Research Article

Open Access

Journal of Annals of Public Health and Preservative Medicine

appm@scientificeminence.com

Evaluation of Two Important Methods, WHO Susceptibility Test and CDC Bottle Bioassay for Determination of Insecticide Susceptibility of The Malaria Vector, Anopheles Stephensi to Malathion, Permethrin, and Propoxur

Morteza Akbari[1,2], Hassan Vatandoost[1,3*], Mohamad Reza Abai[1,3], Ahmad Raeisi[4], Soraya Sheikhi[1]and Mohamad Reza Akbari[5]

[1]*Department of Medical Entomology & Vector Control, School of Public Health and National Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran*

[2]*Department of public health, School of Health, Ilam University of Medical Sciences, Ilam, Iran*

[3]*Department of Chemical Pollutants and Pesticides, Institute for Environmental Research, Tehran University of Medical Sciences, Tehran, Iran*

[4]*Department of Malaria, CDC, Ministry of Health & Medical Education, Iran*

[5]*Health Promotion Research Center, Zahedan University of Medical Sciences, Zahedan, Iran*

*** Corresponding Author**

Hassan Vatandoost, Department of Medical Entomology & Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, Tel: 0098 2188954441, E-mail: hvatandoost1@yahoo.com, vatando@tums.ac.ir

Citation

Morteza Akbari (2022) Evaluation of Two Important Methods, WHO Susceptibility Test and CDC Bottle Bioassay for Determination of Insecticide Susceptibility of The Malaria Vector, Anopheles Stephensi to Malathion, Permethrin, and Propoxur. Ann Public Health Preservative Med 1(1):102

Publication Datess

Received date: March 21, 2022

Accepted date: February 21, 2022

Published date: February 22, 2022

Abstract

Malaria is still an important health problem in the world. The detection of insecticide resistance in natural populations of *Anopheles* vectors is necessary for malaria control. CDC bottle bioassay as new tool has been employed for detecting insecticide resistance by the US Centers for Disease Control and Prevention. WHO's standard tube-test method for the assessment of susceptibility levels of adult *An.stephensi* to the malathion, permethrin, and propoxur were determined and compared two methods.

The larvae of *An.stephensi* were collected from the natural environments of malaria foci, Sistan, and Baluchistan province, Southern Iran. They were colonized at the insectary of the School of Public Health (SPH), Tehran University of Medical Science. The susceptibility tests were carried out on sugar-fed female mosquitoes aged 2-3 days against malathion 5%, permethrin 0.75%, and propoxur 1%. The data were analyzed by probit and t-test using SPSS ver. 18.0. The mortality and knockdown rates as well as the parameters of regression analysis, including LT_{50} and LT_{90} was determined.

The mortality rate was calculated at 99.7% and 100% for malathion, 100%, and 100% for permethrin, and 66.2% and 90.3% were revealed for the propoxur using CDC and WHO methods at 30 and 60 min respectively.

A comparative study of the CDC and WHO methods on *An.stephensi* showed similar results except for the propoxur.

Keywords: Susceptibility; Insecticide; WHO bioassay; CDC bioassay; *Anopheles stephensi*

Introduction

Malaria is a mosquito-borne protozoan disease that remains one of the public health concerns in the world [1,2]. Despite many global efforts to eradicate it, malaria still causes many deaths in the world.

Malaria is one of the important infectious diseases in Iran [3]. More than 80% of malaria cases in Iran are reported from three provinces' southern and southeastern areas of the country [4,5]. Human migration and movement across eastern borders contribute to the spread of malaria in Iran [6].

Some species of *Anopheles* mosquitoes are vectors of malaria in different parts of the world. *Anopheles stephensi* is an important vector of malaria with a geographical range from the Middle East to India and China [7]. In its geographic distribution, this is an important vector for both *Plasmodium falciparum* and *P.vivax* [8-10]. Recent studies on *Anopheline* mosquitoes in Iran have reported the presence of 31 *Anopheles* species including genotypes and sibling species. Eight of them are involved in malaria transmission and among vectors of malaria, *An.stephensi* in Iran is considered as the main vector of malaria in southern Iran [11-13] (Figure 1). Vector control is the main approach to reduce malaria transmission at the community level and in many parts of the world, it is considered the most effective measure for eradicating malaria. It has been reported to be the only measure that can reduce malaria transmission from very high levels to close to zero [14,15]. Resistance to insecticides in vectors of malaria is one of the most important growing concerns in many countries and bioassays allow for the detection and characterization of insecticide resistance in a vector population [16,17]. Pesticide resistance is a decrease in a pest population's susceptibility to the mode of action of a pesticide, causing the pesticide to no longer control the pest population as efficiently. Pesticide resistance is not new or uncommon. It has been a side effect of insect vector control programs since 1914, and insect disease vectors in over 45 countries are resistant to at least one pesticide class. Consequently, there is a risk of pesticide resistance developing in any pest population anywhere. There are several mechanisms of resistance in insects including reduced penetration, target site insensitivity, enzymatic resistance, behavioral change and excretion.

In the Iran region, the WHO insecticide susceptibility test is the most common method for assessing resistance status. (CDC) bottle bioassay is another tool for detecting resistance to insecticides that are not widely used in Iran [18]. CDC bottle bioassay relies on time mortality data, which are measures of the time it takes an insecticide to penetrate a vector, traverse its intervening tissues, get to the target site, and act on that site [19]. It can measure the efficacy of an insecticide formulation. The aim of this bioassay is to measure the mortality of a population at a given dose of insecticide. In WHO test, mosquitoes are exposed to known concentrations of an insecticide for a fixed period at the end of which the number of mortality is recorded [20]. In this study, we compared the WHO tests with the CDC bottle bioassay. Pesticide resistance occurs in a pest, resistance testing must be performed on individuals from the population. For mosquitoes, these assays include the Centers for Disease Control (CDC) bottle bioassay for adults and the World Health Organization's (WHO) susceptibility bioassay. WHO method requires more mosquitoes than CDC method, the comparison between the results of both methods is clear. When the WHO susceptibility kit is not readily available, bottle bioassays can be used to determine insecticide resistance status of mosquito populations. WHO bioassays utilize cylinder plastic tubes whereas CDC bottles bioassays use 250 ml Wheaton bottles which are made of glass. World Health organization (WHO) papers do not need to be treated by oneself before their utilization because they are ordered in the impregnated form. Conversely, CDC bottles need to be coated with insecticide by oneself before each bioassay. In fact, the shelf life and reuse of prepared bottles are still not well documented or studied in laboratory conditions.

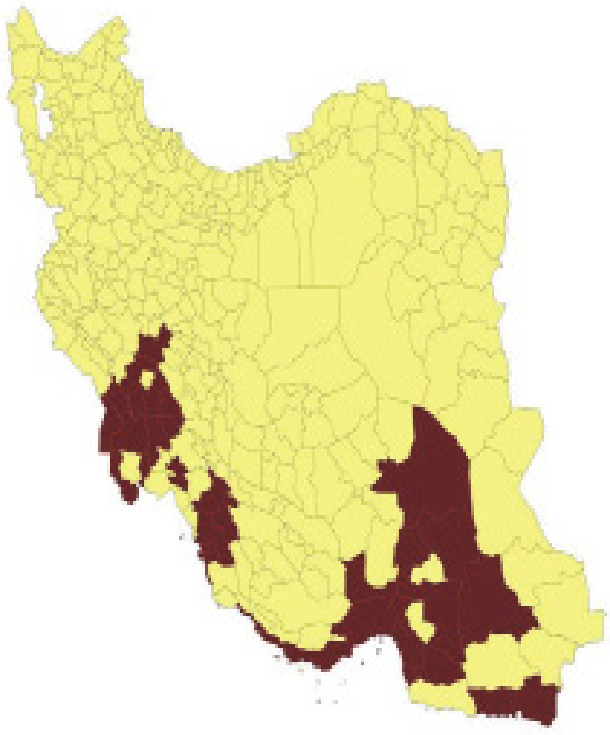

Figure 1: Distribution of *An.stephensi* in Iran

Methods

Study area

The larvae were collected from artificial ponds in the urban area of Chabahar Port of Sistan and Baluchistan province and colonized at the insectary of the School of Public Health (SPH), Tehran University of Medical Science.

Insecticide materials

The insecticide-impregnated papers were purchased with a WHO representative in Penang, Malaysia. The technical active ingredient of malathion, permethrin, and propoxur was provided from the Eco-toxicology Laboratory, School of Public Health, Tehran University of Medical Sciences.

Adult susceptibility test

Adult susceptibility tests were followed according to the current WHO and CDC protocols on unfed female mosquitoes aged 2–5 days old, reared from the larval collections. For each insecticide mortality rate at various times was calculated and then the regression line to each insecticide was plotted using Microsoft Excel (version. 2013). All susceptibility tests were conducted in the (SPH) insectary at 25±−2°C and 70 - 80% relative humidity.

WHO protocol (Insecticide impregnated papers)

The principle of the WHO bioassay is to expose insects to a given dose of insecticide for a given time to assess susceptibility or resistance. The standard WHO discriminating dosages were used [21]. In this study, three insecticides were tested including malathion 5%, permethrin 0.75%, and propoxur 0.75%. An aspirator was used to introduce 20 to 25 unfed female mosquitoes aged 2–5 days from batch into five WHO holding tubes (four tests and one control) that contained untreated papers. They were then gently blown into the exposure tubes containing the insecticide-impregnated papers. After one hour of exposure, mosquitoes were transferred back into holding tubes and provided with cotton wool moistened with a 10% sucrose solution. The number of mosquitoes "knocked down" at 60 minutes and mortalities at 24 hours was recorded following the WHO protocol [21] (Figure 2).

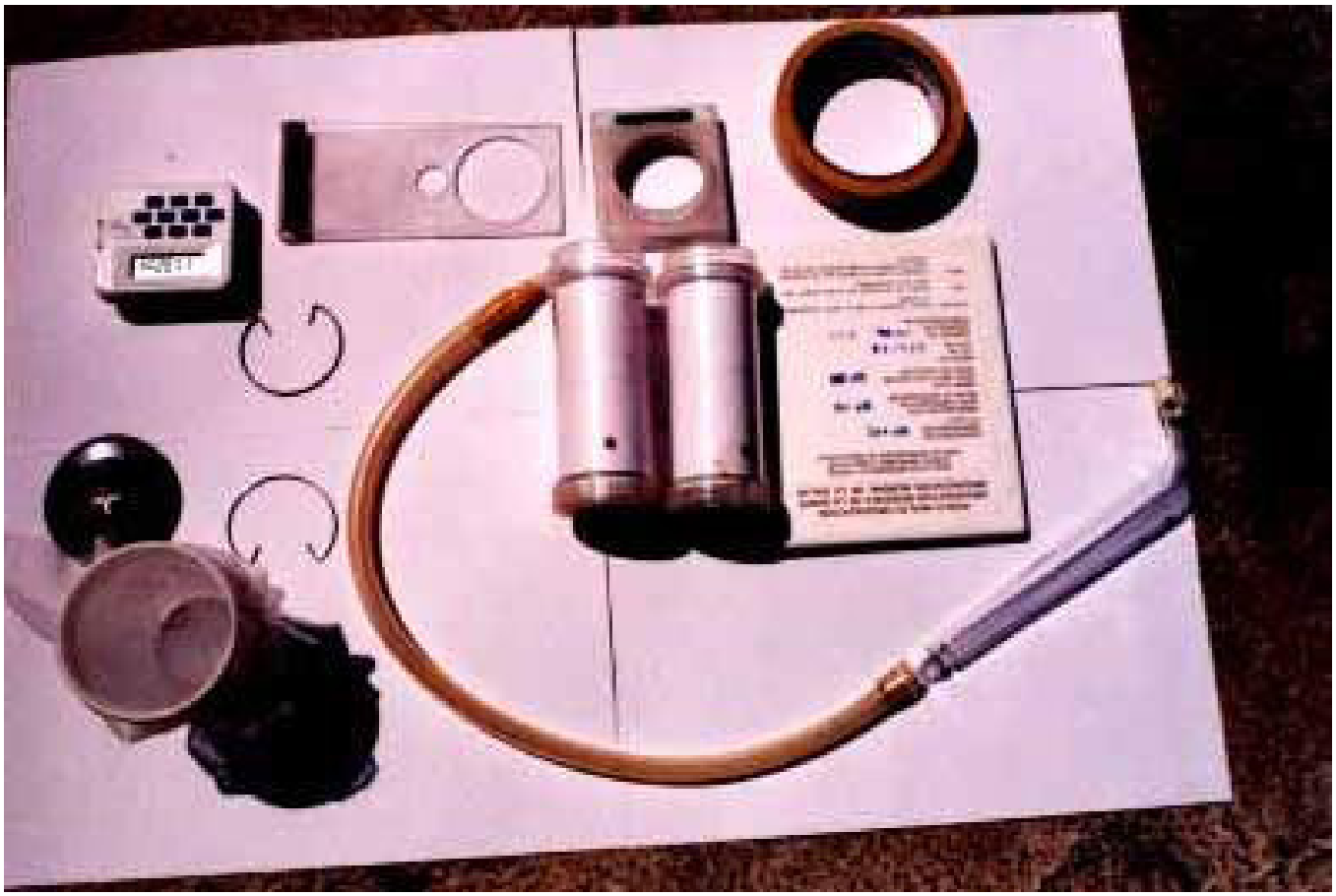

Figure 2: WHO protocol (Insecticide impregnated papers) for evaluation of insecticide resistance in mosquitoes

CDC protocol

CDC bottle bioassay is a surveillance tool for detecting resistance to insecticides in vector populations. The CDC bottle bioassay relies on time mortality data. The diagnostic dose and diagnostic time that was applied in the present study recommended by the CDC. The solutions were prepared and the bottles were coated according to the CDC protocol [19].15 to 30 unfed female mosquitoes aged 2–5 days were fed with 10% sucrose solution were introduced into each test bottle coated with insecticide and one control bottle coated with acetone only. The number of dead or alive mosquitoes was monitored and count at different times (15, 30, 45, 60, 75, 90, 105, and 120 minutes). This allowed us to determine the total percent mortality (Y-axis) against time (X-axis) for all replicates using a linear scale (Figure 3)

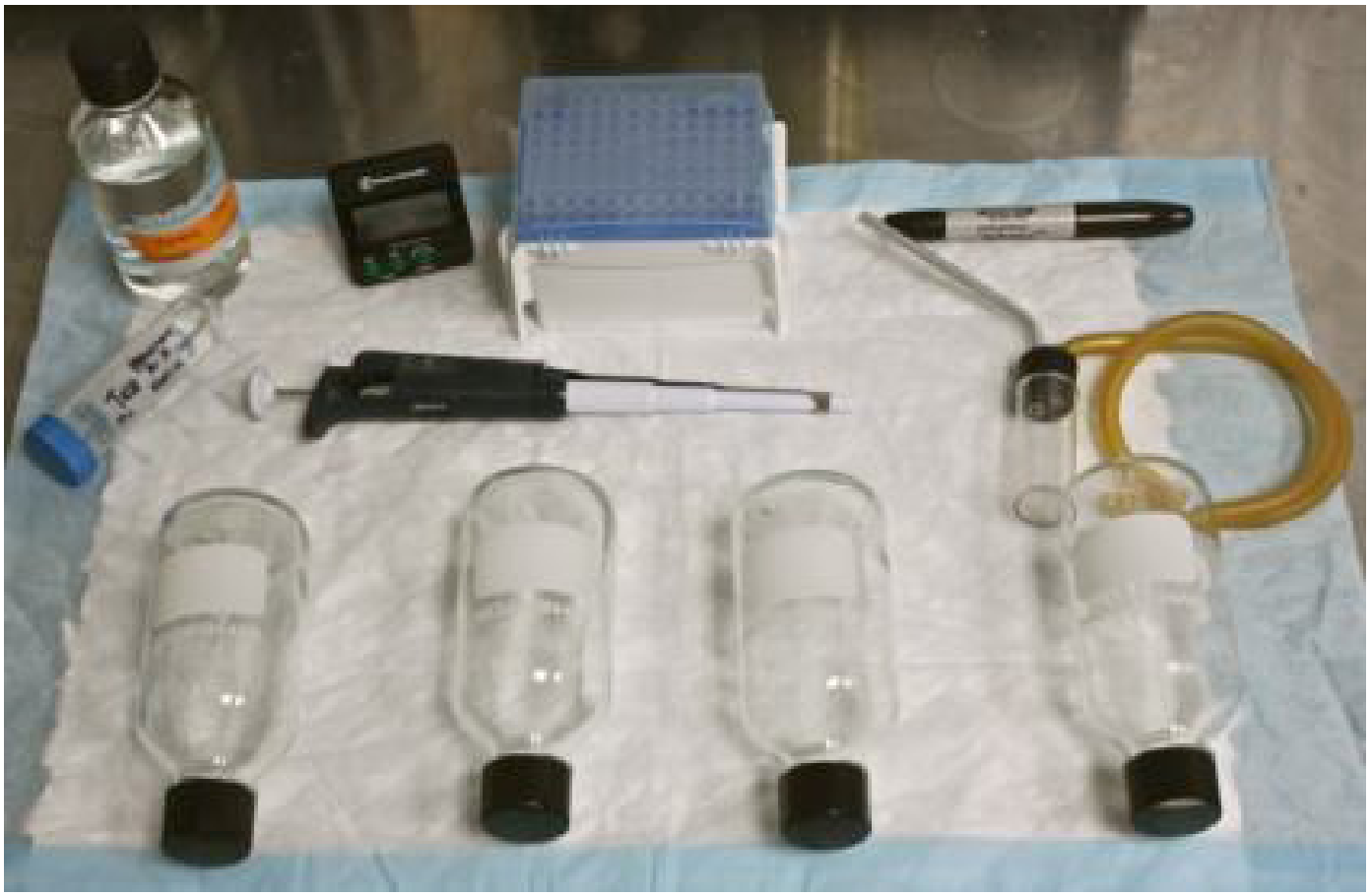

Figure 3: CDC protocol for evaluation of insecticide resistance in mosquitoes

Statistical analysis and data interpretation

The resistance status determined according to the latest WHO criteria (20) as follows:

- When 98%–100% mortality at the recommended diagnostic time indicates susceptibility;
- When 90%–97% mortality at the recommended diagnostic time suggests the possibility of resistance that needs to be confirmed;
- When <90% mortality at the recommended diagnostic time suggests resistance.

The resistance status of mosquito samples was tested by the CDC method determined according to the CDC criteria [19]. The susceptibility thresholds at the diagnostic time of 30 minutes for all insecticides are:

- Mortality rate = 100%: the population is fully susceptible
- Mortality rate < 100%: the population is considered resistant to the tested insecticides.

Results were analyzed by using of *Probit* program (Finney 1971) [22]. Error bars for each mortality were calculated based on the statistical method at α=5%. The lethal Time for 50% and 90% mortality (LT_{50} and LT_{90}) values and their 95% confidence interval also. Probit regression line parameters were estimated and then the regression line of all Insecticides was plotted using Microsoft Excel (version. 2013).

Results

The results of 24 hours mortality recorded after 60 minutes of exposure of mosquitoes to impregnated papers of malathion, Probit analysis and the LT_{50} and LT90 values and confidence interval (95%) were calculated for each of the insecticides. (Table 2).

Insecticides	Total tested		Mortality rate (%) after 24h maintenance		Resistance status	
	WHO	CDC	WHO	CDC	WHO	CDC
Malathion	86	296	100	99.7	* S	S
Propoxur	103	270	90.3%	66.2	** T	*** R
Permethrin	99	104	100	100	S	S

* Susceptible, ** Tolerance, *** Resistance,

Table 1: Susceptibility data recorded according to both WHO and CDC methods against *An.stephensi*

Insecticide	Susceptibility method	A	B±SE	LT_{50}, 95% C.I. (Min)	LT_{90}, 95% C.I. (Min)	X^2(df)	P-value
Malathion-5%	WHO	-2.3423	3.4211 ± 0.869	1.1187 **4.8381** 7.8334	7.2247 **11.4629** 176.2323	11.345 (3)	0.05
Propoxur 0.1%	WHO	-5.3064	3.6879± 0.327	24.5860 **27.4713** 30.4591	53.2056 **61.1502** 73.3081	5.024 (2)	0.05
Permethrin 0.75%	WHO	-0.1205	2.6649± 0.204	0.9737 **1.1098** 1.2531	2.8496 **3.3586** 4.1274	18.475 (6)	0.01

Table 2: Susceptibility data recorded according to both WHO methods against *An. stephensi*

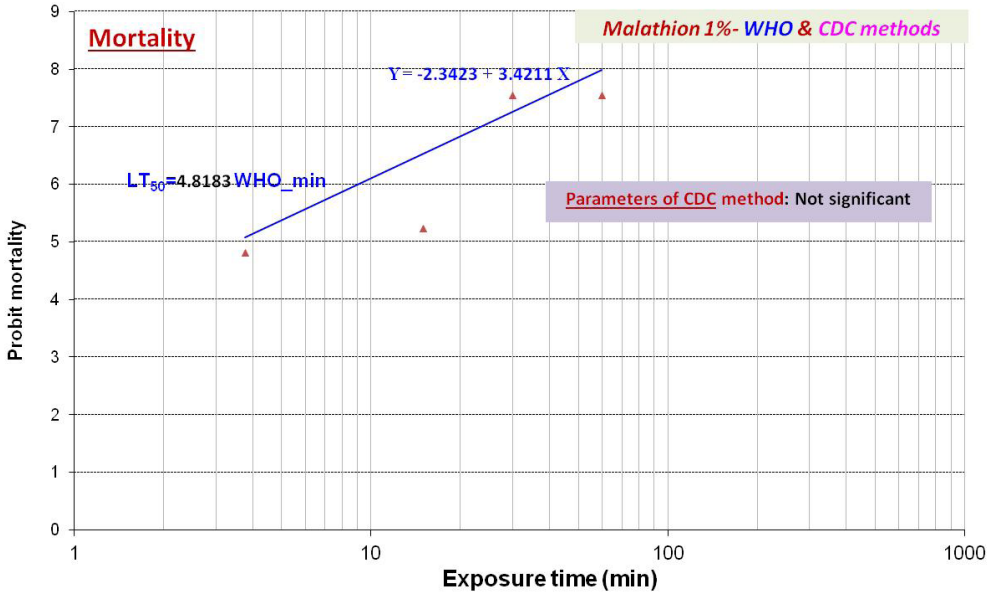

Figure 4: Mortality rate and regression analysis of bioassays of *An.stephensi* exposed to Malathion using WHO and CDC methods

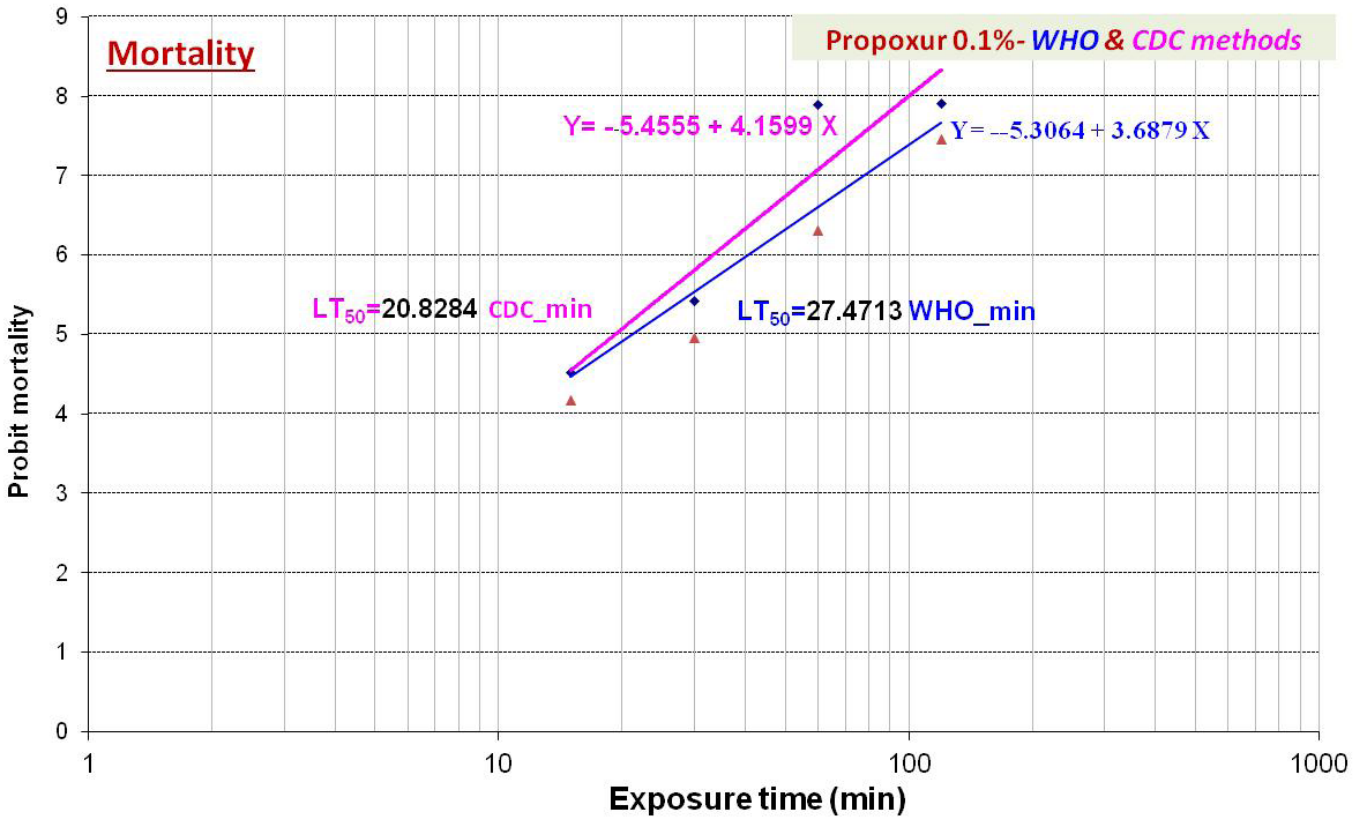

Figure 5: Mortality rate and regression analysis of bioassays of *An. stephensi* exposed to Propoxur using WHO and CDC methods

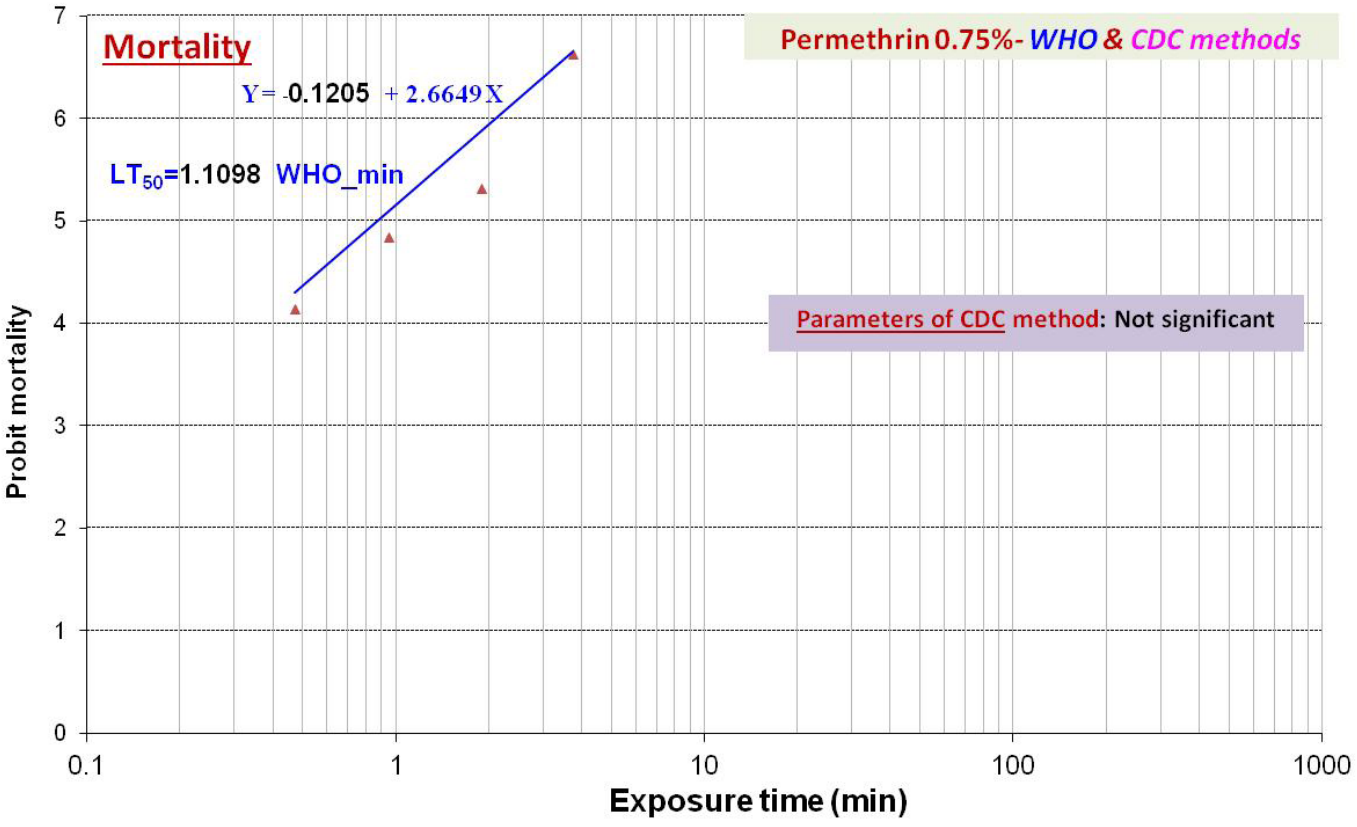

Figure 6: Mortality rate and regression analysis of bioassays of *An. stephensi* exposed to Permethrin using WHO and CDC methods

Discussion

To evaluate the susceptibility level of *An.stephensi* to insecticides, two methods of bioassay testing were used. The WHO standard method is major and widely used, and recently, the CDC Bottle Bioassay method was also used.

Our information about the susceptibility of the vectors to insecticide is essential for chemical interventions, so routine testing of these tests is an integral part of the control of the vectors and is very practical. In this study, we compared two important methods that were used in the world to perform susceptibility tests Malaria vector, *An.stephensi* that colonized at the insectary of School of Public Health (SPH), Tehran University of Medical Science.

The reason for using this strain is previous reports of resistance or tolerance to several types of insecticides [24,25]. WHO and CDC methods indicate that *An.stephensi* is susceptible to malathion. Approximately in all previously conducted studies on *An. stephensi* in Iran, susceptibility to malathion has been reported [26-28].

Permethrin shows significant protection against mosquito bites. This insecticide can be used to protect people from mosquito bites and reduce diseases transmitted by mosquitoes [29].

WHO recommended insecticides for indoor residual spraying against malaria vectors are: DDT, Malathion, Fenitrothion, Pirimiphos- methyl, Bendiocarb, Propoxur, Alpha-cypermethrin, Bifenthrin, Cyfluthrin, Deltamethrin, Etofenprox, Lambda-cyhalothrin, Clothianidin [30].

WHO insecticide susceptibility test is the most common method for assessing resistance status in Iran [18]. WHO method requires more mosquitoes than the CDC method. When the WHO susceptibility kit is not readily available, bottle bioassays can be used to determine the insecticide resistance status of mosquito populations. WHO bioassays utilize cylinder plastic tubes whereas CDC bottles bioassays use 250 ml Wheaton bottles which are made of glass. WHO papers do not need to be treated by oneself before their utilization because they are ordered in the impregnated form. Conversely, CDC bottles need to be coated with insecticide by oneself before each bioassay. The shelf life and reuse of preprepared bottles are still not well documented or studied in laboratory conditions [31]. however, in field conditions, the studies of Perea et al (2009) showed that bottles treated with 10 µg a.i deltamethrin per bottle could be stored for at least 14 days and reused on three occasions [32]. The major advantages of the bottle assays are that any concentration of a custom insecticide (pure or formulated) may be evaluated and the technique is simple, rapid, and economical. One of the disadvantages of the WHO method is the transfer of mosquitoes to the tubes, which can damage the mosquitoes and cause an error in the test results. This problem is partly resolved in the CDC method Because CDC bottles bioassays do not need mosquitoes to be transferred from the exposure bottle. In WHO susceptibility tests mosquitoes must remain in the recovery period (stable conditions of temperature and relative humidity) during the 24 hours after exposure to insecticide-treated paper. The environmental conditions that mosquitoes have in the recovery period can affect the test results, which is one of the disadvantages of this method. In the CDC bottle bioassays method, this problem has been solved. the CDC bottles need to be clean, dry, and coated with insecticide by oneself before each bioassay that takes a long time. If the bottles are contaminated before the coating, there is an error in the test results and this is one of the disadvantages of the CDC bottle bioassays method. The current study emphasizes that the results of the two bioassays methods (WHO and CDC) were almost similar and there was no significant difference between the two methods. Each of the two methods has some specificity. CDC bottle bioassay can be used as part of a broader insecticide resistance monitoring program, which may include the World Health Organization (WHO) paper-based bioassay, and biochemical and molecular methods. . According to this problem that concentrations that proposed by the WHO and CDC is not local, to increase quality of testing and save time and cost. it is recommended that concentrations for the most important vectors of malaria in malarious area in our country is distinction.It is suggested to carry out tests CDC Bottle bioassay used to bottles with narrow openings until when transmitting mosquitoes into the bottle, do not escape into outer space. Similar studies on important species *Culex* and *Aedes t*o be done.To evaluate the efficacy of CDC Bottle bioassay in field conditions and compared with WHO method. If the CDC bottle bioassay is to be used for routine insecticide susceptibility surveillance, the following conditions should be noted: the procedures detailed in the CDC guidelines should be strictly adhered to; in particular, those procedures relating to the use of the recommended insecticide solvents (ethanol or acetone) and the bottle treatment protocols;

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Funding

The research is financially supported by the Tehran University of Medical Sciences under code umber 95-03-27-32784, 18984.

Authors' contributions

All authors were involved

Competing of Interests

The authors declare that there are no competing interests.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Acknowledgments

Tehran University of Medical Sciences for financial support

References

1. Caraballo H, King K. 2014. Emergency department management of mosquito-borne illness: malaria, dengue, and West Nile virus. Emerg. Med . Pract; 16(5):1-23.

2. Fathian M, Vatandoost H, Moosa-Kazemi SH, Raeisi A, Yaghoobi-Ershadi MR, Oshaghi MA, Sedaghat MM.2015. Susceptibility of Culicidae mosquitoes to some insecticides recommended by WHO in a malaria endemic area of southeastern Iran. J. Arthropod-borne. Dis.;9(1):22-28.

3. Vatandoost H, Mesdaghinia AR, Zamani G, Madjdzadeh R, Holakouie K, Sadrizadeh B, Atta H, Beales PF. 2004. Development of the Regional Malaria Training Centre in Bandar-e Abbas, Islamic Republic of Iran. East .Mediterr. Health. J;10(1-2):215-24.

4. Soleimani Ahmadi M, Vatandoost H, Shaeghi M, Raeisi A, Abedi F, Eshraghian MR, Madani A, Safari R, Shahi M, Mojahedi A, Poorahmad-Garbandi F. 2012. Vector ecology and susceptibility in a malaria-endemic focus in southern Islamic Republic of Iran. East. Mediterr .Health. J18 (10): 1034-1041.

5. Khoobdel M, Keshavarzi D, Sobati H, Akbari M. 2020. Species diversity, habitat and abundance of Culicid mosquitoes in Bushehr Province, South of Iran. Biodiversitas. J .Biolog. Divery. 13;21(4).

6. Raiesi A, Hashemi-Shahri SM, Gouya MM, Ansari-Moghaddam A, Shahraki-Sanavi F, Mohammadi M, Okati Aliabad H, Tabatabaei SM, Zanganeh M, Kalan Farmanfarma K. 2019. The experiences of mobile populations about malaria control in southeastern Iran using the PEN-3 Cultural Model: A qualitative study. Health. Scope; 31;8(3).1-6.

7. Pakdad K, Hanafi-Bojd AA, Vatandoost H, Sedaghat MM, Raeisi A, Moghaddam AS, Foroushani AR.2017. Predicting the potential distribution of main malaria vectors Anopheles stephensi, An. culicifacies sl and An. fluviatilis sl in Iran based on maximum entropy model. Acta, Trop; 1;169:93-9.

8. Zahar AR.1974. Review of the ecology of malaria vectors in the WHO Eastern Mediterranean Region. Bull. World. Health. Org. 1974;50(5):427.

9. Vatandoost H, Oshaghi MA, Abaie MR, Shahi M, Yaaghoobi F, Baghaii M, Hanafi-Bojd AA, Zamani G, Townson H.2006. Bionomics of Anopheles stephensi Liston in the malarious area of Hormozgan province, southern Iran, 2002. Acta. Trop;97(2):196-203.

10. Mehravaran A, Vatandoost H, Oshaghi MA, Abai MR, Edalat H, Javadian E, Mashayekhi M, Piazak N, Hanafi-Bojd AA. 2012. Ecology of Anopheles stephensi in a malarious area, southeast of Iran. Acta .Med. Iranica. ;:61-5.

11. Hanafi-Bojd AA, Azari-Hamidian S, Hassan V, Zabihollah C. 2011. Spatio—temporal distribution of malaria vectors (Diptera: Culicidae) across different climatic zones of Iran. Asian Pacific. J. Trop. Med. ; 4(6):498-504.

12. Azari-Hamidian S. 2007. Checklist of Iranian mosquitoes (Diptera: Culicidae). J. Vector. Ecol.;32(2):235-42.

13. Mehravaran A, Oshaghi MA, Vatandoost H, Abai MR, Ebrahimzadeh A, Roodi AM, Grouhi A.2011. First report on Anopheles fluviatilis U in southeastern Iran. Acta. Trop ; 117(2):76-81.

14. Vatandoost H, Dehakia M, Djavadia E, Abai MR, Duchson S. 2006. Comparative study on the efficacy of lambdacyhalothrin and bifenthrin on torn nets against the malaria vector, Anopheles stephensi as assessed by tunnel test method. J. Vector borne. Dis;43(3):133-140.

15. World Health Organization (WHO). 2015. World Malaria Report 2014: Summary. World Health Organization; 2015.

16. Sami Abadi Y, Sanei-Dehkordi A, Paksa A, Gorouhi MA, Vatandoost H. 2021. Monitoring and mapping of insecticide resistance in medically important mosquitoes (Diptera: Culicidae) in Iran (2000–2020): A Review. J. Arthropod-Borne. Dis;15(1):21-40

17. Bagheri A, Vatandoost H, Shayeghi M, Abai MR, Raeisi A, Godwin NG, Akbari M, Sheikhi S. 2017. Evaluation on the bioefficacy of PermaNet® 2.0, a long lasting net against Anopheles stephensi. Asian Pacific. J. Trop. Dis;7:775-7.

18. Vatandoost H, Abai MR, Akbari M, Raeisi A, Yousefi H, Sheikhi S, Bagheri A. 2019. Comparison of CDC bottle bioassay with WHO standard method for assessment susceptibility level of malaria vector, Anopheles stephensi to three imagicides. J. Arthropod-borne Dis.;13(1):17-25.

19. Brogdon W, Chan A.2010. Guideline for evaluating insecticide resistance in vectors using the CDC bottle bioassay. USA: CDC Atlanta.

20. World Health Organization.2018. World Malaria Report: 2012. Geneva: WHO, 2012. Fecha de consulta.;23:247.

21. World Health Organization.2016. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. 2016.

22. Russell RM, Robertson JL, Savin NE. 1977. POLO: a new computer program for probit analysis. Bull ESA.;23(3):209-13.

23. Abbott WS.1987. A method of computing the effectiveness of an insecticide. J. Am. Mosq. Control. Assoc. 3(2):302-3.

24. Vatandoost H, Hanafi-Bojd AA.2012. Indication of pyrethroid resistance in the main malaria vector, Anopheles stephensi from Iran. Asian Pacific. J .Trop .Med;5(9):722-6.

25. Sheikhi S, Vatandoost H, Abai MR, Shayeghi M, Raeisi A, Akbari M, Nikpoor F, Aghdam MS, Bagheri A. 2017. Wash resistance and bio-efficacy of Olyset® Plus, a long-lasting insecticide-treated mosquito net with synergist against malaria vector, Anopheles stephensi. Asian Pacific. J. Trop. Med;10(9):887-91.

26. Gorouhi MA, Vatandoost H, Oshaghi MA, Raeisi A, Enayati AA, Mirhendi H, Hanafi-Bojd AA, Abai MR, Salim-Abadi Y, Rafi F. 2016. Current susceptibility status of Anopheles stephensi (Diptera: Culicidae) to different imagicides in a malarious area, southeastern of Iran. J. Arthropod-borne. Dis;10(4):493-497.

27. Vatandoost H, Mashayekhi M, Abaie MR, Aflatoonian MR, Hanafi-Bojd AA, Sharifi I. 2005. Monitoring of insecticides resistance in main malaria vectors in a malarious area of Kahnooj district, Kerman province, southeastern Iran. J. Vector borne. Dis;42(3):100-8.

28. Abai MR, Mehravaran A, Vatandoost H, Oshaghi MA, Javadian E, Mashayekhi M, Mosleminia A, Piyazak N, Edallat H, Mohtarami F, Jabbari H.2008. Comparative performance of imagicides on Anopheles stephensi, main malaria vector in a malarious area, southern Iran. J .Vector Borne. Di;45(4):307-12.

29. Khoobdel M, Akbari M, Aivazi AA, Moosa-Kazemi SH, Yousefi H, Akbari MR, Keshavarzi D, Moradi M.2020. An investigation on permethrin-treated military uniforms against diurnal mosquitoes under field conditions. Adv. Life. Xci;7(4):247-51.

30. World Health Organization.2015. Global technical strategy for malaria 2016-2030. World Health Organization; Nov 4.

31. Aïzoun N, Ossè R, Azondekon R, Alia R, Oussou O, Gnanguenon V, Aikpon R, Padonou GG, Akogbéto M. 2013. Comparison of the standard WHO susceptibility tests and the CDC bottle bioassay for the determination of insecticide susceptibility in malaria vectors and their correlation with biochemical and molecular biology assays in Benin, West Africa. Parasit Vectors;6(1):1-10.

32. Perea EZ, León RB, Salcedo MP, Brogdon WG, Devine GJ. 2009. Adaptation and evaluation of the bottle assay for monitoring insecticide resistance in disease vector mosquitoes in the Peruvian Amazon. Malar. J.;8(1):1-11.