NANOCIÊNCIAS E NANOTECNOLOGIA:

Pesquisa e Aplicações

Juan Ramón Collet-Lacoste
(Organizador)

EDITORA ARTEMIS
2022

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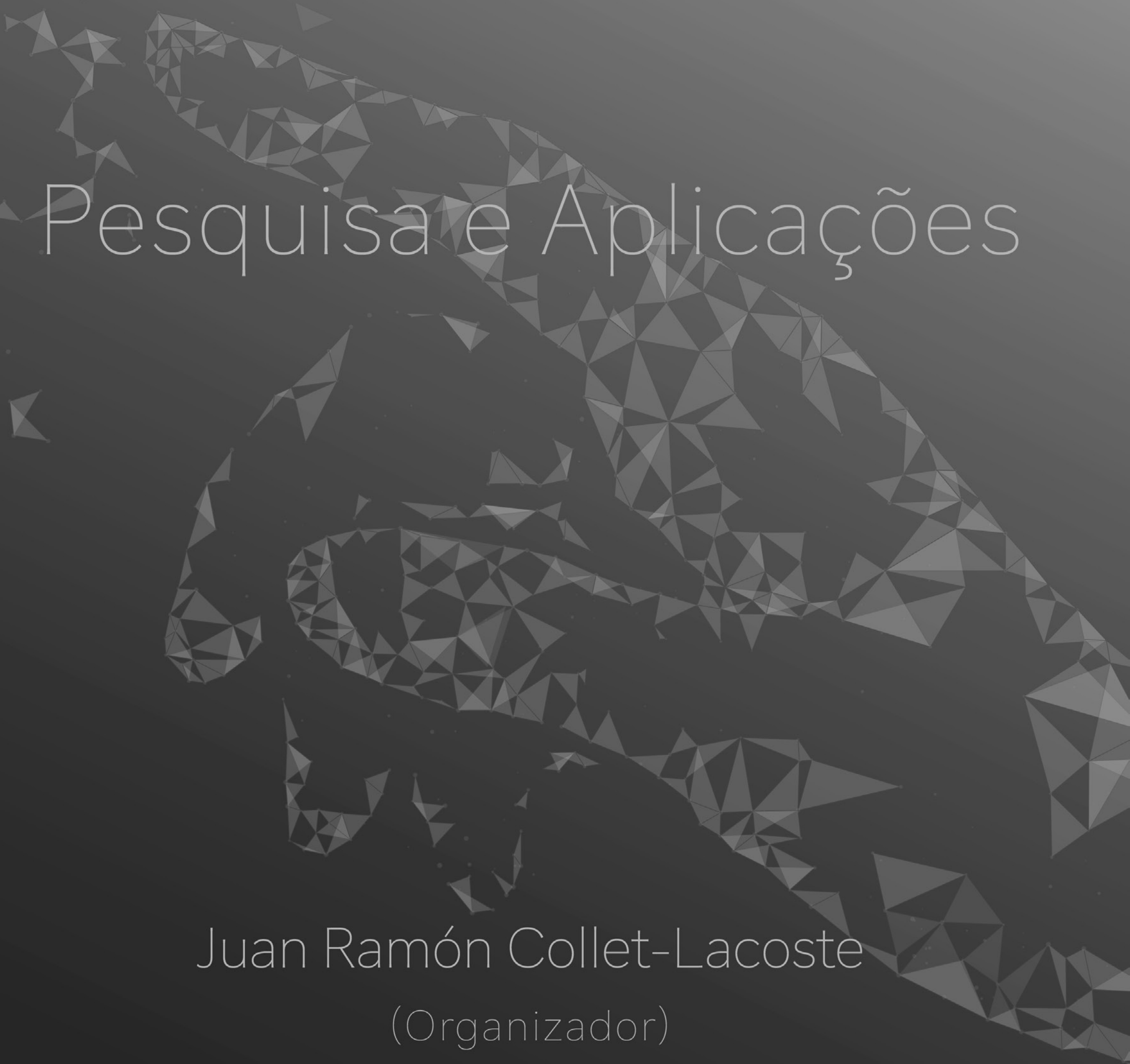

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PRÓLOGO

Las propiedades particulares de las Nps, muy diferentes en muchos aspectos a las de sus sólidos masivos, han abierto nuevos campos de estudio e investigación a todo nivel: teóricos y aplicados. Son más inestables que los sólidos masivos de los que se diferencian principalmente por su estructura electrónica que no suele ser continua. Esto es una ventaja a nivel de su reactividad y suelen presentar superficies específicas altas que son muy propicias para los procesos de catálisis, un ingrediente muy importante en los procesos cinéticos. Otra propiedad interesante es que no presentan defectos estructurales en su volumen como vacancias o dislocaciones, a diferencia de sus correspondientes sólidos masivos.

Las presentes monografías forman parte del título: "Nanociências e Nanotecnologia: Pesquisa e Aplicações". Los artículos están ordenados de lo más general (*e.g.*, producción y caracterización de las Nps) a los relacionados con aplicaciones prácticas (*e.g.*, foto catálisis y a su relación principalmente con aplicaciones de origen biológico).

Estos muestran la potencialidad de las nanotecnologías en la comprensión de nuevas aplicaciones en campos tan variados como la catálisis, fotocatálisis, bio-remediación, contaminantes, ambientes acuáticos, antisépticos, bactericidas, virucidas, compuestos bio-activos, biosíntesis extracelular e intracelular, estudio de suelos, vegetales y probióticos, etc.

Juan Ramón Collet-Lacoste

SUMÁRIO

CAPÍTULO 11

Bacillus thuringiensis AND *Micromonospora echinospora* IN *Lactuca sativa* OPTIMIZE NITROGENOUS FERTILIZER WITH A CRUDE EXTRACT OF CARBON NANOPARTICLES

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ABSTRACT: The healthy growth of *Lactuca sativa* (lettuce) requires nitrogen fertilizer (NIFE) such as NH_4NO_3, to avoid hyperfertilization the dose is reduced and optimized with *Bacillus thuringiensis* and *Micromonospora echinospora* with a crude extract of carbon nanoparticles (CENC). The objective of this work was to analyze the growth of *L. sativa* with *B. thuringiensis* and *M. echinospora* at 50% NH_4NO_3 with a CENC. In that sense *L. sativa* seeds were treated with *B. thuringiensis* and *M. echinospora* at 50% NH_4NO_3: and 10 ppm of CENC and through the phenology/biomass response variables to seedling, the experimental data was validated by ANOVA/Tukey HSD (P< 0.01). The results showed a healthy growth of *L. sativa* with *B. thuringiensis* and *M. echinospora* at NH_4NO_3 50% and 10 ppm CENC. This supports that the positive response of *L. sativa* with *B. thuringiensis* and *M. echinospora* with NH_4NO_3 at 50%, was enhanced by the CENC for the maximum optimization of NH_4NO_3 with preservation of the productivity of soil.
KEYWORDS: Soil. Vegetable. Plant probiotics. Carbon nanoparticles.

1 INTRODUCTION

The healthy growth of *Lactuca sativa* (lettuce) requires nitrogen fertilizer (NIFE) as a NH_4NO_3, applied in excess causes loss of productivity and environmental pollution (De Grazia et al., 2001; Carranza et al., 2009; Sernaqué & López, 2012), an ecological alternative solution to prevent these problems. It is to reduce NH_4NO_3 to 50% applying in *L. sativa* and then optimize it by seed inoculation with *Bacillus thuringiensis* and *Micromonospora echinospora* genera and endophytic species of

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growth-promoting bacteria that transform compounds from seed and root metabolism into phytohormons (Araujo et al., 2012; Chaturvedi et al., 2016; Vurukonda et al., 2018), which can be improved with a crude extract of carbon nanoparticles (CENC), to accelerate and make efficient that *B. thuringiensis* and *M. echinospora* generate phytohormons for enhancing proliferation of radical system to improve the NH_4NO_3 uptake without remaining that deteriorates the soil or the environment (Chung et al., 2011; Chaturvedi et al., 2016; Jordan et al., 2020). In the literature, limited research exists related to inoculate seed with plant growth promoting bacteria and crude or pure carbon nanoparticles, but if the application of carbon nanoparticles with nitrogen fertilization such as reported by Khodakovskaya et al., 2012 in *Nicotiana tabacum* (tobacco) with NIFE and 5-500 ppm multi-walled nanotubes improved by a 55 to 64% plant growth compared to *N. tabacum* with NIFE alone. Therefore, the objective of this work was to analyze the growth of *L. sativa* with *B. thuringiensis* and *M. echinospora* with NH_4NO_3 at 50% and enhanced by CENC.

2 MATERIALS AND METHODS

2.1 COLLECTION AND PREPARATION OF SOIL SAMPLE

A soil collected from a site located at 19° 37' 10" north latitude 101° 16' 41.99" west longitude, with an altitude of 2013 meters above sea level, with a temperate climate of an agricultural area called "Uruapilla" of the municipality was used. of Morelia, Mich., on the highway Morelia-Pátzcuaro, Mexico. Later moved to the Environmental Microbiology Laboratory of the Biological Chemical Research Institute (IIQB) of the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH). Before starting the experiment, a physicochemical analysis of the soil was carried out according to NOM-021-SEMARNAT-2000. The soil was sieved with a No. 20 mesh, then solarized for 40 h to reduce pests and diseases. *L. sativa* seeds were disinfected with 0.2% NaClO for 5 min, then washed 6 times with sterile tap water; they were disinfected in 70% (v/v) alcohol for 5 min, washed 6 times with sterile tap water.

2.2 ORIGEN OF *B. thuringiensis* AND *M. echinospora* FOR *L. sativa*

The strain of *B. thuringiensis* was isolated from the interior of teosinte roots (*Zea mays var. mexicana*) in nutrient agar. The axenic culture of the isolated *B. thuringiensis* was reproduced in nutrient broth for 48h to mix in a 1: 1 ratio with *M. echinospora* that was isolated from alfalfa (*Medicago sativa*) nodules in Congo red mannitol yeast extract agar (Sanchez-Yañez, 2007), the same culture medium to reproduce it before inoculating it into

L. sativa seeds. Then, for every 10 *L. sativa* seeds, they were inoculated with 1.0 mL (v/v) of *B. thuringiensis* and/or *M. echinospora* (Sánchez-Yáñez, 2007).

2.3 CRUDE EXTRACT OF CARBON NANOPARTICLES FOR *L. sativa* SEEDS WITH *B. thuringiensis* AND *M. echinospora*

Obtaining a CENC was made from the leaves of *Albizia julibrissin*, which were disinfected by immersion with 0.5% NaOCl for a minute and rinsed with sterile deionized H_2O, then the leaves were cut into pieces. 5 cm and dried at 80°C/12 h, of which 30 g suspended in 300 mL of deionized H_2O were used, which were heated to 70°C/30 min, then the aqueous extract of *A. julibrissin* was filtered in Whatman No. 1 it was centrifuged at 4000 rpm/10 min, refrigerated at 4°C (Abdelmoteleb et al., 2017). For further characterization of this aqueous extract, field emission scanning electron microscopy (FESEM) (JEOL-JSM5910LV and JEOL-JSM7600F) coupled with an energy dispersive spectrometer (EDS) was used to determine the morphology and chemical composition of the nanostructures in the crude extract of *A. julibrissin.* Then with 1.0 mL of a concentration of 10 ppm of the CENC dispersed in 0.85% NaCl with detergent commercial "La Corona" at 0.5% (p/v). The seeds treated with *B. thuringiensis* and/or *M. echinospora* and the CENC were sown in 100 g of soil in a greenhouse container fed with NH_4NO_3. The experimental design was a randomized block design with 6 treatments, 2 controls and 6 replicates: *L. sativa* irrigated only with water or absolute control (AC); *L. sativa* without *B. thuringiensis* or *M. echinospora* and 100% NH_4NO_3 or relative control (CR); *L. sativa* with *B. thuringiensis* and/or *M. echinospora* NH_4NO_3 enhanced with 10 ppm of a CENC. The response variables used were: phenology: plant height (PH) and root length (RL); and biomass: aerial and root fresh weight (FAW/FRW) and aerial and root dry weight (DAW/DRW) at seedling. The results were validated by ANOVA/Tukey HSD ($P<0.01$) (Sánchez-Yáñez, 2007).

3 RESULTS AND DISCUSSION

Table 1. Physicochemical properties of agricultural for *Lactuca sativa.*

*Parameter	Value	Interpretation
pH	5.67	Moderately acidic
Organic matter (%)	10.44	Very high
Texture (%)	31.8 (clay), 26.92 (sand), 42.0 (silt)	Clayey silt
Total nitrogen ppm	3.20	poor
Phosphorus ppm	21.9	poor
Sodium (Na^+) ppm	153.38	High
Potassium (K^+) ppm	168.61	High

*Parameter	Value	Interpretation
Microelements (ppm):		
Iron (Fe^{2+})	13.91	High
Zinc (Zn^{2+})	0.37	Low
Copper (Cu^{2+})	0.54	Low
Manganese (Mn^{2+})	4.62	Low[1]

*NOM-021-SEMARNAT-2000.

Table 1 shows the physicochemical properties of the soil before the experiment, with a low content of total N with 3200 ppm and P with 21.9 ppm, with a poor C:N:P (carbon:nitrogen:phosphorus) ratio due to the on land use that caused salinity and a low available concentration of metals necessary for plant growth in accordance with NOM-021-SEMARNAT-2000.

Table 2. Effect of *Bacillus thuringiensis* and *Micromonospora echinospora* on the phenology of *Lactuca sativa* at seedling stage with NH_4NO_3 50% plus a crude extract of carbon nanoparticles.

Treatment* *Lactuca sativa*	Plant height (cm)	Radical length (cm)
Absolute control irrigated just water	8.52^{c**}	2.07^{c}
Relative control without *B. thuringiensis* either *M. echinospora* NH_4NO_3 100% no CENC	9.37^{b}	2.17^{c}
T1 = *B. thuringiensis* NH_4NO_3 50% 10 ppm CENC	9.51^{b}	2.48^{b}
T2 = *M. echinospora* NH_4NO_3 plus 10 ppm CENC	9.74^{b}	2.71^{b}
T3 = *B. thuringiensis/M. echinospora* NH_4NO_3 al 50% plus 10 ppm CENC	10.98^{a}	3.23^{a}

*n=6; **Values with different letters indicate statistical difference (P>0.01) according to ANOVA/Tukey.

Table 2 shows the seedling phenology of *L. sativa* with *B thuringiensis* and *M. echinospora* with 50% NH_4NO_3 10 ppm CENC with 10.98 cm of PH and 3.23 cm of RL, both numerical values with statistical difference with the 9.74 cm of PH and 2.71 cm of RL of *L. sativa* with *M. echinospora* and 10 ppm of CENC at 50% of NH_4NO_3; with the 9.51 cm of PH and the 2.48 cm of RL of *L. sativa* with *M. echinospora* and 10 ppm of ECNC at 50% of NH_4NO_3; The results support that *B. thuringiensis* and *M. echinospora* converted organic compounds from metabolism in the roots into phytohormons, improved uptake and optimization of NH_4NO_3 at 50% by including the CENC which enhanced uptake of NIFE (Araujo et al., 2012; Lahiani et al., 2015; Shekhawat et al., 2021). The above is possible because nanomaterials with a size less than 100 nm present a high adsorption capacity, which allows them to improve the retention of NH_4NO_3, then to be released according to the nutritional need of *P. vulgaris*, which through a dense radical system optimized NIFE to 50% (Safdar et al., 2022). In comparation with the 9.37 cm of PH and the 2.07 cm of RL of *L. sativa* without *B. thuringiensis /M. echinospora* with 100% NH_4NO_3 without CENC or relative control.

Table 3. Effect of *Bacillus thuringiensis* and *Micromonospora echinospora* on the biomass of *Lactuca sativa* seedling with 50% NH_4NO_3 with a crude extract of carbon nanoparticles.

Treatment* ***Letuca sativa***	**Fresh weight (g)**		**Dry weight (g)**	
	Aeral	**Radical**	**aereal**	**Radical**
Absolute control irrigated just water	0.086[d**]	0.022[d]	0.017[c]	0.005[b]
Relative control without *B. thuringiensis* either *M. echinospora* NH_4NO_3 100% no CENC	0.114[c]	0.030[c]	0.026[b]	0.007[b]
T1 = *B. thuringiensis* NH_4NO_3 50% 10 ppm CENC	0.145[b]	0.039[b]	0.036[a]	0.010[a]
T2 = *M. echinospora* NH_4NO_3 plus 10 ppm CENC	0.140[b]	0.041[b]	0.035[a]	0.010[a]
T3 = *B. thuringiensis/M. echinospora* NH_4NO_3 al 50% plus 10 ppm CENC	0.156[a]	0.048[a]	0.039[a]	0.012[a]

*n=6, **Values with different letters indicate statistical difference (P>0.01) according to ANOVA/Tukey.

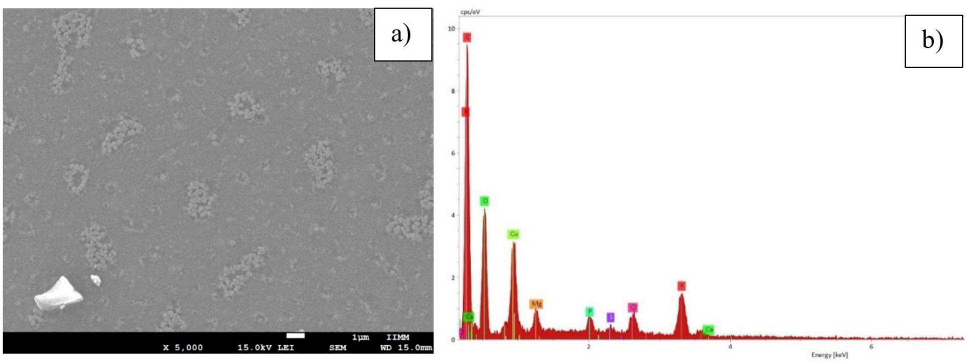
Table 3 shows the seedling fresh weight of *L. sativa* with *B thuringiensis and M. echinospora*, NH_4NO_3 at 50% and 10 ppm CENC where 0.156 g of FAW and 0.048 g of FRW were recorded. The results support that *B. thuringiensis* and *M. echinospora* converted organic compounds from metabolism in the roots into phytohormons, improved uptake by the CENC and optimization of NH_4NO_3 at 50% (Tripathi et al., 2011; Compant et al., 2010; Goryluk-Salmonowicz et al., 2018). According to Siddiqui et al., 2015, carbon-based nanomaterials such as CENC can pass through the cell walls of plants; there release NIFE to maintain the healthy growth of *P. vulgaris*. Those numerical values with statistical difference with the 0.144 g of FAW and the 0.030 g of FRW of *L. sativa* without *B. thuringiensis and M. echinospora*, with 100% NH_4NO_3 without CENC or relative control.

The same way with *L. sativa* and NH_4NO_3 at 50% and 10 ppm of CENC where 0.039 g of ADW and 0.039 g of RDW were recorded, numerical values with statistical difference compared. This supports that it was possible to improve the conversion of organic compounds inside the root of *L. sativa* by *B. thuringiensis* and/or *M. echinospora* with NH_4NO_3 at 50% by CENC for maximum uptake of NIFE without risk of loss of soil productivity or environmental pollution (De Rosa et al., 2010; Kodakvskaya et al., 2012; Vurukonda et al., 2018; Adeleke et al., 2021). The above is supported based on literature, since some nanomaterials such as nanocarbon can adsorb nitrogen from ammonia and release hydrogen ions, which allows plants such as L. sativa to absorb more water and nutrients (Hong et al., 2013; Nair et al., 2010). As a result, the absorption and optimization of NIFE improved and maintained the healthy growth of *L. sativa*, compared to 0.26 g ADW and 0.007 g RDW from *L. sativa* without *B. thuringiensis* and *M. echinospora* with 100% NH_4NO_3 and CENC or relative control.

3.1 PARTIAL CHARACTERIZATION OF CRUDE EXTRACT OF NANOPARTICLES

In **Figure 1a**, the SEM micrograph shows the morphology and size of the particles present in the CENC of *A. julibrissin*, which are spherical with a size of less than 300 nm, where some tended to be dispersed while others formed agglomerates. In **Figure 1b**, the EDS analysis of the CENC recorded carbon as the main element with 64% and oxygen with 35% atomic, in addition, some traces of chemical elements such as K, Ca, Mg, Cl that could be involved in the composition of *A. julibrissin* were also recorded.

Figure 1. SEM (a) and EDS (b) analysis of ECNC of Albizia *pluriforma.*



4 CONCLUSION

The effect of *B. thuringiensis* and *M. echinospora* in *L. sativa* with 50% NH_4NO_3 was improved by the CENC, although the mechanism is not precise, it was evident that both the phytohormonal action related to the optimization of the nitrogen fertilizer by both genus and species was positively influenced by the CENC.

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SOBRE EL ORGANIZADOR

El Dr. Juan Ramón Collet-Lacoste es licenciado en ciencias químicas de la Universidad de Buenos Aires (UBA) y PhD de la Universidad de Paris Sud (XI). Su especialidad es la físico química, en la rama de la termodinámica de los procesos irreversibles (TPI), especialmente en el estudio de los procesos cinéticos en los sistemas electroquímicos.

Ha desarrollado varios trabajos relacionados a los mecanismos de reacción y transporte de materia sobre electrodos metálicos, así como el desarrollo de electrodos para celdas de combustible de baja temperatura (fuel cells).

Es un especialista en la técnica de impedancia electroquímica, en la cual ha publicado varios artículos en revistas internacionales.

Desde el punto de vista experimental, ha trabajado en el desarrollo de celdas de combustible con Nps de platino y paladio y de electrolizadores alcalinos de baja temperatura.

Actualmente realiza trabajos sobre la oxidación acuosa del aluminio en gradientes de temperatura. Este trabajo esta relacionado a los elementos combustibles de los reactores experimentales multipropósito para la fabricación de radioisótopos de uso médico.

ÍNDICE REMISSIVO

A

B

C

E

F

H

T

V