FULL LENGTH RESEARCH ARTICLE

EFFICACY OF PERMETHRIN INSECTICIDE TESTED AGAINST POPULATIONS OF *ANOPHELES* AND *AEDES* FROM DIFFERENT LARVAL HABITATS IN SOUTHERN GUINEA SAVANNA, NIGERIA

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ABSTRACT
The resistance status of *Anopheles gambiae* s.l. Giles, 1902 and *Aedes aegypti* Linnaeus, 1762 collected from different breeding habitats in southern guinea savanna of Kaduna State was investigated. The larvae were collected from swamps, rock and mountain pools, reared and fed with baker's yeast and grounded biscuits in separate containers to adulthood. Susceptibility test was conducted on the adult mosquitoes using 0.75 % permethrin impregnated paper embedded in World Health Organization (WHO) diagnostic test kit following standard procedure. Knockdown rate was recorded at 5 min interval for 1 hr and the final mortality was determined at 24 hr post exposure time. Fifty (50) and ninety five (95) percent knockdown times (KDT_{50} and KDT_{95}) were evaluated using the probit analysis. The result shows that the knockdown for all mosquitoes collected from the three breeding habitats were 100 %. The percentage mortalities for *Anopheles* from swamps, rock pools and *Aedes* from mountain pools were 100 % while 98.7 % was obtained for *Anopheles* from mountain pools. Fifty (50) and ninety five (95) percent knockdown times were between 10.72-12.45 minutes and 21.38-24.56 min respectively. The results show that all mosquitoes tested are susceptible to 0.75 % permethrin which suggest that the insecticide can be used effectively for the control of these vectors in the ecological zone.

Key words: *Anopheles*, *Aedes,* resistance, insecticide, permethrin, breeding habitats,

INTRODUCTION
The most effective way to prevent malaria and other mosquito-transmitted diseases such as yellow fever, dengue haemorrhagic fever, bancroftian filariasis e.t.c. has been to reduce human-vector contact using chemical insecticides (Chareonviriyaphap *et al*. 2000). This is achieved through the control of the vector populations by the use of insecticide treated nets (ITNs) and other material to prevent mosquito biting at the individual and household level (Curtis & Townson 1998). Today, the main thrust for roll back malaria strategy lies in use of insecticide treated nets (ITNs) (Hemingway & Bates 2003). Therefore, the development of resistance by mosquito to conventional insecticides is a threat to the control of mosquito-borne diseases (WHO 1992).

Currently, the only class of insecticides authorized by World Health Organisation (WHO) for use on bed nets are the pyrethroids (Hemingway & Bates 2003). They are preferred because they have been found to be effective with a strong excito-repellent effect on mosquitoes and yet, have lower mammalian toxicity than organochlorine, carbamate and organophosphate compounds and therefore less likely to bioaccumulate (Mittal *et al*. 1991). Pyrethroid insecticides have been effectively used against several species of insects that includes the German cockroach (Pridgeon *et al*. 2002), house fly (Shono *et al*. 2002), Cotton bollworm (Ru *et al*. 1998), *Spodoptera littoralis* Gammon 1980) and tobacco budworm (Park *et al*. 2000) as well as for the control of dipterous insects (Shono *et al*. 2002). Synthetic pyrethroid (also known as emergency insecticide) such as permethrin [(3-phenoxyphenyl)methyl(±)cis,trans-3-(2,2-dicholoroethenyl)-2,2-dimethylcyclopropanecarboxylate], when used to treat nets, played a dual role of killing the mosquitoes in addition to acting as an irritant that repels them from the net (Rozendaal 1989).

Since the insecticide is the key component of Roll Back Malaria, there is concern that emergence of resistance in mosquito populations could reduce the efficacy of ITNs. This paper reports the efficacy of permethrin insecticide on *Anopheles* and *Aedes* populations collected from different breeding habitats in the southern guinea vegetation zone of Kaduna State, Nigeria.

MATERIALS AND METHODS
Collection of immature stages and rearing: Immature stages (larvae and pupae) of *Anopheles* and *Aedes* mosquitoes were collected from swamps, rock and mountain pools which serve as breeding habitats. The larvae of *Anopheles* were distinguished from *Aedes* according to the way they lay horizontally to the water surface due to lack of siphon unlike *Aedes* species (Smart 1956). The larvae and pupae were reared in plastic containers until adult emergence, during which they were fed with baker's yeast or fish meal. Water was changed periodically as it becomes murky.

Susceptibility test: This was performed using standard WHO diagnostic test kit (WHO 1981) on the newly emerged one day old adults on 0.75 % permethrin-impregnated paper. Mosquitoes were first kept in holding tube for one hr, during which all unfit mosquitoes were removed. The mosquitoes were then exposed to the test papers in batches of 20 to 25 per exposure tube for 1 hr in a normal vertical position at room temperature and the knockdown rate recorded at 5 min interval during period of exposure. Thereafter, they were gently transferred to holding tubes with cotton wool moistened with 10 % sucrose solution and final mortality was recorded 24 hr post exposure. Control samples were exposed to untreated papers. All surviving and dead mosquitoes together with the control sample were identified using morphological keys (Huang & Ward 1981, Gillies & Coetzee 1987), the number counted and recorded and the percentage mortality calculated. The KDT_{50} and KDT_{95} were evaluated using the probit analysis (Finney 1971).

RESULTS

Out of the 444 mosquito specimens tested against 0.75% permethrin, 373 (84 %) were *An. gambiae* s. l. and 71 (16 %) *Ae. aegypti*. Of the anopheles, 149 (33.6 %) were from swamps, 74 (16.7 %) from rock pools and 150 (33.8 %) from mountain pools while all the 71 *Ae. aegypti* were from mountain pools.

100 % mortality was recorded for *Anopheles* and *Aedes* from swamps, rock pools and mountain pools and 98.7 % mortality for *Anopheles* from mountain pools. Mortality for control groups was consistently less than 5 %.

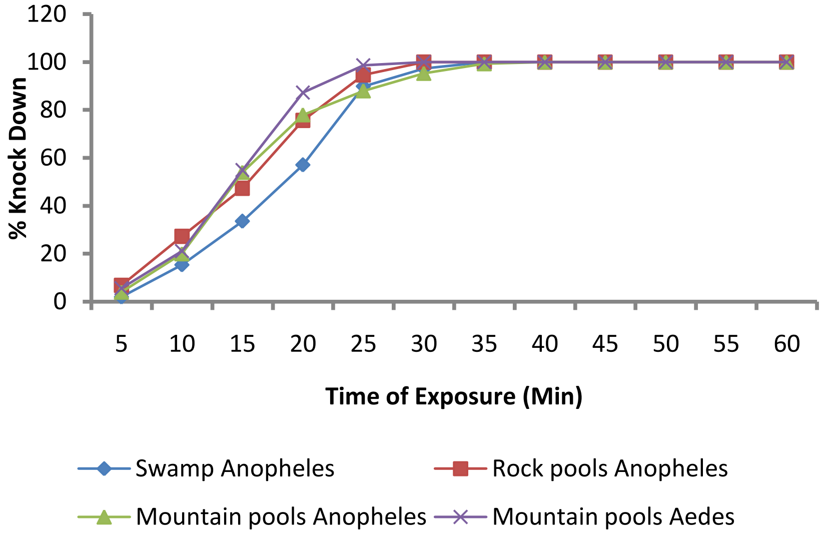

FIG. 1. MORTALITY RATES OF *Anopheles* and *Aedes* MOSQUITOES FROM DIFFERENT BREEDING HABITATS TESTED ON 0.75 % PERMETHRIN.

TABLE I. THE KDT_{50} AND KDT_{95} VALUES AFTER ONE HR EXPOSURE TO PERMETHRIN INSECTICIDE IN EMERGED ADULT MOSQUITOES.

Species	Larval habitats	No. exposed	No. survived	%e Mortality	KDT_{50} (min)	KDT_{95} (min)	% knockdown at 1 hr
An. gambiae	Swamps	149	149	100	10.72	22.91	100
An. gambiae	Rock pools	74	74	100	11.75	21.88	100
An. gambiae	Mountn pools	150	148	98.7	12.45	24.56	100
Ae. aegypti	Mountn pools	71	71	100	11.49	21.38	100

DISCUSSION

In this study, the two species of mosquitoes tested were susceptible to 0.75 % permethrin. The swamps sampling sites have principally shrubs and tall grasses with the surroundings dominated by agricultural crops like yams, cocoyams, maize and guinea corn. The peasant farmers do not use chemical insecticides or pesticides in the control of any pest, so there is less chance that appreciable pesticide residue would be found in the water body that can exert pressure in the insects leading to the development of resistance. Even where it is necessary to apply fertilizer, the method of application is point application and mostly in shallow holes dug by the farmers, so that the fertilizer may not to be washed away by rain.

There was little or no agricultural activities on the mountain areas where samples were collected and since the pools are seasonal, there is less likelihood that heavy metals may accumulate which may exert pressure on the insects leading to resistance. However, it is necessary to conduct water analysis before making deductions.

The knowledge of the effect of breeding habitats on the resistance of insect vectors is significant when controlling immature stages of these insects in the aquatic habitats. Diabate *et al*. (2002) worked in Burkina Faso and showed that resistance was affected by breeding habitats of mosquitoes because insecticides are used less in the rice fields than in cotton-growing areas and the selection pressure was therefore lower in the rice fields. He added that in the dry season, mosquitoes were susceptible to insecticides tested because insecticides for control of pests are not in use at that time. However, in the rainy season, use of insecticides to protect cotton plants was common and this exerts selective pressure on the mosquito population and results in an increase in resistance.

Both species of mosquitoes tested – *An. gambiae* and *Ae. aegypti* were found susceptible to permetrin by WHO standard (Chandre *et al*. 1999) with no appreciable difference between the two even though significant difference in susceptibility to ITNs by some species of mosquitoes has been observed (Lindblade *et al*. 2006). The absence of significant susceptibility to the test insecticide suggest that permethrin when used to treat bed nets will be highly effective for use against mosquitoes in the areas of study

The absence or very low resistance of the mosquito population observed in the present study is similar to the report of Stump *et al*. (2004) on the long term use of insecticide treated nets in western Kenya based on the presence of the knockdown resistance (kdr) gene. The kdr mechanism is a resultant of mutations originating from the voltage-gated sodium channel, the target site for DDT and pyrethroids and is one of the two most important forms of biochemical resistance that occur due to detoxifying activities of enzymes like esterases, mixed function oxidases and transferases (Brodgon & McAllister 1998).

Fifty and ninety five knockdown times (KDT_{50} and KDT_{95}) observed in the present study compare well with those from other studies for *Anopheles gambiae* populations that are categorized as susceptible (Chandre *et al*. 1999; Diabate *et al*. 2002; Kristan *et al*. 2003; Kamau & Vulule 2006). Thus these data suggest that permetrin will have high efficacy when used in the study area. By implication, the bed nets treated with this insecticide will have a dual role preventing the mosquitoes from biting people sleeping under them, but will also kill them as they perch on the nets, thereby reducing mortality and morbidity of humans to mosquitoes-borne diseases.

Brodgon & McAllister (1998) argued that for insecticide resistance to be a concern, the level must be high enough to compromise the efficacy of the intervention programmes employing the insecticides being used for vector control. This being true, the insecticide resistance level in this study is very insignificant to compromise the efficacy of any intervention programmes employing permethrin insecticide for vector control.

The results obtained in this study will guide in the choice of insecticides for use in vector control programmes in the area. In addition, the data obtained provide baseline information needed in the monitoring of the development of resistance to the insecticide arising either due to selective pressure from the use of insecticides and pesticides or through migration to the area of mosquitoes with insecticide resistant genes.

REFERENCES:

Brodgon, W. G. & McAllister, J. C. 1998. Insecticide resistance and vector control. *Emerging Infectious Diseases*, 4(4): 605-13.

Chandre, F., Darriet, F., Manga, L., Akogbeto, M., Faye, O., Mouchet, J., Guillet, P. 1999. Status of pyrethroid resistance in *Anopheles gambiae* s.l. *Bulletin of World Health Organization*, 77:230-234.

Chareonviriyaphap, T., Rongnoparut, P. & Juntarumporn, P. 2002. Selection for pyrethroid resistance in a colony of *Anopheles minimus* species A, a malaria vector in Thailand. *Journal of Vector Ecology*, 27(2): 222-229

Curtis, C. F. & Townson, H. 1998. Malaria: existing methods of vector control and molecular entomology. *British Medical Bulletin*, 54:311-325.

Diabate, A., baldet, T., Chandre, F., Akogbeto, M., Guiguemde, T. R., Darriet, F., Brengues, C., Guillet, P., Hemingway, J. & Small, G. H. 2002. The role of agricultural use of insecticides in resistance to pyrethroids in *Anopheles gambiae* s.l. in Burkina Faso. *American Journal of Tropical Medicine and Hygiene*, 67:617-622.

Finney, D. J. 1971. *Probit analysis*. Cambridge University Press, Cambridge, 3rd edn.

Gammon, D. W. 1980. Pyrethriod resistance in a strain of *Spodoptera littoralis* is correlated with decreased sensitivity of the CNS in vitro. *Pesticide Biochemistry and Physiology,* 13: 53

Gillies, M. T. & Coetzee, M. 1987. *A supplement of the Anopheline of Africa South of the Sahara*. South African Institute for Medical Research, Johannesburg.

Gimnig, T. E., Kolczak, M. S., Hightower, A. W., Vulule, J. M., Schoute, E., Kamau, L., Philips-Howard, P. A., ter Kuike, F. O., Nahlen, B. L. & Hawley, W. A. 2003. Effect of permethrin-treated bed nets on the special distribution of malaria vectors in Western Kenya. *American Journal of Tropical and Medical Hygiene*, 69:115-120.

Hawley, W. A., Phillips-Howard, P. A., ter Kuile, F. O., Terlouw, D. J., Vulule, J. M., Ombok, M., Nahlen, B. L., Gimnig, J. E., Kariuki, S. K., Kolczak, M. S. & Hightower, A. W. 2003. Community-wide effects of permethrin-treated bed nets on child mortality and malaria morbidity in western Kenya. *American Journal of Tropical and Medical Hygiene*, 68 (suppl):121-127.

Hemingway, J. & Bates, I. 2003. Malaria: Past problems and future prospects. *EMBO reports* 2000, 4, Suppl, S29-S31.

Huang, Y. M. & Ward, R. A. 1981. A pictorial key for the identification of the mosquitoes associated with yellow fever in Africa. *Mosquito Systematics*, 13(2):138-149.

Krsistan, M., Fleischmann, H., della-Torre, A., Stich, A. & Curtis, C. F. 2003. Pyrethtoid resistance/susceptibility and differential urban/rural distribution of *Anopheles arabiensis* and *An. gambiae* s.s. malaria vectors in Nigeria and Ghana. *Medical and Veterinary Entomology*, 17:326-332.

Lengeler, C. & Snow, R. W. 1996. From efficacy to effectiveness: Insecticide-treated bed nets in Africa. *Bull World Health Organization*, 74:325-332.

Lindblade, K. A., Gimnig, J. E., Kamau, L., Hawley, W. A., Odhiambo, F., Olang, G., Ter Kuile, F. O., Vulule, J. M. & Slutsker, L. 2006. Impact of sustained use of insecticide-treated bed nets on malaria vector species distribution and culicine mosquitoes. *Medical Entomology*, 43(2):428-32.

Park, Y., Lee, D., Taylor, M. F. J., Holloway J. W., Ottea, J. A., Adams, M. E. & Feyereisen, R. 2000. A mutation Leu 1029 to His in *Heliothis Virescens* F. hscp sodium channel gene associated with a nerve-insensitivity mechanism of resistance to pyrethroid insecticides. *Pesticides Biochemistry and Physiology,* 66*:*1-8

Pridgeon, J. W., Appel, A. G., Moars, W. J. & Liu, N. 2002. Variability of resistance mechanism in pyrethroid-resistant German cockroaches (Dictyopteria:Blatellidae). *Pesticide Biochemistry and Physiology*, 73: 149-156.

Rozendaal, J. A. 1989. Impregnated mosquito nets and curtains for self-protection and vector control. *Tropical Disease Bulletin*, 86:1- 41.

Ru, L., Wei, C., Zhao, J. & Liu, A. 1998 Differences in resistance to fenvalerate and cyhalothrin and inheritance of knock down resistance to fenvalerate in *Helicoverpa armigera. Pesticide Biochemistry and Physiology*, 61:79-85

Shono, T., Kasai, S., Kamiya, E., Kono, Y. & Scott, J. G. 2002. Genetics and mechanics of permethrin resistance in the YPER strain of housefly. *Pesticide Biochemistry and Physiology*, 73: 27-36.

Smart, J. 1956. *A hand book for the Identification of insects of medical importance*. Jarold and Sons Ltd, Norwich

Stump, A. D., Atieli, F. K., Vulule, J. M. & Besansky, N. J. 2004. Dynamics of the pyrethroid resistance allele in Western Kenyan populations of Anopheles gambiae in response to insecticide-*treated net trials. American Journal of Tropical and Medical Hygiene*, 70(6): 591-596.

WHO 1981. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticide-diagnostic test. WHO/VBC/81.806, Geneva, Switzerland

WHO 1992. Vector resistance to insecticides. 15th report of the WHO Expert Committee on Vector Biology and Control. *World Health Organisation Technical Report Series, 818: 1 62.*