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EFFECT OF SILICON ON THE ACTIVITY OF ANTIOXIDANT ENZYMES AND THE PHOTOSYNTHETIC RATE OF CUCUMBER UNDER MITE INFESTATION

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Abstract

The aim of the study was to evaluate the effect of silicon on the activity of antioxidant enzymes and the photosynthetic rate of cucumber plants (Cucumis sativus L.) cv. Gergana under mite infestation. Four variants consisting of 1 – control, 2 – mite-infested plants, 3 – silicon-treated plants, and 4 – mite-infested plants, treated with silicon, were analyzed. Plants were grown hydroponically and silicon (Si) was applied to the nutrient solution in form of Na_2SiO_3*. The plants have been infested by the two-spotted spider mite Tetranychus urticae and 20 days later – analyzed. The results show a decrease in the mite population in the Si-treated plants. The activities of guaiacol peroxidase (GPOD) and syringaldazine peroxidase (SPOD) in the leaves of the mite infested plants increased by 175% and 197% respectively, and the net photosynthetic rate (*P_N*) in the mite-infested plants was reduced by 51%, compared to the control. It was established that Si supply to a nutrient solution of the mite-infested plants decreases the activities of GPOD and SPOD by 62% and 57% respectively and enhances the net photosynthetic rate by 84%.*

Key words*: cucumber, mites, photosynthesis, silicon, stress.*

INTRODUCTION

Silicon (Si) is the second most abundant element on Earth's crust after Oxygen. It has not been considered as an essential element for higher plants, but Si has been proved to be beneficial for the healthy growth and development of many plant species (Epstein, 1999; Ma et al., 2001). The beneficial effects of Si are particularly distinct in plants exposed to abiotic and biotic stresses (Ma, 2004). Over last two decades, more extensive and intensive studies have been performed aiming at better understanding of the possible mechanisms for Si-enhanced resistance and/or tolerance of higher plants to both abiotic and biotic stress (Liang et al., 2003). There are, however, a number of studies of its effects on some phytopathogens – mainly powdery mildew (Shetty et al., 2012). There are also some researches on the effect of Si in insect-induces stress conditions. The increased resistance to herbivore pests caused by silicon treatment has been reported in various sensitive varieties of cereal plants (Keeping & Meyer, 2006; Hou & Han, 2010; Sidhu et al., 2013; Han et al., 2015) and cereal grasses (Massey et al., 2006; Massey & Hartley, 2009).

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 for various pests including *Chilo suppresalis*, *Scirpophaga incertulas*, *Chlorops oryzae*, *Nephotettix bipunctatus cinticeps*, *Nilaparvata lugens*, Spider mites (*Tetranychus* spp.) (Gatarayiha et al., 2010) or mites (Savant et al., 1997; Nikpay et al., 2014).

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is considered one of the most harmful pests in many crops. It is herbivore, with a broad food specialization, developing on over 1200 plant species belonging to more than 140 different families (Van Leeuwen et al., 2010). Small size, rapid development, high reproductive potential, large number of generations, and optional diapause allow the mite to multiply for a short time in high density when the conditions of the environment are favourable. This is accompanied by a rapid decline in the quality of the host plant. During feeding, the mite mechanically damages the leaf epidermis, resulting in a deterioration of the water regime of plants and decrease of the photosynthetic apparatus productivity (changes in CO_2-gas exchange, reduction of chlorophyll content), which can cause a fall of undeveloped blossoms and even death of young plants (Park & Lee, 2002).

Most studies have focused on the effect of silicon on the pathogen or on an enemy and their lifecycle and treatment behaviour. But the information about the effect of Si on the physiological and biochemical changes occurring in the host plant and the involved mechanisms are scarce. This motivated us to conduct this study and gain insight into some of the benefits and effects of Si application.

MATERIALS AND METHODS

Laboratory experiments were performed under controlled environmental conditions in a laboratory at the Department of Entomology, Agricultural University - Plovdiv, Bulgaria: photoperiod 14/10 h (light/dark), photosynthetically active radiation (PhAR) 250 μmol m^{-2} s^{-1}, air temperature 25°C (day)/ 22°C (night) and relative air humidity 50 ± 5%. The seeds 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 ½ Hoagland nutrient solution containing all the necessary macro- and micro elements to phase third true leaf and then infested by *Tetranychus urticae*. At the same time started the treatment with Si in form of Na_2SiO_3 to the nutrient solution. The nutrient solution was replaced every week with a fresh one. Experimental plants were grouped in 4 variants: 1 - control plants; 2 - mite-infested plants; 3 - plants + 1.5 mM Si; 4 - mite-infested plants + 1.5 mM Si. Each variant includes 24 test plants. Twenty days after infestation plants were analyzed.

Mite infestation

All experimental plants were infested on the 15^{th} day of their development with 50 moving stages of the two-spotted spider mite. The infestation was carried out with mobile stages of a laboratory grown mite population. For this purpose, leaf cuts of about 5 cm^2 were examined under a stereomicroscope to ensure the presence of the Tetranychid mite only and to remove other mite families (if any). The number of *T. urticae* mobile forms was recorded and controlled and 10 or 20 mites (mostly adult females) were left on each such cut. The examined leaf cuttings were placed on the 2^{nd} and/or 3^{rd} leaf of each cucumber plant (50 mites/plant respectively).

Methods of analysis

Leaf area

The leaf area of the plants was determined by the electronic digital plotter NEO-2 (TU-Sofia, Bulgaria) (Kerin et al., 1997).

Leaf gas exchange parameters

Leaf gas exchange. The index of the leaf gas exchange rate - net photosynthetic rate (P_N) rate, transpiration intensity (E), stomatal conductance (gs) and intracellular concentration of CO_2 (ci) 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^{-2} s^{-1}, temperature 25ºC and natural external CO_2 concentration of about 400 vpm.

Photosynthetic pigments

The photosynthetic pigments content was determined spectrophotometrically by the Lichtenthaler method (1987) and was recalculated to a unit of fresh weight (mg g^{-1} FW).

Lipid peroxidation

The lipid peroxidation rate (LP) was determined by the method of Heath and Packer (1968). Specific absorbance at 532 and non-specific at 600 nm were recorded at molar extinction coefficient E = 155 mM^{-1} cm^{-1}. The results are presented as malondialdehyde (MDA) content in a unit of fresh weight (nmol MDA g^{-1} FW).

Enzyme extraction

The fresh plant sample (0.5 g) was ground with 5 ml ice-cold extraction buffer (pH 7.8), quartz sand and 200 mg polyvinylpyrrolidone (PVP).

Samples were centrifuged at 4°C for 10 min at 13500 rpm. The resulting clear supernatant was immediately used for analysis.

Guaiacol Peroxidase (GPOD)

The activity of GPOD was determined spectrophotometrically by the method of Bergmeyer et al. (1974). The mixture of 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 was placed in the cuvette. The absorbance was measured at 436 nm against a blank containing the same components without enzyme extract (E = 26.6 mM^{-1} cm^{-1}). The values obtained were expressed as U mg g^{-1} FW.

Syringaldazine peroxidase (SPOD)

The activity of SPOD was determined spectrophotometrically by the method of Imberty et al. (1985). In a cuvette were placed 2.55 ml of 0.1 M Tris-HCl buffer (pH 7.5), 300 µl of 10 mM H_2O_2, 50 µl of 3.5 mM syringaldazine, and 100 µl of the extract. The absorbance was measured at 530 nm against a blank containing the same components without enzyme extract (E = 27 mM^{-1} cm^{-1}). The values obtained were expressed as U mg g^{-1}FW.

Statistical data processing

The data are presented as mean ± SD of 3 replicates. The experimental 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.

RESULTS AND DISCUSSIONS

At the end of the experiment in order to evaluate the effect of Si application, the population density of the two-spotted mite was estimated. It was found that the number of all the mobile stages of the mite was significantly reduced on the leaves of Si-treated plants compared to those which were infested with mites, but untreated with silicon. In larval stages the decrease was about 60%, and the number of adults was reduced by 24%. The decrease in the number of eggs was the lowest - by about 14% (Table 1). Our results are in line with the ones obtained by Nikpay and Nejadian (2012), who reported a decrease in the number of yellow mite *Oligonychus sacchari* in sugar cane. The authors investigated the impact of various Si-containing fertilizers on the yellow mite in four cane varieties and observed a population density on the 10^{th}, 20^{th}, 30^{th} and 40^{th} days after infection. Although they did not observe significant differences among the variants before the 20^{th} day after the infestation, they suggest that the silicon supply is able to decrease the population density of the mite after that period. They measured a less number of mites at the 30^{th} and the 40^{th} day after the infestation, what they associate with the high resistance of this mite species and the need for a longer period for population breeding, as reported in their previous studies (Nikpay et al., 2011; 2012). Our results show a significant decrease in the number of the pest even earlier - on the 20^{th} day after infestation.

Table 1. Number of eggs and mobile stages of *T. urticae* on leaves of cucumber (*Cucumis sativus* L.), cv. Gergana, 20 days after mite infestation

Parameter/Variant	Plants infested with mite	Plants infested with mite + Si
Eggs	208.75	177.25
Larvae	23	9.12
Adults	38.66	29.37

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., 2007). Although the mechanisms of silicon`s action are not quite clear, there are several theories. Toledo and Reis (2018) examined the effect of foliar spraying with potassium silicate on coffee plants infested with red mite *Oligonychus ilicis*. The authors report about a 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 increased hardness and toxic compounds in the plant tissues. About the same effect of the silicon treatment reported Yavas and Unay (2017) in their review article. They suggest that Si could be used to 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.

In their research Gatarayiha et al. (2010) did not observe a direct effect of Si on *T. urticae* mortality, but they established that the Si-supply improves the action of the biological agent *Beauveria bassiana*. They suggest that silicon increases the resistance to spider mites by disturbing their feeding and making them more vulnerable to infestation by the fungus *Beauveria bassiana*. The reduced population density is probably due to the increased hardness of the leaf tissues (epidermal cells), as the silicon is deposited in the cell walls (Ma, 2004; Reynolds et al., 2009), what affects the feeding of the mites. He et al. (2015) found that increased levels of silicic acid in plants shortened the stay of leaf-hoppers on the plants and reduces the pest fertility. Kvedaras et al. (2007) suggest that the positive action of silicon enhances in drought conditions. The authors observed that after treating dried sugar cane with silicon, the content of the element in the stem increased and the number of *Eldana saccharina* (Lepidoptera: Pyralidae) and stalk damage decreased. In the case of application on soil or foliar treatment with silica in the form of $CaSiO_3$, the mortality of *Bemisia tabaci* nymphs was increased (Correa et al., 2005). In another study feeding of lice with Silicon reduces their number in the infested plants (Moraes et al., 2004; Gomes et al., 2005). It is reported that pests with piercing-sucking mouth organs and herbivore caterpillars prefer silicon-poor tissues to those with high silicon content. This element reduces feeding, growth, fertility, and the population of *Sogatella frucifera* (Salim & Saxena, 1992). Silicon reduces the reproductive capacity of the phloem-feeding species (*Myzus persicae*) on potatoes and whitefly (*Bemisia tabaci*) in cucumber (Reynolds et al., 2009).

In order to evaluate the stress levels, induced by the mite feeding, the rate of the lipid peroxidation was measured. Malondialdehyde (MDA) is an indicator of the lipid peroxidation rate (LP) and its concentration is related to the degree of the membrane damages (Hsu & Kao, 2007). The analysis of LP shows strong damage of the cell membranes in the leaves of the infested plants (Figure 1).

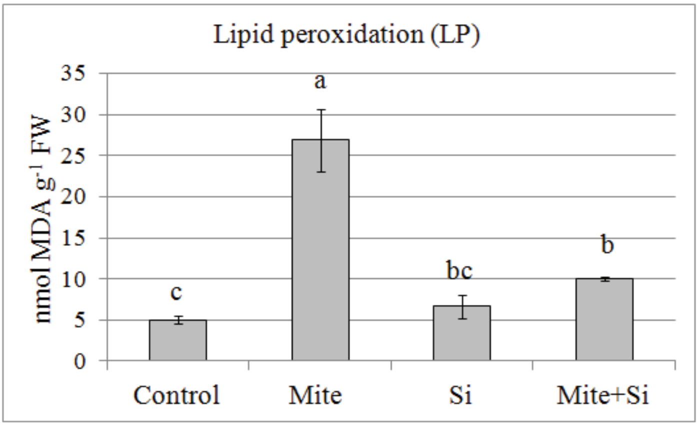

Figure 1. Lipid peroxidation rate (LP) nmol MDA g^{-1} FW in cucumber leaves 20 days after mite infestation

In the stressed plants, MDA content increased more than four times compared to the control. The addition of Si to the nutrient solution of the stressed plants causes a decrease in the MDA content by about 63% compared to the mite-infested plants. Sivritepe et al. (2009) also reported an increase in the LP rate in the leaves of grapevine plants, infested by the two-spotted spider mite. According to Tehri et al. (2014), there is a significant relationship between the resistance of the experimental plants and the LP rate in the leaves. The LP rate increases in parallel with increasing the population of *T. urticae* and is initially higher in the leaves of the resistant variety. According to the authors, a short period of mite feeding on the resistant variety induces subsequent resistance. In another experiment, rice plants were infected with *Pyricularia oryzae* and the authors also reported an increase in MDA content in the leaves of the infected plants (Domiciano et al., 2015). They also observe a decrease in its concentration after treatment of the infected plants with silicon.

The feeding of the two-spotted spider mite significantly increased also the activity of the enzymes of the antioxidant defense system (Figure 2).

The activity of guaiacol peroxidase (GPOD) in the leaves of the infested plants was 175% higher than in the control plants. The Si-supplied plants demonstrate a strong recovery potential. Silicon supply decreases the level of stress and the activity of the guaiacol peroxidase was only 4.31% higher than in the control.

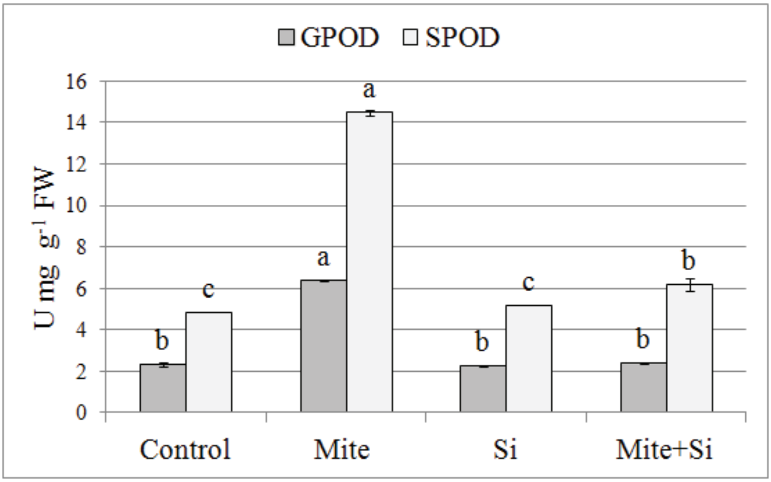

Figure 2. Activity of GPOD and SPOD in cucumber leaves 20 days after mite infestation

Similarly, the activity of syringaldazine peroxidase (SPOD) followed the same tendency. Mite feeding caused a strong increase in SPOD activity by almost 200%. The addition of Si to the nutrient medium of the infested plants leads to a decrease in enzyme activity by 57% compared to the mite-infested, Si-untreated plants.

The mite-induced stress affects negatively the synthetic processes and the biomass accumulation in the infected plants. The data about the effect of the spider mite feeding on the biomass accumulation are presented in Table 2. The mite-infested plants were with reduced growth of all organs. The fresh leaf mass, as well as the fresh mass of the whole plants, were strongly inhibited (by over 60% relative to the control). There was also a decrease in the dry mass of the infected plants, which was more pronounced for the shoots compared to the roots. This effect is explicable as the enemy develops and causes direct damage only to the shoots of the plants, and not to the roots. The leaf area was also significantly reduced in the mite-infested plants compared to the control (by 54%). The Si supply to the nutrient medium of the infested plants resulted in an obvious recovery - the leaf area of these plants was enhanced by 78% in comparison to the infested untreated with silicon plants.

Table 2. Changes in biomass accumulation in cucumber 20 days after infestation with *T. urticae*

Variant	[1]FW Plant (g)	FW Leaves (g)	FW Stems (g)	FW Root (g)	[2]LA (cm^2)	[3]DW Plant (g)	DW Leaves (g)	DW Stems (g)	DW Root (g)
Control	21.072^{a}	11.411^{a}	6.848^{a}	2.813^{a}	599^{a}	1.264^{a}	0.96^{a}	0.222^{a}	0.081^{a}
Mite	8.069^{c}	4.235^{c}	2.608^{d}	1.226^{b}	274^{c}	0.679^{b}	0.525^{b}	0.102^{c}	0.051^{b}
Si	20.701^{a}	11.619^{a}	6.104^{b}	2.976^{a}	655^{a}	1.259^{a}	0.948^{a}	0.21^{a}	0.101^{a}
Mite+Si	15.406^{b}	8.555^{b}	2.031^{ab}	2.031^{ab}	488^{b}	1.105^{a}	0.859^{a}	0.166^{b}	0.078^{a}

[1]FW = fresh weight; [2]LA = leaf area (cm^2); [3]DWpl = dry weight

The increased photosynthetic area was probably responsible for the enhancement of the net photosynthetic rate. The analysis of the net photosynthetic rate is presented in Figure 3. The feeding of the mites reduced the value of P_N by 51%. Plants of Mite+Si variant showed a strong recovery - an increase of 84%.
This suggests that the use of silicon helps infected plants to recover to some extent from the negative effects of mite feeding and to increase their assimilation ability.
A decrease in the rate of P_N in mite-infested plants has also been reported by Sivritepe et al. (2009) in grapevine. Alatawi et al. (2007) also reported a reduced level of CO_2. They suggest that the rate of the damage is in line with the duration of the feeding period and population density of the mite.

Our results are consistent with the reports of Domiciano et al. (2015) who examined the effect of silicon on rice plants infected with the *Pyricularia oryzae* pathogens.
The authors observed a significant decrease in photosynthesis (P_N) in infected plants.
After the addition of Silicon to the nutrient medium of the infected plants, the authors report about a significantly lower decrease in the value of the indicator compared to the phytopathogen-infected plants, but not treated with silicon.
In order to evaluate if the effect of the mite-induced damage and the Si-treatment is related also to stomatal changes, we measured the rate of the transpiration and the stomatal conductance of the test plants.

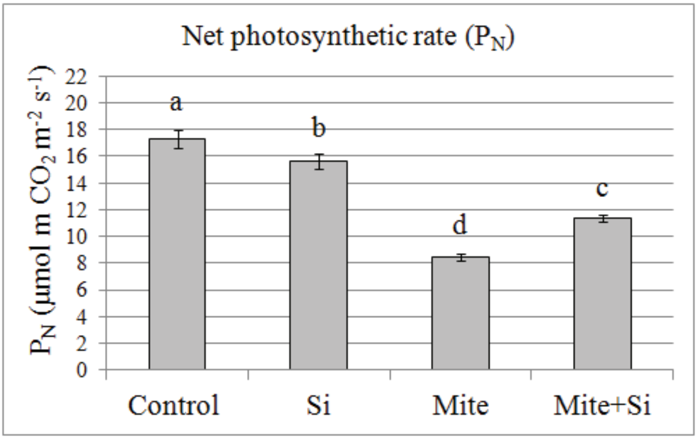

Figure 3. Net photosynthetic rate P_N (µmol CO_2 m^{-2} s^{-1}) in leaves of cucumber (*Cucumis sativus* L.) 18 days after infection with *T. urticae*

Figure 4 presents the data on the transpiration rate. In the plants infested by mites, the process was highly inhibited. The decrease was by 46% relative to the control. After the addition of Si to the nutrient solution of the infested plants, a similar increase (47%) was observed compared to the plants of the mite-infested variant.

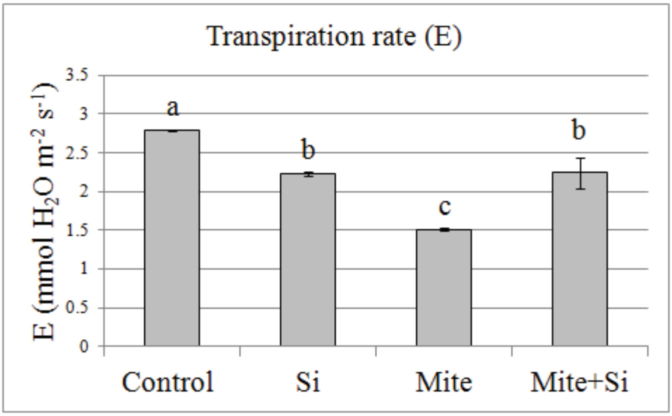

Figure 4. Transpiration rate E (mmol H_2O m^{-2} $s^{-1})$ in cucumber leaves 20 days after infestation with *T. urticae*

During their feeding, the mites mechanically damage the epidermis including the stomata, and the cells lose their ability to open and to close under control. Depending on the degree of attack and the duration of mite feeding, the intensity of transpiration varies greatly. In the first days after the mite infestation, there is usually an increase in the intensity of transpiration. In a severe and prolonged attack transpiration begins to decrease sharply over time. This is observed during the day with regard to stomatal transpiration. During the night, cuticular transpiration increases due to mechanical damage from the mite feeding (Sivritepe et al., 2009). This causes large losses of water from plant tissues and in case of a strong attack leads to a state of permanent water deficit. The analysis of the stomatal conductance is presented in Figure 5. The stomatal conductance in the leaves of the mite infested plants was 64% lower than in the control. The Si-supplied infested plants had two times higher stomatal conductance than the untreated mite infested plants. It shows that the effect of the Si supply on the photosynthesis is related to stomatal changes.

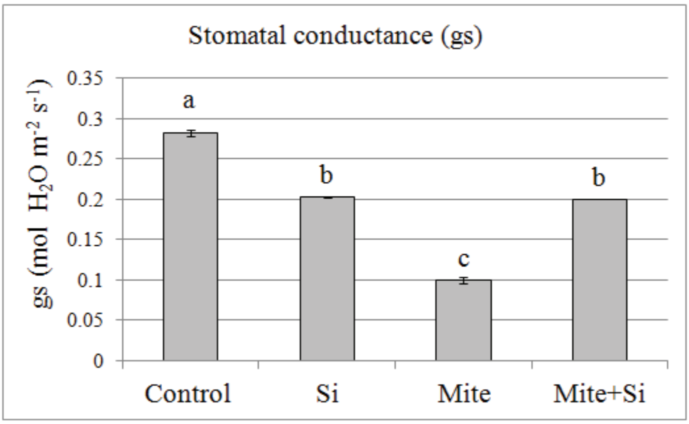

Figure 5. Stomatal conduction gs (mol H_2O m^{-2} s^{-1}) in cucumber leaves 20 days after infestation with *T. urticae*

In order to clarify if the reason for the enhanced net photosynthesis is due only to physiological changes of the leaf anatomy and function, the content of the main photosynthetic pigments was also measured (Table 3). The content of chlorophyll *a* was almost twice reduced in the leaves of the stressed plants. The amounts of chlorophyll *b* and carotenoids were also lower, by 39 and 43%, respectively. In the leaves of the Si-supplied plants, the values of these parameters increased. Chlorophyll *a* content was 45% higher and the increase of the chlorophyll *b* and carotenoids content were by 49 and by 60%, respectively. There is no information about the effect of Si on the pigment content in mite-induced stress conditions, but there are several studies about its positive influence in various abiotic stress conditions like drought (Maghsoudi et al., 2016) or salinity (Rezende et al., 2018). The authors suggest that silicon is able to alleviate the negative effect of low salt concentrations on the photosynthetic pigment content via reducing the production of reactive oxygen species (ROS) or via anatomical changes of the photosynthetic apparatus.

Table. 3. Content of the main photosynthetic pigments (mg g^{-1} FW) in the cucumber leaves 20 days after infestation and treatment with Si

Variant	[1]Chl *a*	Chl *b*	Chl (*a*+*b*)	Carotenoids	Chl *a/b*	Chl (*a*+*b*)/ carotenoids
Control	2.24[a]	0.77[a]	3.02[a]	0.79[a]	2.91[b]	3.82[b]
Mite	1.16[d]	0.47[d]	1.63[d]	0.45[d]	2.46[c]	3.63[c]
Si	1.9[c]	0.63[c]	2.53[c]	0.66[c]	3.02[a]	3.83[b]
Mite+Si	2.13[b]	0.7[b]	2.83[b]	0.72[b]	3.04[a]	3.93[a]

[1]Chl = chlorophyll

CONCLUSIONS

The application of silicon in the form of Na_2SiO_3 in cucumber is able to alleviate the negative effects of spider mite feeding. The results obtained show that Si treatment reduces the population of the spider mite and enhances the net photosynthetic rate. This positive effect is probably due to the increased amount of the main photosynthetic pigments and the protective influence on the cell membranes and the antioxidant defense system. The mechanisms involved are not quite clear. The protective role may be related to the accumulation of silicon 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 photosynthetic activity of the plants, expressed by improved parameters of the leaf gas exchange, high content of photosynthetic pigments, and an increase in biomass and leaf area of the analyzed plants.

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