A novel technique using SWCNTs to enhanced development and root growth of fig plants (*Ficus carica*)

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ABSTRACT

Since the discovery of Carbon Nano Tubes (CNT) by Ijima in 1991 [4], different and novel applications had spun out of the use of this material, from the medical field to electronics and material applications. There is little information about the application of CNT in the field of agriculture and the impact of this material in plants especially in those for human consumption [5, 8]; with this work we provide some insight in the use of Single Walled Carbon Nano Tubes (SWCNT) in agriculture, or better said Nano-Agriculture.

The overall goal of this project is to look at possible mechanisms to explain the effects of SWCNTs in plant growth and its antimicrobial properties in order to reduce the use of harmful chemicals in agriculture. We analyzed how SWCNTs have the versatility to go through the wall and the cellular membrane of plants, helping the plant to have a better system of transport of nutrients to further distances inside of it. The plants we use were fig plants growth in the laboratory. All of the plants used were syngeneic showing about 2.5cm to 3cm in height. After inoculating different concentrations of SWNTs (2µg and 6µg) with the plants, after two weeks we noticed that the root growth and the overall length of the roots plus the length of the stem from the plants treated with SWCNT were significantly better than those from the control group. In this study we also describe a novel method to disperse the tubes using a biological agent. This method of dispersion showed very effective in that it made the tubes hydrophilic and easy to work with and it improved their purity. This work provides more insight on the potential use of SWNTs in agriculture.

Key words: CNT, SWCNT, Agriculture, Nanotechnology, Plant, Growth.

1 INTRODUCTION

In agriculture, nanotechnology is been use creating nanoparticles that help plant growth and the general. Moreover, Carbon Nano Tubes (CNT) has shown properties that help them cross cellular membranes in plants helping increase the transport capacity [1]. In tomato plants, studies show that CNTs are able to cross the outer shell of seeds, helping the growth process and the size of the plants studied [6]. Although, in order to achieved the increment in the transport capabilities the CNTs are required to be of homogenous size (about 46nm), in order to cover long distances inside the plants [1]. In addition, a different study with pumpkin plants show that it is better to inject the plants with CNTs than spray them, because it yield higher concentrations of the material using the first system compare to the lather one [7].

Our study focus in *Ficus carica* (fig plants), and the use of CNTs to observed the development and the root growth of the plants as a biological model to enhanced the species.

2 EFFECT OF SWCNT IN *Ficus carica*

2.1 The fig plants use for this study were in-vitro plants produced by the Centro de Investigación en Biotecnología (CIB), with lengths between 2.5 and 3 cm long. Those plants were grow using and in house medium [2], which was enhanced with 3% sucrose, 0.5mg/L of Benzylaminopurine solution (BAP), 0.125mg/L of Gibberellic Acid (GA3), and with a regulated pH at 5.7. The in-vitro plants were inoculated with two different concentrations of SWCNT, 2µg/ml and 6µg/ml mix inside the medium. About 20ml mix was dispersed in 120ml glass containers having one plant per container incubating the plants at a constant temperature of 26ºC, with 16 hours of continuous light at 100% humidity.

The variables observed in this study were the average length of the stem, average length of roots per plant, the root growth average and the root number average per plant. The test used for the study was ANOVA and Fisher test for each variable with a 95% interval of confidence (IC). The difference in the results for each variable tested can be observed by the different letters shown by the statistical software used, in this case Minitab 16. The study was performed in a sample of 30 plants per concentration of SWCNT plus the controls, given a population of 90 plants in the study.

2.2 The average survival of the in-vitro plants with the SWCNT treatment in both concentrations was of 100% compare to the survival of the controls with 93.33%. Moreover, the best percentage of root growth was obtained with the population treated with the 2.0µg of SWCNT which yield 100% root growth compare to the ones treated with the 6µg that yield 93.33% of root growth and the control with 96.43%. Another important characteristic show by the plants treated with the 2.0µg concentration of SWCNT was the average length of the stem which was more prominent compare to the other ones. Figure 1 shows the results of the observations of the study for both treatments and the controls.

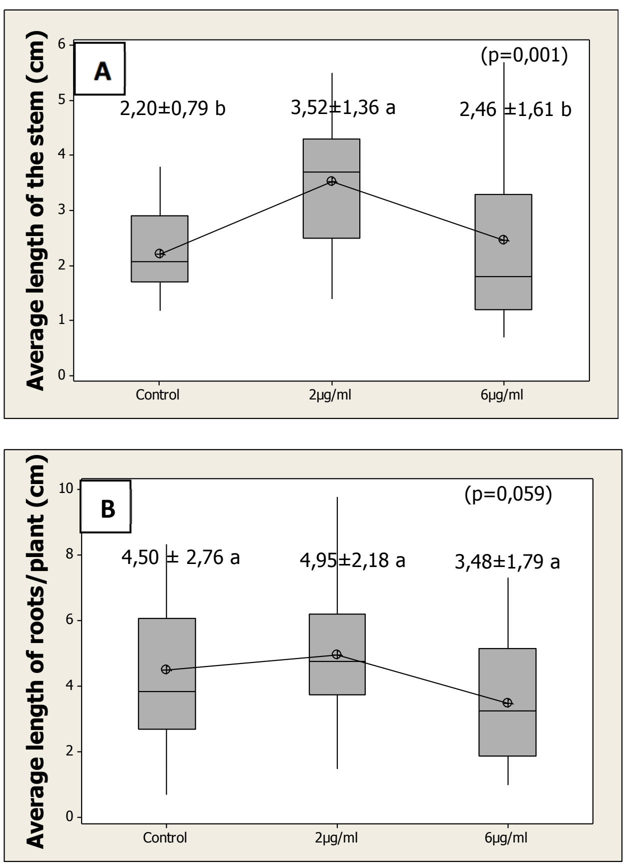

Figure 1: A) Show the average length of the stem in the three populations, and B) shows the average length of the roots per plant of the three samples.

Another interesting result observed in the study, was that the plants treated with a concentration of 2.0µg of SWCNT, were much faster in the root growth than those treated with the 6µg concentration or the control. Figure 2 shows the development of the roots expressed in days in the three populations of plants.

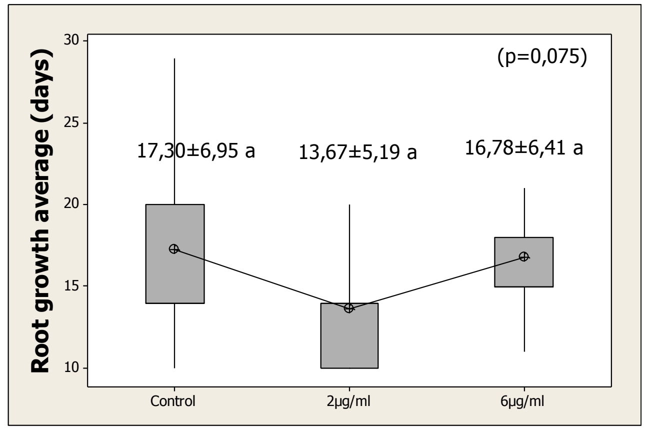

Figure 2: Average growth of roots in days observed in the population.

The root number average per plant showed no significant difference between the treatments given the 2ug, 6ug and control respectively; 8,30±4,59; 8,15±6,48; 8,96±5,54.

2.3 The SWCNT were characterized using RAMAN spectroscopy, and atomic force microscopy (AFM), using a DRX Thermo Scientific Raman microscope using a 532nm laser and a Nanosurf AFM.

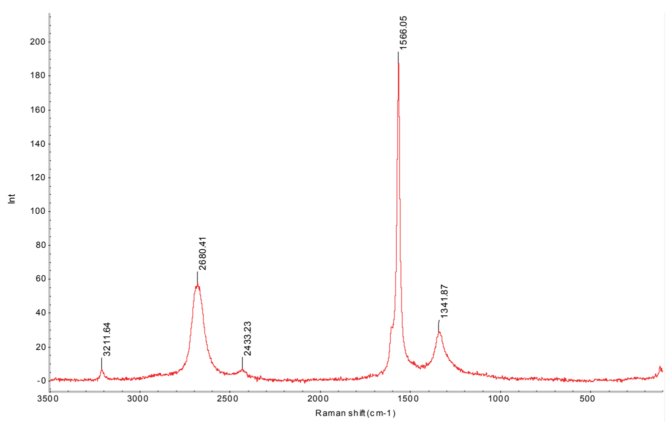

Figure 3: Shows the analysis using Raman spectroscopy for the SWCNT used in this study.

The Raman spectrum using a SWCNT sample placed in a glass slide, shows G band characteristic of the C=C bonds with a magnitude of 1566cm^{-1}, which indicates that the sample analyzed belongs to SWCNT. The dimensions of the

SWCNT were obtained using AFM characterization show in the next figures, and the conductive characteristics of them.

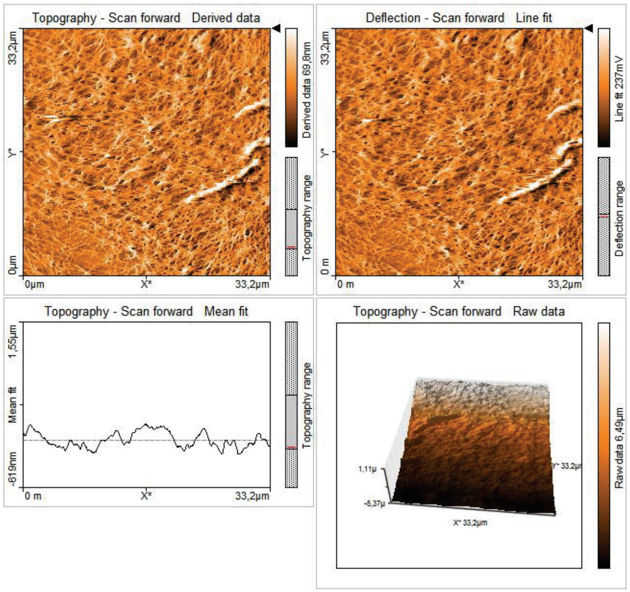

Figure 4: Shows the clusters of SWCNT used in this study.

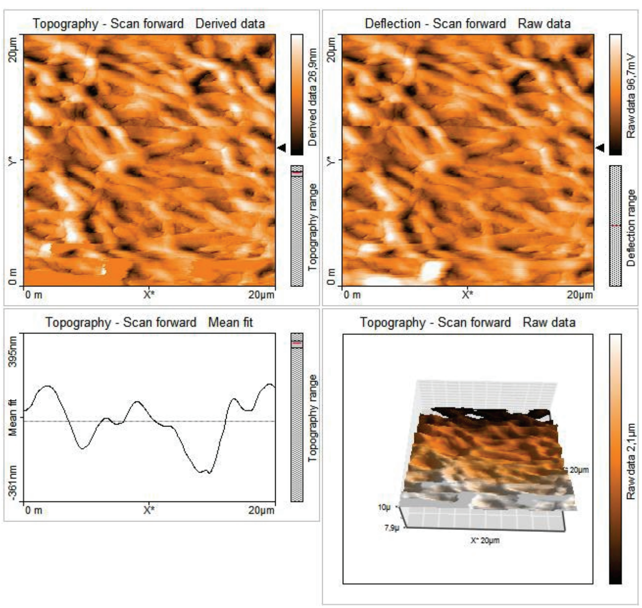

Figure 5: Show the profile analysis performed to the SWCNT to determine their average diameter.

The above image shows that the average diameters of the SWCNT use for this study were between 3.5 and 4nm in diameter. The length of the SWCNT could not be determined due to the clusters that made such analysis difficult to measure.

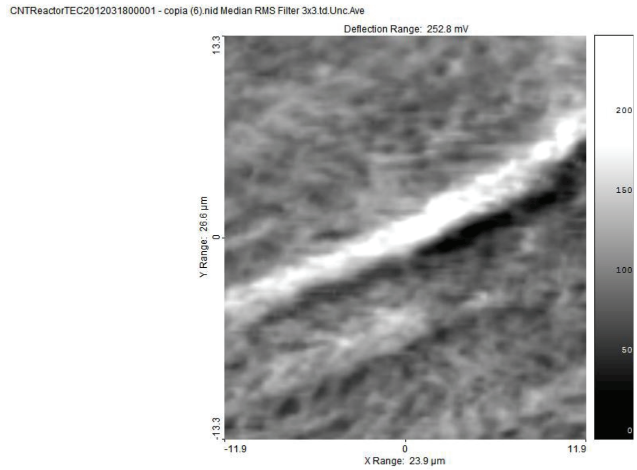

Figure 6: Shows the electrical characteristics in mili volts shown by the SWCNT used

2.4 To observe the presence of SWCNT in the in-vitro plants, the stem of the samples treated with 6µg of SWCNT were analyzed to determine in which part of the plant where allocated. The cross section of the stem of plants were treated with a Karnowsky process for 24 hours as a first stage and in a second stage they were treated with osmium tetroxide for 4 hours at 2%, finally the samples were dehydrated with ethyl alcohol and tert-Butanol at 100%.

A Scanning Electron Microscope from Hitachi TM1000 was used to analyze the images and a Bruker EDS Quantax 50 was used to obtain the spectrograph of the sample.

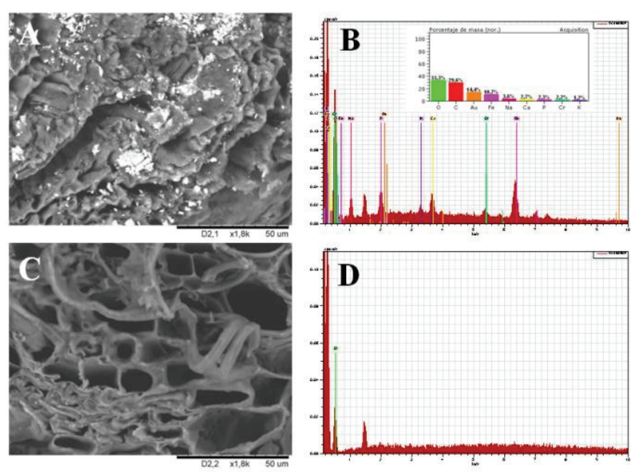

Figure 7: A) Show the sample treated with SWCNT at 6ug concentration. B) Show the EDS analysis of the sample. In micrograph C (the control) it can be observed that the cell walls are clean compare with the one treated with SWCNT, and the D image shows the EDS analysis of the control.

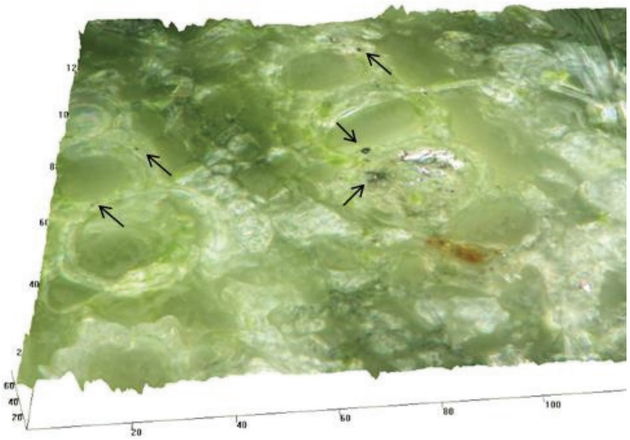

Figure 8: Cross section of the stem showing the SWCNT clusters in the xylem walls of the fig plants treated.

Finally, using a Zeta profiler microscope we observed in the cross sections the presence of SWCNT clusters in the xylem walls of the plant treated, also they were observed in the xylematic cell walls. This was congruent with observations done by other groups using CNT in plants [3].

3 CONCLUSIONS

Based on the observations of this study with Fig plants we concluded that the use of SWCNT does not affect the root growth of the in-vitro fig plants. Hence, the use SWCNT in the plants in this study was observed to help the plants in the development of their root system specially with low concentrations of 2µg were even the stem development was better than with those using higher concentrations. Although, it is our recommendation that a more extensive toxicology study will be needed in order to see the effect of SWCNT in the fruits for human consumption.

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