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Morphological identification of the melon wilting agent in Buein Zahra area and non-chemical control with silicon in the field and laboratory

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ABSTRACT

In order to identify the disease agent of melon plants in Buin Zahra area, sampling carried out from infected melon farmlands. Then, identification steps were done by morphological methods and *Fusarium oxysporum* isolated from infected plants. Effects of sodium silicate application (Na_2SiO_3) were investigated on melon plants against Fusarium wilt in the field condition and lab. *in vitro*, petri dishes subcultured of *Fusarium oxysporum* that were containing 200, 400 and 800 ppm of sodium silicate tested to suppression of mycelial growth. The lab experiments showed that increase concentration of sodium silicate due to decrease mycelial growth of the fungi. In the field, melon plants fed-up by 1000 ppm sodium silicate were showed the lowest disease severity. Also, in field experiments, melon plants irrigated with 250, 500, 750 and 1000 ppm of sodium silicate as soil drench three times in a growing season. The results showed that sodium silicate could reduce the disease severity and increased the vegetative growth in comparison with control plants. Therefore, it can use as an effective eco-friendly to disease control.

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INTRODUCTION

Melon (*Cucumis melo* var. *inodorus*) is an important economic crop in Buin Zahra area, Qazvin Province, Iran. It is useful for health. Fusarium is a soil-borne fungus that is found in all soils and has global distribution. All isolates are able to survive in soil and growth in the area rhizosphere of many plant species. Fusarium wilt of melon caused by *Fusarium oxysporum* Schlecht one of the most serious diseases attacking melon in long-term monoculture in the world. Fusarium can attack the vascular system of the plant and secrete its toxins, which cause the vascular wilting and then death within a few days or weeks (Champaco *et al*., 1993). *Fusarium oxysporum* includes pathogenic and non-pathogenic members that for the pathogenic have been described more than 120 different formae speciales based on host specificity (Armstrong and Armstrong, 1981). The first noticeable symptom is unilaterally wilt in melon plant. Wilting symptoms are the result of fungal spores and mycelium that block the xylem (Beckman, 1987). Infected plants looking healthy at the begining, but the vascular tissue is brown and discolored. The pathogen causes yellowing, stunting, wilting and death of the plant at the end. Symptoms usually appear after flowering and fruit set (Sherf and Macnab, 1986). As long as the host plant is alive, *F. oxysporum* will remain in the xylem tissue. Severely infected plants finally wilt and die, and the chlamydospores will come back to the soil from the infected and decaying host tissue (Di Pietro *et al*., 2003) where they remain for several years (Ploetz and Pegg, 2000) and is thus difficult to control. Control methods that were studied against Fusarium wilt comprise resistant varieties, chemical, biological and cultural control methods. In recent years, the use of biological control agents became popular as an environmentally friendly approach to Fusarium wilt control. One possible alternative to control Fusarium wilt is use of the silicon (Si), considering that this element has decreased the disease severity in different crops (Datnoff *et al*., 2007). Silicon is the second most abundant elements in the earth's crust, occurring in living organisms as amorphous silica (SiO_2nH_2O) and to a lesser extent, soluble silicic acid ($Si(OH)_4$) (Fawe *et al*., 1998). Silicon has caused that vegetative growth and biomass increased in plants (Agarie *et al*. 1998). The objectives of this study were to (i) morphological identification of disease agent (ii) the efficacy determination of sodium silicate on mycelial growth inhibition of *Fusarium oxysporum* in laboratory, and (iii) the efficacy determination of soluble sodium

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silicate against *Fusarium oxysporum* in field to comparison with benomyl-treated melon plants. This study was performed in order to create a way for help to control the fusarium wilt in the field conditions.

MATERIALS AND METHODS

In recent years, prevalence a disease had caused a severe decreasing in melon yield in Buin Zahra area, Qazvin Province, Iran (North of Iran, 35.9000°N-49.7667°E). Disease symptoms in fields was similar to Fusarium wilt that there are in most continental of the world including America, Europe, Asia and especially France.

Sampling and identification:

Isolation of wilting agent was done after surface sterilization. Each sample was rinsed under running water for 15 minutes. Four tissue pieces from root, crown and stem (5mm) were surface sterilized with 1.5 % sodium hypochlorite for 1-2 min and washed two times in sterile distilled water (Akbari *et al.,* 2015). These pieces were plated onto Potato Dextrose Agar (PDA; 200 g potato, 17 g glucose and 17 g agar per liter) supplemented with 1 drop lactic acid. Petri dishes were incubated at 25 °C for 10 days under fluorescent light (12 h photoperiod) (Nirenberg 1976). Morphological identification was done on PDA and Carnation Leaf Agar (CLA) medium according to the morphological criteria keys of Nelson *et al*. (1983).

Pathogenicity test:

Pathogenicity test was proved in Bu-Ali Sina University, Hamedan, Iran, under greenhouse conditions with melon (*Cucumis melo* var. *inodorus*), including 2 cultivar khatooni (endemic in Buein zahra area) and Minoo 095P ergon (Ergon International N.V. The Netherlands) in 2013. Pots were containing of soil, sand and peat (2:1:1; v/v) that they were pre-sterilized for 20 min in an autoclave at 120˚C. Preparation of fungal inoculum was done on a mixture of 5 gr corn flour mixed with 95 gr sand and 50 ml sterile distilled water, that were sterilized for 2 hrs at 120˚C. Fusarium spore suspesion was prepared from purified Fusarium isolate and the concentration adjusted to 4.2×10^{6} spore/ml. 5 ml of spore suspension, was added to the flasks containing 100 g mixture of corn flour and sand. Control flasks contained mixture of corn flour and sand which 5ml sterile water was added to them. These flasks were incubated for 20 days at 27±2°C. Inoculum of fungi was added at a 1:10 ratio into sterile soil of pots. Melon seeds were planted in pots and kept in an environmentally controlled greenhouse at 25±2°C and 12 hours photo period. This was performed in four replicates for each cultivar. Also 4 pots were watered with distilled water as acontrol.

In vitro assessment:

We evaluated effects of sodium silicate inhibition on mycelial growth of *Fusarium oxysporum* in laboratory. This experiment including: (1) petri dishes with different concentration of sodium silicate, inoculated with Fusarium, (2) petri dishes without sodium silicate, inoculated with Fusarium, with four replications. Potato dextrose agar (PDA) was prepared and autoclaved. Soluble sodium silicate was passed through a filter and added to the PDA after autoclaving at a concentration of 200, 400 and 800 ppm of PDA. The Agar-Sodium silicate solutions were blended with magnetic stirrers and then decanted into Petri dishes. In order to determin of sodium silicate efficacy on mycelial growth, the fungus was cultured on PDA Petri dishes at 25°C for 5 days (120 hrs). After incubation at 25°C, the colony diameter of *Fusarium oxysporum* were measured every 24 hrs. Sodium silicate-agar petri dishes were inoculated with fungus by placing a 5 mm diameter disc from an actively growing culture in the centre of each plate. Fungal growth (colony diameter) was measured and percentage inhibition calculated according to the formula:

Percentage inhibition= (C-E)×100/C, Where C= colony diameter (mm) of the control, E= colony diameter (mm) of the experiment petri dishes.

Field experiments:

In this study, pressurized irrigation system was used for transmission of sodium silicate and Benomyl as soil drench for each treatment in field. In the field, was used only melon (*Cucumis melo* var. *inodorus*), cultivar Minoo 095P ergon which is high yield, susceptible to the pathogen and frequently used in the Buin zahra area. These seeds were planted directly into the soil of the field. The field had infected naturally (since 2005) and the pathogen was isolated from this the field. The uniform seedlings were selected at four-leaf stage. Sodium silicate dissolved in water to catch final concentration (250, 500, 750 and 1000 ppm). Disease severity was measured for twenty samples (all treatment was 110 plants in each concentration) by the scale modified by Matsumoto *et al*. (2011) after 5 weeks post four-leaf stage. Soluble sodium silicate as soil drench was carried out at three stages including: four-leaf stage, flowering and raw fruit into pre-ripening. At the end of the growing season (95-105 days after the start of the experiment), twenty samples had taken randomly. Then, their shoot and root dry weight, shoot and root fresh weight and plants length were measured. The field experiments

were conducted; including (1) plants with different concentration of sodium silicate, (2) plants without sodium silicate, (3) plants with benomyl, with three replication.

Evaluate the disease severity:

Disease severity was recorded using a scale containing 4 grades suggested by Matsumoto *et al*. (2011): Grade: 0= no symptoms, 1= small lesions on leaves, 2= leaves strongly affected, 3= plant death.

Preparation of soluble sodium silicate and benomy:

In this study, Si was used as the sodium silicate (Aldrich, Na_2SiO_3; a.i. ~ 27%) at concentrations of 250, 500, 750 and 1000 ppm. HCl was used for modification of pH (pH=6.4). Benomyl (chemical treatment), used for comparison with sodium silicate efficacy (non-chemical treatment). Benomyl was used at a 1.5/1000 ratio (benomyl/water).

Soil analysis:

The soil samples of field had taken in May 2013 (befor the start of the experiment)(Boubakr *et al.,* 2015). The samples were homogenised and then they packaged in plastic boxes. Each box was containing an amount of approximately 2 kg. Soil analysis was performed before planting. Soil properties were measured by different methods: soil texture by hydrometer method (Gee and Bauder, 1986), cation exchange capacity (CEC) by ammonium acetate method (Rowell, 1994), soil extracts pH at a 2/5:1 ratio (water/soil) by pH meter (model 744, Metrohm) (Nguyen and Sukias, 2002) and electrical conductivity (EC) by EC meter (model 712 Metrohm) and 2:1 ratio (water/soil) (Klute, 1986).

Statistical Analysis:

The statistical analysis was done for experiments. The Experimental design was a Completely Randomized Design. The data were analyzed using Procedure GLM (Generalized Linear Models) of the SAS Software v9.1 (SAS Institute Inc., Cary, NC, USA). Comparison of means has been done based on Duncan Test.

RESULTS AND DISCUSSION

Morphological identification and pathogenicity test:

Morphological identification proved *Fusarium oxysporum* was the agent of the disease. Pigmentation of the aerial mycelium of the fongus isolates varied from white to dark red while colony reverse varied from white to cream with a dark brown zonation. Macroconidia were sicklelike, typically four septate. Microconidia were one or two celled. Dimensions of macroconidia ranged from 28.5 µm ~ 39.5 µm x 3.8 µm ~ 5.2 µm. In the greenhouse (pathogenicity test), symptoms were include combinations of wilting, yellowing and chlorosis. Severely infected plants were wilted and died, but plants affected to a lesser degree became unproductive. In some cases, signs of decay on roots was observed. The most prominent internal symptom was vascular browning in plants. Symptoms of Fusarium wilt occurred after 64 days from the start. Reisolation was done from all of the plants.

Effect of soluble sodium silicate on mycelial growth of Fusarium oxysporum:

With regard to mycelial growth in experiment, results showed that soluble sodium silicate was reduced significantly mycelial growth of *Fusarium oxysporum* in all petri dishes containing Si. (Table 1). The silicon efficacy in inhibition the fungal growth depends upon the Si concentration. The results showed that soluble sodium silicate had reduced mycelial growth significantly at all treatments (Table 1). After 120 hours, petri dishes with 800 ppm showed the most inhibition on the mycelial growth of *Fusarium oxysporum* (91.53%). In this study, all petri dishes containing different concentrations of soluble sodium silicate could reduce the mycelial growth of *Fusarium oxysporum in vitro*. Although, their effect seemed to be increasing along with their increasing concentrations. Similar results to this study was previously shown that potassium silicate could inhibit the mycelial growth of *Fusarium oxysporum*, *Fusarium solani*, *Phytophthora cinnamomi*, *P. capsici* and *Pythium* F-group and *Verticillium fungicola* (Kaiser *et al*., 2005).

Table 1: The effect of soluble sodium silicate on mycelial growth of *Fusarium oxysporum* (every 24 hours) on PDA at 4 concentrations (0, 200, 400, 800 ppm).

Treatments	Colony diameter of *Fusarium oxysporum* (mm)					
	24hrs	48hrs	72 hrs	96hrs	120hrs	percentage inhibition(final)
Control	4.32^a	9.45^a	16.78^a	22.05^a	34.25^a	0
200ppm Si	2.1^b	7.4^b	11.85^b	15.1^b	22.5^b	34.3
400ppm Si	1.2^c	4.3^c	7.8^c	10.05^c	17^c	50.36
800ppm Si	0.37^d	0.82^d	1.35^d	2.1^d	2.90^d	91.53

Means followed by common letters are not significantly different at P= 0.01 by Duncan's Multiple Range Test. Data are means of four replicates.

Disease reaction assessment in field:

Effects of different concentrations of sodium silicate in the field indicated in Table (2). The most plants treated with different concentration of sodium silicate were indicated reduction of disease severity in comparison with the control plants (Table 2). Based on our data, when concentrations of sodium silicate was 750 and 1000 ppm, disease severity was reduced considerably. The results showed that application of sodium silicate with concentration of 1000 ppm was similar to applications of benomyl. Disease severity was 90% on the infected melon plants (without sodium silicate), which decrease to 58.33% in the soil amended with sodium silicate (Table 2). Silicon fertilizers have been utilized for crops in multiple countries to increase productivity. The positive effects of silicon have been found in sugarcane, cucumber, citrus, tomato, barley, wheat, corn and other crops (Epstein, 1994). For soilborne pathogens, silicon amended in the soil has decreased the severity of the diseases caused by *Pythium ultimum* and *P. aphanidermatum*, *Ralstonia solanacearum*, *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Phytophthora capsici* (Dannon and Wydra, 2004; French-Monar *et al.*, 2010; Huang *et al.*, 2011). In the field, it was found that sodium silicate application in infested soil, increased the plant length, fresh and dry weight more than the control plants.

Table 2: comparison the different concentrations efficacy of sodium silicate on the disease severity in field.

Treatments	Mean of disease severity	Percentage inhibition	Significant difference
Infected control	2.7	90%	a
250ppm Si	2.55	85%	ab
500ppm Si	2.2	73/33%	bc
750ppm Si	1.9	63/33%	cd
1000ppm Si	1.75	58/33%	d
Benomyl	1.75	58/33%	d

Means followed by common letters are not significantly different at P= 0.01 by Duncan's Multiple Range Test. Data are means of twenty replicates.

Efficacy of different concentrations of sodium silicate on vegetative growth of melon in the field condition:

The results of this study showed that shoot and root dry weight, shoot and root fresh weight and plants length were increased significantly in the most treatments containing sodium silicate in comparison with the infected control. In addition, all treatments indicated that sodium silicate could improve in their roots and shoots dry weight (Table 3). Dry and fresh weight of Infected plants by pathogen (not treated with sodium silicate) was minimum among of all treatments (Table 3). Application of sodium silicate at a concentration of 1000 ppm was similar to application of benomyl. It can be concluded that sodium silicate is able to suppressing growth of pathogen even under soil conditions in a similar way as it controlled in petri dishes assay. The results evidenced that decreasing of disease severity was associated with an increase of the vegetal growth including the plants lenght as well as the plants fresh and dry weights. When adequate amounts of soluble sodium silicate are available in the solution surrounding the roots, melon plants are able of absorbing great amounts of sodium silicate and the Si content in the leaves can reach values as high as those in gramineous plants (Nolla *et al.*, 2006; Mahmoud DIF *et al.,* 2015). Also, root application of sodium silicate at a concentration of 1000 ppm, presented the best results in comparison with benomyl treatment in the field. Thus, based on our data, the increasing of vegetative growth and decreasing of disease severity of plant treated with sodium silicate confirmed with results other researchers (Hadar and Gorodecki, 1991). Singh *et al.* (2006) found that the 180 kg/ha of Silicon increased nitrogen and phosphate levels in the grain and straw of rice. Several studies were earlier reported on the ability of silicon in enhancing the Cucurbitaceae crop growth against *Pythium aphanidermatum* and *P. ultimum* (Cherif *et al.*, 1994; Belanger *et al.*, 1995). This findings confirmed a previous research on the use of Soluble silicon applications for the control of fungal diseases in cucumber (Cherif *et al.*, 1992), peach fruit (Biggs *et al.*, 1997), muskmelon and zucchini squash (Menzies *et al.*, 1992).

Table 3: Comparison of different concentrations efficacy of sodium silicate application on vegetative growth of melon plants in field

Treatments	Shoot fresh weight	Shoot dry weight	Plant length	Roots fresh weight	Roots dry weight
Infected control	309^{f}	38^{f}	93^{f}	4.4^{e}	1.1^{e}
250ppm Si	395^{e}	52.6^{e}	114^{e}	6.4^{d}	1.7^{d}
500ppm Si	409^{d}	56^{d}	127^{d}	7.4^{c}	2^{c}
750ppm Si	429^{c}	63^{c}	145^{c}	8.7^{b}	2.5^{b}
1000ppm Si	454^{b}	70^{b}	159^{b}	10.4^{a}	3.1^{a}
Benomyl	477^{a}	76^{a}	166^{a}	11.1^{a}	3.4^{a}

Means followed by common letters are not significantly different at P= 0.01 by Duncan's Multiple Range Test. Data are means of twenty replicates.

Global Journal of Medicinal Plant Research, 3(6) November 2015, Pages: 1-6

Results of analysis the soil properties:

Analysis of field soil showed that it had the high pH (above 7) as a result was prevented disease spread (Table 4). Also, based on the analysis, low-salinity (EC) of soil was beneficial for plants. Root application of sodium silicate led to a consistent absorption of the Si by the plant, and It has been observed that its effect on fusarium wilt disease was very useful and efficient. Also, positive role of sodium silicate on fusarium wilt may lead to better plant productivity.

Table 4: Comparison soil chemical and physical properties (before planting)

	Properties			
Place	Texture	pH	Ec (ds/m)	CEC (cmolc/kg)
Field	Loam	7.7	0.5	15.9

Conclusion:

In this study, provided evidences that sodium silicate amendments could reduce the fusarium wilt in melon plants and certainly, it to be effective activator of plant defense mechanism. This can lead to use of the sodium silicate as useful tool to control crops diseases.

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