Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(4): 346-350

Received: 23-05-2016
Accepted: 22-06-2016

Zineb Dahchar
Laboratory of Ecology and Terrestrial and aquatic systems and Laboratory of Applied Animal Biology, Department of Biology, Faculty of Sciences Badji Mokhtar University of Annaba 23000-Annaba, Algeria

Fatiha Bendali-Saoudi
Laboratory of Applied Animal Biology, Department of Biology, Faculty of Sciences, Badji Mokhtar University of Annaba 23000-Annaba, Algeria

Noureddine Soltani
Laboratory of Applied Animal Biology, Department of Biology, Faculty of Sciences, Badji Mokhtar University of Annaba 23000-Annaba, Algeria

Correspondence
Fatiha Bendali-Saoudi
Laboratory of Applied Animal Biology, Department of Biology, Faculty of Sciences, Badji Mokhtar University of Annaba 23000-Annaba, Algeria

Larvicidal activity of some plant extracts against two mosquito species *Culex pipiens* and *Culiseta longiareolata*

Zineb Dahchar, Fatiha Bendali-Saoudi, Noureddine Soltani

Abstract
The larvicidal potential of aqueous extracts from leaves of three plants (*Ricinus communis* L., *Daphne gnidium* L. and *Thymus vulgaris* L.) was evaluated against the different larval instars of *Culex pipiens* Linné 1758 and *Culiseta longiareolata* Aitken 1954 mosquitoes. Mortality counts were made after 24, 48 and 72 hours exposure, respectively. The extracts exhibit insecticidal activity against larvae of the two tested mosquito species. Lethal concentrations (LC) values gradually decreased with increased exposure times. The LC_{50} values of the tested plant extracts recorded after 72h of exposure were determined: *D. gnidium* (0.05, 0.03, 0.06, 0.07g/l), *R. communis* (0.03, 0.04, 0.04, 0.06 g/l) and *T. vulgaris* (0.02, 0.03, 0.03, 0.02 g/l) respectively against 1, 2, 3 and 4th instar larvae of *Cx. pipiens*. Concerning *Cs. longiareolata*, the LC_{50}-72h found against 1, 2, 3 and 4th instar larvae were respectively for *D. gnidium* (0.09, 0.07, 0.12, 0.13 g/l), *R. communis* (0.02, 0.06, 0.09, 0.08 g/l) and *T.vulgaris* (0.02, 0.02, 0.03, 0.03 g/l). Plant extracts tested were found more effective against *Cx. pipiens* and the highest activity was found for *T. vulgaris* extracts.

Keywords: Mosquitoes, *Culex pipiens*, *Culiseta longiareolata*, Plant extracts, Toxicity.

Introduction
Insects are a very important part of the biodiversity of terrestrial and aquatic ecosystems. Hematophagous insects play an important role in global disease transmission [1]. Among them mosquitoesare vectors of many human diseases and cause environmental harassment [2]. Mosquitoes, accounts over 3500 species, mostly distributed in three main genres *Aedes*, *Anopheles* and *Culex* [3]. Only females are hematophagous unlike males and vector [4, 5]. Mosquitoes are generally controlled by conventional neurotoxic insecticides [6-7]. However, these chemicals has caused environmental contamination as well as side effects to non-target organisms. Moreover, mosquito control techniques present serious threats because of the emergence of resistance to synthetic insecticides widely used. In this context, there is search for alternative methods for controlling mosquitoes such as biological agents [8-11] or use of insect growth disruptors. The potency of IGRs for mosquito control has been the subject of intensive investigations [6, 12-13].
In Algeria, *Culex pipiens* L. is the most abundant mosquito species, particularly in urban areas [6] and is generally controlled by conventional insecticides such as organophosphates, carbamates and pyrethroids [14], while *Culiseta longiareolata* Macquart is abundant in temporary pools [15] and presents a veterinary interest [16]. Thus, the predatory capacity of several fish species has been tested against different stages of *Cx. pipiens* [7, 9]. Plants are sources of bioactive compounds and can be used as an alternative to conventional insecticides in mosquito control programs. Several plant extracts and isolated compounds from different plant families have been reported for their insecticidal properties [17-18]. Therefore, in the current study we tested the efficacy of extracts from three plants (*Dapnne gnidium*, *Ricinus communis*, *Thymus vulgaris*) against larvae of two mosquito species *Cx. pipiens* and *Cs. longiareolata* vectors.

2. Material and methods
2.1 Collection of plants
Leaves of three species of plants *Daphne gnidium* L. (Thymelaeaceae), *Ricinus communis* L. (Euphorbiaceae) and *Thymus vulgaris* L. (Lamiaceae) were collected from various areas of the region of Annaba. These specimens were identified at the laboratory of Applied Animal

Biology (Badji Mokhtar University, Algeria). Collection of plants was conducted for two months from October to November 2015. These plants were chosen according to their pharmacological properties and also to their traditional uses.

***Ricinus communis*:** It is a robust perennial shrub from 3 to 12 m high. The leaves are long-stalked, palmate, Lobed (5-9 lobes), with green dark color and sometimes traces of red purple. *R. communis* probably originated in Africa and was used in ancient Egypt and by the Romans and Greeks [19].

***Daphne gnidium*:** It is a shrub of Mediterranean scrub and the Atlantic Sands, 60 cm to 2 meters high or more, very thin twigs hardwoods of evergreen, lanceolate linear, 5-7mm wide of at most, very dense. Terminal inflorescences panicle long 5-10cm rower, the fruit is a drupe ovoid, orange red [20].

Thymus vulgaris: It is an aromatic shrub with branched stems, up to 40 cm high.It has small leaves curl at the edges of dark green color, which is covered with hair and glands.Its small zygomorphic flowers are grouped in clusters and their color varies from white to purple through pink. *T. vulgaris* is also characterized by a floral polymorphism that was at least as studied its chemical polymorphism [21].

2.2 Preparation of extracts
Leaves of these three plants (*D. gnidium*, *R. communis* and *T. vulgaris*) were dried in the shade, cut up and then ground in a crusher. The powder material from each species was macerated at room temperature for two days and then filtered through Whatman filter paper (3 mm). The resulting crude extracts were used in the toxicity bioassays [22]. The concentrations (g dry weight/L) stock of different solutions were 0.3, 0.2 and 0.3 g/l for *D. gnidium*, *R. communis* and *T. vulgaris*, respectively.

2.3 Mosquitoes
The larvae of *Cs. longiareolata* and *Cx. pipiens* were obtained from a stock colony and kept as previously described [6]. Pyrex storage jars (80 by 100 mm) containing 200 ml of tap water were maintained at temperature of 25 °C and a photoperiod of 14:10 (L:D). Larvae were daily fed with fresh food consisting of a mixture of Biscuit Petit Regal-dried yeast (75: 25 by weight), and water was replaced every four days.

2.4 Larvicidal bioassays
The crude extracts were tested following the method of World Health Organization [23]. Each plant extract was evaluated at various concentrations against the different larval instars of *Cx. pipiens* and *Cs. longiareolata*. The tested concentrations were: 0.09, 0.18 and 0.3 g/l for *D. gnidium*, 0.06, 0.12 and 0.2 g/l for *R. communis* and 0.0225, 0.045 and 0.09 g/l for *T. vulgaris*, respectively. 20 early larvae from each instar were introduced into each testing cup which contains 200 ml of dechlorinated water. A measured volume of stock solution of plant extracts was added to obtain the desired final concentrations. Controls were exposed to water only. The mortality was recorded after 24, 48 and 72 h of exposure and the assays were conducted with three replicates containing each 20 larvae/instar. The mortality percentages in the different treatments were corrected [24] and LC_{50} together with corresponding 95% confidence limits (95% CL) were calculated.

2.5 Statistical analysis
Results are presented as mean ± standard deviation (SD). The number of individuals tested in each series is given with the results. Data was subjected to analysis of variance (ANOVA) test using MINITAB Software (version 16, PA, State College, USA). Lethal concentrations were determined for each exposure time using the software Graph Pad Prism 2013 v6.01 (for Windows) and $p < 0.05$ was considered statistically different.

3. Results
The results of toxicity bioassays are summarized in tables 1, 2 and 3. All extracts tested exhibited insecticidal activity. The mortality recorded varied as function of the larval instars and the duration of exposure to plant extracts. Based on their LC_{50} values, the effectiveness of extracts decreased with the increasing age of larvae. Table 1 show that the younger instars were more sensitive to *D. gnidium* extracts than the older instars. Moreover, *D. gnidium* extracts appeared more efficient against *Cx. pipiens* larvae than to *Cs. longiareolata* larvae. The LC_{50} obtained against fourth instar larvae after 72 H of exposuretime were 0.06 g/l for *Cx. pipiens* vs 0.13 g/l for *Cs. longiareolata*, respectively.

Table 1: Lethal concentrations (LC_{50}, g/l) of *D. gnidium* extracts against the different larval (L) instars of *Cx. pipiens* and *Cs. longiareolata* (fiducial limits 95%; exposure time in hours; N= 3 replicates containing each 20 larvae/instar/exposure time).

Larval instars		LC50 95%FL (g/l)		
		24h	48h	72h
Cx. pipiens	L1	**0.26** [0.02-0.45]	**0.08** [0.04-0.12]	**0.05** [0.02-1.23]
	L2	**0.33** [0.01-0.69]	**0.09** [0.02-0.42]	**0.03** [0.02-0.57]
	L3	**0.19** [0.07-0.48]	**0.10** [0.07-0.15]	**0.06** [0.02-0.13]
	L4	**0.13** [0.11-0.15]	**0.05** [0.01-0.20]	**0.07** [0.04-0.10]
Cs. longiareolata	L1	**0.15** [0.06 -0.35]	**0.12** [0.03 -0.4]	**0.09** [0.01 -0.13]
	L2	**0.16** [0.08 -0.56]	**0.12** [0.03 -0.41]	**0.07** [0.03-0.18]
	L3	**0.18** [0.13 -0.24]	**0.13** [0.10 -0.17]	**0.12** [0.02 -0.46]
	L4	**0.21** [0.13 -0.34]	**0.15** [0.09 -0.5]	**0.13** [0.06 -0.29]

R. communis was also found more effective against the first larval instars of the two tested mosquito species (Table 2). In addition, *Cx. pipiens* larvae were more sensitive to this plant extract than *Cs. longiareolata* larvae. The LC_{50} values recorded at 72H exposure time for the fourth instar larvae were 0.06 and 0.08 g/L for *Cx. pipiens* and *Cs. longiareolata*, respectively.

Laastly concerning *T. vulgaris* extracts, a similar trend was observed (Table 3). In addition, *Cx. pipiens* larvae were more sensitive to this plant extract than *Cs. longiareolata* larvae. The LC_{50} values recorded at 72H exposure time for the fourth instar larvae were: 0.02 and 0.03 g/L for *Cx. pipiens* and *Cs. longiareolata*, respectively.

Table 2: Lethal concentrations (LC_{50}, g/l) of *R. communis* extracts against the different larval (L) instars of *Cx. pipiens* and *Cs. longiareolata* (fiducial limits 95%; exposure time in hours; N= 3 replicates containing each 20 larvae/instar/exposure time).

Larvae		LC 50 24h	95%FL (g/l) 48h	72h
Cx. pipiens	L1	**0.10** [0.03-0.29]	**0.03** [0.01-0.12]	**0.03** [0.01-0.09]
	L2	**0.11** [0.08-0.18]	**0.05** [0.02-0.10]	**0.04** [0.03-0.05]
	L3	**0.15** [0.03-0.73]	**0.07** [0.01-0.15]	**0.04** [0.03-0.06]
	L4	**0.15** [0.07-0.18]	**0.10** [0.08-0.15]	**0.06** [0.01-0.20]
Cs. longiareolata	L1	**0.08** [0.05 -0.22]	**0.03** [0.02. 0.08]	**0.02** [0.01 -0.08]
	L2	**0.09** [0.02 -0.12]	**0.07** [0.02 -0.10]	**0.06** [0.05 -0.09]
	L3	**0.11** [0.05 -0.12]	**0.10** [0.06 -0.15]	**0.09** [0.03 -0.17]
	L4	**0.12** [0.05 -0.20]	**0.09** [0.02 -0.19]	**0.08** [0.05 -0.13]

Table 3: Lethal concentrations (LC_{50}, g/l) of *T. vulgaris* extracts against the different larval (L) instars of *Cx. pipiens* and *Cs. longiareolata* (fiducial limits 95%; exposure time in hours; N= 3 replicates containing each 20 larvae/instar/exposure time).

Larvae		LC 50 24h	95%FL (g/l) 48h	72h
Cx. pipiens	L1	**0.04** [0.03 -0.12]	**0.03** [0.02-0.04]	**0.02** [0.01 -0.30]
	L2	**0.04** [0.01 -0.09]	**0.03** [0.01 -0.06]	**0.03** [0.01 -0.08]
	L3	**0.03** [0.02 -0.07]	**0.02** [0.01 -0.04]	**0.03** [0.02 -0.08]
	L4	**0.05** [0.04 -0.08]	**0.04** [0.01 -0.10]	**0.02** [0.01 -0.10]
Cs. longiareolata	L1	**0.03** [0.01 -0.50]	**0.03** [0.01 -0.08]	**0.02** [0.01-0.04]
	L2	**0.03** [0.01 -0.07]	**0.03** [0.01 -0.11]	**0.02** [0.01 -0.09]
	L3	**0.04** [0.01 -0.10]	**0.03** [0.01 -0.09]	**0.03** [0.02 -0.08]
	L4	**0.04** [0.02 -0.09]	**0.06** [0.03 -0.12]	**0.03** [0.01 -0.06]

Toxicological data on the potency of three plants extracts against the two mosquito species were subjected to a three way analysis of variance (Concentrations, Plant extracts, Mosquito species). The results of the different ANOVA made for the three considered factors are summarized in table 4. They revealed a significant effect ($P< 0.001$) of concentrations for all instars and exposure times tested and also a significant effect ($P< 0.05$) of plants extracts for all series (except larval instar 3-48 H). In addition, ANOVA indicated a significant ($P< 0.05$) difference between the two mosquito species particularly against the larval instars 1 and 2 (Table 4).

Table 4: Three way ANOVA (concentrations, extracts, mosquito species) for each larval instar ans exposure time: P value of the different factors considered.

Instars - Exposure time	P values		
	Concentrations	Plant extracts	Mosquito species
Larval instar 1-24 H	<0.001	0.003	0.002
Larval instar 2-24 H	<0.001	0.009	0.002
Larval instar 3-24 H	<0.001	0.029	0.190
Larval instar 4-24 H	<0.001	0.001	0.895
Larval instar 1-48 H	<0.001	0.049	0.049
Larval instar 2-48 H	<0.001	0.023	0.013
Larval instar 3-48 H	<0.001	0.757	0.312
Larval instar 4-48 H	<0.001	<0.001	0.020
Larval instar 1-72 H	<0.001	0.023	0.021
Larval instar 2-72 H	<0.001	0.001	0.020
Larval instar 3-72 H	<0.001	0.027	0.196
Larval instar 4-72 H	<0.001	<0.001	<0.001

4. Discussion

Because the secondary effects of conventional neurotoxic insecticides on the environment alternative methods have been [3]. Plant extracts have potential for controlling of mosquitoes in an environmentally-friendly manner to the aquatic ecosystem [25]. More than 2 000 plant species with insecticidal activity are already identified [26]. The larvicidal activity of some legumes was tested against *A. aegypti* and *Cx. pipiens* [27]. As well as the toxicity of *Mentha pulegium* (lipped) was confirmed on mosquito larvae [28]. The larvicidal activity of the extracts from aromatic plants was also reported [29-30]. Several plant species have been tested as aqueous extracts *against Cx. pipiens* such as *Myrtus communis*, *Eucalyptus camaldulensis* and the *Neriumo leander* [31],

Azadirachta indica [15]. *T. vulgaris* used as aqueous extract or in the form of essential oils was found to present larvicidal activity [32]. Essential oils obtained from *T. vulgaris* were also tested against larvae of *Culex quinquefasciatus* [33]. The latter species was found sensitive to an LC_{50} of 0.033 g/l. The essential oil of *T. vulgaris* tested remains the most effective against of *Cx. pipiens*, with an LC_{50} value of 0.103 g/l [34]. *T. vulgaris* presents many biological activities like antispasmodic, antimicrobial, antioxidant, antiplatelet, analgesic and anti-inflammatory, antiviral and antifungal [35]. Our tests focused on the effect of aqueous extracts from three plants of economic and medicinal interests, *D. gnidium*, *R. communis* and *T. vulgaris*. The aqueous extracts of the three plants tested showed an insecticidal activity on the different instar larvae of *Cx. pipiens* and *Cs. longiareolata,* with a higher toxicity obtained with *T. vulgaris* extracts.

R. communis larvicides are promising to fight against harmful mosquitoes (*Cx. pipiens*, *Aedes caspius* and *Cs. longiareolata* and *Anopheles maculipennis*) is a mortality rate of 100% at a concentration of 1%. They are most effective effect on larvae of the second instar (L2) as those of the fourth instar (L4) with LC_{50} after 24h weaker varying between 0. 110 and 0.370 g/ l [36]. Our current experiment also shows that the younger larval instars (L1, L2) of *Cx. pipiens* and *Cs. longiareolata* were more sensitive to extracts compared to other instars. *R. communis* seed extracts have a larvicidal effect, and therefore provide an excellent potential against vectors as

Culex quinquefasciatus, *Anopheles stephensi* and *Aedes albopictus* [36]. After 72 hours, the mortality indicates a rate of up to 100% for the higher dose [37]. *D. gnidium* can be an alternative to harmful chemicals to the environment and health in the biocontrol of fungal flora and mycotoxin [20].

5. Conclusion

This study aims to evaluate the efficacy of aqueous extracts of three plants (*D. gnidium*, *R. communis* and *T. vulgaris*) against the four instar larvae of *Cx. pipiens* and *Cs. longiareolata*. It was observed that these extracts exhibited insecticidal activity against larvae of both species tested and varied as function of mosquito species, larval instars and doses. Plant extracts tested were found more effective against *Cx. pipiens* and the highest activity was found for *T. vulgaris* extracts. These plant extracts have potential for controlling mosquitoes in an environmentally-friendly manner to the aquatic ecosystem.

6. Acknowledgements

This work was supported by the National Fund for Scientific Research to Pr. N. Soltani (Laboratory of Applied Animal Biology) and the Ministry of High Education and Scientific Research of Algeria to Pr. F. Bendali (CNEPRU and PNR projects).

References

1. Marquardt WC. Biology of disease vectors, second edition. Clin. Infect. Dis 2005; 41(11):1692-1693.
2. Tantely ML, Rakotoniaina JC, Tata E. Andrianaivolambo L, Razafindrasata F, Fontenille D *et al.* Biology of mosquitoes that are potential vectors of Rift Valley fever virus in different biotopes of the central highlands of Madagascar. Journal of Medical Entomology. 2013; 50:603-610.
3. Fontenille D. Vecteurs d'arbovirus et indicateurs de lute (WN, RVF, YF, DEN, CHIK). IRD, UR 016. Caractérisation et contrôle des populations de vecteurs, Département Sociétés et Santé 2010. IRD, Montpellier.
4. Rhodain F, Perez C. Précis d'entomologie médicale et vétérinaire. Maloine. 1985. S A Editeur 27, Rue de l'école médecine 75006, Paris.
5. Harbach RE. The Culicidae (Diptera): a review of taxonomy, classification and phylogeny. Zootaxa 2007; 168:591-688.
6. Rehimi N, Soltani N. Laboratory evaluation of alsystin, a chitin synthesis inhibitor, against *Culex pipiens* (Dip., Culicidae): effects on development and cuticle secretion. Journal of Applied Entomology. 1999; 123:437-441.
7. Soltani N, Rehimi N, Beldi H, Bendali F. Activité du triflumuron sur *Culex pipiens* (Diptera: Culicidae) et impact sur deux espèces larvivores non visées. Annales de la Société Entomologique de France (NS) 1999; 35:502-508.

8. Lacey LA, Orr B. The role of biological control of mosquitoes in integrated vector control. American Journal of Tropical Medicine & Hygiene. 1994; 50(6 Suppl.):97-115.
9. Bendali-Saoudi F, Djebbar F, Soltani N. Efficacité comparée de quelques espèces de poissons à l'égard de divers stades de *Culex pipiens L*. dans des conditions de laboratoire. *Parasitica*, 2001; 57(4):255- 265.
10. Bendali-Saoudi F. Etude bioécologique, Systématique et Biochimique des Culicidae (Diptera- Nematocera) de la région d'Annaba. Lutte Biologique anticulicidiènne, 2006. Thèse de Doctorat d'Etat en Science Naturelle, Université Badji Mokhtar Annaba, Algérie.
11. Walton WE, Floore V. Larvivorous fish including *Gambusia*. In: Biorational control of mosquitoes. American Mosquito Control Association, 2007; 23(S2):184-220.
12. Boudjelida H, Bouaziz A, Soin T, Smagghe G, Soltani N. Effects of ecdysone agonist halofenozide against *Culex pipiens*. *Pest. Biochem. Physiol*, 2005; 83(2/3):115-123.
13. Alouani A, Rehimi N, Soltani N. Larvicidal activity of a Neem tree extract (Azadirachtin) against mosquito larvae in the republic of Algeria. Jordan Journal of Biological Sciences. 2009; 2(1):15-22.
14. Hamaidia K, Soltani N. Laboratory evaluation of a biorational insecticide, kinoprene, against *culex pipiens* larvae: effects on growth and development. *Annual Research and* Review in Biology 2014; 4(14):2263-2273.
15. Bouabida H, Tine-Djebbar F, Soltani N. Etude systématique et écologique des Moustiques (Diptera: Culicidae) dans la région de Tébessa (Algérie). Entomologie faunistique – Faunistic Entomology. 2012; 65:99-103.
16. Tine-Djebbar F, Soltani N. Activité biologique d'un agoniste non stéroïdien de l'hormone de mue sur *Culiseta longiareolata* : analyses morphométrique, biochimique et énergétique. Synthèse. 2008; 18:23-34.
17. Markouk M, Bekkouche K, Larhsini M, Bousaid M, Lazrek HB, Jana M. Evaluation of some Moroccan medicinal plant for larvicical activity. J Ethnopharmacol. 2000; 73:93-29.
18. Turell MJ, Dohm DJ, Sardelis MR, O'Guinn ML, Andreadis TG, Blow JA. An update on the potentiel of North American mosquitoes (Diptera; Culicidae) to transmit West Nile Virus. J Med Entomol. 2005; 42(1):57-62.
19. Scarpa A, Guerci A. Various uses of the castor oïl plant (*Ricinus communis* L.). A review. J Ethnopharmacol. 1982; 5:117-137.
20. Mohammedi Z. Etude phytochimique et activités biologiques de quelques plantes médicinales de la région Nord et Sud-Ouest de l'Algérie, 2013. Thèse de Doctorat. Université Abou Bakr Belkaid Tlemcen.
21. Morales R. The history, botany and taxonomy of the genus *Thymus*. *Thyme* the genus *Thymus*. Ed. Taylor & Francis, London, 2002, 1-43.
22. Mordue AJ, Blackwell A. Azadirachtin: an update. J Insect Physiol, 1993; 39:903-24.
23. World Health Organization (WHO), Report of the WHO informal consultation on the evaluation and testing of Insecticides. Geneva, 1996.
24. Abbott WS. A method of computing the effectiveness of an insecticide. J Econ Ent. 1925; 18:265-267.
25. Rehimi N, Alouani A, Soltani N. Efficacy of azadirachtin against mosquito Larvae *Culex pipiens* (Diptera: Culicidae) under laboratory conditions. European J Sci Res. 2011; 57(2):223-229.
26. Maiza A, Aribi N, Smagghe G, Kilani-Morakchi S, Bendjedid M, Soltani N. Sublethal effects on reproduction and biomarkers by spinosad and indoxacarb in cockroaches *Blattella germanica*. Bull. Insectol, 2013; 66(1):11-20.
27. Jacobson M. Botanical pesticides, past present and future In Arnason JT. Insecticides of plant origin. Washington, D.C: American Chemical Society Symposium. 1989; 387:1-10.
28. Jang YS, baek BR, Yang WC, Kim MK, Lee HS. Larvicidal activity of leguminous seeds and grains against *Aedes aegypti* and *Culex pipiens pallens*. J Am Mosq Cntrol. Assoc. 2002a; 18(3):210-213.
29. Michaelakis A, Koliopoulos G, Milonas P, Kontodimas D, Polissiou M, Kimbaris AC *et al.* Activity of nonoxygenated versus oxygenated monoterpenes against mosquitoes. An attempt to correlate toxicity with chemical structure, In: A 7th Joint meeting of AFERP, GA, PSE & SIF, Natural products with pharmaceutical, nutraceutical, cosmetic and agochemical interest, Athens, 2008.
30. Kemassi A, Bouziane N, Boual Z, Ould El Hadj MD. Activité biologique des huiles essentielles de *Peganum harmala* L. (*Zygophyllaceae*) et de *Cleome arabica* L. (*Capparidaceae*) sur *Schistocerca gregaria* (Forskål, 1775). 2014; 12(6):348-353.
31. Mansouri M. Inventaire des Hydrachnidia au Nord-Est Algérien et dosage de la GST au niveau du Lac des Oiseaux. Toxicité de biocides : Le *Bacillus thuringiensis israelensis* et des extraits aqueux végétaux sur les espèces non ciblées: les Hydrachnidia et les Mollusques d'eau douce, 2015. Université Badji Mokhtar-Annaba, Algérie.
32. Tchoumbougnang F, Jazet Dongmo PM, Lambert Sameza M, Nkouaya Mbanjo EG, Tiako Fotso GB, Amvam Zollo PH *et al.* Activité larvicide sur *Anopheles gambiae* Giles et composition chimique des huiles essentielles extraites de quatre plantes cultivées au Cameroun. Biotechnologie, Agronomie, Société et Environnement, 2009; 13(1):77-84.
33. Roman P. Larvicidal property of essential oils against *Culex quinquefasciatus* Say (Diptera: Culicidae), Ind. Crops. Prod. 2009; 30(2)311-315.
34. El-Akhal1 F, Greche H, Ouazzani Chahdi F, Guemmouh R, El Ouali Lalami1 A. Chemical composition and larvicidal activity of *Culex pipiens* essential oil of *Thymus vulgaris* grown in Morocco. 2015; 6(1):214-219.
35. El Bouzidi L, Alaoui JC, Bekkouche K, Hassani L, Wohlmuth H, Leach D *et al.* Chemical composition, antioxidant and antimicrobial activities of essential oils obtained from wild and cultivated Moroccan *Thymus* species. Ind. Crop. Prod. 2013; 43:450-456.
36. Aouinty B, Oufara S, Mellouki F, Mahari S. Evolution préliminaire de l'activité larvicide des extraits aqueux des feuilles du ricin (*Ricinus communis* L.) et du bois de thuya (*Tetraclinis articulate* (Vahl) Mast. sur les larves de quatre moustiques Culicidae: *Culex pipiens* (Linné), *Aedes caspius* (Pallas), *Culiseta longiareolata* (Aitken) et *Anopheles maculipennis* (Meigen). Biotechnol. Agron. Soc. Environ, 2006; 10(2):67-71.
37. Mandal S. Exploration of larvicidal and adult emergence inhibition activities of *Ricinus communis* seed extract against three potential mosquito vectors in Kolkata, India. Asian Pacific Journal of Tropical Medicine. 2010, 605-609.