high temperature and high pressure. The efficiency of tillered onion bulb leaching liquid in controlling of melon wilt disease in potted plants (40% of dry controls was used for comparison of WP after vaccination with pathogenic bacteria) at concentrations of 500 mg/mL, 250 mg/mL, and 125 mg/mL. This experiment adopted a randomized block design, with 3 replicates per variety and 10 seedlings per replicate.

This experiment adopted a randomized block design, with 3 replicates per variety and 10 strains per replicate.

**The preparation of inoculum:** The preparation method for the muskmelon blight pathogen is the same as that described in 2.3.1, and the preparation method for the fresh tillered onion bulb leach liquid is the same as that described in section 2.3.2.

**Melon seedling planting:** Melon seeds previously soaked in lukewarm water were planted in sterilized, sandy soil, and two pieces of cotyledon were transplanted into the nutritional bowl after flattening.

**Vaccination:** After the melon seedling grew leaves, the leaves were pulled out slightly, and the roots were washed with water and soaked in the melon *Fusarium* suspension with a spore concentration of  $10^6$  cell/mL for 5 min. The seedlings were then planted in the nutritional bowl again using different concentrations of tillered onion bulb leaching liquid to submerge the root at 30 mL/strain and then moistened 24 h after inoculation.

**Disease investigation:** After all the seedlings became diseased 20 d later, a survey of incidence was conducted,

and the index of disease was calculated. The degree of melon *Fusarium* wilt degree ranged from 0 ~ 4, with a total of five levels:

Level 0: Plant grows normally;  
 Level 1: Leaf or stem wilting area accounts for 1/4 or less of the whole plant for all strains;  
 Level 2: Leaf or stem wilting area accounts for up to 1/2 of the whole plant;  
 Level 3: Leaf or stem wilting area accounts for 3/4 of the whole plant;  
 Level 4: Plant dies.  

$$\text{Disease index} = \frac{\sum (\text{Condition grade level} \times \text{The disease strains})}{(\text{Series} \times \text{Highest total number})} \times 100$$

$$\text{Prevention effect (\%)} = (\text{Controlled condition index} - \text{Processing condition index}) / \text{Disease index} \times 100$$

**Data analysis methods:** Test results were analyzed using DPS software and compared using Duncan's new multiple range method, with different lowercase letters used to show significant differences at  $P = 0.05$  and different uppercase letters used to show the significant difference at  $P = 0.01$ .

## Results

**The influence of tillered onion bulb leaching liquid on the germination of melon wilt disease spores:** The results of different concentrations of fresh tillered onion bulb leaching liquid on melon wilt pathogen spore germination are shown in Figure 1. The results show that when the concentrations of tillered onion bulb leaching liquid are 1000 mg/mL, 500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL, the spore germination rates are 5.12%, 24.12%, 35.27%, 65.24% and 71.51%, respectively, and the blank control spore germination rate is 88.22%. This result indicates that different concentrations of tillered onion bulb leaching liquid have different inhibitory effects on melon wilt disease spore germination and that the inhibition increased as the concentration of tillered onion bulb leach liquid increased. The inhibition rate ranged from 18.94% ~ 18.94%. When the concentration of tillered onion bulb leach liquid reached 1000 mg/mL, inhibition was the greatest, at 94.19% (spore germination image is shown in Appendix B).

**The influence of tillered onion bulb leaching liquid on the mycelial growth of *Fusarium oxysporum*:** Table 1 and Appendix C show that the tillered onion bulb leaching liquid had significant inhibitory effects on melon *Fusarium* mycelium growth and that inhibition increased as the concentration increased. When the concentrations of tillered onion bulb extract were 62.5 mg/mL and 125 mg/mL, the inhibition rates for mycelial growth were 35.77% and 40.78%. When the concentrations of tillered onion bulb extract were 250 mg/mL and 500 mg/mL, the inhibition rates for mycelial growth were 48.53% and 61.25%. When the concentration of tillered onion bulb extract was 1000 mg/mL, the inhibition was the greatest, reaching 73.15%. Differences between treatments and controls were highly significant (photographs of mycelial growth are shown in Appendix C).

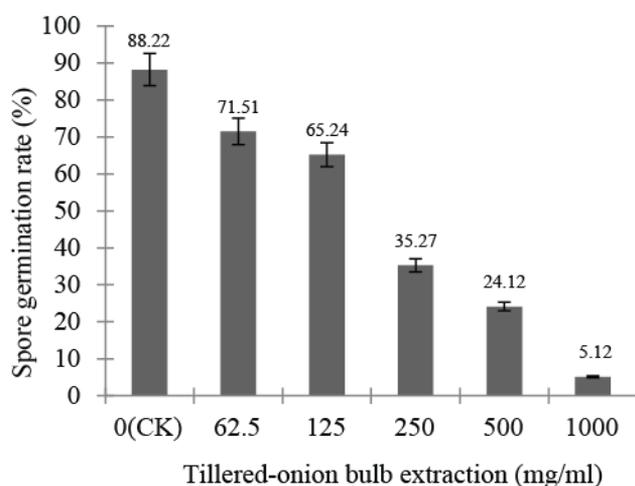


Fig. 1. Effect of different concentrations of tillered onion bulb extract on *Fusarium oxysporum* spore germination.

**Table 1. Inhibitory activity of different concentrations of tillered onion bulb extract on the mycelial growth of *Fusarium oxysporum*.**

Concentration of tillered onion bulb extract (mg/mL)	Average diameter of colony extension (cm)	Inhibition rate (%)
1000	1.1 aA	73.15 aA
500	1.64 bB	61.25 bB
250	1.97 cB	48.35 cC
125	2.28 dC	40.78 dD
62.5	2.47 eD	35.77 eE
0 (CK)	3.60 fE	0.00 fF

Note: Uppercase letters express  $p < 0.01$ ; lowercase letter express  $p < 0.05$ ; different letters in the same column indicate significant differences between treatment groups.

**Table 2. Effect of different concentrations of tillered onion bulb extract on the mycelial dry weight of *Fusarium oxysporum*.**

Concentration of tillered onion bulb extract (mg/mL)	Average mycelial dry weight (mg)
1000	25.2 fE
500	46.4 eD
250	53.1 dD
125	67.6 cC
62.5	81.5 bB
0 (CK)	97.7 aA

**Table 3. Effect of different concentrations of tillered onion bulb extract on sporulation of *Fusarium oxysporum*.**

Concentration of tillered onion bulb extract (mg/mL)	Spore concentration ( $\times 10^3$ units/mL)			
	I	II	III	Means
1000	24.33	24.67	26.67	25.22 c C
500	27.33	25.00	28.33	26.89 bc BC
250	28.00	29.67	28.67	28.78 b ABC
125	27.00	29.33	29.67	28.67 b ABC
62.5	30.33	28.33	29.00	29.22 b AB
0 (CK)	32.00	30.00	33.33	31.78 a A

**Table 4. Effect of different concentrations of tillered onion bulb extract on the germination of a resistant melon cultivar.**

Concentration of tillered onion bulb extract (mg/mL)	Germination rate
1000	41.04 b B
500	84.00 a A
250	86.28 a A
125	89.76 a A
62.5	93.81 a A
0(CK)	94.52 a A

**The influence of tillered onion bulb leaching liquid on melon wilt disease bacterial biomass:** Tillered onion bulb leaching liquid affected melon wilt disease bacterial biomass (shown in Table 2). With increasing concentrations of tillered onion bulb leaching liquid, melon *Fusarium* wilt pathogen biomass showed a gradual decreasing trend. When the concentration of tillered onion bulb leaching liquid reaches 1000 mg/mL, the dry weight of melon *Fusarium* mycelium was the lowest, at only 25.2 mg. Differences between treatments and controls were highly significant.

**Effect of tillered onion bulb extract on the sporulation of *Fusarium oxysporum*:** Tillered onion extracts affected *Fusarium* wilt fungal spore production (Table 3). Tillered onion leaching liquid inhibited the sporulation of melon wilt pathogen bacteria (Table 3), and with increasing concentrations of the leaching liquid, melon *Fusarium* wilt pathogen sporulation reduced gradually, and its inhibition increased gradually. Maximum inhibition occurred at 1000 mg/mL leaching liquid, in which the concentration of spores was only  $25.22 \times 10^3$  units/mL. Differences between treatments and controls were significant.

**Effect of different concentrations of tillered onion bulb extract on the germination of melon cultivars:** The effect of different concentrations of tillered onion bulb extract on the germination of resistant and susceptible melon cultivars is shown in Tables 4 and 5. The result shows that when the concentration of tillered-onion bulb extraction was 1000 mg/mL, the seed germination rate of the resistant and susceptible cultivars and blank control showed significant differences at the 5% significant level and at the 1% significant level. When the concentrations of leaching solution were 250 mg/mL, 125 mg/mL and 62.5 mg/mL, the germination rates of resistant versus susceptible cultivars did not significantly differ from the blank control at the 5% significant level or at the 1% significant level, indicating that a high concentration of tillered-onion bulb extract affects the germination of melon cultivars.

The effect of different concentrations of tillered onion bulb extract on the germination of resistant and susceptible melon cultivars is shown in Tables 4 & 5. The results show that when the concentration was 1000 mg/mL, resistant and susceptible varieties showed significantly different seed germination rates compared to controls at the 5% significance level or at the 1% significant level. In addition, with tillered onion bulb leaching liquid concentrations of 250 mg/mL, 125 mg/mL and 62.5 mg/mL, resistant and susceptible varieties did not have significantly different seed germination rates compared with the controls at the 5% significance level or at the 1% significant level. Therefore, the high concentration of tillered onion bulb extract affects seed germination.

**Effect of tillered onion bulb extract on melon biomass:** The 250 mg/ml bulb extract had the best control effect on muskmelon growth, as shown in Figure 2. The two varieties of melon treated with onion leaching solution had plant height, plant weight, root length, leaf length and leaf width values higher than or near control values, indicating that the 250 mg/ml tillered onion bulb extract does not inhibit melon biomass (photos are shown in Appendix A).

**Table 5. Effect of different concentrations of tillered onion bulb extract on the germination of a susceptible melon cultivars.**

Concentration of tillered onion bulb extract (mg/mL)	Germination rate
1000	55.73 c C
500	73.56 b B
250	79.45 ab AB
125	84.23 a AB
62.5	86.46 a A
0(CK)	88.24 a A

**Protective effect of fresh TOE against melon wilt****Table 6. Field control efficiency of different concentrations of TOE on melon wilt.**

	Infection rate of susceptible variety	Disease index of susceptible variety
0 mg/ml TOE	0	62.67
125 mg/ml TOE	60.64	24.45
250 mg/ml TOE	65.96	21.41
500 mg/ml TOE	53.2	29.45
40% WP	63.2	23.14

**Table 7. Field control efficiency of different concentrations of TOE against melon wilt.**

	Infection rate of susceptible variety	Disease index of susceptible variety
0 mg/ml TOE	0	49.33
125 mg/ml TOE	54.04	22.67
250 mg/ml TOE	63.51	18
500 mg/ml TOE	44.2	27.33
40% Guakuning WP	59.23	21.05

**Effect of tillered onion bulb extract on melon physiology indices:** Resistant and susceptible melon varieties inoculated with melon wilt pathogen differed in the timing of leaf chitinase activity (Figs. 3 & 4). The results show that after pathogen infection in resistant and susceptible varieties, chitinase activity increased rapidly in leaf tissue and was higher than in the control. Changes in chitinase activity in seedlings were the most obvious in plants treated with the 250 mg/mL tillered onion bulb extract, and this treatment showed the highest enzyme activity. Enzyme activity in the resistant variety reached a peak after 72 h post-inoculation, and chitinase activity in susceptible and resistant varieties treated with the 500 mg/mL extract reached a peak after 48 h; in all other treatments, activity reached a peak at 72 h. These results demonstrate that the tillered onion bulb extract can activate protective enzymes.

The results of resistant and susceptible melon varieties inoculated with *Fusarium* wilt pathogen and the timing of leaf  $\beta$ -1,3-glucanase activity are shown in Figs 5 and 6. The results show that after infecting the resistant and susceptible melon cultivars with the pathogen,  $\beta$ -1,3-glucanase activity in leaf tissue increased rapidly and was higher than that of the control. At a concentration of 250 mg/mL, tillered onion bulb extract caused changes in  $\beta$ -1,3-glucanase activity in muskmelon seedling, with the highest activity. At 500 mg/mL, tillered onion extract caused  $\beta$ -1,3-glucanase activity to peak at 48 h in muskmelon seedlings, while  $\beta$ -1,3-glucanase activity peaked at 72 h after other treatments.

The protective effect of fresh TOE against melon wilt is shown in Tables 6 and 7. The results show that different concentrations of TOE (500 mg/mL, 250 mg/mL, and 125 mg/mL) have protective effects against melon wilt. When the concentration of onion extract was 250 mg/mL, its control effect was highest in both resistant and susceptible varieties. The index of disease resistant among varieties was 18%, and control effects reached 63.51%. The disease index for susceptible varieties was 21.41%, while the control effect reached 65.96% (see images in Appendix D, E).

**Discussion**

**Effects of different concentrations of TOE on *Fusarium oxysporum* mycelia growth and spore germination in melon:** Our results showed that different concentrations of TOE differentially affected growth, biomass and spore germination of *Fusarium* wilt in melon. growth, *Fusarium oxysporum* hyphal biomass, and spore germination displaced enhanced inhibition with increasing concentrations of leaching solution. When the concentration of tillered onion bulb leaching solution reached 1000 mg/mL, the maximum inhibitory effect was observed.

**Effects of different concentrations of TOE on melon *Fusarium* wilt disease in potted plants:** High-concentration TOE may exert inhibitory effects on *Fusarium* wilt in melon, while inhibitory effects of low-concentration TOE may be less apparent. Thus, we designed a field experiment to test a concentration gradient with 500 mg/mL, 250 mg/mL and 125 mg/mL TOE. In the 500 mg/mL treatment, the disease control effect in resistant varieties of muskmelon seedling was 44.2%, while in susceptible varieties, it was 53.2%. The 250 mg/mL TOE treatment in resistant varieties of muskmelon seedlings showed a disease control effect of 63.51%, and the disease control effect was 65.96% in susceptible varieties. Effect of the disease on the former was significantly lower than the latter, which indicates that high concentrations of TOE may have negative effects on melon seedlings perhaps by destroying the seedling defense system, thereby reducing prevention of disease. When TOE concentrations were 250 mg/mL the disease index was lowest, at 18% in resistant varieties and 21.41% in susceptible varieties. This result indicates that this TOE concentration may be optimal for use against melon *Fusarium* wilt.

**Effect of TOE on melon seed germination and physiological and biochemical indices:** In tests to determine effects of different concentrations of TOE on melon seed germination, the 1000 mg/mL TOE treatment resulted in low germination rates for both the resistant and susceptible varieties (41.04% in disease-resistant varieties and 55.73% in susceptible varieties), both of which were significantly lower than the blank control, illustrating the high concentration of tillered onion extract had strong inhibitory effects on seed germination in melon.

During the infection of plants by pathogenic microorganisms, resistance-related enzymes and pathogenesis-related proteins of phenolic metabolism in the PR protein family play important roles.

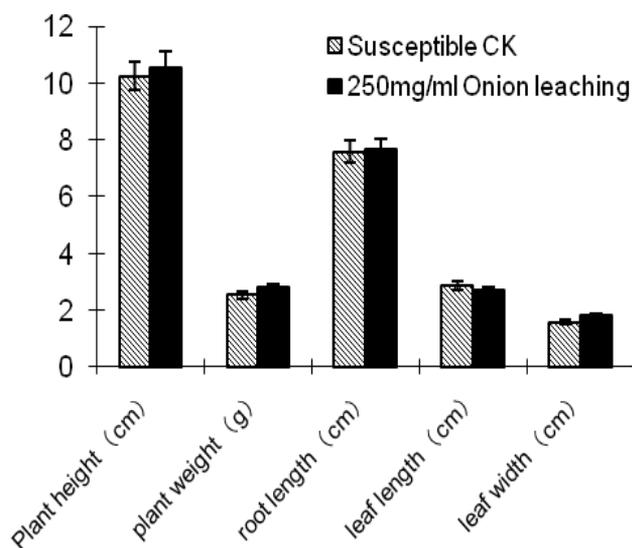
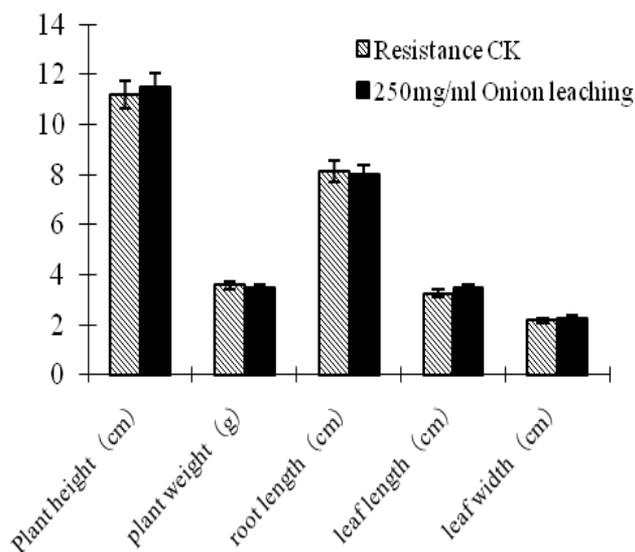


Fig. 2. Effect of tillered onion bulb extract on melon biomass.

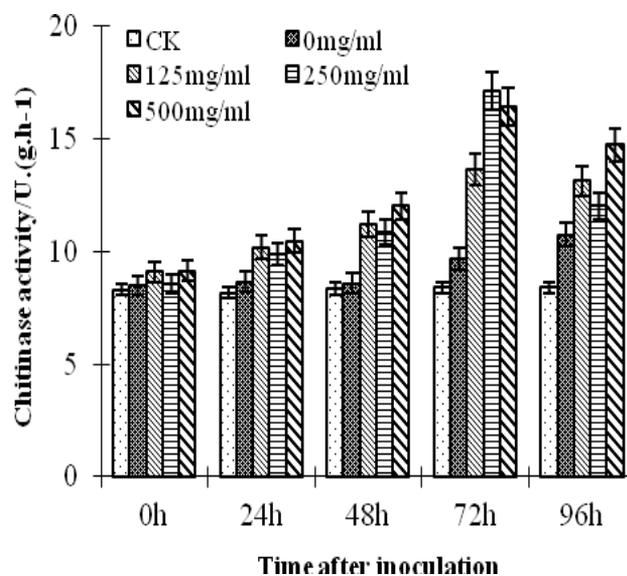


Fig. 3. The activity of chitinase in the leaves of a resistant melon cultivar at different times post-inoculation.

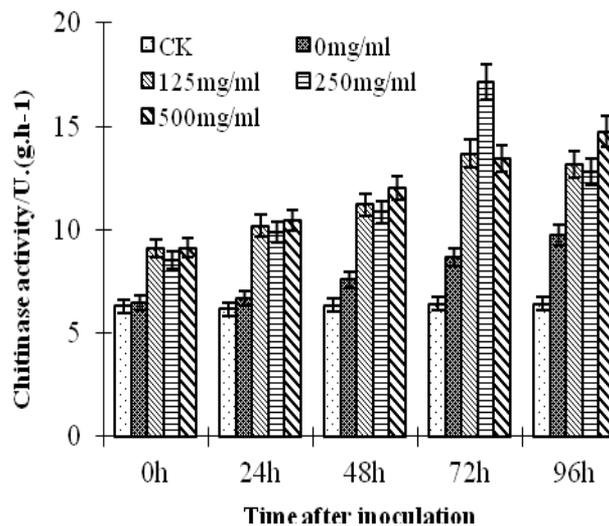


Fig. 4. The activity of chitinase in the leaves of a susceptible melon cultivar at different times post-inoculation.

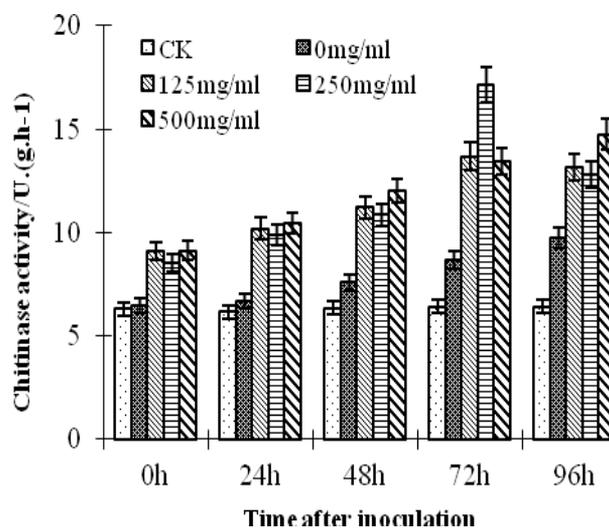


Fig. 5. Changes in  $\beta$ -1,3-glucanase activity in leaves of disease-resistant melon cultivar at various times post-inoculation.

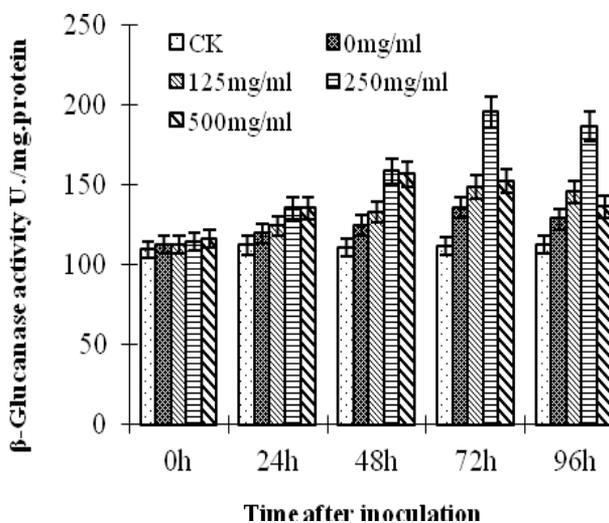


Fig. 6. Changes in  $\beta$ -1,3-glucanase activity in leaves of disease-susceptible melon cultivar at various times post-inoculation.

Chitinase and  $\beta$ -1,3-glucanase exist widely in higher plants and parasitic fungi and catalyze the hydrolysis of chitin and  $\beta$ -1,3-glucan, thereby inhibiting the growth and reproduction of fungi. These proteins are currently the best-studied antifungal proteins.  $\beta$ -1,3-Glucanase and chitinase also important structural components of the fungal cell wall, and occur on the hyphal tip, and  $\beta$ -1,3-glucan and chitin exposed on the surface can be directly attacked by  $\beta$ -1,3-glucanase and chitinase (Selitrennikoff C P2001).

Bera&Purkayastha (1999) studied rice sheath blight and found that when spraying a systemic fungicide,  $\beta$ -1,3-glucanase and chitinase activity in rice was increased and that the disease severity was significantly reduced. Ramachandra (2000) studied the differential resistance of Imperial Valley (pearl millet) varieties and found that  $\beta$ -1,3-glucanase activity level in the seeds was related to downy mildew disease resistance, which means that higher activity shows high resistance to downy mildew and lower activity results in higher susceptibility. It has been suggested that  $\beta$ -1,3-glucanase activity may be used as a biochemical marker for screening Imperial Valley resistance to downy mildew.

Analysis of the effects of tillered onion extract on physiological and biochemical indices in muskmelon showed that after infection with the pathogen, chitinase and  $\beta$ -1,3-glucanase activity was increased in the leaves of both resistant and susceptible melon varieties. Moreover, in the resistant and susceptible plants, the leaves treated with three different TEO concentrations showed higher activity of the two enzymes compared to the control; however, the group with 250 mg/mL tillered onion extract changed the most and had the highest activity. The 500 mg/mL TEO treatment does not have a protective effect.

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### References

- Abdul, M.B., W.B. Abdul, S. Liu, P. Gao, M.J. Baloch, X.Z. Wang, A.R. Davis and F.S. Luan. 2016. Linkage map construction and QTL analysis of fruit traits in melon (*Cucumis melo* L.) based on caps markers. *Pak. J. Bot.*, 48(4): 1579-1584.
- Anonymous. 1999. SIBS. Institute of plant physiology. CAS. Modern plant physiology experiment guide, Beijing: science press.
- Bera, S. and R.P. Purkayastha. 1999. Multicomponent coordinated defence response on rice to *Rhizoctoniasolani* causing sheath blight. *Curr. Sci.*, 76(10): 1376-1384.
- Berrocal-Lobo, M. and A. Molina. 2008. Arabidopsis defense response against *Fusarium oxysporum*. *Trends Plant Sci.*, 13: 145-150.
- Boller, T., A. Gehri, F. Mauch and U. Vogeli. 1983. Chitinase in bean leaves induction by ethylene, purification, properties, and possible function. *Planta*, 157(1): 22-31.
- Boutler, J.I., G.J. Boland and J.T. Trevors. 2000. Compost: A study of the development process and end-product potential for suppression of turfgrass disease. *World J. Microb. Biot.*, 16: 115-134.
- Brimner, T. and G. Boland. 2003. A review of the non-target effects of fungi used to biologically control plant diseases. *Agr. Ecosyst. Environ.*, 100: 3-16.
- Cebolla, B., J. Busto, A. Ferrer, A. Miguel and J.V. Maroto. 2000. Methyl bromide alternatives on horticultural crops. *Acta Hort.*, 532: 237-242.
- Champaco, E.R., R.D. Martyn and M.E. Miller. 1993. Comparison of *Fusarium solani* and *Fusarium oxysporum* as causal agents of fruit rot and root rot of muskmelon. *Hort. Sci.*, 28: 1174-1177.
- Chikh-Rouhou, H., J.M. Alvarez and R.G. Torres. 2007. Differential interaction between melon cultivars and race 1.2 of *Fusarium oxysporum* f.sp. *melonis*. *Comm. Agric. Appl. Biol. Sci.*, 72: 825-829.
- Chikh-Rouhou, H., R. G. Torres, A. Oumouloud and J.M. Alvarez. 2011. Inheritance of race 1.2 *Fusarium* wilt resistance in four melon cultivars. *Euphytica*, 182: 177-186.
- De Cal, A. 1995. Biological control of *Fusarium oxysporum* f. sp. *lycopersici*. *Plant Pathol.*, 44: 909-917
- Fravel, D.R., K.L. Deahl and J.R. Stommel. 2005. Compatibility of the biocontrol fungus *Fusarium oxysporum* strain CS-20 with selected fungicides. *Biol. Cont.*, 34: 165-169.
- Garip, Y., S. Aysel, H.D. Yildiz, A. Ozlem, B. Riza and A. Yelderem. 2017. Effects of salinity stress on chlorophyll and carotenoid contents and stomata size of grafted and ungrafted Galia C8 melon cultivar. *Pak. J. Bot.*, 49(2): 421-426.
- Gong, M., J.D. Wang, J. Zhang, H. Yang, X.F. Lu, Y. Pei and J.Q. Cheng. 2006. Study of the antifungal ability of *Bacillus subtilis* strain PY-1 in vitro and identification of its antifungal substance (iturin A). *Acta Biochim. Biophys.*, Sin38: 233-240.
- Gonzalez, T.R., M.J. Melero, V. J. Gomez and J. Diaz. 1994. *Fusariosis vasculares* del melón y de la sandía (*Fusarium oxysporum* f.sp. *melonis* y *F. oxysporum* f.sp. *melonis*). In: *Enfermedades de las Cucurbitáceas*, vol. 1. Monografía de la Sociedad Española de Fitopatología: 47-50.
- Habiba, N. Rubina, A.A. Syed, S. Viqar, A. Jehan and S. Ehteshamul-Haque. 2016. Evaluation of biocontrol potential of epiphytic fluorescent pseudomonas associated with healthy fruits and vegetables against root rot and root knot pathogens of mungbean. *Pak. J. Bot.*, 48(3): 1299-1303.
- Herman, R. and R. Perl-Treves. 2007. Characterization and inheritance of a new source of resistance to *Fusarium oxysporum* f.sp. *melonis* race 1.2 in *Cucumis melo*. *Plant Dis.*, 91: 1180-1186.
- Hervas, A., B. Linda, L.E. Datnoff and R.M. Jimenez-Diaz. 1998. Effect of commercial and indigenous microorganisms on *Fusarium* wilt development in chickpea. *Biol. Cont.*, 13: 166-176.
- Hoitink, H.A.J. and M.J. Boehm. 1999. Biocontrol within the context of soil microbial communities: A substrate-dependent phenomenon. *Annu. Rev. Phytopathol.*, 37: 427-446.
- Hoitink, H.A.J. and P.C. Fahy. 1986. Basis for the control of soilborne plant pathogens with compost. *Annu. Rev. Phytopathol.*, 24: 93-114.
- Hoitink, H.A.J., M.S. Krause and D.Y. Han. 2001. Spectrum and mechanisms of plant disease control with composts. In: *Compost Utilization in Horticultural Cropping Systems*. (Eds.): Stoffella, P.J. and B.A. Kahn. Lewis Publishers, Boca Raton, Florida: 263.
- King, S.R., A.R. Davis, W. Liu and A. Levi. 2008. Grafting for disease resistance. *Hort. Sci.*, 43: 1673-1676.
- Larena, I., P. Sabuquillo, P. Melgarejo and A. De Cal. 2003. Biocontrol of *Fusarium* and *Verticillium* wilt of tomato by *Penicillium oxalicum* under greenhouse and field conditions. *Phytopathol.*, 151: 507-512.

- Larkin, R.P. and D.R. Fravel. 1998. Efficacy of various fungal and bacterial biocontrol organisms for control of *Fusarium* wilt of tomato. *Plant Dis.*, 82: 1022-1028.
- Louvet, H. 1986. La Fusariose. Fruits et Légumes, 33: 45-47.
- Martyn, R.D. and J. Amador. 1987. Fusarium wilt (*Fusarium oxysporum* f. sp. *melonis* Race 0) of muskmelon in Texas. *Plant Dis.*, 71: 469.
- Ramachandra, K.K., K.U. Vasanthi and H.S. Shetty. 2000. Induction of  $\beta$ -1,3-glucanase in seedlings of pearl millet in response to infection by *Sclerospora graminicola*. *Europ. J. Plant Pathol.*, 106: 373-378.
- Risser, G, Z. Banihashemi and D.W. Davis. 1976. A proposed nomenclature of *Fusarium oxysporum* f.sp. *melonis* races and resistance genes in *Cucumis melo*. *Phytopathol.*, 66: 1105-1106.
- Rojo, F.G., M.M. Reynoso, M. Ferez, S.N. Chulze and A.M. Torres. 2007. Biological control by *Trichoderma* species of *Fusarium solani* causing peanut crown root rot under field conditions. *Crop Protect*, 26: 549-555.
- Sabuquillo, P., A.D. Cal and P. Melgarejo. 2006. Biocontrol of tomato wilt by *Penicillium oxalicum* formulations in different crop conditions. *Biol. Cont.*, 37: 256-265.
- Selitrennikoff, C.P. 2001. Antifungal proteins. *App. Environ. Microbiol.*, 67: 2883-2894.
- Soriano-Martín, M.L., A. Porrás-Piedra and A. Porrás-Soriano. 2006. Use of microwaves in the prevention of *Fusarium oxysporum* f. sp. *melonis* infection during the commercial production of melon plantlets. *Crop Protect*, 25: 52-57.
- Suárez-Estrella, F., C. Vargas-García, C.M.J. López and J.M. Capel. 2007. Antagonistic activity of bacteria and fungi from horticultural compost against *Fusarium oxysporum* f. sp. *melonis*. *Crop Protect*, 26: 46-53.
- Sun, Y.H., G.Q. Zhang, N.H. Du, X.Y. Du and L.X. Meng. 2003. Genetics and breeding of resistance to *Fusarium* wilt of melon. *Yangtze River vegetable*, 1: 1-3.
- Tawfic, A.A. and A.D.A. Allam. 2004. Improving cumin production under soil infestation with *Fusarium* wilt pathogen: II field trial of different landraces and seed treatments. *Assiut Univ. Bull. Environ. Res.*, 7: 47-63.
- Zhao, Q.Y., C.X. Dong, X.M. Yang, X.L. Mei, R. Wei, Q.R. Shen and Y.C. Xu. 2011. Biocontrol of *Fusarium* wilt disease for *Cucumis melo* melon using bio-organic fertilizer. *App. Soil Ecol.*, 47: 67-75.

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