

A Comparative Study on Excito-Repellency Effects of Permethrin, Deltamethrin and Etofenprox Treated Bed Nets against *Anopheles stephensi* Liston, 1901 (Diptera: Culicidae)

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Abstract

Background: Malaria is the most important vector-borne disease in many tropical countries all over the world. Because of the widespread use of pyrethroid insecticide treated mosquito nets in the world, the effects of excito-repellency (ER) phenomenon of pyrethroids against main malaria vector, *Anopheles stephensi* were studied.

Methods: The ER phenomenon of three concentrations of two synthetic pyrethroids (permethrin and deltamethrin) as well as etofenprox was evaluated against *Anopheles stephensi* under laboratory conditions. Female 5-7 day unfed mosquitoes were exposed to animal bait in holder and the animal's back and head were covered with impregnated bed net in -ER test chamber.

Results: Deltamethrin was more effective compared to other insecticides in killing the mosquitoes. The mean of entry to exit trap showed significant differences in all concentrations of insecticides ($P<0.05$).

Conclusion: This study showed that ER phenomenon of insecticides should be noticed in vector control programs. The ranked data indicated the relative potency of both pyrethroids and etofenprox. Deltamethrin repels the female mosquitoes more than other insecticides tested.

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Introduction

Today excito-repellency (ER) phenomenon of insecticides has been introduced as the factor responsible for behavior resistance. Given the inherent complications associated with residual spraying, pyrethroid impregnated bed nets are applied as a simple and cost effective substitute combating against malaria vectors.¹ Additionally, insecticide impregnated bed nets have the advantages of community involvement and the ability to be implemented in the primary health care system.² The excito-repellency profile of some insecticides has proved to be important in interrupting malaria transmission cycle.³ However, some investigators believe that this phenomenon has led

to behavioral changes in a group of endophilic species, leading to the occurrence of secondary exophily which has negative effects on control measures and may cause the mosquitoes to leave the sprayed houses before receiving the lethal dose.² In such circumstances, it is necessary to bring the mosquitoes in close and repeated contact with the surface impregnated bed nets. Also, the repellency profile observed in some insecticides prevents the mosquito to enter the sprayed area and in long term it may lead to changes in the proportion of endophilic and exophilic indexes.⁴ This study was undertaken to design ER chambers with bait compartment and a metal netting holder for guinea pig in order to facilitate the contact between host and vector and an exit trap for entering of

the exited mosquitoes exposed to insecticides. Contact irritability is defined as the irritated exposed mosquitoes and non-contact irritability is the mosquitoes without contact with insecticide induced irritability which can be assessed by ER Chamber.^{2, 11} The main objective of this investigation was to explore the ER phenomenon of permethrin, deltamethrin and etofenprox impregnated nets under laboratory condition against the main malaria vector, *An. stephensi*, using the baited ER chambers equipped to exit trap.

Materials and Methods

The cubic shape excito-repellency test chambers measuring 30 cm in length, width and height and made of aluminum were used as the exposure chamber of mosquito to insecticide impregnated nets. At the center of one side of the cubic chamber, a frame measuring 10×10 cm was devised and an exit trap measuring 10×10×20 cm in length, width and height with adjustable holes was made. The whole surface of ER chambers was covered with polyester net with 25 meshes in square inch. The insecticide impregnated nets in the treated group and untreated nets in the control group were placed on a metal netting holder containing the restrained guinea pig and twenty five 5-7 day old unfed female *An. stephensi* were released to feed on the pigs. The reaction of *An. stephensi* (Liston 1901) against the excito-repellency effects of three concentrations of etofenprox EC10% (50,100 and 200mg a.i./m²), permethrin EC50% (100, 200 and 300 mg a.i./m²) and deltamethrin SC 2.5% (12.5, 25 and 50 mg a.i./m²) was assessed using four indicators including blood feeding rate, entry index, recovery rate and survival rate. Each test was repeated at least six times. The raw data of indicators were transformed to arcsine using the formula ($Y=Arcsin\sqrt{p}$) on SPSS, ver. 16. Following the one-way analysis of variance (ANOVA), the mean of one group was compared with that of the other using Fisher's Least Significant Difference (LSD).

Results

The blood feeding rate of *An. stephensi* indicated that despite the complete physiological readiness of the

released mosquitoes as well as the easy access to the restrained animal bait (guinea pig), there was a significant difference between the treated and untreated chambers ($P<0.05$). The deterrent effects of the insecticides (those factors preventing mosquitoes from entering) showed that the exposed mosquitoes were neither able to sit on the impregnated nets nor able to have blood feeding on the host. They showed excited behavior (entry to exit trap) and rest at the farthest distance away from the nets. Interestingly, as the doses of insecticides reduced, the trend of mosquitoes increased to long rest on nets and at higher doses the mosquitoes tended to leave the exposure chamber through exit traps. A comparison between the effects of blood feeding rate of three insecticides used in this study showed that the mosquitoes feed at the maximum rate on the net impregnated with permethrin while it was minimum for deltamethrin (Figure 1). Generally, the average of blood feeding rate for high concentrations was less. The mean of entry index in exit trap showed the least entry index for the nets impregnated with deltamethrin, and the highest index with etofenprox (Figure 2). This observation further confirms the fast knockdown effect of the applied insecticides. The average of entry into exit trap revealed significant differences in all concentrations of the insecticides ($P<0.05$). The ratio of knockdown mosquitoes which survived after 24 hours and the recovery rate were assessed. The high value of entry index for each insecticide was used as a negative score in its evaluation which led to receiving a sub-lethal dose of mosquitoes which did not kill them and helped them survive more.

In this study, mosquitoes exposed to etofenprox showed the highest recovery rate and those exposed to deltamethrin showed the least value (Figure 3). The survival rate reflects a population of mosquitoes which have remained away from any contact with insecticide impregnated nets and have rested in a corner of chamber or have flown toward the exit trap.

The results showed the least survival rate of mosquitoes in deltamethrin, and the highest rate in etofenprox (Table 1). This index had not varied with three concentrations of etofenprox and no significant differences were indicated. In contrast, there was

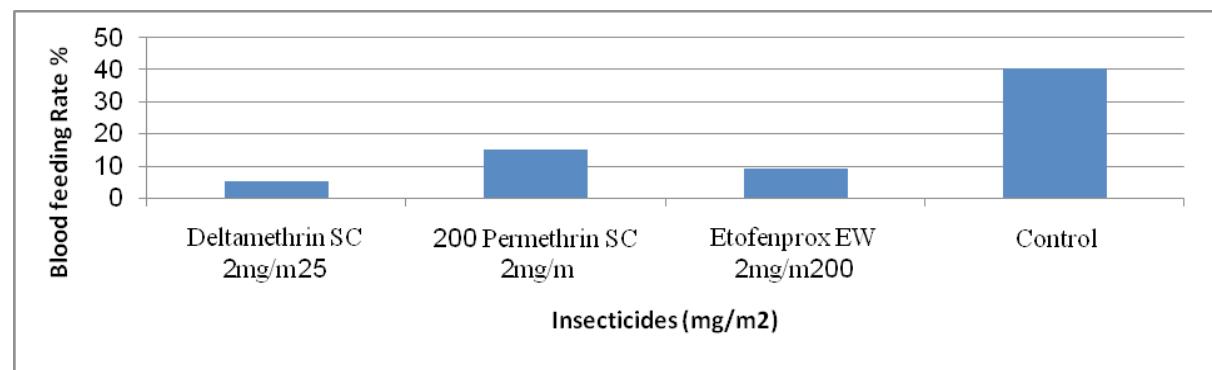


Figure 1: Blood feeding rate of *An. stephensi* on guinea pigs inside the holder covered with nets impregnated with two types of pyrethroids and etofenprox

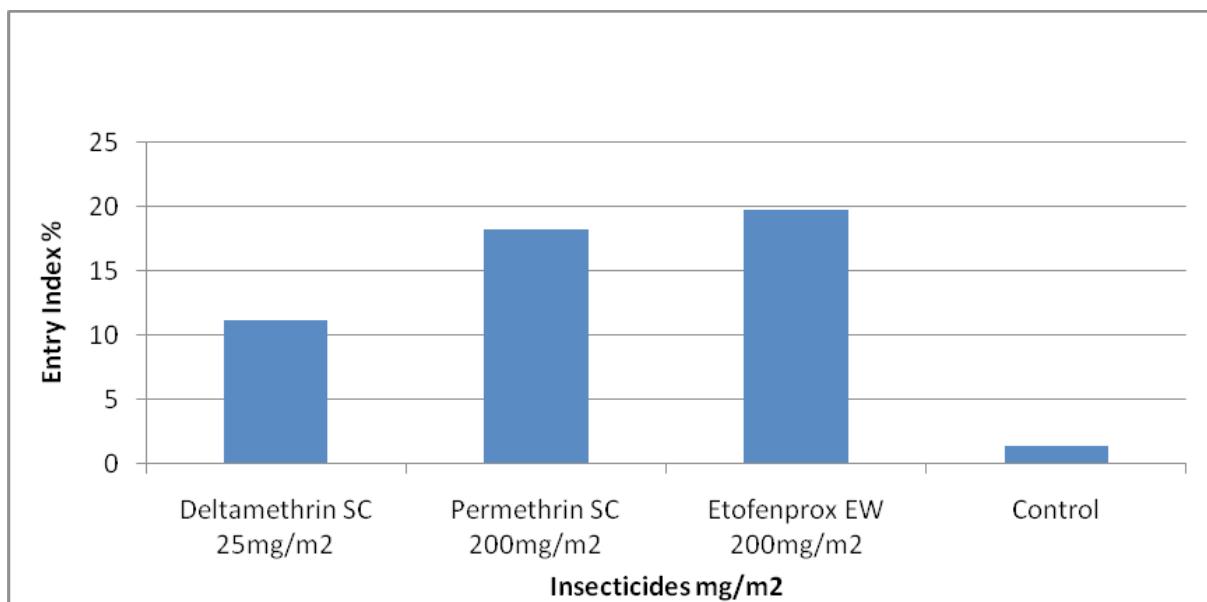


Figure 2: Entry index of *An. stephensi* in exit-trap of ER test chambers exposed to two types of pyrethroids and Etofenprox impregnated nets

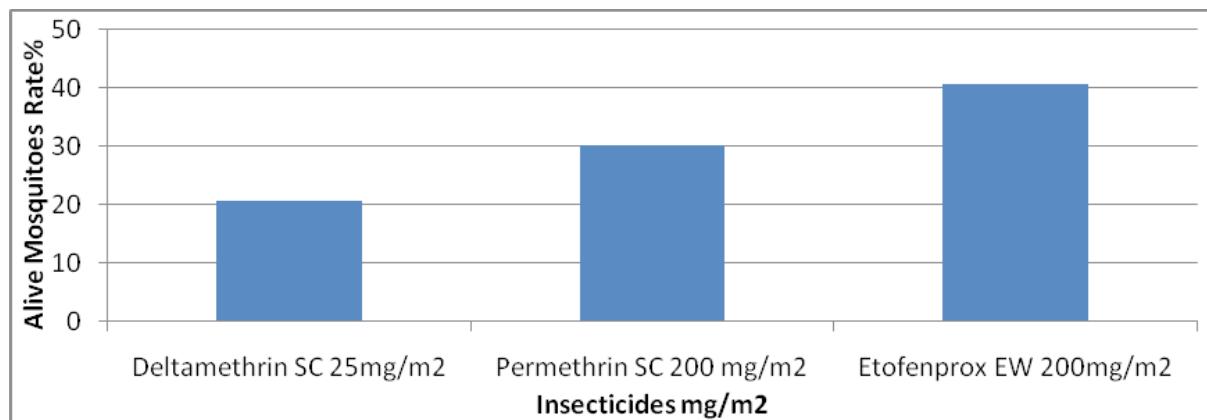


Figure 3: Recovery rate of knock down *An. stephensi* exposed 60 min to nets impregnated with two types of pyrethroids and Etofenprox in ER test chambers

Table 1: Comparison of the survival rates of *An. stephensi* exposed to the nets impregnated with three insecticides in ER test Chambers.

Insecticides	Total Mosquito tested	Survival rate%±SE
Etofenprox (200 mg/m ²)	761	76/7±1/7
Deltamethrin (25 mg/m ²)	450	30/1±2
Permethrin (200 mg/m ²)	700	60/4±1/8
Control	715	98/7±0/42

a significant difference between deltamethrin and permethrin for 12.5 and 50 mg per square meter ($P<0.05$). There was no significant difference in other concentrations of insecticides. The efficacy of mortality rate of the etofenprox, permethrin and deltamethrin was compared; etofenprox was clearly the least effective and deltamethrin was the most effective in killing the exposed mosquitoes.

Discussion

Bed nets impregnated with permethrin reduced the blood feeding rate as much as 91% compared to the control

group.⁵ Another study, conducted by Thomson et al.⁶ has reported a statistically significant reduction in the human blood feeding index. Studies on ITNs have generally yielded a slight reduction in the blood feeding rate on humans; it was not statistically significant.⁷⁻⁹ A reduction of 25% was observed in blood feeding rate of *An. stephensi* on the guinea pig restricted inside the holder. The mortality rate of etofenprox, permethrin and deltamethrin was assessed. The etofenprox was clearly the least effective and deltamethrin was the most effective in killing mosquitoes. These findings were consistent with those of earlier reports in which bioassay results on deltamethrin against vector

mosquitoes showed a significantly greater insecticidal power than alphacypermethrin and cyfluthrin.^{10,11} The recovery rate of knockdown mosquitoes revealed that deltamethrin had the least effects. This is an important factor since many mosquitoes may have an inherent capacity to avoid definite death when exposed for a short time to insecticides. Some investigators reported the least repellency effect of deltamethrin malaria mosquitoes.¹²⁻¹⁴ The ER effect of deltamethrin may also culminate in a ‘mass killing effect’ in the field mosquito populations.^{9,15,16} The observation of excito-repellency phenomenon of two types of pyrethroids and etofenprox has major impacts on malaria transmission as it may lead to secondary exophilicity of vector population and, thereby to failure of control programs. In such conditions, the mosquitoes leave the sprayed houses to either unsprayed one or outdoor shelters before reaching the lethal doses.

The importance of this phenomenon is well defined in relation to the resting habits of local inhabitants in the south of Iran who sleep outdoors in warm seasons and are exposed unprotected to the bite of mosquitoes.

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