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Insecticidal Effect of Controlled Release Formulations of Etofenprox Based on Nano-bio Technique

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The characteristics and the bioassay of controlled release formulations (CRFs) of etofenprox which has been used for pest control using a nano-sized chitosan carrier to control the released amount of the active ingredients were evaluated. The CRF was prepared in three types, ENC1 (M.W. 3000, 0.3%), ENC2 (M.W. 30,000, 0.1%) and ENC3 (M.W. 30,000, 0.3%), according to a difference in release patterns by adjusting the molecular weight and concentration of chitosan. After the release properties of the active ingredients and biological activities of the 3 formulations were evaluated, the ENC2 type was selected. A shape of the ENC2 type was taken by a scanning electron microscope (SEM), and it was confirmed that the ENC2 type had a polygonal shape and ENC2 particles with various sizes of 800 nm or less were mixed. The release of the ENC2 type in a CRF began after it reacted with a solvent, and the ENC2 became smaller, and the shape of ENC2 nanoparticle was changed. However, in the ENC2 type formulation, de-encapsulated active ingredients of etofenprox exhibited initial insecticidal effects, and thus the ENC2 type was modified to be prepared as an ENC2A formulation in which all the active ingredients of etofenprox were encapsulated. The ENC2A formulation exhibited the properties of CRF in the release properties of the active ingredients and the effective activity against Spodoptera litura. These results suggest that such CRF is considered to be used as a method of preventing loss of active ingredients of chemicals and increasing the activity of the chemicals against a target pest, thereby decreasing the use of the chemicals.

INTRODUCTION

In agricultural field, crop protecting agents have contributed to affluent life of mankind by enabling stable growth and development of crops and their yield enhancement. Meanwhile, in line with a trend of well-being culture, increasing customers' needs for high quality and safe agricultural products, and a global diffusion of environmental-friendly agriculture in which agriculture is in harmony with environment, various approaches to decrease the use of the crop protecting agents have been conducted. Accordingly, the development of crop protecting agents is focused on the development of products that are safe to environment and have enhanced insecticidal effects. However, since there are many difficulties in developing novel agricultural chemicals by synthesis, research on development of novel formulations has been intensively conducted. As a result, aqueous formulations replaced by an organic solvent, formulations for seeds and seedling with reduced environment dosage, and directly-treated tablet formulations considering conven-

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ience of users have been developed. However, to achieve successful diffusion of sustainable agriculture, there is still a need to develop a method of crop protection in order to reduce the use of the crop protecting agents.

Spodoptera litura is one of the major agricultural pests in Korea and a polyphagous pest belonging to the genus Spodoptera, the family Noctuidae and the order Lepidoptera (Minamikawa, 1937; Bae et al., 1997), and S. litura is a reportedly an insect pest that attacks approximately 150 kinds of host plants (Rao et al., 1993) and that mainly damages southern areas of Korea (Kim and Shin, 1987; Bae and Park, 1999). S. litura occurs across the country four to five times yearly (Bae et al., 2004), and the insect pest in mixed stages from eggs to adults attacks various crop cultivation areas after August (Bae et al., 2007). In general, at least 3rd-instar larva except for young larva of S. litura is a representative insect pest of which chemical control is very hard (Kim et al., 1998; Bae et al., 2003; Sayyed et al., 2008), and its growth period is so short that the use of insecticides and the best time to spray chemicals during cultivation period are limited, and thus if systematic and intensive management of the larva of S. litura is not implemented in the initial stage of occurrence thereof, it is very hard to control the larva of S. litura.

To control such tobacco cutworm *S. litura*, etofenprox, which was registered in Korea and has been widely used as an insecticide, is used and is known to have effects in insect pests of the order *Lepidoptera* such as *S. exigua* and *Carposina niponensis* and insect pests such as *Hemiptera*, whiteflies and aphids. However, when these insecticides with excellent insecticidal effects

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are sprayed, the residual activity of the insecticides is rapidly reduced due to various factors of natural environments, and thus the insecticides were used to be frequently sprayed at a higher concentration than an appropriate spraying concentration against a target insect pest. In addition, when the amount of chemicals used and the spraying number thereof increase even if the chemicals have excellent insecticidal effects, it may naturally enable the target pest to have a resistance to the chemicals. Therefore, various methods of reducing the spraying number of crop protecting agents, preserving ecosystems and environmentally–friendly controlling insect pests have been studied (Choi *et al.*, 1996; Jin *et al.*, 2009; Kim *et al.*, 2008).

As an example of the methods described above, an encapsulation technology, which is controlled release formulation (CRF) enabling a decrease in the amount of agricultural chemicals used, contributes to the stability of unsteady core materials, provides a protective function from the materials releasing sharp odors and adds biocompatibility to carrier systems having a minimum size distribution. Such encapsulation technology has been widely used in the pharmaceutical drug delivery field and is a considerably potential technology even in the agricultural field (Sonvinco et al., 2006). CRFs prepared using the encapsulation technology protect the drug, maintain the concentration of drug within an optimum range by adjusting the release of active components, and provide the sustainability of the effect. In this method, lecithin, which is widely used as a carrier system and surfactant, is a lipid mixture of phospholipids that has been mainly used for forming liposome that can entrap the hydrophobic or hydrophilic materials inside and in micelles under aqueous conditions (Liu and Park, 2009; Ogawa et al., 2003; Calvo et al., 1997; Batzri and Korn, 1973). Such liposome used as a carrier, loaded with molecules such as small drugs and is extremely versatile due to the variability of their composition (Volodkin et al., 2007). The CRF using the encapsulation technology protects drugs, automatically adjusts the release amount of active components, thereby maintaining the concentration of drugs within an optimum range, and provides the sustainability of the effect for a certain period of time. Some papers disclosing examples of using the properties of the controlled release formulation contributed to the development of the CRF in the agricultural field (Bang et al., 2009; Isiklan, 2006).

This study was conducted to verify a method of reducing the spraying number and the use of insecticides, and enhancing the efficacy of insecticide using the nano–encapsulation technology.

MATERIALS AND METHODS

Preparation of controlled release formulations (CRFs)

CRFs were prepared using the method proposed by Bang et al. (2009). The CRFs of etofenprox-containing using the method were all in different types according to the molecular weight and concentration of a chitosan solution used when liposome was coated with chitosan in order to select optimum coating materials and conditions for encapsulation (Table 1). First, the ENC1 type (MW 3,000, 0.3%), the NEC2 type (MW 30,000, 0.1%), and the ENC3 type (MW 30,000, 0.3%) were prepared by adjusting the molecular weight (MW) and concentration of chitosan to select the coating materials. After ENC2 was selected by bioassay, ENC2A (MW 30,000, 0.1%) was prepared using the same material but a little different method in order to encapsulate all active ingredients of etofenprox because ENC2 type did not encapsulate some of the active ingredients.

4 types of etofenprox CRFs, i.e., ENC1 (MW 3,000, 0.3%), ENC2 (MW 30,000, 0.1%), ENC2A (MW 30,000, 0.1%) and ENC3 (MW 30,000, 0.3%) were prepared in a biopolymer engineering lab at Korea University and provided. As a standard for bioassay, Sebero (ai; 20% EC, Kyung–Nong Co., Ltd.,), a commercial product of which active ingredient is etofenprox, was purchased from an agricultural chemicals shop.

Release characteristics of controlled release formulation (CRF)

The CRF was prepared as a liposome by using the preparation method (Bang *et al.*, 2009) as mentioned above and isolated as a resulting product in a pellet form by centrifugation. Then, it was resuspended with phosphate buffered saline (PBS) to prepare a sample. The prepared nano-liposome sample was stored at room temperature and was stirred at a constant speed. Subsequently, the sample was taken out at predetermined constant time intervals, a small amount of acetone was first added to the sample (this process was performed due to the specific gravity of insecticide used

Table 1. The controlled release type of etofenprox made by Chitosan

Code number	Coating material ^a	First release time	Existence of non-coated etofenprox
ENC1	Chitosan M.W. 3,000 0.3%	14h.	0
ENC2	Chitosan M.W. 30,000 0.1%	24h.	0
ENC2A	Chitosan M.W. 30,000 0.1%	24h.	Х
ENC3	Chitosan M.W. 30,000 0.3%	39h.	0

^a : Diverse chitosan's molecular weight (g/mole=Da) and chitosan solution's concentraion.

and low water solubility thereof), and the sample was centrifuged. Then, it was confirmed how much active ingredients were contained in the sample by using GC with respect to the supernatant of the centrifuged sample, and quantitative analysis of the active components released from the nano-liposome for a certain period of time was performed. GC analysis was conducted using method proposed by Bang *et al.* (2009).

SEM photographing

In order to identify ENC2, which was a chitosancoated CRF, samples were observed in powder state without being subject to any pre-treatment. Meanwhile, in order to identify a change in ENC particles over time after reacted with a solvent, ENC2 was sprayed to a host plant and samples were collected at predetermined time intervals. Leaves of the host plant to which ENC2 was sprayed were cut to a size of $0.5 \text{ cm} \times 0.5 \text{ cm}$ by using a clean scissors, and then immersed in a fixing solution prepared by mixing 5% glutaraldehyde and 4% paraformaldehyde and the leaves-containing solution was preserved at a temperature of 4 °C for 24 hours. Each step was subjected to washing for 5 minutes using distilled water, and then dehydrated using ethanol series and isoamyl acetate. The ethanol series refer to 30, 50, 70, 80, and 100% ethanols, and 100% ethanol was used twice and each step underwent dehydration for 10 minutes. Then, for each step, dehydration is further performed twice by using isoamyl acetate for 10 minutes. Then, the samples were frozen at a temperature of -70 °C for 1 hour and then dried in a vacuum condition for 24 hours. Then, the dried each sample was attached to aluminum stub by using a carbon tape. The samples were coated by using an osmium coater (HPC–1SW, Vacuum device), and then assayed by SEM (S-4800, Hitachi) at 15 kw. The SEM was committed to Common Experimental Lab. of Chungnam National University.

Bioassay

S. litura obtained from National Academy of Agricultural Science of Rural Development Administration was used for the bioassay, and an artificial feed was prepared according to a method disclosed by Goh *et al.* (1990). S. litura was reared for several generations at a temperature of 25 ± 2 °C, in a light condition of 16L:8D, and in a relative humidity of 50 to 60%. Chinese cabbage seedlings obtained from Geumgang Seedling Raising Center (Daejeon, Korea) were used as a host plant in the present experiment. Before used in this experiment, the host plants were transplanted in a plastic port and placed for two to three days for rootage.

The two types of insecticidal effect of the CRFs (ENC1, ENC2, ENC2A, and ENC3) on *S. litura* were conducted. First, each CRF was sprayed at 200 ppm to the Chinese cabbage seedlings and then desired volume of leaves was collected from the Chinese cabbage at each time. The Chinese cabbage used in the test was placed on agar medium having a thickness of 1 to 2 cm in a petri dish to provide moisture. The experiment was performed on every other day, and a residual effect test was per-

formed on Sebero EC and CRFs for about one month. Second, according to the amount of the de-encapsulated active ingredient of etofenprox, a direct contact poison test and a digestive poison were performed using the collected supernatant including the de-encapsulated and released active ingredients of etofenprox 48 hours after CRFs were exposed to identify whether etofenprox was coated when the formulation is prepared or not. For each treatment, 10 2nd-stage-larvae of S. litura were inoculated, each treatment was performed three times, and a mortality rate 48 hours after the inoculation was measured. All the indoor tests were performed at least three times for each and the results are obtained by using Microsoft Excel (Redmend, WA) software. In order to determine the phytotoxicity of CRFs (ENC1, ENC2, ENC2A, and ENC3), and Sebero EC were treated to have a concentration of 400 ppm, twice a conventionally used concentration, and the test of phytotoxicity had been identified for 15 days. The host plant treated with the chemicals was placed in a net cage so as to prevent other insects from invading.

RESULTS AND DISCUSSION

Structure and Preparation of controlled release formulations (CRFs)

There are various pests in crops and in particular, noxious insects exist in the mixed stages of eggs, larvae, pupae, and adults. However, most crop protecting agents for removing insect pests control only the specific stage of insects like larvae or adult. Thus, it is difficult to prevent (control) new population from the uncontrolled pupae and eggs. In addition, in the case of chemicals used for pest control, the active ingredients of the chemicals are decomposed after application by environmental conditions, and thus the effects of the chemicals also decrease Thus, the controlled release formulation over time. (CRF) technology, which can reduce the loss of the active ingredients of chemicals until they reach a target pest, is considered to sustain the effects of the chemicals and decrease the spraying number of the chemicals. In order to appropriately control the amount of active ingredients of an agricultural chemical, research into various purposes of CRFs, such as insecticides or pheromone, based on an encapsulation technique mostly used for medical treatments are being performed (Cao et al., 2005; Cork et al., 2007; Ro et al., 1995; Samson and Harris, 1997). In this experiment, etofenprox was used as a core material and the CRF of etofenprox was encapsulated with a coating material such as chitosan or lecithin.

In regard to the coated particles, etofenprox was surrounded by lecithin, which was hydrophilic and hydrophobic materials, thereby forming a circular structure, that is, a liposome. In regard to the liposome, a hydrophobic group of lecithin was located in an inner part of the liposome and a hydrophilic group of lecithin was located in an outer part of the liposome. The inner part of the liposome had the active ingredient of etofenprox, which was hydrophobic. Since an outer surface of the

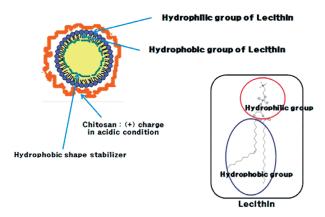


Fig. 1. The schematic of resulting sample.

formed liposome had a negative charge in an acid condition, chitosan, which had a positive charge in the acid condition, was used for the coating (Fig. 1). The CRF of etofenprox had a hydrophilic property due to the chitosan coating, although the property of etofenprox did not have a hydrophilic, thereby having good miscibility with water. Accordingly, the CRF of etofenprox prepared by coating might have a wide size range from about 200 nm to 600 nm (Bang *et al.*, 2009).

The CRFs were prepared as ENC1 (M.W. 3,000, 0.3%), ENC2 (M.W. 30,000, 0.1%), ENC3 (M.W. 30,000, 0.3%) according to the molecular weight and concentration of the coating chitosan (Table 1). According to the amount of chitosan used for coating, a first release time, a cumulative release amount of pesticidal etofenprox, and a particle size might vary. When ENC1 including the smallest amount of coating chitosan was used, the first release time anong the CRFs. When ENC3 including the largest amount of chitosan for coating was used, the first release occurs after 39 hours. The results showed that as higher amount of the chitosan was used, the first release time was increased.

In order to identify nanoparticles of the CRFs, the type and size of the CRFs were observed by SEM (Fig. 2). Referring to Fig. 2, the size range of the controlled formulations is from a small size to 800 nm, and was the

same as the results were similar to the results of Bang et al. (2009). Furthermore, such various sized particles had a polygon shape, not a circular shape which was a normal shape of a liposome. Such results might be due to use of other materials required in the formulation process, such as a surfactant or a dispersant.

Release characteristics of controlled release formulation (CRF)

As shown in Fig. 3, 3 types of the CRF had different release characteristics according to the molecular weight and concentration of the coating chitosan. In regard to ENC1 including the smallest amount of coating chitosan, the active ingredient of etofenprox started to be released the earliest after contact with water, and after 110 hours, the cumulative release amount of the active ingredient of etofenprox was increased at the most rapid rate among the three types of the CRFs and then the cumulative release amount was decreased. On the other hand, in regard to ENC3 including the greatest amount of coating chitosan, the first release occurred later than the others and the cumulative release amount was not decreased even after 160 hours. The cumulative amounts of etofenprox released from the three types of the CRFs did not reach 30 ppm and the release characteristics were gradually reduced. The objective is to maintain the cumulative amount of etofenprox slowly released from the CRF to be 31 ppm for about 15 days so that the activity of LC_{50} appears in 3^{rd} stage instars of S. litura (Bae et al., 2003). Sebero, which is a commercially available etofenprox, persists about 7 days and controls only some larvae and adults of S. litura whose life cycle is from about 27 days to 36 days. Accordingly, additional one week is required to control eggs and flying insects from other fields, excluding the larvae and adults which are controlled by spraying this chemical. Correspondingly, the required characteristic of the CRF is that the effective component of the chemical is slowly released and the drug effect persists for about 14 days (Bae, 1999a; Bae, 1999b).

Among the three types of the CRFs, ENC1 was released too early and on the other hand, ENC3 was released too late. ENC2 showed release characteristics between ENC1 and ENC2. Accordingly, based on the

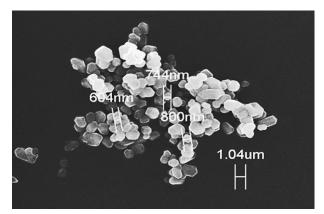


Fig. 2. Scanning electron microscope photograph of ENC2 bead, magnification x 20,000.

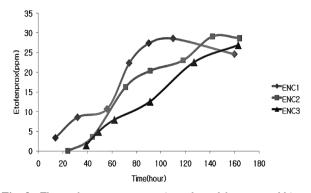


Fig. 3. The etofenprox concentration released from several bionano ENC types.

release characteristic over time and the cumulative amount, ENC2 was selected and a further experiment was performed. According to Fig. 4, the release amount was increased after 71 hours and the cumulative release amount was the greatest at 142 hours. The results suggested that an insecticidal efficacy against *S. litura* persisted for 142 hours. The cumulative release amount continuously increased and at 142 hours, the cumulative release amount was gradually reduced and a certain concentration maintained until 241 hours (Fig. 4).

Meanwhile, since the CRF was prepared by coating etofenprox with liposome and chitosan to encapsulate the active ingredient of etofenprox, the CRF of etofenprox had activity by releasing as it exposed to a solvent. Accordingly, the chemical might show efficacy when pest contacts or feeds the chemical sprayed on crops. A change of the encapsulated CRF when exposed to a highly alkaline midgut of *S. litura* feeding the chemical was identified. The midgut of *S. litura* is known to be basic and produces a basic product (Berenbaum, 1980; Skibbe *et al.*, 1996). The effect of chitosan, which is the major coating material of the CRF, on the release by a chemical reaction in a midgut's basic state was identified (Fig. 5). As a result, though the release occurred in both neutral (pH 7.6) and basic (pH10.6) condition, the release rate was lower in the basic solvent than the neutral solvent. The lower release rate in the basic solvent might be due to the fact that the chitosan and lecithin were dissolved in an acidic solvent in the preparation process. As a similar research result, when calcium alginate which is prepared using an alkaline solvent in the preparation process for a CRF is used as a coating material, the release of an effective material is hindered in an acidic condition (Isiklan, 2006; Roy *et al.*, 2009).

Since a CRF had such a characteristic that a chemical was slowly released over time, a particle shape change was identified by SEM (Fig. 6). The experiment was performed to identify a particle shape when controlled particles began to react a solvent (Fig. 6A), when 44 hours had passed after the spraying (Fig. 6B), when 142 hours, during which the cumulative release amount was continuously increased, have passed (Fig. 6C), and when

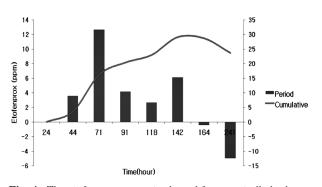


Fig. 4. The etofenprox amount released from controlled release formulation ENC2 type over time.

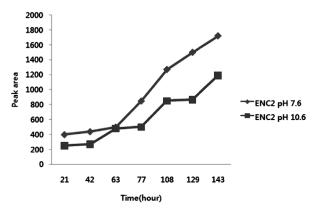


Fig. 5. The release property of ENC2 type under the different pH.

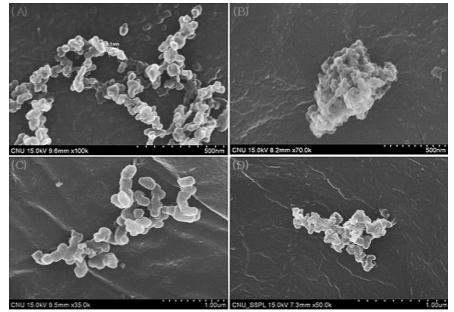


Fig. 6. Scanning electron microscope photographs of treated ENC2. (A) after 0 day, (B) after 44 hours, (C) after 142 hours, and (D) after 14 days.

two weeks had passed (Fig. 6D). Referring to the figures described above, when the CRF began to be exposed to the solvent, the chemical was released and thus, the particle size was reduced. Over time, the group of particles was decreased and particles have smooth surfaces. The sizes of diluted particles were mostly about 100 nm. Even when the release was performed, the particle shape was not or slightly changed. When particle sizes of the experimental group and a control group were compared after two weeks, the particle size of the experimental group was slightly decreased and the number of particles on leaves was reduced.

Biological activity

Since etofenprox used as a material having insecticidal activity in the CRF causes a contact poison and a digestive poison, a new formulation in which etofenprox was coated with chitosan and slowly released was compared with a commercial product in terms of biological activity (Table 2). In regard to the contact poison and the digestive poison characteristic, both ENC2 and a commercial product of etofenprox showed 100% of mortality. This ENC2 of the CRF initially showed a contact poison and the result might be caused by a non-coated pesticide. The objective of the production of the CRF was to entirely coat the pesticide so as to release only a required amount of pesticide at a target time. In order to solve this problem, all active ingredients were coated to prepare ENC2A and the new ENC2A showed a mortality rate of 23% and 27% against a contact poison and

Table 2. The mortality of 2nd instars of Spodoptera litura to the different controlled release formulations (CRFs) by contact poison and digestive poison

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Туре	Contact poison	Digestive poison	
Control	0%1	0%	
Etofenprox	100%	100%	
ENC2	100%	100%	
ENC2A	23%	27%	

¹: Mortality of 2nd instars of *Spodoptera litura* 48 hours after inoculation.

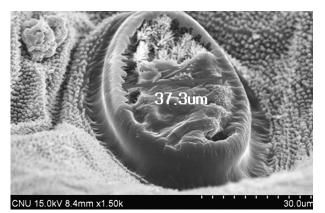


Fig. 7. Scanning electron microscope photograph of *Spodoptera litura's* stoma, magnification x 1,500.

a digestive poison, respectively (Table 2). If ENC2A enables the release amount and release rate to be constant, a desired CRF may be obtained from addition of active ingredients.

Meanwhile, whether sprayed ENC particles, which were a nano CRF, pass through a stigma of S. litura so as to obtain an insecticidal effect was tested (Fig. 7). The size of the stigma of S. litura was about $37.3\,\mu\text{m}$. Thus, it was deemed that ENC particles having a size of 100 to 800 nm of the nano controlled formulation may sufficiently pass. Three types of nano ENC formulations prepared all in different conditions to show characteristics of a controlled formulation were identified by performing an efficacy test (Fig. 8). Based on results of the residual effect test of ENC1, ENC2, ENC3, and etofenprox (Sebero 20% EC) as a standard, it could be identified that all the tree types of formulations showed a substantially higher mortality rate than the standard. In regard to etofenprox used as the standard, 5 days after the spraying, the mortality rate was decreased to 50% or less, and 15 days after the spraying, the mortality rate was almost zero. However, among the three types of CRF, ENC1, of which the initial release occurred the earliest, showed the highest mortality rate until two days after the spraying, and ENC3, of which the initial release occurs most late, showed the lowest mortality rate. In regard to the mortality rate 7 days after the spraying, ENC1 initially showed the highest mortality rate and 9 days after, its mortality rate was rapidly reduced. That is, ENC1 did not show a sustainably effective insecticidal effect. The other types of formulations also initially showed the similar mortality rate, and ENC2 was selected for a further experiment because it showed the highest mortality rate 15 days after the spraying.

The CRFs of etofenprox were needed to improve an inconsistent efficacy as a product according to the amount of etofenprox that was not completely encapsulated in the CRF preparation process. Accordingly, the same material as used for ENC2 was used and some of the method of preparing ENC2 was modified to produce ENC2A in which the active ingredient of etofenprox was completely encapsulated (Table 1 and 2). According to

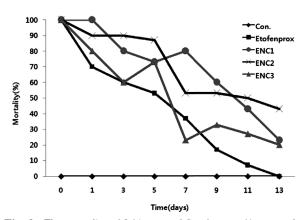


Fig. 8. The mortality of 2nd instars of *Spodoptera litura* to the different controlled release formulations by the residual effect test on the Chinese cabbage.

Table 3, the mortality rate of ENC2A was continuously increased after the spraying, and 9 days after the spraying, the mortality rate reached to 83%. In regard to the commercial etofenprox product, the mortality rate at the spraying date was 100% but 3 days after the spray, the mortality rate was 63%. Compared with time showing more than 50% of mortality rate, the effect of ENC2A lasted up to about 14 days after spraying while the effect of etofenprox (Sebero 20% EC) lasted up to 5 days. The effect of ENC2A might be enhanced by preventing loss of the active ingredients of chemicals. Since the efficacy of ENC2A was maintained twice as long as commercial etofenprox product, the effect of once spraying might be equivalent to that of twice spraying. These results suggest that such CRF is considered to be used as a method of preventing loss of active ingredients of chemicals and increasing the residual activity of the chemicals against a target pest, thereby reducing the use of the chemicals. Therefore, ENC2A might be commercialized through addition of a desired amount of etofenprox to compensate for the low initial insecticidal effect. In addition, etofenprox-containing CRF did not show any symptoms of phytotoxicity on the Chinese cabbage, compared with etofenprox (Sebero, 20% EC) and control.

A variety of chemical pesticide used in agricultural ecosystem are not fully used to control target insects, and are decomposed and lost by various environmental conditions such as sunlight, rainfall, and the like. Thus, there is a need to continuously conduct research on a method of reducing the use of agricultural chemicals by using a controlled release formulation based on nano–encapsulation that can enhance the effect of the chemicals by controlling active components not to be decomposed and lost.

Table 3. The mortality of 2nd instars of Spodoptera litura to
etofenprox (Sebero 20% EC) and ENC2A by the resid-
ual effect test on the Chinese cabbage

Derroften	Mortality $(\%)^1$		
Day after	Etofenprox (Sebero 20% EC)	ENC2A	
3	63	68	
5	50	71	
7	24	74	
9	16	83	
11	11	66	
13	3	53	
15	6	46	
17	0	34	
19	0	28	
21	0	27	
23	0	20	
25	0	11	

 $^{\rm 1}\colon$ Mortality of $2^{\rm nd}$ instars of Spodoptera litura 48 hours after inoculation.

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REFERENCES

- Bae, S. D. 1999a Leaf characteristics of leguminous plants and the biology of tobacco cutworm, *Spodoptera litura* Fabricius:
 I. The larval development and leaf feeding amount. *Korean J. Appl. Entomol.* 38: 217–224
- Bae, S. D. 1999b Leaf characteristics of leguminous plants and the biology of tobacco cutworm, *Spodoptera litura* Fabricius:
 II. Pupal development and adult longevity. *Korean J. Appl. Entomol.* 38: 225–230
- Bae, S. D., B. R. Choi, Y. H. Song and H. J. Kim 2003 Insecticide susceptibility in the different larva of tobacco cutworm, *Spodoptera litura* Fabricius (Lepidoptera:Noctuidae) collected in the soybean fields of Milyang, Korea. Korean J. Appl. Entomol. 42: 225–231
- Bae, S. D., H. J. Kim, Y. K. Hong and H. J. Cho 2004 Effects of sublethal concentration of insecticides on the pupal duration, emergence, adult longevity and oviposition of tobacco cutworm, *Spodoptera litura* (Fab.) (Lepidoptera:Noctuidae). *Korean J. Appl. Entomol.* **43**: 175–180
- Bae, S. D., H. J. Kim, G. H. Lee and S. T. Park 2007 Seasonal occurrence of tobacco cutworm, *Spodoptera litura* Fabricius and beet armyworm, *Spodoptera exigua* Hübner using sex pheromone traps at different locations and regions in Yeongnam district. *Korean J. Appl. Entomol.* 46: 27–35
- Bae S. D. and K. B. Park 1999 Effects of temperature and food source on pupal development, adult longevity and oviposition of the tobacco cutworm, *Spodoptera litura* Fabricius. *Korean J. Appl. Entomol.* 38: 23–28
- Bae, S. D., K. B. Park and Y. J. Oh 1997 Effects of temperature and food source on the egg and larval devlopment of tabacco cutworm, *Spodoptera litura* Fabricius. *Korean. J. Appl. Entomol.* 36: 48–54
- Bang, S. H., Y. M. Yu, I. C. Hwang and H. J. Park 2009 Formation of size-controlled nano carrier systems by self-assembly. J. Microencapsulation 26: 722–733
- Batzri, S. and E. D. Korn 1973 Single bilayer liposomes prepared without sonication. *Biochim. Biophys. Acta*. 298: 1015–1019
- Berenbaum, M. 1980 Adaptive significance of midgut pH in larval Lepidoptera. Am. Nat. 112: 138–146
- Calvo, P., C. Remunan–Lopez, J. L. Vila–Jato and M. J. Alonso 1997 Novel hydrophilic chitosan–polyethylene oxide nanoparticles as protein carrier. J. Appl. Polym. Sci. 63: 125–132
- Cao, Y., L. Huang, J. Chen, J. Liang, S. Long and Y. Lu 2005 Development of a controlled release formulation based on a starch matrix system. *Int. J. Pharm.* **298**: 108–116
- Choi, J. R., W. R. Song, S. Y. Hwang, H. S. Kim and J. O. Lee 1996 Age-related susceptibility of Spodoptera litura larvae to some insecticides. Korean J. Appl. Entomol. 35: 249–253
- Cork, A., K. De Souza, D. R. Hall, O. T. Jones, E. Casagrande, K. Krishnaiah, and Z. Syed 2007 Development of PVC-resincontrolled release formulation for pheromones and use in mating disruption of yellow rice stem borer, *Scirpophaga incertulas. Crop Prot.* 27: 248–255
- Goh, H. G., S. G. Lee, B. P. Lee, K. M. Choi and J. H. Kim 1990 Simple mass-rearing of beef armyworm, Spodoptera exigua. Korean J. Appl. Entomol. 29: 180–183
- Isiklan, N. 2006 Controlled release of insecticide carbaryl from sodium alginate, sodium alginate/gelatin, and sodium alginate/ sodium carboxymethyl cellulose blend beads crosslinked with glutaraldehyde. J. Appl. Polym. Sci. 99: 1310–1319
- Jin, D. Y., S. K. Paek, J. S. Kim, S. Y. Choi, C. Park, T. H. Kim, N. Y. Jin, S. Y. Jung, Y. N. Youn and Y. M. Yu 2009 Environment–

friendly control of beet armyworm, Spodoptera exigua (Noctuidae:Lepidoptera) to reduce insecticide use. Korean J. Appl. Entomol. **48**: 253–261

- Kim, C. H. and H. Y. Shin 1987 Studies on bionomics and control of tobacco cutworm, Spodoptera litura Fabricius in southern part of Korea. J. Inst. Agr. Res. Util. Gyeongsang Natl. Univ. 21: 105–122
- Kim, H. H., S. R. Cho and H. Y. Choo 2008 Biological Control of Tobacco Cutworm, Spodoptera litura (Lepidoptera:Noctuidae) by Steinernematidand Heterorhabditid Entomopathogenic Nematodes. Korean J. Appl. Entomol. 47: 447–456
- Kim, Y. G., J. R. Cho, J. I. Lee, S. Y. Kang, S. C. Han, K. J. Hong, H. S. Kim, J. K. Yoo and J. O. Lee 1998 Insecticide resistance in the tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera:Noctuidae). J. Asia–Pacific Entomol. 1: 115– 122
- Liu, N. and H. J. Park 2009 Chitosan–coated nanoliposome as vitamin E carrier. J. Microencapsulation **26**: 235–242
- Minamikawa, H. 1937 Survey on the tabacco cutworm Spodoptera litura Fabricius. Taiwan Central Res. Int. Agr. Report **70**: 1–66
- Ogawa, S., E. A. Decker and D. J. Mcclements 2003 Influence of environmental conditions on the stability of oil in water emulsions containing droplets stabilized by lecithin–chitosan membranes. J. Agric. Food Chem. **51**: 5522–5527
- Rao, G. V. R., J. A. Wightman and D. V. Ranga Rao 1993 World review of the natural enemies and diseases of *Spodoptera lit*-

ura (F.) (Lepidoptera:Noctuidae). Insect Sci. Appl. 14: 273–284

- Ro A. S., K. W. Han and J. Y. Cho 1995 Effect of different formulations on the biological activity of herbicide Cyhalofop–Butyl. *Agric. Chem. Biotechnol.* 38: 440–446
- Roy, A., J. Bajpai and A. K. Bajapai 2009 Dynamics of controlled release of chlorpyrifos from swelling and eroding biopolymeric microspheres of calcium alginate and starch. *Carbohyd. Polym.* **76**: 222–231
- Samson, P. R. and W. J. Harris 1997 Effectiveness of some controlled–release insecticides against larvae of *Inopus rubriceps* (Macquart) (Diptera: Stratiomyidae), a pest of Australian sugarcane. Crop prot. 16: 653–658
- Sayyed, A. H., M. Ahmad and M. A. Saleem 2008 Cross-resistance and genetics of resistance to indoxacarb in *Spodoptera litura* (Lepidoptera:Noctuidae). J. Econ. Entomol. 101: 472–479
- Skibbe, U., J. T. Christeller, P. T. Callaghan, C. D. Eccles and W. A. Laing 1996 Visualization of pH gradients in the larval midgut of Spodoptera litura using ³¹P–NMR microscopy. J. Insect Physiol. 42: 777–790
- Sonvinco, F., A. Cagnani, A. Rossi, S. Motta, M. T. Di Bari, F. Cavatorta, M. J. Alonso, A. Deriu and P. Colombo 2006 Formation of self–organized nanoparticles by lecithin/chitosan ionic interaction. *Int. J. Pharm.* **324**: 67–73
- Volodkin, D., H. Mohwald, J. C. Voegel and V. Ball 2007 Coating of negatively charged liposomes by polylysine: drug release study. J. Contr. Rel. 117: 111–120