Research Article

Received: 20 October 2017 Revised: 7 May 2018 Accepted article published: 13 May 2018 Published online in Wiley Online Library: 30 June 2018

(wileyonlinelibrary.com) DOI 10.1002/jsfa.9132

# Cinnamaldehyde and related phenylpropanoids, natural repellents, and insecticides against *Sitophilus zeamais* (Motsch.). A chemical structure-bioactivity relationship

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## Abstract

**BACKGROUND: The insecticidal and repellent effects on adult *Sitophilus zeamais* of 12 cinnamaldehyde-related compounds was evaluated by contact toxicity bioassays and a two-choice olfactometer. To determine non-toxicity in mammals, body weight, serum biochemical profiles, liver weight, physiological parameters, sperm motility, and histopathological data were obtained as complementary information in C57BL/6 mice treated with the best natural compound.**

**RESULTS: Based on 24 h $LC_{95}$ and $LC_{50}$ values, $\alpha$-methyl-cinnamaldehyde and cinnamaldehyde exhibited better insecticidal action than the other compounds. The best repellent effect was observed with $\alpha$-bromo-cinnamaldehyde, which even repelled at the lowest concentration studied (0.28 μmol $L^{-1}$). The evaluation of a quantitative structure-activity relationship found a linear relationship between the $LC_{50}$ values for adult weevil toxicity and dipolo with Q values (giving the difference between orbital electronegativity carbon 1 and orbital electronegativity carbon 3 of the molecule) in cinnamaldehyde-related compounds. The polar surface and Log P descriptors also revealed a linear relationship with the *S. zeamais* repellent effect for cinnamaldehyde analogues. Cinnamaldehyde did not show toxicity in the parameters evaluated in mice.**

**CONCLUSION: From the phenylpropanoid components studied, the natural compound that had the best insecticidal and repellent action against *S. zeamais* was cinnamaldehyde. It presented no mammalian toxicity.**


**Keywords:** Cinnamaldehyde-related structures; insecticidal property; quantitative structure-activity relationship; repellent effect; *Sitophilus zeamais*

## INTRODUCTION

The food production chain can involve different regions of the same country or extend to several countries. Production begins in the harvest areas; then the food is distributed to storage centers, then businesses, and then it is sold. Cereals are one of the main groups of food. The use of corn kernels varies according to the different regions of the world and is linked to regional industries and food habits. In South America, 74.9% is used for livestock feed, while in Africa, 65.4% is part of the pubic diet.[1] Throughout the process mentioned above, the exposure of kernels to insects is the principal reason for contamination and dispersion of pests.[2] In fact, pest infestation has negative consequences that go beyond financial losses, because customers and suppliers form a negative image of the products.[3]

Another problem associated with the deterioration of grains is the growth in the world population, which is predicted to increase 30% by 2050. Such growth will increase global populations, resulting in an increased demand for food. However, land suitable for agricultural purposes is limited.[4] Thus, by reducing contamination, insect reproduction and its dispersion through the cereals food

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chain would produce better grain yields and prevent more land from being cultivated.

Nowadays, pest control is carried out with the use of synthetic pesticides but some of these have restrictions on their use at different stages of the food chain. For example, in Argentina, national law 27262 (SENASA) prohibits the application of phosphine in transportation. In California, the Department of Pesticide Regulation listed phosphine and phosphine-generating compounds as toxic air contaminants.[5] In India and Europe, phosphine is used to control insects in places where food is stored.[6,7]

Some synthetic pesticides are consequently being phased out, such as methyl bromide.[8] Moreover, their excessive use has led to the growth of resistant insect populations,[9,10] which in turn require an increased dosage to achieve an effective control. Hence, there is a need to develop alternative insecticides or new methods to control insects in the food supply chain. Essential oils and their main components are considered to be 'generally recognized as safe' (GRAS) substances and could be an effective alternative for insect control as they are molecules that have less impact on human health and they are environmentally friendly.[11]. However, some essential oils components have been reported to bind to ionotropic GABA receptors in human and rodents; others have been reported to cause allergic airway inflammation[12,13] and to have toxic effects at the cellular level.[14] Even though these compounds are considered safe, there is a need for more mammalian toxicity data on essential oils and their compounds used as biopesticides to confirm their safety.

Previous studies have demonstrated strong insecticidal action of essential oils against adult *Sitophilus zeamais*.[15–18] Among the components of essential oils, the benzene derivatives have revealed high insecticidal and repellent action.[19,20] Different factors could be related to the toxic action of phenylpropanoids, such as the distance of the double bond in the side chain in respect to the aromatic ring;[20] LogP values and their relation with cuticular penetration; the polarizability of the molecule; the presence of carbonyl groups and their interactions with the GABA receptor; and the inhibition of AChE activity.[21] Comparison of toxicity values could reveal the potential for structurally dependent toxicity. The connection between toxic effects and the molecular structure of phenylpropanoid analogues can be studied using quantitative structure activity relationship (QSAR) analyses, which correlate chemical structure with well defined processes, such as biological activity. However, to date, there is no information about the molecular features that confer a greater repellency to phenylpropanoid analogues.

Although more than 37 insect species have been reported as being pests associated with stored maize,[19] *Sitophilus zeamais* is the most important one in pre-harvest field drying, transport, and storage in South America,[22] and in the eastern and southern African region.[23] Although a number of recent investigations have shown the effectiveness of phenylpropanoids as insecticides or repellents of insects associated with stored products,[18,24–27] the main objective of this study was to determine which molecular aspects of phenylpropanoids are linked with the insecticidal and repellent actions on *Sitophilus zeamais,* and to evaluate acute toxicological effects in C57BL/6 mice of the best phenylpropanoid compound *in vivo,* as a starting point for the development of a new green pesticide.

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## MATERIALS AND METHODS

### Insects

Adults of *Sitophilus zeamais* Motschulsky (Curculionidae, Coleoptera) were raised in the laboratory under controlled conditions (total darkness, 28 ± 2 °C and 65 ± 5% relative humidity) on insecticide-free maize grains obtained from the breeding program of EEA-Manfredi INTA, Córdoba, Argentina. Unsexed adult insects of about 2–4 weeks old were used in the toxicity and repellent studies.

### Tested compounds

Cinnamic acid (12) ((E)-3-phenylprop-2-enoic acid), ferulic acid (9) ((2E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid), cinnamaldehyde (3) ((2E)-3-phenyl-2-propenal), cinnamamide (4) ((2E)-3-phenylprop-2-enamide), $\alpha$-methyl-*trans*-cinnamaldehyde (7) ((2E)-2-methyl-3-phenyl-2-propenal), *trans*-p-metoxy-cinnamaldehyde (5) ((2E)- 3-(4-methoxyphenyl)-2-propenal, $\alpha$-bromo cinnamaldehyde (1) ((2E)- 3-bromo-3-phenyl- 2-propenal, 4-phenyl-3-buten-2-one (2), eugenol (6) (4-Allyl-2-methoxyphenol), isoeugenol (8) (2-methoxy-4-[(1E)-1-propen-1-yl]phenol), anethole (11) (1-methoxy-4-[(1E)-1-propen-1-yl]benzene) and estragole (10) (methyl chavicol) (1-allyl-4-methoxybenzene) were tested for their contact toxicity and repellent/attraction activity (Fig. 1). All compounds (≥95%) were purchased from Sigma-Aldrich (Buenos Aires, Argentina).

### Bioassays

*Contact toxicity*

The insecticidal action of phenylpropanoids and their analogues against the adults of *S. zeamais* was evaluated with a contact application assay. First, the compounds were diluted with acetone following a serial dilution, and 0.6 mL aliquots of these were applied to a filter paper disc (9 cm) on the bottom of a glass petri dish. Prior to the introduction of adult insects, the acetone was evaporated for 2 min. Groups of ten insects were used for each treatment and controls, with acetone alone being used in controls. The petri dishes were used in a breeding chamber with a temperature of 27 ± 2 °C and 65% relative humidity in total darkness. Each concentration and control was replicated five times, and the bioassay was repeated twice. Mortality was determined after 24 h from the beginning of exposure.[25] Then, the $LC_{50}$ and $LC_{95}$ (lethal concentration 50% and 95%, respectively) values were calculated using probit analysis. Data were considered to be significant at $P < 0.05$.[25,28] Finally, chlorpyrifos was used as a reference compound (positive control).

*Repellent/attraction action*

The repellent/attraction action of phenylpropanoids and their analogues against adults of *S. zeamais* was studied using a bioassay system consisting of a two-choice olfactometer according to Herrera *et al.*[15] A solution of the compounds (treatments) or acetone



  

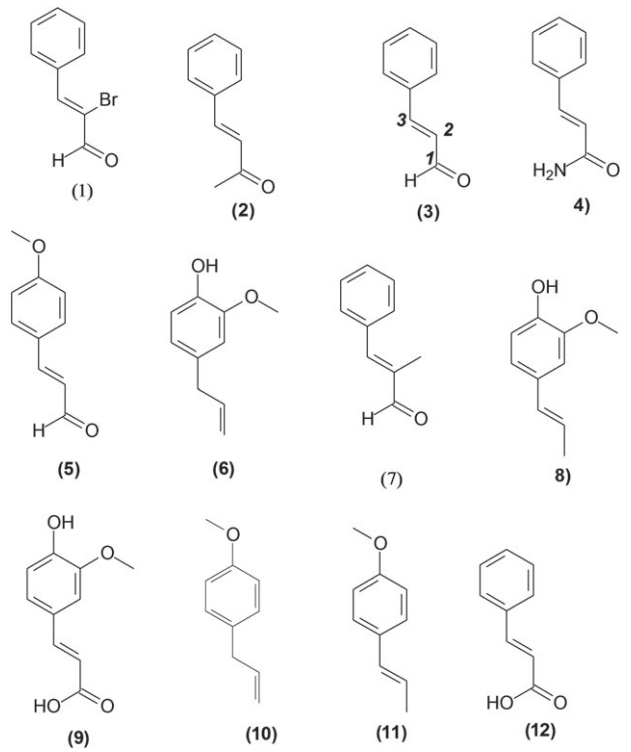


**Figure 1.** Structure of phenylpropanoids studied. Names of the molecules presented: 1) a-brome cinnamaldehyde; 2) 4-phenyl-3-buten-2one; 3) cinnamaldehyde; 4) cinnamamide; 5) para-methoxy cinnamaldehyde; 6) eugenol; 7) a-methyl cinnamaldehyde; 8) isoeugenol; 9) ferulic acid; 10) estragole or methyl chavicol; 11) anethole; 12) cinnamic acid.

(control) was added to a filter paper of 2 cm diameter, which was placed in each flask. Twenty unsexed adult maize weevils were introduced into the olfactomer, with the position of the flasks being randomly changed at every replication to avoid any possible influence of internal or external circumstances. Both the control and treatments were performed in duplicate and repeated five times. The response index (RI) (%) was calculated using the following formula: $RI = [(T - C)/Tot] \times 100$, where $T$ is number of insects responding to the treatment, $C$ is number of insects responding to the control, and *Tot* is the total number of insects released.[29] Attraction is indicated with positive values of RI, whereas repellence is shown by negative values. The positive control used for the repellent effect was propionic acid.[30]

*Quantitative structure activity relationship (QSAR) analysis*

The selection of descriptors is an essential step for determining which molecular characteristics correlated with toxic effects or repellent activity. Here, geometrical, thermodynamic, electrostatic, constitutional, and topological descriptors were used in the QSAR analysis. Specifically, these were: density, boiling point, enthalpy of vaporization, refraction index, molar refractivity, hydrogen bond acceptors, hydrogen bond donors, freely rotating bonds, Log P, Log D pH 5.5, Log D pH 7.4, polar surface area, polarizability, surface tension, molar volume, molecular refractivity, molecular surface area, pi energy (Huckel analysis), Dreiding energy, volume, minimal projection area, maximal projection area, orbital electronegativity, Mulliken charges, Van der Waals COSMO solvation $H_2O$, orbital electronegativity, dipolo, fractional partial negatively charged surface area (FNSA),[31] Latt index, Randic index, Balaban index, Harary index, Winner index, Hyper Wiener index, Wiener polarity, and Szeged index. The difference between the orbital electronegativity of carbon one and three of the propenyl chain was also used as descriptor Q (Table 1).

The molecular modeling program CS Chem3D 3.5.1 was used to draw the phenylpronoids and their analogue molecules, and this was also used to determine the optimal conformers with the lowest energy of these compounds. The molecular descriptors were obtained using the Chemspider, ACD Laboratories, MOL Inspiration, ChemAxon, and Cambridge Soft software packages. The QSAR models were calculated from the best multiple lineal regression analysis (MLR) and revealed which constitutional features had a relevant role in insecticidal action or repellent effects against *Sitophilus zeamais*. The tested $LC_{50}$ and RI values were transformed into Log $1/(LC_{50}/1000)$ and Log 1/(RI/1000), respectively, and were used as dependent variables in the QSAR studies. These models were validated using the root mean square prediction error (RMSPE) obtained by the cross-validation leave-one-out procedure.

In the MLR equations, N is the number of data points, $r$ is the coefficient of correlation between the observed values of the dependent variable and the values calculated from the equation, and $r^2$ is the square of the correlation coefficient and represents the goodness of fit. Results with $P$ values $< 0.05$ were considered to be significant. InfoStat Professional 2010p software was used for the statistical analyses.[21]

## Toxicity

*Animals: mice*

The animals were treated gently to reduce distress. Every effort was made to avoid any unnecessary suffering and the experimental procedure was carried out in strict compliance with the US National Institutes of Health guidelines for the experimental use of animals.[32] C57BL/6 male mice (8 weeks old, $n = 6–10$) were maintained in a specific pathogen-free facility under a 12 h light/dark cycle, at a constant temperature (25 °C) and at 50% relative humidity.

*Acute toxicity*

The phenylpropanoid selected to check the acute toxicity was cinnamaldehyde (Cinn). It is a natural product widely used in food and beverages, and many pesticides/repellents contain it.[33] Cinnamaldehyde showed one of the best repellent and insecticide values in this research.

The toxicity studies were based on the guidelines of the Organization for Economic Cooperation and Development (OECD-guidelines 423 and 407)[34] with minor modifications. According to OECD 423, the method does not aim to enable the calculation of a precise median lethal dose ($LD_{50}$) but, rather, allows for an assessment of the ranking of the test substance in one of a series of classes defined by toxicity $LD_{50}$ values. Animals in the test group received a single dose of 2000 mg $kg^{-1}$ of cinnamaldehyde, dissolved in edible sunflower oil (100 µL), by gavage. The control group received only sunflower oil by gavage. After that, animals were observed 24 h after the treatment and were then sacrificed. Death, occurrence of tremors, convulsions, abdominal contortions, locomotion, salivation, diarrhea, and lethargy were considered. Body weight, drink intake, fecal material (pellets, gr) of the animals and the food consumption were monitored. Blood was collected from the cardiac puncture in tubes with the anticoagulant ethylenediamine-tetraacetate (EDTA). The blood was used for the assessment of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) action, ALkP, direct bilirubin



  

**Table 1.** Molecular descriptors of the phenylpropanoids studied calculated using Chemspider, ACD Laboratories, MOL Inspiration, ChemAxo and Cambridge Soft software

| *Compounds*† *descriptors* | *cinnamaldehyde* | *α-methyl cinnamaldehyde* | *α-bromo cinnamaldehyde* | *4-phenyl-3-buten-2-one* | *estragole* | *eugenol* | *anethol* | *isoeugenol* | *cinnamic acid* | *ferulic acid* | *para-methoxy cinnamaldehyde* | *Cinnamamide* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Q | 1.08 | 1.07 | 1.09 | 1.12 | 0.01 | 0.01 | −0.66 | −0.68 | 0.96 | 0.96 | 1.1 | 0.28 |
| C1OrbEle | 9.62 | 9.65 | 9.87 | 9.67 | 7.9 | 7.9 | 7.56 | 7.56 | 9.65 | 9.65 | 9.62 | 8.89 |
| Average mass | 132.16 | 146.19 | 211.06 | 146.19 | 148.2 | 164.2 | 148.2 | 164.2 | 148.16 | 194.18 | 162.19 | 147.17 |
| Log Max Proy | 1.72 | 1.75 | 1.76 | 1.76 | 1.73 | 1.73 | 1.76 | 1.78 | 1.74 | 1.81 | 1.77 | 1.75 |
| LOG%Ring | 1.81 | 1.75 | 1.75 | 1.75 | 1.85 | 1.86 | 1.85 | 1.86 | 1.78 | 1.83 | 1.85 | 1.77 |
| FNSA | 8.8 | 7.56 | 8.08 | 7.58 | 3.74 | 11.43 | 3.72 | 11.38 | 18.15 | 25.25 | 10.86 | 20.37 |
| Ox Car/Hal OrbEle | 12.88 | 12.9 | 13.01 | 12.95 | 0 | 0 | 0 | 0 | 13.48 | 13.48 | 12.88 | 12.97 |
| C2OrbEle | 8.99 | 9.05 | 9.35 | 9.03 | 7.94 | 7.94 | 8.09 | 8.1 | 9.34 | 9.34 | 8.99 | 9.02 |
| C3OrbEle | 8.54 | 8.58 | 8.78 | 8.55 | 7.89 | 7.89 | 8.22 | 8.24 | 8.69 | 8.69 | 8.52 | 8.61 |
| Dipolo | 3.26 | 2.86 | 3.47 | 3.33 | 1.14 | 1.86 | 0.95 | 1.29 | 4.91 | 4.87 | 4.42 | 3.53 |
| Ox Carb Hetero At | −0.32 | −0.32 | −0.29 | −0.32 | 0 | 0 | 0 | 0 | −0.36 | −0.35 | −0.32 | −0.39 |
| Carbon 1 Mulli | 0.26 | 0.26 | 0.27 | 0.34 | −0.33 | −0.33 | −0.26 | −0.28 | −0.35 | 0.41 | 0.26 | 0.29 |
| Carbon 2 Mulli | −0.35 | −0.22 | −0.32 | −0.32 | −0.22 | −0.22 | −0.23 | −0.21 | 0.42 | −0.32 | −0.36 | −0.28 |
| Carbon 3 Mulli | −0.09 | −0.12 | −0.06 | −0.11 | −0.15 | −0.15 | −0.18 | −0.19 | −0.05 | −0.09 | −0.08 | −0.13 |
| VDW | 82.59 | 86.86 | 93.83 | 88.84 | 90.59 | 94.23 | 90.11 | 92.15 | 86.51 | 100.46 | 93.48 | 88.07 |
| Density | 1 | 1 | 1.5 | 1 | 0.9 | 1.1 | 1 | 1.1 | 1.2 | 1.3 | 1.1 | 1.1 |
| Boiling | 246.8 | 254.6 | 304.4 | 260.8 | 216 | 255 | 237.5 | 266.6 | 265 | 372.3 | 308.7 | 350.3 |
| LOG Boiling | 2.39 | 2.41 | 2.48 | 2.42 | 2.33 | 2.41 | 2.38 | 2.43 | 2.42 | 2.57 | 2.49 | 2.54 |
| Enthalpy | 48.4 | 49.2 | 54.5 | 49.8 | 43.4 | 51.2 | 45.5 | 52.5 | 53.1 | 65.3 | 54.9 | 59.5 |
| Index refraction | 1.58 | 1.56 | 1.63 | 1.56 | 1.5 | 1.54 | 1.54 | 1.58 | 1.62 | 1.63 | 1.56 | 1.61 |
| Molar refractivity | 42.3 | 46.8 | 49.9 | 46.8 | 46.8 | 48.7 | 48.8 | 50.7 | 43.7 | 52.3 | 49 | 45.7 |
| H acceptor | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 4 | 2 | 2 |
| H donor | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 2 | 0 | 2 |
| Log P | 2.12 | 2.68 | 2.44 | 2.17 | 3.15 | 2.2 | 3.17 | 2.45 | 2.41 | 1.64 | 2.07 | 1.35 |
| Log D 5 | 1.76 | 2.04 | 2.39 | 2.16 | 3.14 | 2.48 | 3.08 | 2.55 | 1.13 | 0.4 | 1.85 | 1.14 |
| Log D 7.4 | 1.76 | 2.04 | 2.39 | 2.16 | 3.14 | 2.48 | 3.08 | 2.55 | −0.66 | −1.38 | 1.85 | 1.14 |
| Polar surface | 17 | 17 | 17 | 17 | 9 | 29 | 9 | 29 | 37 | 67 | 26 | 43 |
| Polarizability | 16.8 | 18.6 | 19.8 | 18.6 | 18.6 | 19.3 | 19.4 | 20.1 | 17.3 | 20.7 | 19.4 | 18.1 |
| Surface tension | 39 | 36.9 | 46.5 | 36.9 | 30 | 36.6 | 31.9 | 39 | 49.7 | 56.2 | 37.6 | 47.4 |
| molar volume | 127.7 | 144 | 140.7 | 144 | 157.9 | 156.3 | 154.4 | 152.8 | 125 | 147.5 | 151.7 | 131.2 |
| Carbon 1 | −0.03 | −0.02 | 0 | −0.03 | −0.01 | −0.01 | 0.06 | −0.06 | −0.01 | −0.01 | −0.02 | −0.02 |
| Carbon 2 alpha | 0.02 | 0.03 | 0.06 | 0.03 | −0.09 | −0.09 | −0.08 | −0.08 | 0.06 | 0.06 | 0.03 | 0.02 |
| Carbon 3 bonded to the functional group | 0.09 | 0.09 | 0.11 | 0.09 | −0.1 | −0.1 | −0.05 | −0.05 | 0.09 | 0.09 | 0.09 | −0.01 |
| Log P chem | 1.98 | 2.37 | 2.53 | 2.47 | 2.91 | 2.61 | 2.94 | 2.64 | 2.14 | 1.67 | 1.81 | 1.33 |
| Polarizability chem | 15.78 | 17.63 | 18.44 | 17.63 | 18.16 | 18.73 | 18.24 | 18.81 | 16.19 | 19.35 | 18.27 | 16.9 |
| Polar surface chem | 17.07 | 17.07 | 17.07 | 17.07 | 9.23 | 29.46 | 9.23 | 29.46 | 37.3 | 66.76 | 26.3 | 43.09 |
| Molecular Surface | 194.07 | 225.73 | 211.28 | 225.3 | 247.02 | 257.78 | 248.2 | 258.92 | 205.49 | 264.4 | 242.22 | 211.55 |
| Molecular refract | 42.13 | 46.49 | 49.74 | 46.61 | 46.81 | 48.78 | 47.88 | 49.86 | 43.06 | 51.5 | 48.6 | 44.88 |

**Table 1.** (*Continued*)

| Compounds† descriptors | cinnamaldehyde | α-methyl cinnamaldehyde | α-bromo cinnamaldehyde | 4-phenyl-3-buten-2-one | estragole | eugenol | anethol | isoeugenol | cinnamic acid | ferulic acid | para-methoxy cinnamaldehyde | Cinnamamide |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pi energy | 14.28 | 14.28 | 14.28 | 14.28 | 14.31 | 18.62 | 14.74 | 19.04 | 18.64 | 27.27 | 18.6 | 17.45 |
| Dreidingenerg | 24.07 | 38.34 | 33.48 | 28.1 | 24.87 | 27.22 | 30.2 | 32.18 | 35.25 | 32.32 | 29.76 | 28.53 |
| Volchem | 127.65 | 144.74 | 146.07 | 144.44 | 151.48 | 159.93 | 151.39 | 159.85 | 136.13 | 170.68 | 153.71 | 138.68 |
| Min proy | 21.23 | 24.81 | 24.78 | 22.92 | 29.14 | 34.18 | 26.53 | 30.12 | 20.66 | 30.17 | 23.56 | 22.58 |
| Max proy | 51.96 | 56.19 | 58.1 | 57.24 | 53.84 | 54.21 | 56.99 | 59.66 | 54.89 | 64.83 | 58.85 | 56.66 |
| Relation Min maxproy | 2.4 | 2.3 | 2.3 | 2.5 | 1.8 | 1.6 | 2.1 | 2 | 2.7 | 2.1 | 2.5 | 2.5 |
| Lattind | 22 | 26 | 26 | 26 | 26 | 30 | 26 | 30 | 26 | 36 | 28 | 26 |
| Randicind | 4.93 | 5.33 | 5.33 | 5.29 | 5.36 | 5.77 | 5.36 | 5.77 | 5.29 | 6.63 | 5.86 | 5.29 |
| Balaban | 2.02 | 1.94 | 1.94 | 1.86 | 1.94 | 2.33 | 1.94 | 2.33 | 1.86 | 2.27 | 2.09 | 1.86 |
| Harary | 21.64 | 25.38 | 25.38 | 25.1 | 25.41 | 29.6 | 25.41 | 29.6 | 25.1 | 36.81 | 28.61 | 25.1 |
| Wienner | 133 | 168 | 168 | 174 | 170 | 204 | 170 | 204 | 174 | 330 | 226 | 174 |
| HyperWienner | 323 | 413 | 413 | 446 | 432 | 502 | 432 | 502 | 446 | 945 | 633 | 446 |
| Wienner polarity | 9 | 11 | 11 | 10 | 12 | 15 | 12 | 15 | 10 | 17 | 13 | 10 |
| Szeged | 196 | 240 | 240 | 246 | 260 | 308 | 260 | 308 | 246 | 466 | 331 | 246 |
| Freely | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 2 | 2 | 3 | 3 | 0 |

†Details in material and methods section.

(DBil) and total bilirubin (TBil) by using a commercial kit (Abbott Lab, Buenos Aires, Argentina).

*Hepatic histological analysis*

Liver tissue specimens were fixed in 10% (v/v) neutral buffered formalin (Wako Pure Chemical, Richmond, USA), dehydrated in an ethanol series, cleared in xylene, and embedded in paraffin. The paraffin blocks were cut into sections approximately 5 μm thick, which were defatted with xylene and stained with haematoxylin and eosin (H&E) (Merck, Buenos Aires, Argentina). Sections were viewed under an inverted microscope (Olympus IX-73; Olympus, Tokyo, Japan) (original magnification × 160).

*Sperm collection and analysis of motility*

The epididymis was carefully separated from the testis and cauda severed. The cauda was finely minced with anatomical scissors in 1 mL of isotonic saline at 37.5 °C in a center well at 37.5 °C, then it was completely squashed with tweezers for 3 min to expel the sperms. Sperm concentration and motility were assessed at 23 ± 2 °C in a Makler counting chamber (Sefi Medical Instruments, Haifa, Israel) under an inverted microscope at ×200 magnification. The results are expressed as the percentage of motile cells (progressive plus nonprogressive spermatozoa) and non-motile cells. No fewer than 200 gametes were examined.

*Data analysis*

The dose–mortality response was analyzed by Probit analysis using the POLO–PLUS Software (LeOra software, Northampton, U.K). The significance of the mean RI in each trial of the two-choice olfactometer bioassay was evaluated by the Student's t test for paired comparisons. An ANOVA and Duncan's test were used for the comparison of means, using InfoStat/Professional 2010p with a significance level of $P \leq 0.05$.

In the QSAR analysis, descriptors that had Pearson correlation coefficients with a difference ≥ 0.95 were not included in the QSAR analysis.

## RESULTS AND DISCUSSION

### Insecticidal action

There were two principal groups with respect to insecticidal action (Table 2): those with $LC_{50}$ less than 500 μg cm$^{-2}$ and those with $LC_{50} > 500$ μg cm$^{-2}$. Among the latter, two acids (ferulic and cinnamic acids), an acid derivative (cinnamamide), a phenol (isoeugenol), and *trans*-p-methoxy cinnamaldehyde showed the lowest toxicity. Among the compounds with a $LC_{50}$ less than 500 μg cm$^{-2}$, there were three groups. Of these, the first was built by α-methyl-*trans*-cinnamaldeyde and had a $LC_{95}$ less than 100 μg cm$^{-2}$, with a second group having a $LC_{95}$ between 200 and 400 μg cm$^{-2}$; this included cinnamaldehyde, 4-phenyl-3-buten-2-one and α-bromo-cinnamaldehyde. The third group was represented by phenols and ether and had a $LC_{95}$ higher than 400 μg cm$^{-2}$ (Table 2). Thus, among the phenylpropanoid compounds, the ketones and the aldehydes (with an unsubtitued aromatic ring) were the chemical groups with the best contact insecticidal action against *S. zeamais,* and among these, α-methyl-cinnamaldehyde had the best insecticidal action. Furthermore, α-methyl-cinnamaldehyde did not demonstrate genotoxicity[35] and showed a low acute toxicity for mammals.[36] In contrast, the lowest insecticidal action was shown by p-methoxy-cinnamaldehyde ($LC_{50} > 500$ μg cm$^{-2}$) as



  

**Table 2.** Contact toxicity of phenylpropanoids against *S. zeamais* after 24 h of exposure

| Phenylpropanoids | $LC_{50}$ (95% CL) (μg cm$^{-2}$) | $LC_{95}$ (95% CL) (μg cm$^{-2}$) | Slope ± SEM | $(X^2)^{\dagger}$ |
|---|---|---|---|---|
| cinnamaldehyde | 51.7 (32.8–73.3) | 222.3 (135.3–720.1) | 2.6 ± 0.3 | 4.22 |
| α-methyl-*trans*-cinnamaldehyde | 56.5 (54.3–59.9) | 70.4 (65.9–85.7) | 17.2 ± 2.2 | 2.58 |
| α-bromo-cinnamaldehyde | 67.1 (51.8–96.2) | 378.0 (214.6–1064.7) | 0.2 ± 0.1 | 0.48 |
| 4-phenyl-3-buten-2-one | 69.8 (53.4–85.3) | 295.3 (208.7–568.2) | 2.6 ± 0.5 | 0.17 |
| estragole | 76.1 (24.4–137.9) | 580.9 (257.9–18 020) | 1.9 ± 0.3 | 6.70 |
| eugenol | 186.2 (128.1–301.5) | 3308.5 (1282.5–26 877) | 1.3 ± 0.3 | 0.16 |
| anethole | 324.6 (252.2–430.9) | 795.6 (623.5–1186.8) | 6.5 ± 0.001 | 27.38 |
| cinnamic acid | >500 | | | |
| ferulic acid | >500 | | | |
| cinnamamide | >500 | | | |
| Isoeugenol | >500 | | | |
| *trans*-p-methoxy cinnamaldehyde | >500 | | | |
| chlorpyrifos (+control) | 1.2 (1.0–1.5) | 2.9 (2.1–5.9) | 4.2 ± 0.8 | 0.11 |

$^{\dagger}$Chi-square values significant at $P < 0.05$. $LC_{50}$: lethal concentration 50; $LC_{95}$: lethal concentration 95; 95% CL: confidence limits; (+ control): positive control.

can be seen in Table 2. The introduction of an hydroxyl functional group in cinnamaldehyde was reported to decrease its contact insecticidal action clearly on *S. oryzae*[24].

A notable feature of our findings is the difference between eugenol and isoeugenol with $LC_{50}$ values of 186 and > 500 μg cm$^{-2}$, respectively, despite these compounds only differing in the position of the double bond on the alkenyl group (positional isomers). Similarly, between estragole ($LC_{50}$ = 76.1 μg cm$^{-2}$) and its positional isomer, anethole ($LC_{50}$ = 324.6 μg cm$^{-2}$), estragole was the more toxic, and had a carbon/carbon double bond in the terminal part of the propenyl, as observed in eugenol.[37] Thus it can be hypothesized that there is a better interaction between the propenyl chain and the molecule target when the double carbon-carbon bond is located at the terminal end of the phenylpropanoid.[38] In a previous study, estragole showed a larger toxic effect than anethol on the AChE activity of *S. oryzae,* an enzyme related to insecticidal action.[39]

The introduction of methoxy or phenol groups in the phenyl structure of molecules without an alpha, beta-unsaturated carbonyl group (for example, eugenol, anethole and isoeugenol) had a negative effect on the insecticidal action (Table 2). This finding is consistent with the idea of a steric problem present in the interaction between bioactive compounds and their targets. On the other hand, the low bioactivity of cinnamic acid may be explained by the fact that among the alpha, beta-unsaturated cinnamic acids the Michael addition is greatly decreased by the absence of hydroxyl substituents on the aromatic rings,[24,40] while the introduction of a methoxy group along with a hydroxyl group in ferulic acid produces an antagonistic effect, and thus significantly decreases its insecticidal action (Table 2). Finally, cinnamamide revealed a low toxic effect against *S. zeamais* (Table 2), whereas an opposite effect was shown on *S. oryzae*.[24]

### Effects on behavior, attraction/repellent actions

Although the use of chemicals with stronger repellent properties might reduce the development of resistance among storage-insect pests, the large diversity of storage-insect pests could result in variations in the responses to the same chemical. Taking this into account, a large number of different essential oils and their components were tested for repellence against storage-insect pests.[16,41–44] In previous reports, anethol, estragole, and eugenol have been shown to have repellent action against *S. zeamais,* although anethole also showed some attractive activity at low concentrations.[45,46] However, no critical study has been made of the structural differences occurring in phenylpropanoids or their relationship with repellent action, and this is important in order to contribute to the development of new green pesticides.

From our results, similar repellent effects were shown for α-bromo-cinnamaldehyde; 4-phenyl-3-buten-2-one, cinnamaldehyde and *trans*-p- methoxy-cinnamaldehyde at a dose of 56 μmol L$^{-1}$ (RI between −37.9 and −60.7), with cinnamamide being the least repellent (Table 3). A loss of repellent action against *S. zeamais* was observed with a decrease in the dose for different compounds (with a significance of $P < 0.01$ for the determination of correlation coefficient ($R^2$) of log (dose)–response in linear regression analysis). In fact, at the lowest evaluated dose (0.28 μmol L$^{-1}$), only α-bromo-cinnamaldehyde registered a significant repellent action, with α-bromo-cinnamaldeyde and propionic acid (repellent positive control) having similar RI values at all doses (Table 3).

The compound α-methyl-*trans*-cinnamaldehyde was the only aldehyde to show an attractive effect at almost every dose (Table 3). Among phenylpropanoids, those that attracted *S. zeamais* at all doses were estragole and anethole. Finally, ferulic acid, isoeugenol, cinnamic acid, and eugenol all attracted *S. zeamais* at doses up to 28 μmol L$^{-1}$, with all of these presenting a significant ($P < 0.03$) log (dose)–response in the linear regression analysis. The attraction response of these compounds was not important (< 55%).

### QSAR analysis

In a QSAR study it is assumed that there is a connection between a mathematical function of chemical properties of a set of similar molecules with bioactivity. Although some SAR (structural analysis-relationship) studies on phenylpropanoids-based insecticides have been performed to establish their mechanisms,[24,47] there are no QSAR studies of the insecticidal action or repellent effects of these compounds. The structural characteristics of the molecules involved in these biological properties are thus still unclear. In the present investigation, different descriptors to



  

**Table 3.** Effects of phenylpropanoids on the attraction / repellence response index (RI) of *S. zeamais*

| | RI (mean ± SEM) | | | |
|---|---|---|---|---|
| | Concentration | | | |
| Compounds | 56 (μmol $L^{-1}$) | 28 (μmol $L^{-1}$) | 2.8 (μmol $L^{-1}$) | 0.28 (μmol $L^{-1}$)† |
| *α-bromo-cinnamaldehyde* | −60.7 ± 4.1 ***a1 | −40.4 ± 11.3 *ab1 | −35.1 ± 8.1 *b12 | −18.5 ± 4.6 *b1 |
| *4-phenyl-3-buten-2-one* | −58.6 ± 7.4 **a1 | −35.6 ± 6.7 *b1 | −4.5 ± 2.1 c34 | |
| *cinnamaldehyde* | −49.6 ± 14.3 *a1 | −43.0 ± 15.2 *ab1 | −5.4 ± 4.7 b34 | |
| trans-*p-methoxy cinnamaldehyde* | −37.9 ± 8.1 **a12 | −32.1 ± 6.3 **a1 | −24.9 ± 4.2 **a23 | 16.1 ± 10.0 b4 |
| *Cinnamamide* | −18.3 ± 5.9 *a23 | −18.9 ± 5.9 *a1 | 2.9 ± 4.4 b45 | |
| *α-methyl-*trans-*cinnamaldehyde* | 22.8 ± 3.9 **a45 | 29.1 ± 4.5 **a23 | 27.4 ± 7.6 *a67 | 29.2 ± 11.2 a4 |
| *Eugenol* | 35.4 ± 7.3 * b56 | 17.8 ± 6.6 *ab2 | 9.4 ± 4.1 a456 | |
| *cinnamic acid* | 38.1 ± 6.2 **b56 | 27.0 ± 6.2 **b23 | 3.3 ± 1.3 a45 | |
| *Anethole* | 39.9 ± 8.0 **b56 | 35.1 ± 8.2 ***b23 | 32.7 ± 10.9 *b7 | 8.1 ± 1.5 **a234 |
| *isoeugenol* | 43.5 ± 5.7 **c56 | 26.0 ± 5.2 **b23 | 6.1 ± 3.7 a456 | |
| *Estragole* | 49.1 ± 4.8 **c56 | 29.1 ± 3.8 ***b23 | 22.8 ± 5.1 *ab567 | 14.1 ± 4.6 *a34 |
| *ferulic acid* | 53.3 ± 12.2 *b6 | 46.2 ± 11.5 *b3 | 7.9 ± 1.3 a456 | |
| *propionic acid* (repellent *positive* control) | −55.40 ± 12.06 *a1 | −38.76 ± 13.45 *ab1 | −48.50 ± 16.46 *ab1 | −12.20 ± 4.18 *b12 |

*$P \leq 0.05$.
**$P < 0.01$.
***$P < 0.001$ (significant response to experimental stimulus) T Test paired samples.
†The concentration 0.28 μmol $L^{-1}$ was used to test attraction / repellence only when the compound at 2.8 μmol $L^{-1}$ presented a statically significant response.
Values that have different letters in the same row and different numbers in the same column are significantly different according to Duncan's multiple range test at $P \leq 0.05$ ($n = 5$).

develop two QSAR models with 12 phenylpropanoid analogues against storage pest *Sitophilus zeamais* were used that could explain their insecticide and repellent activities. The experimental values of insecticidal action and the repellent effects were then converted to Log 1/($LD_{50}$/1000) and Log1/(RI56/1000) respectively for modeling purposes. The attraction / repellence Response Index at 56 μM $L^{-1}$ was used in repellent effect (RI56). The optimal models found are expressed in Eqns (1) and (2):

$$Log\ (1/\ (LC_{50}/1000) = -\ 0.47\ \ (Dipole) + 1\ \ (Q) + 2.62 \quad \text{(insecticidal activity)} \qquad (1)$$

N = 12, $r^2 = 0.94$, P = 0.0001, RMSPE = 7.6%
Q = ($carbon_1$ electronegativity − $carbon_3$ electronegativity).

$$Log\ \ (1/RI56/1000) = 0.02\ \ (PS) + 0.51\ \ (Log\ P) + 0.46 \quad \text{(repellent effect)} \qquad (2)$$

N = 12, $r^2 = 0.82$, P = 0.0002, RMSPE = 6.8%
PS = polar surface

The comparison between experimental values and calculated values according to the optimal QSAR models is shown in Figs 2 and 3. The QSAR models were subjected to validation using RMSPE = 7.6% for insecticidal action and 6.8% for repellent effect, and the correlation coefficients were 0.94 and 0.82, respectively. These results showed that statistically significant QSAR models ($P < 0.0002$) were capable of representing the relationship between cinnamaldehyde and related compounds for each bioactivity evaluated.

Good performance as insecticides was prevented by the presence of hydroxyl or ether groups in the aromatic rings, as shown using Eqn (1) for the descriptor dipolo, which revealed negative values. In contrast, the Q descriptor was positively related to the

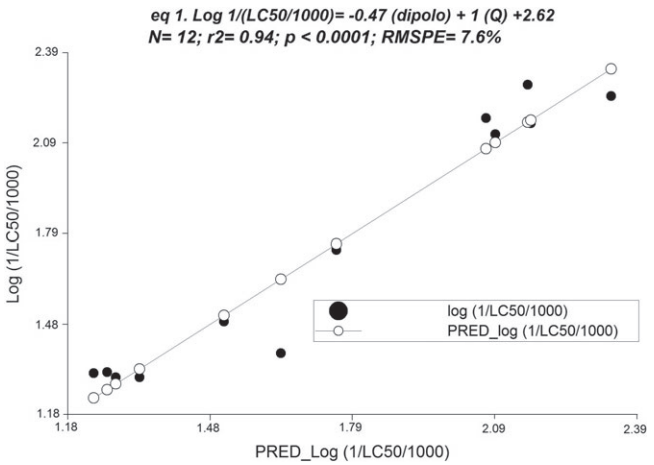


**Figure 2.** Plot of calculated versus experimental insecticidal action (Log 1/($LC_{50}$/1000)) of the 12 phenylpropanoids compounds against *S. zeamais*. N is the number of data points, *r* is the correlation coefficient between observed values of the dependent variable and the values predicted by the equation, and $r^2$ is the square of the correlation coefficient and represents the goodness of fit. The obtained quantitative structure–activity relationship (QSAR) model was validated by the root mean square prediction error (RMSPE), obtained by an across validation leave-one-out procedure.

difference between the orbital electronegativity of carbon one and carbon three of the propenyl chains (see Fig. 1). This is undoubtedly explained by the fact that the α-system of the carbon-carbon double bond of aldehyde and ketones is intrinsically more reactive toward nucleophiles than acids or amides,[48] while the simple carbon–carbon double bonds are subject to electrophilic addition.

The carbonyl groups with a strong polarization due to the presence of an alpha, beta unsaturated structure showed a toxic action



  

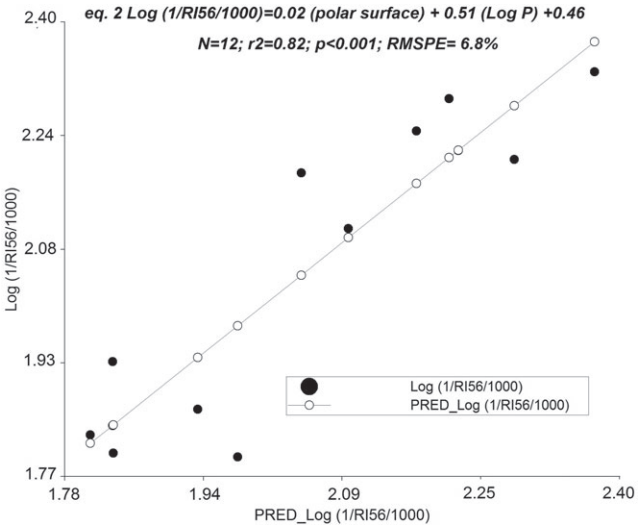


**Figure 3.** Plot of calculated versus experimental repellent effects (Log 1/(RI56/1000)) of the 12 phenylpropanoids compounds against *S. zeamais*. N is the number of data points, *r* is the correlation coefficient between observed values of the dependent variable and the values preddicted by the equation, and $r^2$ is the square of the correlation coefficient and represents the goodness of fit. The obtained quantitative structure–activity relationship (QSAR) model was validated by the root mean square prediction error (RMSPE), obtained by an across-validation leave-one-out procedure. RI56 is the response index at 56 mmol $L^{-1}$.

by a 1.4 addition mechanism (known as a Michael-type addition). However, the structural characteristics of the molecules have a strong effect on the toxic potency of alpha, beta-unsaturated carbonyl compounds.[15,38,49] The molecular mechanism of these alpha, beta-unsaturated carbonyl compounds works by addition on the thiol or amine groups,[49] which induces protein alkylation[50] via a carbanion intermediate. It is also hypothesized that an alpha or beta substitution by a methyl group or bromine atom of the vinyl carbons could result in a reduction in toxic potency because the substitution may alter the electron density.[15,38,49] From the results of $LC_{95}$ (Table 2), it is clear that changes occurred in bioactivity due to the presence of alpha substituents, with halogen (atom of bromine) decreasing the insecticidal bioactivity (378.0 μg $cm^{-2}$), while alkyl substituent increased the toxic property (70.4 μg $cm^{-2}$). Thus, Eqn (1) shows that orbital electronegativity plays an important role in the insecticidal action of phenylpropanoid compounds. However, previous reports[15] have demonstrated that the insecticidal action of cyclic ketones is related to the shape and branching of the skeleton of molecules, although orbital electronegativity of ketone compounds was the principal descriptor found that linked the inhibition of acetylcholinesterase (AChE) with the insecticidal property. Moreover, Lee *et al.*[24] reported that some functional carbonyl groups, rather than LogP, molecular weight or vapor pressure descriptors, seemed to play a very important role in determining the insecticidal action of adult *S. oryzae*.

The repellent effect of α-bromo-cinnamaldehyde, 4-phenyl-3-buten-2-one, cinnamaldehyde and *trans*-p-methoxy cinnamaldehyde can be explained by Eqn (2), which shows a QSAR model of the repellent action of the cinnamaldehyde analogues against *S. zeamais* with two descriptors (polar surface and Log P) that have positive values. The biogenic monoamines, octopamine and tyramine, act as neurotransmitters in invertebrates.[51,52] Recently, Chen *et al.*[53] suggested that three amino acid residues of a beta-adrenergic-like octopamine receptor interact with the phenolic OH and $NH_2$ groups of octopamine. On the other hand, Cui *et al.*[54] were able to demonstrate from docking results that α-bromo-cinnamaldehyde has a strong inhibitory action on tyramine in its interaction with the amino acid residues of the active site center. These two descriptors could thus explain the repellence mechanism (Table 3).

### Acute toxicity study in C57BL/6 mice

The assessment of acute toxicity in C57BL/6 mice, after the administration of 2000 mg kg−1 Cinn, led to no signs of morbidity and mortality. The results of the sperm motility analysis (n: 11, %) were: Control: 61.86 ± 28.27; 28.74 ± 21.97; 9.4 ± 8.45, Cinn: 49.68 ± 28.35; 27.33 ± 18.17; 13.9 ± 12.81, for non-motility, non-progressive, and progressive, respectively. Moreover, there were no significant difference in drink uptake (n: 11, mL): Control 17.00 ± 1.56, Cinn: 18.18 ± 1.83 and in fecal pellets (n: 11, gr) Control: 1.02 ± 0.06, Cinn: 1.08 ± 0.05.

Furthermore, no signals of toxicity were observed, such as behavioral changes, alterations in food intake, or modifications in body weight gain, in liver histopathology (Fig. 4), or in the other parameters evaluated, as show in Table 4. The histological appearance of the control and Cinn mouse liver is typical of the species. All animals survived until their scheduled euthanasia.

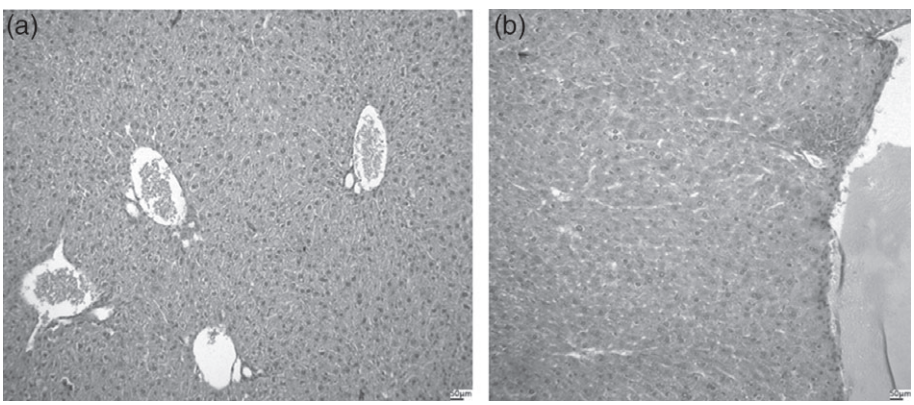


**Figure 4.** Liver segments from mice treated with oral sunflower oil (a) (H&E 100X), and liver section of mice treated with oral cinnamaldehyde (b) (2 g $kg^{-1}$) showing inflammatory infiltrate cells but without hepatocellular degenerative phenomena (H&E 100X).



  

**Table 4.** Effects of the acute administration of cinnamaldehyde (Cinn) on CB57BL/6 mice (n:11)

| Parameters | Control | 2000 mg/kg Cinn |
|---|---|---|
| Food intake (g) | 3.56 ± 0.64 | 3.88 ± 0.63 |
| Body weight (g) | 24.35 ± 0.57 | 24.29 ± 0.59 |
| Liver weight (g) | 1.44 ± 0.15 | 1.38 ± 0.18 |
| AST (U $L^{-1}$) | 155.2 ± 69.22 | 329.5 ± 126.64 |
| ALT (U $L^{-1}$) | 23.00 ± 2.16 | 23.25 ± 2.39 |
| DBil (mg $dL^{-1}$) | 0.11 ± 0.01 | 0.15 ± 0.05 |
| TBil (mg $dL^{-1}$) | 0.100 ± 0.001 | 0.102 ± 0.002 |
| AlkP (U $L^{-1}$) | 5.00 ± 0.001 | 7.83 ± 2.83 |

Data are expressed as mean ± S.D. One way ANOVA followed by the Duncan test showed no significant differences in any measured values.

## CONCLUSION

Comparison of the insecticidal action of cinnamaldehyde and its analogues revealed that the carbonyl compounds (except acids and amide) were the most toxic. Moreover, the greatest reduction in the toxic effects was observed with substitution on the aromatic ring.

For *S. zeamais*, α-bromo-cinnamaldehyde, 4-phenyl-3-buten-2-one, cinnamaldehyde and *trans*-p-methoxy cinnamaldehyde showed better repellent effectiveness than the other compounds. The QSAR model shows the polar surface and LogP descriptors with positive values that may explain the mechanism of the interaction between cinnamaldehyde or its analogues with the tyramine or octopamine receptor, and the result could be a repellent effect on *S. zeamais*.

On the other hand, cinnamaldehyde showed no toxicity in mice, and this study is consistent with other toxicity studies about this compound and the essential oils that contain it.[32,55] These QSAR studies may thus provide guidance for further synthesis investigation.

## ACKNOWLEDGEMENTS

We thank INTA-Manfredi (Manfredi-Córdoba, Argentina) for providing the maize kernels used in the study. We would like to thank native English speaker Dr Paul Hobson for his revision of the manuscript. MPZ and JAZ are career members of CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas). YPZ has a fellowship from FONCYT (Fondo para la Investigación Científica y Técnica)-PICT (Proyecto de Investigación Científica y Técnica) 2012-20146. This work was supported by grants from the CONICET, FONCyT, SECyT-UNC (Secretaría de Ciencia y Técnica – Universidad Nacional de Córdoba) and SECyT-UNLaR (Secretaría de Ciencia y Técnica – Universidad Nacional de La Rioja).

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