

## A New Formulation System for Slow Releasing of Phosphorous Acid in Soil for Controlling Phytophthora Diseases

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Phosphorous acid is known to effectively control various Oomycetes diseases. The phosphoric acid moves upward and downward through the xylem and phloem in plants. The sustainable forms of the slow releasing chemical in rhizosphere would be ideal to be up-taken by plants. Therefore, we developed a new system for phosphorous acid formulation using a carrier coated with polysaccharides. When the product was applied in rhizosphere, the adequate amount of phosphorous acid was consistently released up to 4 weeks in rhizosphere soils. While soil drenching with phosphorous acid at 1,000 µg/ml and metalaxyl at 150 µg/ml were not effective to control pepper Phytophthora blight for 4 weeks, direct application of our formulation product around basal stem of pepper plants resulted in excellent disease control effect against Phytophthora blight over 4 weeks. The application of 4 g of our product per plant was optimum to control the disease, and 8 g product/plant did not cause phytotoxicity. Based on the results, we conclude that the applications of the formulation product once or twice during cropping season can control Phytophthora diseases on various crops.

**Keywords :** curdlan, formulation, phosphorous acid, Phytophthora, sustained-release

Phosphorous acid is used extensively as a fungicide and also sold as a fertilizer (Forster et al., 1998; Jee et al., 2002). The chemical is rapidly absorbed and translocated within the plant (Fenn and Coffey, 1985; Guest et al., 1995). Phosphorous acid is known to effectively control various Oomycetes diseases (Barchietto et al., 1988; Smillie et al., 1989; Forster et al., 1998; Jee et al., 2002). The chemical is an unique vascular system-translocated fungicide that moves upward and downward through the xylem and phloem in plants. The chemical is widely applied for trunk injection, soil drenching, foliar spray, or direct supplement into nutrient solution in hydroponics (Stanghellini and Rasmussen 1994; Jee et al., 2002). However, soil drench-

ing and foliar spray may not be as effective as trunk injection or direct supplement because of run-off, leaching, and inactivation in soils. Consequently, sustainable forms of the slow-releasing chemical in rhizosphere would be ideal to be up-taken by plants to control Oomycetes diseases.

Hence, a number of researches tried actively to regulate the activation period of the agricultural chemicals and to confer a sustained-releasing property on the chemicals. Precisely, the agricultural chemicals in a solid form or in a liquid form have been developed to discharge effective components in a controlled manner and to sustain the efficacies for a long time (Ohtsubo, 2003; Tsuji, 2003). However, these methods include complicated procedures and cause environmental problems, since toxic compounds such as organic solvent were utilized for the preparation. Besides, the cost for this process is extremely high.

The objective of this study is to provide a novel process for preparing sustained-releasing phosphorous acid formulation to enhance the disease control efficacy in soil and to reduce the labor and the cost efficiently.

### Material and Methods

**Material preparation.** For preparing the product, 8.3 g of potassium hydroxide and 2.5 g of polysaccharides (curdlan or pectan) derived from microorganism were dissolved in 200 ml of distilled water. Then, 10 g of phosphorous acid was added and mixed sufficiently to make a colloidal solution containing polysaccharides. One kg of zeolite with 5 mm of diameter was added and mixed homogeneously with the above prepared solution. Then, the resultant was dried at 100°C with hot air. In the final step, the water content was adjusted to less than 10%.

In order to prepare non-sustained-releasing phosphorous acid formulation, phosphorous acid was adsorbed onto zeolite and dried. Ten gram of phosphorous acid was dissolved with 100 ml of distilled water and potassium hydroxide was added in a small amount. The final pH was adjusted to 5.5. One hundred milliliter solution of phosphorous acid salt prepared above was added and mixed

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homogeneously with 1 kg of zeolite with 5 mm of diameter. Finally, the resultant was dried with hot air at 100°C to generate non-sustained-releasing agricultural chemicals.

**Sustained-releasing analysis.** In order to investigate the sustained-releasing property of the product system, 1 g of the product prepared above was mixed with 5 ml of water and stored for 2 hours. Then the supernatant containing the phosphorous acid was recovered and the concentration of phosphorous acid was measured by performing the Ion Chromatography (IC). This procedure was repeated to estimate the practical experimental condition corresponding to the effect of one sprinkling.

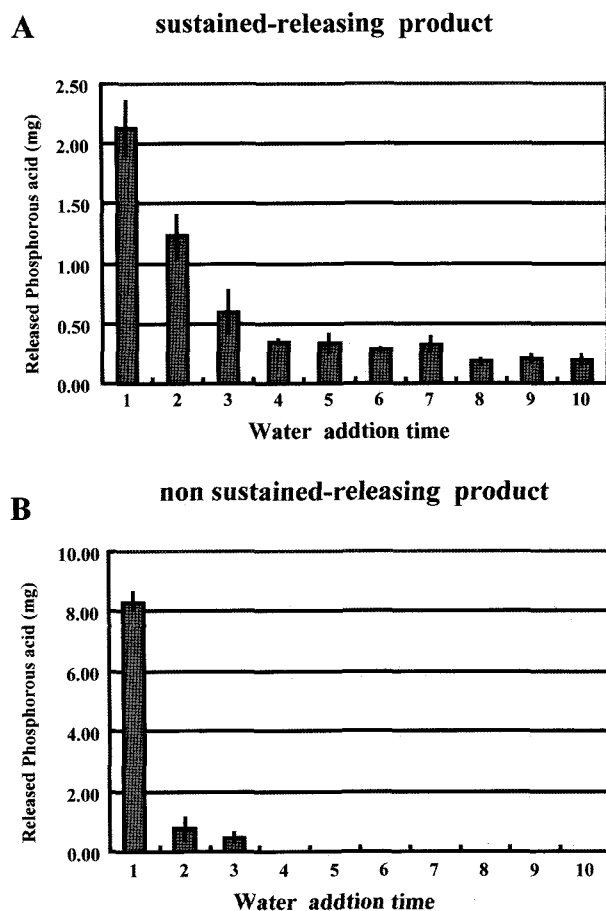
#### Controlling effect of sustained-releasing formulations.

To examine disease control effects of the sustained-releasing phosphorous acid formulations, the mini tomato (variety “koko”) were treated with 1 g of various formulations at a 2-4 leaf stage. Fungal pathogen inoculation was performed by spraying 10 ml of the *Phytophthora infestans* zoospore suspension on the tomato plants at  $4 \times 10^3$  zoospores/ml for 4 days. Through the entire experimental procedure, water was sprinkled sufficiently in a 2-day interval.

The disease control effect of the sustained-releasing product system for the control of red pepper *Phytophthora* disease, the red pepper was treated with the product in greenhouse similarly. The plants were inoculated by the pathogen, *P. capsici*, artificially. Five ml of the zoospore suspension at  $5 \times 10^3$  cfu/ml of the concentration were drenched to each red pepper plant. Through the entire experimental procedure, water was sprinkled sufficiently in a 3-day interval. The disease severity for tomato late blight and disease incidence for pepper blight were investigated by the standard protocol. The disease control value was calculated using the following formula: Disease control value (%) =  $((A-B)/A) \times 100$ , where A is the disease severity triggered by pathogen inoculation alone and B is the disease severity after various treatments.

## Results

**A sustained-releasing analysis.** The release pattern of phosphoric acid was investigated between our formulation and non-sustained-releasing system over time. As illustrated in Fig. 1, the phosphorous acid in the non-sustained-releasing phosphorous system (Fig. 1B) were discharged relatively much in the initial state. However the product system (Fig. 1A) has shown the outstanding sustained-releasing effect. When comparing the released amount of phosphorous acid between two formulations, the non-sustained-releasing type maintained only 10-15% of the



**Fig. 1.** Measurement of sustained-releasing phosphorous acid from product. The product 1 g was contained 10 mg phosphorous acid. Samples were suspended in 5 ml water at a time, separately.

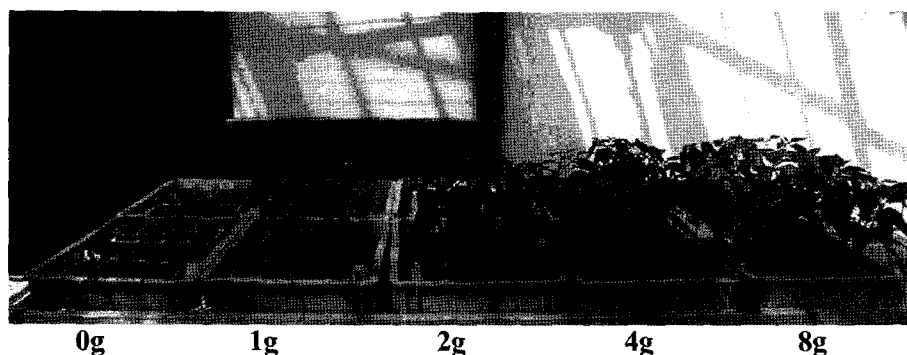
efficacy by our sustained releasing product about 2-3 days after application. The product system discharged the phosphorous acid slowly and consistently in more than 100 µg/ml. The relatively constant amount of phosphoric acid was released toward sprinkling 10 times by our sustained-releasing formulation.

**Control effect on tomato late blight.** Tomato late blight control effects among different formulations were investigated on tomato plants grown in a pot in greenhouse. The treated formulations included a control (non-treatment), a non sustained-releasing product ( $H_3PO_3$ -Zeolite), the curdlan based sustained-releasing product and the pestan based sustained-releasing product. The invasion frequency of the tomato disease was calculated and the controlling effect against the tomato late blight was evaluated in each group (Table 1).

As illustrated Table 1, the sustained-releasing product formulation systems revealed 80-82.5% of the tomato late blight control effect. There was no significant difference on

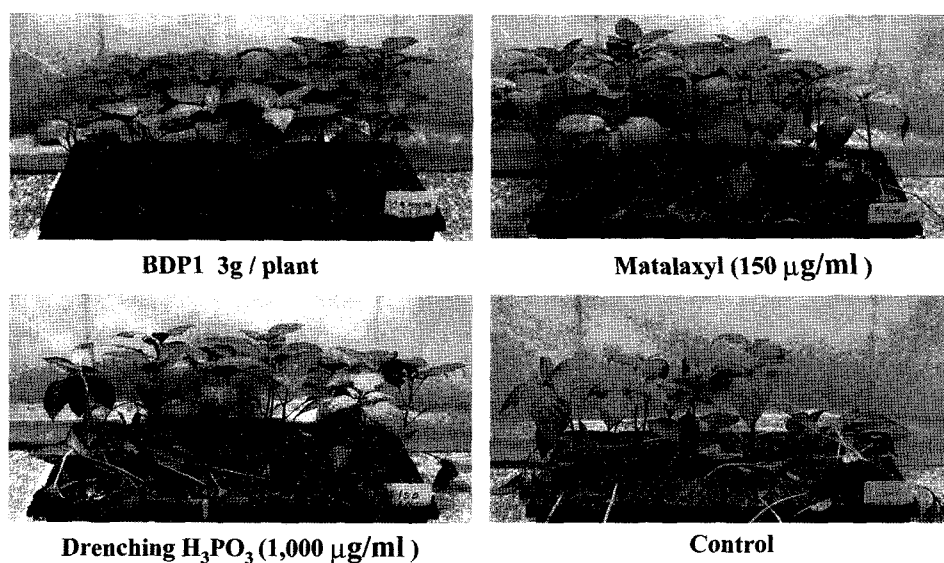
**Table 1.** Effect of the sustained-releasing phosphorous acid formulation on *Phytophthora* late blight of tomato caused by *Phytophthora infestans*

Carrier of $\text{H}_3\text{PO}_3$	Disease severity index					Frequency (plant)	Frequency (%)	Control value (%)
	0	1	2	3	4			
Curdlan based	8	2	1	1	0	4	12.58	82.9
Pestan based	7	4	1	0	0	5	14.58	80.0
$\text{H}_3\text{PO}_3$ -Zeolite	1	3	2	3	3	11	58.33	20.0
Without treatment	1	1	2	2	6	11	72.92	—

**Fig. 2.** Control of *Phytophthora* blight of pepper by sustained-releasing phosphorous acid formulation according to application amount per plant in pots.

the disease control value between two formulations prepared using curdlan or pestan. In contrast, zeolite based non-sustained releasing product was not effective to control the disease with disease control value of 20%. Our result clearly showed that the sustained slow release of the phosphoric acid was highly effective to control the tomato late blight.

**Pepper blight control effect.** Pepper blight control effects among different formulations were investigated on pepper plants grown in a pot in greenhouse. The treated formulations included a control (non-treatment), a non sustained-releasing product ( $\text{H}_3\text{PO}_3$ -Zeolite), the curdlan based sustained-releasing product and the pestan based sustained-releasing product. The disease incidence was investigated 4

**Fig. 3.** Effect of the sustained-releasing phosphorous acid formulation on control of *Phytophthora* blight of pepper compared to metalaxyl and soil drenching of phosphorous acid 1,000  $\mu\text{g}/\text{ml}$ . *Phytophthora capsici* was inoculated 4 days after treatment.

**Table 2.** Effect of the sustained-releasing phosphorous acid formulation on control of Phytophthora blight of pepper 4 weeks after treatments

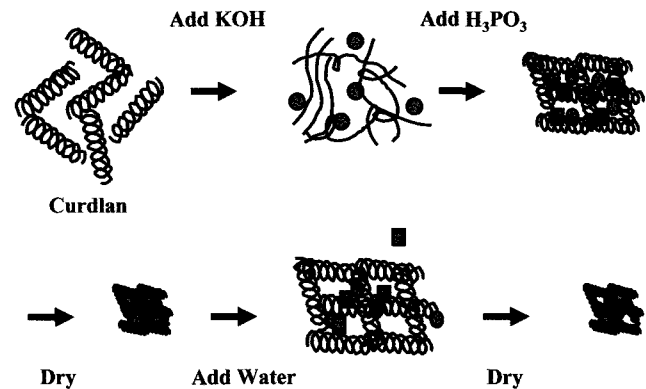
Treatment	Dosage per plant	Disease incidence (%)	Control value (%)
Curdlan based product	3 g	16.70	80.90
Metalaxyl (150 µg/ml)	10 ml	58.30	33.40
H <sub>3</sub> PO <sub>3</sub> (1,000 µg/ml)	30 ml	66.67	23.81
H <sub>3</sub> PO <sub>3</sub> (100 µg/ml)	30 ml	83.33	4.77
Without treatment	–	87.50	–

**Fig. 4.** Phytotoxicity of sustained-releasing phosphorous acid formulation on pepper. The product was treated with 0-8 per plant in pots.

weeks after pathogen inoculation, since our product consistently released adequate amount of phosphorous acid up to 4 weeks in rhizosphere soils. Soil drenching of 1,000 µg/ml of phosphoric acid and 150 µg/ml of metalaxyl did not last its control effect for 4 weeks. The disease control values for drenching application were 23% and 33% for phosphorous acid and metalaxyl, respectively. However, direct application of our curdlan based product on the basal stem of pepper plants resulted in excellent control effect against Phytophthora blight over 4 weeks (Fig. 3 and Table 2). The product 4 g per plant was optimum to control the disease and application of 8 g did not cause phytotoxicity (Fig. 2 and Fig. 4).

## Discussion

The sustained-releasing phosphorous acid formulation system prepared in the present study (Fig. 5) discharged its effective component only in the aquatic condition. The curdlan solution added to acid has unique network structure (Saito et al., 1978). The polysaccharide treated at 95°C lost water solubility (Saito et al., 1977; Stone and Clarke, 1992). Therefore, the network structure was opened in the aquatic condition but closed again in dry condition (Saito et al.,

**Fig. 5.** Process of sustained-releasing phosphorous acid formulation.

1978; Marchessault et al., 1977; Fulton et al., 1980).

Water was sprinkled about every 3 days in the conventional agricultural farming system. About two hours after sprinkling, the water evaporated or absorbed in soil or by crops. Consequently, the moisture was eliminated and soil became dry not to discharge the phosphoric acid from the sustained-releasing phosphorous acid. The product system appeared to extrude its effective components about every three days and two hours toward outer environment (Fig. 1). The discharge of phosphorous acid occurred in a constant ratio equivalent to the 10 direct sprays of phosphoric acid. Therefore, our formulation product system appeared to maintain its biological effect for about 30 days (Fig. 3).

The curdlan-based formulation in our formulation systems showed the remarkable disease control effect (Table 1) and storage ability (data not shown). The formulation product preserved at room temperature maintained the disease control effect against pathogen for 4 years. But the pestan-based formulation system did not have storage ability (data not shown).

The formulation product can maintain its efficacy for about 30-40 days per treating once (Fig. 3 and Table 2). Probably, the effective component can be discharged continuously in a proper and in excessive concentration to maintain the disease control effects. The result showed that the discharge of the effective component was controlled properly in our formulation product. Based on our results, it seemed that once or twice applications of the formulation during a cropping season satisfy to control phytophthora diseases on various crops.

The sustained-releasing phosphorous acid system can be utilized to reduce labor, to decrease yield loss due to successive sparkling and to prevent the environmental pollution. Consequently, the product system saves labor, reduces costs and environmental hazards. Further field trials on several crops to control Oomycetes diseases are being processed.

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