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# PHYTO-TOXICITY BY NANOTUBE OF CARBON MULTI WALLS AND GRAPHITE OXIDE NANOPARTICLES IN PLANTS OF *RAPHANUS SATIVUS*

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**Abstract:** This work consisted in carrying out two bioassays in which multi-walled carbon nanotubes (NTCM) and graphite oxide nanoparticles (NPsOG) were used. In the first, R. sativus seeds were imbibed for 2 h in solutions containing NTCM and NPsOG at concentrations of 0, 100, and 200 ppm. In the second greenhouse bioassay, radish seeds were sown in containers containing a mixed substrate of zeolite and peat. When the plants had a pair of true leaves, foliar applications were started every week with the aforementioned concentrations. The results revealed that in both bioassays, NTCM and NPsOG negatively affected seed germination and caused lower height, leaf area, and plant biomass, indicating a phytotoxic effect.
**Keywords:** Agronanotechnology, nanomaterials of carbon, zeolite, phytotoxicity.

## INTRODUCTION

Advances in nanotechnology (NT) are being integrated into the biology and has led to the emergence of new disciplines such as nanobiotechnology, in which which NPs have interesting applications. NPs are atomic or molecular aggregates with a dimension between 1 and 100 nm [1], with different physical-chemical properties in compared to micron-sized material [2,3]. The applications of the NT in the modern agriculture have great impact through the development of metallic NPs and derivatives of the carbon that they promise improve the germination of seeds and he growth of the plants[4], produce nanofertilizers with controlled release of nutrients [5], nanocapsules for the controlled application of herbicides [6], what is possible that nanoformulations allow to reduce the dose and frequency of application in comparison with the formulations conventional [7].

Carbon-derived nanomaterials, such as NTCMs and NPsOGs, are widely used in biological and material sciences. Due to their nanometric size, they have the facility Once they penetrate cells, they can activate water channels and facilitate the transport of nutrients, improving the germination and growth of plants. However, it is not known the quantities optimal of application, various researchers mention that tall concentrations can cause potential damage to cell membranes; upset the balance of nutrients and loss of electrolytes, causing less vegetative growth [8]. His application in floors of interest agricultural generate effect phytotoxic, already that cause an imbalance of nutrient content and reduction of photosynthetic activity, limiting the plant growth [9]. In order to understand the potential benefits of applying the NT to the agriculture.

Similarly, some materials such as zeolites are being used to improve the retention of water, as carriers of nutrients; and for the soil recovery contaminated by fertilizers, herbicides and pesticides. These materials aluminosilicates promote the germination of seeds, growth of plants, phosphorus fixation and nitrogen [10]. Zeolites are minerals of volcanic origin with a crystalline structure. three-dimensional formed with pores and channels, which can be used to make efficient use of fertilizers [eleven]. By it former, the objective of this study was to investigate through two bioassays carried out in laboratory and greenhouse conditions, the effect of NTCM and NPsOG in the germination of seeds and in the growth of floors of *R. sativus.*

## METHODOLOGY

He performed 2 experiments: the first consisted of imbibition of radish seeds with NPsOG and NTCM (0, 100 and 200 ppm); the second was with applications weekly foliars of the same NPs. It was carried out under protected agriculture conditions in a greenhouse of technology half in the experimental field of the CIQA, in Saltillo, Coahuila.

## NANOPARTICLES USED AND CHARACTERIZATION BY RAMAN SPECTROSCOPY AND TEM MICROSCOPY

Commercial NTCM of 30-50 nm and 90% purity, purchased with the company, were evaluated. NanoLove Inc. New Mexico, USA. NPsOG were synthesized at CIQA and characterized. by TEM techniques and Raman spectroscopy. Charcoal was used as absolute controls. mineral and graphite in size micrometric of origin natural of mines of the region and provided by he Department of Synthesis of polymers.

## PREPARATION OF THE SOLUTIONS WITH NPS

The solutions with the NTCM, NPsOG, microparticles of mineral carbon and graphite were prepared in concentrations of 0, 100 and 200 mg $L^{-1}$ in distilled water and dispersed with a sonicator Branson 2510, by 30 minutes.

## DRIVING OF THE CROP

Germination stage. Sowing the seeds of *Raphanus sativus* cv Champion (Fax of West SA OF CV) was in trays of polystyrene of 200 cavities with substratum of foot moss. Before sowing, the seeds were treated with solutions containing NPs, they left imbibe during 2 h for this stage HE established 5 treatments with twenty repetitions each one. He time of assessment was of twenty days.

Seedling stage. Two different mixtures of substrate were prepared (70% peat moss + 30% perlite and 70% peat moss + 30% zeolite). Planting was carried out in 5 L polyethylene pots. capacity. During the development of the crop, irrigation was applied according to the demand of the plant and every third day it was fertilized with a Hoagland solution, the duration of the experiment was of four days.

## APPLICATION OF NPS TO THE FLOORS

The foliar application of the NPs was manual once a week, three applications throughout the experimental period, with 20 mL capacity sprinklers, 1 mL of the different solutions of NPs and the absolute control was distilled water in the same amount. As controls HE applied the microparticles of graphite and coal mineral in the same concentrations. To the moment of the application HE added a adherent commercial, this product is used in agriculture to facilitate the adherence of any product chemical in the leaves benefiting the absorption.

## DETERMINATION OF THE GROWTH AND BIOMASS

Plant height, leaf area (LI-COR model LI-300, Lincoln, Nebraska, USA), number of leaves, chlorophyll index (Minolta SPAD 502), root length, bulb size (longitudinal and equatorial diameter) and dry biomass (leaves, stem, root). These measurements are they made to the final of the experiment in all the floors.

## ANALYSIS STATISTICAL OF DATA

The experimental design used was completely randomized with five treatments and 20 repetitions in bioassay 1 (germination stage) the treatments were designed according to agreement to the concentration (ppm) of the nanomaterials (NPsOG) either NTCM), as HE sample to continuation:

T1= Control
T2= Graphite (100)
T3= Graphite (200)
T4= NPsOG (100)
T5= NPsOG (200)
T1= Control
T2= Mineral carbon (100)
T3= Mineral coal (200)
T4= NTCM (100)

T5= NTCM (200)

In bioassay 2, six treatments with five repetitions were evaluated in factorial design. AxB. Where factor A was the substrate (peat moss: perlite and peat moss: zeolite, 70:30 v/v); the factor B consisted in the concentrations of NPsOG and NTCM as appropriate (0, 100 and 200ppm). Each pot with 4 plants was a repetition. The distribution is presented in the Board 1. The assessment of the variables physiological HE performed through a analysis of variance and Tukey multiple range test (p≤0.05), with statistical software SAS-JMP version 5.0.1 (SAS Institute, 2002).

| Nanoparticles (ppm) | substrates | |
|---|---|---|
| | 70:30 pm: p (v/v) | 70:30 pm: z (v/v) |
| 0 | T1 | T2 |
| 100 | T3 | T4 |
| 200 | T5 | T6 |

ppm= parts per million, v/v= mixed ratios on a volume/volume basis, pm= peat moss, p= perlite, Z= zeolite, T= treatment.

Table 1. Distribution of treatments of the second bioassay in which NPsOG and NTCM foliarly.

## RESULTS AND DISCUSSION

### CHARACTERIZATION MICROSCOPIC OF THE NANOPARTICLES OF OXIDE OF GRAPHENE

Figure 1 shows the Raman spectrum, which is a technique that allows the study of low-frequency vibrational and rotating modes, giving rise to bands or signals that allow measuring the degree of molecular hybridization (order or disorder) and thereby predicting some of its properties. In this particular spectrum, it is possible to appreciate the D band at 1200 cm-1 with practically the same intensity as the G band at approximately 1600 cm-1, which indicates that the amount of sp2 and sp3 hybridization carbon atoms in the sample is similar, deducing the existence of defects that lead to atomic disorder in the structure. The absence of the M band indicates that the material is not conductive.

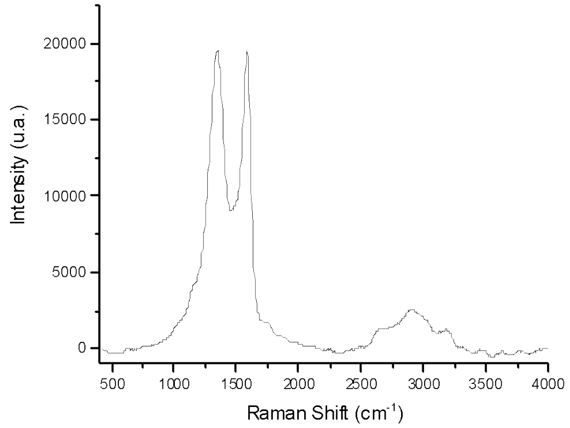


Figure 1. Spectroscopy Raman of nanoparticles of oxide of graphite

Figure 2 shows the image of Transmission Electron Microscopy, a technique that uses the amplifying power of a beam of electrons to display the image of a material. The image is built by observing the trajectory of the electrons that achieved pass through a sample of very low thickness. Graphene sheets are observed in this image with some microns of size deposited about a rack of carbon which are find spliced nails with others.

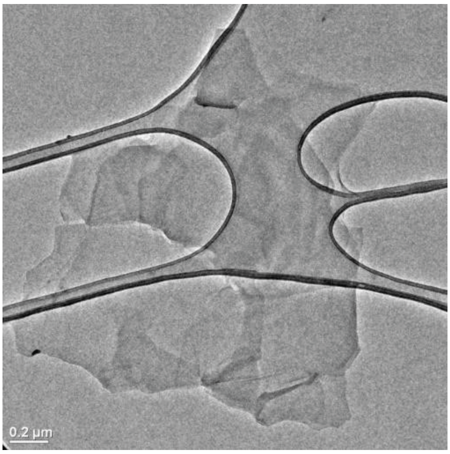


Figure 2. Image of Microscopy electronics of Transmission (TEM) of nanoparticles of oxide of graphite.

## INHIBITION OF SEEDS WITH NTCM AND NPSOG AND HIS EFFECT IN GERMINATION OF SEEDS OF RADISH

Imbibition of radish seeds with NPsOG for 2 h before sowing, significantly caused phytotoxicity ($p \leq 0.05$) (Figure 3A); with 100 ppm (T4), the height was affected in 23.73% (Figure 4A), number of leaves (19.15%, Figure 4B), stem diameter (12.81%), fresh biomass (54.47%, 4C) and dry biomass (41.18%, Figure 4D) with respect to the control (T1). So much micron-sized graphite as graphite oxide NPs caused phytotoxicity, but it can be seen that the NPs from 100 ppm caused significantly elderly damage in the floors.

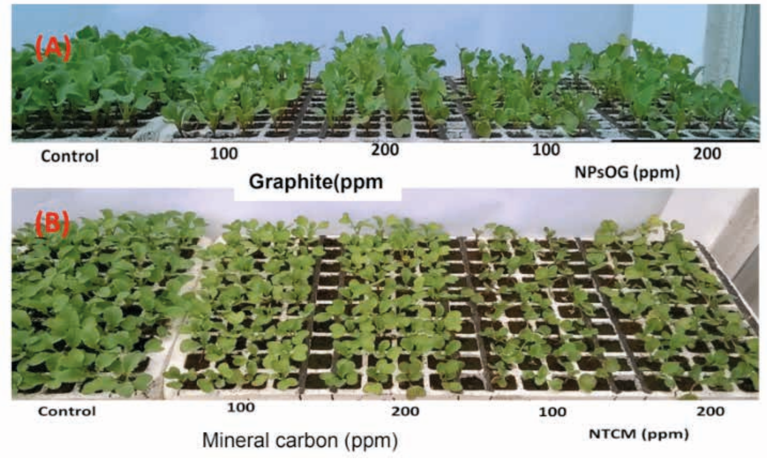


Figure 3. Effect on the growth of radish plants. A) Imbibition of the seeds with microparticles of graphite and NPsOG; B) imbibition of the seeds with coal mineral and NTCM.

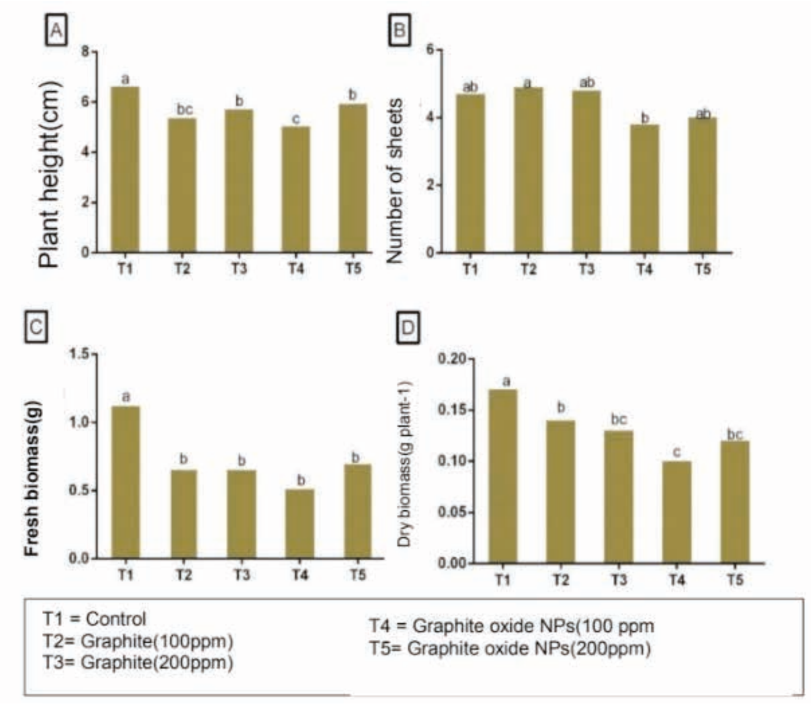


Figure 4. Imbibition of radish seeds with NPsOG and their growth response. A) height of plant, B) number of leaves, C) biomass fresh and D) dry biomass. Different letters in bars indicates significance statistics ($p \leq 0.05$).

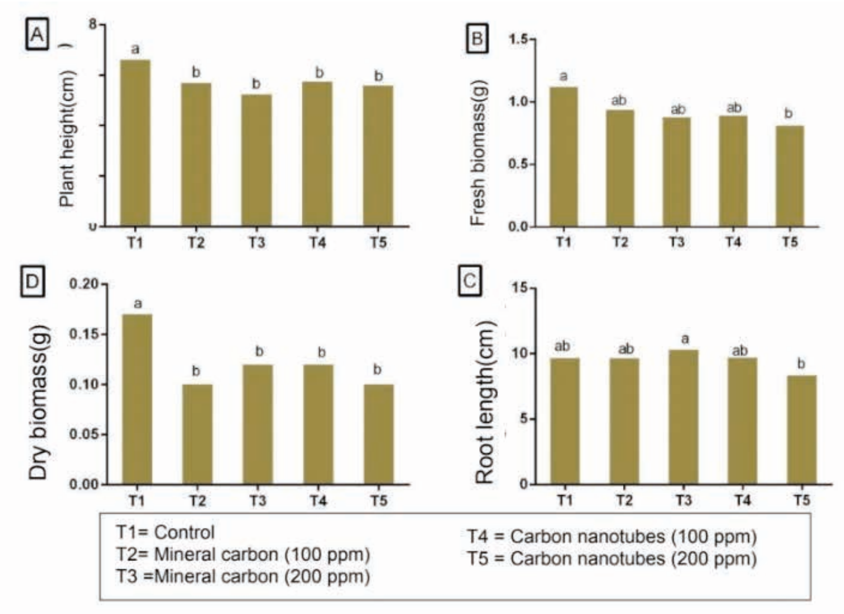


Figure 5. Imbibition of radish seeds with NTCM and its response in the growth of radish plants. *Raphanus sativus*. A) plant height, B) number of leaves, C) biomass fresh and D) dry biomass. Letters different in bars indicates significance statistics (p≤0.05).

The imbibition of radish seeds with NTCM for 2 h before sowing influenced the plant physiology (Figure 3B), observing that the concentration of 200 ppm (T5) caused significantly phytotoxicity (p≤0.05). The height of the plant in Q5 was lower (15.41%, Figure 5A), fresh biomass (27.68%, Figure 5B), root length was also affected (13.57%, Figure 5C) and the biomass dry HE reduced in 41.18% (Figure 5D) in comparison with the treatment control (T1).

**GROWTH OF SEEDLINGS IN GREENHOUSE**

Table 2 shows the average values of all the repetitions of the substrates with zeolite (Factor A), foliar application of NPsOG (Factor B) and the interaction of both factors (AxB). The substrate containing zeolite promoted plant growth by expressing in greater leaf area (7%) and bulb size (14.25%), compared to plants that were cultivated with foot moss: perlite (70:30 v/v).

| Treatments | Area foliar ($cm^2$) | dry weight bulb (g) | biomassdry (g) | number ofleaves | Chlorophyll index(OR Spad) |
|---|---|---|---|---|---|
| substrates | | | | | |
| 70:30 (PM:P) | 365.13 to | 0.69 to | 1.28 to | 9.20 to | 36.37 to |
| 70:30 (PM:Z) | 408.83 to | 0.71 to | 1.37 to | 8.93 to | 34.43 to |
| p≤ 0.05 | 0.257 | 0.836 | 0.572 | 0.385 | 0.332 |
| NTCM (ppm) | | | | | |
| 0 | 386.01 to | 0.88 to | 1.33 to | 9.20 to | 34.30 to |
| 100 | 381.83 to | 0.58 to | 1.28 to | 9.20 to | 37.42 to |
| 200 | 393.10 to | 0.63 to | 1.37 to | 8.80 to | 34.49 to |
| p≤ 0.05 | 0.969 | 0.157 | 0.879 | 0.469 | 0.362 |
| Interaction | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| 0 + PM:P | 441.93 to | 1.14 to | 1.54 to | 9.60 to | 35.14 to |
| 0 +PM:Z | 330.09 to | 0.63 ab | 1.12 to | 8.80 to | 33.46 to |
| 100+MP:P | 321.69 to | 0.39 b | 1.13 to | 9.40 to | 40.44 to |
| 100+MP:Z | 441.96 to | 0.78 ab | 1.43 to | 9.00 to | 34.40 to |
| 200+MP:P | 331.77 to | 0.53 ab | 1.18 to | 8.60 to | 33.54 to |
| 200+MP:Z | 454.43 to | 0.73 ab | 1.56 to | 9.00 to | 35.44 to |
| p≤ 0.05 | 0.025 | 0.022* | 0.058 | 0.274 | 0.273 |

Values with the same literal in columns are not statistically significant (P≤ 0.05). PM= peat moss, P= perlite, Z= zeolite, NTCM= nanotubes of carbon multiwalls. (70:30 indicate the quantities mixed in base to volume-volume).

Table 2. Biomass production of *Raphanus sativus plants* with foliar application of nanoparticles of oxide of graphite.

On the other hand, the plants that were applied foliarly 100 ppm of NPsOG, it was observed a phytotoxic effect (p≤0.05), expressed in lower height (6.94%), bulb diameter (16.48%), bulb size (15.67%), in the same way the leaf area was smaller (30.17%) in the plants that were applied graphite NPs, the dry biomass of the bulb was significantly reduced (51.73%) and the dry biomass of the aerial part was lower (28.58%) compared to the control (0 ppm) (Figure 6).

Table 3 shows the average values of all the repetitions of each of the treatments. For factor A (substrates containing zeolite), for factor B foliar application of NTCM and the interaction of both factors (A x B). The results indicate that the plants cultivated in substrates containing zeolite presented superior growth, observing in greater leaf area (11.96%) and dry biomass of the bulb (2.89%), compared to plants cultivated in substrates without this aluminosilicate.

The index of chlorophyll and number of leaves did not show any change in the different substrates. of sowing. Contrary the application foliar of 100 and 200 ppm of NTCM caused quantitatively phytoxicity in plants (Figure 7), expressed in lower dry biomass of bulbs (34 and 28.40% respectively). The results of the interaction of substrate with zeolite and nanoparticles did not favor growth. Less growth was observed in the floors cultivated with substratum contending zeolite and application of NTCM.

| Treatments | leaf area ($cm^2$) | biomass (g) | | | |
|---|---|---|---|---|---|
| | | Fresh weight bulb | biomassfresh | dry weightbulb | biomass dry |
| substrates | | | | | |
| 70:30 (PM:P) | 332.74a | 9.45b | 16.86a | 0.49b | 1.08a |
| 70:30 (PM:Z) | 358.51a | 13.92a | 17.83a | 0.78a | 1.17a |
| p≤ 0.05 | 0.424 | 0.018** | 0.590 | 0.004** | 0.485 |
| NPs og (ppm) | | | | | |
| 0 | 393.65a | 17.17a | 19.44a | 0.87a | 1.26a |
| 100 | 274.89b | 7.11b | 13.73b | 0.42b | 0.90ab |
| 200 | 368.34ab | 10.79b | 18.86ab | 0.61ab | 1.20b |
| p≤ 0.05 | 0.013** | 0.0004** | 0.028* | 0.002** | 0.050* |
| Interaction | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| 0 + PM:P | 414.37a | 15.68ab | 20.78a | 0.74 abc | 1.36a |
| 0 +PM:Z | 372.93a | 18.65a | 18.09 a | 0.99a | 1.16a |
| 100+MP:P | 245.30a | 5.56c | 12.07a | 0.30c | 0.77a |
| 100+MP:Z | 304.48a | 8.67bc | 15.38a | 0.55abc | 1.04a |
| 200+MP:P | 338.54a | 7.13bc | 17.71a | 0.44bc | 1.11a |
| 200+MP:Z | 398.13a | 14.44abc | 20.02a | 0.79ab | 1.29a |
| $p \leq 0.05$ | 0.342 | 0.001** | 0.354 | 0.002** | 0.257 |

Values with the same literal in columns No are significant statistically ($p \leq 0.05$). PM= foot moss either peat, P = perlite, Z= zeolite (70:30 indicate the quantities mixed in base to volume-volume), OG= nanoparticles of oxide of graphite.

Table 3. Effect on the growth of *R. sativus plants* with foliar application of nanotubes of carbon.


Figure 6. Effect of foliar application of NPsOG on the growth of radish plants. A) Control with application foliar of water distilled and B) application foliar of NPsOG.

The results of this job indicate that the imbibition of seeds with NPs derivatives of the carbon before of the sowing and the application foliar after of the emergency of the seedlings it affects negatively he growth. Although No exists a explanation clear about his effect in plant physiology, various works carried out in other parts of the world have proven that he use of NPs derived from coal have a phytotoxic effect when application of high concentrations (100-1500 mg $L^{-1}$), the authors point out that they may be associates with the induction of stress oxidative that generate the nanomaterials and that jointly cause a unbalance of the content of nutrients and the reduction of the activity photosynthetic, limiting he growth of the floors [9].

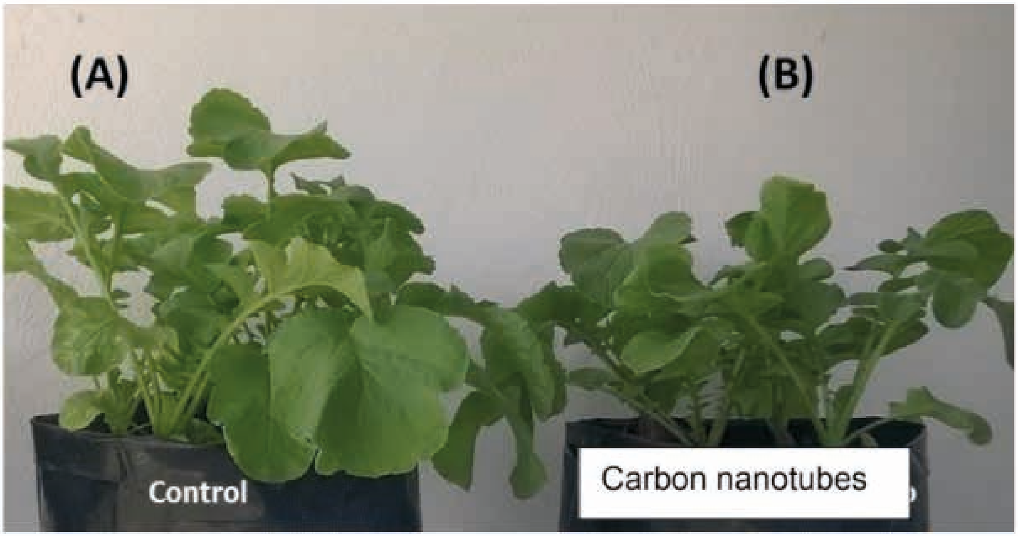


Figure 7. Effect of the application foliar of NTCM in the growth of floors of radish. TO) Control with application foliar of water distilled and B) application foliar of NTCM.

The few works carried out with plants do not present conclusive results, if for example, a study with lettuce, zucchini, tomato and spinach seeds at concentrations of 2000 mg $L^{-1}$ of graphene NPs caused phytotoxicity of the plants. This studio demonstrated that they induce an increase in reactive oxygen species (ROS), causing damage cellular and necrotic lesions due to high bioaccumulation, concluding that the level of damage can be determined by dose and exposure time; Other researchers mention that generally high doses (2000 mg $L^{-1}$) cause strong toxicity [12] due to amounts high levels of bioaccumulation in plant tissues, in this regard they point out that the application of NPs generate an excessive production of ROS that has a potential effect on the synthesis of proteins, lipids and carbohydrates related to growth; In addition, they increase the catalase and peroxidase enzyme activity; and alter acid concentrations indoleacetic and absciss which are closely related to growth [13]. Has been also pointed out that high concentrations of graphene could cause massive damage to the cell membranes and induce an exaggerated leakage of electrolytes, which can affect the transport of water and solutes increasing the oxidative stress of the plants and reflecting in a minor rate of growth [14].

On the other hand, graphene nanomaterials at high concentrations (50, 100 and 200 mg $L^{-1}$) negatively affect plant growth. The toxicity could be an effect of the impaired root growth, increased oxidative stress, imbalance of nutrient homeostasis and inhibition of photosynthesis [9]. The high production of Reactive Oxygen Species (ROS) induced by carbon-derived nanomaterials could be he responsible of the inhibition of the growth and the low production of biomass [12].

The high concentrations and the exposure time of the NPs could be two factors. that inhibit plant growth and seed germination [15], In addition, biochemically they alter some metabolic processes, such as a high accumulation of acid absciss that it affects the concentrations of acid indoleacetic altering negatively he growth of the floors [16].

## CONCLUSIONS

The imbibition of the seeds with NTCM

and NPsOG at concentrations of 100 and 200 mg L $^{-1}$ before of the sowing and his later application foliar weekly during Four. Five days, provoked phytotoxicity. These preliminary results suggest that these NPs affect growth and development of the crop, however, more research is needed regarding this issue.

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