Activity of Some Plant and Fungal Metabolites Towards Aedes Albopictus (Diptera, Culicidae)

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Research

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Abstract

Background: *Aedes albopictus* (Skuse), a globally spread species, vector of important human arboviruses, and of Zika virus, is extremely difficult to control even for the onset of resistances to chemicals insecticides. Ecofriendly biopesticides for vector control are urgently needed. In this study, the activity of some plant metabolites as Amaryllidaceae alkaloids and some their semisynthetic derivatives and 2-methoxy-1,4-naphthoquinones, and two analogues, and of the fungal metabolites cyclopaldic acid and epi-epoformin on the development and survival of *Ae. albopictus* larvae was evaluated.

Methods: A preliminary screening, on first-instar larvae, with lycorine semisynthetic derivatives and cyclopaldic acid at 100 ppm was conducted. The living larvae were recorded 24 and 48 h post treatment. Based on the results, first-instar larvae were exposed to increasing doses of the compounds tested until adult emergence. The mean larval and pupal duration was calculated. Third instar larvae were exposed to increasing doses of naphthoquinones for 72 h. The mortality rates were recorded every 24. The larvicide Device® SC-15 was used as control.

Results: First instar larval exposure to cyclopaldic acid at 50 and 100 ppm for 48 h, resulted in mortality mean percentage of 82.444 and 96.889% respectively; LC_{50} and LC_{90} were 40.119 and 105.092 ppm. 1,2-*O*,*O*-diacetyllycorine at 50 ppm, 48h post-treatment caused 84.667% mean percentage mortality with LC_{50} 27.769 and LC_{90} 88.316 ppm. Significant differences in the larval and pupal duration were proved when larvae were exposed to cyclopaldic acid (H=16.386; df 4; P=0.003; H=31.835; df 4; P=0.000), 1,2-*O*,*O*-diacetyllycorine (H=9.044; df 3; P=0.029; H=18.115; df 3; P=0.000) and *N*-methyllycorine iodide (H=19.457; df 4; P=0.001; H=15.400; df 4; P=0.004).

Statistical analyses revealed that the number of third-instar larvae surviving to naphthoquinones significantly decreases over the time when exposed to 2-methyl-1,4-naphthoquinone 12.5, 25, 50 ppm; 2-hydroxy-1,4-naphthoquinone 12.5, 25 ppm, 2-metoxy-1,4-naphthoquinone 50 ppm. The mean number of surviving larvae exposed to: 2-methyl-1,4-naphthoquinone 12.5, 25, 50, 100 ppm, 2-hydroxy-1,4-naphthoquinone 25, 50, 100 ppm, 2-metoxy-1,4-naphthoquinone 50, 100 ppm was significantly lower than the number of correspondent control larvae, respectively.

Conclusions: This study indicated that 1,2-*O*,*O*'-diacetyllycorine, *N*-methyllycorine iodide, cyclopaldic acid and 1,4-naphtoquinone structural derivatives have good potential to develop bioinsecticides for mosquito control programs.

Background

Aedes albopictus (Skuse) (Diptera: Culicidae), commonly known as "Asian tiger mosquito", is an invasive species which spread globally from its native range in Asia to both tropical and temperate regions [1, 2, 3]. It is vector of several important arboviruses and its role in different outbreaks worldwide has been reported making it a major threat to public health [4, 5, 6, 7, 8, 9]. Even if great efforts are made to control

arbovirus infections, such as dengue, transmission of this disease is increasing [10]. Furthermore, the ability of *A. albopictus* to carry and transmit Zika virus (ZIKV), has been established [8, 11, 12, 13, 14, 15]. Without an efficient vaccine to protect human populations from mosquito-transmitted diseases, a possible way to reduce the transmission risk is mosquito control [7, 16, 17, 18, 19, 20]. This seems an extremely difficult task, especially for *Ae. albopictus*, which exploits a variety of water-collecting containers found in private gardens, backyards, and urban vegetated areas and adapt to different environment thanks to its capacity of producing diapausing eggs [7, 21].

The control of *Ae. albopictus* mainly relies on the reduction of larval breeding sites, chemical interventions (insecticides, chemical larviciding) and non-chemical larviciding. Non-conventional methods, such as the use of irradiated or genetically modified mosquitoes and Wolbachia infection, are under implementation and may be used in the future. Insecticides can be used against both adult mosquitoes and larvae in forms of space treatment, indoor residual spraying, insecticide-treated bed nets, and as larvicides [7, 22, 23, 24]. However, insecticide resistance, already widespread in *Aedes aegypti* (L.), is increasing in *Ae. albopictus*, as resistance can be selected and spread rapidly around the globe also in this species and can compromise control activities. Moreover, considering the very limited number of insecticides available on the market for public health, many concerns raise regarding the global fight against the transmission of diseases [25, 26]. In recent years, a renewed interest in biologically active compounds from natural sources emerged, due to the variety of their biological activities and potential practical applications in different fields [27, 28, 29, 30, 31, 32, 33]. Furthermore, the use of natural ecofriendly biopesticides is a strongly and urgent request coming from the consumers and from the authorities [28, 33]. Natural products of plant and fungal origin may offer a wide source of active compounds to select environmentally friendly alternatives as mosquito control agents. Amaryllidaceae alkaloids and their synthetic derivatives are well known to have a wide range of biological and pharmacological activities, among which, acetylcholinesterase inhibitory, cytotoxic activities and antiproliferative properties [34, 35, 36, 37]. Lycorine, the main Amaryllideaceae alkaloid isolated from *Sternbergia lutea* (L.) Ker-Gawler [38], which is its best source (11g/dried plant kg), shows antitumor and antiviral activity [39] and it acts as a powerful inhibitor of ascorbic acid biosynthesis, and of cell growth and cell division, including antitumor activity in animal and human cell lines [40, 41]. The derivative *N*-methyllycorine iodide completely suppress HeLa cell invasion of type I collagen, in vitro at nontoxic concentrations [35]. Lycorine, 1,2-*O*,*O*'diacetyllycorine, *N*-methyllycorine iodide, α-dihydrolycorine, lycorine hydrochloride lycorin-2-one, all derivatives prepared from lycorine, and ungeremine isolated from *Pancratium maritimum* L. [42], but also synthesized by oxidation from lycorine [43], were evaluated for algicidal, bactericidal, fungicidal, herbicidal, and insecticidal activities [28].

Despite numerous studies concerning the phytochemistry and pharmaceutical activities and applications of Amaryllidaceae alkaloids [for complete reviews see: 44, 45], their potential in control of medical importance insects are much poorly investigated. Only recently, some Amaryllidaceae alkaloids as crinsarnine and sarniensinol, two new crinine and mesembrine type alkaloids isolated from the South African plant *Nerine sarniensis* Herbert, showed activity against the Zika virus vector *Ae. aegypti* [46].

Sarniensine also, a new mesembrine type alkaloids, isolated from the same amaryllidacea showed adulticidal activity against *Ae. aegypti* [47].

Among fungal metabolites, (+)-epi-epoformin, a cyclohexene epoxide isolated from the culture filtrates of *Diplodia quercivora* Linaldeddu & A.J.L. Phillips, a pathogen for cork oak in Sardinia, Italy, showed multiples biological activities, including antifungal, zootoxic and phytotoxic activity [48, 49, 50, 51]. Cyclopaldic acid, produced by several fungi belonging to different genera [52, 53, 54, 55], shows a wide and different range of biological activities, such as antifungal one [56, 57, 58], inhibits electron transport and oxidative phosphorylation in plant mitochondria [59] and also inhibits esterase activity *in vitro* [60]. In addition, cyclopaldic acid showed biting deterrent and larvicidal activity against *Ae. aegypti*, primary vector of dengue, yellow fever and Zika virus [61].

Among the quinones, plant derived substances (1,4-naphthoquinone structural derivatives) showed larvicidal activity against *Ae. aegypti*, *Culex pipiens pallens* Coquillet and *Ochlerotatus togoi* (Theobald) fourth-instar larvae [62]. In particular, among 23 compounds, belonging to different classes of natural compounds, 2-methoxy-1,4-naphthoquinone, isolated together with glanduliferins A and B, two new glucosylated steroids, and a-spinasterol from *Impatiens glandulifera* Royle, a plant native of Himalaya [63], showed larvicidal activity against *Ae. aegypti* larvae [64]. 2-Methoxy-1,4-naphthoquinone showed anticancer activity in vitro in the single digit micromolar range on three cell lines [63]. In order to find new larvicidal biopesticides, some plant and fungal metabolites, belonging to different chemical classes of natural compounds, among which Amaryllidaceae alkaloids, naphthoquinones, some of their derivatives, and the fungal phytotoxins cyclopaldic acid and epi-epoformin were evaluated against *Ae. albopictus* larvae. Moreover, the authors investigated if the alkaloids derivatives, with larvicidal activity, could also affect *Ae. albopictus* development.

Methods

Insects

First and third-instar larvae of *Ae. albopictus*, reared in 2 l plastic jars containing distilled water (1L) and 50 g of insect diet (50% of tuna fish flour, 50% of bovine liver powder and a standard dose of Vitamin Mix equal to 0.4 grams in 100 ml of solution) were purchased from Centro Agricoltura Ambiente “G. Nicoli” (Crevalcore, Bologna, Italy) where the mosquito strain used for the study was reared for 63 generations under controlled conditions. Jars with larvae were maintained at 27±2°C, 90±5% relative humidity (R.H.), 14:10 L:D photoperiod.

Natural compounds tested

Lycorine was obtained from acid extraction of *S. lutea* bulbs collected in Apulia coast [38]; 1,2-*O,O'*-diacetyllycorine, lycorine-2-one and α-dihydrolycorine were prepared from lycorine as previously reported [65] as well as lycorine chlorohydrate [38]. Clivonine hydrochloride was kindly supplied from Professor C. Fuganti, Istituto di Chimica, Politecnico di Milano, Italy as well as *N*-methyl lycorine iodide was a

generous gift of Professor H. M. Fales, Department of Health, Education and Welfare, Bethesda, MD 20014, U.S.A. Ungeremine was extracted from bulbs of Egyptian *P. maritimum* [42] and also obtained by Se_2O oxidation from lycorine [43]. Pseudolycorine was obtained from bulbs of *Narcissus tazetta* subsp. *tazetta* L., collected in Turkey [66]. Epi-epoformin was extracted from the culture filtrates of *D. quercivora* [49]; 2-methoxy-1,4-naphthoquinone was obtained from the organic extract of *I. glandulifera* [63]. 2-methyl-1,4-naphthoquinone and 2-hydroxy-1,4-naphthoquinone were purchased from Sigma-Aldrich, Milan, Italy.

Larvicidal tests

The larvicidal activity of the compounds was evaluated according to WHO standardized procedures and guideline for larvicidal test [67]. An initial screening with epi-epoformin, clivonine hydrochloride, 1-*O*-acetyllicorine, lycorine-2-one, pseudolycorine, ungeremine, lycorine chlorohydrate, cyclopaldic acid, 1,2-*O*,*O*'-diacetyllycorine, *N*-methyllycorine iodide and α-dihydrolycorine, tested at concentration of 100 ppm, was carried out. Twenty-four replicates, each consisting of 5 first-instar larvae, were utilized for each compound concentration as well as for the controls. Since dimethyl sulfoxide (DMSO) 1% was used to solubilize the compounds tested, distilled water and DMSO 1% were used as controls. The larvae were transferred, by using a 20 µl micropipette with a drop of water, in 24-well polystyrene clear flat bottom plate, with lid, provided with 50 µl of 5% insect diet, and exposed to a total volume of 2 ml of compound solutions and controls for each well. The number of living larvae was recorded 24 and 48 h post treatment. The larvae that showed no signs of movements after probing with a needle were considered dead. Bioassays were conducted at 27 ± 1° C, 90 ± 5% relative humidity (R.H.) and photoperiod of 14:10 L:D.

Based on the results of this initial screening, new bioassays were conducted to evaluate the effects of cyclopaldic acid, 1,2-*O*,*O*'-diacetyllycorine, *N*-methyllycorine iodide, and α-dihydrolycorine, tested at increasing dosages, on the development of *Ae. albopictus* until adult emergence. All compounds, except 1,2-*O*,*O*'-diacetyllycorine, were tested at 6.125, 12.5, 25, 50 and 100 ppm, for solubility problems 1,2-*O*,*O*'-diacetyllycorine was not tested at 100 ppm. The insecticide Device® SC-15 (based on Diflubenzuron) for mosquito larvae was used as positive control and tested at 7, 12.5, 25, 50 and 100 ppm.

Twenty-one-instar larvae were transferred in 100-ml beakers, provided with 100 µl of 5% insect diet, and exposed to compound solutions and to controls. Five replicates were utilized for each concentration, for Device® SC-15, as well as for the controls. The number of living insects was recorded every 24 h from the first-instar to adult emergence. The larval mortality percentages, obtained at 24 and 48 h, were reported as an average of values, from five replicates, corrected using Abbotts's formula [68]. For calculating LC_{50} and LC_{90} at 95% confidence interval, the data obtained by the larval mortality at 24 and 48 h were corrected using Abbott's formula, transformed into arcsine/proportion values and then were subjected to probit regression analysis [69, 70].

The total larval and pupal mortality was estimated by counting the dead samples during the entire bioassay. Larval mortality was expressed in percentage according to the initial number of larvae, pupal mortality percentage was estimated according to the total number of obtained pupae. The total number of days, from the start of the bioassay, on which dead larvae were recorded, and the total number of days, from the pupation, on which dead pupae were recorded, were also reported. The mean larval and pupal duration was obtained by multiplying the number of pupae and adults by the number of days exerted to develop in each replicate; these values were summed and the total was divided by the total number of larvae and pupae developed. The mean larval duration values obtained in the control bioassays with distilled water and DMSO 1% were analysed by Student's *t*-test (P = 0.05) for independent samples. The same statistical analysis was carried out on distilled water and DMSO 1% pupal duration values. The non-parametric Kruskal-Wallis test for multiple independent comparisons followed by pairwise Mann–Whitney U-test comparisons (P<0.05) were used to compare the larval and the pupal duration values obtained in the bioassays with DMSO 1% and with each of the compounds tested.

2-Methyl-1,4-naphtoquinone, 2-hydroxy-1,4-naphtoquinone and 2-metoxy-1,4-naphtoquinone were tested at 100, 50, 25, 12.5 and 6.125 ppm towards third-instar larvae. Distilled water and DMSO 1% were used as controls and the insecticide Device® SC-15 as positive control. The larvae were transferred in 100-ml beakers and exposed to test compounds and the number of dead larvae in each beaker was recorded 24, 48 and 72 h after the start of the bioassays. Five replicates, each consisting of 20 three-instar larvae, were utilized for each concentration as well as for the controls. In naphthoquinones tests, no mortality was detected in controls after exposure, so no correction was required based on Abbott's formula. The mean of the mortality percentages, at each concentration, was determined. The values of dead larvae obtained by the bioassays, at different concentrations, were subjected to probit regression analysis for estimation the mean lethal concentration values (LC_{50} and LC_{90}) at 95% confidence interval [69].

The raw data on larval-pupal survival obtained after 24, 48 and 72h after the start of the 1,4-naphthoquinone structural derivatives and Device® SC-15 bioassays were analyzed using the General Linear Model (GLM) for repeated measures (over time) procedure and compared by using a one-way correlated analysis of variance (Tests of within-subjects effects). The differences between the means of the number of survivors in each of the bioassays, carried out using different concentrations of 1,4-naphthoquinone structural analogues and of Device® SC-15, and the means of the number of survived larvae-pupae of related controls over time were analyzed and adjusted with Bonferroni test [71] for multiple comparisons. The Bonferroni test was also used to assess whether the mean number of larvae and pupae surviving to exposure to the same concentration of 1,4-naphthoquinone structural derivatives and of Device® SC-15 and the mean number of larvae and pupae surviving in control solution, at different time of exposure, were significantly different.

All the statistical analyses were performed by Statistical Package for Social Sciences (SPSS), version 20.0 for Windows software (SPSS Inc., Chicago, IL).

Results

The natural compounds, the lycorine semisynthetic derivatives, and the commercially available analogue of 2-methoxy-1,4-naphthoquinone used in this study are reported in Figure 1.

The results of the initial screening obtained by exposure of *Ae. albopictus* first-instar larvae to: epi-epoformin, clivonine hydrochloride, 1-*O*-acetyllycorine, lycorine-2-one, pseudolycorine, ungeremine, lycorine chlorohydrate, cyclopaldic acid, 1,2-*O*,*O*-diacetyllycorine, *N*-methyllycorine iodide, α-dihydrolycorine at the concentration of 100 ppm, showed that only cyclopaldic acid, 1,2-*O*,*O*-diacetyllycorine and *N*-methyllycorine iodide showed a larvicidal activity. In particular, the larval mortality was of 96.66, 80.00 and 68.00% respectively after 48 h of exposure. α-Dihydrolycorine, tested at the same concentration, caused a larval mortality of 40%. No mortality was detected after exposure to distilled water and DMSO 1%.

For what concerned the activity of cyclopaldic acid, 1,2-*O*,*O*-diacetyllycorine, *N*-methyllycorine iodide and Device® SC-15, tested at increasing dosages, on the development of *Ae. albopictus* first-instar larvae and pupae, Table 1 shows their effects on the larval viability at 24 and 48 h post treatment.

Cyclopaldic acid at concentrations of 3.125 to 100 ppm, at 24 h post treatment, showed LC_{50} and LC_{90} of 113.881 and 256.099 ppm respectively. At 48 h LC_{50} and LC_{90} was 40.119 and 105.092 ppm and the larvicidal activity was observed on the larvae treated with 50 and 100 ppm, showing 82.444 and 96.889% mean mortality respectively. 1,2-*O*,*O*-diacetyllycorine caused LC_{50} and LC_{90} of 53.125 and 178.822 ppm respectively after 24 h from the start of the bioassay, and LC_{50} and LC_{90} values of 27.769 and 88.316 ppm at 48h. The compound, at 50 ppm caused 84.667% mean mortality. *N*-methyllycorine iodide, at 24 h, produced LC_{50} and LC_{90} of 177.653 and 380.791 ppm respectively, at 48 h of 78.501 and 176.188 ppm. At 48 h the concentration of 100 ppm caused 68.333% mean mortality. α-Dihydrolycorine at 24 and 48h showed LC_{50} and LC_{90} values of 95.142 and 177.705 ppm; 93.658 and 183.770 ppm respectively. Device® SC-15 larvicidal activity was observed on larvae treated with 50 and 100 ppm, at 24h, the first one caused 80.789% mean mortality and the second 100.00 with LC_{50} and LC_{90} of 18.946 and 104.779 ppm respectively. At 48 h the mean mortality was 100.00 for all the concentrations tested. Observations on the entire larval and pupal duration of *Ae. albopictus* exposed to the above mentioned compounds, showed that at highest concentrations, they produced over 98% larval mortality except for *N*-methyllycorine iodide 50 ppm that was 75% (Table 2). The latter compound, at 50 ppm, caused 48.00% of pupal mean mortality while, cyclopaldic acid caused 38.09%, at the concentration of 25 ppm (Table 1).

The Student's *t*-test carried out on larval duration values, obtained in the control bioassays with distilled water and DMSO 1%, revealed that the difference between the two variables were not statistically significant ($P>0.05$). The same result ($P>0.05$) was obtained comparing pupal duration.

Kruskal-Wallis test followed by pairwise Mann-Whitney U-test comparisons revealed significant differences in the larval duration values obtained in bioassays with DMSO 1% and with each of the three

compounds tested at different concentrations (DMSO 1% - cyclopaldic acid H=16.386; df 4; P=0.003; DMSO 1% - 1,2-*O*,*O*-diacetyllycorine H=9.044; df 3; P=0.029; DMSO 1% - *N*-methyllycorine iodide H=19.457; df 4; P=0.001). The same test showed no significant differences in the larval duration values obtained with DMSO 1% and α-dihydrolycorine (H=4.420; df 3; P=0.220) (Table 2). The same statistical test revealed significant differences in the pupal duration values obtained in bioassays with DMSO 1% and with each of the three compounds tested at different concentrations (DMSO 1% - cyclopaldic acid H=31.835; df 4; P=0.000; DMSO 1% - 1,2-*O*,*O*'-diacetyllycorine H=18.115; df 3; P=0.000; DMSO 1% - *N*-methyllycorine iodide H=15.400; df 4; P=0.004). No significant differences were detected in the pupal duration values obtained with DMSO 1% and α-dihydrolycorine (H=2.952; df 3; P=0.399) (Table.2).

The dose-response mean mortality percentages, the LC_{50} and LC_{90} obtained in naphthoquinones and Device® SC-15 bioassays towards three-instar larvae, are provided in Table 3. 2-metil-1,4-naphthoquinone, 2-hydroxy-1,4-naphthoquinone, and 2-methoxy-1,4-naphthoquinone, at concentration of 50 and 100 ppm led to >94% mortality already 24 h after the start of the bioassays. Moreover, 2-metil-1,4-naphthoquinone at 25 ppm caused >95% mortality after 48 h. While 2-methoxy-1,4-naphthoquinone led to >100% mortality, after 24h, only at 100 ppm. Device® SC-15 caused >80% mortality at 100 ppm 24h after the start of the bioassay, and at 50 and 100 ppm at 48h. 2-Methyl-1,4-naphthoquinone exhibited larvicidal activity with 24, 48 and 72 h LC_{90} values of 24.190, 14.498, 12.139 ppm, and LC_{90} of 39.454, 20.711, 15.814 respectively. The LC_{90} values of 2-hydroxy-1,4-naphthoquinone relating to the same time intervals, were 41.022, 39.192, and 39.627 ppm. While LC_{90} 2-methoxy-1,4-naphthoquinone values were higher than those of the previous compounds (LC_{90} 88.235, 88.431, 80.486 ppm). Device® SC-15 LC_{90} values were 95.307, 90.588, 58.949 ppm.

The raw data obtained by larvicidal bioassays carried out on third-instar larvae with 1,4-naphthoquinone structural derivatives and Device® SC-15 tested at the concentrations: 6.25, 12.5, 25, 50 and 100 ppm were analysed using the GLM repeated measures procedure and Bonferroni test. GLM assessed whether the interaction between both test conditions (treatment and control) and the changes over the time of the number of larvae survived to exposure to compounds tested or the number of survived control larvae was statistically significant.

The analysis of the data obtained with 2-methyl-1,4-naphthoquinone at concentrations 12.5, 25, 50 ppm; 2-hydroxy-1,4-naphthoquinone at 12.5, 25 ppm, 2-metoxy-1,4-naphthoquinone at 50 ppm and Device® SC-15 at all concentrations tested, revealed time × treatment interaction effect (P <0.01) (Table 4). Indeed, the number of larvae surviving to compounds and product exposure significantly decreases over the time of the bioassay.

On the contrary, the analysis of the data obtained with compounds and product tested at all the other concentrations, revealed no time × treatment interaction effect (P >0.05) (Table 3), showing that the number of larvae surviving to compounds exposure does not significantly decrease over the time of the bioassay. In particular, for 2-methyl-1,4-naphthoquinone, at concentrations 6.25 ppm, the difference

between the mean number of larvae survived to exposure of the compound was not statistically significant comparing to control from the beginning to the end of the bioassay. At 12.5, 25, 50 and 100 ppm, the differences between the mean number of larvae survived to exposure of the same compound were statistically significant comparing to control from the beginning to the end of the bioassay. For 2-hydroxy-1,4-naphthoquinone, at concentrations of 25, 50 and 100 ppm the differences between the mean number of larvae survived to exposure of the compound were statistically significant comparing to control from the beginning to the end of the bioassay. At 6.25 and 12.5 ppm, the differences between the mean number of larvae survived to exposure of the compound were not statistically significant comparing to control for the entire duration of the bioassay. For 2-metoxy-1,4-naphthoquinone at 50 and 100 ppm the differences between the mean number of larvae survived to exposure to compound were statistically significant comparing to control from the beginning to the end of the bioassay. At 25 ppm the difference was significant only starting from 72 hours. At concentrations of 6.25, 12.5 and 25 ppm, the differences between the mean number of larvae survived to exposure of the compound were not statistically significant comparing to control from the beginning to the end of the bioassay. For what concerned Device® SC-15, at concentrations of 6.25, the differences between the mean number of larvae survived to exposure to compound was statistically significant comparing to control only starting from the forty-eighth hour. At 12.5, 25, 50 and 100 ppm the differences between the mean number of larvae survived to exposure to product were statistically significant comparing to control from the beginning to the end of the bioassay.

The Bonferroni test was used to assess whether the mean number of survived larvae to exposure to compounds and Device® SC-15 was significantly smaller than the mean number of survived larvae in control solution, over time, indicating a larvicidal effect of the compounds and of the product. This test revealed that the mean number of surviving larvae exposed to: 2-methyl-1,4-naphthoquinone at concentrations 12.5, 25, 50, 100 ppm, 2-hydroxy-1,4-naphthoquinone at 25, 50, 100 ppm, 2-metoxy-1,4-naphthoquinone at 50, 100 ppm and to Device® SC-15 at all concentrations tested, was significantly smaller than the number of correspondent control larvae, respectively, over time (Table 4).

Discussion

In this study, environmentally friendly alternatives to synthetic insecticides towards *Ae. albopictus* larvae have been explored testing natural compounds of different origin and belonging to different chemical classes. We investigated the activity of some Amaryllidaceae alkaloids and some lycorine semisynthetic derivatives, two fungal metabolites epi-epoformin and cyclopaldic acid, and a plant metabolite as 2-methoxy-1,4-naphthoquinone and two of its analogues 2-hydroxy- and 2-methyl-1,4-naphthoquinones on larval viability. As a general note, larvicidal activity demonstrated by the active compounds has been proved to depend on the compound tested, the concentration and on the exposure time, indeed, an increase of concentration and of exposure time determined an increasing larval mortality. Among the alkaloids semisynthetic derivatives tested, only 1,2-*O*,*O*'-diacetyllycorine, *N*-methyllycorine iodide and α-dihydrolycorine showed, for the first time, a mosquito larvicidal activity. They also exhibited different

degrees of effectiveness, the first two compounds proving to be the most active (84.667 and 68.333% mean mortality), toward *Ae. albopictus* first-instar larvae, after 48h of treatment. Amaryllidaceae alkaloids and their derivatives have been reported to exhibit a wide spectrum of bioactivities such as antiproliferative ones, may be by disrupting eukaryotic protein biosynthesis [72, 73, 74], apoptosis inducers [35, 75], antitumor, antiviral, acetylcholinesterase inhibitory and cytotoxic activities [76]. Furthermore, very recently, 2-*O*-acetyllycorine was proved to have a marked antiprotozoal activity against *Trypanosoma brucei brucei* Plimmer & Bradford [77]. Such a broad spectrum of activity could explain the good larvicidal activity demonstrated by 1,2-*O*,*O*'-diacetyllycorine and *N*-methyllycorine iodide toward *Ae. albopictus* larvae. Amaryllidaceae alkaloids are also involved in plant-insect interactions, 3-*O*-acetyl-narcissidine was shown to have antifeedant activity towards *Spodoptera littoralis* (Boisduval) [78]. Han et al. [79] showed that Amaryllidaceae alkaloids, among which lycorine, exhibited considerable aphicidal activity and *N*-allylnorgalanthamine displayed a significant inhibition on AChE in *Aphis citricola* van der Goot both *in vivo* and *invitro*. Among the compounds tested that were found to be not active towards *Ae. albopictus* larvae, ungeremine was also proved to be not active towards *Ae. aegypti* first-instar larvae [47]. The fungal metabolite cyclopaldic acid showed its effectiveness on larvae, not only at the two major concentrations 50 and 100 ppm (82.444 and 96.889% mean mortality), but also at 25 ppm at which larval mortality was 79.00% after 48h from the start of the bioassay. Furthermore, at the higher concentrations tested (50, 100 ppm), the larval mortality values were comparable with these obtained with the Device® SC-15. The results obtained with cyclopaldic acid prove that this fungal metabolite has a good larvicidal activity, not only towards *Ae. albopictus* larvae, but also towards *Ae. aegypti* ones [61]. In addition, this metabolite, as well as some other fungal metabolites, belonging to different classes of natural compounds, as seiridin, sphaeropsidin A and payracillic acid, showed both larvicidal activity and biting deterrent against *Ae. aegypti*, primary vector of dengue, yellow fever and Zika virus [61, 80]. Following these results, cyclopaldic acid could provide different management opportunities in different mosquito species control. Some other, mainly natural phenols, were evaluated as potential attractants of *Ceratitis capitata* (Wiedemann) male, the Mediterranean fruit fly [81]. Recently, a-costic acid, a well-known sesquiterpenoid isolated from the native Mediterranean plant *Dittrichia viscosa* (L.) Greuter, had showed a significant acaricidal activity against *Varroa destructor* Anderson and Trueman, the parasite mite of *Apis mellifera* L., the Western or European honey bee [82], and costic acid isomers contained in n-hexane extracts of the same plant have been held accountable for the contact toxicity against the granary weevil adults *Sitophilus granarius* (L.) [83].

To our knowledge, there are no papers describing the effects of the Amaryllidaceae alkaloids derivatives tested on *Ae. albopictus* larval and pupal development. In this regard, cyclopaldic acid, 1,2-*O*,*O*'diacetyllycorine and *N*-methyllycorine iodide, tested on first-instar larvae, caused a significant increase of the larval stage duration at almost all the concentrations. While, the effect on the pupal stage duration seems to be less marked. Cyclopaldic acid and *N*-methyllycorine iodide, at different concentration, also affected pupal viability, causing 38.09 and 48.00% of mean mortality respectively. The effects on larval and pupal development of these compounds may be due to their growth regulating effects on larvae, which resulted in increasing the larval stage duration and pupal mortality.

The role of alkaloids in insect growth and development was explored by some other authors. Larvicidal, growth-regulating and chemosterilant activities of alkaloids extracted from *Annona squamosa* L. (Annonaceae) were reported against *Anopheles stephensi* Liston, an important vector of malaria. Mortality in the larvae, pupae and adults was reported, and the total developmental period was slightly reduced comparing to control. Furthermore, exposed larvae eclosed adult females with reduced fecundity and fertility [84]. In reports by Sun et al., [85] the treatment of *Spodoptera litura* (Fabricius) with *Cynanchum mongolicum* (Maximowicz) (Asclepiadaceae) extracts led to more than half of the resulting pupae not moulting into adults, and also the developmental time, in particular from third instar to emergence, was increased. Ge et al. [86] also proved that alkaloids from the same plant species had effects on growth and development of *S. litura*, in fact, higher alkaloid concentrations caused a greater developmental disruption and mortality, mainly by 72 h post-treatment. Furthermore, the ecdysone titre of treated larvae and pre-pupae decreased with increasing alkaloid concentration and hormone balances disruption was very similar to that caused by azadirachtin.

The 1,4-naphthoquinones tested proved their larvicidal activity against third-instar larvae. Among the investigated 1,4-naphthoquinone structural analogues, 2-methyl-1,4-naphthoquinone showed a remarkable larvicidal activity, at concentrations of 25, 50 and 100 ppm (75.00, 94.00 and 100% mean mortality respectively), towards *Ae. albopictus* fourth larval stage at 24h of exposure time. 2-Hydroxy-1,4-naphthoquinone also exhibited a good larvicidal activity with LD_{50} and LD_{90} of 33.730 and 41.022 ppm respectively at 24 h of exposure, while 2-metoxy-1,4-naphthoquinone is effective at the highest concentration at 24 and 48 hours. The latter compound caused a comparable larvicidal activity value when tested on *Ae. aegypti* first-instar larvae [64] and its activity was also proved against *Ae. aegypti* fourth-instar larvae [62].

Moreover, the bioassays indicated that larvicidal activity depends not only on concentration and exposure time, but also on functional groups linked to them. Indeed, at the same concentration, the naphthoquinones with different functional groups have shown a different effectiveness on the larval viability. The importance of the functional group in carrying out the activity, has also been highlighted in other papers concerning the toxic activity of naphthoquinones against fourth instar *Ae. aegypti*, and freshwater snail *Biomphalaria glabrata* (Say) [62]. The authors proved the larvicidal activity (LD_{50} 14.952; LD_{90} 23.065 ppm) of 2-methyl-1,4-naphthoquinone and showed the relationship between the bromonaphthoquinones activity and bromine and other substituents. Kim and Lee [87] also determined the structural toxicity relationships of 5-hydroxy-2-methyl-1,4-naphthoquinone and its structural derivatives against the mosquito species *Ae. aegypti*, *Cx. pipiens pallens*, and *Oc. togoi* larvae.

This study also showed that the larvicidal activity of cyclopaldic acid, of some of the alkaloid derivatives and of 1,4-naphthoquinone structural analogues tested was comparable than that of Device® SC-15.

The promising results obtained make these natural compounds worthy of consideration as bioinsecticide to control *Ae. albopictus* larvae, but further studies are required to verify their activity and possible side effects when they are applied to natural habitats. For this purpose, a suitable and effective

bioformulation should also be realized. Such compounds could be utilized in larval breeding sites including rain-water collection areas, peridomestic water, containers etc., both in urban places, where treatment areas are manageable as they are limited, and rural areas. Furthermore, investigations are needed to determine their potential risks on non-target organisms, and on environment in general, including proof of their human safety.

Conclusion

1,2-*O*,*O*'-diacetyllycorine, *N*-methyllycorine iodide, α-dihydrolycorine, cyclopaldic acid and 1,4-naphthoquinone structural derivatives demonstrate strong larvicidal activity against *Ae. albopictus* larvae. Besides causing larval mortality, cyclopaldic acid, 1,2-*O*,*O*'-diacetyllycorine, and *N*-methyllycorine iodide induce a significant increase of the larval stage duration. The obtained results could be useful to develop bioinsecticide-based strategies in mosquito control programs.

Declarations

Ethics approval and consent to participate

Not applicable. This article does not contain any studies with human participants or animals (vertebrates) performed by any of the Authors.

Consent for publication

Not applicable

Availability of data and materials

The datasets supporting the conclusion of this article are included within the article

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

SG, ADC and AE conceived the work and planned the laboratory tests. SG and conducted experiments. SG, AE, drafted the manuscript. SG, PG performed statistical analysis; AE and MM conducted chemical analyses and extraction and provided the figure for the manuscript. ADC and AE critically revised the manuscript. All authors read and approved the final version of the manuscript.

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Tables

Table 1.
Larvicidal activity of cyclopaldic acid, 1,2-*O*,*O*'-diacetyllycorine, *N*-methyllycorine iodide, α-dihydrolycorine, Device®SC-15 against *Ae. albopictus* first-instar larvae.

Cyclopaldic acid				
Concentration (ppm)	Mean mortality (%) ± SE 24 h	LC_{50} (ppm) (LCL-HCL) 24 h	LC_{90} (ppm) (LCL-HCL) 24 h	χ^2 (df)
100	47.474 ± 3.354	113.881	256.099	11.372 (28)
50	25.211± 2.126	(91.312-157.726)	(198.460-374.599)	
25	15.053± 2.691			
12.5	13.053± 3.714			
6.25	9.105± 1.888			
3.125	5.105± 1.666			
	Mean mortality % ± SE 48 h	LC_{50} (ppm) (LCL-HCL) 48 h	LC_{90} (ppm) (LCL-HCL) 48 h	χ^2 (df)
100	96.889 ± 1.274	40.119	105.092	30.630 (28)
50	82.444 ± 3.396	(34.566-46.298)	(92.674-122.370)	
25	65.111± 3.458			
12.5	23.444± 3.321			
6.25	11.222± 1.854			
3.125	6.111± 0.978			
1,2-*O*,*O*'-Diacetyllycorine				
Concentration (ppm)	Mean mortality (%) ± SE 24 h	LC_{50} (ppm) (LCL-HCL) 24 h	LC_{90} (ppm) (LCL-HCL) 24 h	χ^2 (df)
50	53.474 ± 3.135	53.125	178.822	4.460 (18)
25	46.474 ± 2.913	(37.395-135.457)	(112.822-754)	

12.5	30.368 ± 4.589			
6.25	25.158 ± 3.426			
	Mean mortality (%) ± SE 48 h	LC_{50} (ppm) (LCL-HCL) 48 h	LC_{90} (ppm) (LCL-HCL) 48 h	χ^2 (df)
50	84.667 ± 2.759	27.769	88.316	5.055 (18)
25	59.333 ± 3.822	(21.797-35.047)	(70.402-125.478)	
12.5	33.667 ± 5.333			
6.25	29.444 ± 3.768			
N-Methyllycorine iodide				
Concentration (ppm)	Mean mortality (%) ± SE 24 h	LC_{50} (ppm) (LCL-HCL) 24 h	LC_{90} (ppm) (LCL-HCL) 24 h	χ^2 (df)
100	24.158 ± 2.305	177.653	380.791	11.545 (23)
50	16.105 ± 1.788	(126.318-357.493)	(257.376-827.061)	
25	11.053 ± 2.418			
12.5	9.053 ± 1.843			
6.25	4.000 ± 1.871			
	Mean mortality (%) ± SE 48 h	LC_{50} (ppm) (LCL-HCL) 48 h	LC_{90} (ppm) (LCL-HCL) 48 h	χ^2 (df)
100	68.333 ± 2.934	78.501	176.188	23.152 (23)
50	57.000 ± 2.550	(67.368-94.831)	(147.426-224.436)	
25	12.000 ± 3.742			
12.5	13.333 ± 1.394			
6.25	7.00 ± 2.550			
α-Dihydrolycorine				

Concentration (ppm)	Mean mortality (%) ± SE 24 h	LC_{50} (ppm) (LCL-HCL) 24 h	LC_{90} (ppm) (LCL-HCL) 24 h	χ^2 (df)
100	47.421 ± 1.644	95.142	177.705	39.933[a] (23)
50	46.474 ± 1.867	(79.625-121.985)	(144.131-242.831)	
25	8.053 ± 1.981			
12.5	2.00 ± 1.225			
6.25	1.00 ± 1.00			
	Mean mortality (%) ± SE 48 h	LC_{50} (ppm) (LCL-HCL) 48 h	LC_{90} (ppm) (LCL-HCL) 48 h	χ^2 (df)
100	46.778 ± 2.197	93.658	183.770	49.636[a] (23)
50	57.000 ± 4.637	(75.826-128.300)	(143.581-273.272)	
25	9.00 ± 2.449			
12.5	3.00 ± 1.225			
6.25	2.00 ± 1.225			
Device® SC-15				
Concentration (ppm)	Mean mortality (%) ± SE 24 h	LC_{50} (ppm) (LCL-HCL) 24 h	LC_{90} (ppm) (LCL-HCL) 24 h	χ^2 (df)
100	100.00 ± 0.00	18.946	104.779	4.332[a] (23)
50	80.789± 1.910	(8.027-26.273)	(88.228-132.063)	
25	65.579 ± 3.153			
12.5	61.579 ± 2.664			
7	42.474 ± 3.526			
	Mean mortality (%) ± SE			

	48 h
100	100.00
50	100.00
25	100.00
12.5	100.00
7	100.00

Mean mortality percentages and probit regression analysis (LC_{50} and LC_{90}) at 95% confidence interval, at 24 and 48 h post treatment, obtained in the bioassay to evaluate the effects of cyclopaldic acid, 1,2-*O*,*O*'-diacetyllycorine, *N*-methyllycorine iodide, α-dihydrolycorine and Device® SC-15 on the development of *A. albopictus* first-instar larvae.

LC_{50} = lethal concentration (ppm) that kills 50% of the exposed larvae.

LC_{90} = lethal concentration (ppm) that kills 90% of the exposed larvae.

[a] = Since goodness-of-fit test is significant ($P < 0.05$), a heterogeneity factor is used in the calculation of confidence limits (CL).

Table 2.
Effects of cyclopaldic acid, 1,2-*O*,*O*'-diacetyllycorine, *N*-methyllycorine iodide, and α-dihydrolycorine on the development of *Ae. albopictus*.

Compounds Concentration (ppm)	Larval mortality (%) ([a])	Mean larval duration (days)	Pupal mortality (%) ([b])	Mean pupal duration (days)
Control (distilled water)	7.00 (7)	6.40 ± 3.49 a	2.12 (4)	3.51 ± 2.33 a
Control (DMSO 1%)	7.00 (7)	6.58 ± 2.99 a	4.30 (4)	3.85 ± 1.87 a
Cyclopaldic acid				
100	100.00 (3)			
50	100.00 (5)			
25	79.00 (11)	8.57 ± 1.42 b	38.09 (5)	3.15 ± 2.52 b
12.5	38.00 (28)	8.47 ± 2.55 b	16.13 (5)	3.65 ± 1.04 b
6.25	21.00 (16)	7.64 ± 2.53 b	15.19 (9)	3.58 ± 1.24 ab
3.125	13.00 (8)	7.28 ± 2.57 b	11.5 (7)	3.03 ± 1.20 ab
1,2-*O*,*O*'-Diacetyllycorine				
50	98.00 (14)			
25	62.00 (7)	7.34 ± 1.99 b	7.9 (7)	3.34 ± 0.93 b
12.5	44.00 (12)	7.07 ± 2.29 b	7.15 (5)	2.92 ± 0.85 b
6.25	34.00 (7)	7.09 ± 2.54 b	12.3 (7)	4.08 ± 1.34 b
N-methyllycorine iodide				
100	100.00 (10)			
50	75.00 (14)	7.20 ± 1.36 b	48.00 (6)	4.46 ± 1.12 cb
25	25.00 (25)	7.39 ± 2.00 b	12.00 (10)	4.82 ± 1.23 a
12.5	21.00 (22)	7.35 ± 1.71 b	12.66 (7)	3.91 ± 1.30 ab
6.25	16.00 (17)	7.25 ± 1.82 a	13.1 (6)	4.38 ± 1.20 ab
α-Dihydrolycorine				
100	100.00 (9)		0.00	
50	100.00 (9)		0.00	

25	12.00 (15)	6.95 ± 3.06 a	5.68 (7)	4.30 ± 1.68 a
12.5	13.00 (7)	7.21 ± 3.58 a	5.75 (5)	4.08 ± 1.08 a
6.25	10.00 (5)	6.88 ± 2.68 a	4.45 (3)	3.93 ± 1.31 a
Device® SC-15				
100	100.00 (1)			
50	100.00 (2)			
25	100.00 (2)			
12.5	100.00 (2)			
7	100.00 (2)			

Larval and pupal mortality obtained in bioassays with distilled water, DMSO 1%, cyclopaldic acid, 1,2-*O*,*O*'-diacetyllycorine, *N*-methyllycorine iodide, α-dihydrolycorine, and Device® SC-15, tested at different concentrations, on the development of *A. albopictus*. The larval and pupal mean duration values obtained in the bioassays with DMSO 1% and with each of the compounds tested were analysed by a non-parametric Kruskal–Wallis test for multiple independent comparisons, with subsequent pair-wise Mann-Whitney U-test comparisons ($P<0.05$). Different letters indicate significant differences ($P<0.05$).

[a] The number of days, from the start of the bioassay, on which dead larvae were recorded.

[b] The number of days, from the pupation, on which dead pupae were recorded.

Table 3.
Larvicidal activity of 2-methyl-1,4-naphthoquinone, 2-hydroxy-1,4-naphthoquinone, 2-methoxy-1,4-naphthoquinone and Device® SC-15 against *Ae. albopictus* three-instar larvae.

2-Methyl-1,4-naphthoquinone				
Concentration (ppm)	Mean mortality (%) ± SE 24 h	LC_{50} (ppm) (LCL-HCL) 24 h	LC_{90} (ppm) (LCL-HCL) 24 h	χ^2 (df)
100	100 ± 0.00	24.190	39.454	35.801[a] (23)
50	94.00 ± 1.00	(21.609-27.127)	(35.354-45.398)	
25	75.00 ± 0.00			
12.5	15.00 ± 0.00			
6.25	0.00 ± 0.00			
	Mean mortality (%) ± SE 48 h	LC_{50} (ppm) (LCL-HCL) 48 h	LC_{90} (ppm) (LCL-HCL) 48 h	χ^2 (df)
100	100 ± 0.00	14.498	20.711	11.593 (23)
50	100 ± 0.00	(13.498-15.643)	(19.126-22.911)	
25	97,00 ± 1.225			
12.5	41,00 ± 1.00			
6.25	1,00 ± 1.00			
	Mean mortality (%) ± SE 72 h	LC_{50} (ppm) (LCL-HCL) 72 h	LC_{90} (ppm) (LCL-HCL) 72 h	χ^2 (df)
100	100 ± 0.00	12.139	15.814	10.190 (23)
50	100 ± 0.00	(11.492-12.897)	(14.702-17.676)	
25	100.00 ± 0.00			
12.5	55.00 ± 3.536			
6.25	1.00 ± 2.00			
2-Hydroxy-1,4-naphthoquinone				

Concentration (ppm)	Mean mortality (%) ± SE 24 h	LC_{50} (ppm) (LCL-HCL) 24 h	LC_{90} (ppm) (LCL-HCL) 24 h	χ^2 (df)
100	100 ± 0.00	33.730	41.022	1.770 (23)
50	100 ± 0.00	(31.169-38.021)	(37.017-48.527)	
25	8.00 ± 2.00			
12.5	0.00 ± 0.00			
6.25	0.00 ± 0.00			
	Mean mortality (%) ± SE 48 h	LC_{50} (ppm) (LCL-HCL) 48 h	LC_{90} (ppm) (LCL-HCL) 48 h	χ^2 (df)
100	100 ± 0.00	31.946	39.192	0.825 (23)
50	100 ± 0.00	(29.340-38.400)	(34.495-51.990)	
25	12.00 ± 1.225			
12.5	0.00 ± 0.00			
6.25	0.00 ± 0.00			
	Mean mortality (%) ± SE 72 h	LC_{50} (ppm) (LCL-HCL) 72 h	LC_{90} (ppm) (LCL-HCL) 72 h	χ^2 (df)
100	100 ± 0.00	30.879	39.627	13.928 (23)
50	100 ± 0.00	(28.760-34.106)	(35.941-46.092)	
25	18.00 ± 2.00			
12.5	1.00 ± 1.00			
6.25	0.00 ± 0.00			
2-Metoxy-1,4-naphthoquinone				
Concentration (ppm)	Mean mortality (%) ± SE 24 h	LC_{50} (ppm) (LCL-HCL) 24 h	LC_{90} (ppm) (LCL-HCL) 24 h	χ^2 (df)

100	100 ± 0.00	68.658	88.235	
50	5.00 ± 2.575			
25	1.00 ± 1.00			
12.5	0.00 ± 0.00			
6.25	1.00 ± 1.00			
	Mean mortality (%) ± SE 48 h	LC_{50} (ppm) (LCL-HCL) 48 h	LC_{90} (ppm) (LCL-HCL) 48 h	χ^2 (df)
100	100 ± 0.00	66.573	88.431	
50	9.00 ± 3.094	(49.204-104.587)	(69.107-168.779)	
25	1.00 ± 1.00			
12.5	1.00 ± 1.00			
6.25	1.00 ± 1.00			
	Mean mortality (%) ± SE 72 h	LC_{50} (ppm) (LCL-HCL) 72 h	LC_{90} (ppm) (LCL-HCL) 72 h	χ^2 (df)
100	100 ± 0.00	57.969	80.486	46.670[a] (23)
50	30.00 ± 4.949	(52.095-66.044)	(71.271-95.904)	
25	2.00 ± 1.40			
12.5	1.00 ± 1.00			
6.25	1.00 ± 1.00			
Device® SC-15				
Concentration (ppm)	Mean mortality (%) ± SE 24 h	LC_{50} (ppm) (LCL-HCL) 24 h	LC_{90} (ppm) (LCL-HCL) 24 h	χ^2 (df)
100	83.00 ± 3.39	50.639	95.307	60.207[a] (23)
50	77.00 ± 2.550	(42.727-60.124)	(81.901-116.499)	
25	18.00 ± 2.550			

12.5	11.00 ± 1.871			
7	4.00 ± 1.871			
	Mean mortality (%) ± SE 48 h	LC_{50} (ppm) (LCL-HCL) 48 h	LC_{90} (ppm) (LCL-HCL) 48 h	χ^2 (df)
100	87.00 ± 3.742	36.376	90.588	43.440[a] (23)
50	82.00 ± 3.742	(28.809-44.395)	(77.055-112.238)	
25	35.00 ± 4.183			
12.5	29.00 ± 2.915			
7	17.00 ± 2.550			
	Mean mortality (%) ± SE 72 h	LC_{50} (ppm) (LCL-HCL) 72 h	LC_{90} (ppm) (LCL-HCL) 72 h	χ^2 (df)
100	98.00 ± 1.225	15.009	58.949	26.212 (23)
50	88.00 ± 4.062	(9.711-19.471)	(51.339-70.223)	
25	64.00 ± 3.317			
12.5	48.00 ± 3.391			
7	35.00 ± 4.472			

Mean mortality percentages and probit regression analysis (LC_{50} and LC_{90}) at 95% confidence interval, obtained in larvicidal test conducted with 2-metil-1,4-naphthochinone, 2-hydroxy-1,4-naphthoquinone, 2-methoxy-1,4-naphthoquinone and Device® SC-15 against *Ae. albopictus* three-instar larvae

LC_{50} = lethal concentration (ppm) that kills 50% of the exposed larvae.

LC_{90} = lethal concentration (ppm) that kills 90% of the exposed larvae.

[a] = Since goodness-of-fit test is significant ($P < 0.05$), a heterogeneity factor is used in the calculation of confidence limits (CL).

Table 4.
Effect of 2-methyl-1,4-naphthoquinone, 2-hydroxy-1,4-naphthoquinone and 2-metoxy-1,4-naphthoquinone and Device® SC-15, at different concentrations, on survival of *A. albopictus* three-instar larvae-pupae.

Compounds Concentration (ppm)	GLM (time × treatment)[a]		Bonferroni test[b] Mean treatment	Mean control	
2-Methyl-1,4-naphthoquinone					
6.25	$F_{2,16} = 2.667$	$P > 0.05$			
12.5	$F_{2,16} = 55.588$	$P < 0.01$	19.600 ± 0.173	20.00 ± 0.173	n.s. **
25	$F_{2,16} = 297.818$	$P < 0.01$	12.467 ± 0.133	20.00 ± 0.133	**
50	$F_{2,16} = 36.000$	$P < 0.01$	2.200 ± 0.120	20.00 ± 0.120	**
100			0.40 ± 0.047		**
2-Hydroxy-1,4-naphthoquinone				20.00 ± 0.047	
6.25	$F_{2,16} = 1.000$		0.00 ± 0.00		
12.5	$F_{2,16} = 2.667$			20.00 ± 0.00	n.s.
25	$F_{2,16} = 33.778$	$P > 0.05$	19.933 ± 0.047		n.s.
50		$P > 0.05$	19.867 ± 0.058	20.00 ± 0.047	** **
100		$P < 0.01$	17.467 ± 0.191	20.00 ± 0.058	**
2-Metoxy-1,4-naphthoquinone	$F_{2,16} = 1.000$			20.00 ± 0.191	
6.25	$F_{2,16} = 1.000$		0.00 ± 0.00		n.s.
12.5	$F_{2,16} = 28.182$		0.00 ± 0.00	20.00 ± 0.00	n.s.
25				20.00 ± 0.00	n.s.
50		$P > 0.05$	19.800 ± 0.141		**
100		$P > 0.05$	19.867 ± 0.094	20.00 ± 0.141	**
Device® SC-15	$F_{2,16} = 351.455$			20.00 ± 0.094	
6.25	$F_{2,16} = 77.509$	$P < 0.01$	19.733 ± 0.189		**
12.5	$F_{2,16} = 51.118$		16.667 ± 0.654	20.00 ± 0.189	**
25	$F_{2,16} = 10.706$		0.00 ± 0.00	20.00 ± 0.654	**
50	$F_{2,16} = 12.271$	$P < 0.01$		20.00 ± 0.00	**
100		$P < 0.01$	16.000 ± 0.307		
		$P < 0.01$	14.133 ± 0.313	20.00 ± 0.307	

	P <0.01	12.200 ± 0.304	20.00 ± 0.313
	P <0.01	3.533 ± 0.457	20.00 ± 0.304
		2.133 ± 0.338	20.00 ± 0.457
			20.00 ± 0.338

GLM values describe the effect of time on survival of the larvae-pupae.

ns not significant

*P<0.05; **P<0.01

[a] Values of P>0.05 for GLM indicate that the interaction between the two conditions (treated and control) and the change over time was not statistically significant. Values of P<0.05 for GLM indicate that the interaction between the two conditions (treated and control) and the change over time was statistically significant.

[b] Differences between the means of the number of survived larvae-pupae at different 1,4-naphtoquinone structural derivatives and concentrations in each of the experimental treatments and those of the number of related controls over time were analyzed and adjusted with Bonferroni test for the multiple of comparisons.

Figures

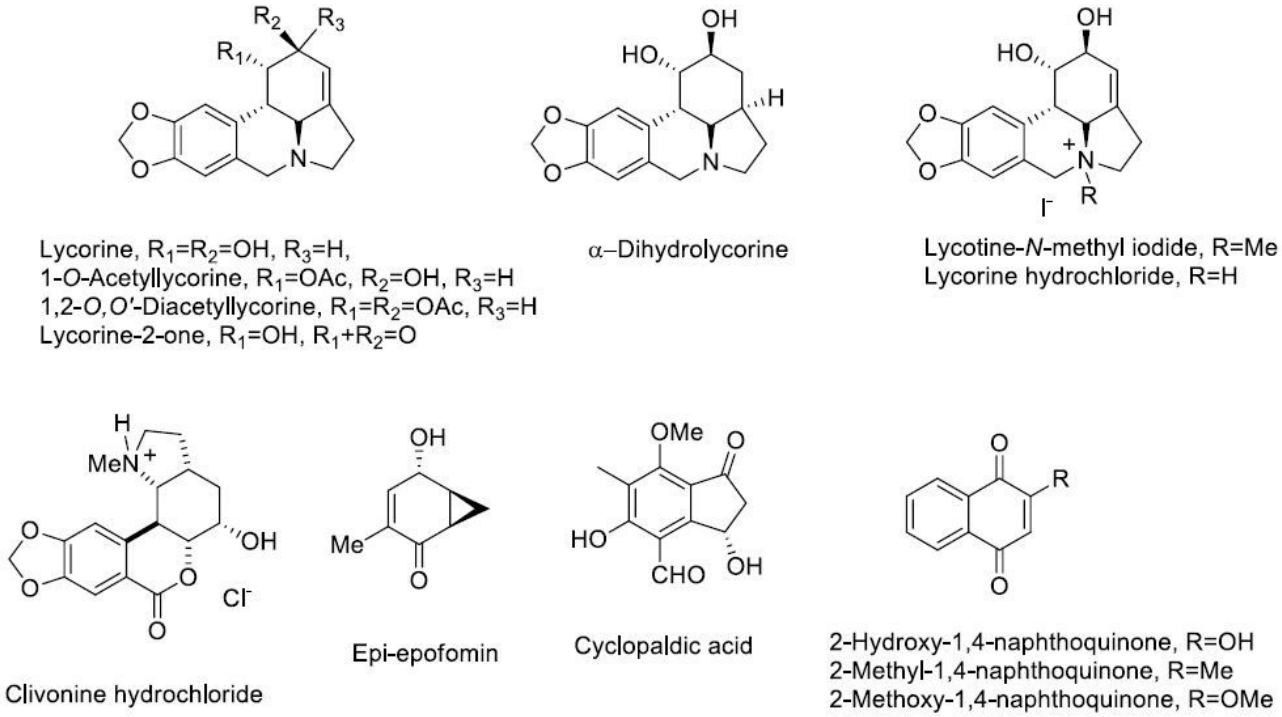

Figure 1

The structure of natural compounds, of the lycorine semisynthetic derivatives, and of the commercially available analogues of 2-methoxy-1,4-naphthoquinone

Supplementary Files

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