International Journal of
Recent Innovations in Academic Research
Open Access, Peer Reviewed, Abstracted and Indexed Journal

E-ISSN: 2635-3040; P-ISSN: 2659-1561
Homepage: https://www.ijriar.com/
Volume-5, Issue-6, June-2021: 1-6

Research Article

Importance of Differed Mortality Recording with Mosquitoes from Window Traps in Resistance Monitoring to Pyrethroids in Malaria Vectors from Mono Department in South-western Benin, West Africa

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Received: May 20, 2021 **Accepted:** May 30, 2021 **Published:** June 6, 2021

Abstract: The current study was aimed to study the importance of differed mortality recording with mosquitoes from window traps in resistance monitoring to pyrethroids in malaria vectors from mono department in south-western Benin, West Africa. *An. gambiae s.l.* adult mosquitoes were collected between 6.00 a.m. to 7.00 a.m in window traps put on windows of four rooms in districts of Grand Popo, Houéyogbé and Bopa surveyed in 2018. Female *An. gambiae* species were morphologically identified using morphological keys. The results showed that there was no dead mosquito when immediate mortality was recorded. But, after six hours, the differed mortality recording was 3.8%, 3% and 5% for mosquitoes from Grand Popo, Houéyogbé and Bopa respectively. These percentages showed that the dead of mosquitoes were not due to a product. That was likely due to tired mosquitoes as they were collected early in the morning between 6.00 a.m. and 7 a.m. and then carried out from the field to insectary. For that, it is important to record the differed mortality with mosquitoes collected in window traps before insecticide susceptibility tests performing using this mosquito sample.
Keywords: Immediate mortality, differed mortality, window traps, malaria vectors, Benin.

Introduction

The resistance of vectors to insecticides is a real handicap to the use of insecticide-treated materials. The recurrent presence on the agenda of most entomological research in Africa of vector resistance, more specifically of *Anopheles gambiae* resistance to insecticides, is due to the fact that insecticide-treated materials remain the principal tool of National Programmes of Malaria Control (NPMC) in the fight against vectors. Pyrethroids resistance is mentioned relatively late, in the 1990s. The first cases of pyrethroid resistance were recorded in Côte d'Ivoire (Elissa *et al.*, 1993), and many other cases have been described in Kenya (Vulule *et al.*, 1994), Benin (Akogbéto and Yakoubou, 1999; Chandre *et al.*, 1999), Burkina Faso (Chandre *et al.*, 1999; Diabaté, 1999), Côte d'Ivoire (Chandre *et al.*, 1999), and Mali (Fanello *et al.*, 2003). In central Africa, cases of pyrethroid resistance have been described in Cameroon and in the Central African Republic (Etang *et al.*, 2003). Pyrethroids and, to a lesser extent, organophosphates and carbamates are increasingly used for malaria vector control in endemic countries (Metcalf, 1973). Resistance monitoring, however, has concentrated mostly on pyrethroids because of the emphasis placed on ITNs for malaria prevention (Coleman *et al.*, 2006). Today, pyrethroid resistance is a major concern in west African countries and is a growing problem in eastern and southern African countries (Casimiro *et al.*, 2006; Hargreaves *et al.*, 2000; Tripet *et* *al.*, 2007 ; Yawson *et al.*, 2004; Stump *et al.*, 2004). The intensive use of pyrethroids for agricultural pest control is linked to the high levels of resistance observed in west African countries (United Nations Millennium Project, 2005). Researches have shown two major mechanisms of resistance in mosquitoes: (1) increased metabolism of insecticides reducing the effective dose of insecticide available at the target site; and (2) reduced target site sensitivity leading to ineffective binding of a given dose of insecticide, also known as knockdown resistance (*kdr*) (Hemingway *et al.*, 2004; Aïzoun *et al.*, 2013). Each mechanism is complex; a diverse range of genes and enzyme families are involved in resistance development. Hence, malaria vectors may have multiple resistance mechanisms, which are field selected under insecticide selection pressure, and may be resistant to one or more classes of insecticides (Hemingway *et al.*, 2004).

The goal of the current study is to study the importance of differed mortality recording with mosquitoes from window traps in resistance monitoring to pyrethroids in malaria vectors from mono department in south-western Benin, West Africa.

Materials and Methods

Study area

The study area is located in Republic of Benin (West Africa) and includes the department of Mono. Mono department is located in the south-western Benin and the study was carried out more precisely in the districts of Grand Popo, Houéyogbé and Bopa. The choice of the study site took into account the economic activities of populations, their usual protection practices against mosquito bites, and peasant practices to control farming pests. These factors have an impact on resistance development in the local vector mosquitoes. We took them into account to determine the importance of differed mortality recording with mosquitoes from window traps with regard to the resistance level. Mono has a climate with four seasons, two rainy seasons (March to July and August to November) and two dry seasons (November to March and July to August). The temperature ranges from 25 to 30°C with the annual mean rainfall between 900 and 1100 mm.

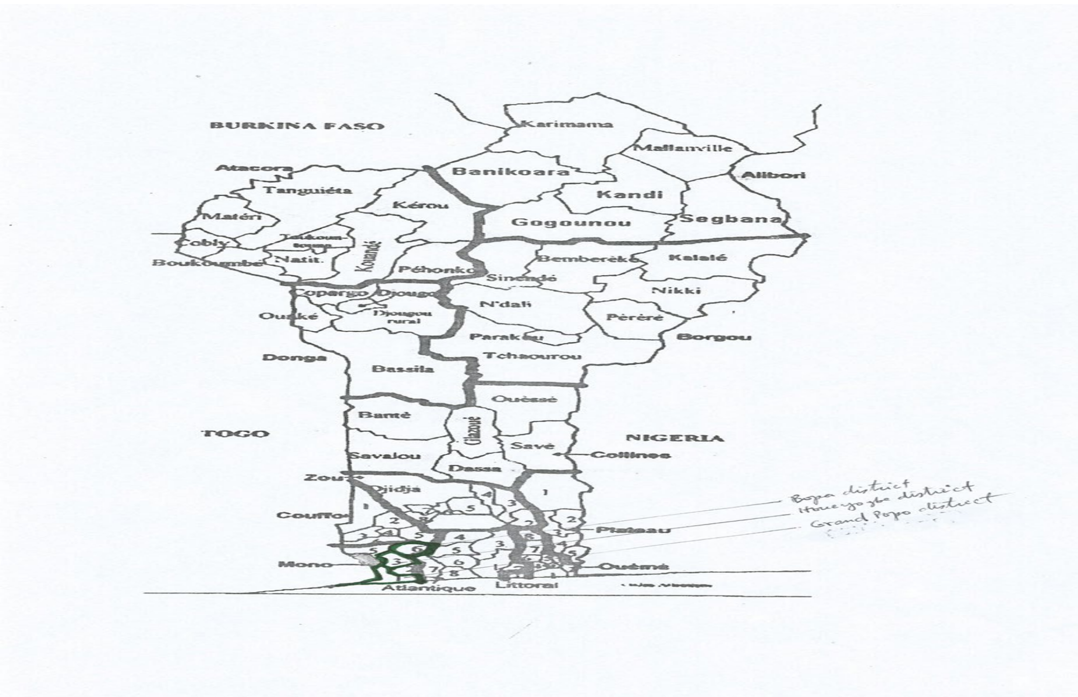

Figure 1. Map of Republic of Benin showing districts surveyed

Mosquito sampling

An. gambiae s.l. mosquitoes were collected in window traps put on windows of four rooms in each locality surveyed in 2018. Between 6.00 a.m. to 7.00 a.m., aspirators were used to collecting mosquitoes from these window traps. They were then put in some plastic cups covered with small cutting untreated net on which was put cotton wool moistened with a 10% honey solution. Female *An. gambiae* species were morphologically identified using morphological keys (Gillies and De Meillon, 1968). Immediate mortality was determined on the field and then plastic cups which contained mosquitoes were covered with wet tissues and carried out to insectary conditions of 27°C +/- 2°C and 75% +/- 10% relative humidity at Department of Sciences and Agricultural Techniques located in Dogbo district in south-western Benin. Immediately, after immediate mortality determination, differed mortality was recorded each hour during six hours and then mosquitoes were transferred into mosquito cages for susceptibility tests performing after dead mosquito elimination. *An. gambiae* Kisumu, a reference susceptible strain was used as a control for the bioassay tests. We used Kisumu more precisely to confirm the quality of treated or impregnated papers. Susceptibility tests were done following WHO protocol. All WHO susceptibility tests were conducted in the Laboratory of Applied Entomology and Vector Control of the Department of Sciences and Agricultural Techniques at 27°C +/- 2°C and 75% +/- 10% relative humidity.

Testing insecticide susceptibility

Females *An. gambiae s.l.* were exposed to WHO diagnostic dosage of permethrin 0.75% according to the WHO protocol (WHO, 2017). Thus, an aspirator was used to introduce 20 to 25 unfed female mosquitoes into six WHO holding tubes (four tests and two controls) that contained untreated papers. They were then gently blown into the exposure tubes containing the insecticide impregnated papers. After one-hour exposure, mosquitoes were transferred back into holding tubes and provided with cotton wool moistened with a 10% honey solution. The number of mosquitoes "knocked down" at every five minutes and mortalities at 24 hours were recorded following the WHO protocol (WHO, 2017). The choice of permethrin was justified by OlysetNet distribution made free recently throughout the entire country by Beninese National Malaria Control Programme to increase coverage of long-lasting insecticidal nets (LLINs).

Data analysis

The resistance status of the used mosquito sample was determined according to the WHO criteria (WHO, 2017) as follows:

- ✓ Mortality rates between 98%-100% indicate full susceptibility.
- ✓ Mortality rates between 90%-97% indicate possible resistance.
- ✓ Mortality rates $< 90\%$, the population is considered resistant to the tested insecticides

Abbott's formula was not used in this study for the correction of mortality rates in the test-tubes because the mortality rates in all controls was always less than 5% (Abbott, 1987). Analysis using Fisher's exact test and test of proportion was performed on the data sets gathered from the localities surveyed to compare immediate and differed mortality and assess the insecticide resistance status of each tested *An. gambiae* population using WHO method.

Results and Discussions

The analysis of table 1 showed that there was no dead mosquito when immediate mortality was recorded. Same results were observed after four hours during differed mortality recording. But**,** after six hours, differed mortality recording showed that 3.8%, 3% and 5% of mosquitoes from Grand Popo, Houéyogbé and Bopa respectively were died. These percentages showed that the dead of mosquitoes were not due to a product. That was likely due to tired mosquitoes as they were collected early in the morning between 6.00 a.m. and 7 a.m. and then carried out from the field to insectary conditions of 25+/-2°C and 70 to 80% relative humidity at Department of Sciences and Agricultural techniques located in Dogbo district in south-western Benin. Also, the age of female *An. gambiae s.l.* mosquitoes collected in the window traps put on windows of four rooms in each localities surveyed was not known. They were likely old mosquitoes. So, differed mortality recording with mosquitoes from window traps is useful in resistance monitoring to insecticide in malaria vectors before mosquito exposure to a product for their susceptibility determination.

The analysis of table 2 showed that the percentage of knock-down at 30 minutes recorded with females *An. gambiae s.l.* populations from Grand Popo (100%) was higher than that recorded at 40 minutes or after ten minutes (88.9%). So, knock-down effect is not synonymous of mortality when susceptibility tests were performed with WHO protocol. Knock down effect is a characteristic of pyrethroids. It happens immediately after the insects are exposed to pyrethroids (Coats, 1982). Therefore, if the time need for insects to be knocked down increases, it indicates that the insects may be resistant to the insecticide (Cochran, 1994). When insects are exposed to pyrethroids, they fall down but will not die immediately. For susceptible insects, they will eventually die. But for resistant insects, after they are knocked down for a while, they will recover and soon be able to fly again after the pyrethroids entering their bodies are detoxified by their metabolism (Cochran, 1994).

The analysis of table 3 showed that after 24 hours mortality recording Kisumu strain (control) confirmed its susceptibility status as a reference strain whereas *An. gambiae s.l.* Grand Popo, Houéyogbé and Bopa populations were resistant to permethrin 0.75%. According to Zaim *et al.,* (2000), pyrethroids have unique modes of action such as fast knockdown and excito-repellent effects.

Table 1. Immediate and differed mortalities recording

Poulations	% Immediate mortality	% Differed mortality					
		1H	2H	3H	4H	5H	6H
Grand Popo	0	0	0	0	0	3.8	3.8
Houéyogbé	0	0	0	0	0	5.9	3
Bopa	0	0	0	0	0	7.6	5

Table 2. 'Knocked-down' time recording with permethrin

Poulations	% Kd at 10 min	% Kd at 20 min	% Kd at 30 min	% Kd at 40 min	% Kd at 50 min	% Kd at 60 min
Grand Popo	0	77	100	88.9	88.9	88.9
Houéyogbé	0	31.6	73.7	89.5	100	100
Bopa	0	0	40	55	60	60

Table 3. Susceptibility status to permethrin in *Anopheles gambiae s.l.* populations

Populations	Number tested	% Mortality	Resistance status
Kisumu (Control)	100	100	S
Grand Popo	12	88.9	R
Houéyogbé	19	86.7	R
Bopa	20	85	R

Conclusion

The current study shows that it is important to record the differed mortality with mosquitoes collected in window traps before insecticide susceptibility tests performing using this mosquito sample. However, the use of the mosquito samples from larvae and pupae collections needs neither immediate nor differed mortality recording before the assessment of insecticide susceptibility tests in malaria vectors.

Acknowledgements

The author would like to thank people from locations surveyed who had helped us in mosquito collection. I would also like to thank KOUASSI Prisca for technical assistance in laboratory during the current study.

Conflicts of interest

I declare no conflicts of interest.

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Citation: Nazaire Aïzoun. 2021. Importance of Differed Mortality Recording with Mosquitoes from Window Traps in Resistance Monitoring to Pyrethroids in Malaria Vectors from Mono Department in South-western Benin, West Africa. International Journal of Recent Innovations in Academic Research, 5(6): 1-6.