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Green Synthesis of Bimetallic Nanoparticles From *Prosopis juliflora* (Sw) DC., and Its Effect Against Cotton Mealybug, *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae)

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Abstract: *Phenacoccus solenopsis* has been recognized as an aggressively invasive species on cotton plants in different countries. This study was conducted to investigate the effect of green synthesized Cu/Zn-nanoparticles using aqueous leaf extract of *Prosopis juliflora* (mezquite) against *P. solenopsis*. The scanning electron microscope (SEM) showed bimetallic nanoparticles of Cu/Zn-NPs with spherical shape with varying size of 74.33 nm to 59.46 nm. More than 30% mortality of *P. solenopsis* was observed with Cu/Zn-nanoparticles (100 ppm) at 96 hours after treatment. Negligible mortality of *P. solenopsis* was recorded with Cu/Zn solution (100 ppm) and aqueous *P. juliflora* extracts. The results of the viability test for Cu/Zn-nanoparticles of *P. juliflora* showed a significant reduction of the cell viability by 50% in insect exposed to Cu/Zn-nanoparticles-*P.juliflora*. Therefore studies about nanotoxicity of Cu/Zn-NPs of *P.juliflora* are needed to reveal the mechanism of toxicity this phytonanoparticles in *P.solenopsis*.

Keywords: Photosynthesis; biocontrol; cotton mealybug; nanoinsecticide

1 Introduction

Phenacoccus solenopsis solenopsis is a polyphagous insect with wide morphological diversity and biological adaptations around the word [1]. This insect has been recognized as an aggressively invasive species on agricultural plants in different countries [2-3]. In recent years, the introduction of Bt cotton in different countries (e.g., Mexico, Pakistan and India), result in an increase of sap feeding insects and emergence of *P. solenopsis* as a pest [2,4]. Some authors reported the use of different control measures for *P. solenopsis* and these measures include the combination between chemical insecticides and biocontrol agents [5-7]. However, recent studies showed that although the insecticides provide high efficiency against *P.solenopsis*. These may cause resistance in this insect and affects their efficiencies [8]. In this sense, the use of bionanotechnology in agriculture has great relevance due to that can promote the development of new bio-pesticides as an alternative to chemical pesticides [9].

Actually, the synthesis of nanoparticles using aqueous leaf extract of plants (green nanotechnology) has great relevance due to that is environmentally friendly and they do not use toxic chemicals in the phytonanoparticles formulation. On the other hand, *Prosopis juliflora* (mezquite) is one of the most important forest resources from northern Mexico to Central and South America constituting an important part of the non-agriculture in America [10]. In recent years *P. juliflora* has been the focus of scientific interest mainly because of its significant content of essential amino acids, tannins, flavonoids,

and polyphenols [11]. However, studies about the biotechnological use of *P. juliflora* for the green synthesis of bimetallic nanoparticles are scarce. Therefore, in the present study, we reported the green synthesis of bimetallic nanoparticles by reduction of copper and zinc (Cu/Zn) by *P. juliflora* extracts and the evaluation of their pesticidal effect against the invasive cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae).

2 Material and Methods

2.1 Biosynthesis of Copper-Zinc Nanoparticles (Cu/Zn-NPs)

The present study was conducted in the Instituto de Ciencias Agrícolas de la Universidad Autónoma de Baja California in Mexicali valley, Baja California, Mexico (lat. 32° 240 26.38" N, long. 115°110 55.43" W). Samples of fresh leaves of *P.juliflora* was collected from a native population in the Mexicali valley, Baja California, Mexico (lat.32° 24′ 6.8394′′ N, long.115° 11′51′′ W). The aqueous extract from *P. juliflora* was prepared with 10 grams of fresh leaves mixed with 100 mL of water and heated at 60°C for 20 min. The homogenate was centrifuged at 4000 rpm for 10 min and the supernatant was decanted into a 50 mL volumetric flask. For Cu/Zn-NPs biosynthesis, 5 mL of supernatant of aqueous extract was added to 50 mL of aqueous solution of 10 mM of $CuSO_4$ and $ZnSO_4$ (50:50 v/v) and heated at 40°C for 30 min. The bio-reduction of Cu/Zn and preliminary identification of the production of Cu/Zn-NPs, was evaluated by the color change of aqueous solution of *P. juliflora*, according to proposal by [9].

2.2 Characterization of Cu/Zn-NPs

The zeta potential and average hydrodynamic size of Cu/Zn-NPs was determined by Dynamic Light Scattering (DLS) in the range of 0.1-1000 μm at 25°C**,** using a nanotrac wave instrument (Microtrac) according to Ruiz-Romero et al. [12]. For EDS analysis, the Cu/Zn-NPs were drop coated on to carbon film and analyzed using instrument Bruker Quantax 400.

2.3 Insecticidal Activity of Cu/Zn-NPs

P. solenopsis was originally collected from cotton plants (Bollgard®) in Mexicali valley, Mexico. Twenty insects' adult females (15 days old) at the same stage were selected and carefully transferred to Petri dishes containing a cotton leaf. Then 2 mL aqueous solution containing 100 ppm Cu/Zn-NPs, (this doses no caused negative impact in foliar tissues, dates not showed) was sprayed on insects in the petri dishes using small hand atomizer. Second group of insects was sprayed with 2 mL aqueous plants extract from *P. juliflora* and third group was only sprayed (2 mL) with metal solution (Cu/Zn) at 100 ppm. All treatments were randomized with five repetitions per treatment. The number of *P. solenopsis* survivors (Mortality percentages) was counted after 24, 48, 72 and 96 hours of initial application by using Abotts formula.

2.4 Determination of Cell Viability

The viability of *P. solenopsis* was determinate using a spectrophotometric assay of Evans blue staining as described previously by Leon-Jimenez et al. [13] with minor modifications. 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 the absorbance at 600 nm. All measurements were carried out in triplicate.

2.5 Data Analysis

Differences between the treatments were analyzed with one-way analysis of variance (ANOVA) and means were compared using Tukey's test ($p \leq 0.05$) using Statistica Version 9.0.

3 Results

The synthesis of Cu/Zn-NPs from *P. juliflora* was primarily confirmed by the change of color from

light yellow to transparent color (Fig. 1) suggests that $CuSO_4$ and $ZnSO_4$ dissolved in water, dissociates into Cu^{2+}, Zn^{2+} and SO_4^{2-}, are reduced to Cu^0 and Zn^0 by reduction action of phytochemicals (e.g., phenols and flavonoids) present in *P. juliflora* forming bimetallic nanoparticles (Zn/Cu-*P.juliflora*) [14].

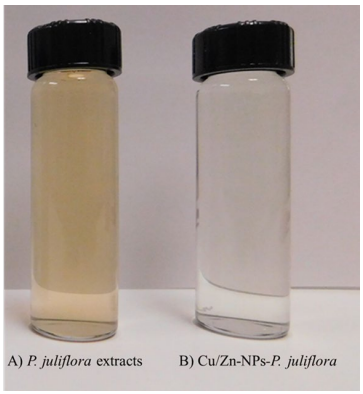

Figure 1: Biosynthesis of Cu/Zn-NPs using aqueous *P. juliflora* extracts: A) aqueous *P. juliflora* extract and B) Cu/Zn-naoparticles of *P. juliflora*

The DLS results for particle size in solution for Cu/Zn nanoparticles are presented in Fig. 2(A). The results showed that Cu/Zn-NPs from *P.juliflora* tendend to form agglomerates with an average hydrodynamic size of 334 nm and zeta potential value of 51.1 mV, when are dispersed in water. On the other hand in the Fig. 2(B), the morphology and size of Cu/Zn-NPs from *P. juliflora* was analyzed using SEM, the results showed that the majority of Cu/Zn-NPs were spherical shape with varying size (74.33 nm to 59.46 nm).

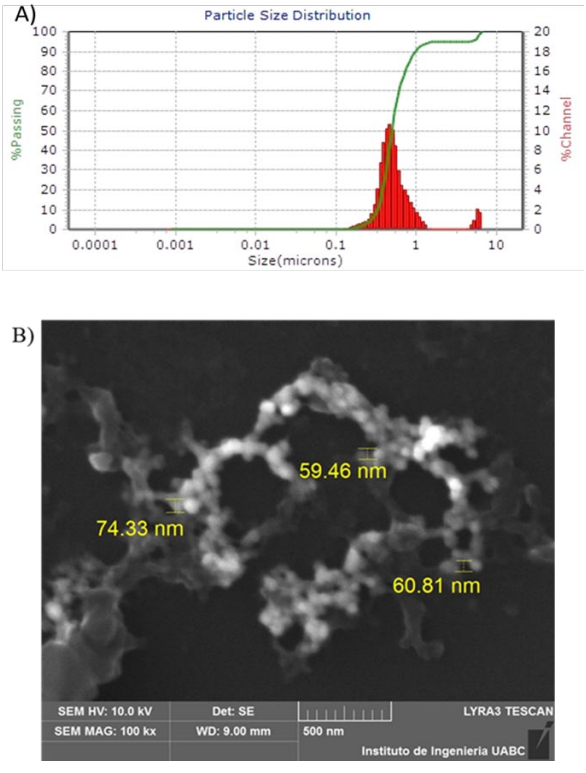

Figure 2: Particle size distribution of Cu/Zn-NPs-*P. juliflora* using A) dynamic light scattering measurements (DLS) and B) scanning electron microscope (SEM)

On the other hand, the Fig. 3, showed that analysis of EDS of Cu/Zn-NPs-*P. juliflora* revealed the presence of copper (1.48%) and zinc (0.45%).

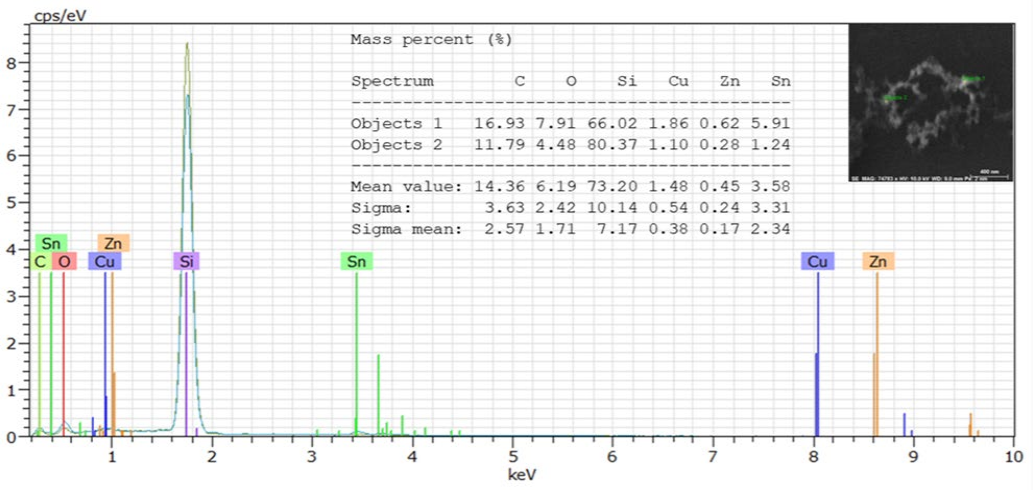

Figure 3: EDS (Energy Dispersive X-ray Spectrometer) of Cu/Zn nanoparticles of *P. juliflora*

As show in the Fig. 4, the changes in the mortality (%) of insects after aplications of Cu/Zn-NPs-*P. julifrora*, solution of Cu/Zn and extract of *P. juliflora*, differed significantly. In this sense, when insects were exposed to Cu/Zn-NPs, the toxicity on *P. solenopsis* was 6, 17, 25 and 33%, after 24, 48, 72 and 96 h of treatment. In contrast, when insects were exposed to Cu/Zn (100 ppm) and extract of *P. juliflora* did not caused a significant increase in toxicity at any time interval.

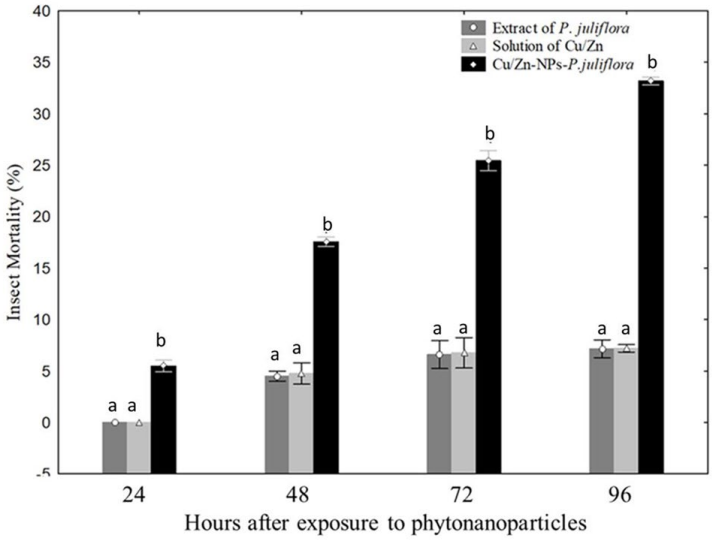

Figure 4: Insect mortality of *P. solenopsis* by Cu/Zn-NPs of P. juliflora after 96 hours of treatment. Means with the same letter were not significantly different according to the Tukey's test ($p \leq 0.05$)

On the other hand, the results of the viability for Cu/Zn-NPs from *P. juliflora* are show in Fig. 5. The results showed a significant reduction of the cell viability (55%) in insect exposed to Cu/Zn-NPs. In

contrast negligible mortality of *P. solenopsis* was recorded with Cu/Zn solution (100 ppm) and extract of *P. juliflora* after 96 hours of exposition.

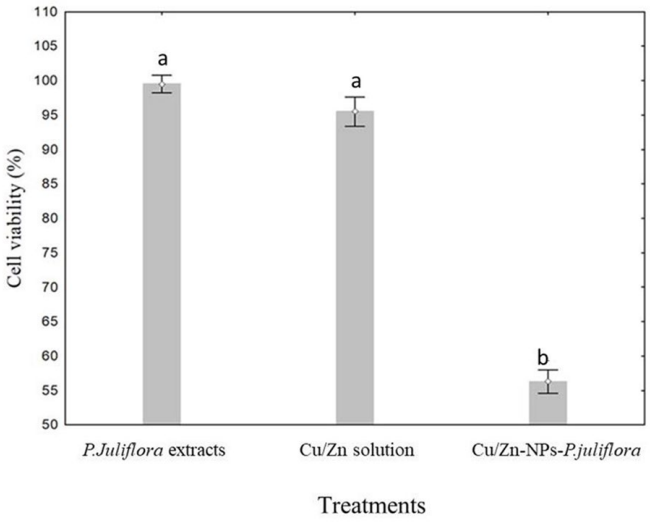

Figure 5: Effect of Cu/Zn-NPs of *P. juliflora* in cell viability of *P. solenopsis* after 96 hours of exposure. Means with the same letter were not significantly different according to the Tukey's test ($p \leq 0.05$)

4 Discussion

According to previous studies on *P. juliflora* the synthesis of monometallic nanoparticles is very frecuently; but scanty reports are available on synthesis of bimetallic nanoparticles which is one of objetives in the present investigation [15]. Our experiments showed that the bioreduction of combination of Cu and Zn to Cu/Zn-NPs of *P. juliflora* was completed within 30 min. Though the mechanism of reduction of Cu/Zn ions by *P.juliflora* under study is not known yet, some studies suggests that the functional groups as phenolic compounds may be involved in the bioreduction and stabilisation process of monometallic nanoparticles [15-16]. However, further detailed studies about role of different metabolites in Cu/Zn-NPs synthesis from *P. juliflora* can will be needed to reveal the mechanism of bimetallic-nanoparticles formation. On the other hand, our results showed that zeta potential was of 51.1 mV for synthesized Cu/Zn-NPs from *P. juliflora* suggested less layer stability formed on the surface of this NPs and functional groups of *P. juliflora*. Similar results was reported by Ruiz-Romero et al. [12] who found positive values of zeta potential for Ag-NPS from *Yucca shidigera* indicating less stability and thus tendency to agglomerate and form large particles. Our results showed that the SEM measured size (74.33 to 59.46 nm) was considerably shorter than DLS size (334 nm). These difference are results of the fact that DLS show the hydrodynamc diameter of Cu/Zn-NPs plus interaction with biomolecules that are attached to surface of phyto-nanoparticles; therefore has large size than SEM [17]. These results are consistent with reported by Bernardo-Mazariegos et al. [18] who found that DLS mean (192 nm) of phytonanoparticles of *Justicia spicigera* was more higher than the SEM mean size (86 nm). On the other hand, the use of bionanotecnology have opened new alternatives in development effective control strategy of insects. In this sense, the present investigation, showed the insecticidal properties of Cu/Zn-NPs of *P. juliflora* against *P. solenopsis*. Cu/Zn-NPs of *P.juliflora* have a significant impact on mortality of *P. solenopsis* in comparasion with some conmonly used chemical insecticides as flubendamide, chlorantraniliprole and imidacloprid [19]. Although however, Huang et al. [20] found that chemical control caused an increased in the mortality of *P. solenopis* on the first instars but not in adult females after 5 days under laboratory conditions. We found that Cu/Zn-NPs caused a significant increase in the mortality of this pest in adult females in similar conditions. In this sense, the present study suggest that interaction of Cu/Zn-NPs with *P. solenopsis* induced membrane damage (cell viability) in cuticule of this

insect and this might be attributable to a distortion of lipid bilayer of membrane as results by the interaction with this bimetallic nanoparticles.In this sense, Kah and Hofman [21] reported that membrane disruption by nanoparticles is likely due to the production of free radicals that cause membrane lipid peroxidation. Similar results were reported by Mao et al. [22] who showed that cell viability were decreased by the generation of reactive oxigen species (ROS) in insects exposed to AgNPs (500 ppm).

5 Conclusion

In the present study our results confirmed the ability of *Prosopis juliflora* capability for the green synthesis of bimetallic nanoparticles. Moreover, biological activity assessment of the bimetallic nanoparticles showed their pesticide effect against *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). Finally studies about nanotoxicity of Cu/Zn-NPs of *P. juliflora* are needed to reveal the mechanism of toxicity of phytonanoparticles in *P. solenopsis*.

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