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Original Article

In Vitro Degradation of Malathion by Organophosphate Resistant and Susceptible Strains of Brown Planthoppers, *Nilaparvata lugens* STÅL*

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Topical toxicity of malathion and fenitrothion to susceptible, malathion resistant and fenitrothion resistant brown planthoppers, *Nilaparvata lugens* STÅL, was determined. LD_{50} values of malathion to susceptible, malathion resistant and fenitrothion resistant strains were 4.42, 1,590 and 71.8, and LD_{50} values of fenitrothion to those strains were 7.79, 71.4 and 336 $\mu g/g$, respectively. An *in vitro* degradation study of ^{14}C-methyl malathion by 900 *g* supernatants showed that malathion resistant and fenitrothion resistant strains degraded ^{14}C-methyl malathion more than susceptible ones and the most of the degradation products were carboxylesterase products. Most of the ^{14}C-methyl malathion degradation activity was found in the 105,000 *g* supernatant fraction. β-Naphthyl acetate hydrolyzing enzymes of the brown planthopper were separated by thin layer agar gel electrophoresis. The aliesterase activity of malathion resistant and fenitrothion resistant strains was significantly higher than that of susceptible one. ^{14}C-Methyl malathion degradation activity was observed only around aliesterase bands which showed a high aliesterase activity.

INTRODUCTION

Recently insecticide resistance of the brown planthopper, *Nilaparvata lugens* STÅL, to organophosphorus and carbamate insecticides has become obvious in Japan, Taiwan and Philippines.[1–6] Brown planthoppers are reported to be unable to overwinter in Japan and migrate every year from foreign breeding sources.[1,7] According to Kilin *et al.*,[3] the immigrant population of the brown planthopper showed resistance to organophosphorus and carbamate insecticides.

Organophosphate resistant strains of the smaller brown planthopper, *Laodelphax striatellus* FALLÉN, and the green rice leafhopper, *Nephotettix cincticeps* UHLER, showed higher β-naphthyl acetate hydrolyzing activity.[8–10]

In this paper, malathion resistance of the brown planthopper and *in vitro* degradation of ^{14}C-methyl malathion by organophosphate resistant and susceptible strains of brown planthoppers will be reported.

MATERIALS AND METHODS

1. *Insects*

Female brown planthoppers from 6 to 7 days after emergence were used throughout this experiment. They were reared at 25°C, 16 hr illumination per day with rice seedlings

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Table 1 Origin of the brown planthopper used in this study.

Strains	Place of collection	Time of collection
S	Kawachinagano, Osaka-Prefecture	1973
Nagoya	Chikusa, Nagoya	1976
Ohkawa	Ohkawa, Kida-District, Kagawa-Prefecture	1975
Marugame	Marugame, Kagawa-Prefecture	1975
Rm	Ohkawa strain has been selected with malathion for 20 generations	
Rf	Marugame strain has been selected with fenitrothion for 20 generations	

renewed weekly. The six strains of the insect used are shown in Table 1.

2. Insecticides

Malathion and fenitrothion were obtained from Sumitomo Chemical Co., Ltd. (Osaka, Japan). ^{14}C-Methyl malathion used here has been described previously.[11] The specific activity was 1,500 dpm/μg as determined by an Aloka LSC-653 liquid scintillation spectrometer.

3. Determination of Susceptibility to Malathion and Fenitrothion

Resistance levels to malathion and fenitrothion were determined by a topical application method. Malathion and fenitrothion in 0.25 μl acetone were respectively applied on the abdomen of adult females of the brown planthopper by means of a micrometer syringe. Treated insects were kept in test tubes with rice seedlings at under 25°C. The mortality was recorded after 24 hr. From these data, LD_{50} values were calculated.[12]

4. Method of Enzyme Preparation

Female adults of the brown planthopper were homogenized in an ice-cold buffer solution by means of a Potter-Elvehjem glass homogenizer. The standard buffer solution was 0.05 M tris-HCl buffer (pH 7.4) containing 0.25 M sucrose. The homogenate was first centrifuged at 900×g for 10 min at 4°C, and the resultant supernatant was used as an enzyme source (i.e., 900 g supernatant). The enzyme concentrations used here were 4% for S and Nagoya strains and 0.5% for *Rm* and *Rf* strains.

Various subcellular fractions were separated by differential centrifugation of the above 900 g supernatant at 4°C in a Hitachi 55P-2 ultracentrifuge. The mitochondrial fraction was separated by centrifuging 900 g supernatant for 10 min at 10,000×g and the sediment was washed with a standard buffer solution twice. Microsomal and soluble fractions were obtained by further centrifugation for 60 min at 105,000×g. The mitochondrial and microsomal fractions were resuspended in the standard buffer.

5. Electrophoresis

Thin layer agar gel electrophoresis followed the method described by Miyata and Saito.[13] Each strain of brown planthoppers (30 and 60 planthoppers for resistant and susceptible strains, respectively) was crushed in 60 μl of distilled water by a glass rod, and the brei was absorbed on Toyo filter paper No. 2 (2×60 mm). The filter paper was put on an agar gel plate (0.7% PVP (K-90) and 0.7% agar in pH 6.8 phosphate buffer (ionic strength 0.015 μm), agar gel thickness 0.8 mm) and the plate was kept at 4°C for 30 min. Then the filter paper strip (2×60 mm) was removed from the agar gel plate and electrophoresis was run for about 90 min at 4°C using a constant current of 2 mA/cm width. After completion of electrophoresis, the middle portion of the plate where the filter paper had been placed was sliced transversely into 5×50 mm pieces to test ^{14}C-methyl malathion degradation. To the remaining portion of the gel, a mixture of 0.1% of β-naphthyl acetate and 2% of naphtanil diazo blue B was sprayed and this was incubated in order to localize esterase activity.

6. *In Vitro Degradation of* ^{14}C*-Methyl Malathion*

The reaction mixture used in the ^{14}C-methyl malathion degradation study contained 0.5 ml each of 900 *g* supernatant and standard buffer. A 10 μl aliquot of ^{14}C-methyl malathion (10^{-3} M) in absolute ethyl alcohol was added to the system, and it was incubated at 37°C for 30 min with shaking. The reaction mixture was extracted with an equal volume of chloroform twice. Radioactivity in the aqueous fraction was determined for its degradation products with Bray's scintillator.[14] To separate carboxylesterase and phosphatase products, the reaction mixture was first twice extracted with an equal volume of chloroform (the aqueous fraction contains both carboxylesterase and phosphatase products). The pH of the aqueous fraction was lowered to pH 2 by adding 2.5% of trichloroacetic acid, and was further extracted with an equal volume of chloroform twice (carboxylesterase products were extracted with chloroform and the phosphatase products remained in the aqueous fraction).[15]

For the degradation study of ^{14}C-methyl malathion on electrophoretically separated enzyme bands, an agar piece (5×50 mm) was incubated with 1.5 ml of standard buffer solution containing 10^{-5} M ^{14}C-methyl malathion. After 2 hr incubation at 37°C, the reaction mixture was extracted with an equal volume of chloroform three times. One ml each of the aqueous fractions was pipetted to a counting vial to determine the radioactivity.

RESULTS AND DISCUSSION

Susceptibility in five strains of brown planthopper to malathion and fenitrothion is shown in Table 2. The Ohkawa strain showed greater resistance to malathion than the susceptible strain. After selection with malathion for 20 generations, the *Rm* strain showed 132 times higher resistance to malathion than the Ohkawa strain. The Marugame strain showed 8.6 times higher resistance to fenitrothion than the susceptible strain. When the Marugame strain was selected by fenitrothion for 20 generations, the *Rf* strain showed 5 times higher resistance to fenitrothion than the Marugame strain.

In vitro degradation of ^{14}C-methyl malathion by 900 *g* supernatants in various strains of the brown planthopper is shown in Table 3. In each strain, most of the degradation products were caused by carboxylesterase as already

Table 3 *In vitro* degradation of ^{14}C-methyl malathion by 900 *g* supernatants in various strains of the brown planthopper.

Strains	Degradation activity (nmol/g/hr)		Ratio of total degradation activity
	Carboxylesterase products	Others	
S	302	21	1
Nagoya	314	65	1.1
Rm	2,642	38	8.3
Rf	2,533	58	8.0

Table 2 Susceptibility of various strains of the brown planthopper to malathion and fenitrothion.

Strains	Malathion		Fenitrothion	
	LD_{50} (μg/g)	R.R.[a]	LD_{50} (μg/g)	R.R.[a]
S	4.42	1	7.79	1
Ohkawa	12.0	2.7 (1)	—	—
Marugame	—	—	66.8	8.6 (1)
Rm	1,588	359 (132)	71.4	9.2 (1.1)
Rf	71.8	16.2 (6.0)	335.8	45.7 (5.0)

[a] Resistance ratio.

Table 4 *In vitro* degradation of ^{14}C-methyl malathion by subcellular fractions in various strains of the brown planthopper.

Enzyme sources	Degradation activity (nmol/g/hr)			
	S	Nagoya	*Rm*	*Rf*
Nuclei and cell debris	11.8	12.7	104	71.2
Mitochondria	31.8	33.5	69.6	110
Microsome	27.7	35.0	88.0	37.6
105,000 *g* Supernatant	319.7	261.8	2,625	2,701

Table 5 Effect of NADPH and GSH on *in vitro* degradation of ^{14}C-methyl malathion by microsome and 105,000 *g* supernatant fractions in various strains of the brown planthopper.

Enzyme source	Degradation activity (nmol/g/hr)			
	S	Nagoya	*Rm*	*Rf*
900 *g* Supernatant	263	273	2,306	2,130
+{NADPH[a], GSH[b]}	254	270	2,288	1,982
Microsome	34.8	38.2	97.5	121
+NADPH[a]	31.0	35.9	77.9	149
105,000 *g* Supernatant	274	289	2,383	2,588
+GSH[b]	251	274	2,189	2,486

[a] Final concentration, 5×10^{-4} M.
[b] Final concentration, 4×10^{-3} M.

reported for the smaller brown planthopper[16] and the green rice leafhopper.[13] ^{14}C-Methyl malathion degradation activity *in vitro* of *Rm* and *Rf* strains was 8.3- and 8.0-fold higher than that of the susceptible strain, respectively. Enhanced degradation of ^{14}C-methyl malathion in resistant strains has been reported in other rice plant and leafhoppers.[13,16] However, the resistance ratio of the *Rm* strain to malathion is about 20 times higher than that of the *Rf* strain. This may indicate that other resistant mechanisms are involved in malathion resistance of the brown planthopper.

In each strain, the highest degradation activity of ^{14}C-methyl malathion was observed in a 105,000 *g* supernatant fraction with a higher activity in resistant strains than susceptible ones (Table 4). No effect of NADPH and GSH on *in vitro* degradation of ^{14}C-methyl malathion was observed (Table 5). According to the degradation activity of ^{14}C-methyl malathion, the Nagoya strain could be classified as a susceptible strain.

β-Naphthyl acetate hydrolyzing esterases were separated by thin layer agar gel electrophoresis (Fig. 1). Seven esterase bands were detected with highest activity in the E_2 band of each strain. All bands from resistant strains showed higher activity than those from susceptible one. High esterase activity in organophosphate resistant strains could also be used for monitoring of organophosphate resistant population as previously reported.[17] *In vitro* degradation of ^{14}C-methyl malathion by the enzymes separated by thin layer agar gel electrophoresis is shown in Fig. 1. This activity was observed on E_1–E_4 bands with the highest activity on E_2 band. *Rm* and *Rf* strains showed higher degradation activity than *S* strain. Further study is necessary to clarify that the enzyme with a high esterase activity also degrades ^{14}C-methyl malathion.

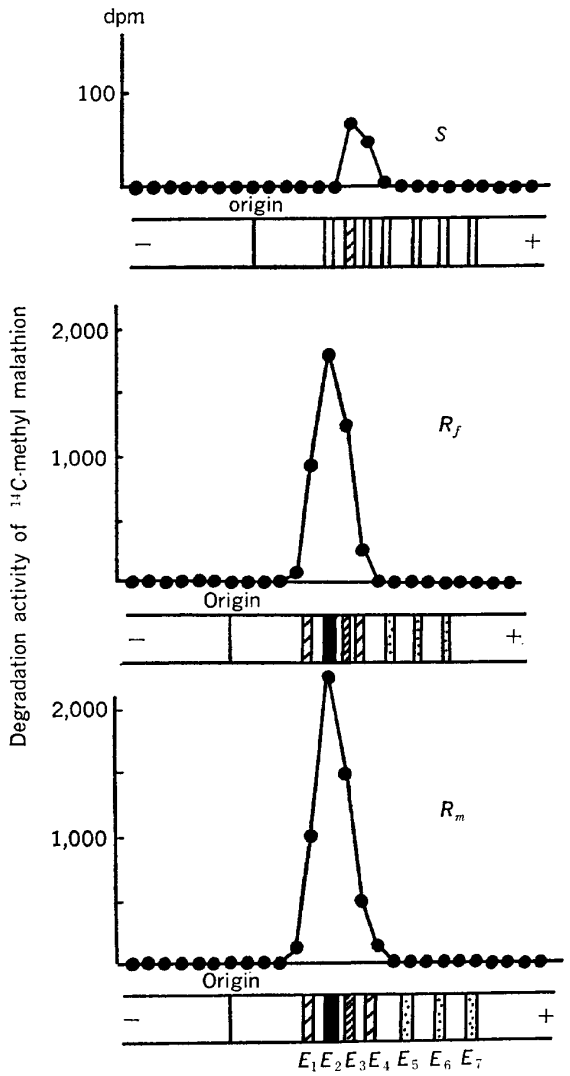

Fig. 1 *In vitro* degradation of ^{14}C-methyl malathion by brown planthopper enzymes separated by thin layer agar gel electrophoresis. The corresponding zymogram for β-naphthyl acetate indicates the relative position of esterase bands against malathion degradation.

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要　　約

有機リン剤抵抗性および感受性トビイロウンカによる *in vitro* でのマラチオンの代謝

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有機リン剤抵抗性および感受性トビイロウンカのマラチオンおよびフェニトロチオンに対する感受性を局所施用法によりしらべた．マラチオン抵抗性およびフェニトロチオン抵抗性系統の両薬剤に対する感受性は感受性の系統にくらべ著しく低かった．900 g 上清分画粗酵素液による *in vitro* での ^{14}C-メチルマラチオンの代謝は，両抵抗性系統では感受性系統にくらべ約8倍高く，代謝物の大部分はカルボキシルエステラーゼによるものであった．また細胞分画して ^{14}C-メチルマラチオン分解酵素の局在性をしらべたところ，105,000 g 上清分画で最も分解活性が高かった．寒天ゲル電気泳動法によって分離された酵素による ^{14}C-メチルマラチオンの分解作用をしらべたところ，高アリエステラーゼ活性を示す泳動帯でのみ分解作用が認められた．