Long-lasting Residual Efficacy of Actellic ® 300CS and Icon ® 10CS on Different Surfaces against Anopheles stephensi, an Invasive Malaria Vector

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Research Article

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Abstract

**Background:** *Anopheles stephensi*, an invasive malaria vector, has developed resistance to several commonly used insecticides, including DDT, Dieldrin, Malathion, and synthetic pyrethroids. In response to this challenge, the World Health Organization (WHO) has recommended the use of Actellic®300CS and Icon®10CS for Indoor Residual Spraying (IRS) to control pyrethroid-resistant mosquitoes. This study aimed to assess the current susceptibility status of *An. stephensi* to the Diagnostic Concentration (DC) of some insecticides in Iran, and the residual effectiveness of Actellic®300CS and Icon®10CS against this malaria vector.

**Method:** Susceptibility of *An. stephensi* populations from the south of Iran was evaluated against Deltamethrin 0.05%, DDT 4%, Malathion 5%, Bendiocarb 0.1%, Synergist assay PBO 4% with Deltamethrin 0.05%, and intensity assay with 5x DC of Deltamethrin (0.25%) and Bendiocarb 0.5%. Laboratory cone bioassay tests for evaluation of the residual efficacy of Actellic®300 and Icon®10CS insecticides on different surfaces commonly found in human dwellings, such as cement, mud, plaster, and wood were conducted by using the WHO test kits and the standard testing protocols.

**Results:** The *An. stephensi* populations in Bandar Abbas were found to be sensitive to Malathion 5% and Deltamethrin 0.25% (5X DC) but resistant to DDT, Deltamethrin 1x DC, and Bendiocarb (1x DC and 5X DC). In laboratory cone bioassay tests, the mortality rates of *An. stephensi* exposed to Actellic®300CS and Icon®10CS on various surfaces were consistently above 80% for Actellic®300CS on all substrates throughout the 300-day after-spraying period. However, for Icon®10CS, the mortality rates were above 80% on plaster and wood substrates for 165 days and on mud and cement substrates for 270 days after spraying. Both Actellic®300CS and Icon®10CS showed 100% mortality 72 hours after each test on all substrates for the entire 300-day after-spraying period.

**Discussion and Conclusion:** The study highlights the varying levels of resistance of *An. stephensi* Hormoodar populations to different insecticides and demonstrates the consistent performance of Actellic®300CS in controlling these mosquitoes on various surfaces. The findings suggest that long-lasting CS formulations may be more effective for malaria vector control compared to the current options. Further research is needed to validate these findings in field settings and assess the impact of these insecticides on malaria transmission.

Introduction

To effectively combat malaria, a strong healthcare system based on primary healthcare is crucial. Almost half of the world's population is at risk of this disease, but WHO-recommended strategies and tools such as vector control and preventative antimalarial drugs have significantly reduced its global burden since 2000 (1–3). Iran has thus embarked on a malaria elimination plan in 2009 with a target to get its certification by 2025 (4–6).
The national Iranian key control prevention policies and strategies implicate four dominant interventions: indoor residual spraying of houses (IRS), free dissemination of long-lasting insecticide-treated mosquito nets (LLINs) to high-risk groups, free malaria diagnosis (Active or passive case recognition) and treatment, fogging in an emergency and eventually source reduction of larval mosquito ecosystem (7). Given these control policies, Iran succeeded in a strategic plan to achieve the 2020 goal of zero autochthonous malaria transmission and thus eliminated this disease early 2 last years ago (8). However, this disease suddenly broke out in 2022. The number of malaria-positive cases was about 10 times higher in 2022 than in 2021 and about 1400 people infected with the disease in 2022 and 5 times more in 9 first months of 2023. According to public health officials, several factors caused the outbreaks, including the presence of foreign nationals, the increased number of cases of malaria in Pakistan (a neighboring country), poor detection of new cases in this country, and, the heavy summer rains in the last two years and the attention of the health department to the Covid-19 pandemic and the invasive Aedes species, the role of sanctions for the purchase of insecticides and vector control requirements (9).

Malaria transmission can be effectively reduced through the use of long-lasting insecticide-treated mosquito nets (LLINs) and indoor residual spraying of houses (IRS). However, the spread of insecticide resistance among Anopheles mosquitoes poses a threat to these interventions. Therefore, there is an urgent need to develop new tools, technologies, and approaches for vector control and evaluate their potential role in a comprehensive malaria control strategy. Regular monitoring of vector species, their susceptibility to insecticides, and the coverage and quality of vector-control interventions are also crucial (10–11).

Anopheles stephensi is a highly efficient vector of Plasmodium falciparum and Plasmodium vivax, causing urban malaria. Its spread from South Asia and parts of the Arabian Peninsula to several African countries is a major potential threat to malaria control and elimination in Africa and southern Asia (12–19). Anopheles stephensi larvae are found in artificial water containers, and it has shown resistance to multiple insecticide classes, posing challenges to its control. The WHO has launched an initiative against its spread in Africa and developed a vector alert to urge immediate action from Member States and implementing partners (19).

The primary malaria vector in southern Iran, An. stephensi, is resistant to DDT, Dieldrin, Bendiocarb, Malathion, and synthetic pyrethroids insecticides and has lost its efficacy against vectors in most parts of the region (20–22). The Iranian government has implemented measures to control the spread of this mosquito species, but continuous monitoring of insecticide resistance and the development of alternative strategies are necessary (23).

Syngenta has repurposed insecticides to tackle the problem of pyrethroid-resistant mosquitoes in malaria control. Actellic®300CS is one such solution, offering long-lasting control of Pyrethroid-resistant mosquitoes through indoor residual spraying of houses (IRS). This approach has shown effectiveness and potential (24). Actellic®300CS is formulated with the active ingredient pirimiphos-methyl, an Organophosphate insecticide introduced by Syngenta in 1970 for broad-spectrum use in crop and non-crop applications. It demonstrates high insecticidal efficacy and long residual activity against pyrethroid-
resistant mosquitoes. The development of Actellic®300CS began in 2008 with funding from the Innovative Vector Control Consortium (IVCC) and was completed by the end of 2012. Modern micro-encapsulation technology was employed to create a formulation that provides at least 9 months of control on surfaces encountered in African habitations. This is a significant improvement compared to current alternatives, which typically last only three months after application, resulting in additional costs and complexities for malaria programs (24–26).

The safety assessment of pirimiphos-methyl 300®300CS for indoor residual spraying, conducted by the Finnish Institute of Occupational Health (FIOH) on behalf of WHOPES, utilized the WHO Generic risk assessment model. Assumptions included compliance with WHO specifications (including highest worst case condition for: dermal absorption rates, breathing volume of the operator, average concentration after spraying, translodgeable part onto the skin, excretion in milk, dissipation of product, air exchange rate, and residents’ indoor stay and ventilation rates). The assessment concluded that when used as directed, pirimiphos-methyl 300CS does not pose undue hazards to spray operators or residents of treated dwellings. However, failure to follow guidelines or use proper equipment may lead to excessive exposure to pirimiphos-methyl (26).

The WHO has recommended the use of lambda-cyhalothrin, a synthetic Pyrethroid insecticide from the alpha-cyano group, for indoor residual spraying of houses (IRS) (27–28). This substance has low vapor pressure and volatility and is essentially insoluble in water. Its formulation is said to have a residual efficacy of 12 weeks. According to the WHO, this insecticide falls into the moderately hazardous class II category of insecticides and is considered biodegradable and non-mobile in the environment. Studies conducted at the village level have indicated that the lambda-cyhalothrin 10% CS spray is well-received by the community (27–28).

The evaluation of lambda-cyhalothrin (Icon®10CS) used a 'capsule suspension' formulation, where the active ingredient is concealed in polymer capsules. When sprayed, the water suspension of this formulation slowly releases the insecticide, extending its residual life. This formulation does not get absorbed by porous sprayed surfaces and adheres easily to insects, increasing insect-insecticide contact. In supervised trials by WHOPES, this formulation showed equal or better efficacy than the WP formulation. Trials carried out elsewhere also reported good efficacy of Icon®10CS indoor residual spraying of houses (IRS) in controlling malaria.

According to WHOPES criteria, an insecticide is considered to have adequate residual efficacy if it elicits ≥ 80% mosquito mortality 24 hours after exposure on sprayed surfaces over a prolonged period (30). Increased or sustained residual efficacy ensures that the population at risk of malaria is protected from malaria infection during peak transmission. Residual efficacy can also affect indoor residual spraying of houses (IRS) operational costs if multiple rounds are not required. Based on the generic model, the hazard assessment concludes that Icon®10CS, when used for indoor residual spraying as per instructions, does not present any excessive risks to either the spray operators or the inhabitants of the treated dwellings. It is not necessary to carry out routine cholinesterase monitoring of the sprayer during indoor residual spraying programs, provided that operational guidelines are adhered to. However, if the operator protection
guidelines specified by WHO and label instructions are not followed, or if inappropriate or faulty equipment is used, exposure to Icon®10CS may surpass safe levels (31).

No documented studies exist regarding the impact of various wall substrates on the residual effectiveness of Actellic®300CS and Icon®10CS against *An. stephensi* for indoor residual spraying of houses (IRS) use in Iran or globally. This study evaluated the residual effectiveness of Actellic®300CS and Icon®10CS on invasive *Anopheles* and common surfaces within human dwellings in southern Iran. Additionally, the susceptibility of this malaria vector to some insecticides was assessed.

**Material and Methods**

**Study area**

Bandar Abbas, the capital city of Hormozgan Province, is situated on the southern coast of Iran along the Persian Gulf. It has an average altitude of 9 meters above sea level and is strategically located in the Strait of Hormoz, serving as the main naval base for Iran. The geographical coordinates for Bandar Abbas are 27°11'11.4'' N and 56°16'50.9'' E (Fig. 1). The city experiences a hot desert climate, classified as BWh according to the Koppen climate classification. Summers can be scorching, with maximum temperatures reaching up to 49°C, while winters can see temperatures drop to as low as 5°C. The annual precipitation in Bandar Abbas is approximately 170 mm, and the average relative humidity stands at 65% (32–34).

**Mosquito collection in experimental huts**

*Anopheles stephensi* larvae collected near the study site (Hormoodar village) from May 2020 were brought to the Bandar Abbas Research Center insectary and reared (Fig. 2). Pupae were collected daily and placed in cages for adult emergence under standard conditions (27 ± 2°C, 70 ± 4% relative humidity). Non-blood-fed females aged 3–5 days (F0) were used for the susceptibility test.

**Susceptibility tests:**

Susceptibility testing of *An. stephensi* populations from Hormoodar village of Bandar Abbas was conducted using the WHO test kits and standard testing protocols (33). The trial test included four classes of insecticides with Diagnostic Concentrations (DC) used for each insecticide: Pyrethroids (Deltamethrin 0.05% (Batch No. 717, Expiry date: August 2021)), Organochlorine (DDT 4% (Batch No. 293, Expiry date: July 2024)), Organophosphate (Malathion 5% (Batch No. 287, Expiry date: August 2020)), and Carbamates (Bendiocarb 0.1% (Batch No. 276, Expiry date: August 2022)). Synergist assay (PBO 4% (Sigma-Aldrich, USA) with Deltamethrin 0.05%), and intensity assay (5x DC of Deltamethrin 0.25% (Batch No. 671, Expiry date: August 2020) and Bendiocarb 0.5% (Batch No. 273, Expiry date: August 2022)) were also performed. The control tests mosquitoes were exposed to papers containing only the carrier oils for PY control (Batch No. 336, Expiry date: August 2020), OC control (Batch No. 141, Expiry date: June 2024) with no insecticide present (33–34).
Four replicates of 20–25 females were exposed for an hour to the diagnostic doses of each insecticide, and 2 replicates for control. In the synergist test, mosquitoes were first exposed to PBO for one hour before being exposed to Deltamethrin for one additional hour. Before exposure to the impregnated paper, mosquitoes were held in tubes for an hour and then transferred to the exposure tubes for 1 hour (Fig. 2). After the 1-hour exposure period, mosquitoes were transferred back to the holding tubes and provided with 10% sugar water. Mosquito mortality rates were recorded after 24 hours. Tests with silicone and olive oil-impregnated papers as controls were conducted in parallel (33).

Mortality rates were used to determine the susceptibility of the mosquitoes to the insecticides. Control mortality rates below 5% were considered acceptable, while rates between 5–20% were corrected using Abbotts’ formula. Tests with control mortality rates above 20% were discarded and repeated with new specimens. Mosquitoes with mortality rates between 98–100% were considered susceptible, those with rates between 90–97% were considered candidates for resistance and required confirmation using specific methods, and those with rates below 90% were considered resistant (33).

For the intensity assays, mortality rates at 5XDC of 98–100% indicated low resistance intensity. Impregnated papers were purchased from WHO (33).

**Preparation and treatment of block substrates**

Four different types of substrates commonly used in housing construction in Bandar Abbas, including cement, mud, plaster, and wood, were transformed into square-shaped blocks with four parts each. These blocks had dimensions of 40*40 cm and a thickness of 5 cm, and they were utilized for laboratory bioassays. The cement blocks were composed of a mixture of commercial cement and sand in a 1:1 ratio. The mud blocks were made by combining mud and straw, following local practices. The plaster surface was created under the same conditions.

Wooden blocks were simply cut from locally available wooden planks. Each of these four surfaces formed blocks measuring 20x20 cm, which were placed side by side to form a larger square measuring 40x40 cm. Before applying insecticides, these blocks were stored at a temperature of 27 ± 2°C and a relative humidity of 70 ± 10% for 30 days. The substrates were treated using a potter tower sprayer to ensure a uniform and precise application of the desired concentration of active ingredients per unit area. All treated blocks were stored at a temperature of 30 ± 2°C and a relative humidity of 70 ± 10% between bioassays. For the Actellic®300 and Icon®10CS insecticides, four replicate blocks of each substrate type were prepared and treated. Additionally, untreated blocks of each substrate type were also prepared and used as controls alongside the treated blocks (Fig. 3).

**Residual efficacy on treated block substrates**

Bioassay tests were conducted to evaluate the residual effect of various concentrations using standard WHO cones. Rubber bands were used to securely attach the cones to different treated and control surfaces. Each cone was populated with approximately 10–12 sugar-fed, 3–5 days old, and F1 female mosquitoes, released gently in a vertical position (Fig. 3). The mosquitoes were exposed to each treated
surface for 30 minutes in five replicates. The same procedures were performed for the control test. After the exposure period, the adult mosquitoes were transferred to clean cups with a cotton wool pad soaked in a 10% sucrose solution. They were then kept in the insectary for 24 and 72-hour recovery periods, during which the mortality rate was recorded. The residual efficacy of the insecticides applied on the treated block substrates was assessed in 12 times tests (including 1, 5, 15, 30, 45, 60, 90, 120, 135, 180, 210, 270, and 300 days after spraying) cone bioassays following WHO guidelines (35–36).

**Statistical analysis**

The raw data were managed with Microsoft Excel and the statistical analyses were performed using SPSS version 24 software. The WHO criteria were used to classify the level of resistance of tested mosquitoes to insecticides (35–36). Other analyses of the significance of each of the bio-efficacy measured parameters between insecticides, block substrates, and test interval times using SPSS software by Kruskal Wallis Test.

**Results**

**Susceptibility testing**

Results of susceptibility test of *An. stephensi* populations collected from Bandar Abbas County to common insecticides are shown in Table 1. This species results obtained sensitive to the insecticides include Malathion 5% and Deltamethrin 0.25% (5X DC) and PBO 4% + Deltamethrin 0.05%. But resistance candidate to insecticide DDT 4%, and Resistant to Deltamethrin 0.05% and Bendiocarb 0.1% and indicated low resistance intensity to Bendiocarb 0.5% (5X DC).
Table 1
The results of *Anopheles stephensi* resistance status to different insecticides in south of Iran, 2020.

<table>
<thead>
<tr>
<th>Diagnostic Concentration</th>
<th>Insecticide Impregnated paper</th>
<th>Exposer Time (Hour/s)</th>
<th>Trial</th>
<th>Control</th>
<th>Resistant Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>Mortality (%)</td>
<td>No.</td>
</tr>
<tr>
<td>1X</td>
<td>Malathion 5%</td>
<td>1</td>
<td>100</td>
<td>99</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>DDT 4%</td>
<td>1</td>
<td>100</td>
<td>97</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Bendiocarb 0.1%</td>
<td>1</td>
<td>200</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Deltamethrin 0.05%</td>
<td>1</td>
<td>200</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>5X</td>
<td>Deltamethrin 0.25%</td>
<td>1</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Bendiocarb 0.5%</td>
<td>1</td>
<td>100</td>
<td>96</td>
<td>50</td>
</tr>
<tr>
<td>1X</td>
<td>PBO 4% + Deltamethrin 0.05%</td>
<td>1 + 1</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
</tbody>
</table>

CR*: Candidate Resistance (It is necessary to test again to confirm the resistance/sensitivity)

Residual efficacy on treated block substrates

Figure 4 shows the results of cone bioassay tests, indicating the mortality rates of *An. stephensi* mosquitoes exposed to Actellic®300CS and Icon®10CS insecticides on different block substrates including mud, cement, plaster, and wood. The mortality rates, assessed 24 hours after each test, were consistently above 80% for Actellic®300CS on all substrates throughout the 300 days after the spraying (Figs. 4 and 5). However, for Icon®10CS, the mortality rates were above 80% on plaster and wood substrates for 165 days and on mud and cement substrates for 270 days after spraying (Figs. 4 and 5). When assessing the mortality rates 72 hours after each test, both Actellic®300CS and Icon®10CS showed 100% mortality rates on all substrates for the entire 300 days after-spraying period (Fig. 6).

The results of the Kruskal Wallis Test indicated a significant difference in the mortality rate among different days after spraying for each insecticide tested, Actellic®300CS and Icon®10CS (p < 0.001). However, there was no significant statistical difference between block substrates and insecticides (Table 2).
Table 2
Results of Kruskal Wallis test between different variables and *Anopheles stephensi* mortality rate

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>Variable</th>
<th>Chi-Square</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actellic®300CS</td>
<td>Block substrate</td>
<td>0.045</td>
<td>3</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Days after spraying</td>
<td>46.63</td>
<td>11</td>
<td>0.001</td>
</tr>
<tr>
<td>Icon®10CS</td>
<td>Block substrate</td>
<td>0.970</td>
<td>3</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Days after spraying</td>
<td>43.63</td>
<td>11</td>
<td>0.001</td>
</tr>
<tr>
<td>Total*</td>
<td>Block substrate</td>
<td>0.49</td>
<td>3</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Days after spraying</td>
<td>86.15</td>
<td>11</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Insecticides</td>
<td>0.16</td>
<td>1</td>
<td>0.68</td>
</tr>
</tbody>
</table>

* Total: Both Actellic®300CS and Icon®10CS cone bioassay tests

Discussion

Despite efforts to eliminate vector-borne diseases in Iran, the disparate ecosystems in different regions have made it challenging. Insecticide-based strategies are key to controlling VBDs, but the resistance of vectors to commonly used insecticides has been a major obstacle to the success of elimination programs (39). Failure to mitigate and manage widespread and increasing insecticide resistance may lead to a higher burden of disease, potentially reversing the gains made in controlling malaria over the last decade (40).

Based on the susceptibility results, the *An. stephensi* samples selected for Bioassay tests have shown resistance to Pyrethroid in 1X DC. The results are consistent with previous studies that reported resistance of *An. stephensi* to DDT, Deltamethrin, Bendiocarb, and Pyrethroid (20–21). Our study also observed the sensitivity of *An. stephensi* to Malathion, which aligns with the findings of Abbasi et al. in 2019 (22). *An. stephensi* resistance to Pyrethroids, Organochlorine, Carbamates, and Organophosphates has also been reported in Afghanistan, India, and Pakistan (41). In Ethiopia, one of the African countries where invasive *Anopheles* was recently discovered, resistance to all 4 groups of insecticides has been reported to be resistant, which is exactly in line with the results of our study (42–43). These findings highlight the importance of continuously monitoring mosquito populations for insecticide resistance and adapting control strategies accordingly.

In Iran, the indoor residual spraying of houses (IRS) program is conducted two times in year. To prevent the rise of insecticide resistance, pyrethroid, and carbamate insecticides are applied periodically. However, the test results have revealed that both groups exhibit resistance at the Diagnostic Dose. Moreover, the carbamate group displayed resistance even at a five times higher dosage. To address this challenge, a
promising approach is to utilize PBO Synergist in combination with Deltamethrin, and the other, new micro-encapsulated insecticides which have shown to be two effective methods.

In terms of residual efficacy, Actellic®300CS demonstrated consistent performance with mortality rates above 80% on all substrates (mud, cement, plaster, and wood) throughout the 300 days after the spraying. This indicates that Actellic®300CS has a long-lasting residual effect, making it an effective tool for malaria vector control. On the other hand, Icon®10CS showed mortality rates above 80% on plaster and wood substrates for 165 days and on mud and cement substrates for 270 days after spraying. While still effective, the residual efficacy of Icon®10CS was shorter compared to Actellic®300CS. The results suggest that Actellic®300CS and Icon®10CS have the potential to be valuable tools in the control of An. stephensi mosquitoes. In comparison with previous studies, our findings regarding the residual efficacy of Actellic®300CS and Icon®10CS on different substrates align with previous studies conducted in various regions. The results of residual efficacy of Actellic®300CS against Pyrethroid-resistant malaria vectors on various surfaces in Benin (West Africa) and Ethiopia reported consistent mortality rates above 80% for up to 9 and 12 months after spraying, respectively (25, 44). Furthermore, in Zanzibar, Actellic®300CS was highly effective in controlling malaria vectors and had a relatively prolonged residual activity compared to other products used for indoor residual spraying of houses (IRS) (45). These findings support our study results about the long-lasting residual efficacy of Actellic®300CS.

Study in Zanzibar and mainland Tanzania with An. gambiae s.s species indicated a significant variation in the residual efficacy of Icon®10CS across different types of wall surfaces. The study found that residual efficacy decreased with increasing pH of the substrate and varied widely across different types of wall surfaces. In areas where malaria transmission is bimodal and wall surfaces with short residual efficacy comprise more than 20% of sprayable structures, the study suggested considering two rounds of indoor residual spraying (IRS) using lambda-cyhalothrin of Anopheles culicifacies control. In India, the persistence of effectiveness of Icon®10CS for 2–3 months was demonstrated in three study areas. This formulation was found to be as effective as or better than other insecticides used in the national program for reducing mosquito densities and interrupting malaria transmission in the study villages. Field trials at three sites established that Icon®10CS was relatively more effective than other insecticides in various evaluation parameters, such as indoor resting mosquitoes, parity rates in vector mosquitoes, and persistence of effectiveness (46–47).

In comparison, our study, which focused on the effectiveness of Icon®10CS, found that the mortality rates were above 80% on plaster and wood substrates for 165 days (5.5 months) and on mud and cement substrates for 270 days (9 months) after spraying. This suggests a longer residual efficacy compared to the other studies, indicating the potential for sustained effectiveness in controlling malaria vectors (An. stephensi). There has been no study on the effectiveness of micro-encapsulated insecticides in Iran. A previous study found that Deltamethrin insecticide was effective for around 2-4.5 months on different surfaces (48). We found that the residual time of insecticides was at least twice as long as Deltamethrin in different substrates. It is important to note that the effectiveness and residual efficacy of insecticides
can vary depending on the mosquito species, geographical location, and local environmental conditions. Therefore, it is essential to consider these factors when implementing vector control strategies.

With this study, the evaluation of the micro-encapsulated formulation of Organophosphorus and Pyrethroid insecticides on Pyrethroid-resistance An. stephensi was done for the first time globally. The insecticides were fully effective against this invasive malaria vector and proved to be a useful alternative in insecticide resistance management plans.

These two microencapsulated insecticides offer long-lasting residual effects, allowing for a reduction in the frequency of spraying from twice a year to just once with these new formulation insecticides. This will result in a significant decrease in costs related to transportation, sprayer vehicles, workers, training, and communication, while also effectively reducing the spread of disease. Unfortunately, the cost of these insecticides is quite high, equivalent to that of Bendiocarb, and five times the cost of Deltamethrin, the most commonly used insecticide in the program. This cost factor poses a challenge in terms of procurement, with the situation exacerbated in Iran due to sanctions, making it difficult to purchase and replace resistant insecticides.

However, it is important to consider the limitations of the study, such as the use of insectarium-reared mosquitoes and the exclusion of field conditions. Further studies should be conducted to validate these findings in field settings and assess the impact of these insecticides on malaria transmission.

**Conclusion**

In conclusion, the study demonstrated the susceptibility of An. stephensi mosquitoes in Bandar Abbas to certain insecticides while also identifying resistance to others. Actellic®300CS exhibited long-lasting residual efficacy on different substrates, providing effective control of An. stephensi populations for up to 300 days after spraying. Icon®10CS also showed promising results, although with a shorter residual efficacy compared to Actellic®300CS. These findings highlight the potential of these insecticides in malaria vector control programs in Bandar Abbas. Further research and field trials are needed to validate these results and assess their impact on malaria transmission in the region.

**Abbreviations**

DDT: Dichloro-Diphenyl-Trichloroethane

WHO: World Health Organization

IRS: Indoor Residual Spraying

DC: Diagnostic Concentration

PBO: Piperonyl butoxide
Declarations

Acknowledgements

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Availability of data and materials

All data on which this article is based are included within the article.

Authors' contributions

FN, AR, and AM conceived the original idea and carried out the implementation, with support from AB, and FK. MA wrote the manuscript with support from AAHB and HV AM, MY helped in field studies in BA county. All authors discussed the results and contributed to the final manuscript.

Ethical consideration
The ethics committee at the Department of Environmental Chemical Pollutants and Pesticides, Institute for Environmental Research, Tehran University of Medical Sciences, Tehran, Iran (Ethics Code: IR.TUMS.VCR.REC.1398.722) approved this protocol.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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**Figures**
Figure 1

Study area map – Bandar Abbas County, Hormozgan Province, south of Iran
Figure 2

*Anopheles stephensi* larvae collection and susceptibility test in south of Iran, 2020
Figure 3

Preparation of block substrates and residual efficacy bioassay tests in south of Iran, 2020
Figure 4

The residual effects of Actellic®300CS and Icon®10CS insecticides 24 hours after tests on various substrates in south of Iran, 2020.
Figure 5

Comparison of the residual effects of Actellic®-300CS and Icon®-10CS insecticides 24 hours after tests on each substrate in south of Iran, 2020.
Figure 6

The residual effects of Actellic®300CS and Icon®10CS insecticides 72 hours after tests on various substrates, Bandar Abbas County, Hormozgan Province, Iran- 2020.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FIG4.xlsx
- FIG5.xlsx
- FIG6.xlsx