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Evaluation of the Efficacy of Commercially Formulated Aerosol Based Pyrethroid Insecticides against *Culex* species (Diptera: Culicidae)

Hafizu, M.S[1*], Tukur, Z[2], Suleiman, K[2], Abdullahi, A.M[1], Aminu, M.A[2]

[1]Department of Biological Sciences,
Federal University of Kashere

[2]Department of Biological Sciences,
Bayero University Kano

Email: hafizdukku13@gmail.com

Abstract

Efforts to control mosquito borne diseases of serious public concern rely largely on the control of vectors using pyrethroids. Emergence of insecticide resistance makes control as well as choice of ideal insecticide difficult. Following standard guidelines effectiveness of twelve (12) commercially formulated aerosol insecticides against Culex species in Kano metropolis, Nigeria was evaluated for knockdown and mortality effect in a Peet-Grady chamber. Highest knockdown efficacy was observed with aerosol insecticide coded K11 containing; transfluthrin 0.15% and permethrin 0.23%, having KT_{50} of 3.7 ± 0.1 and KT_{95} of 10.0 ± 0.2 mins, which differ significantly ($P<0.05$) with aerosol F6, aerosol H8 and aerosol J10. All insecticides with the exception of A1 expressed acceptable limit of mortalities (≥90) for effective aerosol insecticide. This study revealed that; number of active ingredients did not affect formulation performance. Permethrin and Transfluthrin were identified as the most effective pyrethroid isomers in the control of Culex mosquitoes. This study further revealed the susceptibility of Culex species of Kano metropolis to aerosol based pyrethroid insecticides. However, significantly low knockdown effect observed in some of the insecticides, suggests knockdown resistance (kdr) in the population of the tested mosquitoes. Constant monitoring of susceptibility status of other mosquito genera is recommended to address challenges that may face the control of mosquito borne diseases.

Keywords: Aerosols, *Culex*, Pyrethroid, Knockdown, Mortality.

INTRODUCTION

Household chemical insecticides such as aerosols, mosquito coils, mats and vaporizers are the most widely used methods for personal protection against mosquitoes worldwide: because of their availability and cost effectiveness (Chin *et al*., 2017). Aerosol insecticides and mosquito coils are the most commonly used chemical insecticides for personal protections against mosquitoes in Africa (Makwaro *et al*., 2017). In the formulation of commercial aerosol insecticides, technical-grade (concentrated) pyrethrins or pyrethroids are dissolved in solvents like; kerosene, ethanol and petroleum ether (Sarwar, 2015). The World Health Organization (2009) has identified the use of pyrethroid insecticide is an important aspect of resistance management strategy.

Most commercial aerosol insecticides do not use single molecule; rather, a combination of different pyrethroid isomers and in some cases a synergist (Eremina, 2002; Bonnet *et al*, 2009).

**Author for Correspondence*

Commonly used pyrethroid isomers for the formulation of commercial aerosol insecticides include; allethrin, bifenthrin, bioresmethrin, cyfenothrin, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, esbiothrin, dimefluthrin, esfenvalerate, flucythrinate, flumethrin, fluvalinate, fenpropathrin, imiprothrin, permethrin, phenothrin, prallethin, resmethrin, tefluthrin, tetramethrin, and tralomethrin (Khadri *et al*., 2009; Pemba and Kadangwe, 2012; Chukwunwendu, 2015; Makworo *et al*., 2017; Kuri-Morales *et al*., 2018).
Pyrethroids and natural pyrethrins are the most used active ingredients in mosquito coil formulation, due to their rapid effects on insects at minimal dosages and relatively lower mammalian and environmental toxicity (Katsuda *et al*., 2008). Although household insecticides are manufactured and commercialized in some African countries (Makworo *et al*., 2017), data on the use and efficacy of these insecticides on mosquitoes is lacking. Research on the susceptibility status of mosquitoes to commonly used aerosol and mosquito coil insecticides will be of great importance for current and future mosquito borne disease interventions.

However, emergence and spread of insecticide resistance in mosquito populations observed in some parts of the world is threat to vector control success (WHO, 2017).

The genus *Culex* contains species that are among the three most important species of mosquitoes worldwide; *Culex quenquefasciatus* vector of filarial worms the causative agents of lymphatic filariasis (Mullen and Lance, 2009). Lymphatic filariasis is one of the ten (10) neglected tropical diseases considered second leading cause of long-term and permanent disability in humans (Nana-Djeunga, 2017). Efforts to control diseases of serious public concern involve control of vectors responsible for transmission of the disease agents (Sugiura *et al*., 2011). One of such methods involves the use of commercially formulated aerosol based pyrethroid insecticides, which come in various combinations. Increase in chemical insecticides resistance by mosquitoes makes the choice of chemical insecticides difficult and necessitate the formulation of new insecticides, and study of susceptibility status of mosquitoes to insecticides.

MATERIALS AND METHODS

Study Area

Kano metropolis is the business hub of northern Nigeria and the most populous city in the country. It lies between Latitude 12° 25′ and 12° 40′N and longitudes 8° 35′N and 8° 45′E. Kano State lies in the Sahelian savanna area which experiences rainfall for six months per year (May to October) and an average precipitation of 800–900mm annually. The state is typically hot throughout the year with temperatures ranging between 25 and 40°C (with a mean of about 26°C) and reaching as high as 43°C during the dry season, although from December to February the state is noticeably cooler with temperatures as low as 8°C. Entomological situation of Kano metropolis showing the presence of *Culex* species is well established. Mosquito rearing and identification using identification keys by Coatzee (2003) and laboratory evaluation of the efficacy of the insecticides was conducted in the Biological Science Department Post Graduate Laboratory, Bayero University Kano.

Knockdown and Mortality Effect of Aerosols Insecticides

Insecticide knockdown and adulticidal efficacy of twelve (12) commercially formulated aerosols (Table 1) against mosquitoes was conducted in a Peet-Grady chamber (0.7×0.7×0.7 m) as described by Makworo *et al*. (2017). A total of 25, 2-5 days old laboratory reared non-blood fed female mosquitoes were liberated in the Peet-Grady chambers and allowed for 2

minutes to acclimatize. The mosquitoes were then sprayed with 0.3 g of the aerosol. Knockdown effect was observed after every 1 minute up to 10 minutes, then at 10 minutes intervals up to 60 minutes. All mosquitoes were transferred into paper cups containing glucose solution socked in a sterile cotton wool. Mortality was observed 24 hours post exposure to the insecticides. The experiment was replicated three times for each insecticide. Before testing another batch of insecticide, chambers were washed with detergent, followed by ethanol-acetone (19:1). Temperature of 29 °C and relative humidity of 70% were maintained in the chamber during each test.

Table 1: Commercial aerosol insecticides used and active ingredients as described by manufacturers, selected conveniently based on availability.

Brand name	Active ingredient (%w/w)
A1	Imiprothrin 0.05%, prallethrin 0.05%, Cyfluthrin 0.015%
B2	D-phenothrin 0.092%, Prallethrin 0.04%, Imiprothrin 0.025%
C3	Imiprothrin 0.1%, Transfluthrin 0.25%, Permethrin 0.50%
D4	Imiprothrin 0.05%, Cypermethrin 0.10%, Prallethrin 0.9%,
E5	Neo-pynamin 0.25%, Prallethrin 0.04%, Cyphenothrin 0.05%
F6	Imiprothrin 0.02%, D-phenothrin 0.03%, D-transallethrin 0.10%
G7	Permethrin 0.25%, Tetramethrin 0.20%, Phenothrin 0.01%, Pyperonil dioxide 0.34%
H8	D-allethrin 0.25%, Tetramethrin 0.15%, Deltamethrin 0.015%
I9	Transfluthrin 0.20%
J10	Cyfluthrin 0.015%, Prallethrin 0.05%, Imiprothrin 0.05%
K11	Transfluthrin 0.15%, Permethrin 0.23%
L12	Cyfluthrin 0.025%, Transfluthrin 0.04%

Note: Codes were used to replace brand name of the insecticides

Data Analyses

The data for knockdown was subjected to Probit analysis in Statistical Package for Social Sciences 20.0 (SPSS Inc., Chicago, USA) to determined Knockdown Time (KT). Mean knockdown time and mortality values were subjected to Analysis of Variance and significant means were separated using least significant difference in Microsoft Excel (Microsoft Corporation, Washington, USA).

RESULTS

Knockdown Efficacy of Aerosols Insecticides on *Culex* species

Probit analysis of knockdown efficacy of aerosol insecticides against *Culex* species (depicted in Table 2) revealed lower knockdown efficacy with aerosol insecticide J10 having KT_{50} of 477 ± 8.9 mins and KT_{95} of $1.95 \times 10^3 \pm 34.6$ mins. Followed by H8 with KT_{50} of 99.9 ± 8.5 mins and KT_{95} of $1.89 \times 10^3 \pm 22.4$ mins. Then F6 with KT_{50} of 109.8 ± 10.5 mins and KT_{95} of $1.99 \times 10^3 \pm 16.0$ mins. Aerosol insecticide J10 KT_{50} differed significantly ($P<0.05$) with that of F6 and H8 as well as with the rest of the insecticides. Insecticide with the least KT_{50} values was D4 having KT_{50} of 3.6±0.0 mins that differed significantly ($P<0.05$) with F6, H8 and J10.

Table 2: Mean KT_{50} and KT_{95} of aerosol insecticides tested on *Culex* species

Insecticide	KT_{50}(mins) ± S.E	KT_{95} (mins) ± S.E
A1	14.4 ± 0.6^{a}	187.6 ± 2.9^{ab}
B2	14.5 ± 0.0^{a}	78.8 ± 0 .4^{a}
C3	4.8 ± 0.1^{a}	25.6 ± 1.1^{a}
D4	3.6 ± 0.0^{a}	44.4 ± 1.7^{a}
E5	18.4 ± 0.4^{a}	144.2 ± 0.8^{ab}
F6	109.8 ± 10.5^{b}	1.99 × 10^{3} ± 16.0^{d}
G7	45.8 ± 2.1^{a}	339.8 ± 9.0^{c}
H8	99.9 ± 8.5^{b}	1.89 × 10^{3} ± 22.4^{d}
I9	9.7 ± 0.1^{a}	34.5 ± 0.2^{a}
J10	477 ± 8.9^{c}	1.95 × 10^{3} ± 34.6^{c}
K11	3.7 ± 0.1^{a}	10.0 ± 0.2^{a}
L12	9.0 ± 0.1^{a}	35.0 ± 0.9^{a}

Note: Results with different superscript in the same column differ significantly (P<0.05)
KT_{50}: Estimated time in minutes required to knock down 50% of mosquitoes
KT_{95}: Estimated time in minutes required to knock down 95% of mosquitoes
A1; Imiprothrin 0.05%, prallethrin 0.05%, Cyfluthrin 0.015%; **B2**; D-phenothrin 0.092%, Prallethrin 0.04%, Imiprothrin 0.025%; **C3**; Imiprothrin 0.1%, Transfluthrin 0.25%, Permethrin 0.50%; **D4**; Imiprothrin 0.05%, Cypermethrin 0.10%, Prallethrin 0.9%; **E5**; Neo-pynamin 0.25%, Prallethrin 0.04%, Cyphenothrin 0.05%; **F6**; Imiprothrin 0.02%, D-phenothrin 0.03%, D-transallethrin 0.10%; **G7**; Permethrin 0.25%, Tetramethrin 0.20%, Phenothrin 0.01%, Pyperonil dioxide 0.34%; **H8**; D-allethrin 0.25%, Tetramethrin 0.15%, Deltamethrin 0.015%; **I9**; Transfluthrin 0.20%; **J10**; Cyfluthrin 0.015%, Prallethrin 0.05%, Imiprothrin 0.05%; **K10**; Transfluthrin 0.15%, Permethrin 0.23%; **L12**; Cyfluthrin 0.025%, Transfluthrin 0.04%.

Mortality Effect of Aerosol Insecticides on *Culex* species

Mortality of all insecticides (Table 3) with the exception of A1 having 88% was within the specified limit (of ≥90%) for effective insecticide recommended by WHO (2009). Although there was no significant difference (P>0.5) between the insecticides, the least mortality observed was with A1 (88%).

Table 3: Mean mortality effect of aerosol insecticides against *Culex* species

Insecticide	Mean Mortality (%) ± S.E
A1	100 ± 0^{a}
B2	92 ± 0.09^{a}
C3	100 ± 0.0^{a}
D4	100 ± 0.09^{a}
E5	92 ± 0.0^{a}
F6	96 ± 0.0^{a}
G7	100 ± 0.12^{a}
H8	100 ± 0.07^{a}
I9	100 ± 0.0^{a}
J10	100 ± 0.0^{a}
K11	100 ± 0.0^{a}
L12	96 ± 0.06^{a}

Note: Results with the same superscript in the same column do not differ significantly (P>0.05) with each other and with WHO (2009) specified limit for effective insecticide oof 90%
A1; Imiprothrin 0.05%, prallethrin 0.05%, Cyfluthrin 0.015%; **B2**; D-phenothrin 0.092%, Prallethrin 0.04%, Imiprothrin 0.025%; **C3**; Imiprothrin 0.1%, Transfluthrin 0.25%, Permethrin 0.50%; **D4**; Imiprothrin 0.05%, Cypermethrin 0.10%, Prallethrin 0.9%; **E5**; Neo-pynamin 0.25%,

Prallethrin 0.04%, Cyphenothrin 0.05%; **F6**; Imiprothrin 0.02%, D-phenothrin 0.03%, D-transallethrin 0.10%; **G7**; Permethrin 0.25%, Tetramethrin 0.20%, Phenothrin 0.01%, Pyperonil dioxide 0.34%; **H8**; D-allethrin 0.25%, Tetramethrin 0.15%, Deltamethrin 0.015%; **I9**; Transfluthrin 0.20%; **J10**; Cyfluthrin 0.015%, Prallethrin 0.05%, Imiprothrin 0.05%; **K10**; Transfluthrin 0.15%, Permethrin 0.23%; **L12**; Cyfluthrin 0.025%, Transfluthrin 0.04%.

DISCUSSION

Insecticide efficacy testing on *Culex* species mosquitoes yielded positive results in terms of both knockdown and mortality effects, as majority of the insecticides expressed satisfying knockdown and mortalities that were within the acceptable limit of efficacy outlined by WHO (2009). A1 which contains; imiprothrin 0.05%, prallethrin 0.05% and cyfluthrin 0.015% as active ingredients showed KT_{50} value of 14.4 ± 0.6 mins on *Culex* species. This implies that the A1 had effectively knocked down 50% of the mosquitoes in less than 15 mins after exposure of the mosquitoes to the insecticide. However, A1 observed mortality effectiveness of 88% on *Culex* species was lower than the WHO mortality efficacy acceptable limit (of ≥90%), and thus cannot be used as an effective insecticide for the control of *Culex* mosquitoes. However, the mortality values of A1 did not differ significantly (P>0.05) with mortalities of the other insecticides tested on the mosquitoes. Low mortality effectiveness of A1 can be an indication of either resistance by *Culex* mosquitoes to; imiprothrin, prallethrin and cyfluthrin active ingredients or reduced efficacy of the insecticide. Lower efficacy in terms of mortality and high knockdown efficacy of A1 observed in this study is supported by the findings of Mokworo *et al*. (2017) in Kenya, where aerosol insecticide with similar active ingredients in the same proportion as A1 tested against susceptible Kisimu strains of *Anopheles gambiae* was found to have lower mortality efficacy and high knockdown efficacy against the mosquitoes. This suggests reduced efficacy rather than resistance of mosquitoes was responsible for the low mortality effect observed with the insecticide in this study.

High knockdown and mortality effects against *Culex* species were observed with C3 & D4 which are formulation of; imiprothrin 0.1%, transfluthrin 0.25% and permethrin 0.50%; Imiprothrin 0.05%, Cypermethrin 0.10%, Prallethrin 0.9%, respectively. In less than 5 mins, 50% of the mosquitoes were knocked downed and 90-99% of the mosquitoes died 24 hours after exposure to the insecticide. Khadri *et al*. (2009) reported the effectiveness of aerosol formulation containing transfluthrin and another containing permethrin against *Aedes aegypi* mosquitoes, with mortalities of about 99% after 24 hours post exposure to the insecticides. Similarly, Makworo *et al.* (2017) reported the efficacy of aerosol insecticide formulation containing imiprothrin and permethrin against *Anopheles gambiae* with mortalities of over 97% of the mosquitoes; 24 hours post exposure to the insecticide. In contrast, permethrin resistance in mosquitoes from Ghana was reported in a study of Achonduh, *et al.* (2008) with less than 80% recorded mortalities.

Despite low knockdown efficacy on the mosquitoes, F6 expressed satisfying mortality value of 92% against the mosquitoes. However, low knockdown efficacy can play a role in future development of resistance by the mosquitoes to F6 through a Knockdown resistance (kdr) mechanism. Ibrahim *et al*. (2014) reported that this type of mutation confers resistance to pyrethroids in *Anopheles coluzii* in North Western Nigeria. Low knockdown and high mortality effect imply that insecticides act slowly on the mosquitoes to exert their effect. In field conditions this low knockdown effect may give easy room for escape by mosquitoes before the insecticide is fully saturated to exert its effect, and hence can trigger development of resistance overtime. Our finding is in contrast to that of Makwaro *et al*. (2017) where high knockdown efficacy and high mortality effect of aerosol insecticide with same active

ingredient as F6 against *An. gambiae* was observed. Since *An. gambiae* susceptible strains were used in their study low efficacy observed in our findings can be attributed to development of knockdown resistance facilitated by kdr mutation in the mosquitoes. Knockdown resistance to the pyerethroid insecticides; deltamethrin and lambda-cyhalothrin in *Anopheles gambiae* s.l has already been reported in the region (Ibrahim *et al*., 2014). However, this study is the first to report knockdown resistance in *Culex* species to imiprothrin 0.02%, d-phenothrin 0.03% and d-transallethrin which are F6 active ingredients.

In less than 10 minutes of application of H8 containing only transfluthrin as active ingredient to *Culex* species, 50% were knocked down and more than 95% of the mosquitoes died 24 hours after being exposed to the insecticide. One of the striking observations from this study is that number of active ingredients did not affect the performance of aerosols. Sugiura *et al*. (2011) supported this observation where number of active ingredients reported in their study was found to be in-significant factor in aerosols effectiveness. This finding also established susceptibility of *Culex* species to transfluthrin which has never been reported in Northern Nigeria.

Among all the insecticides used in this study G7 having permethrin 0.25%, tetramethrin 0.20%, phenothrin 0.01% and pyperonil dioxide 0.34% as synergist, is the only insecticide having synergist in its formulation. However, presence of the synergist did not make G7 supersedes most of the aerosol insecticides in effectiveness against the mosquitoes. This finding suggests that addition of synergist in aerosol insecticides formulation does not play significant role in aerosol formulation effectiveness; since some insecticides without synergist expressed more effectiveness against the mosquitoes than G7. Pemba and Kadangwe (2012) support this observation where addition of synergist was reported to have no effect on aerosol formulation effectiveness against mosquitoes.

K11 containing transfluthrin 0.15% and permethrin 0.23% only as active pyrethroid isomers in its formulation was effective against *Culex* species with knockdown efficacy of 50% of mosquitoes in less than 4 mins and 100% mortality 24 hours post exposure to the insecticide. Transfluthrin used singly in formulation as seen in I9 had expressed satisfying effectiveness against *Culex* species mosquitoes of this study. Efficacy of aerosol insecticides containing transfluthrin in field conditions has been reported in the study of Khadri *et al*. (2017), where 99% mortality was observed against *Aedes aegypti*. The researchers reported that aerosol insecticide formulations containing transfluthrin as active ingredients were more effective against *Aedes aegypti* than formulations without transfluthrin. Transfluthrin is not among pyrethroid insecticides in the WHO susceptibility diagnostic kit (WHO, 2009). Should transfluthrin be included in the kit many insecticides resistance of mosquitoes to pyrethroid findings may not have been reported.

Having only two pyrethroid isomers cyfluthrin 0.025% and transfluthrin 0.04% in its formulation, L12 expressed a remarkable knockdown and mortality effect against the mosquitoes. In about 9 mins, L12 knocked down 50% of *Culex* species mosquitoes and 97% mortality was also recorded 24 hours post exposure to the insecticide. This further supports the previous findings of this study that revealed number of active ingredients as an unimportant factor for aerosols formulation effectiveness.

In this study, low knockdown efficacy on *Culex* species was observed with H8, F6 and J10 as well as low mortality effect with A1. Common to all the four insecticides mentioned above was presence of imiprothrin as active ingredient. Low efficacy of aerosol insecticide

containing imiprothrin as active ingredient against mosquitoes was equally reported in a study conducted by Pemba and Kwadange (2012). Therefore, in this study imiprothrin was less effective on mosquitoes.

The most active insecticides against *Culex* species were B2, C3, D4, E5, G7, I9, K11 and L12. Permethrin and transfluthrin are common active ingredients in C3, K11 and L12. Transfluthrin is also present in I9. However, none of permethrin or transfluthrin was present in all insecticides that exhibited low potency against the tested mosquitoes. As a result, permethrin and transfluthrin can be used in formulation of aerosol insecticide intended for the control of *Culex* mosquitoes.

CONCLUSION AND RECOMMENDATIONS

Insecticides tested on *Culex* species of mosquitoes were mostly effective, as such they can be used in the control of these mosquitoes in Kano metropolis. Few insecticides with low knockdown effects suggests knockdown resistance among population of the tested mosquitoes. Permethrin and transfluthrin were identified as the most active pyrethroid isomers in aerosol formulation intended for the control of *Culex* mosquitoes.

Evaluation of the effectiveness of technical grade transfluthrin and permethrin as well as effectiveness in impregnated forms such as in mats, mosquito papers, windscreens, curtains, and so on is recommended. Evaluation of the efficacy of aerosols and mosquito coils against other genera of mosquitoes such as *Aedes*, *Mansonia* and *Culiseta* as well as other harmful insects is also recommended.

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