Larviciding Activity of Acroptilon repens Extract against Anopheles stephensi, Culex pипiens and Culex pipiens under Laboratory Conditions

Ramesh Toolabi¹, Mohammad Reza Abai¹*, Mohammad Mehdi Sedaghat¹, Hassan Vatandoost¹, Mansooreh Shayeghi¹, Saeed Tavakoli², Mohammad Sistanizadeh Aghdam¹

ABSTRACT

Introduction: Due to the emergence of insecticide resistance among the vectors of human diseases, there is a need to explore the use of plant extracts which are safe alternatives to conventional chemical larvicides used in control of vector-borne diseases. The aim of this study was to evaluate the larvicidal activity of Acroptilon repens against third instar larvae of Anopheles stephensi, Culex pипiens and Culex pipiens. Materials and Methods: The aerial parts of A. repens plants were dried in the shaded places for 3 days at 26-28°C. The leaves, flowers and stems were blende into a powder using an electric blender. The larvicidal activity of total extract of A. repens was estimated against third instar larvae stage of mosquito vectors, An. stephensi, Cx. pipiens and Cx. pipiens under laboratory conditions with 24h exposure period. Data were subjected to probit regression analysis in order to estimate the lethal concentrations for 50% and 90% mortality values. Result: The extract of A. repens exhibited significant larvicidal activity against third instar larvae of An. stephensi, with 24h LC₅₀ of 0.2970 and LC₉₀ of 2.2097 mg/l. The LC₅₀ and LC₉₀ values were 2.5047 and 24.7374 mg/l for Cx. pипiens and 2.9047 and 16.1459 mg/l for Cx. pipiens. Conclusion: The extract of A. repens can serve as a natural larvicide against An. stephensi, Cx. pipiens and Cx. quinquefaciatus. According to the larvicidal properties of this plant, formulating an extract of A. repens which is known as an abundant agricultural weed in Iran creates an alternative to chemical larvicides and providing a job opportunities. Key words: Acroptilon repens, Anopheles stephensi, Culex pипiens, Culex pipiens, Larvicidal activity, Total extract.

INTRODUCTION

Vector-borne diseases are illnesses transmitted by the vectors which can transmit infectious diseases between humans or from animals to humans and account more than 17% of all infectious diseases, causing more than 1 million deaths annually. One sixth of the illness and disability suffered worldwide is due to vector-borne diseases.¹ Dengue is an acute mosquito-borne viral infection which regarded as the most important arboviral disease and more than 2.5 billion people in over 100 countries live in areas where has a significant socioeconomic and disease burden.² Malaria causes were more than 214 million in 2015 which most of them children under 5 years of age in the world.³ Anopheles stephensi is considered as both urban and rural mosquito in Iran.⁴ Bio pesticides have been found to contain natural constituents of plants, animals, bacteria, viruses and fungi that have been proposed as an alternative to chemical pesticides for insect control. These materials are harmless to humans, animals and the environment and are easily decomposed in soil and are not stored in plants or animals.⁵ Over the past two decades, numerous extracts or essential oils from indigenous plants have been evaluated against larvae of An. stephensi.⁶ Many of vector borne diseases are preventable through informed protective measures. Although there are several methods for controlling the Anopheles mosquitoes, however the environmental effects and resistance are the main human concern. Synthetic pyrethroids which considered as the most effective insecticides against anophelines, are still expensive and beyond the financial resources of some countries.⁷ Members of the plant families including Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Milieaceae, Oocyctaceae and Rutaceae have various types of larval, adulticidal or repellent activities against different species of mosquitoes.⁸ This study aimed to study the larviciding activity of total extract of A.
repens which considered as a dominant and disturbing weeds in the garden and agricultural lands.

MATERIALS AND METHODS
Mosquito rearing
The rearing and maintenance of An. stephensi, Cx. pipiens and Cx. quinquefaciatus were conducted in the Calicidae Insectary of School of Public Health, Tehran University of Medical Sciences. Each species was reared in a separate room. The larvae and adult rearing rooms were equipped with the electronic devices for adjusting temperature, light and humidity. The insectary conditions were fixed at 29±2 °C, dark to light photoperiod of 16:8 h and relative humidity of 70±10%. The late 3rd instar larvae were used for evaluation of larvicidal effects of total extract. Plastic containers with dimension of 35×25 cm was used to rear the immature stages (eggs, larvae and pupae) of the mosquitoes. Two liters of de-chlorinated water with temperature of 24-25 °C were used for immature rearing. Deposited eggs were gently released in the middle of teared paper to prevent the desiccation of mosquito eggs due to attachment to the margin of the containers. The temperature of the immature rooms was adjusted between 29 to 30°C. In these conditions, the eggs were hatched after 1-2 days. Larvae were fed with flaky fish food which was added daily on the surface of the rearing water. Containers for immature stages were thoroughly washed with water and dishwashing liquid, well rinsed and then dried.

MATERIALS
Russian Knapweed, A. repens was collected at the flowering stage (Aug-Sep 2015) from natural habitats in the Makou district, West Azarbaijan province, Iran. A voucher specimen was deposited under code 2731-UT in the herbarium of Department of Medical Entomology and Vector Control. The collected samples were allowed to dry in a shaded room and maintained inside a dark plastic bag.

Extraction
About 250 g crashed plant was transferred into the Buchner funnel of the percolator device. One thousand five hundred mills (1.5L) of pure methanol solvent (99.9%) was added into the Buchner funnel so that the solvent height was about 5 cm above the surface of crushed plant. About 48h, the methanol was drained and collected in the crystalizer. This operation was repeated 3 times to collect the entire extract. After smoothing, using a filter paper, the resulting solution containing methanol-soluble compounds was collected. Concentration was performed by rotary evaporator in vacuum (60-100 rpm and 50°C). After evaporation of the solvent, the condensate extract was transferred to the crystalizer and put on the hood for complete drying. The condensate extract was diluted using pure ethanol as a solvent and the exposure concentration was prepared for the bioassays.

Bioassays and larval mortality
The larvicidal activity of the total extract was assayed according to WHO guideline.9,10 Preliminary tests were carried out to establish concentrations from stock solutions of the total extract. The stock concentration was 5.0 g per 100 ml of pure ethanol (50000 ppm) and the first higher exposure concentration was 781.25 ppm which serially diluted until the lowest concentration 3.05 ppm.

For each concentration, 25 larvae were used. Each test run consisted of 224 ml water, 1 ml of test sample of stock solution and 25 larvae in 25 ml water; so that the final volume was 250 ml. In the control group, 1 ml of pure methanol was added to 249 ml of de-chlorinated water in the 400 ml glass beakers. Mortality was read after 24 h exposure period.

Statistical analysis
In the analysis, both dead and moribund larvae were considered as dead. The lethal concentrations (LC50 and LC90) were calculated using probit regression analysis and related parameters determined. For all bioassays, the mortality rate was adjusted for the mortality in control group by using Abbott’s correction (Abbott 1925) if the control mortality was between 5 and 20%. Significant difference between the means of mortality rate for each mosquito species was assessed by ANOVA method using PASW release 18.

RESULTS
The larvicidal activity of total extract A. repens was tested with 9 logarithmic concentrations from 3.05 to 781.25 ppm with each concentration replicated at least 4 times. The lethal concentrations of 50% and 90% of the total extract of A. repens were estimated to be 0.2970 and 2.2097 ppm for An. stephensi, 2.5074 and 24.7374 ppm for Cx. pipiens and 2.9047 and 16.1459 ppm for Cx. quinquefaciatus Table 1. The line equations of regression were estimated as Y = -0.7753 + 1.4704 X, Y = -0.5147 + 1.2892 X and Y = -0.7967 + 1.7204 X respectively for An. stephensi, Cx. pipiens and Cx. quinquefaciatus. The regression lines of total extract A. repens was compared between third instar larvae of three species of larv-ae (Figure 1).

DISCUSSION
Presently, sources for remedies and control of harmful insects are shifted to the natural resources and medicinal plants. So today, in addition to exploitation of natural habitats, extensive agricultural fields have been allocated for cultivation of medicinal plants, which are used for extraction and production of raw materials used in manufacturing of drugs and for various types of larvicides, insecticides and repellents. In this research, the larvicidal activity of total extract of A. repens was assessed and the value of LC50 and LC90 were found to be in the range of 0.3 to 3.0 ppm and 2.2 to 24.7 ppm respectively.

The only research on insecticidal properties of A. repens extract was carried out in China against some species of Lepidopteran larvae. Contact toxicity of A. repens fractions were assessed against the larvae of Mythimna separata, Platella xylostella, Pieris rapae using immersion method. The results showed that the ethyl acetate fraction from the whole plant of A. repens at flowering stage had very strong contact toxicity against 5th
instar larvae of *M. separate* with corrected mortality of 80% even when the extract was diluted 5 times. The petroleum ether fraction of the extract also had a very strong contact toxicity against 5th instar larvae of *M. separate* with corrected mortality of 86.8% and regression equation as a \( Y=2.5719+2.9107X \) and the LC\(_{50}\) value was 6.83 g extract per liter. Also, the oral toxicity showed 21.28% mortality at the concentration of 96.4 g extract per liter.\(^{11}\)

This is the first trial for the assessment of larvicidal activity of *A. repens* against three important mosquito vectors which showed a promising result for formulating a bio-larvicide from an aggressive weed origin.

### CONCLUSION

The exploration of the potentials for the renewable natural resources requires an accurate information and optimal utilization of the natural sources of herbs from different parts of Iran. Considering the job opportunity this research will guide in order to formulate a bio-larvicide for controlling the immature stages of mosquitoes. This will be resulted as an innovation and entrepreneurship, and a new way to commercialize a larvicde. Considering the fact that this plant is a dominant and unwanted innovation and entrepreneurship, and a new way to commercialize a larvicde controlling the immature stages of mosquitoes. This will be resulted as an opportunity this research will guide in order to formulate a bio-larvicide for the exploration of the potentials for the renewable natural resources

### ACKNOWLEDGEMENT

The authors wish to thank Mr. Naser Ghasemi, the technician of Culicidae Insectary, School of Public Health, Tehran University of Medical Sciences for his kind cooperation during the study. This study was funded and supported under code 95-02-27-32456 by Tehran University of Medical Sciences (TUMS).

---

### REFERENCES

ABOUT AUTHORS

Ramesh Toolabi: MSc student in medical entomology and vector control. This paper is a part of her MSc thesis.

Mohammad Reza Abai: Academic staff who his main interest is vector control of arthropod-borne diseases with especial researches on plants having insecticidal effects.

Mohammad Mehdi Sedaghat: Associate professor and head of department of medical entomology who his main interest is vector control of arthropod-borne diseases with especial researches on plants having insecticidal effects.

Hassan Vatandoost: Professor of medical entomology and vector control who his main interest is vector control of arthropod-borne diseases with especial researches on plants having insecticidal effects.

Mansooreh Shayeghi: Professor of medical entomology and vector control who her main interest is vector control of arthropod-borne diseases with especial researches on plants having insecticidal effects.

Saeed Tavakoli: PhD student in pharmacology who his main interest is pharmacognosy.

Mohammad Sistanizadeh Aghdam: PhD student who his main interest is vector control.