Original Article

The Impact Of Carbon-Nano Tubes On Morphological, Photosynthetic Pigments, Protein Content And Enzyme Activity Of *In-Vitro* Multiplication Of Cucumber

Mohanad T. Meften[1*], A.B. Abdel-Razik[2], S. A. Ibrahim[3] and Ahmed S. Mohamed[4]

[1*,2,3] Genetic Department, Faculty of Agriculture, Ain Shams University, Egypt.
[4]Horticultural Crops Technology Department, Agricultural and Biological Research Institute, National Research Centre,Egypt

***Corresponding Author:** Mohanad T. Meften

*Genetic Department, Faculty of Agriculture, Ain Shams University, Egypt.
DOI:10.47750/pnr.2023.14.S01.185

Abstract

This experiment was performed on cucumber (*Cucumis sativus*) in Ain Shams Center of Genetic Engineering and Biotechnology (ACGEB), Fac. of Agric., Ain Shams Univ., Egypt, during the period from 2020 - 2022 to investigate the responses of cucumber seedlings to different concentrations of multi-walled carbon nanotubes "MWCNT" incorporation into the culture medium. Cucumber seeds were planted in hormone – free agar medium. 21 days' later, stem nots were collected and were divided into 7 groups to be planted in media containing different concentrations of MWCNT (0.5 mg/l, 1 mg/l, 1.5 mg/l, 2 mg/l and 2.5 mg/l MWCNT) in parallel with two other sets, as control, one of them was planted in medium contains 0.5 ml/l of royal water without MWCNT and the other set without either MWCNT or royal water. Results indicated that the applications of different concentrations of MWCNT improved seedling growth. The MWCNT 1.5 and MWCNT 2 mg/l treatments significantly improved the plant length and leaves number respectively comparing to the untreated control. The CNT 2.5 mg/l and CNT 2 mg/l treatments achieved the highest increase in chlorophyll-a chlorophyll-b and total chlorophyll contents. Also the SDS-PAGE total protein showed that the intensity of protein bands increases visibly starting from the concentration 0.1 mg/l moreover, new bands at 100 KD, 75 KD and 20 KD emerged. Peroxidases isozyme analysis showed that peroxidase 1 was produced more intensely at concentrations 1 mg/l, 1.5 mg/l and 2 mg/l of MWCNT while peroxidase 2, 3, 4 and 5 analysis showed contradicting and variable results.

Keywords: Carbon nanotube, MWCNT, In-vitro, Nanoparticle, Gene expression, Cucmber, Peroxidases.

INTRODUCTION

The carbon-nano tubes (CNT's) are cylindrical nanoparticles of carbon atoms that are organized in a hexagonal framework. Multi-walled (MWCNTs) have an outer diameter of 2-100 nm and an inner diameter of 1–3 nm and a length between 100 nm to a few centimeters **(He *et al.*, 2013; Shoukat and Khan 2021)**. Due to their unique properties, the CNTs have the potential to highly biologically reaction (**Azizi *et al.*, 2020**).

The CNT's, including their small size, large surface area, and reactivity, offer excellent potential for their usage in the agricultural industry specially in seed germination, early plant growth, pesticide, and biosensor diagnostics and analysis **(Patel *et al.*, 2020)**. The utilization of CNTs as a plant growth regulator to influence diverse biological functions has been demonstrated in seed germination and early seedling performance **(Joshi *et al.*, 2018; Seddighinia *et al.*, 2019)**, cell cycle **(Khodakovskaya *et al*., 2012; Ghasempour *et al.*, 2019)**, growth **(Oloumi *et al.*, 2018; Joshi *et al.*, 2018; Seddighinia *et al.*, 2020)**, anatomy **(Joshi *et al.*, 2018; Seddighinia *et al.*, 2019)**, flowering **(Seddighinia *et al.*, 2019)**, nutrition **(Joshi *et al.* 2018; Seddighinia *et al.*, 2019)**, and gene expression **(Yan *et al.*, 2013; Ghasempour *et al.*, 2019)**.

The contradicting effect of CNT's has been discussed in many researches, showing how the impact of CNT's is unexpected and complex. It is a complex phenomenon that CNTs interact with plants, causing various physiological and morphological changes in plants; these changes are influenced by plant type, life stage, and concentration of CNT's (**Ghasempour *et al.*, 2019; Patel *et al.*, 2019**). Also it was reported that the effect of CNTs was dependent on the size, concentration, and solubility of the applied CNTs (**Martínez-Ballesta *et al.*, 2016**).

Cucumber (*Cucumis sativus* L.) is one of the most nutritive vegetables rich in vitamins and minerals such as calcium, potassium, silica, phosphorus, iron and magnesium **(Sumathi *et al*., 2008; Merghany *et al.* 2019).** Cucumber growth was enhanced by 1.5- or 2-fold when the soil was mixed with SWCNT or MWCNT (**Tripathi *et al.*, 2019**). Hence, when developing this technology as growth regulator, cucumbers can be used as a reasonable example to demonstrate the technology's applicability to most other crops (**Kim *et al.*, 2019**).

This current study aims to asses CNT utilization as a promising growth regulator of cucumber plants by: 1) Measuring the impact of MWCNT serial concentrations on structural growth of cucumber plant compared to control group. 2) Evaluating the associated changes molecular stimulating growth, and metabolism in the cucumber plants.

MATERIALS AND METHODS

The study was carried out in the Center for Genetic Engineering and Biotechnology (ACGEB) Ain Shams University, Egypt, during the period from 2020 to 2022 to investigate the responses of Cucumber to CNTs incorporation into the culture medium to know the CNT's role in a dose-dependent manner on (I) growth and morphology traits; (II) plant pigments; (III) enzymatic antioxidants; (IV) and total protein expression.

Plant Materials

Cucumber seeds were obtained from the Department of genetics, Fac. of Agriculture, Ain Shams University.

Multiwalled Carbon Nanotube Characterization (MWCNT)

The MWCNTs used in this study was a formulation of powder based carbon material, with a carbon purity of 94.5%, Transmission Electron Microscopy (TEM) characterization corroborates the manufacturing dimensions (Figure 1); Avgerage Size: (Length > 1 µm) & (Outer Diameter: 25 ± 5 nm, Inner Diameter: 10 ± 5 nm).

Application of Carbon Nanotubes *In-Vitro* Cultures

Cucumber seeds were sterelized by soaking in per-acetic acid 3% for 20 minutes, then planted in hormone – free medium; Murashinge and Skoog (MS) medium (3/4x) with 30g sucrose and 6g agar/l. After 21 days, the stem nots were collected and were divided into 7 groups, each group contained 10 nots, five of them were transferred to the agar media with 5 different concentrations of MWCNT which were previously dissolved in 0.5 ml of royal water (0.5 mg/l, 1 mg/l, 1.5 mg/l, 2 mg/l and 2.5 mg/l) in parallel with two other sets, as control, one of them was planted in medium contained 0.5 ml/l of royal water without MWCNT and the other set without either MWCNT or royal water.

Morphological Characteristics

Plant height, leaves number, root number and root length were recorded.

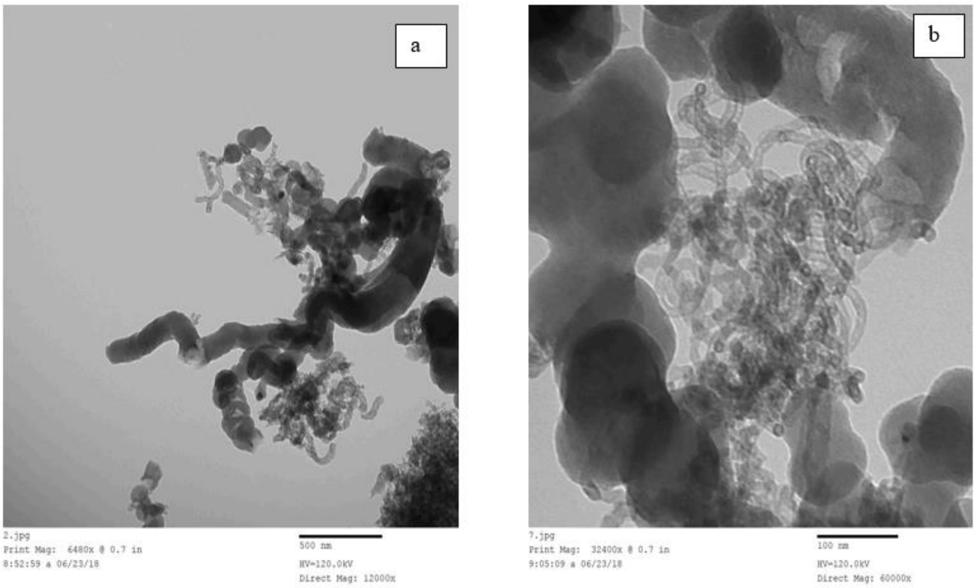


Figure 1. Multi-walled carbon nanotubes (MWCNTs) observed by transmission electron microscopy (TEM) using different magnifications. (a) Black bar = 500 nm. (b) Black bar = 100 nm

Photosynthetic Pigments

The photosynthetic pigments (Chlorophyll A, Chlorophyll *B*, and carotenoids) were determined according to the equations previously represented by **Wettestin (1957)**. Half gram of fresh leaves was extracted by about 15 ml of 85% acetone with 0.5 g $CaCo_3$ salt, the mixture was filtered through a glass funnel (G.H.) of fine porosity and the residue was washed with a small volume of acetone and completed to 25 ml. The optical density of a constant volume of filtrate was measured at a wave length of 662 nm. For chlorophyll A, 664nm for Chlorophyll B and 440nm for carotene using spectrophotometer. The following equations were used:

Chl. A. = 9.784 E. 662 – 0.99 E. 644 = mg/L

Chl. B. = 21.426 E. 664 – 4.65 E. 662 = mg/L

Carotene = 4.695 E. 440 – 0.268 (Chl. A + Chl. B) = mg/L

Where E. = Optical density at the wave length indicated.

Statistical Analysis and Experimental Design

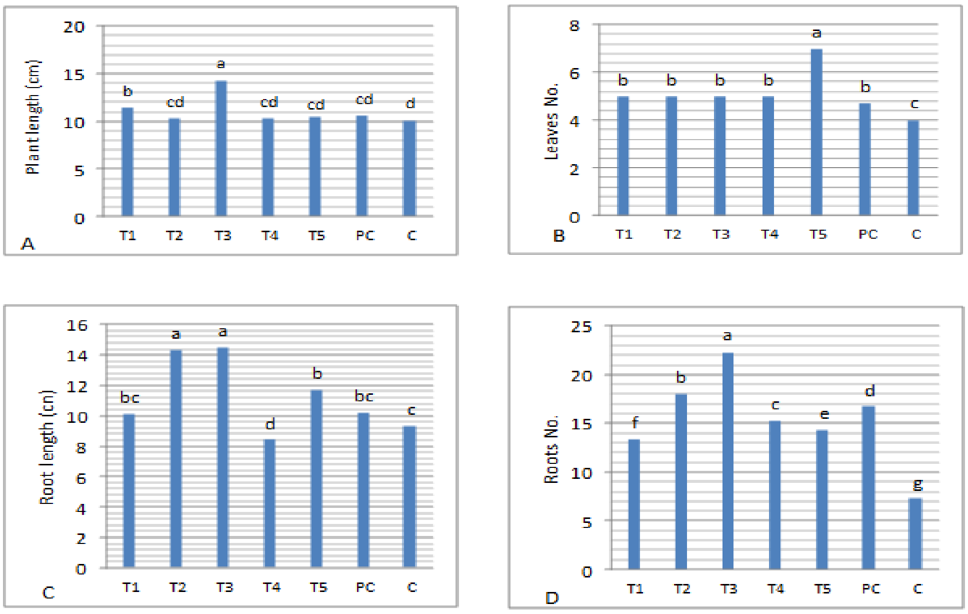
There were 7 different treatment groups of MWCNT-treated seedlings with three replicates. A completely randomized experimental design was used. All experiments were performed in triplicate. Data were subjected to one-way analysis of variance (ANOVA) and LSD $_{0.05}$ mean comparison using SAS statistical software.

SDS- Protein Electrophoresis (Total Protein)
In order to study the protein profile after exposure the plant to the MWCNT and its impact on total protein expression, total proteins were extracted from 21 Dayes plant leaves. Proteins sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to **Laemmli (1970).** Protein fractionations were performed on vertical slab (19.8 cm x 26.8 cm x 0.2 cm) gel using the electrophoresis apparatus manufactured by LABOCONCO.

Isozymes Electrophoresis
Native-polyacrylamide gel electrophoresis (native-PAGE) was used to determine the peroxidase activity. Samples were collected from leaves at 21 days. Isozymes fractions were performed on vertical slab (19.8 cm x 26.8 cm x 0.2 cm) gel using the electrophoresis apparatus manufactured by LABOCONCO according to **Jonathan and Weaden, (1990).**

Enzyme Assay
The gels were stained afterelectrophoresis according to its system and incubated at 37°C peroxidase for complete staining after adding the appropriate substrate and staining solution.

RESULTS AND DISCUSSION
Morphological Characteristics
The effect of multi-walled carbon nanotubes (MWCNT) on the growth of cucumber seedling (Plant length, leaves No., roots length, roots No.) after 21 days from culture in free hormone - MS medium supplemented with different concentrations of MWCNTs are shown in Table (1). The applications of different concentrations of MWCNT improved seedling early growth and performance (Fig.1). Shoots characteristics, including plant length and leaves No. were found to be altered in response to the MWCNT treatments (Fig. 1-A, B). In comparison to the untreated control, the MWCNT 1.5 and MWCNT 2.0 treatments significantly improved the plant length and leaves No., respectively.



T1= 0.5mg/L MWCNT+ 0.5ml Aqua regia **T2= 1mg/L MWCNT+ 0.5ml Aqua regia**
T3= 1.5mg/L MWCNT+ 0.5ml Aqua regia **T4= 2mg/L MWCNT+ 0.5ml Aqua regia**
T5= 2.5mg/L MWCNT+ 0.5ml Aqua regia **PC= 1ml Aqua regia** **C= 0**

Fig. 2 Changes in morphological characteristics for cucumber seedling cultured in free hormone - MS medium supplemented with different concentrations of MWCNTs

Table 1 Effect of multi-walled carbon nanotubes on the growth of cucumber seedling cultured in free hormone - MS medium supplemented with different concentrations of MWCNTs

Treatments	Plant length (cm)	Leaves No.	Roots length	Roots No.
T_1= 0.5mg/L MWCNT+ 0.5ml Aqua regia	11.4^{b}	5.0^{b}	10.1^{bc}	13.3^{f}
T_2= 1mg/L MWCNT+ 0.5ml Aqua regia	10.3^{cd}	5.0^{b}	14.3^{a}	18.0^{b}
T_3= 1.5mg/L MWCNT+ 0.5ml Aqua regia	14.3^{a}	5.0^{b}	14.5^{a}	22.3^{a}
T_4= 2mg/L MWCNT+ 0.5ml Aqua regia	10.3^{cd}	5.0^{b}	8.4^{d}	15.3^{e}
T_5= 2.5mg/L MWCNT+ 0.5ml Aqua regia	10.4^{cd}	7.0^{a}	11.7^{b}	14.3^{de}
PC= 1ml Aqua regia	10.6^{c}	4.7^{b}	10.2^{bc}	16.7^{d}
C= 0	10.1^{d}	4.0^{c}	9.3^{d}	7.3^{g}
L.S.D	0.34	0.38	1.28	1.13

MWCNT interact with plants, causing physiological and morphological changes that are influenced by concentration, plant type, life stage, and CNT type **(Ghasempour *et al.*, 2019; Patel *et al.*, 2019)**. The effect of CNTs was determined by their size, concentration, and solubility **(Martnez-Ballesta *et al.*, 2016)**. Furthermore, CNTs have been shown to improve plant productivity in both soil and hydroponic mediums **(Liné *et al.*, 2021).** Excessive root hair, denser stomata, and longer roots result in faster growth and higher yields in *in vitro* and field studies on bread wheat, *Triticum aestivum* L. by seeds primed with MWCNTs at concentrations of 0, 70, 80, and 90 µg of MWCNTs per mL. The dense root hairs enhanced water and mineral uptake, such as phosphorus and potassium. Cell elongation increased by 80%, while xylem and phloem sizes dilated to nearly 83% and 85% of control, respectively, improving their ability to conduct water and nutrients **(Joshi *et al.* 2018)**.

According to **Seddighinia *et al.* (2020)**, seeds primed with MWCNT showed significant increases in root and shoot lengths, fresh and dry mass, vigor index, and leaf length. **Ghaghelestany *et al.* (2020)** proposed that CNTs improve the plant's ability to absorb water, resulting in healthy root and shoot growth and branch proliferation.

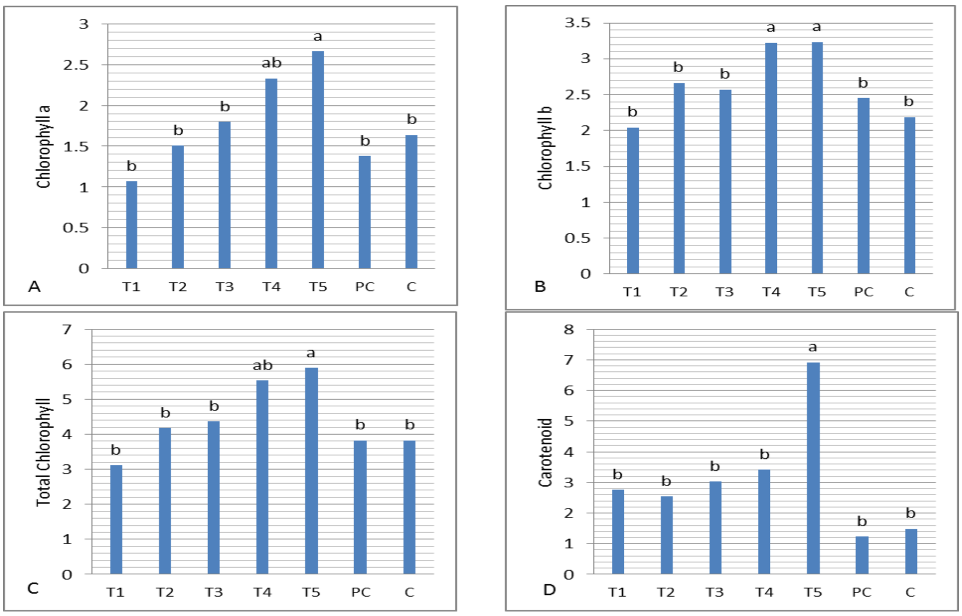
Photosynthetic Pigments

The effect of multi-walled carbon nanotubes (MWCNT) on the photosynthetic pigments of cucumber seedlings (Total carotenoids, chlorophyll-a, chlorophyll-b and total chlorophyll) after 21 days from culture in free hormone - MS medium supplemented with different concentrations of MWCNTs are shown in Table (2). The CNT2.5 and CNT2.0 treatments caused a high increase in chlorophyll-a chlorophyll-b and total chlorophyll contents, whereas the other CNT treatments did not find to make a significant ($P \leq 0.05$) change in this trait relative to the control (Fig. 2-A, B, C). The increases in carotenoid contents were also observed in the 2.5mg CNT/l supplemented seedlings by approximately two folds relative to the untreated control (Fig. 2-D).

With regard to photosynthetic pigments, Chlorophyll content in leaves reflects the plant's physiological status, and chlorophyll content declines faster than carotenoids (**Gitelson *et al.*, 1994; Sims *et al.*, 2002; Liné *et al.*, 2021). Liné *et al.* (2021)** reported a 29% increase in total chlorophyll levels in a comparative study of four plant species (tomato, rapeseed, cucumber, and maize). **Park and Ahn (2016)** investigated the effect of MWCNT's on chlorophyll content in carrot leaves. MWCNTs 500 mg/L were applied to leaf tissues of two- to three-month-old carrot plants for up to 48 hours. The chlorophyll content of Tween 20 (0.05%), a surfactant used in stock solutions to dissolve MWCNTs, was also tested. However according **Giraldo *et al.* (2014)**, SWCNTs were discovered in chloroplast membranes and improved photosynthetic capacity/electron transport, implying that nanomaterials could be used to improve photosynthesis.

Total protein

The effect of MWCNT on the total protein of cucumber seedling after 21 days from culture in free hormone - MS medium supplemented with different concentrations of MWCNTs are shown by SDS-PAGE technique in Figure 3. Also the SDS-PAGE total protein showed that the intensity of protein bands comparing to control, increase of most protein bands visibly starting from the concentration 0.1 mg CNT/l moreover, the heaviest protein bands were between 25 KD and 35 KD and they were at the concentrations 1 mg/l, 1.5 mg/l and 2 mg/l showing that they are the most effective concentration for inducing these proteins.



T1= 0.5mg/L MWCNT+ 0.5ml Aqua regia **T2= 1mg/L MWCNT+ 0.5ml Aqua regia**
T3= 1.5mg/L MWCNT+ 0.5ml Aqua regia **T4= 2mg/L MWCNT+ 0.5ml Aqua regia**
T5= 2.5mg/L MWCNT+ 0.5ml Aqua regia **PC= 1ml Aqua regia** **C= 0**

Figure 3. Changes in photosynthetic pigments for cucumber seedling cultured in free hormone - MS medium supplemented with different concentrations of MWCNTs.

Table 2; Effect of multi-walled carbon nanotubes on the photosynthetic pigments for cucumber seedling cultured in free hormone - MS medium supplemented with different concentrations of MWCNTs

Treatments	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoids
T_1= 0.5mg/L MWCNT+ 0.5ml Aqua regia	**1.07b**	**2.04a**	**3.11b**	**2.76b**
T_2= 1mg/L MWCNT+ 0.5ml Aqua regia	**1.51b**	**2.66a**	**4.17b**	**2.54b**
T_3= 1.5mg/L MWCNT+ 0.5ml Aqua regia	**1.80b**	**2.57a**	**4.37b**	**3.02b**
T_4= 2mg/L MWCNT+ 0.5ml Aqua regia	**2.33ab**	**3.22a**	**5.54ab**	**3.41ab**
T_3= 2.5mg/L MWCNT+ 0.5ml Aqua regia	**2.66b**	**3.23a**	**5.90a**	**6.92a**
PC_1= 1ml Aqua regia	**1.38b**	**2.45a**	**3.83b**	**1.23b**
C= 0	**1.64b**	**2.19a**	**3.83b**	**1.49b**
L.S.D	**1.20**	**1.50**	**1.3**	**3.60**

Comparing to control, new band at 20 KD emerged with all concentrations of CNT starting from 0.5 mg/l to 2.5 mg/l proving the ability of MWCNT to induce expression of proteins which were no expressed usually. The SDS-PAGE total protein showed also that, the treatment with aqua regia alone decreases the total protein expression comparing to all treatments even with the control. In this respect, carrot Hsp17.7 levels were measured during seed germination and seedling development in **Park and Ahn's (2016)** study to see if nanomaterials could affect seed protein stability. Carrot Hsp17.7 levels in seeds treated with Tween 20 (0.05%) remained unchanged on day 5 after imbibition. Protein levels were reduced as a result of the MWCNT treatment. On day 5, only a trace amount of DcHsp17.7 was detected among these seedlings. MWCNTs could reduce seed protein levels, according to the findings. Seedlings with low DcHsp17.7 levels may be more susceptible to abiotic stress conditions because Hsps play a role in stress resistance **(Park and Ahn, 2016).**

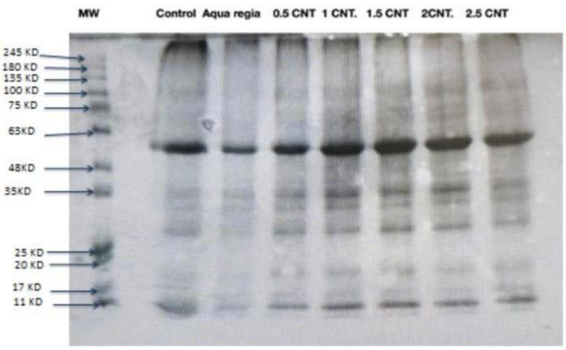


Figure 3. Protein banding pattern of studied species by SDS-PAGE total protein

Peroxidases isozyme

Peroxidases isozyme analysis for the collected leaves showed that, comparing to control sample, peroxidase 1 was produced more intensely at concentrations 1 mg/l, 1.5 mg/l and 2 mg/l of MWCNT, the heaviest expression was at 1 mg/l (Fig., 4). Peroxidase 2 was almost stable in all samples except for the sample of aqua regia where it was less expressed than the other samples making the treatment with only aqua regia decreasing the expression of PO2. The heaviest expression of peroxidase to was at the concentration 1 mg CNT/l. Peroxidase 3, 4 and 5 analysis showed contradicting and variable results. The heaviest expressions are at control, 0.5 mg/l, 1.5 mg/l and 2 mg/l and it decreases dramatically at 2.5mg/l. These results show that MWCNT don't have the same effect on all peroxidases but there is specific optimum concentration for each kind of peroxidase.

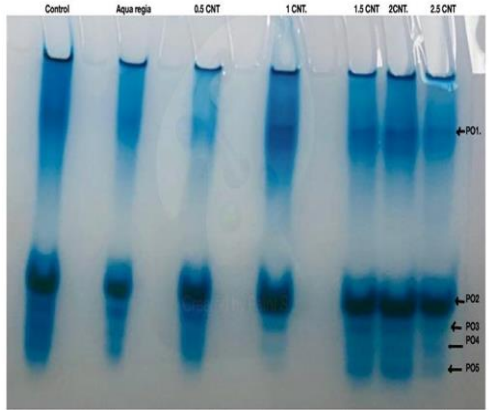


Figure 4. Peroxidase (PO) isozymes in leaves of cucumber plants grown under different concentrations Arrow indicate the PO isozymes detected by staining using 7.5% naïve polyacrylamide gels.

Previous research on peroxidases isozyme analysis by **Tripathi and Prakash (2022)** discussed the benefits of nanotechnology in plant as well as the negative effects of nanotechnology on the ecosystem. A day after inoculation, nanoparticles exhibited strong expression of peroxidase (POX), phenylalanine ammonia-lyase (PAL), and moderate expression of the PR-1 gene, whereas 6 days after inoculation, PAL and PR-1 expression was strong, while POX expression was moderate **(Elsharkawy and Mousa, 2015; Tripathi and Prakash, 2022).**

CONCLUSIONS

In conclusion, our results-demonstrated that cucumber seedlings are affected by water soluble multi walled Carbon nanotubes in a concentration dependent manner. It was demonstrated that the administration of MWCNTs at mediest concentrations produce favorable physiological effects on development in the in vitro multiplication stage of cucumber. It could be concluded that MWCNT (a cost-effective nanomaterial product) can improve plant growth, performance, physiology, antioxidants and secondary metabolism which could have significant economic importance for agriculture.

REFERENCES

1. Azizi-Lalabadi, M., H., Hossein, F., Jianguo and J., Seid (2020). Carbon nanomaterials against pathogens; the antimicrobial activity of carbon nanotubes, graphene/graphene oxide, fullerenes, and their nanocomposites." Advances in Colloid and Interface Science 284 (2020): 102250.
2. Elsharkawy, M., Mohamed, and K. M. Mousa (2015) Induction of systemic resistance against Papaya ring spot virus (PRSV) and its vector Myzus persicae by Penicillium simplicissimum GP17-2 and silica (SiO2) nanopowder." International journal of pest management 61.4: 353-358.
3. Ghasempour M., A. Iranbakhsh, M. Ebadi and Z. O. Ardebili. 2019. Multi-walled carbon nanotubes improved growth, anatomy, physiology, secondary metabolism, and callus performance in Catharanthus roseus: an in vitro study 3 Biotech (2019) 9:404.
4. Giraldo, J.P., Landry, M.P. and Faltermeier, S.M. (2014) Plant nanobionics approach to augment photosynthesis and biochemical sensing. Nat Mater 13:400–408. doi:10.1038/nmat3890
5. Gitelson, A., Mark N. Merzlyak. 1994. Quantitative estimation of chlorophyll-a using reflectance spectra: Experiments with autumn chestnut and maple leaves. Journal of Photochemistry and Photobiology B: Biology 22.3 (1994): 247-252
6. He H, Pham-Huy LA, Dramou P, Xiao D, Zuo P, Pham-Huy C (2013) Carbon nanotubes: applications in pharmacy and medicine.Biomed. Res. Int578290. https://doi.org/10.1016/j.envint.2019.105298.
7. Jonathan, F. W. and Weeden, N.F. (1990) Visualization and interpretation of plant isozymes. Isozymes in plant Biology. D.E.Soltis and P.S.Soltis (eds). London Chpman and Hall, pp.5-45.
8. Joshi A, Kaur S, Dharamvir K, Nayyar H, Verma G (2018) Multiwalled carbon nanotubes applied through seed-priming influence early germination, root hair, growth and yield of bread wheat (Triticumaestivum L.). J Sci Food Agric 98(8):3148–3160
9. Khodakovskaya, M.V., de Silva, K., Biris, A.S., Dervishi, E., Villagarcia, H. (2012) Carbon nanotubes induce growth enhancement of tobacco cells. ACS Nano. 2012 Mar 27;6(3):2128-35. doi: 10.1021/nn204643g. Epub 2012 Feb 29. PMID: 22360840.
10. Kim, T. Y., Sang-Hyo, L., Hara, K., Seung-Yop, L, (2019) Enhancement of drought tolerance in cucumber plants by natural carbon materials." plants 8.11 (2019): 446.
11. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4." Nature (Lond) 227- 680.
12. Liné, C., F. Manenta, A. Wolinskia, E. Flahautb and C. Laruea. 2021. Comparative study of response of four crop species exposed to carbon nanotube contamination in soil." Chemosphere 274 (2021): 129854.
13. Martenez-Ballesta MC, Zapata L, Chalbi N, Carvajal M (2016) Multiwalled carbon nanotubes enter broccoli cells enhancing growth and water uptake of plants exposed to salinity. J Nanobiotechnol 14(1):42
14. Merghany M, ;M. M. Shahein, M. A. Sliem, K. F.Abdelgawad and Amany F.Radwan. 2019. Effect of nano-fertilizers on cucumber plant growth, fruit yield and it' s quality. Plant Archives, 19 (2): 165-172
15. Oloumi H, Mousavi EA, Nejad RM (2018) Multi-wall carbon nanotubes effects on plant seedlings growth and cadmium/lead uptake in vitro. Russ J Plant Physiol 65(2):260–268.
16. Park, Sujin, and Y., Ahn. (2016) Multi-walled carbon nanotubes and silver nanoparticles differentially affect seed germination, chlorophyll content, and hydrogen peroxide accumulation in carrot (Daucus carota L.)." Biocatalysis and Agricultural Biotechnology 8: 257-262.
17. Patel, D. K., Kim, H. B., Dutta, S. D., Ganguly, K., and Lim, K. T. (2020) Carbon Nanotubes-Based Nanomaterials and Their Agricultural and Biotechnological Applications. Materials (Basel, Switzerland), 13(7), 1679. https://doi.org/10.3390/ma13071679
18. Patel, A. Tiwari, S. Parihar, P. Singh, R., Prasad, S. M. 2019. "Carbon nanotubes as plant growth regulators: impacts on growth, reproductive system, and soil microbial community." Nanomaterials in plants, algae and microorganisms. Academic Press, (2019): 23-42.
19. Seddighinia F, Iranbakhsh A, OraghiArdebili Z, NejadSatari T, Soleimanpour S (2019) Seed priming with cold plasma and multi-walled carbon nanotubes modified growth, tissue differentiation, anatomy, and yield in Bitter melon (Momordica charantia). J Plant Growth Regul. https ://doi.org/10.1007/s0034 4-019-09965 -2
20. Seddighinia, F., S., Iranbakhsh, A., Oraghi Ardebili, Z. , Nejad Satari, T. and Soleimanpour, S. (2020) Seed Priming with Cold Plasma and Multi-walled Carbon Nanotubes Modified Growth, Tissue Differentiation, Anatomy, and Yield in Bitter Melon (Momordica charantia). Journal of Plant Growth Regulation, 39 (1). pp. 87-98.
21. Shoukat, R.; Khan, M.I. Carbon nanotubes.(2021) A review on properties, synthesis methods and applications in micro and nanotechnology. Microsyst. Technol. 2021, 1–10.
22. Sims, Daniel A., and John A. Gamon. (2002) Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages." Remote sensing of environment 81.2-3: 337-354.
23. Sumathi, T.; Ponnuswami, V. and Senthamizh-Selvi, B. 2008. Anatomical Changes of Cucumber (Cucumis Sativus L.) leaves and roots as influenced by shade and fertigation. Research Journal of Agriculture and Biological Sciences, 4(6): 630-638.
24. Tripathi, D., Karuna, S., and M. A, Mansoori. (2019) Soil Pollution: Health Implications and Management." Recent Trends and Advances in Environmental Health 1 (2019): 41-64.
25. Wettstein, D. 1957. Chlorophyll – letale und der submikroskopische Formwechsel der Plastiden. Exp. Cell Res., 12: 427–487.
26. Yan S, Zhao L, Li H, Zhang Q, Tan J, Huang M, He S, Li L (2013) Single-walled carbon nanotubes selectively influence maize root tissue development accompanied by the change in the related gene expression. J Hazard Mater 246:110–118.