



**Early diagnosis of poisoning due to
agricultural chemicals in the silkworm
(*Bombyx mori*) larvae**

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1998. 12. 28

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: ()
: ()
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: ()

가 .

1. α -, β -naphthyl acetate 가 esterase
 sunithion (Watanabe
 , 1967) ,
 α -naphthyl acetate 가 esterase
 acetyl cholinesterase(AChE)가
 ,
 가 , , 가
 , 가
 가 . 2.4%(
) , 가

, 가 ,
가 . 가 .

, ,
, , .
, , ,
가 .

2. 가
가가
1976

.
가 .
, , ,
.

di chl orvos,
acephate phenthoate 3 , captan pyrazophos 2 ,
ethoprophos endosulfan 2 , al achl or,
simazine, pendimethalin, paraquat di chl oride 4 .

di chl orvos phenthoate,
 가 feni trothi on
 fenthi on
 .
 가
 가 di chl orvos, acephate, phenthoate
 pyrazophos,
 가 pyrethroi d
 del tamethri n cypermethri n al achlor si mazi ne
 .
 moni tori ng
 가 .
 8 가 가 3 가 3 가
 2
 가
 .

esterase AChE ,
column-gas chromatography/mass spectroscopy system ,
, 3 ('95. 12 - '98. 12)
() ()

1. esterase ,
esterase .

가.

, 가 가
2
5 48
10 μ l , 6
esterase -naphthyl acetate
PAGE(Polyacrylamide gel electrophoresis) IEF(Isoelectric focusing)
, esterase
nitrophenyl acetate 405nm

esterase

3. esterase ,
esterase .

가. , ,
2 . , (alachlor, simazine)

esterase
5 3
10 μ l topical application , 2-3

10
PAGE
esterase -naphthyl acetate PAGE

esterase

-naphthyl acetate PAGE
esterase

4.

가 (8) 3 가 3
(1996, 1997, 1998) (5 6) 가 (9 10) 2

No. 1 3 (), No. 4 6 (), No. 7 9 (), No. 10 12 (), No. 13 15 (), No. 16 18 (), No. 19 21 () No. 22 24 () .

(1) 1 ('96)

1

di chl orvos, acephate
 phenthoate pyrazophos .
 homogeni zer di chl orvos,
 phenthoate pyrazophos . 30 g
 acetone 100 ml 가 , , , di chl ormethane
 2 partitioning florisil silica gel column
 chromatography . acephate 20 g
 ethyl acetate 100 ml 가 , sodium
 sulfate 가 , florisil column
 chromatography . gas chromatograph (HP 5890
 series , Hewlett Packard, U.S.A.) , detector NPD
 (Nitrogen phosphorus detector) FPD (Flame photometric detector)
 , column Ultra-1 (Cross-linked methyl siloxane, 25 m L. ×
 0.32 mm I.D. × 0.17 μm film thickness, Hewlett Packard), HP-1 (Methyl
 silicone gum, 5 m L. × 0.53 mm I.D. × 2.65 μm film thickness, Hewlett
 Packard) SE-30 (Fused silica capillary column, 30 m L. × 0.25 mm
 I.D. × 0.25 μm film thickness, Supelco) .

(2) 2 ('97)

2

가

di chl orvos acephate,

가 pyrethroid deltamethrin
 cypermethrin .
 Dichlorvos acephate ,
 deltamethrin cypermethrin . 20 g
 acetone 100 ml 가 , ,
 acetone 20 ml (H3PO4: NH4Cl: H2O=20: 10: 780, v/w/v) 10
 50 ml 가 30 Celite 545
 . dichloromethane 2 partitioning florisil
 column chromatography . gas chromatograph
 (HP 5890 series , Hewlett Packard, U.S.A.) , dichlorvos
 acephate NPD (Nitrogen phosphorus detector) FPD (Flame
 photometric detector) , deltamethrin cypermethrin ECD (Electron
 capture detector) , column Ultra-1
 (Cross-linked methyl siloxane, 25 m L × 0.32 mm I.D. × 0.17µm film
 thickness, Hewlett Packard), HP-1 (Methyl silicone gum, 5 m L × 0.53
 mm I.D. × 2.65 µm film thickness, Hewlett Packard) SE-30 (Fused
 silica capillary column, 30 m L × 0.25 mm I.D. × 0.25 µm film
 thickness, Supelco) .

(3) 3 ('98)

3 1 2
 dichlorvos acephate
 alachlor simazine 가 .
 Dichlorvos acephate ,
 simazine dichlorvos . Alachlor
 20 g acetone 100 ml 가 ,
 acetone 20 ml (H3PO4: NH4Cl: H2O=20: 10: 780,

v/w/v) 10 50 ml 가 30 Celite 545
 . n-hexane 2 partitioning silica
 gel column chromatography .

(4) peak

phenthoate phenthoate
 retention time No. 6 phenthoate
 1.90 ppm column oven
 peak . Column HP-1 (Methyl
 silicone gum, 5 m L. × 0.53 mm I.D. × 2.65 μm film thickness, Hewlett
 Packard) Ultra-1 (Cross-linked methyl siloxane, 25 m L. × 0.32 mm
 I.D. × 0.17 μm film thickness, Hewlett Packard) , oven
 150 60 retention time
 . peak GC/MSD (HP 5890 A Series
 Gas Chromatograph equipped with HP 5972 Series Mass Selective
 Detector, Hewlett Packard) .

(5)

(0.05, 0.1, 0.5, 1.0
 ppm) , .

5. (蟻蠶)

가. 3 ethyl alcohol
 0.1ml , 20 ,
 . , 가

. 가
10g 50ml ethyl alcohol 24
. , ethyl alcohol
, 20 .

. 가
가 ,
가 ,
가 ,
가 .

6. 가 ,
.

가. 5 5 가 , , ,
1 10 μ l topical application
, .

. ,
,
가 .

7. acetylcholinesterase(AChE)

AChE ,

AChE

AChE

Acetyl cholinesterase Sepharose-6B column
 gel filtration DEAE-cellulose column ion-exchange
 chromatography system

1. esterase
 Column-gas chromatography/ mass
 spectroscopy system

2. 가
 , ,
 , , 가
 가 .
 3. 가 가
 가 .
 가 가

.

Summary

. In order to develop the method to diagnose insecticide intoxication of silkworm exactly in early stage, Seoul national university and chungbuk national university have carried out the research . summary of this research results is like the following

1 . Because esterases in silkworm hemolymph and ganglia, and acetylcholinesterase in head tissue are specifically inhibited by organophosphate insecticides and carbamate insecticides, silkworm intoxication by organophosphates and carbamates used in silkworm rearing farm can be exactly diagnosed with the inhibitory condition of esterase and AChE.

Compared to esterase in ganglia and AChE in head tissue, esterase in hemolymph is most sensitive to organophosphate insecticides and carbamates insecticides and easy to prepare the sample, therefore it is most effective method to diagnose the silkworm intoxication with extraction of esterase in hemolymph

2 . In order to detect whether insecticide is left in mulberry leaves or not, method using the newly hatched silkworm larvae is applied to all insecticide including pyrethroids and easy to use, therefore it is most effective biological assay that can be used in agricultural

extension center, sericulture cooperative association and etc.

3 . We examine the electromyogram change caused by insecticide, muscle strength and muscle amplitude is sharply reduced by pyrethroids as well as organophosphate insecticides and carbamate insecticides, therefore insecticide intoxication of silkworm can be exactly diagnosed with examination of silkworm electromyogram but intoxication silkworm can not be distinguished from non-feeding and diseased silkworm by examination of electromyogram which has to be examined more closely

4 . AChE is partially purified with gel filtration using the sepharos-6B and ion-exchange chromatography system using DEAE-cellulose, specific activity of AChE purified partially increase from 1.3 to 7.6

. In order to evaluate the safety of mulberry leaves for sericultural purpose to the contamination by pesticide residues, the analyses of dichlorvos, acephate, phenthoate, pyrazophos, deltamethrin, cypermethrin, alachlor, and simazine were done for 24 samples collected in spring and fall over three consecutive years (1996, 1997, and 1998), each, from 8 provinces across the country, by selecting three representative farmhouses in each province. In the spring samples of 1996, the residual amounts of dichlorvos, acephate, and phenthoate were 0.018 0.032, 0.013 0.072, and 0.051 ppm respectively, whereas pyrazophos was not detected, and the detection

frequencies were 29, 58, and 4%, respectively. In the fall samples, on the other hand, those of the above pesticides were 0.012 0.048, 0.020 0.156, and 0.018 0.050 ppm respectively, and pyrazophos was not detected either and the detection frequencies were 42, 17, and 8%, respectively. While in the spring samples of 1997, the residual amounts of dichlorvos, acephate, and cypermethrin were 0.014 0.064, 0.033 0.061, and 0.019 0.068 ppm respectively, deltamethrin was not detected. The detection frequencies were 75, 13, and 8%, respectively. In the fall samples, on the other hand, those of the above pesticides were 0.013 0.062, 0.015, and 0.009 0.013 ppm respectively, and deltamethrin was not detected either. The detection frequencies were 88, 4, and 17%, respectively. In the spring samples of 1998, the residual amounts of dichlorvos, acephate, and simazine were 0.011 0.014, 0.012, and 0.018 0.053 ppm respectively, and alachlor was not detected. The detection frequencies were 17, 4, and 13%, respectively. In the fall samples, those of the above pesticides were 0.014 0.016, 0.010 0.034, and 0.018 ppm respectively, and alachlor was not detected either. The detection frequencies were 8, 21, and 4%, respectively. Almost all of the samples turned out to be non-toxic to silkworms, except that one sample (No. 10, spring 1997) contaminated by a high concentration of cypermethrin (0.068 ppm) was judged to do harm to silkworms. In the GC analyses, the selection of columns, the change in column temperature, and the use of MSD made the separation and identification of the vague chemicals possible.

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4	esterase	51
esterase		51
1		51
2		52
3		53
4		56
5		56
5		58
1		58
2		59
3		68
4		77
5		78
6		80
1		80
2		86
3		88
4	acetyl cholinesterase (AChE)	91

1

1

가

가 .

,
가 ,

가 .

, , ,
, , ,
, 가 .

,
가 .

가

가

, . ,

가

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7 10 .

esterase,
(1) acetylcholinesterase (AChE)가
(2)
(3) Column-gas chromatography/ mass spectroscopy system

2

- (1) esterase
, esterase
- (2) AChE
, AChE
- (3) esterase
, esterase
- (4) Column-gas chromatography/ mass spectroscopy system
- (5)
- (6)

AChE .

2

esterase , esterase

.

1

α - β -naphthyl acetate 가 esterase
 , , , , (Eguchi *et al.*, 1965b, 1967). esterase esterase (Khan *et al.*, 1988 ; Yoshitake *et al.*, 1965), esterase
 (Eguchi *et al.*, 1965a, 1975 ; Mon *et al.*, 1993) 가
 , .
 watanabe . (1967) , , ,
 esterase suni thi on ,
 esterase가 가 .
 esterase esterase
 , ,
 esterase major band major band 가 ,
 esterase .
 , esterase ,

.

2

1.

(123 × 124) .

2.

: di chlorvos(50%), phenthonate(40%)

: primi carb(25%), methomyl (45%)

: fenvalerate(5%), cypermethrin(5%)

3.

: di chlorvos 0.001, 0.005, 0.01 (%), phenthonate 0.01, 0.05,
0.1 (%)

: primi carb 0.01, 0.05, 0.1 (%), methomyl 0.001, 0.005,
0.01 (%)

: fenvalerate 0.001, 0.005, 0.01 (%),
cypermethrin 0.001, 0.005 (%)

4.

10 μ l

, 5 48

6

5. Polyacrylamide gel electrophoresis (PAGE)

7.5%

Polyacrylamide gel (gel size ; 8 × 11cm) 2mA/well
2 (Hofer scientific Instruments, 1994),
gel esterase .

6. Isoelectric focusing(IEF)

5% polyacrylamide
gel pH3.5-10.0 IEF (Bi o- Rad
instruction manual, 1993), gel esterase band .

7. Esterase band

gel 0.1% α -naphthyl acetate
(0.05g α -naphthyl acetate 5ml , 0.1M sodium
phosphate buffer, pH6.8 50ml) 3
7 30 . 0.5% fast blue BB salt
10 esterase band .

8. Esterase

Esterase prakaran (1995) 405nm .

3

1. esterase

가. PAGE(Polyacrylamide gel electrophoresis)

1) PAGE 3 esterase major band

. , yoshidake . (1965) agar gel electrophoresis

1 esterase band .

3 esterase major band

. 3

esterase band 가 major band ,

major band , major band .

2) 5 48 , di chlorvos phenthonate

esterase major band pattern Fig.

1 . di chlorvos ,

3 esterase major band 가 가

major band . , Phenthonate

di chlorvos , 3

esterase major band 가 가 major band

.

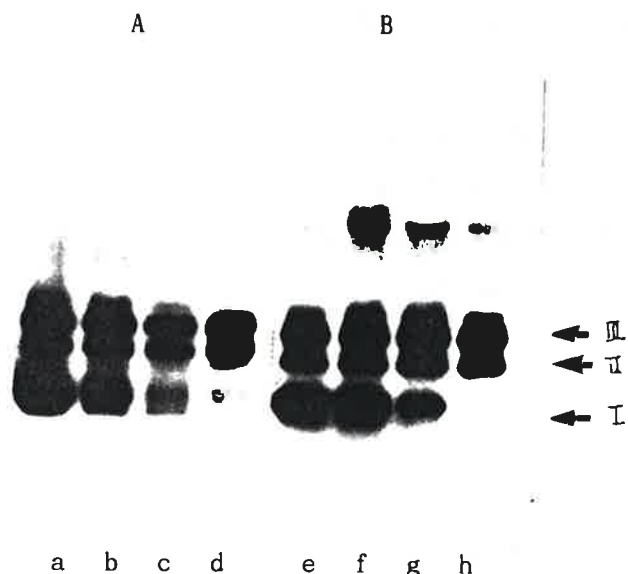


Fig. 1. PAGE analysis of hemolymph esterases in silkworm larvae treated with organophosphates. Band I, II, III : Esterase Major bands. (A) Treatment with dichlorvos ; a : control, b : 0.001%, c : 0.005%, d : 0.01%, (B) Treatment with phenthonate ; e: control, f : 0.001%, g : 0.005%, h : 0.01%.

카바메이트제인 primicarb와 methomyl를 5령 54시간째 누에에 농도별로 처리한 누에의 혈림프 esterase band들의 전기영동적 pattern을 Fig. 2에 표시하였다. 누에에 primicarb, methomyl을 처리한 경우에도 유기인제를 처리한 경우와 같은 경향으로, 누에의 혈림프에 존재하는 3개의 esterase major band들 중에서 가장 이동도가 빠른 Major band I 만이 저해되었다.

일반적으로, esterases는 기질이나 저해제 등에 대한 특이성으로부터 aliesterase, acetylcholinesterase, aromatic esterase의 3개로 분류하고 있다. Eguchi 등., (1967a)은 4종류의 기질을 사용하고, pH indicator법에

의해 기질 특이성을 검토한 결과, 누에의 여러 조직에 존재하는 esterase band들은 대부분 aliesterase에 속하지만, acetylcholinesterase나 aromatic esterase의 성질을 가지는 것이 있다고 보고하였다.

따라서 본 연구에 있어서 유기인제나 카바메이트제에 의해, 누에의 혈림프에 존재하는 3개의 major esterase band 중에서 유일하게 저해되는 major band I은 acetylcholinesterase에 속하거나 acetylcholinesterase의 성질을 가지며, major band II, III은 aliesterase에 속하는 것으로 추정된다.

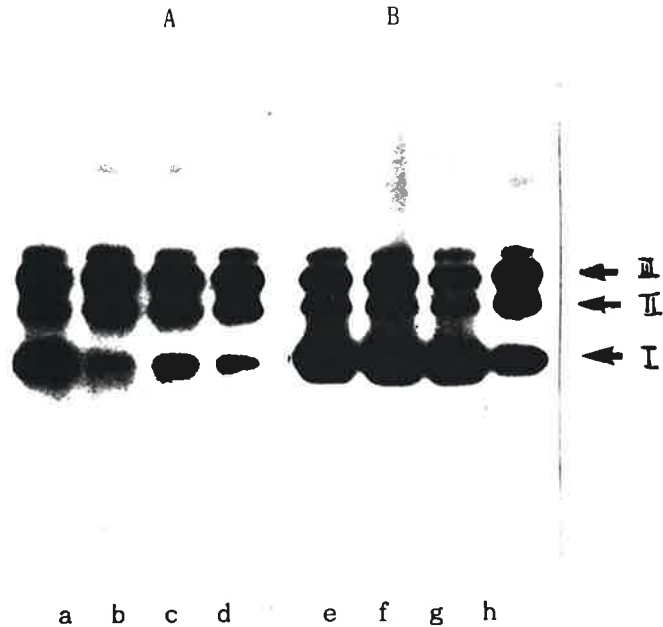


Fig. 2. PAGE analysis of haemolymph esterases in silkworm larvae treated with carbamate. Band I, II, III ; Esterase major bands. (A) Treatment with pirimicarb ; a : control, b : 0.01%, c : 0.05%, d : 0.1%, (B) Treatment with methomyl ; e : control, f : 0.001%, g : 0.005%, h : 0.01%.

5령 54시간째 누에에 fenvalerate, cypermethrin을 농도별로 처리한 누에의 혈림프 esterase major band들의 전기영동적 pattern을 Fig. 3에 표시하였다. Fig. 3에서 보는 바와 같이, 피레스로이드제를 처리한 누에의 혈림프에 존재하는 3개의 esterase major band 들은 유기인제나 카바메이트제를 처리한 것과는 달리, 저해가 나타나는 것을 관찰할 수 없었다.

이와 같이, 피레스로이드제에 의해 누에의 혈림프에 존재하는 3개의 esterase major band들에 있어서 전혀 저해가 나타나지 않는 것은 유기인제나 카바메이트제의 작용기작과는 달리, 피레스로이드제는 곤충의 acetylcholinesterase를 저해하지 않고 신경계의 신경 흥분막에 작용하기 때문이라고 생각된다.

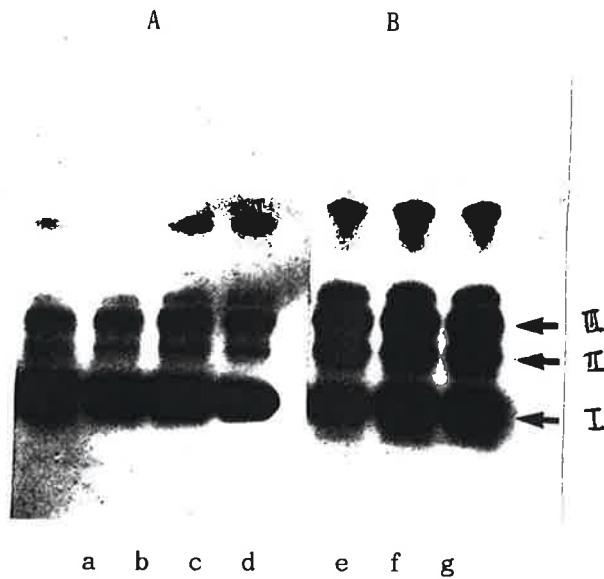


Fig.3. PAGE analysis of haemolymph esterases in silkworm larvae treated with pyrethroids. Band I, II, III ; esterase Major bands. (A) Treatment with fenvalerate ; a: control, b : 0.001%, c : 0.005%, d :

0.01%, (B) Treatment with cypermethrin ; e : control, f : 0.001%, g : 0.005%.

. IEF (Isoelectric focussing)

IEF ,
PAGE 가 3 esterase major band .
3 major band pI major band , ,

1) Fig 4 5 54 di chlorvos ,
pH4.0 - pH6.0 IEF
esterases band . Fig. 4 3
esterase major band가 , pI 가 esterase major
band (pI 4.84) di chlorvos .

2) Fig. 5 ,
di chlorvos 가 3 esterase major band
pI 가 esterase major band . ,
esterase major band acetylchol inesterase 가
esterase , PAGE
esterase major band 1 .

3) Fig. 6 , PAGE
major band 가 .

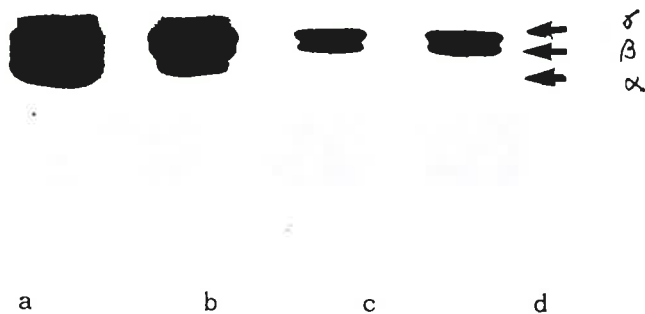


Fig. 4. IEF analysis of haemolymph esterases in silkworm larvae treated with dichlorvos. Band α , β , γ ; Esterase major bands. a : control, b : 0.001%, c : 0.005%, d : 0.01%.



Fig. 5. IEF analysis of haemolymph esterases in silkworm larvae treated with methomyl. Band α , β , γ ; Esterase major bands. a :

control, b : 0.001%, c : 0.005%, d : 0.01%,

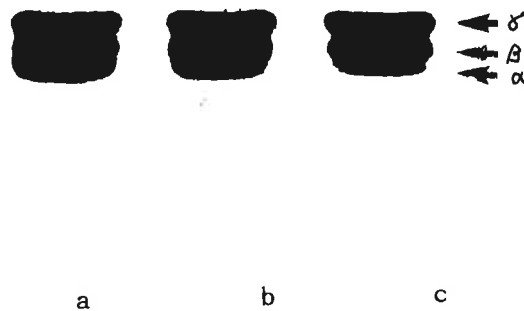


Fig. 6. IEF analysis of haemolymph esterases in silkworm larvae treated with fenvalerate. Band α , β , γ ; Esterase major bands. a : control, b : 0.001%, c : 0.005%, d : 0.01%.

다. Esterase 활성의 측정

살충제에 의한 누에의 혈림프에 있는 esterase활성의 저해정도를 알기 위해, 5령 48시간재 누에에서 혈림프를 채취하여 살충제 용액을 첨가한 후, esterase의 활성을 측정한 결과를 Fig. 7 에 표시하였다.

1) Fig. 7에서 보는 바와 같이, 유기인제 살충제인 dichlorvos를 첨가한 경우의 esterase의 활성은 대조의 $100(431 \pm 4.70)$ 에 비해, 0.001%에서 39, 0.005%에서 25, 0.01%에서 15로서 그 esterase 활성은 dichlorvos의 첨가량에 비례하여 급격하게 감소하였다.

2) 카바메이트제인 methomyl을 첨가한 경우의 esterase활성은 대조의 $100(431 \pm 470)$ 에 비해, 0.001%에서 93, 0.005%에서 86, 0.01%에서 79으로서 그 esterase 활성은 methomyl의 첨가량에 비례하여 완만하게 감소하였다.

3) 한편, 피레스토이드제인 fenvalerate를 첨가한 경우의 esterase활성은

100(431 ± 4.70) 0.001% 99, 0.005% 99, 0.01%
 98 , esterase fenvalerate 가
 가 .
 , esterase
 in vivo (Fig 1 - 6) , in vitro (Fig.
 7)

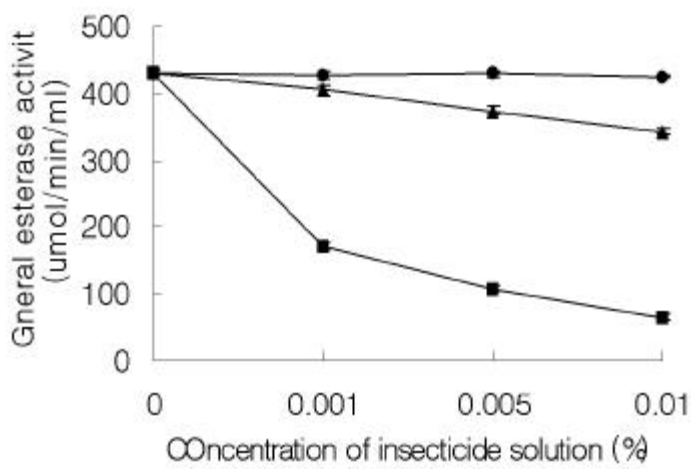


Fig. 7. Effect of some insecticides on esterase activity in haemolymph of the silkworm ■ - ■ ; dichlorvos, ▼ - ▼ ; methomyl, ● - ● ; fenvalerate

esterase

manual.

Eguchi, M and Sugimoto, T (1965a) : Changes in esterase zymogram of the silkworm during development. *J. Insect. Physiol.*, 11, 1145-1149.

Eguchi, M, Yoshitake, N and Kai, H. (1965b) : Types and inheritance of blood esterase in the silkworm. *J. Genetics*, 40(1), 13-15.

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Hoefer Scientific Instruments(1994) : Polyacrylamide gel electrophoresis, in protein electrophoresis application guide, 1-54

Khan, A. A., Moon, J. Y and Lee, S. M. (1988) : Genetic variation of esterase isozymes of larval haemolymph in relation to geographical origin in the silkworm, *Bombyx mori*. *Agri. Res. Seoul National University*, 13(1), 41-49.

Moon, J. Y. and Lee, S. M. (1993) : Change of the haemolymph esterase zymograms during pupal development of the silkworm, *Bombyx mori*. *Korean J. Seric. Sci.*, 35(2), 134-136.

Watanabe, H. and Kobara, R. (1967) : Study on the resistance to insecticides in the silkworm, *Bombyx mori*. . Effect of sumithion on the esterase activities in the blood and some tissues of larvae. *J. Seric. Sci. Jpn.*, 36(3), 183-191.

Yoshitake, M, Eguchi, M. and Tsuchiya, Y. (1965) : Distribution of the blood esterase types in various strains of the silkworm, *Bombyx mori*. *J. Seric. Sci. Jpn.*, 34(2), 95-97.

3

AChE , AChE

1

acetylcholinesterase(AChE) (Ogita *et al.*, 1965 ; Kim *et al.*, 1997 ; Vipawan *et al.*, 1995).

detection ,
AChE biosensor 가
(Francois *et al.*, 1998).

esterase가
1967),
suntion
(Vatanabe *et al.*,
AChE

가
가
AChE 가
AChE

band , AChE band
가 AChE .
, AChE ,
, AChE esterase ,
AChE 가 , AChE
, AChE .

2

1.

가 (123 x 124) 5 .

2.

가 ,
: dichlorvos(50%), : prini carb(25%), methomyl
(45%), : fenvalerate(5%)

3.

acetone ,
: dichlorvos ; 0.001, 0.005, 0.01 (%)
: prini carb ; 0.01, 0.05, 0.1 (%), methomyl ; 0.001,
0.005 0.01 (%)
: fenvalerate ; 0.01, 0.05, 0.1 (%)

4.

5 48 가 microsyringe
10 μ l topical application , 6 .

homogenizing , 10,000g 30 ,

Ellnan (1961) , head extract 30 μ l, 0.075M
acetylthiocholine iodide 10 μ l, 0.1M sodium phosphate(pH7.4) 950 μ l

10 μ l ,
AChE . 37 10

, 0.01M 5,5'-dithio-bis(2-nitrobenzoic acid) 100 μ l ,

405nm . AChE

3

1. AChE

가. AChE 가 PAGE

AChE synaptic plasma membrane detergent dissociation solubilization

가 synaptic plasma membrane AChE solubilization, non-ionic detergent Triton X-100, AChE activity

Fig. 1 0.5% Triton X-100 Triton X-100 1.8, 1.0% Triton X-100 2.4 . 1.5% 1.0% ., Triton X-100 1.0% , homogenizing , phosphate buffer 1.0% Triton X-100 가 .

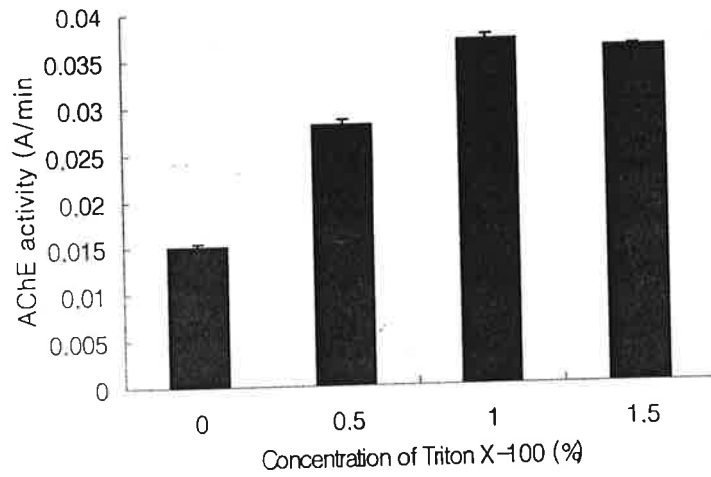


Fig. 1. Solubilization of AChE in silkworm larval head tissue. AChE activity was expressed as absorbance change per minute at 405nm. Mean value \pm SE, n=10

나. 누에의 머리조직에서 AChE band의 검출

누에의 머리조직에 존재하는 esterase 및 AChE의 Polyacrylamide gel electrophoresis(PAGE) 분석결과를 Fig. 2에 표시하였다.



Fig. 2. PAGE analysis of esterase and acetylcholinesterase in larval head tissue of silkworm. A : esterase electrophorogram, B : AChE electrophorogram. Arrow denotes AChE band.

Fig. 2
 1 AChE band . 5-6 esterase band
 acetate esterase , , , , , ,
 , (Eguchi *et al.*, 1967) (yoshitake *et al.* 1965)
 head tissue .
 David . (1987) Tabacco hornworm larval brain 1 AChE band
 가 , Ogita . (1965) house fly tissue
 extract 1 AChE band가 가
 1 AChE band .
 . AChE
 3 5 48 topical application , 6
 , AChE Fig.
 3 . dichlorvos phenthonate()
 , 5 가
 AChE 가 , 0.01%
 AChE 가 , prini carb
 methonyl() dichlorvos
 AChE 가 .
 , fenvalerate cypermethrin()
 AChE
 가 .

이와 같이, 누에의 AChE는 다른 곤충들과 마찬가지로 유기인제나 카바메이트제에 의해 특이적으로 저해됨을 알 수 있다. 그러나, 누에에 있어서 유기인제나 카바메이트제에 의한 AChE의 저해는 혈림프에 존재하는 esterase에 비하여 둔하게 나타난다(1차년도 연구결과 참조). 또한 피레스로이드제에는 전혀 저해되지 않았는데, 이것은 살충제의 작용기작이 서로 다르기 때문이다.

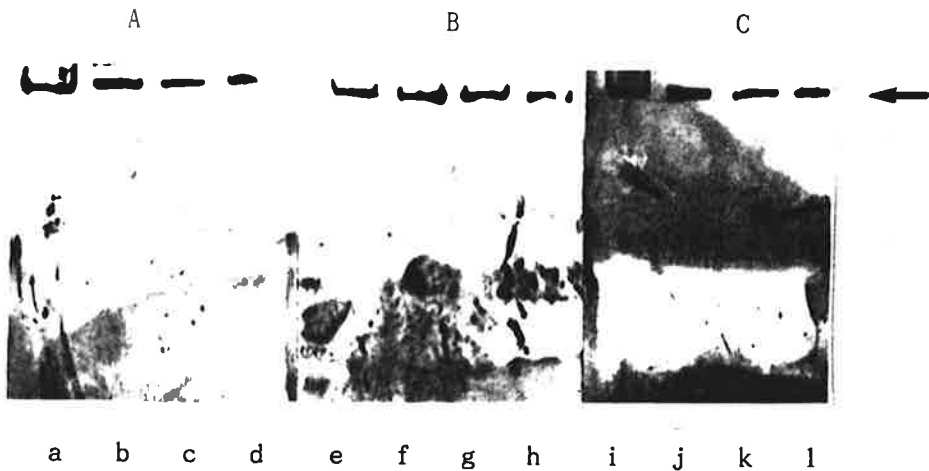


Fig. 3 Effect of some insecticides on AChE inhibition in the larval head tissue of silkworm.

(A) Treatment with dichlorvos ; a: control, b: 0.001%, c: 0.05% d: 0.01% (B) Treatment with pirimicarb ; e: control f: 0.01%, g: 0.05%, h: 0.1% (C) Treatment with Fenvalerate ; i: control, j: 0.001%, k: 0.05%, l: 0.01%.

라. 살충제에 의한 AChE활성의 저해정도 조사

5령 48시간째 누에의 머리를 채취하여 효소액을 제조한 후, 살충제 용액을

, AChE Fig. 4 .
 di chlorvos AChE 100(15.5 ± 0.28)
 , 0.001% 100, 0.01% 92, 0.1% 43
 , AChE dichlorvos , 0.1%
 . methonyl AChE
 100(15.5 ± 0.25) , 0.001% 100, 0.01%
 91, 0.1% 54 , dichlorvos .
 , fenvalerate AChE
 100(15.5 ± 0.28) , 0.001% 100, 0.01% 100,
 0.1% 100 AChE fenvalerate 가

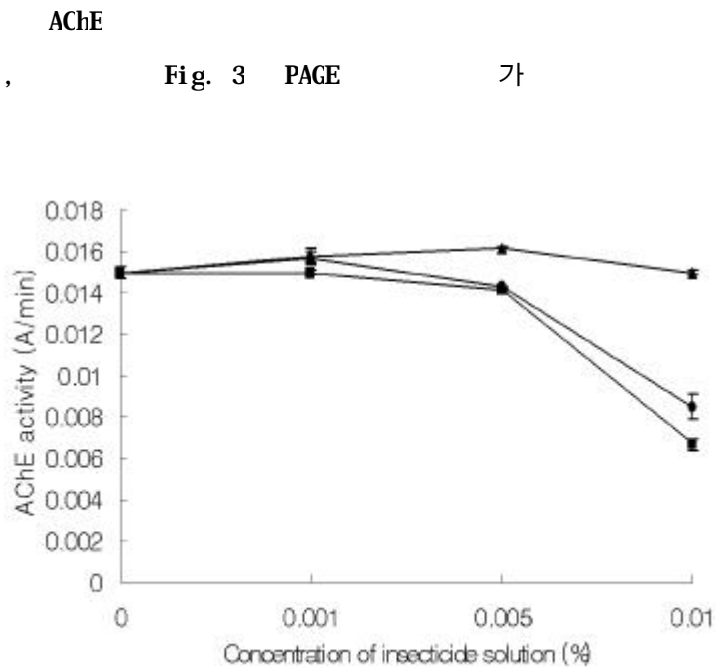


Fig. 4 Effect of some insecticides on AChE activity in head tissue of the silkworm. ■ - ■ ; dichlorvos, • - • ; methonyl, ▽ - ▽ ; fenvalerate. AChE activity was expressed as absorbance change per minute at 405nm. A

; absorbance. Mean value \pm SE, n=5

. ,
AChE ,

. ,
esterase

,

.

4

1. PAGE , 1 AChE가 .
AChE esterase 가

2. , AChE esterase ,

5

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4

esterase

,

esterase

1

- naphthyl acetate 가 esterase
 . (Eguchi
et al., 1965, 1967). Watanabe (1967) suni thi on
 esterase
 agar gel , , ,
 , , , esterase 가
 .
 , esterase가
 esterase 가 ,
 1 esterase band ,
 esterase
 .

2

1.

(123 × 124) .

2.

: dichlorvos, phenthoate

: prnicarb, nethonyl

: fenvalerate, cypermethrin

3.

5 3 10 μ l topical application

2-3 10 .

10 1ml sodium phosphate buffer(pH 7.0) homogenizing

, 10,000g , .

4. PAGE esterase band

, Davis (1964)

7.5% gel . gel 0.1% α

-naphthyl acetate (0.05g α -naphthyl acetate 5ml

, 0.1M sodium phosphate buffer(pH6.8) 50ml

) 37 30 . 0.5%

fast blue BB salt 10 esterase band

.

제 3 절 결과 및 고찰

1. 살충제에 의한 누에의 신경절에 있는 esterase의 저해유무 조사

가. 본 연구에 있어서도 PAGE에 의해, 누에의 중추신경계의 복부신경절로부터 1개의 esterase major band를 검출하였다. Yoshitake등(1965)도 agar gel 전기영동에 의해, 누에의 중추신경계의 복부신경절에서 1개의 esterase major band를 검출하였다.

나. 5령 3일째 누에에 유기인제 살충제인 dichlorvos와 phenthonate의 저해 상태를 조사한 결과를 Fig. 1에 표시하였다. Fig. 1에서 보는 바와 같이, 누에의 신경절에 있는 esterase는 살충제의 농도에 따라 특이적으로 저해되었다.

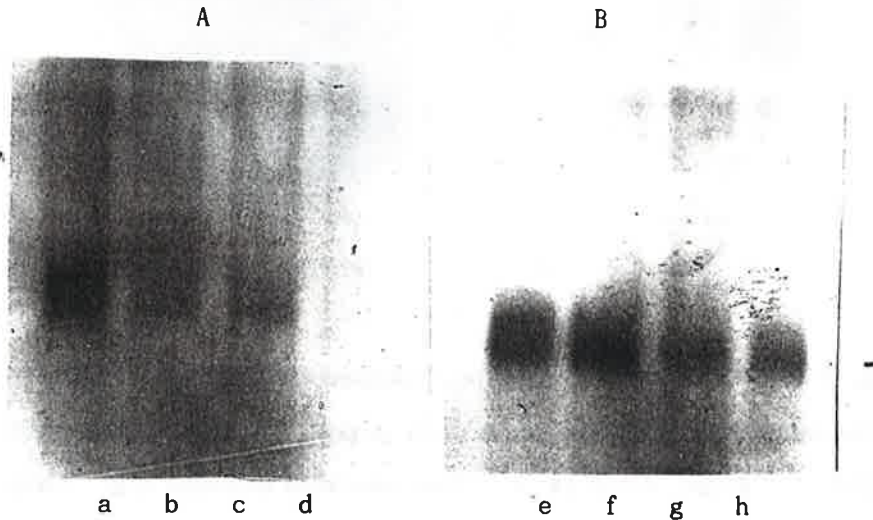


Fig. 1. PAGE analysis of ganglion esterases in silkworm larvae treated with organophosphates. (A) Treatment with dichlorvos : a : control, b : 0.001%, c : 0.005%, d : 0.01%, (B) Treatment with phentionate : e : control, f : 0.001%, g : 0.005%, h : 0.01%.

다. 카바메이트제 살충제인 pirimicarb와 methomyl을 처리한 누에의 신경절에 있는 esterase의 저해상태를 Fig. 2에 표시하였다. Fig. 2에서 보는 바와 같이, 누에의 신경절에 있는 esterase는, 유기인제 살충제를 처리한 경우와 같은 경향으로, 살충제의 농도에 따라 특이적으로 저해되었다.

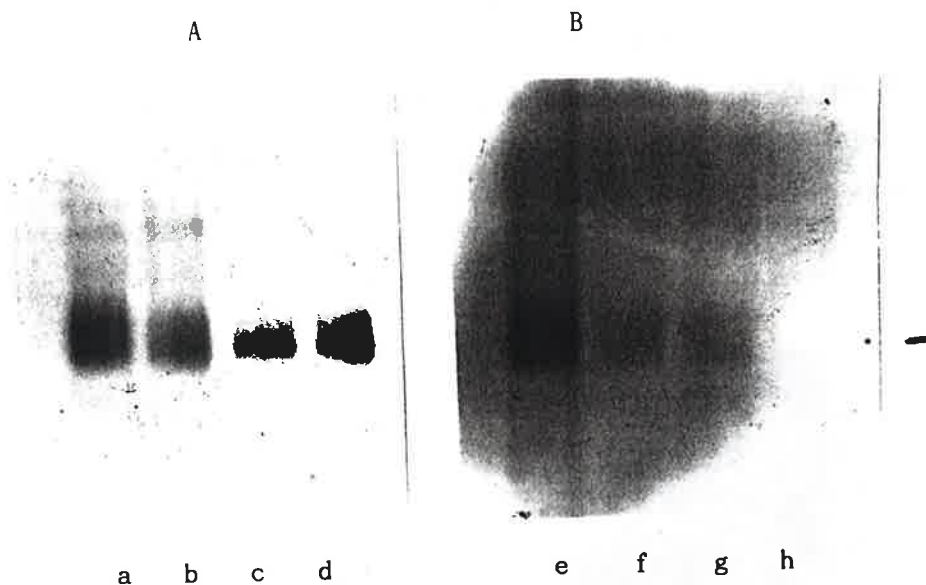


Fig. 2. PAGE analysis of ganglion esterases in silkworm larvae treated with carbamates. (A) Treatment with pirimicarb : a : control, b : 0.01%, c : 0.05%, d : 0.1%, (B) Treatment with methomyl : e : control, f : 0.001%, g : 0.005%, h : 0.01%.

라. 한편, 피레스로이드제인 fenvalerate와 cypermethrin를 처리한 누에의 신경절에 있는 esterase의 저해상태를 Fig. 3에 표시하였다. Fig. 3에서 보는 바와 같이, 누에의 신경절에 있는 esterase는 살충제 처리에도 불구하고 전혀 저해가 관찰되지 않았다.

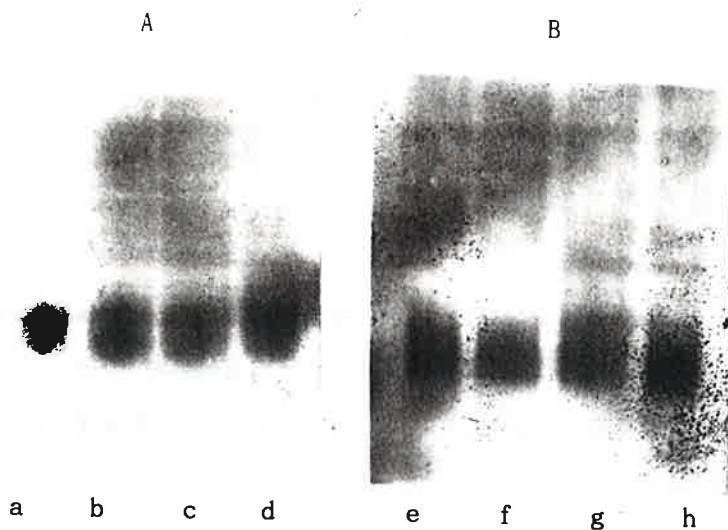


Fig. 3. PAGE analysis of ganglion esterases in silkworm larvae treated with pyrethroids. (A) Treatment with fenvalerarte : a: control, b : 0.001%, c : 0.005%, d : 0.01%, (B) Treatment with cypermethrin : e : contról, f : 0.001%, g : 0.005%.

마. 이상의 연구결과를 종합하여 고찰하여 볼 때, 유기인제나 카바메이트제 살충제에 의해, 누에의 혈림프에 있는 esterase, 신경절에 있는 esterase, 머리조직에 있는 AChE는 다같이 특이적으로 저해되었으나, 합성피레스로이드 살충제에 의해서는 전혀 저해되지 않았다. 따라서, 누에의 신경절에 있는 esterase저해 상태를 기준으로 해서 누에의 유기인제나 카바메이트제

esterase band
esterase
esterase

4

1. PAGE
esterase band

2. esterase가
esterase
AChE

5

Eguchi, M. and Yoshitake, N. (1967) : Interrelation of non-specific esterase zynogram among various tissue in the silkworm, *Bombyx mori*. J. Seri. Sci. Jpn., 36(3), 193-198.

Watanabe, H. and Kobara, R. (1967) : Study on the resistance to insecticides in the silkworm, *Bombyx mori*. . Effect of sumithion on the esterase activities in the blood and some tissues of larvae. J. Seric. Sci. Jpn., 36(3), 183-191.

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5

1

가

가가

1976

1, 2, 3)

2)

가

3).

4)

5).

di chlorvos, acephate phenthoate 3 , captan
pyrazophos 2 , ethoprofos endosulfan 2 ,

alachlor, sinazine, pendinethalin, paraquat dichloride 4

6)

di chlorvos phenthoate,

가

fenitrothion fenthion

가
 dichlorvos, acephate, phenthoate
 pyrazophos

가
 pyrethroid
 deltanethrin cypermethrin alachlor sinazine
 8 가
 가 3 가 2 가 2

가

2

1.

dichlorvos, acephate, phenthoate pyrazophos,

가 pyrethroid deltanethrin cypermethrin,
 alachlor sinazine 8

2.

가 (8) 3 가
 3 (1996, 1997, 1998) (5 6) 가 (9 10) 2

No. 1 3 (), No. 4 6 (), No. 7 9 (), No. 10 12 (), No. 13 15 (), No. 16 18 (), No. 19 21 () No. 22 24 () .

3.

가. 1 ('96)

1) Dichlorvos, phenthoate pyrazophos

30 g acetone 100 ml 10
 20 ml .
 100 ml , 800 ml,
 dichloronethane 100 ml 가 2 0.1 ml 2%
 diethylene glycol/acetone 가 2 ml
 . florasil
 column chromatography . 5 g florasil
 n-hexane slurry glass column (30 cm L. ×
 2 cm I. D.) sodium sulfate 1g
 n-hexane - dichloronethane (8:2, v/v) 15 ml
 n-hexane-acetonitrile-dichloronethane
 (48:5:1.5:50, v/v/v) 50ml phenthoate, dichlorvos
 pyrazophos 30 ml
 n-hexane-acetonitrile-dichloronethane (45:5:50, v/v/v)
 pyrazophos . 0.1 ml 2% diethylene
 glycol/acetone 가 2 ml
 . acetone 3 ml

GC-NPD .

2) Acephate

20 g acetone 100 ml 10
 10 ml 1-butanol 가
 silica gel column chromatography
 3 g silica gel n-hexane slurry
 glass column (30 cm I. × 2 cm I.D.) sodium
 sulfate 1 g n-hexane-dichloromethane (8:2, v/v) 10
 ml
 n-hexane-acetonitrile-dichloromethane (45:5:50, v/v/v) 20 ml
 acetone 10 ml acetone 30 ml
 acetate 2 ml
 acetone 4 ml

GC-NPD

2 ('97)

1) Dichlorvos

30 g acetone 120 ml 가 10
 20 ml acetone 20 ml
 (10 ml 가 sonication, 10 ml 가)
 (H3PO4-NH4Cl-H2O: 20-10-780, v/w/v) 10 50 ml 가 30
 Celite 545 1
 separatory funnel 100 ml, 50
 ml, methylene chloride 100 ml (50+50) 가
 2% diethylene glycol/acetone 0.2 ml 가 1 ml

silica gel column chromatography Silica gel 5 g
 acetone: n-hexane(3:7, v/v)
 acetone 6 ml (3+3) column acetone:

n-hexane(3:7, v/v) 13 ml dichlorvos . 2%
diethylene glycol/acetone 0.2 ml 가 1 ml

2) Acephate

20 g ethyl acetate 100 ml 가 10
sodium sulfate 30 g 가 10
florisil
column chromatography . Florisil 3 g ethyl acetate
ethyl acetate 10 ml column
ethyl acetate 80 ml
acetone 30 ml . acetone 120 ml acephate

3) Deltanethrin Cypermethrin

20 g acetone 100 ml 가 10
20 ml acetone 20 ml
(H3P04-NH4Cl- H2O: 20-10-780, v/w/v) 10 50 ml 가
30 Celite 545 partition
1 separatory funnel 100 ml,
50 ml methylene chloride 100 ml (50+50) 가
florisil column chromatography .
Florisil 5 g n-hexane 7 ml
n-hexane: methylene chloride (8:2, v/v) column
40 ml . n-hexane:
methylene chloride: acetonitrile(49.65:50:0.35, v/v/v) 100 ml
deltanethrin cypermethrin .

. 3 ('98)

1) Dichlorvos Sinazine

20 g acetone 100 ml 가 20 .
50 ml acetone . 10 ml
acetone 20 ml (H3P04-NH4Cl-H2O: 20-10-780, v/w/v) 50 ml 가
30 Celite 545 . 500 ml
50 ml, 150 ml methylene
chloride 50 ml 2 methylene
chloride
methylene chloride 50 ml
. 40 acetone GC-NPD

2) Acephate

20 g ethyl acetate 100 ml 가 10
. sodium sulfate 30 g 가 가 10
florisil column chromatography . mini column
(13 mm I.D., 65 mm L.) 1 g florisil acetone 5 ml
prewashing acetone 10 (5+5) ml column
ethyl acetate 30 ml .
acetone 10 ml acetone 70 ml acephate .
acetone 2 ml GC/NPD 1 µl

3) Alachlor

20 g acetone 100 ml 가 20 .
 50 ml acetone 10 ml
 acetone 15 ml (H3PO4-NH4Cl-H2O: 20-10-780, v/w/v)
 10 40 ml 가 30 Celite 545
 . 500 ml 50 ml,
 100 ml n-hexane 50 ml 2
 n-hexane
 n-hexane 50 ml
 . n-hexane 40 .
 Silica gel 1 g mini-column (13 mm I. D., 65 mm L.) 5
 ml n-hexane n-hexane 5 ml (3+2)
 column 2 ml/min
 . Acetone: n-hexane(1: 99) 7 ml 가
 acetone: n-hexane(5: 95) 13 ml alachlor .

4.

1 .

Table 1. GLC conditions for the analyses of some pesticide residues in mulberry leaves

가. Dichlorvos, phenthoate, and pyrazophos

Column	SE-30 (30 m L. × 0.25 mm I. D. × 0.25 μm film thickness) or HP-1 (5 m L. × 0.53 mm I. D. × 2.65 μm film thickness)
Detector	Nitrogen phosphorus detector
Temperature	Oven : Initial 100 for 0.1 min, 15 /min to 170 maintained for 5 min or initial 130 for 1 min, 5 /min, 140 for 0.1min, and 20 /min to 270

maintained for 5 min.

Injector: 220 , Detector : 250

Injection volume 1 $\mu\ell$

. Acephate

Column SE-30 (30 m L. \times 0.25 mm I.D. \times 0.25 μm film thickness)
or HP-1 (5 m L. \times 0.53 mm I.D. \times 2.65 μm film
thickness)

Detector Nitrogen phosphorus detector or Flame photometric
detector (S-node)

Temperature Oven :
NPD : Initial 110 for 0.1 min, 20 /min to 250
maintained for 5 min. or initial 70 for 2 min,
10 /min to 270 maintained for 5 min.
FPD : Initial 170 for 0.5 min, 20 /min to 250
maintained for 5 min.

Injector : 220 or 230

Detector : 270 (NPD) or 260 (FPD)

Injection volume 1 $\mu\ell$

. Deltanethrin and cypermethrin

Column HP-1 (5 m L. \times 0.53 mm I.D. \times 2.65 μm film
thickness)

5.

(0.05, 0.1, 0.5,

1.0 ppm)

6. peak

phenthoate

phenthoate

retention time

No. 6

phenthoate

1.90 ppm

column oven

peak

. Column HP-1 (Methyl

silicone gum, 5 m L. x 0.53 mm I.D. x 2.65 μ m film thickness, Hewlett

Packard) Ultra-1 (Cross-linked methyl siloxane, 25 m L. x 0.32 mm

I.D. x 0.17 μ m film thickness, Hewlett Packard) , oven

150

60

retention time

. peak

GC/MSD (HP 5890 A Series

Gas Chromatograph equipped with HP 5972 Series Mass Selective

Detector, Hewlett Packard)

3

1.

103% .
 partition 2 ,
 g 0.05 ppm 0.25 ppm
 partition 1 , florisil 5 g
 97% 92%

2

92

deltanethrin

florisil

10

81% 87%

Table 2. Recovery by the analytical methods used

Chemical	Recovery (mean±SD), (%) at different concentrations (ppm)			
	0.05	0.10	0.25	1.00
Di chlorvos		93.3 ± 4.0		95.1 ± 8.2
Acephate		100.3 ± 4.0		94.9 ± 4.2
Phenthoate		99.4 ± 0.8		96.4 ± 2.1
Pyrazophos		91.8 ± 1.5		91.5 ± 2.0
Deltanethrin	97.0 ± 3.8		91.5 ± 5.4	
Cypermethrin	92.7 ± 6.6		91.8 ± 5.4	
Sinazine	92.3 ± 2.9	96.3 ± 2.9		
Alachlor	102.6 ± 4.2			98.3 ± 4.3

a) Standard deviation.

2.

3

4, 5 6 가

가

dichlorvos 1996 , 1997 , 1998 29, 75

17%, 가 42, 88 8%

0.018 0.032, 0.014 0.064 0.011 0.014 ppm, 가

0.012 0.048 , 0.013 0.062 0.014 0.016 ppm . 6)

3 5 dichlorvos (maximum residue limits) 0.11 0.15 ppm .

0.032, 0.064, 0.014, 0.048, 0.062 0.016

ppm 3 0.11 ppm

29, 58, 13, 44, 56 9%

dichlorvos .

acephate

58, 13 4% 17, 4 21%

, 0.010 0.156 ppm

. Phenthoate 1996 , 가 4 8%

, 0.018 0.051 ppm . 6)

3 5 phenthoate

(maximum residue limits) 0.30 0.50 ppm ,

1/6 1/10

phenthoate 가

. Pyrazophos

.

pyrethroid

deltamethrin cypermethrin 1997

가 8 17%

0.019 0.068 0.009 0.013 ppm . No. 10
 0.068 ppm . '89
 가 A
 (,) , B (,) C (,)
 cypermethrin 0.07, 0.05 0.16 ppm 5).
 cypermethrin (0.068 ppm)
 가 .
 alachlor 가 (0.010 ng/kg)
 , sinazine 1998 가 13 4%
 0.018 0.053 0.018 ppm , sinazine
 EPA 7) 4 5000 ng/kg
 body weight , WHO ' "

Table 3. Pesticide residues (ppm)^a in mulberry leaves analyzed over three consecutive years (1996-1998)

Sample No.	Location (Province)	1996							
		Chemical							
		Dichlorvos		Acephate		Phenthoate		Pyrazophos	
Season		Season		Season		Season			
		Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
1	Kangwon	0.024	0.048	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
2		0.018	0.020	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
3		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
4	Kyungki	<0.010	0.017	0.022	<0.010	<0.010	0.050	<0.010	<0.010
5		<0.010	0.030	<0.010	0.041	<0.010	<0.010	<0.010	<0.010
6		0.022	<0.010	0.023	<0.010	<0.010	<0.010	<0.010	<0.010
7	Chunghbuk	<0.010	<0.010	0.017	<0.010	<0.010	<0.010	<0.010	<0.010
8		<0.010	0.014	0.014	<0.010	<0.010	<0.010	<0.010	<0.010
9		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
10	Chungnam	0.032	0.012	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
11		0.028	<0.010	0.025	<0.010	0.051	0.018	<0.010	<0.010
12		0.019	<0.010	0.017	0.156	<0.010	<0.010	<0.010	<0.010
13	Chunbuk	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
14		<0.010	<0.010	0.020	<0.010	<0.010	<0.010	<0.010	<0.010
15		<0.010	0.013	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
16	Chunnam	<0.010	<0.010	0.026	<0.010	<0.010	<0.010	<0.010	<0.010
17		<0.010	0.022	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
18		<0.010	<0.010	0.015	<0.010	<0.010	<0.010	<0.010	<0.010
19	Kyungbuk	<0.010	0.018	0.018	<0.010	<0.010	<0.010	<0.010	<0.010
20		<0.010	<0.010	0.072	<0.010	<0.010	<0.010	<0.010	<0.010
21		<0.010	<0.010	0.023	0.020	<0.010	<0.010	<0.010	<0.010
22	Kyungnam	0.019	0.015	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
23		<0.010	<0.010	0.013	0.035	<0.010	<0.010	<0.010	<0.010
24		<0.010	<0.010	0.041	<0.010	<0.010	<0.010	<0.010	<0.010

a) Figures represent mean of duplication.

Table 3. Continued

Sample No.	Location (Province)	1997							
		Chemical							
		Dichlorvos		Acephate		Deltamethrin		Cypermethrin	
		Season							
		Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
1	Kangwon	0.016	0.040	0.044	<0.010	<0.015	<0.008	<0.015	<0.008
2		0.014	0.036	<0.022	<0.010	<0.015	<0.008	<0.015	<0.008
3		0.018	0.026	0.033	<0.010	<0.015	<0.008	<0.015	0.009
4	Kyungki	0.045	0.013	0.061	<0.010	<0.015	<0.008	<0.015	<0.008
5		0.035	0.022	<0.022	0.015	<0.015	<0.008	<0.015	<0.008
6		<0.013	<0.010	<0.022	<0.010	<0.015	<0.008	<0.015	<0.008
7	Chungbuk	<0.013	0.040	<0.022	<0.010	<0.015	<0.008	0.019	<0.008
8		0.031	0.042	<0.022	<0.010	<0.015	<0.008	<0.015	0.009
9		0.023	0.028	<0.022	<0.010	<0.015	<0.008	<0.015	<0.008
10	Chungnam	0.022	0.062	<0.022	<0.010	<0.015	<0.008	0.068	<0.008
11		0.017	0.035	<0.022	<0.010	<0.015	<0.008	<0.015	<0.008
12		0.020	0.025	<0.022	<0.010	<0.015	<0.008	<0.015	0.009
13	Chunbuk	<0.013	<0.010	<0.022	<0.010	<0.015	<0.008	<0.015	<0.008
14		0.019	0.047	<0.022	<0.010	<0.015	<0.008	<0.015	<0.008
15		0.046	0.036	<0.022	<0.010	<0.015	<0.008	<0.015	<0.008
16	Chunnam	0.015	0.035	<0.022	<0.010	<0.015	<0.008	<0.015	<0.008
17		<0.013	0.022	<0.022	<0.010	<0.015	<0.008	<0.015	<0.008
18		<0.013	0.046	<0.022	<0.010	<0.015	<0.008	<0.015	<0.008
19	Kyungbuk	0.016	0.018	<0.022	<0.010	<0.015	<0.008	<0.015	<0.008
20		0.023	0.034	<0.022	<0.010	<0.015	<0.008	<0.015	<0.008
21		0.038	0.029	<0.022	<0.010	<0.015	<0.008	<0.015	<0.008
22	Kyungnam	0.032	0.024	<0.022	<0.010	<0.015	<0.008	<0.015	0.013
23		<0.013	<0.010	<0.022	<0.010	<0.015	<0.008	<0.015	<0.008
24		0.064	0.034	<0.022	<0.010	<0.015	<0.008	<0.015	<0.008

Table 3. Continued

Sample No.	Location (Province)	1998							
		Chemical							
		Dichlorvos		Acephate		Sinazine		Alachlor	
		Season							
		Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
1	Kangwon	<0.010	<0.010	<0.010	0.016	<0.010	0.018	<0.010	<0.010
2		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
3		<0.010	<0.010	<0.010	<0.010	0.020	<0.010	<0.010	<0.010
4	Kyungki	<0.010	<0.010	<0.010	<0.010	0.018	<0.010	<0.010	<0.010
5		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
6		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
7	Chungbuk	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
8		<0.010	0.016	0.012	0.012	<0.010	<0.010	<0.010	<0.010
9		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
10	Chungnam	<0.010	<0.010	<0.010	<0.010	0.053	<0.010	<0.010	<0.010
11		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
12		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
13	Chunbuk	<0.010	0.014	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
14		<0.010	<0.010	<0.010	0.034	<0.010	<0.010	<0.010	<0.010
15		<0.010	<0.010	<0.010	0.010	<0.010	<0.010	<0.010	<0.010
16	Chunnam	0.014	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
17		0.011	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
18		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
19	Kyungbuk	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
20		0.013	<0.010	<0.010	0.014	<0.010	<0.010	<0.010	<0.010
21		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
22	Kyungnam	0.011	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
23		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
24		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010

Table 4. The detection frequencies and the residual amounts ('96)

Chemical	Detection frequency ^{a)} (%)		Range of residues (ppm)			
	Spring	Fall	Spring		Fall	
Dichlorvos	29	42	0.018	0.032	0.012	0.048
Acephate	58	17	0.013	0.072	0.020	0.156
Phenthoate	4	13	0.051		0.018	0.050
Pyrazophos	0	0	ND ^{b)}		ND ^{b)}	

a) No. of samples detected/No. of samples analyzed × 100.

b) Not detected.

Table 5. The detection frequencies and the residual amounts ('97)

Chemical	Detection frequency ^{a)} (%)		Range of residues (ppm)			
	Spring	Fall	Spring		Fall	
Dichlorvos	75	88	0.014	0.064	0.013	0.062
Acephate	13	4	0.033	0.061	0.015	
Deltamethrin	0	0	ND ^{b)}		ND ^{b)}	
Cypermethrin	8	17	0.019	0.068	0.009	0.013

a) No. of samples detected/No. of samples analyzed × 100.

b) Not detected.

Table 6. The detection frequencies and the residual amounts ('98)

Chemical	Detection frequency ^{a)} (%)		Range of residues (ppm)			
	Spring	Fall	Spring		Fall	
Dichlorvos	17	8	0.011	0.014	0.015	0.016
Acephate	4	21	0.010	0.013	0.010	0.034
Sinazine	13	4	0.018	0.053	0.018	
Alachlor	0	0	ND ^{b)}		ND ^{b)}	

a) No. of samples detected/No. of samples analyzed × 100.

b) Not detected.

3. peak ()

phenthoate 1.90 ppm

2)

peak가 phenthoate가 column oven

phenthoate가 (1).

1 HP-1 column oven 150

phenthoate chromatogram(a) chromatogram

(b) . phenthoate

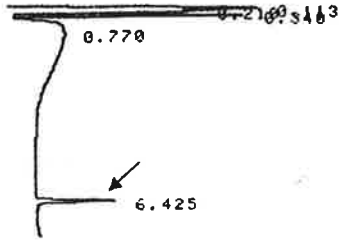
retention time (6.425 min) retention time (6.420 min)

가 . column Ultra-1 oven

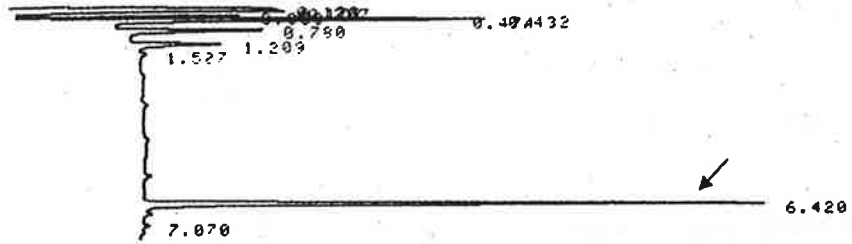
60 retention time 17

phenthoate standard peak (c d). Phenthoate

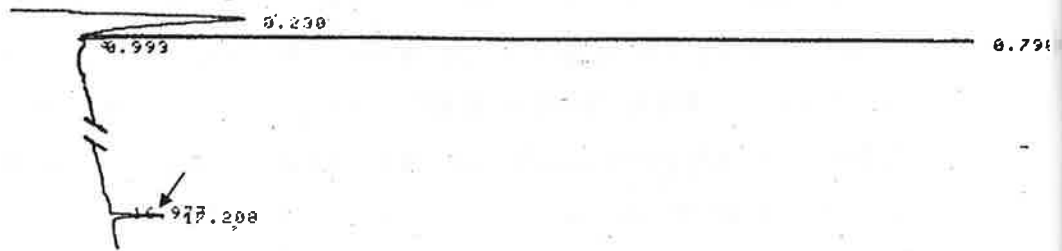
오인했던 peak가 어떤 화합물인지를 알아보기 위하여 GC/MSD를 이용하여 분석해 보았으나 구조는 확인할 수 없었다.



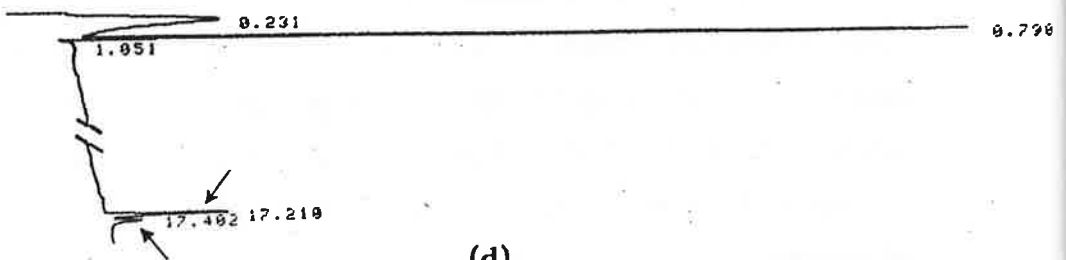
(a)



(b)



(c)



(d)

Fig. 1. Chromatograms of the phenthoate standard and the sample at the different oven temperatures in the different columns. (a) The phenthoate standard at the initial oven temperature of 150 in the HP-1 column, (b) The sample at the initial oven temperature of 150 in the HP-1 column, (c) The phenthoate standard at the initial oven temperature of 60 in the Ultra 1 column, and (d) The mixture of the phenthoate standard and the sample at the initial oven temperature of 60 in the Ultra 1 column.

4

가 가
 dichlorvos, acephate,
 phenthoate pyrazophos,
 가
 pyrethroid deltanethrin cypermethrin,
 alachlor sinazine 3
 . 1996 dichlorvos, acephate
 phenthoate 0.018 0.032, 0.013 0.072, 0.051 ppm
 , pyrazophos , 29, 58 4%
 . 가 dichlorvos, acephate, phenthoate
 0.012 0.048, 0.020 0.156, 0.018 0.050 ppm ,
 pyrazophos , 42, 17 8%

. 1997 dichlorvos, acephate
 cypermethrin 0.014 0.064, 0.033 0.061, 0.019
 0.068 ppm , deltamethrin ,
 75, 13 8% . 가 dichlorvos, acephate,
 cypermethrin 0.013 0.062, 0.015, 0.009 0.013
 ppm , deltamethrin , 88, 4
 17% . 1998 dichlorvos, acephate
 sinazine 0.011 0.014, 0.012, 0.018 0.053 ppm
 , alachlor , 17, 4 13%
 . 가 dichlorvos, acephate, sinazine
 0.014 0.016, 0.010 0.034, 0.018 ppm , alachlor
 , 8, 21 4% . Cypermethrin
 (0.068 ppm) (No. 10, 1997)
 . phenthoate
 GC column

5

1. (1994) 가 .
36(2): 180 181.
2. (1994) 가가 .
36(2): 168 175.
3. (1994) .

36(2): 176 179.

4. (1962)

2: 83 88.

5. (1993) 270 273.

6. , , , , (1984)

3(1): 16 21.

7. (1998) , p. 20.

6 .

1 ,

5 5 (di chlorvos), (methonyl),
(cypermethrin) acetone
topical application ,

1. (muscle power)

Fig. 1 .

Fig. 1 , 3

, 가 , dichlorvos
methonyl cypermethrin 가 .

Fig. 2 . Fig. 2 , 3

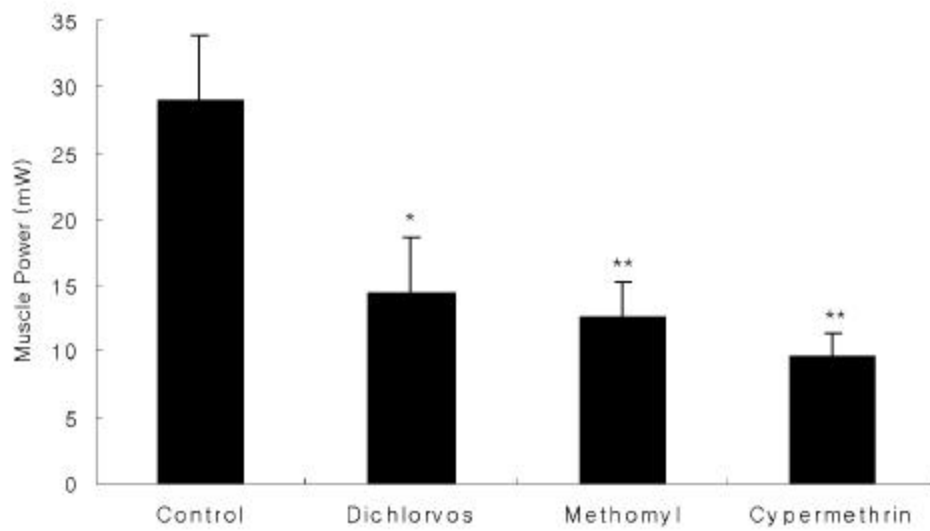


Fig. 1. Effect of pesticides on muscle power. Pesticide solutions ($0.05\mu\ell$ pesticide in 1ml acetone) are treated at $10\mu\ell$ per silkworm by topical application method. mean value \pm SE, $n=10$. (* ; $p \leq 0.05$, ** ; $p \leq 0.01$ vs Control)

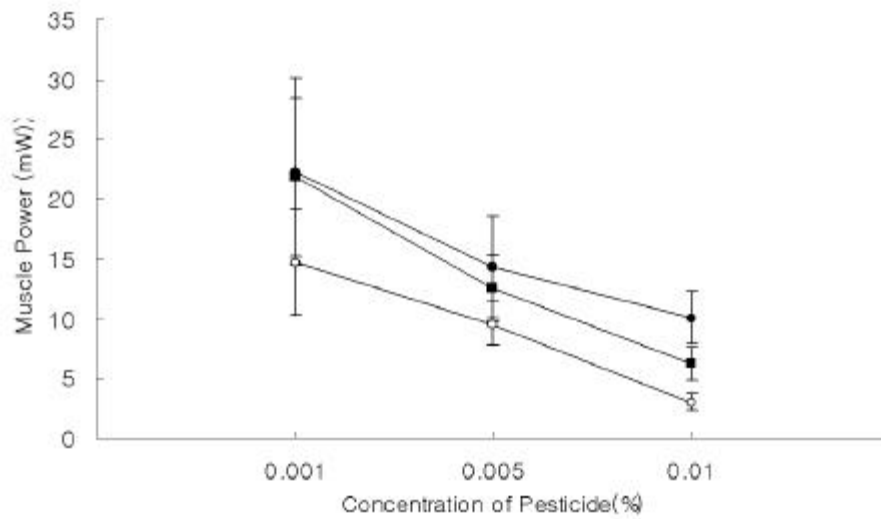


Fig. 2. Effect of pesticides on muscle power. pesticide solutions are treated as described in Fig 11. closed circle ; dichlorvos, closed square ; methionyl, open circle ; cypermethrin.

Mean value \pm SE, n=10.

2. (amplitude)

Fig. 3 .

Fig. 3 , 3

가 ,

.

, 가 methionyl
dichlorvos cypermethrin 가 .

Fig. 4 . Fig. 4 , 3

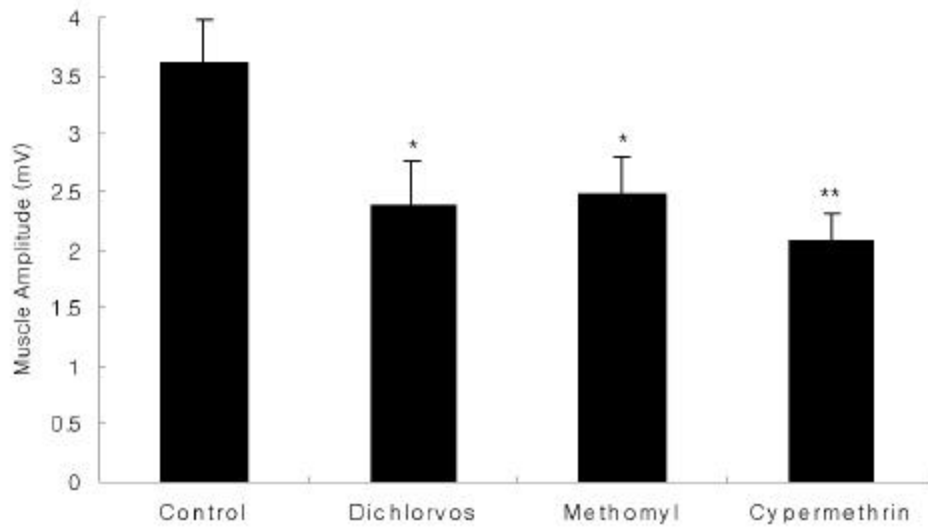


Fig. 3. Effect of pesticides on muscle amplitude. pesticide solution are treated by fig 11. Mean value \pm SE, n=10.

(* ; p 0.05, ** : p 0.01 vs control)

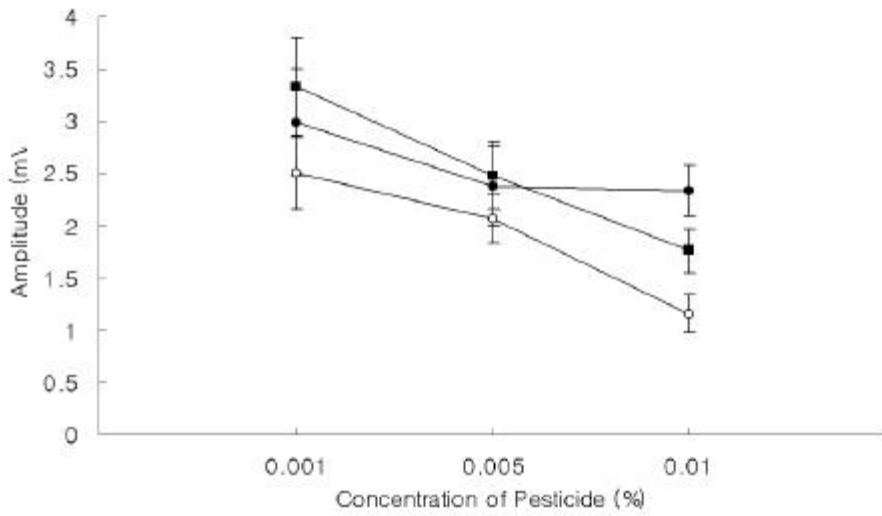


Fig. 4. Effect of pesticides on muscle amplitude. Pesticide solutions are treated as described in Fig 11. closed circle ; dichlorvos, closed square ; methomyl, open circle ; cypermethrin.

Mean value \pm SE, n=10

○ , 0.005%
 50%
 ,
 ,
 non-specific esterase
 acetylcholinesterase 가
 가

2

.

1. 3 0.1ml ,
 , 20
 , DDVP 10ppm 가
 , 10ppm, 5ppm 가 .

2. 8 가
 8 .
 , 10g 50ml , 24 .
 , 20

gas chromatography

가

3. 가
 , 가 ,

가 ,

가

.

3

1. 가

60

2. 20

3. (%)

- : (24.1), (20.6),
(15.5), (39.7)
- : (86.7)
- : (70.0), 1 (5.0),
1-2 (25.0)
- () : (79.3), (70.0), (70.7),
(12.1), (5.2)
- () : (56.9), (56.9),
(12.1), (20.7)
- : > >
- () : DDVP > > >
>

- : > >
- () : > > >
- DDVP >
- 가 가 () :
- > > > >
- 가 가 :
- (20. 0), (14. 3), (54. 3), (11. 4)
- :
- (63. 8), (36. 2)
- :
- (69. 0), (31. 0)
- :
- 가 (81. 8), (15. 6), (2. 6)
- ? :
- (53. 4), (46. 6)
- 가 ? :
- (85. 5), (14. 5)
- 가
- : (91. 1), (8. 9)
- 가
- 가 ,
- 가 . 가
- 가 ,
- , 가
- .

4 acetylcholin -esterase (AChE)

(, .)

1.

Acetylcholinesterase(AChE) , cholinergic synapse
, acetylcholine(ACh) choline acetic
acid 가 . post-synaptic membrane
ACh 가
, ACh choline , 가
(Eldefrawi, 1985).

, AChE target
, 가 AChE , ACh가
, ACh가
(O'brien, 1978). AChE ,
biosensor

(Fennouh *et al.*, 1997 ; Francois *et al.*, 1998).
AChE 가 , ,
, electric fish 가 , ,
, X 가
(Rosenberry *et al.*, 1984 ; Lee *et al.*, 1982 ; Sussman *et al.*,
1991).

가 AChE

가 ,
 (Brestkin *et al.*, 1985 ; Zhu *et al.*, 1994). , AChE
 가
 (Rumpf *et al.*, 1997 ; Zhu *et al.*, 1990).
 , 가 AChE
 . ,
 (Watanabe, 1978), 가 가
 AChE . ,
 AChE .

2.

가.

(123 × 124) ,

0.1g 1ml 1.0% Triton X-100 0.1M sodium
 phosphate buffer(pH 7.0) honogenizing , 10,000g 1
 , AChE

. Sepharose 6B chromatography

Sepharose 6B column 0.1M sodium phosphate buffer(pH 7.0) (0.5%
 Triton X-100) equilibration . 5ml column
 1.0% Triton X-100 0.1M sodium phosphate buffer(pH 7.0)
 , flow rate 0.3ml/min . Fraction 4ml , AChE
 fraction , DEAE-cellulose chromatography

. DEAE-cellulose chromatography

DEAE-cellulose column 0.1M sodium phosphate buffer(pH 7.0) (0.5% Triton X-100) equilibration . buffer
, NaCl 0M 1.0M linear . Flow rate
0.3ml/min , fraction 5ml .

.
Lowry (1951) , BSA (bovine serum albumine) .

3.

가. AChE

AChE , 1g 1% Triton X-100
0.01M sodium phosphate buffer(pH 7.0) 5ml honogenizing ,
10,000×g 1
. 157.6 unit specific
activity 1.30 .

Sepharose 6B chromatography ,

Fig. 4 . Sepharose 6B chromatography

buffer 0.5% Triton X-100 가 ,

AChE .

Fig. 4 fraction No. 25 30 AChE

, fraction 62.4unit,
specific activity가 5.2 39.5% .

Sepharose 6B chromatography , AChE fraction

No. 27-37 najor peak ,

fraction No. 25-30 . , AChE activity

가 , 가 .

Sepharose 6B chromatography 가 ,
 DEAE-cellulose chromatography , Fig. 5
 . DEAE-cellulose chromatography Sepharose 6B chromatography
 가 Triton X-100 buffer ,
 gradient maker 0M 1.0M NaCl
 .
 , AChE NaCl 가 0.2M 0.3M , major peak
 , 12.6unit,
 specific activity가 7.6 crude extract 8.0%
 . -20 , AChE 가
 .
 Table 1 , DEAE- cellulose
 chromatography Sepharose 6B chromatography가 AChE
 , crude extract
 , specific activity가 1.3 7.6 가
 가 .
 AChE
 , 가 , Sepharose 6B
 chromatography 가
 가 . Sepharose Sephadex
 chromatography AChE peak Fig. 4
 peak . (Guilbault *et al.*, 1970 ; Huang *et*
al., 1973 ; Lee *et al.*, 1974 ; Breskin *et al.*, 1984)
 AChE , affinity column
 가 , column ,

가, AChE 가
(Gnagey *et al.*, 1987 ; Zhu *et al.*, 1993).

AChE affinity column
가 .

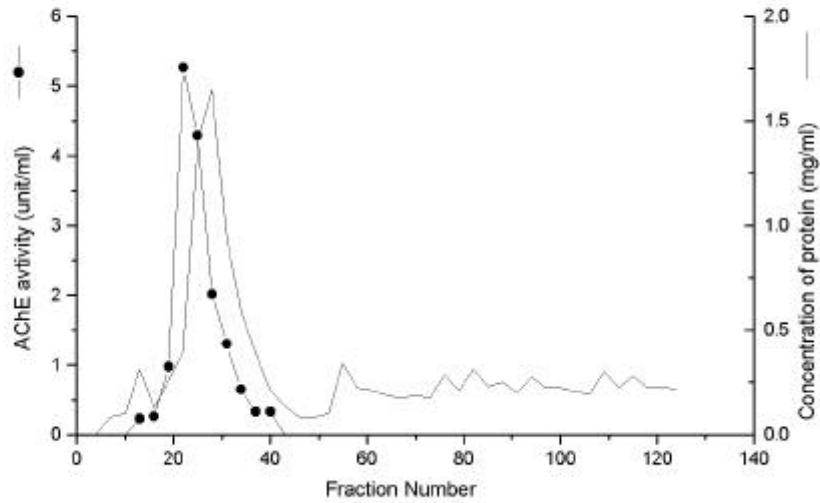


Fig. 3. Sepharose 6B chromatography of AChE in Silkworm larval head tissue. Column(1.7 × 35cm) was equilibrated with 0.01M sodium phosphate buffer, pH7.0, containing 0.5% Triton X-100. Flow rate was 0.3ml per min. 4ml fractions were collected at 4 . One unit of AChE is defined as that amount which will catalyze the of 1 μM of acetylthiocholine per min at 25 and pH 7.5.

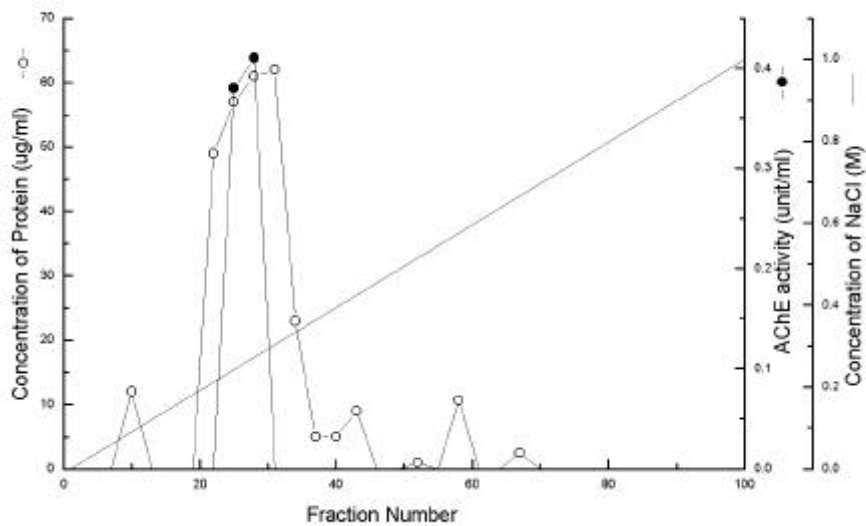


Fig. 4. DEAE- cellulose chromatography of AChE in silkworm larval head tissue. The column(1.5 × 25cm) was equilibrated with 0.01M sodium phosphate buffer, pH 7.0, containing 1% Triton X-100. Elution was with the same buffer, which contained a linear gradient of NaCl from 0M to 1M. Flow rate was 0.3ml per min. and 5ml fractions were collected.

Table 1. Summary of purification procedure for AChE from head tissue of silkworm

Procedure	Total volume (ml)	Total protein (mg)	Protein (mg/ml)	Total activity (unit*)	Activity (unit/ml)	Yield (%)	Specific activity (unit/mg)
Crude extract	5	120.5	24.1	157.6	31.52	100	1.3
Sepharose 6B	24	12.09	0.5	62.4	2.6	39.5	5.2
DEAE-cellulose	30	1.65	0.055	12.6	0.42	8.0	7.6

* One unit of AChE is defined as that amount which will catalyze the of 1 μ M of acetylthiocholine per min at 25 and pH 8.0.

AChE
 가
 (Fig. 5).
 Fig. 5 (a)
 , (b) esterase . Fig. 5
 1-2 가 , 1 esterase band
 (Fig. 2 (a)),
 5-6 esterase가 1 esterase
 band , esterase AChE
 , AChE 5-6 esterase
 , esterase
 AChE 가 ,

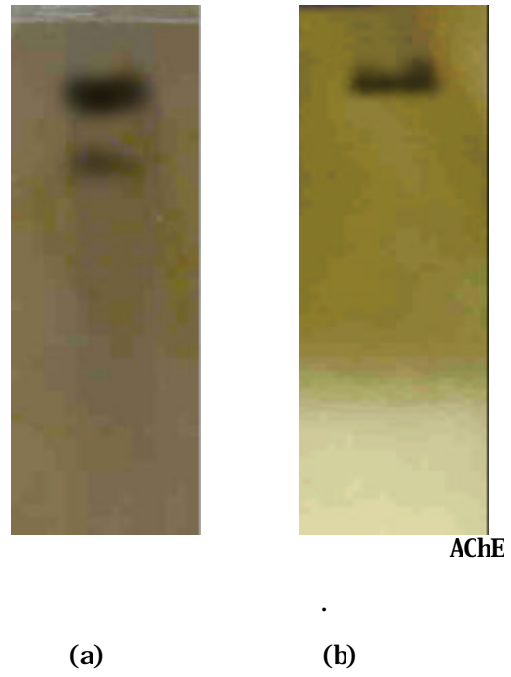


Fig. 5. PAGE analysis of partially purified AChE from silkworm. (a) Silver staining of protein band (b) Staining of esterase band.

Lee A. Metcalf R. L. and Kearns C. W. (1973) Purification and some properties of house cricket (*Acheta domestica*) acetylcholinesterase. *Insect biochem.*, 4, 267-280.

Zhu K. Y., Brindley W. A. and Hsiao T. H. (1991) Isolation and Partial purification of acetylcholinesterase from *Lygus hesperus* (Hemiptera: Miridae). *J. Econ. Entomol.* 84(3), 790-794.

Zhu K. Y. and Clark M. (1994) Purification and Characterization of Acetylcholinesterase from the Colorado Potato Beetle, *Lepidoptera decemlineata*. *Insect Biochem. Molec. Biol.* Vol. 24, No. 5, 453-461.

1.

.

2.

.

3. 가

.