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SILICON TREATMENT EFFECTS ON *TETRANYCHUS URTICAE* AND PHYSIOLOGY OF ZUCCHINI PLANTS

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Abstract

The aim of the study was to examine the effect of Silicon in the form of stabilized orthosilicic acid (H_4SiO_4) on the population of the two-spotted spider mite Tetranychus urticae Koch. on zucchini (Cucurbita pepo L.) cv. Izobilna and on the physiology of the infested plants. The experiment was conducted in a laboratory under controlled conditions at the Department of Entomology, Agricultural University - Plovdiv, Bulgaria. The plants were grown hydroponically and separated into four variants consisting of 1 - control, 2 - mite-infested plants, 3 - Silicon-treated plants and 4 - mite-infested plants, treated with Silicon. The results obtained suggest that Si could be successfully used for reducing the population density of the two-spotted spider mite on zucchini cv. Izobilna and to improve the physiological status of the plants. Several biochemical parameters concerning the main physiological processes in the infested plants were also measured. The results showed a decrease in the population density of T. urticae on the Si-treated leaves. The net photosynthetic rate and the content of the main photosynthetic pigments increased in the Si-supplied infested plants compared to the Si-unsupplied.

Key words: *mites, photosynthesis, pigments, silicon, zucchini.*

INTRODUCTION

Silicon (Si) is the second most abundant elements on Earth`s crust after Oxygen. Most of the Si is present in the soil in the form of insoluble oxides and silicates, but there are also water-soluble forms. Si is the only element that does not damage plants when it is accumulated in excess due to its non-dissociative properties at physical pH and polymerization (Mahdieh et al., 2015). The influence of silicon on growth-enhancing and plant-resistance metabolic processes is recorded under stress conditions mainly. Several studies have shown that silicon is able to reduce the effect of stress caused by abiotic (Zhu et al., 2004; Feng et al., 2010; Harizanova et al., 2014; Harizanova and Koleva-Valkova, 2019) and biotic factors (plant pests and diseases). There are several studies on the effect of silicon on herbivore insect pests and noninsect pests (Massey et al., 2006; Massey and Hartley, 2009; Kvedaras et al., 2010; Harizanova et al., 2019).

Tetranychus urticae Koch (Acari: Tetranychidae) is one of the most harmful pests in the world and belongs to the group of the spider mites (Van Leeuwen et al., 2010; Tehri et al., 2014a). The mite damages crops directly by feeding which reduces the photosynthetic leaf area and biomass of the plants. This leads to disturbance of water balace, reduction of dry matter, CO_2 gas exchange, reduction of chlorophyll content and necrosis (Gorman et al., 2001; Park and Lee, 2002; Alatawi et al., 2007; Tehri et al., 2014b).

Different studies have shown that silicon is involved in the defense system of plants in several ways. According to some authors silicon provides an indirect protection, based on the increased release of volatile substances that attract natural enemies of pests (Kvedaras et al., 2010). Other researchers report that silicon`s positive effect is based on a direct protection through increased (passive) resistance due to the deposition of amorphous silicon in the tissues of the cells. Silicon acts as a mechanical barrier (Guntzer et al., 2012) and reduces the digestibility and/or increase the hardness and abrasion of plants (Massey et al., 2006; Massey and Hartley, 2009). Some hypothesis researches the regulation of genes observed in the infection with powdery mildew in *Arabidopsis* sp. (Fauteux et al., 2005) and infection of rice with *Magnaporthe oryzae*

(Brunings et al., 2009). One of the possible ways of action is also the activation of plant defense system. This triggers protective mechanisms including the accumulation of lignin, phenolic compounds and phytoalexins (Epstein, 1999; Ma and Yamaji, 2006), formation of papillae, deposition of callose and H_2O_2 and stimulation of system stress signals (salicylic acid, jasmonic acid and ethylene) (Shetty et al., 2012) and activation of enzymes such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL), which are key enzymes regulating the formation and accumulation of secondary metabolites, phytoalexins and momilactones (Gomes et al., 2005; Ye et al., 2013).

There is scarce information about the effect of Si on plants which are infested by non-insect pests like spider mites. Most of the results obtained are connected to the silicon effect on the pest. The authors study the longevity and mortality of the herbivore enemy and there is a lack of information about the physiological status of the infested plants. The aim of the current study was to investigate the effect of silicon in form of orthosilicic acid on photosynthesis and the biomass of laboratory grown zucchini plants infested by the two-spotted spider mite *Tetranychus urticae*.

MATERIALS AND METHODS

Laboratory tests were conducted to study the effect of H_4SiO_4 on the physiological status of zucchini plants and on the polulation of the two-spotted spider mite were carried out in the phytostatic boxes of the Department of Entomology, Agricultural University - Plovdiv. Plants were grown in inert substrate under controlled environmental conditions: photoperiod 14/10 hours (light/dark), photosynthetically active radiation (PhAR) – 250 µmol/m/s/, air temperature 24±2°C/17±2°C (day/night) and relative air humidity 65±5%. After 24 hours in a thermostat germinated seeds from zucchini (*Cucurbita pepo* L.), cv. Izobilna were transferred to plastic pots filled with inert material (perlite) for a period of 5-7 days. Then, plants with the same developmental phase were selected and transferred to plastic containers. The experimental design consisted of four variants: 1 - control plants; 2 - plants infested with *Tetranychus urticae* Koch; 3 - plants treated with silicon if form of H_4SiO_4; 4 - plants infested with mites and treated with H_4SiO_4. All test plants were grown in an inert substrate (perlite) supplied with a nutrient solution containing all the necessary macro- and microelements. Ten days after germination, which coincided with the appearance of a third mature leaf, the plants were infested with 50 adult mites per plant. The first foliar Si treatment was 2 days after the infestation. Plants were treated with ones a week for four weeks and analyzed at the end of the period. Small leaf discs with 50 females in total were placed on the second and/or third true (mature) leaf of each zucchini plant. The population density was checked 30 days after infestation. Infested leaves from each plant were removed and checked under the stereomicroscope. The number of eggs, larvae, nymphs and adults of *T. urticae* was counted and recorded on twenty-four leaf discks (2 cm in diameter).

Experiment for study the duration of developmental stages of *T. urticae* was carried out under the same laboratory conditions. Small leaf discs (3 cm in diameter each) of zucchini plants from two variants (treated and untreated with Si respectively) were placed on wet cotton in Petri dishes (9 cm in diameter). Five females from the plants, treated and untreated respectively, were individually isolated and transferred to each leaf disc for egg laying. The discs containing adult females were checked every two hours after mite transfer. The mites were removed if at least one egg was found. Immediately after the new egg deposition females were transferred to new leaf discs. The discs were checked twice a day and the duration of developmental stages was recorded. The immature stages were transferred to new discs very carefully with the help of a tin hair brush.

Analysis

Guaiacol Peroxidase (GPOD)

The activity of GPOD was determined spectrophotometrically by the method of Bergmeyer et al. (1974). First 2.3 ml of KH_2PO_4 buffer (pH 7.0), 300 µl of H_2O_2, 300 µl of 8 mM guaiacol and 100 µl of extract are placed in the cuvette. Absorbance was measured at 436 nm against a blank with the same components without enzyme extract (E = 26.6 mm/cm^2). The values obtained are expressed as U mg/g FW.

Leaf gas exchange parameters

The rate of net photosynthesis (P_N), stomatal conductance (g_s) and transpiration rate (E) were determined with LCpro+ portable photosynthetic system [Analytical Development Company Ltd., Hoddesdon, England]. Measurements were performed under the following conditions: Photosynthetically active radiation (PhAR) of 500 μmol/m/s, temperature 25°C and natural external CO_2 concentration of about 400 vpm.

Content of photosynthetic pigments

The content of photosynthetic pigments was determined spectrophotometrically by the Lichtenthaler method (1987) and was expressed as mg/g fresh leaf material.

Statistical Analysis

The data were presented as mean ± SD of 3 replicates. The experimental results were statistically processed with the SPSS program using a one-way ANOVA dispersion analysis using Duncan's comparative method, with the validity of the differences determined at a 95% significance level. The different letters (a, b, c, d) after the average show statistically significant differences between the analyzed variants. In the analysis of the mite population the differences between two variants with mites were analyzed and the significance was determined by independent samples t-test with a $p<0.05$. All data were analyzed using IBM SPSS Statistics 20 software.

RESULTS AND DISCUSSIONS

In order to analyze the effect of the silicon treatment on the mite population, the number of the different developmental stages of *T. urticae* was counted and recorded (Table 1). It is obvious that the Si treatment affects the number of almost all developmental stages of the mite, especially the number of mobile immature stages - their number is reduced by 29%. The reduction of the number of larvae on the plants treated with silicon is 33% and for nymphs these values are between 70 and 73%.

Table 1. Number of developmental stages of *T. urticae*, on Si-treated and Si-untreated plants

Developmental stages of *T. urticae*	Variant	Mean	Std. Error Mean
Eggs	Mite+Si	105.00	2.97
	Mite	82.25	3.23
Larvae	Mite+Si	31.75	3.72
	Mite	47.13	2.42
Protonymjphs	Mite+Si	3.25	0.73
	Mite	12.13	1.20
Deutonymphs	Mite+Si	9.25	1.18
	Mite	30.50	3.81
All mobile immature stages – larvae and nymphs	Mite+Si	44.25	4.47
	Mite	77.25	7.22
Females	Mite+Si	20.50	4.01
	Mite	14.13	1.57
Males	Mite+Si	9.25	0.81
	Mite	9.38	1.12
Adults (females and males)	Mite+Si	29.75	3.98
	Mite	23.50	1.31

The results of the independent samples t-test show that the difference between mean number of immature mobile stages from two variants (Mite and Mite+Si) is statistically significant ($t_{31.541}=4.090$; $p=0.000$).

Larvae and nymphs, as actively feeding mobile stages of the pest, are smaller than adults and this could explain some possible difficulties in their feeding and development and the reduction of their number. As it is mentioned by many authors (Reynolds et al., 2016; Alhousari and Greger, 2018) silicon treatment provides mechanical barrier and could reduce the tissues digestibility to some vertebrates.

Table 2. Duration of the different developmental stages (in days) of the two-spotted spider mite *T. urticae* on Si-treated and Si-untreated zucchini plants

Developmental stage	Variant	N	Mean	Std. Deviation	Std. Error Mean
Egg	Mite+Si	647	4.48	0.66	0.03
	Mite	307	4.19	0.78	0.04
Larvae	Mite+Si	131	2.76	0.67	0.06
	Mite	119	3.35	0.55	0.05
Protonymph	Mite+Si	103	2.17	0.51	0.05
	Mite	112	2.04	0.64	0.06
Deutonymph	Mite+Si	97	2.44	0.90	0.09
	Mite	96	2.44	0.65	0.07
Life cycle – egg to adult	Mite+Si	75	10.47	1.11	0.13
	Mite	95	10.44	1.18	0.12

The Si-treatment does not affect significantly the life cycle of the pest, although there are some differences between two variants regarding the duration of each developmental stage of the pest (Table 2). The number of mobile immatures, reaching the next stage is lower on Si-treated plants (Figure 1). Eighty percent of the larvae on Si-untreated plants reach adulthood compared to 57% on Si-treated ones.

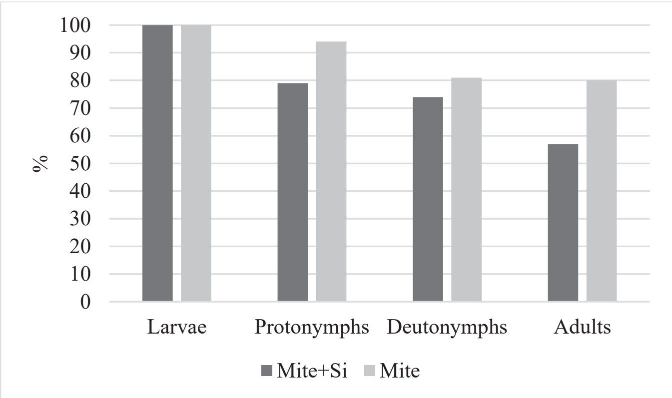

Figure 1. Percentage of the mobile immatures of *T. urticae*, reaching next developmental stage on Si-treated and Si-untreated plants zucchini plants after 30 days mite infestation and treatment with Si

In order to determine the degree of damage by the pest, the activity of guaiacol peroxidase (GPOD) was analyzed. The results show that the mite infestation increased the activity of GPOD in the leaves of the mite-infested plants more than five times compared to the control plants (Table 3). It is related to the higher number of the pest on Si-untreated plants. In the leaves of the Si-supplied mite infested plants the GPOD activity is reduced by 35%. The sole Si application led to a slight increase by only 13% compared to the control.

Table 3. Activity of guaiacol peroxidise GPOD (U mg/g FW) in the leaves of zucchini plants (*Cucurbita pepo* L.), cv. Izobilna, after 30 days mite infestation and treatment with Si

Variant	Control	Si	Mite	Mite+Si
GPOD	6.302^{c}	7.145^{c}	35.57^{a}	23.16^{b}

The parameters of the leaf gas exchange are presented in Table 4. The results show a significant decrease of the net photosynthetic rate (P_N) of the infested plants compared to the control (by 55%). The transpiration rate is also reduced (by 44% compared to the control). The Si application increased P_N of the mite-infested Si-treated plants by 132% in comparison with the infested plants which were not treated with Si. There is also an enhancement of the transpiration rate by 49%. There are some differences in the rate of the stomatal conductance, although they were not statistically proven.

Table 4. Leaf gas exchange parameters of zucchini plants (*Cucurbita pepo* L.), cv. Izobilna, after 30 days mite infestation and treatment with Si

Variant	P_N[1]	g_s[2]	E[3]
Control	6.47^{ab}	0.1^{a}	1.12^{ab}
Mite	2.88^{b}	0.05^{a}	0.74^{b}
Si	5.04^{ab}	0.13^{a}	1.72^{a}
Mite+Si	6.68^{a}	0.08^{a}	1.1^{ab}

[1]P_N = net photosynthetic rate (μmol/m^2/s); [2]g_s = stomatal conductance (mol/m^2/s); [3]E = transpiration rate (mmol/m^2/s).

The content of the main photosynthetic pigments is presented in Table 5. The infested plants were dramatically affected by the feeding of the pest. A reduction of chlorophyll a, chlorophyll b, chlorophyll (a+b) and carotenoids in the mite-infested plants by 27%, 40%, 30%, and 22% respectively was observed. Silicon supply to the infested plants led to an increase of the pigment content by 28%, 16%, 26%, and 35% respectively. In a previous research Harizanova et al. (2019) reported about a significant increasment of the main photosynthetic pigment content in cucumber plants infested by the two-spotted spider mite *T. urticae*. The net photosynthetic rate was enhanced too.

Table 5. Content and ratio of photosynthetic pigments (mg/g) in zucchini leaves (*Cucurbita pepo* L.), cv. Izobilna, after 30 days mite infestation and treatment with Si

Variant	Chl[1] a	Chl b	Chl (a+b)	Carotenoids	Chl a/b	Chl (a+b)/ carotenoids
Control	1.71^{b}	0.7^{b}	2.4^{b}	0.56^{ab}	2.46^{b}	4.3^{a}
Mite	1.35^{c}	0.5^{c}	1.84^{c}	0.46^{c}	2.7^{ab}	4^{b}
Si	2.5^{a}	1.02^{a}	3.52^{a}	0.81^{a}	2.46^{b}	4.37^{a}
Mite+Si	1.74^{b}	0.58^{c}	2.33^{b}	0.62^{b}	3.03^{a}	3.75^{c}

[1]Chl = chlorophyll.

The biomass of the analyzed plants was also measured. The fresh weights of the leaves, stems and the roots of the infested plants were strongly affected. The decrease in leaf biomass was by 51%. Stems were 25% lighter than the control and the weight of the roots was reduced by 52%. The mite infested and Si-treated plants demonstrated a significant increase in the biomass of all the plant organs. There is evidence of the positive relationship between high silica concentrations and plant resistance to herbivores (Gatarayiha et al., 2010; Yavas and Unay, 2017). The authors applied potassium silicate of different doses on the nutrient solutions of maize, eggplant, beans, and cucumber which were infested by the two-spotted spider mite. During their experiment, they observed that the Si-supplied plants suffer less damage than the untreated infested plants which is in line with the observations of the current study. Toledo and Reis (2018) examined the effect of foliar spraying with potassium silicate on coffee plants infested with red mite *Oligonychus ilicis*.

Table 6. Biometry of zucchini (*Cucurbita pepo* L.), cv. Izobilna, after 30 days mite infestation and treatment with Si

Variant	Leaves		Stems		Roots	
	FW[1] (g)	LA[2] (cm^2)	FW (g)	L[3] (cm)	FW (g)	L (cm)
Control	21.6^{a}	1003.4^{a}	6.13^{bc}	39.8^{ab}	4.97^{a}	29.5^{ab}
Mite	14.27^{b}	671.2^{b}	4.9^{c}	27.4^{c}	2.39^{b}	25.3^{b}
Si	24.9^{b}	1079.8^{a}	8.6^{a}	43.6^{a}	4.16^{ab}	35.7^{a}
Mite+Si	21.4^{b}	949.7^{a}	6.81^{b}	34.6^{b}	2.47^{b}	30.6^{ab}

[1]FW = fresh weight; [2]LA = leaf area; [3]L = length.

The authors report about the reduced population of red mite and suggest that this effect may be due to chemical and physical changes in the plant tissues. They also observed the increased content of lignin and tannins which probably makes plant tissues less attractive to herbivores because of the increased hardness and toxic compounds in the plant tissues. About the same effect of the silicon treatment reported Yavas and Unay (2017). They suggest that Si could improve plant growth and resistance under stress conditions as it acts as a mechanical barrier for pests and diseases and prevents of oxidative stress via enzyme activation. The increased resistance to herbivore pests caused by silicon treatment has been reported also in various sensitive varieties of cereal crops (Keeping and Meyer, 2006, Hou and Han, 2010, Sidhu et al., 2013) and grasses (Massey and Hartley, 2009). He et al. (2015) found that increased levels of silicic acid in plants shortened the stay of cicadas on them and reduced the fertility of the pest. In another research it was established that after treatment with $CaSiO_3$, the mortality of *Bemisia tabaci* nymphs increased (Correa et al., 2005).

Higher silicon concentration in the soil or in the nutrient medium causes a decrease in the number of insect and non-insect pests of crop plants (Liang et al., 2005). The positive effect of silicon application has been reported by various pests including *spider mites* (*Tetranychus* spp.) (Gatarayiha et al., 2010) or mites (Savant et al., 1999; Nikpay et al., 2014). In the case of silicon feeding, the number of pests in the infested plants was reduced (Gomes et al., 2005). It is reported that pests with piercing-sucking mouth organs and herbivore caterpillars prefer silicon-poor tissues. Silicon reduces food intake, growth, fertility, and ultimately the population of *Sogatella frucifera* (Salim and Saxena, 1992). Silicon also reduces the reproductive capacity of the phloem-feeding species (*Myzus persicae*) on potatoes and whitefly (*Bemisia tabaci*) in cucumber.

CONCLUSIONS

Silicon supply in form of H_4SiO_4 is able to alleviate the negative effect of mite infestation in zucchini plants.

The number of the larvae and nymphs on the silicon supplied plants is reduced and the duration of some developmental stages of the two-spotted spider mite increased.

The net photosynthetic rate, the pigment content and the biomass accumulation of Si-treated mite-infested plants increased.

The positive effect of the Si-treatment is probably due to activation of the enzymatic defense system of the plants or to the increased hardness and abrasiveness of the plant tissues which makes plants tissues less attractive to the herbivores.

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