Tropical Biomedicine 40(2): 241-249 (2023)
https://doi.org/10.47665/tb.40.2.016

RESEARCH ARTICLE

Insecticidal activity and physiopathological effects of *Cotula cinerea* crude extract against *Culex pipiens*

Demouche, L.[1*], Acheuk, F.[1], Mokrane, K.[1], Abdellaoui, K.[2], Lakhdari, W.[1-3], Bensouici, C.[4], Dehliz, A.[3]

[1]Laboratory of Valorization and Conservation of Biological Resources, Faculty of Sciences, University M'Hamed Bougara of Boumerdes, Boumerdes 35000, Algeria
[2]Department of Biological Sciences and Plants Protection, Higher Agronomic Institute of Chott Mariem, Sousse University, Chott Mariem 4042, Tunisia
[3]National Institute of Agronomic Research, Station of Sidi Mehdi, Touggourt 30200, Algeria
[4]Laboratory of Biochemistry, Biotechnology and Health Division, Center for Research in Biotechnology (CRBT), Constantine 25000, Algeria
*Corresponding author: l.demouche@univ-boumerdes.dz

ARTICLE HISTORY

Received: 10 February 2023
Revised: 26 May 2023
Accepted: 26 May 2023
Published: 30 June 2023

ABSTRACT

The development of new alternatives strategies to synthetic insecticides aimed at reducing pest populations by developing pesticides based on plant extracts without negative effects in non target organisms and environment. The present study was undertaken in order to assess the insecticidal activity of the crude methanolic extract of the Algerian Asteraceae *Cotula cinerea*, against the larval and the pupal stage of *Culex pipiens* (Diptera: Culicidae). It is also to determine the chemical composition of the used extract, and to understand the mechanism of toxic action of the tested extract. Based on the preliminary tests, five concentrations of the crude methanolic extract of *C. cinerea* (0.62, 1.25, 2.50, 3.75, and 5 mg/mL) were tested for their insecticidal activity according to the protocol recommended by the World Health Organization. The chemical profile of the extract was also obtained by high performance liquid chromatography (HPLC). Histopathological effects and inhibition of acetylcholinesterase activity in treated mosquitoes with LC_{90} were examined to elucidate the mechanism of the toxic effect of the tested extract (48 h post treatment). Eight compounds have been identified by HPLC. That includes four flavonoids (rutin, quercetin, myrcetin and cathechin), three phenolic acids (benzoic acid, vanillic acid, *p*-coumaric acid) and one alkaloid (berberine). *C. cinerea* methanolic extract showed good larvicidal and pupicidal activities with LC_{50} and LC_{90} values of 1.10 and 4.37 mg/mL respectively against pupae, 24h post treatment and 1.26, 2.35 mg/mL respectively against the fourth instar larvae. Data of enzymatic assay performed on LC_{50} and LC_{90} pupae and larvae revealed prominent neurotoxic effects. *C. cinerea* extract reduced the activity of acetylcholinesterase (AChE) enzyme in a concentration dependent manner. Obtained inhibition percentages, 48 h after treatment, were 35.11 ± 7.44 and 51.83 ± 4.04% for pupal stage and 30.98 ± 2.97 % and 48.77 ± 4.72% for the fourth instar larvae for LC_{50} and LC_{90} values respectively. Treated larvae and pupae showed also histopathological damages in the pupal cuticle and larval midgut. The results of this study showed that *C. cinerea* crude methanolic extract could be considered as an eco-friendly alternative for mosquito control.

Keywords: *Cotula cinerea*; *Culex pipiens*; methanolic extract; insecticidal activity; physiopathological effects.

INTRODUCTION

Culex pipiens L.1758 (Diptera: Culicidae) is an important species of mosquito of the family Culicidae. This is the most frequent type of mosquito around the world and it is also a carrier of many diseases such as West Nile fever, lymphatic filariasis, and Japanese encephalitis (Hamama *et al*., 2022; Abutaha *et al*., 2023; Zhu *et al*., 2023). In addition, it has been reported to be the most abundant mosquito species in Algeria (Tine-Djebbar *et al*., 2016; Arroussi *et al*., 2021; Hafsi *et al*., 2021).

Therefore, reducing mosquitoes is a major global public health priority. Chemical control is a practical strategy that is often employed in daily life. The most effective mosquito-controlling substances are already synthetic pesticides. However, extensive usage of these chemicals has caused insecticide resistance in mosquitoes, health problems for human and animals, and environmental damage (Şengül Demirak & Canpolat, 2022). In order to effectively control mosquitoes utilizing biodegradable and target-specific substances, it is necessary to conduct research and innovate new alternative methods.

In various regions of the world, human communities have traditionally employed plant products to control pests and vector insects (Madhiyazhagan *et al*., 2014). They serve as the biochemical factories of nature. Through their structural processes, they biosynthesize a wide range of various natural molecules to prevent insect attacks, including flavonoids, phenolic compounds, terpenes and terpenoids, alkaloids, and coumarins (Croteau *et al*., 2000).

Several plant extracts were effective against a variety of insect species as potential insecticides (El Haddad *et al*., 2018; Al-Solami, 2021; Rodzay & Zuharah, 2021; Mir *et al*., 2022), antifeedants (Acheuk & Dounandji-Mitiche, 2013), oviposition deterrence (Zhao *et al*., 1998), insect growth regulators (Gaur & Kumar, 2020), reduction of fecundity and fertility (Hafsi *et al*., 2022), and suppression of calling behavior (Khan & Saxana, 1986). Recently, the utilization of essential oils and other plant-based products has been viewed as a very promising candidate component in insecticidal formulations, both against economically significant insects and against other arthropod pests or vectors (Benelli *et al.,* 2019; Yaméogo *et al*., 2021).

AChE is known as a target enzyme for insect control chemicals insecticides such as organophospahtes and carbamates, which can block the neurotransmitter acetylcholine at the synaptic cleft (Lopez & Pascual-Villalobos, 2010). Many plant secondary metabolites such as essential oils, alkaloids and extracts from aromatic plants were shown to act as efficient AChE inhibitors in various insect species (Acheuk *et al*., 2022). Understanding of the mechanism of action of plant extracts and their biochemical effects on insects may provide safe strategies of insect's pest control.

Cotula cinerea L. (Asteraceae), also known as *Brocchia cinerea* Del., is a typical Saharan plant that develops in sandy environments in the Saharan desert (Quezel & Santa, 1963). This plant includes a variety of chemical components with medicinal effects, including terpenes, essential oils (Bouziane *et al*., 2013; Kasrati *et al*., 2015; El Abdouni *et al*., 2016), and flavonoids (Dendougui *et al*., 2012). A few publications related to its insecticidal activity have been reported. Only the studies of Markouk *et al*. (2000), Kasrati et al. (2015), Acheuk *et al*. (2020), and Agour *et al*. (2022) described its insecticidal activity. Until now, for the best of our knowledge there is no study that proves the insecticidal effect of this plant on mosquito *C. pipiens*.

In this context, the purpose of this study was to identify the phytochemical component of the crude methanolic extract of *C. cinerea* utilizing high performance liquid chromatography (HPLC). We are also looking to evaluate the bio-insecticidal efficacy of the plant extract on larval and pupal stages of *C. pipiens*, the most abundant and interesting mosquito species in Algeria. It is also important to understand this extract's possible mode of action on acetylcholinesterase (AChE) as an enzymatic biomarker of neurotoxicity and its histopathological effect on the midgut of larvae (4th instar larvae) and the pupal cuticle.

MATERIALS AND METHODS

Plant material

Cotula cinerea was collected from Touggourt region (Algerian Sahara Desert; 31° 56' 57'' North, 5° 19' 30'' East) during the spring season of 2017. Botanical researcher from Algeria's National High College of Agriculture and El Oued University taxonomically identified and validated the plant under voucher number (039_35). Without any direct sunlight, the aerial portion of *C. cinerea* was dried at room temperature for two weeks. After drying, the plant became finely powdered and kept at 4 °C until it was time for extraction.

Insects rearing

The egg rafts were found at the facilities of the University of Boumerdes' faculty of sciences (36° 45' 37.23" N, 3° 28' 20.52" E) in standing water. The rafts were brought to the laboratory, and each one was carefully placed in a plastic tray with around 250 mL of tap water. Until they reached the pupal stage, sugar and biscuits were given daily to mosquito larvae. The condition of the laboratory was maintained at a temperature of 25 to 30 °C with a relative humidity of 80 to 97% and a 12h photoperiod of light and darkness. The taxonomic identification of *Culex pipiens* was made at the National High School of Agronomy (ENSA), Algiers by Professor Baba Aissa.

Crude methanolic extract preparation and phytochemical screening

Exhaustion extraction was carried out according to the modified method of Owen & Johns (1999), by macerating 100 g of plant powder in 400 ml of methanol for 72 hours. After filtration, the residue was extracted a second time with 200 ml of methanol for 48 hours and a third maceration was made with 100 ml of methanol for 24 hours. The maceration was carried out at room temperature (28 °C) with magnetic agitation. The three solutions were then combined and filtered using Whatman No. 1 filter paper. A rotary evaporator was used to evaporate the methanol at 45 °C. The crude methanolic extract was weighed and stored in vials that were tightly sealed with parafilm at 4 °C until it was needed.

The methanolic extract was examined for secondary plant metabolites, alkaloids, flavonoids, coumarins, sugar, phenolic compounds, tannins, iridoids, and saponins. The extract was subjected to a conventional procedure for phytochemical screening (Dohou *et al*., 2003). The presence (+) or absence (-) of certain active compounds was thought to be indicated by a visible color change or precipitate formation.

HPLC analyses

HPLC analyses were carried out according to the modified method of Boumaza *et al*. (2018). An HPLC system (YL 9100 HPLC) was used to separate certain phenolic components from crude methanolic extract. A reversed phase C18 analytical column with a 150 mm length, 4.6 mm diameter, and 5 µm particle sizes was installed in this system (AGILENT C18). 35 °C was the constant column temperature. 20 µl of the sample were injected. Mobile phase B was methanol, whereas mobile phase A was water containing 1% acetic acid. The optimum chromatographic conditions were: 95% (A) and 5% (B) from 0 to 55 min; 5% (A) and 95% (B) from 55 to 56 min; and return to initial conditions from 56 to 60 min. A flux of 1 mL/min was utilized. At 280 nm, the UV VIS detector was calibrated. By comparing them to authentic compounds (standards), the phenolic compounds were identified.

Insecticidal activity

Biological tests were realized according to World Health Organization (2005) protocol, with some modifications. Based on the preliminary tests, the dried crude methanolic extract was used in the bioassay at five different concentrations: 0.62, 1.25, 2.50, 3.75, and 5 mg/mL tap water. Test solutions were prepared using a drop of tween 20 and then water as a solvent. For each experiment, groups of twenty larvae of the 1st generation (one of the four larval stages tested: 1st, 2nd, 3rd, or 4th) or pupae were used in four duplicates. As a control, the same number of larvae or pupae is placed in a control cup containing only tap water and a drop of tween. A correction was applied to the mortality percentage (Abbott, 1925).

Acetylcholinesterase activity

Enzymatic tests were performed on larval and pupal stage samples, taken from the control and LC_{50} and LC_{90}-treated groups 48 h after treatment. In a buffer solution of cell lysis (0.25 g lauroylsarcosine, 23.6 g guanidine-HSCN, and 0.37 g citric acid in 50 mL of water RNase-free, the pH was adjusted to 7 with NaOH (1 M)), larval or pupal stage (pool of 5 larvae or pupae) were homogenized. At 4 °C and 9000 g for 5 minutes, the homogenates were centrifuged. An enzymatic assay was conducted using the resultant supernatant.

The approach reported by Rhee *et al*. (2001), based on Ellman's method (Ellman *et al*., 1961), was used to quantify AChE inhibitory activity using a quantitative colorimetric test employing a 96-well microplate reader. Acetylthiocholine is the substrate that the enzyme hydrolyzes, releasing thiocholine as product. Thiocholine then interacts with 5,52 -dithio-bis [2-nitrobenzoic acid] (DTNB) to create 2-nitrobenzoic-5-mercaptothiocholine and 5-thio-2-nitrobenzoate, which can be detected at 412 nm. In this procedure, the total reaction volume is 200 µL consisting of 160 µL (0.1 mol/L) sodium

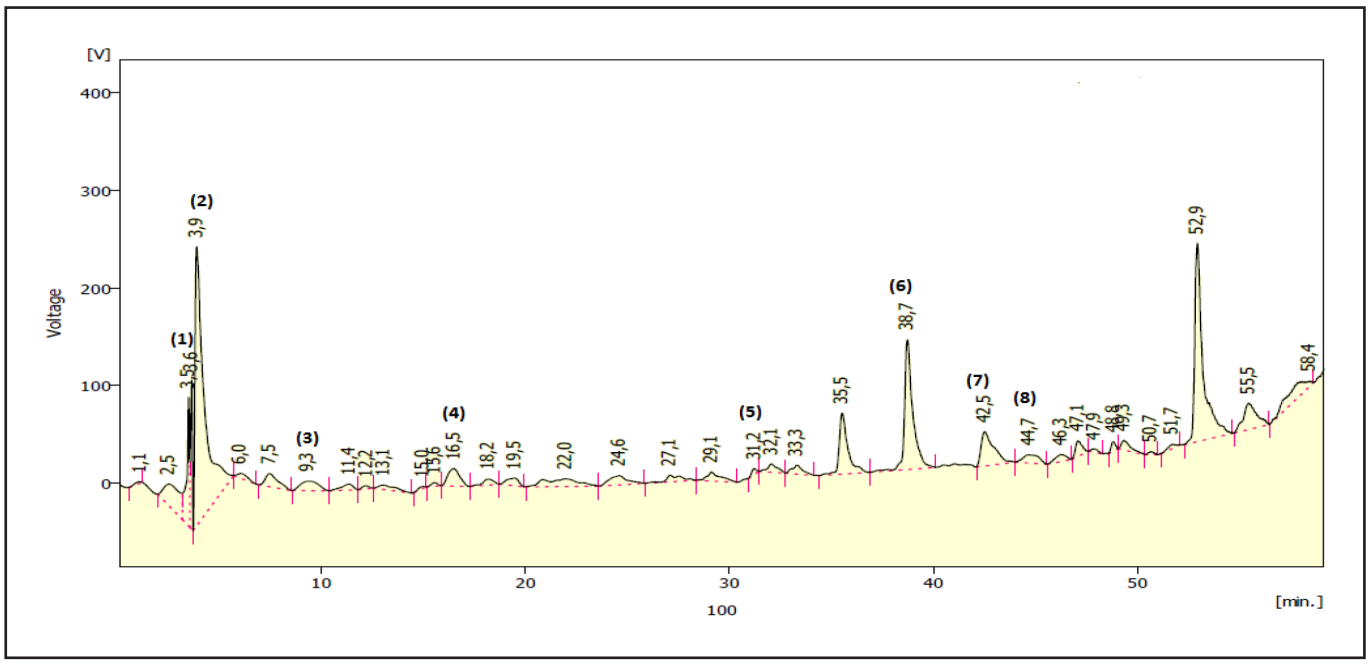

Figure 1. HPLC chromatogram profile of the crude methanolic extract of the aerial part of *C. cinerea*. The numbers presented in the chromatogram correspond to the compounds identified in Table 1.

phosphate buffer (pH 8.0), 20 µL of AChE (the supernatant), 10 µL DTNB (0.03 mmol/L), and 10 µL of acetylthiocholine iodide (a final concentration of 0.68 mmol/L).

As a positive control, galantamine hydrobromide was utilized (reference compound). The percentage of inhibition was based on the following formula:

$$\text{Inhibition (\%)} = (E - S/E) \times 100$$

E is the activity of the enzyme in control larvae or pupae without extract, and S is the activity of the enzyme in treated larvae or pupae with extract.

Histopathological studies

The midgut and cuticle of the pupal stage of *C. pipiens* treated with LC_{90} (48 h post treatment) and control were histologically evaluated with the method described by Martoja and Martoja Pierson (1967) and slightly modified. The formalin solution (10%) was applied immediately to fix the treated and control samples. To dehydrate them, gradually increasing alcohol baths were used. Following an embedding bath, samples were subsequently submerged in blocks of paraffin. A rotating microtome was used for cutting blocks. Haemotoxylin and eosin were used to stain the resulting sections (3 mm), which were then placed on glass slides. Moreover, a stereomicroscope was used to investigate the impact of *C. cinerea* extract on pupal (cuticle) and larval (midgut) tissues.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) 26 is used to analyze the variance (ANOVA) of the results, which are presented as means standard deviation (SD). Tukey's HSD test ($P < 0.05$) was used to compare the means. SPSS 26 was also employed to determine the LC_{50} and LC_{90}, as well as the 95% upper and lower confidence limits, slope, and Chi-square values.

RESULTS

Extraction, yield and phytochemical screening

The yield of extraction of the crude methanolic extract of areal part of *C. cinerea* was 12.42 ± 4.64 % (dry matter of the plant). A phytochemical study of *C. cinerea* showed the strong presence of glycosides, tannins, alkaloids, and flavonoids. A low presence of coumarins was noted. However, saponins, anthocyanins, and iridoids were not detected.

Table 1. Chemical composition of the crude methanolic extract of the aerial part of *C. cinerea*

Peak	Ret time (min)	Area (%)	Height (%)	Compounds
(1)	3.53	0.3	2.7	Cathechin
(2)	3.90	3.1	5.9	Berberine
(3)	9.30	1.5	1.2	Benzoic acid
(4)	16.5	1.6	1.7	P-coumaric acid
(5)	32.1	2.0	2.3	Rutin
(6)	38.7	6.4	5.1	Quercetin
(7)	42.5	7.6	3.3	Vanillic acid
(8)	44.77	3.1	2.8	Myrcetin

HPLC analyses

The chemical profile of the obtained chromatogram (Figure 1) showed the presence of eight identified compounds (Table 1), including flavonoids, phenolic acids, and alkaloids.

Four flavonoids were identified in the aerial part of *C. cinerea*. These compounds were: quercetin (peak 6) as the major compound, myrcetin (peak 8), cathechin (peak 1), and rutin (peak 5). The phenolic acids were: vanillic acid (peak 7) as the major compound, p-Coumaric acid (peak 4), and benzoic acid (peak 3). Only one alkaloid (berberine) was identified (peak 2).

Insecticidal activity

Figure 2 show the results of *C. cinerea*'s crude methanolic extract's insecticidal activity against *C. pipiens* larvae (1st, 2nd, 3rd, and 4th instars) and pupae. *C. cinerea* extract had significant larvicidal and pupicidal effects on *C. pipiens* ($P< 0.05$). The sensitivity of the larval and pupal stages to the tested extract was dose and time dependent. For the larval stage, the two highest concentrations used (3.75 and 5 mg/mL) had strong and early effects on all instar larvae. After 24

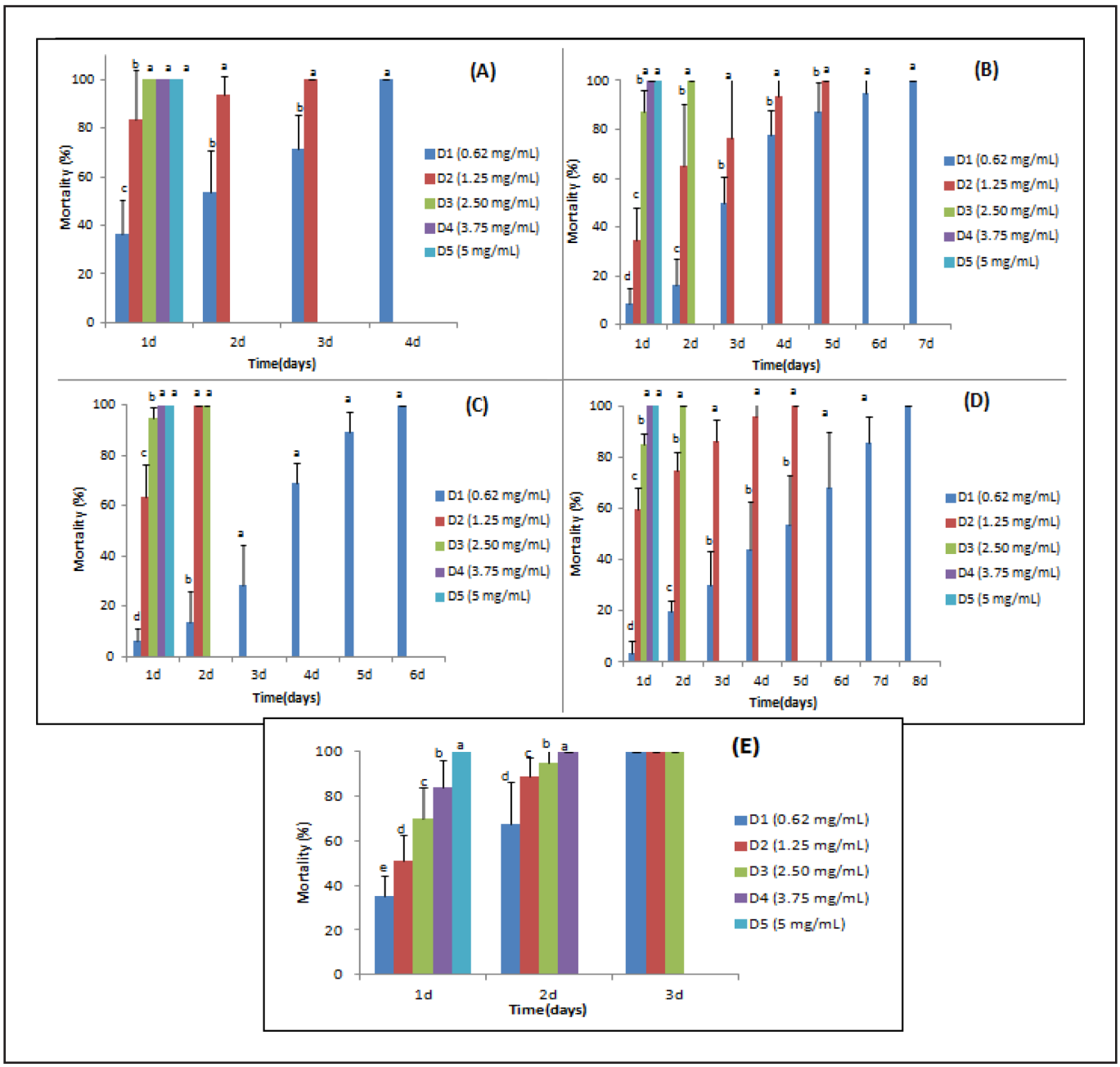

Figure 2. Insecticidal activity of the crude methanolic extract of *C. cinerea* against the larval and the pupal stages of *C. pipiens* (Mean ± SD). N = 20 insects/replicate. Values followed by the same letter are not significantly different at $P < 0.05$ according to Tukey's test. A: 1st instar larvae, B: 2nd instar larvae, C: 3rd instar larvae, D: 4th instar larvae, E: pupae.

hours of treatment, total mortality for all larval stages was shown (Figures 2A, 2B, 2C and 2D). The first, second, third, and fourth instar larvae, respectively, reported 100% mortality after four, seven, six, and eight days of treatment at the lowest dose (0.62 mg/mL) (Figures 2A, 2B, 2C and 2D).

For the pupal stage, the highest dose (5 mg/mL) generated 100% of deaths after 24 h after exposure, while the low dose (0.62 mg/mL) revealed 100% of deaths 72 h after treatment (Figure 2E). In the 24 hours following treatment, the mortality rates are 35 ± 9.12, 51.25 ± 11.08, 70 ± 14.14, and 83.75 ± 12.50%, respectively, for the doses of 0.62, 1.25, 2.5, and 3.75 mg/mL. Total mortality (100%) was achieved 48 h after treatment for the concentration of 3.75 mg/mL and 72 h after treatment for the concentrations of 0.62, 1.25, and 2.50 mg/mL. Mortality was not observed in the control group.

In Table 2, the tested extract showed the lowest LC_{50} and LC_{90} against the 1st instar larvae. However, pupae (after 24 h exposure) were less susceptible, with the highest LC_{90} value compared to the other instar larvae.

Acetylcholinesterase activity

The results for AChE were represented as percentages of inhibitory activity at a concentration of 200 µg/mL for the treated larvae and pupae with LC_{50} and LC_{90} 48 h after treatment, in Figure 3 (a and b). Results revealed that *C. cinerea* extract reduced considerably ($p < 0.05$) the activity of AChE in both of the larval and pupal stages. AChE inhibition was 30.98 ± 2.97 and 48.77 ± 4.72% for larvae and 35.11 ± 7.44 and 51.83 ± 4.04% for pupae, respectively, for the LC_{50} and LC_{90}, two days after treatment. Galanthamine (the standard compound) showed a high inhibitory activity of AchE (94.8 ± 0.3%).

Table 2. LC_{50} and LC_{90} values of the crude methanolic extract of *C. cinerea* tested against the larval and pupal stage of *C. pipiens*, 24 h after treatment

Insect stage	LC_{50}(LCL-UCL) (mg/ml)	LC_{90}(LCL-UCL)(mg/ml)	Slope	Intercept	X^2	Df
L1	0.75 (0.67–0.82)	1.38 (1.23–1.62)	4.44	0.55	1.02	3
L2	1.38 (1.26–1.49)	2.57 (2.32–2.92)	4.16	0.6	7.19	3
L3	1.13 (1.04–1.22)	1.94 (1.75–2.21)	5.28	0.36	2.81	3
L4	1.26 (0.83–1.73)	2.35 (1.72–4.94)	4.68	0.62	14.78	3
Pupae	1.10 (0.32–1.80)	4.37 (2.51–35.83)	1.72	0.08	14.89	3

LC_{50} lethal concentration that kills 50% of the exposed larvae or pupae, LC_{90} lethal concentration that kills 90% of the exposed larvae or pupae, LCL lower confidence limit, UCL upper confidence limit (95% fiducial limit), X^2 chi-square, df degrees of freedom.

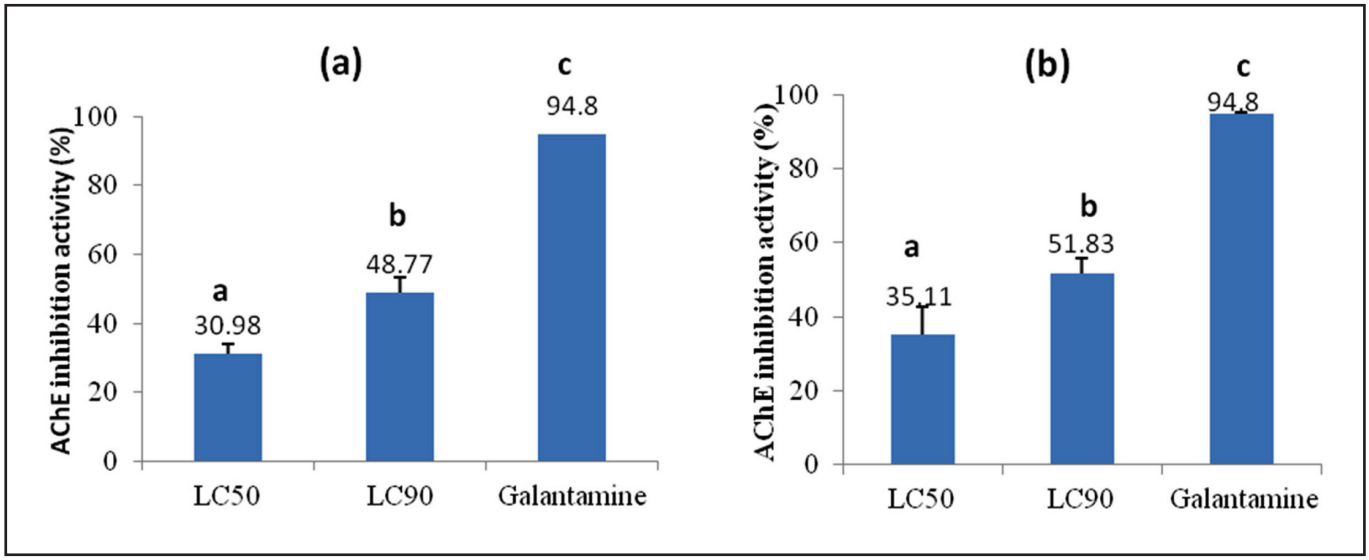

Figure 3. Effect of LC_{50} and the LC_{90} of crude methanolic extract of *Cotula cinerea* on AChE activity of fourth instar larvae and pupal stage of *Culex pipiens* 48h after treatment (Mean ± SD). Different letters denote significant differences (Tukey's test, $p < 0.05$). (a): 4th instar larvae, and LC_{50}=0.90 mg/mL; LC_{90}=1.55 mg/mL, (b): pupal stage and LC_{50}= 0.41 mg/mL; LC_{90}= 1.36 mg/mL.

Histopathological effect

The histopathological impact of *C. cinerea* methanolic extract (Figures 4a and 4b) revealed serious alterations, deformation, and massive disintegration of tissues and bodies of *C. pipiens* pupae treated with LC_{90} (48 h post treatment). The cuticle of treated pupae showed a degeneration of tegument (tgd) and muscular fibers (mfd), as well as nerve ganglion necrosis (ngn).

In the untreated pupae (Figures 4c and 4d), the mosquito's body and cuticle structure were normal, and the cuticle was totally distinct into layers called the epicuticle and procuticle.

The midgut appeared severely damaged by the crude methanolic extract of *C. cinerea*. Epithelial cell disorganization and destruction (ECd), and basal lamina cell detachment (BLd) were observed in treated larvae. Cuts and separations of cells have been observed in some parts. The brush border was completely disorganized and altered (Bbd) in some regions (Figure 5a). In contrast, the midgut epithelium of control larvae displayed flattened regular cells, regular microvilli along the apical surface, and was tightly linked to the basal lamina (Figure 5b).

DISCUSSION

In the present investigation, *C. cinerea* extract was revealed to be potentially harmful to *C. pipiens* larvae and pupae. The presence of several bioactive substances in the crude methanolic extracts, including flavonoids, phenolic acids, and alkaloids, may be the cause of this toxicity. When using complex combinations of bioactive components, such as essential oils or plant extracts comprising terpenes and fatty acids in addition to alkaloids, synergistic effects (also known as "entourage effects") may arise (Acheuk *et al*., 2022). Very certainly, these substances primarily affect specific biological and physiological features of insects (Edriss *et al*., 2013). A similar study was conducted in Morocco against *Anopheles labranchiae* by Markouk *et al*. (2000). The authors demonstrated that a concentration of 1 000 ppm of *C. cinerea* ethyl ether and ethyl acetate extracts was required to generate larvicidal activity, with LC_{50} values of 310 and 325 ppm, respectively. The n-butanolic and chloroformic extracts at the same concentration did not exhibit any activity. Similar to this study, Agour *et al*. (2022) demonstrated that *C. cinerea* essential oil has strong insecticidal activity and moderate repellency against *Callosobruchus maculatus*. The LC_{50} value in the contact test was 0.61 µL/L air and was therefore lower than in the inhalation test (0.72 µL/L of air). *Foeniculum vulgare* and *Matricaria chamomilla* combined hexane extract showed larvicidal activity on *C. pipiens*, with LC_{50} and LC_{90} values of 148.3 and 242.17 mg/mL, respectively, after 24 h of exposure (Al-Mekhlafi *et al*., 2021).

Al-Mekhlafi *et al*. (2017) reported that *Xanthium strumarium* (Asteraceae) shown a harmful impact on larvae of *C. pipiens* with an LC_{50} of 502.32 µg/mL, and a LC_{90} of 867.63 µg/mL. The extract also had remarkable pupicidal toxicity for *C. quinquefasciatus*, with LC_{50} value of 39.21ppm and LC_{90} value of 49.12, ppm.

Phytochemical investigation of *C. cinerea* showed the existence of a number of bioactive substances. It is rich in glycosides, flavonoids, tannins, and alkaloids, which have been shown to have

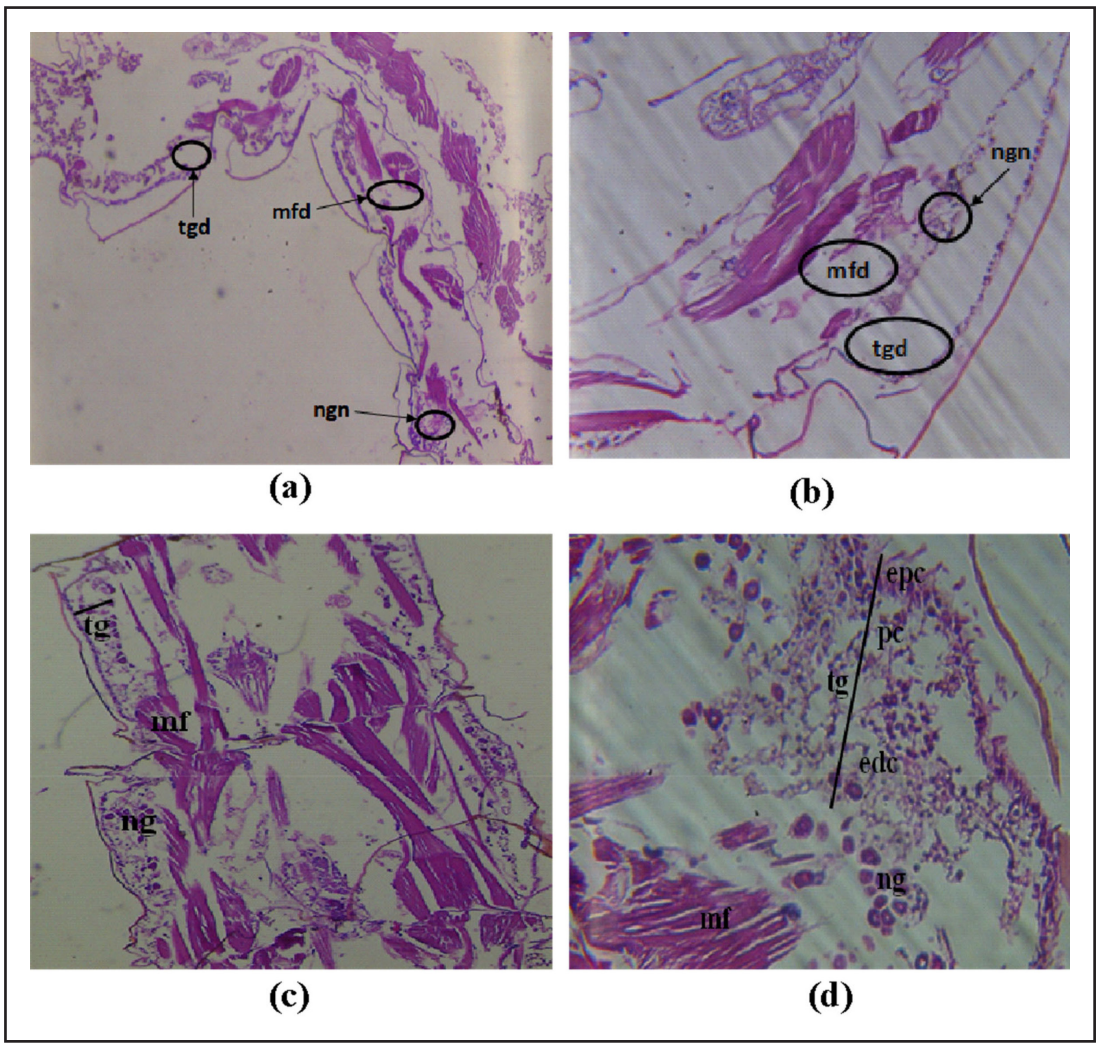

Figure 4. Longitudinal section of abdominal cuticle of *Culex pipiens* nymphs treated (a-b) and control (c-d) with methanolic extract of *C. cinerea* at concentration of LC_{90}. 40' (a-c) and 100' (b-d), epicuticle (epc), procuticle (prc), tegument (tg), tegument degenerating (tgd), muscular fibers (mf), muscular fibers degenerating (mfd), nerve ganglion (ng), nerve ganglion necrosis (ngn).

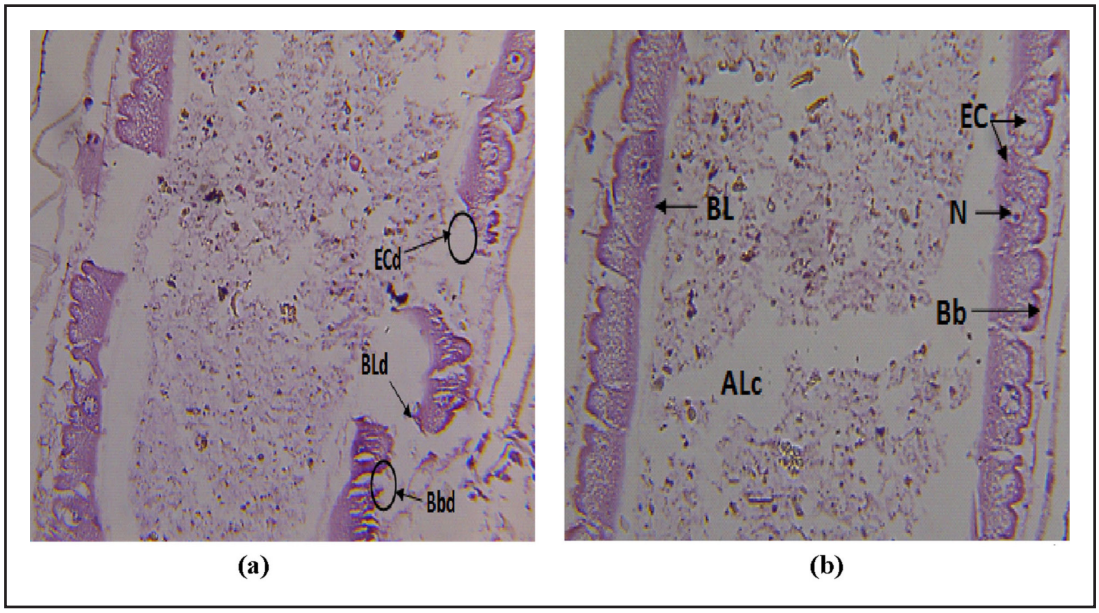

Figure 5. Longitudinal section of midgut of fourth instar larvae of *Culex pipiens*. (40X). (a) The midgut of treated larvae with crude methanolic extract of *C. cinerea* at concentration of LC_{90}. (b) the midgut of control larvae. Nucleus (N), Brush border (Bb), Brush border disorganization (Bbd), Epithelial Cell (EC), Epithelial Cell disorganization (ECd), Basal lamina (BL), Basal Lamina detachment (BLd), Alimentary canal (ALc).

biological effects. A previous study was carried out on *C. cinerea*, which was harvested in the Bechar area of southwest Algeria. It showed the important presence of tanins, terpenes, flavonoids, heterosids, glycosidic flavonoids, steroids, and the average presence of cardenolids, free flavonoids, saponins, and the weak presence of alkaloids (Djellouli *et al*., 2013).

From HPLC analysis of the crude methanolic extract of *C. cinerea*, eight compounds have been identified: rutin, quercetin, myrcetin, cathechin, p-Coumaric acid, benzoic acid, vanillic acid, and berberine. For this plant, Ahmed *et al*. (1987) noted the presence of many flavonoids, including 7-O-β-D-glucoside of luteolin, 7-O-β-D-diglucoside and luteolin itself, as well as apigenin-7-O-α-Lrhamnoside. The ethanolic extract of *C. cinerea* collected in Merrara near Touggourt (Southern Algeria) was found to contain germacranolide, tatridin A, and seventeen flavonoid derivatives, including quercetin glycosides, apigenin, and luteolin (Dendougui *et al*., 2012). Khallouki *et al*. (2015) found one flavonoid (luteolin-4'-O-glucoside) and two phenolic acids (derivatives of chlorogenic acid and dicaffeoylquinic acid) in Moroccan *C. cinerea*. The difference in chemical composition results might potentially be caused by several factors, including the period at which the plant was harvested, extraction techniques, geographic distribution, plant parts utilized, environmental conditions, and genetic factors (El Jemli *et al*., 2017).

Plant extract is a complicated combination comprising hundreds of bioactive molecules, some of which are recognized to possess the strongest biological effects and often act on insects in diverse manners (Pavela *et al*., 2019). Many naturally occurring plant compounds used to manage insect pests have an impact on the enzymatic profiles (Zhang *et al*., 2013). The results of our study demonstrated that the crude methanolic extract of *C. cinerea* inhibited considerably ($P < 0.05$) the enzymatic activity of AChE on treated pupal stage and fourth instar larvae. It's known that AChE regulates nerve impulse transmission across cholinergic synapses. Inhibition of AChE leads to a concentration of acethylcholine at the synaptic, which induces insect paralyze mortality. Thus, the results of our study suggest that the insecticidal activity noted for *C. cinerea* could be related to the extract's capacity to block AChE. This inhibition could result from one of the compounds or from the synergistic action of several compounds in the extract. The presence of alkaloids in the extract (berberine) could be the cause of this inhibition. Several plant extracts have been found to have their principal mechanism of action in insects through the inhibition of AchE (Carreɒo Otero *et al.,* 2018; Sandhanam *et al.,* 2018).

In a similar study, Shahat *et al*. (2020) showed that extracts of *Otostegia fruticose*, *Origanum syriacum*, *Senna italica*, and *Pergularia tomentosa* significantly inhibited AchE activity of third instar larvae of *C. pipiens*. Our results are also in agreement with those of Farag *et al*. (2021), who reported that pomegranate peel petroleum ether extract demonstrated the greatest level of inhibition against AChE recovered from *C. pipiens*. Khaldi *et al*. (2022) demanstrated that extracts of *Melia azedarach L.* decreased AChE activity of fourth instar larvae of *C. pipiens.*

The impact of intoxication with this plant extract also manifests through aberration (necrosis of epidermal cells) and histopathological changes in the cuticle of the pupal stage and in the midgut of the fourth larvae stage. The histopathological modifications in treated insects with botanical insecticides were previously investigated (Bakkali *et al.,* 2008; Sandhanam *et al*., 2019). The cuticle of the pupal stage was disorganized by the *C. cinerea* extract and did not appear to be separated between an epicuticle and a procuticle layer. Necrosis of epidermal cells has also been detected, indicating the direct toxicity of this extract.

Simple diffusion allows toxic chemicals to penetrate the skin's membrane pupae. These substances subsequently destroy skin cells, reducing the impermeability of the skin barrier and permitting other poisonous substances to infiltrate the pupae's body. Additionally, toxic substances harm the skin's membrane proteins, impairing the skin's ability to protect the body (Lu & Kacew*,* 2002). Larvae of *C. pipiens* treated with LC_{50} of pomegranate peel petroleum ether extract showed great damage in cuticle structure and muscles (Farag *et al*., 2021). Many alterations and malformations have been shown by Azmy *et al*. (2021) on the body and tissues of the third larval stage of *C. pipiens* exposed to *Citrus sinensis* essential oil, which mostly damaged the muscles, tissues of the midgut, and cuticle. *C. pipiens* larvae treated with *Zizyphus jujube* oil revealed cuticle, gut, and muscles damages (El Husseiny *et al*., 2014).

Spectacular degenerative reactions in the midgut to *C. cinerea* methanolic extract have also been shown through epithelial destruction and alterations of epithelial cells. The area of digestion and absorption in mosquito larvae is thought to be in the midgut. It is recognized that the midgut epithelium performs a variety of processes, including ionic and osmotic control, lipid and carbohydrate accumulation, pH modulation of the midgut fluid, release of digestive enzymes, and nutrient absorption (Chalom *et al*., 2019). Our results agree with those of Al-Mekhlafi (2018), who found that the midgut epithelium of *C. pipiens* larvae exhibited to *Carum copticum* extract showed cell deterioration and separation. Liu *et al*. (2020) demonstrated that ar-turmerone extracted from *Curcuma longa* L. induced disorder of myofibrils in abdominal muscle cells, atrophy of nucleolus in the malpighian tubule cells, and brush border detachment in midgut epithelial cells on fourth instar larvae of *C. pipiens.* Similar results have been observed in several previous researches utilizing *Foeniculum vulgare* and *Matricaria chamomilla* combined extract on *C. pipiens* (Al-Mekhalafi *et al*., 2021) and *Melia azedarach* extracts on *C. quinquefasciatus* (Al-Mehmadi & Al-Khalaf, 2010).

CONCLUSION

In conclusion, these results showed that *C. cinerea* crude methanolic extract possesses potent insecticidal activity on larval and pupal stages of *C. pipiens*. The biological effect of this plant may result from the particular combination of its various components, such as flavonoids, phenolic acids, and alkaloids, which may act synergistically or separately to cause the death of insects by contact toxicity. Moreover, the crude methanolic extract of *C. cinerea* exhibited neurotoxic effect as an inhibitor of acetylcholinesterase activity. The extract also caused alterations in the cuticle pupae and the midgut of larvae. This plant could be an interesting candidate for botanical insecticide formulations. However, more studies are still required to characterize and appreciate the additional modes of action of this extract. Evaluation of the bio-insecticidal effect of the phytochemical components of the plant extract identified by high performance liquid chromatography and further testing should be performed against *C. pipiens* and other important mosquito vector species.

Conflict of Interest

The author declares that they have no conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to the Algerian research centre of Biotechnology (CRBt) for facilities. We thank Mr. Taoudiate Mohamed and Mrs. Bounadi Leila for manuscript's English review.

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**: 265-267. https://doi.org/10.1093/jee/18.2.265a

Abutaha, N., Al-Mekhlafi, F., Wadaan, M.A., Rady, A.M., Baabbad, A.A.A. & AL-Khalifa, M.S. (2023). The molecular interplay of known phytochemicals as *Culex pipiens* and Rift Valley fever virus inhibitors through molecular docking. *Saudi Journal of Biological Sciences* **30**: 103611. https://doi.org/10.1016/j.sjbs.2023.10361

Acheuk, F., Abdellaoui, K., Lakhdari, W., Chahbar, N., Dehliz, A., Belaid, M., Baouche, N. & Bouazouz, H. (2020). Potentials of the extracts of Algerian Saharan plant *Cotula cinerea* for the management of two insect pests, *Aphis fabae* and *Tribolium castaneum*. *Tunisian Journal of Plant Protection* **15**: 41-57. https://doi.org/10.52543/tjpp.15.2.2

Acheuk, F., Basiouni, S., Shehata, A.A., Dick, K., Hajri, H., Lasram, S., Yilmaz, M., Emekci, M., Tsiamis, G., Spona-Friedl, M. *et al.* (2022). Status and prospects of botanical biopesticides in Europe and Mediterranean Countries. *Biomolecules* **12**: 311. https://doi.org/10.3390/biom12020311

Acheuk, F. & Doumandji-Mitiche, B. (2013). Insecticidal activity of alkaloids extract of *Pergularia tomentosa* (Asclepiadaceae) against fifth instar larvae of *Locusta migratoria cinerascens* (Fabricius 1781) (Orthoptera: Acrididae). *International Journal of Science and Advanced Technology* **3**: 8-13.

Agour, A., Mssillou, I., Mechchate, H., Es-safi, I., Allali, A., El Barnossi, A., Al Kamaly, O., Zuhair Alshawwa, S., El Moussaoui, A., Bari, A. *et al*. (2022). *Brocchia cinerea* (Delile) Vis. Essential Oil Antimicrobial Activity and Crop Protection against Cowpea Weevil *Callosobruchus maculatus* (Fab.). *Plants* **11**: 583. https://doi.org/10.3390/plants11050583

Ahmed, A.A., El-Sayed, N.H., El-Negoumy, S.I. & Mabry, T.J. (1987). Flavonoids of *Cotula cinerea. Journal Natural Product* **50**: 519-520. https://doi.org/10.1021/np50051a037

Al-Mehmadi, R.M. & Al-Khalaf, A.A. (2010). Larvicidal and histological effects of *Melia azedarach* extract on *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). *Journal of King Saud University (Science)* **22**: 77-85. https://doi.org/10.1016/j.jksus.2010.02.004

Al-Mekhlafi, F.A. (2018). Larvicidal, ovicidal activities and histopathological alterations induced by *Carum copticum* (Apiaceae) extract against *Culex pipiens* (Diptera: Culicidae). *Saudi Journal of Biological Sciences* **25**: 52-56. https://doi.org/10.1016/j.sjbs.2017.02.010

Al-Mekhlafi, F.A., Abutaha, N., Al-Doaiss, A.A., Al- Keridis, L.A., Alsayadi, A.I., El Hadi Mohamed, R.A., Wadaan, M.A., Ibrahim, K.E. & Al-Khalifa, M.S. (2021). Target and non-target effects of *Foeniculum vulgare* and *Matricaria chamomilla* combined extract on *Culex pipiens* mosquitoes. *Saudi Journal of Biological Sciences* **28**: 5773-5780. https://doi.org/10.1016/j.sjbs.2021.06.024

Al-Mekhlafi, F.A., Abutaha, N., Mashaly, A.M.A., Nasr, F.A., Ibrahim, K.E. & Al-Wadaan, M. (2017). Biological activity of *Xanthium strumarium* seed extracts on different cancer cell lines and *Aedes caspius*, *Culex pipiens* (Diptera: Culicidae). *Saudi Journal of Biological Sciences* **24**: 817-821. https://doi.org/10.1016/j.sjbs.2016.07.003

Al-Solami, H.M. (2021). Larvicidal activity of plant extracts by inhibition of detoxification enzymes in *Culex pipiens*. *Journal of King Saud University (Science)* **33**: 101371. https://doi.org/10.1016/j.jksus.2021.101371

Arroussi, D.E.R., Bouaziz, A. & Boudjelida, H. (2021). Mosquito survey reveals the first record of *Aedes* (Diptera: Culicidae) species in urban area, Annaba district, Northeastern Algeria. *Polish Journal of Entomology* **90**: 14-26. https://doi.org/10.5604/01.3001.0014.8065

Azmy, R.M., El Gohary, E., Salem, D.A.M., Abdou, M.A., Salama, M.S. & Mahmoud, D.M. (2021). Biochemical and histopathological effect of the essential oil of *Citrus sinensis* (L.) Osbeck on larvae of *Culex pipiens* Linnaeus, 1758 (Diptera: Culicidae). *Aquatic Insects* **42**: 78-90. https://doi.org/10.1080/01650424.2020.1871025

Bakkali, F., Avebeck, S., Averbeck, D. & Idaomar, M. (2008). Biological effects of essential oils- a review. *Food and Chemical Toxicology* **46**: 446-475. https://doi.org/10.1016/j.fct.2007.09.106

Benelli, G., Pavela, R., Maggi, F., Nkuimi Wandjou, J.G., Yvette Fofie, N.B., Koné-Bamba, D., Sagratini, G., Vittori, S. & Caprioli, G. (2019). Insecticidal activity of the essential oil and polar extracts from *Ocimum gratissimum* grown in Ivory Coast: Efficacy on insect pests and vectors and impact on non-target species. *Industrial Crops & Products* **132**: 377-385. https://doi.org/10.1016/j.indcrop.2019.02.047

Boumaza, S., Bouchenak, O., Yahiaoui, K., Toubal, S., EL Haddad, D. & Arab, K. (2018). Effect of the aqueous extract of *Euphorbia guyoniana* (Euphorbiaceae) on pathogenic bacteria from land-based sources. *Applied Ecology and Environmental Research* **16**: 3767-3781. https://doi.org/10.15666/aeer/1604_37673781

Bouziane, M., Badjah-Hadj-Ahmed, Y. & Hadj-Mahammed, M. (2013). Chemical composition of the essential oil of *Brocchia cinerea* grown in southeastern of Algeria. *Asian Journal of Chemistry* **25**: 3917-3921.

Carreño Otero, A.L., Palacio-Cortés, A.M., Navarro-Silva, M.A., Kouznetsov, V.V. & Duque, L.J.E. (2018). Behavior of detoxifying enzymes of *Aedes aegypti* exposed to girgensohnine alkaloid analog and *Cymbopogon flexuosus* essential oil. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* **204**: 14-25. https://doi.org/10.1016/j.cbpc.2017.11.002

Chalom, S., Jumpatong, K., Wangkarn, S., Chantara, S., Phalaraksh, C., Dheeranupattana, S., Suwankerd, W., Pyne, S.G. & Mungkornasawakul, P. (2019). Utilization of electrocoagulation for the isolation of alkaloids from the aerial parts of *Stemona aphylla* and their mosquitocidal activities against *Aedes aegypti*. *Ecotoxicology and Environmental Safety* **182**: 109448. https://doi.org/10.1016/j.ecoenv.2019.109448

Croteau, R., Kutchan, T.M. & Lewis, N.G. (2000). Natural products (secondary metabolites). In: Buchanan, B., Gruissem, W. & Jones, R., (Eds.), Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, MD, pp. 1250-1318.

Dendougui, H., Seghir, S., Jay, M., Benayache, F. & Benayache, S. (2012). Flavonoids from *Cotula cinerea* Del. *International Journal of Medicinal and Aromatic Plants* **2**: 589-595.

Djellouli, M., Moussaoui, A., Benmehdi, H., Ziane, L., Belabbes, A., Badraoui, M., Slimani, N. & Hamidi, N. (2013). Ethnopharmacological study and phytochemical Screening of three plants (asteraceae family) from the region of south west Algeria. *Asian Journal of Natural and Applied Sciences* **2**: 59-65.

Dohou, W., Yamni, K., Tahrouch, S., Idrissi Hassani, L.M., Bado, A. & Guira, N. (2003). Screening phytochimiques d'une endemique ibére-macrowave, *Thymelaea lythroides*. *Bulletin de la Société de Pharmacie de Bordeaux* **142**: 61-78. https://doi.org/10.24310/abm.v29i0.7221

Edriss, A.E., Satti, A.A. & Alabjar, Z.A. (2013). Larvicidal properties of two asclepiadaceous plant species against the mosquito *Anopheles arabiensis* Patton (Diptera: Culicidae*). Journal of the Saudi Society of Agricultural Sciences* **12**: 59-66. https://doi.org/10.1016/j.jssas.2012.06.004

El Abdouni, K.M., Jamali, C.A., Kasrati, A., Hassani, L., Leach, D., Markouk, M. & Abbad, A. (2016). Antibacterial activity of essential oils of some Moroccan aromatic herbs against selected food-related bacteria. *Journal of Essential Oil Bearing Plants* **19**: 1075-1085. https://doi.org/10.1080/0972060X.2015.1004123

El Haddad, D., Bitam, I., Bouchenak, O., Toubal, S., Yahiaoui, K., Arab, K. & Boumaza, S. (2018). Acaricidal activity of flavonoids extract of *Borago officinalis* L. (Boraginaceae) against brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806). *Tropical Biomedicine* **35**: 383-391.

El Jemli, M., Kamal, R., Marmouzi, I., Doukkali, Z., Bouidida, E., Touati, D., Nejjari, R., El Guessabi, L., Cherrah, Y. & Alaoui, K. (2017). Chemical composition, acute toxicity, antioxidant and anti-inflammatory activities of Moroccan *Tetraclinis articulata* L. *Journal of Traditional and Complementary Medicine* **7**: 281-287. https://doi.org/10.1016/j.jtcme.2016.06.006

El Husseiny, I.M., El Kholy, S.E. & Othman, A.A. (2014). Laboratory testing of the toxicity of jujube (*Zizyphus jujuba*) oil and leaf extracts against *Culex pipiens* (Diptera: Culicidae). *African Entomology* **22**: 755-761. https://doi.org/10.4001/003.022.0411

Ellman, G.L., Courtney, D., Andres, J.R.V. & Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* **7**: 88-95. https://doi.org/10.1016/0006-2952(61)90145-9

Farag, S.M., Hussein, M.A., Hafez, S.E., Khaled, A.S., Kamel, O.M. & Zyaan, O.H. (2021). Larvicidal, biological, and histopathological alterations induced by pomegranate peel extract, *Punica granatum* against *Culex pipiens* L. (Diptera: Culicidae). *Egyptian Journal of Aquatic Biology & Fisheries* **25**: 139-161.

Gaur, S.K. & Kumar, K. (2020). Toxicity and insect growth regulatory activities of medicinal plant, *Withania somnifera*, in flesh fly, *Sarcophaga ruficornis* (Diptera: Sarcophagidae). *Journal of Basic and Applied Zoology* **81**: 30. https://doi.org/10.1186/s41936-020-00154-2

Hafsi, N.E.H., Hamaidia, K., Barour, C. & Soltani, N. (2021). Asurvey of Culicidae (Insecta: Diptera) in some habitats in Souk-Ahras province (Northeast Algeria). *Biodiversity Journal* **12**: 3-16. https://doi.org/10.31396/Biodiv.Jour.2021.12.1.3.16

Hafsi, N.E.H., Hamaidia, K. & Soltani, N. (2022). Chemical screening, insecticidal and reprotoxic activities of *Tecoma stans* ethanolic leaf extract against the vector mosquito *Culex pipiens*. *Physiological Entomology* **47**: 176-187. https://doi.org/10.1111/phen.12386

Hamama, H.M., Zyaan, O.H., Ali, O.A.A., Saleh, D.I., El-Akkad, H.A., El-Saadony, M.T. & Farag, S.M. (2021). Virulence of entomopathogenic fungi against *Culex pipiens*: impact on biomolecules availability and life table parameters. *Saudi Journal of Biological Sciences* **29**: 385-393. https://doi.org/10.1016/j.sjbs.2021.08.103

Kasrati, A., Alaoui Jamali, C., Bekkouche, K., Wohlmuth, H., Leach, D. & Abbad, A. (2015). Comparative evaluation of antioxidant and insecticidal properties of essential oils from five Moroccan aromatic herbs. *Journal of Food Science Technology* **52**: 2312-2319. https://doi.org/10.1007/s13197-014-1284-z

Khaldi, R., Rehimi, N., Kharoubi, R. & Soltani, N. (2022). Phytochemical composition of almond oil from *Melia azedarach* L. and its larvicidal, ovicidal, repellent and enzyme activities in *Culex pipiens* L. *Tropical Biomedicine* **39**: 531-538. https://doi.org/10.47665/tb.39.4.008

Khallouki, F., Sellam, K., Koyun, R., Ricarte, I., Alem, C., Elrhaffari, L. & Owen, R.W. (2015). Phytoconstituents and in vitro Evaluation of Antioxidant Capacities of *Cotula Cinerea* (Morocco) Methanol Extracts. *Records of Natural Products* **9**: 572-575.

Khan, Z.R. & Saxana, R.C. (1986). Effect of steam distillate extracts of resistant and susceptible rice cultivars on behaviour of *Sogatella furcifera* (Homoptera: Delphacidae). *Journal of Economic Entomology* **79**: 928-935. https://doi.org/10.1093/jee/79.4.928

Lopez, M.D. & Pascual-Villalobos, M.J. (2010). Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. *Industrial Crops and Products* **31**: 284-288. https://doi.org/10.1016/j.indcrop.2009.11.005

Liu, J., Fernandez, D., Gao, Y., Silvie, P., Gao, Y. & Dai, G. (2020). Enzymology, Histological and Ultrastructural Effects of Ar-Turmerone on *Culex pipiens* pallens Larvae. *Insects* **11**: 336. https://doi.org/10.3390/insects11060336

Lu, F.C. & Kacew, S. (2002). Lu's Basic Toxicology: Fundamentals, Targets Organ and Risk Assessment, 4th edition. New York: Taylor and Francis, pp. 386-392.

Madhiyazhagan, P., Murugan, K., Kumar, A.N. & Nataraj, T. (2014). Extraction of mosquitocidals from *Ocimum canum* leaves for the control of dengue and malarial vectors. *Asian Pacific Journal of Tropical Disease* **4**: S549-S555. https://doi.org/10.1016/S2222-1808(14)60675-7

Markouk, M., Bekkouche, K., Larhsini, M., Bousaid, M., Lazrek, H.B. & Jana, M. (2000). Evaluation of some Moroccan medicinal plant extracts for larvicidal activity. *Journal of Ethnopharmacology* **73**: 293-297. https://doi.org/10.1016/s0378-8741(00)00257-9

Martoja, R. & Martoja Pierson, M. (1967). Animal histology techniques. Masson and Cie edition, Paris. 345p.

Mir, S., Bouchenak, O., Aït kaci, K., Rouane, A., Alliliche, M. & Arab, K. (2022). Chemical composition and insecticidal activity of *Origanum floribundum* Munby essential oil endemic plant from Algeria. *Tropical Biomedicine* **39**: 215-220. https://doi.org/10.47665/tb.39.2.005

Owen, P.L. & Johns, T. (1999). Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout. *Journal of Ethnopharmacology* **64**: 149-160. https://doi.org/10.1016/S0378-8741(98)00119-6

Pavela, R., Maggi, F., Iannarelli, R. & Benelli, G. (2019). Plant extracts for developing mosquito larvicides: From laboratory to the field, with insights on the modes of action. *Acta Tropica* **193**: 236-271. https://doi.org/10.1016/j.actatropica.2019.01.019

Quezel, P. & Santa, S. (1963). Nouvelle flore de l'Algérie et des régions désertiques méridionales (No. 581.965 Q8). France, Paris: CNRS.

Rhee, K., Van de Meent, M., Ingkaninan, K. & Verpoorte, R. (2001). Screening for acetylcholinesterase inhibitors from Amaryllidaceae using silica gel thin-layer chromatography in combination with bioactivity staining. *Journal of Chromatography A* **915**: 217-223. https://doi.org/10.1016/s0021-9673(01)00624-0

Rodzay, R. & Zuharah, W.F. (2021). The determination of effective concentration of acethonilic *Ipomoea cairica* leaves extract against laboratory and field strains of *Aedes albopictus* and *Aedes aegypti* mosquito larvae. *Tropical Biomedicine* **38**: 446-452. https://doi.org/10.47665/tb.38.3.087

Sandhanam, S.D., Ganesan, P., Stalin, A., Michael, G.P., Balakrishna, K., Pandikumar, P., Ignacimuthu, S. & Al-Dhabi Naif, A. (2018). Effect of compound isolated from *Lawsonia inermis* (L.) (Myrtales: Lythraceae) on the immature stages of filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae) and its docking analysis with Acetylcholinesterase (AChE). *Biocatalysis and Agricultural Biotechnology* **15**: 210-218. https://doi.org/10.1016/j.bcab.2018.06.004

Sandhanam, S.D., Pathalam, G., Antony, S., Samuel, R., Michael, G.P., Kedike, B., Pandikumar, P., Savarimuthu, I. & Abdullah Al-Dhabi, N. (2019). Effect of tiliamosine, a bis, benzylisoquinoline alkaloid isolated from *Tiliacora acuminata* (Lam.) Hook. f. & Thom on the immature stages of filarial mosquito *Culex quinquefasciatus* say (Diptera: Culicidae). *Experimental Parasitology* **204**: 107719. https://doi.org/10.1016/j.exppara.2019.107719

Şengül Demirak, M.S. & Canpolat, E. (2022). Plant-Based Bioinsecticides for Mosquito Control: Impact on Insecticide Resistance and Disease Transmission. *Insects* **13**: 162. https://doi.org/10.3390/insects13020162

Shahat, M.A.M., El-Sheikh, T.M.Y., Hammad, K.M., Hasaballah, A.I. & Shehata, A.Z.I. (2020). Effect of some plant extracts on the biochemical parameters, AChE and GST activities of the mosquito, *Culex pipiens* L. (Diptera: Culicidae). *Egyptian Academic Journal of Biological Sciences* **12**: 69-80.

Tine-Djebbar, F., Bouabida, H. & Soltani, N. (2016). Répartition spatiotemporelle des Culicidés dans la région de Tébessa. Allemagne: Editions Universitaires Européennes, pp. 1-82.

WHO. (World Health Organization) (2005). Guidelines for laboratory and field testing of mosquito larvicides (WHO/ CDS/ WHOPES/ GCDPP/ 2005.13).

Yaméogo, F., Wangrawa, D.W., Sombié, A., Sanon, A. & Badolo, A. (2021). Insecticidal activity of essential oils from six aromatic plants against *Aedes aegypti*, dengue vector from two localities of Ouagadougou, Burkina Faso. *Arthropod- Plant Interactions* **15**: 627-634. https://doi.org/10.1007/s11829-021-09842-4

Zhang, M., Fang, T., Pu, G., Sun, X., Zhou, X. & Cai, Q. (2013). Xenobiotic metabolism of plant secondary compounds in the English grain aphid, *Sitobion avanae* (F.) (Hemiptera: Aphididae). *Pesticide Biochemistry and Physiology* **107**: 44-49. https://doi.org/10.1016/j.pestbp.2013.05.002

Zhao, B., Grant, G.G., Langevin, D. & MacDonal, D.L. (1998). Deterring and inhibiting effects of quinolizidine alkaloids on spruce budworm (Lepidoptera: Tortricidae) oviposition. *Environmental Entomology* **27**: 984-992. https://doi.org/10.1093/ee/27.4.984

Zhu, Y., Chen, S., Lurong, Q. & Qi, Z. (2023). Recent advances in antivirals for Japanese Encephalitis Virus. *Viruses* **15**: 1033. https://doi.org/10.3390/v15051033