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# Activities of esterase and acetylcholinesterase on the diamond backmoth, *Plutella xylostella* (Lepidoptera : Yponomeutidae) and beet armywarm, *Spodoptera exigua* (Lepidoptera : Noctuidae) and inhibitions of acetylcholinesterase with flupyrazofos

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**Abstract** : The activities of esterase and acetylcholinesterase(AChE) on the Diamond backmoth (DBM), *Plutella xylostella* (Lepidoptera: Yponomeutidae) and Beet armywarm (BAW), *Spodoptera exigua* (Lepidoptera: Noctuidae) and inhibitions of AChE with flupyrazofos were clarified from the results of a series of experiments. These findings are described in brief as follows.

The AChE activities of DBM and BAW in heads were 1.5~11.1 μmol/g/min in 1st~4th instar larvae of DBM and 1.7~45.2 μmol/g/min in 1st~6th instar larvae of BAW, respectively. Those were 25~30 times higher in above 4th instar larvae of BAW than that of the 1st instar larvae of DBM. The activities of aliesterase in heads were 1.7~4.7 times higher in 2nd~4th instar larvae of DBM and 8~55 times higher in 3rd~6th instar larvae of BAW than 1st instar larvae of DBM. In abdomens, those were 3~17 times higher in 2nd~4th instar larvae of DBM and 12~30 times higher in 3rd~6th instar larvae of BAW than 1st instar larvae of DBM. Median AChE inhibition concentration ($I_{50}$) of flupyrazofos to the 2nd instar larvae of DBM and BAW were 92 nM and 15 μM, respectively, and those to the 4th instar larvae of DBM and BAW were 1.8 μM and 3.1 mM, respectively. Insensitivity ratio of flupyrazofos in the 2nd instar BAW larvae showed ca. 162 times higher than that in the 2nd instar larvae of DBM, and that of the 4th instar BAW larvae showed ca. 1,720 times higher insensitivity to flupyrazofos than that of the 4th instar DBM larvae. AChE activities in the 2nd instar larvae of DBM and BAW at 32 h after applicaton of flupyrazofos decreased from 67.6% to 32.4% of the activity of the untreated control. That of the 4th instar larvae of DBM increased for 0.5 h after application flupyrazofos up to 75% of the untreated control, and after that it decreased to 34.5% of the untreated control at 32 h. In contrast, in the 4th instar larvae of BAW AChE activities increased for 8 h gradually up to 102% of the activity of the untreated control, and then the activity decreased to 97% of the untreated control at 16 h after treatment.
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Keywods : flupyrazofos, esterase, AChE, *Plutella xylostella*, *Spodoptera exigua*.

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## INTRODUCTION

In recent years, the diamondback moth (DBM), *Plutella xylostella,* and the Beet armyworm (BAW), *Spodoptera exigua* (Hübner), have become the most important insect pests of crops in the world. Although these species are believed to have originated in the south-southeast parts of Europe and Asia, their present status are in most parts of the world which is attributed to the extended cultivation of host plants and their superb migratory habit (Chu, 1986; Mikkola, 1970). Furthermore, continuous growing of host plants and favorable climatic conditions result in DBM and BAW attaining high population densities with overlapping generations all the year round. If not managed properly at the early growth stage of the crop, these insects could cause a serious yield loss with excessive feeding on the leaves by larvae.

Since 'mutant aliesterase hypothesis' was reported by Oppenoorth and van Asperen (1960), many researchers have investigated detoxication enzymes as factors of resistance in insects. Esterase, mixed function oxidase, glutathione S-transferase, dehydrochlorinase etc. were summarized as detoxication enzymes. Their role as resistance mechanism have been extensively reviewed by Plapp (1976) and Terriere (1984).

Decreased AChE sensitivity as a factor of resistance was revealed first in the two-spotted spider mite by Smissaert (1964), and followed by the cattle tick, mite, green rice leafhopper, house fly and mosquito. Mechanism of resistance to the organophosphorus and pyrethroid-resistant brown planthopper is mainly due to increase in esterase activity (Miyata *et al.*, 1983; Dai & Sun, 1984; Park and Choi, 1991). However, resistance to the carbamate insecticides is attributable to increase of AChE insensitivity rather than incrased esterase activity (Chung & Sun, 1983; Hama & Hosoda, 1983).

Flupyrazofos(Fig 1), an organophosphorus insecticide, {O,O-diethyl O-1-[U-C] phenyl-3-trifluoromethyl pyrazol-5-yl phosphorothioate} has been developed by Korea Research Institute of Chemical Technology (Hwang, 1989). Flupyrazofos could be an effective alternative because of its outstanding insecticidal activity against DBM (Lee *et al.*, 1997). This insecticide has been recently investigated regarding its absorption, retention and vapor pressure and is of increasing importance in the control of DBM (Kim *et al.*, 1997; Yang *et al.*, 1997). Flupyrazofos was much more potent to DBM than BAW, and flupyrazofos is very specific to the DBM larvae (Lee *et al.*, 1977). However, little work has been done on the mechanisms of flupyrazofos.

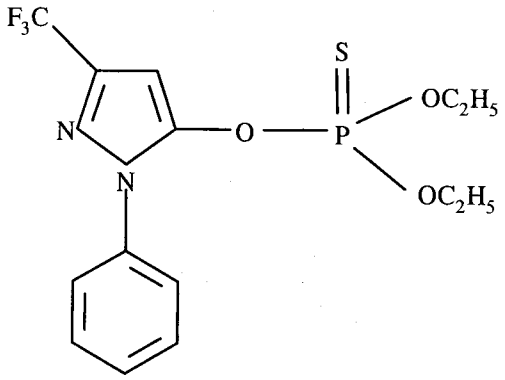


Fig. 1. Chemical structure of flupyrazofos.

In the laboratory study described herein, we dealt with esterase activity for the purpose of elucidation of activity characteristics of flupyrazofos {O,O-diethyl O-1-[U-C]phenyl-3-trifluoromethylpyrazol-5-yl phosphorothioate} to the diamondback moth, as a susceptible insect and the Beet armyworm, as a tolerance insect.

## MATERIALS AND METHODS

### Insects

Two lepidopterous insect species were used in this study. *P. xylostella* larvae was reared on 6- to 9-day-old rape seedling in a cage (40×40×45㎝) under conditions of controlled temperature (25±1°C), 50-60% relative humidity, and a photoperiod of 16 : 8 (L : D) h. Adults were maintained in 20% sucrose solution. The susceptible laboratory population was obtained from Korea Research Institute of Chemical Technology at Daejeon, Korea in 1996, and maintained through 19th generations in the laboratory without exposure to any

insecticide. *S. exigua* was collected from welsh onion field at Suwon, Korea in September 1994. These were reared on artificial diet (Shorey and Hale, 1965) in a plastic cage (22×16×9㎝), and maintained under same conditions mentioned above through 25th generations in the laboratory without exposure to any insecticide.

**Chemicals**

Flupyrazofos technical grade (>95% purity) was provided by Sungbo Chemicals CO., LTD, Seoul, Korea.

**Esterase activity**

Esterase activity assays were performed with homogenate prepared from the head and body of each larval stage of DBM and BAW. For AChE assay, 150 heads were homogenized in 5 mL of 0.1M phosphate buffer (pH 7.4) by tissue tearor (Model 985-370, Biospec Products, Inc.). For aliesterase assay, 30-whole-body were homogenized in 5 mL of the phosphate buffer by tissue tearor. After centrifugation at 15,000*g* for 15 min at 4℃, the supernatant was used for assays (Park *et al.*, 1991). AChE activity in supernatant was measured with acethylthiocholine iodide as a substrate according to the method described by Ellman *et al.* (1961). The 0.5 mL of enzyme solution was mixed with 2.5 mL of 0.1 M phosphate buffer (pH 7.4), 0.2 mL of 3 mM DTNB (5,5'-dithio bis (2-nitrobenzoic acid)) and 0.2 mL of 30 mM acetylthiocholine iodide and incubated at 30℃ for 30 min. AChE activity was based on the change in absorbance at 412 nm with an Ultrospec spectrophotometer (Pharmacia Biotech, Cambridge, England) every 5 min. AChE activity was calculated using a standard regression line (Ellman *et al.*, 1961). The reaction was stopped with 0.2 mL of 5 mM eserine salicylate.

For the aliesterase assay, α- and β- naphthylacetate were used as substrates according to the method of van Asperen (1962). Fast blue RR salt (1%) was used as a coloring agent. The reagent for stopping the reaction was 5% solution of sodium lauryl sulfate. The aliesterase activities were calculated from the absorbance of the reaction mixtures at 600 nm for α-naphthylacetate and 550 nm for β-naphthylacetate (van Asperen, 1962). For both assays, two samples were run for each homogenate. All tests were duplicated.

**AChE inhibition by flupyrazofos**

To test the AChE inhibition effect of flupyrazofos on the 2nd and 4th instar of DBM and BAW, heads were removed from the frozen DBM and BAW and honogenized immediately with 20 mL of 0.1 M phosphate buffer(pH 7.4). Homogenates were centrifuged at 15,000*g* for 15 min at 4℃. The supernatant was decanted and used as the enzyme solution. The reaction mixture, 0.5 mL of the enzyme solution, 2.5 mL of 0.1 M phosphate buffer, 0.2 mL of 3 mM DTNB and 0.05 mL ethanol solution of flupyrazofos, was preincubated at 30℃ for 10 min. After preincubation, a 0.2 mL of 30 mM acetylthiocholine iodide solution was added to the reaction mixture and incubated at 30℃ for 20 min. The reaction was terminated by adding a 0.2 mL of 5 mM eserine salicylate solution. AChE inhibition was calculated from the absorbance of the reaction mixture at 412 ㎚ every 5 min. Median inhibition concentration ($I_{50}$) was estimated from the relationship between AChE inhibition and insecticide concentration using linear regression. Two samples were analyzed per mass homogenate.

**Change of AChE activity**

Second and 4th instar larvae of DBM and BAW were used in this study. Topical insecticide applications were made with an hand micro-applicator (Burkard, Ricksmanworth, England) equipped with a 1 mL glass syringe. Hundreds of each instar were treated with flupyrazofos at the concentration of 0.01～0.02 ㎍/DBM and 5～10 ㎍/BAW, equivalent to $LD_{30}$ of each insect. On each sample hour, live larvae of DBM and BAW(≈ 150 larvae per each sample time) were collected from the funnel and AChE activities were measured using the method as described above. The sample intervals were 0.5, 1, 2, 4, 8, 16, 32 h. Relative percentage of inhibition were calculated as 100 x (AChE activity in

treatment / AChE activity in control). All tests were duplicated.

## RESULTS and DISCUSSION

### Esterase activity

AChE activities in heads and abdomens of DBM and BAW are shown in Table 1. AChE activities with acetylthiocholine were 1.5, 2.9, 5.2 and 11.1 μmol/g/min in the 1st, 2nd, 3rd and 4th instar of DBM, respectively, and 1.7, 3.6, 7.2, 37.8, 41.1 and 45.2 μmol/g/min in the 1st, 2nd, 3rd, 4th, 5th and 6th instar of BAW, repectively. The relative AChE activities of 2nd~4th instar of DBM ranged from 1.9 to 7.4. In BAW, the relative activities of the 2nd~6th instar were from 2.1 to 26.6 (Table 1). In heads, the aliesterase activities of 2nd instar DBM larvae were 1.7 times with α-naphthylacetate and 1.7 times with β-naphthylacetate higher in the 2nd instar than those of 1st instar of DBM. Furthermore, the activities were 3.2 and 2.7 times higher in the 3rd instar, and 4.7 and 3.7 times higher in the 4th instar than the 1st instar of DBM when α -naphthylacetate or β-naphthylacetate was used as a substrate. In the abdomens, the aliesterase activities of 2nd instar DBM larvae were 3.2 and 3.9 times higher in the 2nd instar, and 9.3 and 12.1 times higher in 3rd instar and 14.1 and 17.0 times higher in 4th instar than 1st instar of DBM. The AChE and aliesterase activities in DBM increased slightly as developing larval stages from the both of head and abdomen.

Unlike DBM, changes of esterase activity in BAW were dramatically different among larval stages (Table 1). The relative activities of aliesterase in head of BAW were 1.8 and 1.8 times higher in 2nd instar and 7.0 and 6.5 times higher in 3rd instar than 1st instar of BAW. However, the activities increased rapidly over the 4th instar of BAW, 23.6 and 24.0 times higher in 4th instar, 35.2 and 36.5 times higher in 5th instar and 36.4 and 41.8 times higher in 6th instar than 1st instar of BAW when α-naphthylacetate or β-naphthylacetate was used as a substrate. Similar activity changes were observed in the abdomen of BAW, regardless of the substrates.

### AChE inhibitions by flupyrazofos

The results of AChE inhibition of the 2nd and 4th

Table 1. Esterase activities on substrates, acetylthiocholine, α- and β-naphthylacetate, in each instar of DBM and BAW

| Insects | instar | Esterase activities | | | | |
|---|---|---|---|---|---|---|
| | | Acetylthiocholine[a] | α-Naphthylacetate[b] | | β-Naphthylacetate[b] | |
| | | Heads | Heads | Abdomens | Heads | Abdomens |
| DBM | 1st | 1.5( 1 )[c] | 0.4( 1 ) | 2.5( 1 ) | 0.3( 1 ) | 1.6( 1 ) |
| | 2nd | 2.9( 1.9) | 0.7( 1.7) | 8.1( 3.2) | 0.5( 1.7) | 6.2( 3.9) |
| | 3rd | 5.2( 3.5) | 1.3( 3.2) | 23.2( 9.3) | 0.8( 2.7) | 19.3(12.1) |
| | 4th | 11.1( 7.4) | 1.9( 4.7) | 35.2(14.1) | 1.1( 3.7) | 27.2(17.0) |
| BAW | 1st | 1.7( 1.1) | 0.5( 1.2) | 2.9( 1.2) | 0.4( 1.3) | 1.9( 1.2) |
| | 2nd | 3.6( 2.4) | 0.9( 2.2) | 8.9( 3.6) | 0.7( 2.3) | 6.3( 3.9) |
| | 3rd | 7.2( 4.8) | 3.5( 8.7) | 31.5(12.6) | 2.6( 8.7) | 27.6(17.2) |
| | 4th | 37.8(25.2) | 11.8(29.5) | 43.2(17.3) | 9.6(32.0) | 35.2(22.0) |
| | 5th | 41.1(27.4) | 17.6(44.0) | 69.2(27.7) | 14.6(48.7) | 41.2(25.7) |
| | 6th | 45.2(30.1) | 18.2(45.5) | 76.3(30.5) | 16.7(55.7) | 46.7(29.2) |

[a]Specific enzyme activity is expressed as μmole of hydrolyzed acetylthiocholine/g/min. [b]Specific enzyme activity is expressed as μmole of hydrolyzed naphthyl acetate/25㎎/min. [c]Esterase activity of designated instar/esterase activity of 1st instar of DBM and BAW.

Table 2. Inhibition of acetylcholinesterase of 2nd and 4th instar of DBM and BAW by flupyrazofos

| Instars | $I_{50}(x10^{-4}M)$ | | Insensitivity Ratio (BAW/DBM) |
|---|---|---|---|
| | DBM | BAW | |
| 2nd | 0.00092 | 0.14869 | 162 |
| 4th | 0.01791 | 31.42204 | 1,720 |

instar of DBM and BAW by flupyrazofos are shown in Table 2. Median inhibition concentrations ($I_{50}$) of flupyrazofos to the 2nd instar of DBM and BAW were 92 nM and 15 μM, respectively. For the 4th instar of DBM and BAW, $I_{50}$ values were 1.8 μM and 3.1 mM, respectively. AChE of the 2nd and 4th instar of BAW showed ca. 162 and ca. 1,720 times less sensitive to flupyrazofos than that of 2nd and 4th instar of DBM, respectively. However, Lee *et al.* (1997) reported that toxicities of flupyrazofos ($LD_{50}$'s) to the 2nd and 4th instar of BAW were only 80 times lower than those to the 2nd and 4th instar of DBM, respectively. This result indicates that AChE is involved partially in flupyrazofos tolerance.

**Changes of AChE activities with exposure time**

Changes of AChE activity with exposure time were different between DBM and BAW larvae. As shown in Table 3, AChE activity of the 2nd instar of DBM and BAW decreased gradually. Activity ratio in percentile scale compared to control ranged from 67.6% at 0 h to 32.4% at 32 h. Similar changes were observed in the 4th instar of DBM. In contrast, in the 4th instar of BAW, AChE activity increased gradually till 8 h and maintained >100%. It is clear that inhibiton of flupyrazofos against AChE appeared much more slow in the 4th instar of BAW than in the 2nd and 4th instar of DBM, and 2nd instar of BAW.

These results explain why the 4th instar of BAW need more insecticide to kill than the DBM larvae and the 2nd instar of BAW.

Oppenoorth and Welling (1976) said that phosphorothionate compounds become the substrates of esterase after converted to phosphate and oxon forms. Flupyrazofos can also serve as a substrate of esterase after converted into an activated oxon form. Aliesterase may play a role in tolerance of insect to flupyrazofos in similar pattern with pyraclofos which was previously shown by Kono and Manabe (1983). Lee (1995) also reported that aliesterase is closely related to the

Table 3. Change of AChE activity in the 2nd and 4th instar of DBM and BAW after flupyrazofos application

| Hours After Treatment | AChE activities[a)] | | | |
|---|---|---|---|---|
| | DBM | | BAW | |
| | 2nd | 4th | 2nd | 4th |
| 0 | 2.3(67.6)[b)] | 11.3(67.3) | 11.4(74.5) | 28.5(53.5) |
| 0.5 | 2.1(61.8) | 12.7(75.6) | 12.6(82.4) | 33.1(62.1) |
| 1 | 1.9(55.9) | 11.4(67.9) | 10.6(69.3) | 41.7(78.2) |
| 2 | 2.1(61.8) | 9.7(57.7) | 10.1(66.0) | 41.3(77.5) |
| 4 | 1.6(47.1) | 7.2(42.9) | 10.0(65.4) | 49.9(93.6) |
| 8 | 1.3(38.2) | 6.2(36.9) | 7.0(45.8) | 54.5(102.3) |
| 16 | 1.1(32.4) | 5.7(33.9) | 6.8(44.4) | 51.7(97.0) |
| 32 | 1.1(32.4) | 5.8(34.5) | 6.9(45.1) | 53.5(100.3) |
| control | 3.4(100) | 16.8(100) | 15.3(100) | 53.3(100) |

[a)]Specific enzyme activity is expressed as μmole of hydrolyzed acetylthiocholine/g/min.

[b)]Figures in parentheses are percentages of AChE activity at each time against the control AChE.

metabolism of pyraclofos in resistant insect species. On the basis of this hypothesis, aliesterase might be related to the metabolism of flupyrazofos in tolerant insect species.

A difference in AChE inhibition in DBM and BAW larvae was also clearly observed for flupyrazofos. This compound is converted into an activated oxon as flupyrazofos-oxon form. Flupyrazofos-oxon is activated and more potent in the insect body, but unstable (Segall and Casida, 1982; Kono *et al.*, 1983; Wing *et al.*, 1984; Miyamoto, 1992). In order to establish the contribution of insensitivity of AChE to flupyrazofos tolerance, it is preferred to use the activated oxon metabolite of flupyrazofos. However, this activated compound is very unstable and rarely obtained. Even though direct comparison could not be made on sensitivity of AChE to activated compound, large differences in sensitivity of AChE to fluyrazofos between DBM and BAW larvae indicate that insensitivity of the AChE plays an important role in tolerance mechanisms to flupyrazofos in above 3rd instar of BAW.

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## 배추좀나방과 파밤나방의 효소활성 및 flupyrazofos에 의한 AChE활성 저해

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**요약 :** 배추좀나방과 파밤나방에 대한 flupyrazofos의 작용기작을 구명하기위하여 한국화학연구소에서 분양을 받아 실내에서 19세대 누대 사육한 배추좀나방과 포장에서 채집하여 실내에서 인공사료를 이용하여 25세대 누대사육한 파밤나방을 대상으로 효소활성도 및 flupyrazofos에 의한 AChE 활성 저해정도를 조사한 결과는 다음과 같다. 배추좀나방과 파밤나방의 머리에 있는 AChE의 활성도는 배추좀나방 1령~4령에서 1.5~11.1 ng/larva/min이었고, 파밤나방 1령~6령에서 1.7~45.2 ng/larva/min로 나타나 4령이상의 영기에서는 25~30배이상 높았다. 배추좀나방과 파밤나방에 대한 aliesterase의 활성도는 배추좀나방 1령충에 비하여 배추좀나방 2령~4령에서는 머리와 복부에서 각각 1.7~4.7배와 3~17배 이하였으나, 파밤나방 3령~6령에서는 8~55배와 12~30배 이상이었다. Flupyrazofos에 의한 AChE 반수저해농도($I_{50}$)는 배추좀나방 2령과 4령에서 각각 92 nM과 1.8 μM이었고 파밤나방 2령과 4령에서 각각 15 μM과 3.1 mM로 나타나 파밤나방은 배추좀나방보다 약 162~1,720배 이상 높은 농도를 보였다.

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