

Larvicidal Properties of Botanical Extracts of *Lawsonia inermis* against *Anopheles stephensi*

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Received 29 August 2014; revised 15 October 2014; accepted 19 November 2014

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Abstract

The aim of this study was to determine the larvicidal activity of *Lawsonia inermis* against *Anopheles stephensi* as the main malaria vector in Iran. This study was carried out from February to July 2011. Larvicidal activity of *L. inermis* was studied in the range of 4 - 4000 PPM in the laboratory against early and late stages of larvae of *An. stephensi*. The larvae were reared in the insectarium. The LC₅₀ and LC₉₀ values of the larval stages of *An. stephensi* were calculated by probit analysis and regression line draw using Microsoft office excel 2003 software. The highest toxic effect of *L. inermis* was found at 4000 PPM and the lowest at 4 PPM against larval stages I and II. The same result was found against larval stages III and IV. The LC₅₀ and LC₉₀ was found as 413.8 and 3366.3 respectively against larval stages I and II while against late stages found as 696.9 and 3927.7 respectively. This study suggests that *L. inermis* extract can be used as an alternative larvicidal compound during the IPM programs for the *An. stephensi* control. It is recommended to investigate the competency of other similar plants to malaria control.

Keywords

Anopheles stephensi, *Lawsonia inermis*, Larvicidal Properties

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1. Introduction

The vector borne diseases is known to be important due to Arboviral, bacterial, parasitological and other pathogens they transmit. Some diseases transmitted by arthropods are important in Iran [1]. Manouchehri in 1992 indicated the role of Anopheles genus, and other Culicinae mosquitoes to transmit the malaria, encephalitis and Dirofilariasis in Iran. By now, eight species have identified as proven and suspected vectors of malaria in Iran [2]-[12]. *An. stephensi* is considered as one of the main malaria vectors in Iran. Resistance of the species to the organochlorine, organophosphorus, carbamates and some pyrethroids insecticides reported. Plant extracts are environmental-friendly and the alternative compounds of vector control agent.

An. stephensi is known to be one of the most important vectors of malaria in the Middle East and India [2] [13]. This species is considered as zoophile [14]. Feeding behavior of vectors is the most important indicator in the epidemiological studies due to determine the vectorial capacity and transmission capacity [15]. Environmental factors effect on the vectorial capacity [16]. The olfactory systems of mosquitoes determine the host preferences [17] [18]. Application of plants such as *Lawsonia inermis* to treatment and prevention of some infectious diseases is reported [19]-[24].

Since susceptibility of *An. stephensi*, after they are exposed to different insecticides, their larvae have been evaluated for susceptibility [9] [10]. Some tree oils were investigated previously such as Neem tree extract [8]. *L. inermis* is the scientific name of the henna plant (native) in Iran. This compound has been used in the painting of hairs, hands and legs traditionally. Henna also has been used in painting some agents in industry and its use in health sciences due to antibacterial and pathogens effects [25]-[29]. By now this compound is used in the traditional medicine, leprosy and eczema (itchy skin) and burn [19] [20] [22]-[24]. *L. inermis* is also very important in using as a freshener because of its nice odor and it is used for body painting especially in Muslim region [30]. Many reports stated the effects of *L. inermis* on gram-positive bacteria such as *Staphylococcus aureus*, *Enterococcus faecium* and *Bacillus subtilis* while no evidence was observed due to effects on gram-negative bacteria [31]. So far, only a therapeutic effect on wound healing associated with cutaneous leishmaniasis has been reported on mice [32].

The use of plant extracts is useful due to their no side effects on the environment and its bio cycle. Although, there are many studies of the effectiveness of these compounds on the lethal and repellent aspects of plant extracts, scatter studies have been done that relates to henna extract. Therefore, it was necessary to evaluate the lethal effect of this compound on *An. stephensi* larvae. Despite numerous applications that are used in control of *Culicidae* mosquitoes, the results is not yet effective in relating to vector resistance and socio economic agents [33]. Madhu et al. in 2010 reported the effectiveness of *Curcuma aromatica* extracts on malaria vectors [34]. Some plant extracts have been used against Japanese encephalitis vectors in the Far East [35].

Some plant extracts such as IGR compounds have been used against mosquito larvae in Tanzania [36]. In South America, the extracts of native plants have been used against *Aedes aegypti* larvae [37]. *Etonia rozmaryi* plant extract found a significant impact on the immature stages of *Culicidae* mosquitoes [38]. Repellency effect and excito repellency effect of herbal compounds properties have been studied against *Culicidae* mosquitoes in Egypt [39]. Recent studies indicated the attract effect of *Siliense otites* extract against *Culicidae* mosquitoes [40].

Vatandoost and Vaziri in 2004 reported the effect of *Azadirachtin indica* against *An. stephensi* larvae in southern Iran [17]. The effectiveness of the plant Myrtle, *Myrtus communis* (Myrtaceae) against *Ph. papatasi* was carried out in Iran [41].

Although, there has been many studies on the chemical components of the Henna, but yet its compounds have not been distinguished completely. Recent studies indicated at least seven to eight percent Tanoan, 6% fatty acid, 1.2% essence, 2% - 3% resin, 0.2% Lawson, mannitol and the mucilage. So far only one case has been reported in the efficacy of these extracts on wound healing and treatment of Antroponotic coetaneous leishmaniasis in mice [32]. Accordingly, the efficacy of *L. inermis* plant extract was evaluated during the study on immature stages of *An. Stephensi*.

2. Materials and Methods

In this study the extract of the plant has been used. This compound comprised the active ingredient of *L. inermis* and non-effective compound of methanol. The concentration prepared according to WHO instrument. The different concentrations prepared as 4, 40, 400 and 4000 PPM. The concentration diluted in methanol. Methanol is

a solvent that can be used for allelochemicals. Methanol has the ability for extracting both polar and non-polar compounds [42]. The different concentrations of 4, 40, 400 and 4000 PPM prepared according to WHO instrument. The concentration diluted in methanol and then serial solution prepared in the lab.

A laboratory study was includes both extraction and clean up. This study was carried out from February to July 2011 in 2 labs. The extraction preparing and clean-up was occurred in the pharmacology lab and the susceptibility test in the insectariums, Tehran University of Medical Sciences, Iran. The fresh leaves of *L. inermis* were cut from the nature trees in Dalghan area, Iranshahr County, Sistan and Baluchestan Province, Southeast Iran. These leaves were transmitted quickly to insectarium and kept in small packs, coded and sent to the pharmacy lab, Tehran University of Medical Sciences. The packs were sealed by Nescofilm and kept in 4°C temperature [43]. The fresh leaves were dissolved in ethanol 80% and then were extracted using percolation method [20]. The extraction was concentrated under vacuum distillation condition. The concentrate extract were kept in -20°C [19] [20]. 300 cc capacity of beaker used in this research. One cc of stock solution added to 224 cc of de-chlorinated water and also 24 ml of de-chlorinated water with 25 *An. stephensi* IND strain larvae aged 1 - 2 and 3 - 4 inserted to finally solution 250 cc. The final solution shacked gently to prepare the homogenized solution. The mortality rate calculated after 24 hours recovery at the standard condition in 25°C and more than 60% relative humidity. The result was corrected by abbot formula [44] [45].

$$\text{Improved Mortality \%} = \frac{\text{Test Mortality \%} - \text{Mortality of Control \%}}{100 - \text{Mortality of Control \%}} \times 100$$

The mortality less than 5% in control group means the result is acceptable. The mortality between 5% - 20% means that the results should be corrected by Abbott formula. The mortality more than 20% means the results should be rejected. Different extract solutions from treatment and control samples as well as standard solution were used. The susceptibility test was according to WHO standard method. Soluble components are removed with a solvent flow. Extracts collected from this level of concentration insert to distiller under vacuum and 35°C to 40°C. In order to preparing the extract, chopped herb were inserted to Erlenmeyer flask and incubated in water bath at 50°C - 60°C. Add the solvent to chopped herbs to cover it up. Acetone is polar solvent and N-hexane considered as non-polar solvent. The mixture were mixed gently inside the water bath with the warm water till the oil dissolved in N-hexane completely [19] [20]. The data were analyzed by using SPSS 11.5 and STATA 8.0 a three-way ANOVA test was used to compare the mortality of *An. stephensi* larvae for 3 groups. The mortality was considered significantly when the *P value* was less than 0.05 [46]. LC₅₀ and LC₉₀ values and probit regression line parameters were prepared from plotting the regression line using Microsoft office Excel software (Figures 1-3).

3. Results

Probit analysis of the data done and the results are presented in **Table 1**. Results of the tests against larval stages I, II of *An. stephensi* revealed the LC₅₀, and LC₉₀ values to *L. inermis* were 559.7, 5315.3 PPM. The LC₅₀ and LC₉₀ values to *L. inermis* found 696.6 to 3927.2 PPM against larval stages III, and IV.

In this study, the LC₅₀ calculated as 4, 40, 400 and 4000 PPM were exposed to larval stages. The LC₅₀ calculating in this study indicated that the younger stages are more sensitive to old stages. LC₅₀ values calculated by examining the effect of Henna extract on the larvae of different age's shows that the correlation between the increasing of larval age and larval resistance to the *L. inermis* extract Logarithmic relationship between dose and probit mortality of feeding larvae of different ages and different concentrations of the extracts are shown in **Table 1** to **Table 2**. Also probit regression line parameters of *Lawsonia inermis* extract against larval stages of *An. stephensi* is shown in **Table 3**. Our study indicated that the mortality increased with increasing extract concentration in all larval stages.

4. Discussion and Conclusions

Vector resistance was reported to DDT in 1957, Dieldrin in 1960, and then Malathion in 1976. Propoxure was used after reports of Malathion resistance, in 1978. Twelve insecticides were recommended by WHO for IRS currently, which belong to four chemical groups that include an organochlorine, six pyrethroids, three organophosphors and two carbamates [47].

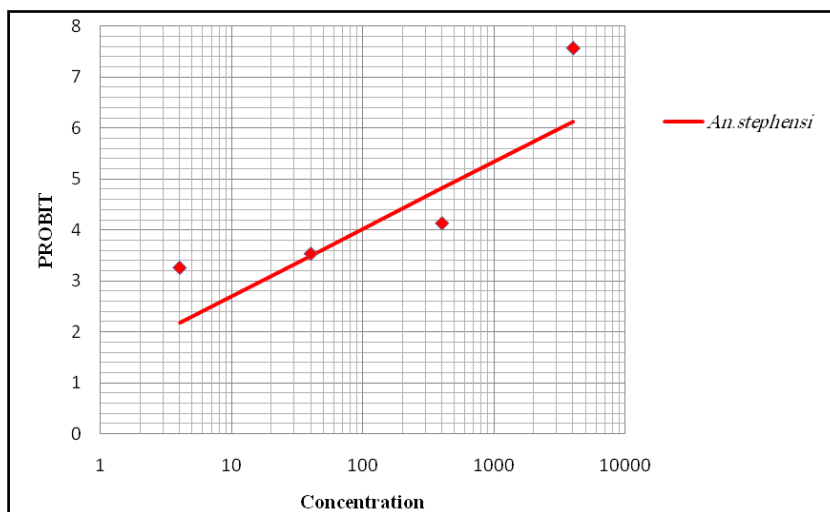


Figure 1. Probit regression line of *An. stephensi* larval stages I, II exposed to different concentration of *Lawsonia inermis* L., 2011.

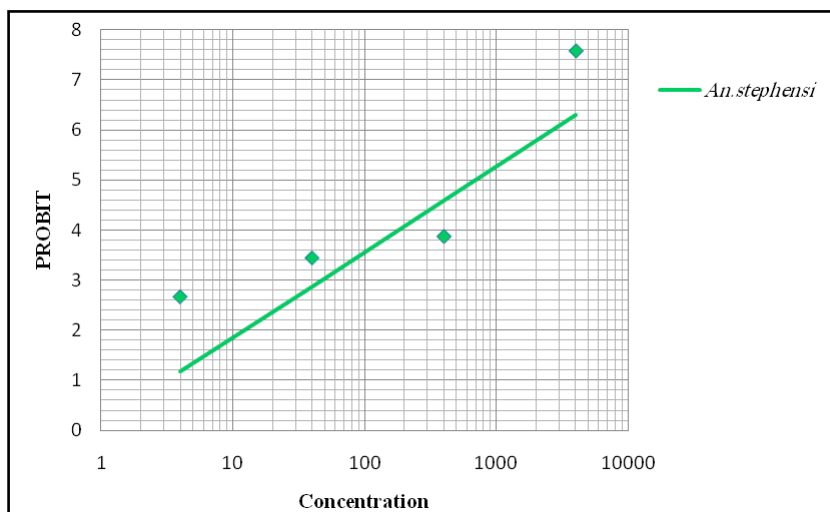


Figure 2. Probit regression line of *An. stephensi* larval stages III, IV exposed to different concentration of *Lawsonia inermis* L., 2011.

Table 1. The mortality of *An. stephensi* larvae I, II stages after 24 hr exposed to different concentration of *L. inermis*.

Treatment	Replicate	Larvae number	Alive No. after 24 hr	Mortality % after 24 hr
4 PPM	4	100	96	4%
40 PPM	4	100	93.5	6.5%
400 PPM	4	100	81.5	18.5%
4000 PPM	4	100	0	100%
Control	4	100	98.5	1.5%

Anopheles stephensi is the main malaria vector in Iran. By now, seven species of *Anopheles* were reported as the malaria vectors in the country including: *An. fluviatilis* s.l., *An. culicifacies* s.l., *An. sacharovi*, *An. maculipennis* s.l., *An. superpictus*, *An. stephensi*, and *An. dhali* [48]. In addition, Zaim et al. reported the *An. pulcher-*

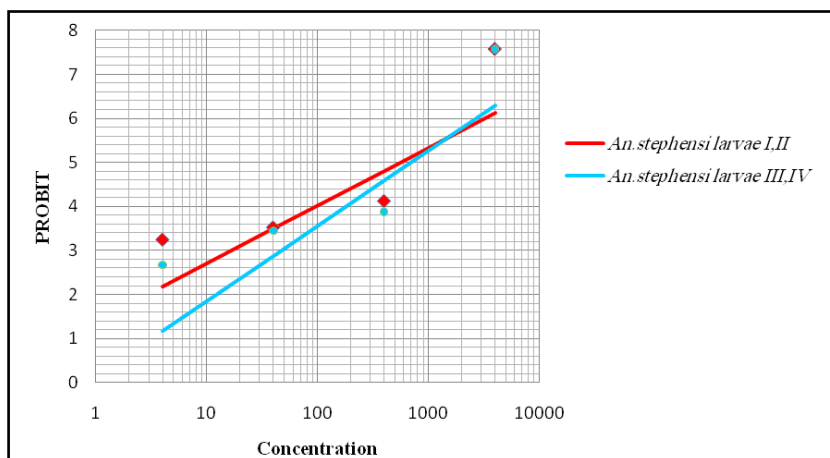


Figure 3. Probit regression line of *An. stephensi* larval stages I, II, III, IV exposed to different concentration of *Lawsonia inermis* L., 2011.

Table 2. The mortality of *An. stephensi* larvae III, IV stages after 24 hr exposed to different concentration of *L. inermis*.

Treatment	Replicate	Larvae number	Alive % after 24 hr	Mortality % after 24 hr
4 PPM	4	100	99	1%
40 PPM	4	100	94.5	5.5%
400 PPM	4	100	87.5	12.5%
4000 PPM	4	100	0	100%
Control	4	100	99	1%

Table 3. Probit regression line parameters of *Lawsonia inermis* L. extract against larval stage of *An. stephensi* Liston IND strain, 2011.

Species	A	B ± SE	UP		X ² (df)	P value	Y = A + BX
			LC ₅₀	LC ₉₀			
			LOW	LOW			
<i>An. stephensi</i> Larvae I, II	-3.6027	1.3110 ± 0.116	766.0236	9754.3132	95.157 (2)	0.05%	-3.6027 + 1.3110X
			559.7239	5315.3032			
			413.8599	3366.3809			
<i>An. stephensi</i> Larvae III, IV	-4.851	1.7064 ± 0.159	909.4682	6409.2969	192.938 (2)	0.05%	-4.8513 + 1.7064X
			696.6730	3927.2168			
			535.5461	2721.6514			

rimus as secondary vectors of malaria in the South East of Iran [49]. Oocyst of *Plasmodium* was found at the first time in *An. multicolor*, while not in salivary glands [50]. Avian malaria was reported in Iran by Ghaffari, 1985 [51].

Against *An. stephensi* from the pooled results, World Health Organization is able to provide a specific guideline for mosquitoes and this guideline will help the countries with monitoring and evaluation of insecticide resistance for implementation of control measures. The LC₅₀ of larval stages III, IV calculated as 2000 PPM. This indicates that there is a significant difference in mortality rate of larval stages. The result of our study show that the mortality of larvae stages I, II are more than III, IV with 99% confidence interval $P < 0.05$. Bernays et al. in

1980 reported that tannin and tannic acid cause the damage on epithelial membrane of gut of mosquito larvae. So this compound is responsible for mortality of mosquito larvae. In fact, extract of *L. inermis* is comprised of tannin and tannic acid and agent to damage the epithelial membrane and suppress the immunity system of mosquito larvae. Govindarajan *et al.* in 2011 reported delaying the mortality of mosquito larvae after they exposed to plant extracts [52]. Tannin and tannic acid is considered as a non-crystallized compound. It seems that this compound causes the delaying to mortality of the mosquito's larvae. The result of this study can be useful in malaria control program as IPM programs.

In conclusion, we recommend the same procedure in different parts of the world to check the results and reach the unique conclusion about criteria for susceptibility status.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgements

The authors would like to express our appreciation to the insectary staff of the school of public health, Tehran University of Medical Sciences.

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