Persistence and residue activity of deltamethrin on indoor residual spraying surfaces against malaria vectors in southeastern Iran

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1. Introduction

Malaria is one of the most important insect borne diseases in tropical and sub tropical regions in the world[1,2]. In Iran, disease was existed from past ages until now and according to the latest reports, 61% of cases occur in southeastern of Iran[3]. Proven and preliminary vector in this area is Anopheles stephensi (An. stephensi) and disease is called obstinate malaria[4,5]. Different methods are used for malaria control in this region and indoor residual spraying (IRS) is one of the most important of them. According to its name, IRS consists of spraying on indoor surfaces (e.g. walls of rooms, warehouses, stable, shed, etc) with the insecticides that keep their efficiency in transmission period and kill or repel vectors[6].

Numerous factors affect the mortality rate of mosquitoes that have contact with treated surfaces, e.g. degradation of insecticide as the result of reaction with alkaline, soil alkaline or other factors in different surfaces, resistance to insecticide in mosquitoes, environmental factors, etc. Evolution of these factors is important to choose appropriate insecticides and to determine times of use of this insecticide in activity periods of vectors in implicated regions.

In this study we considered and compared two major causes of decrease of mortality. So that, mortality rate of An. stephensi on different sprayed surfaces was investigated by conical test as a bioassay method and chromatographic method as an analytical method were used for evolution of persistence and residue of deltamethrin insecticide. Results were investigated statistically by ANOVA and Tukey–HSD tests for determining relations or differences between residue and persistence of deltamethrin. Results: According to the results, there was no significant difference between mortality rates from bioassay tests on different surfaces, and deltamethrin kept its utility to malaria vector control until 120 days after indoor residual spraying on these surfaces. In the case of residue, there was no significant relation between residue amounts and mortality rates on different surfaces, whereas this relation existed between residual amounts on filter papers and mortality rates from bioassay tests. Conclusions: This study shows that measurement of residue in filter papers is a suitable tool for evolution and dictum of efficiency of deltamethrin insecticide in indoor residual spraying for malaria control.
2. Materials and methods

2.1. Deltamethrin

This insecticide is a cyanogroup pyrethroid and is among the first photo stable synthetic pyrethroids[7]. In this work deltamethrin, with commercial name K-Othrin®, as wettable powder (5%) was sprayed at the rate of 25 mg.a.i/m² on the surfaces (plaster, muddy and wooden surfaces) and on the filter papers that installed on these surfaces.

2.2. Indoor residual spraying (IRS)

IRS was carried out by a standard X–Pert Hudson® pump with 10 liter capacity and nozzle 8002. Pressure of solution in pump was 25–55 pond/inch² and outcome rate was 757 c.c/min. For better evolution of pesticide residue in similar condition, filter papers were erected on surfaces before spraying and with each sampling from surfaces (gratage), papers were also tested for deltamethrin residue.

2.3. Bioassay test

Persistence of insecticides was evaluated by the method of Raghavendra et al[8]. Tests were carried out on the basis of suggested method by WHO named conical test[9]. Laboratory blood fed strain of An. stephensi females was used in the tests. This Anopheles was resistant to dichlorodiphenyltrichloroethane (DDT), dieldrin and malathion in Iran[10].

Thus after IRS in the beginning of vector’s pick in this region (September), tests were carried out every 15 day and continued until 4 months later that in this time mortality rate was decreased under 50%[11]. In this method, we set three standards conical on each surface and released 10 blood fed mosquitoes into them with fresh aspirators. The exposure time was 30 minutes and after that, mosquito was removed into the fresh caps and mortality was checked out for 24 hours. Also one conical was set for each three conical on the fresh surfaces as control. If mortality rate of control group was 3 to 5% the results were corrected by Abbott’s formula, and if this was more than 20%, tests were repeated.

2.4. Quantitative analysis of pesticide residue by thin layer chromatography

In this survey, HPTLC was used to determine deltamethrin residue in prevalent surfaces (plaster and mud surfaces) and filter papers. In numerous studies this method had been used to determine residue of pyrethroid pesticides[12].

For sampling, at the same time of bioassay tests, 3 samples from up, down, and median of each surfaces with dimension (10 cm × 10 cm × 1 cm) were picked up. Before spraying, filter papers were also set up on surfaces at sufficient numbers, and at the same time of sampling from surfaces, papers were also picked up randomly from 3 point of surfaces.

Samples preparation was carried out in 3 phases: extraction, partition and clean-up and concentration.

2.4.1. Extraction

For pesticide extraction from plaster and mud, samples were homogenized by Chinese mortar and then in the course of stages were performed by shaking and filtering with acetone as a extraction solvent[7,13,14].

2.4.2. Partition and clean-up

Pyrethroids were co-extracted with a wide variety of other lipophilic compounds during extraction, therefore different solvents were used to decrease or remove these co-extracts[7], in this study hexane was used to remove these compounds[13].

Different materials were used for clean-up e.g. florisil, alumina and silica and in this work silica gel was used[7]. Because of detection limit in TLC and other chromatographic methods, samples were concentrated at last.

Paper samples were also extracted with acetone and because of lack of co-extract, two procedures were only carried out i.e. extraction and concentration.

The organic solvent (as mobile phase) for developing deltamethrin spot was 90:10 hexane-ethyl acetate mixture. This solvent was poured in chamber tank and the prepared plates were put in it after saturation of tank space (about 30 minutes).

For quantitative measurement, HPTLC was used.

In this test, development of spots and running of solvent was done in 20–25 minutes. Then the plates were exited from the tank and the spots were seen by fluorescent light in UV cabinet at 254 nm. After development, retardation factor (Rf) value was calculated for each insecticide. The chromatographic zones corresponding to spots of deltamethrin were scanned by using of TLC scanner 3 (V.1.14 S/N: 080320) (CAMAG company, Switzerland) and CATS4 software (version 4.06, S/N: 0805A007), in reflection / absorption measurement mode, the source of radiation utilized was the deuterium lamp. At the end, the amounts of deltamethrin of each spot and their Rf values were determined. The position of a substance zone (spot) in a thin layer chromatogram can be described by Rf. This is defining factor (Rf) value was calculated for each insecticide.

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2.4.1. Extraction

For pesticide extraction from plaster and mud, samples
The results of bioassay tests on plaster, muddy and wooden surfaces were show in Figure 1–3. These results were mortality ratio of *An. stephensi* that used arcsin formula for analysis data and for normalization them, and these ratios were compared in error level 5% (α =0.05) together. According to the obtained results, there was significant differences between mortality rate from bioassay tests on plaster surfaces for 120 days after spraying with 30, 45, 60 and 70 days. There was also significant differences between 30 days after spraying with 105 and 120 days (α <0.05). All of these results obtained were statistically different by ANOVA and Tukey–HSD tests. Moreover, mortality rate in two first rounds was less than next 5 round (Figure 1).

![Figure 1](image1.png)

**Figure 1.** Mortality rate of *An. stephensi* exposed to plaster surfaces treated with deltamethrin (5%) at 25 mg/m².

3.2. Comparing persistence of deltamethrin on three surfaces

Tests were carried out in different times on surfaces, therefore two ways ANOVA was used to determine the relation between different surfaces and different times. Effect of surface, time and reciprocal effect of these two factors was evaluated, and determined that there was a reciprocal effect between type of surfaces and different times (\(P<0.001, df=16, F=3.039\)). Totally, on the strength of mortality rate on the first 60 days of survey, the highest persistence of deltamethrin 5% in rate of 25 mg/m² was on wooden surfaces as non absorbent surface and muddy and plaster surfaces as absorbent surfaces were in the next ranks.

![Figure 4](image2.png)

**Figure 4.** Residue of deltamethrin on plaster surfaces as compared with mortality rate on these surfaces.

3.3. Determination of residue of deltamethrin in plaster and muddy surfaces and filter papers

On the muddy surfaces, analysis of results showed that there was a significant difference between the 5th day (100%) against days of 30, 60, 105 and 120 (α <0.05) (Figure 2). On muddy surfaces, mortality rate decreased at 63.33% 4 month later after spraying showed high persistence in contrast of plaster surfaces.

Figure 3 showed the results of bioassay tests on wooden surfaces. Extent of mortality rate was 90%–100% until 45 days after spraying and at the end of study it was approximately 66% that showed higher persistence against two other surfaces (mud and plaster surfaces) among absorbent surfaces. On wooden surfaces there was a significant difference between the 30th day after spraying with 90, 105, and 120 days after spraying (α <0.05),

![Figure 3](image3.png)

**Figure 3.** Mortality rate of *An. stephensi* exposed to wooden surfaces treated with deltamethrin (5%) at 25 mg/m².

On the 120th day, the highest persistence of deltamethrin in rate of 25 mg/m² was on wooden surfaces as non absorbent surface and muddy and plaster surfaces as absorbent surfaces were in the next ranks.
3.4. Evolution of relation between residue of deltamethrin in plaster and mud and filter papers

Statistical analysis by analysis of variances showed that there was no significant difference between plaster and muddy surfaces but it was significant between papers and two mentioned surfaces (P<0.002), and regression formula for relation between them was: \( R_{\text{paper}} = 0.741(R_{\text{surface}}) + 10.35. \)

3.5. Evolution of relation between persistence and residue of deltamethrin

By statistical analysis it was determined that there was no significant relation between pesticide residue on plaster and muddy surfaces and mortality rate of An. stephensi on these surfaces (P=0.122). But in the case of filter papers, if the results of 15 and 30th days were omitted (because of exo-to-repellency effect of deltamethrin that caused decrease mortality rate) it was determined that there was a significant relation between pesticide residue in papers with persistence of deltamethrin on different surfaces as plaster, mud and wood (r=0.301, P=0.017). Regression formula of this relation was:

\[
\text{Mortality rate} = [0.002(R_{\text{paper}}) + 0.761] \times 100
\]

Figure 5. Residue of deltamethrin on muddy surfaces as compared with mortality rate on these surfaces.

Figure 6. Residue of deltamethrin in filter papers as compared with average of mortality rate on all surfaces.

4. Discussion

According to the results, there were no significant differences between mortality rate of An. stephensi in bioassay tests on prevalent surfaces (plaster, mud and wood surfaces). This difference is only about times of tests and after 120 days of spraying, this insecticide kept its utility in the malaria vector control. Thus after 120 days after spraying, average of mortality rate on these surfaces reached to 60%.

In the case of all the three surfaces, there is a decrease of mortality rate in the 1st, 2nd and 3rd round of tests as compared with 45th and 60th days. This reduction in mortality rate probably is because of high exito-repellency effect of this insecticide that caused to avoid the mosquitoes to rest on these surfaces. This phenomenon was previously tested in laboratory condition on An. stephensi by Er-test box method[18-24]. In laboratory and field study of permethrin-impregnated clothes against An. stephensi, 4 species of Culex and Plebotomus papatasi, exi-to-repellency effect has also been seen[19-28]. This subject also is cleared by investigation of pesticide residue on surfaces in our survey, because there is no reduction in residue in the 1st, 2nd, and 3rd round in comparison with 4th and 5th round. This specification is higher on absorbent surfaces (plaster and mud surfaces) and it is not high on non absorbent surfaces (wood surface). It is probably due to be left over high concentration of pesticide as a thin layer on surfaces.

There are many of living and non living environmental agents that affected insecticide durability on different surfaces. One study has shown that Bacillus cereus (strain L12), is able to degrade cypermethin to 3-phenoxybenzoic acid (3-PBA)[29-31]. Laboratory studies and fate model predictions suggest photolysis will be an important process in the overall degradation of etofenprox in a rice field environment[32]. Insecticides durability also depended on kinds of sprayed surfaces. Pyrethroids are more resistance on impervious surfaces, such as concrete[33,34].

In the case of residue, especially for filter papers, notable results were obtained. Considering quality, type, compound and usage type of soil that are different, therefore outcome results generality of insecticides residue on the different surfaces of wall didn’t seem correct very much and it is not a suitable tool for prediction bioassay methods and/or other measurement methods for insects resistance to insecticides; but survey of residue in filter papers is a suitable tool for dictum and result generality. Furthermore, measurement of residue in filter papers is very cheaper, simpler and more specific than soil. Also best fitness is between mortality rate and pesticide residue in papers and by investigation of correlation coefficient in regression formula related to this conformity, it is observed that the highest number of this coefficient is about relation between residue in papers and its mortality rates. Reduction of deltamethrin insecticide on the plaster, mud and papers has significant variance at the different times as the same as mortality rates in bioassay tests and because of significance relation between deltamethrin on papers and insecticide residue on the prevalent surfaces as plaster and mud as well as mortality rate in bioassay tests in these surfaces, this method (residue of insecticides in papers) can be used nicely for measurement of persistence and residual effects of this insecticide.

Conflict of interest statement

We declare that we have no conflict of interest.
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