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THE SYNERGISTS ACTION OF PIPERONYL BUTOXIDE ON TOXICITY OF CERTAIN INSECTICIDES APPLIED AGAINST *HELOPELTIS THEIVORA* WATERHOUSE (*HETEROPTERA: MIRIDAE*) IN THE DOOARS TEA PLANTATIONS OF NORTH BENGAL, INDIA

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Abstract: Higher efficacy of mixture of insecticides with synergists piperonyl butoxide (PB) was reported in controlling in the Dooars population of *Helopeltis theivora* Waterhouse. Therefore, the use of synergists as one of the countermeasures against the insecticide resistance problem of *H. theivora* is recommended. The combination of deltamethrin +PB (piperonyl butoxide), quinalphos +PB and imidacloprid +PB showed 44.60, 16.01 and 11.14 folds increase of toxicity (synergistic ratio) than the respective insecticide alone. Piperonyl butoxide acted as an oxidase inhibitor. The addition of PB to some extent suppressed the resistance of *H. theivora* to these insecticides, suggesting that the P450 enzyme complex may be involved in the mechanism of toxicity.

Key words: Tea mosquito bug, piperonyl butoxide (PB), synergists, insecticide toxicity

INTRODUCTION

The tea mosquito bug, *Helopeltis theivora* Waterhouse is an important pest of the tea (*Camellia sinensis*) plantation causing substantial (10–50%) loss in crop. In northeast India out of total 436 thousand hectares, 80% of tea plantations have been suffering from *H. theivora* infestation. This insect pest has been exposed mainly to organochlorine and organophosphorus insecticides for many years, and lately to pyrethroid and neonicotinoid insecticides. Recently, *H. theivora* populations resistant to commonly used insecticides were found in some parts of India, such as Assam (Gurusubramanian and Bora 2007; Gurusubramanian *et al*. 2008) and Dooars (Roy *et al.* 2008a, 2008b). As one of the countermeasures against the insecticide resistance problem, the use of synergists was studied by Liu *et al.* (1982, 1984) and Ho *et al*. (1983). High efficacy of mixture of insecticides with synergists was reported for the control of several insect species which have developed resistance to insecticides, such as *Musca domestica* (Farnham 1973), *Culex pipiens fatigans* (Ranasinghe and Georghiou 1979), *Heliothis virescens* (Plapp 1979) and *Spodoptera littralis* (EI-Sebae *et al.* 1978; Riskallah *et al.* 1984).

The purpose of our study was to evaluate the toxicity of commonly used insecticides like endosulfan, deltamethrin, quinalphos and imidacloprid alone and in mixture with piperonyl butoxicide (synergists) to *H. theivora* in the field in the Dooars, northern part of Bengal.

MATERIALS AND METHODS

H. theivora adults or nymphs were collected from tea plantation estate of Kalchini subdistrict in the Dooars, North Bengal. Field collected insects were preconditioned for seven days in a laboratory (temperature of 27±2°C, 70–80% RH and a 16:10 LD photoperiod). A stock solution of technical grade piperonyl butoxide (PB; 90% w/v supplied by Aldrich Chemical Company, Inc.) was mixed with each tested insecticide namely endosulfan (Thiodon 35 EC, Hoechst), quinalphos (Ekalux 25 EC, Sandoz), deltamethrin (Decis 2.8 EC, Alkali) and imidacloprid (Confidor 17.5 SL Bayer India Ltd.) at the ratio of 1:5. Blends were tested against *H. theivora* using the leaf dip method recommended by FAO Method No. 10a (FAO 1980). Healthy shoots of tea were collected from the experimental garden. The leaves were washed thoroughly with distilled water and air-dried. Five tea shoots for each treatment were dipped up-to five seconds in the insecticide and synergist mixtures to ensure complete welting and stem part of the treated shoot was inserted in a glass tube containing water and wrapped with cotton. The treated tea shoots were kept under ceiling fans for 15 minutes to evaporate the emulsion. This arrangement was caged in a glass chimney. The mouth of which was covered with muslin cloth. Ten field-collected and preconditioned *H. theivora* were released separately into each glass chimney containing treated tea shoots. Observations of adult mortality were recorded in all the five replications of each

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treatment after 24 hours of the treatment. Moribund insects were counted as dead. Graded concentrations of insecticide and synergists mixtures were prepared in distilled water. Five to seven concentrations of each insecticide with synergist mixture were tested to obtain concentration – probit mortality curve. The mortality data were converted to percent of mortality and subjected to probit analysis to obtain LC_{50} values (Finney 1971). Synergistic ratio was calculated by the formula (Hsu *et al.* 2004):

$$\text{Synergistic ratio} = \frac{LC_{50}\text{ of insecticide alone}}{LC_{50}\text{ of insecticide plus synergist}}$$

RESULTS AND DISCUSSION

The bioassay tests against *H. theivora* with insecticides alone and insecticide with synergist mixtures showed the lowest LC_{50} value of 0.016 ppm for deltamethrin mixed with PB, while it was 0.731 ppm for deltamethrin alone, similarly for imidacloprid plus PB it was 1.787 ppm, and for imidacloprid alone 19.907 ppm, quinalphos plus PB was 13.397 ppm, and for quinalphos alone (214.47 ppm); endosulfan plus PB it was 354.667 ppm and for endosulfan alone it was 1580.7 ppm (Table 1).

The data on mortality-dosage response of *H. theivora* collected from Kalchini subdistrict, in the Dooars marked to have less susceptible population (Roy *et al.* 2008) revealed good fit of probit responses in all the bioassays showing significant chi-square values as such there was no heterogeneicity between observed and expected responses (Table 1).

It was further evident that addition of the oxidase inhibitor (PB) to deltamethrin, quinalphos, imidacloprid and endosulfan resulted in a remarkable synergism against *H. theivora* population in the Dooars (Kalchini), which significantly ($p < 0.01$) increased the toxicity of these insecticides when compared to the toxicity of insecticides alone against the concerned pest. The mixture of deltamethrin + PB, quinalphos + PB, imidacloprid + PB and endosulfan + PB proved 44.60, 16.01, 11.14 and 4.45 fold more toxic than the respective insecticide alone (synergistic ratio) (Table 1). The use of synergists to enhance insecticide toxicity (Abd-Elghafar *et al.* 1993) especially PB to inhabit the defense enzymes mixed function oxidase (Wilkinson 1976) are well established strategies to manage resistant insect pest.

Treatments using endosulfan, imidacloprid, deltamethrin, and quinalphos mixed with PB suppressed the resistance in the *H. theivora* populations indicating that P450 complex of MFO (monooxygenases) is a factor responsible for resistance to these insecticides.

Screening of literature suggested that mixed function oxidase (MFO) are a big family of enzymes and many P450s are involved in insecticide resistance (Lloyd 1973; Wilkinson 1976; Scott 1996; Yu and Terriere 1979) playing a significant role in degradation of pyrethroid insecticides (Yamamoto 1973) or organophosphorus insecticides (Attia and Frecker 1984) and neonicotinoids (Nauen *et al.* 1996; Mota-Sanchez *et al.* 2000).

Beside monooxygenases, qualitative and quantitative changes of esterases (Sarker and Mukhopadhyay 2003) and glutathione S-transferase and acetylcholinesterase (Sarker and Mukhopadhyay 2006) in insecticide-exposed *H. theivora* specimens could also be responsible for low sensitivity.

Further in-depth studies are needed with oxidative metabolism, synergists, and receptor binding sites to get more insights about the role of P450 and other resistance mechanisms functional in *H. theivora*.

Table 1. The synergistic effects of piperonyl butoxide (PB) on the relative toxicity of endosulfan, quinalphos, deltamethrin and imidacloprid against *H. theivora*

Treatment	Regression equation	Chi squire value [X^2]	LC_{50} Average	Fiducial limit [95%]	Synergism ratio SR
Endosulfan 35 EC	y = 4.428 x – 22.455	2.38	1580.77	1756.22 1422.48	–
Endosulfan + PB (1:5)	y = 2.5577 x – 9.1952	7.34	354.667	421.118 298.702	4.457
Quinalphos 25EC	y = 2.564 x – 8.671	7.10	214.47	254.542 180.708	–
Quinalphos + PB (1:5)	y = 3.429 x – 9.155	5.22	13.397	15.227 11.787	16.01
Deltamethrin 2.8 EC	y = 5.509 x – 10.781	3.65	0.731	0.818 0.683	–
Deltamethrin + PB (1:5)	y = 2.621 x + 1.816	1.17	0.016	0.020 0.013	44.60
Imidacloprid 17.5 SL	y = 3.641 x – 10.654	1.52	19.907	22.499 17.616	–
Imidacloprid + PB (1:5)	y = 1.756 x – 0.713	1.87	1.787	2.395 1.334	11.14

In none of the cases the data was found significantly heterogeneous at p = 0.05, y = mortality; x = dosage, LC_{50} = mediul lethal concentration

CONCLUSIONS

Piperonyl butoxide, a synergist, was blended with some commonly used insecticides (endosulfan, quinalphos, deltamethrin and imidacloprid) at a ratio of 1:5 and tested under laboratory conditions against tea mosquito bug adults, *H. theivora* in comparison with test insecticides alone in terms of concentration probit mortality to delay the resistance problem in North Bengal tea plantations, India. Synergist with the test insecticides increased the toxicity significantly ($p < 0.01$) to the tune of 4.45–44.60 fold than insecticide alone. Higher synergism of PB with all insecticides indicates the impending resistance to these insecticides in *H. theivora* and microsomal monooxygenases may play a role in the metabolism or detoxification of these insecticides. This suggests that PB may be effective in preventing or retarding the tea mosquito bug from developing resistance of these insecticides in North Bengal tea plantation.

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POLISH SUMMARY

WPŁYW SYNERGISTYCZNEGO DZIAŁANIA PIPENORYLO BUTOKSYDU NA SKUTECZNOŚĆ WYBRANYCH INSEKTYCYDÓW ZASTOSOWANYCH PRZECIW *HELOPELTIS THEIVORA* WATERHOUSE *(HEMIPTERA: MIRIDAE)* NA PLANTACJACH KRZEWÓW HERBACIANYCH W PROWINCJI DOOARS W PÓŁNOCNYM BENGALU W INDIACH

Przedstawione wyniki badań dotyczą wyższej skuteczności mieszaniny insektycydów z synergetykiem – piperonylo butoksyd (PB) w zwalczaniu populacji *Helopeltis theivora* Waterhouse w prowincji Dooars. W oparciu o wyniki badań zaleca się wykorzystywanie synergetyków jako środków ograniczających odporność *H. theivora* na insektycydy. Następujące mieszaniny: deltametryna + PB, kwinalfos + PB i imidachlopryd + PB wykazały odpowiednio 44,60, 16,01 oraz 11,14-krotny wzrost skuteczności (stopień synergizmu) w porównaniu do insektycydów zastosowanych samodzielnie. Piperonylo butoksyd działał jako inhibitor oksydazy. Dodatek synergetyku PB w pewnym stopniu ograniczał odporność *H. theivora* na testowane fungicydy, jednocześnie sugerując, że grupa enzymów P450 może warunkować mechanizm odporności.