Indian J Med Res 149, April 2019, pp 554-557
DOI: 10.4103/ijmr.IJMR_144_18

Quick Response Code:

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Synergist piperonyl butoxide enhances the efficacy of deltamethrin in deltamethrin-resistant *Anopheles culicifacies* sensu lato in malaria endemic districts of Odisha State, India

Sir,

In India, *Anopheles culicifacies* sensu lato (Diptera: Culicidae) is a major malaria vector contributing nearly 65 per cent of malaria cases[1,2]. Insecticides play a vital role in controlling the population of malaria vectors in the country. The national malaria control programmes of various countries all over the world rely on pyrethroids, used in indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) for control of malaria vectors[1]. Due to continuous use of insecticides, there is a rapid development of resistance in malaria vectors[3].

Odisha State of India has been badly affected with a high incidence of malaria for many years and contributed >54 per cent of the total *Plasmodium falciparum* cases recorded in India during 2016[4]. Of the total 30 districts of Odisha, eight southern districts were most seriously affected by malaria. These eight southern districts contributed 40.7 per cent of the total malaria cases (n=432,375) and 33.3 per cent of the total malaria deaths (n=78) during 2015[5]. *A. culicifacies* has been reported to be resistant against DDT, malathion and synthetic pyrethroids (SPs)[2,5].

The application of insecticide mixtures with two active ingredients has been proven to be effective in case of pyrethroid resistance[6]. It has also been shown that the use of synergists like piperonyl butoxide (PBO) with the insecticides is a good alternative for controlling resistant mosquitoes[7]. PBO can enhance the effect of pyrethroid insecticides by decreasing the detoxifying ability of enzymes such as mixed-function oxidases which is also known as the cytochrome P450 monooxygenase[6,8]. Thus, PBO plays a vital role in enhancing the efficiency of pyrethroids against pyrethroid-resistant mosquitoes[6]. The present study was undertaken to evaluate the efficacy of deltamethrin with PBO against field-collected pyrethroid-resistant *A. culicifacies* in five malaria endemic districts of Odisha State, India.

The study was carried out from August to September 2017 in the five districts, namely Kalahandi, Koraput, Malkangiri, Nowrangpur and Rayagada of Odisha. The districts have been hyperendemic for *P. falciparum* since many years[5,9]. One Community Health Centre (CHC) from each of the five districts was randomly selected. From each CHC, three villages were selected based on the relatively higher density of *A. culicifacies* for collection of the mosquitoes to determine their susceptibility/resistance status.

Diurnal resting collections, indoors (0600-0730 h) were made using an oral aspirator and flash light from human dwellings and cattle sheds for collection of female *A. culicifacies* in the study villages. All mosquitoes were brought to the field laboratory at the Vector Control Research Centre, Field Station, Koraput, Odisha, in a mosquito cage and identified morphologically to species level based on morphological characters using a standard key[10] and separated according to their gonotrophic conditions.

Susceptibility tests were performed following the WHO insecticide susceptibility testing procedure[6]. The temperature and relative humidity (RH) in the field laboratory were maintained at 27°C±2°C and 75±10 per cent RH, respectively, while conducting the test. Papers impregnated with deltamethrin (0.05%) and PBO (4%) and silicone oil control papers were obtained from the Universiti Sains Malaysia, Penang, Malaysia. Twenty five wild-caught blood-fed female *A. culicifacies* mosquitoes were exposed to each of the four exposure tubes: PBO only, PBO followed by

deltamethrin, deltamethrin only and solvent control for one hour. After 24 h of holding, the number of dead mosquitoes was counted and recorded. The test process was repeated for three times. A corrected mortality of <90 per cent was considered 'resistant', 98-100 per cent as 'susceptible' and between 90 and 97 per cent as possible resistance[6]. Data were analyzed by using SPSS version 16.0 (IBM Corporation, Armonk, New York, USA). The corrected mortality of PBO only, PBO followed by deltamethrin and deltamethrin only were compared between the five districts using Chi-square test. All analyses were carried out at 5 per cent level of significance and 95 per cent confidence interval.

A total of 300 female *A. culicifacies* mosquitoes from each district were exposed to synergist insecticide susceptibility assay. The corrected mortality and resistance suppression data are given in the Table. In all the five districts, the corrected mortality of *A. culicifacies* after exposure to PBO ranged from 1.3 to 5.3 per cent and was not significant between the districts. The corrected mortality of *A. culicifacies* after exposure to deltamethrin ranged from 73.3 to 85.3 per cent, which showed that the population of *A. culicifacies* was resistant to deltamethrin. There was no significant difference in the corrected mortality of *A. culicifacies* between the five districts. When exposed to PBO followed by deltamethrin, the mosquitoes showed restoration of susceptibility to deltamethrin as the corrected mortality of *A. culicifacies* ranged between 98.7 and 100 per cent. There was no significant difference in the corrected mortality on exposure to PBO followed by deltamethrin, between the five districts (Table).

The spread of insecticide resistance, especially pyrethroid resistance, is a risk for the vector control programme in India, as it is being extensively used for impregnation of bed nets[1]. Pyrethroid resistance has also become widespread among anopheline mosquitoes in Africa[11]. Thus, for an effective control of pyrethroid-resistant malaria vectors, interventions involving synthetic pyrethroid with PBO (synergistic approach) need to be developed.

It has been reported that pyrethroid resistance in mosquitoes is due to the involvement of monooxygenases and not because of mutations in the voltage-gated sodium channels (VGSC) gene[1]. Therefore, nets impregnated with a synthetic

Table. Response of *Anopheles culicifacies* to piperonyl butoxide (PBO), PBO followed by deltamethrin and deltamethrin only in adult susceptibility tests in five districts of Odisha State

District	Control			Deltamethrin only			PBO only			PBO + Deltamethrin		
	Number exposed	Number dead after 24 h	Per cent mortality	Number exposed	Number dead after 24 h	Per cent mortality	Number exposed	Number dead after 24 h	Per cent mortality	Number exposed	Number dead after 24 h	Per cent mortality
Koraput	75	1	1.3	75	55	73.3	75	4	5.3	75	74	98.7
Malkangiri	75	2	2.7	75	63	84.0	75	3	4.0	75	74	98.7
Nabarangpur	75	1	1.3	75	64	85.3	75	1	1.3	75	74	98.7
Rayagada	75	0	0.0	75	60	80.0	75	1	1.3	75	75	100.0
Kalahandi	75	1	1.3	75	56	74.6	75	1	1.3	75	75	100.0

pyrethroid together with a synergist was considered a better alternative against pyrethroid-resistant malaria vectors[12]. PBO is one of many synergists, when added to insecticides, can increase their lethality and effectiveness against resistant vector mosquitoes[13]. Having no insecticidal property of its own, PBO increases the efficacy of certain insecticides such as carbamates, pyrethrins and pyrethroids[14,15].

Our study showed that when pyrethroid-resistant *A. culicifacies* were exposed to PBO before the exposure to deltamethrin, the susceptibility of mosquito to deltamethrin got enhanced in all the districts. However, the field evaluation of PBO-deltamethrin combination along with the cone bioassays in various sprayed surfaces is required to find the difference in the enhancement of the efficacy of deltamethrin in the presence of PBO. The results of the current study were in line with the findings in *Anopheles gambiae* sensu lato in Ghana[16], where enhancement of susceptibility of the resistant *A. gambiae* sensu lato was observed when exposed to PBO followed by deltamethrin[16].

Considering the pyrethroid resistance of the malaria vector in India, the vector control strategy needs modification. The use of synergist (PBO) with deltamethrin could be one of the options for the management of *A. culicifacies*-transmitted malaria.

***Acknowledgment*:** The authors thank the staff of Indian Council of Medical Research-Vector Control Research Centre, Field Station, Koraput, Odisha, for the support provided in this study.

***Financial support & sponsorship*:** None.

***Conflicts of Interest*:** None.

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Received January 22, 2018

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