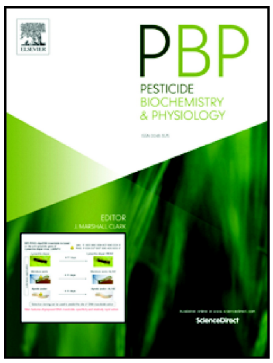

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Valeria Tapia Mattar, José Luis Borioni, Axel Hollman, Sergio A. Rodriguez

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Insecticidal activity of the essential oil of *Schinus areira* against *Rhipibruchus picturatus* (F.) (Coleoptera: Bruchinae), and its inhibitory effects on acetylcholinesterase.

Valeria Tapia Mattar[1], José Luis Borioni,[2] Axel Hollman[3] and Sergio A. Rodriguez[1]*

[1]*Facultad de Agronomía y Agroindustrias* (FAyA), *Universidad Nacional de Santiago del Estero* (UNSE) , *Consejo Nacional de Investigación Científicas y Técnicas* (CONICET).

[2]*Instituto de Investigaciones en Físico Química de Córdoba (INFIQC-CONICET). Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.*

[3]*Laboratorio de Compuestos Bioactivos, Centro de Investigación de Biofísica Aplicada y Alimentos (CIBAAL)-CONICET- Universidad Nacional de Santiago del Estero (UNSE).*

* Corresponding author SAR: drsergiorod@gmail.com

Insecticidal activity of the essential oil of *Schinus areira* against *Rhipibruchus picturatus* (F.) (Coleoptera: Bruchidae), and its inhibitory effects on acetylcholinesterase.

Abstract

During the storage of *Prosopis alba* pods, substantial quantitative and qualitative losses were observed. One of the main factors is the seed beetle *Rhipibruchus picturatus*. A key strategy to develop new pest control management is the use of essential oils (EOs) due they are efficient, less toxic, and less persistent in the environment compared to synthetic pesticides. In this context, seeds and leaves of *Schinus areira* L. (Anacardiaceae) EOs and *Citrus* spp. EO were studied in the present work. In the leaves of *S. areira* EO, 1-epi-cadinol, sesquiterpenoid alcohol, was the major compound. On the other hand, the main compounds of the EO extracted from *S. areira* seeds are the monoterpenes sabinene, and α-pinene. Finally, in the *Citrus* EO, limonene is the principal component. The three EOs obtained exhibited insecticidal activity against *R. picturatus*, being the first report of the use of EOs against this insect pest. The best insecticidal results were obtained with the leaves of *S. areira* EO. Moreover, this EO inhibits the acetylcholinesterase enzyme *in vitro* assays. Molecular docking studies on acetylcholinesterase (AChE) suggest that the main components of the leaves of *S. areira* EOs, bind to the active site of the enzyme, in good agreement with *in vitro* competitive inhibition against AChE observed for this EO. The data obtained demonstrate the potential use of *Schinus areira* EOs in the development of new storage pest control strategies.

Keywords: *Schinus areira*, Essential Oil, *Rhipibruchus picturatus*, insecticidal activity, acetylcholinesterase.

1. Introduction

Stored product pest infestation is the cause of most postharvest economic losses related to the storage of food grains and its related issues like quality and safety (Bhavya et al., 2018). For instance, considerable quantitative and qualitative losses are observed while the pods of *Prosopis alba* Grisebach (algarrobo blanco) are stored. One of the main factors is the seed beetles, also known bruchid beetle (Chrysomelidae: Bruchinae). In the case of *Prosopis* species, these beetles can devastate nearly all the pods stored reaching up to 90 % (Beatley, 1978; Zimmermann, 1991). *Riphibruchus picturatus* is the main pest of *P. alba* pods in north-western Argentina (Kingsolver, 1982; Ramos et al., 2007). This level of destruction is due that oviposition occurs on or inside the seeds and later the larvae feed on a single seed (Muruaga de L'Argentier, 1986). After several larval stages, they pupate, generally killing the embryo, or eat a significant amount of the endosperm, suppressing its germination.

The bruchids infestation is managed with insecticides (pyrethroids and organophosphate) and fumigant phosphine (PH_3) (Iturralde-García et al., 2016). However, the heavy and indiscriminate use of these pesticides has allowed the growth of resistant insect populations over time (Koul et al., 2008). Also, exposure to expensive chemical insecticides can pose a potential health hazard due to insufficient equipment and technical expertise for producers to use safely (Pourya et al., 2018).

All of that reinforces the need to develop alternative methods for bruchid insects control under stored conditions. In this regard, insect management using vegetal material is an ancient practice throughout the world. Furthermore, botanical pesticides such as essential oils (EO) are effective, less toxic, and less persistent in the environment, such as synthetic pesticides (Adenubi et al., 2018; Isman and Grieneisen, 2014). Several

investigations have been carried out aiming at the use of EO in integrated management programs of Bruchinae species (e.g. Adak et al., 2020; Matos et al., 2020; Mauti et al., 2019; Regnault-Roger et al., 1993; Ebadollahi et al., 2012; Heydarzade and Moravvej, 2012; Kanda et al., 2017; Pourya et al., 2018; Wang et al., 2019). EO works through contact, ingestion, and fumigation, causing repellency, feeding and oviposition deterrence, alterations in growth, reduction in oviposition and emergence of adults, and mortality (Guerra et al., 2009; Kanda et al., 2017). EOs toxicity is due to its ability to damage or alter the normal functioning of the nervous system of insects (Kim et al., 2003; Sarwar, 2012). In this sense, the inhibition of acetylcholinesterase (AChE) is the principal route of action described for the EOs and its components like α-pinene, β-pinene, 1,8-cineole, among others (Jankowska et al., 2018).

Schinus areira L. [syn. *S. molle* L. var. areira (L.) DC.] commonly known as '*aguaribay'*, pepper tree, or pink pepper, is a native species from Argentina and presents many traditional uses. A few studies describing the insecticidal activity and repellent effect of *S. areira* extracts against some insect pests have been published (Abdel-Sattar et al., 2010; Chiffelle et al., 2013; De Batista et al., 2016; Deveci et al., 2010; Ferrero et al., 2007, 2006; López et al., 2014; Machado et al., 2019). However, there are no reports of the activity of EOs against *R. picturatus*.

In this work, we aimed to evaluate the chemical composition and insecticidal action of leaves and seeds EO of *S. areira*, located in the North-West of Argentina. EO of *Citrus* spp. was evaluated as a reference insecticide. Also, *in vitro* inhibition of the EO of *S. areira* against acetylcholinesterase was evaluated. In addition, the inhibition mechanism was studied and molecular docking of main organic compounds of EO on both *Torpedo*

californica (*Tc*AChE) and *Anopheles gambiae* (*Ag*AChE) was performed to understand the binding modes.

2. Material and Methods

2.1 Plant material and isolation of essential oil

Samples of *Schinus areira* were collected from July to September 2018 in Villa El Zanjón, Santiago del Estero, Argentina (27°52'21.5"S 64° 14'33.2"W). The species was identified, and the voucher specimens (LIL 617.117 A and B) were deposited at the herbarium of Fundación Miguel Lillo, Tucumán, Argentina (Cutro et al., 2021). Samples of air-dried leaves and seeds (ca. 100 g in each batch) from three plants were extracted using hydrodistillation for 4 hours in a Clevenger-type apparatus. The same procedure was followed for the obtention of *Citrus* spp. peel EO.

2.2 EO analysis

EOs were analyzed in a chromatograph GC Konik 3000 series equipped with a ZB-5 capillary column (30 m x 0.25 mm x 0.25 μm) (Phenomenex, Inc., Torrance, CA, USA) and a flame ionization detector (FID). Initially, the temperature of the column oven was maintained at 50 ºC for 4 min, then it was increased at a rate of 7 °C min^{-1} until 250 °C and then held for 10 min. Also, the volatile compounds were studied by gas chromatography-mass spectrometry (GC-MS) using a Thermo Scientific Focus GC coupled with a DSQ II electron ionization mass detector. The GC injector was operated in splitless mode. A TR-5MS capillary column (30 m x 0.25 mm x 0.25 μm) (Thermo Fisher Scientific) was used under the same analytical conditions described for GC-FID runs. The ratios of compounds

were calculated based on GC peak areas, considering that the total peak areas of interest are equal to 100. Quantification of compounds was achieved using external standards in the GC-FID. The percentages and concentrations correspond to the mean value of three independent analyses. The compounds were identified using the calculated retention indices and their mass spectra analysis. Retention indices were calculated using linear interpolation relative to retention times of C7–C25 of n-alkanes and then compared with those commercial standards and data from the literature. Mass spectra were compared with the NIST 08 library and the spectral data reported in the literature. In some cases to improve the identification, the essential oils were subject to co-chromatography with commercial standards.

2.3 Insect colonies and rearing conditions

The *Rhipibruchus picturatus* colonies were established from infested *Prosopis alba* pods located in Villa El Zanjón, Santiago del Estero, Argentina. The emerging insects were placed in plastic boxes (6 × 12 cm^2) and fed with uninfected *Prosopis alba* pods. Insects were maintained at 26 ± 2 °C under a 12:12 h light/day (L/D) photoperiod.

2.4 Contact toxicity assay

The insecticidal activity of the EOs against adults of *R. picturatus* was evaluated using a contact toxicity assay described by Herrera et al., (Herrera et al., 2015). A series of dilutions of the EOs were prepared in acetone, and then 200 µL of each dilution was applied to filter paper disks placed in glass Petri dishes (9 cm diameter). The EO of *S. areira* seed was tested at concentrations of 400, 600, 800, 1000, and 1200 µg/cm^2. The EO of *S. areira* leaves was tested at concentrations of 20, 50, 100, 150, and 250 µg/cm^2.

Finally, The *Citrus spp.* EO was tested at concentrations of 600, 800, 1000, 1200, and 1400 µg/cm^2. The solvent was allowed to evaporate for 2 min before the introduction of ten adult insects in each Petri dish. Control insects were treated only with acetone. All treatments were replicated five times and mortality was checked after 24 h, 48 h, and 72 h. Insects were considered dead when they showed no movement when touched with tweezers. The concentration-mortality data were subjected to Probit analysis to determine lethal concentrations, those causing 50 % of mortality (LC_{50}), as well as their confidence limits at 95 %. The values of LC were considered to be significantly different if their confidence limits did not overlap. All analyses were conducted using InfoStat 2017 (UNC).

2.5 In vitro inhibition of Acetylcholinesterase (AChE)

Inhibition of AChE was estimated by following the modified method of (Ellman et al., 1961). First acetylcholinesterase enzyme homogenates were prepared from 10 whole bruchids insects (crude preparation), after maceration in cooled Tris-HCl buffer (100 mM, pH 8.0), the sample was centrifugated at 8000 rpm and the supernatant was used**.** The homogenates were incubated for 1 h with different amounts of EO of *S. areira* leaves. After incubation, the reaction was carried out in a 96 vial microplate, and a reaction mixture containing a suitable amount of homogenate with and without EO, DTNB in Tris-HCl buffer (100 mM, pH 8.0), and acetylcholine iodide (52.8 mM) was monitored over 30 min recording the absorbance at 405 nm and 37 °C. The percentage of AChE inhibition due to EO was calculated according to the following formula:

$$Inhibition\ (\%) = (1 - \frac{\Delta\ Absorbance_{\ 405}\ of\ sample\ with\ EO}{\Delta\ Absorbance_{\ 405}\ of\ control\ sample})x\ 100$$

The nature of inhibition was determined from kinetics data by double-reciprocal or Lineweaver-Burk plot obtained with different amounts of substrate and leaves *S. areira* EO.

2.6 Molecular docking studies

The complexes between the ligands evaluated and AChE from *Torpedo californica* (*Tc*AChE) and *Anopheles gambiae* (*Ag*AChE) were obtained by molecular docking assays. In these studies, the structures for the receptor were taken from the complexes *Tc*AChE-donepezil (PDB code: 1EVE) and *Ag*AChE-4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (PDB code: 5X61) (Han et al., 2018; Kryger et al., 1999).

The procedure for preparing the receptors consisted of treatment of the titratable residues within the publicly accessible *H++* server (Anandakrishnan et al., 2012; Myers et al., 2006). There were just a few missing residues at the C and N terminal positions of the chain, with no effect on the binding of any ligand. In the case of *Tc*AChE, glycosides at residues Asn 59, 416, 457, and 533 were removed as well as the ligand, donepezil, and crystallographic waters. Besides, ligand and crystallographic waters were removed from *Ag*AChE. Finally, sulfide bonds were made between cysteines in both cases.

The docking runs were performed with Autodock Vina *v1.1.2* software (Trott and Olson, 2010). The search space was defined using *AutoGrid*. A maximum number of 10 geometries were taken from each run and exhaustiveness of 100 was employed. The visualization of the docking result was performed with Discovery Studio Visualizer *v21.1.0*.

3. Results and Discussion

The present study was dedicated to inquiring about the insecticidal potential of EO of *S. areira* against storage pests like *R. picturatus* because of increasing demands for information about efficacious control tactics and their public health risks especially concerning eco-friendly approaches such as natural volatile oils.

The EOs of *S. areira* previously characterized showed different chemical compositions from plants collected in Europe, North Africa, and America (Cutro et al., 2021; Gomes et al., 2013; Martins et al., 2014; Salem et al., 2013; Solis-Quispe et al., 2016). In particular, in Argentina, the chemical constitution of EOs from *S. areira* was described for samples collected in Córdoba, where α-pinene, limonene, and ocimenone were reported as the main compounds (Scrivanti et al., 2003), in Jujuy where monoterpenes as myrcene, limonene, α-phellandrene, β-phellandrene, sabinene, and camphene were the predominant constituents (Celaya et al., 2014) and in Santiago del Estero where sesquiterpenoid alcohol 1-epicubenol was the main component (Cutro et al., 2021). These differences are attributed to the influence of extrinsic conditions based on geographic origin (climatic and soil-growth conditions), and the effect of intra-specific differences (Solis-Quispe et al., 2016). This high variability in the chemical composition in the genera *Schinus* encourages obtaining EO of *S. areira* seeds from the same specimens used to obtain leaves EOs for this study (Cutro et al., 2019). In addition, recently, the insecticidal effects of *Citrus* spp. EOs were tested against *Callossobruchus maculatus* insect pests of storage beans with excellent results (Dutra et al., 2016). *C. maculatus* belong to the Bruchinae family such as *R. picturatus*, moreover, the citrus essential oil industry is well

developed in the North-Western Region of Argentina. In this sense, the *Citrus* spp. EO was also obtained with the purpose to act as a reference in this study.

3.1 Essential oil characterization

As expected, the chemical composition of EO from leaves and seeds of *S. areira* showed differences. While in the EOs from *S. areira* leaves, monoterpenes and sesquiterpenes hydrocarbons were the most represented class of volatiles (Table 1), with the sesquiterpenoid alcohol 1-epi-cadinol as the mayor representative compound (34.6 %). On the other hand, eighteen compounds were identified in the EO extracted from *S. areira* seeds (Table 1). In these cases, monoterpenes such as sabinene (39.15 %), α-pinene (14.96 %), and β-pinene (8.75 %) are the main compounds. Finally, a total of 20 compounds were found in the *Citrus* spp. EO (Table 1) where limonene (68.64 %) and β-elemenene (13.14 %) are the major ones. Similar results were reported previously (Dutra et al., 2016). This is the first report on the chemical composition of EO from *S. areira* seeds and *Citrus* spp from Santiago del Estero.

EOs were extracted from *S. areira* populations in Brazil and four groups were proposed by Average linkage cluster analysis based on their chemical composition (Gomes et al., 2013). That groups are characterized by sabinene; α- and β-pinene; α-cadinol; and myrcene. In this regard, the leaves EO obtained in this work matches with the α-cadinol group, while the seeds EO matches with the sabinene group.

Table 1. Chemical composition of the EOs extracted in the present work.

Compound[a]		***S. areira* seed**	***S. areira* leaves** [d]	***Citrus spp.***
	RI[b]	**Area%**[c]	**Area%** [c]	**Area%** [c]
α-Pinene	932	14.96		
Sabinene	978	39.15		
β-Pinene	983	8.75	5.85	1.96
α-Terpinene	1020	2.68		
Limonene	1033	1.09	0.75	68.64

β-Phellandrene	1035	2.27		
Linalool	1040			0.48
Ocimene	1055		0.16	
γ-Terpinene	1062	4.48		0.93
Terpinolene	1088	0.98		
Linalool	1105	0.76		
α-Terpineol	1197	6.31	0.24	
4-terpinenyl acetate	1198		0.23	
Geranial	1261			0.31
α-Cubebene	1330		1.71	
δ-Elemene	1339			0.14
β-cubebene	1341		0.40	
Neryl acetate	1355			0.79
Geranyl acetate	1379			0.19
β-Elemene	1388		0.10	1.40
α-Gurjunene	1393		1.30	
Z-Caryophyllene	1400		0.62	
α-(Z)-Bergamotene	1409			0.23
β-Caryophyllene	1420	2.14	5.1	0.63
cis-muurola-3,5-diene	1437		2.48	
Aromadendrene	1446		0.50	
α-Humulene	1460		0.23	4.71
Alloaromadendrene	1474		6.99	
Germacrene-D	1475			0.33
cis-α-Bisabolene	1479	1.33	0.76	0.58
(Z,Z)-α-Farnesene	1490		2.95	
γ-Cadinene	1499		4.63	
δ-Cadinene	1514	1.63	15.52	
Germacrene-B	1533	3.61	1.68	
Spathulenol	1555		3.27	
Viridiflorol	1570		0.21	
Epiglobulol	1587			0.50
D-nerolidol	1597	1.07	2.12	0.31
α-Humulene oxide	1603			1.51
δ-Cadinol	1628		1.24	0.17
β-Eudesmol	1634		4.31	13.14
α-Cadinol	1642	0.83	0.47	1.73
1-epi-Cadinol	1653	7.63	34.06	
α-Bisabolol	1673	0.66	3.59	

[a] Compound identification based on RI, NIST 08 library, and bibliography. [b] RI, experimental linear retention indices on TR5-ms column. [c] Media and standard deviation of three independent experiments. [d] (Cutro et al., 2019).

3.2 Insecticidal activity.

In the contact toxicity tests, the EOs activity was proportional to their concentration; the toxicity was poor at low concentrations, and the opposite effect occurred at high

concentrations (Fig. 1 A). Furthermore, the LC_{50} in 24 h for the EO of leaves of *S. areira* was 89.16 μg/cm^2 (χ^2 3.12), for the EO of *S. areira* seeds the value was 765.50 μg/cm^2 (χ^2 1.40), and for the EO of *Citrus* spp. 932.43 μg/cm^2 (χ^2 1.00) (Fig. 1 B). The p value, obtained by one-way ANOVA for multiple comparisons, between each pairs of LC_{50} values was < 0.0001. In the case of *C. maculatus*, the reported activity of EO of *Citrus* was between 943.9 to 1037.7 μg/cm^2 (Dutra et al., 2016) in good agreement with the results found in this study. All EOs tested present activity until the 72 h post-contact; being EOs from *S. areira* the more potents.

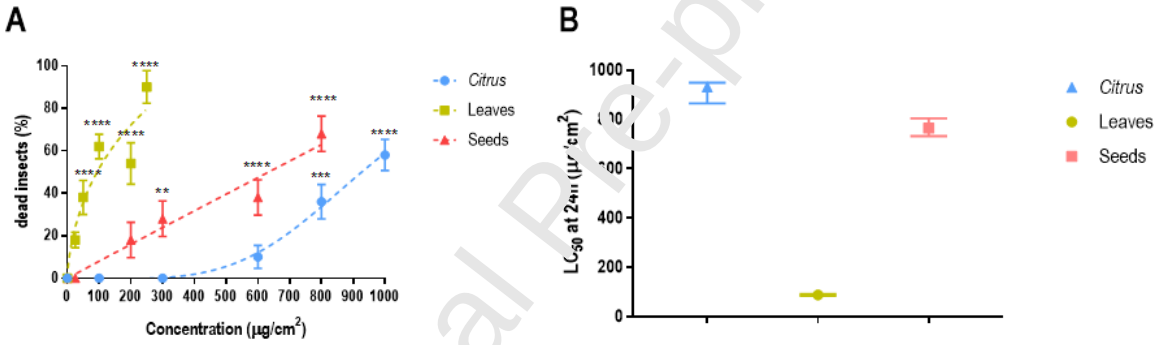

Figure 1. A) Insecticidal activity as a function of EO concentration. Each point represents the averages of five measurements; Error bars indicate standard deviations. ** p < 0.01; *** p < 0.001; **** p < 0.0001 one-way ANOVA followed by a Dunnett post-test for multiple comparisons vs. 0 of EO. B) LC_{50} values at 24 h for the studied EO obtained from concentration-mortality data subjected to Probit analysis. Error bars indicate the upper and lower confidence limits.

The EOs of *S. areira* seed and *Citrus*, richer in monoterpenes, showed lower activity in comparison with the leaves *S. areira* EO richer in sesquiterpenoids. It is

important to note that these results represent the first report of EO toxicity against *R. picturatus*.

3.3 Potential mechanism of action

Damaging the nervous system of insects is one of the main causes described by EOs toxicity (Kim et al., 2003; Sarwar, 2012). In this way, inhibition of acetylcholinesterase (AChE) is the principal mechanism of action studied of EOs, α-pinene and β-pinene, β-phellandrene, carvacrol, limonene, menthol, menthone, 1,8-cineole, cis-ocimene, and niloticin, showed activity at mM concentrations (Jankowska et al., 2018). In this sense, the effect of EO of *S. areira* leaves on *AChE* activity was evaluated, since it was the most active in the insecticidal assays.

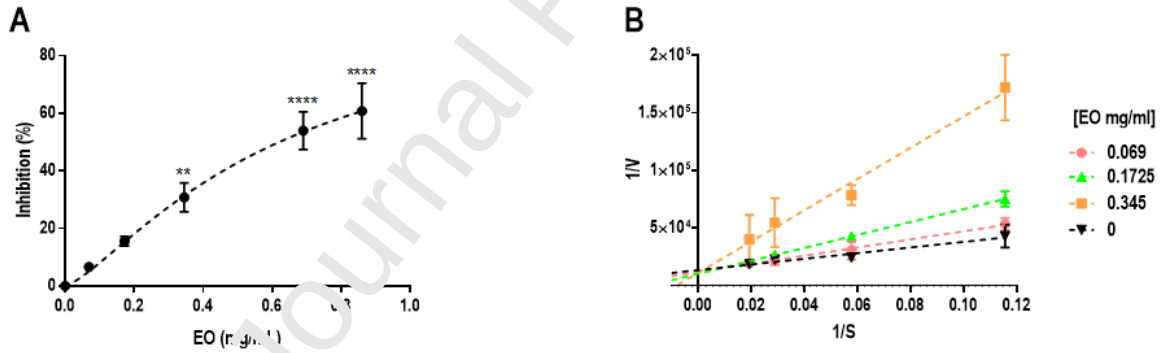

Figure 2. *In vitro* inhibition of AChE by EO of *S. areira* leaves. A) Inhibitory effect on AChE activity as a function of EO concentration. Each point represents the averages of at least two measurements; Error bars indicate standard deviations of the means. ** $p < 0.01$; **** $p < 0.0001$ one-way ANOVA followed by a Dunnett post-test for multiple comparisons vs. 0 of EO. B) The double reciprocal (Lineweaver-Burk) plot at different EO concentrations. Each point represents the averages of at least two measurements; Error bars indicate standard deviations of the means.

The EO of *S. areira* leaves demonstrates an *in vitro* inhibition of insect AChE homogenate. The EO showed a dose-dependent AChE inhibitory effect, with an IC_{50} value of 0.62 mg/mL (Fig. 2A). Unraveling the type of inhibition of AchE is important to understanding the effectiveness of the EOs (Jankowska et al., 2018). In this sense, the reciprocal plots at different substrate concentrations were obtained. As it can be seen in Fig 2B the enzyme inhibition due to EO decrease when substrate concentration increase. Furthermore, the *K*m shifted with the increased concentration of the inhibitor (EO) indicating that the inhibition was competitive. The results obtained are consistent with previously available studies that showed some EOs or their constituents act as competitive inhibitors (Jankowska et al., 2018).

However, should be pointed out that explaining the EOs' mode of action is not an easier task because the activity of Eos, as complex compounds, could differ from the activity of their single components. With this in mind, to establish the possible compounds responsible for the enzymatic inhibition a molecular docking analysis was carried out. The main compounds present in EOs of *S. areira* and EO of *Citrus* were used as AChE ligands. Also, galantamine, a well-known AChE inhibitor, was used as a reference (Thomsen and Kewitz, 1990). It is known that this inhibitor can bind to the active site of the AChE enzyme (Greenblatt et al., 1999). Since the AutoDockVina program has been successfully applied for publishing docking studies with AChE, it is utilized for our binding study (Borioni et al., 2020; Singh et al., 2017). *Tc*AChE and *Ag*AChE were selected as receptors. The last one is present in mosquitoes and will serve as a model of study for insect families.

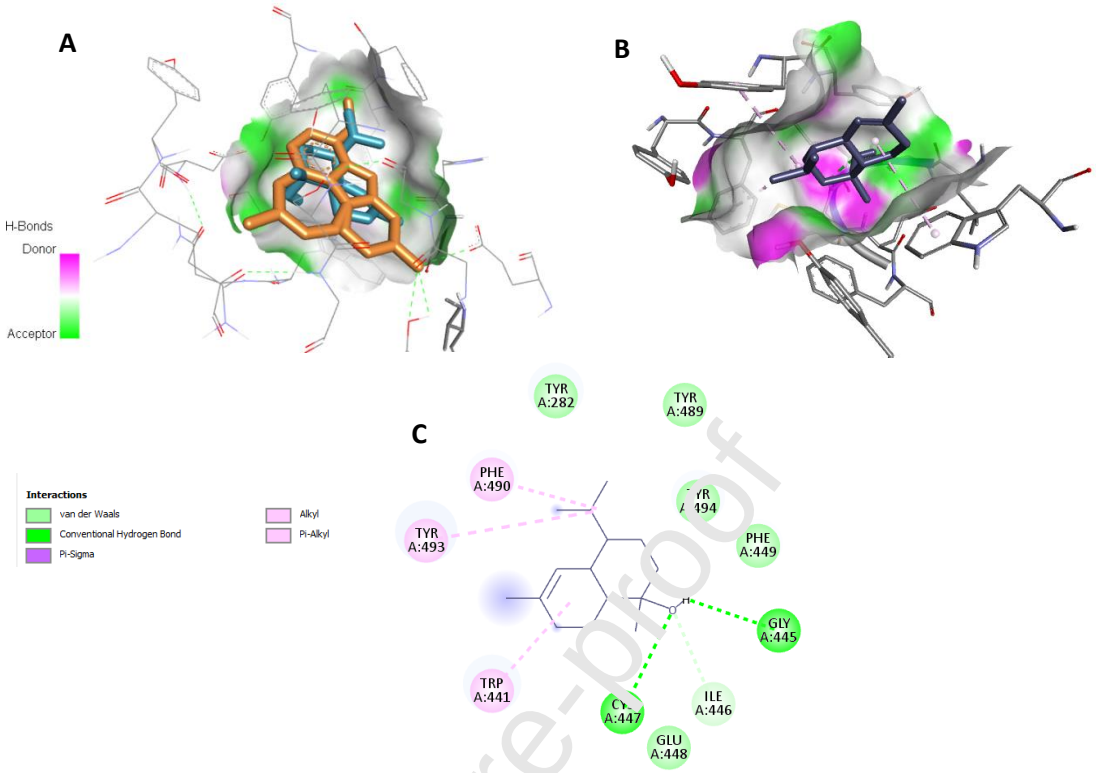

Figure 3. A) Docking results for 1-epi-cadinol (blue) and galantamine (brown) as reference complexed with *Tc*AChE receptor. B) Complex *Ag*AChE with 1-epi-cadinol. C) Representation of main interactions of 1-epi-cadinol with amino-acidic residues of *Tc*AChE.

The AChE has a narrow catalytic cavity of 20 Å deep. The catalytic active site (CAS) is located at the bottom of this cavity, where neurotransmitter acetylcholine (Ach) is hydrolyzed. Moreover, AChE has the peripheral anionic site (PAS) located at the entrance of this gorge. Finally, there is a region called a "bottleneck", between CAS and PAS, which function is to allow the entrance of natural ligands and inhibitors when is open (Dvir et al., 2010; Silman and Sussman, 2008).

The obtained docking results suggested that all selected ligands interact with the CAS. Figure 3 presents docking results for 1-epi-cadinol and galanthamine and showed that the main interactions with AChE active site are H-Bonds and hydrophobic type. Table 2 shows a similar trend in the docking binding energies (DBE) for ligands and both receptors. The lowest DBE was found for galantamine followed by sesquiterpenes and finally monoterpenes. All DBE are negatives, this result implies that the coupling between molecules is energetically favorable and is located between the Van der Waals bond energy level (< -1 kcal/mol) and the covalent chemical bond level (< -100 kcal /mol). Moreover, these binding may be considered favorable and reversible (it is non-covalent). It was recently demonstrated that at docking scores (DBE) of −8 kcal/mol or lower, the probability of identifying active compounds was 61 %, while the probability of misidentifying decoy compounds as active with DBE < −8 kcal/mol was 36 % (Guterres and Im, 2020). β-caryophyllene, alloaromadendrene, δ-cadinene, germacrene-B, spathulenol, β-eudesmol, 1-epi-cadinol, and α-bisabolol (sesquiterpenoids) presents the lowers DBE, being the 1-epi-cadinol isomer found in high quantity in the EO.

Table 2. Docking binding energies (DBE expressed in kcal /mol unit).

compound	*Tc*AChE	*Ag*AChE	*ΔDBE*[a]
α-Pinene	-6.8	-6.2	-0.6
Sabinene	-7.0	-6.1	-0.9
β-Pinene	-6.6	-6.2	-0.4
Limonene	-7.3	-6.2	-1.1
β-Phellandrene	-7.3	-6.2	-1.1
γ-Terpinene	-7.5	-6.1	-1.4
Terpinolene	-7.2	-6.2	-1
Linalool	-6.5	-5.9	-0.6

α-Terpineol	-7.1	-6.3	-0.8
β-Caryophyllene	-8.5	-8.1	-0.4
Alloaromadendrene	-8.9	-8	-0.9
δ-Cadinene	-8.4	-7.6	-0.8
Germacrene-B	-8.5	-8	-0.5
Spathulenol	-9.0	-7.9	-1.1
β-Eudesmol	-9.0	-8.1	-0.9
1-epi-Cadinol	-9.1	-7.8	-1.3
α-Bisabolol	-8.7	-6.9	-1.8
Galantamine	-9.6	-9.5	-0.1

[a] Docking energy differences for both AChE enzymes

Overall, these results suggest that 1-epi-cadinol, among other sesquiterpenoids present in the EO of *S. areira* leaves, binds to the CAS, which is mainly used to bind the substrate (Ach), in good agreement with the competitive inhibition founded in the *in vitro* assays (Fig. 2B). These results are in good agreement with the hypothesis that the best insecticidal activity of the EO of *S. areira* leaves is due to its high sesquiterpenes content compared to the OE of *Citrus spp*.

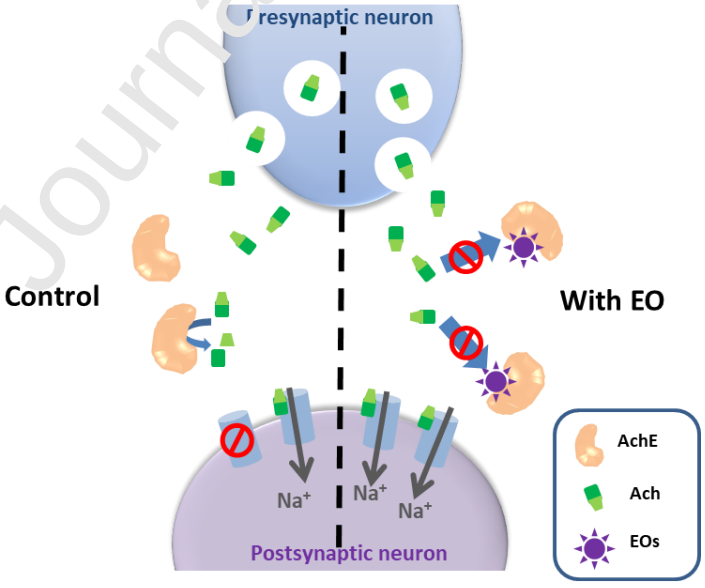

Figure 3. Proposed action mechanism of AChE inhibition

3. Conclusions

The main composition of the EO of seeds and leaves of *S.areira* were represented by monoterpenes and sesquiterpenes, respectively. *Citrus* spp EO was composed mainly of limonene. The order of insecticidal activity was: leaves EO >> seeds EO > *Citrus* spp EO. *In vitro* inhibition AChE assay demonstrate that the leaves EO reduce the activity of AChE from *R. picturatus* in a competitive way. That means that the inhibitor binds to the active sites in AChE and prevents the attaches of Ach (Fig. 3). Moreover, the molecular docking study allows concluding that the best ligands for two AChE enzymes (one represents insect enzyme) are the sesquiterpenoids molecules including the 1-epi-cadinol the main component of the leaves *S.areira* EO.

The fact that the leaves *S. areira* EO exhibited a higher insecticidal effect against *R. picturatus* is an important milestone for the development of a new pest management tool for *Prosopis alba* storage pests from the natural flora of Northern Argentina.

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References

Abdel-Sattar, E., Zaitoun, A.A., Farag, M.A., El Gayed, S.H., Harraz, F.M.H., 2010. Chemical composition, insecticidal and insect repellent activity of Schinus molle L. leaf and fruit essential oils against Trogoderma granarium and Tribolium castaneum. Nat. Prod. Res. https://doi.org/10.1080/14786410802346223

Adak, T., Barik, N., Patil, N.B., Govindharaj, G.P.P., Gadratagi, B.G., Annamalai, M., Mukherjee, A.K., Rath, P.C., 2020. Nanoemulsion of eucalyptus oil: An alternative to synthetic pesticides against two major storage insects (Sitophilus oryzae (L.) and Tribolium castaneum (Herbst)) of rice. Ind. Crops Prod. 143, 111849. https://doi.org/10.1016/j.indcrop.2019.111849

Adenubi, O.T., McGaw, L.J., Eloff, J.N., Naidoo, V., 2018. In vitro bioassays used in evaluating plant extracts for tick repellent and acaricidal properties: A critical review. Vet. Parasitol. https://doi.org/10.1016/j.vetpar.2018.03.008

Anandakrishnan, R., Aguilar, B., Onufriev, A. V, 2012. H++ 3.0: automating pK prediction and the preparation of biomolecular structures for atomistic molecular modeling and simulations. Nucleic Acids Res. 40, W537–W541. https://doi.org/10.1093/nar/gks375

Beatley, J.C., 1978. Mesquite: Its Biology in Two Desert Scrub Ecosystems . B. B. Simpson Creosote Bush: Biology and Chemistry of Larrea in New World Deserts . T. J. Mabry , J. H. Hunziker , D. R. DiFeo, Jr. . Q. Rev. Biol. https://doi.org/10.1086/410869

Bhavya, M.L., Chandu, A.G.S., Devi, U.S., 2018. Ocimum tenuiflorum oil, a potential insecticide against rice weevil with anti-acetylcholinesterase activity. Ind. Crops Prod. 126, 434–439. https://doi.org/10.1016/j.indcrop.2018.10.043

Borioni, J.L., Cavallaro, V., Pierini, A.B., Murray, A.P., Peñéñory, A.B., Puiatti, M., García, M.E., 2020. An activity prediction model for steroidal and triterpenoidal inhibitors of Acetylcholinesterase enzyme. J. Comput. Aided. Mol. Des. 34, 1079–1090. https://doi.org/10.1007/s10822-020-00324-y

Celaya, L.S., Alabrudzińska, M.H., Molina, A.C., Viturro, C.I., Moreno, S., 2014. The inhibition of methicillin-resistant Staphylococcus aureus by essential oils isolated from leaves and fruits of Schinus areira depending on their chemical compositions. Acta Biochim. Pol. 61, 41–46. https://doi.org/10.18388/abp.2014_1921

Chiffelle, I., Huerta, A., Celis, M., Araya, J.E., 2013. Proximal analysis and insecticidal effects of extracts from pepper tree (Schinus molle) leaves on elm leaf beetle

(Xanthogaleruca luteola) larvae. Ind. Crops Prod. https://doi.org/10.1016/j.indcrop.2012.07.062

Cutro, A.C., Castelli, M.V., López, S.N., Rosales, M.A., Hollmann, A., Rodriguez, S.A., 2021. Chemical composition of Schinus areira essential oil and antimicrobial action against Staphylococcus aureus. Nat. Prod. Res. 35, 2931–2936. https://doi.org/10.1080/14786419.2019.1675065

Cutro, A.C., Castelli, M.V., López, S.N., Rosales, M.A., Hollmann, A., Rodriguez, S.A., 2019. Chemical composition of Schinus areira essential oil and antimicrobial action against Staphylococcus aureus. Nat. Prod. Res. 35, 1–6. https://doi.org/10.1080/14786419.2019.1675065

De Batista, L.C.S.O., Cid, Y.P., De Almeida, A.P., Prudencio, E.R., Riger, C.J., De Souza, M.A.A., Coumendouros, K., Chaves, D.S.A., 2016. In vitro efficacy of essential oils and extracts of Schinus molle L. against Ctenocephalides felis felis. Parasitology. https://doi.org/10.1017/S0031182016000061

Deveci, O., Sukan, A., Tuzun, N., Kosabas, E.E.H., 2010. Chemical composition, repellent and antimicrobial activity of Schinus molle L. J. Med. Plants Res. https://doi.org/10.5897/JMPR10.326

Dutra, K. de A., de Oliveira, J.V., Navarro, D.M. do A.F., Barbosa, D.R. e. S., Santos, J.P.O., 2016. Control of Callosobruchus maculatus (FABR.) (Coleoptera: Chrysomelidae: Bruchinae) in Vigna unguiculata (L.) WALP. with essential oils from four Citrus spp. plants. J. Stored Prod. Res. 68, 25–32. https://doi.org/10.1016/j.jspr.2016.04.001

Dvir, H., Silman, I., Harel, M., Rosenberry, T.L., Sussman, J.L., 2010. Acetylcholinesterase: From 3D structure to function. Chem. Biol. Interact. 187, 10–22. https://doi.org/https://doi.org/10.1016/j.cbi.2010.01.042

Ebadollahi, A., Nouri-Ganbalani, G., Hoseini, S.A., Sadeghi, G.R., 2012. Insecticidal activity of essential oils of five aromatic plants against callosobruchus maculatus f. (coleoptera: Bruchidae) under laboratory conditions. J. Essent. Oil-Bearing Plants 15,

256–262. https://doi.org/10.1080/0972060X.2012.10644044

Ellman, G.L., Courtney, K.D., Andres Jr, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95.

Ferrero, A.A., Sánchez Chopa, C., Werdin González, J.O., Alzogaray, R.A., 2007. Repellence and toxicity of Schinus molle extracts on Blattella germanica. Fitoterapia. https://doi.org/10.1016/j.fitote.2006.11.021

Ferrero, A.A., Werdin González, J.O., Sánchez Chopa, C., 2006. Biological activity of Schinus molle on Triatoma infestans. Fitoterapia. https://doi.org/10.1016/j.fitote.2006.03.004

Gomes, V., Agostini, G., Agostini, F., Atti dos Santos, A.C., Rossato, M., 2013. Variation in the essential oils composition in Brazilian populations of Schinus molle L. (Anacardiaceae). Biochem. Syst. Ecol. 48, 222–227. https://doi.org/10.1016/j.bse.2013.01.003

Greenblatt, H.M., Kryger, G., Lewis, T., Silman, I., Sussman, J.L., 1999. Structure of acetylcholinesterase complexed with (−)-galanthamine at 2.3 Å resolution. FEBS Lett. 463, 321–326. https://doi.org/https://doi.org/10.1016/S0014-5793(99)01637-3

Guterres, H., Im, W., 2020. Improving Protein-Ligand Docking Results with High-Throughput Molecular Dynamics Simulations. J. Chem. Inf. Model. 60, 2189–2198. https://doi.org/10.1021/acs.jcim.0c00057

Han, Q., Wong, D.M., Robinson, H., Ding, H., Lam, P.C.H., Totrov, M.M., Carlier, P.R., Li, J., 2018. Crystal structure of acetylcholinesterase catalytic subunits of the malaria vector Anopheles gambiae. Insect Sci. 25, 721–724. https://doi.org/10.1111/1744-7917.12450

Herrera, J.M., Zunino, M.P., Dambolena, J.S., Pizzolitto, R.P., Gañan, N.A., Lucini, E.I., Zygadlo, J.A., 2015. Terpene ketones as natural insecticides against Sitophilus zeamais. Ind. Crops Prod. https://doi.org/10.1016/j.indcrop.2015.03.074

Heydarzade, A., Moravvej, G., 2012. Contact toxicity and persistence of essential oils from Foeniculum vulgare, Teucrium polium and Satureja hortensis against Callosobruchus maculatus (Fabricius) (Coleoptera: Bruchidae) adults. Turkiye Entomoloji Derg. 36, 507–518. https://doi.org/10.16970/ted.56583

Isman, M.B., Grieneisen, M.L., 2014. Botanical insecticide research: Many publications, limited useful data. Trends Plant Sci. https://doi.org/10.1016/j.tplants.2013.11.005

Iturralde-García, R.D., Borboa-Flores, J., Cinco-Moroyoqui, F.J., Riudavets, J., Del Toro-Sánchez, C.L., Rueda-Puente, E.O., Martínez-Cruz, O., Wong-Corral, F.J., 2016. Effect of controlled atmospheres on the insect Callosobruchus maculatus Fab. in stored chickpea. J. Stored Prod. Res. https://doi.org/10.1016/j.jspr.2016.06.004

Jankowska, M., Rogalska, J., Wyszkowska, J., Stankiewicz, M., 2018. Molecular targets for components of essential oils in the insect nervous system—a review. Molecules. https://doi.org/10.3390/molecules23010034

Kanda, D., Kaur, S., Koul, O., 2017. A comparative study of monoterpenoids and phenylpropanoids from essential oils against stored grain insects: acute toxins or feeding deterrents. J. Pest Sci. (2004). https://doi.org/10.1007/s10340-016-0800-5

Kingsolver, J.M., 1982. Taxonomic studies in the genus Rhipibruchus Bridwell (Coleoptera: Bruchidae), with description of four new species. Proc. Entomol. Soc. Wash. 84, 661–664.

Koul, O., Walia, S., Dhaliwal, G., 2008. Essential oils as green pesticides: Potential and Constraints. Biopestic. Int 4, 63–84.

Kryger, G., Silman, I., Sussman, J.L., 1999. Structure of acetylcholinesterase complexed with E2020 (Aricept®): implications for the design of new anti-Alzheimer drugs. Structure 7, 297–307. https://doi.org/10.1016/S0969-2126(99)80040-9

López, A., Castro, S., Andina, M.J., Ures, X., Munguía, B., Llabot, J.M., Elder, H., Dellacassa, E., Palma, S., Domínguez, L., 2014. Insecticidal activity of microencapsulated Schinus molle essential oil. Ind. Crops Prod.

https://doi.org/10.1016/j.indcrop.2013.12.038

Machado, C.D., Raman, V., Rehman, J.U., Maia, B.H.L.N.S., Meneghetti, E.K., Almeida, V.P., Silva, R.Z., Farago, P. V., Khan, I.A., Budel, J.M., 2019. Schinus molle: anatomy of leaves and stems, chemical composition and insecticidal activities of volatile oil against bed bug (Cimex lectularius). Brazilian J. Pharmacogn. https://doi.org/10.1016/j.bjp.2018.10.005

Martins, M.D.R., Arantes, S., Candeias, F., Tinoco, M.T., Cruz-Morais, J., 2014. Antioxidant, antimicrobial and toxicological properties of Schinus molle L. essential oils. J. Ethnopharmacol. 151, 485–492. https://doi.org/10.1016/j.jep.2013.10.063

Matos, L.F., Barbosa, D.R. e. S., Lima, E. da C., Dutra, K. de A., Navarro, D.M. do A.F., Alves, J.L.R., Silva, G.N., 2020. Chemical composition and insecticidal effect of essential oils from Illicium verum and Eugenia caryophyllus on Callosobruchus maculatus in cowpea. Ind. Crops Prod. 145, 112088. https://doi.org/10.1016/j.indcrop.2020.112088

Mauti, G.O., Kasigwa, P.F., Munissi, J.J.E., Onguso, J.M., 2019. Evaluation of the repellent effect of Dioscorea sansibarensis Pax (Dioscoreaceae) leaf essential oil against Bruchus chinensis Linnaeus, 1758 (Coleoptera: Bruchidae). Polish J. Entomol. 88, 119–128. https://doi.org/10.2478/pjen-2019-0009

Muruaga de L'Argentier, S., 1986. Especies de Bruchidae (Coleoptera) asociadas con semillas de Prosopis (Leguminosas) en el NOA. Morfología y biologla de los estadios preimaginales. Tesis Doctoral, San Miguel de Tucumán.

Myers, J., Grothaus, G., Narayanan, S., Onufriev, A., 2006. A simple clustering algorithm can be accurate enough for use in calculations of pKs in macromolecules. Proteins Struct. Funct. Bioinforma. 63, 928–938. https://doi.org/https://doi.org/10.1002/prot.20922

Pourya, M., Sadeghi, A., Ghobari, H., Taning, C.N.T., Smagghe, G., 2018. Bioactivity of Pistacia atlantica desf. Subsp. Kurdica (Zohary) Rech. F. and Pistacia khinjuk stocks essential oils against Callosobruchus maculatus (F, 1775) (Coloeptera: Bruchidae)

under laboratory conditions. J. Stored Prod. Res. https://doi.org/10.1016/j.jspr.2018.03.007

Ramos, R.Y., Kingsolver, J.M., Romero-Nápoles, J., 2007. Sobre el estatus taxonómico actual de los brúquidos (Coleoptera: Bruchidae) en los Chrysomeloidea. Dugesiana 14, 1–21.

Regnault-Roger, C., Hamraoui, A., Holeman, M., Theron, E., Pinel, R., 1993. Insecticidal effect of essential oils from mediterranean plants upon Acanthoscelides Obtectus Say (Coleoptera, Bruchidae), a pest of kidney bean (Phaseolus vulgaris L.). J. Chem. Ecol. 19, 1233–1244. https://doi.org/10.1007/BF00987383

Salem, M.Z.M., El-Shikh, M.S., Ali, H.M., 2013. Chemical characterization of the volatile oil isolated from leaves of schinus molle L. and its antioxidant activity. J. Pure Appl. Microbiol. 7, 355–360.

Scrivanti, L.R., Zunino, M.P., Zygadlo, J.A., 2003. Tagetes minuta and Schinus areira essential oils as allelopathic agents. Biochem. Syst. Ecol. 31, 563–572. https://doi.org/10.1016/S0305-1978(02)00202-8

Silman, I., Sussman, J.L., 2008. Acetylcholinesterase: How is structure related to function? Chem. Biol. Interact. 175, 3–10. https://doi.org/https://doi.org/10.1016/j.cbi.2008.05.035

Singh, K.D., Labala, R.K., Devi, T.B., Singh, N.I., Chanu, H.D., Sougrakpam, S., Nameirakpam, B.S., Sahoo, D., Rajashekar, Y., 2017. Biochemical efficacy, molecular docking and inhibitory effect of 2, 3-dimethylmaleic anhydride on insect acetylcholinesterase. Sci. Rep. 7, 12483. https://doi.org/10.1038/s41598-017-12932-0

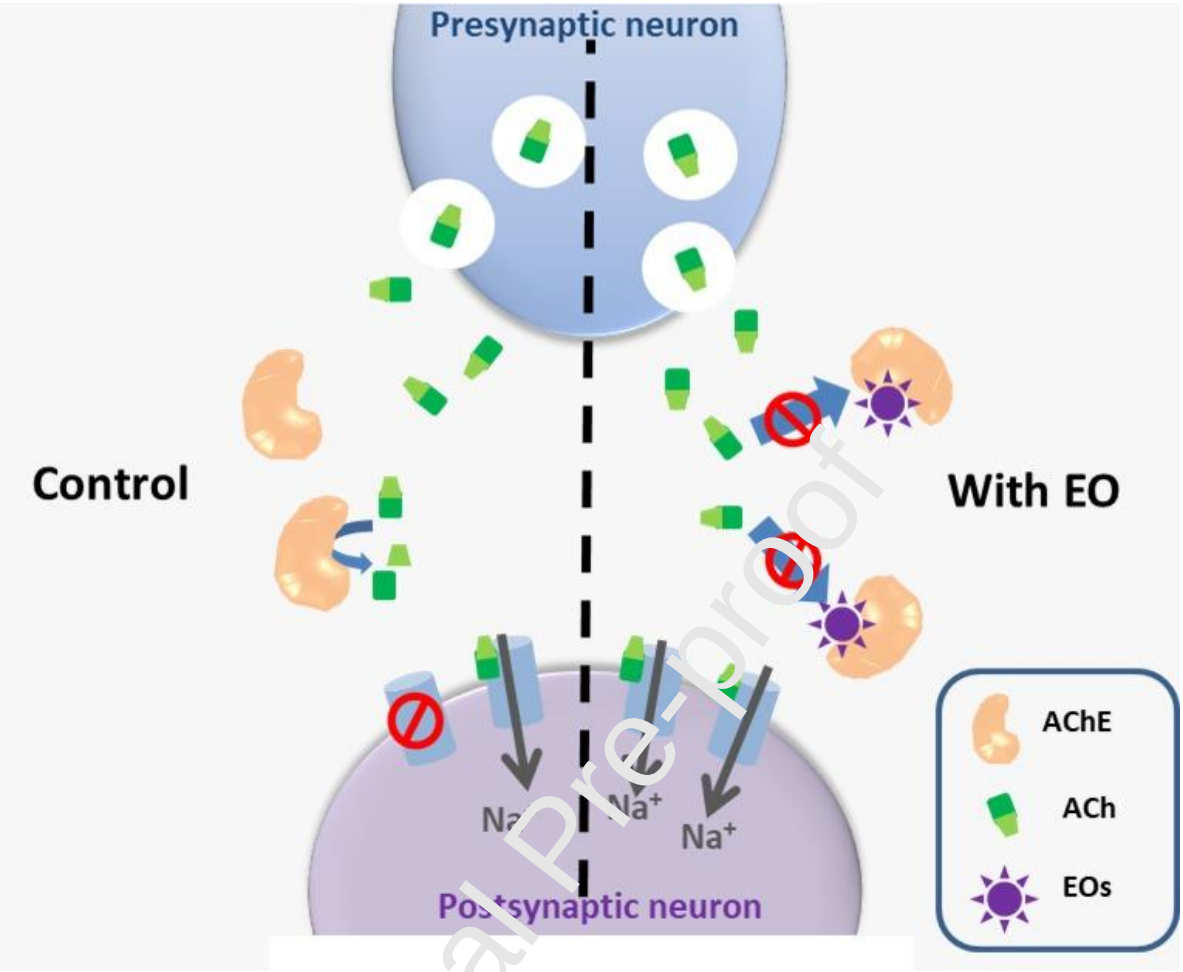
Solis-Quispe, L., Tomaylla-Cruz, C., Callo-Choquelvica, Y., Solís-Quispe, A., Rodeiro, I., Hernández, I., Fernández, M.D., Pino, J.A., 2016. Chemical composition, antioxidant and antiproliferative activities of essential oil from Schinus areira L. and Minthostachys spicata (Benth.) Epl. grown in Cuzco, Peru. J. Essent. Oil Res. 28, 234–240. https://doi.org/10.1080/10412905.2015.1120691

Thomsen, T., Kewitz, H., 1990. Selective inhibition of human acetylcholinesterase by galanthamine in vitro and in vivo. Life Sci. 46, 1553–1558. https://doi.org/https://doi.org/10.1016/0024-3205(90)90429-U

Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 31, 455–461. https://doi.org/10.1002/jcc.21334

Wang, Y., Zhang, L.T., Feng, Y.X., Zhang, D., Guo, S.S., Pang, X., Geng, Z.F., Xi, C., Du, S.S., 2019. Comparative evaluation of the chemical composition and bioactivities of essential oils from four spice plants (Lauraceae) against stored-product insects. Ind. Crops Prod. 140, 111640. https://doi.org/10.1016/j.indcrop.2019.111640

Zimmermann, H.G., 1991. Biological control of mesquite Prosopis spp. (Fabaceae), in South Africa. Agric. Ecosyst. Environ. https://doi.org/10.1016/0167-8809(91)90145-N



The EOs from leaves and seeds of *Schinus areira*, and EO from *Citrus* spp, were studied.

All EOs present insecticidal activity against *Rhipibruchus picturatus*, being the leaves EO the more active.

The EO of *S. areira* inhibits the AChE enzyme from insect homogenates in a competitive way.

Docking assays allow concluding that sesquiterpenoid compounds are good ligands from AgAChE and TcAChE.

These EOs are potential bioinsecticides for the control of stored insect pests.