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To cite this article: Ganesh Elango, Selvaraj Mohana Roopan, Naif Abdullah Al-Dhabi, Mariadhas Valan Arasu, Kasinathan Irukatla Damodharan & Kuppuswamy Elumalai (2016): Cocos nucifera coir-mediated green synthesis of Pd NPs and its investigation against larvae and agricultural pest, *Artificial Cells, Nanomedicine, and Biotechnology*, DOI: [10.1080/21691401.2016.1262382](https://doi.org/10.1080/21691401.2016.1262382)

To link to this article: <http://dx.doi.org/10.1080/21691401.2016.1262382>



Published online: 01 Dec 2016.



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## Cocos nucifera coir-mediated green synthesis of Pd NPs and its investigation against larvae and agricultural pest

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### ABSTRACT

In recent decades, several scientists focused their process towards nanoparticles synthesis by using various sustainable approaches. *Cocos nucifera* (*C. nucifera*) was one of the versatile trees in tropical regions which also can act as a thrust quencher in all over the world. *Cocos nucifera* coir was one of the waste by-products in all coconut-refining industries and with the help *C. nucifera* coir, Palladium nanoparticles (Pd NPs) were synthesized. Green-synthesized spherical-shape Pd NPs were over layered by secondary metabolites from *C. nucifera* coir extract and with an average particle size of  $62 \pm 2$  nm, which were confirmed by morphological analysis. Eco-friendly mediated Pd NPs were further subjected to several biological applications like larvicidal against *Aedes aegypti* (*A. aegypti*) and anti-feedent, ovicidal, and oviposition deterrent against agricultural pest *Callasobruchus maculatus* (*C. maculatus*) and compared with *C. nucifera* coir methanolic extract, which results in LC<sub>50</sub> value of 288.88 ppm and LC<sub>90</sub> value of 483.06 ppm using LSD-Tukey's test against dengue vector (*A. aegypti*). *Cocos nucifera* coir methanolic extract shows significant output while compared with Pd NPs towards anti-feedent assays; ovicidal activity and oviposition deterrent were discussed here.

### ARTICLE HISTORY

Received 14 September 2016  
Revised 15 November 2016  
Accepted 15 November 2016

### KEYWORDS

Phytosynthesis; *Cocos nucifera*; Pd NPs; larvicidal; anti-feedent; ovicidal; oviposition deterrent

### Introduction

The only accepted species in *Cocos* genus was said to be *Cocos nucifera* (*C. nucifera*) that belongs to Arecaceae family. It is one of the most important fruit trees in tropical and sub-tropical regions which contains several by-products like coconut water, toddy, oil, etc. (Gao et al. 2014). It is endorsed with 51.9% of endosperm of water, 26.1% of fats, and 15.1% of traces of vitamins, carbohydrates, and several minerals which are most essential for day-to-day life (Ochoa-Velasco et al. 2014). This fruit contains major fractions of proteins like globulins, albumins, glutelins 1 and 2. This plant is utilized in the treatment of several biological applications like anti-oxidants, anti-malarial, antimicrobial, leishmanicidal, and insecticidal activities (Roopan and Elango 2015, Roopan et al. 2013). Various solvent extracts of *C. nucifera* coir were used as secondary metabolites for greener synthesis of metal nanoparticles, which play a major role nowadays and are to be exploited further in future.

The combination of two major fields like nanotechnology and biotechnology is said to be nanobiotechnology (Akhtar et al. 2013). Phyto or green-mediated syntheses of nanoparticles were considered under higher exploration technique to avoid toxic nature of nanoparticles related to environment (Kumar et al. 2014, Madhumitha et al. 2015, 2016,

Madhumitha and Roopan 2013, Roopan et al. 2012, Surendra and Roopan 2016). This green synthesis of nanoparticles has several benefits by decreasing the usage of reducing agents like hydrogen gas or hydrazine, sodium borohydride, etc., which mostly result in advantages over environment (Nasrollahzadeh et al. 2016).

Transition metal nanoparticles were rated as one of the important metallurgies in periodic elements because of biocompatibility, greener approach, eco-friendly adoptable nature, and phyto-synthesizing property (Elango et al. 2015). Many metals like Pd, Au, Ag, Pt, and Cu were frequently utilized for the synthesis due to their colloidal stability, which is majorly utilized in several fields: catalysis, photo thermal therapy, optoelectronics, and labeling the biological substances. Here, we plan to synthesize palladium nanoparticles (Pd NPs) using agricultural wastes of *C. nucifera* coir due to its important applications like hydrogenation, low temperature in pollutants of automobile reduction, and also majorly as primary catalyst in many organic reactions (Chowdary et al. 2016).

There are several physio-chemical methods for fast and rapid synthesis of metal nanoparticles like hydrothermal, microwave, nano-micro-emulsion, sol-gel and sonochemical-mediated synthesis (Jia et al. 2009). But, when compared to physio-chemical methods which are available for Pd NPs synthesis, eco-friendly synthesis of Pd NPs plays a major role in

environment by avoiding some toxic defects and also has several advantages like easy reaction setup, usage of non-toxic solvents, mild reaction progress, less cost, and suitable for biological and pharmaceutical applications (Nasrollahzadeh et al. 2015). These Pd NPs also play a major role in several medical applications without destructing the morphology and bind with single-strand DNA, which also has a property to act as a sensor for glucose-sensing instruments (Kalaiselvi et al. 2015). The green synthesis by using various plant sources does not require higher temperature maintenance and is an easiest process yet to be explored (Elango and Roopan 2015). Hence, we focused towards *C. nucifera* coir-mediated synthesis of Pd NPs, subjected to various applicational studies. The present study completely deals about the green synthesis of Pd NPs and applicational study towards various biological activities like anti-feedent, oviposition deterrent, and ovicidal activity by cow pea adult bruchid *Callasobruchus maculatus* (*C. maculatus*); further, it was tested against a dengue vector named *Aedes aegypti* (*A. aegypti*) (Erick and Padmanabhan 2015).

## Materials and methods

### Resources

*Cocos nucifera* coir was aggregated from Gudiyatham, Vellore district, Tamil Nadu, India, and its coordinates are 12.9397°N, 78.8644°E. The collected sources were further subjected for authentication process in Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India, with the authentication no: BSI/SRC/5/23/2013–14/Tech 1118 and kept for future reference. Palladium acetate was purchased from Sigma Aldrich Chemicals Ltd, Mumbai, India, and CH<sub>3</sub>OH from AVRA Laboratories, Hyderabad, India. Throughout this experimental process, double-distilled water was utilized without any modifications.

### Methanolic extract collection of *C. nucifera* coir

Collected samples of *C. nucifera* coir were washed and cleaned with double-distilled water and subjected to air-drying in shade conditions at room temperature for 72 h. Further, it was grinded using mechanical mixer and powdered particles were stored in room temperature for future experimental process. Preliminarily collected fine-powdered materials were subjected for pet ether extraction for hydrocarbons removal. Once hydrocarbons were removed by checking thin-layer chromatography (TLC) method, further fine-powdered material was placed in room temperature for the evaporation of pet ether solvents for 2–3 h. After this process, samples were re-packed in a Soxhlet apparatus for methanol extraction and it was distilled and stored in a refrigerator for further process.

### Bio-approach for the production of Pd NPs

We have followed the protocol of Elango and Roopan (2015) with slight modifications for the synthesis of Pd NPs. About 100 ml of 1 mM palladium acetate solution was prepared

using double-distilled water and placed on stirrer under room temperature for one hour. 20 mL of methanolic extract (1000 ppm) was collected and mixed with 80 mL of palladium acetate solution, then placed under 60° C for 4 h with the help of water bath and observed using UV-Vis at 1 h interval. Once the highest absorbance was confirmed by UV-Vis spectroscopy, further collected sample was subjected for centrifugation for 30 min at 3000 rpm and further pellets were placed in muffle furnace for 2 h at 350 °C for calcination process.

### Characterization

Here, the agricultural waste-mediated synthesis of Pd NPs were monitored and confirmed by various analytical techniques like UV-Vis spectroscopy (UV-1800-Schimidzu, Shimadzu Analytica, Mumbai, India), Bruker D8 make XRD for the analysis of crystalline data of sample, Bruker Alpha T-FT-IR for the functional group analysis, Philips make TEM for structural analysis; to identify the phytoconstituents, samples were analyzed using GCMS, Zeta potential (Horiba Nanoparticle Analyzer, Horiba Corp., Chennai, India).

### Pd NPs activity against larvae's

*Aedes aegypti* dengue vector were segregated from stagnant water area near Cooum river, Saidapet, Chennai, its coordinates are 13.0681°N, 80.2858°E and were identified by Dr. Kuppasamy Elumalai, Department of Advanced Zoology and Biotechnology, Government Arts College (Autonomous), Nandanam, Chennai. With various concentrations like 100, 200, 300, 400, 500 ppm, synthesized Pd NPs were subjected towards 20 larvae which were placed in 10 mL of distilled water. This experimental setup was processed using re-cyclable paper cups, as shown in Figure 1 with five replicates. Each test having a control contains distilled water and larva in the absence of Pd NPs. We identified mortality percentage, dose-response bioassay for various concentrations ranging 100, 200, 300, 400, and 500 ppm. With the help of LSD Tukey's test, we have also identified the LC<sub>50</sub> and LC<sub>90</sub> values reported here.

### Adulticidal bioassay of *Callasobruchus maculatus* (*C. maculatus*) by anti-feedent method

We have collected *C. maculatus* from infected cow pea seeds. The collected pests were processed before emergence and



Figure 1. Experimental setup for larvicidal activity.

transferred it into 35 mm petri dishes which contain 100 cow pea seeds, as shown in Figure 2. We further separated the two groups in a set of three, in which one group of petri dishes was coated with extract and another with Pd NPs in the presence of seed and pests with different concentrations like 1.25, 2.5, 5, 10, and 20 mg/L. In each petri dish, we introduced 10 pairs of adults. Dishes will be separated and placed in laboratory ( $28 \pm 2^\circ\text{C}$ ,  $75 \pm 10\%$  (rh) conditions at 12 h dark and 12 h light. Mortality rate was observed 1, 3, 5, 7, and 14 d (Erick and Padmanabhan 2015, Roopan et al. 2013). Further, we utilized distilled water as a control. The mortality of adults was observed from the 24 h of exposure and the results were reported with replicates of five. We compared this process with *C. nucifera* coir methanolic extract and reported.

### Ovicidal activity using *Vigna unguiculata* (cow pea) seeds on *C. maculates* eggs

Everyone knows that ovicidal means nothing but to kill eggs of insects, mites, nematodes, etc. Here, we collected *C. maculates* egg from cow pea seeds, as shown in Figure 3, and placed in petri dishes with various concentrations of green-synthesized Pd NPs and *C. nucifera* coir methanolic extracts (1.25, 2.5, 5, 10, and 20 mg/L). We placed the petri dishes under laboratory conditions and recorded the ovicidal rate of the eggs at different intervals of time (24, 48, 72, and 96 h) with five replicates by following the protocol of Adedire et al. (Krishnaraju et al. 2006) with slight modifications.



Figure 2. Adulticidal bioassay (anti-feedent method).



Figure 3. Collection of *C. maculates* egg for ovicidal activity.

### Oviposition deterrent activity of *C. maculates* on *Vigna unguiculata*

We processed oviposition deterrent activity using cow pea seeds against *C. maculates* egg by following the protocol of Prajapati et al. (Su et al. 1998) with slight modifications with different concentrations of Pd NPs at 1.25, 2.5, 5, 10, and 20 mg/L, then later compared with *C. nucifera* coir methanolic extract. Five replications were studied and are reported here.

## Results and discussion

### Pd NPs analysis results

#### Pd NPs–UV–visible spectroscopy

Reaction progress of conversion of  $\text{Pd}(\text{OAc})_2$  to Pd NPs was observed using UV–Vis spec at different time intervals. Further progress was noted at the wavelength of 200–800 nm. Result in peak exactly at 264 nm and clearly indicates the formation of Pd NPs by surface plasmon resonance. The optimized absorbance for nanoparticle formation was said to be 90 min as clearly shown in Figure 4.

#### Pd NPs–XRD pattern

Figure 5 clearly depicts the XRD crystalline pattern of prepared Pd NPs at  $60^\circ\text{C}$  for 90 min. Obtained XRD pattern was matched with Joint Committee Powder Diffraction Standards (JCPDS) data and the resulted number was 89–4897, which

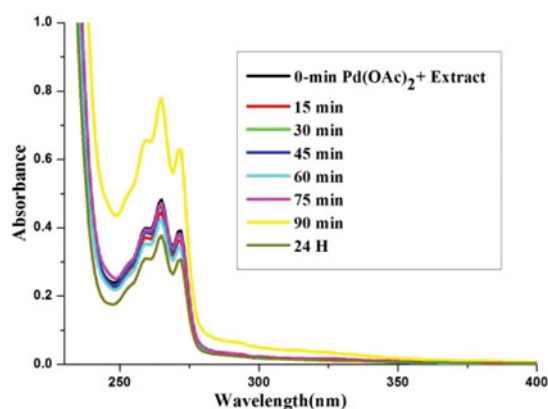


Figure 4. UV–visible spectrum of Pd NPs at various intervals.

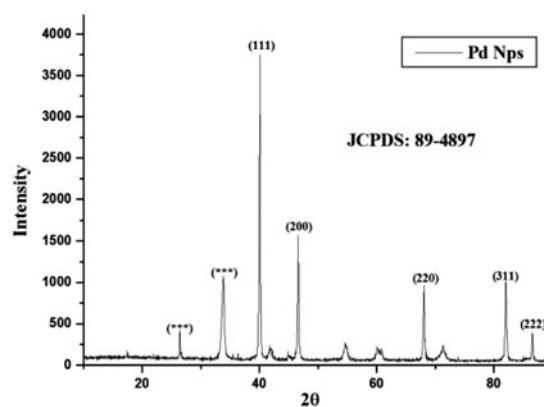


Figure 5. XRD pattern of Pd NPs.

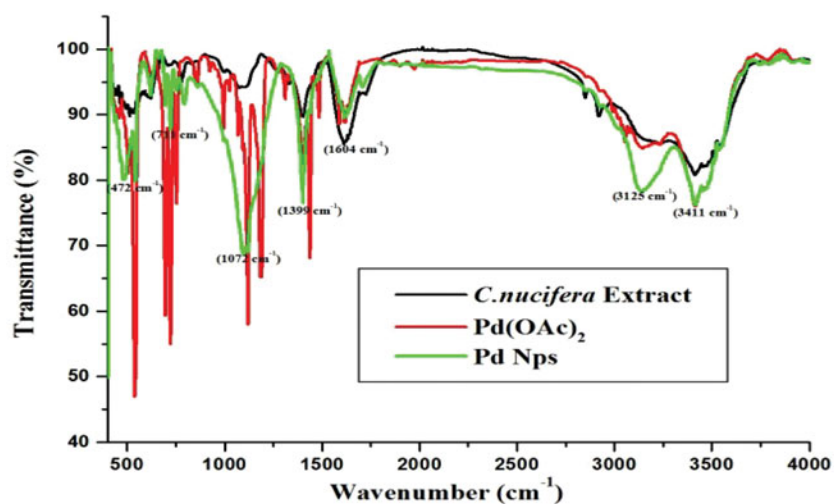


Figure 6. FT-IR spectrum of Pd NPs.

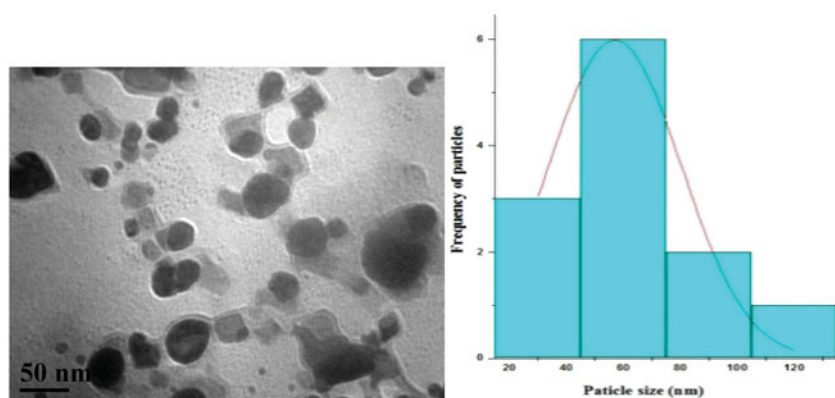


Figure 7. TEM and particle size histogram of Pd NPs.

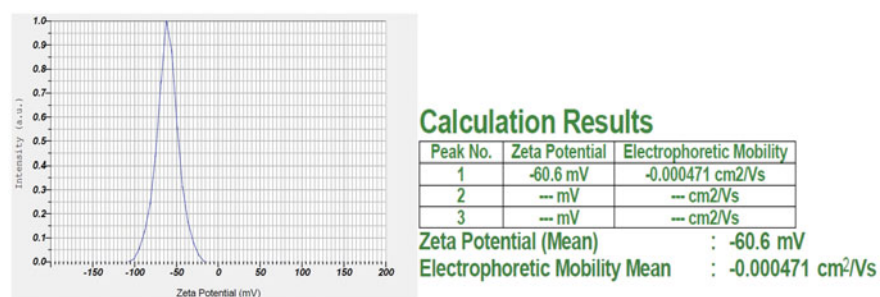


Figure 8. Zeta potential of Pd NPs.

Table 1. Larvicidal activity of synthesized Pd NPs against dengue vector *A. aegypti*.

Concentration (ppm)	Mortality <sup>a</sup> (%)	LC <sub>50</sub> (ppm)	95% Confidence limits (ppm)		LC <sub>90</sub> (ppm)	95% Confidence limits (ppm)		$\chi^2$ value
			LCL	UCL		LCL	UCL	
100	12.8 ± 1.4	250.13	233.39	266.86	412.35	387.94	442.34	2.417
200	38.4 ± 1.2							
300	62.8 ± 2.5							
400	86.3 ± 2.6							
500	98.6 ± 2.4							

LC<sub>50</sub>: lethal concentration brings out 50% Mortality and LC<sub>90</sub>: lethal concentration brings out 90% mortality. LCL: lower confidence limit; UCL: upper confidence limit. Values in a column with a different superscript alphabet are significantly different at  $p < 0.05$  (ANOVA; LD- Tukey's Test). Values represent the mean ± SD of five replications.

<sup>a</sup>Mortality of the larvae observed after 24 h of exposure period WHO (2005).

clearly reveals that green-synthesized Pd NPs were crystalline in nature. (\*\*\*) indicates the presence of some organic compounds in the extract. The observed result was identified as  $d=2.24061$  with  $2\theta$  plane=40.048. With the help of the Scherrer equation, the particle sizes average was found to be 64.43 nm (Prajapati et al. 2005).

$D=k\lambda/\beta \cos\theta$  where the particle size is denoted by  $D$ , (0.94) constant of Scherrer denoted by  $k$ ,  $\lambda$  value can be evacuated from the equation derived by Bragg's ( $2d \sin \theta=n\lambda$ ),  $\lambda$  is the wave length,  $\beta$  is the width half and maximum full, and  $\theta$  is the angle of diffraction.

### Pd NPs-FT-IR

FT-IR was done for palladium salt precursor, *C. nucifera* coir methanolic extract, and green synthesized Pd-NPs. The results clearly state that stretching of palladium precursor and *C. nucifera* coir methanolic extract at  $3411 \text{ cm}^{-1}$  is because of the hydroxyl groups (-OH). Then, researchers identified a stretching exactly at  $1604 \text{ cm}^{-1}$  due to the presence of C=O and also we noticed that in the peak range of  $3125 \text{ cm}^{-1}$  two different peaks are separated which resulted in the Pd NPs formation, as illustrated in Figure 6.

### TEM analysis

We processed the synthesized sample for its morphological studies which was performed using transmission electron microscope and its results show that the particles were

spherical in shape, the average size was  $62 \pm 2 \text{ nm}$  as shown in Figure 7. Here, we are able to conclude that the methanolic extract of *C. nucifera* was used to convert Pd (OAc)<sub>2</sub> into Pd NPs as a reducing agent. With the help of Image J software, histogram was calculated and the resultant particle size was averaged to be 60 nm.

### Pd NPs-Zeta potential

Pd NPs were processed in Horiba nanoparticles analyzer to identify the stability which resulted in  $-60.6 \text{ mV}$  of high stability, as shown in Figure 8.

### GC-MS prediction of C. nucifera coir

We processed our *C. nucifera* coir methanolic extract for gas chromatography and mass spectroscopy analysis to identify the secondary metabolites for the formation of Pd (OAc)<sub>2</sub> to Pd NPs as a reducing agent. We have identified that 3-methoxy cinnamic acid has higher area percentage compared with other secondary metabolites. So, we confirmed that 3-methoxy cinnamic acid can be used as a reducing agent for the conversion of Pd (OAc)<sub>2</sub> to Pd NPs. These GC-MS processes were already studied and our research group has already been reported in another work (Elango and Roopan 2015).

### Pd NPs-larvicidal activity

With different concentrations like 100–500 ppm, larvicidal activity of Pd NPs was tested in the presence of larvae

**Table 2.** Anti-feedent activity of Pd NPs and *C. nucifera* methanolic extract using *C. Maculates*.

Concentration (mg/L)	Anti-feedent activity (%)	
	Pd NPs	<i>C. nucifera</i> coir methanolic extract
1.25	53.09 ± 0.28	54.6 ± 0.31
2.5	66.59 ± 0.24	68.47 ± 0.35
5	86.12 ± 0.32	86.12 ± 0.42
10	97.35 ± 0.27	91.98 ± 0.31
20	92.31 ± 0.31	99.01 ± 0.18

Anti-feedent activity assayed by the N/P-NPs with 0.5% DMSO with distilled water at different concentrations. Values in a column with a different superscript alphabet are significantly different at  $p < 0.05$ .

**Table 4.** Oviposition deterrent activity using Pd NPs and *C. nucifera* coir methanolic extract against *C. maculates*.

Concentration (mg/L)	% Oviposition deterrent	
	Pd NPs	<i>C. nucifera</i> coir methanolic extract
1.25	81.81 ± 0.66	83.63 ± 0.52
2.5	83.91 ± 0.43	91.48 ± 0.41
5	95.45 ± 0.55	97.52 ± 0.23
10	97.52 ± 0.21	100.0 ± 0.00
20	100.0 ± 0.00	100.0 ± 0.00

Values represent mean ± SD of five replications.

**Table 3. (a)** Ovicidal activity of Pd NPs.

Exposure (h)	%Ovicidal activity concentrations tested (ppm)				
	1.25	2.5	5	10	20
24	65.84 ± 0.68	72.65 ± 0.41	74.22 ± 0.75	82.44 ± 0.41	92.84 ± 0.12
48	73.66 ± 0.58	77.39 ± 0.57	77.92 ± 0.22	85.93 ± 0.37	98.16 ± 0.14
72	92.11 ± 0.16	88.91 ± 0.40	93.91 ± 0.36	100.00 ± 0.00	100.00 ± 0.00
96	97.39 ± 0.10	98.54 ± 0.21	95.62 ± 0.31	100.00 ± 0.00	100.00 ± 0.00

**Table 3. (b)** Ovicidal activity of *C. nucifera* coir methanolic extract.

Exposure (h)	%Ovicidal activity concentrations tested (ppm)				
	1.25	2.5	5	10	20
24	61.57 ± 0.67	65.26 ± 0.32	67.25 ± 0.28	76.29 ± 0.72	92.21 ± 0.32
48	62.51 ± 0.84	70.39 ± 0.90	69.11 ± 0.33	81.99 ± 0.81	96.52 ± 0.41
72	74.26 ± 0.31	83.41 ± 0.25	77.39 ± 0.51	95.22 ± 0.54	100.00 ± 0.00
96	85.11 ± 0.24	88.26 ± 0.22	85.24 ± 0.23	100.00 ± 0.00	100.00 ± 0.00

causing dengue *A. aegypti*. The results revealed are shown in Table 1.

### Anti-feedent activity of Pd NPs against *C. maculatus*

Mortality of the adults treated with nanoparticles was recorded under laboratory conditions after 1, 3, 5, 7, and 14 d. Here, we kept distilled water as a control and replicated five times for optimization of different concentrations of 1.25, 2.5, 5, 10, and 20 mg/L were studied for both Pd NPs and *C. nucifera* methanolic extract and reported in Table 2. We finally concluded that: compared with Pd NPs, *C. nucifera* coir methanolic extract shows better anti-feedent activity (Kumari et al. 2015, Petla et al. 2012).

### Ovicidal activity of Pd NPs

Ovicidal activity of the synthesized Pd NPs and *C. nucifera* coir methanolic extract is compared and is reported in Table 3(a, b). The obtained result clearly indicates that Pd NPs have less efficiency to kill eggs of *C. maculatus* compared with *C. nucifera* coir methanolic extract.

### Oviposition deterrent activity against gravid of *C. maculatus*

*Cocos nucifera* coir methanolic extract and Pd NPs were tested for its oviposition deterrent activity against gravid of *C. maculatus*, as reported in Table 4. Hence, we identified that *C. nucifera* coir methanolic extract has high efficacy of oviposition deterrent activity while compared with Pd NPs from the obtained results.

### Conclusion

This article summarizes that the *C. nucifera* coir mediated the synthesized Pd NPs of  $62 \pm 2$  nm average size with spherical shape. This methodology is one of the cheapest methods for synthesizing Pd NPs. Further larvicidal activity using Pd NPs against dengue vector (*Aedes aegypti*) resulted in LC<sub>50</sub> value of 288.88 ppm and LC<sub>90</sub> value of 483.06 ppm using LSD-Tukey's test and shows 98% of mortality rate in 500 ppm of Pd NPs. We conclude that *C. nucifera* coir methanolic extract shows better output while compared with Pd NPs towards anti-feedent assay, ovicidal activity, and oviposition deterrent.

### Acknowledgements

Elango express his gratitude towards DBT for Research Assistant fellowship. Dr. S. M. Roopan prompts his thank to the Department of Biotechnology, Government of India, for providing the research grant to carry out this work (DBT-RGYI scheme no. BT/PR6891/GBT/27/491/2012). Prof. M. Valan Arasu and Naif Abdullah Al-Dhabi encompass their heartfelt obligation to the Deanship of Scientific Research (King Saud University) for its finance to Prolific Research Group (PRG-1437–28).

### Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

### Funding

Elango express his gratitude towards DBT for Research Assistant fellowship. Dr. S. M. Roopan prompts his thank to the Department of Biotechnology, Government of India, for providing the research grant to carry out this work (DBT-RGYI scheme no. BT/PR6891/GBT/27/491/2012). Prof. M. Valan Arasu and Naif Abdullah Al-Dhabi encompass their heartfelt obligation to the Deanship of Scientific Research (King Saud University) for its finance to Prolific Research Group (PRG-1437–28).

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