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Development of Sustainable Releasing Micro Formulation System using γ-Irradiation Technique to Control Phytophthora Blight Disease

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Abstract − We introduced a novel sustainable slow-releasing agrochemical formulation, a biopolymer bound to silica, for controlling plant diseases. The formulation was obtained through the following process. Curdlan, sodium silicate (Na_2SiO_3) and isopropyl alcohol were dissolved in DDW (Deionized-distilled water). The resultant solution was then irradiated using a ^{60}Co γ-irradiator (150 TBq of capacity; ACEL, Canada) at KAERI. The resultant solution was treated with phosphorous acid (H_3PO_3). Finally, we obtained a novel biopolymer-silica microsized formulation containing phosphorous acid (H_3PO_3) from the solution. The morphology of the complex was characterized using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM and TEM images revealed that the curdlan-silica formulation has a particle size ranging from 1 to 3 μm with high stability. We also detected that H_3PO_3 was distributed within the formulation through energy dispersive X-ray spectroscopy (EDX) analysis. H_3PO_3 was sustain-released from the formulation in water. Based on our results, it seems effectively that one or two applications of the formulation during a cropping season will assist in controlling various plant diseases.

Key words : Micro formulation, Sustainable releasing, γ-irradiation, Plant disease, Phosphorous acid

INTRODUCTION

Presently, the agrochemicals used for preventing damage to vegetables fruits, flowering plants and grains caused by plant pathogens and noxious insects are usually in a liquid form, or in a particle form blended with a carrier. The effective constituents can be released out of the region easily or make the soil acidic. They can also be evaporated and thus be rapidly reduced. Therefore, the duration of plant disease control is usually short, and thus a large quantity of agrochemicals should be applied over the recommended dose. It seriously has a bad effect on the health of farmers or the users of farm products. Agrochemicals can also cause serious environmental pollution if they are dispersed continuously, since salts of agrochemicals are accumulated in the soil.

A number of researchers are trying to regulate the activation period of agrochemicals and to create a sustained-releasing property. In particular, agrochemicals in a solid or liquid form have been developed to discharge their effective components as a controlled pattern and to sustain their effectiveness for a long time, even when spread only once at a recommended concentration. Some of these demonstrated methods include the process for inserting the constituents into a microcapsule, converting the ingredients into cyclodextrin, and methods for blending effective constituents of

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agrochemicals into a particle or powder form independently or with an extender or other device, and then covering the nucleus of the particle with wax or various kinds of resins (Ohtsubo 2003; Tsuji 2003). Unfortunately, these methods have some disadvantages like i) procedures are too complicated; ii) the materials used are expensive and iii) a harmful effects on the environment.

To overcome these disadvantages, sustained-releasing agrochemicals composed of eco-friendly materials need to be developed. Some studies have illustrated a method for the conferring a sustained-releasing property in a pesticide, in which the agrochemicals are dissolved with a solvent and introduced into a biodegradable resin molded in a plate form. However, these methods also include complicated procedures and create environmental problems, since toxic compounds such as organic solvents are employed for the preparation. In addition, they require considerable cost.

Phosphorous acid (H_3PO_3), which effectively controls various oomycetic diseases, is a unique phloem-tanslocated fungicide that moves upward and downward through the xylem and phloem in plants (Fenn *et al.* 1984; Stanghellini and Rasmussen 1994; Jee *et al.* 2002). H_3PO_3 is widely applied for trunk injection, soil drenching, foliar sprays, or direct supplements as nutrient solutions in hydroponics (Barchietto *et al.* 1988; Smillie *et al.* 1989; Feorster *et al.* 1998; Jee *et al.* 2002). However, soil drenching and foliar spray may not be as effective as trunk injection or a direct supplement because of run-off, leaching, and inactivation in soils. Consequently, sustainable forms of the chemical releasing slowly in the root zone are ideally taken up by plants to protect against oomycetic diseases.

The aim of the present study was to provide a novel process for preparing sustained-releasing microformulation using γ-irradiation for enhancing the efficacy of the agrochemical in field and reducing the labor costs.

MATERIALS AND METHODS

Chemicals

Sodium silicate solution (Na_2SiO_3) and phosphorous acid (H_3PO_3, 99.8%) were prepared using analytical reagent grade. Curdlan was purchased from the Kirin-Food Tech Company (Japan) and isopropyl alcohol from the Aldrich-Sigma chemical company. The solutions were prepared with purified water in a Milli-Q Plus water purification system (Millipore Co. Ltd. USA; final resistance of the water was 18.2 MΩ cm^{-1}) and degassed prior to each measurement.

Preparation of the products

A sustainable-releasing micro-formulation was obtained as follows Curdlan (0.5 g), sodium silicate (Na_2SiO_3) (1.0 g) and isopropyl alcohol (20 ml) were dissolved in DDW (Deionized-distilled water) and made the total volume to 200 ml. The resultant solution was irradiated with γ-rays using a ^{60}Co γ-irradiator (150 TBq of capacity; ACEL, Canada) at ARTI-KEARI; Korea. The resultant solution was treated with H_3PO_3 (1.0 g), and then gently shaken for 10 minutes. After 1 hour, the colloidal solution was dried by a freeze dryer to obtain the final product. The surface morphology of the products was determined using field emission-scanning electron microscopy (FE-SEM, SU-70, HITACHI, Japan) and transmission electron microscopy (TEM, HD-2300, HITACHI, Japan).

Analysis of sustained-releasing pattern

To elucidate the sustained-releasing property of the prepared formulation containing H_3PO_3 prepared in the present study, the aquatic discharge property was investigated under the same condition as that of the practical use. Concretely, 0.1 g of the prepared formulation was added to 200 ml of DDW and stored for 15 minutes in room temperature. The supernatant containing the ingredient was then recovered and the concentration of H_3PO_3 was measured by performing ion chromatography (IC, ICS 2000, Dioxe, USA).

Preparation of effective preventive test to control plant pathogen

The preventive effects of the sustained-releasing product system were examined in a green house for control of red pepper phytophthora blight disease (a kind of oomycetic disease). The experiment was performed by inoculating the pathogen, *Phytophthora capsici*, artificially. Twenty five ml of the zoospore suspension (1×10^4 cfu ml^{-1}) was drenched onto each pepper plant. Through the entire experimental procedure, water was supplied sufficiently in a 3-day interval. The effects of the pathogen inoculation were examined after

3 weeks.

Four experimental groups, each containing 12 plants of red pepper (variety "Rockwang") were adopted for the experiment. A control group that was not treated with phosphorous acid salt was maintained. A comparative group of red pepper that was treated with 100 and 1,000 ppm of phosphorous acid salt in liquid state (20 ml plant^{-1}) around the region adjacent to the soil and stem. The experimental groups were treated with the sustained-releasing formulation product (50 mg plant^{-1}). The invasion frequency of red pepper disease (phytophthora blight) was calculated at 3 weeks after treatment and the preventive effects against phytophthora blight were evaluated in each group. The disease control value was calculated using the following formula: Disease control value (%)=((A−B)/A)×100, where A is the disease severity triggered by pathogen inoculation alone and B is the disease severity after various treatments.

RESULTS AND DISCUSSION

Curdlan-silica micro formulation

In the present study, the term "sustainable-releasing micro formulation" means a complex comprised of microsized formulations that are combined with a network of curdlan and silica molecules which allow the sustainable-release of chemicals.

SEM images and EDX (Energy dispersive X-ray spectroscopy) analysis show uniform spherical type morphology with an average size of 2 μm, and the formulation is contain-

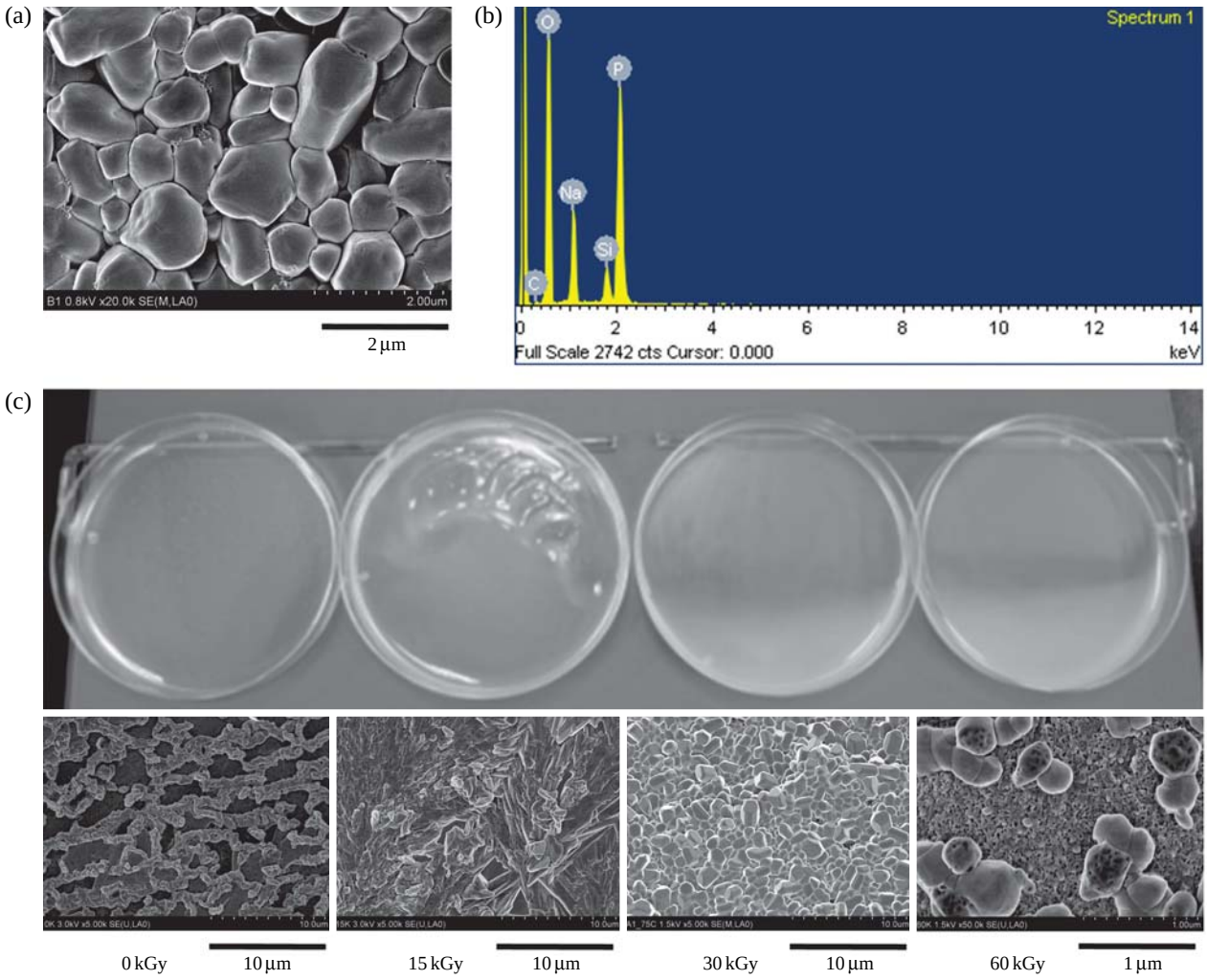

Fig. 1. FE-SEM analyses of a sustainable releasing microformulation containing H_3PO_3: (a) FE-SEM analysis, (b) EDX spectrum, (c) FE-SEM analysis of the formulations irradiated with different dose of γ-irradiation.

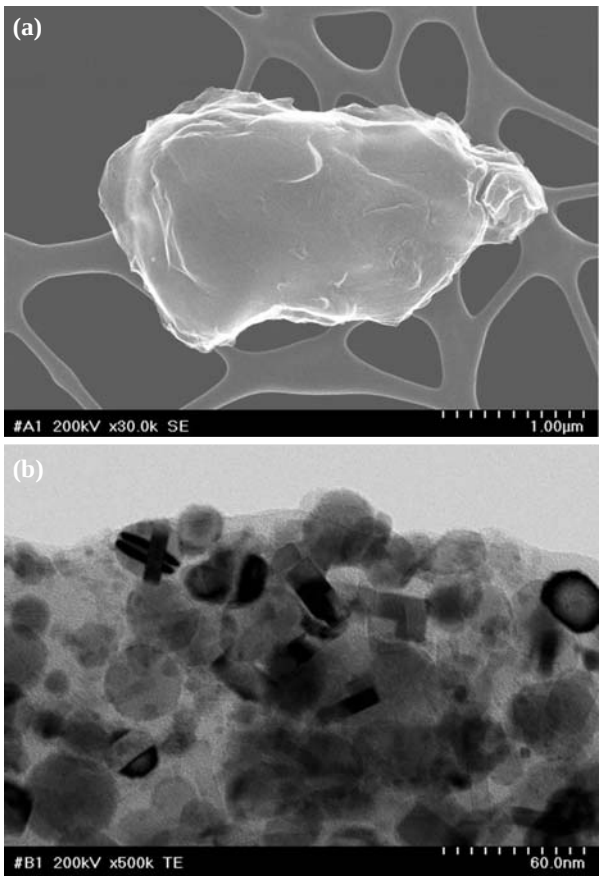

Fig. 2. TEM analysis of a sustainable slow-releasing micro-formulation containing H_3PO_3 (a) 1 μm scale and (b) 60 nm scale.

ed in H_3PO_3 (Fig. 1a, b). The curdlan-silica compound polymers, which were irradiated by γ-ray (30 kGy), built up a micro formulation complex after the addition of H_3PO_3 (Fig. 1c). The polymers irradiated with 15 kGy γ-ray was made only into a matrix after the treatment with H_3PO_3. A 60 kGy treatment caused the many nanoparticles with microparticles. Unfortunately, the formulation using 60 kGy γ-ray irradiation is not able to be used in the agriculture field because nanoparticles are not yet permitted for agrochemical formulation. The morphology of the formulation complex was also imaged by TEM, as shown in Fig. 2. Structural characterization showed that there were significant interactions observed among the silica, curdlan, and H_3PO_3 molecules. Each curdlan-silica compound polymer was networked by H_3PO_3 in the formulation.

We suggest an illustration to understand the reaction mechanism for the formation of the formulation complex containing phosphorous acid through γ-irradiation (Fig. 3). It was reported that an organic-inorganic hybrid matrix was formed from the polymer and silicate solution containing isopropyl alcohol by heat treatment (Bae 2001). In the present study, the formulation represents a structure in which microsized particles are formed from curdlan-oligomer which is curdlan-polymer cut by γ-ray of 30 kGy or more (Byun *et al.* 2008; Park and Kim 2010), and silicate simultaneously

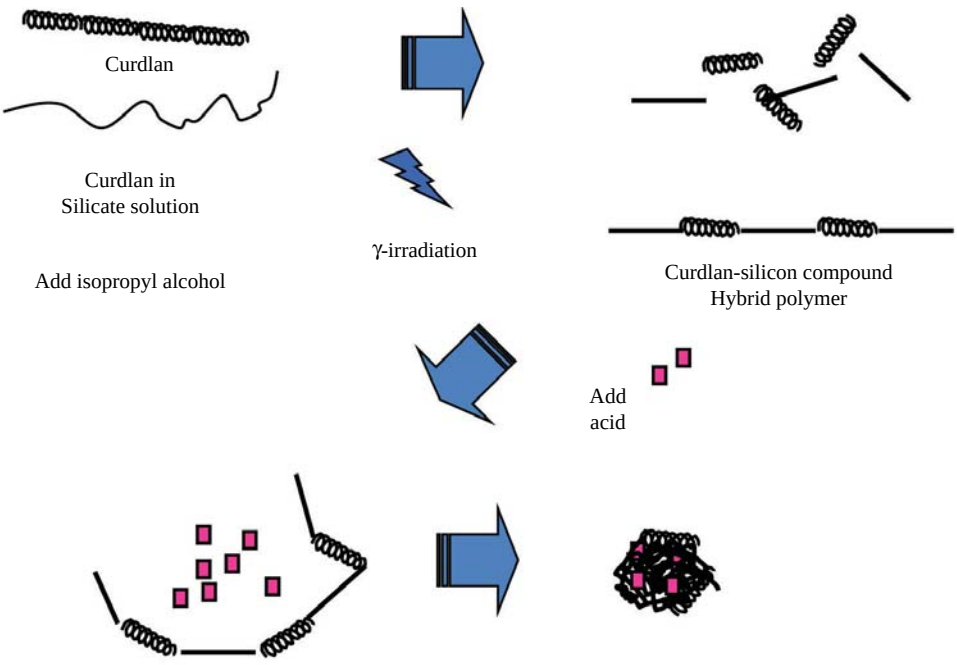

Fig. 3. Preparation of a sustainable slow-releasing micro-formulation using γ-irradiation.

polymerized with isopropyl alcohol by γ-irradiation, and through a networking interaction with the addition of acid (H_3PO_3) inside the formulation.

A sustained-releasing analysis

The sustained-releasing property of the prepared formulation containing H_3PO_3 was investigated in a one-time water supply in field. Only 5% of the H_3PO_3 was released from the formulation at 3 hours after being added the formulation in the water. The concentration of H_3PO_3 gradually increased in water, and about 25% was released during at least a day after the treatment (Fig. 4). It is observed that the formulation slowly released H_3PO_3 at the rate of 2～3% per a day.

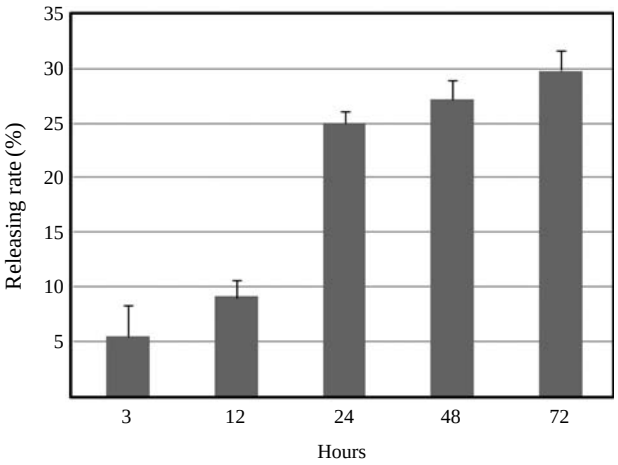

Fig. 4. Sustained-releasing rate of H_3PO_3 phosphorous acid from the prepared micro formulation. The bars indicate the standard deviation from the mean in three experiments.

The sustained-releasing micro-formulation system prepared in the present study discharged its effective component (H_3PO_3) only in the aquatic condition. The curdlan in system has a unique networking structure if acid is added into the KOH-curdlan solution (Sato *et al.* 1978; Park *et al.* 2007). Therefore, we confirmed that the curdlan networking structure was constituted in the formulation by the above method. The networking structure was opened only in the aquatic condition, and the network closed again in a dry condition (Marchessault *et al.* 1977; Sato *et al.* 1978; Fulton *et al.* 1980; Stone and Clark 1992; Park *et al.* 2007). Therefore, the formulation system is able to prevent or reduce phosphorous acid run-off in soils.

Formulation system to control plant pathogens effectively

As illustrated in Table 1, the highest control effect (88.9%) was observed in sustained-releasing product formulation

Table 1. Effects of the sustainable slow releasing micro formulation on the control of phytophthora blight in red pepper at 3 weeks after treatment

Treatment	Dosage /plant	Disease incidence (%)	Disease control value (%)
Micro formulation (40% H_3PO_3 product^{-1})	50 mg 20 ml^{-1}	10.00	88.90
H_3PO_3 (1,000 ppm)	20 ml	35.00	53.81
H_3PO_3 (100 ppm)	20 ml	66.67	24.77
Control	–	80.00	–

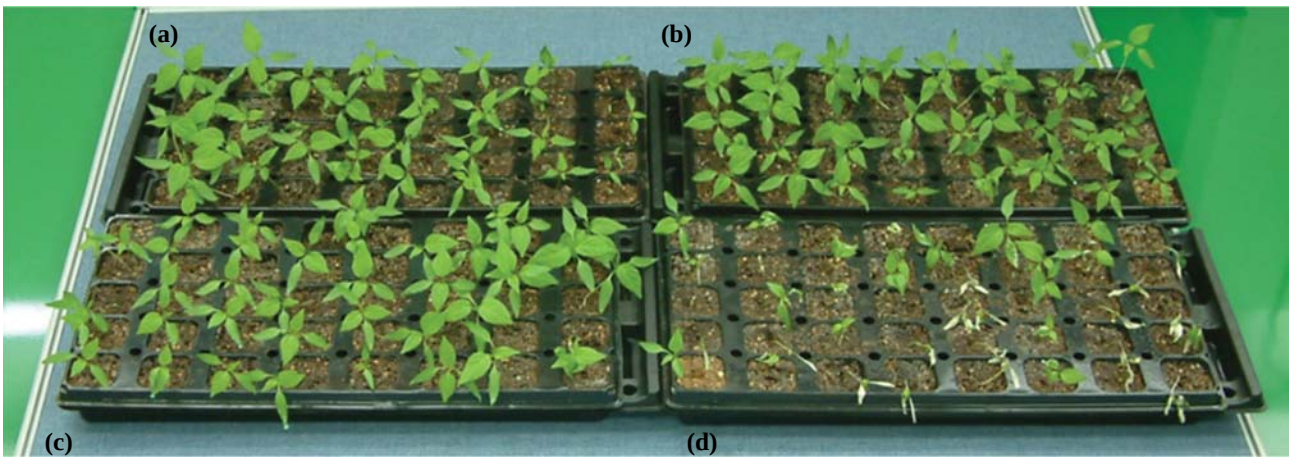

Fig. 5. The chemical injuries of red pepper plants caused by a high concentration of commercial product containing H_3PO_3. (a) water treatment (20 ml), (b) 25 mg 20 ml^{-1}, (c) 50 mg 20mL^{-1}, (d) 500 mg 20 ml^{-1} of the formulation product (40% H_3PO_3 product^{-1}).

system (40% H_3PO_3 product^{-1}) which showed the controlled release of the effective component (H_3PO_3). It is observed that the disease control effect (88.9%) of the formulation systems increased by 1.65 and 3.59 times of the comparative group treated with 1,000 ppm and 100 ppm of H_3PO_3 salts, respectively.

In the present study, it is observed that the micro formulation system can maintain its efficacy during about 20 ~ 30 days of treatment (Table 1), and has no phytotoxicity in the formulation after treatment (Fig. 5). The results showed that the discharge of the effective component was controlled properly in the micro formulation system and that preventive effects against the phytophthora blight were detected to be complete. Based on these results, it seems that one or two applications of the formulation during a cropping season may satisfy the control of phytophthora disease on various crops. This sustained-releasing phosphorous acid system can be also beneficial for reducing the human labor costs and environmental pollution. Further field trials on several crops for the control of plant diseases by the sustained-releasing micro formulation system are underway.

CONCLUSION

A new microsized curdlan-silica formulation containing phosphorous acid was prepared and conveniently manufactured through γ-irradiation at room temperature. The formulation released phosphorous acid sustainably in a solution and controlled phythphora blight in red pepper effectively in 3 weeks. Based on our results, it seems that one or two applications of the formulation during a cropping season will be helpful in controlling phytophthora diseases in red pepper plants.

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