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The Effects of Innovative Silicon Applications on Growth and Powdery Mildew Control in Cucumber (*Cucumis sativus* L.) and Zucchini (*Cucurbita pepo* L.) Soilless Grown

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Abstract

Silicon (Si) is one of the most beneficial microelement for several plants including the growth regulation in horticultural species. The research evaluated the effects of innovative Si-applications on *Cucumis sativus* L. and *Cucurbita pepo* L. soilless grown. Crop growth, powdery mildew incidence and abiotic stress resistance were evaluated. Two experiments were carried out in a not heated glasshouse on benches. Two new Si treatments (Si-Nanosponge complex, and one experimental fertilizer) were compared with the traditional K_2SiO_3. Topas® EC 10 was used as control fungicide treatment. Biometric parameters, and incidence and severity of powdery mildew were measured. *C. sativus* plants showed a severe powdery mildew infection and no significant effect of the Si treatments was found. *C. pepo* plants were initially grown under lower disease pressure conditions and the positive effect of Si treatments was found. The innovative use of Si-Nanosponge complex and the new experimental fertilizer can be considered a good alternative to traditional compounds for plant growth stimulation.

Keywords: Nanosponge complex, fertilization, disease control, hydroponics, greenhouse

INTRODUCTION

Silicon (Si) is one of the most beneficial elements for several plants although it is not considered as an essential plant nutrient (Marschner 1995). Silicon is abundant in the earth's crust and hence a sub-optimal supply of this element is more likely in soilless cultivation rather than in soil-grown crops (Savvas et al. 2009). Si is not used routinely in nutrient solution preparations for soilless cultivation, however the Si beneficial effects has been demonstrated by many studies carried out in hydroponic experiments (Guntzer et al. 2012; Miyake and Takahashi 1983; Voogt and Sonneveld 2001) and in different horticultural plants subjected both to abiotic and biotic stresses (Cho et al. 2013; Datnoff et al. 2001; Garg et al. 2015; Soundararajan et al. 2015). The effects on biometric parameters in Si-treated plants were observed by Sivanesan et al. (2013). Si fertilization has the potential to mitigate environmental and pathogenic stresses being a suitable alternative to the extensive use of conventional disease control tools and fertilizers thus complying current sustainable agriculture EU regulations (Directive 2009/128/EC) and related National recommendations and regulations (National Action Plans 2009). Particularly in the field of disease control, Silicon induces mechanism for broad-spectrum plant disease resistance (Van Bockhaven et al 2013). Si may also contribute to limit rates and number of application of fungicides and it is now considered as an environment-friendly fertilizer being to limit powdery mildew epidemics as widely recognized (Hammerschmidt 2005). Different types of Si fertilizers exist, which have been compared in several studies (Gascho 2001; Mecfel et al. 2007; Meyer and Keeping 2001; Rodgers-Gray and Shaw 2004). The most popular fertilizers contain inorganic silicates (potassium or sodium silicates) and can be applied through the irrigation water delivered via drip or sprinkler irrigation. In order to ameliorate the Si-efficiency, considering that plants have low Silicon requests, controlling its release is fundamental. Recently there has been development of β-Cyclodextrin-based nanosponges (NS) as a delivery system capable of slowing the release of active ingredients (Cavalli et al. 2006; Roggero et al. 2011; Trotta and Tumiatti 2003; Sharma and Pathak 2010; Swaminathan et al. 2010). In previous researches NS complexes have been investigate in cut flowers postharvest conservation (Seglie et al. 2008) and in hydroponic Fe-fertilization for horticultural plants (Vercelli et al., 2015). Based on the Si content of the plant tops, plants are separated into high Si-accumulators [10 – 15% (w/w) DW], intermediate Si-accumulators [1 – 3% (w/w) DW], and non-accumulators [< 1% (w/w) DW]. Cucurbitaceae, are classified as high Si-accumulators (Ma et al. 2001). Moreover this species is subjected to heavily powdery mildew infections (agent: *Podosphaera xanthii* syn *Sphaerotheca fuliginea*) particularly on leaves and, particularly in Italy, is considered economically important. The aim of this study was to compare the effects of innovative Silicon nanosponge complex, and new commercial fertilizer with traditional applications, supplied via the nutrient solution, on crop growth, powdery mildew (*Podosphaera xanthii*) incidence and abiotic stress resistance (low temperature exposure) in *Cucumis sativus* L. and *Cucurbita pepo* L. grown in a soilless system.

MATERIALS AND METHODS

Nanosponges synthesis and Si-loading

Nanosponges (NS) was synthetized as the procedure mentioned in the patent (Roggero et al. 2011). For this study Si-Nanosponge complex (Si-NS) were developed. Si was dispersed as K_2SiO_3in aqueous suspension of the NS and was stirred for 24 h in the dark and at acidic pH. Si-NS was micronized at 63µm to avoid the possible clogging problems in the drippers. A preliminary phytotoxicity test using NS on plants was carried out excluding problems for their use (data not shown).

Experimental trials

Centro di Sperimentazione ed Assistenza Agricola (CeRSAA), Albenga, Savona (Northern Italy) was the test site were the trials were carried out in a glasshouse (High technology greenhouse in accordance with EFSA 2008) on benches. In order to simulate a abiotic stress and due to the high sensitivity to low temperature of Cucurbits plants (Schwarz et al. 2010) the trial was organized between October and December in a not heated greenhouse. Two experiments were conducted using *Cucumis sativus* L. (experiment 1: 23/09/2013-18/10/2013) and *Cucurbita pepo* L. (experiment 2: 21/10/2013-25/12/2013) as model plants. Basically the experimental design was the same for both experiments. A randomized experimental design was organized. The total number of experimental units was 40 slabs (i.e., 5 nutrients X 8 replications). Each experimental plot contained 18x8 plants. Seeds were sown in perlite for two weeks. Seedlings were directly transplanted into slabs (18 plants) and hydroponically grown. Rockwool slabs (Cutilene) were adopted as growing substrate. Each treatment consisted of two slabs per each single replicate and totally four replicates were set up. Seeds were sown in perlite for two weeks; furthermore seedlings were directly transplanted into slabs and hydroponically grown. In Table 1 the treatments and the application timing are reported. In particular the fungicide (Topas® 10 EC) was supplied *via* foliar application once each two weeks, the other treatments (K_2SiO_3, Si-NS, OSK) were supplied *via* drip irrigation twice a week.

According to Datnoff et al. (2001), the amount of chemicals was calculated to ensure 100 ppm of Silicon needed for plant nutrition. All plants were fertilized with a standard nutrient solution delivered via drip irrigation system. Considering that the addition of Si compounds (K_2SiO_3) determined a strongly basic nutrient solution, an equivalent amount of acid was necessary (Voogt and Sonneveld 2001). The pH of all nutrient solutions was adjusted to 5.5 – 6.0 using 1 M H_2SO_4 (Buttaro et al. 2009). The control treatment without Si was balanced using K_2SO_4.

Assessments

During the cultivation period biometric parameters (chlorophyll content, mortality, dry weight) and incidence and severity of powdery mildew were measured on all plants. Chlorophyll content was estimated by a Chlorophyll Meter (Field Scout CM 1000, Spectrum Technologies Inc., Plainfield IL, USA), that provides a sensitive and accurate index of plant response to the treatments. Mortality (as effect of a plant stress) and plant dry weight were evaluated at the end of the cultivation period. Disease incidence and severity were assessed evaluating on all plants the percentage of infected leaves and the percentage of the leaf surface affected in accordance with EPPO Standard scale (EPPO 2005). The evaluation of powdery mildew incidence and severity was carried out during 3 assessments carried out 7 days after last treatment (DAT). On *C. sativus* only chlorophyll content and disease incidence were assessed. Moreover molecular analysis were performed on powdery mildew mycelia and conidia to characterize the disease specie.

Statistical analysis

All data were analyzed using by one-way ANOVA with SPSS statistical package (version 21.0; SPSS Inc., Chicago, Illinois). Mean values were compared using the Ryan-Einot-Gabriel-Welsch-F test (REGW-F). The critical value for statistical significance was $P \leq 0.05$. Arcsine transformation was performed on all percent incidence data (dry weight, mortality, incidence and severity) before statistical analysis in order to improve homogeneity of variance.

RESULTS

Experiment 1. The evaluation of chlorophyll content on *C. sativus* gave similar evidences as the disease assessments: plants treated with Topas® 10 EC showed greener leaves, while other treated and not treated plants suffered for a high presence of fungal mycelium and sporulation characterized by a whitish color. The infections caused by *Podosphaera xanthii* occurred immediately after plant emergence, causing fast plant decay. Nevertheless plots treated with Topas® 10 EC showed the lowest disease incidence and severity. The application of Si based compounds did not highlight any advantage (Table 2).

Experiment 2. Among the chlorophyll content significant differences emerged between treatments on *C. pepo* (Table 3). Considering the initial mean value of 137.61, the highest increase was observed in OSK while the lowest in (-Si). The evaluation of the indoor air temperature (15^{th} - 29^{th} November) showed, as expected, a temperature decrease as well as a decrease in chlorophyll content. Nevertheless persistent leaf greening was observed on plants treated with OSK. The average temperature dropped from 20 to 15°C and considering optimal *C. pepo* growing conditions, such temperature are considered too low for the crop growing. Particularly the minimum temperature level was from 21^{st} November to 21^{st} December rather below the minimum optimal growing temperature. Comparing Si-treatments in terms of dry weight OSK plants grew more than Topas® EC 10 and K_2SiO_3 while plants treated with Si-NS did not statistically differ from the other treatments. Topas® 10 EC showed a smaller size (Table 3). Plant mortality, as effects of the exposure to unpleasant climatic conditions occurred between 15th and 29th November, increased after 05th December. Even if no statistical differences were observed, lowest mortality was recorded in plots treated with Si based compounds (Si-NS, K_2SiO_3 and OSK) (data not shown). In term of disease incidence *P. xanthii* spread showed statistical differences starting from the first date (Table 4). The untreated control exhibited a widespread presence of infected leaves. Treated plants showed lower disease infections than control plants to powdery mildew infection in terms of the percentage of infected leaves and in terms of the percentage of leaf surface infected. Topas® 10 EC and K_2SiO_3 performed better; particularly Topas® 10 EC showed the best control in term of disease incidence reduction, nevertheless, both treatments maintained across the whole growing period statistical differences compared with the untreated control. The evaluation of the severity of powdery mildew infection gave for all the tested treatments statistical differences respect with the untreated control. Best disease control was guaranteed both by Topas® 10 EC and K_2SiO_3. At the end of the trial K_2SiO_3 and Topas® 10 EC were different from (-Si) for both the incidence (respectively 72.40% and 89.43% *vs* 100%) and severity (respectively 18.53% and 37.53% *vs* 62.53%); nevertheless both Si-NS and OSK statistically reduced the disease incidence, but less efficiently as Topas® 10 EC and K_2SiO_3.

Among the various edible horticultural plants, the most efficient Si accumulators are some species of the *Cucurbitaceae* family. The two experiments shows that the use of Si compounds in the nutrient solution increased Cucurbits plant biometric parameters and health. Concerning powdery mildew responses no differences between *C.sativum* and *C.pepo* can be highlighted. As Savvas and Ntatsi (2015) confirmed, the Si application through by roots is much more effective in increasing the Si levels in plant tissues, but moreover the use of nanosponges to can provide a more effective Si translocation in the plant.

DISCUSSION

In the first experiment, thanks to the mild climatic conditions, plants showed a severe powdery mildew infection: under such conditions no significant effect of the Si treatments was found. This is in agreement with Seebold et al. (2004): the Authors observed that under conditions relatively unfavorable to rice blast, the application of 1000 kg ha-1 of silicon reduced symptoms better than did the fungicide tricyclazole in Colombia. Pagani et al. (2014) found that efficacy of silicate treatments is more valuable when conditions for the wheat blast development are less favorable. When disease pressure was high, the effects of silicate treatments were less marked. In the second experiment plants were initially grown under lower disease pressure conditions and the effects of Si treatments (K_2SiO_3, and OSK) were shown in plant growth and disease control, thus confirming previous achievements on rice (Seebold et al. 2004; Pagani et al. 2014). Moreover these results agree with the findings reported by Walters and Bingham (2007) in several species, by Fawe et al. (1998) and Liang et al. (2005) in *Cucumis sativus* L., and by Savvas et al. (2009) in *Cucurbita pepo* L.. Application of Topas® EC 10 was effecting in reducing powdery mildew infection. Nevertheless plants showed a smaller size and the lowest dry weight, as widely known for several triazoles (Benton and Cobb 1997; Fletcher et al. 2010; Petit et al. 2012; Rademacher 2000). Fungicide constitute one of the most effective and integrative method to control diseases phytophatogenic fungus in agriculture. However, the potential toxicity and pollution generate by fungicide use cannot be neglected. Some pesticides interfere with the metabolic pathways of plants and some interfere specifically with the photosynthetic process (Petit et al. 2012). Better agronomic achievements were observed in OSK treatment. As expected

(Soundararajan et al. 2014) these data confirmed that Si supply in the nutrient solution is positive for plants soilless cultivated, because of the nitrogen content in the product, confirmed also by the higher plant dry weights.

The innovative use of Si-NS complex can be considered a good alternative to traditional compounds for plant growth stimulation. Otherwise Si release in nutrient solution and Si plants kinetic in different climates is not yet clear, therefore further experiments have to be carried out.

Silicon supplied via nutrient solution to hydroponically grown plants allowed a good plant development and reduced the incidence of powdery mildew. Si provided a rapid and long term fungal control. Considering that *C. pepo* should be grown with a minimum air temperature of 12°C and an optimal air temperature of 25°C (Maynard et al. 2007; Tesi 2008), under low temperature conditions occurred in experiment 2 Si provided additional advantages: after 15th November better plant growth in plots treated with Si-based compounds (excluding Si-NS) were assessed. Comparing results of the two experiments performed, the fungicide treatment is necessary when the disease appears in the first plant cultivation period, otherwise Si-treatments can be much effective if used for the disease prevention and its slow development. The data collected in this study made possible to increase the knowledge about the potential use of Si to limit negative effect of unpleasant climatic condition on specific crops as Cucurbits. Future investigations will be devoted to understand Si efficacy for fruit yield and qualitative parameters. Moreover a combination of silicate and synthetic fungicides, not tried here, may display additive or even synergistic effects.

In conclusion, our results contribute to clarify the multiple role of silicon as a biostimulant in horticulture, increasing plant resistance to multiple stresses. While, as researches largely demonstrated, he use of silicon is not toxic to humans and the environment (Savvas and Ntatsi 2015), opening a good perspective in using Si in soilless systems particularly for minor crops and for organic farming, where no conventional active chemical ingredients are allowed.

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TABLES

Table 1 Treatments and application timing accordingly with standard practice

Treatment[z]	Treatment acronym	Treatment description	Active ingredient	Rate/l	Timing
Blank	(-Si)	Drip irrigation	-	-	-
Fungicide	Topas®10 EC	Foliar application	10.15%Penconazole	0.5 ml	Once each two weeks
Mineral nutrient	K_2SiO_3	Drip irrigation	26.5% SiO_2	0.81 g	Twice a week
Nanosponge Complex	Si-NS	Drip irrigation	18% SiO_2	1.19 g	Twice a week
Experimental fertilizer	OSK	Drip irrigation	16% SiO_2	1.34 g	Twice a week

[z] *C. sativus*: Topas® 10 EC: 08/10, 23/10; treatments via drip irrigation 08/10, 10/10, 15/10, 17/10. *C. pepo*: Topas® 10 EC: 12/11, 26/11, 10/12; treatments via drip irrigation 12/11, 14/11, 19/11, 21/11, 26/11, 28/11, 03/12, 05/12, 10/12, 12/12, 17/12, 19/12

Table 2 Effects of treatments on chlorophyll content, and incidence (% infected leaves) and severity (% of withered leaves) of powdery mildew on *Cucumis sativus* L.

	Chlorophyll content			Incidence		Severity
Treatment	18 October	25 October	31 October	10 October	14 October	14 October
(-Si)	164.17 a	154.31	154.87 b	40.65 a	66.47 b	35.60 ab
Topas®10 EC	169.80 a	160.73	178.48 a	21.90 bc	41.20 c	27.47 c
K_2SiO_3	151.98 b	152.72	140. 84 b	36.57 ab	70.27 b	51.70 b
Si-NS	143.42 b	145.00	151.98 b	40.65 a	89.75 a	51.82 b
OSK	162.85 a	157.77	159.55 ab	9.77 c	81.27 a	75.60 a
P [z]	***	ns	***	***	***	***

[z] ns non-significant and *** significant at $P \leq 0.001$

Table 3 Effects of treatments on chlorophyll content during the experiment (ns, no significant differences between treatments; different letters, significant differences at P ≤ 0.001) and dry weight [Index 0-100: (-Si) = 100] at the end of the trial (different letters, significant differences at P ≤ 0.05) on *Cucurbita pepo* L.

	Chlorophyll content			Dry weight
Treatment	15 November	29 November	10 December	25 December
(-Si)	154.83 b	165.08	155.54 b	100 a
Topas®10 EC	159.41 b	175.58	155.10 b	83.15 b
K_2SiO_3	147.26 c	174.38	150.77 b	91.88 b
Si-NS	178.14 a	172.52	153.75 b	109.95 ab
OSK	164.5 ab	187.17	192.98 a	128.03 a
P [z]	***	ns	***	*

[z] ns non-significant, * and *** significant at $P \leq 0.05$ and $P \leq 0.001$, respectively

Table 4 Effects of treatments on incidence (% infected leaves) and severity (% infected leaf surface) of powdery mildew on *Cucurbita pepo* L.

	5 December		12 December		19 December	
Treatment	Incidence	Severity	Incidence	Severity	Incidence	Severity
(-Si)	98.60 a	53.48 a	97.68 a	57.73 a	100 a	62.53 a
Topas®10 EC	49.98 b	20.23 b	26.88 c	20.80 bc	20.55 c	16.80 c
K_2SiO_3	44.05 b	12.95 b	55.90 b	15.28 c	72.40 b	18.53 c
Si-NS	78.00 a	14.20 b	92.93 a	31.85 b	95.25 a	41.83 b
OSK	82.05 a	20.90 b	94.38 a	29.05 bc	89.43 ab	37.53 b
P [z]	***	***	***	***	***	***

[z] ***, significant at $P \leq 0.001$