



WHOPES methods to test insecticide susceptibility of 4 *Aedes aegypti* field populations in Cambodia

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Context

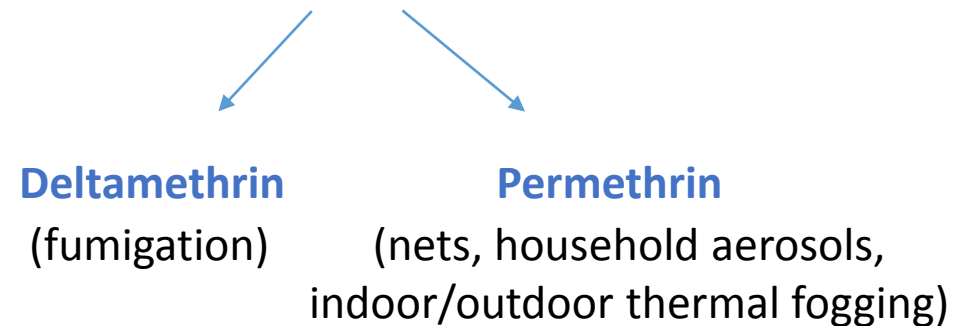
- Dengue is endemic in Cambodia
- No vaccine yet !
- The best control method = Vector control
- Actual control : larval and adult insecticides
- Question: methods are still effecient ?

Questions

Do resistance to insecticide exists in the field for the main dengue vector ?

Is there larval resistance to **temephos** in endemic areas ?

Is there adult resistance to the two main insecticides ?



Methods (1/5)

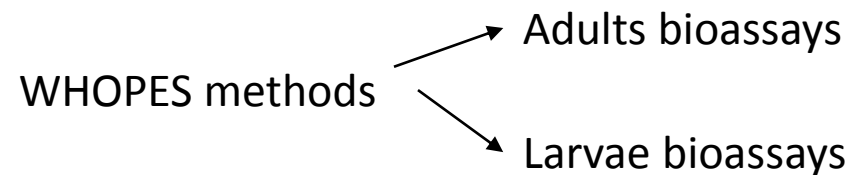
WHO methodology ^[1]

Sampling and larvae collections in the field

Rearing F1 generation for bioassays

World Health Organization (WHO) Pesticide (WHOPES)

Use of standardized WHOPES methods

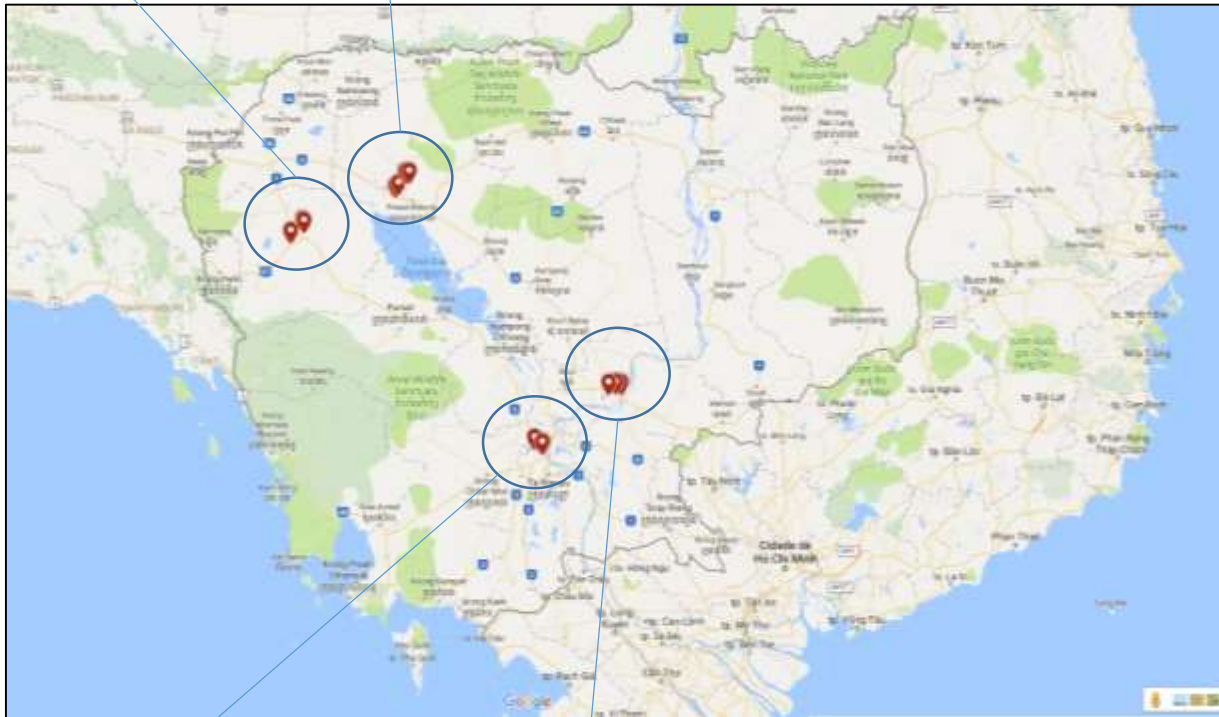


^[1] World Health Organization (WHO). 1981. Instruction for determining the susceptibility or resistance of mosquito larvae to insecticide. Geneva : World Health Organization

Methods (2/5)

Field collection of immature stages

Battambang Siem Reap



Kampong Cham

Phnom Penh



Methods (3/5)

Mosquito rearing



Larvae ready !



Adults ready !



Rearing F1 for bioassays tests



F1 generation eggs



Rearing for F1 generation

Methods (4/5)

Larval bioassays

WHO & WHOPES protocol standard

Tested insecticide : temephos (Abate ©)

- 25 larvae / cup
- 4 cups (=4 replicates)/ concentration
- 7 concentrations (including 0 for control)

} 700 3rd-instar larvae per population

Serial Temephos concentrations used (mg/L)

- 0
 - 0.004
 - 0.01
 - 0.02
 - 0.03
 - 0.05
 - 0.2
-



Methods (5/5)

Adult bioassays

WHO & WHOPES protocol standard

Tested insecticides : deltamethrin (0.03%) and permethrin (0.25%)

- 25 adults / tube
 - 4 tubes (=4 replicates)
 - One WHO discriminating dose per insecticide
- } 100 adult per insecticide



Methods (5/5)

Adult bioassays

WHO & WHOPES protocol standard

Tested insecticides : deltamethrin (0.03%) and permethrin (0.25%)

- 25 adults / tube
 - 4 tubes (=4 replicates)
 - One WHO discriminating dose per insecticide
- } 100 adult per insecticide + 100 adult for synergist



Data analysis

Larval bioassays

Determination of LD50 and LD90 with log probit analysis
(performed with R)

$$\text{RR ratio} = \frac{\text{LD50 field population}}{\text{LD50 sensitive strain}}$$

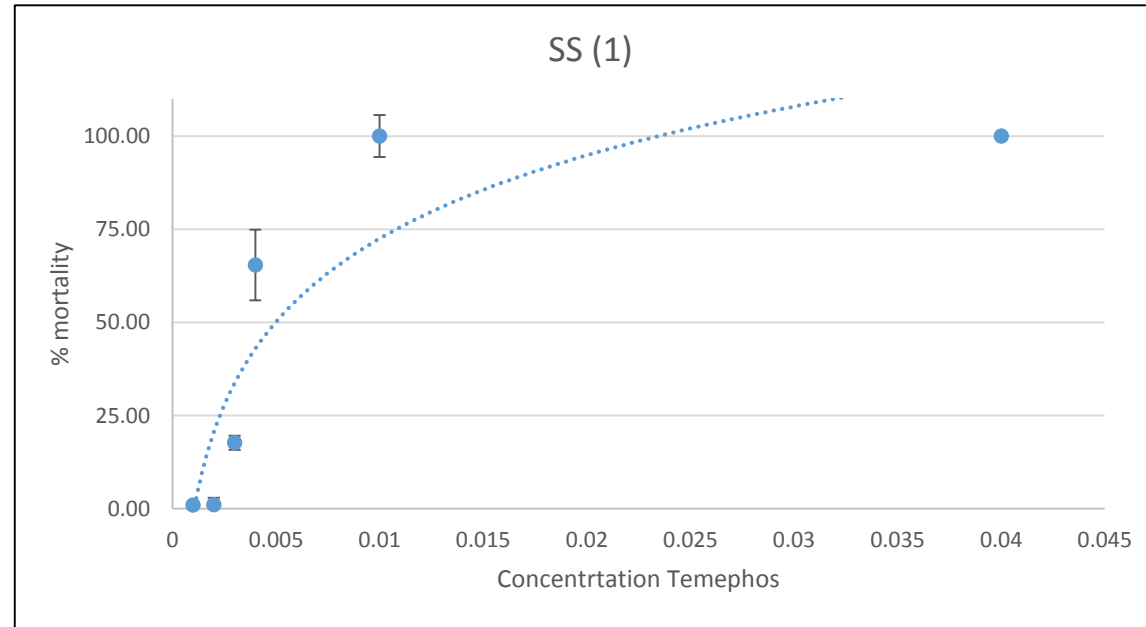
Adult bioassays

Mean comparison

Results

Larval bioassays

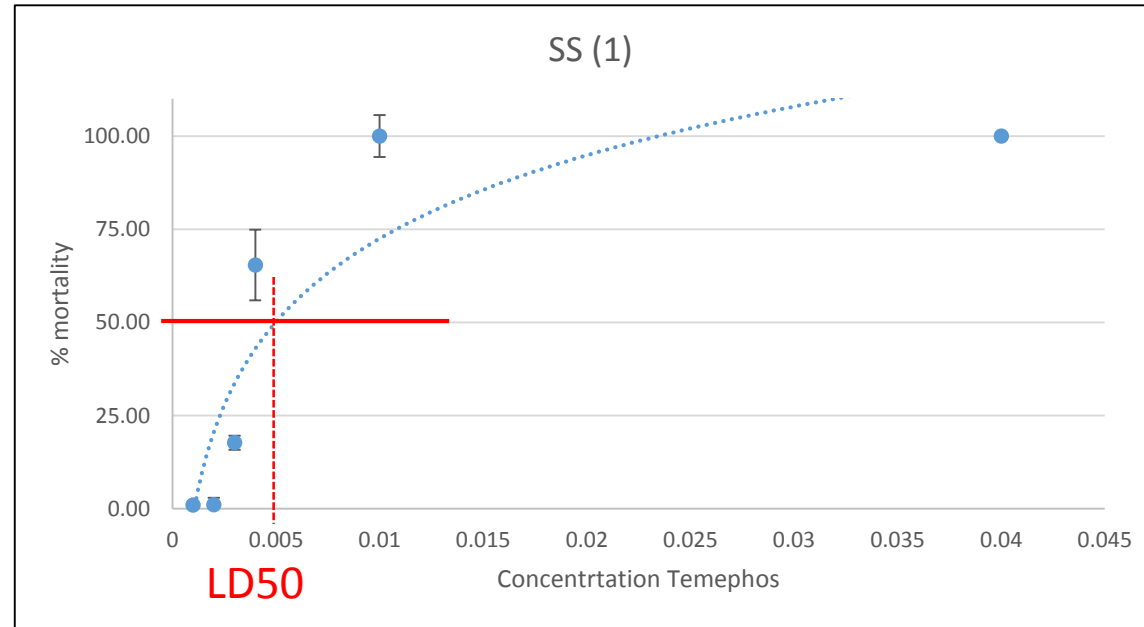
Sensitive strain (*Aedes aegypti* USDA strain)



Results

Larval bioassays

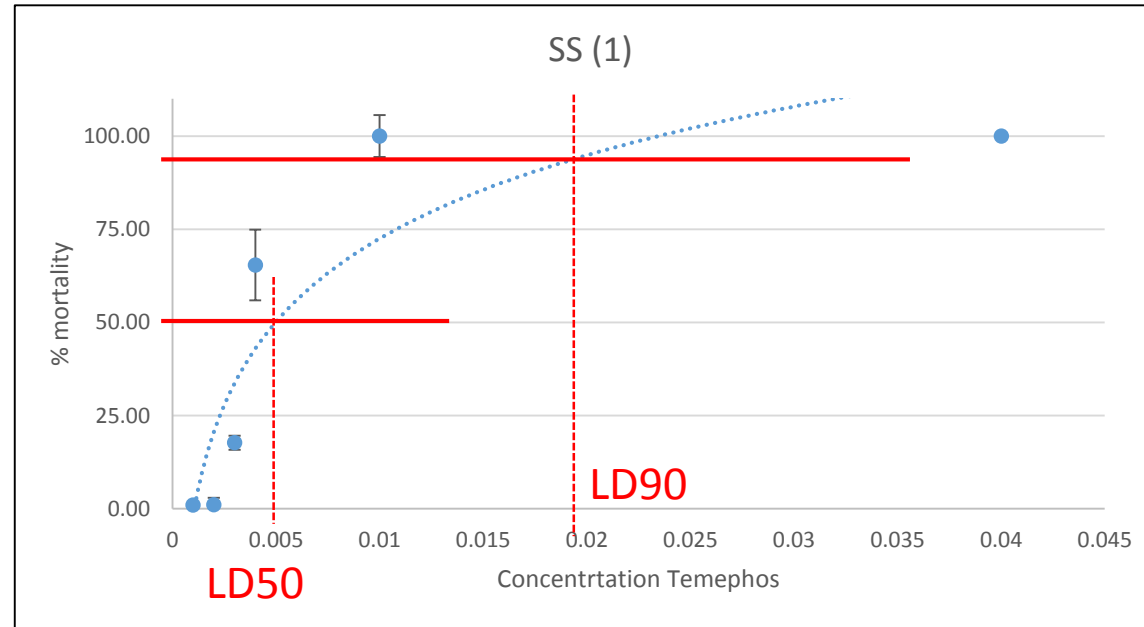
Sensitive strain (*Aedes aegypti* USDA strain)



Results

Larval bioassays

Sensitive strain (*Aedes aegypti* USDA strain)



Results

Larval susceptibility to Temephos



Populations	Urban		Rural	
	LD50	LD90	LD50	LD90
Phnom Penh	0.020	0.028	0.020	0.032
Siem Reap	0.014	0.020	*	
Kampong Cham	0.031	0.052	0.048	0.066
Battambang	0.125	0.221	0.041	0.064

*Insufficient F1 larvae to conduct testing

Results

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Battambang	0.125	0.221	0.041	0.064

*Insufficient F1 larvae to conduct testing

Sensitive strain : LD50 = 0.0037 mg/L & LD90= 0.0047 mg/L

Results

Larval susceptibility to Temephos

Resistance ratio

Populations	Urban	Rural
Phnom Penh	5.3	5.3
Siem Reap	3.8	-
Kampong Cham	8.4	13.0
Battambang	33.6	11.2

Values of resistance ratio (RR) greater than 5 is an indication of resistance and values less than or equal to 5 are considered as susceptible (WHO 2016)

Results

Larval susceptibility to Temephos

Resistance ratio

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Siem Reap	3.8	-
Kampong Cham	8.4	13.0
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Status resistance

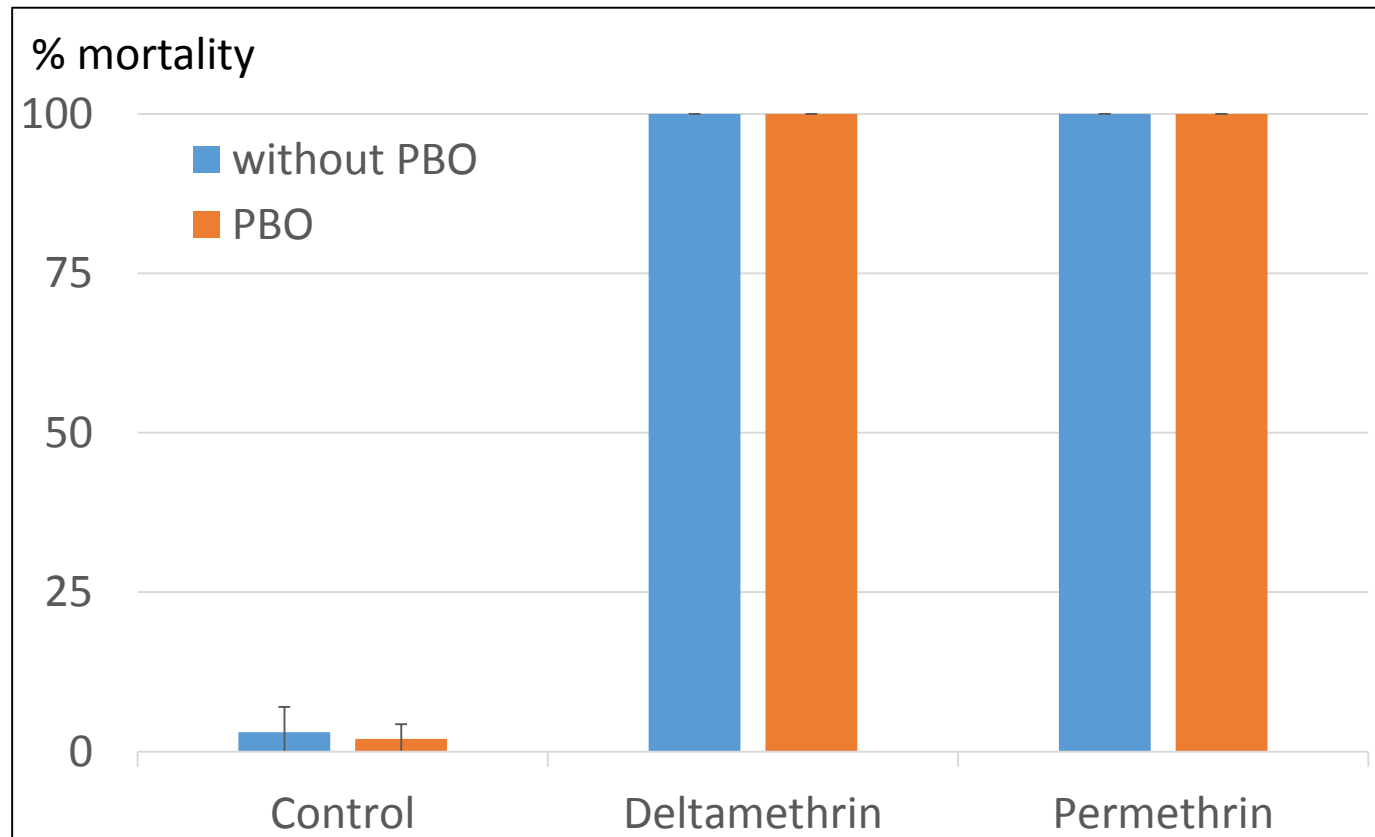
Populations	Urban	Rural
Phnom Penh	Resistant	Resistant
Siem Reap	Susceptible	-
Kampong Cham	Resistant	Resistant
Battambang	Resistant	Resistant

Values of resistance ratio (RR) greater than 5 is an indication of resistance and values less than or equal to 5 are considered as susceptible (WHO 2016)

Results

Adult susceptibility to two pyrethroids

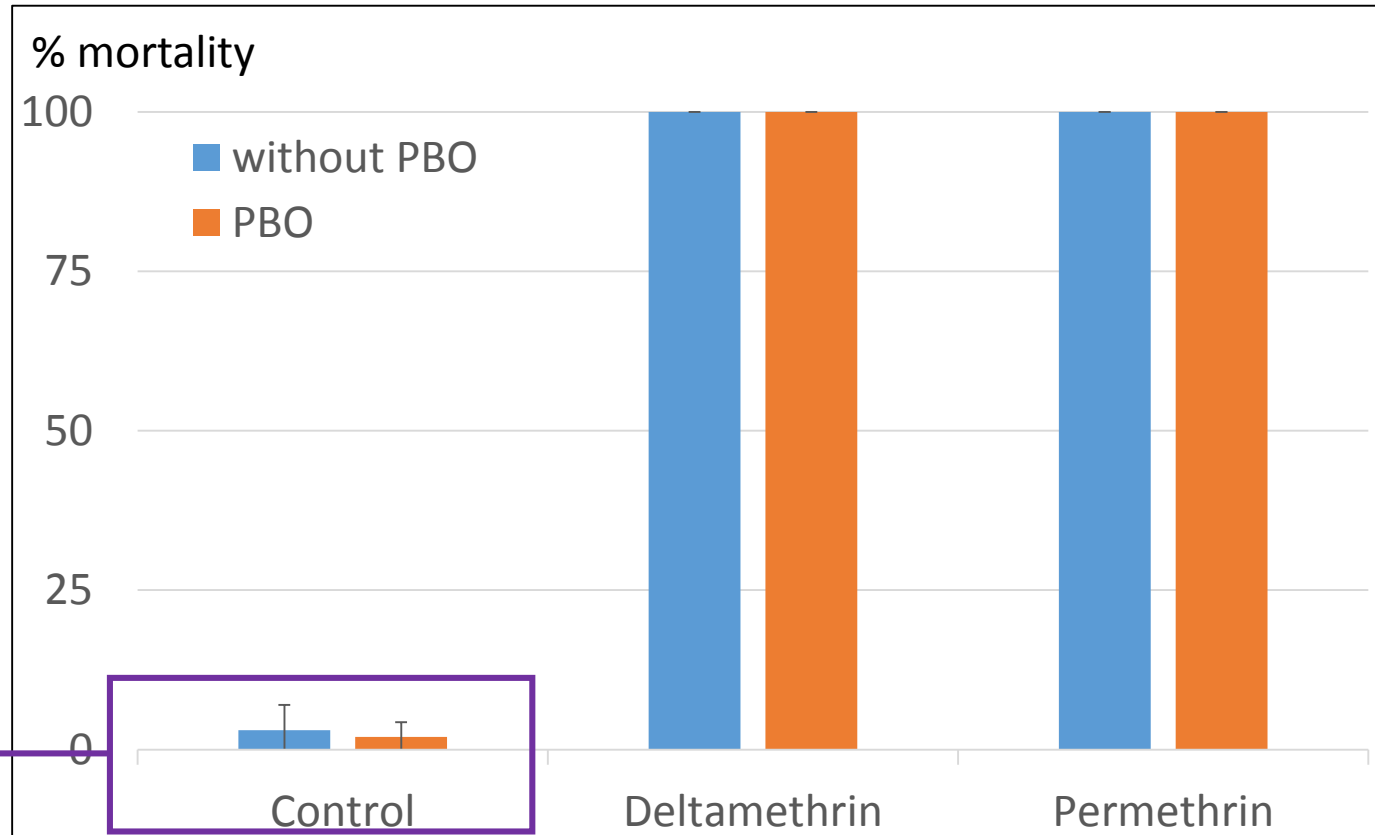
USDA strain



Results

Adult susceptibility to two pyrethroids

USDA strain

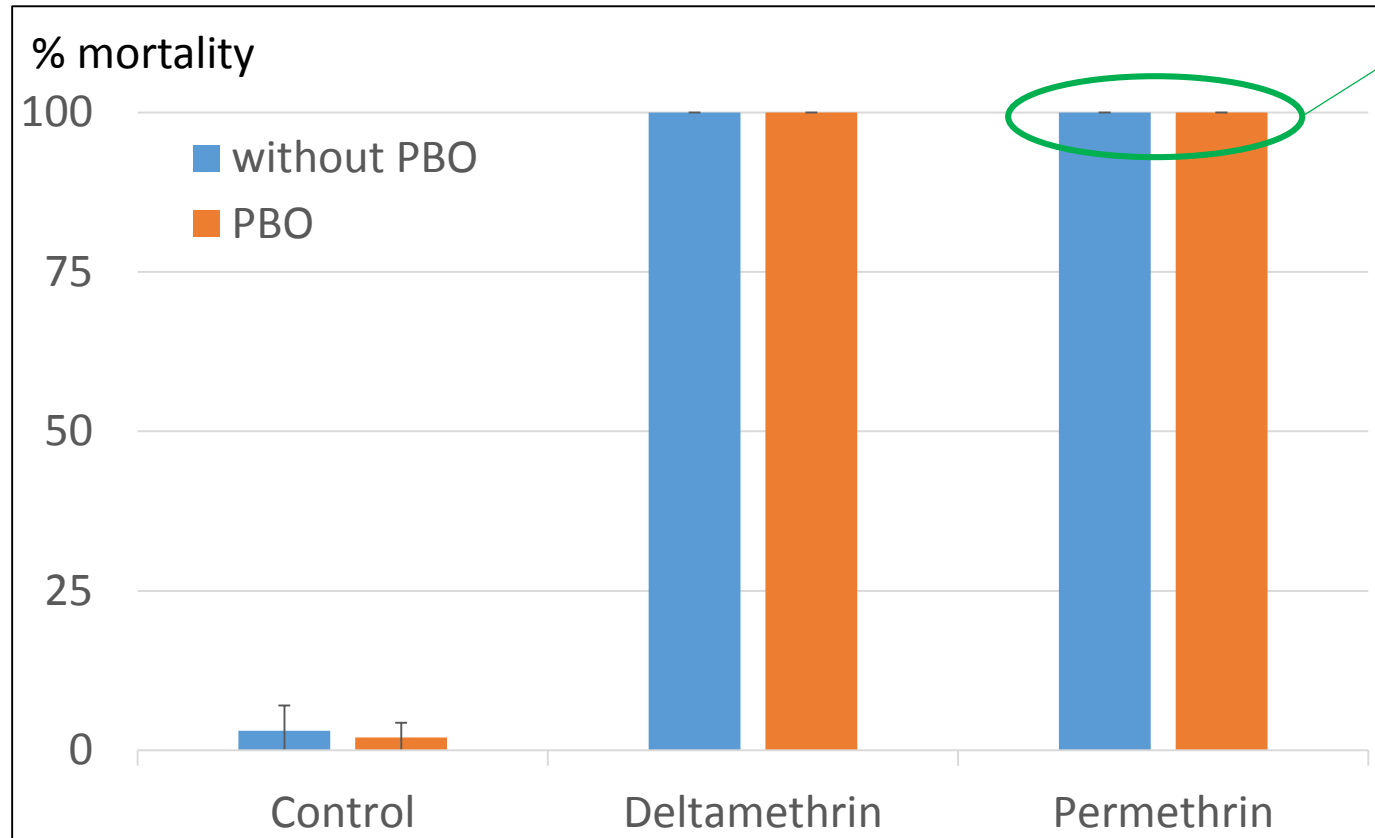


Negative control :
essential to control the
conditions of the test

Results

Adult susceptibility to two pyrethroids

USDA strain

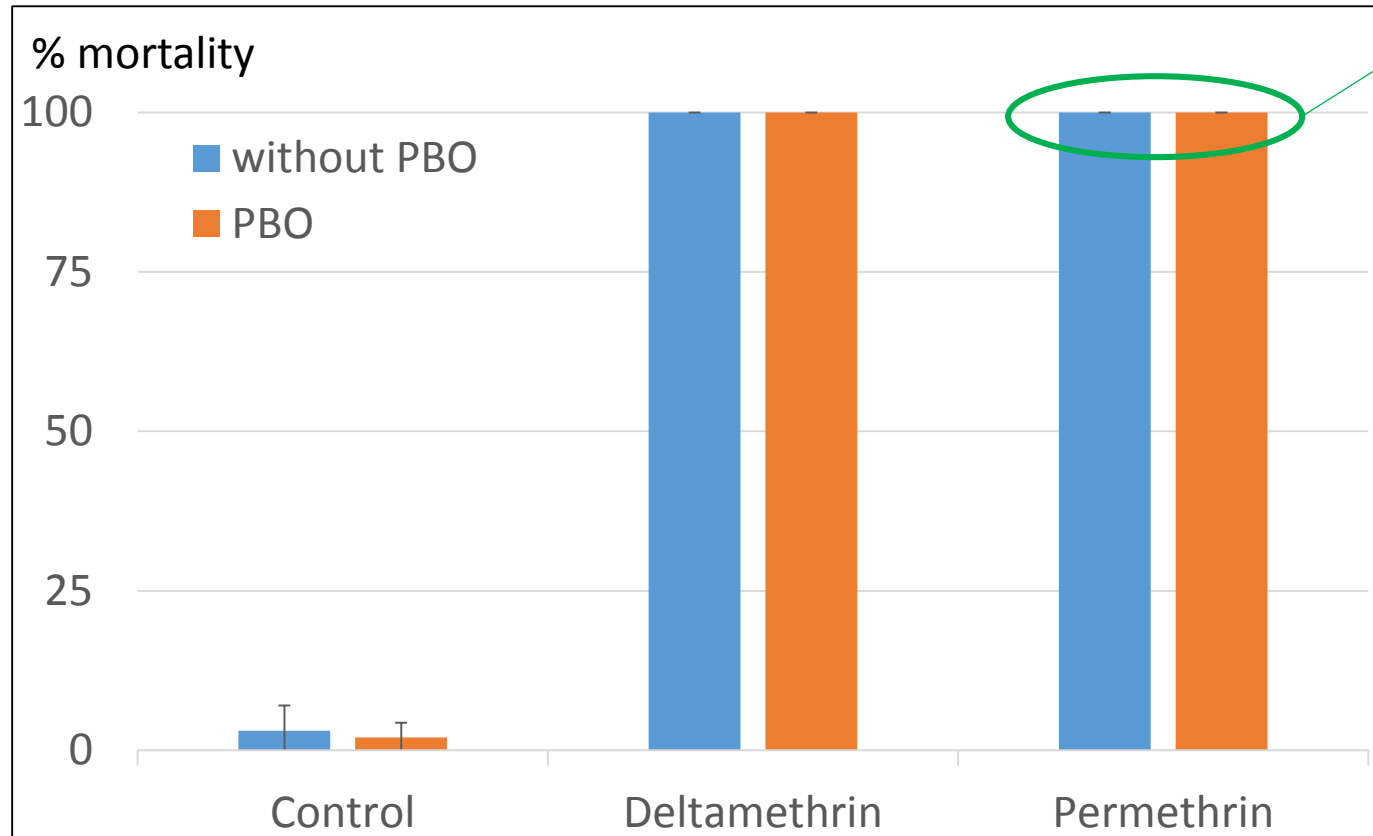


98-100% of mortality
= sensitive
population

Results

Adult susceptibility to two pyrethroids

USDA strain



98-100% of mortality = sensitive population

90-98% of mortality : apparition of resistance to monitore

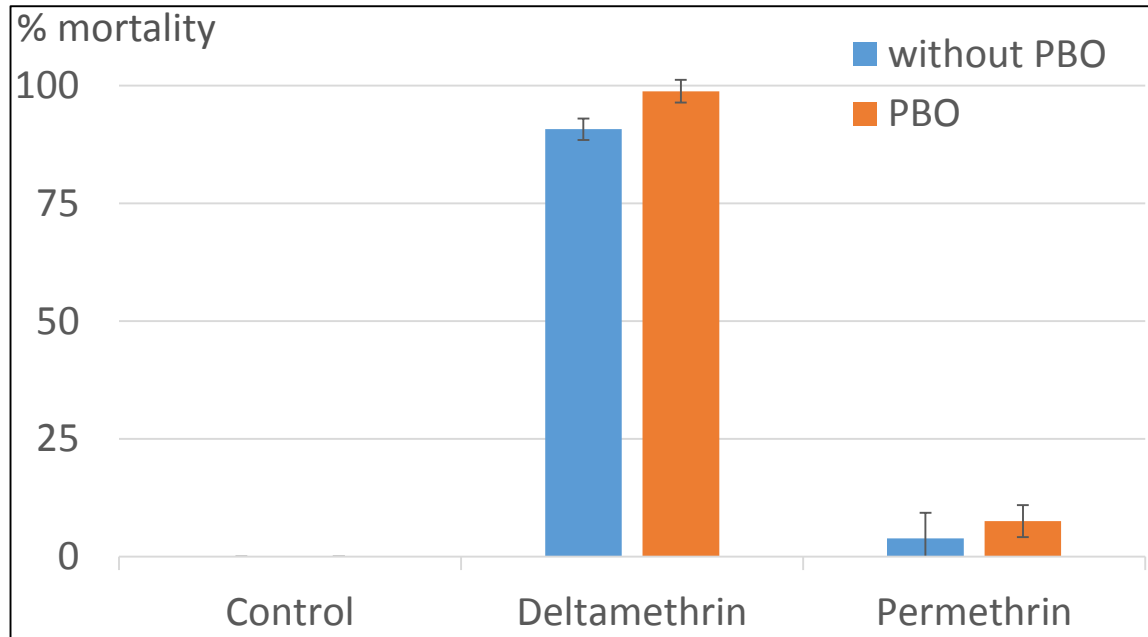
0-90% mortality : Resistant population

Results

Adult susceptibility to two pyrethroids by Province

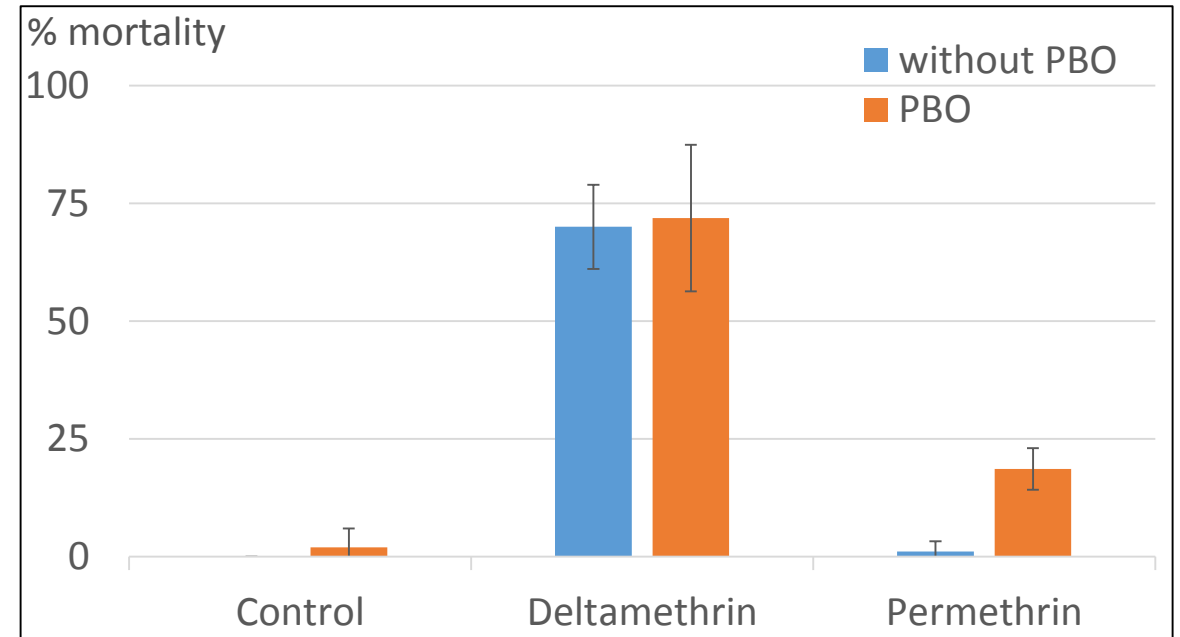
Kampong Cham

Kampong Cham urban population



Resistance to Permethrin
Resistance to Deltamethrin

Kampong Cham rural population



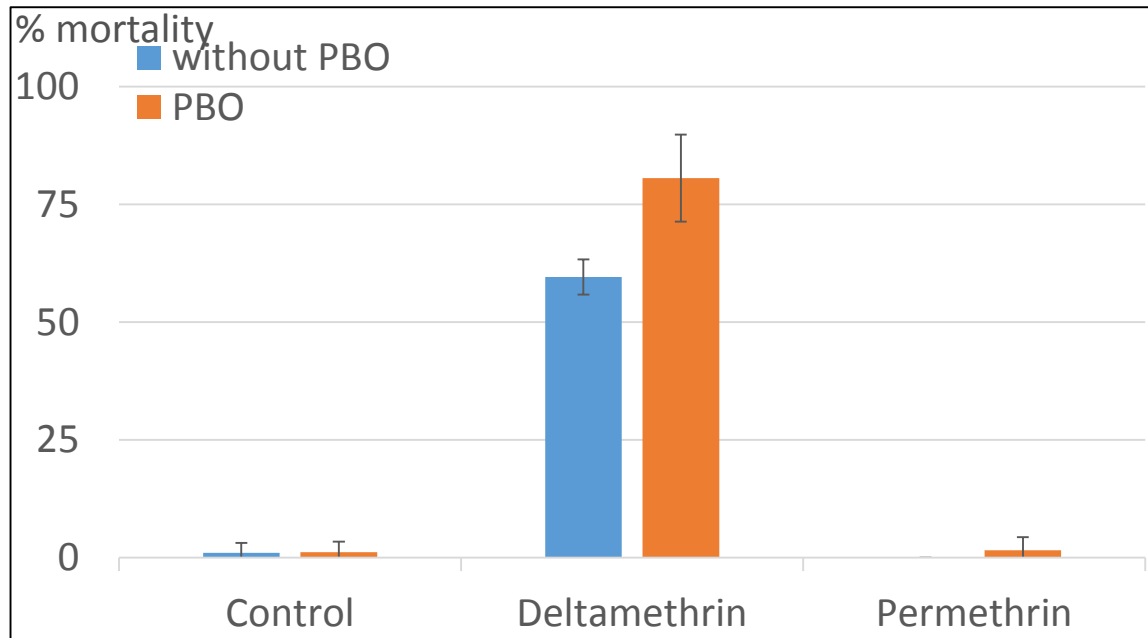
Resistance to Permethrin
Resistance to Deltamethrin

Results

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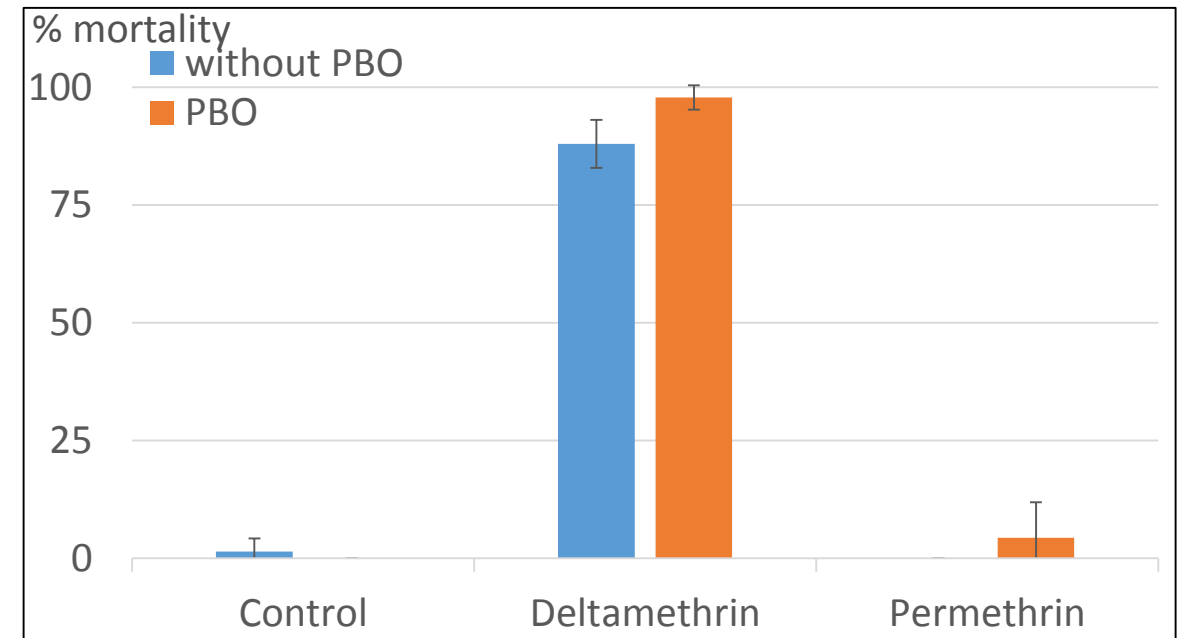
Battambang

Battambang urban population



Resistance to Permethrin
Resistance to Deltamethrin

Battambang rural population



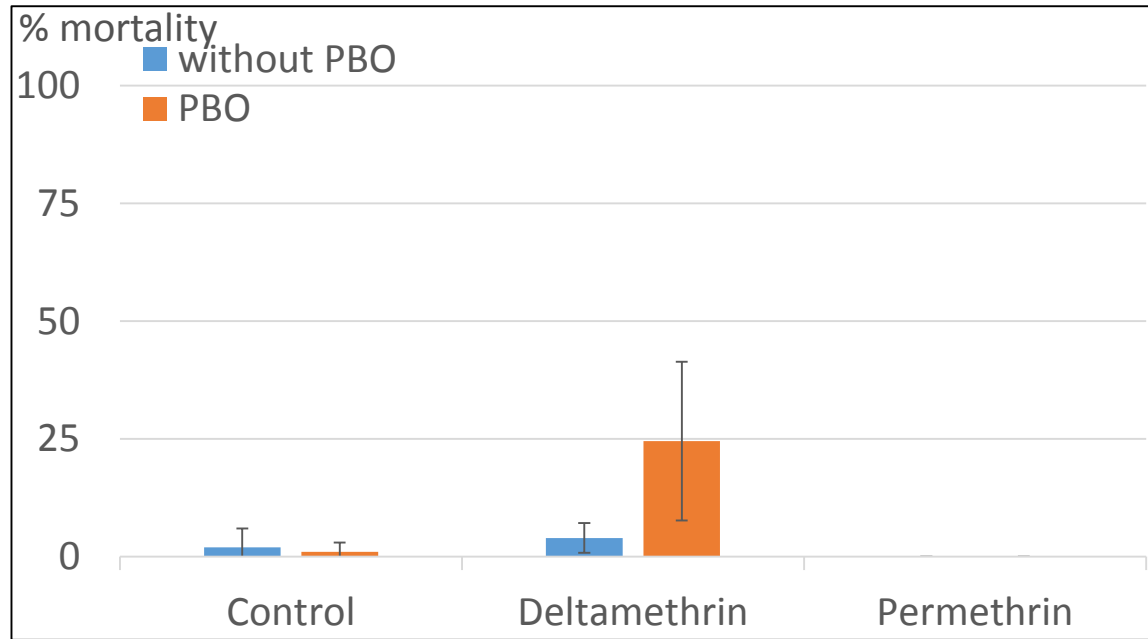
Resistance to Permethrin
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Results

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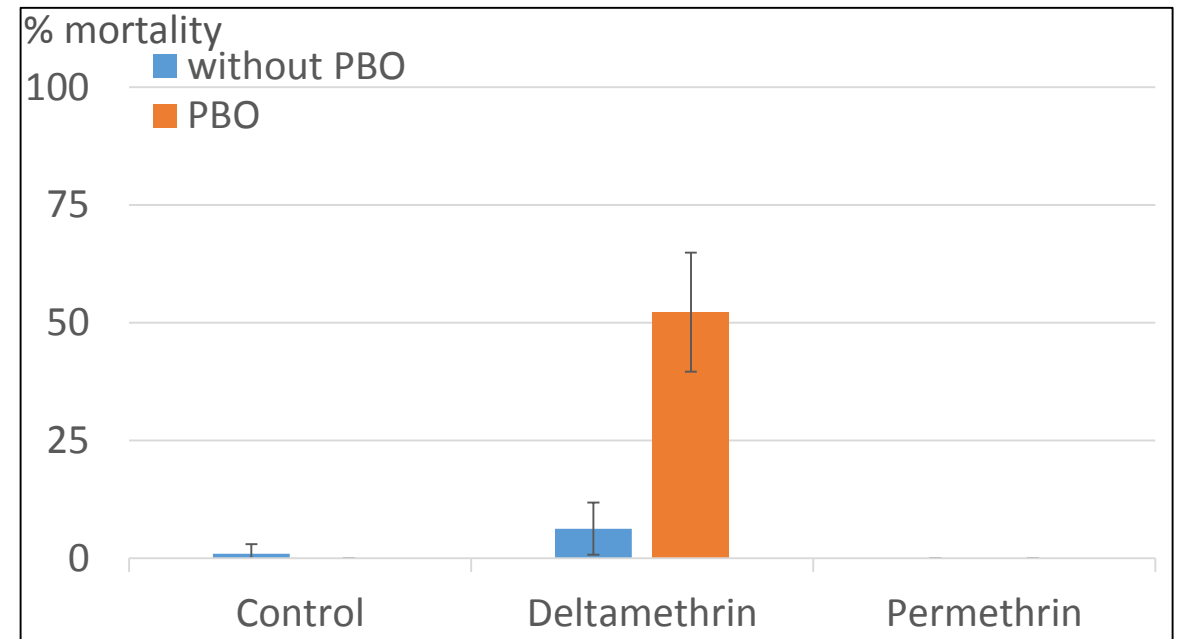
Siem Reap

Siem Reap urban population



Resistance to Permethrin
Resistance to Deltamethrin

Siem Reap rural population



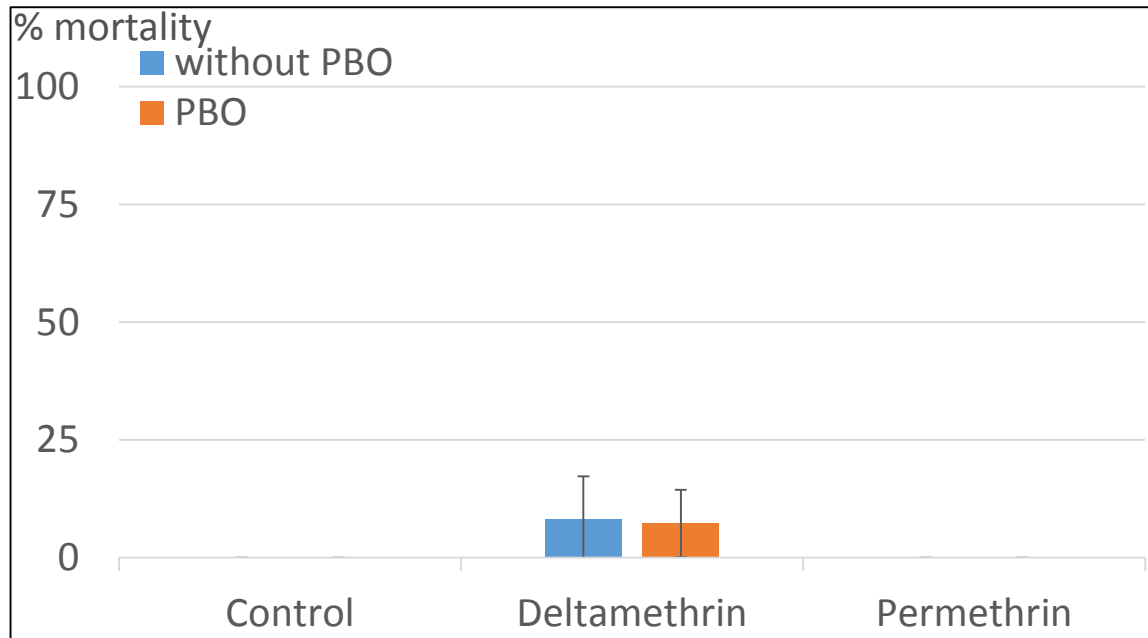
Resistance to Permethrin
Resistance to Deltamethrin

Results

Adult susceptibility to two pyrethroids by Province

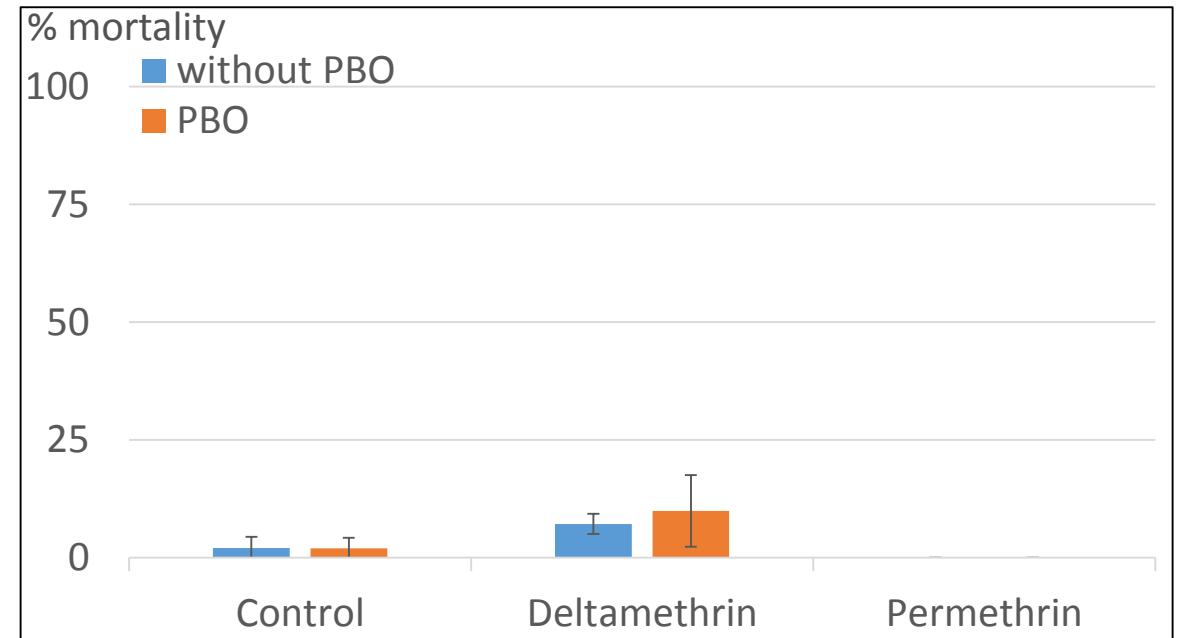
Phnom Penh

Phnom Penh urban population



Resistance to Permethrin
Resistance to Deltamethrin

Phnom Penh rural population



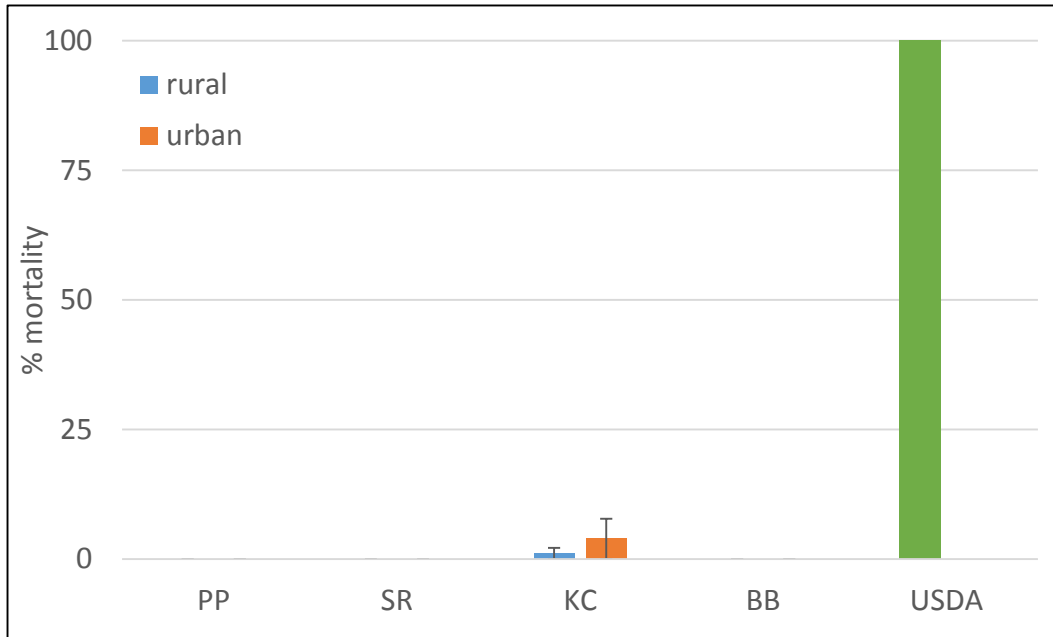
Resistance to Permethrin
Resistance to Deltamethrin

Results

Adult bioassays

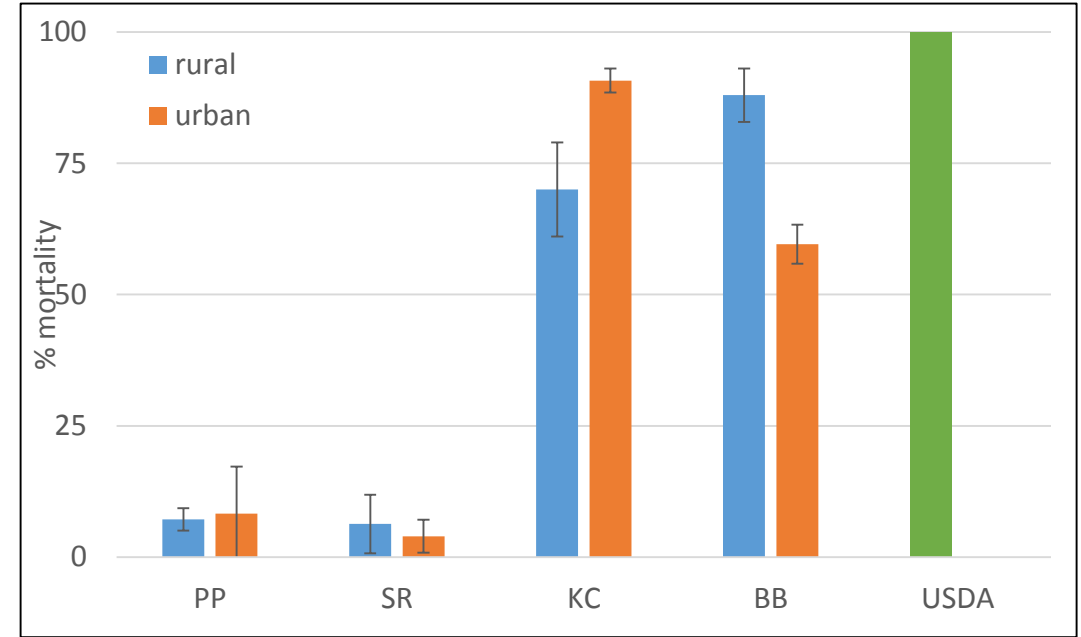
Synthesis

Permethrin



Strong resistance

Deltamethrin



Resistance

Main conclusions

Do resistance to insecticide exists in the field for the main dengue vector ?

Is there larval resistance to **temephos** in endemic areas ?

Is there adult resistance to the two main insecticides ?



Deltamethrin
(fumigation)

Permethrin
(nets)

Main conclusions

Do resistance to insecticide exists in the field for the main dengue vector ?

Is there larval resistance to **temephos** in endemic areas ? **YES**

Is there adult resistance to the two main insecticides ? **YES**

Deltamethrin
(fumigation)

Permethrin
(nets)

Perspectives after this work

Public Health

Need a change in vector control methods

Mapping insecticide resistance in Cambodia = same tests in all province

Testing resistance to other insecticides : *Bacillus thuringiensis* ser. *israelensis*, *Bacillus sphaericus*, spinosad, pyriproxifen, methoprene...

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