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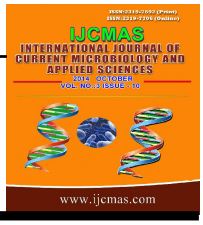

Original Research Article

Susceptibility status to malathion in *Anopheles gambiae s.l.* populations from Toffo district in southern Benin, West Africa

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A B S T R A C T

In order to seek for alternative insecticides to pyrethroids for malaria vector control, it is important to investigate susceptibility to malathion, an organophosphate compound in *Anopheles gambiae s.l.* populations from Benin. Larvae and pupae of *Anopheles gambiae s.l.* mosquitoes were collected from the breeding sites in Atlantic department. WHO susceptibility tests were conducted on unfed female mosquitoes aged 2–5 days old. WHO bioassays were performed with impregnated papers with malathion 5%. PCR techniques were used to detect species and *Ace-1* mutations. *Anopheles gambiae s.l.* Toffo populations were susceptible to malathion. The mortality rate observed was 98.96%. PCR revealed that all specimens tested were *Anopheles gambiae s.s.* The *Ace-1R* frequency observed in *Anopheles gambiae* Toffo populations was 0%. The current study clearly shows that even if organophosphate resistance is already present in *Anopheles gambiae s.l.* populations from northern Benin, those from southern Benin are still susceptible to this compound. Further studies are need to investigate the use of malathion for Indoor Residual Spraying in Benin.

Keywords

Anopheles gambiae; *Ace-1*; Resistance; malathion; Benin

Introduction

Overall protection of at risk populations with IRS decreased globally from 5% in 2011 to 4% in 2012; in the African Region the proportion protected by IRS decreased from 11% to 8% during the same time period. The reasons for the decrease in IRS implementation are not clear. Some countries appear to have decreased use of pyrethroids and increased their use of non-pyrethroid insecticides, either in direct response to insecticide resistance monitoring data or as part of a plan to use insecticides in rotation to minimize the development of resistance. Since most of the non-pyrethroid insecticides used in rotation are more costly than pyrethroids, control programmes with funding constraints may have reduced the target population to be protected by IRS, and provided vector control coverage through ITNs in areas previously covered with IRS (WHO, 2013a).

IRS is applicable in many epidemiological settings, provided that its operational and resource feasibility is considered in policy and programming decisions. IRS requires specialized spray equipment and techniques, and given the difficulty of carrying out spray operations, it also requires scrupulous maintenance of the equipment, timing and quality of application, and monitoring and disposal capabilities (WHO, 2013a). National vector control programmes should annually rotate the insecticides used for IRS in order to preserve the effectiveness of current compounds. In places where this recommendation can only be implemented in stages, the first priority should be to introduce rotations in areas of identified resistance and in those with the highest malaria transmission (WHO, 2013a).

Carbamate and OP are the main alternatives for indoor residual spraying or larval treatments against mosquitoes in case of pyrethroid resistance. These insecticides inactivate acetylcholinesterase (AChE). Acetylcholinesterase (AChE) is a synaptic enzyme that hydrolyzes the neurotransmitter acetylcholine to terminate nerve impulses. It is also involved in the development of the nervous system in vertebrates and invertebrates (Grisaru *et al.*, 1999; Cousin *et al.*, 2005).

The Benin National Malaria Control Programme has implemented indoor residual spraying (IRS) campaign under the financial support of the PMI (President's Malaria Initiative) using bendiocarb in Oueme department in southern Benin (2008-2010).

The present study propose was to assess the resistance status of malaria vectors from Toffo to malathion in order to check if there is already resistance to this product and also to check if the insensitive acetylcholinesterase (*ace-1R*) is already present in these *Anopheles gambiae s.l.* populations.

Materials and methods

Study area

The study area is located in the Republic of Benin (West Africa) and includes the department of Atlantic. The Atlantic department is located in the south of Benin and the study was carried out more precisely in Toffo district, cereals and fruit growing area. 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 a direct impact on the development of insecticide resistance in the local mosquito vectors. Toffo is characterized by a sub-equatorial type climate with two rainy seasons (March–July and September–November). The mean annual rainfall is over 1,500 mm.

Mosquito sampling

Anopheles gambiae s.l. mosquitoes were collected during the rainy seasons (March-July and September-November 2012) across Toffo district selected in southern Benin. Larvae and pupae were collected in this district within both padding and town using the dipping method on several breeding sites (brick pits, pools, marshes, streams, ditches, pits dug for plastering traditional huts, puddles of water, water pockets caused by the gutters).

Once, larvae and pupae collected, they were then kept in labeled bottles related to the Toffo district surveyed. Otherwise, larvae collected from multiple breeding sites were pooled together then re-distributed evenly in development trays containing tap water. Larvae were provided access to powdered

TetraFin® fish food, and were reared to adults under insectary conditions of 25+/-2°C and 70 to 80% relative humidity at Centre de Recherche Entomologique de Cotonou (CREC) located in Akpakpa, in Cotonou district. The samples were reared up to adult emergence at the CREC insectary. *An. gambiae s.l.* Kisumu, a reference susceptible strain was used as a control for the bioassay tests. Susceptibility tests were done following WHO protocol on unfed females mosquitoes aged 2-5 days old reared from larval and pupal collections. All susceptibility tests were conducted in the CREC laboratory at 25+/-2°C and 70 to 80% relative humidity.

Testing insecticide susceptibility

Females *An. gambiae s.l.* aged 2 to 5 days old were exposed to WHO diagnostic dosage of malathion 5% according to the WHO protocol (WHO, 1998). Thus, an aspirator was used to introduce 20 to 25 unfed female mosquitoes into five WHO holding tubes (four tests and one control) 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 60 minutes and mortalities at 24 hours post treatment were recorded following the WHO protocol (WHO, 1998). Dead and surviving mosquitoes were separately stored in individual tubes with silicagel and preserved at -20°C in the laboratory, for further molecular characterization. We used malathion, an organophosphate compound to check if there was already resistance to this product in the district surveyed.

PCR detection of species and *Ace-1* mutations

Specimens of *An. gambiae* from the WHO bioassay tests were subjected to the *An. gambiae* species specific PCR assays for species identification (Scott *et al.*, 1993). The PCR-Restricted Fragment Length Polymorphism (PCR-RFLP) diagnostic test was used to detect the presence of G119S mutation (*ace.1R* gene) as described by Weill *et al.* (2003). Mosquito genomic DNA was amplified using the primers Ex3AGdir 5′GATCGTGGACACCGTGTTCG3′ and Ex3AGrev 5′AGGATGGCCCGCTGGAA CAG3′ according to Weill *et al.* (2003). One microlitre of total DNA extracted from a single mosquito was used as a template in a 25 µl PCR reaction containing Taq DNA polymerase buffer, 0.2 mM dNTP and 10 pmol of each primer. The PCR conditions were 94°C for 5 min and then 35 cycles of (94°C for 30 s, 54°C for 30 s and 72°C for 30 s) with a final 5 min extension at 72°C. Fifteen microlitres of PCR product were digested with 5U of AluI restriction enzyme (Promega) in a final volume of 25 µl. The PCR fragments were fractionated on a 2% agarose gel stained with ethidium bromide and visualized under UV light.

Statistical analysis

The resistance status of mosquito samples was determined according to the latest WHO criteria (WHO, 2013b) as follows:

– Mortality rates between 98%-100% indicate full susceptibility

– Mortality rates between 90%-97% require further investigation

– 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 test-tubes because the mortality rates in all control tubes was less than 5% (Abbott, 1987).

To compare the status of insecticide resistance, Fisher's exact test was carried out to determine if there was any significant difference between mortality rates of populations of *An. gambiae s.s.* of districts using Statistica 6.0. Allelic frequency of G119S mutation was analysed using the version 1.2 of Genepop (Raymond and Rousset, 1995). The software R-2.15.2. (R Development Core Team, 2011) was used for the statistical analysis.

Ethical approval

This study was approved by the Ministry of Health and the Center for Entomological Research of Cotonou, Benin.

Result and Discussion

Susceptibility of *An. gambiae s.l.* populations to malathion

Kisumu strain (control) confirmed its susceptibility status with 100% mortality as a reference strain. All female mosquitoes of *Anopheles gambiae s.l.* Kisumu, which were exposed to WHO papers impregnated with malathion 5% showed a total mortality with no survivors after 24hours mortality recording. This result confirmed that they were susceptible to this product. Regarding *An. gambiae s.l.* populations from Toffo, they were also susceptible to malathion 5% with the mortality rate of 98.96% (Table 1).

Species of *Anopheles gambiae* and *Ace-1* genotype

PCR revealed 100% of mosquitoes tested were *Anopheles gambiae s.s.* The frequency of *Ace-1R* in *Anopheles gambiae s.l.* Toffo was 0% (Table 2).

Organophosphates (OPs) are one of the main alternatives for indoor residual spraying or larval treatments against mosquitoes in case of pyrethroid resistance. These insecticides inactivate acetylcholinesterase (AChE), an enzyme responsible for neurotransmitter degradation at the cholinergic nerve synapse. So, acetylcholinesterase (AChE) is a synaptic enzyme that hydrolyzes the neurotransmitter acetylcholine to terminate nerve impulses. It is also involved in the development of the nervous system in vertebrates and invertebrates (Grisaru *et al.*, 1999; Cousin *et al.*, 2005).

An. gambiae s.l. populations from Toffo were susceptible to malathion and this result was corroborated with *Ace-1* mutation frequency. This result may be explained by the low amount of insecticidal products (OP) applied in agriculture and public health in this district. The susceptibility to malathion in *An. gambiae s.l.* populations from southern Benin was already reported by Corbel *et al.* (2007) more precisely in Ladji and Asecna localities in Cotonou district. This study by Corbel *et al.* (2007) suggested that there were no malathion-specific carboxyesterases in Benin.

The susceptibility to organophosphates was also recently reported in *Anopheles gambiae s.l.* from southern Benin such as *An. gambiae* Seme populations in Oueme department (Aïzoun *et al.*, 2013).

Similar to *An. gambiae s.l.* populations from southern Benin, those from the central part of the country were also susceptible to organophosphate compounds (Aïzoun *et al.*, 2014) whereas resistance to OPs was already detected in northern Benin in Atacora department more precisely in Tanguieta district and in Alibori department more precisely in Malanville district (Aïzoun *et al.*, 2013).

Table.1 Percentage of dead *Anopheles* mosquitoes observed after 1hour exposure to WHO papers impregnated with malathion 5% in Toffo district

Population	Insecticide	Number tested	% Mortality	Resistance status
Kisumu (Control)	Malathion	102	100	S
Toffo	Malathion	94	98.96	S

Table.2 *Ace-1* mutation frequency in *An. gambiae* populations issue from WHO bioassays tests

			Ace-1 mutation			
Locality	Number tested	Species Ag	RR	RS	SS	F(*Ace-1*)
Toffo	49	49	0	0	49	0

Ag: *An. gambiae s.s.*

In the current study, PCR revealed that 100% of mosquitoes from Toffo district tested were *Anopheles gambiae s.s.* No *An. arabiensis* mosquitoes were found. Similar results were already found by Djogbenou *et al.* (2008) (unpublished data), who have shown the absence of *Anopheles arabiensis* populations within *An. gambiae* complex in Toffo district. So, this specie is not present in this district.

The current study clearly showed that even if organophosphate resistance is already present in *Anopheles gambiae s.l.* populations from northern Benin, those from southern Benin are still susceptible to this compound. Further studies are need to investigate the use of malathion for Indoor Residual Spraying in Benin.

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