Article

Synthesis and Insecticidal Activity of Lactones Derived from Furan-2(5*H*)-one

Milena G. Teixeira,[a] Elson S. Alvarenga,*,[a] Mirian F. Pimentel[b] and
Marcelo C. Picanço[b]

[a]Departamento de Quimica and [b]Departamento de Entomologia,
Universidade Federal de Viçosa, 36570-900 Viçosa-MG, Brazil

Ten 4,7-methanoisobenzofuran-1(3*H*)-ones were synthesized and their insecticidal activities against the insect pest *Diaphania hyalinata* were evaluated. The most active substances have been selected from the initial screening to find the dose to kill 50 (LD_{50}) and 90% (LD_{90}) of the melonworm. Time-mortality curves of the three selected formulations at the LD_{90} concentration have been made against *D. hyalinata*. From the time-mortality curves we found that the formulation (3a*R*,4*R*,5*S*,6*S*,7*S*,7a*S*)- and (3a*S*,4*S*,5*R*,6*R*,7*R*,7a*R*)-5,6-dichlorohexahydro-4,7-methanoisobenzofuran-1(3*H*)-one + (3a*R*,4*R*,5*R*,6*R*,7*S*,7a*S*)- and (3a*S*,4*S*,5*S*,6*S*,7*R*,7a*R*)-5,6-dichlorohexahydro-4,7-methanoisobenzofuran-1(3*H*)-one has killed 50% of the melonworm after 2 h, presenting the best knockdown effect. Bioassays against *Solenopsis saevissim*a and *Tetragonisca angustula* were carried out for the lactones selected in the initial screening against *D. hyalinata*. The formulation (3a*S*,4*R*,5*S*,6*S*,7*S*,7a*R*)- and (3a*R*,4*S*,5*R*,6*R*,7*R*,7a*S*)-5,6-dibromohexahydro-4,7-methanoisobenzofuran-1(3*H*)-one + (3a*S*,4*R*,5*R*,6*R*,7*S*,7a*R*)- and (3a*R*,4*S*,5*S*,6*S*,7*R*,7a*S*)-5,6-dibromohexahydro-4,7-methanoisobenzofuran-1(3*H*)-one has killed 31.25 and 68.30% of the pest natural enemy and the pollinator bee, respectively. At the same concentration this formulation killed 90% of *D. hyalinata*. The selectivity in favor of the non-target organisms has rendered this formulation a position as a promising agrochemical.

Keywords: *Diaphania hyalinata*, *Tetragonisca angustula*, *Solenopsis saevissima*, insecticide

Introduction

The melonworm *Diaphania hyalinata* (Lepidoptera: Crambidae) is an important pest of plants of the family *Curcubitaceae* and occurs in the Americas.[1,2] The leaves are first attacked by these insects, limiting plant photosynthesis, therefore, growth and development. After this the stems and shoots are damaged leading to the plant death. When attacked by the caterpillar, fruits become unfit for human consumption.[3]

Solenopsis saevissima, an ant widely distributed in South America, is among the natural enemies of *D. hyalinata*. Worker individuals of this species are aggressive and have a painful sting.[4] Predation by *S. saevissima* contributes significantly to the biological control of agricultural crops.[5] Species of the genus *Solenopsis* have been considered important predators contributing to insect pest reduction in the tropics and subtropics.[6]

Bees are another important non-target group of organisms that contribute to the biodiversity of the ecosystems. However, an alarming fact is that during the last decades there were losses in the number of colonies in locations around the world.[7] Multiple environmental factors can potentially contribute to these losses, including biotic ones like pathogens, parasites, availability of resources due to habitat fragmentation and loss; and abiotic ones like climate change and pollutants.[8-10] Although the causes of the loss of pollinators are still under study, which currently go through a screening process, extensive use of chemical pesticides against insect pests for crop protection may have contributed to the decline of pollinators.[11] One hypothesis for the disappearance of bees relates to the use of neonicotinoid insecticides.[12-17] Concern for bees is justified by the fact that they play a vital role in agriculture.[18] Nearly 35% of the world's food crops depend on pollination mostly by insects,[19] especially bees and butterflies. The pollinator insects can increase genetic biodiversity in the cultivated species by the cross pollination leading to better fruits, seeds and plants.[20]

In recent years, several species are rapidly going into extinction because the insecticides have been planned to be very effective against pests in general, but with great

**e-mail: elson@ufv.br

potential to affect beneficial organisms.[21] This concept has fueled concerns about the ecological consequences of biodiversity loss, leading to a flurry of studies examining how changes in diversity affect the ecosystems, particularly those that provide goods and services that humans depend upon.[22,23]

Pest control should be based on equilibrium between the use of chemical agents and survival of the natural enemies.[24] Therefore, to avoid the possible interruption of the natural control of pests it is important to evaluate the effects of pesticides against the beneficial organisms.[25]

The idea that beneficial organisms and insecticides were incompatible has been modified in recent years. The outdated broad-spectrum insecticides have been superseded by more modern agrochemicals like spinosads,[26] and novel pyrethroids that present selectivity in favor of the beneficial organisms and a desirable ecotoxicology.[27] The novel pyrethroids have been evaluated for their toxicological effect against target insects and adverse activity on natural enemies.[28,29]

The conservation of beneficial organisms is an essential component in integrated pest management.[22,23,30] The most frequent source of mortality for phytophagous arthropods in ecosystems is attack by natural enemies.[5]

The synthesis of new lactones has been inspired by small molecules from natural products that have been the mainstay of research in organic chemistry since its initial development.[31] Within this context, phthalides or isobenzofuranones, natural volatile compounds present in apiaceous plants have attracted the attention of many research groups.[32] This class of compound has presented a wide spectrum of activities such as vasodilatation,[33] analgesic,[34] anti-inflammatory,[35,36] and protection effect against neuronal impairment induced by deprival of oxygen and glucose.[37]

The focus on the use of natural products for agrochemicals is widely used, because the resulting secondary metabolites of plant interaction with the environment during its evolution play an important function in plant protection against herbivores.[38-40]

Phthalide derivatives possess potential as agrochemicals because recent research indicates that a large number of compounds display insecticidal,[41,42] nematicidal,[43] and acaricidal[44] activities. These plant-based agrochemicals have presented themselves as an important tool to combat the evolution of resistance to pesticides.[45]

Therefore, the aim of this work was to investigate the toxicity of compounds of the class of phthalides against *D. hyalinata* and non-target organisms: bee pollinator (*T. angustula*) and the pest natural enemy (*S. saevissima*). The insecticidal activities of these compounds have been evaluated against the important pest of *Curcubitaceae* from which three of them have shown stunning insecticidal activities. Besides that we have been involved in functional group modification and/or stereochemistry aiming for novel compounds which could play an important role with respect to the biological activity.

Results and Discussion

Synthesis

Cycloaddition reaction of furan-2(5*H*)-one **1** and cyclopentadiene has been employed in first stage of our work for the synthesis of 4,7-methanoisobenzofuran-1(3*H*)-ones. The synthesis of the *endo* **2** and *exo* **3** adducts using this methodology is represented in Scheme 1. This reaction has been carried out with regio- and stereoselective control but without asymmetric induction. Therefore, to simplify we have opted to use only one of the enantiomers to represent each adduct.

The most distinct signals of adduct **2** in the ^{13}C nuclear magnetic resonance (NMR) spectrum were the carbonyl and the alkene carbons at δ 178.0, 134.3, and 136.7, respectively. The double bond is confirmed by the multiplet at 6.25-6.33 ppm in the ^{1}H NMR spectrum. These distinct signals have also been observed in the ^{13}C NMR of adduct **3** but at slightly different chemical shifts (δ 177.6, 137.3, and 137.6). The olefinic protons H5 and H6 of **3** are displayed as doublet of doublets at δ 6.18 and 6.23, respectively. Long distance detection experiments in the NMR (nuclear Overhauser effect (NOE)) have enabled us to differentiate these isomers. Irradiation of the signal of H8 of adduct **2** has resulted in 1.32 and 1.87% increase in the signals of
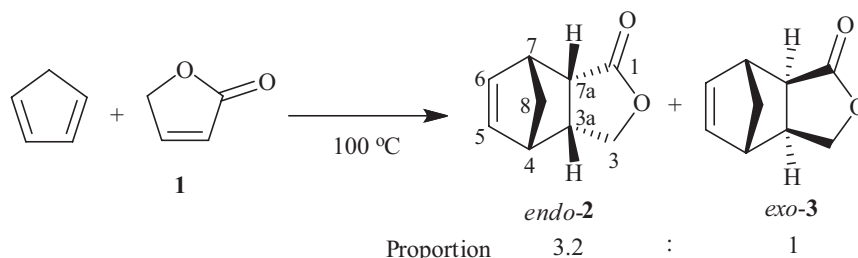
Scheme 1. Preparation of adducts **2** and **3** using Diels-Alder reaction.

H7a and H3a, respectively. However, irradiation of H8 of adduct **3** has resulted in 0.85% NOE in the signal of H3' which is close in space to H8.

In the next stage, the double bond of lactones **2** and **3** was oxidized with *meta*-chloroperbenzoic acid to give only epoxides **4** (98% yield) and **5** (83% yield) with stereospecific control (Scheme 2). In the ^{1}H NMR of **4** and **5**, H5 and H6 (δ ca. 3.2 ppm) were chemically shielded compared to H5 and H6 (δ ca. 6 ppm) at the double bond in compounds **2** and **3**, which is a strong evidence of the epoxide formation. The oxygen at the *exo* position in epoxides **4** and **5** was established by NOE experiments. Irradiation of the signal of H5/H6 of epoxide **4** has resulted in 1.17% increase in the signal of H3'. However, irradiation of the signal of H5 of epoxide **5** has led to 0.9% increase of H3a. To reinforce the *exo* position of the oxygen in the epoxide **5** we have irradiated the signal of H6 resulting in 2.03% increase of H7a.

Following the idea of preparing novel isobenzofuranone derivatives we have also performed hydrogenation, chlorination, and bromination reactions of these adducts (Scheme 2). Bromination and chlorination of unsaturated lactone **3** have proceeded stereoselectively to give only the *trans*-1,2-dihalogenated products, as expected. The structures of all compounds have been established by using the spectrometric methods, including in these methods the bidimensional NMR experiments such as heteronuclear single quantum coherence (HSQC),

correlation spectroscopy (COSY), and nuclear Overhauser effect spectroscopy (NOESY).

Insecticidal activity

In this study, we have assessed the efficacy of compounds **1-7**, **9a**, and **9b** against second-instar larvae of *D. hyalinata*. Furthermore, the insecticidal activities of the mixtures of **8a/8b** (2:1 ratio), and **9a/9b** (1:1 ratio) have also been evaluated. Compounds **8a** and **8b** have not been evaluated separately because only a small quantity of **8b** was isolated and purified (57.5 mg).

There was a significant difference in the mortality of larvae of *D. hyalinata* as a function of the treatments (analysis of variance (ANOVA), $F_{14.75} = 48.24$, $p < 0.001$). The mortality of *D. hyalinata* as a result of the treatments is presented in Figure 1 as a histogram with standard deviation bars and assignment letters according to the Scott-Knott grouping analysis test at $p < 0.05$. The compounds **1**, **9a**, **9a + 9b**, **9b**, and **8a + 8b** have presented significant insecticidal activities killing more than the negative control. The lactones **9a + 9b**, **9b**, and **8a + 8b** at the concentration of 45.2 µmol g^{-1} insect were the most potent, killing 84.8, 91.3 and 96.3% of *D. hyalinata*, respectively. These compounds were more active than the commercial piperine, which has presented 64.5% mortality.[46] Compounds **1** and **9a** have shown moderate activities of 23.1 and 40.0%, respectively.
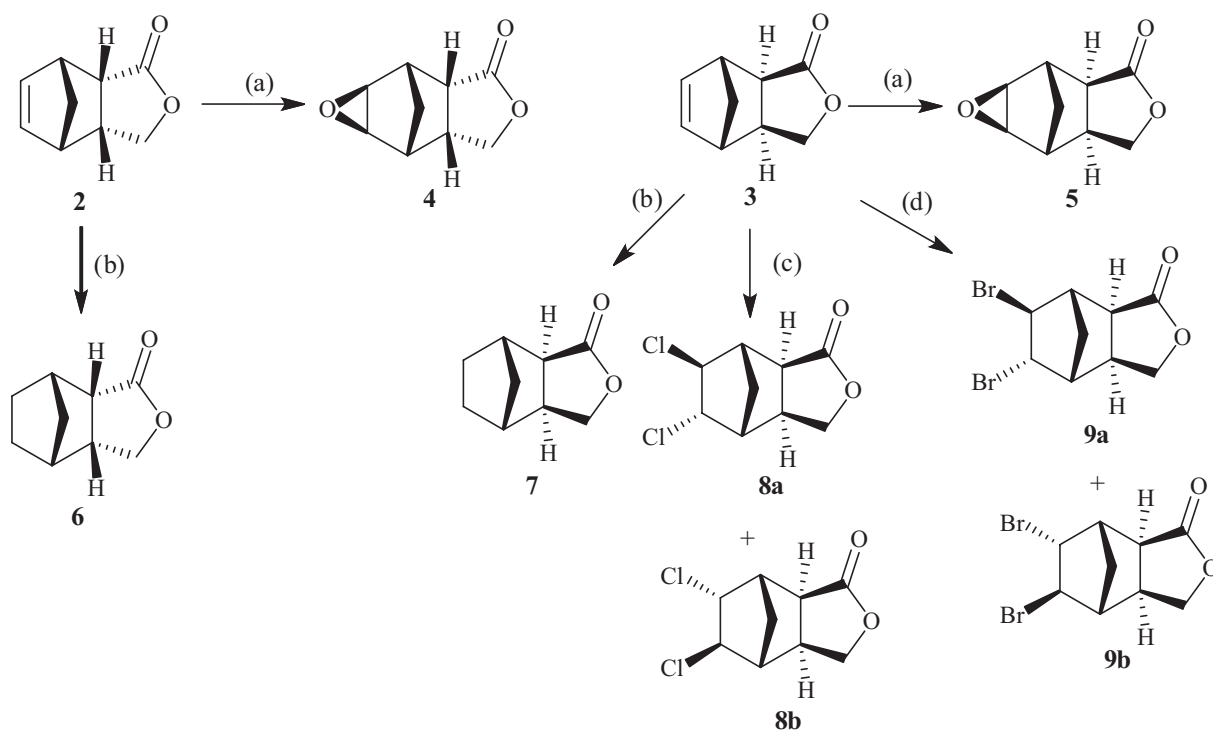
Scheme 2. Reagents: (a) *meta*-chloroperbenzoic acid (MCPBA), CH$_2$Cl$_2$; (b) H$_2$, Pd/C (10%), EtOH; (c) Cl$_2$, CH$_2$Cl$_2$; (d) Br$_2$, CH$_2$Cl$_2$.
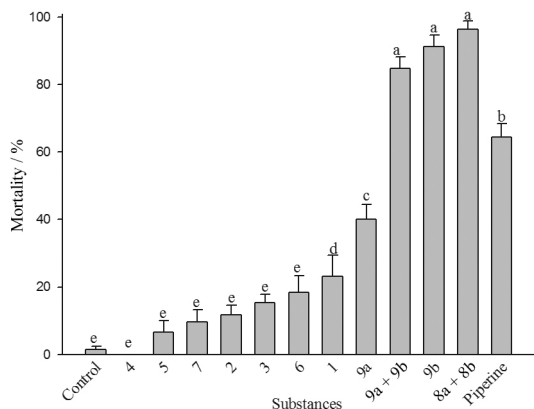
Figure 1. Mortality (mean ± standard error) of second-instar larvae of *D. hyalinata*. Columns followed by the same letter are not significantly different by the Scott-Knott grouping analysis test at $p > 0.05$.

Piperine was used as the positive control because the insecticidal activity of this substance has been extensively described in the literature.[47-51] Furthermore, extracts of black pepper *Piper nigrum* L. (Piperaceae), whose main constituent is piperine are toxic to Lepidoptera insects, including *Ascia monuste orseis* Latreile (Lepidoptera: Pieridae)[52] and *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae).[53]

The isolated isomers **9a** and **9b**, in the concentration of 45.2 μmol g^{-1} insect and a period of 48 h, have caused 40.0 and 91.3% mortalities of the insects, respectively. According to Ahern and Whitney,[54] many secondary metabolite classes are responsible for the plant resistance against herbivore. Therefore, totally different compounds could play an important defense mechanism for the plants. To the best of our knowledge we have not found many references describing the ecological effects played by modifying the stereochemistry of the active ingredients.[55-59]

In some cases, individual isomers have identical bioactivities, but in other cases different isomers can drastically differ in bioactivity.[60]

A significant difference in insecticide activity has been observed for stereoisomers **9a** and **9b**. The isomer **9b** is the most active killing 91.3% of the insect pest. However the formulation **9a + 9b** at the ratio of 1:1 has killed 84.8% of the pest, which does not differ significantly from that obtained for the pure isomer **9a**. Therefore, the laborious

separation of the chemicals **9a** and **9b** is not necessary considering only the difference in insecticidal activity.

The most active lactones **8a + 8b**, **9a + 9b**, and **9b** were selected for a more thorough investigation after the general insecticidal bioassay. This selection is in line with the Brazilian Health Surveillance Agency (ANVISA) for tests of efficacy on pest control products, which recommends that only values between 90 ± 10% mortality should be considered satisfactory.[61]

The dose-mortality results from the lactones application in larvae of *D. hyalinata* have shown low chi-square test (χ^2) and high p values (< 2.39 and > 0.05, respectively) indicating the suitability of the probit model for fitting the dose-response curves. Curves with acceptance probability of the null hypothesis by χ^2 greater than 0.05 were accepted. The doses to kill 50 (LD$_{50}$) and 90% (LD$_{90}$) of the insects were obtained from these curves (Figure 2).
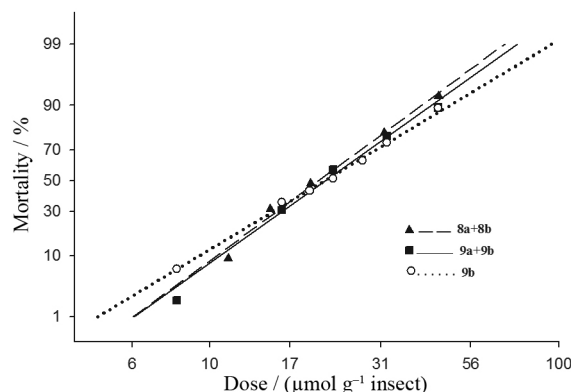

Figure 2. Dose-mortality curves of the compounds **8a + 8b**, **9a + 9b**, and **9b** against *D. hyalinata* caterpillars.

According to Table 1, the formulation **8a + 8b** exhibited the highest toxicity to *D. hyalinata*, with lethal doses of 20.58 (LD$_{50}$) and 40.37 (LD$_{90}$) μmol g^{-1} insect. However, lactones **9a + 9b** and **9b** have shown similar insecticidal activities.

The curves with the activity rates for the most promising chemicals at the LD$_{90}$ concentration against *D. hyalinata* are presented in Figure 3. The survival analysis of *D. hyalinata* exposed to lactones indicated significant differences among treatments (log-rank test, $\chi^2 = 190.28$, d$f = 3$, $p < 0.001$).

Table 1. Toxicity of the chemicals **8a + 8b**, **9a + 9b** and **9b** against second instar *D. hyalinata* caterpillars 48 h after application

Lactones	LD$_{50}$[a] / (μmol g^{-1} insect)	LD$_{90}$[a] / (μmol g^{-1} insect)	y	χ^2	df	p
8a + 8b	20.58 (19.37-21.86)	32.03 (29.57-35.39)	−0.75 + 4.38x	2.39	4	0.67
9a + 9b	21.49 (19.95-23.08)	33.90 (31.10-37.67)	−0.66 + 4.25x	1.88	3	0.60
9b	21.40 (19.97-22.86)	36.73 (33.55-41.21)	0.24 + 3.58x	0.72	5	0.98

[a]The numbers in parentheses are the confidence intervals. LD$_{50}$ and LD$_{90}$: lethal dose to kill 50 and 90% of the insects, respectively; y: curve equation; χ^2: chi-square test; df: degree of freedom; p: probability.

The chemicals **8a + 8b**, **9a + 9b** and **9b** have killed 50% of the population of the pest in intervals of 2, 4 and 3 h, respectively. The rate of activity of the insecticide is very important to control the outbreak of pests in the agriculture. The fast population growth of pests should be controlled to avoid great damages to the crops. The formulation **8a + 8b** has presented the best time mortality result, which is adequate to control the outbreak of pests because it has killed 50% of the population of *D. hyalinata* in the shortest period (2 h).
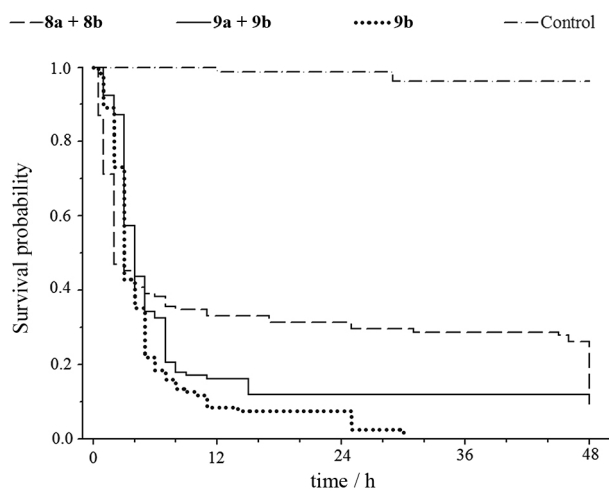

Figure 3. Survival curves of *D. hyalinata* subjected to the application of the chemicals **8a + 8b**, **9a + 9b**, and **9b** at the LD_{90} concentration.

The insecticides have been planned to be very effective against pests in general, but with great potential to affect beneficial organisms. Thus we have evaluated the toxicity of eleven chemical formulations against the pest *D. hyalinata*, and the three most promising formulations have been tested against the beneficial bee pollinator and the natural enemy fire ant.

The formulations **8a + 8b**, **9a + 9b**, and **9b** have been evaluated against the beneficial organisms *S. saevissima* and *T. angustula* (Table 2). The results from this study showed that lactones **9b** (t_{10} = 6.35, $p < 0.001$) and

Table 2. Mortalities of the pest natural enemy *S. saevissima* and the pollinator *T. angustula* 48 h after application of the formulations **8a + 8b**, **9a + 9b**, and **9b** at 40.37, 43.03, and 48.74 mmol g^{-1} of insect, respectively

Lactones	Average mortality ± standard error / %	
	S. saevissima	*T. angustula*
8a + 8b	91.37 ± 4.24	82.61 ± 5.74
9a + 9b	31.25 ± 12.81[a]	68.30 ± 7.03[a]
9b	46.99 ± 6.79[a]	65.22 ± 6.59[a]

[a]Significantly different from the mortality caused by these chemicals to *D. hyalinata* by *t*-test at $p < 0.05$.

9a + 9b (t_{10} = 4.59, $p < 0.001$) were selective in favor of *S. saevissima* and *T. angustula* (more toxic to the pest than to the natural enemy).

Besides the efficiency against insect pests, novel agrochemical agents should preferably provide selectivity to non-target species, especially predators and pollinators. This is important because natural enemies can aid in the control of the population of pests in agricultural crops.[62] Moreover, the conservation and enhancement of natural enemy activity in agroecosystems is one of the key elements of sustainable agricultural production.[63-67]

However, the natural enemies alone are not able to prevent any damage caused by the pest.[24] Because of this, finding a balance between the use of chemical insecticides and the survival of natural enemies is a priority of integrated pest management.

Furthermore, pollination is essential to ensure the reproduction of most plants.[68] Thus pollinator should be retained, so that the biodiversity of the ecosystem is maintained.[69,70]

The International Organization for Biological Control (IOBC) uses a standardized classification for the impact of pesticides on natural enemies for experiments in laboratory which consists of four categories: harmless (< 30% effect), slightly harmful (30-79% effect), moderately harmful (80-99% effect), and harmful (> 99% effect).[71] The formulation **9a + 9b** has killed 31.25 and 68.30% of *S. saevissima* and *T. angustula*, respectively. However, the isolated compound **9b** has killed 46.99 and 65.33% of *S. saevissima* and *T. angustula*, respectively. The selectivity of these lactones in favor of *S. saevissima* and *T. angustula* surpasses the negative impact of the outdated broad-spectrum insecticides which kill the insects without selectivity. The selectivity provided by lactones (**9a + 9b** and **9b**) to *S. saevissima* and *T. angustula* suggests that the use of these compounds to control *D. hyalinata* presents a low risk to these non-target insects according to the IOBC classification.

To sum up we have found that a number of structural features have influenced the insecticidal activity, particularly the presence of halogens and the *cis*-relationship between the lactone ring and the methylene bridge. The most active compounds were the *exo* adducts and the ones containing halogens which is in agreement with literature reports where various compounds with biological activities such as fungicide, herbicide, antibiotic, and insecticide have been described.[72-74]

Conclusions

In summary, we have synthesized and determined the insecticidal activity of ten lactones. The agrochemicals

9a + 9b, **9b**, and **8a + 8b** at the concentration of 45.2 µmol g⁻¹ insect were the most active against *D. hyalinata* killing 84.8, 91.3 and 96.3% of the worms, respectively. The activities presented by these lactones were better than the commercial positive control. The lactones **9a + 9b** and **9b** were selective in favor of non-target organisms. Therefore, these lactones are promising as potential novel agrochemicals for the integrated pest management.

Experimental

General

Reagents and solvents were purified, when necessary, according to the procedures described by Perrin and Armarego.[75] The reactions were followed by visualizing the thin layer chromatography (TLC) plates coated with silica-gel in an ultraviolet chamber at 254 nm.[76] The furan-2(5H)-one was obtained as previously described.[77] The cyclopentadiene was obtained by distillation of dicyclopentadiene commercially available (Sigma-Aldrich) before being used in the Diels-Alder reaction. The ¹H, ¹³C NMR, COSY, HSQC, heteronuclear correlation (HETCOR), heteronuclear multiple-bond correlation (HMBC), NOE difference (NOEDIFF), and NOESY spectra were recorded on a Varian Mercury 300 instrument (300 MHz), using deuterated chloroform as solvent. Infrared (IR) spectra were recorded on a Varian 660-IR, equipped with GladiATR scanning from 4000 to 500 cm⁻¹. Mass spectra (MS) were recorded on a Shimadzu GCMS-QP5050A instrument under electron impact (70 eV) conditions. Melting points are uncorrected and were obtained using MQAPF-301 melting point apparatus (Microquímica). Column chromatography was performed over silica gel (60-230 mesh).

Synthesis of lactones

(3aR,4S,7R,7aS)- and (3aS,4R,7S,7aR)-3a,4,7,7a-tetrahydro-4,7-methanoisobenzofuran-1(3H)-one (2) and (3aS,4S,7R,7aR)- and (3aR,4R,7S,7aS)-3a,4,7,7a-tetrahydro-4,7-methanoisobenzo-furan-1(3H)-one (3)

Furan-2(5H)-one **1** (1.0012 g, 11.9 mmol) and cyclopentadiene (8.1 g, 0.12 mol) have been added to a sealed tube. The resulting reaction mixture has been magnetically stirred and heated at 100 °C for 72 h. The excess of cyclopentadiene has been evaporated, and the crude product was purified by column chromatography (eluent: hexane:ethyl acetate 2:1 v/v) to give 1.139 g (66% yield) of **2** and 0.363 g (18% yield) of **3**.

Data for compound 2

White solid; TLC R_f = 0.44 (hexane:ethyl acetate 2:1 v/v); m.p. 119.8-120.6 °C; IR (film) ν_{max} / cm⁻¹ 3065, 2097, 2873, 1765, 1481, 1381, 1345, 1185, 1001; ¹H NMR (300 MHz, CDCl₃) δ 1.46 (d, 1H, *J* 8.6 Hz, H8), 1.64 (d, 1H, *J* 8.6 Hz, H8'), 3.04-3.15 (m, 2H, H3a and H4), 3.25 (dd, 1H, *J* 9.2, 4.6 Hz, H7a), 3.30-3.36 (m, 1H, H7), 3.79 (dd, 1H, *J* 9.7, 3.1 Hz, H3'), 4.28 (dd, 1H, *J* 9.7, 8.3 Hz, H3), 6.25-6.33 (m, 2H, H5 and H6); ¹³C NMR (75 MHz, CDCl₃) δ 40.1 (C3a), 45.6 (C7), 46.0 (C4), 47.4 (C7a), 51.7 (C8), 70.2 (C3), 134.3 (C5), 136.7 (C6), 178.0 (C1); MS *m/z* (%) 91 (7), 85 (11), 66 (100).

Data for compound 3

Colorless oil; TLC R_f = 0.56 (hexane:ethyl acetate 2:1 v/v); IR (film) ν_{max} / cm⁻¹ 3061, 2974, 2909, 1761, 1485, 1381, 1185, 1005; ¹H NMR (300 MHz, CDCl₃) δ 1.47 (dt, 1H, *J* 9.8, 1.7 Hz, H8), 1.55 (dquint, 1H, *J* 9.8, 1.7 Hz, H8'), 2.51-2.60 (m, 1H, H3a), 2.65 (dt, 1H, *J* 8.5, 1.7 Hz, H7a), 2.90 (sl, 1H, H4), 3.27 (sl, 1H, H7), 3.96 (dd, 1H, *J* 9.8, 3.5 Hz, H3'), 4.48 (dd, 1H, *J* 9.8, 8.7 Hz, H3), 6.18 (dd, 1H, *J* 5.7, 3.0 Hz, H5) 6.23 (dd, 1H, *J* 5.7, 3.0 Hz, H6); ¹³C NMR (75 MHz, CDCl₃) δ 41.7 (C3a), 43.1 (C8), 46.2 (C7), 47.8 (C7a), 48.0 (C4), 71.7 (C3), 137.3 (C6), 137.6 (C5), 177.6 (C1); MS *m/z* (%) 150 ([M]⁺, 1), 91 (7), 85 (10), 66 (100).

(3aR,4R,5R,6S,7S,7aR)- and (3aS,4S,5S,6R,7R,7aS)-5,6-epoxyhexahydro-4,7-methanoisobenzofuran-1(3H)-one (4)

meta-Chloroperbenzoic acid (MCPBA) (2.6366 g of MCPBA 70%, 15.2 mmol) was added portionwise to a round-bottom flask containing phthalide **2** (1.1386 g, 7.59 mmol) and dichloromethane (100 mL). The reaction mixture was stirred for 15 h and quenched with aqueous Na₂SO₃ solution (20%, 100 mL). The mixture was extracted with diethyl ether (3 × 100 mL), and the combined organic layers were washed with aqueous NaHCO₃ solution (10%, 100 mL) and brine until neutral. The organic layer was dried with anhydrous MgSO₄ and filtered. After evaporation of solvent, the product mixture was purified by column chromatography with silica-gel using hexane:ethyl acetate 1:1 as eluent to give 1.238 g of **4** (98% yield): white solid; TLC R_f = 0.36 (hexane:ethyl acetate 1:1 v/v); m.p. 120.9-121.7 °C; IR (film) ν_{max} / cm⁻¹ 2997, 2961, 1761, 1381, 1193, 1001, 849; ¹H NMR (300 MHz, CDCl₃) δ 0.96 (d, 1H, *J* 10.2 Hz, H8), 1.56 (d, 1H, *J* 10.2 Hz, H8'), 2.70-2.74 (m, 1H, H4), 2.95-2.99 (m, 1H, H7), 2.99-3.06 (m, 1H, H3a), 3.11 (dd, 1H, *J* 10.3, 5.0 Hz, H7a), 3.32-3.36 (m, 2H, H5 and H6), 4.26-4.41 (m, 2H, H3 and H3'); ¹³C NMR (75 MHz, CDCl₃) δ 29.3 (C8), 39.2 (C7), 39.9 (C4), 41.3 (C3a), 45.5 (C7a), 47.3 (C5)*, 48.5 (C6)*, 67.6 (C3), 176.7 (C1); MS *m/z* (%) 138 (11), 110 (13), 109

(20), 92 (17), 91 (25), 82 (42), 81 (100), 80 (11), 79 (49), 78 (19), 77 (45), 70 (27), 69 (57), 66 (17), 65 (14), 55 (17), 54 (27), 53 (29), 51 (21), 41 (22), 40 (12). *These assignments could be reversed.

(3a*S*,4*R*,5*R*,6*S*,7*S*,7a*S*)- and (3a*R*,4*S*,5*S*,6*R*,7*R*,7a*R*)-5,6-epoxyhexahydro-4,7-methanoisobenzofuran-1(3*H*)-one (**5**)

Epoxide **5** was obtained (137.5 mg) in 83% yield by reaction of lactone **3** (150 mg, 1.0 mmol) with 70% MCPBA (346.8 mg, 2.0 mmol), by following the same procedure described for lactone **4**: white solid; TLC R_f = 0.30 (hexane:ethyl acetate 1:1 v/v); m.p. 112.9-113.4 °C; IR (film) v_{max} / cm^{-1} 2969, 1769, 1385, 1189, 1001, 853; ^{1}H NMR (300 MHz, CDCl$_3$) δ 0.87 (dm, 1H, *J* 11.2 Hz, H8), 1.42 (dquint, 1H, *J* 11.2, 1.9 Hz, H8'), 2.57 (sl, 1H, H4), 2.61 (ddt, 1H, *J* 8.9, 3.6, 1.9 Hz, H3a), 2.65-2.70 (m, 1H, H7a), 3.94 (sl, 1H, H7), 3.16 (d, 1H, *J* 4.4 Hz, H5), 3.26 (d, 1H, *J* 4.4 Hz, H6) 3.97 (dd, 1H, *J* 9.7, 3.6 Hz, H3'), 4.49 (dd, 1H, *J* 9.7, 8.9 Hz, H3); ^{13}C NMR (75 MHz, CDCl$_3$) δ 21.3 (C8), 39.1 (C3a), 41.6 (C7), 43.2 (C4), 45.6 (C7a), 50.5 (C5), 50.9 (C6), 71.4 (C3), 177.3 (C1); MS *m/z* (%) 93 (21), 91 (27), 82 (92), 81 (100), 79 (34), 77 (43), 66 (12), 65 (11), 54 (22), 53 (21), 51 (18), 41 (15), 40 (9).

(3a*R*,4*R*,7*S*,7a*S*)- and (3a*S*,4*S*,7*R*,7a*R*)-hexahydro-4,7-methanoisobenzofuran-1(3*H*)-one (**6**)

To a 25 mL round-bottom flask were added compound **2** (0.1024 g, 0.68 mmol), ethanol (10 mL) and Pd/C 10% (7.3 mg). The resulting mixture was stirred under hydrogen atmosphere for 1 h. After the solvent evaporation the residue was purified by column chromatography with silica-gel using hexane:ethyl acetate 1:1 as eluent to give 73.1 mg of **6** (70% yield): white solid; TLC R_f = 0.63 (hexane:ethyl acetate 1:1 v/v); m.p. 64.8-65.6 °C; IR (film) v_{max} / cm^{-1} 2961, 2881, 1761, 1485, 1381, 1172, 1069, 997; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.50-1.63 (m, 6H, H5, H6, H8 and H8'), 2.34-2.40 (m, 1H, H4), 2.64-2.69 (m, 1H, H7), 2.80-3.92 (m, 1H, H3a), 2.98 (dd, 1H, *J* 11.2, 5.7 Hz, H7a) 4.21-4.34 (m, 2H, H3 and H3'); ^{13}C NMR (75 MHz, CDCl$_3$) δ 21.5 (C5)*, 25.4 (C6)*, 39.8 (C7), 40.3 (C4), 41.9 (C8/C3a), 46.7 (C7a), 68.4 (C3), 178.8 (C1); MS *m/z* (%) 93 (13), 91 (12), 85 (40), 80 (33), 79 (31), 77 (15), 68 (12), 67 (32), 66 (100), 65 (10), 53 (9), 41 (19). *These assignments could be reversed.

(3a*S*,4*R*,7*S*,7a*R*)- and (3a*R*,4*S*,7*R*,7a*S*)-hexahydro-4,7-methanoisobenzofuran-1(3*H*)-one (**7**)

Product **7** was obtained (197.8 mg, 1.30 mmol) in 92% yield by reaction of lactone **3** (0.2132 mg, 1.42 mmol) and Pd/C 10% (14.2 mg) under hydrogen atmosphere, by using the same procedure described for the synthesis of lactone **6**: white solid; TLC R_f = 0.30 (hexane:ethyl acetate

1:1 v/v); m.p. 112.9-113.4 °C; IR (film) v_{max} / cm^{-1} 2969, 1769, 1385, 1189, 1001, 853; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.15-1.38 (m, 4H, H5, H6, H8 and H8'), 1.45-1.67 (m, 2H, H5' and H6'), 2.24-2.28 (m, 1H, H4), 2.42 (tdd, 1H, *J* 8.7, 3.6, 1.3 Hz, H3a), 2.51 (dt, 1H, *J* 8.7, 1.3 Hz, H7a), 2.65-2.69 (1H, m, H7), 3.89 (dd, 1H, *J* 9.5, 3.8 Hz, H3'), 4.43 (dd, 1H, *J* 9.5, 9.0 Hz, H3); ^{13}C NMR (75 MHz, CDCl$_3$) δ 27.1 (C5)*, 27.8 (C6)*, 33.4 (C8), 40.5 (C7), 41.7 (C3a), 42.6 (C4), 48.0 (C7a), 72.9 (C3), 179.6 (C1); MS *m/z* (%) 152 ([M]$^+$, 14), 93 (19), 91 (11), 85 (10), 81 (10), 80 (100), 79 (72), 78 (11), 77 (20), 68 (17), 67 (39), 66 (86), 64 (15), 54 (14), 53 (13), 51 (10), 41 (27), 40 (10). *These assignments could be reversed.

(3a*R*,4*R*,5*S*,6*S*,7*S*,7a*S*)- and (3a*S*,4*S*,5*R*,6*R*,7*R*,7a*R*)-5,6-dichlorohexahydro-4,7-methanoisobenzofuran-1(3*H*)-one (**8a**) and (3a*R*,4*R*,5*R*,6*R*,7*S*,7a*S*)- and (3a*S*,4*S*,5*S*,6*S*,7*R*,7a*R*)-5,6-dichlorohexahydro-4,7-methanoisobenzofuran-1(3*H*)-one (**8b**)

Chlorine was bubbled for 20 min into a solution of lactone **3** (537.1 mg, 3.58 mmol) dissolved in dichloromethane (20 mL). The chlorine was produced by adding concentrated HCl to a two-neck round-bottom flask containing MnO$_2$ under vigorous stirring. The progress of the reaction was checked by TLC, and the mixture was stirred until all the substrate was fully consumed. The solvent was evaporated and the pure product was obtained after column chromatography packed with silica-gel using hexane:diethyl ether 1:1 as eluent to give 0.2761 g (35% yield) of **8a** and 0.0575 g (7% yield) of **8b**.

Data for compound 8a

Colorless oil; TLC R_f = 0.37 (hexane:ether 1:1 v/v); IR (film) v_{max} / cm^{-1} 2981, 2913, 1769, 1485, 1385, 1273, 1189, 1052, 1009, 945, 812, 776, 689, 641, 480; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.55-1.64 (m, 1H, H8), 2.00 (dquint, 1H, *J* 12.1, 1.6 Hz, H8'), 2.53-2.58 (m, 1H, H4), 2.70 (d, 1H, *J* 8.9 Hz, H7a), 2.86 (sl, 1H, H7), 3.20 (tdd, 1H, *J* 8.9, 3.7, 1.6 Hz, H3a), 3.74 (t, 1H, *J* 3.0 Hz, H6), 3.97 (dd, 1H, *J* 9.7, 3.7 Hz, H3'), 4.24 (dd, 1H, *J* 4.0, 3.0 Hz, H5), 4.56 (t, 1H, *J* 9.7 Hz, H3); ^{13}C NMR (75 MHz, CDCl$_3$) δ 30.4 (C8), 33.6 (C3a), 45.6 (C7a), 49.3 (C4), 49.6 (C7), 66.5 (C6), 67.4 (C5), 71.6 (C3), 177.3 (C1); MS *m/z* (%) 224 ([M+4]$^+$, 0.22), 222 ([M+2]$^+$, 1.21), 220 ([M]$^+$, 1.70), 141 (12), 105 (29), 91 (25), 80 (100), 79 (52), 78 (11), 77 (23), 66 (38), 65 (42), 63 (19), 53 (17), 52 (15), 51 (44), 50 (15), 49 (10), 41 (15), 40 (13).

Data for compound 8b

Colorless oil; TLC R_f = 0.31 (hexane:ether 1:1 v/v); IR (film) v_{max} / cm^{-1} 2975, 2911, 1769, 1485, 1385, 1189,

1048, 1009, 821, 785, 653, 493; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.57-1.66 (m, 1H, H8), 2.03 (dquint, 1H, *J* 12.1, 1.7 Hz, H8'), 2.49 (sl, 1H, H4), 2.61 (tdd, 1H, *J* 9.0, 3.7, 1.7 Hz, H3a), 2.93-2.97 (m, 1H, H7), 3.27 (dt, 1H, *J* 9.0, 1.7 Hz, H7a), 3.70 (t, 1H, *J* 2.7 Hz, H5), 3.97 (dd, 1H, *J* 9.9, 3.7 Hz, H3'), 4.30 (dd, 1H, *J* 4.1, 2.7 Hz, H6), 4.54 (dd, 1H, *J* 9.9, 9.2 Hz, H3); ^{13}C NMR (75 MHz, CDCl$_3$) δ 30.5 (C8), 39.3 (C3a), 47.5 (C7a), 47.6 (C7), 52.1 (C4), 66.3 (C5), 67.2 (C6), 71.5 (C3), 178.6 (C1); MS *m/z* (%) 224 ([M+4]$^+$, 0.31), 222 ([M+2]$^+$, 1.88), 220 ([M]$^+$, 2.87), 141 (13), 105 (50), 91 (40), 80 (100), 79 (72), 78 (16), 77 (31), 75 (12), 66 (66), 65 (65), 63 (31), 53 (25), 52 (22), 51 (74), 50 (27), 49 (20), 41 (23), 40 (24).

(3a*S*,4*R*,5*S*,6*S*,7*S*,7a*R*)- and (3a*R*,4*S*,5*R*,6*R*,7*R*,7a*S*)-5,6-dibromohexahydro-4,7-methanoisobenzofuran-1(3*H*)-one (**9a**) and (3a*S*,4*R*,5*R*,6*R*,7*S*,7a*R*)- and (3a*R*,4*S*,5*S*,6*S*,7*R*,7a*S*)-5,6-dibromohexahydro-4,7-methanoisobenzofuran-1(3*H*)-one (**9b**)

The phthalide **3** (420.3 mg, 2.80 mmol) was dissolved in dichloromethane (20 mL) in a round-bottom flask with a magnetic stirring bar. Then a solution of bromine in dichloromethane was added dropwise using a Pasteur pipette. The reaction mixture was stirred until the substrate was fully consumed. When the reaction was completed by TLC (1 h), the mixture was concentrated. The product mixture was purified by column chromatography with silica-gel using hexane:diethyl ether 1:1 to give 0.1952 g (23% yield) of **9a** and 0.1858 g (21% yield) of **9b**.

Data for compound **9a**

Colorless oil; TLC R$_f$ = 0.31 (hexane:diethyl ether 1:1 v/v); IR (film) ν_{max} / cm^{-1} 2976, 2917, 1761, 1475, 1457, 1371, 1180, 1052, 996, 754, 677, 605, 543, 466; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.56-1.64 (m, 1H, H8), 2.08 (d, 1H, *J* 12.1 Hz, H8'), 2.55 (d, 1H, *J* 3.7 Hz, H4), 2.72 (d, 1H, *J* 8.9 Hz, H7a), 2.92 (s, 1H, H7), 3.22 (tdd, 1H, *J* 8.9, 3.7, 1.9 Hz, H3a), 3.88 (t, 1H, *J* 3.0 Hz, H6), 3.96 (dd, 1H, *J* 9.9, 3.6 Hz, H3'), 4.43 (t, 1H, *J* 3.7 Hz, H5), 4.56 (t, 1H, *J* 9.6 Hz, H3); ^{13}C NMR (75 MHz, CDCl$_3$) δ 30.6 (C8), 35.7 (C3a), 46.2 (C7a), 50.0 (C4/C7), 56.0 (C6), 58.0 (C5), 71.7 (C3), 177.0 (C1); MS *m/z* (%) 231 (33), 229 (35), 106 (31), 105 (100), 92 (11), 91 (60), 80 (19), 79 (48), 78 (22), 77 (40), 66 (86), 65 (85), 63 (36), 53 (28), 52 (32), 51 (78), 50 (34), 41 (26), 40 (33).

Data for compound **9b**

Colorless oil; TLC R$_f$ = 0.29 (hexane:diethyl ether 1:1 v/v); IR (film) ν_{max} / cm^{-1} 2977, 2911, 1761, 1478, 1380, 1180, 1053, 1009, 757, 613, 485; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.60-1.66 (m, 1H, H8), 2.13 (dquint, 1H, *J* 12.1,

1.8 Hz, H8'), 2.55 (sl, 1H, H4), 2.63 (tdd, 1H, *J* 8.7, 3.6, 1.8 Hz, H3a), 2.92-2.96 (m, 1H, H7), 3.31 (ddd, 1H, *J* 8.7, 1.8, 1.8 Hz, H7a), 3.85 (t, 1H, *J* 2.9 Hz, H5), 3.97 (dd, 1H, *J* 10.0, 3.6 Hz, H3'), 4.47-4.56 (m, 2H, H3 and H6); ^{13}C NMR (75 MHz, CDCl$_3$) δ 30.8 (C8), 40.0 (C3a), 43.0 (C7a), 48.3 (C7), 52.6 (C4), 55.9 (C5), 57.6 (C6), 71.6 (C3), 178.6 (C1); MS *m/z* (%) 231 (40), 229 (43), 106 (29), 105 (100), 91 (60), 80 (16), 79 (45), 78 (21), 77 (34), 66 (79), 65 (73), 63 (31), 55 (14), 53 (23), 52 (24), 51 (68), 50 (28), 41 (26), 40 (30).

Biological assays

Biological assays were conducted with second-instar larvae of *D. hyalinata* and adults of *S. saevissima* and *T. angustula*. Larvae of *D. hyalinata* were obtained from a laboratory rearing and adults of *S. saevissima* and *T. angustula* were collected from nests located at the University campus.

Screening bioassay for *D. hyalinata*

The experimental design was completely randomized with six replications. Each experimental unit consisted of a glass petri dish (9.5 cm × 2.0 cm) containing ten insects. The average weights of the insects were obtained by measuring, on an analytical balance, the mass of ten insects and taking the average. Bioassays were conducted by topical application. A 10 μL Hamilton micro syringe was employed to apply, on the thoracic tergite of each individual insect, 0.5 μL of a solution of the test compound, dissolved in acetone, corresponding to a concentration of 45.2 μmol g^{-1} insect. In a negative control experiment, carried out under the same conditions, 0.5 μL of acetone was applied on each insect. After the application, the insects were kept in individual petri dishes containing discs of chayote leaf as food. The petri dishes were placed in an incubator at 25 ± 0.5 ºC and 75 ± 5% relative humidity with a photoperiod of 12 h. The mortality counts were made after 6, 12, 24 and 48 h of treatment. Mortalities included dead individuals as well as those without movements. The insecticidal activity of piperine (Sigma-Aldrich) was taken as positive control.

Dose-mortality bioassays for *D. hyalinata*

The most active lactones against *D. hyalinata* were subjected to experiments to obtain dose-mortality curves. The experimental design was completely randomized with six replications. Each experimental unit consisted of a glass petri dish (9.5 cm × 2.0 cm) containing ten insects. Initially, four doses of each compound were tested to identify the range of concentrations that would provide mortalities greater than zero and less than 100%. Once the ranges of

concentrations were defined, other doses were tested for each compound. The number of doses used to obtain the dose-mortality curves varied from five to seven. Bioassays were conducted by topical application using the same procedure described above.

Time-mortality bioassays for *D. hyalinata*

The most active lactones were subjected to experiments to obtain curves of survival. The experimental design was completely randomized with twelve replicates. Each experimental unit consisted of ten insects kept on a glass Petri dish (9 cm diameter × 2 cm height) covered with organza. Bioassays were conducted by the same procedure as that described above. The dose used was equivalent to LD_{90} obtained for the three most active lactones. Insect mortality was observed every 30 min during the initial 12 h exposure followed by 5 h intervals of observation until the death of approximately 90% of insects' populations.

Risk assessment to non-target insects

To determine the magnitude of selectivity of the compounds, LD_{90} for the most active lactones against *D. hyalinata* were applied to beneficial insects. The experimental design was completely randomized with six replications. Bioassays were conducted using the same procedure to that described above. *T. angustula* was subjected to a photoperiod of 12 h while *S. saevissima* was kept in the dark during the experiment. After application, the insects were kept in individual Petri dishes, containing a mixture of sugar (85%), honey (15%) and water as food. The mortality counts have been made after 6, 12, 24 and 48 h after treatment.

Data analysis

Mortality data were subjected to analysis of variance, and the averages were compared by the Scott-Knott grouping analysis test ($p < 0.05$). Dose-mortality data of active compounds were corrected by Abbott's method[78] and then subjected to probit analysis[79] using PROC PROBIT procedure of Statistical Analysis System (SAS) program[80] to estimate dose-mortality curves. The curves that presented probabilities greater than 0.05 by the χ^2 test[81] were accepted. These curves were used to estimate the lethal dose (LD) that causes 50 and 90% mortalities. Time-mortality data were subjected to survival analysis ($p < 0.05$) with non-parametric Kaplan-Meier estimator[82] using LIFETEST procedure.[80] The survival curves constructed were compared by log-rank test ($p < 0.05$) and the median survival times (LT_{50}) of the larvaes were estimated. To evaluate the selectivity, mortality of non-target species

were compared with pest mortality by *t*-test for independent samples ($p < 0.05$).

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

References

1. Moreira, M. D.; Picanço, M. C.; Barbosa, L. C. A.; Guedes, R. N. C.; Barros, E. C.; Campos, M. R.; *Pest Manage. Sci.* **2007**, *63*, 615.

2. Paula, V. F.; Barbosa, L. C. A.; Teixeira, R. R.; Picanço, M. C.; Silva, G. A.; *Pest Manage. Sci.* **2008**, *64*, 863.

3. Bavaresco, A.; *Acta Sci., Agron.* **2007**, *29*, 309.

4. Pesquero, M. A.; Vaz, A. P. A.; Arruda, F. V.; *Sociobiology* **2013**, *60*, 484.

5. Moreno, S. C.; Carvalho, G. A.; Picanço, M. C.; Morais, E. G. F.; Pereira, R. M.; *Pest Manage. Sci.* **2011**, *68*, 386.

6. Way, M. J.; Khoo, K. C.; *Annu. Rev. Entomol.* **1992**, *37*, 479.

7. Ratnieks, F. L. W.; Carreck, N. L.; *Science* **2010**, *327*, 152.

8. Decourtye, A.; Mader, E.; Desneux, N.; *Apidologie* **2010**, *41*, 264.

9. Neumann, P.; Carreck, N. L.; *J. Apic. Res.* **2010**, *49*, 1.

10. Johnson, R. M.; Ellis, M. D.; Mullin, C. A.; Frazier, M.; *Apidologie* **2010**, *41*, 312.

11. Blacquiere, T.; Smagghe, G.; van Gestel, C. A. M.; Mommaerts, V.; *Ecotoxicology* **2012**, *21*, 973.

12. Laycock, I.; Lenthall, K. M.; Barratt, A. T.; Cresswell, J. E.; *Ecotoxicology* **2012**, *21*, 1937.

13. Whitehorn, P. R.; O'Connor, S.; Wackers, F. L.; Goulson, D.; *Science* **2012**, *336*, 351.

14. Gill, R. J.; Ramos-Rodriguez, O.; Raine, N. E.; *Nature* **2012**, *491*, 105.

15. Goulson, D.; *J. Appl. Ecol.* **2013**, *50*, 977.

16. Feltham, H.; Park, K.; Goulson, D.; *Ecotoxicology* **2014**, *23*, 317.

17. Gill, R. J.; Raine, N. E.; *Funct. Ecol.* **2014**, *28*, 1459.

18. Breeze, T. D.; Bailey, A. P.; Balcombe, K. G.; Potts, S. G.; *Agric., Ecosyst. Environ.* **2011**, *142*, 137.

19. Klein, A. M.; Vaissiere, B. E.; Cane, J. H.; Steffan, D. I.; Cunningham, S. A.; Kremen, C.; Tscharntke, T.; *Proc. R. Soc. London, Ser. B* **2007**, *274*, 303.

20. Hajjar, R.; Jarvis, D. I.; Gemmill, H. B.; *Agric., Ecosyst. Environ.* **2008**, *123*, 261.

21. Thomas, J. A.; Telfer, M. G.; Roy, D. B.; Preston, C. D.; Greenwood, J. J. D.; Asher, J.; Fox, R.; Clarke, R. T.; Lawton, J. H.; *Science* **2004**, *303*, 1879.

22. Hooper, D. U.; Chapin, F. S.; Ewel, J. J.; Hector, A.; Inchausti, P.; Lavorel, S.; Lawton, J. H.; Lodge, D. M.; Loreau, M.; Naeem, S.; Schmid, B.; Setala, H.; Symstad, A. J.; Vandermeer, J.; Wardle, D. A.; *Ecol. Monogr.* **2005**, *75*, 3.

23. Desneux, N.; Decourtye, A.; Delpuech, J. M.; *Annu. Rev. Entomol.* **2007**, *52*, 81.

24. Stara, J.; Ourednickova, J.; Kocourek, F.; *J. Pest Sci.* **2011**, *84*, 25.

25. Bengochea, P.; Amor, F.; Saelices, R.; Hernando, S.; Budia, F.; Adán, A.; Medina, P.; *Chemosphere* **2013**, *91*, 1189.

26. Gentz, M. C.; Murdoch, G.; King, G. F.; *Biol. Control* **2010**, *52*, 208.

27. Preetha, G.; Stanley, J.; Suresh, S.; Samiyappan, R.; *Chemosphere* **2010**, *80*, 498.

28. Alvarenga, E. S.; Silvério, F. O.; Picanço, M. C.; Moreno, S. C.; *Br PI 0705674-5A2*, **2009**.

29. Silvério, F. O.; Alvarenga, E. S.; Moreno, S. C.; Picanço, M. C.; *Pest Manage. Sci.* **2009**, *65*, 900.

30. Metcalf, R. L.; *Annu. Rev. Entomol.* **1980**, *25*, 219.

31. Wilson, R. M.; Danishefsky, S. J.; *J. Org. Chem.* **2006**, *71*, 8329.

32. Logrado, L. P. L.; Santos, C. O.; Romeiro, L. A. S.; Costa, A. M.; Ferreira, J. R. O.; Cavalcanti, B. C.; Moraes, O. M.; Lotufo, L. V. C.; Pessoa, C.; Santos, M. L.; *Eur. J. Med. Chem.* **2010**, *45*, 3480.

33. Chan, S. S. K.; Cheng, T. Y.; Lin, G.; *J. Ethnopharmacol.* **2007**, *111*, 677.

34. Du, J.; Yu, Y.; Ke, Y.; Wang, C.; Zhu, L.; Qian, Z. M.; *J. Ethnopharmacol.* **2007**, *112*, 211.

35. Shao, M.; Qu, K.; Liu, K.; Zhang, Y.; Zhang, L.; Lian, Z.; Chen, T.; Liu, J.; Wu, A.; Tang, Y.; Zhu, H.; *Planta Med.* **2011**, *77*, 809.

36. Huang, J.; Lu, X. Q.; Zhang, C.; Lu, J.; Li, G. Y.; Lin, R. C.; Wang, J. H.; *Fitoterapia* **2013**, *91*, 21.

37. Wei, Q.; Yang, J.; Ren, J.; Wang, A.; Ji, T.; Su, Y.; *Fitoterapia* **2014**, *93*, 226.

38. Dayan, F. E.; Cantrell, C. L.; Duke, S. O.; *Bioorg. Med. Chem. Lett.* **2009**, *17*, 4022.

39. Xu, H.; Xiao, X.; Wang, Q. T.; *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5009.

40. Sreelatha, T.; Hymavathi, A.; Rao, V. R. S.; Devanand, P.; Rani, P. U.; Rao, M.; Babu, K. S.; *Bioorg. Med. Chem. Lett.* **2010**, *20*, 549.

41. Miyazawa, M.; Tsukamoto, T.; Anzai, J.; Ishikawa, Y.; *J. Agric. Food Chem.* **2004**, *52*, 4401.

42. Tsukamoto, T.; Ishikawa, Y.; Miyazawa, M.; *J. Agric. Food Chem.* **2005**, *53*, 5549.

43. Momin, R. A.; Nair, M. G. J.; *J. Agric. Food Chem.* **2001**, *49*, 142.

44. Kwon, J. H.; Ahn, Y. J.; *Pest Manage. Sci.* **2002**, *59*, 119.

45. Copping, L. G.; Duke, S. O.; *Pest Manage. Sci.* **2007**, *63*, 524.

46. Alvarenga, E. S.; Teixeira, M. G.; Pimentel, M. F.; Picanço, M. C.; *Br PI 102014031753 8*, **2014**.

47. Park, I. K.; Lee, S. G.; Shin, S. C.; Park, J. D.; Ahn, Y. J.; *J. Agric. Food Chem.* **2002**, *50*, 1866.

48. Scott, I. M.; Gagnon, N.; Lesage, L.; Philogene, B. J.; Arnason, J. T.; *J. Econ. Entomol.* **2005**, *98*, 845.

49. Krchnak, V.; Waring, K. R.; Noll, B. C.; Moellmann, U.; Dahse, H. M.; Miller, M. J.; *J. Org. Chem.* **2008**, *73*, 4559.

50. Tavares, W. S.; Cruz, I.; Petacci, F.; Freitas, S. S.; Serrao, J. E.; Zanuncio, J. C.; *J. Med. Plants Res.* **2011**, *5*, 5301.

51. Qu, H.; Yu, X.; Zhi, X.; Lv, M.; Xu, H.; *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5552.

52. Paula, V. F.; Barbosa, L. C. A.; Demuner, A. J.; Pilo-Veloso, D.; Picanço, M. C.; *Pest Manage. Sci.* **2000**, *56*, 168.

53. Batista-Pereira, L. G.; Castral, T. C.; Silva, M. T. M.; Amaral, B. R.; Fernandes, J. B.; Vieira, P. C.; Silva, M. F. G. F.; Corrêa, A. G.; *Z. Naturforsch., C: J. Biosci.* **2006**, *61*, 196.

54. Ahern, J. R.; Whitney, K. D.; *Ann. Bot.* **2014**, *113*, 731.

55. Bidart-Bouzat, M. G.; Kliebenstein, D. J.; *J. Chem. Ecol.* **2008**, *34*, 1026.

56. Berenbaum, M. R.; Zangerl, A. R.; Nitao J. K.; *Evolution* **1986**, *40*, 1215.

57. Agrawal, A. A.; *Evol. Ecol. Res.* **2005**, *7*, 651.

58. Shonle, I.; Bergelson, J.; *Evolution* **2000**, *54*, 778.

59. Macel, M.; Klinkhamer, P. G. L.; *Evol. Ecol.* **2010**, *24*, 237.

60. Patel, B. K.; Hutt, A. J. In *Chirality in Drug Design and Development*; Reddy, I. K.; Mehvar, R., eds; Marcel Dekker Inc.: New York, 2004.

61. Agência Nacional de Vigilância Sanitária (ANVISA); *Manual de Protocolo para Testes de Eficácia de Produtos Desinfestante*s; Anvisa: Brasília, 2004.

62. Mills, N. J.; Beers, E. H.; Shearer, P. W.; Unruh, T. R.; Amarasekare, K. G.; *Biol. Control, in press*, DOI: 10.1016/j.biocontrol.2015.05.006.

63. Symondson, W. O. C.; Sunderland, K. D.; Greenstone, M. H.; *Annu. Rev. Entomol.* **2002**, *47*, 561.

64. Pretty, J.; *Philos. Trans. R. Soc., B* **2008**, *363*, 447.

65. Shennan, C.; *Philos. Trans. R. Soc., B* **2008**, *363*, 717.

66. Biondi, A.; Desneux, N.; Siscaro, G.; Zappalà, L.; *Chemosphere* **2012**, *87*, 803.

67. Jones, V. P.; Horton, D. R.; Mills, N. J.; Unruh, T. R.; Baker, C. C.; Melton, T. D.; Milickzy, E.; Steffan, S. A.; Shearer, P. W.; Amarasekare, K. G.; *Biol. Control, in press*, DOI: 10.1016/j.biocontrol.2015.03.009.

68. Garibaldi, L. A.; Steffan-Dewenter, I.; Winfree, R.; Aizen, M. A.; Bommarco, R.; Cunningham, S. A.; Kremen, C.; Carvalheiro, L. G.; Harder, L. D.; Afik, O.; Bartomeus, I.; Benjamin, F.; Boreux, V.; Cariveau, D.; Chacoff, N. P.; Dudenhöffer, J. H.; Freitas, B. M.; Ghazoul, J.; Greenleaf, S.;

Hipólito, J.; Holzschuh, A.; Howlett, B.; Isaacs, R.; Javorek, S. K.; Kennedy, C. M.; Krewenka, K. M.; Krishnan, S.; Mandelik, Y.; Mayfield, M. M.; Motzke, I.; Munyuli, T.; Nault, B. A.; Otieno, M.; Petersen, J.; Pisanty, G.; Potts, S. G.; Rader, R.; Ricketts, T. H.; Rundlöf, M.; Seymour, C. L.; Schüepp, C.; Szentgyörgyi, H.; Taki, H.; Tscharntke, T.; Vergara, C. H.; Viana, B. F.; Wanger, T. C.; Westphal, C.; Williams, N.; Klein, A. M.; *Science* **2013**, *339*, 1608.

69. Larsen, T. H.; Williams, N. M.; Kremen, C.; *Ecol. Lett.* **2005**, *8*, 538.

70. Ollerton, J.; Winfree, R.; Tarrant, S.; *Oikos* **2011**, *120*, 321.

71. Sterk, G.; Hassan, S. A.; Baillod, M.; Bakker, F.; Bigler, F.; Blümel, S.; Bogenschütz, H.; Boller, E.; Bromand, B.; Brun, J.; Calis, J. N. M.; Coremans-Pelseneer, J.; Duso, C.; Garrido, A.; Grove, A.; Heimbach, U.; Hokkanen, H.; Jacas, J.; Lewis, G.; Moreth, L.; Polgasr, L.; Roversti, L.; Samsoe-Petersen, L.; Sauphanor, B.; Schaub, L.; Stäubli, A.; Tuset, J. J.; Vainio, A.; Van de Veire, M.; Viggiani, G.; Viñuela, E.; Vogt, H.; *BioControl* **1999**, *44*, 99.

72. Jeschke, P.; *Pest Manage. Sci.* **2010**, *66*, 10.

73. Gribble, G. W.; *Naturally Occurring Organohalogen Compounds - A Comprehensive Update*; Springer: Vienna, 2010.

74. Smith, D. R. M.; Gruschow, S.; Goss, R. J. M.; *Curr. Opin. Chem. Biol.* **2013**, *17*, 276.

75. Perrin, D. D.; Armarego, W. L. F.; *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: Oxford, 1988.

76. Alvarenga, E. S.; Saliba, W. A.; Milagres, B. G.; *Quim. Nova* **2005**, *28*, 927.

77. Näsman, J. H.; *Org. Synth.* **1990**, *68*, 162.

78. Abbott, W. S.; *J. Econ. Entomol.* **1925**, *18*, 265.

79. Finney, D. J.; *Probit Analysis*; Cambridge University Press: London, 1971.

80. SAS Institute Inc.; *SAS/STAT User's Manual, version 9.4*; SAS Institute Inc.: Cary, NC, 2013.

81. Young, L. J.; Young, J. H.; *Statistical Ecology: A Population Perspective*; Kluwer Academic: Boston, 1998.

82. Kaplan, E. L.; Meier, P.; *J. Am. Stat. Assoc.* **1958**, *53*, 457.