Potential and pitfalls in the use of mixtures for the control of *Helicoverpa armigera* in cotton

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The cotton bollworm, *Helicoverpa armigera*, is a very serious cotton pest in China. This pest has developed very strong resistance to several classes of insecticides, especially pyrethroids.

A lot of mixtures of pyrethroid+organophosphate have been widely used to control the cotton bollworm in China since 1990.

But the role of mixture on delaying insecticide resistance development is very controversial.

Some popular mixtures used to control resistant cotton bollworm in China in 1990s:

Cyhalothrin+Phoxim (26% Mielinghuang EC)

Fenvalerate+Phoxim (50%Xinqing EC)

Cypermethrin+Profenofos (44% Polytrin EC)

Cypermethrin+Chlorpyrifos (44%Superkill EC)

Theoretical studies suggest that mixtures of pesticides could retard resistance development only under certain conditions. These conditions include:

* No cross resistance between components in the mixture (**?**)
* Resistance to each component is functionally recessive (X)
* Doubly resistant individuals are rare (**?**)
* The pesticides have similar persistance (X)
* Refugia for susceptible individuals (✓)

In reality, these conditions are highly unlike to be met.

The interests of our work

- ***Identifying joint action patterns of mixtures of insecticides***

- ***Investigating mechanisms of synergistic action***

- ***Evaluation the role of mixture on delaying resistance development by lab selection***

Part One

Joint action patterns of mixtures

Methodology of joint action analysis

Toxicities of each insecticides to *Helicoverpa armigera* were tested to establish LD-P lines. On the basis of LD50 values of each insecticides, the ratio of two insecticides in mixtures was decided to make each insecticide play the same role in the mixture and co-toxicity coefficient (**CTC**) of mixtures was determined according to Sun's method (Sun and Johnson,1960).

CTC>200	**Significant synergism**
CTC>120	**Synergism**
CTC 80-120	**Addition**
CTC<80	**Antagonism**

Background of strains tested for mixtures

Fen-R: Very high level resistance to Pys
Low level resistance to other classes

SUS2: Low level resistance to all classes

YG: field strain, high level resistance to Pys

LH: field strain, high level resistance to both Pys and OPs.

Synergistic patterns of mixtures between pyrethroids and OPs in Fen-R strain

	Phoxim	Chlorpyrifos	Profenofos	Monocrotophos
Cypermethrin	206.8	104.0	99.3	77.1
Alpha-cypermethrin	205.4	341.5	497.7	157.5
Fenvalerate	235.2	121.2	488.6	<50
Esfenvalerate	158.4	92.7	245.4	94.0
Cyhalothrin	188.6	82.5	369.1	92.0
Deltamethrin	110.8	292.3	245.6	142.7

Synergistic patterns of mixtures between pyrethroids and methomyl or endosulfan in Fen-R strain

	Methomyl	Endosulfan
Cypermethrin	126.7	<50
Alpha-cypermethrin	158.1	<50
Fenvalerate	159.1	<50
Esfenvalerate	179.2	<50
Cyhalothrin	107.1	49.8
Deltamethrin	227.7	100.9

Synergistic patterns of mixtures between OPs and methomyl or endosulfan in Fen-R strain

	Methomyl	Endosulfan
Phoxim	99.7	149.9
Chlorpyrifos	87.9	83.1
Profenofos	109.6	102.4
Monocrotophos	57.2	75.6

Synergistic patterns of mixtures between Pyrethoids in Fen-R strain

	Cyhalothrin	Deltamethrin
Cypermethrin	<50	<50
Fenvalerate	<50	<50
Alpha-cypermethrin	62.4	<50
Esfenvalerate	149.1	64.7
Deltamethrin	85.1	-----
Cyhalothrin	-----	<50

Synergistic patterns of mixtures between OPs in Fen-R strain

	Phoxim	Profenofos	Chlorpyrifos
Phoxim	---	135.1	---
Profenofos	135.1	---	---
Chlorpyrifos	107.3	283.6	---
Monocrotophos	55.9	154.4	78.4

Synergistic patterns of Fenvalerate+phoxim and Fenvalerate+monocrotophos in different strains of *H.armigera*

Strains	Combinations	CTC
SUS2	Fenvalerate+phoxim	150.3
	Fenvalerate+monocrotophos	104.4
LH	Fenvalerate+phoxim	367.8
	Fenvalerate+monocrotophos	176.5
YG	Fenvalerate+phoxim	628.0
	Fenvalerate+monocrotophos	140.0
Fen-R	Fenvalerate+phoxim	235.2
	Fenvalerate+monocrotophos	<50

Conclusions:

(1) Most pairs of a pyrethoid and a thiophosphorate (phoxim and profenofos) are significantly synergistic.

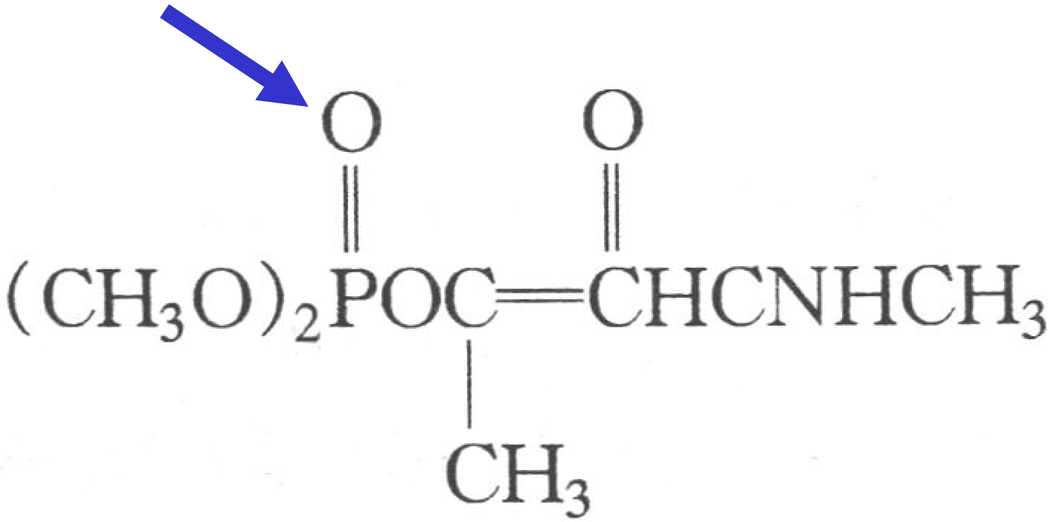
(2) Toxicity of mixtures between pyrethroids and methomyl show moderate synergism.

(3) Toxicity of mixtures between pyrethroids and endosulfan show antagonism.

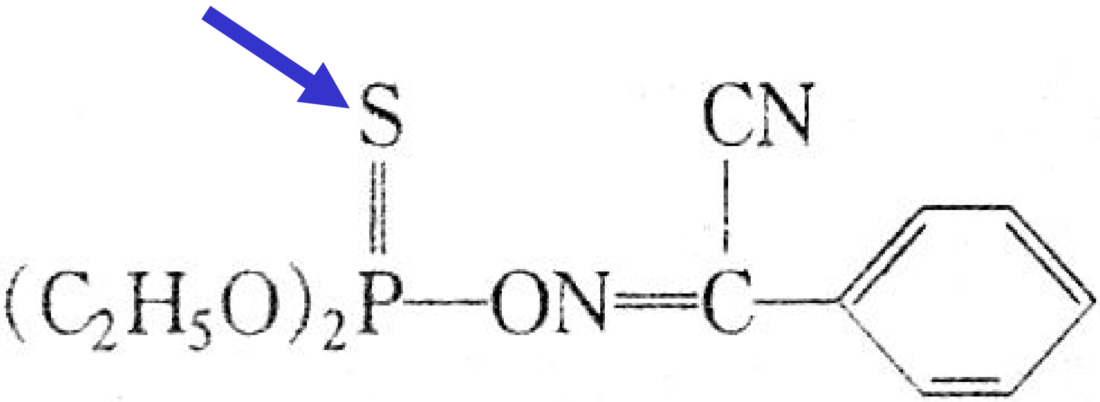
(4) The same mixture in strains with different resistance profiles have different joint action patterns.

Part Two

Investigating mechanisms of synergistic action in mixtures of pyrethroid+ thiophosphorate



Monocrotophos



Phoxim

Possible synergistic mechanisms:

Metabolic interaction----important

MFO: increased activation of P=S

or competing binding of OP

Esterase:OP inhibit esterase which can sequester or detoxify pyrethroids

Target site interaction----not important???

Different sites (AChE, Na^+channel)

Possible partition of MFO between Pyrethroid and OP metabolism

	MFO for Pys degrading	MFO for OP Activation (P=S)	MFO for OP degrading
Fenvalerate	100%	0%	0%
Fenvalerate+ phoxim	40%	30%	30%
Fenvalerate+ monocrotophos	70%	0%	30%

The effect of up and down of MFO activity on the toxicity of phoxim and monocrotophos

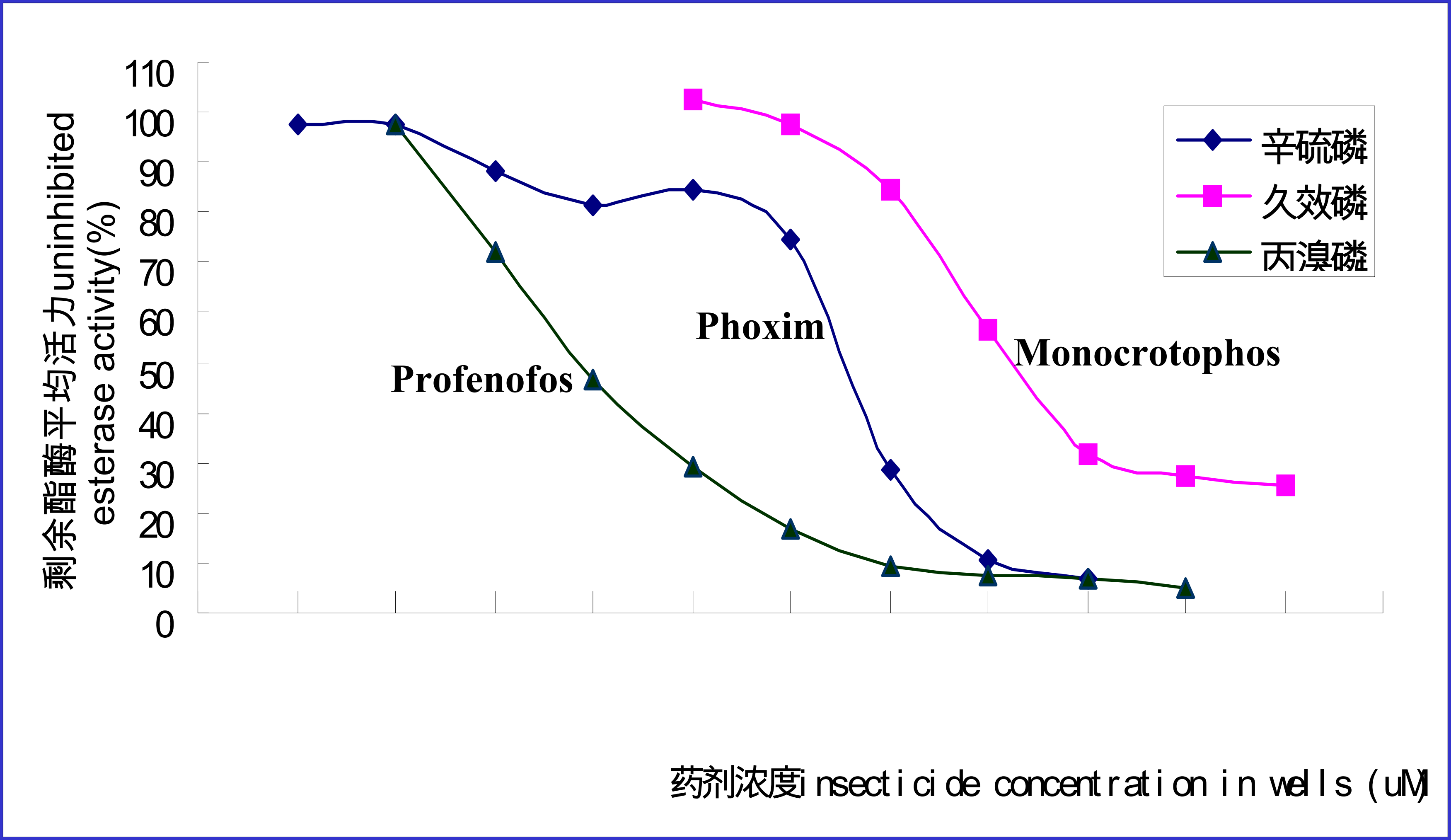
Effect of piperonyl butoxide(PBO) on the toxicity of phoxim and monocrotophos in the LH and YG strains of *H. armigera*

Strain	Insecticide	LD-P line	LD_{50} μg/larva	95% C L	SR
LH	Phoxim	Y=5.3153+1.4222X	0.600	0.427~0.900	—
	Phoxim +PBO	Y=5.3785+1.6209X	0.584	0.434~0.766	1.03
	Monocrotophos	Y=4.2789+1.0399X	4.937	3.081~7.482	—
	Monocrotophos+PBO	Y=4.7900+0.4854X	2.708	1.471~6.245	1.82*
YG	Phoxim	Y=5.8500+2.0143X	0.378	0.286~0.540	—
	Phoxim +PBO	Y=5.5005+1.2831X	0.407	0.296~0.545	0.93
	Monocrotophos	Y=4.6995+1.7354X	1.490	1.097~1.982	—
	Monocrotophos+PBO	Y=5.0563+1.7190X	0.927	0.701~1.180	1.61*

Effect of phenobarbital (PB) induction on the toxicity of phoxim and monocrotophos in SUS2 strain of *H.armigera*

Insecticide	LD-P line	LD_{50} µg/larva	95% C L	LD_{50}(PB) /LD_{50}(No PB)
Phoxim	Y=5.3121+1.9708X	0.694	0.559~0.904	——
Phoxim (PB induced)	Y=5.4094+1.9033X	0.609	0.492~0.777	0.88
Monocrotophos	Y=4.8864+1.5664X	1.182	0.720~1.680	——
Monocrotophos (PB induced)	Y=4.5923+0.8959X	2.851	1.511~5.170	2.41*

Phoxim and Profenofos are better esterase inhibitors than monocrotophos



IC_{50}:	**Profenofos**	**0.18uM**
	Phoxim	**1.76uM**
	Monocrotophos	**147.7uM**

Conclusions:

(1) The activation of the thiophosphate leaves less MFO available for detoxifying pyrethroids. This may be an important factor for the synergistic action between Py+thiophosphate.

(2) Strong inhibition of esterases by the thiophosphate is probably another factor responsible for the synergism.

Part Three

Evaluation the role of mixture on delaying resistance development by lab selection

8 published reports in China suggested that the binary mixture between pyrethroid and organophosphate can delay resistance development more efficiently than using single insecticide according to the results of laboratory selection experiment.

These laboratory selection experiments were designed as:

A insect population was divided into four subpopulations. Then compare the resistance development of the insecticide used for selection in each subpopulation. The conclusions were generally that the resistance development of the mixture was slower than that of a single insecticide.

Subpopulation 1:as control without any selection.

Subpopulation 2: selected with insecticide A

Subpopulation 3: selected with insecticide B

Subpopulation 4: selected with the mixture of A+B

?

Our research program was designed at a different angle to evaluate the role of mixture in delaying resistance development in the cotton bollworm.

A insect population was divided into three subpopulations as follows:

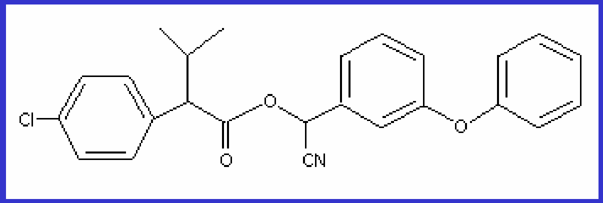
Subpopulation 1: no selection.

Subpopulation 2: selected with a mixture of fenvalerate+phoxim

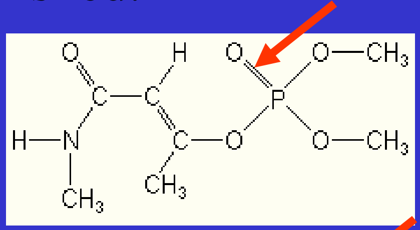
Subpopulation 3: selected with a mixture of fenvalerate+monocrotophos

The resistance development of both the components and mixture was monitored every second generation during the selection.

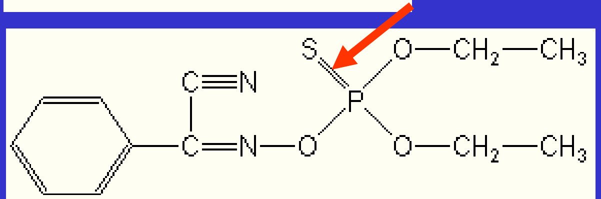
Cross resistance patterns and biochemical mechanisms were also evaluated after the selection was finished.



Fenvalerate



Monocrotophos



Phoxim

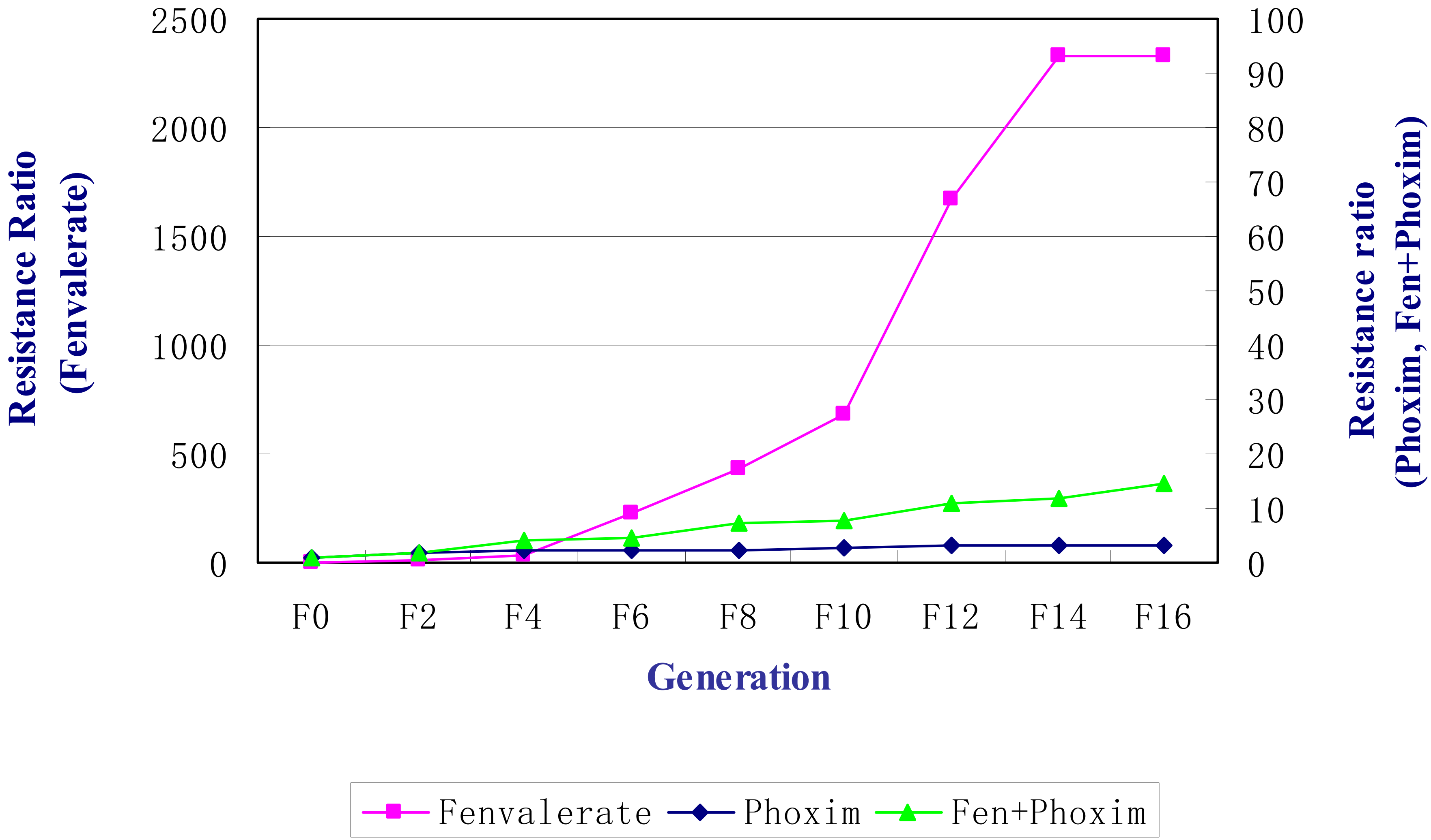
Resistance development of both components and mixtures

YS-FP: selected with Fenvalerate+Phoxim (a.i. 1:10)

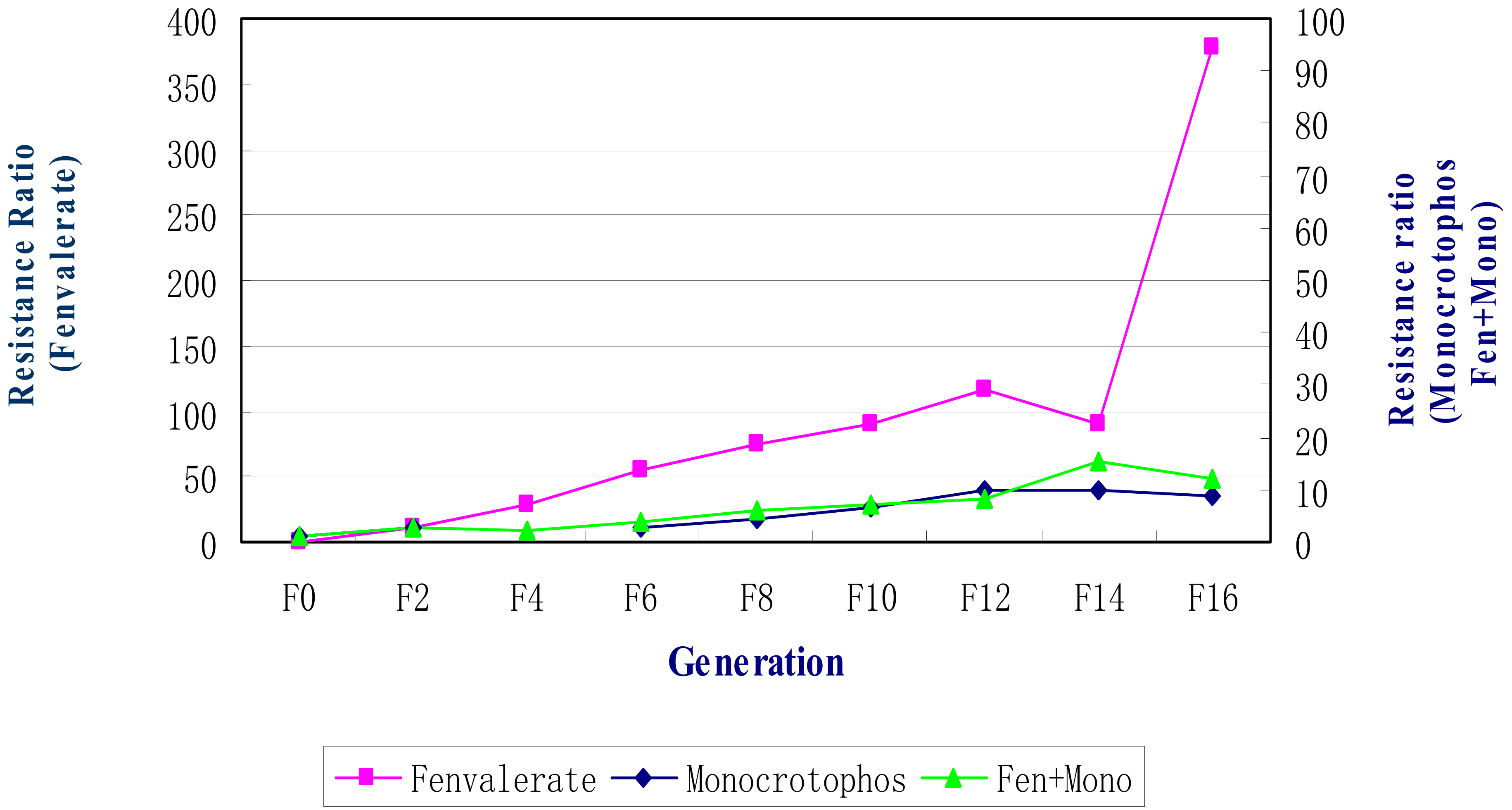
YS-FM: selected with Fenvalerate+Monocrotophos (a.i. 1:50)

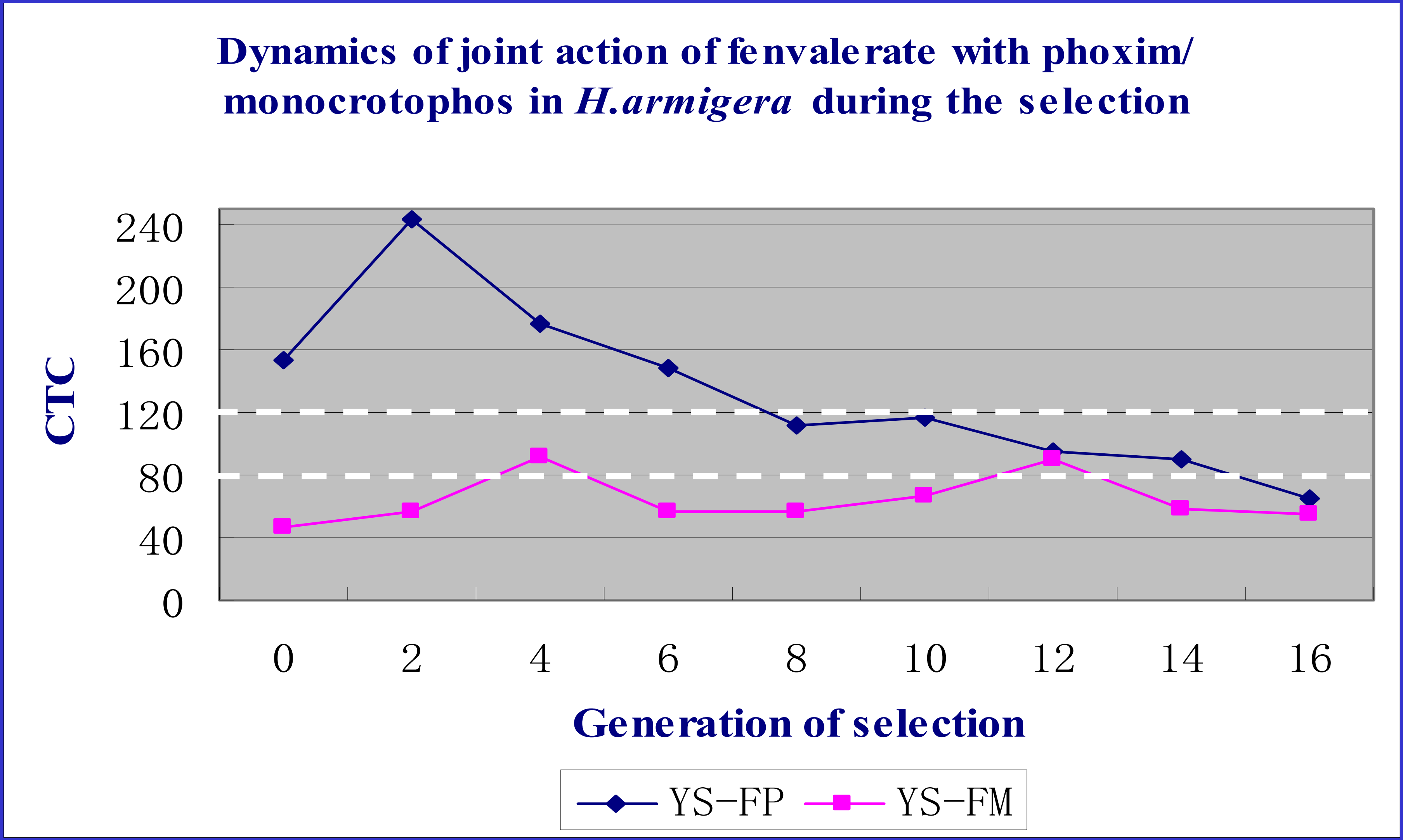
YS: control strain

Resistance development in the YS-FP strain of *H.armigera* (selected with Fen+Phoxim)



Resistance development in the YS-FM strain of *H.armigera* (selected with Fen+Monocrotophos)



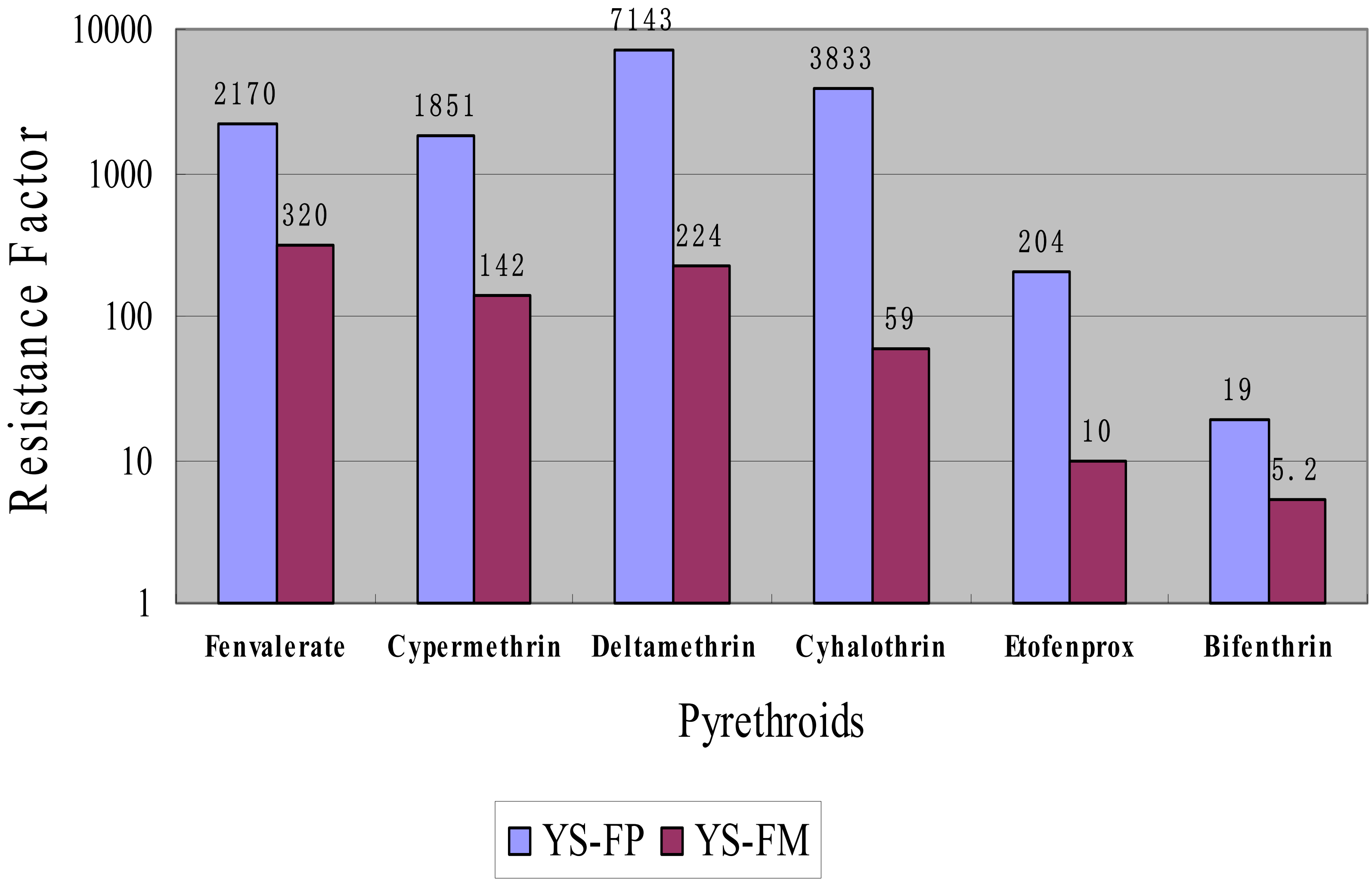


CTC means Co-toxicity coefficient (Sun et al, 1960).

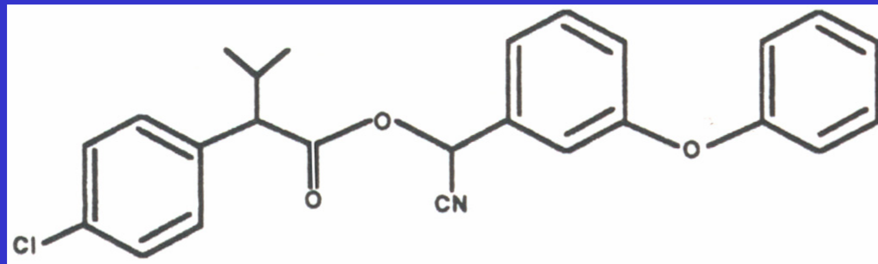
CTC>120: synergism; 80<CTC<120: additive action; CTC<80: antagonism.

Patterns of cross resistance in the YS-FP and YS-FM strains of *H. armigera*

Cross resistance to pyrethroids of the YS-FP and YS-FM strains compared with the YS strain

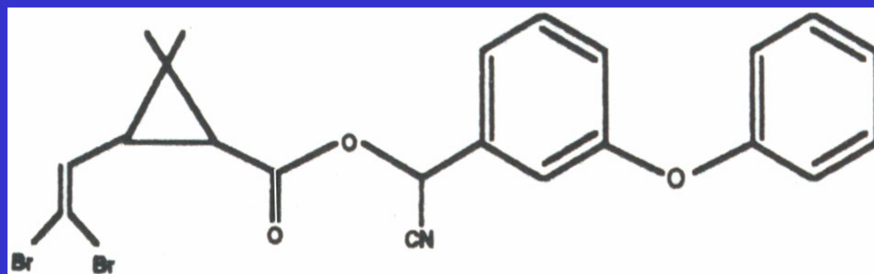


Fenvalerate

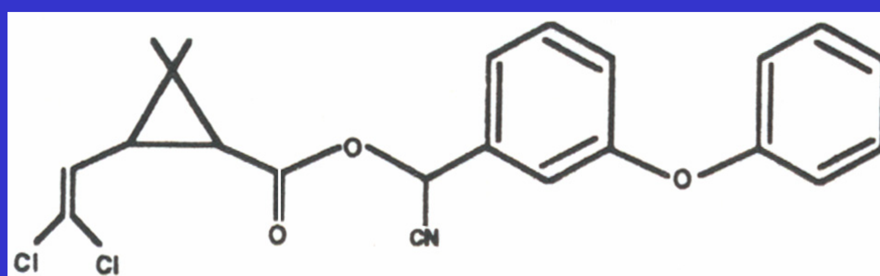


Ester bonded, phenoxybenzyl alcohol, aromatic acid

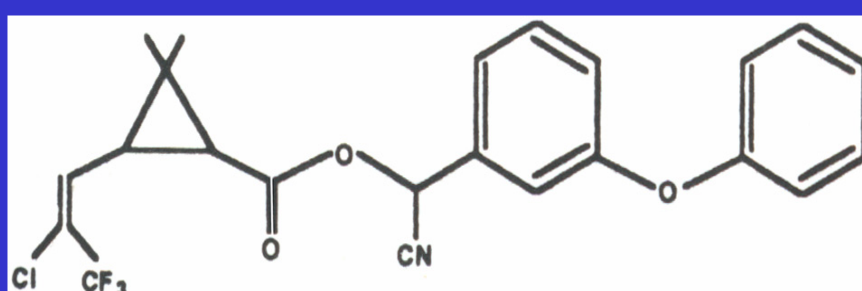
Deltamethrin



Cypermethrin



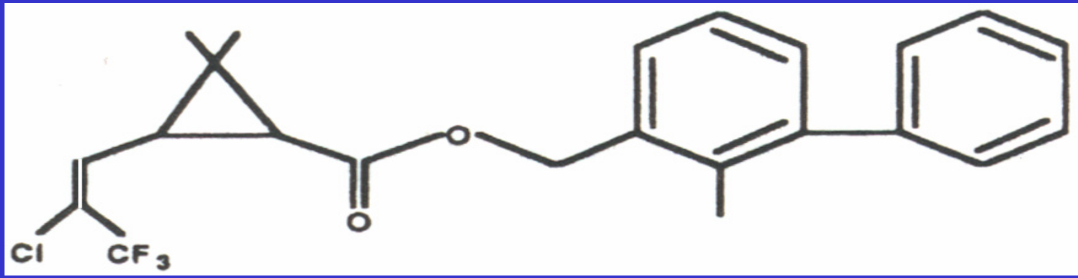
Cyhalothrin



Ester bonded, phenoxybenzyl alcohol, aliphatic acid

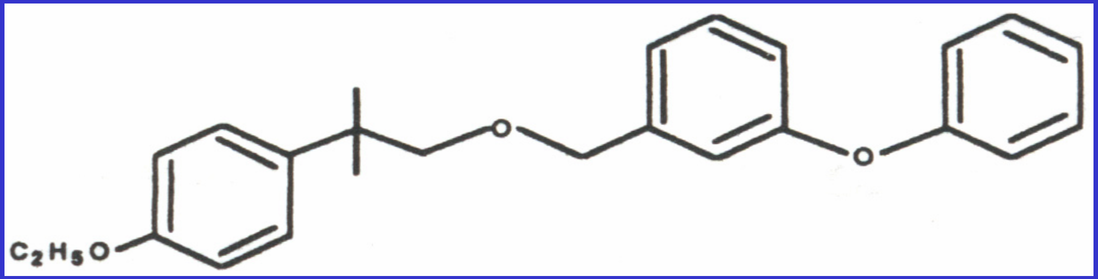
Ester bonded, methylated biphenul alcohol, aliphatic acid

Bifenthrin

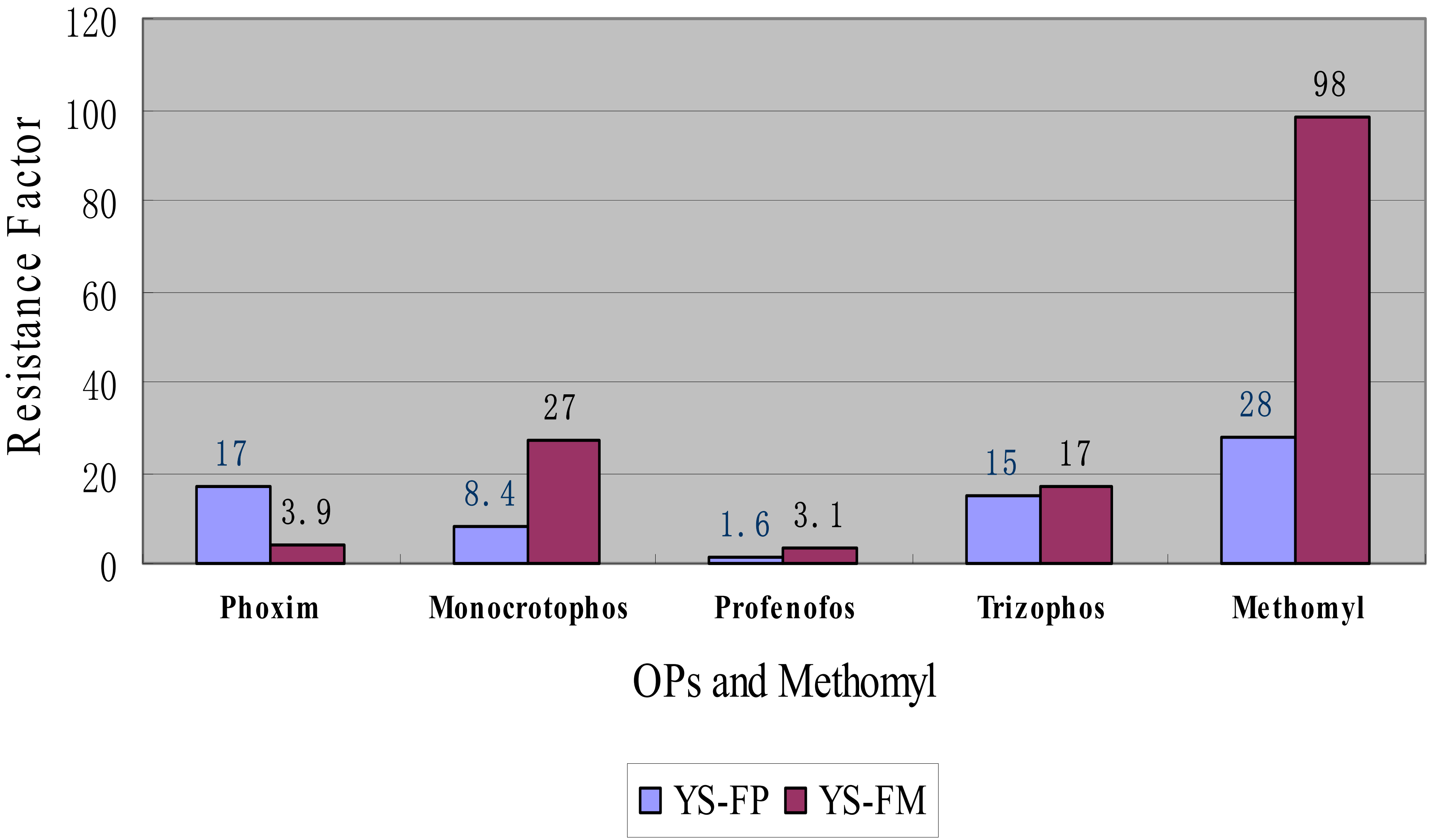


Non ester, phenoxybenzyl alcohols

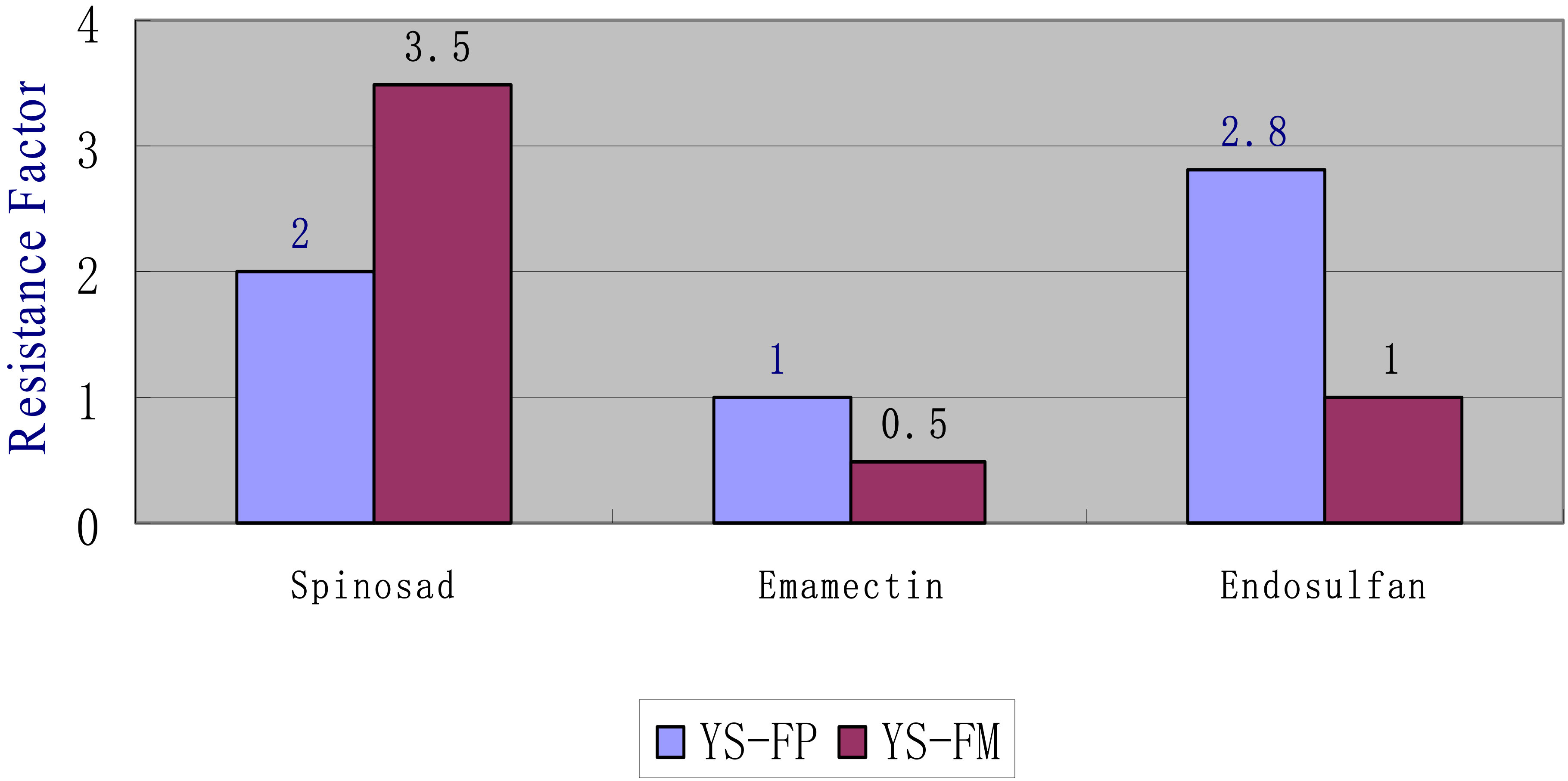
Etofenprox



Cross resistance to OPs and methomyl of the YS-FP and YS-FM strains compared with the YS strain of *H.armigera*



Cross resistance to other types of insecticide of the YS-FP and YS-FM compared with the YS strain of *H.armigera*

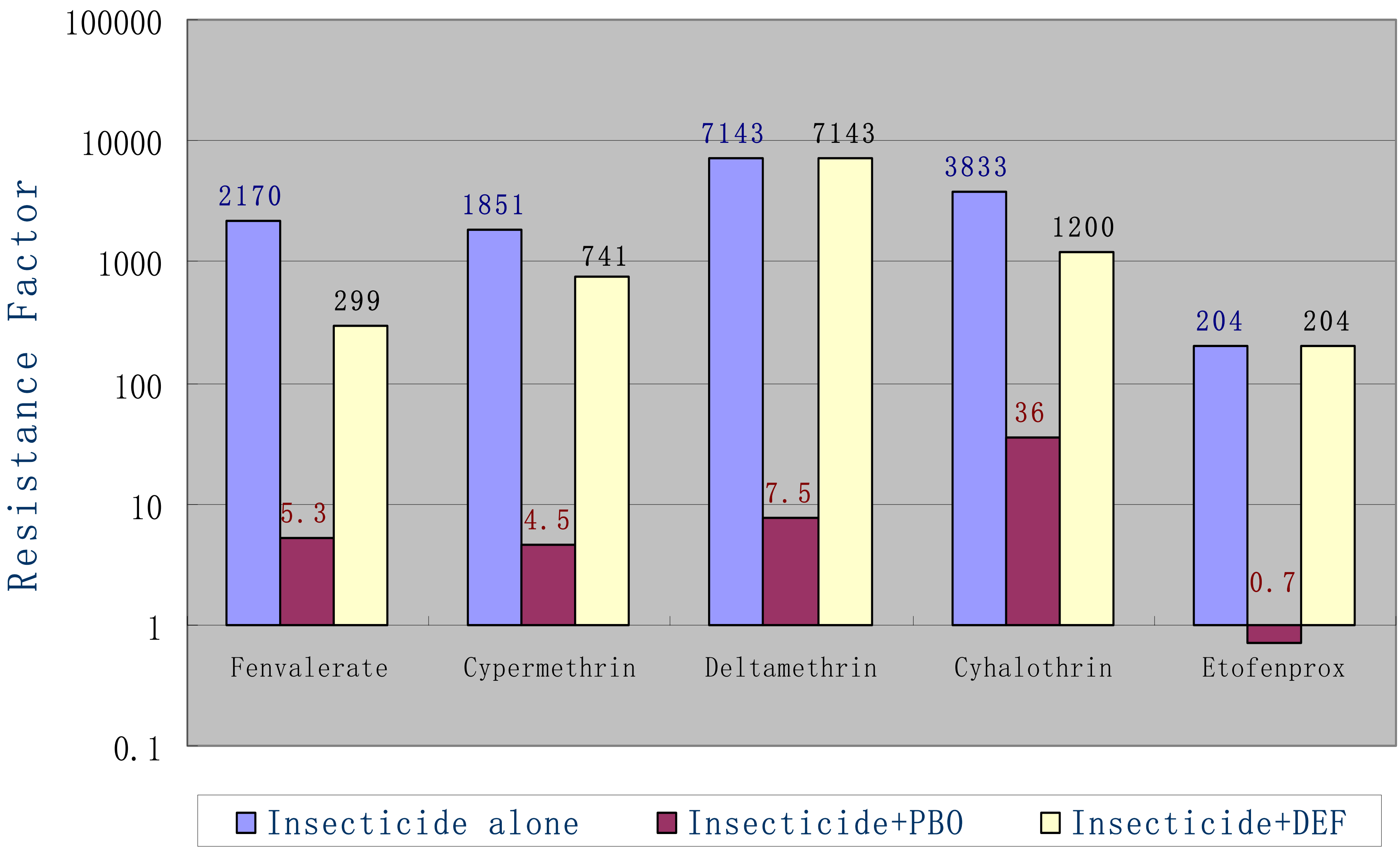


Synergism of PBO and DEF to different insecticides

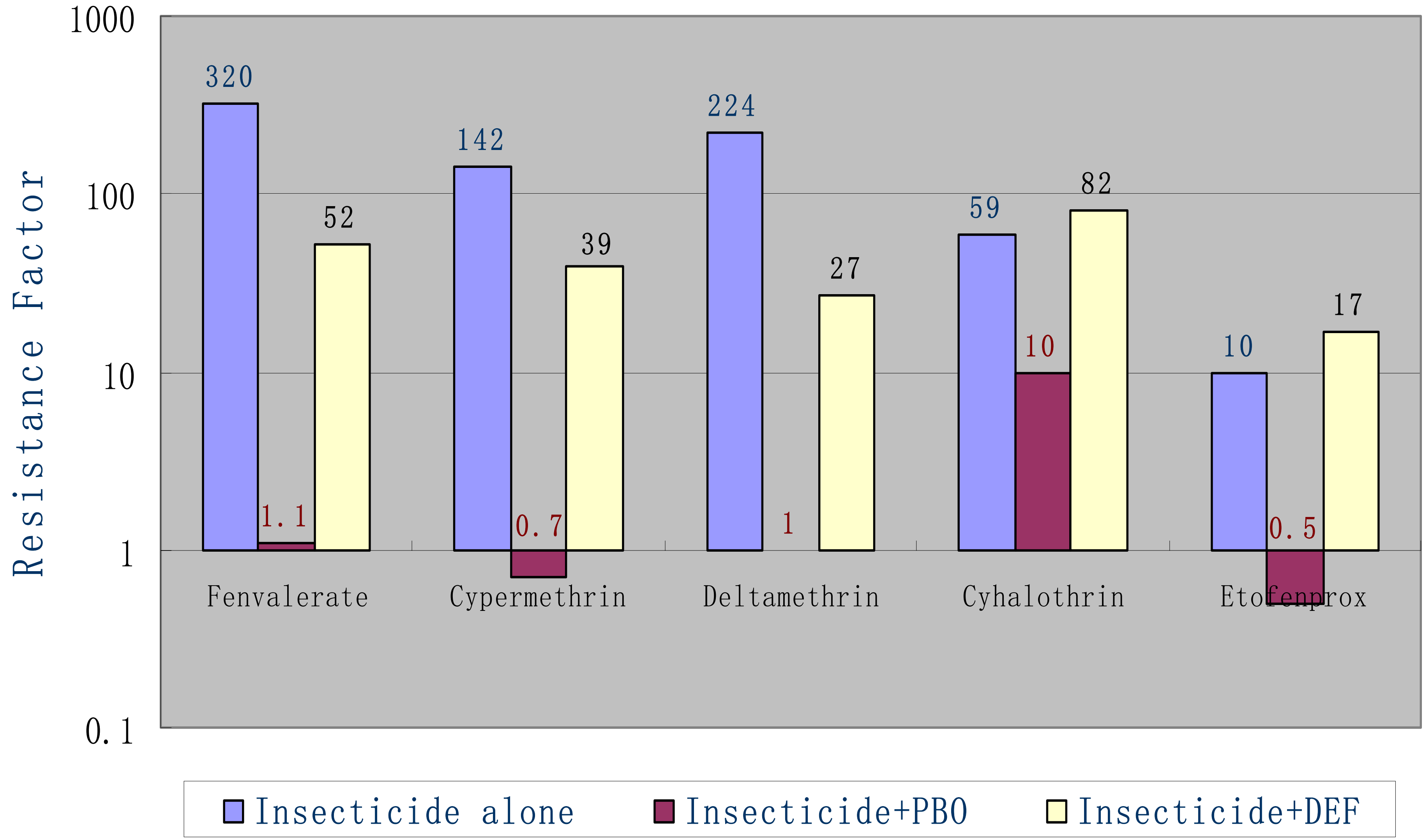
Piperonyl butoxide (PBO): Oxidase inhibitor

S,S,S-tributylphosphorothioate (DEF): Esterase inhibitor

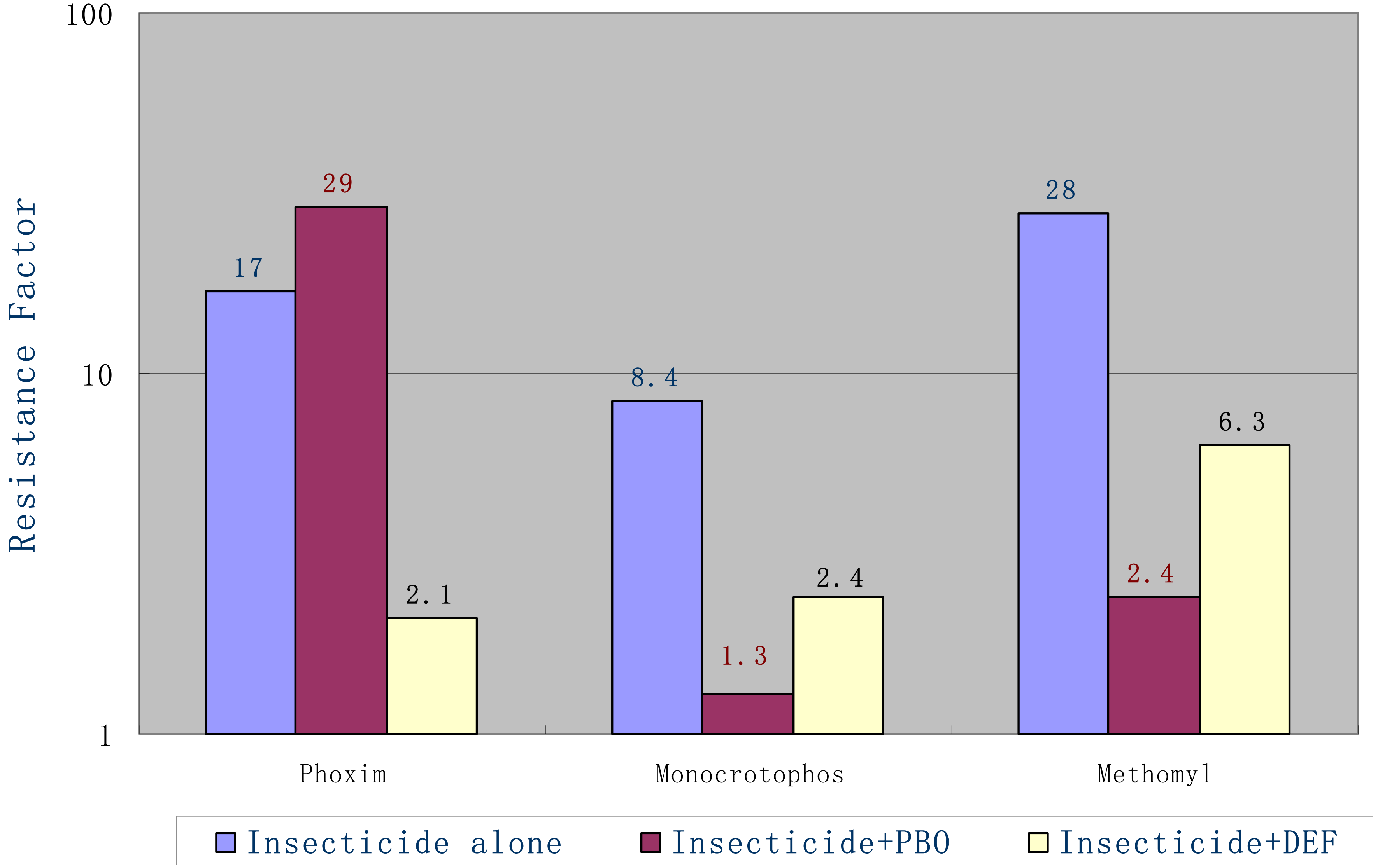
Synergism of PBO to pyrethroids in the YS-FP strain of *H.armigera*



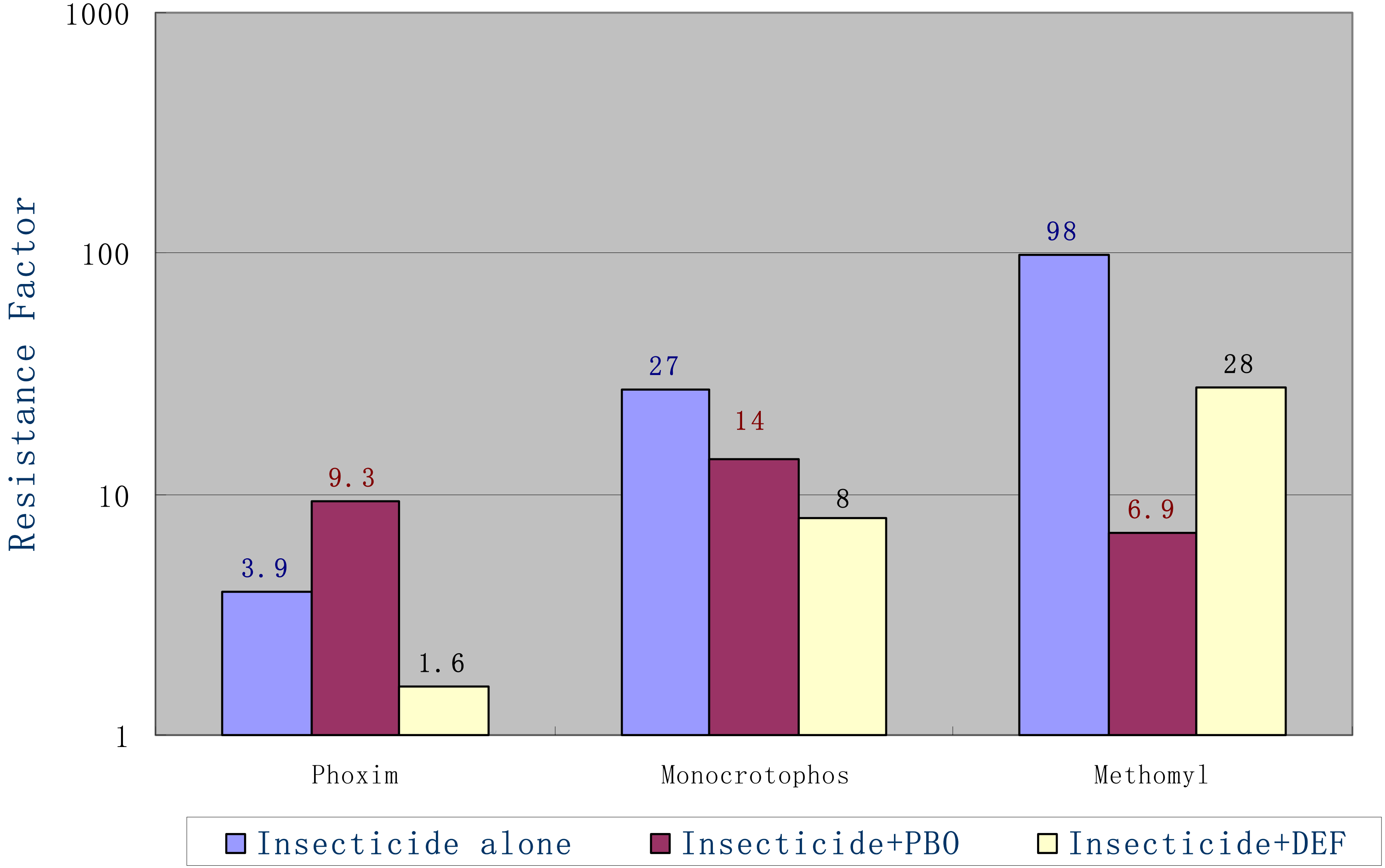
Synergism of PBO to pyrethroids in the YS-FM strain of *H.armigera*



Synergism of PBO to OPs and methomyl in the YS-FP strain of *H.armigera*



Synergism of PBO to OPs and methomyl in the YS-FM strain of *H.armigera*



Biochemical Mechanisms

Enzyme assays

Metabolic enzymes

(Midgut of last instar larvae)

Cytochrome monooxygenases (MFO):

PNOD: O-demethoxylation to *p*-nitroanisole

ECOD: O-deethoxylation to ethoxycoumarin

MCOD: O-demethoxylation to methoxycoumarin

Esterases (EST):

1-Naphthal acetate

Glutathione S-transferases (GST):

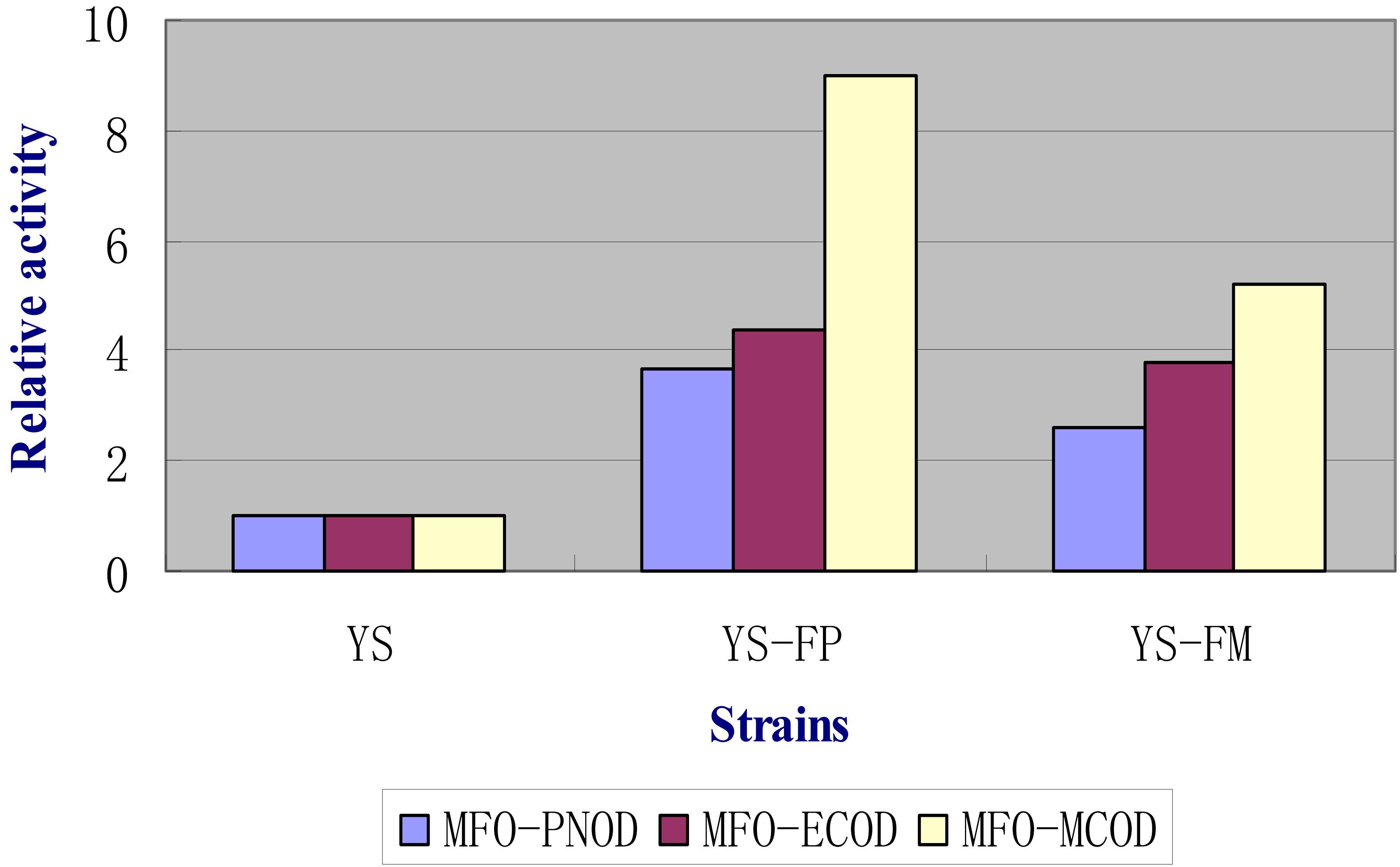
CDNB, DCNB

Target insensitivity

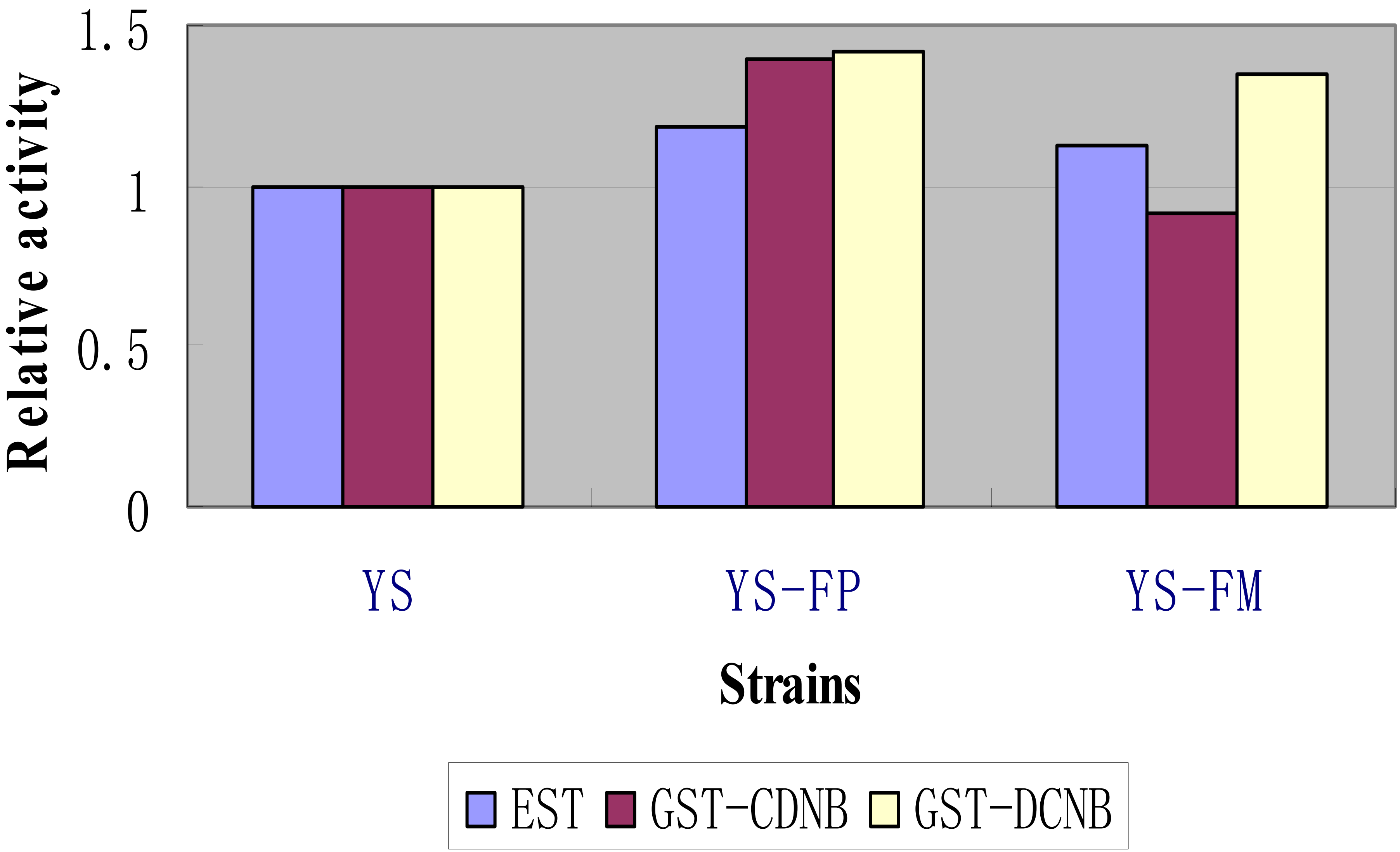
(3rd instar larvae)

AChE (the target site of OPs and methomyl)

Relative activities of cytochrome monooxygenases against different substrates in *H.armigera*



Relative activities of EST and GST in *H.armigera*



I_{50} of three insecticides to AChE from the YS, YS-FP and YS-FM strains of *H.armigera*

Strains	Monocrotophos		Phoxim-oxon		Methomyl	
	I_{50}(μmol/L)	Ratio	I_{50}(μmol/L)	Ratio	I_{50}(μmol/L)	Ratio
YS	93.5± 12.9 b	1	5.3±0.6 b	1	3.0±0.5 b	1
YS-FP	172.6±28.8 a	1.8	7.5±0.6 a	1.4	7.4±1.2 a	2.5
YS-FM	212.5±21.7 a	2.3	9.6±0.9 a	1.8	8.7±1.1 a	2.9

Results are shown as Mean±SE. Means in the column followed by the same letter are not significantly different (α =0.05, ANOVA).

Monocrotophos: $F=8.716$; df=2, 12; $P=0.0046$. Phoxim-oxon: $F=9.714$; df=2, 6; $P=0.0131$; Methomyl: $F=14.575$; df=2, 12; $P=0.0006$.

Conclusions

I. **Relatively slow development of resistance to a mixture does not mean slow development of resistance to each component in the mixture.**

II. **The binary mixtures of pyrethroid+organophosphate select intensely for metabolic mechanisms, especially oxidases in *H. armigera*.**

III. **The employment of mixtures in controlling *H. armigera* from Asia could result in the simultaneous development of multiple resistance mechanisms and significant cross resistance to other compounds.**