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Insecticide susceptibility profile of the principal malaria vector, *Anopheles gambiae* s.l. (Diptera: Culicidae), in north-central Nigeria

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Malaria remains the most important and endemic parasitic disease all over Nigeria, with >90% of ~140 million population is at risk of infection[1]. It accounts for 63% of the diseases reported in health care facilities across the six geographical zones of the country[2], costing the country >1 billion US$ annually; thus, it constitutes a huge burden to the already depressed Nigerian economy. This unacceptably high malarial burden could have been informed, by the unfortunate incidental occurrence of the most efficient malarial pathogen/vector combination complex, i.e. *Plasmodium falciparum* and *Anopheles gambiae* complex, supported by optimum environmental conditions across the entire country[3]. The continued desire to abate this heavy burden resulted in the initiation of aggressive control interventions directed at the parasite and vectors. The success of the parasite approach was, however, hindered by the advent and rapid spread of drug-resistant plasmodial strains worldwide, thus, vector control remains the only viable approach to reducing malaria burden in the country[4]. McCaffery and Nauen[5] confirmed this by noting that malaria incidence cannot be significantly reduced in Africa, if, in addition to chemotherapy, transmission is not mitigated through very effective vector control.

For decades, the Nigerian National Malaria Control Program (NNMCP) has prioritized the use of residual chemical insecticides, as the key tactic for vector control. Notable successes were reported as drastic reduction of malaria prevalence rates were observed across the country, following the large-scale indoor residual spraying of DDT in the 1950s and 1960s[6]. The incessant and unguarded use of insecticides for agricultural and public health purposes in our ecosystem, no doubt, puts anopheline vectors of malaria under continuous resistance selection pressure; a trend that does not augur well for the continued success of the chemical tactic. To forestall the loss of efficacy of the insecticidal approach, an informed and guided use of insecticides for this purpose is required. This must, however, be wittingly informed by the trend of noticeable anopheline insecticide susceptibility profile. Therefore, the susceptibility of the principal malarial vector *An. gambiae* to various insecticides in Minna, north-central zone of Nigeria was assessed amidst unpublished reports of reduced efficiency of common mosquito insecticides and compared with susceptibility of a similar population of laboratory cultured and insecticide-free mosquitoes.

Four insecticides, namely permethrin (pyrethroid), propoxur (carbamate), fenitrothion (organophosphate) and DDT (organochlorine), representing the most frequently mentioned in each of the four groups of WHO recommended insecticides for vector control were selected for investigation from a long list of insecticides commonly used in Minna, generated through a questionnaire administered at the advent of the 2008 rainy season. Minna the capital city of Niger State, Nigeria, is located between 6°33´and 6°37´ E Longitudes and 9°37´ and 9° 40´ N Latitudes, with an estimated human population of 1.2 million, on an estimated land area of 88 km^2.

The adult mosquitoes used for the bioassay were obtained from an F_1 generation of *An. gambiae* larvae collected from temporary ground pools in Minna during the rainy season in 2008. The identity of the larval specimen was confirmed as *An. gambiae* s.l. using the keys of Gillies and Coetzee[7]. The larvae were reared in the laboratory following standard procedures[8]. Technical grade of the various insecticides were impregnated individually on filter papers (Whatman No. 1) at operational field concentrations of 2 g/m^2 of DDT, 0.02 g/m^2 permethrin and 0.05 g/m^2 propoxur and fenitrothion, prepared using acetone diluents, according to WHO protocol[9].

The susceptibility of *An. gambiae* to four test insecticides was assessed by exposing 3-day old adult female mosquitoes to the insecticide-treated test papers following standard procedures and exposure times. Ten replicate tests were put up for each insecticide each time. Test papers in the control experiments were impregnated with acetone only. Ten randomly selected 3-day old female mosquitoes were monitored per tube. Exposure of the mosquitoes to insecticides was at 27 ± 2°C temperatures and 65 ± 3.22% relative humidities, respectively. Knockdowns were recorded at 10 min interval over a 1 h exposure period and these were compared statistically for variance at $p = 0.05$ confidence limit. Mortality rate was confirmed at the end of 24 h recovery time. The susceptibility status, i.e. resistant, susceptible or tolerant of the mosquito species to each of the test insecticide was evaluated according to the World Health Organization's criteria[10].

Table 1 shows the mean total number of 3-day old adult female *An. gambiae* mosquitoes knocked down at 10 min interval during an hour exposure to various insecticides and the mean total mortality rates recorded after 24 h recovery exposure. All the four insecticides showed encouraging knockdown capabilities at various times during the 1 h exposure. DDT, permethrin and propoxur showed statistically comparable and insignificantly different ($p > 0.05$) instant knockdown numbers. Fenitrothion, however, knocked down significantly higher ($p < 0.05$) number of mosquitoes, i.e. 209.40 ± 24, instantly. Permethrin and propoxur put up their best knockdown performances in the first 10 min of exposure but their knockdown capabilities declined considerably subsequently. Knockdowns due to DDT exposure were restricted to the first 40 min and these were consistently significantly higher ($p < 0.05$) than other insecticides particularly between 10 and 20, and 30 and 40 min. There was, however, no knockdown after 40 min and an appreciable number of mosquitoes survived after the 24 h recovery period. Fenitrothion consistently knocked down about 30 mosquitoes every 10 min during 1 h exposure duration, leaving just about 20 mosquitoes, all off which died after 24 h recovery exposure.

Whereas all the insecticides had a commendable instant impact, particularly within the first 10 min, on the *An. gambiae* population from Minna, the mosquitoes were quite responsive to fenitrothion, as instant knockdown featured prominently and it subsequently persisted throughout the 1 h exposure period affecting 97.88% and eventually killing all the mosquitoes after the recovery period. Permethrin and propoxur showed slightly delayed knockdown activities restricted majorly to the first 10 min and these subsequently tapered as the hour progressed with 95.70 and 91.57% eventually knocked down, respectively, and 99.30 and 98.14% killed eventually, respectively. The instantaneous activity of DDT was evident but subsequent knockdown records that accounted for 66.26% knockdown were not commendable and did not last beyond 40 min and 78.36% mortality was eventually recorded.

The findings of this study indicated that in Minna *An. gambiae* population is resistant to DDT, as <80% mortality was recorded after 24 h recovery period. This could have been due to the intense and preferred use of

Table 1. Progressive mean number of 3-day old wild adult female *Anopheles gambiae* s.l. mosquito population from Minna, north-central Nigeria, knocked down at 10 min interval during an hour exposure to standard operational concentrations of some insecticides and the mean total mortality rates after 24 h recovery exposure

Insecticide (Conc.)	Mean number of knockdown (kD) mosquitoes at 10 min time interval							Mean total mortality (%) 24 h
	Instant 0 min	10 min	20 min	30 min	40 min	50 min	60 min	
DDT (2 g/m^2)	119.60 ± 20.53^{b*}	40.64 ± 27.31^{b}	57.83 ± 9.40^{c}	14.00 ± 1.20^{b}	32.96 ± 8.40^{c}	0 ± 0^{a}	0 ± 0^{a}	78.36 ± 2.98^{b}
Permethrin (0.02 g/m^2)	111.93 ± 32.87^{b}	132.25 ± 14.00^{c}	75.80 ± 5.32^{d}	45.96 ± 9.63^{d}	8.60 ± 1.29^{b}	0 ± 0^{a}	7.96 ± 1.60^{b}	99.30 ± 1.56^{c}
Fenitrothion (0.05 g/m^2)	209.40 ± 24.00^{c}	25.80 ± 7.72^{b}	28.52 ± 6.68^{b}	41.00 ± 8.88^{d}	30.07 ± 15.60^{c}	32.72 ± 9.24^{c}	24.00 ± 6.64^{c}	100.00 ± 0^{d}
Propoxur (0.05 g/m^2)	117.75 ± 18.81^{b}	150.40 ± 23.88^{c}	55.50 ± 9.73^{c}	24.81 ± 3.12^{c}	5.32 ± 1.20^{b}	6.40 ± 0.96^{b}	6.08 ± 1.40^{b}	98.14 ± 1.72^{c}
Control	0 ± 0^{a}	0 ± 0^{a}	0.63 ± 0.6^{a}	1.05 ± 0.33^{a}	0.97 ± 0.92^{a}	0.54 ± 0.29^{a}	0.69 ± 0.48^{a}	2.16 ± 0.29^{a}

*Values followed by same superscript alphabet in a column are not significantly different at $p = 0.05$ level of significance.

organophosphates over a long period in Minna, as the intensive use of a particular insecticide over long periods selects resistance in mosquito populations[11]. A prior survey to this study conducted at Minna confirmed DDT to be the most available and preferred insecticidal option for >70% households that regularly use insecticides for mosquito control, as it is affordable.

The resistant status of Minna population of *An. gambiae* to DDT no doubt negates the accomplishment of the goals of the Roll Back Malaria Program being implemented in Minna, as the programme's malaria vector control approach is at variance with the residents' insecticide use pattern. Whereas the National Malaria Control Program recommends and promotes the use of pyrethroids as indoor residual sprays and in the treatment of bed nets, the residents rely more on organochlorines (DDT), despite the fact that the use of DDT has been banned in most of the countries of the world due to resistance and environmental concerns[12]. Today, reports indicate that pyrethroids are the most cost-effective insecticides for malaria vector control. The other three insecticides tested, namely permethrin, propoxur and fenitrothion, induced very high mortality rates (>98%) in the Minna population of *An. gambiae*, indicating full susceptibility, i.e. lack of resistance to the insecticides. Thus, agreeing with the choice of any of the three insecticides in the ongoing malaria vector control drive in Minna, the pyrethroids, including permethrin, being the most cost-effective insecticides for malarial control and highly effective at low dosage with low mammalian toxicity, were recommended for large-scale use in the treatment of mosquito bed nets for malaria prevention. The active distribution of pyrethroid-treated mosquito bed nets all over Nigeria commenced in 1992[1]; yet the *An. gambiae* population in Minna is still highly susceptible to the chemical. This finding contradicts that of Awolola *et al*[13] who reported pyrethroid insecticide-resistance in *An. gambiae* population from the south-western part of Nigeria. This welcome situation, should, however, be guarded and handled with caution, as pyrethroid resistance development in *An. gambiae* has been reported from other African localities that have had long time exposure to pyrethroids[24, 26-28]. The availability of other less suitable options with equal level of susceptibility, such as propoxur and fenitrothion could go a long way to assist the situation.

CONCLUSION

The *An. gambiae* population in Minna is still susceptible to most of the commonly used insecticides in the area, despite a long period of exposure and, therefore, vector control interventions utilizing any of such insecticides may not be jeopardized by resistance. However, there is a disconnect between the National Malaria Control Program's insecticide-use policy and the insecticide-use pattern of the residents of Minna, and it is hoped that proper education and enlightenment campaign will guide stakeholders to informed choice of insecticides for use in malaria vector control in the area.

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REFERENCES

1. *Malaria control in Nigeria: a strategy for behavior change communication.* Abuja, Nigeria : Roll Back Malaria Secretariat, Federal Ministry of Health 2004; p. 1–58.
2. *Malaria desk situation analysis, Nigeria 2000.* Federal Government Publication, Aligeria. Abuja, Nigeria: Federal Ministry of Health, Govt. of Nigeria 2000; p. 1–27.
3. Onyabe DY, Conn JE. The distribution of two major malaria vectors, *Anopheles gambiae* and *Anopheles arabiensis*, in Nigeria. *Memorias Instituto Oswaldo Cruz, Rio de Janairo* 2001; *96*(8): 1081–4.
4. Okogun GRA. Life-table analysis of *Anopheles* malaria vectors: generational mortality as tool in mosquito vector abundance and control studies. *J Vector Borne Dis* 2005; *42:* 45–53.
5. McCaffery A, Nauen R. The insecticide action committee (IRAC): public responsibility and enlightened industrial self interest. *Outlooks on Pest Management* 2006; *2:* 11–4.
6. Sadasivaiah S, Tozan Y, Breman JG. Dichloro diphenyl trichloroethane (DDT) for indoor residual spraying in Africa: how can it be used for malaria control? *Am J Trop Med Hyg* 2007; *77*(6-Suppl): 249–63.
7. Gillies MT, Coetzee M. Supplement to the anophelinae of Africa, South of the Sahara (Afrotropical region). *Pub South African Inst Med Res* 1987; *55:* 143.
8. Olayemi IK, Ande AT. Colony maintenance and bionomics of *Anopheles gambiae* (Diptera: Culicidae) in Ilorin, Nigeria. *African J Biosci* 2009; *2*(1): 59–63.
9. Busvine JR. Experiments concerned with the development of World Health Organization test for resistance in adult mosquitoes. *Indian J Malariol* 1958; *12:* 279–86.
10. Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. *WHO/CDS/MAL/98.12.* Geneva: World Health Organization 1998.
11. Chareonviriyaphap T, Rongnoparut P, Juntarumporn P. Selection for pyrethroid resistance in a colony of *Anopheles minimus* species A, a malaria vector in Thailand. *J Vector Ecol* 2003; *27*(2): 222–9.

12. Roberts DR, Manguin S, Mouchet J. DDT house spraying and re-emerging malaria. *Lancet* 2000; *356:* 330–2.
13. Awolola TS, Brooke BD, Hunt RH, Coetzee M. Resistance of the malaria vector *Anopheles gambiae* s.s. to pyrethroid insecticides, in south-western Nigeria. *Annals Trop Med Parasitol* 2002; *96:* 849–52.

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