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# Biosynthesis of silver nanoparticles using *Argemone mexicana*: A novel route of malarial fever vector mosquito control

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#### Abstract

The impact of this nano-mosquitocidal against biological control agents of mosquito larval populations has been poorly studied. In this research, we biosynthesized silver nanoparticles (Ag NP) using the *Argemone mexicana* leaf extract as a reducing and stabilizing agent. Ag NPs were characterized using a variety of biophysical methods, including UV-vis spectophotometry, SEM, XRD and FTIR analyses. Moreover, the *A. mexicana* aqueous extract and the biosynthesized Ag NP were tested for acute toxicity against I, II, III, IV instars larvae of *An. stephensi*. The LC<sub>50</sub> values achieved by the *A. mexicana* aqueous extract from 176.52 ppm (I instar), 218.67 ppm (II instar), 235.18 ppm (III instar), 257.56 ppm (IV instar), and synthesized silver nanoparticles were 28.53 ppm (I instar), 31.72 ppm (II instar), 37.47 ppm (III instar), 42.28 ppm (IV instar), respectively. Overall, this research pointed out that *A. mexicana* fabricated Ag NPs are easy to produce, stable over time, and can be employed at low doses to reduce populations of malarial vector.

Keywords: Argemone mexicana, UV, SEM, XRD, FTIR, larvicidal

## Introduction

Mosquitoes are disturbance bothers and a noteworthy vector for the transmission of a few life debilitating illnesses (Baranitharan *et al*, 2019). *Anopheles stephensi* mosquito is determination of risk of the most important vector, malaria and it transmission requires quick and accurate methods of identification with especially when targeting vector control in India and other West Asian countries, then the relics one of the most prevalent diseases in the equatorial world, among 200 million to 450 million infectivity's annual global, this causes up to 2.7 million deaths (WHO, 2010; Baranitharan *et al*, 2017). Malaria death charge with child in Africa has been abated by an expected 58%

for 2000 (WHO, 2014). Moreover, it slew a possible 306 000 under-fives widely, including 292 000 child's in the African Region (WHO, 2015). Death rates has fallen by 61 per cent for 2000 and 2015, with a more 13 countries "approaching elimination" (WHO, 2016). Presence of the report, India statement for 6 per cent of all malaria cases in the world, 6 per cent deaths, and 51 per cent of the cases in world. The statement estimates the total cases in India found in 1.31 million and deaths at 194 (WHO, 2017). The approach to combat malaria is regard, plant derived natural products have played an important role as is exemplified by the development of quinine and

artemisinin. The importance of the development of artemisinin is reflected by the Nobel Prize in medicine (2015) that was awarded for the discovery of this drug (Nobel Prize, 2015). However, the development and spread of drug resistant remain a threat to the eradication of the disease and the investigation of the phytochemical and mosquito biological control effects of plant metabolites is still of importance. Medicinal plant products have been making use of conventionally by human being population in variety part rural areas global against vector-borne diseases and parasitology diseases (Pavela and Benelli, 2016). Biological activity components produced by overall medicinal plants can act as larvicides, repellence and toxin against insects (Baranitharan et al, 2014).

Nanotechnology has the potential to revolutionize a wide array of applications, including drug delivery, diagnostic, imaging, sensing, gene delivery, artificial implants, tissue engineering, pest management, and parasitology (Elechiguerra et al, 2005). The plantmediated biosynthesis (i.e., green synthesis) of metal nanoparticles is advantageous over chemical and physical methods, since it is cheap, single-step, and does not require high pressure, energy, temperature, and the use of highly toxic chemicals. In latest years, biological routes for fabrication of nanoparticles have been suggested as possible eco-friendly alternatives to classic chemical and physical methods (Rajan et al, 2015). In particular, green synthesized silver nanoparticles are emerging as multipurpose materials, since their biosynthesis is easy and cheap; they are stable over time and effective against different mosquito vectors (Roni et al, 2015; Subramaniam et al, 2015), arboviruses (Sujitha et al, 2015) and human pathogenic bacteria (Dinesh et al, 2015).

In latest year, extensive research has been carried out to investigate the efficacy of botanical products against mosquito vectors (Gokulakrishnan *et al*, 2016; Baranitharan *et al*, 2016; Dhanasekaran *et al*, 2018; Veena *et al*, 2019; Kavitha *et al*, 2019). People entering into regions mostly dengue, malarial and yellow fever risks exist may protect them using plant derived repellents (Kovendan *et al*, 2012; 2013; Mahesh Kumar *et al*, 2012; Benelli, 2015). On the other hand, people living in endemic regions have to protect themselves using several strategies at the same time, since infection rates of mosquitoes may be extremely high (Semmler *et al*, 2009). Americana mexicana, is a species of poppy found in Mexico and now in the United States, India and Ethiopia. It is reported to have antimicrobial activity, wound healing capacity in rat, larvicidal and chemosterilant activity, nematicidal and allelopathic potential (Dash and Murthy, 2011) and chemical investigations of this plant have revealed the presence of alkaloids, amino acids, phenolics and fatty acids. Furthermore, it has been investigated in terms of modern pharmacology for its anti-malarial activity (Bertrand et al, 2010), molluscicidal and nematicidal activity, anticancer activity, antimicrobial activity, hepatoprotective activity, anti-HIV activity and neuropharmacological activity (Capasso et al, 2002). (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and energy-dispersive X-ray analysis (EDX). The aqueous extract of A. mexicana and the biosynthesized Ag NP were tested for their larvicidal and ovicidal potential against the malarial vector An. stephensi.

# **Materials and Methods**

### Materials

Silver nitrate was procured from Merck, India. The glassware was acid washed thoroughly and then rinsed with Millipore Milli-Q water. Healthy and fresh leaves of *A. mexicana* (Fig. 1) were collected from Pagasalai villege (11.17'N to 12°14'N latitude and 79°71'E to 80°77'E longitude), Nagapattinam District, Tamil Nadu State, India. The identity was confirmed at the Department of Plant Biotechnology, Poompuhar College (Autonomous), Tamil Nadu. Voucher specimens were numbered (Authentication Number AU/BOT/129) and kept in our laboratory and are available upon request.

#### **Preparation of plant extract**

Leaves of *A. mexicana* were dried in the shade and ground to fine powder in an electric grinder. Aqueous extract was prepared by mixing 30 g of dried leaf powder with 300 mL of water (boiled and cooled distilled water) with constant stirring on a magnetic stirrer. The suspension of dried leaf powder in water was left for 3 h and filtered through Whatman no. 1 filter paper, and the filtrate was stored in an amber-colored airtight bottle at 10 °C temperature until testing.

#### **Mosquito rearing**

Laboratory bred pathogen free stains of mosquito are reared in the vector control laboratory, Department of Zoology, Poompuhar college. At the time of adult feeding, these mosquitoes were 3-4 days old after emergences (maintained on raisins and water) and were starved for 12 h before feeding. Each time, 100 mosquitoes per cage were fed on blood using a feeding unit fitted with Parafilm as membrane for 4 h. An. stephensi are fed during 6.00 p.m. to 10.00 p.m. A membrane feeder with the bottom end fitted with Parafilm was placed with 2.0 mL of the blood sample (obtained from a slaughter house by collecting in a heparinized vial and stored at 4 °C) and kept over a netted cage of mosquitoes. The blood was stirred device, and a constant temperature of 37 °C was maintained using a water jacket circulating system. After feeding, the fully engorged females were separated and maintained on raisins. Mosquitoes were held at 28±2 °C, 70-85 % relative humidity, with a photoperiod of 12 h light and 12 h dark.

#### Larvicidal activity

Larvicidal activity of the aqueous extract and Ag NP from *A. mexicana* was evaluated according to WHO protocol (2005). Based on the wide-range and narrow-range tests, aqueous crude extract was tested at 50, 100, 150, 200 and 250  $\mu$ g mL<sup>-1</sup> concentrations and Ag NP was tested at 10, 20, 30, 40 and 50  $\mu$ g mL<sup>-1</sup> concentrations. Twenty numbers of late III instar larvae were introduced into a 500 mL glass beaker containing 250 mL of dechlorinated water plus the desired concentrations of leaf extract or Ag NP. For each concentration, five replicates were recorded at 24 h after exposure, during which no food was given to the larvae. Each test included a set control group (silver nitrate and distilled water) with five replicates for each concentration.

# Biosynthesis and characterization of silver nanoparticles

The broth solution of fresh leaves was prepared by taking 10 g of thoroughly washed and finely cut leaves in a 300 mL Erlenmeyer flask along with 100 mL of sterilized double-distilled water and then boiling the mixture for 5 min before finally decanting it. The extract was filtered with Whatman filter paper no. 1, stored at -15 °C, and tested within a week. The filtrate was treated with aqueous 1 mM AgNO<sub>3</sub> (21.2 mg of AgNO<sub>3</sub> in 125 mL of Milli-Q water) solution in an Erlenmeyer flask and incubated at room temperature.

Eighty-eight milliliters of an aqueous solution of 1 mM silver nitrate was reduced using 12 mL of leaf extract at room temperature for 10 min, resulting in a brown-yellow solution indicating the formation of Ag NP.

The bioreduction of Ag<sup>+</sup> ions was monitored using UV-visible spectrophotometer (UV-160v, Shimadzu, Japan). Analysis of size, morphology and composition of Ag NP was performed by scanning electron microscopy (Hitachi S3000 H SEM), and energydispersive X-ray spectrum (EDX). The purified Ag NP were examined for the presence of biomolecules using FTIR spectrum (Thermo Scientific Nicolet 380 FTIR spectrometer) KBr pellets and crystalline Ag NP were determined by single crystalline XRD analysis.

#### Data analysis

Mortality data were subjected to probit analysis.  $LC_{50}$  and  $LC_{90}$  and other statistics at 95% fiducial limits of upper confidence and 50% fiducial lower confidence limit, slope, regression and chi-square values were calculated using the software developed by Finney (1971). A probability level of *p*<0.05 was used for the significance of differences between values.

## **Results and Discussion**

# Larvicidal activity of aqueous extract and synthesized AgNPs

The results, larvicidal activity of *A. mexicana* aqueous leaf extract and synthesized AgNPs against I, II, III and IV instars larvae, An. stephensi was noted and presented in Table 1 and 2. Considerable mortality was evident after the treatment of A. mexicana for four instars larvae of important vector mosquito. The  $LC_{50}$ and LC<sub>90</sub> values of A. mexicana aqueous extract appeared to be effective against I instars (LC<sub>50</sub>= 176.52  $\mu$ g/mL and LC<sub>90</sub>= 258.71  $\mu$ g/mL), II instars  $(LC_{50}= 218.67 \ \mu g/mL \text{ and } LC_{90}= 337.36 \ \mu g/mL)$ , III instars (LC<sub>50</sub>= 235.18  $\mu$ g/mL and LC<sub>90</sub>= 361.64  $\mu$ g/mL), IV instars (LC<sub>50</sub>= 257.56  $\mu$ g/mL and LC<sub>90</sub>= 370.82 µg/mL) larvae of An. stephensi. Most considerable mortality was evident after the treatment of silver nanoparticles, synthesized AgNPs, had the following LC<sub>50</sub> and LC<sub>90</sub> values: I instars larvae had  $LC_{50}$  and  $LC_{90}$  values of 28.53 and 46.67 µg/mL; II instars larvae had LC<sub>50</sub> and LC<sub>90</sub> values of 31.72 and 57.35  $\mu$ g/mL; III instars larvae had LC<sub>50</sub> and LC<sub>90</sub> values of 37.47 and 59.70 µg/mL; IV instars larvae had LC<sub>50</sub> and LC<sub>90</sub> values of 42.28 and 65.73  $\mu$ g/mL. A control showed nil mortality in the concurrent assay.

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Plant names	Instars	LC <sub>50</sub> (mg/L)	95% Confidence limits		LC <sub>90</sub> (mg/L)	Slope	Regression	2
			LCL	UCL	$LC_{90}$ (IIIg/L)	Stope	Regression	
A. mexicana	Instar I	176.52	145.75	199.47	258.71	3.513006	y=3.513×-2.669	5.678
	Instar II	218.67	183.29	251.34	337.36	2.704522	y=2.704×-1.352	4.107
	Instar III	235.18	211.26	262.72	361.64	2.511838	y=2.511×-1.084	3.351
	Instar IV	257.56	229.48	280.51	370.82	2.582189	y=2.582×-1.467	2.393

#### Table 1. Larvicidal activity of A. mexicana aqueous extract against An. stephensi

Values represent mean of five replications. Mortality of the after 24 h of exposure period  $LC_{50}$ = Lethal Concentration brings out 50% mortality and  $LC_{90}$ = Lethal Concentration brings out 90% mortality. LCL= Lower Confidence Limit, UCL= Upper Confidence Limit, <sup>2</sup> = Chi-squire, <sup>a</sup>Significant at *p* 0.05

Table 2. Larvicidal activity of silver nanoparticles synthesized using A. mexicana against An. stephensi

Plant names	Instars	LC <sub>50</sub> (mg/L)	95% Confidence limits		LC (mall)	Slope	Decreasion	2
			LCL	UCL	$LC_{90} (mg/L)$	Slope	Regression	
A. mexicana	Instar I	28.53	23.24	32.52	46.67	3.946671	y=3.946×-0.048	5.464
	Instar II	31.72	25.17	36.96	57.35	2.940226	y=2.940×+0.989	0.651
	Instar III	37.47	30.56	41.24	59.70	2.848000	y=2.848×+0.935	0.484
	Instar IV	42.28	38.57	46.73	65.73	2.853371	y=2.853×+0.659	0.494

Values represent mean of five replications. Mortality of the after 24 h of exposure period  $LC_{50}$ = Lethal Concentration brings out 50% mortality and  $LC_{90}$ = Lethal Concentration brings out 90% mortality. LCL= Lower Confidence Limit, UCL= Upper Confidence Limit,  $^2$  = Chi-squire, <sup>a</sup>Significant at *p* 0.05.

<sup>2</sup> value was significant at p < 0.05 level. Recently, a growing number of plant extracts have been found effective against vector mosquito [Gokulakrishnan et al, 2015; Kovendan et al, 2016]. The mortality effect evoked by Ag NP on mosquito larvae and pupae may be due by the small size of the Ag NP, which allows their passage through the insect cuticle and into individual cells, where they interfere with molting and other physiological processes (Roni et al, 2015). Ramanibai and Velavutham, (2015) reported that the Ag NP biosynthesized using the 2,7,bis [2-[diethylamino]-ethoxy]fluorence isolate from the Melia azedarach leaves did not show acute toxicity against Mesocyclops pehpeiensis copepods. The report toxicity effects of Vina rosa synthesized Ag NP from P. reticulata against An. stephensi and Cx. quinquefasciatus (Subarani et al, 2013), with did not detect toxicity of Ag NP produced using dried green fruits of Drypetes raxburghii against P. reticulata, LC<sub>50</sub> of IV instar larvae of An. stephensi (Haldar et al, 2013).

#### Syntheses and characterization of Ag nanoparticles

The aqueous silver nitrate solution turned brown within 2 min with the addition of A. mexicana aqueous extract and control AgNO<sub>3</sub> solution (without aqueous extract) showed no change of color (Figure 1a and b), and there was no absorption peak in the UV-vis spectrum. It plant, could be able to reduce silver ions and produce silver nanoparticle of incubation at higher pH values of 6 and 7 (Fig. 2). The production of the silver nanoparticles synthesized from leaf extract of A. mexicana was evaluated through spectrophotometer in a range of wavelength from 200 to 1200 nm. This revealed a peak at 350 nm in leaf extract indicating the production of silver nanoparticles. This is similar to the synthesis of Ag NPs using various plant extracts (Kumar and Yadav, 2009). This behavior may be due to the excitation of the surface Plasmon resonance (SPR) effect and to the chemical reduction of AgNO<sub>3</sub> (Mulvaney, 1996; Krishnaraj et al, 2010).

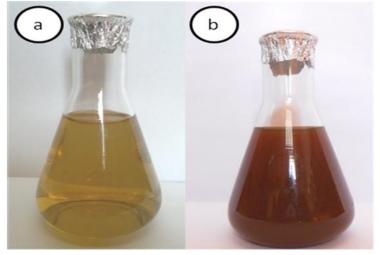


Figure 1: Photograph showed changes in colour after adding AgNO<sub>3</sub> a before reaction and 6 h after reaction.

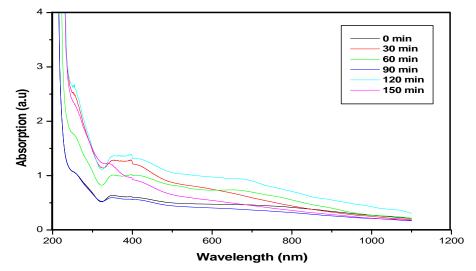


Figure 2. UV-vis spectra of aqueous silver nitrate with Argemone mexicana leaf extract

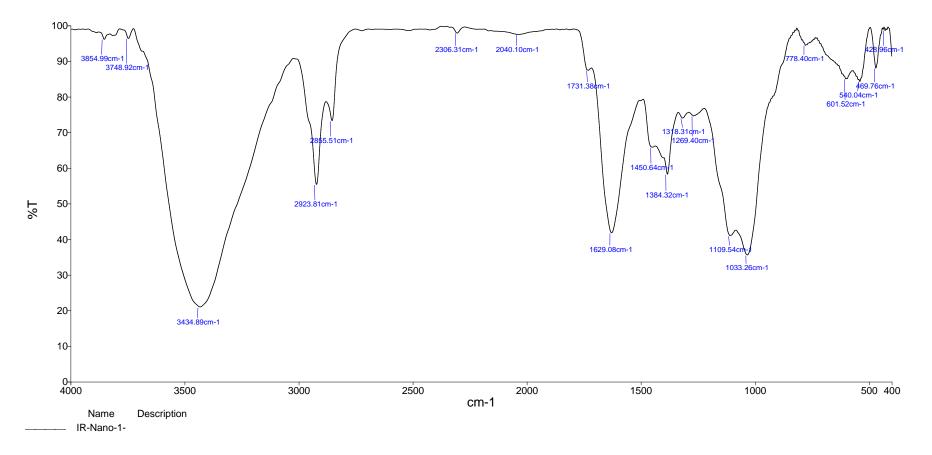


Figure 3: FTIR spectrum of synthesized AgNPs using Argemone mexicana leaf extract

FTIR analysis supports our hypothesis that the bioreduction of  $Ag^+$  ions to  $Ag^{\circ}$  carried out by A. mexicana cake borne metabolite. Indeed, the FTIR spectrum showed major peaks at 3854.99, 3748.92, 2923.81, 2855.51, 2306.31, 2040.10, 3434.89, 1629.08, 1450.64, 1384.32, 1318.31, 1731.38. 1269.40, 1109.54, 1033.26, 778.40, 601.52, 540.04, 469.76, 428.96 cm<sup>-1</sup> (Fig. 3). Above the peak value they corresponded to functional groups like alcohols, phenols (O-H stretch, H-bonded, 3434.89 cm<sup>-1</sup>). These result peaks are very similar to the results of previous studies by Mohan et al, (2007) and Kora et al, (2010). Shanmugam et al, (2014) suggested that these bonds could be due to the stretching of -OH in proteins, enzymes, or polysaccharides present in the extract. Alkanes (C-H stretch, 2923.81, 2855.51 cm<sup>-1</sup>), carboxylic acids (C=O stretch, 1731.38 cm<sup>-1</sup>), 1\* amines (N-H bend, 1629.08 cm<sup>-1</sup>), nitro compounds (N-O asymmetric stretch 1450.64 strong, 1318.31 cm<sup>-1</sup> medium), alcohols, carboxylic acids, esters, ethers (C-O stretch, 1269.40, 1109.54, 1033.26 cm<sup>-1</sup>). Previous report, Li *et al*,(2007) the peak at 2962 cm<sup>-1</sup> indicated carboxylic acid. Alkyl halides (C-Cl stretch, 778.40 cm<sup>-1</sup> and C-Br stretch, 601.52 cm<sup>-1</sup>). Benelli, (2016) reported that the peak near 659 cm-1 was assigned to CH out of plane bending vibrations of substituted ethylene systems –CH=CH.

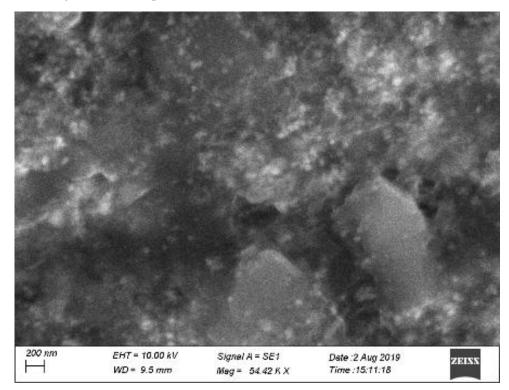


Figure 4: Scanning electron micrograph of AgNPs synthesized with *Argemone mexicana* leaf extract and 9.5 mM AgNO<sub>3</sub> solution and incubated at 60 °C for 6 h at pH 7.0; magnified ×30000 kV, in set bar 200nm.

SEM showed that the Ag NP biosynthesized using the *A. mexicana* cake extract were mostly spherical or cubic in shape, with a mean size ranging from 9.5 to 54.42 nm (Fig. 4). Similarly, spherical and cubic nanoparticles are the most common products of nanosynthesis green routes (Benelli, 2016), with the exception of neem-febricated ones, which yielded polydispersed particles both with spherical and flat plate-like morphology 5-35 nm in size (Shankar *et al*, 2004). However, we did not detected spherical Ag NP among the nanoparticles synthesized using the *A*.

*mexicana* cake extract and this can be due to different reducing agents present in *A. mexicana* cake products. Also, previous studied the *Sargassum muticum* mediated synthesis of Ag NP, indicating that they were well dispersed and with a size range of 43-79 nm (Madhiyazhagan *et al*, 2015). Furthermore, SEM images of Ag NP fabricated using *Emblica officinalis* were also predominantly spherical with aan average size of 16.8 nm ranging from 7.5 to 25 nm (Ankamwar *et al*, 2005).

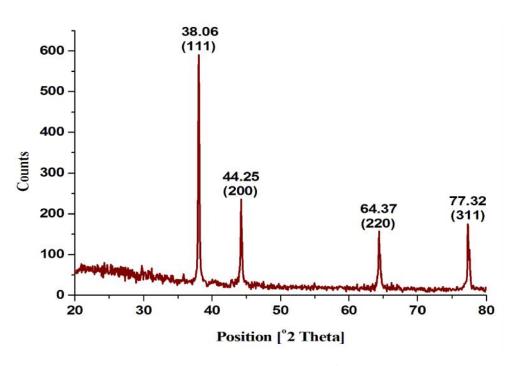


Figure 5: XRD showing synthesized AgNPs from Argemone mexica

The X-ray diffraction pattern of silver nanoparticles produced by leaf extract is shown in Fig. 5. XRD analysis showed intense peaks at 2 values of 38.06°, 44.25°, 64.37°, and 77.32° corresponding to 111, 200, 220, and 311 planes of the cubic face-centered silver. The lattice constant calculated from this pattern was =4.026 Å. The obtained data was matched to the database of the Joint Committee on Powder Diffraction Standards (JCPDS) file No. 04.0783. This result is in agreement with a previous result, where Ag NPs were synthesized using leaf extract Acalypha indica against waterborne pathogens was investigated (Krishnaraj et al, 2010) and Bragg's reflection based on the fcc structure of silver nanopaeticles (Borchert et al, 2005). Thus, XRD spectrum confirmed the formation of silver nanoparticles (Balaji et al, 2009). A few unassigned peaks were also noticed in the vicinity of the characteristic peaks. These sharp Bragg peaks might have resulted due to the capping agent stabilizing the nanoparticle. The XRD pattern of pure silver ions is known to display peaks at  $2 = 7.9^{\circ}$ , 11.4°, 17.8°, 30.38°, and 44° (Gong et al, 2007). Therefore XRD results also suggest that crystallization of the bioorganic phase occurs on the surface of the silver nanoparticles.

#### Conclusion

In conclusion, green synthesis shows that the environmentally benign and renewable source of A. mexicana is used as an effective reducing agent for the synthesis of AgNPs. This biological reduction of silver nanoparticles would be a boon for the development of clean, nontoxic and environmentally acceptable green approach to produce AgNPs involving organisms even renging to higher plants. The formed AgNPs are highly stable and have significant mosquito larvicidal activity of An. stephensi. This is the first report on the larvicidal mosquito activity synthesized of nanoparticles from A. mexicana.

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