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Designing of a laboratory model for evaluation of the residual effects of deltamethrin (K-othrine WP 5%) on different surfaces against malaria vector, *Anopheles stephensi* (Diptera: Culicidae)

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Abstract

Background & objectives: Deltamethrin plays an important role in controlling malaria vectors, and is used in indoor residual spraying and manufacture of long-lasting insecticidal mosquito nets. The residual activity of deltamethrin (K-othrine WP 5%) @ 25 mg/m^2 was studied in laboratory conditions on different surfaces.

Methods: The surfaces were made artificially with different building materials such as mud, plaster, cement and wood in the laboratory. The surfaces were mounted inside petri-dishes (diam: 20 cm) and wood surface was prepared separately. The prepared surfaces were attached to the walls and deltamethrin was applied using standard Hudson pump sprayer with a discharge rate of 757 ml/min or 0.2 gal/min. The spraying was conducted at standard rhythm as recommended by WHO. After application all the sprayed surfaces were transferred into the special wooden boxes designed for this purpose. The surfaces were maintained at laboratory conditions. The WHO's recommended bioassay kit and method was used during this study.

Results: Bioassays on *Anopheles stephensi* Liston showed that the persistence of deltamethrin on different surfaces (>70% mortality) was around 4 months on plaster (Mortality = 77±6.2%), 2 months on mud (76.9±6.8%), 4.5 months on cement (79±3.2%), 4 months on wood (71.7±6.8%) and 4 months on filter papers (82.3±5.4%).

Interpretation & conclusion: The results of this study on residual effects of deltamethrin WP 5% (25 mg/m^2) are highly concordant with two field bioassays carried out in a malarious area at south-eastern Iran. This method can be replaced by the field bioassay tests which are time consuming and costlier.

Key words *Anopheles stephensi* – bioassay – deltamethrin – Iran – malaria – vector

Introduction

Indoor residual spraying (IRS) is one of the primary vector control interventions for reducing and interrupting malaria transmission. In recent years, however, it has received relatively little attention. Recent data reconfirm the efficacy and effectiveness of IRS in malaria control in countries where it was implemented well. IRS can be used in unstable, epidemic-prone malaria transmission areas, in stable-endemic malaria areas with moderately intense but seasonal transmission, in stable-hyperendemic areas where very intense seasonal or perennial transmission occurs. There are currently 12 insecticides recommended by WHO for IRS, belonging to four chemical groups (one organochlorine, six pyrethroids, three organophosphates and two carbamates)[1]. Deltamethrin [(S)-alpha-cyano-3-phenoxybenzyl (1*R*, 3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate] is a synthetic pyrethroid insec-

ticide. Deltamethrin is not mobile in the environment. With the current usage pattern and under normal conditions of use, environmental exposure is expected to be low. Deltamethrin has a high to moderate acute oral toxicity and the International Programme on Chemical Safety (IPCS) has classified it as 'moderately hazardous'[2].

According to the Department of Malaria Control Programme (unpublished data) there have been a total of around 15,000 malaria cases in 2008 in Iran. Several measures have been employed for malaria vector control including IRS, larviciding and use of pyrethroid-treated bednets, especially in southern part of the country. In southern part of the country, five anopheline mosquitoes, *Anopheles stephensi, An. dthali, An. fluviatilis, An. superpictus* and *An. culicifacies* (Diptera: Culicidae) are known to be malaria vectors[3]. *An. stephensi* is the main vector responsible for transmission of malaria in this area, and is known to be endophilic. Resistance to DDT was first recognized in 1957 and subsequently to dieldrin in 1960, and then to malathion in 1976. After report of malathion resistance in *An. stephensi,* the propoxur was used in 1978 and it was used for about 13 years. In recent years, pyrethroids are currently receiving considerable attention for residual spraying in malaria control programmes. Lambda-cyhalothrin was introduced in malaria control programmes from 1992. From 2003 onwards, deltamethrin has been used for IRS. However, no resistance has been detected in *An. stephensi* to these two latter insecticides so far. Adult resistance to DDT, dieldrin and malathion was reported in *An. stephensi,* which has been widely distributed in Persian-Gulf, Middle-east and Indian subcontinent causing operational problems for control programmes[3–5]. According to WHO[1,6], deltamethrin is recommended for IRS @ 20–25 mg a.i./m^2 for malaria vector control and its residual efficacy is estimated to be 3–6 months. Other insecticides have relatively shorter residual effect (pyrethroids: 4–6 months; organophosphates and carbamates: 2–6 months)[1]. Numerous factors such as humidity, temperature, ultraviolet light, the type of sprayed surface will affect the residual activity of pesticides which are being used for IRS. The evaluation of residual effect of any insecticide on sprayed surfaces in the field requires time and cost as well as establishing an insectary at the field condition with susceptible strain. The main objective of this study was to design a laboratory model for assessing the residual activity of insecticide on simulated surfaces which are being used by the people for construction of their dwellings. The results of laboratory test will reduce the cost and time of doing such experiments under field conditions and the results can be utilised for field conditions. This method will provide a simple method for evaluation of some insecticides prior to application in the field for malaria vector control.

Material & Methods

Preparation of the artificial surfaces: Three sorbent surfaces like cement, plaster and mud were prepared with a thickness of 1.5 cm in 20 cm diam Petri dishes. The blocks in each Petri dish were allowed to dry in laboratory conditions. The wood and filter papers as non-sorbent surfaces were cut in a situation where the conical test can be fitted on the surfaces, in a way that the bioassay test can be carried out easily. All the prepared samples were then stored inside a dark wooden box inside the laboratory. Seven blocks of each type of surfaces were prepared, from which two surfaces remained as untreated control. Five replicates of each surface were treated with insecticide. The filter paper (Whatman No.1) was attached to the sprayed walls normally and then removed and packed inside the kit for bioassay tests (Fig.1). The K-othrin WP 5% has been used at the dosage of 25 mg a.i./m^2 and the samples were collected from the Ministry of Health, Iran.

Mosquito species tested: An. stephensi (lab strain susceptible to all insecticides), 2–3 days-old females reared in the insectary at the School of Public Health & Institute of Health Research, Tehran University of Medical Sciences were used for experiments.

Residual spraying: Hudson® X-Pert compression

Fig. 1: Preparing surfaces in Petri dishes for bioassay test of deltamethrin @ 25 mg a.i./m^2

sprayer which is recommended by WHO for IRS fitted with pressure gauge and HSS-8002 nozzle tips equipped with regulator set at 24–55 PSI pressure was used. The discharge rate of sprayer was measured as 755–780 ml/min. The duration for spray was set to spray 19 m^2 in one minute. The operation was done by an expert under supervision. The dried blocks were fixed on to the vertical surfaces of the wall. One sachet (125 g) of deltamethrin was dissolved in 10 L of water into the tanks to obtain 25 mg a.i./m^2. After spraying, all the blocks were collected and stored in the box for further bioassay tests.

Insecticide susceptibility tests: Insecticide susceptibility tests were carried out in the laboratory conditions against *An. stephensi* with deltamethrin 0.05% impregnated paper provided by WHO. The procedure of test was followed according to WHO[7].

Bioassay tests: The bioassay tests were carried out for evaluation of residual effect of deltamethrin using standard WHO cones. The cone was fitted on the surface of each replicate using rubber band. About 10–12 sugar-fed female mosquitoes were gently released into each cone at the vertical position. The mosquitoes were exposed for 30 min to the treated surfaces in three replicates. The same procedures were carried out for control. At the end of exposure

Fig. 2: Bioassay test on different surfaces using deltamethrin @ 25 mg a.i./m^2 against *An. stephensi*

time, the adults were transferred into the clean cup with cotton wool pad with 10% sugar solution and the mortality was recorded after 24 h recovery period. Contact bioassay tests were carried out on Day 1, 5, 15, 30, 45, 60, 105, 120, 135, 150 after application. Relative humidity and temperature of the laboratory were recorded during the bioassay experiments[8] (Fig. 2).

Statistical analysis: Data obtained from different replicates were pooled for each surface. Additionally, in order to compare the sorbant with non-sorbant surface (wood) all the data obtained from sorbant surfaces were pooled and then compared with wood. The mortality >70% was considered as threshold level. The mortality rates were transformed into the Arc Sin $\sqrt{P}$. ANOVA tests were used for comparison. If the mortality of the control was between 5 and 20%, then the mortality of the treatment was corrected using Abbott's correction[7].

Results

The results of susceptibility tests on *An. stephensi* against impregnated paper of deltamethrin 0.05% indicated that this species is susceptible to this insecticide (mortality 98–100%).

Results of bioassay test on plaster showed 88.6–100% mortality of *An. stephensi* during the first month of application. The results are shown in Table 1. Mortality rate after 135 days of application

Table 1. Results of bioassay tests on different surfaces in ambient temperature with deltamethrin at 25 mg a.i./m^2 against *An. stephensi*

Day after application	Mortality ± S.E.				
	Plaster	Mud	Cement	Wood	Filter paper
1	88.6±1.32	81.6±5.38	100	74.31±5.91	87±4.1
5	100±0	96.44±2.34	100	92.18±3.69	91.5±3.88
15	94.54±3.13	91.52±3.8	100	89±4.5	98.33±1.79
30	98.33±3	97.5±2.24	100	100±0	95.55±3.13
Monthly mean	95.36^{a}	91.76^{a}	100^{a}	88.89^{a}	93.12^{a}
45	98±2.16	59.61±7.22	100	68.33±7.5	88.18±6.24
60	83±5.64	78.85±6.88	100	85.78±5.7	95.83±4.35
Monthly mean	91.5^{ab}	68.23^{b}	100^{a}	77^{abc}	91^{a}
105	79±5.93	50.45±6.62	82.9±75.76	80.41±6.1	91.55±4.46
120	77±6.31	55.35±6.21	78.79±4.33	71.7±6.8	83.39±5.47
Monthly mean	80.68^{abc}	53.25^{bc}	89.52^{b}	75.43^{bc}	86.9^{ab}
135	52.3±5.65	28.28±7.1	76.66±8.1	62.62±6.66	69.2±7.4
150	52.25±7.52	–	52.26±7.41	64.76±7.68	66±9
Monthly mean	64.46^{c}	28.28^{c}	58.41^{c}	63.69^{bc}	67.65^{b}

Monthly means sharing similar superscripts do not differ significantly.

reduced to 52.3% indicating that deltamethrin has a residual effect of about 4.5 months on plaster surface. Results of Tukey-HSD test revealed a significant difference between mortality of mosqutoes one month after application compared to five months after application (p <0.05).

Mortality of *An. stephensi* on mud surface ranged from 81.6–97.5% during the first month of application. Mortality after 120 days of application reduced to 50.45% indicating that deltamethrin has a residual effect of about four months on mud surface. Statistical test revealed a significance different between mortality of mosqutoes one month after application compared to four months after application (p <0.05).

Mortality of *An. stephensi* on cement surface was 100% during the second month of application and after 150 days of application it reached to 52.26% indicating that deltamethrin has a residual effect more than that of mud and plaster surfaces. There was no significant difference between mortality of mosquitoes recorded after first and second month after application (p >0.05). Similarly, to the previous surfaces significant difference between mortality of mosqutoafter one month and five months of application was observed.

Bioassay tests on wood showed that mortality of *An. stephensi* was 74.3–100% during the second month of application and after 150 days of application it reduced to 64.76%. There was no significant difference between mortalities after first and second month after application (p >0.05). There was a significant difference between mortality after one month and five months after application (p <0.05).

Mortality of *An. stephensi* on filter paper showed 87–98.33% during the second month of application and after 150 days of application it reduced to 66%. There was no significant difference between mortalities after first and second month after application (p >0.05). There was also a significant difference between mortality after one month and five

months after application (p <0.05).

Discussion

The results of susceptibility tests on *An. stephensi* against deltamethrin (0.05%) impregnated paper indicated that this species is susceptible (mortality 98–100%). Insecticide susceptibility tests on laboratory and field collected adults of *An. stephensi* from malarious areas in Hormozgan province showed that they are susceptible to bendiocarb, propoxur, malathion, fenitrothion, deltamethrin, permethrin, cyfluthrin, and lambda-cyhalothrin, but are resistant to DDT and showed low level of tolerance to dieldrin[4]. The resistance to DDT and dieldrin in *An. stephensi* in other parts of malarious area in Iran were also reported[3]. However, there is no evidence of resistance to other insecticides at the larval stages of other malaria vectors in Iran[9].

Pyrethroid resistance in *An. gambiae* and *An. arabiensis* had been reported from different parts of Africa[10–23]. Different methods of malaria vector control have been employed in Iran[24,25]. Due to occurrence of insecticide resistance the monitoring and evaluation of resistance have been documented since malaria eradication in the country [26]. Prior to application of any insecticide for malaria control programme it is recommended that durability and residual effect of the insecticide should be evaluated.

Our results of bioassay tests on different surfaces showed a significant decrease in mortality over five months after application on all surfaces. However, results of Tukey-HSD test revealed a significant difference between mortality rates on mud surfaces compared to others. There is also significant difference between mortalities of mosquitoes expused to wood and cement surfaces (p <0.05) (Table 2).

In a study carried out by Abtahi *et al*[27] in Sistan and Baluchistan Province in Iran against *An. stephensi,* the persistency and residual effect of deltamethrin 5% at the dosage of 25 mg/m^2 was three months at the most. Afterwards persistence and residual effect decreased on absorbent and non-absorbent surfaces. The residual effect of deltamethrin 10% @ 20 mg/m^2 on different surfaces was estimated to be 2.5 months. Field assessment of the residual effect of deltamethrin (20 mg/m^2) against *An. arabiensis* on mud and cement-walled surfaces in Mpumalanga Province, South Africa showed that its bioefficacy was up to seven consecutive months after treatment with 100% mortality[28].

Table 2. Results of bioassay tests on different surfaces in ambient temperature with deltamethrin e 25 mg a.i./m^2 against *An. stephensi* at different interval months

Month ofter spray	Filter paper	Wood	Cement	Mud	Plaster
1	93.12	88.89	100	91.76	95.36
2	91	77	100	68.23	91.5
4	86.9	75.43	89.52	53.25	80.68
5	67.65	63.69	58.41	28.28	64.46

A village-scale trial of deltamethrin (K-othrin) at a dosage of 25 mg/m^2, carried out against both anopheline and culicine mosquitoes in India reported to be effective in reducing the overall vector population for about 10 to 12 wk. Contact bioassays also indicated that the insecticide has a residual life of about 12 wk both on mud and cement plastered surfaces. Survival rate of the vector population was observed to be below 50% up to 8–10 wk[29].

There are several reports on the efficacy of deltamethrin on different surfaces against different species of malaria vectors worldwide[30–35]. Results showed that the efficacy of deltamethrin mainly depend on location, concentration of insecticide used, formulation, the surface, humidity, temperature and the method of evaluation; and the residual effect ranged from 2.5 to 9 months. The results of the present study suggested varied durability of deltamethrin on different surfaces. Therefore, persistence of insecticides should be considered in spray round and spry cycle.

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