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Effect of soluble silicon and *Trichoderma harzianum* on the *in vitro* growth of *Pythium aphanidermatum*

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With respect to the environmentally friendly sustainable agricultural practices, this research was therefore conducted to evaluate the interaction effect of soluble silicon (3 kinds with 3 concentrations) and *Trichoderma harzianum* on the *in vitro* growth of *Pythium aphanidermatum* using bi-culture antagonistic test. First, effect of soluble silicon was determined on growth of *P. aphanidermatum* as well as *T. harzianum* using 3x5 factorials in CRD with 5 replications. Factor A and B were the 3 kinds of soluble silicon (sodium silicate, potassium silicate and Phyton) and their 5 concentrations (0, 250, 500, 750 and 1000 ppm). The results showed that all tested soluble silicon significantly reduced mycelial growth and sporangial production of *P. aphanidermatum*. Moreover, soluble silicon could also retard the vegetative and reproductive growth of *T. harzianum* significantly. The retardation effect of potassium silicate on both fungi was greater than those of sodium silicate and Phyton, respectively. Besides, their effect seemed to increase along with their increasing concentrations. The combination of soluble silicon and *T. harzianum* on the *in vitro* growth of *P. aphanidermatum* was consequently evaluated employing 3x3 factorials in CRD with 5 replications. Factor A, B and C were 3 kinds of soluble silicon, 3 concentrations (0, 250 and 500 ppm) and *T. harzianum*, respectively. It showed that all tested soluble silicon as well as *T. harzianum* could reduce the growth of *P. aphanidermatum*. However, no additional inhibition effect of soluble silicon and *T. harzianum* was noted. The combination of *T. harzianum* either with Phyton at 250 and 500 ppm or 250 ppm sodium silicate gave the best inhibition effect on *P. aphanidermatum* as that of using *T. harzianum* alone.

Key words: soluble silicon, *Trichoderma harzianum*, *Pythium aphanidermatum*

Introduction

Damage by *Pythium* spp. is particularly severe in the early stages of seedling growth to plants growing in soil or soilless system (Blancard *et al*., 1994 and Moulin *et al*., 1994). These polycyclic pathogens can destroy plants tissues within a few days and then produce motile zoospores to initiate further cycles of disease (Duniway and Gordon, 1986). The resulting root rot can cause considerable damage to the plant crop. Of the number of *Pythium* spp.

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that can cause root rot of greenhouse-grown plant crop, *P. aphanidermatum* (Edson) Fitzp. is the most widely reported (Favrin *et al*., 1988; Paternotte, 1992 and Moulin *et al*., 1994). Besides, they have wide host ranges and can cause serious economic losses under conditions favorable for disease development. Although fungicides are available for the control of such diseases, any technology that involved little risk to the pollution of the environment or to human health would be highly desirable.

Silicon is thought to play an important role in plant growth and development (Epstein, 1994 and Datnoff *et al*., 2001). Biologically, it is a common but generally minor element in the majority of living organisms, occurring as amorphous silica (SiO_2 nH_2O) and soluble silicic acid ($Si(OH)_4$) (Epstein, 1994). Silicon is amended to soil or nutrient solution low in soluble silicon, plants exhibit improved growth and yield, better disease and insect resistance, and reduced mineral toxicities (Belanger *et al*., 1995; Savant *et al*., 1999 and Saigusa *et al*., 2000). Soluble silicon has shown potential for the increased resistance to fungal diseases such as powdery mildew and root rot (Epstein, 1994 and Belanger *et al*., 1995) and its control of fungal diseases in cherry fruit, cucumber, muskmelon, zucchini squash and peach fruit (Menzies *et al*., 1992; Bigg *et al*., 1997 and Qin and Tian, 2005).

Trichoderma spp. are common inhabitants of the rhizosphere and are well recognized as biocontrol agents of soilborne plant pathogens (Harman and Lumsden, 1990; Chet *et al*., 1997 and Howell *et al*., 2000). A commendable amount of research has focused on the mycoparasitic nature of *Trichoderma* and its contribution to plant health (Chet, 1987). Several mechanisms have been considered to be key factors in antagonistic interactions: lysis of host cell walls, antibiosis, competition for nutrients, induced resistance in plants, and inactivation of host enzymes (Harman, 2000). Thus, strains of *T. harzianum* have been commonly used as agents for the biocontrol of plant pathogenic fungi such as *Rhizoctonia solani*, *Lentinus lepideus*, *Fusarium solani*, *Botryosphaeria berengeriana* f.sp. *piricola*, *Phytophthora capsici* and *Pythium aphanidermatum* (Sivan *et al*., 1984; Ghisalberti and Sivasithamparam, 1991; Kexiang *et al*., 2002; Ezziyyani *et al*., 2007 and Rojo *et al*., 2007).

The objective of this study was to (i) determine the effect of soluble silicon on growth of *P. aphanidermatum* and *T. harzianum*, and (ii) examine the interaction effect of soluble silicon and *T. harzianum* on growth of *P. aphanidermatum*.

Materials and methods

Isolations of Pythium aphanidermatum and Trichoderma harzianum

Isolation for *P. aphanidermatum* was made from root tissue of kale (*Brassica oleracea* L. var. *acephala* DC.) showing root rot symptoms. Diseased root tissue was surface disinfested for 1 min. in a 10% clorox, rinsed twice in sterile water, and placed on potato dextrose agar (PDA; 200 g potato, 17 g glucose and 17 g agar per liter) +BNPRA+rb (Masago *et al*. 1977) prepared by adding 10 ppm benomyl, 25 ppm nystatin, 25 ppm of PCNB, 10 ppm rifampicin, 500 ppm ampicillin and 5 ppm rose Bengal per 1 liter of PDA after autoclaving. Culture plates were incubated at 25°C in the dark and *P. aphanidermatum* was purified by making repeated hyphal tip transfers as required. Identification of *P. aphanidermatum* was based on cultural morphology and microscopic observation as originally described by Plaats-Niterink (1981).

Isolation for *T. harzianum* was made from wettable powder, commercial bioproduct. Bioproduct was soaked in sterile water for 24 hrs, and spread on Martin's medium [prepared by 15 g agar, 1 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 5 g peptone, 10 g dextrose, 3.3 ml rose bengal (1% in alcohol), 1,000 ml distilled water and 1 g streptomycin] (Johnson and Curl, 1972). Culture plates were incubated at 25°C in the dark and *T. harzianum* was purified by hyphal tip transfer. Confirmed identification of *T. harzianum* was based on cultural morphology and microscopic observation as originally described by Bissett (1991a and b).

Determination of the effect of soluble silicon on growth of Pythium aphanidermatum and Trichoderma harzianum

Effect of soluble silicon on vegetative and reproductive growth of *P. aphanidermatum and T. harzianum* was conducted by using 3x5 factorials in CRD with 5 replications. Factor A and B were the 3 kinds of soluble silicon [sodium silicate ($Na_2Si_3O_7$; a.i. ~ 27%), potassium silicate ($K_2Si_3O_7$; a.i. ~ 25%) and Phyton (commercial product; 73.9% SiO_2, 12.9% Al_2O_3, 1.4% Fe_2O_3, 2.1% FeO, 0.8% MgO, 0.8 of CaO, 2.2% Na_2O, 5.7% K_2O and 0.2% P_2O_5)] and their 5 concentrations (0, 250, 500, 750 and 1000 ppm).

For their effects on vegetative growth of both fungi, each fungus was cultured on PDA dishes at 25°C for 3 days. Mycelial discs (5 mm Ø) were cut from the growing edge of each fungus and transferred onto PDA dish added with tested soluble silicon. Sodium silicate and potassium silicate were added to PDA after autoclaving while Phyton was added to PDA before autoclaving.

After incubation at 25°C, the colony diameter of *P. aphanidermatum* and *T. harzianum* were measured every 6 and 12 hrs, respectively.

For their effects on reproductive growth, the amount of sporangia of *P. aphanidermatum* and conidia of *T. harzianum* were consequently determined at the end of the above mentioned experiments. Regarding *P. aphanidermatum*, a mycelial disk (5 mm Ø) was cut from the growing edge of each treatment and transferred into 5 cm Ø plate containing 3 ml sterile distilled water to induce sporangial production. Amount of sporangia was counted under microscopy after incubation in the light at 25°C for 24 hrs. For *T. harzianum*, culture plates were flooded with sterile distilled water then the suspension was filtered through two layers of cheesecloth to remove debris. Conidia concentration was determined using a haemacytometer.

Evaluation of the interaction effect of soluble silicon and Trichoderma harzianum on growth of Pythium aphanidermatum

Interaction effect of soluble silicon and *T. harzianum* on mycelial growth of *P. aphanidermatum* was consequently evaluated using bi-culture antagonistic test on PDA added with soluble silicon. 3x3 factorials in CRD with 5 replications were employed. Factor A, B and C were 3 kinds of soluble silicon, 3 concentrations (0, 250 and 500 ppm) and *T. harzianum*, respectively. Regarding the concentrations of soluble silicon were selected from previous experiments given that it would be the most effective on *P. aphanidermatum* but less effect on *T. harzianum*.

With respect to bi-culture antagonistic test, a mycelial disk of *P. aphanidermatum* and *T. harzianum* was placed on the opposite side of the plate (6 cm away from either fungus). After 60 hr-incubation at 25°C, growth of *P. aphanidermatum* was measured and percent growth inhibition was calculated. Besides, antagonistic action between *T. harzianum* and *P. aphanidermatum* was studied under the microscope.

Statistical analysis

An analysis of variance was performed on the data with SAS. The level of significance was determined at P=0.01.

Results

Effect of soluble silicon on growth of Pythium aphanidermatum

With regard to vegetative growth experiment, the results showed that all tested soluble silicon significantly reduced mycelial growth of *P.*

aphanidermatum (Table 1). Significant interaction between kinds and concentrations of soluble silicon was noted at 18 hrs. Potassium silicate gave greater inhibition on *P. aphanidermatum* than those of sodium silicate and Phyton, respectively. Besides, the retardation effect of tested soluble silicon seemed to increase along with their increasing concentrations (Table 1). At 36 hrs, potassium silicate at 750 and 1,000 ppm as well as 1,000 ppm of sodium silicate gave the most inhibition effect on the mycelial growth of *P. aphanidermatum* (21.67, 18.11 and 18.11%, respectively) while at 250 ppm of both kinds of soluble silicon resulted in less retardation effect (only 1.56 and 1.22%). For Phyton, no retardation effect was noted at 250 ppm while the rest of the concentrations resulted in quite less inhibition effect compared to the control (only 0.33, 0.78, 2.33 and 2.44%, respectively) (Table 1, Fig. 1a and 2).

For reproductive growth, the result was in line with that of vegetative growth (Table 1). That is, any kinds or concentrations of soluble silicon gave the most inhibition effect on mycelial growth of *P. aphanidermatum* and could significantly reduce its sporangial production as well (Table 1). After inducing the sporangia by distilled water (at 24 hrs), potassium silicate at 1,000 ppm gave the greatest inhibition effect (86.71%) on sporangial production of *P. aphanidermatum* while 1,000 ppm of sodium silicate and 750 ppm of potassium silicate were amongst the second best (69.62 and 67.09%, respectively). Regarding Phyton, 250 ppm resulted in the least retardation effect (only 14.56%) (Table 1 and Fig. 1b).

Effect of soluble silicon on growth of Trichoderma harzianum

It is pointed out that the tested soluble silicon could also significantly retard the growth of biological control agent as well as pathogen. For vegetative growth experiment, all tested soluble silicon reduced mycelial growth of *T. harzianum* significantly. Interaction among kinds and concentrations of soluble silicon was significantly noted at 12 hrs (Table 2). At 72 hrs, potassium silicate gave significantly greater inhibition effect on *T. harzianum* than those of sodium silicate and Phyton, respectively. Besides, the mycelial growth of *T. harzianum* had been significantly decreasing along with the increasing concentrations of soluble silicon (Table 2). Potassium silicate at 1,000 ppm was the best in retarding the mycelial growth of *T. harzianum* (46.44%) while 1,000 ppm of sodium silicate was the second best (41.33%). With regard to Phyton, at any tested concentrations revealed the least effect on growth of *T. harzianum* (only 0.78-5%) compared to the other tested soluble silicon and control (Table 2, Fig. 3a and 4).

Table 1. Effect of soluble silicon on growth of *Pythium aphanidermatum* (every 6 hours) on PDA with added 3 kinds of soluble silicon (sodium silicate, potassium silicate and Phyton) at 5 concentrations (0, 250, 500, 750 and 1,000 ppm) and its sporangia amount after induced by distilled water at 24 hours.

Treatments		Colony diameter of *Pythium aphanidermatum* (cm)						amount of sporangia at 24 hours (sporangia/field)[2/]
Kind of soluble silicon	Concentration (ppm)	6 hrs	12 hrs	18 hrs	24 hrs	30 hrs	36 hrs	
sodium silicate	0	1.25a[1/]	2.79a	4.22a	5.95a	7.62a	9.00a	15.80a
	250	1.19ab	2.42b	3.76bc	5.53ab	7.24ab	8.89a-c	12.50c
	500	1.09a-c	2.23b-d	3.43cd	5.19bc	6.85b	8.54bc	9.20f
	750	1.05a-d	2.07cd	3.05e	4.57de	6.14c	7.86d	7.70g
	1000	1.00b-e	2.07de	2.95ef	4.19e	5.64d	7.37e	4.80h
potassium silicate	0	1.25a	2.79a	4.22a	5.95a	7.62a	9.00a	15.80a
	250	1.12a-c	2.31b-d	3.64bc	5.40b	7.08b	8.86a-c	10.50e
	500	0.99b-e	2.07cd	3.18de	4.85cd	6.88b	8.50c	7.70g
	750	0.84de	1.75ef	2.69fg	4.10e	5.61d	7.37e	5.20h
	1000	0.79e	1.66f	2.56g	3.59f	5.31d	7.05e	2.10i
Phyton	0	1.25a	2.79a	4.22a	5.95a	7.62a	9.00a	15.80a
	250	0.98b-e	2.46b	3.84b	5.60ab	7.27ab	8.97a	13.50b
	500	0.87de	2.36bc	3.70bc	5.47ab	7.09b	8.93ab	11.50d
	750	0.91c-e	2.30b-d	3.68bc	5.46ab	7.06b	8.79a-c	9.80ef
	1000	0.84de	2.34bc	3.67bc	5.48ab	7.15ab	8.78a-c	7.60g
Average of kind of soluble silicon								
sodium silicate		1.12a	2.31b	3.48b	5.09b	6.70b	8.33b	10.00b
potassium silicate		1.00b	2.11c	3.26c	4.78c	6.50c	8.16c	8.26c
Phyton		0.97b	2.45a	3.82a	5.59a	7.24a	8.89a	11.64a
Average of concentration								
0		1.25a	2.79a	4.22a	5.95a	7.62a	9.00a	15.80a
250		1.09b	2.40b	3.75b	5.51b	7.20b	8.91a	12.17b
500		0.98c	2.22c	3.44c	5.17c	6.94d	8.66b	9.47c
750		0.93c	2.04d	3.14d	4.71d	6.27d	8.01c	7.57d
1000		0.88c	2.01d	3.06d	4.42e	6.03d	7.73d	4.83e
C.V. (%)		14.47	9.50	7.61	6.92	5.02	3.39	6.55
Kind of soluble silicon (A)		**	***	***	***	***	***	***
Concentration (B)		***	***	***	***	***	***	***
A x B		ns	ns	***	***	***	***	***

[1/] Average of five replications. Means followed by common letters in a column are not significantly different at P = 0.01 by Duncan's Multiple Range Test.

[2/] Average of four fields in one replication.

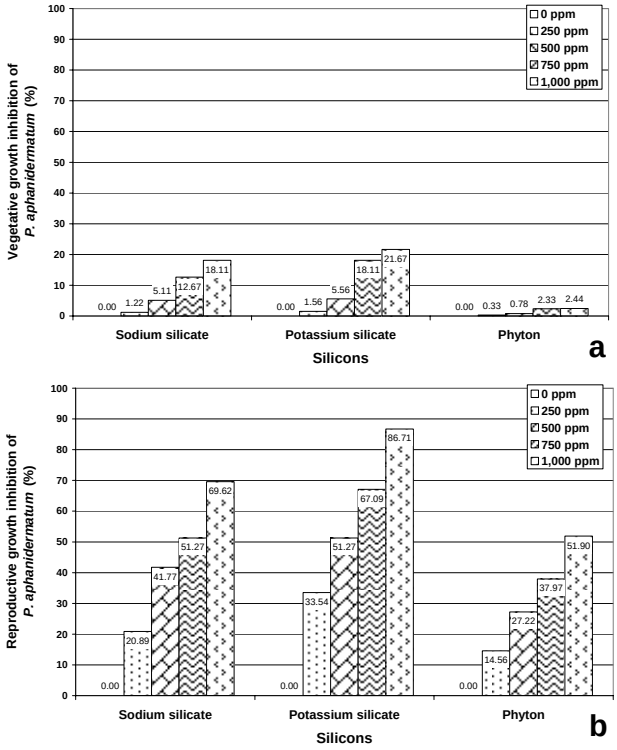

Fig. 1. Growth inhibition of *Pythium aphanidermatum* on PDA added with 3 kinds of soluble silicon (sodium silicate, potassium silicate and Phyton) at 5 concentrations (0, 250, 500, 750 and 1,000 ppm); a.: vegetative growth inhibition (at 36 hours), b.: reproductive growth inhibition (sporangia induced by distilled water at 24 hrs).

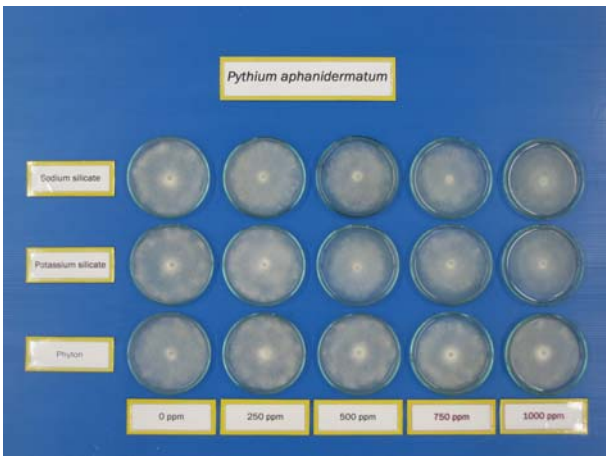

Fig. 2. Growth of *Pythium aphanidermatum* on PDA added with 3 kinds of soluble silicon (sodium silicate, potassium silicate and Phyton) at 5 concentrations (0, 250, 500, 750 and 1,000 ppm) at 36 hrs.

Table 2. Effect of soluble silicon on growth of *Trichoderma harzianum* (every 12 hrs) on PDA added with 3 kinds of soluble silicon (sodium silicate, potassium silicate and Phyton) at 5 concentrations (0, 250, 500, 750 and 1,000 ppm) and its conidial amount at 72 hours.

Treatment		Colony diameter of *Trichoderma harzianum* (cm)						Amount of conidia ($x10^7$ conidia/ml)
Kind of soluble silicon	Concentration (ppm)	12 hrs	24 hrs	36 hrs	48 hrs	60 hrs	72 hrs	
sodium silicate	0	1.50a[1/]	2.77a	4.25a	5.78a	7.57a	9.00a	71.44a
	250	1.11b	2.24b	3.57b	4.86c	6.40b	7.77d	40.76cd
	500	0.78c	1.63c	2.93c	4.08d	5.47c	6.74e	29.92e
	750	0.58d	1.15d	2.21d	3.34e	4.54d	5.85f	14.62f
	1000	0.50d	0.94ef	1.81e	2.86f	4.09e	5.28g	9.16f
Potassium silicate	0	1.50a	2.77a	4.25a	5.78a	7.57a	9.00a	71.44a
	250	1.09b	2.22b	3.56b	4.78c	6.24b	7.59d	37.92cd
	500	0.77c	1.61c	2.89c	4.05d	5.34c	6.49e	25.86e
	750	0.54d	1.09de	2.07d	3.16e	4.40de	5.56fg	11.88f
	1000	0.50d	0.78f	1.62e	2.57g	3.72f	4.82h	7.98f
Phyton	0	1.50a	2.77a	4.25a	5.78a	7.57a	9.00a	71.44a
	250	1.49a	2.76a	4.18a	5.68ab	7.47a	8.93ab	48.36b
	500	1.48a	2.76a	4.13a	5.58ab	7.29a	8.76a-c	43.74bc
	750	1.47a	2.76a	4.13a	5.57ab	7.23a	8.66bc	39.86cd
	1000	1.44a	2.72a	4.05a	5.40b	7.21a	8.55c	36.56d
Average of kind of soluble silicon								
Sodium silicate		0.89b	1.75b	2.95b	4.18b	5.61b	6.93b	33.18b
potassium silicate		0.88b	1.69b	2.88b	4.07b	5.45c	6.69c	31.02b
Phyton		1.48a	2.75a	4.15a	5.60a	7.35a	8.78a	47.99a
Average of concentration								
0		1.50a	2.77a	4.25a	5.78a	7.57a	9.00a	71.44a
250		1.23b	2.41b	3.77b	5.11b	6.70b	8.09b	42.35b
500		1.01c	2.00c	3.32c	4.57c	6.03c	7.33c	33.17c
750		0.86d	1.67d	3.80d	4.02d	5.39d	6.69d	22.12d
1000		0.81d	1.48e	2.49e	3.61e	5.01e	6.22e	17.90e
C.V. (%)		7.20	6.19	5.49	4.51	4.31	3.09	13.37
Kind of soluble silicon (A)		***	***	***	***	***	***	***
Concentration (B)		***	***	***	***	***	***	***
A x B		***	***	***	***	***	***	***

[1/] Average of five replications. Means in a column followed by common letters are not significantly different at P = 0.01 by Duncan's Multiple Range Test.

For reproductive growth, the result was in line with that of vegetative growth. Any tested concentrations and kinds of soluble silicon showing the greatest retardation effect on mycelial growth of *T. harzianum* could also significantly reduce its conidial production (Table 2). 1,000 ppm and 750 ppm of potassium silicate and sodium silicate gave the greatest inhibition effect on

conidial production of *T. harzianum* by 88.83, 83.37, 87.18 and 79.54%, respectively. Amongst the tested soluble silicon, Phyton gave the least retardation effect (Table 2 and Fig. 3b). Especially at its 250 ppm, retardation effect was shown only 32.31%.

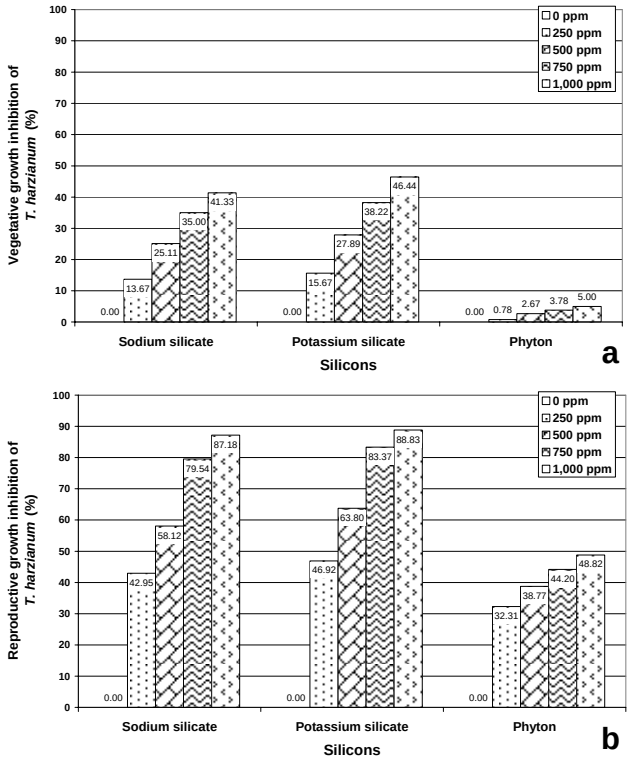

Fig. 3. Growth inhibition of *Trichoderma harzianum* (at 72 hrs) on PDA added with 3 kinds of soluble silicon (sodium silicate, potassium silicate and Phyton) at 5 concentrations (0, 250, 500, 750 and 1,000 ppm); a.: vegetative growth inhibition, b.: reproductive growth inhibition.

Interaction effect of soluble silicon and Trichoderma harzianum on the in vitro growth of Pythium aphanidermatum

Interaction effect of soluble silicon (3 kinds and 3 concentrations) and *T. harzianum* on growth of *P. aphanidermatum* was determined at 60 hrs by bi-culture antagonistic test. There was insignificant interaction with kinds and concentration of soluble silicon. *P. aphanidermatum* was significantly more inhibited by *T. harzianum* alone than that by *T. harzianum* in combination with soluble silicon (Table 3 and Fig.5). Furthermore, the combination of *T. harzianum* either with 250 or 500 ppm of Phyton as well as *T. harzianum* with 250 ppm sodium silicate resulted in the same retardation effect by 41.11, 40.89

and 40.67, respectively. Nevertheless, the other combinations gave less retardation effect on *P. aphanidermatum* compared to that of control (using *T. harzianum* alone) (Table 3 and Fig. 5).

Under light microscopic observations, it showed that *T. harzianum* could completely colonize over colony of *P. aphanidermatum* on PDA either added with soluble silicon or without. Hyphae of *T. harzianum* grew with coiling over the surface of *P. aphanidermatum* hyphae causing / leading to cell disruption (Fig. 6).

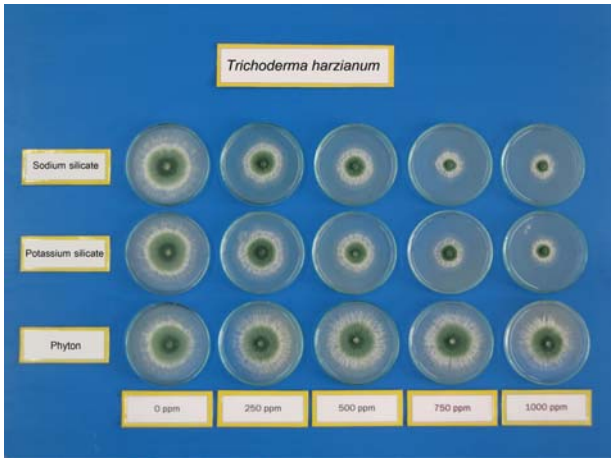

Fig. 4. Growth of *Trichoderma harzianum* on PDA added with 3 kinds of soluble silicon (sodium silicate, potassium silicate and Phyton) at 5 concentrations (0, 250, 500, 750 and 1,000 ppm) at 72 hrs.

Discussion

In this study, all tested soluble silicon could reduce the vegetative and reproductive growth of *P. aphanidermatum* and *T. harzianum in vitro*. The retardation effect of potassium silicate on both fungi was greater than those of sodium silicate and Phyton, respectively. Besides, their effect seemed to be increasing along with their increasing concentrations. Similar result to this research was previously shown that potassium silicate (20.7% silicon dioxide) could inhibit the mycelial growth of Pythiaceae fungi (e.g. *Phytophthora cinnamomi*, *P. capsici* and *Pythium* F-group) and Moniliaceae fungi (*Verticillium fungicola*) (Kaiser *et al*. 2005). Several studies were earlier reported on the ability of silicon in enhancing the Cucurbitaceae crop growth against root rot disease caused by *P. aphanidermatum* and *P. ultimum* (Cherif and Belanger, 1992; Cherif *et al*., 1994 and Belanger *et al*., 1995).

Table 3. Interaction effect of 3 kinds of soluble silicon (sodium silicate, potassium silicate and Phyton) at 3 concentrations (0, 250, and 500 ppm) and *Trichoderma harzianum* on growth of *Pythium aphanidermatum* by bi-culture antagonistic test at 60 hrs.

Biological control agent + Silicon		Growth inhibition of
Kind of soluble silicon	Concentration (ppm)	*Pythium aphanidermatum* (%) [1/]
sodium silicate	0	42.22a[2/]
	250	40.67ab
	500	38.22c
potassium silicate	0	42.22a
	250	39.78b
	500	38.00c
Phyton	0	42.22a
	250	41.11ab
	500	40.89ab
Average of kind of soluble silicon		
sodium silicate		40.37b
potassium silicate		40.00b
Phyton		41.41a
Average of concentration		
0		42.22a
250		40.52b
500		39.04c
C.V. (%)		2.98
Kind of soluble silicon (A)		**
Concentration (B)		***
A x B		ns

[1/] Growth inhibition (GI), GI = [(R1 – R2) / R1] x 100; R1 = colony diameter of *Pythium aphanidermatum* in control, R2 = colony diameter of *Pythium aphanidermatum* in bi – culture antagonistic plates.

[2/] Average of five replications. Means followed by common letters are not significantly different at P = 0.01 by Duncan's Multiple Range Test.

Regarding the interaction effect of soluble silicon and *T. harzianum,* it showed that no additional retardation effect of soluble silicon and *T. harzianum* on growth of *P. aphanidermatum*. Surprisingly, *T. harzianum* in combination with 250 ppm and 500 ppm of potassium silicate as well as *T. harzianum* with 500 ppm sodium silicate gave less inhibition effect on *P. aphanidermatum* growth than that of using *T. harzianum* alone. On this regard, there have been some studies on using *T. harzianum* for controlling *P. aphanidermatum* damping-off of pea, cucumber, tomato, pepper, gypsophila and Chinese kale (Sivan *et al*., 1984; Kanjanamaneesathian *et al*., 2003 and Jayaraj *et al*., 2006).

Further research on the retardation effect of soluble silicon and BCAs should be conducted for controlling *P. aphanidermatum* damping-off with thevarious crops *in vivo.*

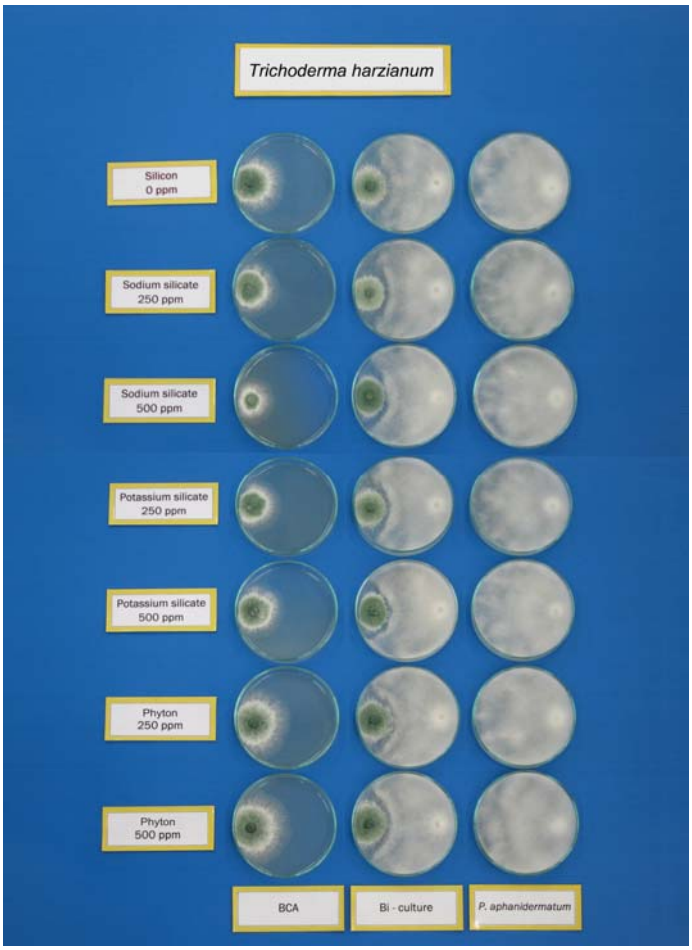

Fig. 5. Bi-culture antagonistic test (at 60 hrs) between *Trichoderma harzianum* and *Pythium aphanidermatum* grown on PDA added with 3 kinds of soluble silicon (sodium silicate, potassium silicate and Phyton) at 3 concentrations (0, 250, and 500 ppm).

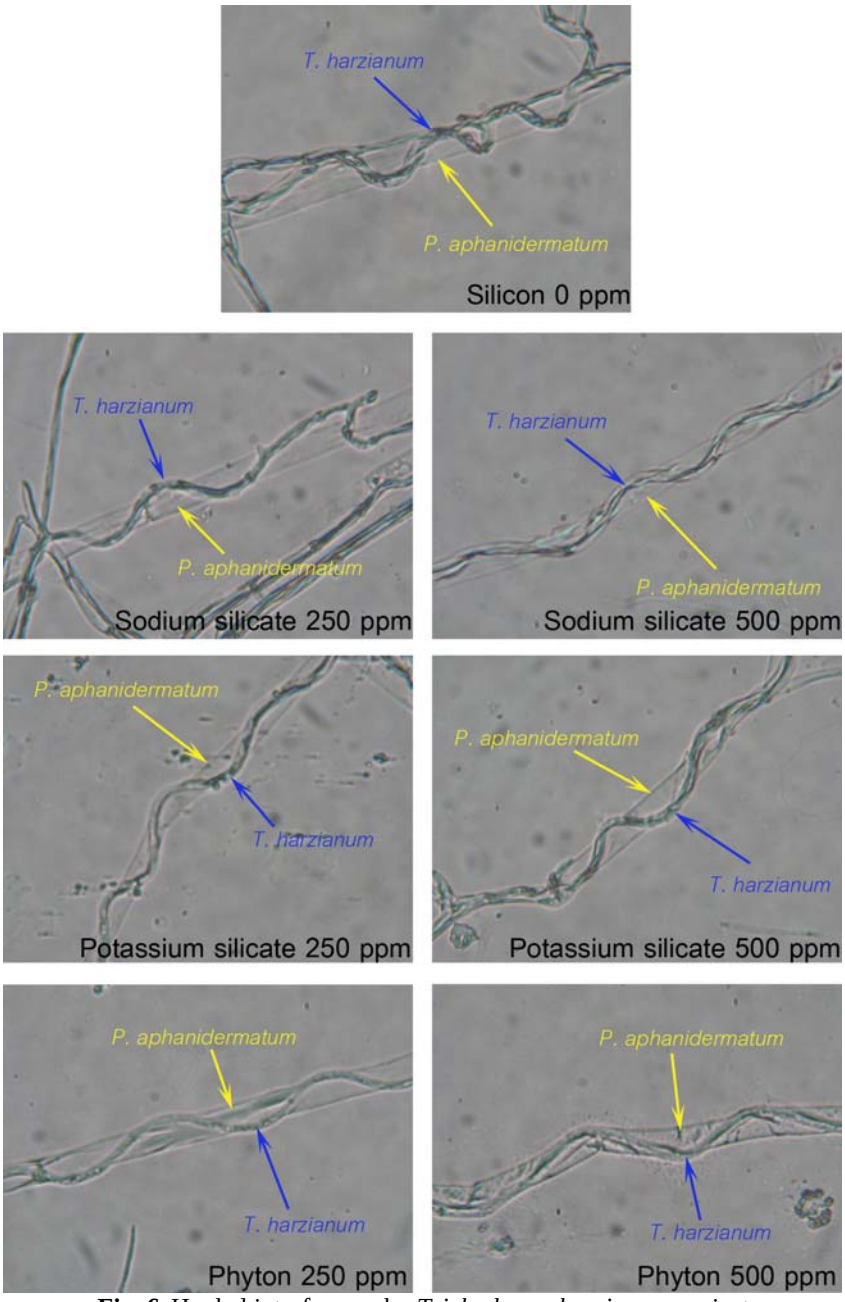

Fig. 6. Hyphal interference by *Trichoderma harzianum* against *Pythium aphanidermatum* (40X).

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