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Asian Pacific Journal of Tropical Biomedicine

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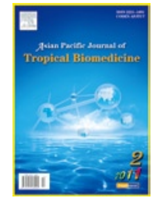

Document heading

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

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ARTICLE INFO

Article history:
Received 19 August 2011
Received in revised form 5 September 2011
Accepted 28 September 2011
Available online 15 October 2011

Keywords:
Deltamethrin
Persistence
Pesticide residue

Malaria vector
Indoor residual spraying
Insecticide
Malaria

ABSTRACT

Objective: To evaluate the efficacy of deltamethrin and find a relation between persistence and residue of this insecticide on the prevalent surfaces against malaria vectors in southeastern Iran. **Methods:** After indoor residual spraying on prevalent surfaces in studied areas (plaster and mud as absorbent surfaces, wood as non absorbent surface and filter paper as control) for malaria control, conical tests 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.

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 in transmission period of malaria in this area and degradation procedure of this insecticide in the length of time in more prevalent surfaces (plaster and muddy surfaces) and filter papers was measured with high performance thin layer chromatography (HPTLC) as a chromatographic method. Insecticide resistance was evaluated by bioassay method, and degradation process by chromatographic methods on prevalent surfaces. At last, for determination of relations between two mentioned factors, these factors were compared with each other.

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Foundation Project: This work was financially supported by Tehran University of Medical Scinces (grant No. TUMS-HFI/2008/898821).

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/m^2 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/$inch^2$ 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 60%[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 5%–20% 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 deltamethrine 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 (R_f) 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 R_f values were determined. The position of a substance zone (spot) in a thin layer chromatogram can be described by R_f. This is defining as the quotient obtained by dividing the distance between the substance zone and the starting line by the distance between the solvent front and the starting line[15,16]. To prevent from spot distribution during development in plaster and muddy samples, 0.2 cc acetic acid was added in mobile phase also[17].

In this spot development's system, R_f was in 0.3–0.7 that it was an ideal range for the spot scanning. UV was utilized as a reagent for developed spots by UV cabinet. The final determination of developed spots on plates was based on measuring in a HPTLC scanner. The best wavelength for track scanning was 206 nm that deuterium lamp provides this wavelength[17].

Because of presence of other materials, non haemogenesis samples, kind of solutions and other factors, there is usually no perfect extraction and measurement of investigated compounds from samples, therefore we should determine recovery rates in all stages for proper compound(s). In this study, recovery rate for soil samples (plaster and mud) was (25±5)% and for filter papers was (95±5)%.

3. Results

3.1. Bioassay tests

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).

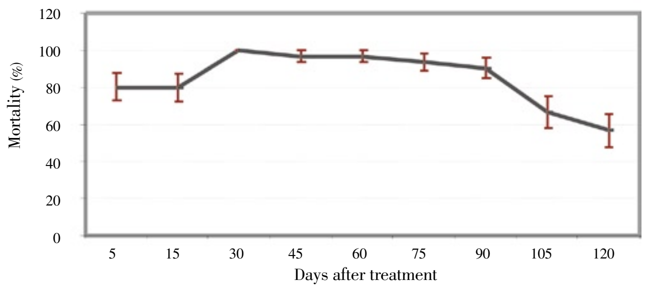

Figure 1. Mortality rate of *An. stephensi* exposed to plaster surfaces treated with deltamethrin (5%) at 25 mg/m^2.

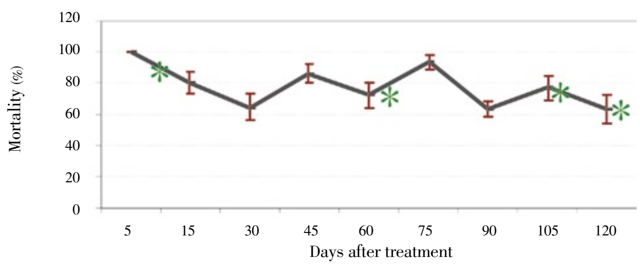

Figure 2. Mortality rate of *An. stephensi* exposed to muddy surfaces treated with deltamethrin (5%) at 25 mg/m^2.

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).

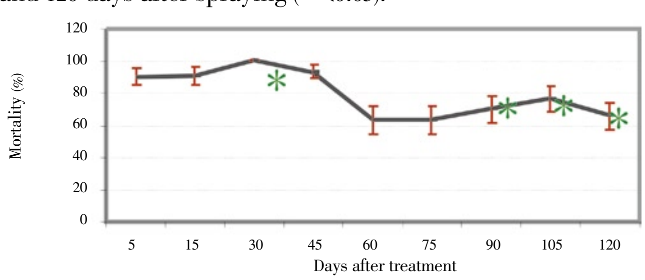

Figure 3. Mortality rate of *An. stephensi* exposed to wooden surfaces treated with deltamethrin (5%) at 25 mg/m^2.

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/m^2 was on wooden surfaces as non absorbent surface and muddy and plaster surfaces as absorbent surfaces were in the next ranks.

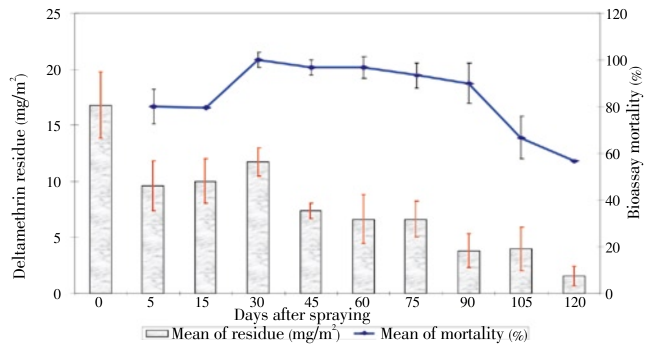

Figure 4. Residue of deltamethrin on plaster surfaces as compared with mortality rate on these surfaces.

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

Range of changes of residue in the 1st month on plaster surfaces in the course of four sampling (with three repetitions in each sampling) was between (6.93-22.75 mg/m^2) with average of 12.05 mg/m^2.

Statistically analysis of pesticide residue showed that there was a significant difference between one month after spraying with 3 months after that (P<0.05). After 4 months also average of residue was 1.54 mg/m^2 that conformed with 56.67% of mortality rate on plaster surfaces (Figure 4).

Average of residue in muddy surfaces was 11.57 mg/m^2 and there was significant differences between 1-60 days after spraying with 3 and 4th months (α<0.05). On the 120th day also the amount of residue was 1.61 mg/m^2 that conformed with 63.33% mortality rate of bioassay tests (Figure 5).

Average of residue in filter papers was 20.38 mg/m^2 that conformed with (90.00$\pm$5.53)% mortality on all surfaces. According to the statistical analysis of pesticide residue in filter papers, there were no significant differences between residue and times of sampling. Also 4 month after spraying average of residue in papers was 12.74 mg/m^2 that conformed with (61.98$\pm$2.58)% mortality on all surfaces in bioassay tests with *An. stephensi* at the same times (Figure 6). According to the Figure 6, competition of average mortality of bioassay tests during all of study with pesticide residue in filter papers at the same times, showed a high fitness on one graph, and subsequently mortality rate would be predictable by having amount of residue in papers at the same times. Also, because of higher recovery rate of papers in TLC method (90$\pm$5)%, pesticide residue in papers was more than that in soil samples.

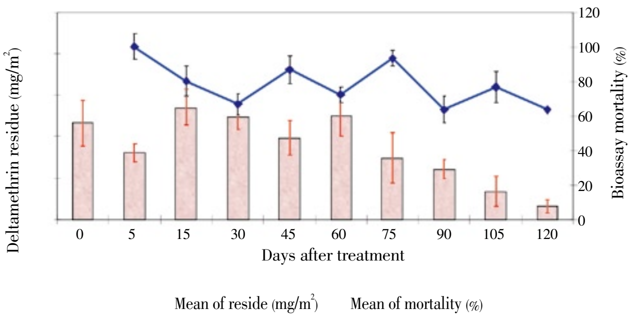

Figure 5. Residue of deltamethrin on muddy surfaces as compared with mortality rate on these surfaces.

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 differences 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: $R_{paper}=0.741(R_{surface})+10.35$.

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 exito-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:

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

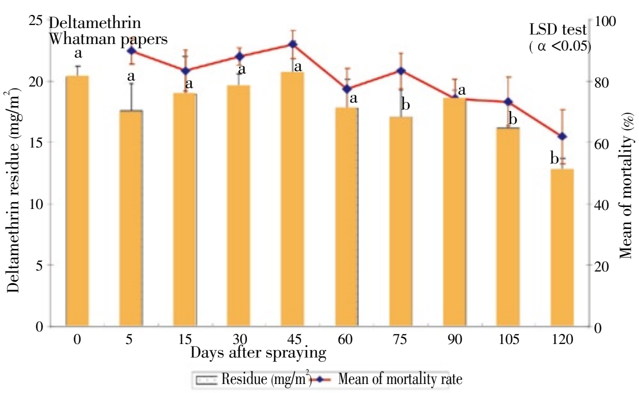

Figure 6. Residue of deltamethrin in filter papers as compared with average of mortality rate on all surfaces.

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*, exito-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 this 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.

Acknowledgments

We would like to thank all staff of Iranshahr Center of Education and Public Health Research, Tehran University of Medical Sciences, Iranshahr, southeast of Iran for their kind cooperation. This study was financialy suported by Tehran university of Medical Scinces, Grant No. and Project No. is TUMS-HFI/2008/898821.

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