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QSAR Study of Biologically Active Essential Oils against Beetles Infesting the Walnut in Catamarca, Argentina

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Supporting Information

ABSTRACT: Essential oils from six species of aromatic plants collected in the Catamarca Province of Argentina were evaluated for their chemical composition and repellent and insecticidal activities against beetles of the genus *Carpophilus* (Coleoptera: Nitidulidae) and *Oryzaephilus* (Coleoptera: Silvanidae) that infest the local walnut production. Experimental data were analyzed using generalized estimating equations, with normal distribution and the identity link function. From the spectral information from the tested essential oils, we worked their molecular modeling as mixtures by developing mixture descriptors (D_{mix}) that combined the molecular descriptor of each component in the mixture (d_i) and its relative concentration (x_i), i.e., $D_{mix} = f(d_i, x_i)$. The application of chemoinformatic approaches determined that a combination of mixture descriptors related to molecular size, branchedness, charge distribution, and electronegativity were useful to explain the bioactivity profile against *Carpophilus* spp. and *Oryzaephilus* spp. The reported models were rigorously validated using stringent statistical parameters and essential oils reported with repellent activity against other beetle species from the Nitidulidae and Silvanidae families. This model confirmed each essential oil as a repellent with a comparable performance to the experimental reports.

KEYWORDS: *walnuts, Carpophilus spp. (Coleoptera: Nitidulidae), Oryzaephilus spp. (Coleoptera: Silvanidae), essential oils, repellent and insecticide activity, generalized estimating equations, chemoinformatics, principal component analysis*

INTRODUCTION

International demand for walnuts has been on the rise for the last few decades due to their protective effects against some aspects of age-related behavioral and cellular dysfunction.[1,2] Argentina is one of the largest walnut producers in South America. The Argentine provinces that produce walnuts are Catamarca (in the northwest), followed by Mendoza, La Rioja, and San Juan (in the central-west) and Río Negro (in Northern Patagonia). In Catamarca, small-scale farmers produce the dominant share of Argentina's output. They produce walnuts with very low inputs and little or no management.[3] Under these circumstances, dried fruit beetles of the family Nitidulidae (*Carpophilus* spp.) and Silvanidae (*Oryzaephilus* spp.) have been observed attacking walnuts in pre- and post-harvest situations.[4] The economic value of losses of walnuts ranges between 30% and 74% in the main walnut producing area of the Catamarca Province (Andalgalá, Belén, Ambato, Santa María, Pomán, and Capayán departments). These species are cosmopolitan and polyphagous and are the major secondary pests of stored products throughout the world.[5]The available approach to protecting walnuts from damage by beetles is the use of chemical insecticide (fumigation with phosphine). However, such an approach is not appropriate and is environmentally unfriendly in walnut small-scale farming systems.

Research aiming at the development of natural products for pest control is increasingly important.[6] Phytochemicals from aromatic plants (mainly essential oils) can be used as pesticides themselves, or they can serve as model compounds for the development of chemically synthesized, easily biodegradable derivatives, with low plant and human toxicity.[7−9] Essential oils are natural extracts responsible for the specific flavor and scent of aromatic plants. In the last few years, many plant essential oils and major constituents have been tested against a wide range of stored product pests.[10−12] Particularly, there are reports on repellent and insecticidal activities, lethal doses, and time to achieve lethal effects of essential oils come from Asteraceae, Apiaceae, Lauraceae, and Verbenaceae plant families against beetle pests.[13−15] (see Scheme 1). Moreover, they show insecticidal effects against a wide range of arthropods at several stages of their life (e.g., *Pieris rapae* L.

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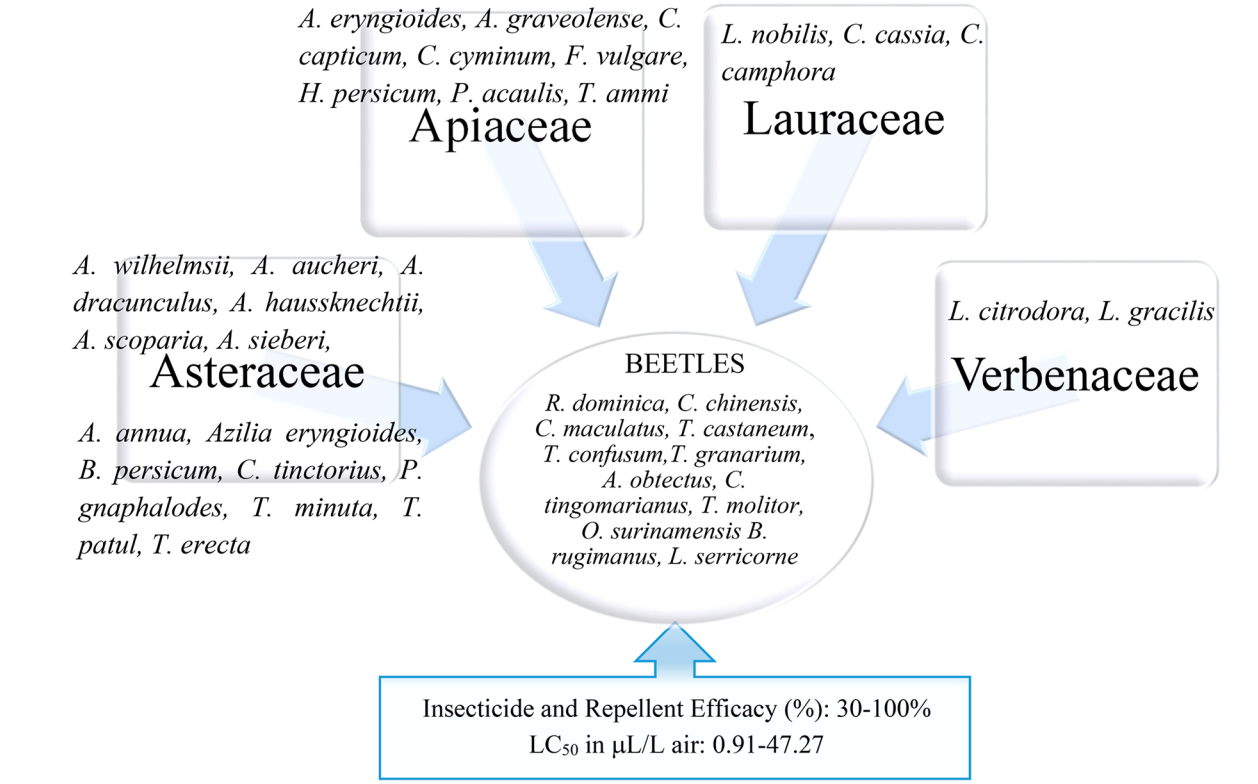
Scheme 1. Essential Oils of Plants Belonging to Asteraceae, Apiaceae, Lauraceae, and Verbenaceae Plant Families As Insecticides and Repellents against Beetle Pests



(Lepidoptera: Pieridae), *Plutella xylostella* L. (Lepidoptera: Plutellidae), *Ephestia kuehniella* Zell. (Lepidoptera: Pyralidae), *Sitotroga cerealella* Olivier (Lepidoptera: Gelechidae), etc; Scheme 1).[13,14,16] The main components of essential oils related with repellent and insecticidal activity against beetles are summarized in Scheme 1. A wide diversity of compounds contribute to the overall activity of each essential oil individually as well as by interacting among them. Then, as no chemical constituent accounted for the tested bioactivity of the essential oils, there is marked interspecific differences in bioactivity among insects, even among closely related compounds, and synergistic and antagonistic effects were reported among constituents.[17] Furthermore, the molecular properties related to their insecticidal activity are not well understood.

Many Argentinian plant-product extracts have been reported with insecticidal activity against the major stored grain and cereal insect pests in Argentina (*Sitophilus oryzae, Rhizopertha dominica, Tribolium castaneum, Sitotroga cerealella*, and *Tenebrio molitor*).[18] Others papers documented the insecticidal and repellent effects of essential oils extracted from some members of Asteraceae (*Tagetes terniflora* Kunth,[19] *Tagetes filifolia*,[20] *Eupatorium buniifolium* Hook. and Arn., *Eupatorium inulaefolium* Kunth, *Eupatorium arnottii* Baker, *Eupatorium viscidum* Hook. and Arn.,[21] *Baccharis salicifolia* (Ruiz and Pavon) Pers,[22] and *Flourensia oolepis*[18]) and Verbenaceae (*Aloysia polystachia*

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(Griseb), Moldenke,[23,24] and *Lippia alba*[25]) against *T. castaneum* and *R. dominica*. However, there are no reports on the chemical composition and potential biological activity of essential oils against beetles of the genus *Carpophilus* and *Oryzaephilus*.

As we were interested in the development of knowledge on the selection and rational use of essential oils to address walnut crop protection problems in pre- and post-harvest situations, the objective of this study was the evaluation of the repellent and insecticidal activities against adult beetles of the genus *Carpophilus* and *Oryzaephilus* of six aromatic species used as flavoring agents in Catamarca, Argentina. Then, the parameters that explain and predict the toxicological profile observed experimentally were determinated using several statistical and chemoinformatics tools. Dose−response data, chemical composition, and different molecular properties of essential oils as mixtures[26] were correlated with the toxicity end points. The models were rigorously validated using stringent statistical parameters to ensure their external predictivity for untested new chemicals.

MATERIALS AND METHODS

Catamarca Province in Argentina. The province is located in northwestern Argentina between 25° and 30°S latitude and 65° and 69°W longitude, covering an area of 102602 km^2. Here, variations in altitude and soil with average thermal amplitudes of 16 °C and annual precipitation between 150 to 1500 mm lead to the establishment of a diverse and intricate vegetation pattern with high abundance and diversity of aromatic plants.[27] However, the ecological services of aromatic plants are underutilized, and their socio-economic potentials have been scarcely characterized by scientific research. The lack of public awareness on their importance combined with inadequate knowledge about sustainable production and marketing of their products are the major constraints to converting local natural resources into income-making advantage that draws on the social, physical, and human assets available.

Plant Material. The aromatic plants used were *Laurus nobilis* (Lauraceae), *Lippia integrifolia* (Verbenaceae), *Aloysia polystachya* (Verbenaceae), *Tagetes minuta* (Asteraceae), *Pimpinella anisum* (Apiaceae), and *Cuminum cyminum* (Apiaceae). These aromatic species were selected based on their local availability, essential oils yield, and because they are widely used as flavoring agents in northern Argentina.

Aerial parts of Asteraceae, Lauraceae, and Verbenaceae were collected at full flowering stage, in different regions of Catamarca in September−December 2015. Fruits of the two Apiaceae species were collected at ripeness in November 2015. The location of collection sites, organs employed for essential oil extraction, and traditional uses are summarized in Table S1 in the Supporting Information. Collected plant material was shade dried at room temperature. The identity of the plants was confirmed by Ing. Agr. Alejandro Quiroga of the Ecology Department of the Faculty of Agrarian Sciences of the University National of Catamarca.

Essential Oil Extraction and Analysis. Essential oils from all plants (300 g) were extracted by hydrodistillation using a Clevenger-type apparatus for 3 h.[28] Then, they were dried over anhydrous sodium sulfate to remove traces of moisture and stored in a refrigerator at 4 °C in dark brown bottles until analysis.

Essential oils component analysis was performed by gas chromatography/mass spectroscopy (GC/MS) using an Agilent 6890 GC Plus automatic sampler system coupled to a quadrupole mass spectrometer (Agilent Technologies, Diegem, Belgium) and equipped with a PerkinElmer Elite-5MS capillary column (5% phenyl methyl siloxane, 30 m × 0.25 mm i.d. × 0.25 μm d_f) in the split mode 1:80. The oven temperature was programmed at 60 °C for one min, increased from 60 to 300 °C at the rate of 3.0 °C/min, and held at 300 °C for 10 min. Helium was used as carrier gas at a flow rate of 1 mL/min. All analyses were performed at constant flow. The injector and detector temperatures were kept at 230 and 150 °C, respectively. Quantification of each compound was performed using percentage peak area calculations. The components were identified on the basis of comparison of their retention indices and mass spectra with published data,[29] and computer matching was performed with the WILEY 275 and National Institute of Standards Technology (NIST 3.0) libraries provided with the computer controlled GC-MS systems.

Insect Rearing. Insects of the genus *Carpophilus* and *Oryzaephilus* were reared in a glass container (0.5 L) covered with a fine mesh cloth for ventilation in growth chambers at 27 ± 2 °C and relative humidity of 70 ± 5% with alternating light and dark periods of 12 h. *Carpophilus* spp. were maintained on an artificial diet containing 46.5% wheat flour, 46.5% cornstarch, and 7% beer yeast.[30] *Oryzaephilus* spp. were cultured with wheat germ.[31] Parent adults were obtained from infested walnuts stored at small farmer storehouses from the Andalgalá Province of Catamarca. Adults were placed in the rearing medium for 10−15 days. Then, they were removed and placed in a new medium to obtain a new progeny and avoid generation overlap. The medium containing the eggs was placed in the growth chamber until adult emergence. This process was repeated with the aim of obtaining homogeneous beetle generations.

Repellent Activity. The repellent effects of the essential oils against adults of *Carpophilus* spp. and *Oryzaephilus* spp. were evaluated on filter papers[32] using, in each experimental unity, 10 unsexed adults (5−14 days old). Test solutions were prepared by dissolving different aliquots (1.52, 3.04, 6.08, 12.16, and 18.24 μL) of each oil in 0.5 mL of acetone corresponding to the doses of 0.05, 0.10, 0.20, 0.40, and 0.60 μL/cm^2. The treatments were repeated 5 times and replicated in triplicate. The numbers of insects present on the control (N_c) and treated (N_t) areas of the discs were recorded at 2 and 4 h of treatment. Data were transformed into repellency percentage (RP) as RP = $[(N_c - N_t)/(N_c + N_t)]\times 100$. The mean repellency value of each phytochemical was calculated and assigned to repellency classes[33] from 0 to V: class 0 (PR < 0.1%), class I (PR = 0.1−20%), class II (PR = 20.1−40%), class III (40.1−60%), class IV (60.1−80%), and class V (80.1−100%).

Toxicity/Mortality Assay. The toxicity of the essential oils was assayed following the method reported in ref 32 using 10 adults (5−14 days old) into each experimental unity. All treatments were replicated five times and repeated in triplicate. Insect mortality (number of dead beetles from a total of 10 live beetles per dish) was recorded daily up to 5 days and expressed as percentages using the Abbott correction formula[34] for natural mortality in untreated controls. Insects that were recorded as knocked down or dead-plus-moribund were those that did not move or that responded only feebly when probed lightly.

DATA ANALYSIS

Generalized Estimating Equations (GEE). Dose−response data observed at different times on a same group of insects exposed to different concentrations of stimulus were analyzed by the method of generalized estimating equations (GEEs)[35] with the statistical software SPSS 23.0 (GEE; IBM SPSS Statistics 23.0). Through this study, we examine how dose−response data in both the individual and the average group of bioassays changes over time. For modeling we assume that the response variables had a normal distribution and used the identity link function, the model-based estimator (the negative of the generalized inverse of the Hessian matrix), to provide an estimate of the covariance and the unstructured working correlation matrix to treat the intrasubject correlation. The dependent variable was the mean bioactivity value of 5 repetitions. Predictors entering into the model were the oil treatments, employed doses, and the time points and all two-way interactions between them.

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Molecular Modeling and Chemoinformatic Study of Essential Oils As Mixtures. Investigations of structure−activity relations for the prediction of the bioactivity of essential oils from their chemical composition are scarce due to the experimental complexity of characterizing especially possible chemical interactions between their components.[36] Some investigations about the toxicological activities of monoterpenoid compounds against arthropods revealed that the activities are dependent upon the nature and position of the functional groups and molecular configuration rather than volatility and molecular size.[37,38]

From the well-established idea on that, the variation of biological activity within a group of compounds or mixtures can be correlated with the variation of their respective structural and chemical features; herein, we applied techniques of molecular modeling to identify the molecular properties of the essential oils that can be used for explaining the average toxicological profile reported in this work. For the study, we described the chemical space of the tested essential oils using (i) diverse descriptors of the molecular structure of chemicals present in an essential oil and (ii) the assumption that the behavior of a mixture can be estimated by combining the molecular descriptors d_i of each component individual and their relative abundance in the mixture. Thus, if the precise composition of the mixture is known, the descriptor of an essential oil as a mixture can be developed by combining the molecular descriptors d_i of each component and their relative concentration in the mixture x_i (eq 1).

$$D_{\text{mix}} = \sum (x_i d_i) \tag{1}$$

Since the number of essential oils investigated for their activity on *Carpophilus* spp. and *Oryzaephilus* spp. is limited and the activity of their constituents has not been described on such insect models to take into account possible biochemical interactions (synergisms and antagonisms), D_{mix} is a measure for the contributions of the components of an essential oil to its overall activity. In fact, the D_{mix} general expression assumes that all oil components (i), according to their relative concentration (x_i), in the mixture shall contribute to the predictive descriptor value and, hence, to the overall activity of the mixture.[39]

For computational treatment, the structures of the compounds were generated in both SMILES notations and 2D structures drawn with *ACD/ChemSketch* package, without performing geometrical optimizations, and saved in MDL-MOL format afterward. In total, 14464 theoretical conformation-independent molecular descriptors and fingerprints were calculated using the freely available software PaDEL-Descriptor (version 2.20), leading to 1444 1D and 2D descriptors and 9 fingerprint types. For a more detailed description about these molecular descriptors refer to ref 40. The average experimental response was transformed into a logarithmic scale. The whole molecular descriptor data set, measured on different scales, was centered and scaled to zero mean and unit-variance. In order to minimize redundant information from the original matrix of variables, the descriptors pool was reduced by applying a procedure written in MATLAB for eliminating constant values and descriptors found to be linearly dependent. Thus, 1926 molecular descriptors were identified as acceptable for model development.

Building models from chemical−analytical data suitable for predicting future observations is challenging and often requires the reduction of dimensionality. In lots of situations, too many variables can dramatically increase the noise in the data and thereby decrease the descriptive and predictive power of a model. So, the selection of the optimal subset of X variables is a key step for the development of mathematical models. In this work, to find the most appropriate subset of descriptors that correlated well with the experimental average bioactivity profile against *Carpophilus* spp. and *Oryzaephilus* spp. and that carry most of the relevant information for predicting future observations, we used the reshaped sequential replacement (RSR) algorithm.[41−43] In this approach the coefficient of determination in cross-validation (Q^2_{cv}) is used as a parameter to be optimized in regression problems.

In order to handle the large number of calculated descriptors, i.e., 1926, and to avoid potential overfitting, the most appropriate combinations of molecular descriptors were chosen from linear regression models calibrated by using ordinary least squares (OLS). In all these calculations, the smallest and highest model size, and the number of models for each model size, were set to 1, 6, and 10, respectively. Cross validation was performed by dividing samples in 2, 5, and 6 cancellation groups. Since the data set comprised a few samples, a small and high number of cross validation groups allowed for testing the stability of the selected models upon inclusion/exclusion of mixtures and the overestimation of the model predictive ability. The coefficient of determination in cross validation (Q^2_{cv}) was used as a parameter to be optimized, while the final model population was validated by using y-scrambling, R functions, and the nested criterion.[41,43] For more details about the selected options to run the RSR algorithm, refer to Table S2.

The best models were judged on the basis of their predictive power using external test sets composed of 9 and 4 essential oils having measured repellent activity against the beetle *Meligethes aeneus* (*Fabricius*), a Coleoptera specie of the Nitidulidae family, Cucujoidea superfamily,[44] and *Oryzaephilus surinamensis*.[45,46] Further details on the test sets used in this study appear in Table S3. To the best of our knowledge, these data were the only reports found about essential oils bioactivity against weevils of the families Nitidulidae and Silvanidae (the chemical composition data and the bioactivity assays protocol were similar to the one employed in this work).

In an attempt to understand the underlying correlation among the variables selected by the RSR algorithm, identified descriptor sets were projected into a lower-dimensional space through a principal component analysis (PCA). We think that such descriptors encode structural information that, in a first approximation, may constitute the basis to advance toward generating testable hypotheses about purported bioactive volatile mixtures, by combining in a linear way the information on the RSR models. In this regard, PCA is a powerful and versatile method capable of providing an overview of complex multivariate data and can be used for revealing relations between variables and samples (e.g., clustering), detecting outliers, finding and quantifying patterns, generating new hypotheses, as well as many other things. For these calculations, we used the collection of MATLAB freely available modules from the Milano Chemometrics and QSAR Research Group web site.[47] Descriptor sets that correlated with the experimental response against *Carpophilus* spp. and *Oryzaephilus* spp. were grouped and arranged in two

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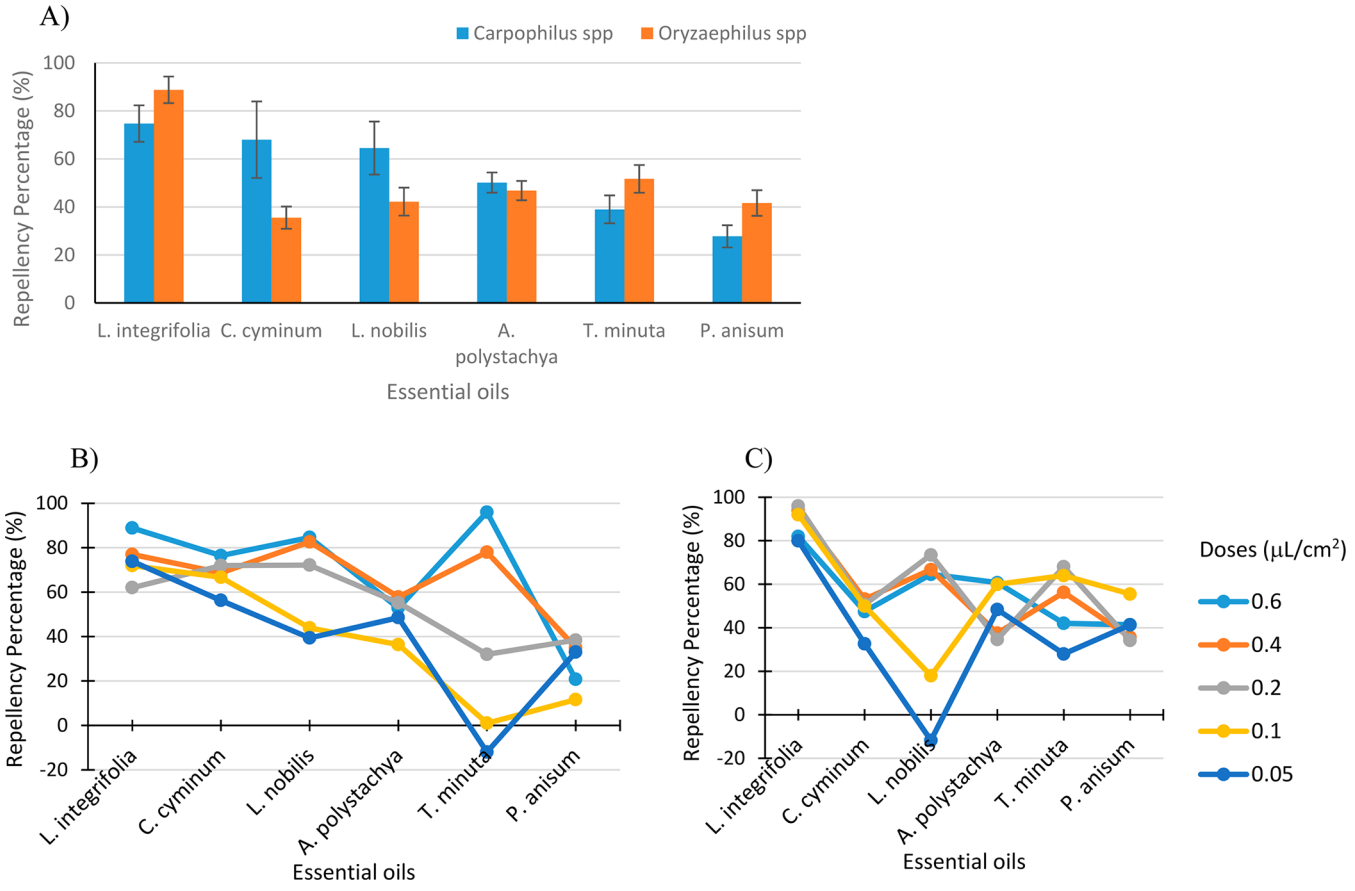

Figure 1. Average repellency percentage of essential oils against: (A) *Carpophilus* spp. and *Oryzaephilus* spp, (B) *Carpophilus* spp. and, (C) *Oryzaephilus* spp. as a function of tested essential oils doses.

matrices of 6 × 4 size. Then, these data were autoscaled, and the selection of the optimal number of principal components (PCs) was carried out on the basis of the cross validation procedure, with 5 groups divided according to the venetian blinds procedure. Eigenvalue-based selection criteria, i.e, the average eigenvalue criterion (AEC, also known as Kaiser's criterion) and the corrected average eigenvalue criterion (CEC), were considered for selecting the optimal number of principal components. Afterward, the obtained PCA results (numerical values of eigenvalues, explained and cumulative variances, scores, loadings, Hotelling's T^2 and Q residuals of samples) were analyzed and plotted as a function of the retained components. In addition, we determine whether the lack-of-fit of the sample in the test set is due to systematic deviation of that sample or simple random variation. For them, we check the conditions proposed in ref 48 from the Q contributions statistic, a measure of PCA residuals which defines how much each variable contributes to the overall Q statistic for the sample. More evaluations are not possible due to the fact that it is not a simple task to introduce degrees of freedom in the calculation of the mean squared error after PCs and PCA models are developed.[49]

RESULTS AND DISCUSSION

Oil Yields and Chemical Composition. The oil yield, calculated as the volume (mL) of essential oil per 100 g of dry plant matter (i.e., % v/m), and color were as follows: *C. cyminum* (4.0%, light yellow oil), *A. polystachya* (2.5%, pale yellow oil), *L. integrifolia* (2.5%, reddish orange oil), *T. minuta* (2.5%, reddish orange oil), *L. nobilis* (1.0%, pale yellow oil), and *P. anisum* (1.0%, pale yellow oil).

The chemical constituents of six different essential oils are given in Table S4.A. Gas chromatography−mass spectrometry analysis led to the identification of 91 components. According to the total amount of compound types identified in the essential oils tested (Table S4.B.), the oils characterized by a high percentage of hydrocarbons and oxygenated monoterpenes were *L. nobilis, A. polystachya*, *T. minuta*, and *C. cyminum* (19.48−76.75%). In contrast, *P. anisum* essential oil is rich in phenylpropanoids, pyranoids, and furanoids (97.34%). Other compounds (aldehydes and esters) present in more than 10% in *C. cyminum* and *L. nobilis* essential oils were cuminaldehyde (28.91%), α-terpinen-7-al (18.01%), and α-terpinyl acetate (11.81%). In contrast, the chemical composition of *L. integrifolia* essential oil is characterized by a high content of sesquiterpenoids. The hydrocarbon sesquiterpenes accounted for 12.76%. Compounds present in more than 2% of the total oil were african-1-ene (2.08%), african-5-ene (4.88%), and asterisca-3(15),6-diene (2.63%). Then, the oxigenated sesquiterpenes (61.84%) represent the main fraction of the essential oil being lippifoli-1(6)-en-5-one (43.31%) along with caryophyllene oxide (3.78%), 3α-hydroxy-6-asteriscene (3.78%), and african-5-en-1α-ol (3.18%), the components more abundant in the sample analyzed.

According to our data all oils are rich in the compounds showing insecticidal activity against beetles.[9] Then, the selected aromatic plant essential oils could have insecticidal

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and/or repellent properties against insects of the genus *Carpophilus* and *Oryzaephilus*.

Repellent Activity of Essential Oils on Adults of the Genus *Carpophilus* spp. and *Oryzaephilus* spp. The repellent activity at 2 and 4 h after treatment against adults of the genus *Carpophilus* spp. and *Oryzaephilus* spp. of the tested six essential oils are summarized in Tables S5.A and S5.B. The average repellency values as a result of the treatments and tested doses are presented in Figure 1.

Average repellency percentage as a result of the treatments is presented in Figure 1 (A) as a histogram with standard deviation bars of the mean. The data indicate that all the treatments tested were active toward both coleopteran species. In the range of concentrations applied, the average repellency values of the essential oils were assigned to repellency classes II−V. For both beetles, *L. integrifolia* essential oil showed the higher repellency activity (74.73% at *Carpophilus* spp. and 88.80% at Oryzaephilus spp.), while *P. anisum* essential oils were less repellent (27.75% at *Carpophilus* spp. and 41.64% at *Oryzaephilus* spp.).

According to the GEE, treatments and doses were significant predictors of repellent activity (p-value < 0.05) (Table S6.A and S6.B). Particularly, in the average repellency model calculated from *Carpophilus* spp. data (Table S6.A), treatment from *L. integrifolia*, *C. cyminum*, *L. nobilis*, *A. polystachya*, and *T. minuta* and doses at 0.60 and 0.10 μL/cm^2 were significant predictors of the bioactivity. Doses at 0.40, 0.20, and 0.05 μL/cm^2 and time were not significant as main effects (p-value > 0.05), but their interaction with the treatments mentioned above were significant (p-value < 0.05). *P. anisum* treatment was not included in the model because this parameter is considered redundant. In fact, for this treatment and in the range of assayed doses, average repellency values were not significantly different of the other treatments. Calculations indicated that such a parameter is not of importance to explain the modeled activity.

Likewise, in Table S6.B the parameters *A. polystachya*, *P. anisum*, doses at 0.60 and 0.05 μL/cm^2, and the interaction parameters with p-value > 0.05 were not of importance to explain the observed average repellency on *Oryzaephilus* spp. In particular, it is worth noting that in the calculated statistical model any relationship with time was included (p-value > 0.05). This fact revealed that observations made from a treatment on the same group of organisms and between subjects were not significantly different with time. Then, this parameter was not of importance to explain the observed repellent effects.

Estimates of the repellence values with a 95% CI for the different essential oils and doses against *Carpophilus* spp. and *Oryzaephilus* spp. are shown in Table S7. For all essential oils, analysis showed that the percentage repellency increased with increasing concentration of treatments and that the mean repellency did not differ significantly across the time points observed (Figure 1 (B and C)).

Toxicity/Mortality Assay on Adults of the Genus *Carpophilus* spp. and *Oryzaephilus* spp. The average mortality percentages of each insect species after 5 days exposure to increasing doses of volatile oils are shown in Figure 2. The scarce variability of mortality percentage with the types of essential oils, doses, and time limited their study with GEE. At this point, it is worth mentioning that in experiments where all dose levels produce a 100% response, it is due to the dose levels being too high and that they all

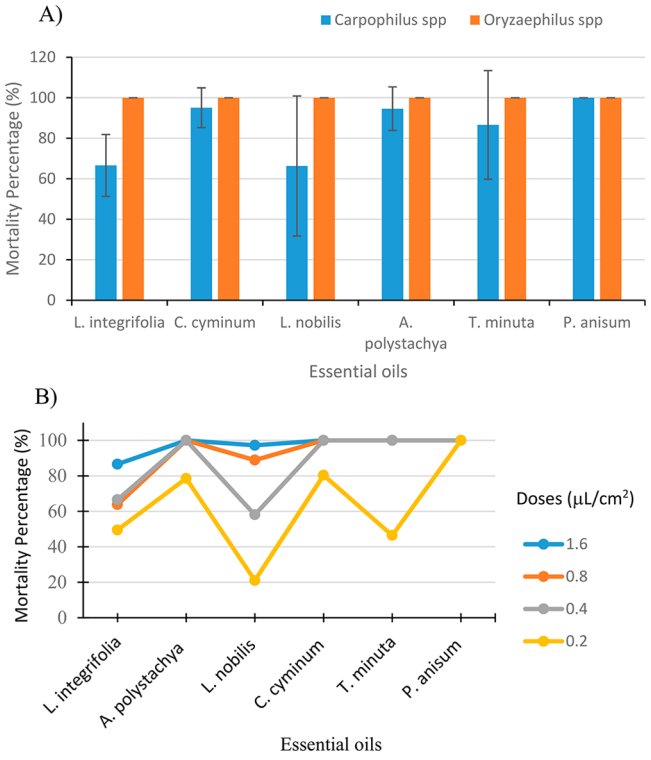

Figure 2. Average mortality percentage of essential oils against (A) *Carpophilus* spp. and *Oryzaephilus* spp. and against (B) *Carpophilus* spp. as a function of tested essential oils doses.

produce in a spontaneous occurrence the toxic response under consideration (death). In this context, if there is not much information to the estimation and analysis of the dose−response models, dosages of the tested agents that will not produce toxic effects should be explored below the lowest dose tested (0.2 μL/cm^2). In contrast, toxicological data on a given host showing variability from a given stimulus at different levels may depend on several factors among which are the chemical composition of the essential oil and insect susceptibility. Thus, once the proper experimental data have been obtained, we interpret that essential oils with different toxic effects against similar insects reveal different modes of interaction with the organism (and its tissues) and warns us over change in tolerance to the agent.

Accordingly, a histogram with standard deviation bars of the mean of the average mortality percentage as a result of the treatments indicates that all the essential oils have strong insecticidal activity against both beetle species. In the range of concentrations tested (Figure 2 (B)), the essential oils of *C. cyminum* and *P. anisum* caused 100% mortality on *Carpophilus* spp. Analog results were observed for *A. polystachya* and *T. minuta* essential oils in the range of 0.4−1.6 μL/cm^2. At the lowest tested dose (0.2 μL/cm^2) both oils caused 78.55% and 46.40% mortality, respectively. Regarding *L. integrifolia* and *L. nobilis* toxicity, the mortality of *Carpophilus* spp. was dose-dependent in the range of concentrations applied. A dose of 1.6 μL/cm^2 was able to induce 86.6% and 97.23% mortality of insects within 5 days of exposure of each oil. At low concentrations, *L. integrifolia* oil was more toxic to *Carpophilus* spp. than *L. nobilis* oil.

In contrast, mortality on *Oryzaephilus* spp. was not treatment or dose dependent. Results indicate that *Oryzaephilus* spp. was more susceptible to the tested essential oils than

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Table 1.A. RSR Model Parameters

database	R^{2a}	Q^{2}_{cv} [b]	$\overline{Q}^{2c}_{y}$	descriptors		b_i [d]
Carpophilus spp.	0.9979	0.9905	−2.1322	AATSC0c	maxsssCH	[−0.1485; 0.8881]
	0.9977	0.9891	−2.5609	ATS8m	PubchemFP697	[−0.5702; 0.5962]
Oryzaephilus spp.	0.9929	0.9864	−4.9909	ATSC7e	AATSC0m	[0.3830; −0.6547]
	0.9898	0.9565	−20.4572	WTPT-2	KRFPC3721	[−0.4759; 0.57071]

[a]Coefficient of determination, $R^2 = 1 - \sum_{i=1}^{n\text{train}} \frac{(y_i - \hat{y}_i)^2}{\text{TSS}}$ where *n*train is the number of training objects, y_i and $\hat{y}_i$ represent the calculated response and the real response of the *i*th object, respectively, and TSS (total sum of squares) is the sum of squared deviations from the data set mean. It determines the ability of the model to fit the training data. [b]Coefficient of determination in cross-validation, $Q^2_{cv} = 1 - \sum_{i=1}^{n\text{train}} \frac{(y_i - \hat{y}_i)^2}{\text{TSS}}$ where y_i is the real response value of the *i*th object and $\hat{y}_i$ is the value of the *i*th object predicted by the model in which the *i*th object was not taken into consideration. [c]Average correlation coefficient of *Y*-scrambling, $\overline{Q}^2_y$. [d]Standardized regression coefficients of the selected descriptors.

Table 1.B. Optimal Descriptor Subsets Selected through the RSR Algorithm for *Carpophilus* spp. and *Oryzaephilus* spp. Datasets

database	ID subset	descriptors	meaning
Carpophilus spp.	I	AATSC0c	Average centered Broto−Moreau autocorrelation − lag 0/weighted by charge. A measure of the distribution of atomic charge on the molecule topology
		maxsssCH	Maximum atom-type *E* state: >CH−
	II	ATS8m	Broto−Moreau autocorrelation − lag 8/weighted by mass
		PubchemFP697	PubChem Substructure Fingerprint that tests the presence of simple CCCCCC(C)C SMARTS pattern, regardless of count
Oryzaephilus spp.	III	ATSC7e	Centered Broto−Moreau autocorrelation − lag 7/weighted by charges
		AATSC0m	Average centered Broto−Moreau autocorrelation − lag 0/weighted by mass
	IV	WTPT-2	Molecular ID/number of atoms
		KRFPC3721	PubChem Substructure Fingerprint that tests the presence of simple CCCCCCC═O SMARTS pattern, regardless of count

Carpophilus spp. within the exposure period and that the total mortality (100%) was achieved at all ranges of concentrations assayed.

Molecular Modeling and Chemoinformatic Study of Essential Oils As Mixtures. Table 1.A reports the best models found by the RSR method validated using *y*-scrambling, cross-validation validation, *R* functions, and nested criterion. Each model is comprised of two molecular descriptors that encoded information about lipophilicity and polarizability of mixtures to a certain extent. A brief description of such descriptors is provided in Table 1.B.

In PCA calculations, the eigenvalue-based selection criterion suggested retaining one PC (Figure S1). Score and loadings plots for analyzing the data structure are summarized in Figure 3 (A) and (B), respectively. According to these results, only one PC explains 77.6% of the data variability related to the average repellent activity against *Carpophilus* spp. The score plot in Figure 3 (A) revealed a systematic data distribution such that an observation with a high score was related to an essential oil with a high repellent activity, and vice versa. Therefore, the relevant information for separating samples with high and low scores was contained in four variables (see loading plot). In fact, the PC contained high and negative loadings on molecular attributes that described the average distribution of atomic charge on the molecular topology (AATSC0c) and the presence of a branched chain (maxsssCH).[50] In contrast, the PC had significant and positive loadings from the ATS8m and PubchemFP697, variables that suggested the importance of molecular size and the contribution of flexible nonpolar substructures in describing and discriminating the analyzed experimental data.

Since such information on molecular size, branchedness, flexibility, and charge distribution encoded information on molecular lipophilicity and polarizability to a certain extent, it was then possible to interpret that essential oils with molecules containing flexible substructures with aliphatic carbon chains and that a number of reduced non-hydrogen substituents were associated with increased activity. In contrast, mixtures associated with a decreased bioactivity contained small molecules with certain intrinsic reactive character of the atoms constituting the molecules (a negative charge on atoms characterized their nucleophilic nature whereas a positive charge characterized their electrophilic nature).

On the other hand, a PC constructed as a linear combination of descriptors correlating to the average repellent activity against *Oryzaephilus* spp. explained 90.7% of the data variance. By comparing the score and loading plots (Figure 3 (B)), the most important differences between *P.anisum* and *L. integrifolia* were attributed to dissimilarities between the ATSC7e/KRFPC3721 and AATSC0m/WTPT-2 descriptor pairs. Although the PC was strongly correlated with the four original variables, it increased with increasing the ATSC7e and KRFPC3721 variables and decreased with decreasing the AATSC0m and WTPT-2 variables. These descriptor combinations, which encoded the electronic environment and topology of molecular fragments, suggested that the biological activity increased in mixtures with small molecules represented by aliphatic fragments with low branching and electron-rich moiety, which could favorably act as organic bases. In contrast, low biological activity values were related to mixtures constituted by molecules with nonpolar and branched moieties.

The above interpretation revealed that the different coleopterans reacted differently to the same essential oils probably due to the fact that volatile compounds acted through different mechanisms of action. In this context, it should be

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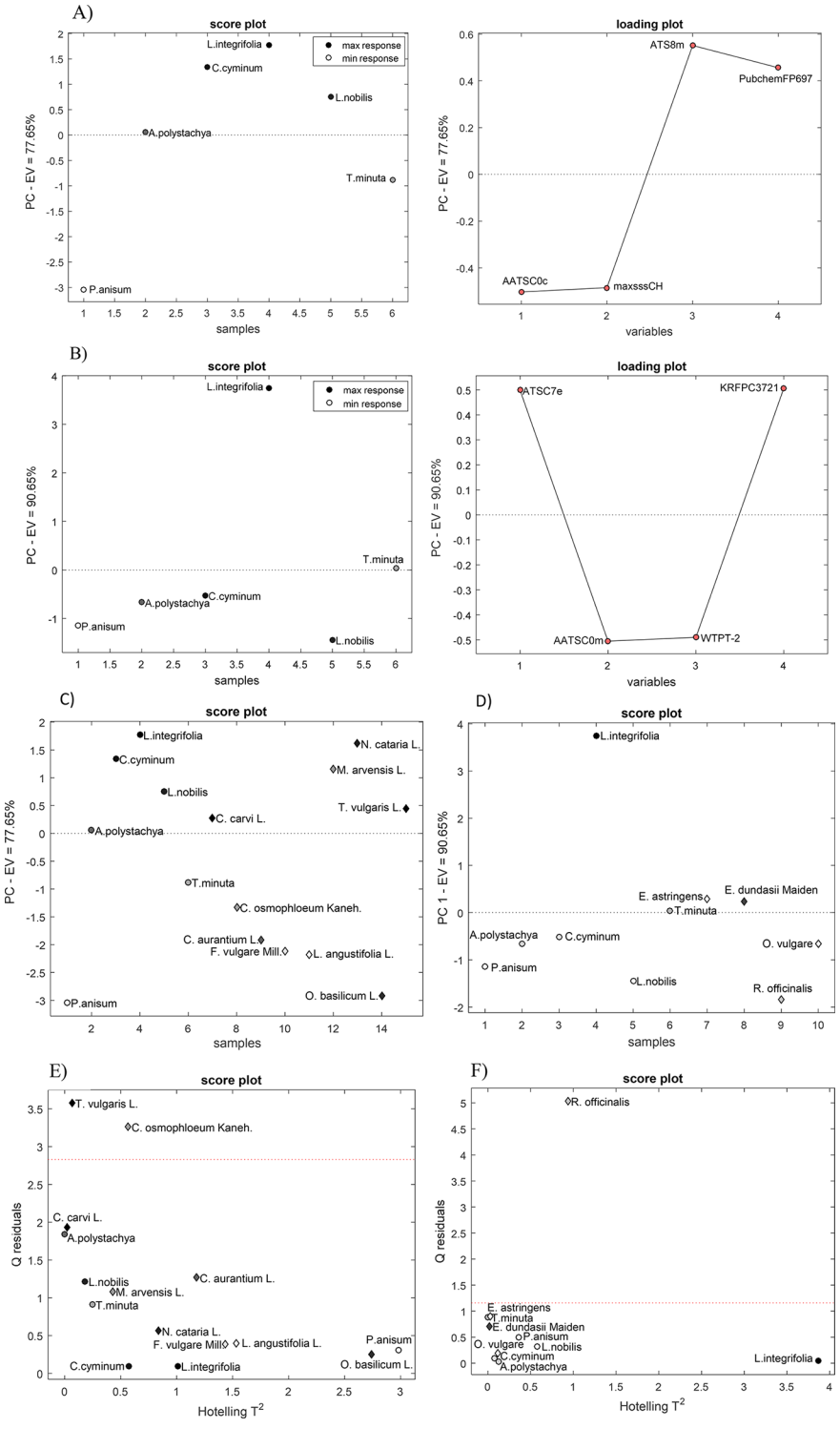

Figure 3. PCA score and loading plots for the average repellent activity against (A) *Carpophilus* spp. and (B) *Oryzaephilus* spp. In the score plot, each sample is identified and colored with a gray scale on the basis of its experimental response (logPR). In the loading plot, each variable is identified by its label. Score plots with training samples (circles) and projected test samples (diamonds) for the PCA model of (C) *Carpohilus* spp. and (D) *Oryzaephilus* spp. Each sample is colored with a gray scale on the basis of its experimental response. Influence plots with training samples (circles) and projected test samples (diamonds) for the PCA model of (E) *Carpohilus* spp. and (F) *Oryzaephilus* spp. Each sample is colored with a gray scale on the basis of its experimental response.

noted the stereoelectronic profile of the *L. integrifolia* essential oil satisfactorily responded to the balance of hydrophobicity and polarity that explain the observed repellent property against *Carpophilus* spp. and *Oryzaephilus* spp. Likewise, it was valid to highlight that the mixture of phenylpropanoids, pyranoids, and furanoids in *P. anisum* defined a poor combination of chemical features (aliphatic and aromatic hydrophobic moieties and ionizable group) to interact with the recognition sites in receptors of the insects under study. In fact, the charge distribution and the nonpolar regions of the

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molecules present in the essential oils provided a moderate measure of the required stereoelectronic properties to allow maximum interaction with the receptor and to have potent activity. Such interpretation was based on the assumption of the degree of complementarity between the molecular fields of a bioactive mixture and its receptor, which should be directly related to the binding strength and relative activity of the agent.

Our outcomes were in agreement with previous knowledge on the physicochemical characteristics of small molecules to have potent activity as insect repellents.[37,38] Particularly, it was confirmed that a favorable lipophilicity and a localized negative electrostatic potential region by the oxygen atom played a definite role in the repellent activity of a compound. Then, large hydrophobic regions and weakly polar sites were considered as key features through which a molecule fitted into a receptor at longer distances and, accordingly, promoted interaction between complementary sites with the receptor.

On the other hand, the predictive capability of the reported models were evaluated through the application of 9 and 4 mixtures defined as test sets in Table S3. Such mixtures were projected into the PCA space and their distribution analyzed in the score and influence plots (Figure 3 (C−F)). According to our calculations, each PCA model confirmed each test set mixture as a repellent with a performance comparable to the one achieved on samples belonging to the calibration of the PCA models (training set, samples with high scores had high bioactivity, and vice versa). Q contribution statistics calculated for the predicted data of each independent test set are presented in Table 2.A. The parameters calculated to judge the

Table 2.A. Values of Q Contribution (Q_i) Obtained during External Validation of PCA Models

Q_i *Carpophilus* spp. PCA Model			
−0.7985	−0.1932	0.0281	−1.1190
−1.5418	0.6623	−0.6190	−0.2468
0.9657	−0.2221	0.4523	0.2813
0.3773	0.1509	0.0926	0.4640
−0.5010	0.2526	−0.2838	0.0594
−0.3629	0.8491	0.0184	0.4798
0.4806	−0.5635	0.0287	−0.1037
0.0137	0.3648	0.0511	0.3408
−0.7261	1.3961	−0.2730	1.0129
Q_i *Oryzaephilus* spp. PCA Model			
−0.1675	−0.7558	0.0508	−0.5401
0.0196	−0.6168	0.0658	−0.5712
0.7115	0.5186	1.5709	1.3379
0.1496	−0.2611	0.2862	−0.1309

presence of systematic errors and/or model bias are summarized in Table 2.B. According to our calculation, predicted data are devoid of systematic error and the model predictions are ascertained since the error values, both positive and negative, are in proportional amounts. Likewise, influence plots at the 95% confidence level (Figure 3 (E and F)[49] indicated that the samples from the *Carpophilus* spp. and *Oryzaephilus* spp. test sets had Hotelling's T^2 values comparable with values of the training samples. With respect to the position in the influence plots of the samples *T. vulgaris* L., *C. osmophloeum* Kaneh, and *R. officinalis*, they had a relatively high Q residual. Analyzing the variable contributions to the Q residual of sample *T. vulgaris* L. and *C. osmophloeum*

Table 2.B. Lack-of-Fit and Predictive Ability of PCA Models

	PCA model	
parameter	*Carpophilus* spp.	*Oryzaephilus* spp.
nPE/nNE	1.5714	1.2857
nNE/nPE	0.6363	0.7777
|MPE/MNE|	1.1411	1.0529
|MNE/MPE|	0.8763	0.9497
AAE − |AE|	0.4197	0.3804
R^2 (residuals; serial correlation)	0.1619	0.0047
R^2 (residuals and Y_{obs} values)	0.0146	0.0003
systematic error result[a]	absent	absent
Metric of Fit and Predictive Ability of PCA Models[b]		
R^2	0.7765	0.9065
Q^2_{test} (100% data)	0.6919	0.5572

[a]There are systematic errors when one or more of the following conditions are true: nPE/nNE > 5* or nNE/nPE > 5*, where nPE is the number of positive errors and nNE is the number of negative errors; |MPE/MNE| > 2* or |MNE/MPE| > 2*, where MPE is the mean of positive error and MNE is the mean of negative errors; AAE − |AE| < 0.5* × AAE, where AAE is the average of absolute prediction errors and AE is the average of prediction errors; R^2 (ith vs $(i-1)$th residuals) > 0.5* for residuals sorted on experimental response values (Y_{obs}); and R^2(Y_{obs} and residuals) > 0.5**. Threshold values are set as default. [b]Explained variation on calibration set (training set), R^2. Explained variation on new observations (test set), Q^2_{test}.

Kaneh (Figure S2.A and S2.B), it could be seen that the contributing variables to a high residual were maxsssCH, PubchemFP697, AATSC0c, and ATS8m, respectively. Likewise, *R. officinalis* had high residual variations on all the PCA model variables (Figure S2.C). We understand that the PCA models were not able to correctly fit the original values of the samples due to the absence of such information in the training set. Further studies are necessary in order to complement the training data to confirm the causal association here reported.

In conclusion, the information here reported is of great interest since this is the first report that describes two essential oils from aromatic species widely used as flavoring agents in northern Argentina as repellents and insecticides against beetles infesting the walnut production in Catamarca. An interesting result was the high mortality caused by the volatiles from cultivated aromatic species *C. cyminum* and *P. anisum*. Actually, such crops are cultivated at a low level of industrialization and the products are traded at a relatively low value. In addition, from the application of chemometric approaches we determined the stereoelectronic properties that contribute to potent repellent activity. This analysis provides key information that can be used to more effectively solve the tasks related to the search of ecologically friendly templates for the management and control of the Nitidulidae and Silvanidae families of beetles without expensive laboratory analysis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b04161.

Selected aromatic plants, locations of collected sites, plant parts used for extraction of essential oils, and traditional uses (Table S1); input data to run the RSR algorithm (Table S2); test sets for the external validation of linear models in this study (Table S3); chemical composition and relative concentrations of the essential

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oils obtained from six aromatic plants grown in Catamarca, Argentina and the relative amounts of compound types identified (Table S4); repellent effect of essential oils from *L. integrifolia*, *C. cyminum*, *L. nobilis*, *A. polystachya*, *T. minuta*, and *P. anisum* against *Carpophilus* spp. and *Oryzaephilus* spp. (Table S5); parameter estimates of the GEE model from repellency data against *Carpophilus* spp. and *Oryzaephilus* spp. and predicted values from GEE linear predictor (Tables S6 and S7); plots of eigenvalues as a function of PCA principal components for *Carpophilus* spp. and *Oryzaephilus* spp. (Figure S1); and normalized Q contributions of *T. vulgaris* L., *C. osmophloeum* Kaneh, and *R. officinalis* (Figure S2) (PDF)

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Notes

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