Hindawi
Journal of Nanotechnology
Volume 2018, Article ID 6917938, 8 pages
https://doi.org/10.1155/2018/6917938

Research Article

Larvicidal Activity of Silver Nanoparticles Synthesized Using Extracts of *Ambrosia arborescens* (Asteraceae) to Control *Aedes aegypti* L. (Diptera: Culicidae)

Bianca Morejón,[1] Fernanda Pilaquinga,[2,3] Flavia Domenech,[2] Danny Ganchala,[2] Alexis Debut,[4] and Marco Neira[1]

[1]*Exact and Natural Sciences Department, Center for Research on Health in Latin America, Pontificia Universidad Católica del Ecuador, Quito, Ecuador*
[2]*Exact and Natural Sciences Department, Laboratory of Nanotechnology, Pontificia Universidad Católica del Ecuador, Quito, Ecuador*
[3]*Department of Chemistry, University of the Balearic Islands, Palma de Mallorca, Balearic Islands, Spain*
[4]*Universidad de las Fuerzas Armadas ESPE, Sangolquí, Ecuador*

Correspondence should be addressed to Marco Neira; mvneira@puce.edu.ec

Received 1 November 2017; Accepted 4 March 2018; Published 18 April 2018

Academic Editor: David Quesada

The mosquito species *Aedes aegypti* is the primary vector of dengue, chikungunya, and Zika infections worldwide. Since effective vaccines or drugs are not available for the prevention and/or treatment of these pathologies, vector control has been adopted as the main approach to reduce their transmission. To control *Aedes* populations, the most commonly used tool is the application of chemical insecticides and, despite their effectiveness, indiscriminate use of these chemicals has led to high operational costs, appearance of resistant populations, and adverse nontarget effects. Plant-derived insecticides may be an eco-friendly, cost-effective, and safe biocontrol alternative. The present study was carried out to evaluate the larvicidal activity of leaf extracts of *Ambrosia arborescens* and green-synthesized silver nanoparticles (AgNPs) using aqueous extracts obtained from this plant against third instar larvae of *Ae. aegypti*. To test this, larvae were exposed for 24 h to the aqueous plant extract at 1500, 3000, 4500, and 6000 ppm and the plant-synthesized AgNPs at 0.2, 0.3, 0.4, and 0.5 ppm. In laboratory assays, AgNPs were more toxic (LC_{50} = 0.28 ppm; LC_{90} = 0.43 ppm) than the plant extract (LC_{50} = 1844.61 ppm; LC_{90} = 6043.95 ppm). These results suggest that *A. arborescens* aqueous extract and green-synthesized silver nanoparticles produced from those extracts have the potential to be developed into suitable alternative tools useful for the control of *Ae. aegypti* populations.

1. Introduction

Over the last decades, climate change, population growth, deforestation, habitat invasion, and insecticide resistance have contributed to the emergence, reemergence, and dispersion of several vector-borne diseases. Among these, those transmitted by the mosquito *Aedes aegypti* (Diptera: Culicidae), which include dengue fever, yellow fever, chikungunya, and Zika, represent some of the major challenges of public health in a vast region of our planet, affecting the lives of hundreds of millions of people every year [1, 2].

Originally native to Africa, *Ae. aegypti* is a strongly anthropophilic and sinanthropic species which, due to its capacity to thrive in human settlements, has been able to expand its distribution all along the tropical and subtropical regions of the globe [3]. Female *Ae. aegypti* become arbovirus vectors after ingesting an infectious blood meal. Once the pathogen starts being secreted with the insect's saliva, the mosquito becomes infective and will transmit the pathogen every time it blood feeds on a susceptible host, for the rest of its life [4, 5].

With the exception of yellow fever, for which an efficient vaccine has been available since the 1940's [6], no vaccine is

currently commercially available against the viral diseases transmitted by *Ae. aegypti*. Therefore, prevention of these diseases is mainly achieved through mosquito population control. Traditionally, most control programs have relied heavily on the use of chemical pesticides, targeting both adults and larvae [7, 8]. Unfortunately, the persistent and in some cases indiscriminate use of these chemicals has resulted in a reduction of their efficacy due to the dramatic emergence of resistant insect populations during the last decades [9].

Larvicides are among the main tools in our arsenal to control mosquito populations. The most widely used larvicides are organophosphates such as temephos, growth inhibitors, and bacterial insecticides such as *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* [8, 10, 11]. Because larvicides must be applied to either natural or artificial bodies of water, they must be harmless to fish and other nontarget organisms, including humans, potentially associated with these water reservoirs [12]. Therefore, the development of new biodegradable, eco-friendly, and specific larvicides is of paramount importance for future control strategies.

Larvicides derived from botanical extracts constitute a new and promising category of pesticide, due to their reduced toxicity for nontarget species and low environmental pollution [13]. Unlike conventional insecticides, which normally contain one specific active agent, plant-derived larvicides usually contain a combination of several chemical compounds that work synergistically, targeting different biological processes and therefore reducing the likelihood of resistance development in their targets [14]. An interesting alternative to strictly botanic larvicides is the production of silver nanoparticles (AgNPs) synthesized using plant extracts as reducing, stabilizing, and capping agents [15]. This technology combines the microbicide properties of silver, the insecticidal activity of the selected plant, and a high efficiency due to the favorable surface area to volume ratio due to the small size of the particles (1–100 nm) [16, 17]. In combination, these characteristics allow AgNPs to achieve their insecticide effect at very low concentrations (often ≥30 mg/L) [14].

Due to its location, geography, and climate, Ecuador is widely regarded as a hotspot for biodiversity [18]. Among the large variety of plant species present in this country, at least 34 have been reported to be used as insecticides by local populations [19]. Among these, *Ambrosia arborescens* (Mill.) stands out as a species used by various populations for insecticidal purposes, including the elimination of fleas, lice, flies, and other insects [19]. This plant, commonly known as "marco," "marku," "altamisa," or "artemisia," is a species native to Ecuador that belongs to the family Asteraceae and is found in the Andean region of the country, between 2000 and 3500 m.a.s.l [20]. The plant can range in size from a subshrub to a small tree and presents a pungent odor, and its stems contain an aqueous bitter latex [21].

In this context, we attempted to evaluate the potential of *A. arborescens* as a larvicide against *Ae. aegypti*, both as an aqueous extract and in combination with AgNPs.

Figure 1: *Ambrosia arborescens* (Mill.) voucher.

2. Materials and Methods

2.1. Plant Specimens. Fresh leaves of *A. arborescens* were collected in a small, family-owned farm in the Calacalí parish (geographic coordinates: 78°30′55.7″S, 00°00′32.7″O), which is located approximately 17 km north of Ecuador's capital city, Quito, at an altitude of 2980 m.a.s.l. The plot where the plant was growing had not received any kind of pesticide or fertilizer for at least one year previous to our collection date (R. Zambrano, personal communication).

A botanical voucher specimen (Figure 1) was prepared and deposited at the Center for Research on Health in Latin America (CISeAL) as a reference.

2.2. Preparation of A. arborescens Extract. The leaves of *A. arborescens* were cleaned of any visible debris and dried in shade for seven days at room temperature. Subsequently, they were dried in an oven at 40°C for 48 hours; dried sample was ground in a mortar. To prepare the extract, 1 g of vegetable material and 20 mL of distilled water were added. The mixture was placed under constant stirring for one hour at 60°C ± 1°C; after this time, the extract was filtered and stored at 4°C until use.

2.3. Synthesis of Silver Nanoparticles. The best conditions were previously optimized using 1 mM silver nitrate Sigma-Aldrich® solution. Twenty mL of this solution was taken, and 2 mL of the extract of *A. arborescens* were added dropwise. Two to three drops of 1% NaOH were added to adjust the pH to 8. This mixture was stirred for 50 minutes at a temperature of 50°C ± 1°C. The colloidal solution was cooled and stored in amber containers at 4°C for further analysis.

2.4. Characterization of Silver Nanoparticles

2.4.1. UV-Vis Spectroscopy. The nanoparticles were analyzed on a Cary 60 double beam spectrophotometer from Agilent Technologies. The resolution of the equipment was one nm, and the measurement range was from 350 to 800 nm. Dilutions 1 to 10 were made with distilled water from colloidal solutions obtained from the synthesis process.

2.4.2. FTIR Spectroscopy. Infrared spectroscopy analyses were performed on a Spectrum BX PerkinElmer with ATR Miracle Pike coupling under 4 cm^{-1} resolution conditions, ranging from 4000 cm^{-1} to 500 cm^{-1}, and ten scans per sample. Both the extract and the synthesized nanoparticles were dried in the oven at 60°C for 24 hours.

2.4.3. STEM Analysis. The STEM analysis was carried out on a FEG-SEM Tescan Mira 3 device, using a voltage of 30 kV. Nanoparticles were observed in colloidal solution.

2.4.4. SEM-EDX Analysis. SEM analysis was performed in a Phenom ProX scanning electron microscope equipped with an EDX-detector operating at 10 kV and Prosuite-EDS software. Nanoparticles were read in solid state over a pin of carbon.

2.4.5. Flame Atomic Absorption Spectroscopy for Silver Quantification. The silver content was analyzed on a PerkinElmer AAnalyst 400 atomic absorption spectrophotometer. A flux of 10.0 L/min of air and 2.5 L/min of acetylene were used for the flame. For the reading of the samples, once the performance of the method was verified, and a 1 : 100 dilution with distilled water of the obtained colloidal solutions was made.

2.4.6. Mosquito Rearing. The strain of *Ae. aegypti* used for this work was collected in 2015 in the Ecuadorian city of Puerto Francisco de Orellana, Orellana Province, and has since been continuously maintained under standard insectary conditions (28 ± 1°C temperature, 80 ± 10% relative humidity, and 12 h light/12 h darkness photoperiod) at CISeAL [22].

Eggs were hatched in water that had been previously boiled and allowed to cool down to room temperature. Larvae were fed on finely ground fish food flakes, following the feeding regime developed by Carvalho et al. [23]. The larvae were maintained at the aforementioned standard insectary conditions until reaching the 3rd instar, when they were used for experimentation [24].

2.4.7. Larvicidal Assays. In order to test the larvicidal activity of the aqueous extract and the plant-synthesized AgNPs of *A. arborescens*, we followed the protocol proposed by the WHO [24]. Briefly, each bioassay consisted of a set of four experimental groups (one for each concentration tested) and a control group. Within each one of these groups, the basic testing unit (i.e., technical replicate) was a plastic well containing 25 third instar larvae in either 200 mL of the test solution at the desired concentration (for experimental groups) or distilled water (for control groups). Each group contained four technical replicates, for a total of 500 larvae per bioassay ((100 larvae per concentration ×4 concentrations) + (100 larvae per control group)). The entire bioassay was repeated five times.

During bioassays, larvae were maintained at standard insectary conditions (28 ± 1°C temperature, 80 ± 10% relative humidity, and 12 h light/12 h darkness photoperiod) [22]. No food was provided during this period. Mortality was recorded 24 hours after the beginning of each bioassay. Specimens were considered as dead when, following stimulation by touch, they either did not move at all or moved sluggishly and were unable to rise towards the surface of the rearing medium [24].

To establish the concentration that results lethal to 50% of individuals (LC_{50}), concentration gradients were established for both the aqueous plant extract and the plant-synthesized AgNPs. The upper and lower limits of these gradients were established by preliminary experiments (data not shown). Concentrations tested for the aqueous plant extract were 1500, 3000, 4500, and 6000 ppm. Concentrations tested for the plant-synthesized AgNPs tests were 0.2, 0.3, 0.4, and 0.5 ppm.

2.4.8. Data Analysis. Using the mortality results obtained from the five bioassay replicates, we calculated the mean mortality per dose. With these values, we performed dose-mortality regressions using a log-probit model [25] in order to calculate the LC_{50} and LC_{90} values. Calculations were done using the R-software for statistical computing [26], and codes within the package MASS containing the material are discussed in [27].

3. Results

3.1. UV-Vis Spectroscopy. Parameters for this assay were previously optimized using 1.0 mM silver nitrate heated at 50°C for 50 minutes, at pH 8, with 2 mL of aqueous *A. arborescens* extract [28]. The UV spectrum of the synthesized nanoparticles is shown in Figure 2. The wavelength of maximum absorbance was 414 nm, and this value, according to Pradeep's description [29], corresponds to an average nanoparticle-core shell size of 10 to 14 nm. It can be evidenced by the shape of the spectrum that there is a high dispersion of particle size, since the peak is wide but symmetrical. Peak spreading may also indicate that there are wide spaces between the nanoparticles [30].

3.2. FTIR Spectroscopy. Fourier transform infrared spectroscopy (FTIR) was used to verify that the silver nanoparticles were indeed coated with the *A. arborescens* extract used for their synthesis. This is important because the extract does not only act as a reducing agent, but its organic fraction overlays or functionalize nanoparticles. Thus, the compounds responsible for the reduction of silver nitrate can be

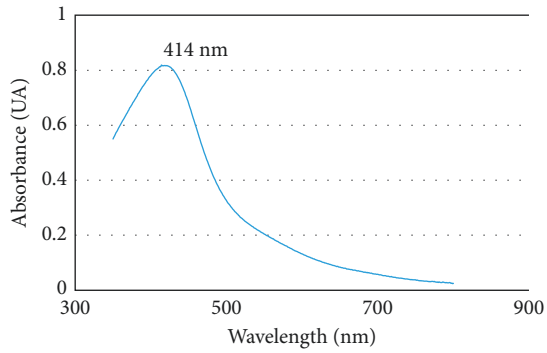


Figure 2: UV-Vis spectra of silver nanoparticles in optimal conditions.

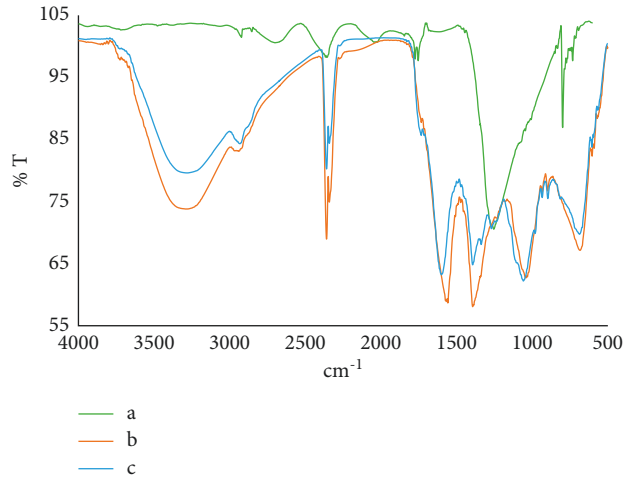


Figure 3: FTIR spectra of silver nitrate (a), *A. arborescens* extract (b), and silver nanoparticles-*A. arborescens* extract (c).

identified. In Figure 3, we compare the spectra of silver nitrate, *A. arborescens* extract, and nanoparticles synthesized with *A. arborescens* extract.

The similarity between the spectra of the synthesized nanoparticles and the extract indicates that the organic residue of the extract is kept intact. Similar bands are present in both spectra around 1570 cm^{-1} corresponding to the stretching of the C=C group of the aromatic rings, in addition to a band at approximately 1050 cm^{-1} of CO bonds within the alcohols. These data confirm the adequate coating of nanoparticles with the extract. Once again, the extract not only fulfills the function of reducing agent but also acts as a stabilizing medium. This saves additional use of other reagents.

In the spectra of the *A. arborescens* extract, there is a band at 1337.47 cm^{-1} that corresponds to the O–H bond characteristic of the phenolic compounds [31]. In the nanoparticle spectra, this band is not present; however, the high intensity band around 3280 cm^{-1} is maintained. This difference occurs because the OH groups of the phenolic compounds do not react in their entirety. Rais [32] indicates that only one out of four hydroxyl groups react, which is why the band of the OH groups at 3200 cm^{-1} is not affected by the reaction. The remaining hydroxyl groups are attached to the nanoparticles by electrostatic attraction, coating them. A colorimetric test using $FeCl_3$ showed the color difference between the extract and the silver nanoparticles-*A. arborescens* extract.

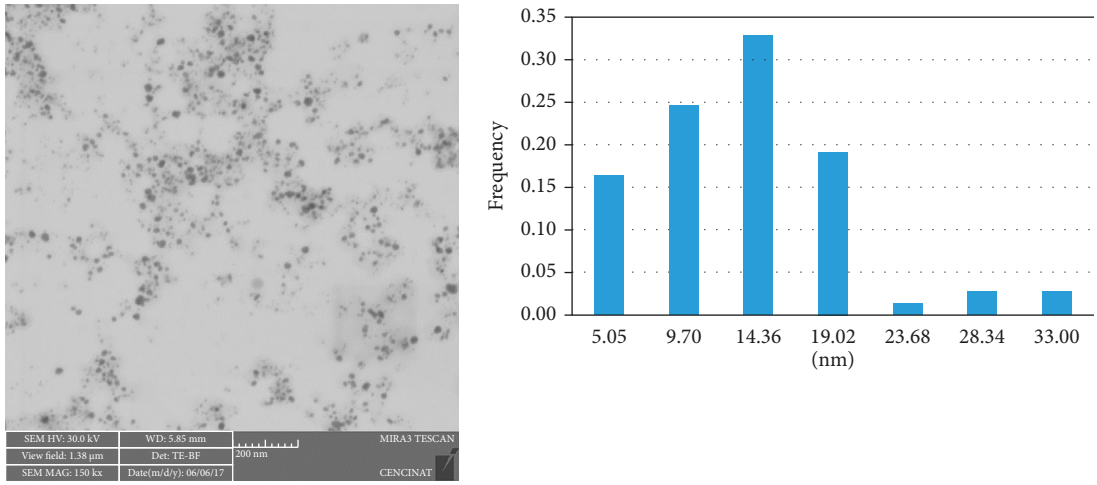
3.3. STEM Analysis. As shown in Figure 4, the silver nanoparticles obtained are spherical and dispersed with an average size of 14 ± 6 nm. STEM image shows the action of aqueous extract on the nanoparticles as a stabilization agent. Anandalakshmi et al. [33] reported similar results in terms of particle size with diameters of 13 to 61 nm; this study was carried out with natural extract whose predominant components were polyphenols, which is why it resembles the synthesis performed with *A. arborescens* extract. Ndikau et al. [34] not only got similar size particles but also they found out that silver nanoparticles synthesized with plant extracts have smaller sizes than those synthesized with sodium citrate.

In STEM, a direct projection of the nanoparticles was observed and due to the low diffraction of the conjugated molecule these do not appear on the electron microscope images and so you only observe the core shell of the nanoparticles. This is quite useful in green chemistry as normally the UV-Vis methods give an overestimation of the nanoparticle size and then the difference in size between UV-Vis and STEM gives the average size value of the capping agent. A complete description of this effect and an extensive list of relevant references can be found in [35].

3.4. SEM-EDX. Figure 5 shows the micrograph of silver nanoparticles-*A. arborescens* extract powder measured by SEM. The image shows the dispersion of the nanoparticles onto the carbon pin. EDX spectrum shows three peaks of silver located below 4 kV. The rest of elements are present in the organic *A. arborescens* extracts, confirmed by FAAS.

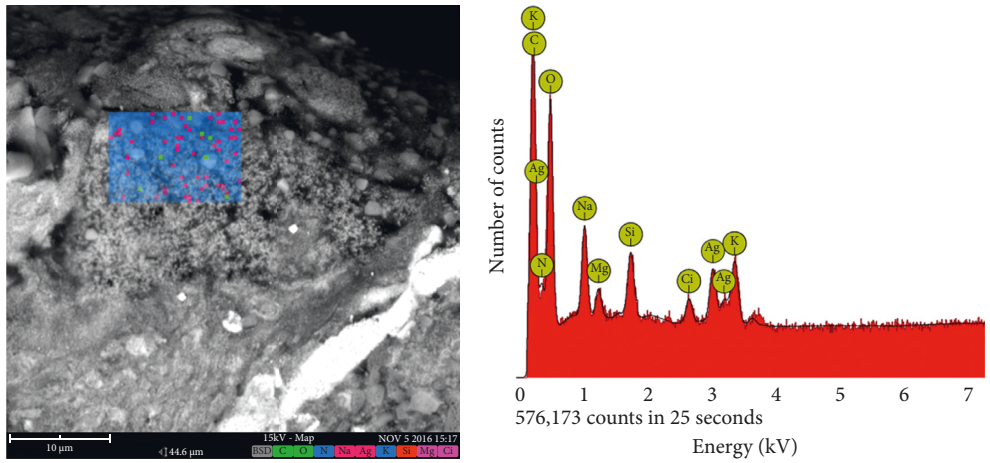
3.5. Flame Atomic Absorption Spectroscopy for Silver Quantification. The silver content of silver nanoparticles synthesis with *A. arborescens* extract was determined in order to verify that the silver content did not change to determine the yield of the reaction. The amount of theoretical silver that should be obtained was calculated as a function of the amount of silver nitrate in the initial solution and the volume of extract added. An average concentration of 90.73 ppm silver in the synthesized nanoparticles was obtained compared with initial 98.01 ppm present in the silver nitrate. This amount of silver equals a reaction yield of 92.52%, which is acceptable for synthesis on a smaller scale. In his doctoral thesis, Li [36] tested different phenols and mixtures of phenols as reducing agents and obtained yields in the range of 75 to 90%.

3.6. Larvicidal Activity of Aqueous Extract and Synthesized AgNPs. Mortality values observed following all bioassays are shown in Table 1. Both the aqueous extract and the plant-synthesized AgNPs showed a dose-dependent toxic effect against *Ae. aegypti* larvae. No mortality was observed in the control groups.



(a) (b)

Figure 4: STEM image (a) and frequency histogram (b) of average shape of silver nanoparticles-*A. arborescens* extract.



(a) (b)

Figure 5: SEM micrograph (a) and EDX spectrum (b) of silver nanoparticles-*A. arborescens extract.*

Table 1: Larvicidal activity of aqueous extracts and silver nanoparticles synthesized using *Ambrosia arborescens* against *Aedes aegypti* third instar larvae.

Treatment	Dose (ppm)	Larval mortality[a] ± SD
Plant aqueous extract	1500	43.4 ± 4.9
	3000	66.6 ± 2.1
	4500	81.2 ± 0.8
	6000	92.8 ± 1.3
Control	0	0 ± 0.0
AgNPs	0.2	17.6 ± 3.2
	0.3	60.6 ± 3.2
	0.4	82.6 ± 3.1
	0.5	96.4 ± 1.8
Control	0	0 ± 0.0

SD: standard deviation; [a]values are mean ± SD of five trials.

The LC_{50} and LC_{90} values of the aqueous extract were 1844.61 ppm and 6043.95 ppm, respectively (Table 2). However, the combination of this extract with silver nanoparticles greatly potentiated its toxicity to *Ae. aegypti* larvae: LC_{50} and LC_{90} values for plant-synthesized AgNPs were 0.28 ppm and 0.43 ppm (Table 2), respectively. χ^2 value was not significant at $p \leq 0.05$ level.

Furthermore, exposure to aqueous extract at a concentration of 6000 ppm resulted in an average mortality of 97% of individuals, while exposure to 0.5 ppm plant-synthesized AgNPs resulted in an average mortality of 93.4% of individuals (Table 1).

4. Discussion

Our study suggests that the leaves of *A. arborescens* contain water-soluble chemicals which are toxic to *Ae. aegypti* larvae. Furthermore, our results show that the toxicity of such chemicals is greatly enhanced when they are combined with AgNPs.

It has been reported that plants belonging to the Asteraceae family can present an elevated toxicity due to the

Table 2: Probit values of plant aqueous extracts and silver nanoparticles synthesized using leaves of *Ambrosia arborescens* against *Aedes aegypti* third instar larvae.

Treatment	LC_{50} (ppm)	95% fiducial limits (LCL–UCL)	LC_{90} (ppm)	95% fiducial limits (LCL–UCL)	χ^2
Plant aqueous extract	1844.61	1489.16–2146.46	6043.95	5031.51–7970.18	2.01^{NS}
AgNPs	0.28	0.26–0.29	0.43	0.41–0.48	0.9^{NS}

LC_{50}: lethal concentration that kills 50% of the exposed larvae; LC_{90}: lethal concentration that kills 90% of the exposed larvae; LCL: lower confidence limit; UCL: upper confidence limit; χ^2: chi-square test; ^{NS}not significant at $p \leq 0.05$ level.

presence of a wide range of defensive chemical agents against herbivores, such as sesquiterpenic lactones, monotherpenes, terpenoids, and polyacetylenic resins [37]. Within this family, the genus *Ambrosia* presents a remarkable physiological plasticity that allows it to adapt to a wide range of environmental conditions [38], a characteristic that might be explained, at least partially, by the secondary metabolites produced by the species of this taxon [39]. All members of the *Ambrosia* genus present a high content of sesquiterpenic lactones which possesses antibacterial, cytotoxic, and antifungal properties [40, 41]. Regarding *A. arborescens*, former studies have reported damsines, coronophilines, and psilostachynes as the main sesquiterpenic lactones in this species [39, 42, 43]. It seems plausible that the lethal effect observed is, at least in part, due to these kinds of compounds.

Several studies have reported on the larvicidal activity of plants of the Asteraceae family against *Ae. aegypti*. For example, Govindarajan and Karuppannan [44] used the methanolic extract of *Eclipta alba* against third instar larvae, obtaining a LC_{50} of 127.64 ppm. Álvarez et al. [45] reported that the LC_{50} of the ethereal extracts of *Heli oppositifolia* and *Jaegeria hirta* are 41 ppm and 24 ppm, respectively. Sukhthankar et al. [46] used a methanolic extract of the leaves of *Chromolaena odorata*, obtaining a LC_{50} of 138 ppm, and Tennyson et al. [47] tested hexane and ethyl acetate extracts of *Ageratum houstonianum*, obtaining LC_{50} values of 8889.13 ppm and 1952.12 ppm, respectively. Furthermore, and as far as we are aware, only two previous reports exist regarding the toxicity of plants within the *Ambrosia* genus against mosquito larvae: (a) Eisa [48] tested the aqueous extract of *A. marítima* leaves against field-collected *Culex* larvae, obtaining a LC_{50} of 1349 ppm, and (b) De La Torre et al. [49] tested the ethereal extract made with the aerial parts of *A. confertifolia* against *Ae. aegypti*, obtaining a LC_{50} of 185.6 ppm.

In this context, the LC_{50} value of 1844.61 ppm observed in our study for *A. arborescens* aqueous extract is within the range observed with similar plant extracts against mosquito larvae. However, one of the most interesting and striking aspects of our study was the exponential potentiation of the lethal effect we observed when the aqueous extract was used to synthetize AgNPs, reaching a LC_{50} of 0.28 ppm, which represents a >6 000-fold increase in toxicity. Although this is consistent with other reports which have reported an enhanced efficiency of plant-synthesized AgNPs in comparison with the plant extracts by themselves [50], it is worth mentioning that the LC_{50} reported in our work is, as far as we are aware, the lowest reported for AgNPs synthesized from any member of the Asteraceae family [51, 52].

The physiological basis for the high toxicity of plant-synthesized AgNPs remains an open question. It has been suggested that a key factor is their ability to permeate through the invertebrate exoskeleton and penetrate into the insect's cells, where they bind macromolecules such as proteins and DNA, altering their structure and therefore their functionality [53, 54]. Interestingly, it has also been reported that doses of plant-synthesized AgNPs which result lethal to several species of mosquito larvae have little or no effect on other nontarget species, including other aquatic arthropod species and fish [55, 56], suggesting that at least some mosquito species are particularly susceptible to the lethal effect of AgNPs. At the moment, the reasons behind this phenomenon remain unknown.

It is, therefore, important to stress that more research is needed to identify the mechanisms by which AgNPs exert their toxic effect on their intended target species. This information will be crucial to establish whether the use of nanoparticles for mosquito control could have any unintended negative impact on either the environment or the health of the human population. Furthermore, any field application of this technology must be preceded by extensive laboratory and semifield research, exploring aspects such as residuality, bioaccumulation, and long-term effects of exposure to AgNPs on relevant biological systems.

5. Conclusions

In summary, our study shows that the aqueous extract obtained from the leaves of *A. arborescens* presents a clear insecticidal effect against *Ae. aegypti* larvae. Furthermore, this effect is greatly potentiated when this extract is used for the synthesis of AgNPs.

Therefore, our results suggest that *A. arborescens* has the potential to be used for the development of novel pesticides. However, the said development must be complemented by studies aimed at defining key aspects, such as the nature of the specific molecules responsible for the insecticidal effect observed, the potential effects of these chemicals in both the environment and in human health, and the field logistics required for the use of such a novel insecticide.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Bianca Morejón conducted the bibliographical research that lead to the selection of *Ambrosia arborescens* for this work, performed all bioassays, and analyzed experimental results.

Marco Neira supervised the experimental design and performance of bioassays and contributed to the analysis of experimental results. Fernanda Pilaquinga, Flavia Domenech, and Danny Ganchala performed the synthesis and characterization of silver nanoparticles. Alexis Debut performed STEM analysis. Bianca Morejón, Marco Neira, Fernanda Pilaquinga, and Flavia Domenech wrote the manuscript. All authors reviewed and approved this manuscript. Bianca Morejón and Fernanda Pilaquinga contributed equally to this work.

Acknowledgments

This research was supported in part by PUCE's Research Grants N13409 and N13438. The authors would also like to thank Mr. Ricardo Zambrano for his help in obtaining suitable plant samples and Dr. Svetlana Ivanova from the University of Sevilla for her support in the characterization of nanoparticles.

References

[1] World Health Organization (WHO), "Dengue y dengue grave," 2016, http://www.who.int/mediacentre/factsheets/fs117/en/.

[2] S. Bhatt, P. Gething, O. Brady et al., "The global distribution and burden of dengue," *Nature*, vol. 496, no. 7446, pp. 504–507, 2013.

[3] G. Cook and A. Zumla, *Manson's Tropical Diseases*, Saunders, Philadelphia, PA, USA, 2008.

[4] R. Chamberlain and W. Sudia, "Mechanism of transmission of viruses by mosquitoes," *Annual Review of Entomology*, vol. 6, no. 1, pp. 371–390, 1961.

[5] T. Sardar, S. Rana, and J. Chattopadhyay, "A mathematical model of dengue transmission with memory," *Communications on Nonlinear Science and Numeric Simulation*, vol. 22, no. 1, pp. 511–525, 2015.

[6] J. Frierson, "The yellow fever vaccine: a history," *Yale Journal of Biology and Medicine*, vol. 83, no. 2, pp. 77–85, 2010.

[7] WHO and TDR, *Dengue Guidelines for Diagnosis, Treatment, Prevention and Control*, WHO Publications, Geneva, Switzerland, 2009.

[8] J. Bisset, M. Rodríguez, and D. Fernández, "Selection of insensitive acetylcholinesterase as a resistance mechanism in *Aedes aegypti* (Diptera: Culicidae) from Santiago de Cuba," *Journal of Medical Entomology*, vol. 43, no. 6, pp. 1185–1189, 2006.

[9] M. Govindarajan, A. Jebanesan, and D. Reetha, "Larvicidal effect of extracellular secondary metabolites of different fungi against the mosquito, *Culex quinquefasciatus* Say," *Tropical Biomedicine*, vol. 22, no. 1, pp. 1–3, 2005.

[10] J. da Silva and J. Mendes, "Susceptibility of *Aedes aegypti* (L) to the insect growth regulators diflubenzuron and methoprene in Uberlândia, State of Minas Gerais," *Revista da Sociedade Brasileira de Medicina Troipcal*, vol. 40, no. 6, pp. 612–616, 2007.

[11] S. Poopathi and S. Abidha, "Mosquitocidal bacterial toxins (*Bacillus sphaericus* and *Bacillus thuringiensis* serovar *israelensis*): mode of action, cytopathological effects and mechanism of resistance," *Journal of Physiology and Pathophysiology*, vol. 1, no. 3, pp. 22–38, 2010.

[12] T. Hurst, B. Kay, P. Ryan, and M. Brown, "Sublethal effects of mosquito larvicides on swimming performance of larvivorous fish *Melanotaenia duboulayi*; (Atheriniformes: Melanotaeniidae)," *Journal of Economic Entomology*, vol. 100, no. 1, pp. 61–65, 2007.

[13] P. Thiyagarajan, P. Kumar, K. Kovendan, and K. Murugan, "Effect of medicinal plant and microbial insecticides for the sustainable mosquito vector control," *Acta Biologica Indica*, vol. 3, no. 1, pp. 527–535, 2014.

[14] G. Benelli, A. Caselli, and A. Canale, "Nanoparticles for mosquito control: challenges and constraints," *Journal of King Saud University-Science*, vol. 29, no. 4, pp. 424–435, 2017.

[15] A. Priyadarshini, K. Murugan, C. Panneerselvam, S. Ponarulselvam, J. Hwang, and M. Nicoletti, "Biolarvicidal and pupicidal potential of silver nanoparticles synthesized using *Euphorbia hirta* against *Anopheles stephensi* Liston (Diptera: Culicidae)," *Parasitolology Research*, vol. 111, no. 3, pp. 997–1006, 2012.

[16] H. Borase, C. Patil, R. Patil et al., "Phyto-synthesized silver nanoparticles: a potent mosquito biolarvicidal agent," *Journal of Nanomedicine & Biotherapeutic Discovery*, vol. 3, no. 1, 2013.

[17] U. Muthukumaran, M. Govindarajan, M. Rajeswary, and S. Hoti, "Synthesis and characterization of silver nanoparticles using *Gmelina asiatica* leaf extract against filariasis, dengue, and malaria vector mosquitoes," *Parasitology Research*, vol. 114, no. 5, pp. 1817–1827, 2015.

[18] N. Myers, R. Mittermeier, C. Mittermeier, G. da Fonseca, and J. Kent, "Biodiversity hotspots for conservation priorities," *Nature*, vol. 403, no. 6772, pp. 853–858, 2010.

[19] L. De La Torre, H. Navarrete, P. Muriel, M. Macía, and H. Balslev, *Enciclopedia de las Plantas Útiles del Ecuador*, Herbario QCA de la Escuela de Ciencias Biológicas de la Pontificia Universidad Católica del Ecuador & Herbario AAU del Departamento de Ciencias Biológicas de la Universidad de Aarhus, Quito, Ecuador, 2008.

[20] P. Jørgensen and S. León-Yánez, "Catalogue of the vascular plants of Ecuador," *Monographs on Systematic Botany from Missouri Botanical Garden*, vol. 75, pp. 1–1182, 1999.

[21] C. Ruales, *Plantas de Quito-La Vegetación Original de una Ciudad Siempre Verde*, Universidad San Francisco de Quito, Quito, Ecuador, 2013.

[22] E. Gerberg, D. Barnard, and R. Ward, *Manual for Mosquito Rearing and Experimental Techniques*, American Mosquito Control Association, Inc., Lake Charles, LA, USA, 1994.

[23] D. Carvalho, D. Nimmo, N. Naish et al., "Mass production of genetically modified *Aedes aegypti* for field releases in Brazil," *Journal of Visualized Experiments*, no. 83, p. e3579, 2014.

[24] WHO, *Guidelines for Laboratory and Field Testing of Mosquito Larvicides*, World Health Organization, Geneva, Switzerland, 2005.

[25] D. Finney, *Probit Analysis*, Cambridge University Press, Cambridge, UK, 1971.

[26] R Core Team, *R: A Language and Environment for Statistical Computing*, Foundation for Statistical Computing, Viena, Austria, 2013.

[27] W. Venables and B. Ripley, *Modern Applied Statistics with S*, Springer, New York, NY, USA, 4th edition, 2002.

[28] F. Domenech, *Síntesis y caracterización de nanopartículas de plata usando extracto de hojas de Ambrosia arborescens (marco) como reductor químico*, B. Sc. thesis, Pontificia Universidad Católica del Ecuador, Quito, Ecuador, 2017.

[29] T. Pradeep, *A Textbook of Nanoscience and Nanotechnology*, McGraw Hill, New Delhi, India, 2012.

[30] M. Aguilar, *Síntesis y caracterización de nanopartículas de plata: efecto sobre Collectotrichum gloesporioides*, Doctoral dissertation, Instituto Politécnico Nacional de México, Mexico City, Mexico, 2009.

[31] S. Trifunschi, M. Munteanu, V. Agotici, S. Pintea, and R. Gligor, "Determination of flavonoid and polyphenol compounds in viscum album and allium sativum extracts," *International Current Pharmaceutical Journal*, vol. 4, no. 5, pp. 382–385, 2015.

[32] M. Rais, "Preparation of silver nanoparticles," Patent WO2012074355 A1, 2012, https://www.google.com/patents/WO2012074355A1?cl=en.

[33] K. Anandalakshmi, J. Venugobal, and V. Ramasamy, "Characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity," *Applied Nanoscience*, vol. 6, no. 3, pp. 399–408, 2015.

[34] M. Ndikau, N. Noah, D. Andala, and E. Masika, "Green synthesis and characterization of silver nanoparticles using *Citrullus lanatus* fruit rind extract," *International Journal of Analytical Chemistry*, vol. 2017, Article ID 8108504, 9 pages, 2017.

[35] S. Ramesh, M. Grijalva, A. Debut, B. De la Torre, A. Albericio, and L. Cumbal, "Peptides conjugated to silver nanoparticles in biomedicine–a "value-added" phenomenon," *Biomaterials Science*, vol. 4, no. 12, pp. 1713–1725, 2016.

[36] V. Li, *Advancing silver nanostructures towards antibacterial applications*, Doctoral dissertation, Royal Melbourne Institute of Technology, Melbourne, Australia, 2014.

[37] J. Mauseth, *Botany: An Introduction to Plant Biology*, Jones and Bartlett Publishers, Burlington, MA, USA, 2003.

[38] A. Parkhomenko, E. Oganesyan, O. Andreeva, E. Dorkina, E. Paukova, and Z. Agadzhanyan, "Pharmacologically active substances from *Ambrosia artemisiifolia*. Part 2," *Pharmaceutical Chemistry Journal*, vol. 40, no. 11, pp. 627–632, 2006.

[39] M. Vera, *Estudio Fitoquímico de una Planta de la Flora del Ecuador: Ambrosia arborescens*, Escuela Politécnica del Ejército, Quito, Ecuador, 2008.

[40] O. Spring, J. Kupka, B. Maier, and A. Hager, "Biological activities of sesquiterpene lactones from *Helianthus annuus*: antimicrobial and cytotoxic properties; influence on DNA, RNA, and protein synthesis," *Zeitschrift für Naturforsch C*, vol. 37, no. 11-12, pp. 1087–1091, 1982.

[41] M. Neerman, "Sesquiterpene lactones: a diverse class of compounds found in essential oils possessing antibacterial and antifungal properties," *International Journal of Aromatherapy*, vol. 13, no. 2-3, pp. 114–120, 2003.

[42] W. Herz, G. Anderson, S. Gibaja, and D. Raulais, "Sesquiterpene lactones of some *Ambrosia* species," *Phytochemistry*, vol. 8, no. 5, pp. 877–881, 1969.

[43] M. De Leo, M. Vera, B. Naranjo, N. De Tommasi, and A. Braca, "Sesquiterpenes and diterpenes from *Ambrosia arborescens*," *Phytochemistry*, vol. 71, no. 7, pp. 804–809, 2010.

[44] M. Govindarajan and P. Karuppannan, "Mosquito larvicidal and ovicidal properties of *Eclipta alba* (L.) Hassk (*Asteraceae*) against chikungunya vector, *Aedes aegypti* (Linn.) (Diptera: Culicidae)," *Asian Pacific Journal of Tropical Medicine*, vol. 4, no. 1, pp. 24–28, 2011.

[45] J. Álvarez, I. Duarte, O. Aguirre, and J. Jiménez, "Control del vector del dengue utilizando fracciones etéreas de dos plantas (*Asteraceae*) como larvicidas," *Revista de Salud Pública*, vol. 15, no. 2, pp. 227–236, 2013.

[46] J. Sukhthankar, H. Kumar, M. Godinho, and A. Kumar, "Larvicidal activity of methanolic leaf extracts of plant, *Chromolaena odorata* L. (*Asteraceae*) against vector mosquitoes," *International Journal of Mosquito Research*, vol. 33, no. 13, pp. 33–38, 2014.

[47] S. Tennyson, J. Ravindran, A. Eapen, and J. William, "Larvicidal activity of *Ageratum houstonianum* Mill. (*Asteraceae*) leaf extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinque*fasciatus (Diptera: Culicidae)," *Asian Pacific Journal of Tropical Diseases*, vol. 5, pp. S73–S76, 2015.

[48] A. Eisa, *Toxicity of Ambrosia maritima L. leaves aqueous extract and abate aqueous solutions, on Culex species*, University of Khartoum, Khartoum, Sudan, 2010.

[49] Y. De La Torre, F. Martínez, A. Flores, N. De Torres, and R. Salazar, "Larvicidal and cytotoxic activities of extracts from 11 native plants from northeastern Mexico," *Journal of Medical Entomology*, vol. 50, no. 2, pp. 310–313, 2013.

[50] G. Benelli, "Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: a review," *Parasitology Research*, vol. 115, no. 1, pp. 23–34, 2016.

[51] G. Rajakumar and A. Rahuman, "Larvicidal activity of synthesized silver nanoparticles using *Eclipta prostrata* leaf extract against filariasis and malaria vectors," *Acta Tropica*, vol. 118, no. 3, pp. 196–203, 2011.

[52] M. Nalini, M. Lena, P. Sumathi, and C. Sundaravadivelan, "Effect of phyto-synthesized silver nanoparticles on developmental stages of malaria vector, *Anopheles stephensi* and dengue vector, *Aedes aegypti*," *Egyptian Journal of Basic and Applied Sciences*, vol. 4, no. 3, pp. 212–218, 2017.

[53] J. Subramaniam, K. Murugan, C. Panneerselvam et al., "Eco-friendly control of malaria and arbovirus vectors using the mosquitofish *Gambusia affinis* and ultra-low dosages of *Mimusops elengi*-synthesized silver nanoparticles: towards an integrative approach?," *Environmental Science and Pollution Research*, vol. 22, no. 24, pp. 20067–20083, 2015.

[54] N. Arjunan, K. Murugan, C. Rejeeth, P. Madhiyazhagan, and D. Barnard, "Green synthesis of silver nanoparticles for the control of mosquito vectors of malaria, filariasis, and dengue," *Vector Borne Zoonotic Diseases*, vol. 12, no. 3, pp. 262–268, 2012.

[55] K. Haldar, B. Haldar, and G. Chandra, "Fabrication, characterization and mosquito larvicidal bioassay of silver nanoparticles synthesized from aqueous fruit extract of putranjiva, *Drypetes roxburghii* (Wall.)," *Parasitology Research*, vol. 112, no. 4, pp. 1451–1459, 2013.

[56] A. Rawani, A. Ghosh, and G. Chandra, "Mosquito larvicidal and antimicrobial activity of synthesized nano-crystalline silver particles using leaves and green berry extract of *Solanum nigrum* L. (Solanaceae: Solanales)," *Acta Tropica*, vol. 128, no. 3, pp. 613–622, 2013.