The antifungal activity of different ZumSil concentrations towards plant pathogens. Trial conducted by University of the Free State, of South Africa.

Introduction

ZumSil is a liquid silicon product of monosilicic acid [$Si(OH)_4$] which is absorbable for plants and shows promise as a fertilizer supplement. However, in this study ZumSil was tested for *in vitro* antifungal activity against *Altenaria alternata subspecies* 1 (Alt 1.0855), *Altenaria alternata subspecies* 2 (Alt1), *Botryosphaeria dothidea subspecies* 1 (12JB6), *Botryosphaeria dothidea subspecies* 2 (Bot), *Fusarium oxysporum subspecies* 1 (CCP178), *Fusarium oxysporum subspecies* 2 (FUS 1), *Pythium ultimum* (Phyt) and *Rhizoctonia solani* (DE4). The following ZumSil dilutions (with distilled water) were tested: 1:500, 1:300, 1:200 and 1:100.

Materials and Methods

Materials

Malt extract agar (ME) was purchased from Astro Labs (South Africa) and Potassium hydroxide from Merck, Germany. All fungal mother cultures were obtained from the Department of Plant Sciences, Pathology section, University of the Free State, South Africa.

Microorganisms

Five common South African plant fungal pathogens, in some cases more than one strain, were chosen to test for the antifungal properties of ZumSil. These included *Altenaria alternata* (Fr.) Keissl *(Dothideomycetes) subspecies* 1 (Alt 1.0855), *Altenaria alternata subspecies* 2 (Alt1), *Botryosphaeria dothidea* (Moug.: Fr.) Ces. & De Not. (Loculoascomycetes) *subspecies* 1 (12JB6), *Botryosphaeria dothidea subspecies* 2 (Bot), *Fusarium oxysporum* Schlechtend.*:Fr.* (Hyphomycetes) *subspecies* 1 (CCP178), *Fusarium oxysporum subspecies* 2 (FUS 1), *Pythium ultimum* Trow (Oömycetes). (Phyt) and *Rhizoctonia solani* Kühn (Agonomycetes) (DE4).

Preparation of fungal mother cultures

Mother cultures of fungi were prepared separately on pre-sterilized malt extract (ME) agar and stored at 4°C until being used in this study: Separate inoculation of the ME agar with a mycelium sample from each fungus was done in a laminar flow hood under complete sterile conditions. In each case a 6mm mycelium sample was placed upside down on the agar contained in a Petri dish and allowed to grow at 25°C in a growth cabinet for six days before using pure uncontaminated mother cultures in standard antifungal activity tests.

Screening for antifungal properties

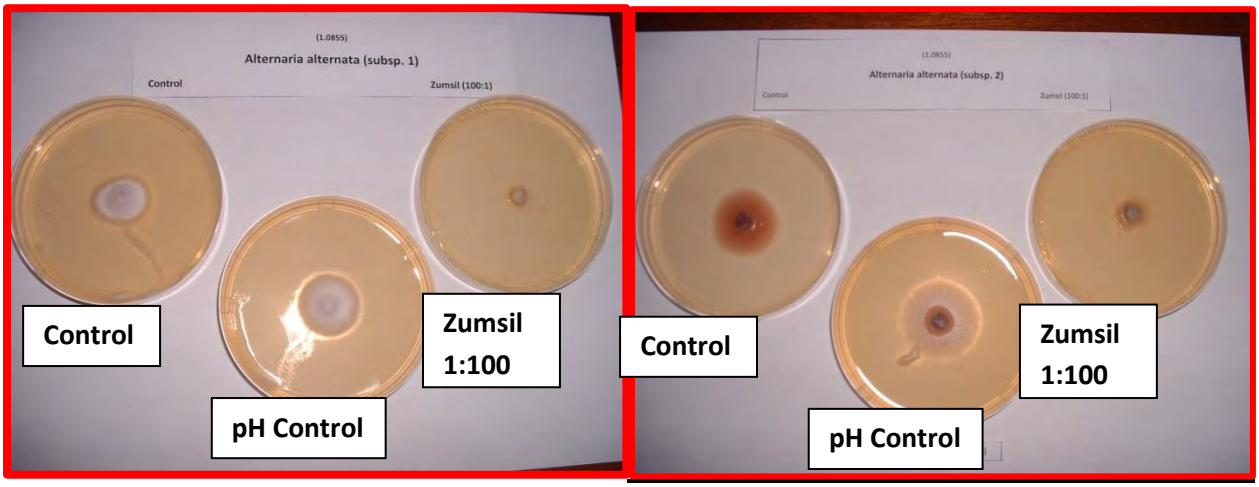
A modified agar dilution method (Rios *et al.* 1988) was used for determining the inhibition of mycelial radial growth of the test organisms by ZumSil. All plant pathogenic test fungi were cultured on 2% (m/v) ME agar, prepared according to the specifications of the manufacturers, and autoclaved for 20 min at 121^{o}C. On cooling to 45^{o}C in a water bath, 300 µl of a 33% (m/v) Streptomycin solution was added to the basal medium for controlling bacterial growth. ZumSil was dissolved in 100 ml sterile distilled water and amended in the agar to yield a final concentration series of 1:100, 1:200, 1:300 and 1:500. Working in a laminar flow cabinet, the medium was poured into 90 mm sterile plastic Petri dishes, to a thickness of 2-3 mm, and allowed to set. The center of each test plate was subsequently inoculated with a 5 mm size plug of 7-10 day old cultures, for each of the pathogens separately. Plates containing only the basal ME agar medium, and inoculated with the different pathogens, served as controls. Additionally, a plate containing the basal ME agar medium with pH amended to 12.2 by Potassium hydroxide, was used to test the effect of pH. A pH of 12.2 corresponded with the pH of ZumSil previously determined.

Plates were incubated for four days at 25 ± 2 ^{o}C (March *et al.,* 1991) in a growth cabinet. Each assay was performed in triplicate. Radial mycelial growth was determined after four days by calculating the mean of two perpendicular colony diameters for each replicate. The measurement included the assay wells (March *et al.,* 1991; Pfaller *et al.,* 1992) and was expressed as percentage mycelial growth inhibition by calculating according to the formula of Pandey *et al* (1982): (*dc -dt*)/*dcx100*, where *dc* = average diameter of the fungal colony of the controls and *dt* = average diameter of the fungal colonies treated with ZumSil. Photos represented the qualitative effects.

Results and Discussion

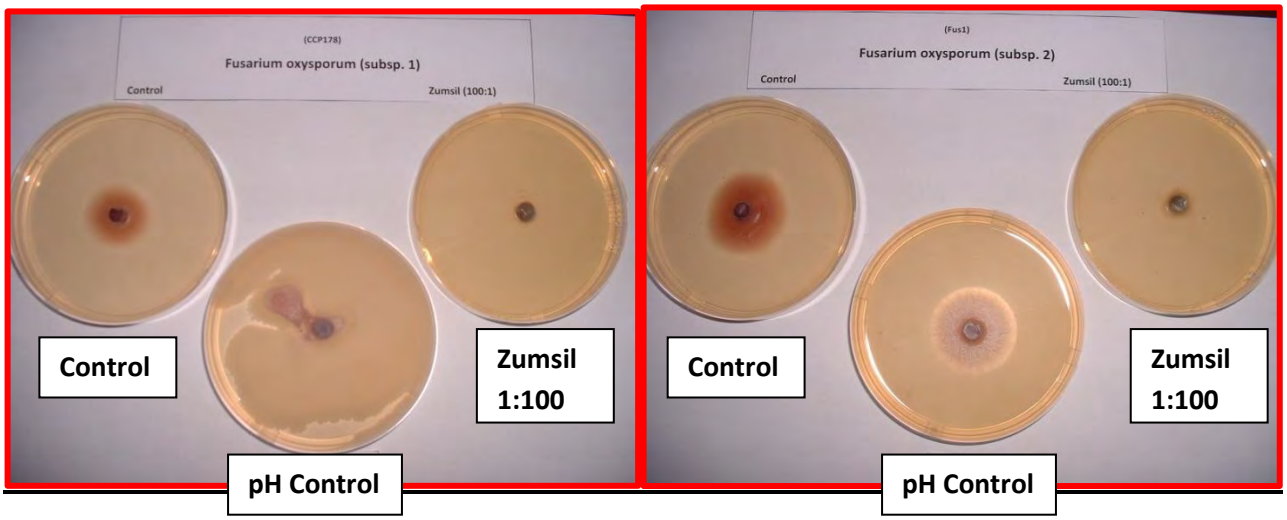
The 1:500, 1:300 and 1:200 ZumSil had no inhibitory effect on mycelium growth of either of the test pathogens (results not shown). However, the 1:100 dilutions had a marked, and in most cases significant *in vitro* inhibitory effect on all of the test pathogens (Photos and Table 1) and this is the only result shown.

***Alternaria alternata* (Subsp. 1)** ***Alternaria alternata* (Subsp. 2)**



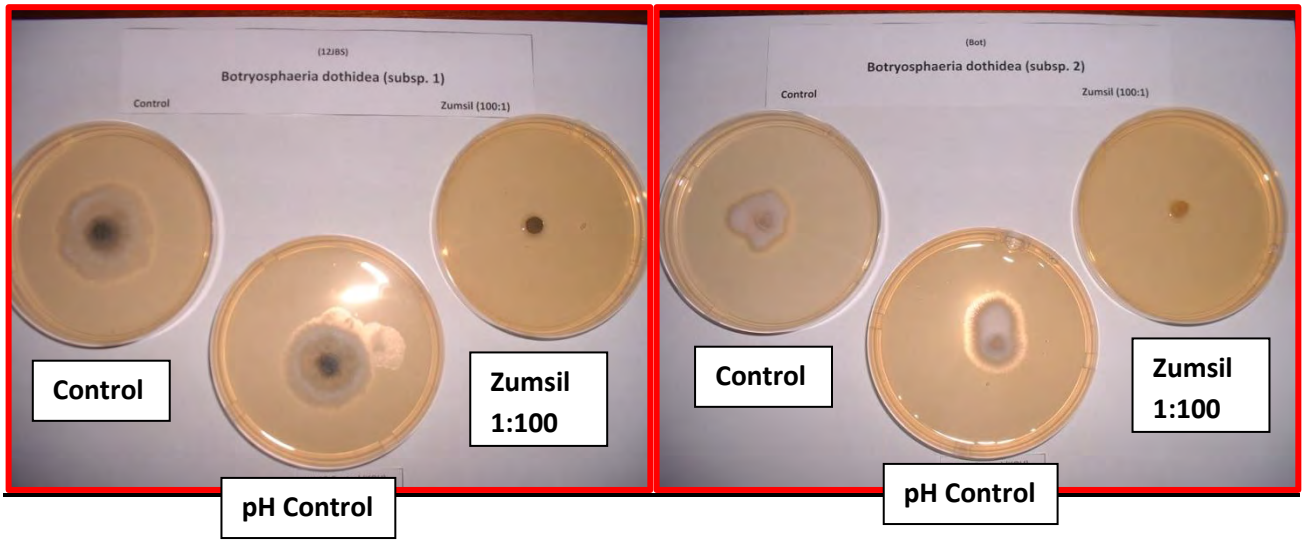
With ZumSil at 1:100 complete (100%) mycelial growth inhibition of *Alternaria alternata* subspecies 1 and 81.3% with subspecies 2 was obtained, while pH had no significant effect.

***Fusarium oxysporum* (Subsp. 1)** ***Fusarium oxysporum* (Subsp. 2)**



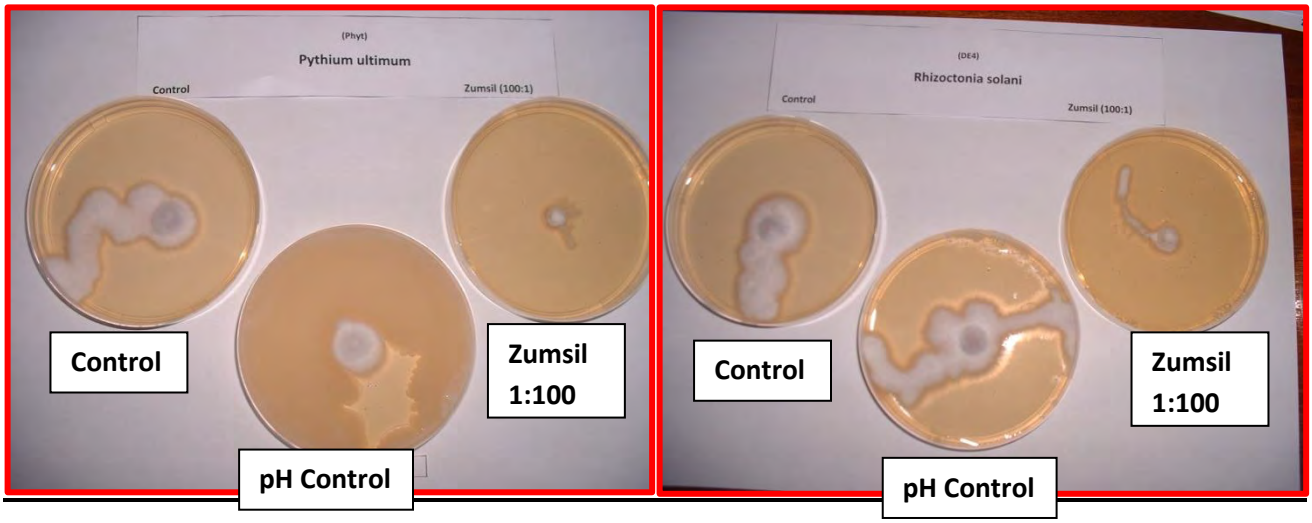
With ZumSil at 1:100 complete (100%) mycelial growth inhibition of *Fusarium oxysporum* subspecies 1 and 83.7% with subspecies 2 was obtained, while pH had no significant effect.

***Botryosphaeria dothidea* (subsp 1)** ***Botryosphaeria dothidea* (subsp 2)**



With ZumSil at 1:100 complete (100%) mycelial growth inhibition of both *Botryosphaeria dothidea* subspecies was obtained while pH had no significant effect.

Pythium ultimum ***Rhizoctonia solani***



With ZumSil at 1:100 80.7% mycelial growth inhibition of *Pythium ultimum* and 68.5% with *Rhizoctonia solani* was obtained, while pH had no significant effect.

Table 1: *In vitro* mycelial growth inhibition of different plant pathogens treated with ZumSil at a concentration of 1:100

Pathogen	% Mycelium growth inhibition
Alternaria alternata subspecies 1	**100.00**
Alternaria alternata subspecies 2	**81.25**
Fusarium oxysporum subspecies 1	**100.00**
Fusarium oxysporum subspecies 2	**83.70**
Botryoshaeria dothidea subspecies 1	**100.00**
Botryoshaeria dothidea subspecies 2	**100.00**
Pythium ultimum	**80.67**
Rhizoctonia solani	**68.53**

Conclusion

From the results (Photos and Table 1) it is clear that *Pythium ultimum* (80.7% inhibition) and especially *Rhizoctonia solani* (68.5% inhibition) showed the most resistance towards treatment with ZumSil when tested *in vitro*, but only at the highest concentration (1:100). It must, therefore, be expected that not all fungi is sensitive towards treatment with a Silicon product such as ZumSil and care must be taken not to generalize in terms of the potential antifungal activity contained in ZumSil. Proper *in vitro* screening trials must be conducted before large scale field trials are considered in terms of specific pathogens. It seems that the rather high pH of ZumSil played no significant role in terms of the inhibitory activity observed in this study.

In soil silicon is usually found in its rather insoluble form [$Si(O_2)$]. In ZumSil silicon is in its water soluble form [$Si(OH)_4$] that has been proven previously to contribute towards plant nutrition and even yield increases when diluted 1:500 or even 1:1000 with water (Matichenkov, 2010). However, at high concentrations it might inhibit plant growth (Roy *et al.* 2006) and care should therefore be taken to apply ZumSil at a 1:100 dilution for its antifungal property while a negative effect on plant growth and/or production is obtained.

It is recommended that ZumSil should also be tested as a seed treatment at a 1:100 dilution because of its antifungal property observed in this study. It is postulated that ZumSil has the potential to act as an antifungal seed treatment while its fertilizer properties might contribute to seedling growth improvement, seedling established and possibly also the final yield.

References

Matichenkov, V.V. 2010. Silicon containing mixture. US patent 2010/0275666

Roy, R.N., Finck, A., Blair, J. and Tandon, H.L.S. 2006. Plant Nutrition for Food Security, A Guide for Integrated Nutrient Management, Fertilizer and Plant Nutrition Bulletin, 16, Rome Italy.