# Lethal and Residual Effects of Lambdacyhalothrin, Deltamethrin and Cyfluthrin Insecticides on Adult Mosquitoes of *Anopheles stephensi* Liston (Diptera: Culicidae) on Different Surfaces

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## Abstract

**Background:** Human malaria remains a major malady in Eastern Iran. Vector control interventions such as indoor residual spraying are used to fight with the disease. This study was undertaken to determine the lethal and residual effects of three different pyrethroid insecticides on adult mosquitoes of *Anopheles stephensi* on different surfaces in Iran, as part of a national program to monitor insecticide resistance in endemic areas.

**Methods:** Two main endemic foci were selected as collection sites. Wild adult females of *An. stephensi* (*mysoriensis* strain) from the first focus were subjected to standard susceptibility tests, using lambdacyhalothrin, deltamethrin and cyfluthrin insecticides within holding tubes according to the method proposed by WHO. In Kazerun, the residual effects of these compounds were examined by conical bioassay tests of *An. stephensi* (*type* strain) on plaster and cement walls. Data were analyzed using Mann-Whitney test to determine the differences in susceptibility and residual effects of *An. stephensi* mosquitoes to these insecticides.

**Results:** The susceptibility of females of *An. stephensi* to three concentrations of lambdacyhalothrin, deltamethrin and cyfluthrin insecticides culminated in full scale mortality at the highest diagnostic dose. The maximal residual time of these three insecticides on plaster and cement walls was estimated to be about three months. There was no significant difference in the mortalities of *An. stephensi* on different sprayed surfaces ($P$=0.653).

**Conclusion:** All field-collected *An. stephensi* populations exhibit gross susceptibility to all diagnostic doses of the three evaluated insecticides. In endemic areas, lambdacyhalothrin reveals a slightly longer residual activity than the other two insecticides.

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## Introduction

Human malaria caused by the infectious bites of adult female *Anopheles* mosquitoes is the most important vector-borne infection in Iran, though it is on the verge of elimination in the endemic oriental parts of this country.[1] Although a total of five malaria vectors including *Anopheles stephensi*, *An. culicifacies*, *An. fluviatilis*, *An. superpictus*, *An. dthali* and a suspected one *An. pulcherrimus* have regionally been incriminated,

only the first species is presently considered since it is a predominantly endophilic and endophagic vector of malaria in most of the Eastern Mediterranean regions and the Indian subcontinent.[2]

The use of indoor residual spraying (IRS) and long lasting insecticidal nets (LLIN) are but two mainstay interventions in malaria control in this region.[3,4] The latter (LLIN) is reported elsewhere.[5] The former method (IRS) is an important vector control intervention to reduce/interrupt malaria transmission. Finding residual activity of pyrethroid insecticides, which are currently sprayed in malaria control operations in south and southeast Iran, is crucial for use in IRS. The pyrethroid insecticides are appropriate in that they have swift knock-down effect, high insecticidal property and low mammalian toxicity.[6] Despite application of lambdacyhalothrin and deltamethrin in Iran since 1992 and 2003, respectively, their residual effects have not been considered under field condition in endemic areas.[7] According to the WHO criteria, mortality rates of 98-100%, 80-97%, and <80% represent susceptibility, tolerance and resistance, respectively.[8]

Insecticide resistance is also increasingly prevalent. The recent evolution and distribution of anopheline mosquitoes resistant to the pyrethroid insecticides is threatening, leading to reduction of the potency of vector control efforts.[9] Further selection of pyrethroid resistance seems inevitable as malaria elimination goal is fortified.[10,11] This problem is more exacerbated in endemic regions where other concomitant infectious vectors, pathogens, or pests prevail.[12-15]

Numerous parameters, such as reaction and subsequent degradation of insecticides on exposure to alkaline soil, humidity, temperature, UV light or other factors on different surfaces, environmental factors, insecticide susceptibility of mosquitoes, *etc*., influence the mortality of vectors that have been in contact with treated surfaces.[16] Evaluation and analysis of these factors is critical for selecting the appropriate insecticides and finding times of application on different surfaces during activity periods of vectors in endemic areas.

The extensive use of pyrethroids and the challenges of mosquito resistance to these chemical compounds are the main reasons that prompted the researchers to undertake this study. As part of an investigation to determine the level of resistance among the predominant malaria vectors in Iran, this study was conducted to examine the lethal and residual effects of three well-known pyrethroid insecticides (namely lambdacyhalothrin, deltamethrin, and cyfluthrin) against adult female mosquitoes of *Anopheles stephensi* Liston (Diptera: Culicidae) on plaster and cement surfaces in endemic parts of eastern and southern Iran.

## Materials and Methods

*Study Area*

The first part of this study for susceptibility tests of three different pyrethroid insecticides on field-collected adult female mosquitoes of *An. stephensi* (*mysoriensis* strain) was carried out in the city of Nikshahr which is located at 60°12`E, 26°12`N at an altitude of 510 meters above the sea level (Figure 1).


**Figure 1:** Map of Iran shows the location of study sites in Nikshahr and Kazerun cities, south Iran.

It is the third largest city (Area=23930 km$^2$) with five districts and a population of 148901. The mean annual ambient temperature and relative humidity of Nikshahr are 32°C and 36.8%, respectively. This focus is located in the Sistan-Baluchistan province bordering the neighboring country of Pakistan. It has a tropical climate and the people are mainly involved in agricultural activities.

The second part of the present study for residual activity of three different pyrethroid insecticides on adult female mosquitoes of *An. stephensi* (*type* strain) was carried out in the city of Kazerun (51°39`E, 29°37`N) at an altitude of 860 meters above the sea level, located in the southern Fars province of Iran. It is the third most populous city (population=254704) with four districts (Area=4062 km$^2$) in the province. The mean annual ambient temperature and precipitation of Kazerun are 28°C and 522mm, respectively. This focus has a subtropical climate and agricultural activities are the main source of outcome.

*Mosquito Collection and Rearing*

The field collection of larval *An. stephensi* mosquitoes at Nikshahr was conducted during the malaria transmission season. Wild *An. stephensi* larvae were transferred from their natural habitats to the local insectarium. They were reared to adults at 27±2 °C at a relative humidity of 80±10%. First generation adults were reared from the larvae which were collected from the study area.

*Insecticides*

Three different synthetic pyrethroid insecticides are formulated as wettable powder (WP) and known as:

• lambdacyhalothrin 10% at concentrations of 0.02, 0.03 and 0.0625 mg active ingredient/m$^2$,

• deltamethrin 5% at concentrations of 0.02, 0.05 and 0.0075 mg a.i./m$^2$, and

• cyfluthrin 10% at concentrations of 0.02 and 0.05 mg a.i./m$^2$ produced by Levant Overseas Developments Ltd. (France), dated 2009, were examined in the following experiments.

*Susceptibility Tests*

A total of 1200 adult (2-3 days old) female mosquitoes of *An. stephensi* (*mysoriensis* strain) fed with 10% sugar solution were used in experiments. Insecticide susceptibility tests with deltamethrin-, lambdacyhalothrin-, or cyfluthrin-impregnated Whatman filter papers (12x15cm) against *An. stephensi* (*mysoriensis* strain) adult unfed females using standard WHO holding tubes were conducted under laboratory conditions. The exposure period for 25 mosquitoes in each replicate was one hour at a diagnostic dose. Following the exposure, the mosquitoes were allowed to feed again. Mortality was scored after 24 hours of recovery period. Each insecticide dose had 4 repeats and a total of 32 replicates were recorded.

*Residual Spraying*

A standard X-Pert® Hudson compression pump sprayer (10 liters capacity) as recommended by WHO for IRS operation fitted with HSS-8002 nozzle and a regulator-adjusted pressure gauge set at 25-45 psi pressure was used. The discharge rate of the insecticide was 757 ml/min. This operation carried out at Kazerun, Fars province, southern Iran, was supervised by an expert.

*Bioassays*

Three imagicides were tested at the diagnostic doses to determine their residual effects according to the WHO procedure.[8] They were deltamethrin 5% (concentration of 0.05 mg a.i./m$^2$), cyfluthrin 10% (0.05 mg a.i./m$^2$) and lambdacyhalothrin 10% (0.0625 mg a.i./m$^2$). To spray on cement and plaster surfaces, 5280 adult (4-5 days old) blood-fed female mosquitoes of *An. stephensi* (*type* strain) were used. These tests were conducted to evaluate the residual effects of the three above-named pyrethroid insecticides using standard WHO cones. The tests were started from October 2012 and repeated every fortnight up to 105 days later so that the mortality rate dropped to <80%. For each replicate, 10 adult female mosquitoes were aspirated into each cone. After half an hour exposure on each surface, 10 replicates from the top, middle and bottom of each wall were recorded. Two control groups from unsprayed surfaces >100 m away from treated walls were also considered. Mortality was scored after 24 hours of recovery period.

*Data Analysis*

If the control mortality was between 5 and 20%, the treatment mortality was then corrected using Abbott's formula.[17] The data were analyzed using Mann-Whitney test.

## Results

The present data indicated that there was full scale susceptibility to all studied pyrethroid insecticides at the higher diagnostic doses (*i.e.* 0.05-0.06) (Table 1). In the case of cyfluthrin and deltamethrin, tolerance developed in mosquitoes during the first 35 days post-spraying. *An. stephensi* (*mysoriensis* strain) mosquitoes exhibited tolerance to the lower doses of lambdacyhalothrin and deltamethrin, but not to cyfluthrin.

The outcome of bioassay tests on *An. stephensi* adult female mosquitoes (*type* strain) to evaluate the residual effects of the above mentioned insecticides is shown in Figures 2 and 3. No significant difference is found between

**Table1:** Mortality of *An. stephensi* to different pyrethroid insecticides at diagnostic dose after one hour exposure and 24 h recovery period, in Nikshahr, Iran.

| Insecticide | Applied Dose mg a.i./m² | Replicates | Tested mosquito no. | Mortality | %Mortality | Standard error |
|---|---|---|---|---|---|---|
| Lambdacyhalothrin | 0.02 | 4 | 100 | 94 | 94 | 2.58 |
| | 0.03 | 4 | 100 | 95 | 95 | 1 |
| | 0.0625 | 4 | 100 | 100 | 100 | 0 |
| Cyfluthrin | 0.02 | 4 | 100 | 98 | 98 | 1.15 |
| | 0.05 | 4 | 100 | 99 | 99 | 1 |
| Deltamethrin | 0.02 | 4 | 100 | 96 | 96 | 0 |
| | 0.05 | 4 | 100 | 100 | 100 | 0 |
| | 0.0075 | 4 | 100 | 97 | 97 | 1.91 |
| Control | --- | 32 | 400 | 14 | 3.5 | 0.32 |

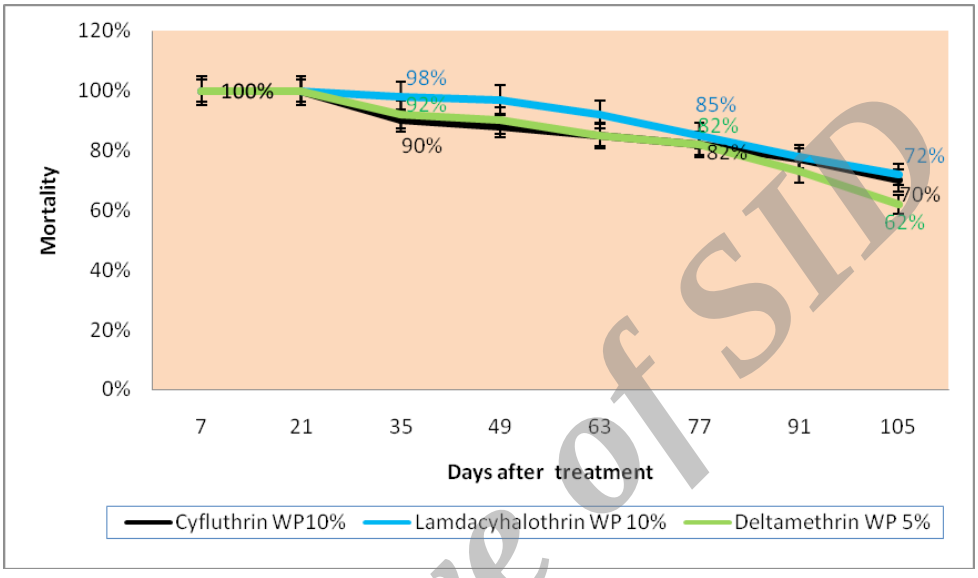


**Figure 2:** Plots show efficacy of experimental dosages (0.05, 0.0625, 0.05 mg ai/m²) of cyfluthrin WP 10%, lambdacyhalothrin 10% and deltamethrin 5% on plaster surfaces, Kazerun, southern Iran, 2012.

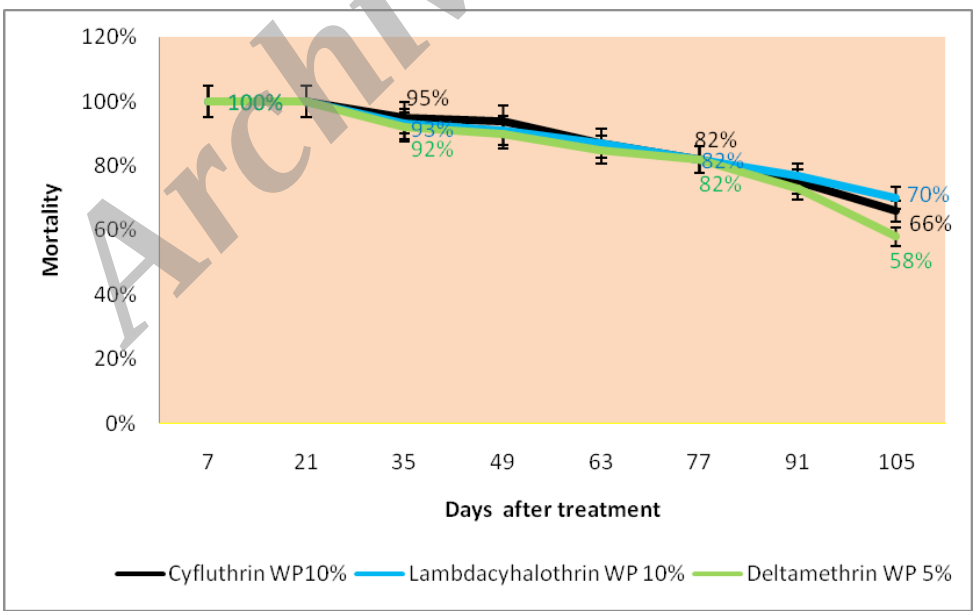


**Figure 3:** Plots show efficacy of experimental dosages (0.05, 0.0625, 0.05 mg ai/m²) of cyfluthrin WP 10%, lambdacyhalothrin 10% and deltamethrin 5% on cement surfaces, Kazerun, southern Iran, 2012.

the treated plaster ($P$=0.7) and cement surfaces ($P$=0.89). It is evident that full scale susceptibility was preserved for the first 35 days post-spraying of lambdacyhalothrin on plaster but not on the cement surfaces.

## Discussion

The susceptibility of adult female mosquitoes of *An. stephensi* to different concentrations of

lambdacyhalothrin, deltamethrin and cyfluthrin insecticides led to full scale mortality at the highest diagnostic doses. All field-collected first generation *An. stephensi* (*mysoriensis* strain) mosquito populations exhibited full scale susceptibility to the highest diagnostic doses of the three evaluated insecticides over the first three weeks post-exposure. At the lower diagnostic doses, tolerance and then resistance were noted in due course. The order of potency was lambdacyhalothrin> cyfluthrin> deltamethrin.

The maximal residual time of these three insecticides on plaster and cement walls was estimated to be similar (about 90 days). There was no significant difference between the mortalities of *An. stephensi* on different sprayed surfaces ($P$=0.653). All adult female mosquitoes died at the highest diagnostic doses of the three tested insecticides in the first three weeks post-exposure. At the lower diagnostic doses, tolerance and then resistance were noted over longer periods. This was particularly exemplified in the case of those *An. stephensi* mosquitoes exposed to deltamethrin insecticide which demonstrated only 58% mortality (*i.e.* 42% survival or resistance) on cement surfaces about 105 days post-exposure (Figure 3). Despite earlier reports on the susceptibility of malaria vector mosquitoes to these three insecticides in other endemic foci within the oriental region of Iran,[18,19] the present study demonstrated a three month persistence of the relative efficacy of these chemicals *in situ*. The present study confirms earlier reports on the longest residual activity of lambdacyhalothrin, which is a third generation insecticide, on plaster compared with cement surfaces,[20] and it gives rise to a higher mortality of malaria vector mosquitoes, *An. stephensi*, compared with cyfluthrin and deltamethrin insecticides.

## Conclusion

It is concluded in the present study that adult *An. stephensi* mosquito populations in the south of Iran are fully susceptible to higher doses of lambdacyhalothrin, cyfluthrin and deltamethrin insecticides over the first three weeks post-exposure. Lambdacyhalothrin revealed a slightly longer residual effect than the other two insecticides in endemic areas. Its use is thus recommended in future control operations in oriental parts of Iran. The maximal period of persistence or stability for all the three tested insecticides in WP formulation is about three months.

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**Conflict of Interest**: None declared

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