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Effect of Potassium Phosphonate on the Control of Phytophthora Root Rot of Lettuce in Hydroponics

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The effect of potassium phosphonate (KH_2PO_3 or K_2HPO_3) on the control of Phytophthora root rot of lettuce was evaluated in a liquid hydroponic culture. Phosphonate 100 ppm strongly inhibited mycelial growth of *Phytophthora* species *in vitro* but did not affect normal growth of lettuce in a greenhouse test. Application of the chemical before infection showed over 94% control value, while it was less than 35% when applied after infection. In a field trial, phosphonate 100 ppm, which was directly supplemented into the nutrient solution, satisfactorily controlled the disease as it did not develop until 28 days after transplanting and remained at less than 2% infection rate at the end of cultivation. Meanwhile, in the control plot, the disease initiated at 7 days after transplanting and developed rapidly reaching over 70% infection rate at 28 days. Population density of the causal pathogen, *P. drechsleri*, in a heavily infested farm was 22.0-25.0 cfu/100 ml of nutrient solution. However, it decreased to 1.3-2.0 cfu/100 ml at 7 days after treatment with phosphonate 200 ppm.

Keywords : control, hydroponics, lettuce, phosphonate Phytophthora root rot.

Phytophthora root rot of lettuce caused by *P. drechsleri* severely occurs in liquid hydroponic culture in Korea. The disease develops in all seasons and appears to be the major limiting factor in cultivation, showing over 90% infection rate in heavily infested farms (Jee et al., 2001). In hydroponics, once an aquatic pathogen like *Phytophthora* has been introduced into the unit, disease losses are often greater than in soil because of the rapid dispersal mechanism of the pathogen and since cultural environments favor the disease development (Jee et al., 2000; Stanghelline and Rasmussen, 1994).

Cultural and physical methods such as sanitation, filtration, sonication, ozonation, ultraviolet irradiation, and thermal inactivation are being practiced to control diseases in hydroponics. Biological methods using antagonistic microorganisms are also applicable to suppress diseases under the system (Jee et al., 2000; Stanghelline and Rasmussen, 1994). However, no effective strategies are available to growers for the control of Phytophthora root rot of hydroponically-grown lettuce especially with heavy contamination by the pathogen.

Direct addition of fungicides into the re-circulating nutrient solution could be an effective method to control the disease. However, it is apparently dangerous because of the lag period between the chemical application and the lettuce harvest, which is being done every day in commercial farms. Also, no fungicides are registered for use in hydroponics in Korea. As experienced by the authors, metalaxyl was detected in lettuce leaves up to 7 days at a concentration of over the maximum residue limit (MRL: 2 ppm) when the chemical was directly amended into the nutrient solution with 10-folds dilution of field dose (unpublished data).

Phosphorous acid (H_3PO_3) is known to effectively control various diseases caused by Oomycetes (Forster et al., 1998; Guest et. al., 1995; Jee et al., 2000). The chemical is primarily responsible for the fungicidal activity of fosetyl-Al as a breakdown product in plant tissues and is the only available phloem-translocated fungicide that moves upward and downward through the xylem and phloem (Fenn and Coffey, 1985; Guest et al., 1995; Ouimette and Coffey, 1989a). Since the chemical showed high preventive and curative effects on the control of various *Phytophthora* diseases and exhibited low mammalian toxicity similar to that of phosphate and less than that of aspirin (Dunhill, 1990), the possibility of its use in hydroponics to control the Phytophthora root rot of lettuce was explored in this study. Recently, similar studies on the control of Phytophthora root and crown rot of tomato and pepper in hydroponics have been reported by Foster et al. (1998) to have excellent control effects.

Materials and Methods

Effects of phosphonate on the growth of *Phytophthora* and

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lettuce in hydroponics. Phosphorous acid (Aldrich, 99%) used in this study was partially neutralized with potassium hydroxide (KOH) to yield mono- and dipotassium phosphonate ions (KH_2PO_3 and K_2HPO_3). The phosphonate stock solution adjusted to pH 6.0 was filtered through a Millipore filter (0.2 μm) and stored in a refrigerator in the dark. The corn meal agar (CMA, Difco, 17 g/*l*) was supplemented with aliquot amount of the stock solution after autoclaving to make 0, 50, 100, 200, 500 ppm. Isolates of *Phytophthora drechsleri, P. capsici*, and *P. nicotianae* tested in this study were obtained from the authors previous studies (Jee et al., 2000). These isolates grown on CMA were cut into small pieces, inoculated onto the center of each medium, and incubated at 24°C in the dark. Radial mycelial growth was measured every 2 days.

A simple hydroponic system was prepared by using a rectangular plastic box 50 × 40 × 10 cm in size in a greenhouse. Twenty-one-day old lettuce seedlings grown in 128-hole tray were transplanted into the hydroponic system: seedlings with soils were pulled out from the holes, wrapped with thin non-woven fabric sheets (12 × 12 cm), and inserted into the holes (28 mm in diameter) on a styrofoam plate floating on nutrient solution. Nutrient solution used in this study was prepared following the standard of the National Horticultural Research Institute (NHRI), Korea. The phosphonate was supplemented into the nutrient solution to make 0, 50, 100, 200, and 500 ppm, respectively. Sixteen plants were used for each treatment and the experiment was repeated once.

Application timing of phosphonate on the control of lettuce Phytophthora root rot. Two- to three-week old lettuce seedlings growing in the hydroponic system as described above were tested in this study. Neutralized phosphonate was directly added to the nutrient solution at three different application times: 4 days before, on the same day, and 4 days after inoculation with *P. drechsleri,* 5.5 zoospores/ml of nutrient solution. The disease was recorded at 14 days after inoculation and plants showing wilt due to root decay were counted as infected.

Effect of phosphonate on population changes of *P. drechsleri* in a naturally infested hydroponic farm. A hydroponic farm located at Sangil-dong, Gangdong-gu, Seoul was heavily infested by the lettuce root rot pathogen, *P. drechsleri,* showing over 90% infection rate (Table 4). In order to determine population density of the pathogen in the nutrient solution, about 2 liters of nutrient solution was randomly sampled from several places in a hydroponic unit. Twenty milliliters (20 ml) of the nutrient solution was directly flooded onto a *Phytophthora* selective medium (PSA) modified by Jee et al. (2000). The plate (9 cm in diameter) containing 20 ml of the solution was incubated for 48 h at 24°C in the dark. Plates were washed with tap water after incubation and dried in a clean bench. Colonies that formed on the medium were observed under a microscope. Mycelial tips growing out from the colony were transplanted on a new PSA for identification. The PSA consisted of 5% clarified V8 juice agar supplemented with pimaricin 10 ppm, ampicillin 100 ppm, rifampicin 10 ppm, hymexazol 25 ppm, and PCNB 50 ppm.

To determine the effects of phosphonate on population changes of the pathogen and the disease severity in the farm, phosphonate 200 ppm was directly added into the nutrient solution tank. Population density of the pathogen was determined as described above at 7 and 14 days after treatment. In each test, at least 100 ml of nutrient solution was examined and the experiment was repeated at least once. Incidence of the disease was also recorded at 7 and 14 days after treatment, with the plants showing wilt considered as diseased.

Field trial of phosphonate for the control of lettuce Phytophthora root rot. This experiment was conducted in a naturally infested hydroponic farm at Sangil-dong, Gangdong-gu, Seoul. Neutralized phosphonate was directly amended into a nutrient solution tank prior to transplanting the 3-week old lettuce seedlings on beds of the liquid hydroponic system. Final concentration of the chemical in the nutrient solution was adjusted to 100 ppm. Another hydroponic unit in the same farm was used as control. Plants in both units were grown by conventional cultivation practice under the same condition and greenhouse.

Results

Effect of phosphonate on growth of *Phytophthora* and lettuce in hydroponics. Mycelial growth of *Phytophthora drechsleri, P. capsici*, and *P. nicotianae* was greatly inhibited by phosphonate. Phosphonate 50 ppm and 100 ppm inhibited mycelial growth of the pathogen 45.5-60.2% and 80.0-87.5%, respectively, and none of the isolates grew at 200 ppm (Table 1). However, lettuce growth was not significantly affected at concentrations of up to 200 ppm in the hydroponic system. The weight of lettuce leaves at concentrations of up to 200 ppm from the first and second harvest ranged from 8.8-9.0 g/plant and 11.3-12.1 g/plant, respectively, which did not differ statistically (Table 2). Meanwhile, lettuce plants grown in the nutrient solution containing phosphonate 500 ppm were slightly inhibited.

Effect of application timing of phosphonate on the control of the disease. When phosphonate was applied into the nutrient solution 4 days before inoculation with *P. drechsleri*, control efficacy was highest showing over 76% and 94% at 50 ppm and 100 ppm, respectively. Meanwhile, the values were only 0 and 33.3% at the same concent-

Table 1. Effect of phosphonate on mycelial growth of *Phytophthora* species

Concentration of H_3PO_3 (ppm)	Mycelial growth (mm/24 h)		
	P. drechsleri	*P. capsici*	*P. nicotianae*
0	6.5±0.4	8.8±0.4	4.0±0.4
50	3.5±0.3	4.7±0.3	2.2±0.3
100	1.3±0.3	1.8±0.3	0.5±0.3
200	0	0	0
500	0	0	0

*Values are mean of two replicates. Isolates were cultured on CMA at 25°C in the dark.

Table 2. Effect of phosphonate on growth of lettuce in hydroponics

Concentration of H_3PO_3 (ppm)	Fresh weight (g)/plant		
	Leaf harvest		Root
	1st	2nd	
0	8.8a	11.8a	4.0a
50	8.8a	12.0a	4.1a
100	9.1a	11.3a	4.2a
200	9.0a	12.1a	4.2a
500	8.3ab	10.2b	3.9a

*Different letters in the column mean significant difference at 5% level by Tukey-Kramer Honestly Significant Difference (HSD).

Table 3. Effect of application timing of phosphonate on the control of Phytophthora root rot of lettuce in hydroponics

Concentration of H_3PO_3 (ppm)	*Application time and disease incidence (%)*		
	4 days before	0 day	4 days after
0	100a	100a	100a
50	23.5b	41.5b	100a
100	5.9c	16.7bc	66.7b
200	0c	0c	26.7c
500	0c	0c	20.0c

*Different letters in the column mean significant difference at 5% level by Tukey- Kramer Honestly Significant Difference (HSD).

rations when the chemical was applied 4 days after inoculation (Table 3). When the chemical and the pathogen were inoculated at same time, Phytophthora root rot of lettuce did not develop at 200 ppm and showed only 16.7% infection rate at 100 ppm. However, non-treated control plots showed 100% infection rate (Table 3).

Effect of phosphonate on population changes of *P. drechsleri* in a naturally infested hydroponic farm. Infection rates of 95.8% and 97.2% in two naturally infested units were observed in lettuce showing severe wilt due to root rot caused by *P. drechsleri* (Fig. 2A). Population of the pathogen in the nutrient solution, expressed as colony forming unit (cfu), were measured as 22.0-25.0 cfu/100 ml. However, the fungal population decreased to 1.3-2.0 cfu/100 ml while the disease incidence decreased to 15.9% and 18.5% one week after supplementing phosphonate 200 ppm into the nutrient solution (Fig. 2B). Two weeks after treatment, the pathogen population slightly increased to 3.0-4.4 cfu/100 ml, but the disease remained similar or slightly decreased to 14.2-17.1% (Table 4).

Control of Phytophthora root rot of lettuce by phosphonate in a farm. In the control plot, Phytophthora root rot of lettuce started to develop 7 days after transplanting into the hydroponic system showing 3.3% infection rate. The disease increased drastically after 3 weeks showing over 60% and reached to 75% infection rate after 5 weeks (Fig. 1). Meanwhile, the disease did not occur until 3 weeks after transplanting in the plot treated with phosphonate 100 ppm and remained at less than 2% infection rate until 6 weeks (Figs. 2C and 2D). From the phosphonate-treated plot and the control plot, lettuce leaves were harvested four times and yielded 53.6 kg and 9.3 kg, respectively.

Table 4. Effect of phosphonate on population changes of lettuce root rot pathogen, *Phytophthora drechsleri*, and suppression of the disease in a naturally infested hydroponic farm

Hydroponic unit	Disease incidence (%)			No. of cfu of *P. drechsleri*/100 ml		
	0	7	14d	0	7	14d[a]
A	95.8	15.9	14.2	22.0±13.0	1.3±0.8	3.0±2.1
B	97.3	18.5	17.1	25.0±15.8	2.0±0.5	4.4±2.5

[a]Days after phosphorous acid 200 ppm was supplemented into the nutrient solution.

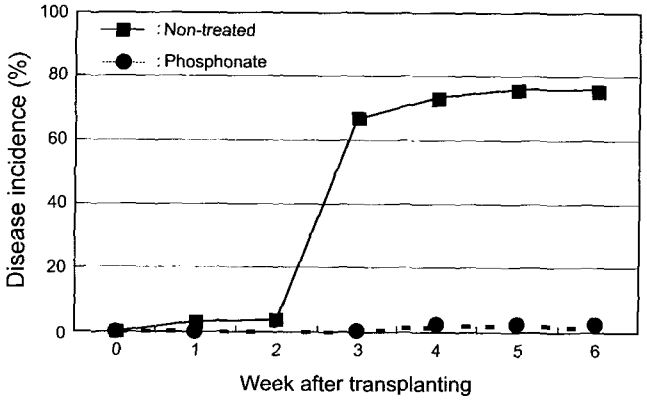

Fig. 1. Control of Phytophthora root rot of lettuce by phosphonate in a farm trial. The nutrient solution was supplemented with *phosphonate 100 ppm prior to transplanting of lettuce seedlings* into the hydroponic system.

Discussion

The term 'phosphonate' refers to compounds containing a phosphorous-hydrogen (P-H) bond that confers biological activity against Oomycetes and host plants (Guest et al., 1995). The mode of action of phosphonate has not been clearly understood yet. However, it is generally conceived that complex mechanisms are both directly and indirectly responsible in the disruption of phosphorous metabolism of pathogens and in stimulating the defense mechanism of the host (Guest and Grant, 1991; Guest et al., 1995; Smillie et al., 1989). Recently, Jackson et al. (2000) reported that the chemical directly inhibited growth of *P. cinnamomi* in plant at high concentrations, while it stimulated host defense

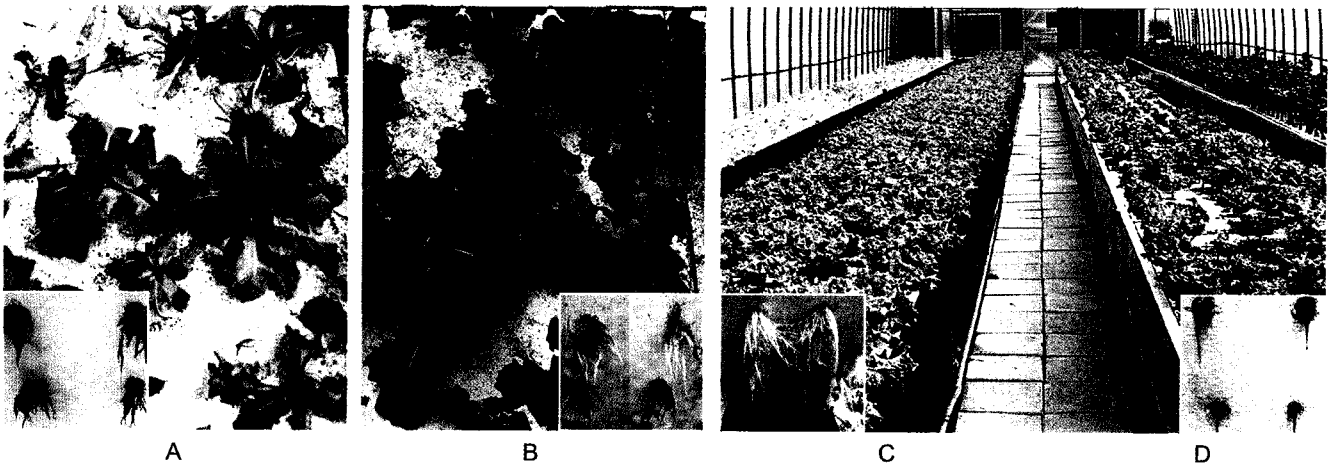

Fig. 2. A lettuce hydroponic farm naturally infested by *Phytophthora drechsleri* and effects of phosphonate on the control of lettuce root rot. A: Heavily infected plants showing wilt, B: Wilted plants became healthy looking at 7 days after treatment with phosphonate 200 ppm. New roots (white) are growing out. C: Phosphonate 100 ppm was supplemented into the nutrient solution prior to transplanting lettuce seedlings into the hydroponic unit, D: Plants growing in non-treated control plot showing severe wilt or death. Root systems of corresponding plants in the pictures are shown inside the bottom boxes.

enzymes at low concentrations. In any case, various forms of phosphonates noticeably inhibited *Phytophthora* growth (Ouimette and Coffey, 1989b). On the other hand, phosphoric acid (H_3PO_4) that consisted of phosphorous-hydroxide (P-OH) instead of P-H bond showed no inhibitory effect on the fungal growth up to 1000 ppm (unpublished data).

In this study, potassium phosphonate over 50 ppm strongly inhibited mycelial growth of *Phytophthora* species (Table 1) and effectively suppressed the fungal population in the nutrient solution (Table 4). Fortunately, it did not affect lettuce growth in hydroponics consisting of standard nutrient solution (Table 2). Forster et al. (1998) reported that tomato and pepper treated with phosphonate, instead of phosphate, were deficient in phosphate and showed phosphorous-deficiency symptoms. Consequently, phosphonate cannot substitute for phosphate for plant growth as it is a poor source of phosphorous nutrient (Guest et al., 1995). As well, over 200 ppm of phosphonate in hydroponics impeded normal development of lettuce since plants grown at this concentration showed light and yellowish green leaves compared with those grown in plots containing phosphonate 0-100 ppm. This was possibly due to competition in absorption or transportation among inorganic nutrient elements in the plant. Consequently, direct amendment of the phosphonate 100 ppm into nutrient solution before infection is recommended to control the lettuce Phytophthora root rot in hydroponics (Fig. 1).

Phosphonate is a phloem-located fungicide that actively moves upward and downward in plant tissues (Guest et. al., 1995). In various plants, phosphonate is readily detected in leaves and roots within a few minutes to hours after soil drenching, foliar spray or trunk injection, and persists for a substantial period to contribute biological activity in plant tissues (Guest et al., 1995). Ouimette and Coffey (1989a) reported that sufficiently high level of phosphonate accounting for a direct antifungal effect on *P. cinnamomi* was detected in leaves and roots of avocado for 8 weeks. However, it is important to apply phosphonate before infection to effectively control the disease because control values varied significantly depending on application time (Table 3). Nevertheless, application of phosphonate 200 ppm after infection could also be practical since about 80% severely wilted plants recovered vitality and grew new healthy roots (Table 4).

Currently, phosphonate is registered as a fungicide for use against various Oomycete diseases of avocado, pineapple, citrus, ornamentals, and many others in Australia and a few other countries (Guest et al., 1995). In Korea, however, this was the first attempt to study the effect of phosphonate on the control of Phytophthora disease. Further studies to investigate its bio-spectrum and to develop optimum application techniques in accordance with cultivation systems and crops are in progress.

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