Relative Efficacy of Different Synthetic Pyrethroid Impregnated Fabrics (ITNs) on Knock Down Time of *Anopheles Stephensi*

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We aimed to evaluate the efficacy of different fibers of bednets impregnated with various pyrethroids and the efficacy of LD50,LD90 and diagnostic dose of pyrethroids on median knock down time of susceptible strain of *An.stephensi*. The other aim of the present study was to determine distribution of knock down time values (Standard Division). Bioassey test was carried out with simple netting apparatus under laboratory condition using a new method of WHO proposed by Curtis. 11 female mosquitoes were released into net apparatus and median knock down time of them were calculated for each type of pyrethroids and fabrics, their standard division (SD) and Standard error (SE) were calculated exactly by Probit analysis of Finney.

Results showed Median knock down time in 10 mg.m-2 dosage of lambdacyhalothrin on polyester, nylon and cotton net was calculated as 3.1, 3.5 and 6.3 minutes respectively. It was calculated in 20 mg.m-2 dosage of deltamethrin on polyester, nylon and cotton net, as 6.6, 8.7 and 21.7 minutes respectively. In 40 mg.m-2 dosage of cyfluthrin on polyester, nylon and cotton net, It was calculated as 6.3, 7.5 and 19.9 minutes respectively. Finally in 200 mg.m-2 dosage of etophenprox on polyester, nylon and cotton net, It was calculated as 10.6, 12.5 and 7.4 minutes. It was seen direct relationship between LD50, LD90 and diagnostic dose of pyrethroids and median knock down time. Lambdacyhalothrin had the least value of LD50 (10 mg.m-2), LD90 and diagnostic dose, created the least value of knock down time in comparison with other pyrethroids. Etophenprox had the highest value of LD50 (200 mg.m-2), LD90 and diagnostic dose, created the highest value of knock down time. Deltamethrin and cyfluthrin had intermediate values. Also there was a reverse relationship between dosage of pyrethroids and median knock down time. Polyester net need the least concentration of pyrethroid, shown the least values of knock down time and the least LD50, LD90 and diagnostic dose. So it is the best suitable net for impregnation. In all doses of all pyrethroids on all type of nets, median knock down time was similar to the knock down time of the 6th mosquito (mode). These values were the closest value to the KDT50.

**Keywords:** Pyrethroid impregnated bednet, knock down time, *Anopheles stephensi*, I.R.Iran., Standard Division

**Introduction**
The world strategy of malaria control in the last decade is concentrated on using of pyrethroid impregnated bednets. They are the most suitable replace for residual insecticide treatments. The use of (ITNs) is an important part of Roll Back Malaria (RBM) programs which is recommended by the WHO. Because of variationes in types of nets, insecticides and their formulations and species of mosquitoes, determination of diagnostic dose of insecticides and their created knock down time on mosquitoes is through necessary [1, 2].
Pyrethroids are today the only insecticides recommended for treatment of mosquito nets [3]. This is due to the rapid knockdown effects and high insecticidal potency of pyrethroids at low dosages combined with relative safety for human contact and domestic handling *Anopheles stephensi* is the main malaria vector, mainly can be found in south urban part, flat and the coastal areas. It considers being endophilic, but biting outdoor during summer months. This species is absent during the cold winter months, but has high density during spring and autumn in mountainous and coastal region, respectively. This mosquito is resting outdoor in animal house as well as human dwelling [4]. This species in some areas showed resistance to pyrethroids. For example *An. stephensi* from Dubai (where much insecticide is used against nuisance insects) showed resistance to pyrethroids as well as to other insecticides [5]. This resistance in *An. stephensi* might due to target site insensitivity. In this regards, approved that susceptible strain responded to the lowest concentration of permethrin, but resistant strain required a much higher dose to produce the same rise in frequency of action potentials [6].

For treatment of nets WHO (2002) lists some insecticide products which have been passed the World Health Organization Pesticide Evaluation Scheme (WHOPES).

Impregnated mosquito nets have been welcomed by people at various level, it was been introduced in 2003 as ITNs and nowadays LLITNs are used in malarious areas of Iran [7]. Treated mosquito nets were used in May 2005 as ITNs when epidemic started in southern area of Iran, but now LLITNs are using [8]. Some pyrethroids are more effective than the others. For example Bifenthrin, lambdacyhalothrin and DEET were respectively more effective on mortality rate and blood-feeding inhibition [9]. Also bifenthrin is to be recommended as an effective insecticide for net treatment in comparison with lambdacyhalothrin against susceptible strain of *Anopheles stephensi* [10]. WHO [11] in comparison the efficacy of seven pyrethroids on knock down time of mosquitoes, six currently recommended by WHO and one candidate, bifenthrin, has announced that bifenthrin have a pyrethroid resistance, because of its stronger impact, despite its slower knock down effect. Rafinejad et al. [12] used three methods of bioassay tests against *An. stephensi*. They found that Permanet was more efficient than Olysetnet net. Results on ITNs showed that deltamethrin and permethrin were more effective than bifenthrin and etofenprox.

Even at low dosage of insecticides treated torn pyrethroids they are able to be effective against main malaria vector in Iran. Tests of torn nets impregnated with permethrin revealed the best Individual Protection (PI >90%) [10]. There is some factors affect on the residual insecticidal activity of impregnated nets. For example some pyrethroids have lost their effects after being washed. It has been carried out many bioassay tests to evaluate these factors. In this regards approved that lambdacyhalothrin has saved its insecticidal impact after being washed. Whereas deltamethrin has lost its activity after than other insecticides [13]. It was concluded that the effect of sun is much smaller than that of washing, and drying nets for a few hours in the sun is not harmful [14].

Reported studies in 2008 shown that out of 11460 malaria cases of Iran, 8% was due to *P. falciparum*, while 90% were infected by *P. vivax* (Ministry of Health 2008). In WHO malaria report 2009, Iran showed evidence of a sustained decrease in the number of cases associated with wide scale implementation of malaria control activities. This country is classified as in the pre-elimination stage [15].

**Materials and Methods**

*Mosquitoes:* This study was carried out in School of Public Health and Institute of Health Research in Tehran University of Medical Science, Department of Medical Entomology and Vector Control in 2006 and revised in 2010. Laboratory strain of *An. stephensi*, a principal vector of malaria in Iran, was used. This species is highly susceptible to pyrethroid insecticides and provided by London School of Tropical Medicine and Hygiene. Mosquitoes were reared to the pupal stage on a diet of BEMAX. Adults were kept at, 75±5% RH and 28±2°C in a photophase of 12:12 (L:D) h with unlimited access to 10% glucose.

*Pyrethroid insecticides:* Emulsifiable Concentration formulation (EC) of deltamethrin (25%) (Russel Uclaff company), Suspension Concentration formulation (SC) of lambdacyhalothrin (2.5%) (Zeneca company), Emulsifiable oil – in Water formulation (EW) of cyfluthrin (5%) (Bayer company) and Emulsifiable Concentration formulation (EC) of etofenprox(10%) (Mitsui company) were used during the study. The range of seven concentration of each of these pyrethroids was used to examine the relationship between dose and knock down time.
Fabrics: local fabrics were used as polyester; with 21 horizontal and 17 vertical threads per inch², 350 mesh. One meter square of this fabric absorbed 27 ml water. Nylon; with 39 horizontal and 24 vertical threads per inch², 940 mesh. Each meter square of this fabric absorbed 43 ml water. Cotton; with 35 horizontal and 26 vertical threads per inch², 910 mesh. One meter square of this fabric absorbed 75 ml water.

Impregnation technique: The absorption rate was determined by dipping one meter square of each fabric into water. The volume of insecticide required to give the target dosage as mg m⁻² was added to the water absorbed by one meter square of soaked fabric and so one meter square of the same fabric was soaked in this solution in a non-absorbent container tube and then were rubbed and squeezed to obtain uniform distribution of the insecticide over the entire piece of fabric.

The target dose was exactly calculated. The impregnated fabric was then spread on a nylon surface and allowed to dry in the shade. The dried and impregnated fabrics were labeled and maintained into black sacks in the shade away from heat and sunshine. Bioassay tests were conducted the nets within one week after impregnation of the fabrics.

Statistical analyses: Median knock down time for each of pyrethroids and nets were calculated. In all cases, these values were similar to the knock down time of the 6th mosquito (Mode). These values were similar to the KDT₅₀ too. Knock down time of the first (1st), the middle (6th) and the last (11th) mosquito were calculated. Also their Standard Division (SD) that exposed their genetic variation or distribution of knock down values, were calculated exactly by Probit analysis of Finney.

Bioassay test: Bioassay test was carried out with simple netting apparatus proposed by WHO [16]. A sperical metal wire frame was covered by pyrethroid impregnated net. In each test 11 unfed female mosquitoes were transmited into the apparatus net. Release time of mosquitoes was written. They were maintained into that until their knock down time. Each mosquito which was knocked down, deleted from apparatus net by aspirator, before again flying. In this manner knock down time of each mosquito was recorded exactly. They were transmited into clean caps, maintained in laboratory conditions (75±5% RH and 28±2º C). Next day, the numbers of alive mosquitoes were counted and their recovery rate were scored. Tests were replicated ten times for each dose, lambdacyhalothrin, deltamethrin and cyfluthrin 0.625, 1.25, 2.5, 5, 10, 20, 40 and 80 mg.m⁻², Etofenprox 6.25, 12.5, 25, 50, 100, 200 and 400 mg.m⁻², and control untreated netting.

Results
There was reverse relationship between dosage of pyrethroids and median knock down time. Median knock down time was increased when the insecticide concentration was decreased, and reverse (Table 1 & Diagram 1).

There was direct relationship between knock down time and LD₅₀, LD₉₀ and diagnostic dose of insecticides. With increase of LD₅₀, LD₉₀ and D.D, median knock down time was increased. Lambdacyhalothrin had the least value of LD₅₀ (10 mg.m⁻²), created the least knock down time. Etofenprox had the highest value of LD₅₀ (200 mg.m⁻²), created the highest knock down time (Table 1 & Diagram 3).

It was seen increasing in LD₅₀ value of pyrethroids from polyester to the cotton. Polyester nets in comparison with nylon and cotton had minimum quantity of LD₅₀, LD₉₀ and diagnostic dose. In definite concentration, mosquitoes exhibited maximum mortality. Therefore polyester nets need the minimum concentration of insecticide, and showed the least KD time, the least LD₅₀, LD₉₀ and diagnostic dose and the least recovery rate. In the contrary, the cotton acts differently (Table 1 & Diagram 4).

As shown in tables 1 & 2 and diagrams 1 & 2, we should have special notice that median knock down time was similar to the knock down time of the 6th mosquito (mode). It was obtained similar results in all doses of all pyrethroids on all type of nets. Exhibition all of them was not possible in this paper. All results were prepared in original paper at Department of Entomology and Vector Control, Tehran University.

HETEROGENY: In high dosage there was no significant differences between knock down time of the first(1st), the 6th and the last(11th) mosquito. In this case all of mosquitoes were knocked down in a short time one by one rapidly. There was no enough time to show their heterogeny or genetic variation in knock down time. In fact differences between knock down time of the first (1st), the middle(6th) and the last(11th) mosquito determine heterogeny or Standard Division(SD) in knock down times. Distribution of knock down time values of 11 mosquitoes (SD) was decreased when the insecticide concentration was increased. Therefore there was reverse relationship between
insecticide dosage and standard division or differences between the knock down time values of 11 mosquitoes (diagram 2).

In low dosage, there was significant differences between knock down time of the first(1st), the 6th and the last (11th) mosquito. In this case mosquitoes were knocked down in a long period of time one by one slowly. There was enough opportunity to show their heterogeny or genetic variation in knock down time. With decreasing of the dosage of insecticides, distribution of knock down time of 11 mosquitoes (SD) was increased(diagram 2).

Finally there was direct relationship between LD$_{50}$ of insecticides and heterogeny. When LD$_{50}$, LD$_{90}$ and D.D values were decreased, differences between knock down time values of the first and the last mosquito (SD) were decreased too, and reverse ( Table 1&2 ) .

It should be noted that there was some exceptions in cotton net impregnated with etofenprox. LD$_{50}$, LD$_{90}$ and diagnostic dose of etofenprox was highly decreased. So median knock down time was decreased highly. Etofenprox was the most effective insecticide on cotton nets, with the least LD$_{50}$ value, even in comparison with lambdacyhalothrin, deltamethrin and cyfluthrin (Table 1).

**Discussion**

There was direct relationship between the concentration of lambdacyhalothrin, bifenthrin and DEET and mortality rate of *An.stephensi* [9]. In the current study, this relationship was reverse in knock down time rate. In China,Tanzania and Kenya , median knock down time of different species of *Anopheles* (depond on geographical areas) were calculated during exposure to nets treated with deltamethrin (25 mg.m$^{-2}$) or permethrin (500 mg.m$^{-2}$) (Curtis et al. 1998) . In China KDT$_{50}$ of *An.sinensis* was calculated as 11 minutes (Kang et al. 1995). In Tanzania KDT$_{50}$ of *An.gambiae* was calculated as 10.1 – 12.2 minutes (Curtis et al. 1996). In Kenya KDT$_{50}$ of *An.gambiae* was calculated as 13-36 minutes [17].

Singh et al. [18] in India attractively concluded that field species showed a high knock down resistance to deltamethrin. KDT$_{50}$ and KDT$_{90}$ were calculated as 74 - 81 and 217 - 297 minutes respectively in comparison with pyrethroid-susceptible laboratory strain of *An.culicifacies* subspecies B & C , as 8.8 - 10.7 and 14.2 - 15.7 minutes respectively. Both B and C complexes have no significant differences in knock down susceptibility to deltamethrin . Batra et al. (2005) stated that median knock down time of *An.culicifacies* in 25 mg.m$^{-2}$ dosage of lambdacyhalothrin (CS) was about 3.1 to 11.4 minutes . Jinijiang et al. [19] conducted that KD$_{50}$, and KD$_{95}$ during the tests of nylon bednets impregnated with permethrin after seven weeks of impregnation against *An. Sinensis*, were 13.9 and 21.1 minutes respectively. In this regards, knock-down starting time was seven minutes after exposure.

In our study median knock down time of susceptible strain of *An.stephensi* created with lambdacyhalothrin (25 mg.m$^{-2}$) was about 2.1 minutes , in comparison with the result of Batra et al. (2005) which was 3.1 minutes. In our study , median knock down time of susceptible strain of *An.stephensi* created with deltamethrin (25 mg.m$^{-2}$) was calculated about 6 minutes, in comparison with the result of Singh et al. [18] in India which was 8 minutes, in comparison with the result of Kang et al. [20] in China which was 11 minutes, and the result of Vulule et al. (1996) in Kenya which was 13-36 minutes, and the result of Curtis et al. [1] in Tanzania which was 10.1 – 12.2 minutes. There was close correlation between our results and above-mentioned studies. Some differences are due to different geographical areas, different species of mosquitoes, different types of nets, insecticides and their Formulations. So in each study, the specific knock down values must have been determined.

In the other study median knock down time of *An.stephensi* exposed to deltamethrin treated unwashed nets was calculated as 4.3 minutes [12]. Therefore different species of *Anopheles* have different susceptibility and knock down time to one specific concentration of insecticides. On the other hands differences in knock down time and diagnostic dose in different studies is due to different species of *Anopheles* and their susceptibility status [21].

It should be noted that some pyrethroids have lost their effects after being washed. In fact washing of pyrethroid impregnated bed nets increased knock down time value. In this regards, Watandoost et al. [22] found that Washing of deltamethrin impregnated bed net with soap increased the KD$_{50}$ . They reported that about 80% of mosquitoes were Knocked Down in any case after 60 min. Frances et al. [23] using military shirt fabric impregnated with permethrin against *An.farauti* in Australia found < 20% of knock down after three washes. They reported only 25% of knock
down using bifenthrin after two washes. In another study, Knock down time on unwashed and 6 times washed nets impregnated by permethrin using Tube test, were calculated about 0.72 and 5.44 min respectively. Using tunnel test, it was increased to 53.35 min after 20 washes. Using Cone test, it was significantly increased from 4.16 to 37.2 minutes after 6 washes. On etophenprox-treated nets, KD time after 6 washes was 48.3 min which was 9-fold more than unwashed one [12]. In all of above-mentioned cases, Knock down time slightly increased when ITNs were washed. The results of above studies indicate that in addition to washing, there was another factor affect on KD time, such as resistance of mosquitoe to different pyrethroids. For example KDT95 of sensitive strain of An.gambiae exposure to nets treated with deltamethrin (25 mg.m-2) or permethrin (500 mg.m-2) was less than 1 hour. But KDT95 of resistance wild strain of An.gambiae exposure to nets treated with deltamethrin (25 mg.m-2) or permethrin (500 mg.m-2) was 22 hours respectively.

Different pyrethroids have no the same effect on knock down or mortality rate. In this regards Hougard et al. [24] has declared that alpha-cypermethrin had the fastest knock down effect against susceptible strain of An. gambiae. For resistance strain of An. gambiae, knock down effect of alpha-cypermethrin was slow and it was reduced by exposure to the other insecticides, particularly bifenthrin and permethrin. Also in susceptible strain of Culex, knock down effect was significantly slower than in An.gambiae, specially with bifenthrin [24].

Pyrethroids in different formulations have different knock down effect. In this regards 25 mg.m-2 dosage of deltamethrin (WP) was sprayed on both smooth and rough surface of curtains against An.maculatus in Kuala Lumpur, gave the least KD50 value. In this study Rohani et al. [25] found that WP formulation of deltamethrin in 25 mg.m-2 induced lower KD50 in comparison with WG formulation of deltamethrin in 25 mg.m-2 (P<0.05).

It should be stressed that in all of above-mentioned studies, "knock down" phrase was applied when mosquitoe fall to the floor rapidly, even again flying after falling. On the other hands, falling is different from landing. In landing case, mosquitoe has enough control on its leg and wing muscles, so lands precautiously. But in knock down case, mosquitoe fall to the floor without any control, its muscles paralyze completely even for a side-glance. In many cases, knocked down mosquitoe can rise up and fly again, because its paralyze is happened just a moment.

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References
1. Curtis CF., Myamba J. and Wilkes TJ. 1996. Comparison of different insecticides and fabrics for anti-mosquito bednets and curtains. Med.Vet. Entomol.,1996; 10: 1-11.
2. Curtis CF. 2005. Insecticide treatment bednets to prevent malaria. Sci. Dev. November 2005.
3. Zaim M., Aitio M. and Nakashima N. 2000. Safety of pyrethroid-treated mosquito nets. Medical & Veterinary Entomology. 2000; 14 : 1-5.
4. Vatandoost H., Golizadeh S., Abai M.R. and Djavadian E. 2006. Laboratory Efficacy of Protection Rate of Torn Nets Treated With Pyrethroids, Cyfluthrin, Deltamethrin, nd Permethrin Against Anophelles stephensi (Diptera: Culicidae). Journal of Biological Sciences. 2006, 6(2): 331-336.
5. Ladonni, H. & Townson, H. 1998. A major gene conferring permethrin resistance in the larvae of the malaria vector Anopheles stephensi. Bull. Entomol. Res.
6. Vatandoost, H., McCaffery, A. & Townson, H. 1998. An electro-physiological investigation of target-site insensitivity in permethrin-resistant and permethrin-susceptible strains of Anopheles stephensi. Iranian Journal of Arthropod-Borne Diseases. 1998 ; 27(3-4) : 29-44.
7. Moosa-Kazemi SH., Vatandoost H., Raeisi A. and Akbarzadeh K. 2007. Deltamethrin Impregnated Bed Nets in a Malaria Control Program in Chabahar, Southeast Baluchistan, I.R. Iran. Iranian J. Arthropod-Borne Dis. 2007; 1(1): 43–51
8. Hanafi-Bojd AA, Vatandoost H., Philip E., Stepanova E., Abdi A., Safari R., Mohseni GH., Bruhi MI., Peter A., Abdulrazag SH. And Mangal G. 2010. Malaria Situation Analysis and Stratification in Bandar Abbas County, Southern Iran, 2004–2008. Iranian J. Arthropod-Borne Dis, 2010; 4(1): 31–41.
9. Vatandoost H., Dehaki M., Djavadian E. and Abai MR., 2005. Comparative performance of torn impregnated nets by bifenthrin, lambdacyhalothrin and DEET against malaria vector An.stephensi in Iran using tunnel test. *Medicine and Health in the Tropics, Marseille*, 11-15 September 2005; France.

10. Vatandoost H., Dehakia M., Djavadian E., Abai MR. & Duchon 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. Vec. Borne Dis.* 43 September 2006, PP.133-135.

11. WHO, 2002. Malaria vector control, decision making criteria and procedures for judicious use of insecticides. Najera NJ. and Zaim M. (Eds), *WHO/CDS/WHOPES/2002*.

12. Rafinejada J., Vatandoosta H., Nikpoora F., Abaia M.R., Shaeghia M., Duchenb S. and Rafia F. 2008. Effect of washing on the bioefficacy of insecticide-treated nets (ITNs) and long-lasting insecticidal nets (LLINs) against main malaria vector *Anopheles stephensi* by three bioassay methods *J. Vector Borne Dis.* 45, June 2008; PP: 143–150.

13. Vatandoost H., Ramin E., Rassi Y. 2009. Stability and Wash Resistance of Local Made Mosquito Bednets and Detergents Treated with Pyrethroids against *Anopheles stephensi* . *Iranian Journal of Arthropod-Borne Diseases*. 2009;3(1) : 19-28.

14. Kayedi M.H., lines JD., Haghdooost A.A., Vatandoost MH. Eassi Y. and Khamisabadi K. 2008. Evaluation of the effects of repeated hand washing, sunlight, smoke and dirt on the persistence of deltamethrin on insecticide-treated nets. *Transactions of Royal Society of Tropical Medicine and Hygiene*. 2008; 102(8): 811-816.

15. WHO, 2009. World malaria report 2009. WHO Press, World Health Organization, Geneva. 66 pages.

16. WHO, 1997. The impact of permethrin and deltamethrin resistance in *Anopheles gambiae* S.S on the efficacy of insecticide-treated mosquito nets. Darriet F., Guillette P., N’Guessan RN., Doannio JMC., Koffi AA., Carnevale P. *WHO/MA1/99.1088*. WHO/WHO/99.1002 / 1997.

17. Vulule J., Beach JM., Atieli FK., Mount DL., Roberts JM. & Mwangi RW. 1996. Long term use of permethrin-impregnated nets does not increase *Anopheles gambiae* tolerance. *Med.Vet. Entomol.*. 10: 71-79.

18. Singh OP., Raghavendra K., Nanda N., Mittal PK. and Subbarao SK. 2002. pyrethroid resistance in *Anopheles Culicifacies* in Surat district, Gujarat,west India . *Research Communications current science*. 2002; 82 (5): P:47-550.

19. Jingjiang X Li Z, Banquan L., Taihua Z. and Mingxin L. 1987. Mosquito nets impregnated whit deltamethrin against malaria vectors in China. *WHO/WHO/87-93/1987*.

20. Kang W., Gao B., Jiang H., Wang H., Yu T., Yu P., Xu B. and Curtis CF. 1995. Tests for possible e:fects of selection by domestic pyrethroids for resistance in culicine and anopheline mosquitoes in Sichuan and Hubei. *China. Ann. Trop. Med. Parasitol.*. 1995; 89: 677–84.

21. Sharma S.K., Upadhyay AK., Haque MK., Padhan K., Tyagi PK., Batra CP., Adak T., Dash AP. and Subbarao SK. 2005. Village-Scale evaluation of mosquito nets treated with a tablet formulation of deltamethrin against malaria vector. *med. Vet. Entomol.*, 2005; 19: 286-292.

22. Vatandoost H., Ramin E., Rassi Y. 2009. Stability and Wash Resistance of Local Made Mosquito Bednets and Detergents Treated with Pyrethroids against *Anopheles stephensi*. *Iranian Journal of Arthropod-Borne Diseases*. 2009;3(1) : 19-28.

23. Frances SP, Watson K, Constable BG. Comparative Toxicity of permethrin and bifenthrin-treated cloth fabric for *Anopheles farauti* and Aedes aegypti. *J Am. Mosq Control Assoc.*. 2003; 9(3): 275–8.

24. Hougard JM., Duchon st., Darrie , Fredric et al., 2003. Comparative Performances,under laboratory conditions, of seven pyrethroids used for impregnation of mosquito nets. *Bull World Health Organ.*. 2003; 81 (5) , P:324-333.

25. Rohani A., Saadiyah I., Walgun A. and Lee HL. 2007. Laboratory study on the effect of Deltamethrin WG and WP formulations against An.maculatus on rough and smooth surface of bamboo wall . *Tropical Biomedicine*. 2007; 24(2) : 77-82.
Appendixes

Diagram 1. Revised relationship between dosages of cyfluthrin (EW, 5%) impregnated on polyester net and median knock down time.
Diagram 2. Heterogeneity or differences between the knockdown time of the first (1st) and the last (11th) mosquito. At low dosages, standard division or heterogeneity is high, and reverse.
Table 1. Relative efficacy of different synthetic pyrethroids and different fabrics on knock down time of *An.stephensi*.

| Insecticide         | Lambdacyhalothrin | Deltamethrin | Cyfluthrin | Etophenprox |
|---------------------|-------------------|--------------|------------|-------------|
| Fabric              | 1th   | 25   | 10   | 20  | 40  | 80  | 12.5 | 25 | 200 | 400 | Polyester |
| 10th mos. KD. time (m:s) |       |      |      |     |     |     |      |    |     |    |  
| 1st                  |       |      |      |     |     |     |      |    |     |    |  
| 6th                  | 14:06 | 11:57 | 03:04 | 02:00 | 01:01 | 15:28 | 12:00 | 06:35 | 04:55 | 03:20 | 23:30 | 19:06 | 06:21 | 02:18 | 01:00 | 24:00 | 14:01 | 10:24 | 02:54 |
| 11th                 | 17:24 | 15:00 | 05:24 | 04:01 | 02:04 | 21:12 | 16:54 | 10:42 | 09:54 | 06:38 | 27:42 | 23:54 | 09:51 | 04:20 | 02:01 | 32:36 | 20:06 | 15:00 | 04:10 |
| Median KD. time      | 14:07 | 11:56 | 03:03 | 02:01 | 01:02 | 15:30 | 12:00 | 06:36 | 04:55 | 03:24 | 22:30 | 19:05 | 06:20 | 02:20 | 01:02 | 24:00 | 14:00 | 10:40 | 02:54 |
| 1st                  |       |      |      |     |     |     |      |    |     |    |  
| 6th                  | 14:04 | 10:30 | 03:31 | 02:24 | 01:28 | 17:40 | 13:47 | 08:41 | 06:17 | 04:18 | 24:55 | 20:19 | 07:35 | 02:43 | 01:21 | 14:01 | 15:03 | 12:27 | 04:30 |
| 11th                 | 18:59 | 15:30 | 04:53 | 03:37 | 02:30 | 25:38 | 20:53 | 12:19 | 10:25 | 08:19 | 28:01 | 29:30 | 11:36 | 06:41 | 03:00 | 18:00 | 22:30 | 17:29 | 08:30 |
| Median KD. time      | 14:03 | 10:29 | 03:30 | 02:25 | 01:30 | 17:40 | 13:48 | 08:40 | 06:17 | 04:19 | 25:00 | 20:19 | 07:35 | 02:42 | 01:20 | 14:00 | 15:04 | 12:25 | 04:30 |
| 1st                  |       |      |      |     |     |     |      |    |     |    |  
| 6th                  | 18:54 | 13:54 | 06:17 | 04:48 | 03:36 | 80:00 | 79:54 | 21:42 | 24:32 | 16:13 | 67:36 | 51:12 | 19:54 | 17:01 | 14:54 | 13:01 | 10:20 | 07:19 | 04:12 |
| 11th                 | 26:24 | 19:54 | 10:19 | 08:24 | 06:30 | NS   | 121:00 | 25:06 | 30:20 | 24:38 | 121:00 | 82:12 | 29:48 | 26:12 | 22:45 | 15:48 | 14:12 | 10:57 | 07:18 |
| Median KD. time      | 19:00 | 14:00 | 06:20 | 04:49 | 03:35 | 81:00 | 79:50 | 21:41 | 24:33 | 16:10 | 67:35 | 51:14 | 19:55 | 17:00 | 14:55 | 13:00 | 10:20 | 07:20 | 04:15 |

Dosages (mg.m-2), Knock Down time (minute:second).

Table 2. The result of knock down test on polyester nets impregnated by different dosages of deltamethrin (EC) (%) against *An.stephensi*.
| Dosage | No. of a.i. Mos. | First Mos. | 6th Mos. | 11th Mos. | Mean KD. time | SD KD. time | SE KD. time | Mean Median KD. time | SD Median KD. time | SE Median KD. time | Total KD. time | Mortality & survival of contacted mosquitoes |
|--------|-----------------|------------|----------|-----------|---------------|-------------|-------------|---------------------|---------------------|---------------------|-------------|--------------------------------------------|
| mg/m²  | Tested KD. time | time       | time     | time      |               |             |             |                     |                     |                     |             | (until total KD. time )                |
| 0.625  | 110             | 09:06      | 18:00    | 26:00     | 18:00         | 00:49       | 00:31       | 17.51               | 05:33               | 01:31               | 110          | 51 59 53.6 46.3                             |
| 1.250  | 110             | 08:24      | 15:23    | 21:12     | 15:30         | 01:36       | 00:30       | 15:06               | 04:16               | 01:16               | 110          | 25 85 77.2 22.7                             |
| 2.500  | 110             | 06:36      | 12:00    | 16:54     | 12:00         | 00:36       | 00:12       | 11:47               | 03:18               | 00:59               | 110          | 0 110 100 0                                  |
| 5.000  | 110             | 05:30      | 10:30    | 14:42     | 10:31         | 00:30       | 00:09       | 10:20               | 03:02               | 00:54               | 110          | 0 110 100 0                                  |
| 10.000 | 110             | 04:54      | 09:06    | 12:18     | 09:08         | 00:21       | 00:07       | 08:46               | 02:30               | 00:45               | 110          | 0 110 100 0                                  |
| 20.000 | 110             | 03:12      | 06:35    | 10:42     | 06:36         | 00:25       | 00:08       | 06:30               | 02:18               | 00:42               | 110          | 0 110 100 0                                  |
| 40.000 | 110             | 01:43      | 04:55    | 09:54     | 04:55         | 00:25       | 00:08       | 05:23               | 02:28               | 00:45               | 110          | 0 110 100 0                                  |
| 80.000 | 110             | 00:59      | 03:20    | 06:38     | 03:24         | 00:31       | 00:10       | 03:40               | 01:48               | 00:31               | 110          | 0 110 100 0                                  |

There are more similar tables showing other pyrethroids impregnated on the other type of nets in Institute of Health Research, Tehran University, Expose of all of them in this paper is not possible.
Diagram 3: The comparison of 4 pyrethroids in crested KD time on polyester net. Direct relationship between LD50 and median knockdown time. Lambda-cyhalothrin had the lowest LD50, LD90 and D.D., so it created the lowest median knockdown time.
Diagram 4. The comparison of three types of nets in created KD time. Cotton creates the highest knock down time.

Note: There are similar diagrams that show regular relationships in other types of nets or other pyrethroids in principal documents at the Department of Medical Entomology and Vector Control, Tehran University. Exposure to all of them in this paper is not possible.