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# Effects of Organic and Inorganic Silicon Compounds on the *In vitro* Growth of Some Plant Pathogenic Fungi

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**Abstract:** Plant pathogenic fungi were shown to solubilise silicic acid and a range of other inorganic silicon compounds *In vitro*. Such solubilisation was generally associated with a statistically significant increase in fungal biomass. Silicic acid caused a marked formation of black pigmentation in *Aspergillus citri*, but not other fungi. Electron dense bodies of elemental silicon were found, both in the cell wall and inside the cell, of *A. citri* grown in media containing silicic acid. Finally, the organic silicon compound dimethlysiloxane also stimulated fungal growth while hexamethyldisiloxane did not, thereby showing that organic silicon compounds vary in their ability to stimulate fungal growth. The results are discussed in relation to the use of silicon compounds as fungicides and plant fertilizers.

**Key words:** Silicon · Plant pathogens · Inorganic silicon · Organic silicon

## INTRODUCTION

After oxygen, silicon is the most common element on the Earth's surface and is released in soil by both chemical and biological processes. Most microbiologists never consider the element silicon, probably because it is thought to be largely biologically un-reactive and is not microbiologically transformed [1]. Bacteria can however, solubilise insoluble silicon compounds (e.g. silicic acid, rock potash) and the growth of *Penicillium Spp. In vitro* has been reported to increase the solubilisation of sodium silicate [2]. Microorganisms play an important role in the formation of the Earth's surface including the accelerated dissolution of silicates and the release of mineral nutrients from feldspars involves the microbial dissolution of the silicate matrix [3]. Some microorganisms are also able to degrade polymerized silicon into the monomeric form. It is also possible that silicon can act as an alternative, or additional energy source, for several simpler forms of life, particularly members of the *Mycobacteria* and *Nocardiae;* as a result, these bacteria may scavenge silicon from the media and environment [2]. Silicon compounds increase bacterial growth and have been implicated in aggravating tubercular infection of the lung in patients suffering from silicosis [4]; they may even also have been important to the early formation, or establishment of life on Earth [5].

Silicon amendments are effective in controlling several important plant diseases, notably both foliar and soil-borne diseases of cucumber and other cucurbits [6].

Research continues to show that silicon nutrition in terrestrial plants is much more important than was previously believed [7, 8] and that treating crops with silicon helps reduce the severity of fungal infections [9].

This study investigated the effects of some organic and inorganic silicon compounds on the growth of some important plant pathogenic fungi and showed that these organisms have the ability to solubilise insoluble silicon.

## MATERIALS AND METHODS

The following fungi, *Alternaria citri* 3309583, *Botrytis cinerea* 322230 and *Cladosporium cladosporioides* 381056 were obtained from the CABI Bioscience CAB International and *Fusarium oxysporum* from the Department of Animal and Plant Sciences, University of Sheffield. The following inorganic and organic silicon compounds (Sigma) were used: Silicic acid, calcium silicate, colloidal silica, sodium silicate, silicon nitride, potassium silicate and the organic silicon compounds, dimethylpolysiloxane and hexamethyldisilane.

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**Effect of Silicic Acid on Fungal Growth:** *Alternaria citri, Botrytis cinerea* and *Cladosporium cladosporioides* were grown on Czapek Dox agar (Oxoid), for 10 days at 25°C, discs (11mm) were then cut from the leading edge of the colonies using a flame-sterilized cork borer. These were transferred (1 disc per flask) to unbuffered Czapek Dox liquid medium., Liquid medium (100 ml in a 250 ml Erlenmeyer flask) was amended with 0.5, 1, 1.5 and 2g of silicic acid, or a range of inorganic silicon compounds, (Sigma) in sealed dialysis tubing (size 14 mm) bags. Controls lacking silicic acid and containing silicic acid but no fungus were also included. All media were autoclaved at 121°C for 20 min. Three replicates were used throughout and all flasks were incubated on a rotary shaker (150 rpm) for 8 days at 25°C. After 7 days, the contents of the flasks were then filtered (through Whatman No.1 filter paper) and the dry weight was determined. The soluble silicon content of the medium was determined colorimetrically. The fungal biomass was determined after heating at 40°C overnight. In all cases triplicates were used.

**Effect of Hexamethyldisilane and Dimethylpolysiloxane on Fungal Growth:** Dimethyldichlorosilane ($C_2H_6C_{12}Si$) and hexamethyldisilane ($C_6H_{18}OSi_2$) 0.5 and 1.5 ml were added, (using a sterile syringe) directly to autoclaved 250ml Erlenmeyer flasks containing (100 ml) nutrient rich medium (Czapek Dox liquid medium). The fungi were grown in above media for 7 days at 25°C. At the end of growth period, the dry weight was determined after filtration through Whatman filter No. 1.

**Determination of Soluble Silicon:** Soluble silicon in the medium was determined colorimetrically by adding the following to 1ml of filtrate: ammonium molybdate (2 ml, 10%w/v); ascorbic acid (2 ml, 5%w/v); oxalic acid (1 ml, 10%w/v); and hydrochloric acid (5 ml, 1:1 dilution of concentrated HCl). After 15 min. incubation at room temperature, without shaking, the absorbance of the blue colour was measured spectrophotometrically at 600 nm. The concentration of soluble silicon (as $SiO_2$) in the filtrate was then determined by reference to a standard curve (0-100 μg $SiO_2$ $ml^{-1}$), prepared using EIL silica (sodium fluorosilicate standard 1000 ppm).

**Determination of Protein Content of Fungal Mycelium:** *Alternaria citri, Botrytis cinerea and Cladosporium cladosporioides,* were grown in (250 ml) Erlenmeyer flasks containing nutrient-rich medium (100 ml Czapek Dox liquid). Silicic acid 1% (Sigma) was added into sealed dialysis tubing (size 14 mm). The medium was autoclaved at 15 psi for 20 minutes. Three replicates were used and all flasks were incubated on a rotary shaker (150 rpm) for 8 days in batch culture at 25°C. After 8 days, the culture was removed and filtered. Mycelium (dry weight, 0.1 g), (control and treatment) was mixed with 0.5ml of NaOH (1M) in test tube which were transferred to hot plates and heated at 90°C for 10 min. after tubes were cooled in ice. The tubes were centrifuged at high speed (4000 rpm) for 10 min. The suspension (0.1 ml) was mixed well with Bradford reagent 3 ml (Sigma). Distilled water (0.1 ml) mixed with Bradford reagent was used as the blank. The tubes were left at room temperature for 5 min. when the optical density was read at 595 nm and the amount of protein determined by plotting the absorbency of standard solution containing 0 - 100 μg protein (bovine serum albumin) $ml^{-1}$.

## RESULTS AND DISCUSSION

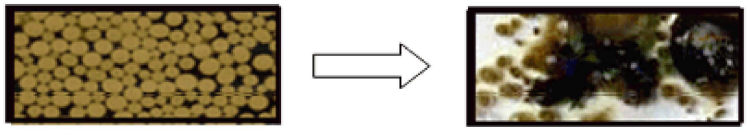
The solubilization of insoluble silicic acid by the fungi used here and the resultant effect on growth is shown in Table 1. The presence of this silicon compounds led to an increase in fungal dry weight for all of the fungi tested with the amount of biomass produced generally increasing with increasing weight of added silicic acid. The increased fungal biomass varied with the individual species and was particularly marked in the case of *C.cladosporoides* (Table 1). Increased biomass production was associated with an increase in the amount of soluble silicon in the medium, showing that all of the fungi are able to solubilize the insoluble silica acid. Fungal protein content was also stimulated by the presence of silicic acid in the medium, particularly in the case of *B.cinerea* (Table 2). The presence of silicic acid had a marked effect on the pigmentation of *A. citri*, but not the other fungi used here.

As is shown in Fig. 1, the biomass of this fungus turned darker in colour in the presence of silicic acid (when added directly to the medium), ultimately becoming black. This pigmentation is presumably associated with the presence in the medium of free silicon, but it is unclear what may cause this effect or why it occurs in *A. citri* and not in the other fungi tested. It should be emphasized that the above growth stimulus and silicic acid solubilization occurred when this insoluble silicon compound was placed in a dialysis tubing bag which prevented any direct contact between the fungus employed and

Table 1: Effect of silicic acid on fungal growth and solubilisation

| Silicic acid (g.100ml $^{-1}$) | 0.5 | 1.0 | 1.5 | 2.0 |
|---|---|---|---|---|
| Fungal dry weight (increase over control value, g.100ml$^{-1}$) | | | | |
| *A. citri* | 3.5* | 5.5* | 1.2* | 1.3* |
| *B. cinerea* | 0.8* | 0.9* | 1.4* | 0.95* |
| *C. cladosporoides* | 2.0* | 2.2* | 2.3* | 3.2* |
| Soluble silicon (increase over control, value (µgml$^{-1}$) | | | | |
| *A. citri* | 165* | 161* | 132 | 110 |
| *B. cinerea* | 176* | 142* | 136* | 120 |
| *C. cladospoiroides* | 182* | 158* | 159* | 175* |

*Significantly different from control value, $p \leq 0.05$



A) Control B) Treatment

Fig. 1: Effect of silicic acid on pigmentation in *A. citri* (1.5g) added directly to the Czapek Dox liquid medium, at 25°C)

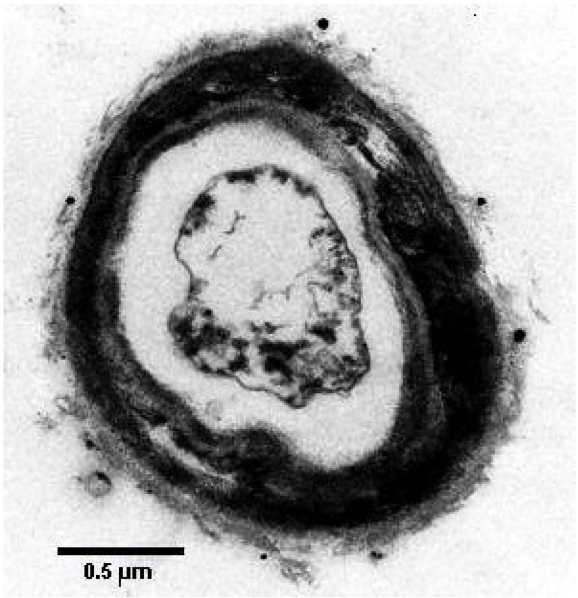



Fig. 2: Electron micrograph of the hyphae of *A. citri* grown in the presence of silicic acid, showing electron-dense accumulations of elemental silicon

the silicic acid, thereby suggesting the involvement of a relatively low molecular weight compound in silicic acid solubilization and the associated increase in fungal biomass. It is clear from Fig. 2 that fungi (in this case of *A. citri*) accumulate electron dense bodies located in the cell wall and within the hyphae; EDAX analysis showed that these bodies are composed of elemental silicon. Presumably, such silicon accumulation is a means of localizing and thereby isolating, the element in order to prevent any possible toxic effects.

Table 2: Effect of silicic acid on fungal protein content (Percentage increase over control value)

| *Fungus* | *A. citri* | *B. cinerea* | *C. cladosporioides* |
|---|---|---|---|
| *Protein Content* | 56* | 98* | 20* |

(Protein content: mg protein g$^{-1}$ fungal dry weight mycelium)

*Significantly different from control value, $p \leq 0.05$

Table 3: Effect of a range of inorganic silicon compounds on fungal growth (Percent increase or decrease over control value)

| *Fungus* | *A. citri* | *B. cinerea* | *C. cladospoiroides* |
|---|---|---|---|
| *Inorganic silicon* | | | |
| Calcium silicate | 260* | 300* | 312* |
| Silicon nitride | 100* | 250* | 290* |
| Colloidal silica | 150* | 200* | 210* |
| Silicic acid | 105* | 250* | 280* |
| Sodium silicate | -40* | -30* | -49* |

*Significantly different from control value, $p \leq 0.05$

Table 4: Effect of organic silicon compounds (added directly to the medium) on the growth of fungi (Percent increase or decrease over control value)

| Dimethylpolysiloxane | | |
|---|---|---|
| *Added organic silicon* (g 100ml$^{-1}$) | 0.5 | 1.0 |
| *A. citri* | 5.0* | 52* |
| *B. cinerea* | 20* | 63* |
| *C. cladospoiroides* | 6.0* | 28* |
| Hexamethyldisiloxane | | |
| *A. citri* | -60* | -83* |
| *B. cinerea* | -2.0 | -3.0 |
| *C. cladosporoides* | 1.5 | 10* |

*Significantly different from control value, $p \leq 0.05$

Table 3 shows that the stimulatory effect of silicon was not restricted to silicic acid, but occurred in the presence of a range of other silicon compounds, notably calcium silicate; the only exception to this rule being sodium silicate. In the case of calcium silicate, the observed growth stimulation may, in part at least, have been due to an increase in medium pH, but this cannot provide an explanation for increased fungal biomass brought about by the presence of the other silicon compounds.

Finally, Table 4 shows that an increase in the biomass of all of the fungi studied is also brought about by the addition, to the medium, of the organic silicon compound, dimethylpoysiloxane. This stimulatory effect is not however, produced by all organic silicon compounds as is evidenced by the fact that hexamethylsiloxane caused a reduction in the growth of *A citri* and *B. cinerea* and only a small increase in the growth of *C. cladosporoides* (Table 4).

As was mentioned in the Introduction, silicon compound are increasingly being used as fertilizers, as well as to help reduce the severity of fungal infections in crop plants [10]. If the *In vitro* stimulation in the growth of the pathogenic fungi studied here are translated to the field then the use of such silicon compound might stimulate, rather than inhibit, the growth of fungal pathogens, thereby leading to detrimental effects on crop plant growth.

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