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Nano-structures in plant protection

Nano-estructuras en la protección de plantas

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Foreword

There is no accepted international definition of a nanoparticle, but it's generally accepted that those particles have one or more dimensions of the order of 100 nm or less. Many nanoparticles compromise novel physical and chemical properties. The properties are entirely dependent on the fact that at the nanoscale, the physics of nanoparticles are different from the properties of the bulk material. This makes the size of particles or the scale of its features the most important attribute.

What is different between a nanoparticle and nano-structures? There is no strict dividing line between nanoparticles and non-nanoparticles. The size at which materials display different properties to the bulk material is material dependant. However, some materials in the size larger than 100 nm display properties based on their structures. Those structures can be on a nanoscale. All definitions become difficult when materials are not round shaped. E.g. such materials can be nano-tubes with a diameter of a few nanometers but a length much longer than 100 nm. Therefore, the definition of a nanoparticle depends mainly on their physic-chemical properties to be different from that of the bulk material.

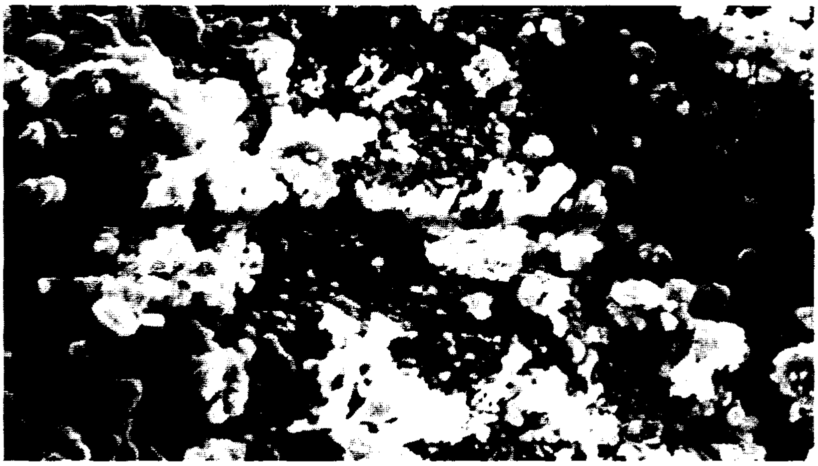

Figure 1. SiO_2-nanoparticle interacting with insect cuticle.

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Introduction

The field of nanoparticle research covers a wide range of interests in the areas of chemistry, physics, biological-, agricultural-, and materials science. Agricultural engineers are seeking knowledge of nano-materials for engineering bio-pesticides, fertilizer coatings, and other purposes (Ulrichs *et al.*, 2006a; 2006b).

Various forms of amorphous silica dusts of natural origin have been successfully used in the past against insect pests. The biggest source for natural occurring silicon dioxide is diatomaceous earth (DE). Diatomaceous earth consists of hard shells of fossilized diatom algae. DE has got a promising potential as a grain protectant. Most diatom algae are greater than 0.01 mm in length. Diatomaceous earths particles are often smaller than living algae. Commercial products are additionally mechanically fractured to particles about 10 µm in size. On the other side, many pores in diatoms are within a few nm diameters, enlarging the surface area of each particle.

Although nanotechnology promises much potential in different fields, some researchers are raising cautionary flags, finding that nanoparticles, because of their size and configuration, may cause problems to human health. Because of their small size, they are deposited preferentially in the lung periphery where they can come into contact with alveolar macrophages and epithelial cells (Kleinman *et al.*, 1995). Some researchers suggest the nano-material itself may be benign but, given its size and configuration, may catalyze other chemical reactions which may be harmful. Agricultural researchers even found that some nanoparticles can stunt plant growth and cause cells to die. These and other published studies prove that problems exist and show the need to know more about the behavior and effects of nanoparticles and nano-structures.

Amorphous DE provides good protection when grain is stored properly, can be separated partially from the grain, and possibly recycled in storage bins. According to The International Agency for Research on Cancer (IARC) amorphous silica dusts are contrary to crystalline silica not rated as carcinogen. IARC detected no association mesothelioma with biogenic amorphous silica fibres (IARC, 2007). Natural DE contains mostly amorphous silica with below 1-5 % of crystalline particles.

The primary action of DE is the destruction of the insects cuticle by absorption of the protective wax layer resulting in body water loss and death of insect due to desiccation (Mewis and Ulrichs, 2001a; Prashanta, 2003; Völkl *et al.*, 2004; Weisshaupt *et al.*, 2004). One major disadvantage of DE is the ineffectiveness against arthropods at higher relative humidity levels (Mewis and Ulrichs, 2001a, Völk *et al.*, 2004, Weishaupt *et al.*, 2004). Control of the nanoscale morphology enables precise control of the properties of the end product. Particle size, morphology and composition can be manipulated to produce materials of different properties. There are different routes to making nano materials: lithography, vacuum coating, spray coating, gas phase synthesis including chemical vapor synthesis, laser ablation, wet chemistry, and mechanical top-down technologies. This paper describes studies leading to improved silica-based insecticides, based on the nano-structure of the substance. Additionally, we evaluate the possibility of electrostatic application of different natural and synthetic nano-structured particles onto cruciferous crops.

Material and methods

Test substances and application method

The silica based materials tested were Fossil Shield® 90.0s, Advasan®, Biobeck® PA910, and a formulation newly developed by the Urban Horticultural Section at Humboldt University called "Al-06". FS90.0s is an improved DE, which is supposed to work under relative humidity's above 80%. Advasan® has been obtained from the same company but is of volcanic origin and not DE-based. Biobeck® PA910 is amorphous synthetically derived silica. AL-06 is based on a natural occurring amorphous mineral. All materials have been amorphous and contain above 86 % silica (SiO_2). We assessed the materials for their suitability for electrostatic application. Furthermore, the powders were tested for their effect on plant photosynthesis and efficacy against the mustard beetle, *Phaedon cochleariae* (F.) and the granary weevil *Sitophilus granarius* L. Experiments were conducted in the experimental greenhouse at Humboldt University Berlin under controlled conditions in 2005 and 2006.

For aphids and other under-leaf insects only insecticides deposited on leaf undersides are effective. Conventional sprayers rely on gravity and inertia to deliver pesticides. By some estimates, only half of the applied pesticides adhere to the plant. Therefore, we tested a new application technique and used electrostatic sprayers. This technique reduces

Surface chemistry	Fully hydrophobic	Medium hydrophobic	Medium hydrophobic	Medium hydrophobic
Main particle size	(40 - 60 Å h·lo) 5µm	7 µm	7,89 µm	7,56 µm
Particle shape	Spherical and granular	Spherical	Rectangle	Fragments of spherical shapes
Occurrence	Naturally	Synthetically produced	Naturally	Naturally but modified*
Surface	300 - 350 m^2/g	160 m^2/g	unknown	unknown

Table 1. Properties of different SiO_2-products tested.

[*We combined ball milling of natural silica particles and coating with synthetic silica in a 2nd step. The synthetic silica has been produced over a wet route and is commercially available. The coating results in complete hydrophobic properties of the new material with a hydrophilic DE core.]

spraying time and can improve insect and disease control per unit of material applied. The electrostatic application of dry powder has many benefits versus traditional wet application methods.

Several methods of electrostatic charged pesticide spraying have been developed to improve deposition of active materials, and the principles and techniques have been extensively reviewed (Law, 2001). These methods employ electrostatic forces of attraction that place a surface charge on the spray particles and theoretically will cause a greater proportion of the spray to reach and remain on the target. Under laboratory conditions, these systems have shown increased spray deposition on model targets and plant surfaces. Although greater deposition of pesticides on leaf surfaces is generally observed with electrostatic charged spraying, control of insect and mites may not necessarily be improved. The electrostatic charger we used has been especially developed for fine particulate matter applications by the Bein Company in Eiterfeld, Germany. All substances have been applied to four week old crucifer crops: *Brassica rapa ssp. chinensis* (L.) Hanelt.

Insect bioassays

In order to test the efficacy different hydrophobe materials we conducted biotests with the granary weevil *Sitophilus granarius* L. Experiments were conducted at three different rel. humidity's in climate chambers. Beetles were treated with Fossil Shield® (FS) and mortality checked over time.

Greenhouse experiments have been conducted with the mustard beetle, *Phaedon cochleariae* (F.). *P. cochleariae* belongs to the leaf beetle family Chrysomelidae and is specialized on plants in the mustard family (Brassicaceae). Adult and larval leaf beetles feed on all sorts of plant tissue. They were cultured at Humboldt University on pak-choi, *B. chinensis* and savoy cabbage, *Brassica oleracea* convar. *capitata* var. *sabauda* L.

Feeding experiments were conducted with pak-choi. This vegetable is one of the most important leafy crops in Asia. Pak-choi has Chinese origins and is spread throughout Asia and beyond, developing a wide range of varieties. The most typical pak-choi features dark green leaves atop white spoon-shaped upright stems. Stems vary considerably in thickness and shape, and in some varieties they are green.

Two different types of insect bioassays were conducted. Repellent effects of different silica materials (FS90.0s®, AL-06, and Advasan®) were evaluated in choice feeding experiments. Experiments were carried out in 100x100x200 cm cages in a greenhouse (25°C, 16:8 h light:dark cycle) for seven days. The plants were randomized arranged in sets of 3 treated and 3 untreated plants in 6 replications. 200 beetles were released in each cage and percentage leave damage was estimated after four and seven days.

Insecticidal efficacy of materials was tested in forced (feeding) contact experiments. *P. cochleariae* beetles, ten each, were placed one single treated plant and caged with gauze covered foil cylinders. Beetle mortality was recorded after 2, 4, 7, and 9 days. The statistical analysis of insecticidal effects obtained from experiments involving repeated measures was done using Tukey's HSD test.

Measurement of photosynthetic activity

The gas exchange of pak-choi after silica dust application has been determinate using a gas analyser (evaporimeter). The measuring equipment composed of 8 leaf cuvette, flux sampler and heating system, twin diaphragm pump, solenoid valves and a Dewar vessel with a sensor unit (temperature and humidity sensor, CO_2-Scanner). Air samples were sucked through the leaf cuvette in a Dewar vessel. In this vessel temperature, humidity, and CO_2-content is measured. By using solenoid valves the airflow switched continuously from cuvette to the reference air and back to measure the gas differences. Based on humidity and temperature the transpiration was calculated. Considering CO_2-differences, leaf area, and rate of airflow the net photosynthesis was calculated. Furthermore, the light intensity was recorded. The net photosynthesis was related to corresponding light intensity which was measured at the same time to elucidate differences. Photosynthesis was measured for two days after silica application and for another two days after silica has been washed off carefully using tab water.

Results and discussion

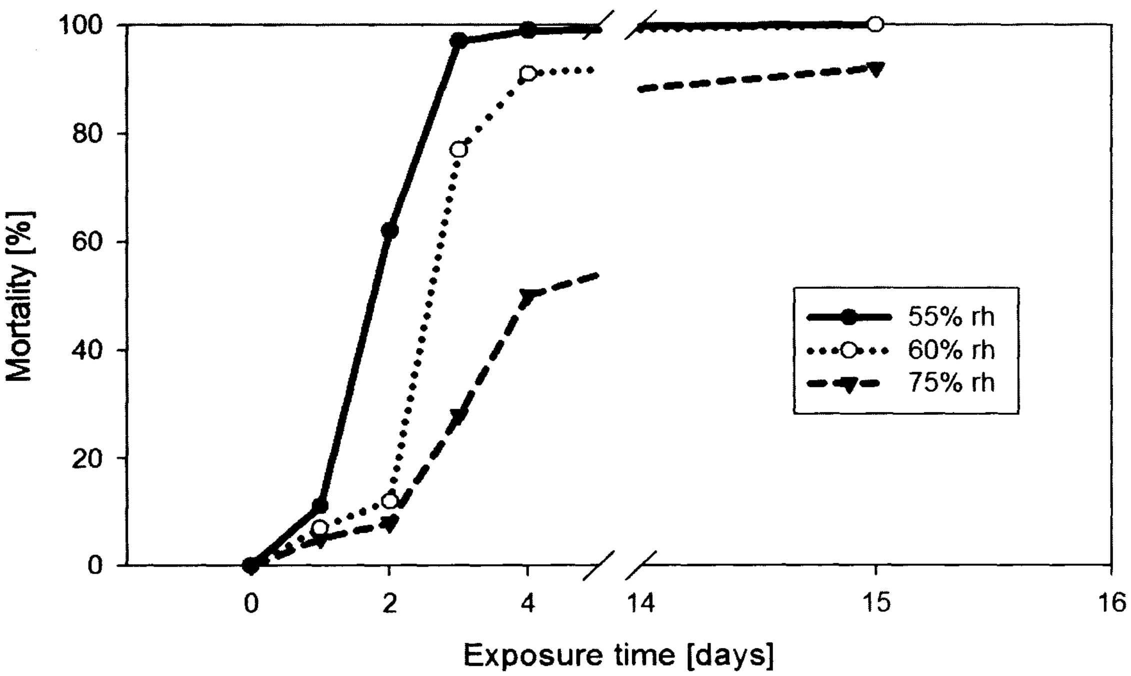

Figure 2. Mortality of Sitophilus granarius adults after treatment with silica at a temperature of 25°C; significant differences between treatments starting from day 2 (Tukey's HSD, P = 0.05).

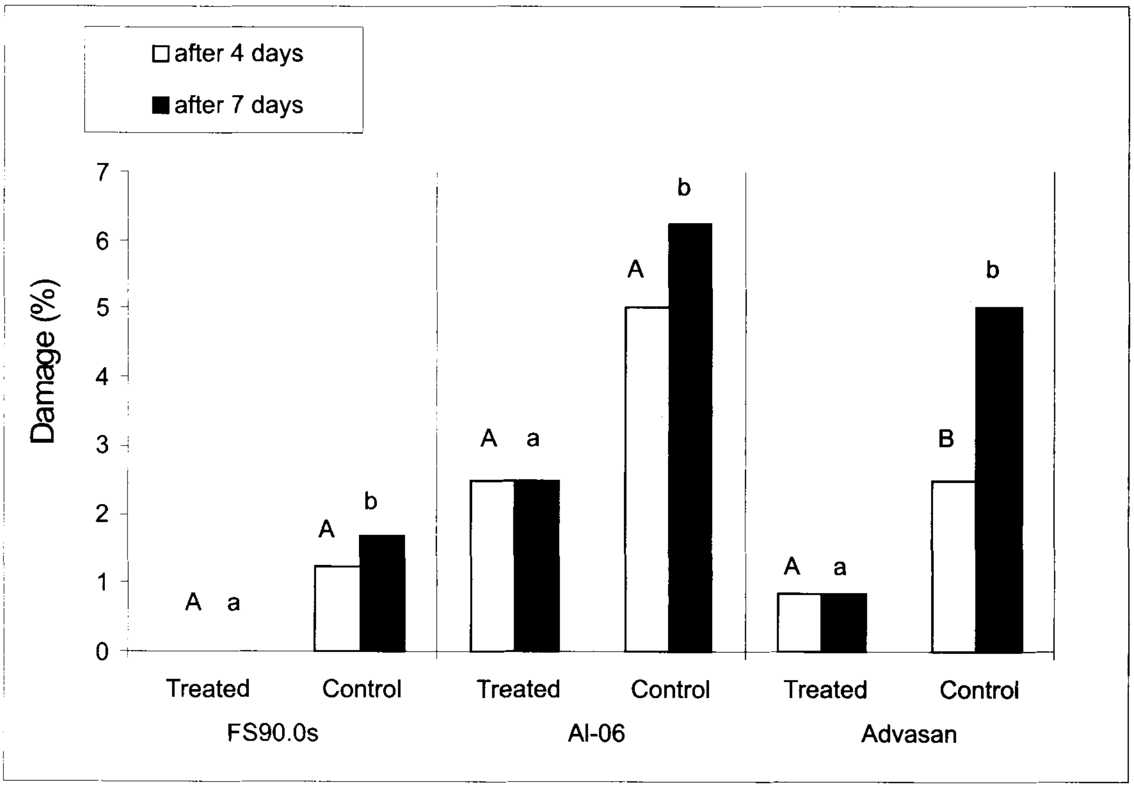

Figure 3. Pak-choi leave damage 4 and 7 days after treatment with different amorphous silica dusts and exposition to the mustard leave beetle Phaedon cochleariae (Different letters indicate significant differences (Tukey's HSD test $P < 0.05$) between treatments for each substance, capital letters are used for the comparison after 4 days, small letters for the comparison after 7 days).

Insecticidal efficacy

The medium hydrophobe material FS90.0s® has been tested at different relative humidity's. Under all humidity conditions insect mortality increased over time (Fig. 2). As expected the mortality rate was higher at lower rel. humidity's. This because the water pressure deficiency between insect's body and the surrounding atmosphere is here greater.

In greenhouse trials all materials have been effective in contact experiments against tested insects. However, significant differences were observed between materials after application onto plant leaves. Fossil Shield®, Advasan®, and Al-06 application resulted in a good coverage of both leaf sides. Biobeck® PA910 was easily removed by wind from leaf surfaces and did not protect the plants as well. In choice experiments with P. cochleariae, pak-choi leave damage was significantly reduced 7 days after plant treatment whereby best results, no damage, was obtained with FS90.0s® followed by, Advasan®, and Al-06 (Fig. 3).

Mortality rate of adult *P. cochleariae* increased significantly after treatment with Advasan®, FS90.0s®, and PA910® (Fig. 4). The material Al-06 did not show a good insecticidal

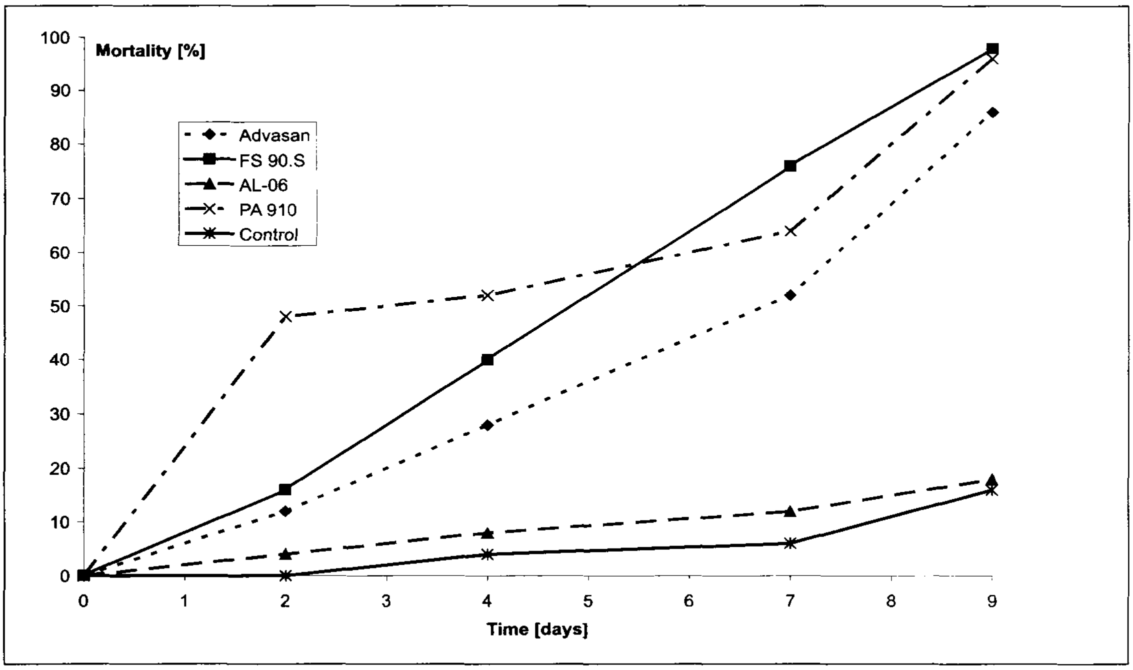

Figure 4. Phaedon cochleariae adult mortality in forced contact experiments after plant treatment with four different amorphous silica dusts.

efficacy at the current state of development. Most effective materials have been FS90.0s® and PA910®. However, since PA910® did not result in a good coverage after being electrostatically applied the best material has been FS90.0s®. The later material is contrary to the other dusts a hydrophobe DE formulation which may explain the good result in the green house at comparable high rel. humidity.

Phytotoxicity

Dusts can have both a physical and a chemical effect on plant physiology. The absolute level of dust deposition might be important for physical effects. Fluckiger *et al.* (1979) found, that while 1 mg cm^{-2} of silica dust was necessary to cause a decrease in stomatal diffusive resistance in Populus tremula, only 0.5 mg cm^{-2} was necessary to cause an increase in leaf temperature. In our experiments photosynthetic rate was significantly reduced after application of FS90.0s (Fig. 4). At a medium light intensity the reduction was about 50 % compared to the control. Such layer of silica dust is likely to cause direct stress to the plants by shading the leaves. Previous studies have shown that shading by dust can cause about a 20 % reduction in leaf photosynthesis. Fine dust particles have also been reported to clog up stomata (Oblisami *et al.*, 1978; Hirano *et al.*, 1995), reduce photosynthesis (Borka, 1980), increased leaf temperature (Steinhubel & Halas, 1967, Guggenheim *et al.*, 1980), and increase transpiration (Eveling, 1969). Krajickova and Mejstrik (1984) reported that the stomata diameter was 8-12 µm for a range of crops. This particle size is important if stomata functions are affected. Materials applied had a particle size of 5-10 µm and could therefore affect the stomata. This might also explain why rate of photosyn-

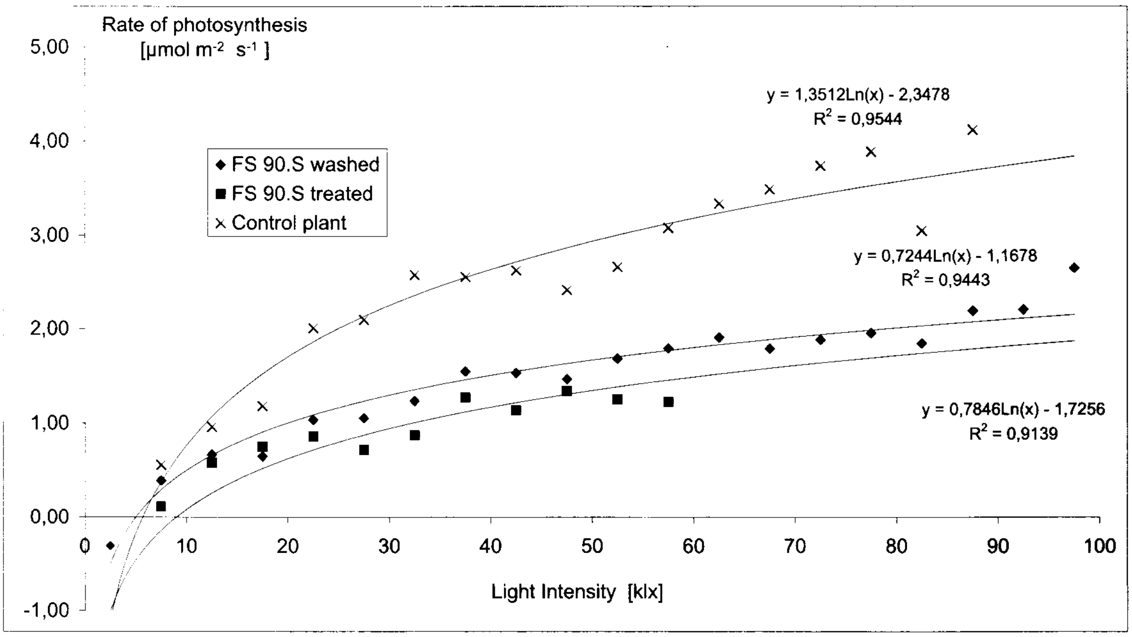

Figure 5. Rate of photosynthesis of pak-choi after treatment with FS90.0s, after removing FS90.0s by washing, and in untreated control plants.

thesis stayed at a reduced level even after the removal of silica dust particles by washing them off. However, we could not find any rests silica particles blocking stomata under the light microscope. There we assume that the photosynthesis rate has not been reduced, but instead the respiration rate increased. Since we calculate photosynthesis out of CO_2 and O_2 level in the ambient air increased respiration would look like a decrease in photosynthesis. Those findings will be currently analyzed in new experimental setting.

Conclusion

Nano-strcutured silica kills arthropod pests based on physicosorption of lipids from the insect cuticle. In general, the smaller the particles size of porous silica, the greater the rate of adsorption to the cuticle and absorption of lipids. However, this is not the case for AL-06. Here we are having ultra fine particles but insecticidal efficacy is less effective compared to FS90.0s. This most likely because it is a natural hydrous material and the absorption capacity for lipids is reduced in comparison to the other silicates tested.

Based on the physical mode of action and the current knowledge that amorphous silica is not toxic for humans, the group of materials offers potential for new insecticides. However, to apply the current available silica onto plant surfaces cannot be recommended. Even if some materials showed in contact experiments very good insecticidal effects, coverage after electrostatically application was uneven for some materials like the synthetic silica PA910®. Additionally, silica reduced significantly photosynthetic activity of pak-choi plants and affected plants grow (data not presented). This reduction could be acceptable under green house conditions for very short durations only.

It is important to understand, that silica particles in its current state of development can not replace synthetic pesticides. Foremost if offers an additional tool to complete the range of substances available for pest control. It can be envisioned, that silica treatments become useful when resistances towards chemical insecticides have been established. Additionally, materials can be used for plant treatments when synthetically pesticides can not be applied because of possible residue effects shortly before harvesting. Additionally this group offers potential as biocide where it is not necessarily applied to plant surfaces. Currently at Humboldt University Berlin admixture experiments with silica and different synthetic pesticides are conducted. Some materials show even a synergistic effect and could be applied in combination in pest management programs.

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