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SILICON - AN ALTERNATIVE WAY TO CONTROL THE POPULATION OF THE TWO-SPOTTED SPIDER MITE ON CUCUMBER

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Abstract

The need for biological control of pests is increasing every day. Science is trying to minimize the use of conventional pest-control products. The aim of the current study was to examine the effect of Silicon on the population of the two-spotted spider mite Tetranychus urticae Koch. on cucumber (Cucumis sativus L.) cv. Gergana. The experiment was conducted in a laboratory under controlled conditions at the Department of Entomology, Agricultural University of Plovdiv, Bulgaria. Plants were infested with mites and treated with Si in form of orthosilicic acid. The results showed an effect of Si on the male/female ratio of the mite population, as well as the duration of the development of the different stages of the pest. Some biochemical parameters of the infested plants were also measured. There was an increase of the salicylic acid content and the activity of phenylalanine ammonia-lyase. The results obtained suggest that Silicon could be successfully used for reducing the population density of the two-spotted spider mites on cucumber and to improve the physiological status of the plants.

***Key words**: biotic stress, cucumber, mites, salicylic acid, silicon.*

INTRODUCTION

Producing vegetables under variable biotic and abiotic stresses is a big challenge for the farmers nowadays. The need for more and more high-quality produce is rising along with the rise of the population worldwide. It is really a challenge for the common farmer to grow healthy and pesticide-free yield. The overuse of chemical pesticides led to the fast-growing development of resistance in targeted insect pests, as well as to severe effects on non-target organisms and human health as well (Lucchi and Benelli, 2018). In the last years, there is a sustainable tendency for reducing the levels not only of the pesticides but also of the mineral fertilizers. That's why scientists together with the leading producers of crop protection products discover new horizons in agriculture. Their efforts aim at testing new generation of plant protection and fertilization using substances that act as biostimulants. Some of these products not only have positive effect on the quantity and quality of crop production but also decrease the level of pest invasions.

Pest control is a serious issue especially in the greenhouse vegetable growing. Tetranychid mites (family Tetranychidae), some of which are the spider mites, along with thrips, whiteflies and nematodes, are major pests of crops. They are the most important herbivore mites around the world (Hoy, 2011) and are among the most economically dangerous pests of greenhouse crops (Zhang, 2003). More than a thousand species of plants, representing more than a hundred families, are attacked (Van Leeuwen et al., 2010).

Before World War II, spider mites were a minor problem for crops. This changed dramatically in the 1950s and 1960s, when the use of synthetic organic pesticides and fertilizers increased sharply (Hoy, 2011). Tetranychid mites quickly manage to build up resistance to intensively applied pesticides, especially in greenhouse vegetables and flowers.

The small size of mites, rapid development, high reproductive potential, large number of generations and diapause (optional) allow mites to multiply for a short time in high density when environmental conditions are favorable. The attack of the common spider mite *Tetranychus urticae* represents a potential biotic stress for the host plant and seriously affects the physiological and biochemical processes in it (Sivritepe et al., 2009).

During feeding, the mite mechanically damages the epidermis of the leaves. This disturbs the water regime of the plants and effects on the productivity of the photosynthetic apparatus (changes in CO_2-gas exchange, reduction of the chlorophyll content), which can cause premature foliage and flowering, even the death of whole plants (Park and Lee, 2002).

Meteorological conditions in Bulgaria are favorable for the development and intensive multiplication of mites, which is a problem both in the field and in greenhouses. In Bulgaria, the pest multiplies rapidly at the end of July and the beginning of August as the temperatures rise and the relative humidity decreases. In greenhouses under favorable conditions and in the presence of a host plant, the species develops without diapause (continuously). This makes the two-spotted spider mite one of the most dangerous pests of many vegetable crops, including cucumber and other species of the Cucurbitacea family.

Many synthetic organic pesticides kill not only the herbivores but also the beneficial species (predatory insects and mites) that control populations of pests. On the other hand, it is well known fact that the overuse of some fertilizers stimulates the multiplication of herbivore mites. Moreover, some pesticides have a stimulating effect on mite breeding when applied at low concentrations, leading to higher reproductive potential. This stimulating effect, called "hormoligosis," has been described as a physiological phenomenon that exhibit living organisms exposed to very low concentrations of essentially toxic substances (Hoy, 2011).

All the problems described above necessitate the research, development and implementation of alternative pest control approaches. A pest control strategy that relies only on plant protection chemicals for mites is unsustainable. To all these disadvantages of the use of chemical products for plant protection, we can add their negative effects on soil, water and crops. Consumers of plant products are also becoming increasingly concerned about pesticide residues in food.

Inspired by the lack of alternative of the chemical pest control researchers tested many unknown properties of some substances during the last years. Some of these substances are Silicon-containing products like different silicates (K_2SiO_3, Na_2SiO_3, Ca_2SiO_3), rice hulls, H_4SiO_4, etc. According to some researchers (Ma et al., 2001; Shetty et al., 2012; Debona et al., 2017; Harizanova and Koleva, 2019), silicon is able to alleviate negative effects of variable abiotic and biotic stress factors. Plants commonly use monosilicic acid (H_4SiO_4) as the source of Si and H_4SiO_4 exists in soil in form of liquid (Meena et al., 2014; Carpinteri et al., 2013). Although there is a plenty of publications reporting about Silicon application advantages in agriculture, this element is not considered as an essential one. Silicon is classified as a quasi-essential element for plants (Epstein, 1999). Si application is recognized as an eco-friendly approach for crop production therefore for example the use of Si is commonly recommended under package and practices for cereals. Likewise, in vegetables, Si application has been documented to reduce the attack of diseases (Bakhat et al., 2018).

The efficacy of Si application has been reported against many fungal pathogens including powdery mildew, *Fusarium* sp., *Pythium* sp. etc. (Fauteux et al., 2005) especially in vegetable crops. Studies have shown that Si application is able also to increase the resistance of host plants to insect and non-insect pests. Several authors report about the effect of Si against *Bemisia tabaci* in tomato and cucumber (Correa et al., 2005; Callis-Duehl et al., 2017) and *Tetranychus urticae* also in cucumber (Harizanova et al., 2019). According to Bakhat et al. (2018) Si increases tolerance against insect pests such as brown plant hopper, stem borer, green leaf hopper, white backed plant hopper, and non-insect pests like spider mites (Debona et al., 2017).

MATERIALS AND METHODS

The experiments were performed under controlled environmental conditions in a laboratory at the Department of Entomology, Agricultural University of Plovdiv, Bulgaria: photoperiod 14/10 h (light/dark), photosynthetically active radiation (PhAR) - 250 μmol/m/s, air temperature 25°C (day)/ 22°C (night) and relative air humidity 50±5%. The cucumber seeds, cv. Gergana, were subjected to the process of imbibition for 24 h. They were then placed in inert material (perlite). After the full development of the cotyledons and the appearance of the first true leaf, the young plants were transferred in plastic containers with nutrient solution. Plants of all variants were grown in perlite containing ½ Hoagland nutrient solution with all the necessary macro and microelements to phase third true leaf and then artificially infested with *Tetranychus urticae*. At the same time started the treatment with Si in form of H_4SiO_4 via leaf spraying (3 applications for 30 days). Experimental plants were grouped in 4 variants: 1 - control plants; 2 - mite-infested plants; 3 - Si-treated plants; 4 - mite-infested Si-treated plants. Each variant included 24 test plants. Thirty days after mite infestation test plants were analyzed.

Mite infestation

All plants from variants Mite and Mite + Si were artificially infested with mites from the stock colony reared under described above laboratory conditions. For infestation, each plant received small discs of cucumber leaves with 50 females in total, which were placed on the second and/or third true (mature) leaf of each cucumber plant. Infestation was done 15 days after planting. 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 cucumber 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 these two variants were individually isolated and transferred to each leaf disc for laying eggs. 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 leaf discs were changed to ensure their freshness. The immature were transferred to new discs very carefully with the help of a tin hair brush.

Phenylalanine ammonia-lyase

Phenylalanine ammonia-lyase (PAL) activity is determined spectrophotometrically by following the formation of trans-cinnamic acid which exhibits an increase in absorbance at 290 nm according to Brueske (1980). One g leaf sample was homogenized in sodium borate buffer (pH = 7.0). After a 10 minutes centrifuge at 10,000 rpm 0.2 ml of the supernatant was mixed with 0.5 ml borate buffer (pH = 8.7) and 1.3 ml of distilled water to prepare the reaction mixture. In each test tube was added 0.5 ml 1-phenylalanine followed by 30 minutes of incubation. The reaction was terminated by 0.5 ml of trichloroacetic acid (1 M). Finally the absorbance was recorded at 290 nm and PAL activity was measured in terms of amount of t-cinnamic acid (t-CA) formed.

Salicylic acid

Content of Salicylic acid in leaves of infested plants was determined according to Warrier et al. (2013) using 1 g fresh plant material. The sample extraction was made using distilled water followed by centrifuge at 10,000 rpm for 10 min and 100 μl of the supernatant was mixed with 0.1% freshly prepared ferric chloride. The volume of the reaction mixture was made up to 3.0 ml and the complex formed between Fe^{3+} ion and SA, which is violet in color was determined by spectrophotometry, measuring the absorbance of the complex in the visible region (at 540 nm). The results were calculated using standard curve.

Statistical Analysis

The results were statistically processed with the SPSS program using a one-way ANOVA dispersion analysis and 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 mean value show statistically significant differences between the variants. The analysis of the mite population included 2 variants and independent sample t-test with $p < 0.05$ was used for data processing. All data were analyzed using IBM SPSS Statistics 20 software.

RESULTS AND DISCUSSIONS

In order to evaluate the population build-up of the two-spotted spider mite on Si-treated and Si-untreated plants the number of eggs and mobile stages was recorded and analyzed. Living and dead individuals of each stage were separately recorded (Table 1). There is a decrease by 17% of the total number of the mobile stages on the leaves of Si-treated plants compared to the Si-untreated. The number of the males is almost 15% less, and the number of the females is reduced by 72% on the silicon sprayed leaves. There is also a reduction of the number of the protonymphs - by 52%. The number of the other stages is higher in the Si-treated variant. Silicon treatment resulted in a higher number of dead mites. The highest number of dead individuals is recorded for mobile immature stages - average number of dead larvae on Si-treated plants is 11.63, compared to 0 on Si-untreated plants. For adults the difference between two variants is not so significant but the number of dead mites on the Si-treated plants is still higher than the number on the Si-untreated ones.

Table 1. Number of living and dead individuals of each developmental stage of *T. urticae* on Si-treated and Si-untreated plants

Developmental stages of *T. urticae*	Variant	Living mites		Dead mites	
		Mean	Std. Error Mean	Mean	Std. Error Mean
Eggs	Mite+Si	24.63	7.91	0.00	0.00
	Mite	18.08	4.55	0.00	0.00
Larvae	Mite+Si	20.25	4.94	11.63	2.12
	Mite	19.42	5.50	0.00	0.00
Protonymphs	Mite+Si	3.13	0.94	0.50	0.21
	Mite	6.58	2.08	0.00	0.00
Deutonymphs	Mite+Si	12.00	2.37	0.12	0.07
	Mite	11.67	1.42	0.00	0.00
Mobile immature stages	Mite+Si	35.38	5.81	12.25	2.20
	Mite	37.67	8.10	0.00	0.00
Females	Mite+Si	2.25	0.86	4.12	0.58
	Mite	8.08	3.19	0.75	0.19
Males	Mite+Si	10.87	1.29	2.00	0.68
	Mite	12.75	1.39	0.25	0.12
Adults (females and males)	Mite+Si	13.13	2.01	6.13	1.06
	Mite	20.83	4.31	1.00	0.21
All mobile stages	Mite+Si	48.50	6.95	18.38	2.17
	Mite	58.50	8.88	1.00	0.21

Generally, the average number of all dead individuals from the mobile stages is 18.38 in the Si-treated variant compared to 1.00 in the Si-untreated variant. It is obvious that Si-treatment leads to a high mortality and respectively to a visible reduction of the number of mobile stages of the mite at the end of the experiment.

The percentage of dead individuals of each developmental stage is different for the two variants of the experiment. In the variant without Si only dead adults are recorded and the average number is very low, while in the Si-treated variant 67% of the dead mites are for mobile immature stages (Figure 1). One of the possible explanations of the higher mortality of larvae and nymphs of *T. urticae* in the Si-treated variant could be that these stages cannot feed normally on the leaves treated with Si. The results of Independent sample t-test show that the differences between mean number of dead individuals from the two variants (with and without Si treatment) are statistically significant ($t_{23 \div 24.776} = 1.813 \div 5.559$; $p = 0.000 \div 0.025$). The Si-treatment affects also the life cycle of the pest (Table 2). The analysis of the duration of each developmental stage of the pest shows differences among the two variants. More than 80 % of the mites in the Si-treated variant did not reach adulthood because they died as immature stages. Almost 70 % of them died at larval stage and 18 % died as nymphs. Only 11% of the hatched larvae on the Si- treated leaves reached adult stage.

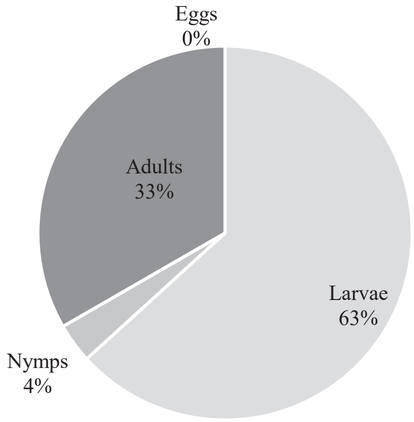

Figure 1. Percentage of dead eggs, larvae, nymphs and adults of *T. urticae* on Si-treated plants

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

Developmental stage	Variant	Number	Duration	Std. Error Mean
Egg	Mite+Si	53	3.60	0.08
	Mite	45	3.29	0.07
Larva	Mite+Si	16	3.19	0.26
	Mite	22	2.41	0.13
Protonymph	Mite+Si	12	2.08	0.15
	Mite	15	1.73	0.18
Deutonymph	Mite +Si	6	3.33	0.42
	Mite	11	2.09	0.16
Egg to adult	Mite+Si	6	10.50	0.56
	Mite	13	9.85	0.19

After analyzing the time needed for the individuals to enter the next developmental stage it was noted that on the Si-treated plant the mites needed a little bit longer to reach the next developmental stage compared to those feeding on Si-untreated plants. The differences are statistically significant for all stages ($t_{6,184 \div 24,186} = 0.03 \div 0.312$). According to some authors 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).

Some of the researchers suggest that the Si-treatment affects the feeding of the pests via mechanical injuries of the digestive system that makes them unable to feed and eventually leads to death (Han et al., 2017; Bakhat et al., 2018).

Some other researchers suppose that silicon treatment triggers some biochemical mechanisms that activate the plant defence system. It is observed also that silicon supply affects the duration of each developmental stage. In the Si-treated plants the time for entering the next developmental phase is longer in comparison to the Si-untreated plants. The reduced number of the feeding mites is probably related to the level of the mechanical and physiological injury, respectively of the physiological status of plant leaf tissues. Many studies confirm that Silicon treatment helps in alleviating plant stress. Most of the studies present positive results about the silicon properties in abiotic stress conditions (Ma et al., 2004; Song et al., 2014).

Most of them analyzed some abiotic stress factors as salinity (Harizanova et al., 2014; Harizanova and Koleva-Valkova, 2019), metal toxicity, nutrient imbalances etc.). There is also information about heat, freezing, and drought stress (Ma et al., 2004; Liu et al., 2009). According to Harizanova and Koleva-Valkova (2019) one of the possible effects of Si-treatment is the enhancement of the photosynthesis and the activation of the enzymatic defence system of the plants. In the last years the number of manuscripts about the silicon influences in biotic stress conditions is increasing (Gomes et al., 2005; Han et al., 2017; Harizanova et al., 2019). Some authors suggest that the effect is caused by physical strengthen of the plant tissues that makes them unfavourable for feeding (Massey et al., 2006; Massey and Hartley, 2009). Others suppose that the silicon could be involved in the plant defence system (Gomes et al., 2005; Kvedaras et al., 2010; Ye et al., 2013). There are also many evidences about the ability of Si pre-treatment to improve the activities of the defense related enzymes such as peroxidase, phenylalanine ammonia-lyase (PAL) and polyphenoloxidase (Bakhat et al., 2018). Rahman et al. (2015) reported about higher activity of PAL in Si-treated plants infested with pathogens. All phenylpropanoids are derived from cinnamic acid, which is formed from Phenylalanine by the action of PAL. Phenylalanine ammonia-lyase is the branch-point enzyme between primary metabolism and the branch of secondary metabolism leading to the phenylpropanoid pathway, which is considered to be one of the most important metabolic pathways due to its responsibility for the synthesis of a large range of secondary metabolites, including phenolic acids and flavonoids (Shetty et al., 2011). Plants with low PAL activity have thinner cell walls in the secondary xylem (Elkind et al., 1990) and reduced lignin content. Twenty-five days after the mite infestation the activity of PAL in the plants was analyzed. The results are presented in table 4. The activity of the enzyme is lowest in the leaves of the control. The activity is higher in the mite infested plants but the difference is not statistically proven. Si treatment affected the activity of PAL in the leaves of mite-infected plants where its value increased by 2% compared to the mite infested untreated plants and the sole Si-treated plants had the highest activity of the enzyme. Kim et al. (2011) analyzed the effect of short-term treatment with Si combined with wounding stress in rice plants. The authors observed that the levels of endogenous salicylic acid level was significantly higher in sole Si-treated plants. However, a combined application of wounding stress and Si induced a significantly small quantity of endogenous salicylic acid (SA). In our research the mite feeding induced the production of relatively low amount of SA compared to the control. But when treated with silicon the content of that phytohormone increased almost twice in mite-infested plants (Table 3). One of the possible ways of silicon action is namely the activation of plant defense system. It 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 stimulation of system stress signals (salicylic acid, jasmonic acid and ethylene) (Shetty et al., 2012).

Table 3. Activity of phenylalanine ammonia-lyase (PAL) (μM t-cinnamic acid/g FW/min) and content of Salicylic acid (SA) in the leaves on the test plants (μg/g)

Variant	PAL	SA
Control	154.6^{c}	$1{,}228^{b}$
Si	170.3^{a}	627^{d}
Mite	157^{c}	804^{c}
Mite+Si	161^{b}	$1{,}430^{a}$

CONCLUSIONS

The application of silicon in the form of H_4SiO_4 on cucumber is able to alleviate the negative effects of two-spotted spider mite feeding.

The data obtained show that Si treatment reduces the population of the spider mite. The number of larvae, nymphs and adults is reduced. The duration of the all of the analyzed developmental stages of the mite increased on the Si-supplied cucumber plants. This effect of Silicon on pest`s life cycle could be due to the enhancement of the activity of some phytohormones involved in the biochemical defense response of the infested plants.

Silicon application enhances the content of salicylic acid in the infested plants but decresed in the Si-treated plants not infested with mites.

The activity of phenylalanine ammonia-lyase in the Si-supplied plants also increased.

The mechanisms which are involved in way of silicon action are not quite clear. The positive effect of silicon may be related to the accumulation of the element in the cell walls of the plants, which makes the tissues harder and less attractive to the pest, as well as to its stimulating effect on the formation of some protective substances activation of the enzymatic defense system of the plants.

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