J Clust Sci (2018) 29:27–39
https://doi.org/10.1007/s10876-017-1300-3

ORIGINAL PAPER

Poly(Styrene Sulfonate)/Poly(Allylamine Hydrochloride) Encapsulation of TiO_2 Nanoparticles Boosts Their Toxic and Repellent Activity Against Zika Virus Mosquito Vectors

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Received: 17 July 2017 / Published online: 27 September 2017

Abstract Green fabricated nanoparticles often need to be encapsulated and stabilized, to ensure uniform dispersion in the aquatic environment and relevant larvicidal activity over time. However, recent research showed that nanoencapsulation processes led to a reduction of nanoparticle larvicidal efficacy. We used an extract of *Argemone mexicana* to reduce TiO_2 nanoparticles, which were then capped with PSS/PAH (poly(styrene sulfonate)/poly (allylamine hydrochloride)). The toxic and repellent potential of the nanoparticles were compared to elucidate their potential effects against the Zika virus vector *Aedes aegypti*. Nanoparticles were characterized by biophysical methods including UV–Vis, EDX and FTIR spectroscopy, SEM, TEM, XRD and DLS analyses. In larvicidal and pupicidal experiments, TiO_2 nanoparticles achieved LC_{90} values from 41.648 (larva I), to 71.74 ppm (pupa). Nanoencapsulated TiO_2 achieved LC_{90} values from 39.16 (I), to 69.12 ppm (pupa). In adulticidal experiments, LC_{90} of TiO_2 nanoparticles on *Ae. aegypti* was 10.31 ppm, while LC_{90} of nanoencapsulated TiO_2 was 9.54 ppm. At 10 ppm, the repellency towards *Ae. aegypti* was 80.43% for TiO_2 nanoparticles, and 88.04% for nanoencapsulated TiO_2. This research firstly highlighted the promising potential of PSS/PAH encapsulation, leading to the production of highly effective titania nanostructures, if compared to titania nanoparticles synthesized with eco-friendly routes without further stabilization.

Keywords *Aedes aegypti* · *Argemone mexicana* · Dengue fever · Encapsulation · Repellent activity

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Introduction

Insects are vectors of key diseases leading to significant outbreaks and epidemics in the increasing global populations of humans and animals [10, 58]. Mosquitoes include more than 3500 species. However, less than 100 species, mostly belonging to *Aedes, Anopheles* and *Culex* genera, are reported as vectors for diseases that affect humans and other vertebrates. Good examples are malaria, dengue, yellow fever, West Nile, filariasis and Zika virus [16, 78]. These mosquito-borne diseases cause high morbidity and mortality worldwide, and represent a major economic burden within endemic countries [32, 55].

Dengue is primarily transmitted by females of the yellow fever mosquito *Aedes aegypti*, and the Asian tiger mosquito *Aedes albopictus* [18, 76]. *Ae. aegypti* is also a vector for other viral diseases such as yellow fever, chikungunya, and—as showed by recent outbreaks—Zika virus [14]. It is a highly anthropophilic, endophilic, and endophagic day-biting species with an autogenous feeding behavior. This cosmotropical species can rapidly adapt to different anthropogenic environments. *Ae. aegypti* mosquitoes lay eggs in various aquatic habitats—including small-size ones—such as water-filled plastic containers and tires, tree holes, wells, temporary and permanent pools, and marshy areas, which are often close to human settlements [79].

The first clinically recognized dengue epidemic occurred almost simultaneously in Asia, Africa, and North America in the 1780 s [80]. The World Health Organization estimates that dengue infects approximately 50–400 million people annually in the tropical and subtropical regions [81]. Dengue fever is endemic in Southeast Asian countries including India, Bangladesh, and Pakistan, and its spread is associated with population growth and uncontrolled urbanization in tropical countries, and has become an important public health problem as the number of reported cases continues to increase [14]. Dengue fever is characterized by fever, headache, muscle and joint pains, rash, nausea, and vomiting [58, 59], while more severe forms include dengue hemorrhagic fever and dengue shock syndrome [56].

One of the approaches for the management of mosquito-borne infections is the interruption of disease transmission by killing vectors or preventing mosquitoes from biting [5]. Moreover, due to their low mobility in breeding habitats, young mosquito instars are attractive targets for pest control operations, even if larvicidal treatments are not recommended for rural areas [16]. However, these operations are weakened by the emerging resistance of mosquitoes to synthetic insecticides [35], 49], thus botanical insecticides may be suitable alternative control strategies to pursue [3, 19, 20, 26, 45, 46, 54, 63, 64, 73]. The massive screening of plant materials and fungi as sources of metabolites for parasitological studies is worthy of attention, as elucidated by the recent example given by Y. Tu, who received the Nobel Prize for her discovery of artemisinin [14]. Notably, plant-borne molecules are often effective at a few parts per million against young instars of *Aedes, Anopheles* and *Culex* mosquitoes [11, 52, 53].

Nanotechnology can revolutionize the biomedical, agricultural and veterinary industry [17, 40], leading to the development of novel tools for pest and vector management [9, 15, 50, 57, 72]. Nanoparticles are usually smaller than 100 nm in each spatial dimension, and can be synthesized using top-down and bottom-up strategies. In recent years, the application of nanotechnology in pest management has revolutionized the application of pesticides [6–8, 12, 13, 67, 68]. As arthropod vectors rapidly gain pesticide resistance, there is an urgent need to synthesize novel products via eco-friendly nanosynthesis routes [9, 20].

Titanium dioxide nanoparticles have attracted considerable attention because of their unique physico-optical properties, and are widely used for sunscreen and toothpaste production, surface coating, and water treatment [42]. Constituents from plant extracts can be used to reduce metal ions to nanoparticles in a green single-step synthesis. Recently, researchers proposed the green synthesis of nanomaterials with methods using naturally occurring components such as vitamins, sugars, plant extracts, biodegradable polymers and microorganisms acting as reducing and capping agents [17].

Argemone mexicana L. (Papaveraceae), commonly known as the prickly poppy, is used in folk medicine in several countries [23] for its analgesic, antibacterial, antimalarial, antispasmodic, sedative and narcotic effects. *A. mexicana* seeds are useful for treating cough and asthma [38] and possess anti-HIV, antioxidant, anxiolytic, hepatoprotective, and sedative activities [4]. Among its main secondary metabolites, benzylisoquinoline alkaloids appear to be the most important for the biological activities, namely antimicrobial, antiparasitic, antimalarial, pesticide and neuroprotective ones [60].

Green fabricated nanoparticles often need to be encapsulated and stabilized, to ensure uniform dispersion in the aquatic environment and relevant insecticidal activity over time [21, 22]. Nanoencapsulation is a process through which a chemical such as an insecticide is slowly and efficiently released for insect pest control, allowing proper absorption of the chemical into plants as well as in the aquatic environment [62]. The release mechanisms of nanoencapsulated pesticides include diffusion, dissolution, biodegradation and pH-specific osmosis [25, 75]. Nanoencapsulation has been widely used in biomedicine, since some of the products are highly tissue-compatible. Besides their potential application as scaffolds in tissue engineering, environmentally sensitive hydrogels, or as sustained-release delivery systems, nanoencapsulated molecules or cells can be used as biosensors, drug carriers and insecticides [44].

However, recent research showed that several nanoencapsulation processes led to a serious reduction of larvicidal efficacy of metal and metal oxide nanoparticles [48]. To face the above mentioned challenge, in this study, we developed a novel titanium dioxide nanocomplex with mosquitocidal potential using *A. mexicana* extracts encapsulated with the polymers poly(styrene sulfonate)/poly(allylamine hydrochloride) (PSS and PAH). Both *A. mexicana*-synthesized TiO_2 nanoparticles and PSS/PAH encapsulated TiO_2 nanoparticles were characterized using UV–visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD),energy-dispersive X-ray analysis (EDX), and field emission scanning electron microscopy (SEM). Furthermore, the toxicity of *A. mexicana*-synthesized TiO_2 nanoparticle sand PSS/PAH encapsulated TiO_2 nanoparticles was comparatively assessed on larvae, pupae and adults of the dengue and Zika virus vector *Ae. aegypti*. We also investigated the repellent activity of *A. mexicana*-synthesized TiO_2 nanoparticles and PSS/PAH encapsulated TiO_2 nanoparticles on *Ae. aegypti* adults over different exposure times.

Materials and Methods

Green Synthesis, Encapsulation, and Characterization of TiO_2 Nanoparticles

Fresh *A. mexicana* leaves were collected from the Bharathiar University Campus (Coimbatore, Tamil Nadu, India). The *A. mexicana* leaves were gently washed with tap water and dried in the shade at room temperature, cut into fine pieces, grinded, and sieved to produce a fine powder. Extraction of 50 g of the plant material was performed in 300 mL of methanol for 8 h in a Soxhlet apparatus [77]. The crude plant extract was evaporated to dryness at room temperature.

TiO_2 nanoparticles were synthesized in an Erlenmeyer flask by reacting 0.4 M of titanium tetra-isopropoxide with the *A. mexicana* leaf extract. After 4 h of continuous stirring at 50 °C, the mixture was centrifuged at 5000 rpm for 15 min to obtain a colloidal solution of TiO_2 nanoparticles, which was washed with ethanol and centrifuged at 5000 rpm for 10 min. TiO_2 nanoparticles were separated via annealing at 400 °C in a muffle furnace for 3 h to yield a nanopowder. Experimental concentrations (10, 20, 30, 40, and 50 ppm) were prepared by dilution in distilled water. All stocks and dilutions were stored at − 4 °C.

Calcium carbonate($CaCO_3$) particles with nano-dispersed diameters were prepared by mixing 0.21 g of Na_2CO_3 and 0.29 g of $CaCl_2 \cdot 5H_2O$ in 20 mL of H_2O under magnetic agitation with 0.29 g of polystyrene sulfate (PSS). After 30 min, the $CaCO_3$ particles were centrifuged at 5000 rpm for 10 min and washed in water 3 times. Polyelectrolytes (2 mg/mL) were adsorbed onto the $CaCO_3$ particles by immersing the particles in 0.1 M of Tris–HCl buffer (pH 7.0) for 15 min followed by three washes in distilled H_2O. The mixture was incubated for 15 min under gentle shaking, and excess electrolytes were removed by centrifugation at 5000 rpm for 10 min and washing in water for 3 h. After assembly of the four subsequent polyelectrolytes bilayers of PSS/PHH/PSS/PAH, the particles were formed by dissolving. The $CaCO_3$ core was immersed in 0.2 M EDTA solution (100 mL in Tris–HCl buffer at pH 7.0) for 30 min under agitation, and subsequently centrifuged (5000 rpm for 10 min). After washing in water, the hollow microcapsules were re-dispersed in 10 mL of sample solution at a different pH, and the mixture was incubated at room temperature for 15 min. Then, the particles were centrifuged at 5000 rpm for 10 m to remove the suspension.

The presence of *A. mexicana*-synthesized TiO_2 nanoparticles and PSS/PAH encapsulated TiO_2 nanoparticles in the tested preparations was confirmed by sampling the colloidal solutions at regular intervals for UV–Vis analysis using a Shimadzu UV-3600 spectrophotometer scanned from 200 to 700 nm with a resolution of 1 nm. The mixture was then centrifuged at 15,000 rpm for 20 min, the pellet was dissolved in distilled water, then filtered through a 0.45-mm Millipore filter. The morphology of the *A. mexicana*-synthesized TiO_2 nanoparticles and PSS/PAH encapsulated TiO_2 nanoparticles was analyzed using a 10-kV ultra-high-resolution FEI Quanta 200 SEM, where 25 μL of sample were sputter-coated onto a copper stub. The surface groups of the *A. mexicana*-synthesized TiO_2 nanoparticles and PSS/PAH encapsulated TiO_2 nanoparticles were qualitatively confirmed by FTIR spectroscopy [66] using a Perkin-Elmer Spectrum 2000 FTIR spectrophotometer. XRD and EDX were shed light on the crystalline structure and elemental composition of the samples, respectively [67, 68]. TEM was performed using a JEOL 1200 EX microscope operating at an accelerating voltage of 120 kV. Samples were prepared by placing a drop of colloidal solutions of *A. mexicana*-synthesized TiO_2 nanoparticles and PSS/PAH encapsulated TiO_2 nanoparticles on carbon-coated TEM grids. The film on the TEM grid was dried for 5 min under laboratory conditions. The DLS particle size measurements were carried out using a Malveern Zetasizer Nano ZS. Samples were diluted to

0.1 wt% using C12-C15 alkyl benzoate. The measurement duration was set to automatic, and five repeated measurements were taken at 25 °C. The TiO_2 and capped TiO_2 samples were run using the refractive index obtained using Malveern Mastersizer 2000 [2].

Larvicidal, Pupicidal and Adulticidal Activity

Aedes aegypti mosquitoes were reared as reported by Sujitha et al. [70]. Larvicidal and pupicidal assays were conducted in laboratory conditions [27 ± 2 °C, 75–85% R.H., 14:10 (L:D) photoperiod] by testing both *A. mexicana*-synthesized TiO_2 nanoparticles and PSS/PAH encapsulated TiO_2 nanoparticles. Following the method by Murugan et al. [46], in the toxicity tests, 25 first, second, third, fourth and instar larvae and pupae were kept in glass beakers containing 250 mL of dechlorinated water plus the desired concentration of *A. mexicana*-synthesized TiO_2 nanoparticles or PSS/PAH encapsulated TiO_2 nanoparticles. Each dose, as well as the negative control (where no nanoparticles were added) was replicated 5 times. Control mortalities were corrected as indicated by Abbott [1].

In adulticidal assays, based on wide and narrow range tests, *A. mexicana*-synthesized TiO_2 nanoparticles and PSS/PAH encapsulated TiO_2 nanoparticles were tested at 2, 4, 6, 8, and 10 ppm (formulated in 5 ml of aqueous solution), applied on Whatman no. 1 filter papers (size 12 cm × 15 cm). Control papers were treated with distilled water; 20 *Ae. aegypti* females were collected and gently transferred into a plastic holding tube. *Ae. aegypti* females were allowed to acclimatize in the holding tube for 1 h and then exposed to the test paper for 1 h. At the end of the exposure period, the mosquitoes were transferred back to the holding tube for a 24 h recovery period. A cotton pad soaked with 10% of glucose solution was placed on the mesh screen. Each test included a set control groups with five replicates.

Repellent Activity

In repellent assays, a treated and a control cotton pad were soaked with goat blood and placed in opposite directions inside a glass container; then the treated pads were soaked in different concentrations of *A. mexicana* extracts; 20 *Ae. aegypti* females were released into each container, and the number of females landing on each pad was recorded with the slightly modified protocol by Govindarajan and Sivakumar [34]. The repellency of treated and control pads were calculated by the following formula:

$$\frac{C - T}{C} \times 100$$

where C and T is the number of mosquitoes on the control and treated pad, respectively.

Statistical Analysis

Toxicity and repellent data were subjected to ANOVA, then the means were separated by Tukey's HSD test ($P < 0.05$); mosquito mortality data were subjected to probit analysis. LC_{50} and LC_{90} were calculated using the method reported by Finney [30]; Chi square values were not significant [15]. Data were analyzed using the SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). A probability level of $P < 0.05$ was used to evaluate the statistical significance of differences between values.

Results

Green Synthesis, Encapsulation, and Characterization of TiO_2 Nanoparticles

The formation and stability of the *A. mexicana*-synthesized TiO_2 nanoparticles was monitored by UV–Vis spectrophotometry, where the UV–Vis spectrum showed a maximum absorbance at 370 nm (Fig. 1a). PSS/PAH encapsulated TiO_2 nanoparticles showed a minimum and maximum absorbance at 148 nm and 370 nm (Fig. 1b), respectively; it was also observed that absorbance increased with the incubation time.

The FTIR spectrum of *A. mexicana*-synthesized TiO_2 nanoparticles indicated several potential functional groups from the *A. mexicana* extracts which probably acted as capping and reducing agents in titania nanosynthesis. Peaks were found at 668 and 1320 cm^{-1} (Fig. 2a). The stretching frequencies at 1658, 3120, 3468, and 3660 cm^{-1} can be linked to the presence of organic components in the poppy extract, which were attached to or adsorbed on the surfaces of PSS/PAH encapsulated TiO_2 nanoparticles (Fig. 2b).

The XRD spectrum for *A. mexicana*-synthesized TiO_2 nanoparticles (Fig. 3a) shows five intense peaks with 2θ values of 27°, 41°, 54°, 63°, and 69°, which were assigned to the (101), (100), (111), (211), and (200) planes of the face centered cubic (fcc) phase, which are comparable with the standard (JCPDS 89-3722) TiO_2 lattice. A few unassigned peaks were also seen near these characteristic peaks. For the PSS/PAH encapsulated TiO_2 nanoparticles (Fig. 3b), peaks with 2θ values of 19°, 29°, 26°, 43°, and 54° were assigned to the (100), (101), (111), (201), and (202) planes of the face centered cubic (fcc) phase.

According to SEM and TEM analyses, the morphology of *A. mexicana*-synthesized TiO_2 nanoparticles was mostly rod-shaped; the titania nanoparticles formed aggregates

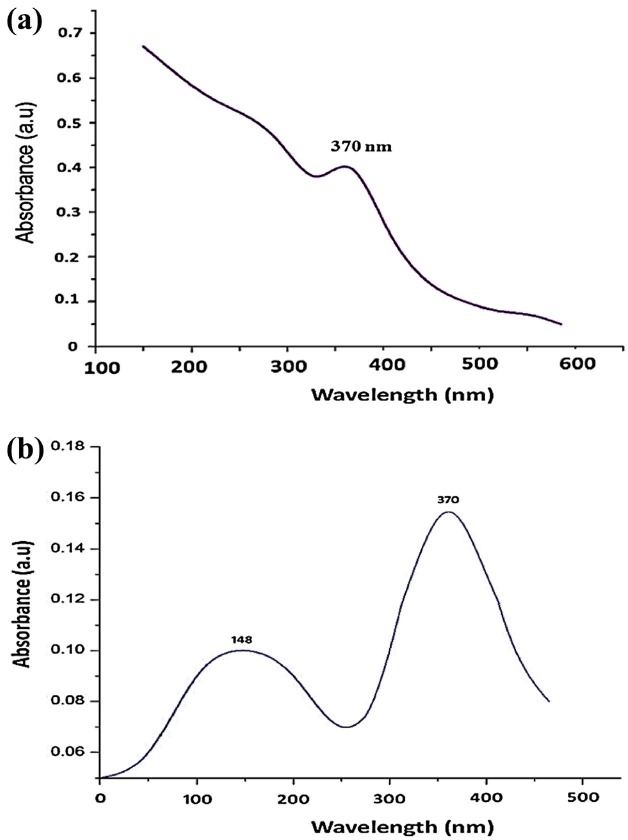

Fig. 1 UV-visible spectra of **a** *Argemone mexicana*-fabricated TiO_2 nanoparticles (120 min after the reaction) and **b** PSS/PAH encapsulated TiO_2 nanoparticles

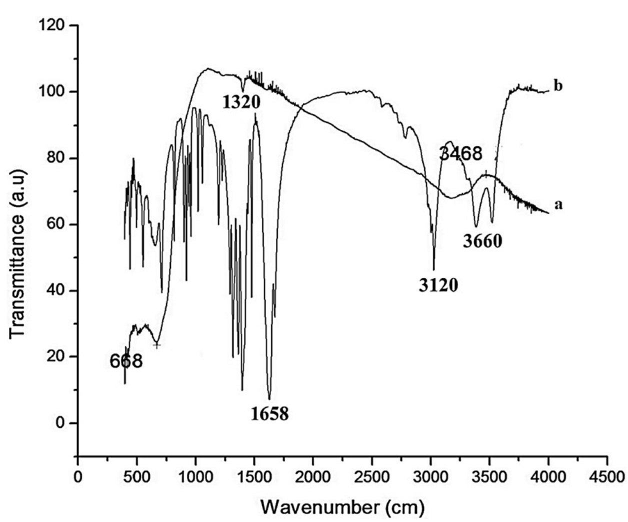

Fig. 2 FT-IR spectra of *a Argemone mexicana*-fabricated TiO_2 nanoparticles and *b* PSS/PAH encapsulated TiO_2 nanoparticles

that were about 50 nm in diameter (Figs. 4 and 5). The rod-shaped *A. mexicana*-synthesized TiO_2 nanoparticles (Figs. 4a and 5a) had cubic structures and increased in size to about 65 nm after the encapsulation with PSS/PAH (Figs. 4b and 5b). Figure 5b shows the morphology of the PSS/PAH encapsulated TiO_2 nanoparticles, formed aggregates of about 40 nm in diameter and TiO2 exhibited 23 nm.

TheTiO_2 nanoparticles generally have a typical absorption peak at 4.9 and 0.1 keV due to the SPR phenomenon. Figure 6 shows a representative EDX profile of the sample spot, showing strong titanium and oxygen signals along with calcium peaks at approximately 0.1, 1, 2, and 4 keV. Overall, strong signals in EDX spectra for Ti and O atoms confirmed the presence of TiO_2 (Fig. 6) in the analyzed samples.

Lastly, from the DLS results given in Fig. 7, it is evident that the particles were dispersed fully and exhibited nanosize lower than 25 nm for *A. mexicana*-synthesized TiO_2 nanoparticles (Fig. 7a) and 45 nm for the PSS/PAH encapsulated TiO_2 nanoparticles (Fig. 7b).

Toxic and Repellent Activity Against *Aedes aegypti*

In larvicidal and pupicidal experiments, *A. mexicana*-synthesized TiO_2 nanoparticles achieved LC_{50} values of 17.880 (larva I), 21.708 (II), 26.115 (III), 30.045 (IV), and 35.298 ppm (pupa), while the LC_{90} values were 41.648 (I), 51.148 (II), 56.51 (III), 62.97 (IV), and 71.74 ppm (pupa) (Table 1). Furthermore, PSS/PAH encapsulated TiO_2 nanoparticles achieved LC_{50} values of 16.154 (I), 21.302 (II), 24.265 (III), 27.550 (IV), and 32.881 ppm (pupa), while the LC_{90} values where 39.16 (I), 49.94 (II), 53.82 (III), 61.36 (IV), and 69.12 ppm (pupa) (Table 2).

In adulticidal experiments, LC_{50} and LC_{90} values of *A. mexicana*-synthesized TiO_2 nanoparticles on *Ae. aegypti* were 3.55 and 10.31 ppm, respectively, while the LC_{50} and LC_{90} values of PSS/PAH encapsulated TiO_2 nanoparticles were 3.06 and 9.54 ppm, respectively (Table 3).

In repellence assays, at the maximum concentration tested (10 ppm), the repellency rates calculated on *Ae. aegypti* was significantly lower ($P < 0.05$) for *A. mexicana*-synthesized TiO_2nanoparticles (80.43%), if compared to that achieved by a single treatment with PSS/PAH encapsulated TiO_2 nanoparticles (88.04%) (Table 4).

Discussion

Green Synthesis, Encapsulation, and Characterization of TiO_2 Nanoparticles

UV–Vis spectroscopy is an essential tool to evaluate the production and stability of metal oxide nanoparticles [31]. The formation of *A. mexicana*-synthesized TiO_2 nanoparticles was confirmed by an absorption peak at 370 nm, while PSS/PAH encapsulated TiO_2 nanoparticles showed a

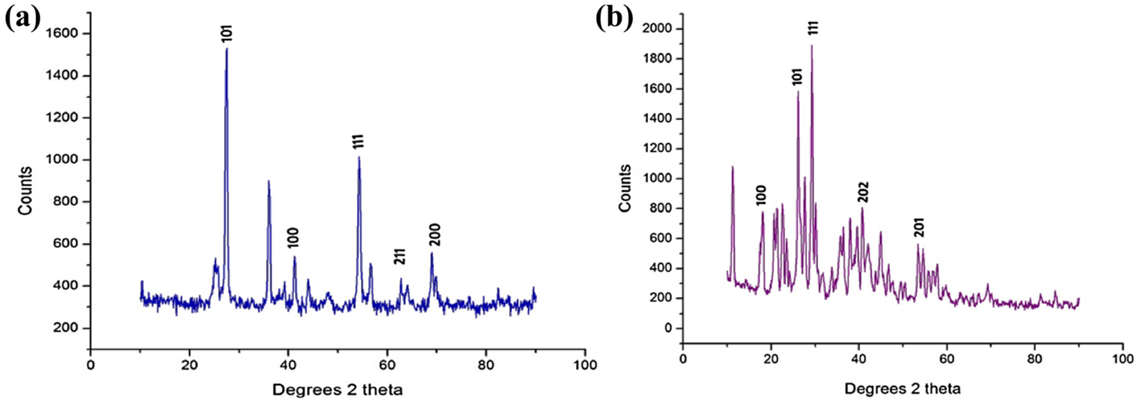

Fig. 3 XRD patterns of **a** *Argemone mexicana*-fabricated TiO_2 nanoparticles and **b** PSS/PAH encapsulated TiO_2 nanoparticles

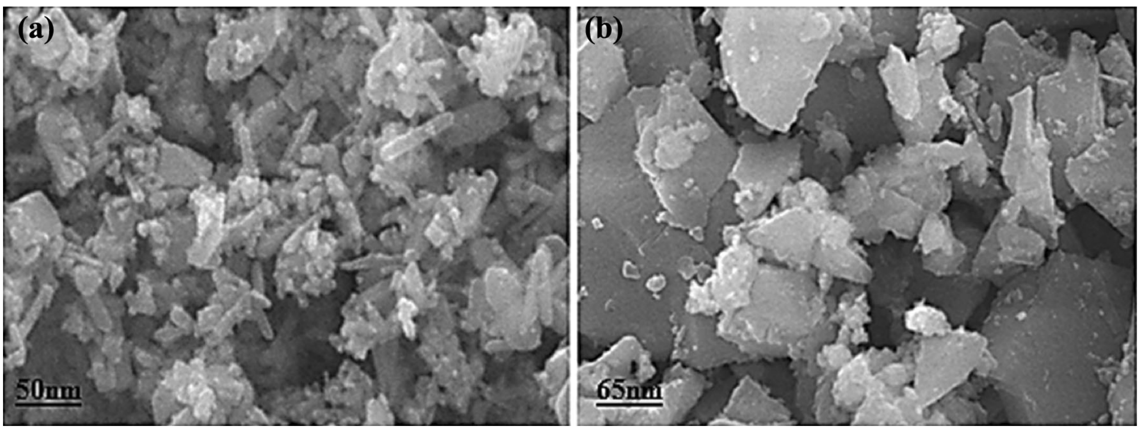

Fig. 4 SEM (20 kV, X30,000) of **a** *Argemone mexicana*-fabricated TiO_2 nanoparticles and **b** PSS/PAH encapsulated TiO_2 nanoparticles

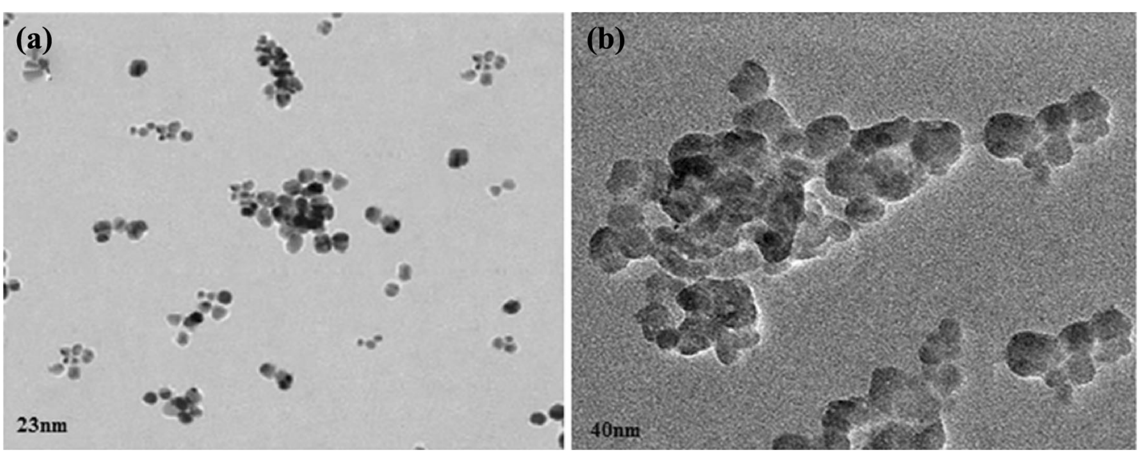

Fig. 5 TEM of **a** *Argemone mexicana*-fabricated TiO_2 nanoparticles and **b** PSS/PAH encapsulated TiO_2 nanoparticles

minimum absorption peak at 148 nm and a maximum absorption peak at 370 nm. Very recently, UV–Vis has been used to study the green synthesis of other metal nanoparticles, including silver and gold ones. About the latter, a recent example has been provided by Murugan et al. [46], which studied the UV–Vis spectrum of gold nanoparticles synthesized using *Cymbopogon citratus* (DC.) Stapf, where the formation of Au nanostructures was confirmed by the presence of an absorption peak at 540 nm.

Concerning the functional groups from the *A. mexicana* extract, which were probably involved in the reduction of

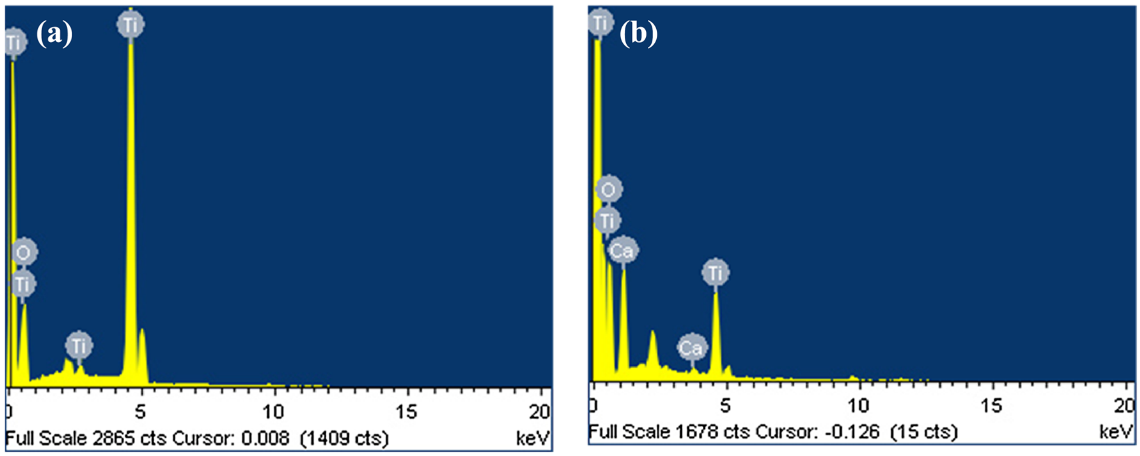

Fig. 6 EDX profiles of **a** *Argemone mexicana*-fabricated TiO_2 nanoparticles and **b** PSS/PAH encapsulated TiO_2 nanoparticles

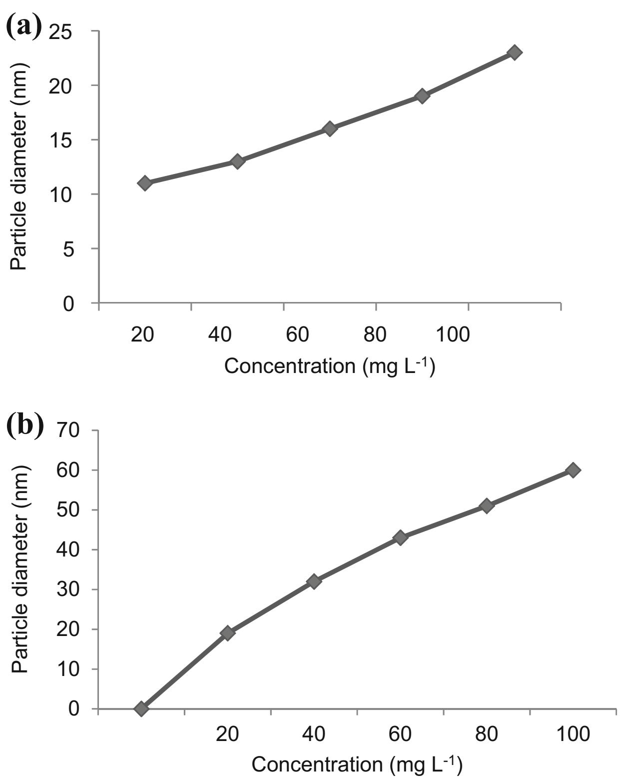

Fig. 7 Dynamic light scattering (DLS) showing particle size of **a** *Argemone mexicana*-fabricated TiO_2 nanoparticles and **b** PSS/PAH encapsulated TiO_2 nanoparticles

TiO_2 nanoparticles, the FTIR peak at 668 cm^{-1} may be linked with the formation of C–Br bonds, while the peak at 1320 cm^{-1} may indicate C=O bonds. The peak sat 1658, 3120, 3468, and 3660 cm^{-1} were probably due to the formation of C=C, C–H, O–H, and O–H bonds, respectively [41, 69]. Based on this information, it was argued that various concentrations of alcohols, phenols, and alkenes were still present in the colloidal solution after the formation of PSS/PAH encapsulated TiO_2 nanoparticles.

Furthermore, the XRD pattern of *A. mexicana*-synthesized TiO_2 nanoparticles showed 5 intense peaks at 27°, 41°, 54°, 63°, and 69°, while the XRD pattern of PSS/PAH encapsulated TiO_2 nanoparticles had peaks at 19°, 29°, 26°, 43°, and 54°. Recently, several XRD studies have been carried out to shed light on the crystalline structure of mosquitocidal nanoparticles [13, 71, 74], including titania nanostructures [47]. Concerning other metal nanoparticles, several studies are available. For example, Madhiyazhagan et al. [41], studied the XRD pattern of Ag nanoparticles synthesized using the extract of the seaweed *Sargassum muticum;* results showed five high diffraction peaks at 45.6°, 51.3°, 58.1°, 66.5°, and 71.1°, indexing the Bragg reflection planes (004), (112), (103), (104), and (006), respectively.

SEM studies pointed out that the reduced *A. mexicana*-synthesized TiO_2 nanoparticles were observed to be mostly rod-shaped, with aggregates always lower of 50 nm in size; on the other hand, encapsulated PSS/PAH encapsulated TiO_2nanoparticles were cubic, with a size of about 65 nm. In both samples, EDX assays revealed the presence of strong Ti and O peaks. The increase in particle diameter confirmed the encapsulation of TiO_2 nanoparticles by PSS/PAH. Notably, the present green synthesis protocol led to the production of titania nanoparticles with different features if compared with the ones synthesized via classic methods, such has hydrothermal synthesis. Indeed, at variance with our results, Murugan et al. [47] showed that titania nanoparticles fabricated via hydrothermal synthesis and analyzed by FESEM showed spherical shapes, not rod-like ones, with a wider size range (i.e. from 20 to 100 nm). These data represent a further evidence of the great potential of plant-mediated green synthesis routes, that can be easily carried out to produce nanoparticles with different features if compared with those obtained with chemical and physical methods (see [21, 22] for recent reviews).

Table 1 Larvicidal and pupicidal toxicity of *Argemone mexicana*-synthesized TiO_2 nanoparticles against the dengue and Zika virus vector *Aedes aegypti*

Target	LC_{50} (LC_{90}) (ppm)	95% Confidence limit LC_{50} (LC_{90})		Regression equation	χ^2 (*d.f.* = 4)
		95% LCL	95% UCL		
Larva I	17.880 (41.648)	14.769 (38.232)	20.428 (46.294)	$y = -0.964 + 0.054x$	$3.042^{n.s.}$
Larva II	21.708 (51.148)	18.337 (46.425)	24.538 (57.946)	$y = -0.945 + 0.044x$	$0.598^{n.s.}$
Larva III	26.115 (56.514)	23.071 (51.156)	28.900 (64.327)	$y = -1.101 + 0.042x$	$0.359^{n.s.}$
Larva IV	30.045 (62.971)	26.996 (56.448)	33.100 (72.825)	$y = -1.169 + 0.039x$	$0.325^{n.s.}$
Pupa	35.298 (71.742)	32.004 (63.292)	39.133 (85.195)	$y = -1.241 + 0.035x$	$0.834^{n.s.}$

n.s. Not significant ($P > 0.05$)

Table 2 Larvicidal and pupicidal toxicity of PSS/PAH encapsulated *Argemone mexicana*-synthesized TiO_2 nanoparticles against the dengue and Zika virus vector *Aedes aegypti*

Target	LC_{50} (LC_{90}) (ppm)	95% Confidence limit LC_{50} (LC_{90})		Regression equation	χ^2 (*d.f.* = 4)
		95% LCL	95% UCL		
Larva I	16.154 (39.165)	12.907 (35.920)	18.750 (43.568)	$y = -0.900 + 0.056x$	$5.203^{n.s.}$
Larva II	21.302 (49.946)	17.980 (45.420)	24.083 (56.410)	$y = -0.953 + 0.045x$	$1.486^{n.s.}$
Larva III	24.265 (53.823)	21.155 (48.844)	27.011 (61.009)	$y = -1.052 + 0.043x$	$0.705^{n.s.}$
Larva IV	27.550 (61.361)	24.282 (54.887)	30.615 (71.223)	$y = -1.044 + 0.038x$	$0.381^{n.s.}$
Pupa	32.881 (69.120)	29.622 (61.129)	36.433 (81.750)	$y = -1.163 + 0.035x$	$1.120^{n.s.}$

n.s. Not significant ($P > 0.05$)

Concerning other metal nano-complexes obtained with green synthesis routes, Suresh et al. [71] reported that SEM of green synthesized Ag^0 nanoparticles showed spherical shapes with an average size of 30–60 nm.

Various attempts have been conducted also to microencapsulate plant-borne products. For instance, Hsieh et al. [37] applied thermal treatments to study the controlled release properties of chitosan-microencapsulated citronella oil, while Mourtzinos et al. [43] encapsulated olive leaf extracts in β-cyclodextrin substrates. In the present investigation, the adoption of DLS showed the nano-particle size of both green synthesized and capped nanoparticle. DLS is an efficient and convenient method to analyze the particle size of nanoparticles [48]. Hence, here DLS has been applied to support TEM data on the size of synthesized nanotitania. Notably, suspensions tested in DLS measurements are often treated with a standard dispersant to give good or at least reproducible dispersions [28]. If this is not carried out, and the sample is simply dispersed in deionized water, the measured size may vary significantly with the tested particle concentration, and because the particle concentration is often adjusted to give optimum obscuration, significant variability of the results may occur. A similar dependence has been reported for 5 nm TiO_2 particles by Fatisson et al. [29]. Moreover, Patri et al. [51] have reported large differences for bigger TiO_2 particles, i.e., about 120 nm at low ionic strength (10 mM NaCl) and 1000–2000 nm in solutions showing high ionic strength.

Toxic and Repellent Activity Against *Aedes aegypti*

The experiments conducted on larval and pupal stages of the dengue and Zika virus vector *Ae. aegypti* showed a relevant toxicity of both *A. mexicana*-synthesized TiO_2 nanoparticles and PSS/PAH encapsulated TiO_2nanoparticles. Notably, the LC_{50} values of *A. mexicana*-synthesized TiO_2 nanoparticles (i.e., from 17.880 ppm on first instar larvae to 35.298 ppm on pupae) were slightly higher if compared with PSS/PAH encapsulated TiO_2nanoparticles (i.e., from 16.154 ppm on first instar larvae to 32.881 ppm on pupae). Moreover, the same trend was confirmed in adulticidal experiments, where *A. mexicana*-synthesized TiO_2 nanoparticles had a LC_{50} of 3.55 ppm, while PSS/PAH encapsulated TiO_2 nanoparticles had slightly lower LC_{50} (3.06 ppm). Notably, also in repellence assays, the repellency rates calculated on *Ae. aegypti* were lower for *A. mexicana*-synthesized TiO_2 nanoparticles (80.43%) if compared to PSS/PAH encapsulated TiO_2 nanoparticles (88.04%).

To our mind, these findings represent a rather rare evidence in the field of green nanosynthesis of mosquitocidal nanoparticles. Indeed, earlier attempts failed to maintain the high insecticidal activity of green synthesized nanoparticles after the encapsulation process; very recently, Murugan et al. [48] proposed the bio-encapsulation of chitosan-Ag nanocomplex, showing activity against *Anopheles stephensi* malaria vectors in laboratory and in the field. Both nano-products also reduced longevity and fecundity of *An. stephensi*. The bioencapsulated chitosan-Ag nanocomplex showed a lower larvicidal and pupicidal toxicity (LC_{50} range: 54.65–98.17 ppm) if compared with non-encapsulated chitosan-fabricated Ag nanoparticles (LC_{50} range: 4.43–7.64 ppm) [48].When comparing the toxicities of non-encapsulated and encapsulated *A. mexicana*-TiO_2 nanoparticles, the increase of toxicity detected here was in accordance with the findings by Cheung and Hammockm [24], who carried out the micro-lipid droplet encapsulation of *Bacillus thuringiensis* subsp. *israelensis* endotoxins, reporting its toxicity against the larvae of four mosquito species, i.e., *Ae. aegypti, An. freeborni, Cx. pipiens* and *Cx. tarsalis.* Later, Hadapad et al. [36] evaluated the residual activity of sustained-release biopolymer-based formulations for *B. sphaericus* strains against *Cx. quinquefasciatus* larvae. The present work reports an eco-friendly and simple method allowing the encapsulation of titania nanoparticles without lowering their mosquitocidal activity against the dengue and Zika virus vector *Ae. aegypti*.

More generally, in the latest years, a wide number of green routes aimed at the production of nanoparticles toxicity to various mosquito species have been developed. Most of the studies focused on the toxicity of plant-synthesized metal nanoparticles, with special reference to silver ones [12, 39, 65]. On the other hand, only few researches focused on the toxicity of titania nanoparticles against mosquito young instars [47]. Besides plant-based fabrication processes, bacterial and fungal extract have also been employed to prepare various metal nanoparticles. For example, Ag nanoparticles have been fabricated using extracts of the filamentous fungus *Cochliobolus lunatus* and then tested against *Ae. aegypti* II–IV larval instars, achieving LC_{50} of 1.29, 1.48, and 1.58 ppm, respectively [61].

In addition to being used as reducing and capping agents for nanosynthesis, a number of phytochemicals have been tested for their mosquito larvicidal, adulticidal and repellent properties [27, 33]. The review by Pavela [52] recently highlighted that several plant-borne compounds showed relevant activity as mosquito larvicides. Also, Amerasan et al. [3] studied the adulticidal activity of

Table 3 Adulticidal activity of *Argemone mexicana*-synthesized titanium nanoparticles and PSS/PAH encapsulated TiO_2 nanoparticles against the dengue and Zika virus vector *Aedes aegypti*

Treatment	Mortality (%) (mean ± SD)						LC_{50} (95% LCL–UCL)	LC_{90} (95% LCL–UCL)	χ^2 ($d.f = 4$)
	Control	2 ppm	4 ppm	6 ppm	8 ppm	10 ppm			
TiO_2 nanoparticles	0.0	40.6 ± 2.41	53.2 ± 1.92	64.6 ± 2.07	78.8 ± 1.92	91.2 ± 2.59	3.55 (2.62–4.24)	10.31 (9.23–11.96)	$1.310^{n.s.}$
PSS/PAH encapsulated TiO_2 nanoparticles	0.0	44.2 ± 2.39	56.4 ± 2.70	68.8 ± 2.59	82.6 ± 2.07	93.6 ± 2.41	3.068 (2.08–3.78)	9.546 (8.574–11.000)	$1.420^{n.s.}$

$^{n.s.}$Not significant ($P > 0.05$)

Table 4 Repellent activity (%) of *Argemone mexicana*-synthesized titanium nanoparticles (NP) and PSS/PAH encapsulated titanium nanoparticles (eNP) against the dengue and Zika virus vector *Aedes aegypti*

Activity (h)	Control	2 ppm		4 ppm		6 ppm		8 ppm		10 ppm	
		NP	eNP	NP	eNP	NP	eNP	NP	eNP	NP	eNP
10.00–11.00	30 ± 3.60	27 ± 2.64	26 ± 1.73	22 ± 2.64	20 ± 2.00	17 ± 5.29	14 ± 4.35	12 ± 1.00	10 ± 2.82	8 ± 4.00	5 ± 0.70
12.00–13.00	25 ± 0.57	22 ± 1.73	21 ± 2.00	19 ± 5.29	16 ± 4.58	15 ± 4.00	11 ± 1.73	10 ± 2.64	7 ± 0.00	5 ± 1.00	4 ± 1.41
14.00–15.00	18 ± 3.60	15 ± 4.58	14 ± 1.41	12 ± 2.00	10 ± 2.82	10 ± 1.00	6 ± 1.41	8 ± 2.64	3 ± 1.41	3 ± 1.00	2 ± 1.41
16.00–17.00	12 ± 0.00	10 ± 1.00	7 ± 3.00	8 ± 1.73	4 ± 0.00	5 ± 2.64	2 ± 0.70	3 ± 1.73	1 ± 0.70	2 ± 0.00	0 ± 0.00
18.00–19.00	7 ± 2.00	6 ± 0.00	5 ± 2.64	4 ± 0.00	4 ± 2.64	2 ± 1.00	2 ± 0.00	1 ± 0.57	0 ± 0.00	0 ± 0.00	0 ± 0.00
Fed mosquitoes	92 ± 2.00	79 ± 1.00	73 ± 1.00	65 ± 4.00	54 ± 3.46	49 ± 1.00	35 ± 0.70	34 ± 3.60	21 ± 2.82	18 ± 2.00	11 ± 2.82
Unfed mosquitoes	8 ± 2.64	21 ± 1.00	24 ± 4.58	35 ± 1.00	46 ± 2.0	51 ± 3.60	65 ± 2.82	66 ± 3.60	79 ± 1.41	82 ± 2.00	89 ± 2.82
Protection (%)	–	14.13[a]	20.6[b]	29.35[c]	41.30[d]	46.74[e]	61.96[f]	63.04[f]	77.17[g]	80.43[g]	88.04[h]

Within the row, means followed by the same letter(s) are not significantly different (ANOVA, Tukey's HSD test, $P < 0.05$)

Senna tora (L.) Roxb. extracts in hexane, chloroform, benzene, acetone, and methanol against *Ae. aegypti*. The adulticidal activity of the essential oil of *Lantana camara* L. was evaluated against five mosquito species, *Ae. aegypti, Cx. quinquefasciatus, An. culicifacies, Ancylus fluvialitis*, and *An. stephensi*, on impregnated papers at concentrations of 0.208 mg/cm^2, where the KDT_{50} and KDT_{90} values of the oil were 20, 18, 15, 12, and 14 min and 35, 28, 25, 18, and 23 min, respectively; mortality percentages for each species were 93.3, 95.2, 100, 100, and 100%, respectively [26]. However, on the other hand, our knowledge about the adulticidal potential of green synthesized nanoparticles remains scarce, if compared with research efforts on their larvicidal and pupicidal potential [12, 39].

Conclusions

Overall, here we used the poppy species *A. mexicana* to reduce titanium dioxide nanoparticles, which were then capped with PSS/PAH. The toxicity and repellent potential of both nanoparticles were compared to elucidate their potential effects against the dengue and Zika virus vector *Ae. aegypti*. At variance with earlier studies, this research firstly highlighted the highly promising potential of PSS/PAH encapsulation. This process led to the production of highly stable encapsulated titania nanostructures showing higher toxicity and repellent potential against *Ae. aegypti,* if compared with titania nanoparticles synthesized with eco-friendly routes without further stabilization.

Acknowledgements The authors extend their appreciation to the International Scientific Partnership Program ISPP at King Saud University for funding this research work through ISPP# 0062. Dr. Anitha Jaganathan is grateful to the University Grant Commission (New Delhi, India), Project No. PDFSS-2014-15-SC-TAM-10125.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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