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FULL PAPER

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Toxicity and Sublethal Effects of Phthalides Analogs to *Rhyzopertha dominica*

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Phthalides and their precursors have demonstrated a large variety of biological activities. Eighteen phthalides were synthesized and tested on the stored grain pest *Rhyzopertha dominica*. In the screening bioassay, compounds *rac*-(2*R*,2a*S*,4*R*,4a*S*,6a*R*,6b*S*,7*R*)-7-bromohexahydro-2,4-methano-1,6-dioxacyclopenta[*cd*]pentalen-5(2*H*)-one (**15**) and *rac*-(3*R*,3a*R*,4*R*,7*S*,7a*S*)-3-(propan-2-yloxy)hexahydro-4,7-methano-2-benzofuran-1(3*H*)-one (**17**) showed mortality similar to the commercial insecticide, Bifenthrin® ($\geq$90%). The time (LT_{50}) and dose (LD_{50}) necessary to kill 50% of the *R. dominica* population were determined for the most efficacious phthalides **15** and **17**. Compound **15** presented the lowest LD_{50} (1.97 $\mu g\,g^{-1}$), being four times more toxic than Bifenthrin® (LD_{50} = 9.11 $\mu g\,g^{-1}$). Both compounds presented an LT_{50} value equal to 24 h. When applied at a sublethal dose, both phthalides (especially compound **15**), reduced the emergence of the first progeny of *R. dominica*. These findings highlight the potential of phthalides **15** and **17** as precursors for the development of insecticides for *R. dominica* control.

Keywords: Bostrichidae, insecticidal activity, *Rhyzopertha dominica*, sublethal effect, γ-lactones, biological activity.

Introduction

Modern agriculture is highly dependent on the use of synthetic chemicals, including the insecticides.[1][2] However, due to the excessive use of these chemicals, insecticide-resistant pest populations have been developed.[3][4] Therefore, for agriculture, there is a continuous need for the development of new molecules with insecticidal effects to be complementary or to substitute the existing insecticides.[5]

Stored-product insects are serious pests of dried, stored, and durable agricultural commodities.[6] The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), is one of the major pests of stored products worldwide.[7] This insect is an internal grain feeder which infests wheat and other cereals in silos such as corn, rice, and other substrates containing starch.[8] The females oviposit outside the grain kernel, but when the eggs hatch, the larvae bore into the grain kernel to complete development to the adult stage, damaging the grain.[7]

Synthetic insecticide use is the major method adopted for *R. dominica* control. However, in the last decades, control failures of this pest due to development of *R. dominica* resistant strains have been reported.[8] In attempts to incorporate new molecules in the management of this pest, the development of

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Table 1. Summary of the results of Probit analysis.[a]

Compound	*y*	χ^2	*df*	*P*	LD_{50} [μg g^{-1}]	LD_{90} [μg g^{-1}]
Bifenthrin®	$-4.70+4.91x$	5.22	3	0.16	9.08 (8.12–10.07)	16.56 (14.55–19.65)
15	$-1.06+3.60x$	1.32	4	0.26	1.97 (1.74–2.19)	4.47 (3.75–5.89)
17	$-6.22+5.33x$	4.79	5	0.44	14.66 (13.68–15.70)	25.50 (22.87–29.67)

[a] *y* = curve equation; χ^2 = chi-square test; *df* = degrees of freedom; *P* = probability; LD = lethal dose with the 95% confidence interval (in parenthesis).

efficacious compounds is encouraged.[5] In addition to the acute toxicity, it is desirable that the candidate molecules present sublethal effects in order to reduce the pest progeny in silos and storages.[9][10]

In this context, natural products have been used as models for the development of synthetic insecticides.[11–14] For instance, phthalides compounds have shown great potential for agriculture in recent years.[15–17] Phthalides are members of a group of secondary metabolites comprising a benzene ring fused to a γ-lactone.[18] These compounds have been reported to exhibit a variety of phytotoxic and insecticidal activities.[19][20]

Considering the phthalides' potential and the need for new insecticides molecules to be applied in the management of stored-product pests, the insecticidal activity of 18 synthetic compounds against *R. dominica* was assessed. In addition, the effect of the most efficacious phthalides in the progeny production of *R. dominica* was also evaluated.

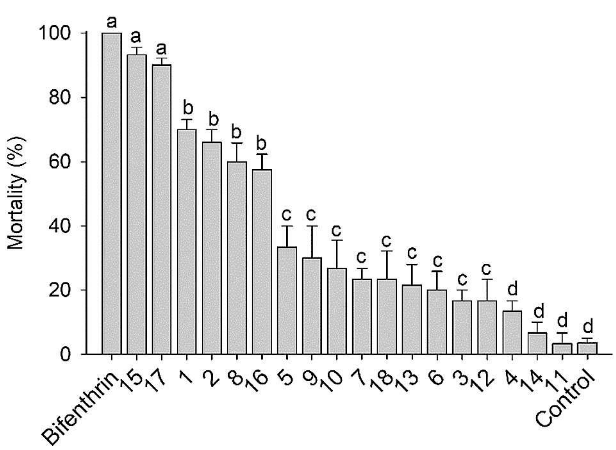

Figure 1. Mortality (mean ± standard error) of adults of *Rhyzopertha dominica* after 72 h exposure to compounds **1**–**18** at 30 μg g^{-1}. Histograms bars with the same lower-case letter do not differ by Scott–Knott test at the 5% level. Control = acetone. Bifenthrin® was used as positive control.

Results and Discussion

Significant differences in the mortality data of *R. dominica* were observed after 72 h exposure to the compounds ($F_{19,58}=57.15$; $P<0.001$; *Figure 1*). Chemical structures of the tested compounds are presented in *Figure 2*. Compounds **15** and **17** were the most active, causing 93 and 90 % of mortality, respectively. Mortality caused by these compounds did not differ from the commercial insecticide Bifenthrin®. Compounds **1**, **2**, **8**, and **16** caused mortality ranged from 57 to 70 %, and compounds **3**, **5**, **6**, **7**, **9**, **10**, **12**, **13**, and **19** caused mortality ranged from 16 to 33 %. At last, compounds **4**, **11**, and **14** presented low insecticidal activity (3–13 % mortality rates). Although compounds **1**, **2**, **8**, and **16** caused reasonable mortality (57–70 %) in the screening bioassay, according to Brazilian legislation, products to be considered effective for pest control are required to lead mortalities ≥80 %.[21][22] Therefore, the mortalities caused by compounds **15** and **17** (>80 %) indicate that these compounds have the potential to be used in *R. dominica* control.

Dose–mortality curves for *R. dominica* were estimated for the most active compounds (**15** and **17**) and for the commercial insecticide Bifenthrin® (*Figure 3*). Compound **15** showed the lowest slope (3.60), while **17** presented the highest slope (5.33) of the dose–mortality curves (*Table 1*). The lethal doses 50 (LD_{50}) and 90 (LD_{90}) of the sample population of *R. dominica* were also estimated. The LD_{50} ascending order for *R. dominica* adults was **15** < Bifenthrin® < **17**. Compound **15** ($LD_{50}=1.97$ μg g^{-1}) was seven times more toxic than compound **17** ($LD_{50}=14.66$ μg g^{-1}) and four times more toxic than Bifenthrin® ($LD_{50}=9.11$ μg g^{-1}). Since the slope of the dose–mortality curve for compound **17** was higher than that observed for compound 1**5**, a more homogeneous response of the *R. dominica* populations exposed to compound **17** is expected.[23] Therefore, a small variation in the dose of compound **17** promotes wide variations in pest mortality, increasing the risk of failures in the pest

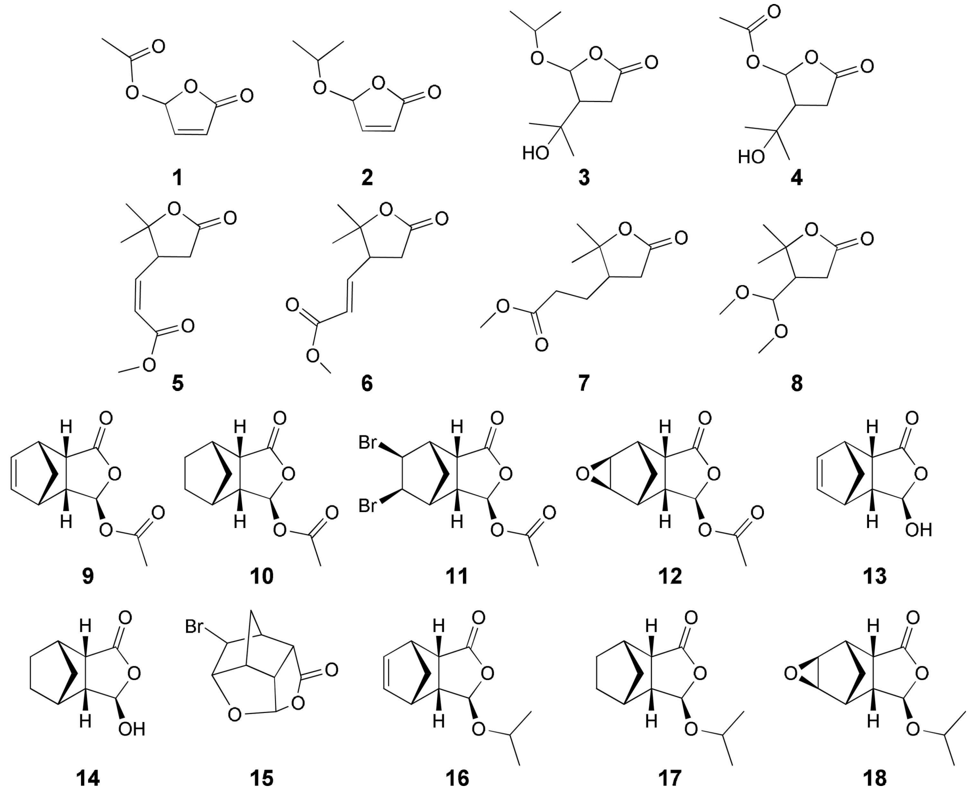

Figure 2. Structures of the tested γ-lactones (compounds **1**–**8**) and phthalides analogs (compounds **9**–**18**). All evaluated substances were synthesized in the racemic form but compounds **9**–**18** were represented only by one enantiomer since it was possible to establish the relative configurations based on NOE experiments.

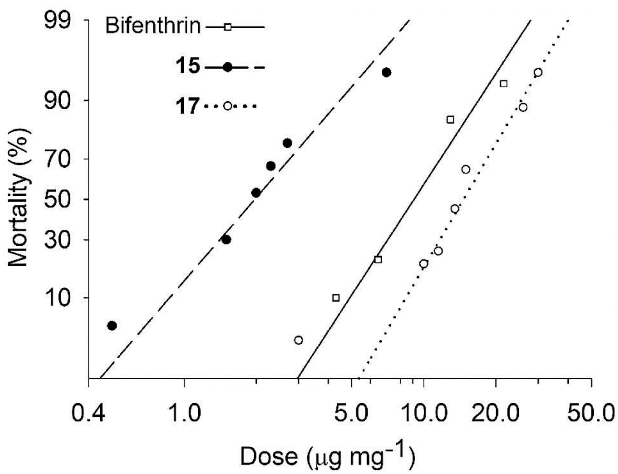

Figure 3. Dose–mortality curves of compounds **15** and **17** for adults of *Rhyzopertha dominica*.

control.[15] Moreover, compound **15** was more toxic than compound **17** and the insecticide Bifenthrin® (LD_{50}), indicating a saving cost for the pest control promoted by the phthalide **15**. The commercial product of a pesticide usually tends to be more toxic than their primordial molecules because the product is a mixture of molecules and adjuvants that increase its activity.[24][25] The results obtained from this study indicate that compound **15** is a more potent insecticide than Bifenthrin® since it causes higher pest mortality at smaller doses.

Survival curves of *R. dominica* treated with compounds **15** and **17** were determined (*Figure 4*). Significant difference in the curves was not observed (log-rank test, $\chi^2=0.42$, $df=1$, $P<0.52$; *Table 2*). For both phthalides, a period of 24 h was required to kill 50% (LT_{50}) of the insects and, after 72 h, mortality was

Table 2. Summary of the results of survival analysis on adults of *Rhyzopertha dominica* exposed to LD_{90} of compounds **15** and **17**.[a]

Compound	LT_{50} [h]	χ^2	*df*	*P*
15	24.00 (21.00–27.00)	0.42	1	0.52
17	24.00 (20.40–27.60)			

[a] LT = lethal time with the 95% confidence interval; χ^2 = chi-square test; *df* = degrees of freedom; *P* = probability.

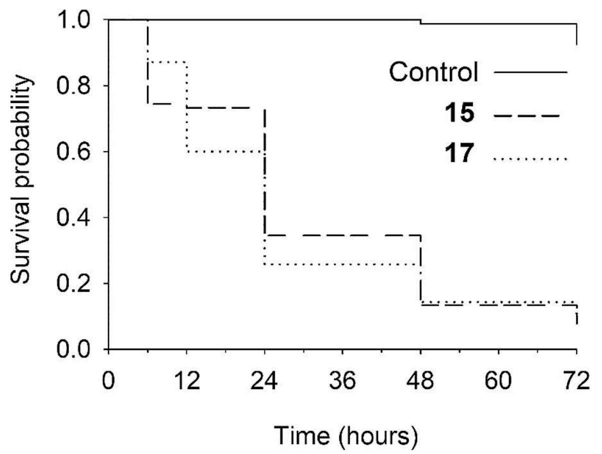

Figure 4. Survival curves estimated by the method of Kaplan–Meier product-limit of adults of *Rhyzopertha dominica* exposed to LD_{90} of compounds **15** and **17**.

above 85 %. The majority of the conventional insecticides (organophosphorus and pyrethroids) adopted currently for management of stored grain pests require at least seven days to promote effective control.[8] Due to the high reproductive potential of stored product pests,[26] their control is recommended to be carried out quickly to prevent economic losses.[6][27] In some industrialized countries like Canada and Australia, there is zero tolerance for insects in food grains.[28][29] Therefore, compounds that promote fast control should be considered as potential molecules to be explored for the management of stored grain pests. Moreover, *R. dominica* has developed resistance to many conventional insecticides such as organophosphorus and pyrethroids, and this fact supports the replacement of these products with new insecticidal molecules.

The parental mortality of *R. dominica* was not affected 25 days after exposure to the LD_{20} of the treatments ($F_{3,16}=3.67$; $P=0.035$; *Figure 5A*). Conversely, there was a significant effect of the treatments on the F1 progeny produced ($F_{3,16}=3.14$; $P=0.055$). Bifenthrin® and the phthalides **17** and **15** reduced adult progeny as compared to control. Compound **15** caused a 13-fold reduction in the number of emerged adults (*Figure 5B*). To sum up, a sublethal dose of compounds **15** and **17** reduced the emergence of adults in F1. In addition, the reduction of parental longevity, factor affecting the progeny of several insects,[30] was not affected by the treatments. The mechanism underlying the reduced emergence of adults exposed to the phthalides was not studied in further details here. Compounds **15** and **17** may have affected progeny production of *R. dominica* by the disruption of mating behavior leading to reproductive failure,[31][32] reduction in the number of eggs laid,[33] disturbance in egg hatchability and immature physiology.[34] This result is relevant since insects exposure to sublethal doses is common as a consequence of degradation of the used insecticide formulation.[35][36]

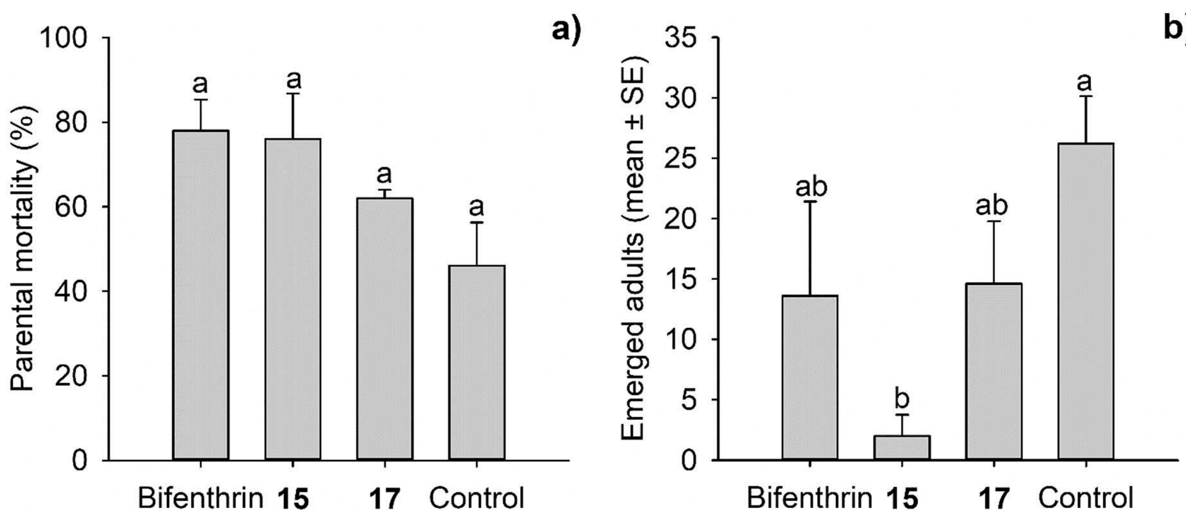

Figure 5. a) Parental mortality (mean ± standard error) and b) F1 progeny production of adults of *R. dominica* treated with the LD_{20} of compounds **15** and **17**, and Bifenthrin® (positive control). Histograms bars with the same lower-case letter do not differ by Tukey's test at the 5% level. Control = acetone.

Conclusions

The insecticidal activity of 18 synthetic compounds, including phthalides and their precursors, on the lesser grain borer *R. dominica* was evaluated. The results support the potential of compounds *rac*-(2*R*,2a*S*,4*R*,4a*S*,6a*R*,6b*S*,7*R*)-7-bromohexahydro-2,4-methano-1,6-dioxacyclopenta[*cd*]pentalen-5(2*H*)-one (**15**) and *rac*-(3*R*,3a*R*,4*R*,7*S*,7a*S*)-3-(propan-2-yloxy)hexahydro-4,7-methano-2-benzofuran-1(3*H*)-one (**17**) to be used in this pest control. Both are effective in controlling the lesser grain borer after 72 h of exposure. The cage compound **15** is a more potent insecticide than Bifenthrin® because it provides effective pest control in smaller doses, which can lead to cost savings for lesser grain borer control. The sublethal doses of compounds **15** and **17** contribute also to the reduction of populations of *R. dominica.* These findings support the importance of investigating new molecules with potential insecticide, which could contribute to the replacement of the conventional insecticides that are already inefficient.

Experimental Section

Compounds

In this study, γ-lactones (compounds **1**–**8**) and phthalides analogs (compounds **9**–**18**) were tested on *R. dominica.* The complete synthesis of these compounds has been described previously.[15][37–39] 5-Hydroxyfuran-2(5*H*)-one was synthesized from the photooxidation of furfural and used to prepare compounds 5-oxo-2,5-dihydrofuran-2-yl acetate (**1**) and 5-(propan-2-yloxy)furan-2(5*H*)-one (**2**). These lactones were then submitted to additional reactions, followed by other chemical modifications, to afford the saturated lactones 4-(2-hydroxypropan-2-yl)-5-(propan-2-yloxy)dihydrofuran-2(3*H*)-one (**3**), 3-(2-hydroxypropan-2-yl)-5-oxotetrahydrofuran-2-yl acetate (**4**), methyl (2*Z*)-3-(2,2-dimethyl-5-oxotetrahydrofuran-3-yl)prop-2-enoate (**5**), methyl (2*E*)-3-(2,2-dimethyl-5-oxotetrahydrofuran-3-yl)prop-2-enoate (**6**), methyl 3-(2,2-dimethyl-5-oxotetrahydrofuran-3-yl)propanoate (**7**), and 4-(dimethoxymethyl)-5,5-dimethyldihydrofuran-2(3*H*)-one (**8**).

The Diels–Alder reaction between cyclopentadiene and α,β-unsaturated γ-lactones (5-hydroxyfuran-2(5*H*)-one and compounds **1** and **2**) was chosen as the key step for achievement of the bicyclic framework of the phthalides, leading to formation of the adducts, *rac*-(1*R*,3a*S*,4*R*,7*S*,7a*R*)-3-oxo-1,3,3a,4,7,7a-hexahydro-4,7-methano-2-benzofuran-1-yl acetate (**9**), *rac*-(3*R*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxy-3a,4,7,7a-tetrahydro-4,7-methano-2-benzofuran-1(3*H*)-one (**13**), and *rac*-(3*R*,3a*R*,4*S*,7*R*,7a*S*)-3-(propan-2-yloxy)-3a,4,7,7a-tetrahydro-4,7-methano-2-benzofuran-1(3*H*)-one (**16**). These adducts were then subjected to hydrogenation (to give compounds *rac*-(1*R*,3a*S*,4*S*,7*R*,7a*R*)-3-oxooctahydro-4,7-methano-2-benzofuran-1-yl acetate (**10**), *rac*-(3*R*,3a*R*,4*R*,7*S*,7a*S*)-3-hydroxyhexahydro-4,7-methano-2-benzofuran-1(3*H*)-one (**14**), and *rac*-(3*R*,3a*R*,4*R*,7*S*,7a*S*)-3-(propan-2-yloxy)hexahydro-4,7-methano-2-benzofuran-1(3*H*)-one (**17**)); epoxidation (to give compounds *rac*-(1a*R*,2*R*,2a*R*,3*R*,5a*S*,6*S*,6a*S*)-5-oxooctahydro-2,6-methanooxireno[*f*][2]benzofuran-3-yl acetate (**12**) and *rac*-(1a*R*,2*R*,2a*R*,5*S*,5a*S*,6*S*,6a*S*)-5-(propan-2-yloxy)hexahydro-2,6-methanooxireno[*f*][2]benzofuran-3(1a*H*)-one (**18**)); and bromination reactions (to give compounds *rac*-(1*R*,3a*R*,4*S*,5*S*,6*R*,7*R*,7a*S*)-5,6-dibromo-3-oxooctahydro-4,7-methano-2-benzofuran-1-yl acetate (**11**) and *rac*-(2*R*,2a*S*,4*R*,4a*S*,6a*R*,6b*S*,7*R*)-7-bromohexahydro-2,4-methano-1,6-dioxacyclopenta[*cd*]pentalen-5(2*H*)-one (**15**)). Physical data and NMR spectra of all compounds are available in the *Supporting Information.*

Insects

Rhyzopertha dominica adults were reared in the laboratory in 1.5 L glass bottles at 28±2 °C, 70±5% relative humidity and 24 h scotophase, as described elsewhere.[40] Whole wheat grains (13% moisture content, wet basis), previously expurgated and kept under refrigeration (−5 °C) to avoid contamination, were used as substrate.

Bioassays

Screening. The treatments were carried out with the 18 compounds, the insecticide Bifenthrin® (92.2% w/w, FMC, Campinas, Brazil), and control (acetone only). The substances were diluted in acetone PA (99.5%, Vetec, Rio de Janeiro, Brazil) at the 30 $\mu g\, g^{-1}$ dose and topically applied at the dorsal thorax (0.5 µl per insect) using a microsyringe (Hamilton, model 701N, Reno, USA). The design was completely randomized with at least three replicates. Each replicate consisted of a round plastic container (6 cm diameter×5 cm height, with lid) containing ten insects.

Mortality was evaluated after 72 h of exposure to the compounds. Insects were considered dead when they did not move while touched by a fine brush.

Mortality data were first checked for normality (Shapiro–Wilk test) and for homoscedasticity of residuals (Bartlett test) and then subjected to ANOVA followed by Scott–Knott cluster analysis at 5% probability.[41][42]

Dose–Mortality Curves

Dose–mortality curves of the compounds selected in the previous assay and of Bifenthrin® (positive control) were determined for the pest. The same procedure described in the previous test was used. Five to six doses causing mortalities between 1 and 99% were used for each treatment. Mortality data were submitted to Probit analysis (PROC PROBIT, SAS 9.2, SAS Institute Inc., Cary, USA) to estimate the dose–mortality curves.

Speed of Action

In order to estimate the speed of action of the selected compounds, 100 adults of *R. dominica* were treated with the LD_{90} of phthalides **15** and **17** (4.47 and 25.49 $\mu g\,g^{-1}$, respectively). The procedures were similar to the previous bioassays. The death of the larvae was monitored for 72 h by noting the time at which each insect died. The mortality was assessed every 10 min during the first hour of the experiment, every 60 min up to 24 h and subsequently, every 240 min up to 72 h. Experimental data were submitted to survival analysis using Kaplan–Meier estimators (PROC LIFETEST, SAS 9.2) to obtain survival curves and estimates of median lethal times (LT_{50}s). Overall similarity among the survival curves and LT_{50}'s values was tested using the Log-Rank test, and pairwise comparisons among the curves were tested using the Holm–Sidak's test at 5% level.

Sublethal Effect

In this bioassay, fifteen-day-old *R. dominica* adults were treated with the LD_{20} of compounds **15**, **17**, and Bifenthrin® (1.15, 10.19, and 6.12 $\mu g\,g^{-1}$, respectively). Following application, the insects were transferred to 250 mL round plastic containers containing 50 g of expurgated wheat and kept at 28 ± 2 °C, 70 ± 5% RH, and 24 h scotophase. The experimental design was completely randomized with five replicates (containing 10 adults each) per treatment. After 25 days, wheat grains were sieved, mortality of parental adults was assessed and these were removed. The containers were kept under the same experimental conditions until the emergence of F1 adult progeny (55 days after bioassay setup). Parental mortality and progeny emergence data were subjected to ANOVA followed by Tukey's test (PROC GLM, SAS 9.2).

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Author Contribution Statement

E.S.F. and T.A.A. participated in study concept and design, acquisition of data, analysis and interpretation of data, and in the manuscript writing. J.N.D.C. and M.F.P. participated in the acquisition of data. G.C.R. and E.S.A. synthesized the tested compounds and revised the manuscript. M.C.P. participated in study concept and design and revised the manuscript.

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