Plant Pathology Bulletin 18: 155-165, 2009

A Simple Technique, Concentration and Application Schedule for Using Neutralized Phosphorous Acid to Control Phytophthora Diseases

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Accepted for publication: September 19, 2009

ABSTRACT

Ann, P. J., Tsai, J. N., Wong, I. T., Hsieh, T. F., and Lin, C. Y. 2009. A simple technique, concentration and application schedule for using neutralized phosphorous acid to control Phytophthora diseases. Plant Pathol. Bull. 18: 155-165.

A simple method for application of phosphorous acid to control Phytophthora diseases was developed. The proposed method involves measuring equal weight of phosphorous acid and potassium hydroxide and dissolving phosphorous acid in water before adding potassium hydroxide. The pH of the neutralized phosphorous acid solution (NPA) is 6.2-6.7. The NPA concentration at 5000 mg/L was effective in controlling tomato seedling blight caused by *Phytophthora capsici* when using soil drench method. For suppression of lily leaf and blossom blight caused by *Phytophthora parasitica*, foliar application is much more effective than soil drench. However, NPA concentrations exceeding 2000 mg/L were phytotoxic to young plant tissues. The best results were achieved by applying NPA at the rate of 1000 mg/L for 3 times at one week intervals. Applying NPA to mother lily plants also effectively protected bulblets from infection by *P. parasitica* during storage periods.

Keywords: phosphorous acid, potassium hydroxide, *Phytophthora parasitica*, *Phytophthora capsici*, disease control, induced resistance

INTRODUCTION

In the 1980s, a systemic fungicide, fosetyl-Al (aluminum tris-o-ethyl phosphonate, commercially known as Aliette), which could effectively control many oomycete diseases, was developed [(3)]. The researchers further found that the major active component of the fungicide for disease control is its metabolites phosphorous ions [(3)]. This indicated that phosphorous acid itself can be used as a control agent. However, a water solution of phosphorous acid is strongly acidic with a pH value of 2-3, which is toxic to plant tissues if applied directly. Therefore, phosphorous acid must be neutralized with alkaline compounds before use. Today several commercial products of phosphorous compounds and its derivatives [(2, 20)] have been developed as fungicides or fertilizers which can be used to control a wide spectrum of

Phytophthora diseases [9, 11,13] and downy mildew diseases [2, 12, 20] in the field.

However, direct use of a neutralized phosphorous acid solution prepared by growers themselves for field disease control is rare because the procedure for neutralizing phosphorous acid by titration with alkaline compounds such as sodium hydroxide (NaOH), potassium hydroxide (KOH) or calcium hydroxide ($Ca(OH)_2$) is difficult for most farmers. This laboratory recently developed a very simple and practical method enabling farmers to prepare their own neutralized phosphorous acid solutions for field use. This study describes the neutralization procedure, application method and disease control effects of the neutralized phosphorous acid solution.

MATERIALS AND METHODS

Preparation of neutralized phosphorous solution (NPA) and pH measurement

High purity phosphorous acid (H_3PO_3, 99% white crystal, Aldrich Co.) and crude phosphorous acid (92-95% H_3PO_3, white crystal, industrial grade, Nippon Soda Co., Japan) were used. Experimental grade (≥85% KOH pellet GR, Merck Co.) and industrial grade (≥85% purity, white, thin piece, Nippon Soda Co., Japan) potassium hydroxide were used to neutralize highly purified and crude phosphorous acid, respectively. In a preliminary study, the pH values of 1000-5000 mg/L neutralized phosphorous acid solution were approximately 6.2 to 6.7 when an equal weight of potassium hydroxide was added to the phosphorous acid water solution (1-5 g in 1000 ml distilled water). To prepare a neutralized phosphorous acid solution, phosphorous acid and potassium hydroxide were separately dissolved in equal amounts of distillated water. Phosphorous acid solution was then added to the potassium hydroxide solution to produce the end concentrations of phosphorous ion ($^{-1}H_2PO_3$ plus $^{-2}H_2PO_3$) at 500, 1000, 2000, 3000 and 5000 mg/L. The pH values of the neutralized phosphorous acid solution at different concentrations were measured by a pH meter. Because potassium hydroxide releases heat in the dissolving process whereas phosphorous acid absorbs heat, for the safe reason in further use, the phosphorous acid was first dissolved in water before directly adding equal weights of potassium hydroxide to the phosphorous acid solution. The neutralized phosphorous acid solution (NPA) was applied to the tested plants within few hours to avoid oxidation of phosphorous ion in the air.

Pathogen used and preparation of inocula

The main pathogen used in this study was a single-zoospore isolate PPL15-1 (TARI P97024, A^1 mating type) of *Phytophthora parasitica* Dastur which was isolated from the diseased stem tissue of a lily (*Lilium* Oriental hybrid cv. 'Star Gazer') collected in Taichung, Taiwan. An additional isolate PCa 35 (TARI P97016, A^1 mating type) of *Phytophthora capsici* Leonian isolated from sweet pepper (*Capsicum annuum* L. cv. 'Blue star') in Nantou, Taiwan was also used to inoculate tomato seedlings.

Phytophthora isolates were cultivated on fresh 5% V8A at 24℃ for 3-5 days before use. Five percent V8A contains 5% V8 juice (Campbell Co.), 0.02% $CaCO_3$ and 1.5% agar (Hwei Shen Co., Taiwan). The method described by Hwang *et al.* [10] was used to produce large amounts of sporangia. Zoospore suspension was prepared by chilling the mycelial mats with sporangia at 15℃ for 30 min and moving back to 24℃ for another 30 min. Zoospore suspension was used as inocula for all inoculation studies and its concentration was adjusted to 5,000-10,000 zoospores ml^{-1} then placed in an icebox before inoculation. Inoculation was done within 30 min after preparation.

Test plant preparation, NPA application, pathogen inoculation and statistic analysis

Lily and tomato were used in the study of the effectiveness of NPA in controlling Phytophthora diseases in a greenhouse. The tested lily (*Lilium* Oriental hybrid cv. 'Star Gazer') bulbs with young shoots were imported from the Netherlands. Each bulb was grown in one plastic pot (13.5 cm diam. × 15 cm high) containing 1.2 L BIO-MIX (Tref Group Co., Netherlands) culture medium in the greenhouse at 25 to 28℃. Each lily plant was thoroughly sprayed with 50 ml NPA or distilled water 2 to 3 times at one week intervals unless indicated otherwise. The final NPA application was performed 10 days before the average time of initial flowering. Controls were sprayed with distilled water. Plants were inoculated 7 days after the last application. Each test plant with 3 to 5 flower buds

was sprayed with 20 ml zoospore suspension then covered with a translucent cellophane bag to maintain a highly moist condition for 3 days at room temperature (24-25℃). Disease incidence was recorded daily for up to 7 days by counting the numbers of infected flowers and leaves.

For tomato (*Lycopersicum esculentum* M. cv. Nung-Yu No.301' and cv. AVRDC No. 4) study, each germinating seed was planted in a plastic pot (9 diam.× 9 cm high) containing BIO-MIX culture medium. One-month-old seedlings were individually drenched with 50 ml NPA or distilled water once or twice at one week intervals. Seven days after the last application, a pipette was used to apply 0.5 ml of zoospore suspension of *P. capsici* to the stem base of each seedling. Disease infection rates were recorded by counting the numbers of wilted and dead seedlings every two days for 14 days after inoculation.

Effect of different concentrations of NPA on control of Phytophthora diseases of lily and tomato

Concentrations of NPA were prepared at 500, 1000, 2000, 3000 and 5000 mg/L. For lily study, different concentrations of 50 ml NPA were sprayed on each test plant two times at one week intervals. Disease incidence was determined 7 days after inoculation. At least 10 plants were treated with each NPA concentration, and the experiment was repeated once. For tomato study, each seedling was drenched with 50 ml NPA one or two times at one week intervals. Percentages of wilted seedlings were recorded 14 days after inoculation. Each treatment consisted of 36 to 40 seedlings and the experiment was repeated twice.

Effect of number of NPA application on control of lily Phytophthora blight

A NPA concentration of 1000 mg/L was used for the following study. Fifty milliliters of NPA or distilled water was sprayed on each test plant 1, 2 and 3 times at one week intervals. Each treatment contained 20 plants, and the experiment was repeated once.

Residual effect of NPA on control of lily Phytophthora blight

In order to understand the residue effect of phosphorous acid on control of lily Phytophthora blight, the test plants were inoculated 7, 14 and 21 days after NPA application. Fifty milliliters of NPA at 1000 mg/L was applied only once. Ten plants at least were used for each treatment and the experiment was repeated once.

Effect of NPA application sites on control of lily Phytophthora blight

The translocation ability of phosphorous ions in the treated lily plants was examined by applying NPA to different parts of plants or soil surface. The areas treated with NPA were (i) upper leaves of plant (about 10 leaves + flower buds), (ii) lower leaves of plant (about 10 leaves from the basal stem), (iii) whole plants and (iv) soil surface. The NPA at 1000 mg/L was applied to the label portion of the tested lily plants twice at one week intervals. Fifty milliliters of NPA were used for whole plant treatment and 25 ml was used for treating upper or lower areas. Soil drench application was done by pouring 100 ml of NPA on the soil surface around each tested lily plant. Each treatment consisted of 10 plants, and the experiment was repeated once.

Pretreatment of mother plants with NPA to control Phytophthora bulb blight of lily

This study is to examine the protective effect of NPA pretreatment on bulblets produced by mother plants. After all flowers withered, the healthy mother lily plants without phosphorous application and pathogen inoculation were kept in pots for 2 to 3 months until development of bulblets. Before bulb harvesting, each mother lily plant was drenched with 100 ml NPA at 1000 mg/L twice at one week intervals. The bulblets were harvested 7 days after the last NPA application and stored in plastic bags at 2 to 5℃ for 1 month. Afterwards, bulblets were inoculated with *P. parasitica* by dipping them in the zoospore suspension for 1 min. The inoculated bulblets were kept in a moist chamber at 24℃, and infection rates were counted 10 days after inoculation. Twenty bulblets were inoculated, and the experiment was repeated once. To elucidate the effect of NPA on detached plant tissues, bulb scales picked from the mother bulbs were also inoculated and investigated at the same time. The number of inoculated scales, inoculation method and number of repeated tests were identical to that of bulblet inoculation.

Statistic analysis

Data were analyzed by analysis of variance (ANOVA), and means were compared by least significant difference test (LSD) at P=0.05.

RESULTS

Phosphorous acid solution preparation

The pH values of the water solution of phosphorous acid, potassium hydroxide and neutralized phosphorous acid (NPA) at different concentrations were 1.85 to 2.34, 11.98 to 12.88 and 6.26 to 6.96, respectively (Table 1), when equal weights of phosphorous acid and potassium hydroxide were separately dissolved in water before adding phosphorous acid solution to the potassium hydroxide solution. The pH value of NPA at 1000 mg/L was 6.28 to 6.32 when NPA was prepared by dissolving high purity chemicals (experimental grade) in distilled water whereas the pH value was 6.65 to 6.71 when crude chemicals (industrial grade) and tap water were used. Similar pH values of neutralized phosphorous acid solution could be achieved by dissolving phosphorous acid crystals in the water before adding the potassium hydroxide pellets to the phosphorous acid solution, and *vice versa*.

Effect of different concentrations of NPA on control of Phytophthora diseases of lily and tomato

NPA at all tested concentrations could significantly decreased lily Phytophthora blight (Table 2). The effectiveness of NPA in controlling blossom and leaf Phytophthora blight of lily increased as the applied concentration was increased. Applying 5000 mg/L NPA twice at one week intervals to lily plants completely inhibited lily Phytophthora blight induced by *P. parasitica*. For other treatments, two applications of 500, 1000, 2000 and 3000 mg/L of NPA decreased flower infection rates by 40 to70%, 70 to 85%, 75 to 90% and 90 to 95%, respectively. The infection rates of lily blossom blight in controls with pathogen inoculation were 50% and 75.8% in the two tests. Leaf blight in this study was less serious than blossom blight. Although high concentrations of NPA (such as 3000 and 5000 mg/L) were much more effective in control of lily Phytophthora blight, it also caused damage to above ground young tissues if concentrations were equal to or higher than 2000 mg/L. Higher NPA concentrations, *e.g.*, 5000 mg/L, suppressed the height of lily plant by up to 10% in comparison with control plants. In a preliminary study, the mature leaves of many plant species, including lily, orchids, gerbera, peppers, tomato, potato, citrus, papaya and lychee were not sensitive to high

Table 1. The pH values of the water solution of phosphorous acid, potassium hydroxide and neutralized solution

Chemical	Concentration (mg/L)	pH ranges	
		Experimental grade	Industrial grade
Distilled water[1]		6.40 - 6.73	
Tap water[1]			7.77 - 7.80
H_3PO_3	1000	2.15 - 2.34	2.41 - 2.46
KOH	1000	12.13 - 12.26	11.98 - 12.05
H_3PO_3 + KOH	500	6.28 - 6.30	6.63 - 6.71
H_3PO_3	2000	2.00 - 2.09	2.17 - 2.18
KOH	2000	12.42 - 12.43	12.36 - 12.39
H_3PO_3 + KOH	1000	6.28 - 6.32	6.65 - 6.71
H_3PO_3	4000	2.00 - 2.03	2.01 - 2.02
KOH	4000	12.69 - 12.72	12.63 - 12.65
H_3PO_3 + KOH	2000	6.26 - 6.35	6.64 - 6.96
H_3PO_3	6000	1.81 - 1.83	2.00 - 2.02
KOH	6000	12.82 - 12.89	12.73 - 12.79
H_3PO_3 + KOH	3000	6.35 - 6.45	6.40 - 6.52
H_3PO_3	10000	1.85 - 1.87	1.95 - 2.02
KOH	10000	12.85 - 12.88	12.83 - 12.88
H_3PO_3 + KOH	5000	6.30 - 6.31	6.51 - 6.56

[1] Distilled water was used as solvent for experimental grade of phosphorous acid and potassium hydroxide, whereas tap water for industrial grade of chemicals.

concentrations (up to 2000 mg/L) of NPA. However, high concentrations (>1000 mg/L) of NPA were toxic to the young tissues of some species of fruit trees, flowers and vegetables (Table 2 and unpublished data). Spraying NPA at 1500 mg/L caused browning of the fruit skin of treated sweet orange, papaya and lychee.

The effectiveness of NPA in controlling tomato seedling blight also increased with the increase of the number of applications and concentration (Tables 3). Two applications of 2000, 3000 and 5000 mg/L NPA on the soil surface were equally effective in decreasing basal stem rot. They were 0 and 8.3% of treated seedlings died in the two tests. Two applications of NPA at 1000 mg/L was the next effective with deaths of 10% and 22% seedlings, respectively. The treatment of 500 mg/L NPA, which resulted in 20% and 33.3% seedlings killed, was less effective. The controls were worst with mortality rates of 41.7% and 66.7% in the two tests.

Effect of number of NPA application on control of lily Phytophthora blight

The effect of NPA on controlling lily Phytophthora blight increased as number of applications (up to 3 times) increased (Table 4). Spraying lily plants with 1000 mg/L NPA three or four times completely inhibited Phytophthora leaf blight and almost completely inhibited Phytophthora blossom blight (Fig.1). One or two applications of NPA at the same concentration also significantly decreased Phytophthora infection in lilies and the disease was decreased by about 60% and 80 to 99% in comparison with controls in the two tests (Table 4).

Table 2. Effect of concentrations of neutralized phosphorous acid solution (NPA)[1] on control of Phytophthora blight of lily (*Lilium* Oriental hybrid cv. 'Star Gazer')[2]

NPA concentration	Infected flowers[3] (%)		Infected leaves[3] (%)	
(mg/L)	Test 1	Test 2	Test 1	Test 2
500	15.6 b[4] (69.2)[5]	44.8 b (40.9)	5.5 a (75.9)	4.5 a (82.1)
1000	7.4 ab (85.2)	25.0 ab (67.2)	0.0 a (100)	2.6 a (89.9)
2000	4.5 ab (91.0)	18.8 a (75.6)	0.0 a (100)	0.0 a (100)
3000*[6]	3.3 a (93.4)	9.8 a (87.2)	0.0 a (100)	0.0 a (100)
5000*	0.0 a (100)	0.0 a (100)	0.0 a (100)	0.0 a (100)
Control	50.0 c	75.8 c	22.6 b	25.3 b

[1] Phosphorous acid (H_3PO_3) was neutralized with equal weights of potassium hydroxide (both are of experimental grade).
[2] NPA was applied twice with one week intervals and inoculation was done 7 days after the second application.
[3] Infected flower and leaves were recorded 7 days after inoculation.
[4] Means followed by the same letters within each column are not significantly different at $p \leq 0.05$ according to LSD test.
[5] Data in parenthesis were disease reducing rate (%).
[6] *: Treated plants were stunting.

Table 3. Control of Phytophthora seedling blight of tomato var. "Nung-you No. 301' with neutralized phosphorous acid solution (NPA)[1]

NPA concentration	Application[2]	Seedling killed (%)[3]		
(mg/L)	(times)	Test 1	Test 2	Average
500	2	20.0 (52.9)[4]	33.3 (50.0)	26.7 b[5]
1000	2	10.0 (76.5)	22.2 (66.7)	16.1 ab
2000	2	0.0 (100)	8.3 (87.5)	4.2 a
3000	2	0.0 (100)	8.3 (87.5)	4.2 a
5000	2	0.0 (100)	8.3 (87.5)	4.2 a
Control	0	42.5	66.7	54.6 c

[1] Phosphorous acid (H_3PO_3) was neutralized with equal weight of potassium hydroxide (both are of experimental grade).
[2] One-month-old pot seedlings were, individually, pre-drenched with 50 ml of NPA for 3 times at one week intervals and then inoculated with 0.5 ml of zoospore suspension of *Phytophthora capsici*.
[3] Wilt and dead seedlings were recorded 14 days after inoculation.
[4] Data in parenthesis were disease reducing rate (%).
[5] Means followed by the same letters are not significantly different at $p \leq 0.05$ according to LSD test.

Residual effect of NPA on control of lily Phytophthora blight

The effect of shorter intervals (such as 7 days) between 1000 mg/L NPA application and pathogen inoculation on controlling lily blossom and leaf blight was better but not significantly different (p=00.5) from those of longer intervals (such as 14 or 21 days) (Table 5). Application of 1000 mg/L NPA 7 days before pathogen inoculation achieved best protection results followed by treatments of 14 and 21 days before pathogen inoculation. Inoculated lily flowers were infected at rates of 25.1% and 30.1%, 36.2% and 45.6%, and 45.1% and 45.2% at 7-, 14- and 21days intervals, respectively, between NPA treatment and pathogen inoculation. The two tests produced highest infection rates of 55.9% and 58.5% in control flowers, which were significantly higher (p=0.05) than any NPA treatment. Spraying NPA 14 and 21 days before pathogen inoculation could still significantly protect more than 90% of leaves from infection by *Phytophthora* compared to the leaves of controls, which were only about 70% disease free.

Effect of NPA application sites on control of lily Phytophthora blight

Table 6 shows the effect of translocation of NPA in lily tissues on control of Phytophthora blight. Spraying NPA at 1000 mg/L 3 times to the entire lily plants completely inhibited the development of Phytophthora disease. Spraying leaves 3 times in the upper half of plants plus flowers also completely inhibited upper leaf blight and blossom blight and partially decreased blight (51.2%) in the lower half of the plants. Spraying leaves 3 times in the lower half of the plants could effectively protected leaves in both parts of plants with reducing rates of 90.6% and 83.9% and were slightly decreased blight of flowers (60%), indicating the gradual upward movement of NPA in

Table 4. Effect of application times of neutralized phosphorous acid solution (NPA)[1] on control of Phytophthora blight of lily (*Lilium* Oriental hybrid cv. 'Star Gazer')

NPA treatment[2] (times)	Infected flowers[3] (%)		Infected leaves[3] (%)	
	Test 1	Test 2	Test 1	Test 2
1	25.5 b[4] (62.2)[5]	27.5 b (63.1)	15.8 b (35.2)	6.1 a (78.7)
2	12.8 ab (81.2)	3.3 a (95.6)	1.0 a (99.8)	3.6 a (87.4)
3	0.0 a (100)	0.0 a (100)	0.0 a (100)	0.0 a (100)
4	0.0 a (100)	0.6 a (99.2)	0.0 a (100)	0.0 a (100)
Control	67.5 c	74.5 c	24.4 b	28.6 b

[1] Phosphorous acid (H_3PO_3, conc. 1000 mg/L) was neutralized with equal weight of potassium hydroxide (both are of experimental grade).
[2] NPA was applied to the whole plants every 7 days and inoculation (at the same day for all treatments) was done 7 days after the last application.
[3] Infected flower and leaves were recorded 7 days after inoculation.
[4] Means followed by the same letters within each column are not significantly different at $p \leq 0.05$ according to LSD test.
[5] Data in parenthesis were disease reducing rate (%).

Table 5. Effect of duration between neutralized phosphorous acid solution (NPA)[1] application and pathogen inoculation on control of Phytophthora blight of lily (*Lilium* Oriental hybrid cv. 'Star Gazer')

Duration between NPA treatment and pathogen inoculation (days)	Infected flowers[2] (%)		Infected leaves[2] (%)	
	Test 1	Test 2	Test 1	Test 2
7	25.1 a[3] (55.1)[4]	30.1 a (48.5)	6.3 a (77.1)	4.9 a (80.9)
14	36.2 ab (35.2)	45.6 ab (22.1)	8.3 a (69.8)	6.6 a (73.2)
21	45.1 ab (19.3)	45.2 ab (22.7)	8.9 a (67.6)	6.9 a (74.3)
Control	55.9 b	58.5 b	27.5 b	25.7 b

[1] Phosphorous acid (H_3PO_3, conc. 1000 mg/L) was neutralized with equal weights of potassium hydroxide (both are of experimental grade).
[2] NPA was applied to the whole plants and infection rates were recorded 7 days after inoculation.
[3] Means followed by the same letters within each column are not significantly different at $p \leq 0.05$ according to LSD test.
[4] Data in parenthesis were disease reducing rate (%).

lily plants during the growth stage. Soil drench with NPA was ineffective in controlling the aboveground Phytophthora diseases of lily. Tested plants showed 23.3% blighted flower and 24.7% rotted leaves. The infection rates of flower, upper leaves and lower leaves in controls were 43.8, 35.0 and 20.5%, respectively.

Two applications of NPA on different areas of lily plants were less effective in control of lily Phytophthora blight compared to three applications (Table 6). The infection rates of flower, upper leaves and lower leaves were 3.3, 2.8 and 2.3%, respectively, for entire plant treatment, 5.3%, 4.7% and 22.5%, respectively, for upper half plant treatment, and 50.0%, 17.0% and 11.9%, respectively, for lower half plant treatment. Soil drench produced 52.0% infected flower and 26.3% infected leaves which were not significantly different from those of controls. The infection rates of flowers and whole leaves were 73.7% and 31.5%, respectively, in controls.

Fig. 1. Control of Phytophthora flower blight of pot lily with neutralized phosphorous acid (NPA) at 1000 mg/L. (NPA was sprayed to the whole plants 3 times at one week intervals before flowering)

Effect of pretreatment of mother plants with NPA on controlling Phytophthora bulb blight in lily plants

Applying NPA at 1000 mg/L to the soil surface of growing mother lily plants effectively decreased bulblet infection by *P. parasitica* during storage periods (Table 7, Fig. 2). In the two tests, infection rates in inoculated bulblets were 30% and 0% after one application of NPA and 20% and 0% after two applications, in contrast with infection rates of 100% and 60% in controls. The percentages of disease-free bulblets were significantly different between NPA treatments and controls but didn't differ between the two NPA treatments. However, the same treatment was ineffective in controlling Phytophthora blight in individual detached scales already separated from the mother bulbs (Table 7). The infection rates were 65% and 80% after one application of NPA, 45% and 70% after two applications and 80% and 80% for controls.

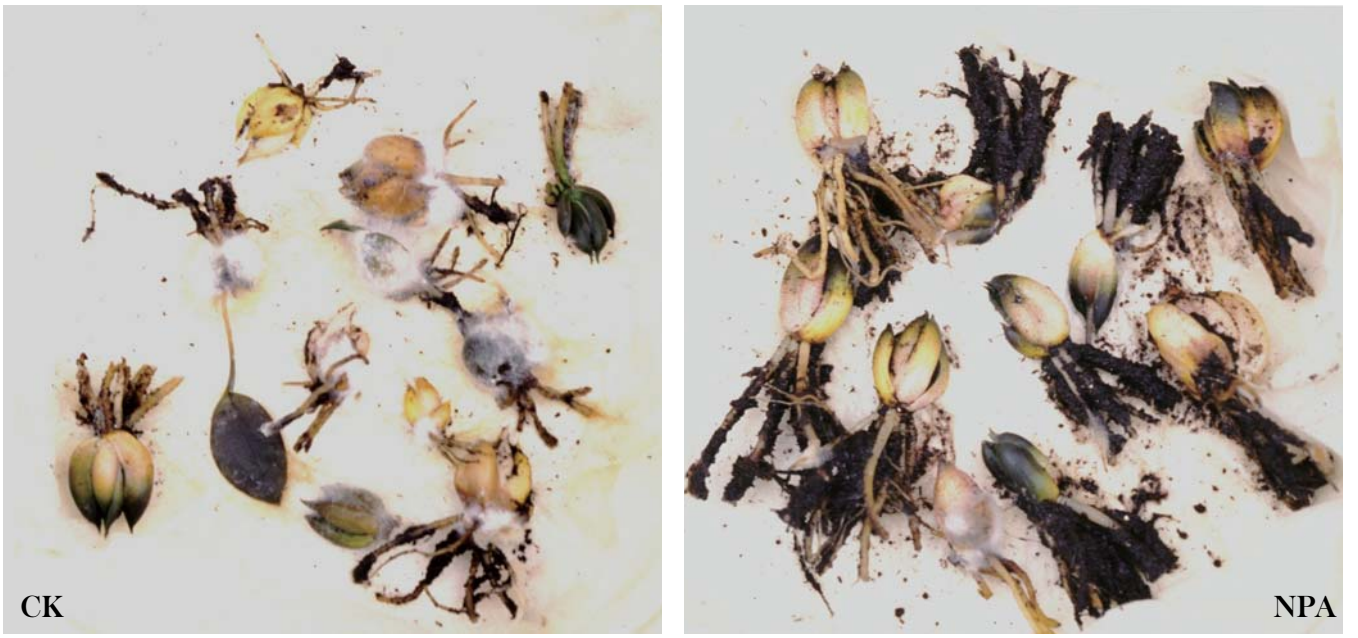

Fig. 2. Control of Phytophthora bulb blight of lily with neutralized phosphorous acid (NPA) at 1000 mg/L. (NPA was drenched to soil surface 2 times at one week intervals before harvest)

Table 6. Effect of different part treatments with neutralized phosphorous acid solution (NPA)[1] on control Phytophthora blight of lily (*Lilium* Oriental hybrid cv. 'Star Gazer')

Portions of lily Treated	NPA (application & times)[2]	Infected flowers (%)[3]	Infected leaves (%)[4]		
			Upper leaf of plant	Lower leaf of plant	Whole plant
Test 1					
Whole plant	Spray, 2	3.3 a[5] (95.5)[6]	2.8a (92.8)	2.3 a (90.3)	2.5 a (92.1)
Upper half of plant	Spray, 2	5.3 a (92.8)	4.7 ab (87.9)	22.5 b (5.5)	12.2 ab (61.8)
Lower half of plant	Spray, 2	50.0 b (31.8)	17.0 b (56.3)	11.9 ab (50.0)	13.6 ab (56.8)
Soil surface	Soil drench, 2	52.0 b (29.3)	—[z]	—	26.3 bc (16.5)
Control	0	73.7 b	38.9 c	23.8 b	31.5 c
Test 2					
Whole plant	Spray, 3	0.0 a (100)	0.0 a (100)	0.0 a (100)	0.0 a (100)
Upper half of plant	Spray, 3	0.0 a (100)	0.0 a (100)	10.0 a (51.2)	5.0 a (81.8)
Lower half of plant	Spray, 3	17.7 ab (60.0)	3.3 a (90.6)	3.3 a (83.9)	3.3 a (88.0)
Soil surface	Soil drench, 2	23.2 b (47.0)	—	—	24.7 b (10.2)
Control	0	43.8 c	35.0 b	20.5 b	27.5 b

[1] Phosphorous acid (H_3PO_3, conc. 1000 mg/L) was neutralized with equal weights of potassium hydroxide (both are of experimental grade).
[2] NPA was applied every 7 days and inoculation was done 7 days after the last application.
[3] Infected flowers and leaves were recorded 7 days after inoculation.
[4] Means followed by the same letters within column within the same test are not significantly different at $p \leq 0.05$ according to LSD test.
[5] Data in parenthesis were disease reducing rate (%).
[6] Not measured.

Table 7. Effect of pretreatment of neutralized phosphorous acid solution (NPA)[1] to mother plants on control of Phytophthora bulb blight of lily (*Lilium* Oriental hybrid cv. 'Star Gazer')

NPA application[2] (times)	Infected bulblets[3] (%)			Infected detached scales[3] (%)		
	Test 1	Test 2	Average	Test 1	Test 2	Average
1	30 (70)[4]	0 (100)	15 a[5]	65 (18.8)	80 (0)	72.5 a
2	10 (90)	0 (100)	5 a	45 (43.8)	70 (12.5)	57.5 a
None, Control	100	60	80 b	80	80	80.0 a

[1] Phosphorous acid (H_3PO_3, conc. 1000 mg/L) was neutralized with equal weights of potassium hydroxide (both are of experimental grade).
[2] Mother plants were, individually, drenched with 100 ml of NPA once or twice at one week intervals before bulb harvesting.
[3] Bulblets and scales picked from mother bulbs were dipped in zoospore suspension of *Phytophthora parasitica* and infected bulblets and scales were recorded 10 days after inoculation.
[4] Data in parenthesis were disease reducing rate (%).
[5] Means followed by the same letters within each column are not significantly different at $p \leq 0.05$ according to LSD test.

DISCUSSION

Phytophthora species are distributed worldwide and have caused serious economic losses in many crops including fruit trees, vegetables, flowers and forests (4). In Taiwan, Phytophthora diseases are very common due to the warm and moist weather, which is favorable for the pathogen propagation and dissemination and disease development (8). Synthetic fungicides are generally used by growers to control Phytophthora diseases. In order to reduce the impact of synthetic fungicides, searching for effective, safe and cheap materials as well as convenient and simple measures to control Phytophthora diseases have been the goals of many researchers for a long time. Commercial products containing phosphorous compounds, such as Foli-R-Fos 400 (20), Nutri-Phite P Foliar (6), Akomon-40 (2) and Potphos (2), could partially reach the purpose of replacing some of synthetic fungicides for Phytophthora and other oomycete disease control. However, none of those products are commonly used in Taiwan due to their high price or other reasons. An

alternative method for directly using phosphorous acid has been researched in this laboratory for many years. Because the water solution of pure phosphorous acid is approximately pH 2, which is phytotoxic to plant tissues, a neutralizing alkaline chemical is required. Potassium hydroxide was found suitable, and addition of equal weight (W/W) of this chemical to phosphorous acid solution could adjust the pH value to a weakly acidic condition. The pH values of the neutralized phosphorous acid solution (NPA) at 1000-5000 mg/L remain at approximately pH 6.2-6.7 whether experimental or industrial grade of chemicals was used. Therefore, NPA at this pH value range is, at appropriate concentrations, not phytotoxic to any of the plants tested by the authors (unpublished data), indicating that it might be safe for most other plant species. Growers can easily prepare a neutralized solution at 1:1 ratio by weight at concentrations of 1000, 2000 and 5000 mg/L (approximating 1000, 500 and 200 times dilution, respectively).

In Taiwan, lily Phytophthora blight is very common, and the entire lily plant is susceptible to *P. parasitica* [1]. Therefore, the lily was selected as the major model plant for testing the effectiveness of NPA in controlling Phytophthora diseases. Additionally, basal stem rot of tomato seedlings is also very serious in Taiwan and the plants were used for testing the effectiveness of NPA in soil drench study. In this study, NPA showed good results in reducing the incidence and severity of lily blight and tomato seedling blight caused by inoculation with *P. parasitica* and *P. capsici*, if right concentration, application schedules and application methods were used. These findings strongly indicate that the simple phosphorous acid preparation used in this study is practical and effective for field disease control. Generally, concentration and application methods significantly affected the effectiveness of phosphorous compounds in controlling diseases caused by *Phytophthora* and other oomycetes [3, 6, 11, 19]. NPA at higher concentrations (*e.g.*, 2000-5000 mg/L) plus multiple applications (such as two or three applications) proved more efficient in reducing incidence of lily and tomato Phytophthora diseases than lower concentrations (such as 500-1000 mg/L) in a single application. However, foliar application of NPA at concentrations equal to or exceeding 2000 mg/L damaged young tissues of most tested plants, and 5000 mg/L particularly stunted lily plant growth (Table 3). Walker [18] and Washington *et al.* [19] also reported that foliar application of phosphorous compounds at high concentrations caused plant phytotoxicity. In this case, increase of number of applications was more important for increasing the effectiveness of lower NPA concentrations in controlling foliar, blossom and fruit diseases. Two or three applications of 1000 mg/L NPA were more effective in controlling lily Phytophthora blight than one application at the same concentration. The disease was decreased by about 40-60, 80-90 and 90-100% after one, two and three applications, respectively, of NPA at 1000 mg/L in most tests. In terms of methods of applying NPA, soil drench was suitable for controlling root and basal stem diseases whereas foliar spray was more efficient in reducing leaf, blossom and fruit diseases caused by *Phytophthora* species. In studies by this research team, applying NPA by soil drench rather than foliar spray (unpublished data) effectively decreased the Phytophthora basal stem rot of tomato seedlings. However, foliar spray with NPA rather than soil drench was more efficient in controlling the aboveground Phytophthora diseases in lily plants (Table 6).

Many reports have indicated that phosphorous compounds at high concentrations can function as fungicides capable of killing pathogens and protecting host plants directly [5]. At the same time, substantial evidences showed that the chemicals indirectly induce host resistance against pathogen attacking [15]. Currently, most researchers believed that phosphorous acid has both direct (plant protection) and indirect (host defense) modes of action [7, 16, 17]. The indirect effect of phosphorous acid in controlling Phytophthora diseases was also noted by the authors. When the mother lily plants were treated with NPA twice, their bulblets were almost completely free from infection (0-10% infected) by *Phytophthora* whereas the detached scales were 45% and 70% infected. These experimental results indicate that the resistant systems in bulblets could be strongly activated after inoculation with *Phytophthora* but less in their detached scales. Increasing plant resistance induced by phosphorous acid treatment is another important reason for recommending NPA to growers; and preventive measures during pre-infection are more important than post-infection control measures. Due to the

mechanism of disease control, NPA should be applied before symptoms appear during the rainy season rather than after occurrence of the diseases.

Additionally, NPA may serve as a nutrient for plant use since phosphorous acid ions (as PO_3H^{-2}or $PO_3H_2^{-1}$) in the soil are slowly oxidized to phosphoric acid, which provides phosphates for further plant growth [14]. Meanwhile potassium hydroxide provides plants with potassium ion. In addition, industrial grade phosphorous acid and potassium hydroxide are relatively cheaper than commercially available phosphorous compound products. Therefore, NPA is suitable highly promising for disease control in the field in terms of efficiency, ease of application, economy, safety and wide-spectrum protection.

ACKNOWLEDGMENTS

The authors thank Dr. Wen-hsiung Ko and Lih-Ling Chern for review of the manuscript and Dr. Lu, Hsiou-Ying for assistance of statistic analysis.

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摘 要

安寶貞[1, 4]、蔡志濃[1]、王姻婷[1]、謝廷芳[2]、林俊義[3]. 2009. 利用亞磷酸簡便調配技術、合適濃度及施用方法防治作物疫病. 植病會刊 18: 155-165. ([1]台中霧峰 行政院農委會農業試驗所植物病理組，[2]雲林古坑 行政院農委會農業試驗所花卉研究中心，[3]台中霧峰 亞洲大學健康學院，[4]聯絡作者，電子郵件：pjann@tari.gov.tw；傳眞：+886-4-2330-2803)

研發一種簡便調製中和亞磷酸水溶液的技術，可用來防治作物疫病，該方法將亞磷酸 (H_3PO_3) 與氫氧化鉀 (KOH) 以 1:1 (w/w) 等重稱量後，先將亞磷酸溶解於水中後，再將氫氧化鉀加入溶解，該中和後的亞磷酸水溶液 (neutralized phosphorous acid solution, 簡稱 NPA) 的酸鹼值爲 pH6.2~6.7。防治由 *Phytophthora capsici* 引起的番茄幼苗根莖部疫病時，以較高濃度的 NPA (如 5000 mg/L) 灌注根圈土壤的防治效果較佳。而防治由 *P. parasitica* 引起的百合地上部花器與葉片疫病時，以葉面噴部的效果較土壤灌注爲佳。但 NPA 施用於地上部時，濃度超過 2000 mg/L 時易造成花器與幼嫩組織藥害。試驗中最佳的組合施用方法爲，NPA 的施用濃度爲 1000 mg/L，每隔 7 天施用一次，連續三次，防治百合疫病的效果最佳。此外，NPA 施用於百合母株及其根圈土壤，亦能保護貯藏期的小種球，降低被疫病菌感染的機率。

關鍵詞： 亞磷酸、氫氧化鉀、*Phytophthora parasitica*、*Phytophthora capsici*、疫病防治、誘導性抗病