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Effects of *Persea americana* Mill. seed extracts on the postembryonic development of *Musca domestica* (Diptera: Muscoide)

[Efectos del extracto de semilla de *Persea americana* Mill. sobre el desarrollo postembrionario de *Musca domestica* (Diptera: Muscoide)]

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Abstract

Context: The synthetic insecticides used to control Diptera are harmful to the environment and humans. Extracts and compounds from plants are a more sustainable source for the development of bio-insecticides.

Aims: To evaluate the efficacy of a hydroalcoholic extract of *Persea americana* Mill seeds as an alternative control of the species *Musca domestica*.

Methods: The extracts were obtained by two methods, the Shaker (S) and the Soxhlet extraction (SE) method, using 94% ethanol as the solvent. Also, the qualitative chemical composition was determined by phytochemical screening. The effect of the two extracts on the post-embryonic development of the fly as well as the adulticidal effect was evaluated.

Results: Phytochemical analysis revealed the presence of metabolites such as alkaloids, coumarins, tannins, flavonoids, sugars and amino acids. The influence on the post-embryonic development of *M. domestica* was demonstrated, especially on the viability of larvae and neolarvae to adults; however, the effect on the weight and duration of each period was low. The adulticidal effects of the extracts were determined by the lethal concentration 50(LC_{50}) of 2.910 mg/100 mL and 3.944 mg/100 mL for the S and SE extracts, respectively.

Conclusions: Both extracts showed their insecticidal effects against *Musca domestica*, but the extract elaborated by S method showed greater influence diminishing viability and better adulticidal effect.

Keywords: biological control; Diptera; *Musca domestica; Persea americana* Mill.

Resumen

Contexto: Los insecticidas sintéticos empleados en el control de Dípteros resultan perjudiciales para el medio ambiente y los seres humanos. Los extractos y/o compuestos derivados de las plantas constituyen una fuente para el desarrollo de bio-insecticidas más viables.

Objetivos: Evaluar la eficacia del extracto hidroalcohólico de semillas de *Persea americana* Mill. para el control alternativo de la especie *Musca domestica*.

Métodos: Los extractos se obtuvieron mediante dos métodos, maceración/agitación con zaranda (S) y extracción con Soxhlet (SE), empleando como solvente el etanol al 94%. A los dos extractos se les determinaron la composición química cualitativa a través de los ensayos descritos en la técnica de tamizaje fitoquímico. Se evalúo el efecto de los dos extractos sobre el desarrollo post-embrional de la mosca, así como el efecto adulticida.

Resultados: Se informan los resultados del análisis fitoquímico, revelando la presencia de metabolitos como: alcaloides, cumarinas, taninos, flavonoides, azúcares y aminoácidos. Se demostró la influencia en el desarrollo post-embrionario de *M. domestica*, especialmente sobre la viabilidad de larvas y en el periodo neolarva a adultos y sobre la duración de cada período. Se evidenció el efecto adulticida de los extractos, mediante la determinación de la concentración letal 50 (LC_{50}) de 2.91 mg/100 mL y 3.944 mg/100 mL para los extractos S y SE, respectivamente.

Conclusiones: Ambos extractos mostraron sus efectos insecticidas contra *Musca domestica*, pero el extracto elaborado por el método de zaranda mostró mayor influencia sobre la reducción de la viabilidad y mejor efecto adulticida.

Palabras Clave: control biológico; Dipteros; *Musca domestica; Persea americana* Mill.

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INTRODUCTION

Muscoid dipterans are recognized as vectors of viruses, enterobacteria, fungi, helminths, and protozoans. Such transmissions are mediated through the consumption of food and water. These insects are responsible for the appearance of episodes of child diarrheal in the young (Sulaiman et al., 2000; Sukontason et al., 2007). Moreover, some species can produce myiasis, in both animals and humans (Borges et al., 2014).

Musca domestica (Diptera: Muscidae) is a vector for more than 100 pathogens (Forster et al., 2007; Malik et al., 2007), which can cause diseases in humans and animals, including typhoid fever, cholera, bacillary dysentery, tuberculosis, anthrax, and ophthalmia (Akinboade et al., 1984; Iwasa et al., 1999; Bertoni, 2013).

Moreover, dipterans are very important during the decomposition of organic matter from animals and plants. Also, many dipterans actively participate in pollination and are an important link in the trophic chain because of their nutritive value for many insectivores (Mendoça et al., 2011).

There are diverse methods of control of harmful dipterous insects. The most important system is the use of chemical insecticides in the form of adulticides and larvicides. However, chemical insecticides have been shown to affect the biological diversity of the environment through air, water and soil contamination. In addition, the persistent use of insecticides can lead to the bioaccumulation of such chemicals in the food chain and cause severe damage to humans and animals (Siriwattanarungsee et al., 2008). On the other hand, resistance to insecticides has been demonstrated in several species of insects, which is an adaptive response to prolonged exposure. Another aspect of great importance generated by this resistance is the cost for the farmer (Bisset et al., 2016).

As pollution caused by insecticides constitutes a threat to biodiversity, there is a need for ecofriendly alternatives. Biopesticides are an option to replace synthetic pesticides as they are non-persistent in the environment. In addition, they confer less resistance to pests and are non-toxic. At the same time, they biodegrade rapidly, do not pollute the environment, and they have a relatively low cost (Cortinhas, 2012).

Various studies with *Persea americana* Mill. (Laureaceae), known as avocado, have been carried out showing different medicinal properties, such as: antioxidant, hypolipidemic, antimicrobial (Mohammad et al., 2010; Dabas et al., 2013), cicatrizing (García et al., 2015), among others. In the bibliography consulted, the insecticidal activity of the avocado seed has been little explored; there are only reports of such activity from seed extracts on different species of mosquitoes. Adesina et al. (2016) studied the insecticidal properties of the seed of this fruit for the alternative control of *Anopheles gambiae* (Diptera: Culicidae). Several seed extracts have been tested against larvae of the genus *Aedes* (Diptera: Culicidae) (Torres et al., 2014; Nzelibe and Albaba, 2015). These studies demonstrate the potential of *P. americana* in the research and development of new bioinsecticides. This study was carried out to evaluate the efficacy of the hydroalcoholic extract of *P. americana* seeds as alternative control of *Musca domestica*.

MATERIAL AND METHODS

Vegetal material

The fruits of avocado were collected in Caney town, municipality of Santiago de Cuba, during the period from October-November, 2015. The seeds of *Persea americana* Mill. var. americana were taxonomically identified by Jainer Costa Acosta, specialist at the Eastern Center for Ecosystems and Biodiversity (BIOECO) in the province of Santiago de Cuba and a vegetal sample was settled at the herbarium of the said institution with the registration number 21 510.

The seeds of the plants were used in fresh conditions, for which the skin that covered them was removed and then they were striped with a grater. Finally, 100 g of the striped seed were weighted in an analytical balance (Nagema Avius 2429) for the immediate preparation of the extracts.

Extraction methods

Two extracts were made by two extractive methods Shaker (S) method and Soxhlet extraction (SE) method. In both extractions, a 94% hydroalcoholic solution was used as the solvent.

Soxhlet Extraction (SE) method

It was performed by a Soxhlet apparatus for 6 h after the start of the first reflux. In this process 100 g of the previously scratched seed and 200 mL of the hydroalcoholic solution were used.

Shaker (S) method

A Zaranda JP Selecta 3000974 (Spain) was used. For the process, 100 g of the grated seeds were used, placed into two Erlenmeyer flasks, each adding 100 mL of the hydroalcoholic solution. The extraction was performed for 24 hours.

The extracts were filtered using a Buchner funnel and filter paper and then concentrated in a Kirka-Werke rotary evaporator (Germany) at 45°C until 50 mL at a ratio of 1 g of solids/0.5 mL of solvent (stock solution). Five concentrations were prepared: 0.153 mg/100 mL, 0.383 mg/100 mL, 1.917 mg/100 mL, 3.835 mg/100 mL, 7.67 mg/100 mL by Shaker method and 0.136 mg/100 mL, 0.34 mg/100 mL, 1.70 mg/100 mL, 3.40 mg/100 mL, 6.80 mg/100 mL by Soxhlet extraction method, using distilled water as a solvent.

Quality control of extracts

Qualitative chemical control tests, such as phytochemical screening, were performed according to (Ochoa et al., 2002) to determine the secondary metabolites present in these extracts:

Test for alkaloids

Mayer' s test: To a few mL of plant sample extract, two drops of Mayer's reagent were added along the sides of the test tube. The appearance of creamy white precipitate indicated the presence of alkaloids.

Wagner's test: A few drops of Wagner's reagent are added to few mL of plant extract along with the sides of the test tube. A reddish- Brown precipitate confirmed the test as positive.

Test for amino acids

Ninhydrin test: Two drops of ninhydrin solution (10 mg of ninhydrin in 200 mL of acetone) were added to 2 mL of aqueous filtrate. The appearance of purple color indicated the presence of amino acids.

Test for carbohydrates

Molish' s test: To 2 mL of plant sample extract, two drops of an alcoholic solution of α- naphthol were added. The mixture was shaken well, and few drops of concentrated sulphuric acid was added slowly along the sides of the test tube. A violet ring indicated the presence of carbohydrates.

Test for saponins

Foam test: The extract (50 mg) is diluted with distilled water and made up to 20 mL. The suspension was shaken for 15 minutes. A two-cm layer of foam indicated the presence of saponins.

Test for phenolic compounds and tannins

Ferric chloride test: The extract (50 mg) was dissolved in 5 mL of distilled water. To this solution, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenolic compounds.

Test for mucilage

The extract (100 mg) was dissolved in 10 mL of distilled water, and to this solution, 2 mL of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of mucilage.

Test for reducing sugars

Benedict's test: Filtrates were treated with Benedict's reagent and heated gently. Orange-red precipitate indicated the presence of reducing sugars.

Fehling's test: Filtrates were hydrolyzed with diluted HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Test for triterpenes

Salkowski's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. The appearance of golden yellow color indicated the presence of triterpenes.

Liebermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulfuric acid was added.

A positive test was due to a rapid change of coloration: pink-blue: very fast; intense green: visible but fast.

Test for flavonoids

Assay with concentrated sulfuric acid: An aliquot (1 mL) of the extract was concentrated to dryness in test tubes, and a few drops of concentrated H_2SO_4 were added. A color other than light brown indicates a positive test result.

Rosenheim's test: It allows to recognize in an extract the presence of leucoanthocyanidins and anthocyanidins. An aliquot (1 mL) of the extract in ethanol is heated 10 min with 1 mL of concentrated HCl. Allow to cool and add 1 mL of water and stir with 2 mL of amyl alcohol. The two phases are allowed to separate. The appearance of red to brown in the amylic phase is indicative of a positive test.

Colonies of Diptera

Adults of *M. domestica* were collected from the garbage located in an area of locality of Castillito, (Santiago de Cuba, Cuba) by using an entomologic network and transported into a small cage to Department of Pharmacy of the Universidad de Oriente, following the methodology of Queiroz and Milward-de-Azevedo (1991). The colonies were kept in cages at room temperature and were fed on water and sugar *ad libitum*. The ovarioles and the oviposition were stimulated placing rotten poultry inside the cages. In the experiment was used the second generation of larvae.

Bioassay to measure insecticidal effect

Bioassay for larvicidal effect

The extracts were applied on 30 individuals of the newborn larvae grouped in a Petri dish. An aliquot of 30 µL of each extracts concentration from *P. americana* was applied topically. Each concentration was assayed on 30 larval bodies at a final concentration about 1 µL/larva using an automatic pipette. Bioassays were performed in triplicate.

Two controls were used. A control group, only containing flies without any extract, was used to compare with the flies treated with extracts. A control group was also used with the solvent (distilled water) used to prepare the dilutions. After application, the larvae were transferred to a vessel containing rotten poultry (50 g). These containers (50 mL) were then placed in larger containers (500 mL) containing sand for pupation and were covered with a nylon cloth fastened with rubber bands.

The experiment was performed at room temperature and humidity, with light-dark cycles of 12 hours. The larvae spontaneously abandon their diet on reaching maturity and then they were collected. These larvae were weighed individually and transferred to glass tubes containing sand and sealed with cotton thread. Adults were separated by gender, using as a criterion for classification the distance between the eyes of the insect, which basically identify female individuals those having a wider distance between eyes than male ones (Dübendorfer et al. 2002).

During the experiment, the duration of each stage of the larval cycle (larval, pupal and newly-hatched larvae to adult) was analyzed. The influence of extracts on the sex of fly was determined by the calculation of the sexual ratio (SR). Finally, the viability of the insect was determined for each treatment.

$$SR = \frac{F}{F + M}$$

Where F: Female and M: Male

Bioassay for adulticidal effect

To evaluate the insecticidal effect of the extract on the adult fly, groups of 10 flies were placed in a 6-cm diameter, 13 cm high and 30 mL capacity glass flasks. A 12-cm cotton thread, which was fixed to the top of each flask, hung downwards, reaching the bottom of the flask. Once the flies had been introduced into each flask, 100 µL of the extract S at 0.076 µg/µL and SE at 0.068 µg/µL were applied to the cotton thread using a micropipette. The flasks were then immediately closed and observed for 2 h. A flask without the extract was used as a control. There were three replicates of each treatment and a control. The number of dead flies was determined

after two hours for each concentration, and the flies that experienced knock-down effect (muscle paralysis) for more than 5 minutes were quantified. The adulticidal effect of the extracts against adult individuals of *M. domestica* was evaluated by determining the LC_{50} (lethal concentration for 50% of the sample values declared as the concentration of the extract, present in cotton, which kills 50% of the fly population) at room temperature.

Statistical analysis

Results were analyzed by ANOVA ($p \leq 0.05$). Statistical analysis was performed through the multiple range contrast by the method of Fisher's least significant difference (LSD) at 95% confidence level. This method establishes comparisons between the means of the groups defining the difference between them. For calculations of the lethal concentration for 50% (LC_{50}) in the adulticidal assay, a Probit correlation was used by calculating the Pearson correlation factor. SPSS version 18.1 for Windows was used for the statistical analyses. A significance level of 95% was used.

RESULTS AND DISCUSSION

Chemical characterization of *Persea americana* seed extracts

No appreciable differences were observed in the qualitative chemical composition between the two extracts, showing that both methods extracted most of the metabolites present in the *P. americana* seeds. Saponins were the only group of metabolites that were not detected in the extract obtained by the SE method. These compounds are generally found in the form of glycosides (polar portion) and are thermostable. However, during the SE process, due to the thermal conditions, the glucosides may have been hydrolyzed, thus losing the surfactant properties, which are the basis of the test used for the determination of these metabolites (Table 1).

Among the main metabolites detected in both extract were alkaloids, terpenes, quinones, coumarins, phenols, tannins, amino acids, and flavonoids. The qualitative chemical composition of both extracts agrees with those reported in the literature for this plant species (Torres et al., 2001; Mendoça et al., 2011).

Table 1. Results of the phytochemical screening of *Persian americana* Mill hydroalcoholic extract.

Metabolites	Test	S	SE
Alkaloids	Mayer' s test	+++	+++
	Wagner' s test	+++	+++
Triterpenes	Lieberman-Burchard' s test	-	-
	Solkowski' s test	+	+
Saponins	Foam test	+	-
Mucilage	Mucilage' s test	-	-
Reducing sugars	Fehling' s test	+	+
	Benedict' test	+	+
Phenolic compounds and tannins	Ferric chloride test	+	+
Amino acids	Ninhydrin test	+	+
Flavonoids	Assay with concentrated sulfuric acid	+	+
	Rosenheim' s test	+	+
Carbohydrates	Molish' s test	-	-

(+) Positive evidence; (+++) Positive evidence; (-) Negative evidence; SE: Soxhlet extraction; S: Shaker method.

Effects of *Persea americana* seed extracts on the *Musca domestica* sex ratio

The effect of S and SE extracts on sex ratio indicates a predominance of male individuals, this phenomenon is known as arrenótoca or arrenogenic; and according to Ullerich (1963), male individuals cannot influence the sex of their offspring. Other investigations do not show alterations in the sexual reason of the offspring, after applying the vegetal extracts (Pinto et al., 2015, Dutok, 2015), showing a very close relation to 1:1 between the number of female individuals in relation to the number of male individuals. The result of this investigation is favorable because the predominance of male individuals, which would result in an increase of competition among male individuals by mating. As female individuals are responsible for reproduction, when they are diminished in number, this can negatively influence in the number of eggs that will give way to the offspring (Table 2).

Effects of *Persea americana* seed extracts on the life cycle periods of *Musca domestica*

Seed extracts S and SE showed little influence on the duration of the larval period in relation to the control groups. Only significant differences were observed in three treatments when compared to controls (SE 0.136 mg/100 mL, SE 1.7 mg/100 mL and S 0.153 mg/100 mL (Fig. 1). In general, there was a tendency to prolong the larval period. This increase in the larval period, due to the application of the seed extracts, lead to an increase in the time the larvae were exposed to specific pests (Fig. 1). It also increased the duration of each generation. Moreover, as the period exposed to the environment is longer, they are more susceptible to predators, parasitoids, and pathogens, which naturally help reduce the population of this species (Cabral et al., 2007).

Other reports in the scientific literature have shown similar behavior. Mendonça et al. (2011) reported that *Chrysomya megacephala* larvae treated

Table 2. Effects of the hydroalcoholic extract of *Persea americana* Mill on the sexual ratio of *Musca domestica*.

Treatments	Concentration (mg/100 mL)	F	M	SR
Control group		34	10	0.77
Solvent control		20	19	0.51
S	0.153	18	21	0.46
	0.383	15	9	0.63
	1.918	9	18	0.33
	3.835	14	17	0.45
	7.670	9	9	0.5
SE	0.136	21	35	0.38
	0.340	16	19	0.46
	1.700	15	21	0.41
	3.400	15	21	0.41
	6.800	14	14	0.5

SR: Sexual ratio (determined as $SR = \frac{F}{(F+M)}$); F: Female; M: Male; SE: Soxhlet extraction; S: Shaker method; C: Control (this control group only contains the flies without applying the plant extract); Cs: Solvent control (in this control the solvent used to make the dilutions was applied on the larvae, in this case distilled water).

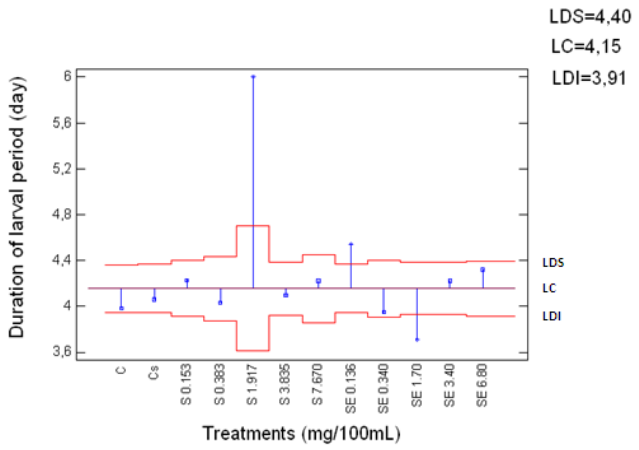

Figure 1. Duration (day) of larval development period of *Musca domestica* (Diptera: Muscidae) topically treated with different concentrations of seed extracts from *Persea americana* Mill.

Duration of the larval period (days) under the influence of the applied treatments of Soxhlet extracts (SE) and Shaker extract (S). Statistically significant differences were found between the means of the 10 treatments and the controls used, with 95% confidence level ($F_{11, 468}$ = 14.23, p <0.001). The multiple range test showed that there are significant differences in the length of larval period between flies from control group (4 days) and those treated with SE 0.136 mg/100 mL (4.5 days), SE 1.7 mg/100 mL (3.7 days) and S method 1.917 mg/100 mL (6 days).

SE: Soxhlet extraction; S: Shaker method; C: Control (this control group only contains the flies without applying the plant extract); Cs: Solvent control (in this control the solvent used to make the dilutions was applied on the larvae, in this case distilled water); LSD: Upper confidence limit; LC: Central limit; LDI: Lower confidence limit.

with 3.0% *Parahancornia amapa* latex showed an increase in the larval period when compared to the control group (4.8 and 4.3 days, respectively). Other researchers have obtained a similar result, which indicates the potential of this type of treatment to stimulate a (natural) reduction of this species (Cabral et al., 2007).

There was also a tendency to prolong the pupal stage (Fig. 2) in the groups treated with the extracts, with statistically significant differences being observed for SE 0.136 mg/100 mL, S 0.153 mg/100 mL, S 1.918 mg/100 mL and SE 6.80 mg/100 mL, compared to the other groups and the control groups. The elongation of the pupal stage is favorable for the insecticidal activity because in this stage the flies are static and are more susceptible to changes in temperature, humidity, predators, parasitoids, and pathogens. This behavior agrees with those reported by (Cabral et al., 2007) where the effect of the *Melia azedarach* extract on *M. domestica* was associated with a slow development in the pupal stage.

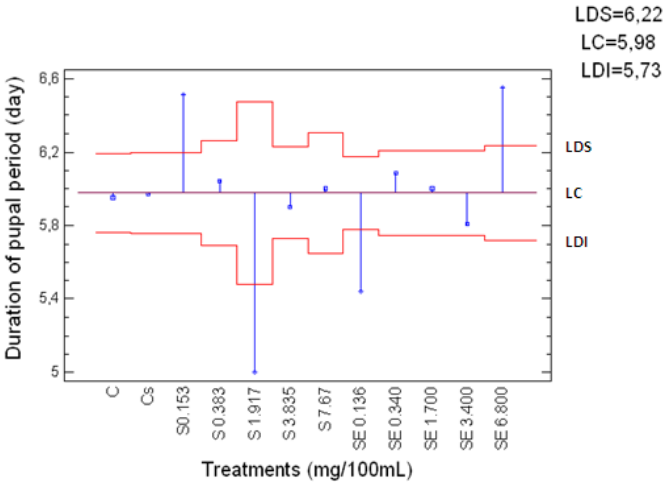

Figure 2. Duration (day) of pupal development period of *Musca domestica* (Diptera: Muscidae) topically treated with different concentrations of seed extracts from *Persea americana* Mill.

Duration of the pupal period (days), there were statistically significant differences ($F_{11, 368}$ = 15.32, p = <0.001) between control group (C), control with solvent (Cs) and SE 6.80mg/100 mL (6.6 days) and S method 0.153 mg/100 mL (6.5 days), in which there is an increase in the duration of the pupal period and, in contrast to this behavior, the concentrations of S 1.917 mg/100 mL (5 days) and SE 0.136 mg/100 mL (5.4 days) evidenced a decrease in the pupal period, with statistically significant differences when compared with the control.

SE: Soxhlet extraction; S: Shaker method; C: Control (this control group only contains the flies without applying the plant extract); Cs: Solvent control (in this control the solvent used to make the dilutions was applied on the larvae, in this case distilled water); LSD: Upper confidence limit; LC: Central limit; LDI: Lower confidence limit.

Finally, in the newly hatched larvae to the adult stage, the tendency of the two extracts to increase the duration was maintained, with significant differences between the control group and some of the treatments (S 0.153 mg/100 mL, S 1.918 mg/100 mL), increasing the duration of this stage by one day (Fig. 3). Similar results have been reported for extracts from other plant species. For example, Begum et al. (2010) reported that for groups of *M. domestica* treated with ethanolic extracts of *Calotropis procera* and *Annona squamosal,* adult emergence was delayed 10-11 days. Phytochemical analysis of the two extracts revealed the presence of alkaloids and phenols in the extract of *C. procera* leaves. In the *A. squamosa* extract these two latter groups, as well as flavonoids, were detected. Reports in the literature show that the partially purified flavonoids from the aqueous extract of *A. squamosa* leaves have antimi-

crobial and insecticidal activity. Similarly, the alkaloids reported for *C. procera* latex have been shown to contain insecticidal properties. These groups of metabolites were also identified in *P. americana* Mill seed extracts. Hence the activity observed may be related to the presence of these metabolites (Begum et al., 2010).

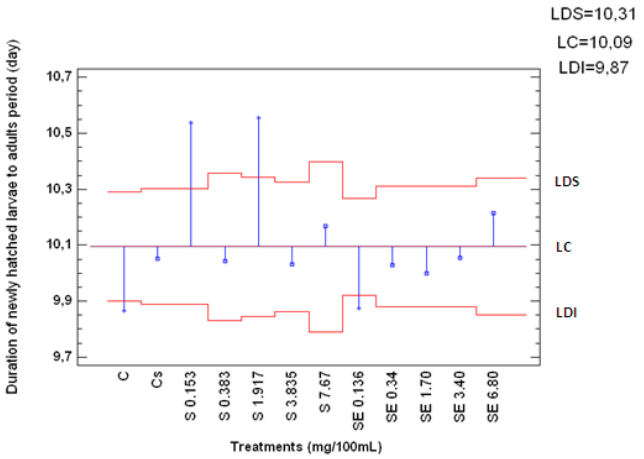

Figure 3. Duration (day) of newly hatched larvae to adult's development period of *Musca domestica* (Diptera: Muscidae) topically treated with different concentrations of seed extracts from *Persea americana* Mill.

Duration (day) of newly hatched larvae to adult's development period of *Musca domestica* ($F_{11, 403} = 8.08$ $p < 0.001$) shows differences between the averages of the S 0.153 mg/100 mL (10.5 days), 1.917 mg/100 mL (10.6 days) and the rest of the treatments, including the control group (9.9 days).

SE: Soxhlet extraction; S: Shaker method; C: Control (this control group only contains the flies without applying the plant extract); Cs: Solvent control (in this control the solvent used to make the dilutions was applied on the larvae, in this case distilled water); LDS: Upper confidence limit; LC: Central limit; LDI: Lower confidence limit.

Cabral et al. (2007) suggested that compounds extracted from plants and tested to control insects could modify specific physiological processes such as the endocrine control of insect growth, the neuroendocrine system or the production of some hormones.

In general, for the three stages (larval, pupal and newly hatched larvae to adults) of the life cycle of *M. domestica* both extracts (S and SE) showed a tendency to extend these periods, which may be related to the fact that the two extracts have very similar chemical compositions. The alteration in the duration of the stages contributes indirectly to the natural reduction of adult individuals of the species *M. domestica*.

Effects of *Persea americana* seed extracts on the viability of *Musca domestica*

P. americana seed extracts affected the larval viability of the species of *M. domestica*, with favorable results being observed since, in eight of the ten treatments applied, the viability percentage reached values lower than 50%. These results show that both extracts directly affect the post-embryonic development, which leads to a decrease in the population of this Diptera.

However, the results for pupal viability for each treatment were very similar to the controls. These results suggest that all larvae that arrived at the third instar stage and abandon the diet became pupae. Also, those larvae that reach the third instar are probably more resistant and able to continue their life cycle. Finally, a similar behavior to larval viability was observed in the newly hatched larvae to adults, since the viability values were below 50%, in most of the treatments (Table 3).

These results indicate the strong influence of the extracts prepared from the *P. americana* seeds on the viability in the life cycle of *M. domestica*. However, the S extract showed a stronger effect, since independently of the fact that both extracts presented a similar qualitative chemical composition, the concentration at which these metabolites can be extracted may be different according to the extraction method.

The observed activity is probably related to the presence of metabolites in the extracts with potential insecticide activity. In a study of *Anopheles gambiae*, the larvicidal effect of a *P. americana* seed extract was associated with the presence of several compounds such as fatty acids, flavonoids, triterpenes, anthocyanins and abscisic acid (Adesina et al., 2016), many of which were identified in the S and SE extracts.

Similar results have been reported for extracts from other plant species. An ethanolic extract of *Azadirachta indica* (neem) containing 0.24% azadirachtin was evaluated on *M. domestica* and *C. megacephala*. The tests showed a significant dose-dependent reduction in the larval and pupal viabil-

ity of adult birth. The species *M. domestica* was the most susceptible. Moreover, the female adult individuals of this species that survived to adulthood did not produce eggs after being in contact with neem extract at concentrations equal to or greater than 0.1 and 0.2% (Siriwattanarungsee et al., 2008).

Many authors argue that the effect of plant extracts on viability is the result of some secondary metabolites present in these extracts (Adesina, 2016) showing that the high mortality of insects, when avocado seed extracts were applied, is related to the mixture of chemical constituents present in the seed. These compounds inhibit the developmental stages of insects by interacting with the cuticle.

Metabolites such as lectin obtained from *Ricinus communis*, with insecticidal effect against populations of *M. domestica*, owe their effect to biochemical mechanisms based on their inhibitory action of protein synthesis (Álvarez et al., 1996).

Effect of *Persea americana* seed extracts on adult flies

The results of the adulticidal activity showed that both extracts were effective against *M. domestica* (Fig. 4A-B), in the control group the death of any fly inside the bottle is not observed. The test showed that the dead flies had previous contact with the cotton thread worsted yarn containing the different concentrations of the extracts, indicating that the highest toxicity of the extracts was by contact and not by inhalation. The above results demonstrate that *P. americana* extracts can be classified as a contact insecticidal poison. The mean values of inhibitory concentration (LC_{50}) showed that the S extract (LC_{50} = 2.91 mg/100 mL) was more potent than the SE extract (LC_{50} = 3.944 mg/100 mL), which agrees with results observed in the viability test.

After 2 h of exposure to the extract, the flies that remained alive inside the flask lost the ability to fly leaving them practically immobile, mainly at higher concentrations than 3.50 mg/100 mL. On the other hand, flies inside the bottle in the control sample continued flying after the planned test time (2 h), eliminating the idea that oxygen depletion in the bottle was the cause.

This result could be related to a neuroinhibition process (Rattan, 2010), which is one of the most common effects of insecticides and generally causes immobility and paralysis due to the possible deprivation of oxygen and that ultimately leads to death.

Table 3. Viability of the different periods of *M. domestica* after a topical administration of *P. americana* Mill. seed extracts.

Treatments	Concentration (mg/100 mL)	Larval period (%)	Pupal period (%)	Newly hatched larvae to adult periods (%)
Control group		93.3	100.0	73.3
Solvent control		88.3	100.0	65.0
S	0.153	44.4	97.5	43.3
	0.383	50.0	96.7	40.0
	1.918	50.0	96.7	45.0
	3.835	47.8	100.0	34.4
	7.670	30.0	100.0	20.0
SE	0.136	65.6	98.3	62.2
	0.340	65.0	100.0	60.0
	1.700	50.0	97.8	40.0
	3.400	54.4	100.0	46.7
	6.800	46.1	92.7	31.5

SE: Soxhlet extraction; S: Shaker method; C: Control (this control group only contains the flies without applying the plant extract);
Cs: Solvent control (in this control the solvent used to make the dilutions was applied on the larvae, in this case distilled water).

Vergara et al. (1997) indicated that the combined effects of the compounds in fruits are more potent than the effects individually of such components. This event infers that the total extract of a part of the whole plant is more suitable than isolating the main active ingredient, since, in the presence of a group of compounds, it might not only increase the mortality of the insects but would decrease the probability that they develop resistance to a mixture of active ingredients. Isman (1997) pointed out that it is more difficult for insects to react to a complex of substances than to a single molecule. The mixture of secondary metabolites may deter insects and herbivores for a more extended period than the single compound, and different physical properties prevent or harass the development of the resistance mechanisms.

Mechanisms of action have been described for some of the commonly used synthetic and botanical insecticides to explain how they exert such effect. Among them, the inhibition of the acetylcholinesterase enzyme (AChE) plays a role in the cholinergic synapses, which is essential for insects and higher animals. Inhibition of AChE causes accumulation of the neurotransmitter acetylcholine in the synaptic spaces; thus, the post-synaptic membrane is in a state of permanent stimulation, resulting in a general lack of coordination in the neuromuscular system, and thereby causing death (Rattan, 2010).

The ability of aqueous extracts obtained from *P. americana* seeds and leaves to inhibit AchE has been studied (Oboh et al., 2016). According to the reviewed literature, the inhibition of AChE by *P. americana* extracts could be attributed to its phenolic contents, as these have been shown to inhibit AChE activity (Oboh et al., 2012). In addition, alkaloids are potent cholinergic enzyme inhibitors; therefore, their presence in the extracts could be related to a dose-dependent inhibition of AChE. It was reported that an aqueous extract of avocado seeds has a higher alkaloid content concerning the leaves. Therefore, the highest inhibitory effect was observed in the seed extracts (Arukwe et al., 2012).

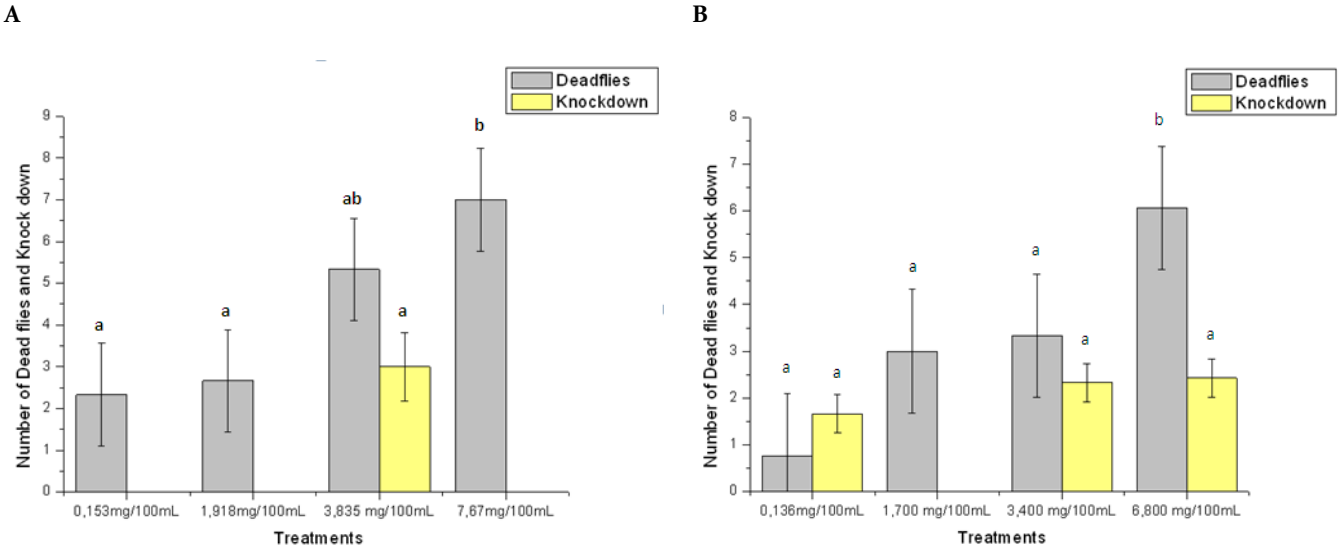

Figure 4. Adulticide effects of the extract of by Shaker method **(A)** and Soxhlet **(B)** of *Persea americana* Mill. in *Musca domestica*.

The results are presented as mean ± SEM (n=30). Values with different superscripts are statistically significant at $p \leq 0.05$ using ANOVA and the multiple range contrast by the method of Fisher's least significant difference (LSD) at 95% confidence level. SE: Soxhlet extraction; S: Shaker method; Dead flies (Number of dead flies after two hours); Knock down (Flies that experienced muscle paralysis for more than 5 minutes).

Based on these observations, the adulticidal effect observed for the seed extracts on the species *M. domestica* in this work is probably associated with a mechanism of AChE inhibition, since the phytochemical analysis of the extract indicates the presence of phenolic compounds (flavonoids and tannins) and alkaloids, to which this activity is attributed.

A comprehensive analysis of the results shows that the two extracts of *P. amerinaca* seeds are candidates for the development of a natural insecticide; however, the S extract was shown to be the most effective, more potent and more viable for the alternative control of the species *M. domestica*.

CONCLUSIONS

The hydroalcoholic extracts S and SE of the seeds of *P. americana* did not show significant differences in the qualitative chemical composition, revealing the presence of alkaloids, phenols, tannins, flavonoids, sugars and amino acids in both extracts. It was demonstrated a greater influence of the extracts on the viability of larvae and in the neolarva period to adults, but also on the post-embryonic development of *M. domestica*, to a lesser extent. The hydroalcoholic extracts S and SE of *P. americana* Mill have adulticide effect on *M. domestica* obtaining LC_{50} values of 2.910 mg/100 mL and 3.944 mg/100 mL, respectively.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Concepts or ideas	X	X			X		
Design	X	X	X	X	X	X	X
Definition of intellectual content					X		
Literature search	X					X	
Experimental studies	X	X					
Data acquisition	X	X					
Data analysis	X	X	X	X	X	X	X
Statistical analysis	X				X		
Manuscript preparation	X					X	X
Manuscript editing	X	X	X	X	X	X	X
Manuscript review	X	X	X	X	X	X	X

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