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Chemical characterization and mosquito larvicidal activity of essential oil from leaves of *Persea americana* Mill (Lauraceae) against *Culex quinquefasciatus* (Say)

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ABSTRACT

Objective: To determine chemical composition and mosquito larvicidal activity of essential oil from dried leaves of *Persea americana* against *Culex quinquefasciatus*.
Methods: About 20 larvae in each group were used for larvicidal assays. The mortality, relative growth index, larval and pupal duration and viability were estimated. The essential oil was analyzed by solid phase microextraction using gas chromatography coupled to mass spectrometry.
Results: The most abundant compounds were estragole (61.86%), sabinene (15.16%) and 1R-α-pinene (14.25%). The essential oil inhibited mosquito larvae growth up to 26.73% [relative growth index (RGI) = 0.74)] and 16.83% (RGI = 0.84) at 800 and 50 mg/L respectively, while the untreated control and group treated with polysorbate 20 showed the RGI of 1.01. The viability of larvae to pupae decreased 53.75% when used 800 mg/L essential oil causing prolongation of development of 14.14 days, while the control had durability in its development of 12 days. In the pupae-adult phase, when used 800 and 50 mg/L of essential oil, 22.36% and 21.81% adults were formed, and there was prolongation of 15.88 days and delay of 13.62 days respectively; however the control showed duration of 14.63 days. Mortality at the end of the experiment was recorded as 57.50% with treatment of 800 mg/L and gradually decreased to 40% with treatment of 50 mg/L essential oil.
Conclusions: The study demonstrated that the larvicidal activity of essential oil of Mexican avocado inhibited the normal growth and development of mosquito larvae, prolonged larval and pupal duration.

1. Introduction

Persea americana Mill (Lauraceae) (*P. americana*) known as Mexican avocado and "Aguacatillo" is native to Mexico and Central America[1], and is found in most tropical and subtropical countries[2]. The Lauraceae family includes 50 genera and about 2 200 species[3]. The bark, fruit and leaf are used in traditional medicine in South and Central America for the treatment of various ailments. Researchers have focused on various parts of the plants, and have demonstrated that leaf extract has antidiabetic activity[4], hypoglycaemic effect[5], antispastic effect[6], and analgesic and antiinflammatory properties[7]. People have used various plant parts, products and secondary metabolites of plant origin in pest control since early historical times; in the last decades, there has been a significant increase in the use of natural products focused on their potential applications in agriculture

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and mosquito borne-disease control. Insect-transmitted diseases remain a major cause of illness and death worldwide[8], but their control has become complicated because of their resistance to synthetic insecticides, as well as the toxicity on non-target organisms[9].

Plant essential oils have been suggested as alternative sources for insect control, because some are selective and biodegrade to nontoxic products[10,11]. Various essential oils obtained from different parts of the variety of plants have also been documented to exhibit mosquito larvicidal potential[12-14]. Some members of Lauraceae family are important sources of bioactive substances with properties that act as insecticides[15,16]. Biological activity studies performed with ethanolic extracts and compounds isolated from species of this family have shown the potential insecticide against mosquitoes[17].

Culex quinquefasciatus (Say) (Diptera: Culicidae) (*Cx. quinquefasciatus*) is a member of the *Culex pipiens* complex and one of the most widespread mosquitoes in the world found in tropical and subtropical countries, and insecticide resistant populations have been reported worldwide. Mosquitoes can be vectors of various human diseases. *Cx. quinquefasciatus* is a vector of lymphatic filariasis commonly known as elephantiasis regarded as the second most common global mosquito-borne infectious disease, which is a neglected tropical disease[18]. For that reason, the present study investigated the mosquito larvicidal effect of the essential oil from dried leaves of *P. americana* on *Cx. quinquefasciatus* and determine the chemical composition of the essential oil.

2. Materials and methods

2.1. Mosquito larvae population

The larvae of *Cx. quinquefasciatus* were obtained from the insectarium located at the Center for Interdisciplinary Research on Regional Development, National Polytechnic Institute, Oaxaca, Mexico. In the laboratory, *Cx. quinquefasciatus* produced numerous mosquito larvae. Here, a colony of mosquitoes are nourished by a live chicken every third day.

2.2. Preparation of plant material

Fresh leaves of *P. americana* were collected from Santa María Huitepec, Oaxaca, Mexico. The plant was selected according to its aromatic properties, availability and frequency of use among the inhabitants. Taxonomic identification was made by the curator of the herbarium of Center for Interdisciplinary Research on Regional Development, National Polytechnic Institute, Oaxaca, Mexico and a sample copy was deposited in their research laboratory for future reference. The plant was washed with tap water, and then placed on sheets of newspaper into a solar dryer on the roof of the laboratory.

2.3. Extraction of essential oil

The dried material was powdered and rehydrated for 45 min before the extraction. Essential oil was obtained by subjecting 300 g of powdered leaves to conventional hydrodistillation for 3 h using Clevenger-type apparatus. The essential oil layer was separated from the aqueous phase with a separatory funnel and the resulting essential oil was dried using anhydrous Na_2SO_4 and preserved in an amber colored bottle at 4 ℃ until used.

2.4. Gas chromatography-mass spectrometer (GC-MS) and identification of volatile compounds

Solid phase microextraction was used to identify volatile compounds. Fibers coated with polydimethylsiloxane of 100 μm were used. The fibers were conditioned before use by heating them in the chromatographic injector port performing a complete run on the system. For contacting the volatiles with the fiber, 5 mL amber colored sterilized jars were used. About 4 μL of essential oil were deposited into the jars and kept inside the fiber for 4 min. The analysis was performed on a HP 6890-5973 GC-MS system. The compounds were separated on a capillary column HP-5MS. The oven temperature was programmed at 40-250 ℃ with increasing intervals of 10 ℃/min with a full run of 21 min. The relative percentages of the essential oil compounds were obtained using helium as a carrier gas with a flow rate of 1 mL/min. The compounds were identified by GC retention time and mass spectra library National Institute of Standards and Technology (NIST) 02; also, a comparison of the spectra with those stored in the library NIST 14 was made.

2.5. Preparation of the essential oil concentrations

From the stock solution 0.008 mL of essential oils were diluted in 10 mL of distilled water with 0.01% polysorbate 20, which served as an emulsifier to the solution. Subsequently, volumetric dilution series concentrations were prepared and used for a bioassay on early second instar mosquito larvae. The concentrations used in the bioassay were 800, 400, 200, 100 and 50 mg/L.

2.6. Larvicidal activity and growth inhibition bioassays

The partial and total mortality of early second instar larvae of *Cx. quinquefasciatus* was determined at the moment to obtain the relative growth index (RGI) and at the end of the experiment respectively. For the establishment of bioassays, groups of 20 larvae were selected and placed in a plastic beaker with 99 mL of distilled water. The larvae were considered to be dead when the larvae had no normal movements compared to the control, and when the larvae were disturbed with a brush on the siphon in the cervical region and didn't show any reaction. Each experimental group received 1 mL of the concentrations used with four replications. Two controls were used, one treated with 0.01% polysorbate 20 and another without application of treatment (distilled water only). When the control treatments presented 90%-93% of formed pupae, dead and alive pupae were counted in each stage and the number of adults emerged. Adults trapped in the pupal exuviae were considered dead, and the larvae and pupae were considered dead if they did not show normal movements when disturbed with a dissecting needle[19]. With the information gathered, a growth inhibition index (GII) was estimated according to the following formula[20]:

$$GII = \frac{\sum_1^4 (\text{No. alive insects} \times \text{insect phase}) + \sum_1^4 [\text{No. dead insects} \times (\text{insect phase-1})]}{\text{No. total evaluated insects} \times \text{total insects plases}}$$

Where 1, 2, 3 and 4 represent the insect phase of 2nd, 3rd, 4th instar and pupae formed respectevily.

About 80 insects were used for each concentration and the total number of stages of the insect were four (three larval and pupae). The RGI was determined by the formula: RGI = GII treatment / GII control without application.

2.7. Larval and pupal viability and duration

The larval and pupal duration was obtained by multiplying the percentage of pupae and adults developed by the number of days in that each mosquito developed; these values are summed and divided by the total percentage of pupae and adults developed. The larval and pupal viability was estimated by counting the number of mosquito that became pupae and adult and expressed in percentage according to the initial number of mosquitoes treated.

2.8. Statistical analysis

In all tests, no mortality of control group was detected after exposure, so no correction was required based on Abbott's formula. No calculation of lethal concentrations was requeried because essential oil did not reach more than 90% of biological effectiveness on mosquito larvae. The RGI, larval and pupal duration results were expressed as mean ± SE (n = 4). ANOVA followed by Duncan's test was utilized to analyze the significant difference among the tested essential oils against the mosquito larvae. A P value of 0.01 was considered significant. Analysis of the data was performed using SAS 9.0 software.

3. Results

3.1. Identification of volatile compounds

The microwave-assisted hydrodistillation of 300 g of powdered dried leaves of *P. americana* in 1 000 mL of water, showed a transparent oil with 0.24% yield. Volatile compounds are shown in Table 1. By comparing the mass spectra of each compound with the data reported in the library NIST 14, 11 compounds were identified representing 99.99%. The most abundant compounds were estragole (61.86%), sabinene (15.16%) and 1R-α-pinene (14.25%).

Table 1

Chemical composition of volatile extracted from dried leaves of *P. americana*.

Compounds	Retention time (min)	Composition (%)
1R-α-pinene	6.05	14.25
Camphene	6.24	0.66
Sabinene	6.78	15.16
β-Pinene	6.85	2.10
Eucalyptol	7.55	3.08
1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	7.74	0.37
Linalool	8.63	1.96
Estragole	10.32	61.86
Anethole	11.51	0.15
Methyleugenol	12.99	0.24
Caryophyllene	13.34	0.16
Total (%)		99.99

3.2. Larvicidal activity and growth inhibition

Table 2 shows the results of susceptibility of second instar larvae of *Cx. quinquefasciatus* to the essential oil of *P. americana*; over 50% mortality of mosquito larvae and pupae were recorded in the group treated with 800 mg/L of the essential oil, presenting a proportional decrease in biological effectiveness with the decrease in the essential oil concentrations.

The essential oil inhibited the larvae growth up to 26.73% (RGI = 0.74) and 16.83% (RGI = 0.84) at 800 and 50 mg/L respectively, while the untreated control and polysorbate 20 group recorded RGI of 1.01 respectively.

Table 2

Number and partial percentage of larvae and pupae dead and RGI of second instar larvae of *Cx. quinquefasciatus* treated with essential oil of *P. americana*.

Concentration (mg/L)	No. of larvae (4 replicates)	No. of larvae and pupae by instar				Partial mortality (%)	RGI
		II	III	IV	Pupae		
800	80	0	1	40	3	55.00 ± 12.24^{a}	0.74 ± 0.07^{c}
400	80	0	0	34	3	46.25 ± 12.50^{ab}	0.78 ± 0.07^{bc}
200	80	0	0	34	1	43.75 ± 4.78^{ab}	0.81 ± 0.01^{bc}
100	80	0	0	31	4	43.75 ± 6.29^{ab}	0.80 ± 0.03^{bc}
50	80	0	0	23	5	35.00 ± 8.16^{b}	0.84 ± 0.05^{b}
Control	80	0	0	0	0	0.00 ± 0.00^{c}	1.01 ± 0.02^{a}
Polysorbate 20	80	0	0	0	0	0.00 ± 0.00^{c}	1.01 ± 0.02^{a}

$^{a, b, c}$: Statistically significant at $P < 0.01$ (ANOVA followed by Duncan's test).

3.3. Larval and pupal viability and durability

Table 3 shows the viability of second instar larvae of *Cx. quinquefasciatus* treated with essential oil from dried leaves of *P. americana*; the viability of larvae to pupae decreased by 53.75% when used 800 mg/L of essential oil causing prolongation of development in 14.14 days, while the control had a durability in its development of 12 days. In the pupae-adult phase, when treated with 800 and 50 mg/L of essential oil, 22.36% and 21.81% adults were formed respectivly, and there was a prolongation of 15.88 days and 13.62 days in development, while the control recorded duration of 14.63 days. Mortality at the end of the experiment was 57.50% with 800 mg/L treatment, gradually decreased to 40% with treatment of 50 mg/L essential oil.

Table 3

Duration of larval and pupal phase and total mortality of larvae of *Cx. quinquefasciatus* treated with essential oil of *P. americana*.

Concentration (mg/L)	Larval (larvae to pupae)		Pupal (pupae to adult)		Mortality (%)
	Formed (%)	Duration (day)	Formed (%)	Duration (day)	
800	46.25^{c}	14.14 ± 0.60^{a}	22.36^{a}	15.88 ± 0.91^{a}	57.50^{a}
400	55.00^{bc}	13.33 ± 1.87^{ab}	20.19^{a}	15.08 ± 1.77^{ab}	47.50^{ab}
200	57.50^{bc}	12.79 ± 0.56^{ab}	24.45^{a}	14.14 ± 0.88^{b}	43.75^{ab}
100	61.25^{b}	12.44 ± 0.72^{b}	22.95^{a}	14.42 ± 0.90^{ab}	43.75^{ab}
50	68.75^{b}	13.04 ± 0.31^{ab}	21.81^{a}	13.62 ± 0.19^{b}	40.00^{b}
Control	100.00^{a}	12.47 ± 1.18^{b}	25.00^{a}	14.63 ± 1.05^{ab}	0.00^{c}
Polysorbate 20	100.00^{a}	12.53 ± 0.99^{b}	25.00^{a}	14.45 ± 0.71^{ab}	0.00^{c}

$^{a, b, c}$: Statistically significant at $P < 0.01$ (ANOVA followed by Duncan's test).

4. Discussion

Generally, the studies on effectiveness of natural products for mosquito control were quantified at the effect on mortality

of mosquito larvae in a period of three to five days, however it should be considered that mode of action of botanical products can provide alternatives for management in stages of development of insect. In this study it was observed that in the phase of larvae-pupae the mosquito prolongs its development in 1.67 days with the concentration of 800 mg/L, reducing the mosquito population in 57.50%. These results are similar to those obtained by Cabral *et al*., who evaluated the effect of a neolignans (yangambin) isolated from various Lauraceae species on the development of larval and pupal stage of the blowfly *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae), and obtained results of 6.07 and 5.48 days respectively, showing a significant difference ($P < 0.001$) when compared with control group that showed 5.51 and 4.39 days respectively[21]. Additionally, Regnault-Roger and Hamraoui used aromatic essential oils from *Cinnamomum verum* (Lauraceae) to control the biological development of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) larvae[22]. The toxicity of essential oils to stored-product insects is influenced by the chemical composition of the oil, which in turn depends on the source, season and ecological conditions, method of extraction, time of extraction and plant part used[23]. The degree of effectiveness of a natural product is considerable, because each investigator raises different objectives in each study, but it is important to mention that with the reduction of more than 50% of the population of an organism, the natural product demonstrated the potential of a treatment evaluated and serves as a reference for future research. Lagunes mentioned that 1 600 species of plants studied in Mexico belonging to 159 botanical families have toxic effect on 112 species of arthropods and are promising plants that cause mortality equal to or greater than 40%[24].

The results regarding the evaluation of the chemical composition can be various. The work done by Ogunbinu *et al*., who investigated the composition of the essential oils of *P. americana* from Nigeria[25], determined β-caryophyllene (43.9%) and valencene (16%) as the most abundant compounds, and Pino *et al*. determined estragole (53.9%) as the major component in the oil of *P. americana* var. *drymifolia* cv. Duke from Cuba[26].

Larijani *et al*. investigated the component in leaves of *P. americana* cultivated in Iran, and the most abundant compounds were methyl eugenol (31.2%), β-caryophyllene (16.9%) and estragole (methyl chavicol) (9%)[27]. These results are similar to that of our work in the determination of compounds but not in percentage because in our study estragole (61.86%) is the most abundant compound and methyl eugenol (0.24%) is the least.

Sagrero-Nieves and Bartley investigated the chemical composition of the volatile oil of avocado leaves from Mexican race (*P. americana*) grown in the highlands of Mexico by GC and GC-MS[28]. Thirty compounds were identified accounting for 92.45% of the oil, and estragole (78.12%), α-cubebene (3.58%), methyl eugenol (3.37%) and β-caryophyllene (2.1%) were the major components representing more than 87% of the oil. It has been reported that estragole and its related phenolic compounds have significant insecticidal properties[29].

There is an evidence regarding the potential of *P. amerciana* for mosquito control, but the potential has been mainly exhibited by extracts of different plant parts and with solvents of varying polarity. This was similar to the work of Leite *et al*. in which LC_{50} of hexane and methanol extract from avocado seed against *Aedes aegypti* (*Ae. aegypti*) larvae were determined[30], and the work of Torres *et al*. where the toxicity of the crude ethanol and hexane extracts of different parts of *P. americana* toward third and fourth instars larvae of *Ae. aegypti* were evaluated[31]; the mortality at 24 and 48 h after treatment was observed and both the hexane and ethanol extracts from different parts of *P. americana* exhibited larvicidal toxicity. The hexane extract from the seeds exhibited the highest toxicity with LC_{50} and LC_{90} values of 9.82 mg/L and 22.19 mg/L respectively, while the ethanol seed extract exhibited LC_{50} of 16.48 mg/L and LC_{90} of 45.77 mg/L. This was closely followed by the LC_{50} (10.35 mg/L) and LC_{90} (26.29 mg/L) of ethanol extract from the peels. The pulp extracted with ethanol also yielded great larvicidal toxicity with LC_{50} of 21.32 mg/L and LC_{90} of 59.45 mg/L. Carvalho estimated LC_{50} of seed and stem bark extracts of *P. americana* on *Aedes albopictus* (Skuse) of 3.5 and 4.2 mg/L for larvae and 75.2 and 68.9 mg/L for pupae respectively[32]. Several reports indicate that monoterpenoids cause insect mortality by inhibiting acetylcholinesterase enzyme activity[33].

Ramos-Casillas *et al*. evaluated the methanolic extract from seed of this plant that showed the effect on 3rd and 4th larval instar of *Ae. aegypti* with LC_{50} and LC_{95} of 20.39 and 41.64 mg/L respectively at 24 h[34]. These studies report that biological effectiveness varies according to the botanical species, the part of plant used and extraction methods[35,36].

Conflict of interest statement

We declare that we have no conflict of interest.

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