Polymer(Korea). Published by the Polymer Society of Korea
Vol. 12. No. 2. 1988

페로몬의 효과적인 사용법으로서의 微少 캡슈울化

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(1987년 10월 29일 접수)

Microencapsulation as an Effective Release Method for Pheromone

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(Received October 29, 1987)

요 약 : 요소-포름알데히드를 사용한 微少 캡슈울化를 응용하여 이화명충 방제를 위한 페로몬 약효의 지속효과를 연구 하였고 아울러 벼에 분무하기 좋은 제제조건도 개발하였다. 유인실험 결과, 微少 캡슈울化된 페로몬의 약효가 8일간 지속됨으로 微少 캡슈울化시키지 않은 페로몬 보다 약 3배의 효과를 나타내었다.

Abstract : Urea-Formaldehyde microencapsulation was studied as a slow release system for the synthetic sex pheromone of the striped rice borer. The formulation which gives applicable size for spray was also developed in this study. In the result of the attraction test, the microencapsulated pheromone showed 8 days of releasing effect which is 3 times more effective than the unencapsulated pheromone.

INTRODUCTION

In 1959, Karlson and Lüscher[1] found that insect produces a secretion which attracts the other sex of the insect and they named it pheromone.

The pheromones are being utilized in three ways for pest control[2] : Detection and surveillance of insect species, Mass trapping of male moths, Communication disruption between sexes to prevent mating. Disruption method is the most effective for pest control in a wide range. In 1960, it was reported that a certain concentration of pheromone in the air caused disruption of communication of insects.[3]

The main pest of rice crop in Korea is rice stem borer[4] of which female attracts male and breeds. In 1975, Nesbitt and Beevor isolated, identified and synthesized (Z)-11-hexadecenal(1) and (Z)-13-octadecenal(2) as a pheromone of rice stem borer by using gas chromatography and electroantennogram at Tropical Products Institute (TPI) in London. They, also, reported that the olefinic aldehydes, (1) and (2) in a ratio of 4.5 : 1, showed 70% attraction relative to virgin female rice stem borer.

$$CH_3(CH_2)_3CH=CH(CH_2)_9CHO \quad (1)$$

$$CH_3(CH_2)_3CH=CH(CH_2)_{11}CHO \quad (2)$$

It is difficult and expensive to synthesize these pheromones, so the development of slow release system is required for persistency of mating disruption using small amounts of pheromone.[5]

Microencapsulation[6] which is made from synthetic polymer is used as one of the slow release methods, but this sometimes causes pollution problem.

In this respect, this study reported microencapsulation technique using urea-formaldehyde condensation[7] and formulation for spray.

EXPERIMENTAL

Most of the organic compounds used in this study were purchased from Aldrich Chemical Co. and (Z)-11-hexadecenal and (Z)-13-octadecenal were synthesized by Dr. J. H. Kim in KAIST. GLC analysis were performed using a Varian Aerograph series 2700 gas chromatography equipped with 5'×1/8", 1.5% OV-101 column.

Preparation of Xanthate : To a suspension of 81g of starch in 500ml of water is added 20ml of carbon disulfide, followed by 20g of sodium hydroxide dissolved in 200ml of water. The solution has changed the color to a reddish brown after 1 hour stirring at room temperature. This xanthate is stable for 30 days at 5℃.

Preparation of Xanthide : To a mixture of 100g of xanthate and 1g of pheromone is added 18ml of sodium nitrate(50%) with vigorous stirring and changed the pH to 5 with glacial acetic acid. The color is changed to white and white solid which has sponge character is formed. Salt has removed by washing with water and the white solid is filtered and dried and resulted in 94.1g of reddish yellow solid.

Urea-Formaldehyde Microencapsulation : The mixture of 6.08g (0.1 mol) of urea and 16.44g(0.2 mol) of 37% formalin is stirred for 20 min. at room temperature and made the pH to 8 with 0.2g of 10% triethanolamine. This mixture is heated to 60-65℃ for 1 hr and then cool to 31℃ and added 25ml of water. To this mixture is added 0.6g of pheromone, followed by 0.3g of Tween 80(HLB 15.0) as a emulsifier with vigorous stirring. The pH is changed to 4-4.5 by adding 5% oxalic acid and this mixture is heated to 60-65℃ for 1 hour. The resulting solid can be sprayed directly or reencapsulated with precondensate which is made from urea and formalin at pH 8.

RESULTS AND DISCUSSION

Starch Encapsulation[8]

Starch and carbon disulfide in basic condition produce starch xanthate. Pheromone is encapsulated through cross-linking[9] of starch xanthate by oxidation. Namely, pheromone is encapsulated inside of starch xanthide matrix.[10]

$$ROH + CS_2 \xrightarrow[NaOH]{} ROC(=S)SN_a \text{ (Xanthate)}$$

$$ROC(=S)SN_a + \text{Pheromone} \xrightarrow{[O]}$$

$$ROC(=S)SSC(=S)OR \quad \text{(Xanthide)}$$

R : starch

The polymerization occurs on the surface of the small particles of dispersed pheromone by stirring with oxidation. The particle size of this polymer depends on stirring rate to some degree, but this technique does not allow the constant size of the particles.

In this starch encapsulation, the polymer is swollen after 1 hour soaking in water, even latex and polystylene is added for capsule wall material. So this is not good method for rice stem borer, but it can be utilized for the pest of tree.

Urea-Formaldehyde Microencapsulation

Urea reacts with 2 equivalents of formaldehyde in water to give precondensate which is dispersed to get proper size(1-500μm) by stirring.

Condensate polymerization occurs on the surface of the pheromone in acidic condition(pH=4.5).[11]

$$NH_2C(=O)NH_2 + 2\ HC(=O)H \longrightarrow$$

$$HOCH_2NHC(=O)NHCH_2OH \text{ (precondensate)}$$

$$\text{precondensate} + \text{pheromone} \xrightarrow{pH=4.5} (\text{-}CH_2NHC(=O)NHCH_2O\text{-})_n \text{ (encapsulation)}$$

$$\text{encapsulated pheromone} + \text{precondensate} \xrightarrow{pH=4.5} \text{reencapsulation}$$

In order to get homogeneous condition from two layers when pheromone reacts with precondensate, emulsifier is required. Viscosity and the particle size of the encapsulated products are determined by these emulsifiers. For instance, Briz 92(HLB 4.9) or Tween 80(HLB 15.0) gives milkish colloidal condition which has applicable size for spray. But Briz 92(HLB 9.7) gives sticky gum which can not spray.

To identify the degree of encapsulation of pheromone, the encapsulated product is filtered and the filtrate is extracted with methylene chloride and resulted in 88.1% of encapsulation relative to 0.6g of starting pheromone. The solid particle size is 1-104μm and this solid is degradable in soil and also can be used as a fertilizer.[12]

Comparison of Slow Release Rate : Unencapsulation, Encapsulation, Reencapsulation

Unencapsulation ; After 0.2g of pheromone gets stained on polymer which does not contain pheromone, the polymer is stirred for 3 min. in 30 ml of *n*-heptane and then filtered. The filtrate is evaporated and dissolved in 1ml of *n*-heptane, and takes 2μl to GC. The integration of this result is compared to the integration of 0.4mg of pheromone in 2 μl of *n*-heptane. The filtered polymer is stirred and treated again in the same way as the above during the indicated time intervals in Table 1.

Table 1. Comparison of Slow Release Rate.

Stirring hour	1 3 min	2 7 min	3 30 min	4 80 min	5 16 hrs
Unencapsulation	86	12	2	0	0
Encapsulation	77	8	2	8	5
Reencapsulation	64	4	4	16	12

This data indicates the amount of pheromone(%) released by solvent extraction.

Encapsulation ; Encapsulated polymer which contains 0.2g of pheromone is treated as same as the unencapsulation.

Reencapsulation ; Reencapsulated polymer which contains 0.2g of pheromone is treated as same as the unencapsulation.

After 2 hours of solvent extraction, there is nothing coming out from the unencapsulation, but still shows the pheromone even after 18 hours of extraction in the encapsulation and the reencapsulation. This result indicates that the reenapsulation and the encapsulation are more effective than the unencapsulation as a slow release system.

Another Measurement for Slow Release Rate

Nitrogen gas is passed through, with 50 ml / min of flow rate, two same glass tubes and one of those contains 0.6g of encapsulated pheromone on filter paper and the other contains 0.6g of neat pheromone on filter paper. Each outlet of glass tubes has installed with carbowax 20M trap (10cm) which collects the vaporized pheromone every 6 hours and compares the amount vaporized in GC.

In the result, unencapsulated pheromone is not detected after 9 days in GC, but encapsulated pheromone still shows in GC even after one month of releasing test. This experiment also indicates that the encapsulated pheromone is much more effective for slow release than the unencapsulated pheromone.

Laboratory Test of Rice Stem Borer

The bottom of lure box(100cm×200cm×70cm) is made of plywood and the sides and the top are all surrounded by nylon net. Small rices are planted on the pot in lure box which is similar to the field. The lure box is installed in green house which has ventilation and maintained at 25℃. Sticky traps(20 cm×28 cm) are installed diagonally at four top corners, two controls and two test traps, of lure box.

Attraction Test : In order to test the activity of synthetic pheromone, synthetic pheromone dissolved in hexane(0.2mg / 1ml) is absorbed in filter paper and tried attraction test at different concentrations from 0.5ml (0.1mg of pheromone) to 4.0ml (0.8mg

Table 2. Attraction Activity of Synthetic Pheromone.

Test	Control	Concent. of syn. pheromone	The No. of added male rice stem borer
13(56.5%)	10(43.5%)	0.1mg / 0.5mL	40
24(75.0%)	8(25.0%)	0.2mg / 1.0mL	40
23(70.6%)	9.6(29.4%)	0.4mg / 2.0mL	40
18(64.3%)	10.4(36.6%)	0.6mg / 3.0mL	40
20(59.5%)	13.6(40.5%)	0.8mg / 4.0mL	40

The numbers of this results are the average of 5 repetitions.

Table 3. Confusion Effect of Unencapsulated Pheromone*.

Day	Test	Control	The No. of added male rice stem borer
1	48.9%	51.1%	40
2	53.7%	46.3%	40
3	45.2%	54.8%	40
4	69.3%	30.7%	40
5	73.2%	26.8%	40
6	73.5%	26.5%	40
7	72.6%	27.4%	40

*The numbers of this results are the average of 5 repetitions.

of pheromone), and compared with control containing 2 virgin females.

As shown in table 2, 0.2mg of synthetic pheromone shows the best result of attraction. The attraction activity is getting decreased with higher concentration and even shows evasion effect at a certain point.

Confusion Effect of Unencapsulated Pheromone: In order to test the confusion effect and persistency of unencapsulated synthetic pheromone, 30mg of synthetic pheromone dissolved in 1ml of hexane is sprayed all over the seedbed in lure box. Each test trap contains 3 virgin females which are replaced every day and the control is the blank sticky trap.

As shown in table 3, confusion effects of the test and the control traps are almost same until 3rd day, but confusion effect is disappeared after 4th day and male insects are getting much more attracted at test trap.

Confusion Effect of Encapsulated Pheromone:

Encapsulated pheromone which contains 30mg of pheromone is sprayed all over the seedbed and the experiment is carried out same as the above and resulted in table 4.

Table 4. Confusion Effect of Encapsulated Pheromone.

Day	Test	Control	The No. of added male rice stem borer
1	55.6%	44.4%	40
2	43.8%	56.2%	40
3	54.2%	45.8%	40
4	48.0%	52.0%	40
5	55.6%	44.4%	40
6	61.1%	38.9%	40
7	51.7%	48.3%	40
8	55.6%	44.4%	40
9	72.2%	27.8%	40
10	62.5%	37.5%	40
11	64.3%	35.7%	40
12	66.7%	33.3%	40
13	62.5%	37.5%	40
14	72.2%	27.8%	40

This data indicates that the encapsulated pheromone is effective for 8 days of communication disruption.

In conclusion, urea-formaldehyde microencapsulation of pheromone is very effective for mating confusion of 8 days relative to the unencapsulated pheromon of 3 days. Also, this formulation is directly applicable to spray.

Acknowledgement: Financial support of the Korean Ministry of Education for the free research project (1987) is gratefully acknowledged.

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