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In vivo Metabolism of Flupyrazofos into *Plutella xylostella* (Lepidoptera: Yponomeutidae) and *Spodoptera exigua* (Lepidoptera: Noctuidae)

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Abstract : *In vivo* metabolism study was carried out to find out the biochemical or metabolic tolerance mechanism between Diamond backmoth (DBM), *Plutella xylostella* and Beet armywarm (BAW), *Spodoptera exigua* to flupyrazofos. They showed some differences between the DBM and BAW. About 20% of flupyrazofos applied to the 3rd instar larvae of DBM was metabolized within 1 h and about 50% of that was metabolized within 4 h. The metabolites of flupyrazofos-oxon in 3rd instar larvae of DBM were increased 10 times more at 4 h than 1 h after application. The amounts of flupyrazol were nearly same between at 1 h and 4 h. The amount of unknown and origin increased 2 and 3 times more at 1 h than 4 h after application, respectively. In the 4th instar BAW larva, about 50% of flupyrazofos was metabolized within 1 h and about 70% of that was metabolized within 4 h. As metabolites, the amounts of flupyrazofos-oxon increased 2 times more at 4 h than 1 h after application. The amounts of flupyrazol increased 4 times more at 4 h than 1 h after application. The amount of unknown and origin increased 2.5 and 2 times more at 4 h than 1 h after application, respectively. From the study, it is supposed that hydrolytic enzyme, esterase, cleave the alkyl bond of flupyrazofos and conjugates with flupyrazofos. This seems to be the main tolerance mechanism of BAW to flupyrazofos. (Recived September 5, 2002; accepted September 30, 2002)

Key words : flupyrazofos, *In vivo* metabolism, *Plutella xylostella* (L.), *Spodoptera exigua*..

Introduction

In recent years, *Plutella xylostella* (L.) (DBM) and *Spodoptera exigua* (BAW) have become one of the most important insect pests of cruciferous plants in the world. Its present status is in most parts of the world which is attributed to the extended cultivation of host plants and its superb migratory habit (Chu, 1986). 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 from the early growth stage of the crop, these insects could cause a serious yield loss with excessive feeding on the leaves by larvae.

Control is primarily dependent on continued or

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repeated applications of insecticides. Although they have effectively controlled this pest, their extensive use for several decades has disrupted control ability of natural enemies and has led to outbreak of these pests and development of resistance to various types of insecticides (Miyata et al, 1986; Sun et al, 1986; Lee et al, 1993). Although many insecticides have been registered for the control of BAW, new methods for the control of this pest in Korea are still in the process of development. Decreased efficacy and increasing concern over adverse effects of the earlier types of insecticides have brought about the need for the development of new types of selective alternatives or new methods of protection with reduced use of conventional insecticides (Brown, 1978; Hayes and Laws, 1991; Ripper, 1956; Georghiou and Saito, 1983.

The orgaophosphorus insecticide, flupyrazofos (Fig. 1), has been developed by the Korea Research Institute of Chemical Technology (Hwang, 1989). Flupyrazofos could be an effective alternative because of its outstanding insecticidal activity against DBM. This insecticide has been recently investigated for 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 is very specific to the DBM larvae (Lee et al, 1997).

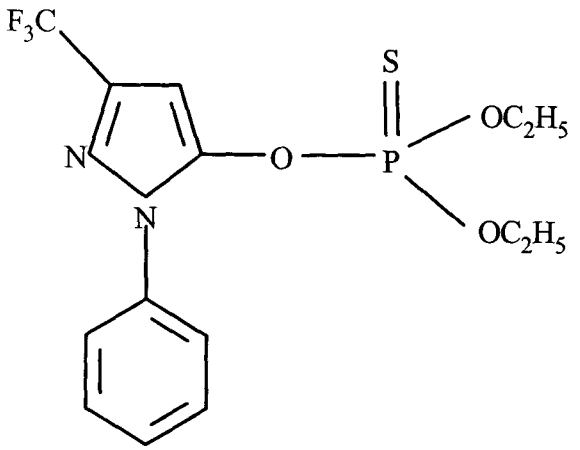

Fig. 1 Chemical structure of flupyrazofos.

However, little work has been done on the metabolism of flupyrazofos. In this study, we dealt with the metabolism for the purpose of elucidation of activity characteristics of flupyrazofos {O,O-diethyl O-1-[U-C]phenyl-3-trifluoromethylpyrazol-5-yl phosphorothioate} to *P. xylostella* as a susceptible insect and *S. exigua* 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 cm×40 cm×45 cm) 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 initially 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 initially obtained from welsh onion field at Suwon, Korea in September 1994. These were reared on artificial diet in a plastic cage (22 cm×16 cm×9 cm), and maintained under conditions of controlled temperature(25±1°C), 50-60% relative humidity, and photoperiod of 16 : 8 (L : D) h through 25th generations in the laboratory without exposure to any insecticide (Shorey and Hale, 1965).

Chemicals

^{14}C-Labelled flupyrazofos was a gift from Korea Research Institute of Chemical Technology, Daejeon, Korea. The specific radioactivity was 185.7 MBq (5.02 mCi)/mmol, and the radiochemical purity was more than 99% by silica gel TLC. The solvents used for rinsing the larva body were purchased from Wako Pure Chem. Ind, Ltd. and of the highest grade.

Analysis of metabolites

Third instar larvae of DBM and 4th instar larvae of BAW were used, and ^{14}C-flupyrazofos was prepared in optima-grade acetone. Doses were adjusted to the levels more than caused no mortality. A droplet containing 4,000 dpm equivalent to 0.1 $\mu\ell$ (below LD_{30} of the DBM 3rd instar larva) of flupyrazofos was topically applied to the dorsal plate of 3rd instar of DBM with a micro-topical applicator. For the 4th instar of BAW, an 20,000 dpm equivalent to 0.5 $\mu\ell$ (below LD_{30} of BAW 4th instar larva) of flupyrazofos was topically applied to the dorsal plate. The samples were subsequently homogenized in the mixture of ethyl-acetate (10 ml)/ methanol (10 ml) with a glass hand mixer (50 ml capacity) in ice water at one and four hours after treatment. After centrifugation at 15,000×*g* for 15 min at 4℃, the supernatant was decanted and used for assays (Park *et al*, 1991).

These fractions were concentrated and separated by TLC. Precoated silica gel 60 F_{254} thin layer chromatoplates (20×20 cm, 0.25 mm thickness, Merck, F.R.G.) and three dimensional chromatography were conducted using the following solvent systems for separation and identification of metabolites of flupyrazofos, *n*-hexane/acetone (8/2, v/v), ethyl-acetate/ methanol(5/1, v/v) and *n*-hexane/ethyl-acetate (2/1, v/v). Identification of metabolites was carried out by co-chromatography with authentic compounds (Fig. 2). Radioactive spots on TLC plates were separated, and then put in the scintillation counter vial (20 ml). The vials were determined by liquid scintillation counter (Beckmann, LS6000). Non labelled authentic compounds were detected under 254 nm UV-light (Topcon FI-31, Tokyo Kogaku Kikai K.K.).

Results and Discussion

The metabolites of ^{14}C-flupyrazofos were detected and identified with authentic compounds with developing by TLC. The amount of metabolites were measured by scraping and counting the dpm of each spot. The amount of these three metabolites are listed in Table 1~4. The recovery rates of labelled compounds were 50% or more in all experiments.

To 3rd instar DBM larva, about 20% of flupyrazofos was metabolized for 1 h and about 50% of flupyrazofos was metabolized for 4 h (Table

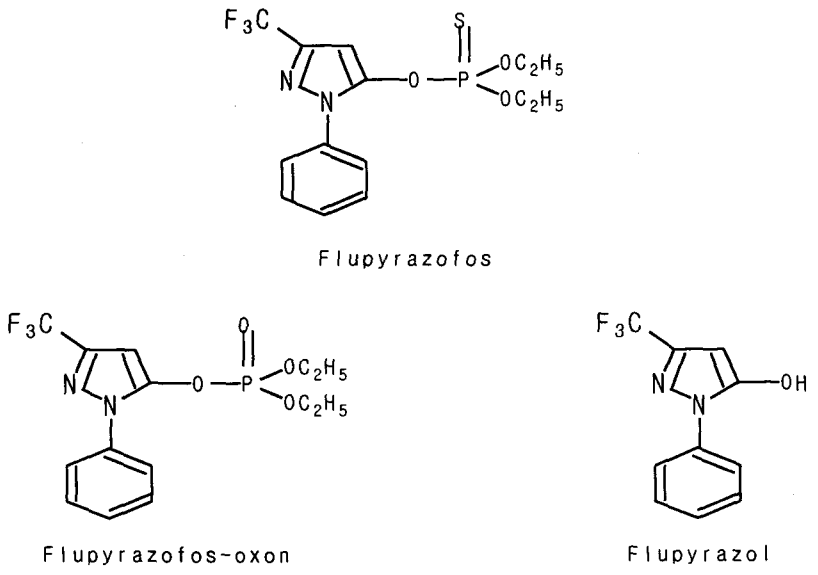

Fig. Metabolites of flupyrazofos

Fig. 2. Metabolites of flupyrazofos.

Table 1. Fate of flupyrazofos in 3rd instar larvae of DBM treated with ^{14}C-flupyrazofos 1 h and 4 h after application

Metabolites	RI amount (dpm)		% of RI		S.E. of %	
	1 h	4 h	1 h	4 h	1 h	4 h
Flupyrazofos	135,729	55,193	80.2	53.5	0.45	0.54
Flupyrazofos-oxon	841	5,344	0.5	5.2	0.43	0.63
Flupyrazol	1,810	1,385	1.1	1.3	0.63	0.35
Unknown	7,577	12,000	4.5	11.6	0.67	0.87
Origin	8,993	19,018	5.3	18.5	0.17	0.46
Unextracted ^{14}C	14,180	10,140	8.4	9.8	0.14	0.42
Total	169,130	103,080	100.0	100.0	-	-
Recovery rate (%)	92	56	-	-	-	-

[a]Standard error

1). The metabolite, flupyrazofos-oxon, was increased more 5,344 dpm at 4 h than 841 dpm at 1 h after application. The amount of flupyrazol at 1 h was slightly higher than that at 4 h. The amount of unknown and origin was increased more 2 times and 3 times at 4 h than 1 h after application, respectively. In the 4th instar BAW larva, about 50% of flupyrazofos was metabolized for 1 h and about 70% of that was metabolized for 4 h (Table 2). The amount of flupyrazofos-oxon was increased more 1,256.8 dpm at 4 h than 752.9 dpm at 1 h. The amounts of flupyrazol was 6,279.4 dpm at 4 h and 2,726.8 dpm at 1 h. The amounts of unknown and origin were increased more 2.5 times and 2 times at 4 h than 1 h after application, respectively.

Tolerance mechanisms of insects such as resistance mechanisms to OP insecticides are known to be: 1) Reduction of penetration rate, 2) Increase in insensitivity of acting site, 3) Enhanced detoxifying enzyme activity, including oxidase (cytochrome P450 monooxygenase), hydrolase (aliesterase, phosphatase), and transferase (grutathione S-tansferase). On the basis of the above insecticide resistant mechanisms, these studies were carried out the point of view of biochemistry. Another tolerance mechanism is due to detoxifying enzymes, aliesterase, and P450 monooxygenase. In vivo studies, the amounts of flupyrazofos and flupyrazofos-oxon are higher in DBM larvae than the amounts of flupyrazol, origin and unknown at 4 h after flupyrazofos treatment, but the cleavaged form of flupyrazol and any conjugated forms of origin and unknown are much higher in BAW larvae than the amounts of flupyrazofos and flupyrazofos-oxon. These results indicate that metabolic rates of flupyrazofos to flupyrazol and any conjugates are much faster in the BAW larva than in the DBM larva, and the tolerance of BAW larva to flupyrazofos is associated with flupyrazol, unknown, and any conjugates.

This explains why the amount of flupyrazofos-oxon are higher in the DBM larva and why the amounts of flupyrazol are higher in the BAW larva. The cleavage of the O-P-alkyl bond for producing flupyrazol is thought to be done by cytochrome p450 monooxygenase and esterase. Cytochrome p450 monooxygenase is well known to cleave the desulfuration and ester bond (Lee, 1995). Kono and Manabe (Kono and Manabe, 1983). studied the importance of aliesterase, which can cleave the P-O ester bond, for detoxification of pyraclofos in resistant insects species (*Spodoptera litura* (Tobacco cutworm), *Plutella xylostella* (Diamondback moth)) to pyraclofos compared with susceptible insects species (*Henosepilachna vigintiocto-punctata* (28 Spotted lady beetle, adult),

Table 2. Fate of flupyrazofos in 4th instar larvae of BAW treated with ^{14}C-flupyrazofos 1 h and 4 h after application

Metabolites	RI amount (dpm)		% of RI		S.E. of %	
	1 h	4 h	1 h	4 h	1 h	4 h
Flupyrazofos	26,686.3	9,370.0	48.0	23.8	0.37	0.32
Flupyrazofos-oxon	752.9	1,256.8	1.3	3.2	0.54	0.33
Flupyrazol	2,726.8	6,279.4	4.8	16.0	0.42	0.75
Unknown	6,236.8	10,432.4	11.0	26.5	0.83	0.67
Origin	7,811.2	9,084.5	13.7	23.1	0.27	0.72
Unextracted ^{14}C	12,622.4	2,904.4	22.2	7.4	0.34	0.28
Total	56,836.4	39,327.5	100.0	100.0	-	-
Recovery rate (%)	76.8	53.1	-	-	-	-

[a]Standard error.

Laodelphax striatellus (small brown planthopper), and *Nephotettix cincticeps* (Green rice leafhopper).

In the tolerance insect such as BAW larva, the major metabolic pathways are considered to be: 1) cleavage of the O-P-alkyl bond to give flupyrazol, 2) conjugation of flupyrazol with unknown materials, and 3) exchange double bond O (oxigen) for the S (sulfate) to give flupyrazofos-oxon. In the susceptible insect such as DBM larvae, the major metabolic pathways are presumed to be: 1) exchange double bond O (oxigen) for the S (sulfate) to give flupyrazofos-oxon, 2) cleavage of the O-P-alkyl bond to give flupyrazol, and 3) conjugation of flupyrazol with unknown materials (Fig. 3).

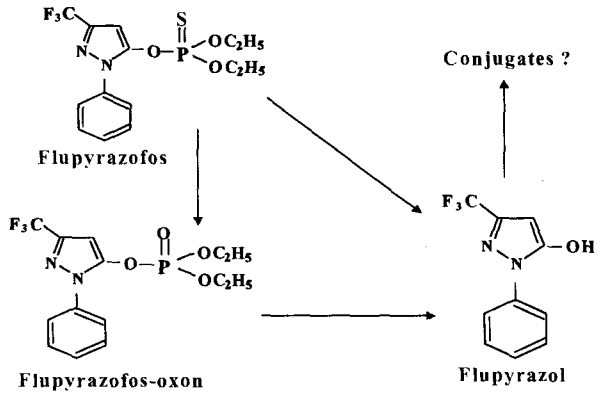

Fig. 3. Proposed metabolic pathway of flupyrazofos in the DBM and BAW.

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배추좀나방과 파밤나방의 체내에서 Flupyrazofos의 대사

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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 누대 사육한 파밤나방을 대상으로 ^{14}C-flupyrazofos를 처리하여 두 종간의 생체내 대사물을 조사한 결과는 다음과 같다. flupyrazofos의 생체내 대사물은 flupyrazofos-oxon, flupyrazol, unknown으로 분리되었으며, 대사량은 약제처리 1시간 후와 4시간 후에 배추좀나방에서 flupyrazofos가 80.3%와 53.5%, flupyrazofos-oxon이 0.5%와 5.3%, flupyrazol이 1.1%와 1.3%, unknown이 4.5%와 11.6%, 전개되지 않은 물질이 5.3%와 18.5%이었고, 파밤나방에서 flupyrazofos가 48.0%와 23.8%, flupyrazofos-oxon이 1.3%와 3.2%, flupyrazol이 4.8%와 16.0%, unknown이 11.0%와 26.5%, 전개되지 않은 물질이 13.7%와 23.1%이었다. 따라서 시간이 경과할수록 flupyrazofos-oxon이 배추좀나방에서는 크게 증가한 반면 파밤나방에서는 적게 증가하였고, 배추좀나방에서는 flupyrazol, unknown, 전개되지 않은 물질 등이 상대적으로 적은 반면 파밤나방에서는 상대적으로 많았다. 이상의 결과로 볼 때 파밤나방에서는 일반적으로 곤충에서 생화학적 저항성 기구로 잘 알려진 가수분해효소의 일종인 esterase의 활성이 증가하여 alkyl 결합을 끊어 무독화 시키거나 flupyrazofos와의 결합체 역할이 강하게 작용하는 것으로 판단된다.

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