Open Access

ORIGINAL ARTICLE

TOXICITY INVESTIGATION OF AQUEOUS EXTRACT FROM DIFFERENT PARTS OF *RICINUS COMMUNIS* AGAINST *CULEX PIPIENS* LARVAE

| Brahim Aouinty *[1-2] | Mohamed Chennaoui [1-3] | Souhail Aboulfadl [2] | and | Fouad Mellouki [2] |

[1.] Laboratoire des Sciences de la Vie et de la Terre | Centre Régional des Métiers de l'Enseignement et de la Formation Casablanca-Settat | El Jadida | Morocco |
[2.] Laboratoire LVMQB / ETB University Hassan II | department of Biology| Faculté des Sciences et Techniques | Mohammedia | Morocco |
[3.] Laboratoire de l'eau et de l'environnement | Université Chouaïb Doukkali | Faculté des Sciences | El Jadida | Morocoo |

| Received | 10 January 2017 | | Accepted | 26 January 2018 | | Published 31 January 2018 |

ABSTRACT

Background: Knowing that chemical insecticides used in pest control have become harmful to the environment and public health, the search for effective and safely alternatives has become a necessity today. Among alternative means, toxic plant extracts are actively sought. **Objective:** This article aims to highlight the efficacy of the aqueous extracts of *Ricinus* communis by investigating its toxicity against larvae of the mosquito. **Methods:** The toxicity of the aqueous extracts from the different parts of the plant *Ricinus communis* (leaves, stems and roots) was studied on *Culex pipiens* larvae. Percentages of larval mortality were analyzed by the A NOVA1 statistical test. Mortality rates and lethal concentrations (LC50 and LC90) were determined after 24 hours of exposure to several aqueous extracts. **Results**: Results obtained in laboratory indicated that all parts of the plant are toxic but to different degrees. The importance of toxicity based on the LC50 and LC90 values are arranged in a decreasing order: young leaves (LC50 = 195 mg/L; LC90 = 398 mg/L) > Roots (LC50 = 224 mg/L; LC90 = 417 mg/L) > Stems (LC50 = 398 mg/L; LC90 = 1820 mg/L). The active substances contained in the extracts and which are not yet identified have a different distribution in the plant. The larvicidal activity of these aqueous extracts is relatively comparable to those obtained in other works using organic extracts and essential oils of medicinal plants. **Conclusion:** The results of this study suggest that the aqueous extracts of the *Ricinus communis* leaves, stems and roots have larvicidal properties and they could be used as a means in mosquito larvae control.
keyswords: *Larvicidal activity, mosquito larvae, mosquito control, lethal concentrations* .

1. INTRODUCTION

In the human environment, several diseases transmitted by mosquitoes cause hundreds of deaths every year in the world. The means of control employed against these insect vectors are usually based on synthetic chemical nevertheless these products have many disadvantages being harmful to the environment, to non-target organisms and to human health. Moreover the abundant use of these conventional insecticides induces appearance and development of pesticides resistant strains [1, 2, 3, 4, 5].

Hence, more attention has been focused on search of eco-friendly compounds which could be possible alternatives to synthetic insecticides. Targeting this objective, many studies on efficacy of phytochemicals against mosquito vectors were conducted around the world and showed their larvicidal, pupicidal, adult emergence inhibition and repellent properties, so phytochemicals can be used as alternatives to the synthetic insecticides or along with other insecticides under the integrated vector control program [6, 7].

In Morocco, many researchers reported the effectiveness of plant extracts against mosquito larvae [8, 9, 10, 11, 12]. In a previous work, Aouinty and *al*. (2006) studied the larvicidal activity of aqueous extracts of twenty local plants against mosquito and showed that *Ricinus communis*, *Tetraclinis articulata*, *Nerium oleander*, *Ammi visnaga* and *Innula viscosa*, had a significant larvicidal activity [13]. Moreover, the aqueous extract of the *R. communis* leaves, which proved to be very toxic, showed a broad spectrum of action by effectively acting on the larvae of 3 other mosquito species *Aedes caspius*, *Culiseta longiareolata* and *Anopheles labranchea*. The lethal LC_{50} of this extract, are ranging between 140 ppm and 375 ppm for the 2nd instar larvae and between 205 ppm and 963 ppm for the 4th instar larvae. Hammiche and al. (2013) suggested that the presence of ricin, a toxic alkaloid substance, with a maximum concentration in seeds, qualifies this plant as a potential source of biological insecticides [14]. This plant now known to contain toxic substances can play a useful role in the control of vectors.

As the efficacy of phytochemicals against mosquito larvae can vary significantly depending on plant parts [15,16], the objective of this study is to provide more information concerning the toxic activity of *R. communis* against *Cx. pipiens* by assessing the killing efficacy of aqueous extract from different parts of this plant, namely young leaves, stems and roots, on fourth-instar larvae of *Cx. pipiens*.

2. MATERIALS AND METHODS

2.1. The mosquito larvae:

The mosquito larvae used in this study are belonging to the species *C. pipiens*, they are identified using the determination key of Himmi and *al*. (1985) [17]. All these larvae were collected from a breeding site rich of organic matter in Mohammedia region at Morocco [18] , then they were conserved in the same water of the origin site under the laboratory conditions (75 ± 5 % RH and 20 ± 2 °C T). After 2 days in rearing, the larvae of 4^{th} instar were selected for experiments testing.

2.2. Aqueous extracts preparation:

The plant targeted in this study, *Ricinus communis,* was harvested from the same region of Mohammedia. The leaves, stems and roots of this plant are brought to the laboratory, washed with tap water and then rinsed with distilled water. After drying in an oven, they are ground to fine powder and the aqueous extracts from leaves (AEL), stems (AES) and roots (AER) are prepared according to the same protocol described by Aouinty and *al*. (2006) [13]. A sample of 100 g of powder from each part of the plant (leaves, stems, roots) is placed in 1 liter of boiling distilled water and allowed to cool with magnetic stirring for 30 minutes. After filtration on Wattman paper, the recovered filtrate is an aqueous extract used as a stock solution at 10%. This procedure has also been adopted in several other studies [10-19-20].

2.3. Toxicity tests:

The toxicity testing realized for all aqueous extracts parts of *R. communis* are performed on fourth instar larvae according to the WHO protocol (2005) [21]. From stock aqueous extracts at 10 %, five concentrations (0,7%; 0,13% ; 0,25% ; 0,5% ; 1%) were prepared for each extract and four repetitions were performed for each bioassay in the presence of twenty larvae. The control was run simultaneously that included only 100 mL of distilled water and received the same larvae number. The numbers of dead larvae, when they become immobile and unable to react to touch stimuli, were counted after 24 h of exposure and the percentage mortality of larvae for all concentrations was reported from the average of four replicates. The LC50 and LC90 values were obtained using the probit method of Finny (1971) [22].

2.4. Data analysis means:

All results, mortality rate (%) and lethal concentrations (LC50 and CL90), were analyzed statistically by ANOVA1, using statistical software XLSTAT 2010. The significance degree was fixed at a probability value lower 5%. Lethal concentrations (LC50, LC90) are calculated according to Finney's mathematical methods. The data is transformed and standardized according to the Bliss table [23].

3. RESULTS

3.1. Mortality rates variation:

After have counted the numbers of dead larvae 24 hours of exposure during, larval mortality percentages of each concentration and those of control are being used to the calculate of mortality averages. The larval susceptibility to aqueous extracts from leaves, stems and roots of *R. communis* are reported in table 1. As it can be seen, compared to the control group, all extracts showed considerable larvicidal activity when tested against 4^{th} instar larvae of *Cx. Pipiens*. The mean larval mortality values calculated were tested by ANOVA 1 at a level of 5% as it's indicated in table 2. The results obtained for the different aqueous extracts are statistically valid on the basis of the F-ratio> F-probability.

Whatever the aqueous extract used from leaves or stems or roots, the percentage of larval mortality increased in perfect linearity with the concentration. This correlation represented on Figure 1, is justified by high correlation coefficients, which are $R^2 = 0.827$; $R^2 = 0.992$ and $R^2 = 0.985$ respectively. The highest mortality percentage (100%) occurred at a concentration 0.5% of AEL whereas this same concentration caused only 60% and 82.25% of mortality with AES and AER respectively. The lowest mortality percentage was 24.62; 19.1 and 10.37 % at the lowest concentrations (0.07%), respectively for AEL, AER and AES of *R. communis*.

Table 1: Larvicidal activity in % mortality of aqueous extracts from *Ricinus communis* parts against fourth-instar larvae of *Culex pipiens*.

Aqueous extrat origin	Concentrations					Control 0%
	0.07%	0.13%	0.25%	0.50%	1%	
Leaves (AEL)	23.5	72.5	92.5	100	100	1
	27.5	65	92	100	100	
	25	67.5	87.5	100	100	0
	22.5	62.5	90	100	100	
Mean ± SD (%)	24.62 ± 2.17	66.87 ± 4.26	90.5 ± 2.27	100 ± 0	100 ± 0	2.5
Stems (AES)	11.5	32.5	40.5	62.5	78	0
	10	34	42	60	75	
	9.5	30.5	45	58.5	72.5	0
	10.5	30	44	59	75	
Mean ± SD (%)	10.37 ± 0.85	31.75 ± 1.84	42.87 ± 2.01	60 ± 1.77	75.12 ± 2.25	0
Roots (AER)	18	50.5	67.5	82.5	100	0
	18.5	48	65	84	100	
	19	47.5	62.5	80	100	1
	20.5	47	67	82.5	100	
Mean ± SD (%)	19 ± 1.08	48.25 ± 1.55	65.5 ± 2.27	82.25 ± 1.65	100 ± 0	2.5

SD: Standard deviation.

Table2: Variance analysis results by ANOVA1 of mean mortality percentages.

	Variation source	Df	SS	SM	F -ratio	F -prob
Leaves aqueous extract	Factorial	4	16336.425	4084.11	726.06	3.05
	Residual	15	84.375	5.625		
	Total	19	16420.8			
Stems aqueous extract	Factorial	4	10026.92	2506.73	762.50	3.05
	Residual	15	49.31	3.29		
	Total	19	10076.23			
Roots aqueous extract	Factorial	4	15597.5	3899.37	1695.38	3.05
	Residual	15	34.5	2.30		
	Total	19	15632			

Df: degree of freedom; **SS**: Sum of squares; **MS**: mean square.

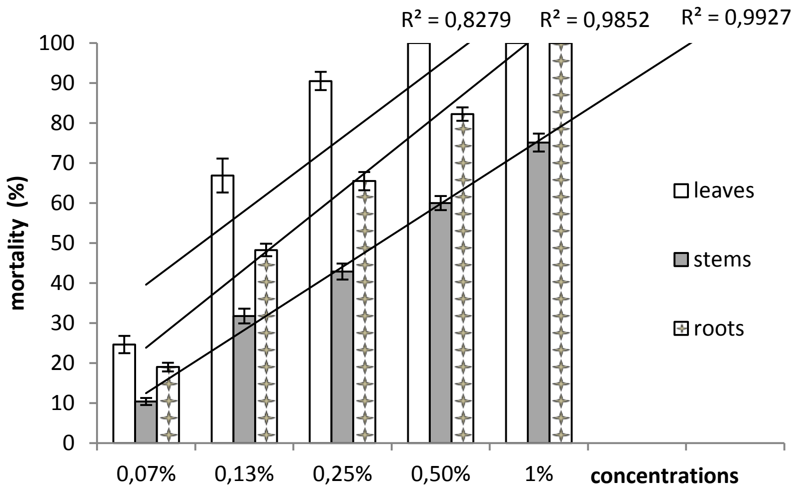

Figure 1: Mortality of *Culex pipiens* larvae according the aqueous extract concentrations from different parts of *Ricinus communis.*

3.2. Lethal concentrations LC50 and LC90:

For more information on the toxicity of *Ricinus communis* on mosquito larvae, biological tests were repeated using the same aqueous extracts, reducing the range of concentrations for determiniting LC50 and LC90 lethal concentrations. Values of concentrations are converted in log C and those of mortality are converted in probits. Results obtained and statistical analyses by ANOVA1 are shown in Tables 3, 4 and 5.

Table 3: The effect of the aqueous extract concentration from leaves of *Ricinus communis* on mortality rate of fourth instar larvae of *Culex pipiens*

C (mg/L)	log (C)	Mortality (M) in %	Probits of (M)		Df	SS	MS	F-ratio	F prob
120	2.07	25	4.33	**Regression**	1	1.42	1.42	27.15	0.006
150	2.17	33	4.5						
180	2.25	42	4.5	**Residus**	4	0.21	0.05		
210	2.32	57	5.18						
240	2.38	65	5.3	**Total**	5	1.42			
270	2.43	78	5.77						

C : Concentration ; **M** : Mean.

Table 4: The effect of the aqueous extract concentration from stems of *Ricinus communis* on mortality rate of fourth instar larvae of *Culex pipiens.*

C (mg/L)	log (C)	Mortality (M) in %	Probits of (M)		Df	SS	MS	F-ratio	F prob
100	2	12	3.82	**Regression**	1	3.44	3.44	108.45	0.001
200	2.30	32	4.53						
400	2.60	48	4.95	**Residus**	3	0.09	0.03		
800	2.90	64	5.36	**Total**	4	3.54			
1600	3.20	91	6.34						

Table 5: The effect of the aqueous extract concentration from roots of *Ricinus communis* on mortality rate of fourth instar larvae of *Culex pipiens.*

C (mg/L)	log (C)	Mortality (M) in %	Probits of (M)		Df	SS	MS	F-ratio	F prob
120	2.07	11	3.77	**Regression**	1	3.48	3.49	277.55	7.6E-05
160	2.20	19	4.12						
200	2.30	38	4.69	**Residus**	4	0.05	0.01		
240	2.38	58	5.23						
300	2.47	71	5.58	**Total**	5	3.53			
340	2.53	82	5.92						

All obtained F-ratio were larger than critical F-value, then the relationship between mortality rates of *Cx. pipiens* mosquito larvae and concentrations of each *R. communis* plant extracts (EAL, EAS and EAR) was validated statistically at the p = 0.05 level of significance. Based on the linear regression of this relationship (R^2 between 0.87 and 0.98) lethal concentrations (LC50 and LC90) values were determined by the linear regression method (Table 6).

Table 6: Calculation results of LC50 and LC90 according the log-probit method.

Extracts of	y = ax +b	R^2	y = 5 probits	y = 50 % (LC50)	y = 6.98 probits	y = 90 % (LC90)
Leaves	3.96x – 4.06	0.7	x = 2.28	x = 195 mg/L	x = 2.60	x = 398 mg/L
Stems	1.95x + 0.08	0.97	x = 2.60	x = 398 mg/L	x = 3.26	x = 1820 mg/L
Roots	4.91x - 6.56	0.98	x = 2.35	x = 224 mg/L	x = 2.62	x = 417 mg/L

The presented LC50 values of aqueous extracts leaves, stems and roots of *R. communis against Cx. pipiens* larvae, calculated, are 195, 398, and 224 ppm, respectively. Concerning LC90 values, they are 398, 1820, and 417 ppm, respectively.

4. DISCUSSION

The sensitivity of larvae L4 of *Cx. pipiens* is variable depending on the nature of the aqueous extract. This variation in sensitivity, expressed as a mortality rate reflects, in fact, a variability of the concentrations of active substances or an unequal distribution of these substances in the different parts of the plant. These findings are similar to those reported by Bream and *al.* (2010) using the ethanolic extract of an aquatic plant *Echinochloa stagninum* [16]. In the same context, Sukmar and *al.* (1991) suggested variations in the toxicity of phytochemical compounds depending on the parts of the plant from which they are extracted [24].

In an analogous study realized by Alaoui Boukhris (2010), aqueous extracts of aromatic plants *Artemisia absinthium* L., *Mentha piperita* L., mortality rates of larvae L4 of *Cx. pipiens* were much lower than those obtained in our study [10]. However, the mortalities recorded using the essential oils of these same aromatic plants were as important as those obtained in this work.

According to our results, leaves aqueous extract from *R. communis* remains the most effective with the lowest lethal concentrations LC50 = 195 mg/L and LC90 = 398 mg/L. The extract of the young leaves of the *R. communis* appears here more toxic than that of old leaves with a lethal concentration LC50 = 589 mg/L (Aouinty et al., 2006) [13]. The least toxic is the stems extract with LC50 = 398 mg/L and LC90 = 1820 mg/L. On the other hand, the toxicity of the roots aqueous extract remains very important, it approaches that obtained using leaves extract with LC50 = 224 mg/L and LC90 = 417 mg/L.

In other works concerning the larvicidal activity of aqueous extracts of solanaceous plants, *Solanum villosum* leaves extract used by Showdhury et al. (2008) and the fruit extract of *S. nigrum* used by Raghavendra et al. (2009), have been tested on larvae of *Culex quinquefasciatus*, LC50 lethal concentrations were 645,44 ppm and 337,2 ppm respectively [25, 26] . Several other works studied the larvicidal activity of extracts from plants belonging to the Euphorbiaceae family. Yadav and *al*. (2002) reported that methanolic extracts of the bark of *Euphorbia tirucalli* against L4 larvae of *Cx. quinquifasciatus* showed lethal concentrations LC50 = 177,14 mg/L and CL90 = 513,38 mg/L [27] Kemmassi and al. (2015) tested aqueous extracts of the aerial part of *Euphorbia guyoniana*, from the Algerian Sahara, against L3 larvae of *Cx. pipiens* and showed very high mortality with low lethal concentrations values (LC50 = 1,5 mg/L and LC90 = 9,4 mg /L) [28]. In fact, it is an aqueous extract of the powder from the all aerial parts of the plant, obtained from a mixture of methanol - distilled water (2/3 - 1/3) and recovered after evaporation of methanol. The values of the lethal concentrations we found in this study are close to those obtained against larvae of *Cx. pipiens* by EL- Akhal and *al*. (2014, 2015) with essential oils obtained from *Thymus vulgaris* (LC50 = 102 ppm and LC90 = 179 ppm), *Citrus sinensis* (LC50 = 280,82 ppm and LC90= 516,25 ppm) and *C. aurantium* (LC50= 139,48 ppm and CL90 = 212,04 ppm) [11,12]

In order to investigate the mode of action of castor plant's extracts on larvae of *Cx. pipiens* we conducted a histopathological study on 4^{th} instars larvae after their exposure to aqueous extracts from leaves of the *R. communis* and we showed significant tissue lesions manifested on the digestive tract, muscles and external integuments (Aouinty and *al*, 2018) [29]. David and *al.* (2000) reported that toxicity of phytochemicals in mosquito larvae affects primarily the midgut epithelium and secondarily the gastric caeca and the malpighian tubules [30]. The induced lesions on larval tissues of *Cx. pipiens* by extracts from *R. communis* may be linked to the presence of several bioactive chemicals in this plant (alkaloids, saponins, tannins, flavonoids, and steroids) that contribute to the extract's efficacy as killing agents against mosquito larvae. Kang and *al*. (1985) showed that the major phytochemicals isolated from leaves of *R. Communis* were phenolic compounds and alkaloids [31]. Hammiche and *al.* (2013) reported the presence of ricin, a toxic glycoprotein, in the seeds of the *R. communis* plant [14]; ricin belongs to the type II group of ribosome inactivating proteins and can be a potential source of biological insecticides suitable for use in mosquito control program.

5. CONCLUSION

This study aimed to investigate the toxicity of aqueous extracts from different parts of *Ricinus communis* against the 4^{th} instar larvae of *Culex Pipiens*. Our results showed that extracts from leaves, stems, and roots exhibited larvicidal activity, nevertheless the strongest toxicity was obtained for leave's extract with the lowest lethal concentration values (LC50 = 195 ppm and CL90 = 398 ppm). Reported literature and reported results here demonstrate that *Ricinus communis* shows significant larvicidal activity against mosquito which is similar to that of certain organic extracts and essential oils of other plant species. This opens the possibility of further investigations of efficacy on larvicidal properties of *R. communis*'s extracts, with the objective of isolating biologically active molecules which could be lead products for bio-insecticides more efficient and safer in usage and potentially suitable for use in mosquito population management.

Acknowledgments

Authors are thankful for the financial support from the University Hassan II of Casablanca and the Moroccan Ministry of National Education, Professional Training, Higher Education and Scientific Research. Authors are also grateful to Mr Youssef Ben Omar from the Municipal Hygiene office of Mohammedia for its help and all its guidelines for the collection of biological material in the larval sites of the Mohammedia wetland.

6. REFERENCES

1. Faraj C., Elkohli M., Elrhazi M., Laqrâa L., Lyagoubi M. Niveau actuel de la résistance du moustique *Culex pipiens* aux insecticides au Maroc. *Science Letters*. 2002; 4: 4 p. Available on: https://www.researchgate.net/publication/258050832
2. El Ouali Lalami A, El-Akhal F, El Amri N, Maniar S, Faraj C. State resistance of the mosquito *Culex pipiens* towards temephos central Morocco . *Bull Soc Pathol Exot*, 2014; 107:194-198. Available on: https://www.ncbi.nlm.nih.gov/pubmed/24827876
3. El Joubari M., Faraj C., Louah A., Himmi O. Sensibilité des moustiques *Anopheles labranchiae, Culex pipiens, Ochlerotatus detritus et Ochlerotatus caspius* de la région de Smir (Nord- Ouest du Maroc) aux organophosphorés utilisés en santé publique. *Environnement, Risque & Santé*. 2015; 14: 1 72-79. Available on: https://www.researchgate.net/publication/272823859

4. Bkhache M, Tmimi FZ., Charafeddine O., Faraj C., Failloux AB., Sarih M. First report of L1014F-kdr mutation in *Culex pipiens* complex from Morocco. Parasit Vectors. 2016; 9(1): 644. Available on: https://www.ncbi.nlm.nih.gov/pubmed/27986090
5. J. BenritVimal and S. Sam Manohar Das, *Euphorbia antiquorum* latex and its mosquitocidal potency against *Aedes aegypti*, *Journal of Entomology and Zoology Studies.* 2014; 2(6): 267-269.
6. Nasir S., Nasir I. And Iqra Y. Medicinal plant extracts: control strategies against Dengue Mosquitoes. *Bangladesh J. Bot*. 2017; 46: 1 27-33.
7. Anupam Ghosh, Nandita Chowdhury, and Goutam Chandra, Plant extracts as potential mosquito larvicides. Indian J Med Res. 2012; 135: 5 581–598.
8. Bouallam Tifnouti S. Ecologie des diptères culicidés de la région de Marrakech: Contribution à l'amélioration des moyens de lutte chimique et biologique. Thèse. doct. es.sci., Fac. Sci. Semlalia., *Uni. cadi ayyad., Marrakech, Maroc*. 2001; 148 p.
9. Alaoui Slimani N.. Faune culicidienne d'une zone marécageuse de Rabat-Salé : Biotypologie et contribution à la lutte par des substances naturelles. Thèse Doct. es. sci. Biol., Fac. Sci. *Univ. Mohammed V., Rabat, Maroc*. 2002; 192p.
10. Alaoui Boukhris M. Activités larvicides des extraits de plantes sur les larves de moustiques vecteurs de maladies parasitaires. Mém Master Sciences et Techniques. *Fac Sci Tech Fez.* 2010; 64 p. Available on: http://www.memoirepfe.fst-usmba.ac.ma/get/pdf/465
11. EL-Akhal F., Guemmouh R., Greche H., El Ouali Lalami A. Valorisation en tant que bioinsecticide de deux huiles essentielles de *Citrus sinensis* et *Citrus aurantium* cultivées au centre du Maroc Valorization as a bio-insecticide of essential oils of *Citrus sinensis* and *Citrus aurantium* cultivated in center of Morocco). *J. Mater. Environ. Sci*. 2014 ; 5 (S1) :2319-2324. Available on: https://www.jmaterenvironsci.com/Document/vol5/vol5_NS1/43-JMES-S1-LAKHAL.pdf
12. El-Akhal F., Greche3 H., Ouazzani F., Chahdi R., Guemmouh, El Ouali Lalami A. Chemical composition and larvicidal activity of *Culex pipiens* essential oil of *Thymus vulgaris* grown in Morocco. *J. Mater. Environ. Sci*. 2015; 6 (1): 214-219. Available on : https://www.researchgate.net/publication/282187069
13. Aouinty B., Mellouki F., Oufara S. et Mahari S. Evaluation préliminaire de l'activité larvicide des extraits aqueux des feuilles du ricin (*Ricinus communis* L.) et du bois de thuya (*Tetraclinis articulata* (Vahl) Mast.) sur les larves de quatre moustiques culicidés: Culex pipiens (Linné), *Aedes caspius* (Pallas), *Culiseta longiareolata* (Aitken) et *Anopheles maculipennis* (Meigen). *Biotechnol. Agron. Soc. Environ*. 2006; 10(2): 67 – 71. Available on: http://popups.ulg.ac.be/1780-4507/index.php?id=1115
14. Hammich V., Merad R., Azzouz M. Plantes toxiques à usage medicinal du pourtour mediterraneen. Paris. 2013; 391 p. Available on: http://www.springer.com/br/book/9782817803746
15. Bream A. S., AlMkhtar O., El-Sheikh T. M.Y., Fouda M. A. and Hassan M. I. Larvicidal and Repellent Activity of Extracts Derived from Aquatic Plant *Echinochloa stagninum* against *Culex pipiens. Tunisian Journal of Plant Protection* 2010; 5: 107-123. Available on: http://www.iresa.agrinet.tn/tjpp/tjpp9/11Bream.pdf
16. Spring P., Nagar S., Ramteke P W. and Mahiban Ross E. Biological Activities of *Solanaceous* Plants Against Vector Mosquitoes: A Review. *IJTEMAS*, 2016; 4 (9): 28-37. Available on: http://www.ijetmas.com/admin/resources/project/paper/f201609221474602741.pdf
17. Himmi O., Dakki M., Trari B., Elagbani M., Les Culicidae du Maroc : Clés d'identification, avec données biologiques et écologiques. *Trav. Inst. Sci. Rabat, série Zoologie*. 1995; 44 - 51 p.
18. Aouinty B., Rihane A., Chennaoui M.. et Mellouki F. Étude des gîtes larvaires de moustiques culicidés de la région de Mohammedia, littoral atlantique *Revue Internationale Afri Sci.* 2017; 13: 120 -129. Available on: http://www.afriquescience.net/numero2_vol_13.html#
19. Merabti B., Lebouz I., Adamou A., Ouakid M. L. Toxic effect of *Citrullus colocynthis* (L.) Schrad fruit aqueous extract against larvae of culicidae. *Revue des BioRessources.* 2015; 5(2): 120 - 130. Available on: https://www.researchgate.net/publication/296485717
20. Aouati A. Etude de la toxicité de certaines plantes sur les larves de *Culex pipiens* (Diptera, Culicidae). *Thèse doct Sci Entomo* 2016; 150 p. Available on: https://bu.umc.edu.dz/theses/biologie/AOU6912.pdf
21. WHO. Guidelines for laboratory and field testing of mosquito larvicides. 2005; 41 p. Available on http://apps.who.int/iris/bitstream/10665/69101/1/WHO_CDS_WHOPES_GCDPP_2005.13.pdf
22. Finney D.J. Probit analysis. *Cambridge University Press*. IBSN 0521080421X, 1971; 3th Ed: 333 p.
23. Greenberg, B. G.. "Chester I. Bliss, 1899-1979". *International Statistical Review*. 1980; 8(1): 135-136. Available on: http://www.jstor.org/stable/1402441?seq=1#page_scan_tab_contents
24. Sukumar K., Perich MJ. and Boobar L.R. Botanical derivatives in mosquito control: *a review. J Am Mosq Control Assoc* 1991; 7: 210-237. Available on: https://www.biodiversitylibrary.org/content/part/JAMCA/JAMCA_V07_N2_P210-237.pdf
25. Showdhury N, Laskar S, Chandra G. Mosquito larvicidal and antimicrobial activity of protein of *Solanum villosum* leaves. *BMC Comple Alter Med*. 2008; 8: 62. Available on: https://bmccomplementalternmed.biomedcentral.com/articles/10.1186/1472-6882-8-62
26. Raghavendra K., Singh SP., Subbarao S.K., Dash A.P. Laboratory Studies on mosquito larvicidal efficacy of aqueous and hexane Extracts of dried fruit of *Solanum nigrum* Linn. *Indian J Med Res.* 2009; 130: 74-7. Available on: https://www.ncbi.nlm.nih.gov/pubmed/19700805
27. Yadav R., Srivastava V.K., Chandra, Singh A. Larvicidal activity of latex and stem bark of *Euphorbia tirucalli* plant on the mosquito *Culex quinquefasciatus. J Commun Dis.* 2002; 34(4): 264 -269. Available on: https://www.ncbi.nlm.nih.gov/pubmed/14710857

28. Kemassi A., Boukhari K., Cherif R., Ghada K., Bendaken N., Bouziane N., Boual Z., Bouras N., Ould Elhadj-khelil A. et Ould Elhadj M.D. Evaluation de l'effet larvicide de l'extrait aqueux d'*Euphorbia guyoniana* (Boiss. & Reut.) (Euphorbiaceae) *El Wahat. Rech Etu.* 2015; 8: 44 – 61. Available on: https://www.asjp.cerist.dz/en/article/1030
29. Aouinty B., Chennaoui M., Rihane A., Mahari S. et Mellouki F. Larvicidal effects of aqueous extract from *Ricinus communis* L. leaves against mosquito *Culex pipiens* : mortality and histopathology of treated larvae. *Journal of Materials and Environmental Science*. 2018; 9(2): 619-623. Available on: https://www.jmaterenvironsci.com/Document/vol9/vol9_N2/68-JMES-3590-Aouinty.pdf
30. David J.P., Rey D. Pautou M.P., Meyran J.C. Differential toxicity of leaf litter to dipteran larvae of mosquito developmental sites. *Journal of Invertebrate Pathology.* 2000; 75: 9-18. Available on: https://www.sciencedirect.com/science/article/pii/S0022201199948863
31. Kang S.S., Cordell A., Soejarto D.D., Fong H.H.S. Alkaloids and flavonoids from *Ricinus communis. J. Nat. Prod*. 1985; 48(1): 155–156. Available on: http://pubs.acs.org/doi/pdf/10.1021/np50037a041

Cite this article: Aouinty Brahim, Chennaoui Mohamed, Aboulfadl Souhail and Mellouki Fouad. TOXICITY INVESTIGATION OF AQUEOUS EXTRACT FROM DIFFERENT PARTS OF *Ricinus Communis* AGAINST *Culex Pipiens* LARVAE. *Am. J. innov. res. appl. sci.* 2018; 6(1): 35-40.