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Original Article

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

Ramesh Toolabi[1], Mohammad Reza Abai[1]*, Mohammad Mehdi Sedaghat[1], Hassan Vatandoost[1], Mansooreh Shayeghi[1], Saeed Tavakoli[2], Mohammad Sistanizadeh Aghdam[1]

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 pipiens* and *Culex quinquefaciatus*. **Materials and Methods:** The aerial parts of *A. repens* plants was dried in the shaded places for 3 days at 26-28°C. The leaves, flowers and stems were blended to crashed form using an electric blender. The larvicidal activity of total extract of *A. repens* were evaluated against third instar larvae stage of mosquito vectors, *An. stephensi*, *Cx. pipiens* and *Cx. quinquefaciatus* 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 LC_{50} of 0.2970 and LC_{90} of 2.2097 mg/l. The LC_{50} and LC_{90} values were 2.5047 and 24.7374 mg/l for *Cx. pipiens* and 2.9047 and 16.1459 mg/l for *Cx. quinquefaciatus*. **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 pipiens*, *Cx. quinquefaciatus*, Larvicidal activity, Total extract.

Ramesh Toolabi[1], Mohammad Reza Abai[1]*, Mohammad Mehdi Sedaghat[1], Hassan Vatandoost[1], Mansooreh Shayeghi[1], Saeed Tavakoli[2], Mohammad Sistanizadeh Aghdam[1]

[1]Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, IRAN.
[2]Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, IRAN.

Correspondence

Dr. Mohammad Reza Abai

Qods Ave, Poursina Ave, Department. of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran 1417613151, IRAN.

Phone no: 00982142933112
Fax: 00982188951393

E-mail: abaimr@tums.ac.ir

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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*, *Miliaceae*, *Oocystaceae 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.*

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repens which considered as a dominant and disturbing weeds in the garden and agricultrural lands.

MATERIALS AND METHODS

Mosquito rearing

The rearing and maintenance of *An. stephensi*, *Cx. pipiens* and *Cx. quinquefaciatus* were conducted in the *Culicidae* 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 Maku 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. After 48h, the methanol was drained and collected in the crystallizer. 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 crystallizer 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.

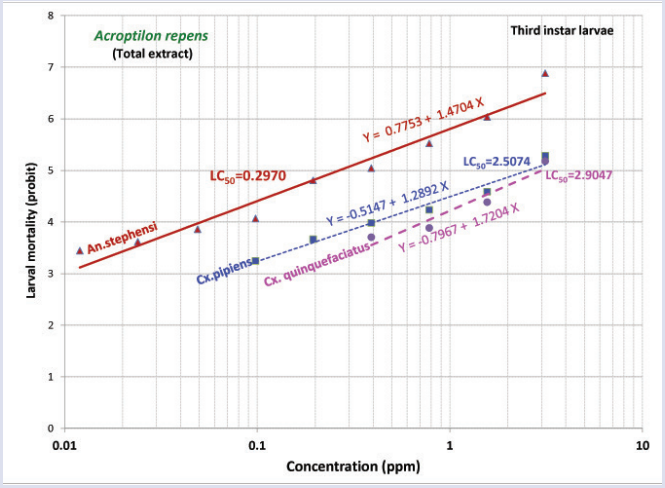

Figure 1: Comparative regression lines of total extract *A. repens* against third instar larvae of three species of mosquito vectors.

Statistical analysis

In the analysis, both dead and moribund larvae were considered as dead. The lethal concentrations (LC_{50} and LC_{90}) 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 (Abbot 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 larvae (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 LC_{50} and LC_{90} 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*, *Plutella 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

Table 1: Lethal concentrations (LC_{50} and LC_{90}) and associated statistics 24 h bioassay tests of total extract *A. repens* against third instar larvae of three species of Culicidae mosquitoes

Mosquito species	a	b ± SE	LC_{50} (ppm) ± 95%C.L.	LC_{90} (ppm) ± 95%C.L.	χ^2 (Heterogeneity)	χ^2 table (df)	p-Value
An. stephensi	0.7753	1.4704 ± 0.110	0.2317	1.5987	29.479*	18.475 (7)	0.01
			0.2970	2.2097			
			0.3720	3.3948			
Cx. pipiens	-0.5147	1.2892 ± 0.133	1.9163	13.5910	3.950**	13.277 (4)	0.01
			2.5074	24.7374			
			3.5957	60.0197			
Cx. quinquefaciatus	-0.7967	1.7204 ± 0.187	2.3039	9.4268	4.528**	9.210 (2)	0.01
			2.9047	16.1459			
			4.0888	39.7364			

**Heterogeneity
** No heterogeneity

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. separata* 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 larvicide. Considering the fact that this plant is a dominant and unwanted weed in agriculture and due to discovering of larvicidal properties of fractions, essential oils and determining the effective compounds of these extracts is necessary and in this regard, an attempt is made to provide a stable formulation for field evaluation, especially against vectors of malaria and arboviral diseases.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

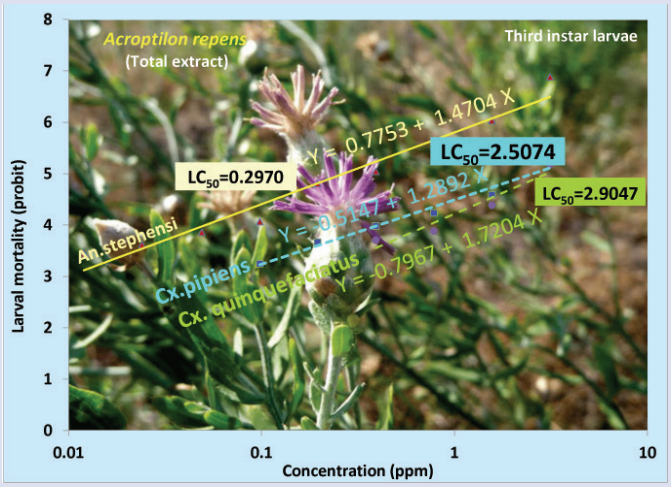
ABBREVIATIONS USED

LC: Lethal concentration; **WHO:** World Health Organization; **ppm:** Part per million; **rpm:** Round per min; **ANOVA:** One-way analysis of variance.

REFERENCES

1. World Health Organization. A global brief on vector-borne diseases. WHO. Geneva, 2014.
2. Murray NA, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and prospects. Clin Epidemiol. 2013;5:299-309.
3. World Health Organization. Global malaria program. World malaria report 2015. Available at: apps.who.int/iris/bitstream/10665/200018/1/9789241565158_eng.pdf.
4. Oshaghi MA, *et al. Anopheles stephensi* biological forms, geographical distribution, and malaria transmission in malarious regions in Iran. Pakistan J Biol Sci. 2006; 9(2):294-8.
5. Maheswaran R, Ignacimuthu S. *Novel biopesticide Ponneem* to control human vector mosquitoes *Anopheles stephensi* L. and *Culex quinquefasciatus* Say. Environ Sci Pollut Res Int. 2015;22(17):13153-66. doi: 10.1007/s11356-015-4586-4.
6. Khanavi M, *et al. Larvicidal activities* of some *Iranian native plants* against the main malaria vector, *Anopheles stephensi*. Acta Medica Iranica. 2013;51(3):141-7.
7. Sedaghat MM, *et al.* Larvicidal activity of essential Oils of *Apiaceae plants* against malaria vector, *Anopheles stephensi*. J Arthropod-Borne Dis. 2011;5(2):51-9.
8. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: a review. J Am Mosq Control Assoc. 1991;7(2):210-37.
9. World Health Organization. Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides. 1981. WHO/VBC/ 81.807.
10. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides, WHO/CDS/WHOPES/GCDPP/2005.13.
11. Wang X, *et al.* Insecticidal activity of fraction from *Acroptilon repens* against larvae of *Lepidoptera*. Southwest China. Journal of Agricultural Sciences. 2015;28(3):1124-9.

GRAPHICAL ABSTRACT



SUMMARY

- Russian Knapweed, *A. repens* is a dominant and unwanted weed in the agriculture and this is the first trial for determining of larvicidal activity against three important mosquito vectors. The lethal concentrations of total extract A. repens ranged from 0.3 to 3.0 pp for *An. stephensi* and 2.2 to 24.7 ppm for *Cx. pipiens* and *Cx. quinquefaciatus*. The findings indicate the significant killing activity against mosquito larvae.

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.

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