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Artículos

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Síntesis y actividad insecticida de nanopartículas de Cu de *Prosopis juliflora* (Sw) DC y *Pluchea sericea* (Nutt.) sobre *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)

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Abstract: *Phenacoccus solenopsis* is an aggressively invasive species on cotton plants in several countries. Hence, the pesticidal effect of copper nanoparticles synthesized from leaf extract of *Prosopis juliflora* (Sw) DC and *Pluchea sericea* (Nutt.) against *P. solenopsis* was investigated. The results showed that the Cu-nanoparticles (Cu-NPs) obtained from *P. juliflora* and *P. sericea* averaged 33.8 and 68.10 nm, respectively. The high stability for both synthetized Cu-NPs was indicated for their zeta potential (-69.7 mV for *P. juliflora* and -53.9 mV for *P. sericea*). Both nanoparticles showed significant toxicity to *P. solenopsis* after 96 hours. Mortality rates of *P. solenopsis* were 14 and 20% when exposed to Cu-NPs extracted from *P. sericea* and *P. juliflora*, respectively. Tests in copper solution showed an insignificant mortality of *P. solenopsis*. Viability tests for Cu-NPs from *P. sericea* and *P. juliflora* indicated a significant reduction of cell viability by 30 and 38% respectively.

Keywords: Cotton mealybug, Green insecticide, Insecticide effect, Nanoparticles.

Resumen: *Phenacoccus solenopsis* es una especie agresivamente invasiva e invasora en plantas de algodón en varios países. Se investigó el efecto pesticida de nanopartículas de cobre, sintetizadas a partir de extracto de hoja de *Prosopis juliflora* (Sw) DC y *Pluchea sericea* (Nutt.), contra *P. solenopsis*. Las nanoparticulas de Cu (Cu-NPs) obtenidas de *P. juliflora* y *P. sericea* promediaron 33.8 y 68.10 nm, respectivamente. La estabilidad alta para ambas Cu-NPs estuvo indicada por el potencial zeta de -69.7 mV de *P. juliflora* y -53.9 mV para *P. sericea*. La toxicidad de las Cu-NPs fue significativa para ambas plantas contra *P. solenopsis* después de 96 horas. Las tasas de mortalidad de *P. solenopsis* fueron de 14 y 20% ante la exposición a Cu-NPs de *P. sericea* y *P. juliflora*, respectivamente. Los ensayos en solución de cobre mostraron que la mortalidad de *P. solenopsis* fue insignificante. Los ensayos de viabilidad para las Cu-NPs de *P. sericea* y *P. juliflora* indicaron reducción significativa de la viabilidad celular de *P. solenopsis* de 30 y 38% respectivamente.

Palabras clave: Cochinilla harinosa del algodón, Efecto insecticida, Insecticida verde, Nanopartículas.

INTRODUCTION

Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) was first reported as a cotton pest in Texas, USA (Wu et al., 2014). This pest is a polyphagous insect with a wide geographical distribution worldwide (Vennila et al., 2010). This pest has a wide morphological diversity, biological adaptations and ecological adjustments that gives it a high capacity to feed on different host plants, including economically important species of Cucurbitaceae, Fabaceae and Solanaceae (Hodgson et al., 2008). *Phenacoccus solenopsis* has been recognized as an aggressively invasive species on both agricultural and ornamental plants in Brazil, China, Pakistan, and India (Wang et al., 2009; Da Silva, 2012; Maruthadurai & Singh, 2015). Control studies in cotton have included the application of chemical insecticides and biopesticides (Saddiq et al., 2017; Ullah et al., 2017). However, the development of resistance to many kinds of chemical insecticides and the unpredictable results of biological control have caused variable results (Zhou et al., 2015; Afzal & Shad, 2016). Therefore, new alternatives are required to reduce populations of *P. solenopsis* on cotton. Recently, the use of nanotechnology in agriculture is achieving great importance as it promotes improved pest management and crop fortification through the excellent efficiency of agrochemicals such as fertilizers, pesticides and growth agents (Kah & Hofmann, 2014). Today, green nanotechnology (synthesis of nanoparticles using biological systems) had great relevance due to the fact that it is environmentally friendly and it does not use toxic chemicals in the nanoparticles formulation.

Although there have been numerous studies on the toxicity of phytonanoparticles on pathogenic bacteria and fungi, research on toxicity of metal phyto-nanoparticles on insects is scarce. Therefore, the present investigation is aimed to evaluate pesticidal effect of copper nanoparticles synthesized from leaf extract of mesquite, *Prosopis juliflora* (Sw.) DC. (Fabaceae) and chachanilla, *Pluchea sericea* Nutt (Asteraceae), against the invasive cotton mealybug, *P. solenopsis.*

MATERIAL AND METHODS

Fresh and healthy leaf samples of *P. juliflora* and *P. sericea* were collected from native plants in the Mexicali valley, Baja California, Mexico (32° 24' 7" N; 115° 11' 51" W). The aqueous extract was prepared taking 30 g of leaves of each plant mixed in 300 mL of distilled water. The mixture was kept in agitation at 2.5 g during 24 hours at 28 °C. The samples were then centrifuged at 5,000 *g* for 10 min to remove particulate matter and to get a clear solution which was stored at 4 °C until use. For Cu-NP synthesis, 10 mL of aqueous extract of *P. juliflora* and *P. sericea* were added to 40 mL of 10 mM solution of $CuSO_4$ in 100 mL erlenmeyer flask and heated at 40 °C for 30 min. The bio-reduction process of Cu+ and the production of NPs, was determined visually by color change of the aqueous solution of the extract. Previous to the addition of $CuSO_4$ the solutions were yellow and after 30 min of reaction they were both brown (Fig. 1). The Cu-NPs were centrifuged 15 min at 11,200 *g* and the precipitates were thoroughly washed with sterile distilled water to get rid of any unwanted impurities and the powders obtained for toxicological assays were transferred to a freeze dryer.

Dynamic Light Scattering (DLS) and laser Doppler velocimetry (LDV) were used for characterization of size and zeta potential of copper-phytonanoparticles in solution from *P. juliflora* and *P. sericea*. These analyses were performed on a nanotrac wave instrument (Microtrac) and DLS data were analyzed with the Microtrac FLEX operating software (Ruíz-Romero et al., 2018). The size, morphology and composite homogeneity of Cu-NPs were studied using a scanning electron microscope (SEM) JEOL 6010 according to Abdelmoteleb et al. (2016).

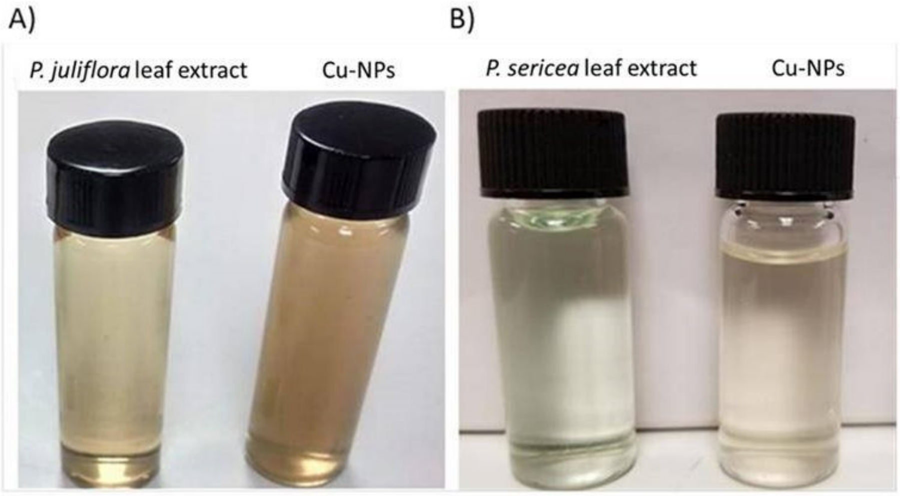

Fig. 1. Green synthesis of copper-nanoparticles using leaf extract of *Prosopis juliflora* (A) and *Pluchea sericea* (B).

For EDS analysis, the Cu-NPs were drop coated on carbon film and analyzed using a Bruker Quantax 400 instrument. Fourier transform infrared spectral measurements (FTIR) were taken to identify the possible biomolecules in *P. juliflora* and *P. sericea* leaf extracts responsible for reducing and capping the bioreduced Cu-NPs.

For evaluation of insecticide activity of Cu-NPs, *P. solenopsis* mealybugs were collected from infested Bollgard® cotton in Mexicali Valley, Mexico during summer 2018. Twenty adult females of the same stage of each species were selected and transferred to 100 x 15 mm Petri dishes containing a cotton leaf. Then, 2 mL of aqueous solution of Cu-NPs at 300 ppm from *P. juliflora* (Treatment 1) or *P. sericea* (Treatment 2), were applied with a small volume hand sprayer. The control group consisted of distilled water (Treatment 3), and $CuSO_4$ at 300 ppm was Treatment 4. A randomized complete block design was used, with four replicates for treatment, plus a water control. The experiment unit was a Petri dish with 20 individuals. All Petri dishes were placed in a growth chamber at 27 ± 2 °C under 16 h photon flux density of 340 µmol m−2 s −1 light intensity and 80% relative humidity. The number of *P. solenopsis* survivors (mortality percentage) was recorded after 24, 48, 72 and 96 hours of initial application by using Abotts formula. Death of insect individuals was determined based on the coordinated muscle response of mealybugs to gentle prodding with an insect needle.

The cell viability levels of the mealybugs were evaluated by Evans blue staining spectrophotometry (Gonzalez-Mendoza et al., 2009), with minor modifications, a test used widely to study cellular membrane permeability in diverse organisms (Yao et al., 2018). For this the insects of each treatment were stained with 0.25% aqueous Evans blue solution for 15 min at room temperature, then washed extensively with distilled water for 20 min to remove excess and unbound dye. The Evans blue trapped on insects cells was released by suspending cells in 50% ethanol with 1% SDS solution at 60 °C for 30 min and quantified by measuring absorbance at 600 nm.

Differences between the treatments were analyzed with the Kruskal–Wallis test (Statistical Package version 5.5, Statsoft, USA). Significant differences were accepted if $p \leq 0.05$ and data was expressed as mean ± S.E.M.

RESULTS

The formation of Cu-NPs from *P. juliflora* and *P. sericea* were very rapid and it was completed within 30 min. The average hydrodynamic size of the Cu-NPs showed that those obtained from *P. juliflora* were polydisperse mixtures with an average mean size of 33.8 nm (Fig. 2a) and, in contrast, the average size of the synthesized Cu-NPs using *P. sericea* leaf extract was around 68.10 nm (Fig. 2b). On the other hand, the zeta potential was found to be -69.7 mV for synthesized Cu-NPs from *P. juliflora* and -53.9 mV for *P. sericea*.

Analysis by SEM showed that the majority of Cu-NPs from *P. juliflora* were spherical with varying size (Fig. 3b), whereas those from *P. sericea* showed non-spherical (ellipsoidal form) particle with different size (Fig. 3a). On the other hand, in the Figure 4a, the EDS of Cu-NPs-*P. sericea* revealed that pure copper (Cu 43.22%) was the major constituent element compared to Cu-NPs-*P. juliflora* that showed 34.85% (Fig. 4b).

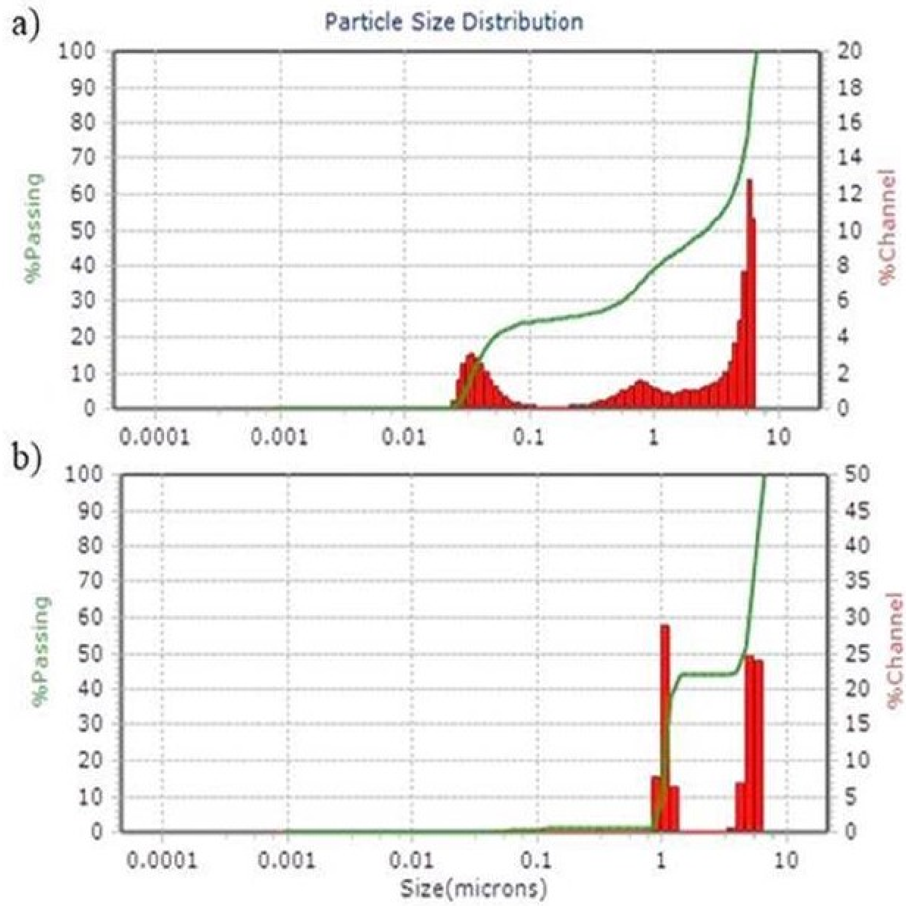

Fig. 2. Particle size distribution of Cu-NPs-*Prosopis juliflora* (a) and *Pluchea sericea* (b) using dynamic light scattering measurements (DLS).

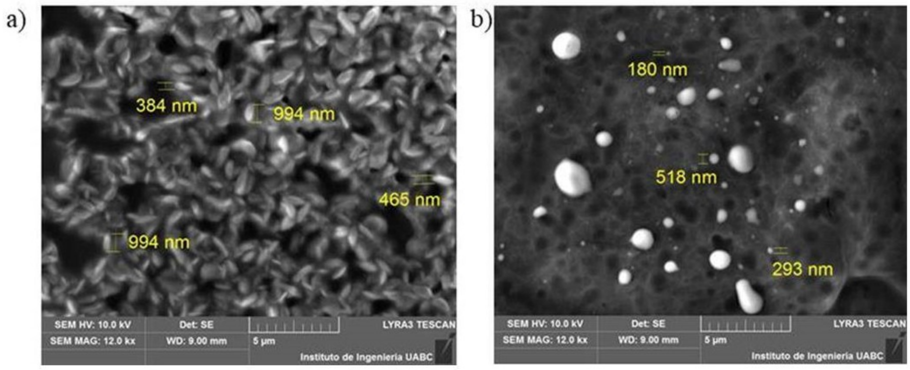

Fig. 3. Scanning electron microscopy (SEM) of Cu-NPs-*Pluchea sericea* (a) and Cu-NPs-*Prosopis juliflora* (b).

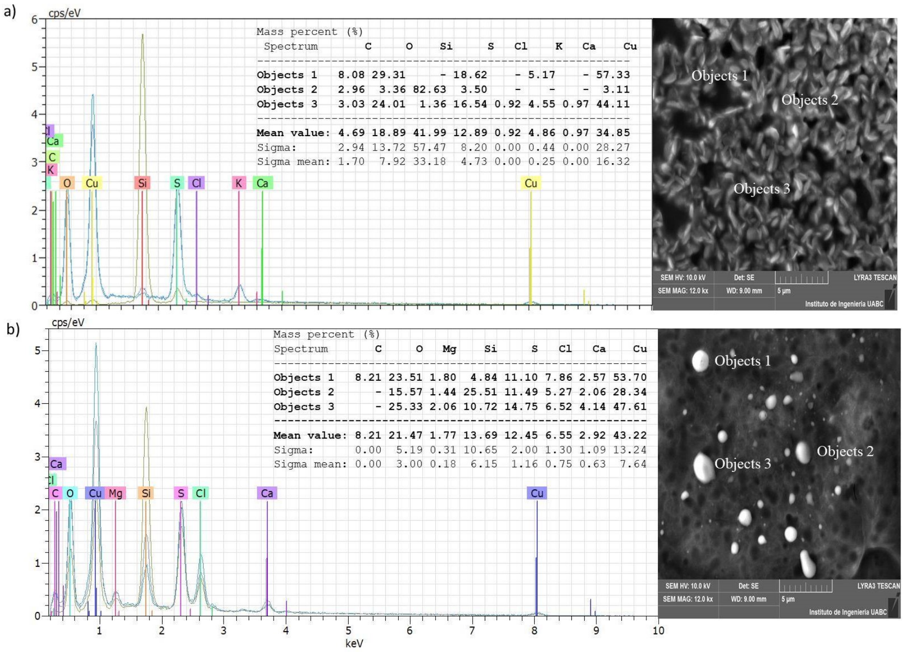

Fig. 4. EDS (Energy Dispersive X-ray Spectrometer) of Cu-NPs of *Prosopis juliflora* (a) and Cu-NPs-*Pluchea sericea* (b).

The FTIR spectrum of aqueous extract of *P. juliflora* and *P. sericea* were used as control. The bands observed in aqueous extract of *P. sericea* before and after bioreduction had similar peaks except for a variation in the size of bands 3341, 2935, 1623, 1410, 1265, 1046, and 662 cm−1 (Figs. 5a and 6a). The band at 3341 cm-1 corresponds to O-H stretching H-bonded alcohols and phenols compounds. The band at 2935 cm-1 can be related to the valence oscillations of the C-H and N-H bonds within the benzene ring. The bands at 1623-1410 cm-1 were principally C=N stretching vibrations. For aromatic amines, one band was observed at 1265 cm-1. The bands close to 1046 cm-1 can be probably related to O-H and C=O bonds. Finally, visible variation in Cu-NPs from *P. sericea* was observed at 662 cm-1, showing a reduction in the size band after bioreduction corresponding to C-H bending of alkynes (Fig. 6b). On the other hand, FTIR spectra of Cu-NPs from *P. juliflora*, exhibited a variation in the size of IR bands in region 3225, 2925, 2853, 1601, 1103,1059, 602 cm-1 indicating the biomaterial bind to alcohol/phenol, silicon, amines, alkenes and halogen functional groups (Fig. 5b). For example: 3225 cm-1 corresponded to O–H hydroxyl group and the band at 2925-2853 was due to C-H stretching of alkanes. The band at 1601 correponded to formation of C=C double bond of alkenes and the bands 1103-1059 indicated C-N alkyl stretching of amides.

Significant toxicity was recorded for Cu-NPs from *P. sericea* and *P. juliflora* to *P. solenopsis* after 96 hours (Fig. 7a). More than 14 and 20% mortality of *P. solenopsis* was observed with Cu-NPs from *P. sericea* and Cu-NPs of *P. juliflora*, respectively. A negligible mortality of *P. solenopsis* was recorded with the 300 ppm Cu solution and water.

The results of the viability test for Cu-NPs from *P. sericea* and *P. juliflora* are presented in Figure 7b, showing a 30 to 38% significant reduction of cell viability in mealybugs exposed to Cu-NPs from *P. sericea* and *P. juliflora*, respectively.

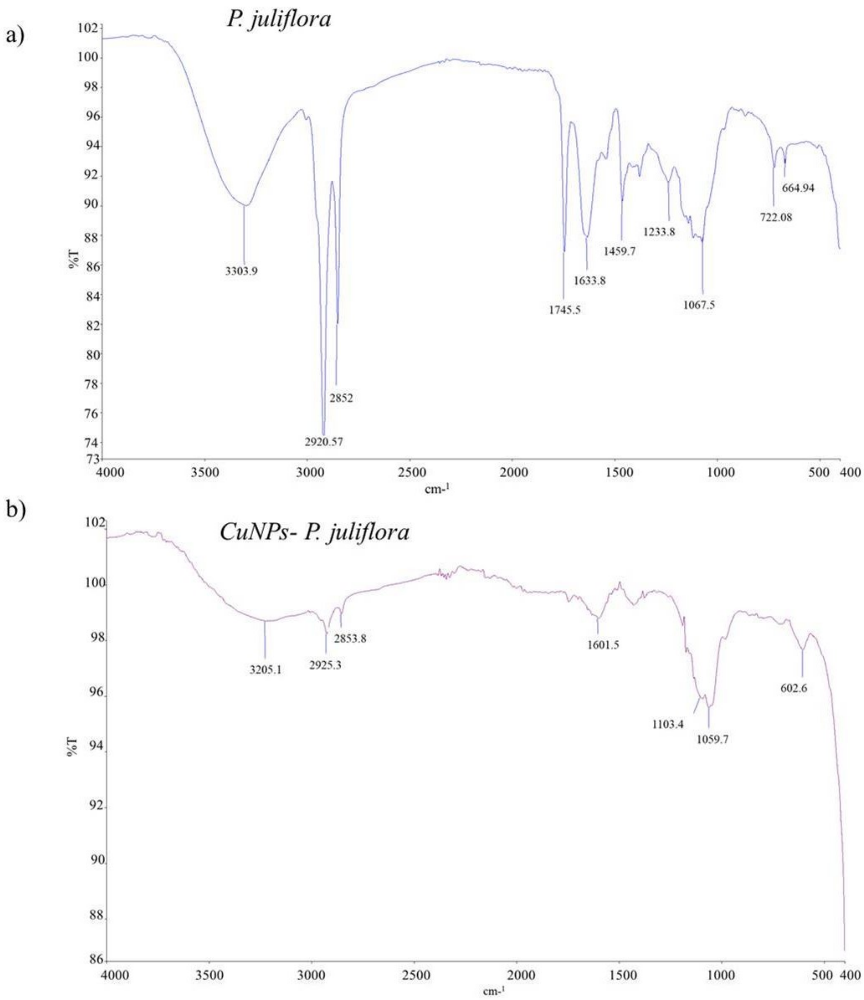

Fig. 5. FTIR spectrum of aqueous extract (a) and Cu-NPs (b), extracted from *Prosopis juliflora*.

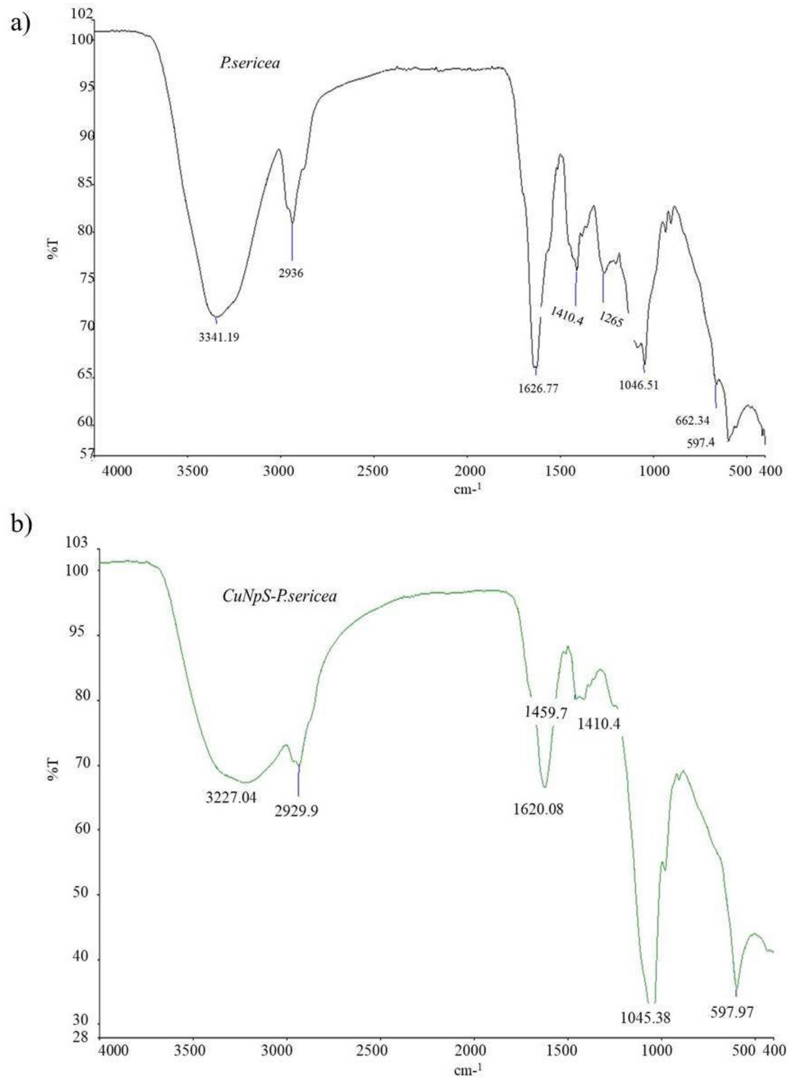

Fig. 6. FTIR spectrum of aqueous extract (a) and Cu-NPs (b), extracted from *Pluchea sericea*.

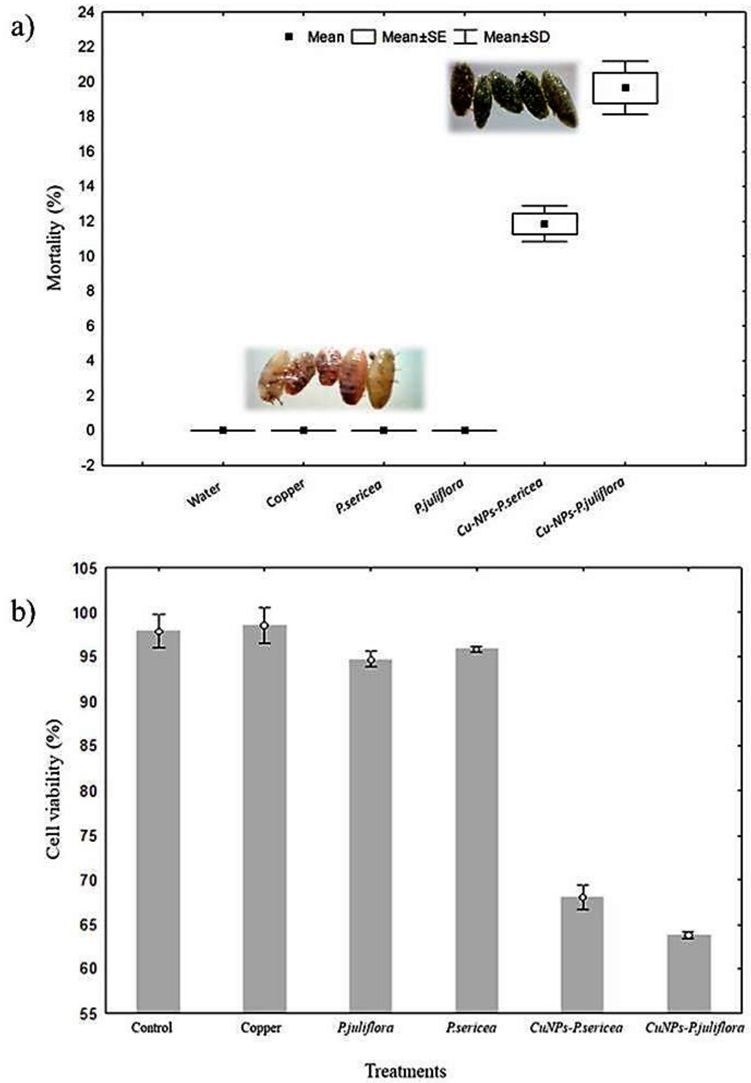

Fig. 7. Insect mortality (a) and cell viability (b) of *Phenacoccus solenopsis* after 96 hours of treatment with Cu-NPs of *Prosopis juliflora* and *Pluchea sericea*.

DISCUSSION

The synthesis of Cu-NPs from *P. sericea* and *P. juliflora* was confirmed by the development of pale yellow colour (Fig. 1), suggesting the formation of Cu nanoparticles due to the interaction of phytochemicals of aqueous extract from *P. sericea* (Hussain et al., 2013) and *P. juliflora* (Gonzalez-Mendoza et al., 2018a), that reduces Cu ions into Cu nanoparticles. In the present study, the formation of Cu-NPs from *P. juliflora* and *P. sericea* was completed within 30 min. This result coincides with experiments in *Sargassum vulgare* C. Agardh (Phaeophyceae: Sargassaceae) (González-Mendoza et al., 2018b) and *Yucca schidigera* Roezl ex Ortgies (Asparagaceae) (Ruíz-Romero et al., 2018) following the aplications of metals to aqueous extract of leaves. Bioreduction studies in other plants have reported the participation of carbonyl, phenolic, aromatic and carboxylate groups involved in the stabilization of nanoparticles (Kah, 2015; Chung et al., 2016). Therefore, the bioreduction and capping of Cu ion into Cu nanoparticles might be due to phenols, flavonoids and proteins according to FTIR analysis. However, further detailed studies about the role of different metabolites in Cu-NPs synthesis from *P. juliflora* and *P. sericea* will be needed to reveal the exact mechanism of nanoparticles formation.

On the other hand, our results showed that zeta potential was found to be -69.7 and -53.9 mV for synthesized Cu-NPs from *P. juliflora* and *P. sericea*, respectively, indicating high stability for both, according to Abdelmoteleb et al. (2016) who found that the stability of the colloidal system is determined by the magnitude of large negative or positive zeta potential. The negative values of zeta potentials for both Cu-NPs obtained indicate a long term stability of the colloids. For example colloidal suspension of Cu-NPs synthesized using Periploca of the Woods (*Gymnema sylvestre* R. Brown (Apocynaceae)) leaf extract was highly stable, with a zeta potential of -57.4 mV (Singh, 2018).

Phenacoccus solenopsis is being studied currently due to its invasiveness, rapid spreading and need for establishing an effective control strategy. We report here the first study of the insecticide properties of Cu-NPs obtained from two plants (*P. sericea* and *P. juliflora*) on *P. solenopsis* and demonstrate their potential as a reducing and stabilising agent to generate Cu-NPs. Both Cu-NPs from *P. sericea* and *P. juliflora* had a significant impact on *P. solenopsis* mortality in comparasion with some commonlyused insecticides as Neem oil, flubendamide, or novaluron (Nagrare et al., 2016).

Our results suggest that Cu-NPs from *P. sericea* and *P. juliflora* induce stress membrane damage in the cuticule of *P. solenopsis* (cell viability) that might be attributable to a distortion of the lipid layers of the membrane proteins by exposition to these nanoparticles. Similar results were reported by Yasur & Usha-Rani (2015) and Mao et al. (2018), who obtained decreased cell viability by the generation of reactive oxigen species (ROS) in insects exposed to 50 to 500 ppm silver nanoparticles (Ag-NPs). At present, the information available about the mode of action of Cu-NPs from plants against insects is less abundant that the information reported for Ag-NPs (Benelli, 2018). Therefore, further studies about nanotoxicity of Cu-NPs from *P. sericea* and *P. juliflora* are needed to reveal the exact mechanism of toxicity of nanoparticles in *P. solenopsis*.

Finally, our study showed the potential of *P. sericea* and *P. juliflora* as reducing and stabilizing agents to generate Cu-NPs using green synthesis. At present, the use of Cu-NPs from plants against *P. solenopsis* is scarce. Therefore, our report is the first study that demonstrates the insecticide potential of both Cu-NPs from two plants on *P. solenopsis.*

Acknowledgements

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