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AVALIAÇÃO DA DISPERSÃO DE NANOMATERIAIS EM CUBA DE ULTRASSOM E ANÁLISE DE SEU POTENCIAL FITOTÓXICO

JUIZ DE FORA

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Trabalho de Conclusão de Curso apresentado ao Curso de Graduação em Ciências Biológicas da Universidade Federal de Juiz de Fora como requisito à obtenção do título de Bacharel em Ciências Biológicas.

Orientador: Prof. Dr. Saulo Marçal de Sousa

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Dedico este trabalho ao Vô Tide, Vó Ana e Breno. Pessoas importantes e queridas que perdi durante o processo da graduação, mas que também fazem parte dessa jornada.

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“Devemos acreditar que somos talentosos para algumas coisas, e que essa coisa, a qualquer custo, deve ser alcançada.” Marie Curie

RESUMO

Devido ao seu tamanho reduzido, as nanopartículas (NP) apresentam uma alta reatividade que pode trazer riscos toxicológicos. A nanofibra de celulose (NFC) e os nanotubos de carbono multicamadas funcionalizados com carboxila (MWCNT-COOH) são exemplos de nanomateriais (NMs) e possuem características físicas e químicas dependentes de tamanho. A dispersão é um processo importante para a suspensão de NMs e o método que geralmente é usado para a dispersão de nanopartículas é o ultrassônico. No entanto, existem diferentes dispositivos para realizar o processo. Este estudo tem como objetivo avaliar a dispersão de NMs, MWCNT-COOH e NFC, em uma dispersão de banho ultrassônico e analisar o potencial citotóxico com estudos de fitotoxicidade usando *Lactuca sativa* como modelo vegetal. Realizamos a técnica DLS para conhecer os valores de Diâmetro Hidrodinâmico (HD), Índice de Polidispersão (PDI) e Potencial Zeta (ZP). A maioria das concentrações dispersou melhor em M199+SFB; PDI foi variável independente do meio de dispersão e ZP foi negativo em todos os grupos com variabilidade entre eles. Também expusemos as sementes de *L. sativa* aos NMs e observamos uma grande influência deles na germinação, no tamanho da raiz e no tamanho do hipocótilo. Concluiu-se que a dispersão em banho ultrassônico apresentou bom potencial de dispersão e os NMs não apresentaram toxicidade, porém apresentaram influência nos parâmetros avaliados à *L. sativa*.

Palavras-chave: *L.sativa*; nanopartículas; diâmetro hidrodinâmico; nanotoxicidade.

ABSTRACT

Due to their reduced size, nanoparticles (NP) present a high reactivity that could bring toxicity risks. The cellulose nanofiber (CNF) and the multiwall carbon nanotubes functionalized with carboxyl (MWCNT-COOH) are examples of nanomaterials (NMs) and possess size-dependent chemical and physical characteristics. Dispersion is an important process for the NMs suspension s and the most common method that is used for the nanoparticles dispersion is the ultrasonic. However, there are different dispositives to do that. This study has the objective to evaluate the dispersion of NMs, MWCNT-COOH and CNF, in an ultrasonic bath dispersion and analyze the phytotoxicity effects using *Lactuca sativa* as a vegetal model. We performed the DLS technique to knowledge Hydrodynamic Diameter (HD), Polydispersity Index (PDI) and Zeta Potential (ZP) values. Most of all the concentrations dispersed better in M199+FBS; PDI was variable independent of dispersion medium and ZP was negative in all the groups with a variability between them. We also exposed the seeds of *L.sativa* to the NMs and we observed a high influence of them in the germination, root size and hypocotyl size. As a conclusion, the ultrasonic bath dispersion presented a great potential of dispersion and the NMs did not present toxicity, however, they had an influence on the evaluated parameters to the *L. sativa*.

Keywords: *L.sativa*; nanoparticles; hydrodynamic diameter; nanotoxicity.

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LISTA DE ABREVIATURAS E SIGLAS

NPs	Nanoparticles
NMs	Nanomaterials
CNF	Cellulose nanofiber
MWCNT-COOH	Carbon nanotubes functionalized with carboxyl
PC	Protein Corona
HD	Hydrodynamic diameter
ZP	Zeta Potential
L.sativa	*Lactuca sativa*
M199	Medium 199
FBS	Fetal bovine serum
DLS	Dynamic light scattering
M199+FBS	Medium 199 supplemented with fetal bovine serum
dH2O	Deionized water
PDI	Polydispersity Index
CNPs	Carbon nanoparticles

SUMÁRIO

Este Trabalho de Conclusão de Curso foi redigido no formato de artigo a ser submetido para os Anais da Academia Brasileira de Ciências. O texto a seguir encontra-se formatado segundo as normas desta revista.

EVALUATION OF NANOMATERIALS DISPERSION IN ULTRASONIC BATH AND ANALYSIS OF ITS PHYTOTOXIC POTENTIAL

Caroline da Silva Almeida Ferreira, Eduarda Rocha de Oliveira, Saulo Marçal de Sousa, Michele Munk Pereira

Abstract: Due to their reduced size, nanoparticles (NP) present a high reactivity that could bring toxicity risks. The cellulose nanofiber (CNF) and the multiwall carbon nanotubes functionalized with carboxyl (MWCNT-COOH) are examples of nanomaterials (NMs) and possess size-dependent chemical and physical characteristics. Nanoparticle dispersion is an important process for the NMs suspension and the most common method used for the nanoparticles dispersion is the ultrasonic. However, there are different dispositives to do that. This study has the objective to evaluate the dispersion of NMs in an ultrasonic bath dispersion and analyze the phytotoxicity effects using *Lactuca sativa* as a vegetal model. We performed the DLS technique to knowledge Hydrodynamic Diameter (HD), Polydispersity Index (PDI) and Zeta Potential (ZP) values. Most of all the concentrations dispersed better in M199+FBS; PDI was variable independent of dispersion medium and ZP was negative in all the groups with a variability between them. We also exposed the seeds of *L.sativa* to the NMs and we observed a high influence of them in the germination, root size and hypocotyl size. As a conclusion, the ultrasonic bath dispersion presented a great potential of dispersion and the NMs did not present toxicity, however, they had an influence on the evaluated parameters to the *L. sativa*.

Keywords: *L.sativa*; nanoparticles; hydrodynamic diameter; nanotoxicity.

INTRODUCTION

The nanoparticles (NPs) are materials with one of its dimensions up to 100 nanometers (VERMA et al., 2019). Due to their reduced size, NPs have a large contact surface and can access cells and biological structures that can not be accessed by traditional methods and it can provide different and interesting properties for many applications (YAN et al., 2016). Despite these advantages, the high reactivity because of the nano size brings toxicity risks with the possibility of crossing the cell membrane and causing damage to the genetic material and metabolism of cells (YU et al., 2020). As an example of nanomaterials (NMs), it is possible to cite the cellulose nanofiber (CNF) and the multiwall carbon nanotubes functionalized with carboxyl (MWCNT-COOH). The CNF is a biomaterial that can be extracted from different renewable sources like cotton, palm, bamboo and rice straw depending on the obtention method (MUNK et al., 2015; FAHMA et al., 2011; ZHANG et al., 2012; LU; POLYMERS; 2012). The CNF has many properties such as biodegradability, low density , high modulus of elasticity, high tensile strength, high rigidity, flexibility and good thermal, electrical and optical properties. Furthermore, it is abundant and has a low-cost and sustainable synthesis (BHAT et al., 2017), (NETO et al., 2013). In other way, the MWCNT-COOH presents a thermal/electric conduction, capillarity, large contact surface, mechanical resistance and are ultralight. In addition, they are more hydrophilic than non-functionalized carbon nanotubes and have greater potential for cytocompatibility and, for this reason, they have potential applications in the biomedical and tissue engineering fields(WANG et al., 2019), (JHA; HATA; SUBRAMANIAM, 2019).

These NMs can be discarded in the environment and, due to their high reactivity, it is necessary to develop studies with plant tissues exposed to NMs to understand the effects of this exposure. The use of *L.sativa* as experimental model is common because this vegetal present a simplicity and low cost and, in addition, has a high sensitivity in toxicity tests, including cytotoxicity, phytotoxicity and genotoxicity (SILVEIRA et al., 2017), (PINHEIRO et al., 2015). *L.sativa* is the most studied horticulture in the world and, because of it, there are a lot of studies about this species (KIM et al., 2016).

NPs possess size-dependent chemical and physical characteristics that enable interesting and correlated approaches for dealing with fundamental biological molecules (S BHATTACHARJEE - JOURNAL OF CONTROLLED; 2016). When a NP encounters a biological fluid, biomolecules spontaneously form adsorption layers around the NP, called a “protein corona” (PC). The corona's composition depends on the time-dependent environmental conditions, which determines the NP's fate within living organisms (RAIJIWALA; PANDYA; SHUKLA, 2019). Because of that, it is important to determine the hydrodynamic diameter (HD). It is also important to determine the Zeta Potential (ZP) value of the nanoparticles because this can explain how the nanoparticles interact with each other and other molecules. The ZP evaluation analyzes the electrophoretic motility and measures the surface charge of NMs through the electrical potential on the shear surface (S BHATTACHARJEE - JOURNAL OF CONTROLLED; 2016). NP dispersion is another important process for the nanomaterials. The dispersion is the proper distribution of in the suspension, which must be further maintained throughout the full process, avoiding aggregates (RENNHOFER; NANOMATERIALS; 2021) and this process is fundamental to determined the ZP, HD and PDI values.

The most common method that is used for the NP dispersion is ultrasonic dispersion, but there are different forms to do that. Independent of the matrix, the most important parameters reported for high dispersion quality are the ultrasonication time and the applied power per volume. The sound on the ultrasound dispersion induces waves because of a compression and decompression front moving through the liquid that interacts with the liquid molecules and small gas bubbles in the liquid (RENNHOFER; NANOMATERIALS; 2021). Therefore, this study has the objective to evaluate the dispersion of NMs in an ultrasonic bath dispersion for phytotoxicity studies using a vegetal tissue from *Lactuca sativa (L.sativa)*.

MATERIALS AND METHODS

Materials

As materials we used Medium 199 (M199) Sigma Aldrich, Co., 3050 Spruce Street, St. Louis and fetal bovine serum (FBS) that was acquired from LGC Biotechnology, Brazil to prepare the suspension for the dispersion of NMs and for the Dynamic Light Scattering (DLS) and Zeta Potential (ZP) evaluation. The MWCNT-COOH was obtained from a collaboration with Laboratório de Nanomateriais, located in the physics department from the Universidade Federal de Minas Gerais (Lote: ROT26271308, Al2O3–Co–Fe catalyst, purity >93%, <2% of other structures, and <5% of contaminants). The CNF was provided by Laboratório de Nanobiotecnologia para o Agronegócio (Embrapa Instrumentação, São Carlos, SP, Brazil) as collaboration.

Dispersion of nanomaterials

The equipment used for the dispersion of the nanomaterials was the ultrasonic bath Cristófoli (Shenzhen Codyson Electrical Co. Ltd, China) in the maximum potential of 160 Watts during three different times for each five concentrations: 20, 30 and 40 minutes. At first, it performed the ultrasonic degasification, as suggested in the manufacturer’s manual. Then, the samples were prepared on a sterilized microtube with MWCNT-COOH or CNF at concentrations of 0,1µg/mL; 1,0µg/mL; 10,0µg/mL; 50,0µg/mL e 100,0µg/mL for the dispersion in three different medium: deionized water (pH 7.1), M199 (pH 7.2-7.4) and M199 with FBS (pH 7.2).

Nanomaterials characterization

Dynamic Light Scattering (DLS) and Zeta Potential (ZP) evaluation

Both analyses were performed using Malvern 3000 ZetaSizer NanoZS (Malvern Instruments, Reino Unido) in the Laboratório de Nanotecnologia para Saúde e Produção Animal da Embrapa Gado de Leite (Juiz de Fora, MG, Brazil). All the five MWCNT-COOH and CNF concentrations were prepared in three types of aqueous medium: deionized water (pH 7.1), M199 (pH 7.2-7.4), and M199 supplemented with FBS (M199+FBS) (10% v/v) (pH 7.2). The refractive index of MWCNT-COOH used in DLS analysis was 1.891 and the refractive index of CNF used was 1.5.

Exposure to MWCNT-COOH and CNF and Phytotoxicity assessment

For this assay, 100 *L.sativa* seeds were arranged randomly in polystyrene Petri dishes (90 x 15 mm) containing filter paper soaked in 6 mL of distilled water (negative control) or in 6 mL of the suspension of MWCNT-COOH and CNF in dH2O in three different concentrations that shows the most relevant PDI and HD results in dispersion: 0.1; 1.0 and 10.0; µg/mL and were exposed for 72 hours in an oven at 25 ± 2°C without photoperiod. The nanomaterials were dispersed for 20 minutes. The procedure followed a completely randomized experimental design (DIC) with three replicates (Petri dishes) of each concentration.

To analyze the phytotoxicity of MWCNT-COOH and CNF, 100 seeds of *L. sativa* were exposed to MWCNT-COOH and CNF. Every 24 hours the average of germinated seed was measured and at the end of the exposure time, germination percentage and root and hypocotyl length were evaluated. The length was measured using a digital caliper (150 mm/MTX). 30 rootlets and hypocotyl out of 100 were measured by plate for the length measurement.

Statistical analysis

The results of the phytotoxicity assessment test were analyzed using the ANOVA means comparison test followed by the Tukey test. Values of $p<0.05$ will be considered significant. The analyzes will be carried out by the PRISM program.

RESULTS

Dynamic Light Scattering (DLS)

The different groups of NM in the five concentrations were dispersed in ultrasonic bath dispersion by Cristófoli (Shenzhen Codyson Electrical Co. Ltd, China). To comprehend how this equipment works on MWCNT-COOH and CNF dispersion, we performed the DLS technique for the hydrodynamic diameter (HD), polydispersity index (PDI) and zeta potential (ZP). It is possible to observe the results on table 1 and table 2.

At first, it is pointed out that most of all the concentrations of the tested NMs dispersed better in M199+FBS. The hydrodynamic diameter values roundabout about 12.5 nm and 3209.2 nm in MWCNT-COOH dispersion and 11.1 nm and 777.2nm in CNF dispersion. It is also possible to notice that the medium with only deionized water presented the highest HD, for both NMs, 3209,8nm in MWCNT-COOH and 777,2 in CNF. The same happened with the group M199 that presented the high value of 1162nm in CNF and 872,5nm in MWCNT-COOH. Another observation is about the time of the dispersion. The values kept the same pattern in the three different times.

The polydispersity index (PDI) was variable independent of dispersion medium. Some samples obtained the highest value possible of 1,0 that characterizes a heterogeneous distribution of nanoparticles. Still, other samples presented good PDI values. The lowest one was 0,27 on the CNF group, showing homogeneity in the suspension .

The zeta potential (ZP) was the lowest in the samples that were dispersed in deionized water: -32,2 in the 10 ug/mL CNF group l and -39,8 in the 50 ug/ml MWCNT-COOH group . On the other hand, the groups dispersed in M199 and M199+FBS presented the least negative values, such as -6,0 in the samples with MWCNT-COOH and -4,4 in the samples with CNF.

Tab.1 – Hydrodynamic Diameter (HD), Polydispersity Index (PDI) and Zeta Potential (ZP) averages of Carboxylated Multiwall Carbon Nanotubes (MWCNT-COOH) dispersed in deionized water (dWater), Medium 199 (M199) and Medium 199 supplemented with Fetal Bovine Serum (M199 + FBS), analyzed by Dynamic Light Scattering (DLS).

0.1µg ml^{-1}									
Media/ Time	**20'**	**30'**	**40'**	**20'**	**30'**	**40'**	**20'**	**30'**	**40'**
	HD			**PDI**			**ZP**		
dWater	786.8	417.4	312.7	0.48	0.410	0.46	-22.9	-24.3	-14.8
M199	305.8	167.6	248.6	0.69	0.57	0.49	-8.7	-8.0	- 6.2
M199 + FBS	12.9	20.0	12.5	0.50	0.40	0.63	-9.8	-9.1	- 9.9
1 µg ml^{-1}									
dWater	491.3	354.8	254.0	0.50	0.39	0.41	-23.9	-28.9	-30.0
M199	299.8	221.9	232.1	0.61	0.49	0.54	-6.3	-7.0	-7.4
M199 + FBS	13.2	29.2	16.9	0.55	0.40	0.62	-10.1	-10.3	-11.3
10 µg/ml^{-1}									
dWater	2363.5	1187.8	563.7	0.91	0.63	0.46	-11.5	-24.0	-25.5
M199	7.27	250.9	373.9	0.51	0.46	0.50	-7.1	-6.0	-6.2
M199 + FBS	137.4	109.6	51.4	0.47	0.67	0.83	-11.5	-11.7	-12.4
50 µg/ml^{-1}									
dWater	1062.4	986.9	798.1	0.73	0.67	0.59	-39,.	-31.8	-27.8
M199	817.8	177.8	213.8	0.611	0.40	0.46	-7.3	-7.1	-6.9
M199 + FBS	121.2	121.6	124.0	0.82	0.65	0.93	-10.7	-11.5	-11.1
100 µg/m^{-1}									
dWater	3209.2	1353.4	552.7	0.76	0.63	0.49	-29.2	-24.6	-21.4
M199	872.5	445.9	309.6	0.33	0.53	0.50	-11.2	-6.9	-6.3
M199 + FBS	203.5	200.2	198.8	0.95	0.86	0.76	-9.8	-10.9	-11.3

Tab.2 – Hydrodynamic Diameter (HD), Polydispersity Index (PDI) and Zeta Potential (ZP) averages of Cellulose Nanofiber (CNF) dispersed in deionized water (dWater), Medium 199 (M199) and Medium 199 supplemented with Fetal Bovine Serum (M199 + FBS), analyzed by Dynamic Light Scattering (DLS).

0.1 µg ml^{-1}

Media/Time	20’	30’	40’	20’	30’	40’	20’	30’	40’
	HD			PDI			ZP		
dWater	777.2	495.0	487.2	0.58	0.45	0.51	-20.8	-29.4	-19.0
M199	659.0	287.9	285.8	0.55	0.59	0.51	-5.9	-8.3	-8.0
M199 + FBS	11.1	13.4	14.3	0.58	0.47	0.48	-10.0	-10.3	-9.4
1 µg ml^{-1}									
dWater	676.8	458.3	531.2	0.50	0.47	0.48	-18.7	-31.5	-29.2
M199	549.7	482.7	510.4	0.50	0.57	0.53	-18.7	-7.2	-6.9
M199 + FBS	16.2	13.6	19.1	0.55	0.58	0.59	-8.7	-8.5	-7.5
10 µg/ml^{-1}									
dWater	393.1	333.3	288.0	0.41	0.43	0.42	-42.3	-32.3	-31.0
M199	683.0	590.6	498.1	0.27	0.42	0.49	-5.2	-5.2	-4.4
M199 + FBS	68.1	71.2	40.5	0.60	0.66	0.72	-6.6	-6.4	-6.3
50 µg/ml^{-1}									
dWater	384.3	285.4	359.8	0.41	0.39	0.44	-47.9	-29.8	-25.2
M199	835.2	867.8	853.9	0.39	0.36	0.30	-5.1	-4.9	-5.2
M199 + FBS	215.6	243.0	192.2	1.0	1.0	1.0	-6.0	-5.6	-6.1
100 µg/m^{-1}									
dWater	368.7	321.5	268.8	0.45	0.45	0.46	-40.8	-26.0	-20.6
M199	973.5	1078.5	1162.0	0.37	0.32	0.28	-5.5	-5.4	-5.1
M199 + FBS	671.5	730.1	630.7	0.83	0.84	0.77	-5.7	-5.4	-6.3

Exposure to MWCNT-COOH and CNF and Phytotoxicity assessment

L.sativa seeds were exposed to these three different concentrations of CNF and MWCNT-COOH to understand how these NMs dispersed in ultrasonic dispersion could interact with vegetal tissue and possibly affect it. The three groups that presented the most relevant PDI and HD results, considering the values in table 1 and table 2, were 0.1 µg ml^{-1}; 1.0 µg ml^{-1} and 10.0 µg ml^{-1}.

At first, on figure 1 it is possible to point out that the exposure to MWCNT-COOH presented a rate increase in germination in comparison to the control group with the most treatments presenting a high average germination value. The same could be observed on figure 2 with the seeds exposed to CNF. However, the exposure of 72 hours did not affect exposed seeds in comparison with the control group. When comparing the influence of time in the same group of NM, figure 3 and 4, there is no difference on seed germination in all the three concentrations of MWCNT-COOH and CNF. However, it is possible to notice that the MWCNT-COOH groups of 1.0 ug ml^{-1} and 10.0 ug ml^{-1} presented a difference in comparison with the control group within 24 hours and the same behavior was observed in 0.1 ug ml^{-1} NFC group.

Another observation was in the hypocotyl and root sizes in 72 hours of exposure. Both values were significant for MWCNT-COOH and CNF exposure. Therefore, an increase in these vegetable tissues occurred. The only exception was in hypocotyl size in 1.0 ug ml^{-1} and 10.0 ug ml^{-1} in the CNF group, such as in the figure 5.

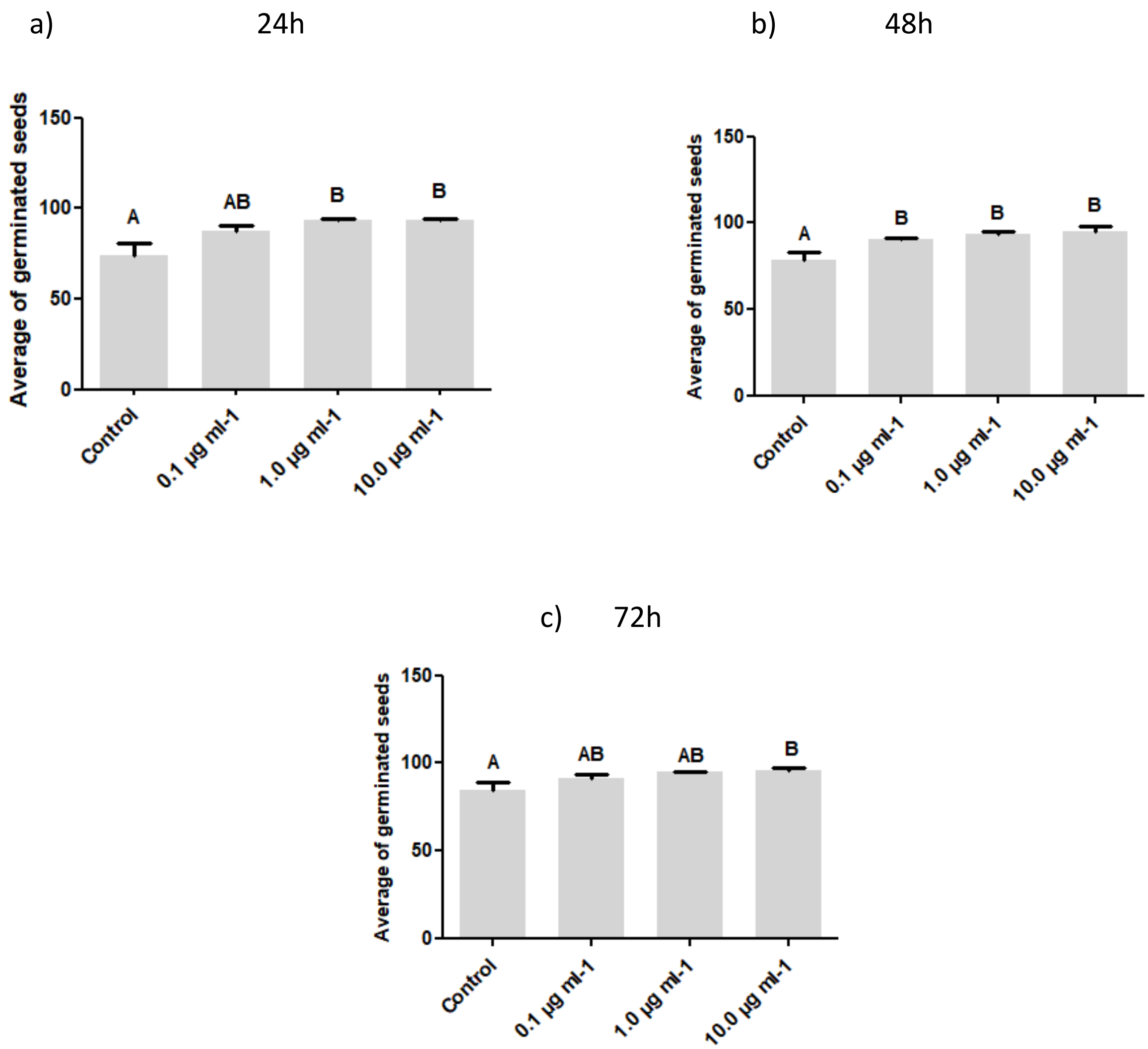

Figure 1: Averages of germinating seeds exposed to 0, 0.1, 1.0 and 10µg ml^{-1} of Carboxylated Multiwall Carbon Nanotube (MWCNT-COOH) for a) 24h, b) 48h and c) 72h. Data analyzed by One-Way ANOVA post-hoc Tuckey ($p<0.05$). Groups assigned with the same letters did not differ statistically.

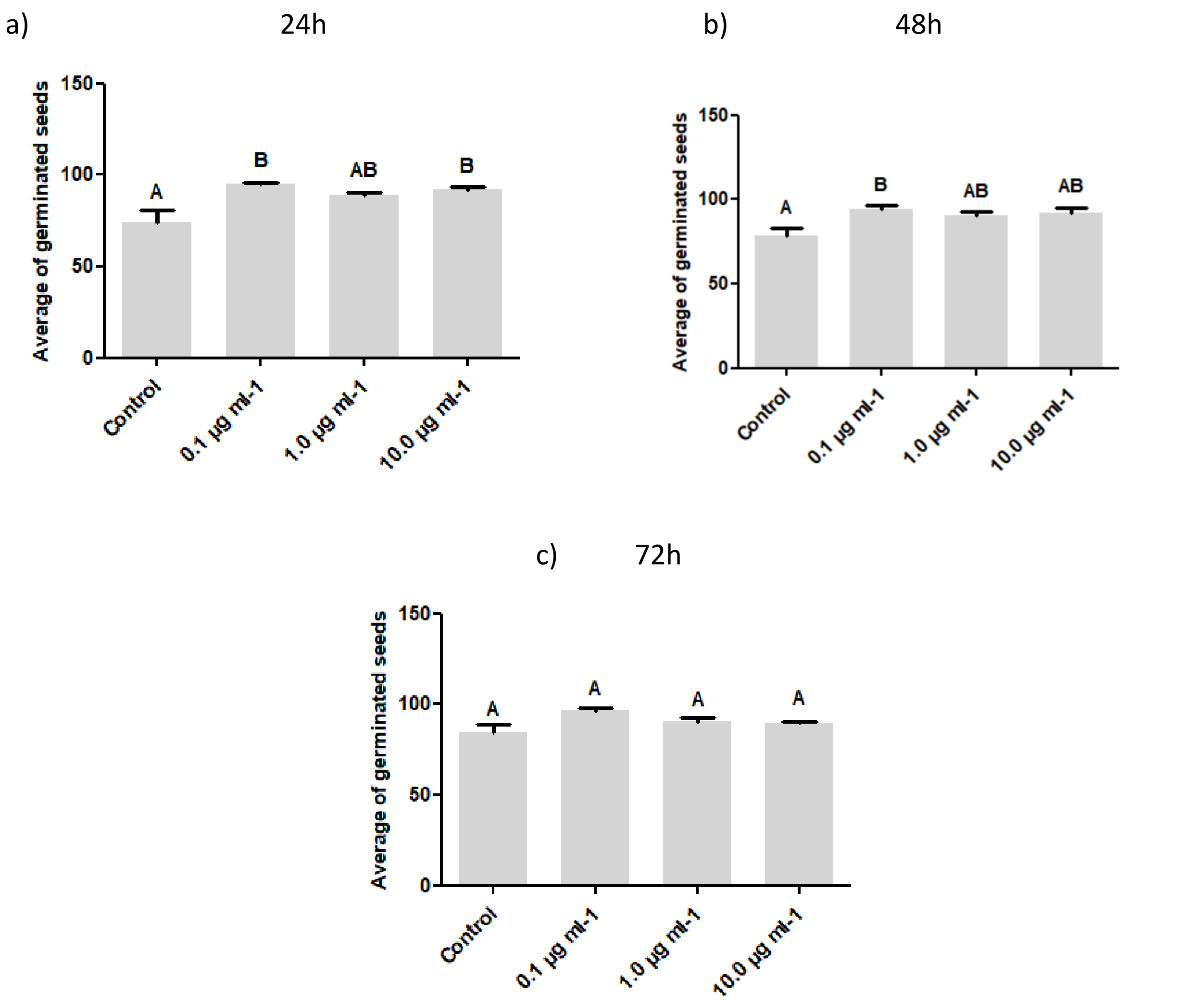

Figure 2: Averages of germinated seeds are exposed to 0, 0.1, 1.0 and 10µg ml^{-1} of Cellulose Nanofiber (CNF) for a) 24h, b) 48h and c) 72h. Data analyzed by One-Way ANOVA post-hoc Tuckey ($p<0.05$). Groups assigned with the same letters did not differ statistically.

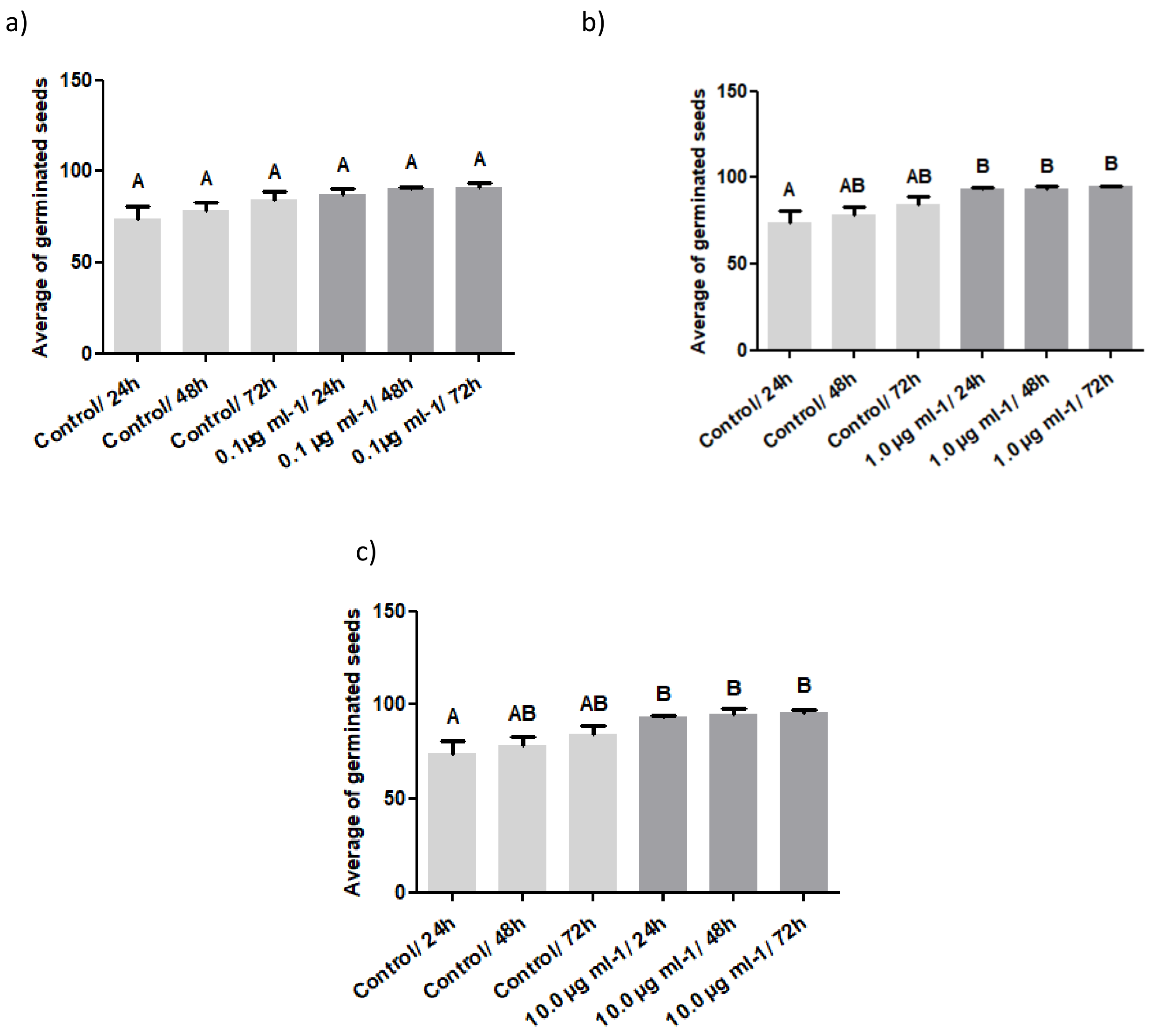

Figure 3: Comparison of average of germinated seeds exposed to a) 0.1 b) 1.0 c) 10.0 µg ml^{-1} of Carboxylated Multiwall Carbon Nanotube (MWCNT-COOH) considering time. Data analyzed by One-Way ANOVA post-hoc Tuckey ($p<0.05$). Groups assigned with the same letters did not differ statistically

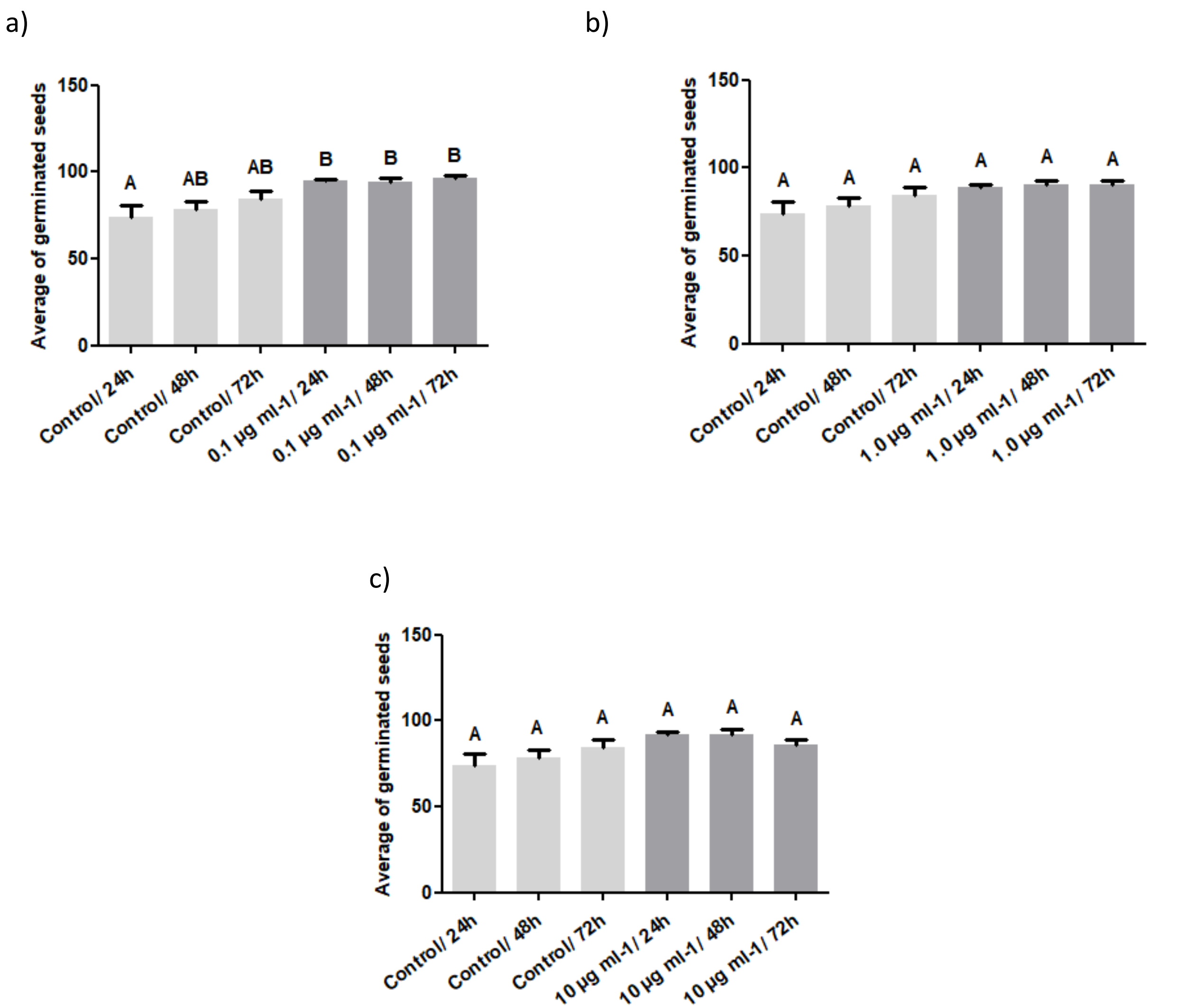

Figure 4: Comparison of averages of germination seeds exposed to a) 0.1 b) 1.0 c) 10.0 in µg ml^{-1} of exposed to Cellulose Nanofiber (CNF) considering time and growth. Data analyzed by One-Way ANOVA post-hoc Tuckey ($p<0.05$). Groups assigned with the same letters did not differ statistically.

a) b)

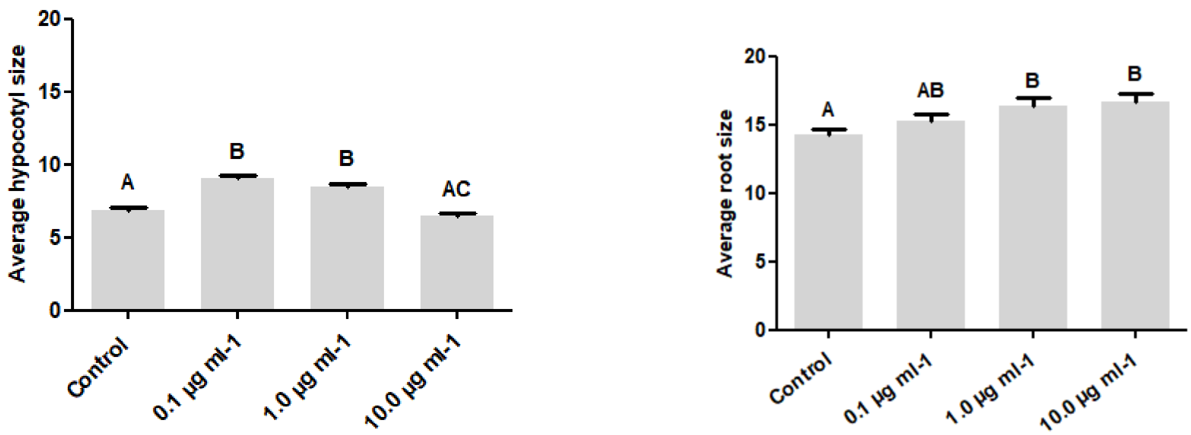

Figure 5: Averages of a) hypocotyl and b) root size development (in mm) of seeds exposed to 0, 0.1, 1.0 and 10µg ml^{-1} of Carboxylated Multiwall Carbon Nanotube(MWCNT-COOH) for 72h. Data analyzed by One-Way ANOVA post-hoc Tuckey ($p<0.05$). Groups assigned with the same letters did not differ statistically.

a) b)

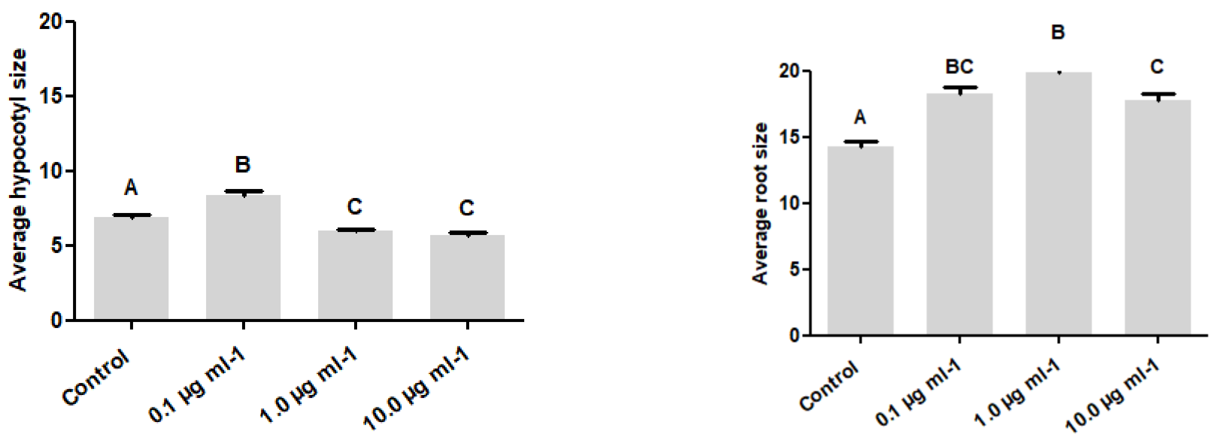

Figure 6: Averages of a) hypocotyl size and b) root size development (in mm) of seeds exposed to 0, 0.1, 1.0 and 10µg ml^{-1} of Cellulose Nanofiber (CNF) for 72h. Data analyzed by One-Way ANOVA post-hoc Tuckey ($p<0.05$). Groups assigned with the same letters did not differ statistically.

DISCUSSION

We performed the DLS technique to knowledge HD and ZP and comprehend the dispersion behavior, approximate size and superficial net charge of the MWCNT-COOH and CNF. These are parameters that can interfere in the interaction of the NM with the cells and vegetable tissues.

In general, the HDs showed aggregation as a function of MWCNT-COOH and CNF concentration in deionized water and in M199. In these cases, Van der Waals overcomes the repulsive electrostatic forces, leading to different sizes of the NMs aggregates (LEE et al., 2007).

The M199 with FBS presented the higher dispersibility and this can be explained by the FBS proteins, mainly albumin and other culture medium constituents, that influence the behavior related to aggregation and dispersion of NMs. Albumin has the potential to form a protein corona that can interact with the NPs avoiding the aggregation of them. In the specific MWCNT-COOH case, the interaction happens with the free carboxylic groups (LIMA et al., 2020). In the case of NFC, the presence of sulfate groups added during the process of production to esterification of hydroxyls is probably able to promote an interaction between the albumin and these salts (ZANETTE et al., 2020).

PDI is an estimator of the average uniformity of a particle solution. Our results show that most of MWCNT-COOH groups had an intermediary polydispersity average level of 0.58 and CNF treatments had an intermediary polydispersity average level of 0.53. These values indicated that in general the dispersion was homogeneous, such as the guidelines suggested with values smaller than 0.05 being mainly highly monodisperse standards and PDI values bigger than 0.7 indicate that the sample has a very broad particle size distribution (DANAEI et al., 2018). Some samples in the dispersion of 50.0 ug ml-1 and 100.0 ug ml-1 groups presented the higher possible value of PDI or values closest to the maximum and these concentrations were disregarded for the phytotoxicity assessment.

In comparison with OLIVEIRA et al., 2021, which used an ultrasonic probe sonicator method for MWCNT-COOH dispersion, the best HD value sizes were in DMEM/F12 supplemented with FBS and roundabout 62.7 - 211.4 nm. These results were similar to the HD results in M199 supplemented with FBS presented in this article that were between 12.9 - 203.5, even with the difference between the methods and the dispersion medium. The PDI values were also close one to another with most samples being homogeneous, considering the values proposed in the literature. This similarity suggests that boths methods can be exchanged without loss of performance in NMs dispersion for use in the same purposes.

The CNF is able to present a different value of DH depending on the biomaterial that the NM was extracted from and the medium that was dispersed. RAVINDRAN; SREEKALA; THOMAS, (2019) presented a HD average for 420 nm in a dH20 using the traditional ultrasonic method of dispersion for a CNF extracted from pineapple, a value aproximated to what was presented in

dH20 dispersion in this article and a greater value if compares the averages for NFC obtained for peanut shell (PUNNADIYIL et al., 2016) and banana peel (HARINI et al., 2018), for example.
The zeta potential is the potential at the slipping/shear plane of a colloid particle moving under an electric field. The usual guidelines classifying NP dispersion with ZP values of +/- 0 a 10mV as highly unstable; +/- 10 a 20 mV, relatively stable; +/- 20 a 30 mV, moderately stable, +/- 30 mV, highly stable. However, the ZP values could be changed by any factors and these references could be changed because of the sum of van der Waals attractive forces and electrostatic repulsive forces (S BHATTACHARJEE - JOURNAL OF CONTROLLED; 2016).

As can be observed, all the ZP potential values in our results were negative, as expected in comparison with Oliveira et al. (2021) and ZANETTE (2019). The negative ZP in CNF could be explained because of the esterification of hydroxyls, with sulfate groups (anionic), during the process of production. On the other hand, the negative ZP in MWCNT-COOH could be explained because of the functionalization with carboxyl groups that presented free ions.

Another result observed was between the stability of the ZP values. The dH2O group presented values classifying as stable. In contrast, the M199 supplemented with FBS, which presented the best HD results, had values classifying as unstable or relatively stable. In comparison with MONTANHEIRO et al. 2014, which also uses an ultrasonic bath for dispersion, MWCNT-COOH presented a high zeta potential for dH20, -21.03 mV, such as our results. It shows that MWCNT-COOH are fairly stable and should present a colloidal stability over the days. This behavior suggested that the same happens with the NFC, such as was observed in our results. On another hand, M199 and M199 supplemented with FBS are able to avoid the aggregate formation but the results indicated that these smaller NPs were not able to keep the stability.

It is also possible to point out, according to JIANG et al. 2009, that when in an aqueous medium, the pH of the samples is considered one of the most important factors that can change the values of the Zeta Potential. SIQUEIRA (2021), in comparison with TOMASZEWKA et al. 2013, presented the concept that ZP varies with pH, becoming more positive in acidic pH and more negative in basic pH. In this sense, the analysis of the Zeta Potential at neutral pH (7.0), such as dH2O and M199, allows a minimal interference of this on the surface charge.

We performed the phytotoxicity assessment to comprehend the impact of the NMs in the vegetal tissue of *L.sativa* and our results showed a root elongation on the three concentrations of MWCNT-COOH and CNF exposure in comparison with the control group. We also observed a hypocotyl elongation on the three concentrations of MWCNT-COOH and on one concentration of CNF. SIQUEIRA et al. (2021) also presented a root elongation in MWCNT-COOH exposure and it is suggested, in comparison with KHODAKOVSKAYA et al 2012; MARTINEZ et al 2014; TIWARI et al 2014; HATAMI et al 2017; GOHARI et al 2020, that this happens because of the increase of water absorption as a result of penetration into the seed coat, creating new pores that may allow the entry of water, oxygen and external molecules and, consequently, affect water channels and protein synthesis.

Another phytotoxicity study with NMs exposure presented a similarity with our results. KUMAR et al. (2018) observed how carbon nanoparticles (CNPs) influence photomorphogenesis and flowering time in *Arabidopsis thaliana* and, as a result, even with the photoreceptor condition, the CNPs still promoted hypocotyl length. According to the authors, these NMs could penetrate and internalize into plant roots serving as nanotransporters that probably facilitated nutrient uptake.

Also, LIANG et al. (2013) observed that applications of CNPs on *N. tobacco L*. plants resulted in enhanced growth at different stages as compared with the plant growth obtained by using conventional fertilizers. Lin et al. 2009 presented the results on the carbon NMs exposure in the rice plants and a tissue growth was also observed.

As CNF and MWCNT-COOH exposure presented similar results, it is suggested that the same behavior that was observed in the carbon nanomaterials can apply for the CNF. LIN et al. 2009 presented the concept that the accumulation and transformation of nanoparticles in plant tissues and cells suggest a plausible mechanism for nanoparticles uptake: a dynamic competition between nanotransport driven by water and NPs conventions and the physical hindrances of plants tissues and NPs aggregation. In the specific cases of the 1.0 ug ml^{-1} and 10 ug ml^{-1} hypocotyl results on the CNF group was presented a reduction in the average hypocotyl size and in a phytotoxicity study it is possible to conclude that in theses concentrations the NMs were toxic to this vegetal tissue.

Another discussion is about the fact that, in general, the average of hypocotyl size results present a different behavior in comparison with the average of root size. Hypocotyl elongation is very plastic and is influenced strongly by factors that regulate cell elongation in the adult plant such as light, plant hormones, temperature, and touch (Collett et al., 2000) and root growth in soil can be limited by physical, chemical, and biological properties of the soil (Bengough et al., 2011). How the factors to determine the elongation on these tissues are similar, it is suggesting that the own properties of these vegetal tissues were different and presented a different interaction with the NMs.

We also observed that, in general, the seeds exposed to the NMs presented a high germination in comparison with the control and, in specific cases, the average of germination stayed the same that the control. This pattern shows that, in this phytotoxicity study, the NMs did not hinder the seeds growth. According to LIN & XING (2007), the NMs may not affect germination if they cannot pass through the seed coat but, after this acrossing happens, the rootlets can come into direct contact with the MWCNT-COOH, making it possible to observe the effects more easily. These explanations suggested that the same is expected for the CNF.

In the specific cases that the treatment groups did not present an increase on the last day of exposure in comparison with the control groups it is possible to point out too that in the middle of the exposure time the germination process was accelerated by the NMs, even if in the end the values started approaching.

As has been demonstrated our findings reveal that the NMs dispersion with the ultrasonic bath Cristófoli (Shenzhen Codyson Electrical Co. Ltd, China) presented a satisfactory result and attended all the parameters of DH, PDI and ZP observed on NMs dispersed on the traditional ultrasonic sonicators.. Furthermore, the NMs provided from this dispersion did not cause phytotoxicity to the *L.sativa* seeds besides to induce germination, root and hypocotyl growth. In addition, the NMs also were presented as an option in the use for agriculture. Therefore, our work evaluated some toxicological parameters of MWCNT-COOH and CNF in *L. sativa* providing essential information for future safe applications of these NMs in other phytotoxicity studies.

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