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New alternative for culicidian fauna control using *Borago officinalis* and *Drimia maritima* plant extracts

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Abstract. *Djeddar H, Boudjelida H, Arroussi DER. 2021. New alternative for culicidian fauna control using* Borago officinalis *and* Drimia maritima *plant extracts. Biodiversitas 22: 5688-5694.* Focus on the development of insecticides based on botanical extracts may represent an alternative environmentally interesting tool to chemical control. The current study aimed to investigate the effects of both extracts of *Drimia maritima* (Asparagales: Asparagaceae) and *Borago officinalis* (Lamiales: Boraginaceae) on larvae sensibility, physiological and biochemical parameters of *Culex pipiens* (Diptera: Culicidae). The larvicidal bioassay exhibits a significant dose-response relationship against the fourth instar larvae of *Cx. pipiens*. Moreover, biochemical analyses revealed a decrease in proteins levels, carbohydrates and lipids in the whole larvae body treated with the lethal concentrations (LC50, LC90) of *D. maritima* extract as compared to the control series. Whereas, using *B. officinalis* herbal plants, the result showed a decrease in lipid and carbohydrate contents and an increase in protein. The ability of *D. maritima and B. officinalis* extracts to inhibit acetylcholinesterase (AChE) activity in a whole body of the treated larvae of *Cx. pipiens* is examined. The estimated specific activity of glutathione S-transferase (GST) at different times was significantly increased under the effects of both plants. The present study demonstrates that the aqueous extracts of *D. maritima* and *B.officinalis* present insecticidal properties with neurotoxic effects against larval stages of the domestic mosquito *Cx. pipiens*. In this context, the use of chemical insecticides for mosquito control is no longer recommended and the alternatives of eco-friendly products are encouraged.

Keywords*:* Acetylcholinesterase, botanical extracts, *Culex pipiens*, Glutathione S-transferase, Toxicity

Abbreviations: *Cx. pipiens: Culex pipiens*; *B. officinalis*: *Borago officinalis; D. maritima*: *Drimia maritima*; GST: glutathione S-transferase; AchE: acetylcholinesterase; LC: lethal concentrations; FL: fiducial limits; UCL: upper confidence limit; LCL: lower confidence limit; SD: standard deviation

INTRODUCTION

Arthropods act as potential vectors of many diseases caused by a huge number of deadly pathogens. Mosquito species are considered among the most medically and important veterinary vectors, responsible for the transmission of many human and animal diseases, such as malaria, yellow fever, West Nile Fever, dengue and other arboviruses (Börstler et al. 2016; Fernandes et al. 2019) which caused millions of death a year (Mayer et al. 2017; Benelli et al. 2018; Nwabor et al. 2019). The persistence of these diseases with the emergence of epidemics or pandemics in many parts of the world has renewed the interest in re-consideration of vector control strategies (Chinnaperumal and Abdul 2010; Alouani et al. 2017). Mosquito vectors are generally controlled by conventional neurotoxic insecticides (Cuervo-Parra et al. 2016). Beyond the pollution for the environment and development of insect resistance (Ranson and Lissenden 2016; Sumarnrote et al. 2017; Main et al. 2018), these insecticides present a toxic effect on other animals, like birds, fish, bees and mammals (Promsiri et al. 2006). These encouraged the scientists to focus their work on searching for new products that could be good alternatives for vectors control to be eco-friendly and cost-effective (Pavela and Benelli 2016). Insecticides based on botanical extracts are also an environmentally interesting tool because they are biodegradable and have minimal side effects on non-target organisms, and are environmentally safe (Govindarajan et al. 2016). Since many molecules were proposed, such as botanical biopesticides, medicinal plant extracts, and essential oils (El-Akhal et al. 2016; Sutthanont et al. 2019; Yaseen 2020).

The ornithophilic mosquitoes in the genus of *Culex* are the most widely distributed species in the world and have been widely implicated as primary vectors of the West Nile Virus, especially *Cx. pipiens* (Linnaeus 1758), *Cx. modestus* (Ficalbi 1889), *Cx. torrentium* (Martini 1925) (Calistri et al. 2010). Up to now, *Cx. pipiens*, *Cx. modestus*, *Cx. theileri*, *Culiseta longiareolata*, *Aedes aegypti* and *Aedes albopictus* (Arroussi et al. 2021), the most abundant mosquito species in Algeria, are controlled by abusive use of conventional pesticides.

The current comprehensive study was designed to evaluate in the laboratory the toxic effect of *D. maritima* bulbs and *B. officinalis* L. stems aqueous extracts against *Cx pipiens* larvae. Furthermore, and in order to explain their mode of action, the effect of the lethal concentrations (LC50 and LC90) were estimated on metabolites and hormonal profile of the mosquito species.

MATERIALS AND METHODS

Mosquito rearing

Culex pipiens larvae were obtained from the reared colonies of the laboratory of applied animal biology, university BADJI Mokhtar Annaba, Algeria. The first larvae genera were collected from untreated wetlands located at Annaba district (Northeast Algeria) 36°49'5.57"N - 7°43'19.51"E. In the laboratory, each larval stage were kept separately in storage jars containing 250 mL of stored tap water and maintained under the laboratory conditions. The environmental conditions of the laboratory were controlled, with a maintained temperature at 26 ± 2°C and a photoperiod of 14: 10 (L: D). Then, they were fed with fresh food consisting of a mixture of biscuit-dried yeast (75: 25 by weight), and water was renewed every three days. Whereas the adult was reared in cages (50 × 50 × 50 cm), contained a jar for laying eggs, and were fed on 10% sucrose solution. Therefore, the blood meal for female adults was supplied by the introduction of baby Wistar rat into the cage for 2 hours in a dark room.

Toxicity bioassay

The vegetable matter, *D. maritima* (Asparagales: Asparagaceae) bulbs and *B. officinalis* (Lamiales: Boraginaceae) stems, were collected from a non-agricultural area; in Souk-ahras district, northeast of Algeria. In the laboratory, the used parts of the plant were washed with distilled water, shade-dried and grounded. The finely ground plant powder (250 g/L) was extracted with infusion using 1L bowling water. The extraction was continued, in-room temperature, till visibly no further extraction is possible, in a glass beaker and decanted for 15 minutes and stored at refrigerator until the bioassays tests. From this stock solution, four concentrations were considered, after preliminary tests (2.5g/L= 10 mL; 5g/L= 20 mL; 10g/L= 40mL; 15g/L= 60 mL and 20 g/L= 80 mL) and were prepared for toxicity treatments.

The bioassays were conducted in the laboratory according to the testing methods for larval susceptibility (WHO 1991). The toxicological essays, using *D. maritima* and *B. officinalis*, were carried out with the four prepared concentrations against the newly exuviated fourth instar mosquito larvae (L4) of *Cx. pipiens*, under laboratory conditions. The bioassays of treated and control series were realized with three repetitions of 25 larvae for each used concentration, prepared in a separate jar containing 100 mL of breeding water. The treated larvae series were exposed to the aqueous extract for 24 hours only, according to the recommendations of the world health organization (WHO 2005). After this, the water is changed and the food is added every three days until adults emergence. The larval mortality of the experimental series is recorded daily and followed during the other developing stages until the adult emergence.

Biochemical composition

The biochemical assay is carried out with the whole body of the fourth larvae of *Cx. pipiens*, after treatment with CL50 and CL90 of the aqueous extracts of the used plants. Extraction of the main metabolites (proteins, carbohydrates and lipids) was done according to the procedure of Shibko et al. (1966). The fourth instar larvae were sampled from the control and treated series for different periods of 1, 3 and 5 days of age. Pooled larvae samples (n: 20 larvae with 3 replicates) were weighted with a precision balance (KERN 770) with a range of 0.1mg and conserved in 1 mL of TCA 20% (trichloroacetic acid). The quantities of the metabolites (proteins, carbohydrates and lipids) were estimated from the equation of the regression lines, expressed the absorbance as a function of the quantity of the using standard. Proteins were evaluated according to Bradford's method (1976), using Coomassie brilliant blue as a reagent (BBC) (G 250, Merck) and bovine serum albumin (BSA) as standard (Sigma). The calibration range was performed using a BSA stock solution (1mg/mL) and BBC. The absorbance was measured with a spectrophotometer at 595 nm wavelength. Lipids were estimated according to Goldsworthy et al. (1972) using a 25 mg/mL lipid stock solution (25 mg vegetable table oil (Afia, brand market in Algeria), 1 mL ether/chloroform (v/v)) as standard and vanillin (0.38 g vanillin, 55 mL distilled water, 195 mL 85% orthophosphoric acid) as a reagent with an absorbance at 530 nm in a visible/UV spectrophotometer. The calibration ranges were obtained using a lipid stock solution in an Eppendorf tube, adding 1 mL chloroform ether (1V/1V). Carbohydrates were quantified according to Duchateau and Florkin (1959) using glucose stock solution (1 g/L) as standard and anthrone as a reagent (150 mg anthrone, 75 ml sulphuric acid and 25 mL water) and the absorbance was measured at 620 nm wavelength.

Biomarkers enzymatic assays

In order to evaluate the mode of action of plants on the treated larvae, the enzymatic activity of GST, AchE were estimated. The assay is conducted with several replicates each containing 20 individuals with a control series. The sampling larvae were made from treated series with the lethal aqueous concentrations of the 2 used plants and control series, during periods of 1, 3 and 5 days of development. The measurement of AChE activity is determined according to the method of Ellman et al. (1961). It is based on the conjugation reaction between the enzyme and a substrate, acetyltiocholine (ASCh), whose hydrolysis releases acetic acid and thiocholine (SCh). The thiocholine, with DTNB (5, 5'-dithio-bis-2-nitrobenzoic acid) forms a yellow complex whose intensity is read at a wavelength of 412 nm. The determination of the GST activity is carried out according to the method of Habig et al. (1974), which is consisted by providing the GST with a substrate, CDNB (1-chloro 2, 4 dinitrobenzene) in the presence of a glutathione cofactor (GSH) and the solution is measured at a wavelength of 340 nm in a visible/UV spectrophotometer.

Statistical analysis

The statistical analysis was conducted using Prism, version 7.03 software. The obtained mortality was corrected (Abbott 1925), when the mortality was observed

in the control series and toxicity data subjected to probit analysis (Finney 1971). Lethal concentrations (LC50 and LC90) and their 95% fiducial limits (95% FL) of upper confidence limit (UCL) and lower confidence limit (LCL) values, regression equation and slope of the concentration mortality lines were estimated (Swaroop et al. 1965). The obtained results of mortality were expressed as the mean ± standard deviation (SD). The significance between different samples, toxicity, biochemical and physiological results, was tested using an ANOVA with repeated measures (prism, 7.03) followed by (post-hoc HSD Tukey's test), in order to examine the effect of the two aqueous extracts at the various concentrations over different periods.

RESULTS AND DISCUSSION

Larvicidal activity

The mortality recorded after the treatment with different concentrations of *D. maritima* and *B. officinalis* extracts applied to the fourth instar larvae was determined (Table 1). The reported results were subjected to the analysis of variance with repeated measures, which reveals a highly significant dose-response effect ($p<0.005$) to both plant extracts. Using the same concentrations, it was found that *B. officinalis is* more toxic than *D. maritima.*

Linear regression was determined (Figure 1) after transforming the concentrations tested in decimal logarithms and the corrected mortalities in probits. The coefficient of determination value (R^2) for each test reveals a positive relationship between the probits and the concentration-test's logarithm (Table 2). The lethal concentrations LC50 and LC90 of *D. maritima* (13.63, 28.25 g/L) and *B. officinalis* (6.86, 17.8 g/L) extracts were estimated from the equation and the linear regression with their fudicial limits (FL) (Table 2).

Biochemical activity

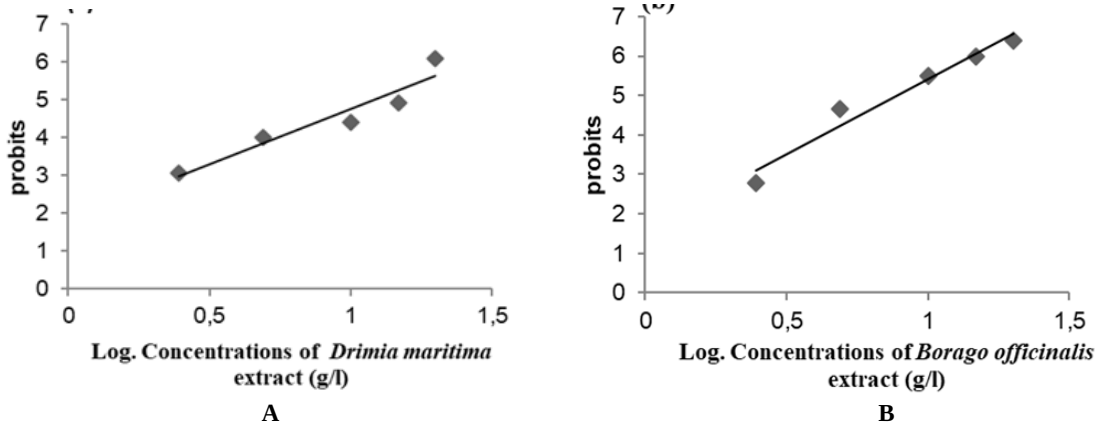
The results of the biochemical analysis showed that the treatment with the lethal concentrations (LC50, LC90) extract of *D. maritima* reduced significantly the protein contents ($p < 0.05$) of the treated fourth instar larvae of *Cx. pipiens* as compared with the control series of the same age (Table 3). The statistical analyses of a two-way ANOVA revealed significant effects of time ($F2, 18= 31.66$, $p < 0.001$), treatment ($F2, 18= 103.4$, $p < 0.001$) and time/treatment interaction ($F4, 18= 49.91$, $p < 0.001$). Whereas a significant increase in protein levels ($p < 0.05$) was recorded in the larvae treated with the lethal concentrations extract of *B. officinalis* at the different sampling time. The same statistical analyses showed significant effects of time ($F2, 18= 33.34$, $p < 0.001$), treatment ($F2, 18= 99.25$, $p < 0.001$) and time/treatment interaction ($F4, 18= 6.16$, $p < 0.005$).

Table 1. Corrected mortality of fourth instars larvae of *Culex pipiens* exposed to different concentration of *Drimia maritima* and *Borago officinalis* extracts during 24 h (Mean ± SD %, n: 75)

Concentration (g/L)	*D. maritima*	*B. officinalis*
	Mortality (%)	Mortality (%)
2.5 g/L	2.66±2.31	1.33±2.3
5 g/L	16±4	37.33±6.11
10 g/L	28±4	69.33±2.31
15 g/L	47.4±6,79	84±4
20 g/L	86.66±4.61	92±4

Table 2. Toxicity of *Drimia maritima* and *Borago officinalis* extracts against *Culex pipiens* larvae (LC50, LC90, LCL, and UCL g/L)

Plant	Equation	R^2	Slope	LCL ≤ LC50 ≤UCL(g/L)	LCL ≤ LC90 ≤UCL(g/L)
Drimia maritima	y = 2,912X+ 1,8481	0.90	3.01	(11.57≤13.63≤15.57)	(21.04≤28.25 ≤49.6)
Borago officinalis	y = 3,8064X+ 1,6021	0.95	2.3	(6.23-7≤6.86≤7.55)	(14.92≤17.8≤21.54)



A **B**

Figure 1. Concentrations-response relationship for treatment by *Drimia maritima* (A) and *Borago officinalis* (B) extracts applied to the newly ecdysed larvae of *Culex pipiens*

The carbohydrate content was also significantly affected ($P < 0.05$) in treated fourth-instar larvae of *Cx. pipiens* at different times, after treatment with the used lethal concentrations extracts of *D. maritima* and *B. officinalis* (Table 3). A significant decrease in carbohydrate cotenant was observed with the both used plant extracts. The time-response ($p < 0.001$, $F2, 18 = 77.78$; $F2, 18 = 45.08$, $p < 0.001$), response-treatment ($F2, 18 = 74.06$, $p < 0.001$; $F2, 18 = 71.03$, $p < 0.001$) and time/treatment interaction ($F4, 18 = 5.19$, $p < 0.005$) ($F4, 18 = 4.52$, $p < 0.001$), were significant respectively.

Finally, the biochemical analysis showed a decrease in lipid levels ($P < 0.05$) in the series of the treated fourth instar larvae with aqueous extract lethal concentrations of *D. maritima* and *B. officinalis* at different times as compared to control series (Table 3). The Anova with two criteria statistical analyses showed a significant effects of time ($F 2, 18 = 13.88$, $p < 0.001$) ($F2, 18 = 91.48$, $p < 0.001$), treatment ($F2, 18 = 105.9$, $p < 0.001$) ($F2, 18 = 91.48$, $p < 0.001$) and time/treatment interaction ($F4, 18 = 3.06$, $p < 0.05$) ($F4, 18 = 6.7$, $p < 0.001$), respectively.

Enzymatic activity

The specific activity of GST and AChE (µM/mn/mg protein) was estimated for fourth instar larvae of *Cx. pipiens* at various times (day 1, 3 and 5) after treatment with *D. maritima* and *B. officinalis* aqueous extracts. A significant increase in GST activity ($p < 0.05$) was recorded starting from the day 1 for the tested lethal concentrations of both plant extracts, as compared to the control of the same age (Figure 2). The highest activity was observed at day 3 for the CL90 of *B. officinalis.* According to the statistical analyses, a significant effect was observed during time ($F2, 18 = 31.6$; $p < 0.001$) ($F2, 18 = 123.7$; $p < 0.001$), treatment ($F2, 18 = 174.3$; $p < 0.001$) ($F2, 18 = 774.3$; $p < 0.001$), and time/treatment interaction ($F 4, 18 = 7.20$; $p < 0.001$) ($F 4, 18 = 30.11$; $p < 0.001$), respectively. Therefore, the results of the AChE activity showed a significant inhibition ($p < 0.005$) (Figure 3) during the 3 sampling period after treatment with *D. maritima* and *B. officinalis* aqueous extracts. The statistical analyses confirm the significant effect of time ($F2, 18 = 16.16$; $p < 0.001$) ($F2, 18 = 17.2$; $p < 0.001$), treatment ($F2, 18 = 662.9$; $p < 0.001$) ($F2, 18 = 375.2$; $p < 0.001$), and time/treatment interaction ($F4, 18 = 18.43$; $p < 0.001$) ($F4, 18 = 54.44$; $p < 0.001$), for both plants respectively.

Table 3. Effects of *Drimia maritima* and *Borago officinalis* aqueous extracts on proteins contents, carbohydrates and lipids (µg.mg -1) in the whole body from the fourth instar larvae of *Culex pipiens*

Sampling day and component	Control	*Borago officinalis*		*Drimia maritima*	
		LC50g/L	LC90g/L	LC50g/L	LC90g/L
day 1					
Proteins	37.98± 2.05 **a**	37.30± 0.93 **a**	47.65 ±1.2 **b**	29.11± 0.80 **b**	26.54±2.41 **c**
Carbohydrates	61.46± 7.41 **a**	47.10± 3.60 **b**	39.70±2.01 **c**	46.13± 3.60 **b**	43.86±3.46 b
Lipids	51.28± 4.23 **a**	38.63± 1.45 **b**	41.34± 2.8 **b**	27.69± 0.03 **b**	30.44± 2.6 **c**
day 3					
Proteins	39.54 ± 2.20 **a**	34.28± 1.7 0 **b**	45.36± 2.90 **c**	28.72± 1.29 **b**	23.54± 1.06 **c**
Carbohydrates	43.36 ± 1.32 **a**	39.65± 2.08 **b**	30.10± 0.50 **c**	32.94± 2.20 **b**	29.10± 0.50 **c**
Lipids	62.43± 4.83 **a**	39.27± 2.46 **b**	38.30± 3.10 **b**	49.27± 2.64 **b**	41.3± 3.10 **c**
Day 5					
Proteins	41.16± 1.20 **a**	43.30± 3.60 **a**	51.30 ± 4.10 **b**	32.07± 1.10**b**	29.44± 2.90 **c**
Carbohydrates	46.13± 2.13 **a**	39.60± 1.90 **b**	35.70 ± 0.20 **c**	43.86± 1.55 **b**	34.28± 0.70 **c**
Lipids	71.03± 8.20 **a**	47.53± 0.30 **b**	39.67 ± 4.00 **b**	51.33± 5.30 **b**	44.69± 2.90 **b**

Mean ± SD, values followed by the same letter are not significantly different at $p > 0.05$

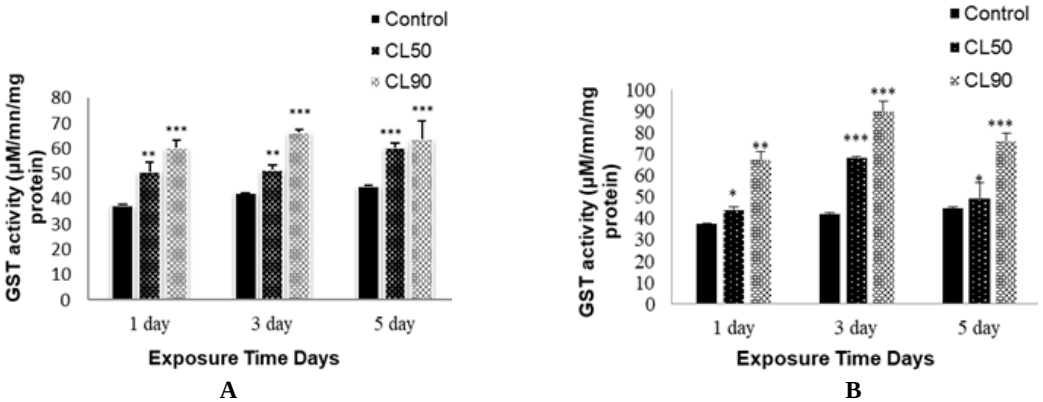


Figure 2. Effects of *Drimia maritima* (A) and *Borago officinalis* (B) aqueous extracts on GST activity (µM/mn/mg protein) in *Culex pipiens* larvae. (Mean ± SD, Asterisks above the treated series indicated a significant difference at $p < 0.05$ with control at the same time)

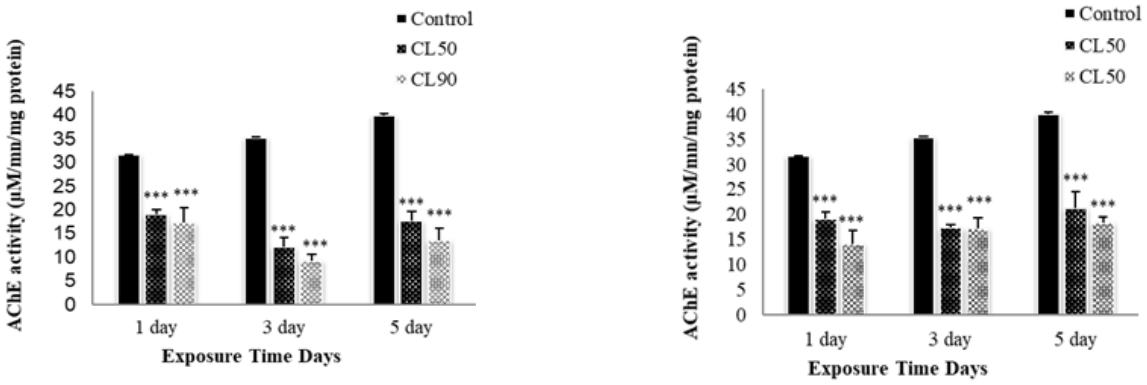


Figure 3. Effects of *Drimia maritima* (A) and *Borago officinalis* (B) aqueous extracts on AChE activity (µM/mn/mg protein) in *Culex pipiens* larvae. (Mean ± SD, Asterisks above the treated series indicated a significant difference at $p < 0.05$ with control at the same time)

Discussion

Plants produce a wide variety of secondary metabolites that have toxicological effects against many insect species such as mosquitoes (Aarthi et al. 2018; Benelli and Pavela 2018; Pavela et al. 2019). This research has focused on the biological control of *Culex pipiens* (Diptera: Culicidae) mosquito, using natural active products, which are less harmful, more reasoned and eco-friendly substances like plant extracts. Many previous studies have reported that the different compounds extracted from *Drimia maritima* (Asparagales: Asparagaceae) and *Borago officinalis* (Lamiales: Boraginaceae) plants showed insecticidal properties against several pests (Hidayat et al. 2014; El Haddad et al. 2018; Hazaa et al. 2021). The effect of *Urginea maritima* bulbs extract used for *Sitophilus oryzae* control was reported by (Maazoun et al. 2017). The acaricidal activity of *D. maritima* bulbs and *Dittrichia viscosa* leaves against *Dermanyssus gallinae* is due to the presence of bufadienolides compounds (Rhimi et al. 2019), previously tested against the third stages larvae of silkworm of *Bombyx mori* (Hidayat et al. 2014). Other study, using *D. maritima* ethanolic extract against *Drosophila melanogaster* exhibited significant effects on the development, sexual and oviposition behavior (Saadan et al. 2021). Similarly, the tested plant *B. officinalis* compounds exhibited significant insecticidal activity against several insect species (Pavela 2009; El haddad et al. 2018; Hazaa et al. 2021). The acaricidal activity of flavonoid extracts of *B. officinalis* against Brown dog tick was previously reported (El haddad et al. 2018). However, the study by using the methanol extract of *B. officinalis* leaves caused very high mortality rates against *Cx. quinquefasciatus* (Pavela 2009). In this study, *D. maritima* and *B. officinalis* extract, although with different efficacy, caused relatively high mortality, with a concentration-response relationship, as a function of exposure time of *Cx. pipiens* fourth instar larvae compared to control. The toxicological effects of *D. maritima* and *B. officinalis* extracts might be linked to the presence of bufadienolides and pyrrolizidine alkaloids compound in the extracts.

During the biochemical analyses, the treated larvae metabolites revealed a decrease in the proteins levels, carbohydrates and lipids in a whole body of larvae treated by *D. maritima* aqueous extract compared to the control series. The use of *B. officinalis* against mosquito larvae showed a decrease in lipid and carbohydrate and an increase in protein contents. The increase in the total protein content in the whole body of larvae could be due to an accentuated synthesis of detoxification enzymes. The same results were reported during the use of hydro-alcoholic extracts from the *Nerium oleander* leaves against *rhizotrogini* white grub larvae compared to the control (Madaci et al. 2008). Also, Askar et al. (2016) reported that the application of clove oil to adults of the three *Sitophilus* species increased the total lipid and protein levels. The reduction in protein reserve content in larvae treated by *D. maritima* may also be due to the physiological adoption of the insect to correct stress caused by insecticides or attributed to an increase in their degradation to detoxify the active ingredients present in plant extracts. Our results indicate a significant reduction in carbohydrate and lipid levels in the larval stages in *Cx. pipiens* after treatment with *B. officinalis* and *D. maritima* aqueous extract. This is in accord with the observations of Bouguerra et al. (2018) where revealed a significant decrease in the proteins levels, carbohydrates and lipids in all development stages of *Cx. pipiens* treated by *Ocimum basilicum* essential oil. Cinnamon and cardamom essential oils reduce the protein, carbohydrate and lipid levels of two insect species *Tribolium castaneum* and *Callosobruchus*, previously described by Tarigan et al. (2016). In *Cx. quinquefasciatus* larvae treated by ethanolic extract of *Catharanthus roseus* revealed a significant decrease on glycogen and carbohydrate levels as compared to control (Shoba 2018). This depletion could be explained by the energy demand and consequently increase metabolism, which may be due to the stress conditions imposed on these insects that require more energy. Exhaustion of tissue lipids under insecticide stress could be due to the lipoproteins formation, used to repair cell damage, energy source, increased lipolysis and/or damage to cell organization and adipokinetic hormone (Sak et al. 2006).

Glutathione S-transferase is an enzyme involved in the detoxification of xenobiotic substances (Walters et al.

2009). In this purpose, the specific activity of GST in *Cx. pipiens* larvae treated with aqueous extracts of *D. maritima* and *B. officinalis* plants increased significantly at different times as compared to controls. Pinho et al. (2014) indicate an increase in GST activity in *Drosophila melanogaster* exposed to essential oil from *Psidium guajava*, which may be related to an adaptive response attached to the elimination of toxic plant derivatives. Similarly, Shojaei et al. (2017) show an increase in GST activity in *Tribolium castaneum* larvae treated by essential oil of *Artemisia dracunculus*. The increase in this activity can come either from an increase in the production of an individual's total protein or from a change in a regulatory gene controlling the degree of enzyme expression and an increase in copies number of the gene that code for these enzymes. The increase in GST activity is associated with its detoxification of xenobiotic (Clark 1989).

Analysis of the results obtained after determination of the AChE activity in the whole body of larvae 4 of *Cx. pipiens*, treated with aqueous extracts from *D. maritima* and *B. officinalis* plants at the lethal concentrations at different times, revealed a significant decrease in AChE activity as compared to control. Maazoun et al. (2017) showed that *Urginea maritima* bulbs extract exhibited a significant effect on inhibitory acetylcholinesterase activity for the control of *Sitophilus oryzae*. Similarly, Singh et al. (2017) showed that AChE activity is strongly inhibited in insects *Persea americana* and *S. oryzae* exposed to 2,3-dimethylmaleic anhydride (a natural molecule isolated from plants) and inhibition was competitive. Oboh et al. (2017) indicate that *Citrus sinensis* orange peel essential oil may be effective in biological control and this may be associated with its inhibitory effects on acetylcholinesterase and Na+ / K+-ATPase activity.

The present study demonstrates that the aqueous extracts of *D. maritima* and *B. officinalis* present insecticidal properties with neurotoxic effect against larval stages of the domestic mosquito *Cx. pipiens.* In perspectives, it will be interesting to explain furthermore the pathway of the effecting process and the mode of action of these components.

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