

CAPÍTULO 4

NUTRITIONAL CHARACTERIZATION, FUNCTIONAL LIPIDS CONTENTS, ANTIOXIDANT PROPERTIES AND LARVICIDAL ACTIVITY FROM FRUITS, FLOUR AND FLOUR RESIDUE FROM PINK PEPPER (*Schinus terebinthifolius* RADDI)

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ABSTRACT: *Schinus terebinthifolius* known as Pink pepper fruit is used as a condiment and has received expressive attention due to its nutritional attributes and potential use as an antioxidant. In this study extracts of fruits and dried flours were analyzed for chemical, fatty acid, phytosterol and total phenolics composition besides antioxidant activity. Essential oils from fruits collected in Seropédica and Arraial do Cabo and as from flours and total fruit dried were tested for its larvicidal

activity against *Aedes aegypti*. The drying process at 30 °C used for flours promoted a significant increase in proteins, fat, unsaturated fatty acids, and antioxidant capacity of the pink pepper flour. The total phenolics from pink pepper flour were quite lower than that from pink pepper fruits. These results showed that other compounds are contributing to the antioxidant effect of flours. The drying process increase in α -phellandrene in the essential oil from the residue flour which reflected on its larvicidal activity. In this way, drying is an excellent alternative for the use of co-products from pink pepper, including the residue flour for *Aedes* mosquito control and food products for pink pepper flour.

KEYWORDS: *Schinus terebinthifolius* Raddi, pink pepper flour, antioxidant activity, larvicidal activity, essential oil flour.

1 | INTRODUCTION

Pink Pepper (*Schinus terebinthifolius* Raddi), Anacardiaceae family, is a popular tree extensively cultivated in Brazil. It is largely found in the Brazilian coast and is distributed from the northeast to the south part of the country (Carvalho et al. 2013). Its fruit is used as a condiment and has received expressive attention due to its nutritional attributes and potential use as an antioxidant, anti-inflammatory, antitumor, and antimicrobial agent (Gomes et al. 2020; Patocka and Diz De Almeida 2017; da Silva et al. 2018).

The fruit essential oil obtained from different places had been analyzed and presented limonene, β -pinene, α -fenchene, α -pinene, δ -3-carene, among others, as major compounds (Bendaoud et al. 2010; Carvalho et al. 2013). The essential oil from the fruit is used to treat respiratory disorders, mycosis and invasive candidal infections (Bendaoud et al. 2010) and it has insecticidal activity against *Rhizopertha dominica*, *Sitophilus zeamais*, *Anopheles gambiae*, *Anopheles arabiensis*, *Culex quinquefasciatus* and larvicidal activity against *Aedes aegypti* (Bortolucci et al. 2019; Kweka et al. 2011; Pratti et al. 2011). This essential oils with activity against *A. aegypti* presented β -pinene and δ -3-carene as major compounds.

Aedes aegypti is an anthropophilic mosquito that has a need to feed on a source of human blood, which serves as an important source of protein necessary for the development of its eggs. As a consequence, it becomes an efficient vector in the transmission of arboviruses. In this sense, the search for tools to control this insect becomes fundamental. Control strategies still focus on the use of organophosphates. However, continued use has contributed to the development of resistant animals (Braga and Valle 2007; Melo-Santos et al. 2010; Polson et al. 2011).

Several studies have sought to identify new insecticides of plant origin in order to control the spread of *A. aegypti* and, consequently, to reduce the transmission of arboviruses. In this sense, interfering with the larval stage of this insect and consequently preventing it from reaching adulthood is an important strategy.

Most studies are based on the use of essential oils as an insect control strategy (Dias and Moraes 2014). The application of these phytochemicals in addition to “biosafety”

can be easily obtained and low cost for the control of *Aedes* and other insect pests in poor communities and rural areas.

Considering the importance of naturally occurring compounds in plants, their proper recovery is a substantial task. The composition and consequent antioxidant and larvicidal capacity of natural extracts are highly influenced by the extraction step, which must allow the total extraction of the bioactive compounds without causing any modification of their chemical composition (Andrade et al., 2017; Yang et al. 2017).

This work aims to evaluate the chemical characteristics of the different derivatives obtained from *Schinus terebinthifolius* fruits, their antioxidant properties and larvicidal activity against *A. aegypti*.

2 | MATERIAL AND METHODS

2.1 Chemicals

Phytosterol standards, including brassicasterol, campesterol, stigmasterol and β -sitosterol were acquired from Sigma-Aldrich (St. Louis, MO, USA). The standards used in the antioxidant activity assays, ((\pm)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Trolox), 2,4,6-tris-2,4,6-tripyridyl-2-triazine (TPTZ), gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), were obtained from Alfa Aesar (Ward Hill, MA, USA), and 2,2'-azinobis(3-ethyl-benzo-thiazolone-6-sulphonate) (ABTS) was obtained from Fluka Chemie (Buchs, Switzerland). A total of 37 saturated, mono-unsaturated and polyunsaturated fatty acids standards (SupelcoTM FAME Mix 18919, Bellefonte, PA, USA) were used. The purities of the standards ranged from 95% to 99%. Sodium methoxide was purchased from Sigma (St. Louis - MO, USA). Tri-Sill reagent was obtained from Pierce (Rockford, IL, U.S.A.). HPLC grade n-hexane was obtained from Mscience (Darmstadt, Germany), and all other analytical grade solvents were obtained from Vetec (Sigma, São Paulo, Brazil).

2.2 Plant Collecting

Fresh Pink Pepper fruits (*Schinus Terebinthifolius* Raddi) were collected at a local farm in Seropédica, Rio de Janeiro, Brazil (latitude 22°45'00.2"S, longitude 43°4'56.5"W), and in Arraial do Cabo, Rio de Janeiro, Brazil (latitude 22° 57' 58" S, 42° 1' 44" W) in February 2016. The plant material from Seropedica was authenticated and a voucher specimen was deposited in the Herbarium of UFRRJ (RBR 15557). The plant from Arraial was identified as the same species.

2.3 Samples

2.3.1 Flour and residue flour preparation

For flour samples the fruits from Seropédica were dried in an air circulation oven

(Solab, SP, Brazil) at 30°C for 48 hours. The pink pepper flour was prepared by grounding the dried fruits in an analytical mill (A11 Basic, IKA) and passing through a sieve (mesh 24, TPL, Brazil) to obtain a particle size lower than 710 μm . The residue corresponds to the dried and ground fruits that do not pass through the sieve. The whole fruit dried was also grounded for the oil composition comparison. Subsequently, each type of sample was stored in multilayer laminated aluminum foil bags at room temperature until analyses.

2.3.2 Extracts

To determine the phenolic contents and antioxidant properties, extracts of fresh fruits, flour and pink pepper residue flour were used at a concentration of 10mg/ml in acetone:ethanol: water solution (40:30:30), respectively. The samples were submitted to agitation, for one hour, in the dark. After this, they were filtered according to Swain and Hillis (1959) and Torres (2002). Absorbance data were means by spectrophotometer Model Nova 2000 UV.

2.3.3 Essential oils

Five different samples of Pink pepper essential oil were evaluated: the whole fruit from Seropédica (EOST) and from Arraial do Cabo (EOAC), pink pepper flour (EOFVA), the residue obtained with the flour preparation (EORA) and the total fruit dried and grounded (EOAF).

2.4 Chemical constituents

2.4.1 Chemical composition

The samples were evaluated as fresh, dry fruits and as flour and residue flour. The samples were analyzed for chemical composition (moisture, ash, fat, proteins, fiber) using the AOAC procedures (AOAC, 1995). Crude protein content ($N \times 6.25$) of samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600°C. Total carbohydrates were calculated by the difference: total carbohydrate = 100- (g moisture +g protein +g fat +g ash+g fiber). Total energy was calculated according to the following equations: Energy (Kcal) = 4 x (g protein + g carbohydrate) + 9 x (g lipid).

2.4.2 Fatty acid composition

The samples (25 mg) were converted into methyl esters by transesterification according to Huang et al. (2006). The fatty acids were determined using a gas chromatograph (Shimadzu GC 2010, Tokyo, Japan), equipped with a split injector (1:50), flame ionization

detector and a workstation. The chromatographic separation was achieved in a fused silica CP-SIL 88 capillary column 50 m × 0.25 mm i.d., 0.20 μm film thickness (Chrompack, Middelburg, The Netherlands). The chromatographic conditions were: initial temperature 100 °C (5 minute) followed by 5 °C/minute up to 160 °C (zero minute), 8 °C/minute up to 230 °C (12 minute); injector and detector temperatures were 250°C and 280 °C. The equipment used hydrogen as the carrier gas at a flow rate of 1 mL/minute. Retention times of FAME standards were used to identify chromatographic peaks of the samples. The quantification was done by external standardization with a concentration range from 0.05 to 7 mg/mL.

2.4.3 *Phytosterol compositions*

Phytosterols were obtained from direct saponification of samples (1g of pink pepper samples, 4 ml of a 50% ethanol solution of KOH and 6 ml of ethanol) at 20°C for 22 h in the dark and the non-saponifiable matter extracts 4 times with hexane, according to Saldanha et al. (2006). The samples and standards were derivatized to trimethylsilyl (TMS) ethers according to Menendez-Carreño et al. (2008). The TMS derivatives were diluted with 1 mL of hexane, filtered through a 22 μm filter (Millipore, Maryland, MD, USA), and injected into a gas chromatograph (Shimadzu GC 2010, Tokyo, Japan), equipped with a split injector (1:20), a flame ionization detector, and a workstation. The TMS-ether derivatives of sterols were separated in a capillary column Rtx-5-MS (30m × 0.25mm × 0.25 μm, Restek, Bellefonte, USA). The oven program was: initial temperature, 230°C (0 min); a heating rate of 2°C/min to a temperature of 264°C (5 min); and then a heating rate of 1°C/min to a final temperature of 275°C (2 min). The injector temperature was 290°C and detector 350°C. The carrier gas was hydrogen at a flow rate 1 mL/min. Identification was done by comparison of the retention times of phytosterols standards. Quantification was done by external standardization with concentrations ranging from 0.01 to 1.0 mg/ml. The regression coefficients of phytosterols by stigmasterol, β-sitosterol, brassicasterol, and campesterol were higher than 0.99.

2.5 Determination of total phenolic contents (TPC)

The total phenolic contents were determined with Folin-Ciocalteu reagent (Quettier-Deleu et al., 2000) using gallic acid as the standard ($R^2=0.99$), and the results were expressed as gallic acid equivalent (mg GAE. g⁻¹, in dry basis). For this method, 7.0 ml of deionized water was added to 0.5 ml of pink pepper extract and then mixed with 0.5 ml of Folin-Ciocalteu phenol reagent. After three minutes, two milliliters of 20% sodium carbonate solution was added to the mixture. Then the mixture was heated in a water bath at 100°C for one minute. The blue color formed was measured at 685 nm using a spectrophotometer (Model Nova 2000 UV). Total phenols were measured using a standard curve by gallic acid equivalent, ranging from 10 to 50 μg/ml ($R^2>0.99$), and the results were expressed as gallic acid equivalent (mg GAE. g⁻¹ dry basis).

2.6 In vitro antioxidant capacity

2.6.1 DPPH free radical scavenging assay

Antioxidant activity was determined by scavenging the radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as described by Rufino et al. (2010). In this study 0.1 mL of extract was mixed with 3.9 mL of 6×10^{-5} M DPPH solution. After 60 min, the absorbance of samples, standard and blanks (methanol) were determined at 517 nm absorbance. Antioxidant activity was expressed as the percentage of DPPH radical-scavenging activity (% DPPH) and μmol Trolox equivalent (ET)/g of the sample. The quantification was performed by external standardization using a calibration curve with concentrations ranging from 100 – 800 μM /mL. The regression of coefficient was higher than 0.98.

2.6.2 Ferric-reducing antioxidant power assay

The reaction started after the addition of distilled water (270 μL) and FRAP reagent (2.7 mL) to the extract (90 μL). The mixture was maintained at 30°C for 30 min and the absorbance was determined at 595 nm. Results were obtained using a calibration curve and expressed as μmol of Fe^{+2} /g of sample (Rufino et al., 2010).

2.7 Essential oils extraction and analysis

The essential oils from fresh fruits and derivatives (200g) were obtained by hydrodistillation in a Clevenger-type apparatus for 4 h. Gas chromatographic analyses were performed using a HP 5890 series II gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and a HP-5 (5% phenyl/95% dimethylpolysiloxane) fused silica capillary column (30 m x 0.25 mm x 0.25 μm). Helium was the carrier gas (1.0 mL min⁻¹). The injector temperature was kept at 220°C and the oven temperature program was from 60° to 290°C at a rate of 5°C min⁻¹. The detector (FID) was operated at 250°C. Pure oils (2 μL) were injected in split mode (30:1). The GC-MS analyses were performed in a CG-MS-QP2010 Plus (Shimadzu), equipped with a HP5-MS capillary column (30 m X 0.25 mm X 0.25 μm), operating in electronic ionization mode at 70 eV, with the transfer line maintained at 260°C, while mass analyzer and ion source temperatures were held at 150°C and 250°C, respectively. Helium (1.0 mL min⁻¹) was used as carrier gas. Oven temperature program, injector temperature and split rate were the same as stated for GC analyses. A standard solution of n-alkanes (C7-C26) was used to obtain the retention indices (Van Den Dool & Kratz 1963). Individual volatile components were identified by comparison of their mass spectra (MS) and retention indices (RI) with those reported in literature (Adams, 2009) and also in the Wiley Registry of Mass Spectral Data, 6th Edition (Wiley Interscience, New York).

2.8 Larvicidal assay of essential oils

The larvicidal assay were developed using methodology recommended by the World Health Organization (WHO, 2005). The population of *A. aegypti* cepe Rockefeller was reared in the laboratory at 27 ± 1 °C and relative humidity of $70 \pm 5\%$. The eggs were placed to hatch in plastic pots with 1 L of dechlorinated water. After hatching, the larvae were fed daily with fish feed (Nutriflakes, Nutriconpet). Larvicidal activity was performed in quadruplicate in 50 ml plastic cups, where 10 larvae of 3rd instar were added in 10 ml of dechlorinated water. The essential oil was diluted in an aqueous solution of 2% DMSO and distributed in the cups. Larval mortality was verified after 24 h of exposure to oil. In parallel, controls were performed with water and 2% DMSO.

2.9 Statistical Analyses

Data analysis was carried out by ANOVA. The Fisher test was used to identify significant differences between the means, using the Statistic® 7.0 software. The level of significance for the difference between means was 5% ($p < 0.05$).

To calculate the percentage of larvae mortality, the program Prisma 5.0 was used and the LC_{50} (defined as the oil concentration necessary to kill 50% of the larvae in 24 hours) was calculated by the Probit Analysis using R.

3 | RESULTS AND DISCUSSION

3.1 Chemical composition

The proximate composition of the samples is shown in Table 1. Moisture is one of the most important parameters for the stability of flours during storage (Silva et al., 2020). With respect to the standard established in the current Brazilian legislation (15g 100 g⁻¹) (Brasil, 2005) the flour presented lower values (13.54 ± 0.02 100 g⁻¹) while the residue presented higher values (15.03 ± 3.4 100 g⁻¹). There were no significant differences between the ash content of pink pepper and flours. The protein content were higher in the flour (8.06 ± 0.26 100 g⁻¹) than that from fruit (7.07 ± 0.31 100 g⁻¹). According to Brazilian legislation, for a product to be considered a source of protein, it must contain a minimum of 6g of protein per 100g (Brasil, 2012; Silva et al.2020) which allows pink pepper flour to be considered a protein source.

Common flours used in bakery products possess different amounts of fat (Villela et al., 2013). The lipid content of the pink pepper flour (15.49 ± 0.76 100 g⁻¹) were higher than the fruits (7.53 ± 1.33 100 g⁻¹). In addition, the fatty acid profile (supplementary material- table 1) from pink pepper flour give 11-eicosenoic acid as majoritarian monounsaturated fatty acid (1.20 ± 0.17 on 1.24 ± 0.21 total monounsaturated fatty acids). The total unsaturated fatty acids have the double content in the flour than in the fruits. Hypothetically, 100 g of pink

pepper flour contributes with 19% of total caloric value of an individual with a diet of 2000 kcal/day (Villela et al., 2013) which can be a reflection of the fat highest content.

Carbohydrates levels include starch, cellulose, hemicellulose, lignin, pectin and other biopolymers present in the fruit were higher in the residue flour (70.66 ± 0.79 100 g⁻¹) than that from the pink pepper flour (54.78 ± 0.58 100 g⁻¹) probably due to flour preparation process.

Fiber content was higher in the pink pepper flour (17.96 ± 0.04 100 g⁻¹) than in the residue flour (15.93 ± 0.02 100 g⁻¹). Compared with conventional flours (Padovani, 2007) the pink pepper flour showed higher values.

Phytosterols content are presented in table 2 and were lower in the flour than in the fruits but as they play an important function in cholesterol absorption and reduce cholesterol levels in blood their presence in some ingredients as pink pepper flour can be applied in many food and dishes (Silva et al, 2019).

3.2 TPC and *in vitro* antioxidant activity

The total phenolic compounds of fruits and flours are present in table 3. The results showed that total phenolics from pink pepper flour are quite lower than that from pink pepper fruits. The *in vitro* antioxidant activity of samples is show in table 3 and was accessed using three methods. The antioxidant activity was higher in flours when compared with fresh fruits extraction. Pink pepper flour showed the best results. The antioxidant activity of fruits is influenced by several factors, including environmental aspects, ripening, fruit variety, type of solvents and extraction conditions (Halliwell, 1996). Nunes et al (2016) reported an increase in antioxidant activity of guavas subjected to oven-drying process as we founded in the present study for flours. Heat induces numerous chemical reactions, such as Maillard reaction, Strecker degradation and hydrolysis of esters and glycosides leading to generation of new antioxidant compounds. Almeida et al. (2011) pointed out that the antioxidant potential of fruits is also influenced by the action of different antioxidant compounds with synergistic and antagonistic effects between them. These results showed that other compounds are contributing to the antioxidant effect of flours and should be investigated.

3.3 Essential oil composition

The essential oils from the fruits of *Schinus terebinthifolius* were obtained in 2.1 and 1.8 % yields, respectively. The main identified compounds are listed in the supplementary material- Table 2. The essential oils from fruits presented a high content of monoterpenes and a low content of sesquiterpenes as described previsously (Bendaoud et al. 2010; Kweka et al. 2011, Cavalcanti et al., 2015). Forty three compounds were identified in the essential oil of Arraial do Cabo representing 95.7% of the total oil composition. Among them, myrcene (26.93%), p-Cimene (18.04%), sylvestrene (17.38%) and a-Pinene (12.72%)

were the major components. All other components were detected at contents below 3.5 %. In the essential oil from Seropedica, forty eight compounds were identified representing 97.95% of the total oil composition. The major components were terpinen-4-ol (35.84 %), elemol (8.77%), α -eudesmol (10.32 %) and β -eudesmol (11.24 %). All other components were detected at contents below 3.58 %. The chemical classes of the studied oils are reported in supplementary material- Table 2. The predominant class was monoterpene hydrocarbons (84.31%) for essential oil from Arraial do Cabo (EOAC) while the essential oil from Seropedica (EOST) presented oxygenated monoterpenes (49.57%) as predominant class. It was observed that essential oils chemical profile obtained from this survey was not overall similar to those ones presented by other authors (Affonso et al. 2012; Bendaoud et al. 2010; Carvalho et al. 2013; Cole et al. 2014; Kweka et al. 2011; Pawlowski et al. 2012; Pratti et al. 2015, Cavalcanti et al., 2015). Variations in essential oils contents from plant tissue can be related to different factors, some of them intrinsic and controlled by the plant genetic traits (Gomes et al., 2013, Cavalcanti et al., 2015), On the other hand, quantitative traits are susceptible to the edaphoclimatic effects, such as seasonality, water availability and soil nutrients (Lima et al., 2003).

The essential oils from the total dried fruits (EOAF) of *Schinus terebinthifolius* were obtained 1.05% yields. Some significant differences were noted in the quantitative and qualitative composition of the oils obtained from fresh and dry fruits (Zardi-Bergaoui et al., 2018) but we can deduce that both of them may be considered as monoterpene rich oils. The main class was formed by the monoterpene hydrocarbons (75.15 %) which characterizing the dry fruits oil, α -pinene (44.90 %), α -phellandrene (9.88 %) and p -cimene (8.79%) represented the major components. However, we noted that the major constituent of fresh fruits essential oil was EOFVA represented only 7.23% in the dry fruits. They were mainly represented by borneol (1.87 %) and carvacrol (1.62 %).

Between the essential oil from the derivatives the EORA and EOFVA were obtained with 0.85% and 0.04% yields, respectively. Fifty eight identified compounds were present for residue flour essential oil (EORA) while for flour essential oil (EFVA) only twenty-five compounds were identified. Monoterpenes hydrocarbons (47.48%) which characterizing the residue oil, α -pinene (9.02 %) and α -phellandrene (18.32 %) while oxygenated sesquiterpenes (93.23%), β -Eudesmol (53.29%), γ -eudesmol (24.42%) and elemol (9.58%) represent major components in the pink pepper flour oil. These compounds could be most present in different tissues of the fruit and its detection was possible by the flour process production. In the odder hand, the flour (EOFVA) has smaller content of monoterpenes probably due to drying process (Zardi-Bergaoui et al., 2018).

3.4 Larvicidal assay

In this work, the effects of essential oils derived from *Schinus terebinthifolius* fruits obtained by different samples on *A. aegypti* L4 larvae were evaluated. All oils showed

mortality against *A. aegypti* larvae after 24 hours of treatment (Table 4). The EORA showed the best larvicidal activity (Table 4), presenting an LC50 of 94.5 ppm (Table 4, Figure 1). Studies using essential oil obtained from *Commiphora leptophloeos* showed α -phellandrene as a major component and presenting an LC50 of 99.4 ppm of larvicidal activity, which corroborates with our result.

4 | CONCLUSIONS

The drying process at 30 °C used for flours promoted a significant increase in proteins, unsaturated fatty acids and antioxidant capacity of the pink pepper flour and an increase in α -phellandrene in the essential oil from the residue flour which reflected on its larvicidal activity.

In this way, drying is an excellent alternative for the use of co-products from pink pepper, including the residue flour for *Aedes* mosquito control and food products for pink pepper flour.

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TABLES

	Fresh Fruits	Flour	Residue flour
Moisture	35.25 ± 0.21 ^A	13.54 ± 0.02 ^B	15.03 ± 3.4 ^B
Ash	3.74 ± 0.28 ^A	3.71 ± 0.15 ^A	3.61 ± 0.16 ^A
Protein	7.07 ± 0.31 ^B	8.06 ± 0.26 ^A	5.47 ± 0.45 ^C
Fat	7.53 ± 1.33 ^B	15.49 ± 0.76 ^A	4.32 ± 1.08 ^B
Carbohydrate	59.07 ± 0.58 ^B	54.78 ± 0.58 ^C	70.66 ± 0.79 ^A
Fiber	22.59 ± 0.35 ^A	17.96 ± 0.04 ^B	15.93 ± 0.02 ^C
Energy	332 ± 5.78 ^B	391 ± 2.26 ^A	343 ± 4.76 ^B

Means values ± SD (standard deviation) (n=3). Means in rows followed by the same letter are not different at 5% probability by Student's t test.

Table 1: Moisture (g100g⁻¹ of fresh weight), nutrients composition (g 100g⁻¹ of dry weight) and energetic values (Kcal/100g dry weight) of fruits, flour and residue flour of pink pepper.

Samples	Campesterol	Stigmasterol	β-Sitosterol	Total phytosterol content
Fresh fruits	3.58 ± 0.44 ^A	26.09 ± 0.36 ^A	166.70 ± 8.44 ^A	196.60 ± 9.00 ^A
Flour	0.77 ± 0.00 ^B	23.67 ± 3.53 ^A	96.46 ± 3.77 ^B	120.90 ± 7.30 ^B
Residue flour	ND	18.34 ± 0.34 ^A	31.23 ± 3.66 ^C	49.50 ± 3.50 ^C

Means ±SD = standard deviation; means in columns followed by the same letter are not different at 5% probability by Student's t test. ND = not detected.

Table 2: Phytosterol compositions of pink pepper fruits, flour and residue flour (mg100g⁻¹ dry weight)

	Fresh Fruits	Flour	Residue flour
Total phenolics (mg gallic acid g ⁻¹ sample dry weight)	14.93 ± 3.14 ^A	13.06 ± 0.76 ^A	3.15 ± 0.09 ^C
FRAP (μmol Fe ²⁺ /g)	151.56 ± 2.19 ^B	189.58 ± 7.39 ^A	108.28 ± 0.82 ^C
DPPH (μmol TE.g ⁻¹ of sample)	246.03 ± 6.43 ^B	301.43 ± 13.40 ^A	204.36 ± 19.22 ^B
ABTS (μmol TE.g ⁻¹ of sample)	225.66 ± 3.72 ^B	348.20 ± 3.07 ^A	333.60 ± 5.60 ^A

Means ± SD = standard deviation (n=3) SFR = Sequester free radicals. Means in rows followed by the same letter are not different at 5% probability by Student's t test.

Table 3: Total phenolic contents and antioxidant activity of the fruits, flour and residue flour extracts of *Schinus terebinthifolius*.

EO	N	Inclination (\pm DP)	CL ₅₀ (ppm)	CL ₉₀ (ppm)	X ²	p
EOAC	90	2.536 (\pm 0,322)	144.27 (123.59; 163.26)	461.81 (386.09; 596.57)	13.06	0.957
EOST	80	3.679 (\pm 367)	219.87 (199.04; 240.48)	490.301 (431.35; 581.33)	11.34	0.921
EORA	100	94.304 (\pm 0.292)	94.30 (81.05; 107.23)	241.00 (204.089; 303.56)	6.27	0.820
EOFVA	120	3.814 (\pm 0.697)	309.96 (278.64; 340.64)	671.00 (591.51; 796.02)	30.46	0.999
EOAF	110	2.688 (\pm 0.730)	289.128 (250.77; 328.205)	866.46 (700.09; 1,193.58)	43.47	1.000

The whole fruit from Seropédica (EOST) and from Arraial do Cabo (EOAC), pink pepper flour (EOFVA), the residue obtained with the flour preparation (EORA) and the total fruit dried and grounded (EOAF).

Table 4: Mosquito larvicidal potential of *Schinus terebinthifolius* EOs from different methods of extraction against *Aedes aegypti*.

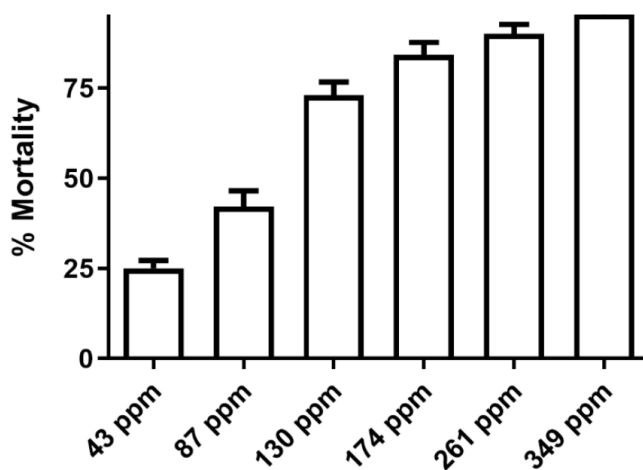


Fig. 1: Larvicidal activity of the EORA extract obtained from *Schinus terebinthifolius* oil fruits after 24h of treatment of larvae in stage A of *A. aegypti*.