CHAPTER 2
IN VITRO ASSESSMENT OF THE ANTIFUNGAL ACTIVITY OF POTASSIUM SILICATE AGAINST SELECTED PLANT PATHOGENIC FUNGI

2.1 ABSTRACT

Silicon is a bioactive element only recently implicated as having fungicidal properties. The present study examines the use of liquid potassium silicate for activity against several phytopathogenic fungi. *In vitro* dose-responses towards soluble potassium silicate (20.7% silicon dioxide) were determined for *Alternaria solani, Colletotrichum gloeosporioides, Curvularia lunata, Drechslera* sp., *Fusarium oxysporum, Fusarium solani, Glomerella cingulata, Lasiodiplodia theobromae, Mucor pusillus, Natrassia* sp., *Pestalotiopsis maculans, Phomopsis perniciosa, Phytophthora capsicii, Phytophthora cinnamomi, Pythium* F-group, *Sclerotinia sclerotiorum, Sclerotium rolfsii, Stemphylium herbarum* and *Verticillium tricorpus*. Inhibition of mycelial growth was dose-related with 100% inhibition at 80ml (pH 11.7) and 40ml (pH 11.5) soluble potassium silicate per litre of agar, for all fungi tested in two replications with the exception of *Natrassia* sp., *G. cingulata, F. oxysporum* and *C. gloeosporioides* at 40ml in one replication. For all replications, *P. cinnamomi, P. perniciosa, P. maculans, L. theobromae, G. cingulata, Natrassia* sp., and *C. gloeosporioides* were only partially inhibited at 5, 10 and 20ml soluble potassium silicate per litre of agar, but percentage inhibition was positively correlated with dosage concentrations. Soluble potassium silicate raised the pH of the agar from 5.6 (unameliorated agar) to 10.3 and 11.7 at concentrations of 5 and 80ml potassium silicate per litre of agar, respectively. Increased pH, in the absence of potassium silicate, only partially inhibited mycelial growth. Clearly, potassium silicate had an inhibitory effect on fungal growth *in vitro* and this was mostly fungicidal rather than a consequence of raised pH or the nutritional value of potassium.

* Part of the data from this chapter has been published:

BEKKER, T.F., KAISER, C., VAN DER MERWE, R., & LABUSCHAGNE, N., 2006. *In-vitro* inhibition of mycelial growth of several phytopathogenic fungi by soluble silicon. *S.A. J. Plant Soil* 26(3), 169-172.

2.2 INTRODUCTION

Numerous studies have shown increased resistance to plant fungal diseases in response to silicon applications, including increased resistance to powdery mildew (*Uncinula necator* (Schwein) Burrill) in grapes (Bowen *et al*., 1992); powdery mildew (*Sphaerotheca fuliginea* (Schltdl. Fr.) Pollacci) in cucumbers (Adatia and Besford, 1986; Belanger *et al.*, 1995); powdery mildew (*Erysiphe cichoracearum* D.C.) in muskmelons (Menzies *et al*., 1992); grey leaf spot (*Magnaporthe grisea* (T.T. Hebert) Barr) in St. Augustinegrass (*Stenotaphrum secundatum* Kuntze.) (Brecht *et al*., 2004), brown spot (*Pyricularia oryzae* Cavara) and neck rot [*Bipolaris oryzae* (Breda de Haan) Shoemaker] in rice crops (Seebold *et al*., 2004).

Wainwright (1993) suggested nutrient-free silica gel supports fungal growth, with the gel itself acting as a nutrient source and stimulating spore formation. Visible mycelial growth was reported by Wainwright *et al*. (1997) to be present on silicic acid amended media. They however ascribed this growth to silicon acting only as a physical contact surface for spores, rather than acting as a nutrient source.

This may have been the case, as recent literature reported on the inhibitory effect of silicon on fungal growth. Biggs *et al*. (1997) reported a 65% growth reduction of *Monilinia fructicola* (G.Wint.) Honey, the causal fungus of brown rot of peach fruit, on Potato Dextrose Agar (PDA) amended with calcium silicate compared to control treatments. Menzies *et al*. (1992) conducted a study to determine the *in vitro* effect of silicon on conidial germination and germ tube growth. They concluded that potassium silicate had no effect on either factors tested. The effect of potassium silicate on mycelial growth was however not mentioned. Wainwright *et al*. (1994) reported that, although fungal mycelia formed when inoculum particles were placed on nutrient free silica gel, no mycelia could be found on the gel surface and growth was therefore most probably supported by nutrients from the inoculum.

The aim of the current *in vitro* study was therefore to assess whether potassium silicate has any fungicidal effect on fungal growth, and if inhibition is found, at what concentration is *Phytophthora cinnamomi* effectively inhibited.

2.3 MATERIALS AND METHODS

2.3.1 Fungal Isolates

Fungal isolates maintained on potato dextrose agar (PDA) were obtained from the Mycological Herbarium of the Plant Protection Research Institute, Agricultural Research Council (ARC), Pretoria, South Africa; Westfalia Technological Services (WTS), Tzaneen, South Africa; and the University of Pretoria, South Africa, culture collections. Fungi were selected on the basis of availability of cultures as well as the importance as pathogens (Table 2.1).

2.3.2 Siliceous Material

Potassium silicate was obtained from Ineos Silicas (Pty) Ltd. (Mean weight ratio SiO_2: K_2O = 2.57; Mean K_2O = 8.0%; Mean SiO_2 = 20.7 %; Mean total solids = 28.75%).

2.3.3 Agar Preparation and Assay

Sterilization of soluble potassium silicate by means of autoclaving resulted in a solidified silicon mass. It was therefore passed through a 0.45µm millipore filter to remove fungal spores and other possible contaminants, and added to autoclaved potato dextrose agar (PDA) prior to solidification to obtain final concentrations of 5, 10, 20, 40 and 80ml soluble potassium silicate per litre of PDA, with non-ameliorated PDA (i.e. with no silicon) as control.

Concentrated soluble potassium silicate has a pH of 12.7, which perceptibly increases the pH of a PDA media. Unamended PDA has a pH of 5.6 but upon addition of 5, 10, 20, 40 and 80ml soluble potassium silicate per litre of agar, the pH of the PDA was raised to 10.3, 10.7, 11.2, 11.5 and 11.7 respectively (Bekker *et al*., 2006). To assess the effect of pH alone on mycelial growth, PDA control plates were adjusted to pH 10.3, 10.7, 11.2, 11.5 and 11.7 using potassium hydroxide. This assessment of the effect of pH was however limited to certain isolates (Table 2.2).

Agar was aseptically dispensed into 9cm diameter plastic Petri dishes. A 5mm diameter mycelial disc taken from a seven-day-old fungal culture on PDA was transferred to the centre of a Petri dish containing PDA either amended with soluble potassium silicate or potassium hydroxide, or unamended control plates. This was performed for each fungus. Ten replicates were included for each treatment. Plates

were incubated at 25˚C in the dark, with colony diameters measured every second day for eight days.

2.3.4 Statistical Analysis

Percentage inhibition was calculated according to the formula (Biggs *et al*., 1997):

$$\textit{Percentage inhibition} = \frac{(C-T) \times 100}{C}$$

Where C = colony diameter (mm) of the control
T = colony diameter (mm) of the test plate

This experiment was repeated for all fungi and results presented are pooled data. Data were subjected to analysis of variance (ANOVA). Mean differences were separated according to Duncan's multiple range test ($P < 0.05$).

2.4 RESULTS AND DISCUSSION

Soluble potassium silicate (20.7% silicon dioxide) inhibited all mycelial growth of *P. cinnamomi* (Figure 2.1), *P. capsicii, S. rolfsii* and *S. sclerotiorum* at all concentration tested from 5 to 80ml.l^{-1} potassium silicate (Table 2.2). Soluble potassium silicate however completely suppressed mycelial growth of all fungi tested at concentrations of 40 and 80ml.l^{-1} potassium silicate, except for *Drechslera* sp. (mean percentage inhibition of 98.35). For all fungi tested, treatments within each fungal group differed with highly significant differences in colony diameter already prevalent within two days after inoculation as can be seen in the data collected for *P. cinnamomi* (Table 2.3). Suppression of mycelial growth at concentrations of 5 - 20ml.l^{-1} potassium silicate varied in the degree of suppression at these low concentrations and was not consistent between replications. This implies a difference in sensitivity of different fungi to potassium silicate inhibition, and effective inhibition concentrations are to be determined for each fungus. Although the concentration gradient in this study resulted in inhibition variability between different fungi tested, the suppressive effect that soluble potassium silicate has on fungal mycelial growth cannot be debated.

While inhibition of mycelial growth on potassium hydroxide amended PDA ranged from 0 – 100%, in some cases mycelia were induced to grow faster and in the case of

Alternaria solani growth was 13.8% faster at pH 11.2 (Table 2.2). Nevertheless, the effect of pH on mycelial growth is inconsistent. Although these results are variable, the effect of added soluble potassium silicate is apparent. The potassium silicate has a direct impact on mycelial growth and the observed inhibition is not solely the result of the potassium silicate changing the pH of the medium. In all fungi tested, mycelial growth continued at high pH in the absence of potassium silicate, although at a slower rate as can be seen in *P. perniciosa* (Figure 2.2), and this implies that soluble potassium silicate has a two-fold effect on fungal growth.

From results obtained in the current study, it can be deducted that, if silicon is applied to plants, it may form an impenetrable barrier to pathogens on the plant surface, preventing colonisation. Ma *et al*. (2001) reported that the applications of silicon to leaves could possibly prevent mildew colonisation and consequent infection, and that this approach may be useful in crops with a passive or rejective silicon uptake mode. Although the current study does not deny the activation of plant defence mechanisms including induced systemic resistance (ISR) by silicon (Fawe *et al*., 1998, 2001), it is proposed that silicon may act as the first protecting barrier in silicon treated plants, and may inhibit pathogen colonisation and consequential infection by inhibiting fungal growth on the plant surface.

Low potassium silicate concentrations resulted in increased fungal growth of *F. oxysporum* (-8.2% inhibition) and *Verticillium tricorpus* (-10.13% inhibition) at 5ml.l^{-1} potassium silicate, and *F. solani* (-8.61%; -11.27% inhibition) at 5 and 10ml.l^{-1} potassium silicate respectively (Table 2.2). This phenomenon has been observed in numerous fungi by Wainwright *et al.* (1997). Although highly improbable, the silicon could also act as additional nutrient supplementation and induce faster mycelial growth. It is however interesting to note that wherever low soluble silicon concentrations induced faster mycelial growth, all pH control groups of the same fungus showed similar results. It is therefore possible that faster mycelial growth in certain fungi is due to an affinity of those fungi for higher pH growing conditions. The precise reason for this is however yet to be determined. This has implications if silicon is to be used as a fungicide and it should therefore be ensured that silicon addition does not increase (water soluble) concentrations in soil to a level conducive to the growth of these fungi.

Seebold *et al*. (1995) and Seebold (1998) reported silicon tested as a fungicide under field conditions resulted in a 40% suppression of rice neck blast (*Magnaporthe grisea*

(T.T. Hebert) Barr). Complete suppression of a particular fungus in the current study however occurred at different concentrations and is variable. The suppression level of each fungus is therefore to be determined *in vitro* before *in vivo* investigations are initiated.

2.5 CONCLUSION

Soluble potassium silicate completely suppresses mycelial growth of all fungi tested at concentrations of 40 and 80ml.l^{-1} potassium silicate. Suppression of mycelial growth at concentrations of 5 - 20ml.l^{-1} potassium silicate vary in the degree of suppression at these low concentrations and is not consistent between replications. This implies a difference in sensitivity of different fungi to potassium silicate inhibition, and effective inhibition concentrations are to be determined for every fungus. In all fungi tested, mycelial growth continued at high pH in the absence of potassium silicate, although at a slower rate which implies that soluble potassium silicate has a two-fold effect on fungal growth, of which the direct effect on fungal growth overrides the effect of pH. The inclusion of potassium hydroxide as a control treatment eliminated any potential role potassium may play in enhancing or suppressing mycelial fungal growth. It is proposed that silicon may act as the first protecting barrier in silicon treated plants, and may inhibit pathogen colonisation and consequential infection by inhibiting fungal growth on the plant surface.

Low potassium silicate concentrations resulted in increased fungal growth in *F solani*, *F. oxysporum* and *V. tricorpus* isolates. This can be due to the presence of nutritional elements present overriding the inhibitory effect of silicon. Although highly improbable, the silicon could also act as additional nutrient supplementation and induce faster mycelial growth. It is however interesting to note that wherever low soluble silicon concentrations induced faster mycelial growth, all pH control groups of the same fungus showed similar results. Complete suppression of a particular fungus in the current study however occurred at different concentrations and is variable. The suppression level of each fungus is therefore to be determined *in vitro* before *in vivo* investigations are initiated. The results of this study clearly indicate that soluble potassium silicate (20.7% silicon dioxide) has fungicidal activity. Effective application of these results in a field trial is however paramount to determine the commercial value of water soluble potassium silicate as a fungicide.

2.6 LITERATURE CITED

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Table 2.1: Fungal pathogens tested for their ability to grow in the presence of soluble potassium silicate (20.7% silicon dioxide).

Fungus	**Isolate nr.**	**Host plant**	**Disease name**
Alternaria solani Sorauer	UPGH100	Tomato	Early Blight
Colletotrichum coccoides (Wallr.) S. Hughes	-	Tomato	Anthracnose
Colletotrichum gloeosporioides (Penz.) Penz. & Sacc.	PPRI3848	Avocado	Anthracnose, Stem end rot
Curvularia lunata (Wakker) Boedijn	UPGH105	Lettuce	Leaf spot
Dreschlera sp.	UPGH106	Tomato	Leaf spot
Fusarium oxysporum Schltdl. W. Snyder & H.N. Hansen	UPGH110	Banana	Wilt
Fusarium solani (Mart.) Appel Wollenw.	UPGH111	Cucumber	Wilt
Glomerella cingulata (Stoneman) Spauld. & H. Schrenk	PPRI6360	Avocado	Anthracnose
Lasiodiplodia theobromae (Pat.) Griffon & Maubl.	PPRI6630	Avocado	Stem end rot
Mucor pusillus	-	Tomato	Postharvest rot
Natrassia sp.	PPRI6718	Avocado	
Pestalotiopsis versicolor (Spreg.) Steyaert	PPRI5564	Avocado	Stem end rot
Phomopsis perseae Zerova	PPRI6005	Avocado	Stem end rot
Phytophthora capsicii Leonian	UPGH118	Tomato	Root Rot
Phytophthora cinnamomi Rands	-	Avocado	Root Rot
Pythium F-group	UPGH009	Lettuce	Root Rot
Sclerotinia sclerotiorum (Lib.) De Bary	UPGH122	Lettuce	Rot
Sclerotium rolfsii Sacc.	UPGH123	Tomato	Wilt
Stemphylium herbarum E. Simmons	UPGH124	Lettuce	Leaf spot
Verticillium tricorpus I. Isaac	-	Lettuce	Wilt

Table 2.2: Mean percentage inhibition of different fungi at different potassium silicate (20.7% silicon dioxide) or potassium hydroxide concentrations on ameliorated potato dextrose agar.

Pathogen	Percentage inhibition										
	5ml Si (pH 10.3)	**10ml Si (pH 10.7)**	**20ml Si (pH 11.2)**	**40ml Si (pH 11.5)**	**80ml Si (pH 11.7)**	**pH 10.3**	**pH 10.7**	**pH 11.2**	**pH 11.5**	**pH 11.7**	**Pr.F.**
Alternaria solani	22.9	100.0	100.0	100.0	100.0	33.5	11.8	-13.8	-13.0	24.8	< 0.001
Colletotrichum coccoides	70.43	77.88	95.16	100.0	100.0						< 0.001
Colletotrichum gloeosporioides	15.7	76.4	100.0	100.0	100.0	20.4	17.1	16.2	26.5	57.8	< 0.001
Curvularia lunata	3.17	41.8	99.03	100.0	100.0						< 0.001
Drechslera sp.	13.25	21.52	62.9	98.35	100.0						< 0.001
Fusarium oxysporum	-8.2	23.7	96.2	100.0	100.0	-8.2	-8.2	-8.2	-8.2	-2.3	< 0.001
Fusarium solani	-8.61	-11.27	72.17	100.0	100.0	-0.07				30.1	< 0.001
Glomerella cingulata	9.3	79.7	100.0	100.0	100.0	0.0	0.0	9.3	14.0	32.0	< 0.001
Lasidiodiploidia theobromae	11.3	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	43.0	< 0.001
Mucor pusillus	50	92.86	100.0	100.0	100.0						< 0.001
Natrassia sp.	46.4	100.0	100.0	100.0	100.0	8.4	22.5	0.0	19.0	34.0	< 0.001
Pestaliopsis maculans	23.6	100.0	100.0	100.0	100.0	18.3	7.7	14.3	44.0	77.0	< 0.001
Phomopsis perniciosa	41.8	100.0	100.0	100.0	100.0	4.6	13.6	18.8	34.9	64.7	< 0.001
Phytophthora capsicii	100.0	100.0	100.0	100.0	100.0	22.0	31.8	19.4	47.7	49.9	< 0.001
Phytophthora cinnamomi	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	< 0.001
Pythium F-group	32.9	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	96.7	< 0.001
Sclerotinia sclerotiorum	100.0	100.0	100.0	100.0	100.0						< 0.001
Sclerotium rolfsii	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	< 0.001
Stemphylium herbarum	5.14	14.89	89.24	100.0	100.0	-0.04				1.5	< 0.001
Verticillium tricorpus	-10.13	25.22	54.78	100.0	100.0	-0.05				11.2	< 0.001

Table 2.3: Mycelial growth of *Phytophthora cinnamomi* incubated for eight days at 25˚C on PDA. Agar was either amended with soluble potassium silicate (20.7% silicon dioxide) to create a concentration range, or potassium hydroxide to increase agar pH, mimicking the pH effect of soluble silicon (20.7% silicon dioxide).

	Mean colony diameter (mm)[x]			
Treatment	Day 2	Day 4	Day 6	Day 8
0ml/l [y]	23.9 a[z]	48.2 a	61.8 a	72.3 a
5ml/l	5.3 c	10.8 d	15.7 e	16.1 e
10ml/l	5.0 c	5.0 e	5.0 f	5.0 f
20ml/l	5.0 c	5.0 e	5.0 f	5.0 f
40ml/l	5.0 c	5.0 e	5.0 f	5.0 f
80ml/l	5.0 c	5.0 e	5.0 f	5.0 f
pH 10.3	17.5 b	28.1 b	45.9 b	55.6 b
pH 10.7	12.4 b	25.2 b	42.1 b	50.3 c
pH 11.3	15.5 b	30.7 b	46.1 b	56.9 b
pH 11.5	7.9 c	19.9 c	31.1 c	39.9 d
pH 11.7	7.9 c	18.68 c	25.6 d	38.2 d

[x] Means of 10 plates/ treatment. Measurements include initial 5mm diam. mycelial disc

[y] Concentration of the active ingredient per litre of PDA growth medium

[z] In each column, values followed by the same letter do not differ significantly (F.Pr < 0.01)

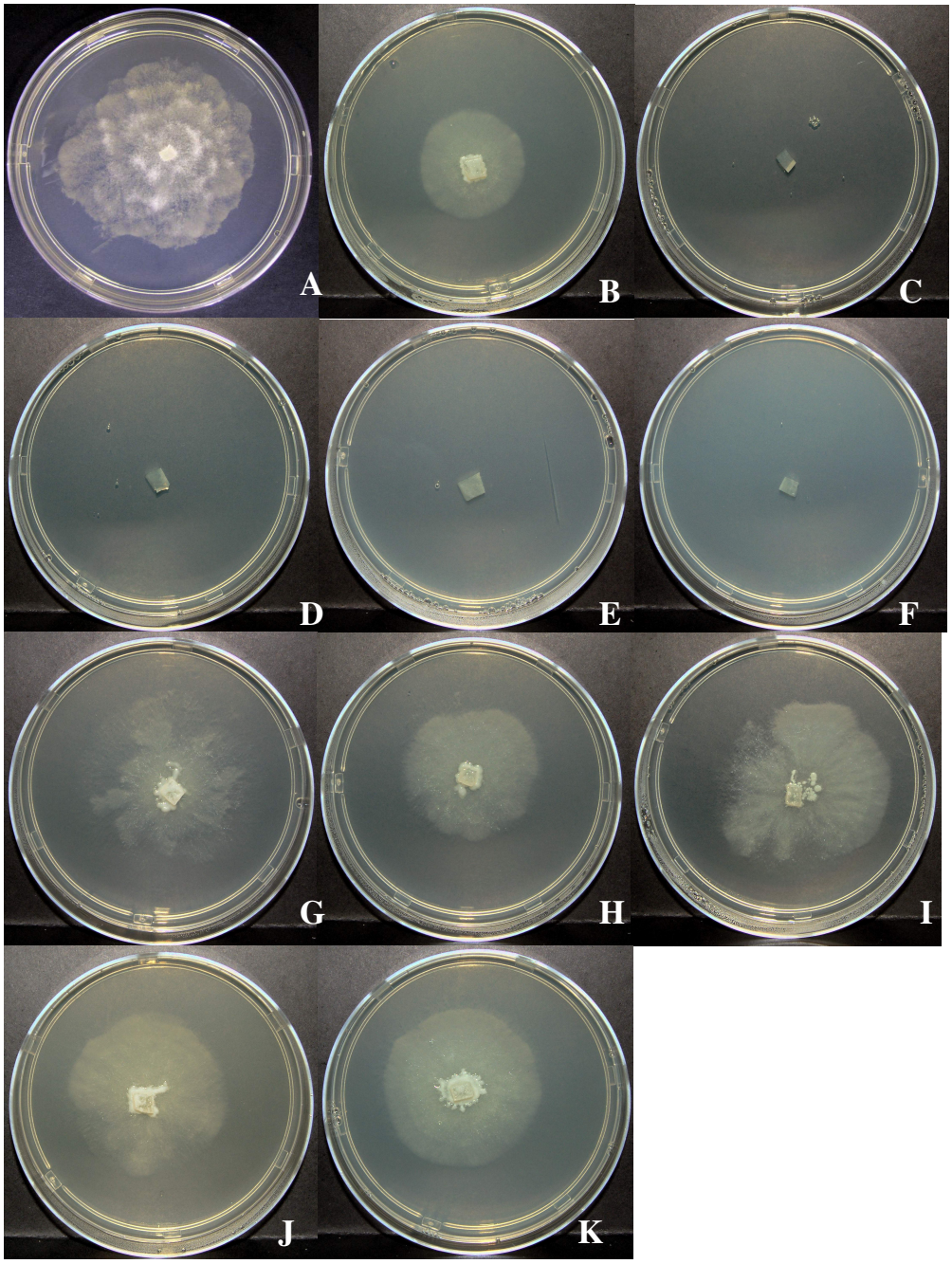

Figure 2.1: Mycelial growth of *Phytophthora cinnamomi* in response to 0ml (pH 5.6)(A), 5ml (pH 10.3) (B), 10ml (pH 10.7) (C), 20ml (pH 11.2) (D), 40ml (pH 11.5) (E), and 80ml (pH 11.7) (F) soluble potassium silicate (20.7% silicon dioxide) per litre of potato dextrose agar (PDA) compared to a potassium hydroxide control group including pH 10.3 (G), pH 10.7 (H), pH 11.2 (I), pH 11.5 (J), and pH 11.7 (K).

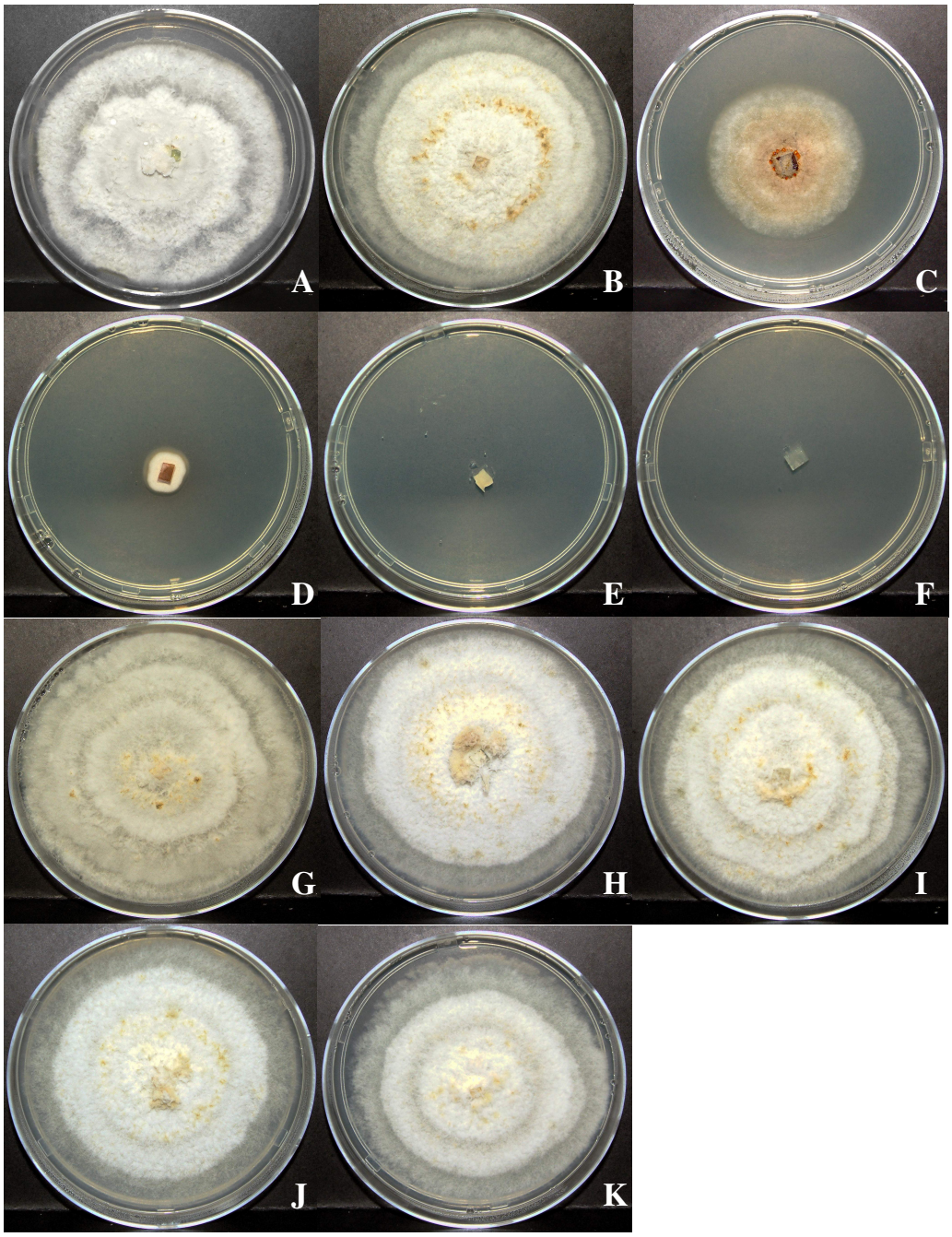

Figure 2.2: Mycelial growth of *Phomopsis perniciosa* in response to 0ml (pH 5.6)(A), 5ml (pH 10.3) (B), 10ml (pH 10.7) (C), 20ml (pH 11.2) (D), 40ml (pH 11.5) (E), and 80ml (pH 11.7) (F) soluble potassium silicate (20.7% silicon dioxide) per litre of potato dextrose agar (PDA) compared to a potassium hydroxide control group including pH 10.3 (G), pH 10.7 (H), pH 11.2 (I), pH 11.5 (J), and pH 11.7 (K).