Effect of Carbon Nanotubes on Plant Growth and Gas Exchange using *Arabidopsis thaliana*

Rajeeva Voleti*, Dr. D. Alexander Wait**

* Graduate Student, Missouri State University, Springfield, MO, USA, rajeeva123@live.missouristate.edu
**Professor and Graduate Co-ordinator, Missouri State University, Springfield, MO, USA, alexanderwait@missouristate.edu

ABSTRACT

There has been increasing interest in the biological and biomedical applications of carbon nanotube. We addressed the novel question: Are pure CNTs produced for distribution to manufacturers "toxic" to plants through their effects on physiological processes? We germinated and grew Arabidopsis thaliana seedlings on basal salt medium in Petri plates with or without pure CNTs and polyhydroxy fullerenes [C60(OH)24] and determined the biomass and also effects of an aged CNTs on chlorophyll fluorescence. We found that photosynthesis, stomatal conducatance or transpiration in Arabidopsis thaliana grown in CNTs was significantly reduced compared to controls and positive controls. Carbon gain was reduced by 15% in plants grown in CNTs versus control plants. The carbon dioxide compensation point was significantly higher in CNT grown plants than control plants. Dry weight was not significantly affected in CNTs or control plants and the dry weight of plants grown in aged CNTs were significantly lower than control plants. A significant reduction in chlorophyll fluorescence (11% reduction) between plants grown in aged CNTs versus control media. The pure CNTs we examined appear to not be "toxic" to plants at the whole plant level.

Keywords: carbon naotubes, photosynthesis, gas exchange, Arabidopsis thaliana

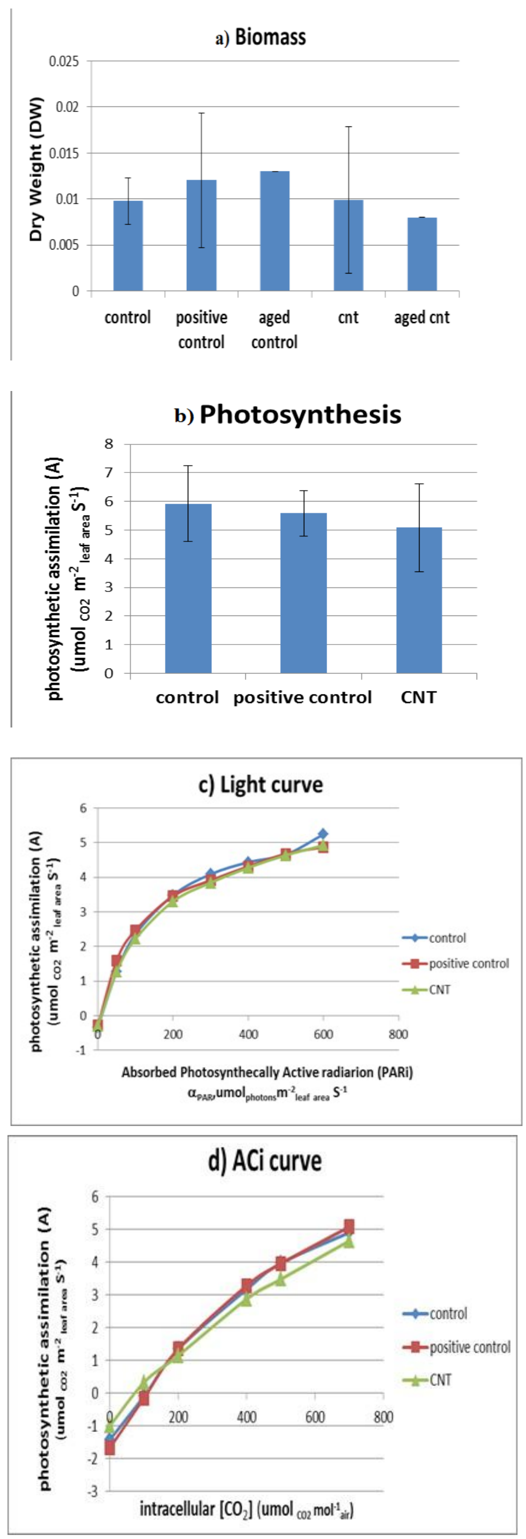
1 INTRODUCTION

Nanotechnology is an emerging science with wide range of applications in fields like physics, electronics, medicine, molecular sciences and technology. Nanomaterials such as fullerenes, single-walled carbon nanotubes (SWNTs) and multi-walled carbon nanotubes (MWNTs) have gained importance due to rapid industrial production and their usage in modern technology [1]. Fullerenes are hollow spheres of carbon and their cylindrical forms are called carbon nanotubes (CNTs). Carbon nanotubes have outstanding physical, chemical and mechanical properties due to which they are believed to have wide range of benefits in research [2]. However, the sustainability of CNTs lies in understanding if there are negative effects of the materials on organisms [3]. CNTs are classified mainly into SWNTs and MWNTs. SWNTs have diameter close to 1 nanometer and can be imagined as wrapping one atom thick layer of graphite known as graphene, into a seamless cylinder and MWNTs are constructed of multiple rolled layers of graphene. Nanomaterials have gained utmost importance due to their ability to interact with biological systems at various levels [2]. This has caused a matter of concern because when these nanomaterials discharge into the environment, their properties might be altered which could result in treat to living organisms [3]. There are some studies which indicate how CNTs might negatively affect animals and plants. For example, carbon nanotubes have been shown to cross membrane barriers and cause inflammation and fibrotic reactions in organs [4]. Administration of SWNTs into lungs of mice has shown to impair lung function, damage mitochondrial DNA in aorta and induce lesions in arteries of its heart [6]. Exposing mesothelial cavity of rodents to MWNTs may lead to inflammation and lesions in its lungs [5]. SWNTs have shown to have very little toxicity on plant cells [7]. The presence of CNTs in the aquatic environment have been shown to inhibit the growth of green alga (Chlorella species) [8]. Arabidopsis T87 cells cultured in media containing MWNTs have shown a reduction in their cell dry weight, chlorophyll content and superoxide dismutase activity [9]. Protoplasts of Arabidopsis and rice leaf exposed to SWNTs had adverse effects and this lead to cell death due to oxidative stress [10].

2 METHODS

We have used *Arabidopsis thaliana* Col-0 strain as my study model. Col-0 is the most widely-used wild type of *Arabidopsis thaliana*. *Arabidopsis thaliana* is a small flowering plant and is a member of the mustard (Brassicaceae) family. Approximately 115 Mb of the 125 Mb genome has been sequenced and annotated. Genetic and physical maps of all 5 chromosomes of *A.thaliana* are available. It has a short life cycle of about 6 weeks from germination to seed maturation and seeds can be cultivated in restricted space and hence it can be easily studied with no restrictions to space and time. A large number of mutant lines and genomic resources are also available. Seeds were obtained from Lehle Seeds, Round Rock, Texas, USA. In order to sterilize these seeds, about 200 seeds of *A.thaliana* were placed in a wax paper and in turn were placed in a jar which contains 100 ml of bleach and 3 ml of HCl. This whole set up was placed in a hood for dessication for about 2 hours. Carbon nanotubes and fullerenes were obtained from Brewers Science, Springfield, MO, USA. These were stored in De-ionized (DI) water as solutions. In order to prepare a medium containing these carbon nanotubes, 0.1625 g of Murashige & Skoog (MS) salts were dissolved in 50 ml of distilled water, followed by adjusting the pH to 7.0 with 100 mM KOH and making up the volume to 60.40 ml with distilled water. This was followed by addition of 0.6 g agar to this solution and heating the solution until agar dissolves completely. This solution was poured into culture tubes and the tubes were autoclaved for 20 minutes. Then a sonicator was prepared to contain water at 55^oC temperature. A volume of 1,108 uL of CNT preparation (135 ug/mL) was added to the culture tubes containing 4,826 uL of sterile MS medium. The CNTs were mixed with the MS medium thoroughly by turning the tube up and down. The tube was then sonicated by submerging it in warm water in the sonicator for about 2 minutes. After complete sonication, 60 uL of fungicide and 6 uL of carbenicillin was added to the culture tube. Sonication wase continued until we see no aggregation in the tube; this was an indication that CNTs were uniformly dispersed. Later this medium was poured in a 6-well plate, followed by solidification and labeling. The same procedure will be repeated with aged nanotubes. Before plating the seeds in the wells containing medium, wax papers containing seeds were removed carefully from the desiccating jar and were taken to laminar air flow chamber where the wells were present. With the help of index finger, seeds were pressed and evenly spread on one of the wells and this was repeated until the 200 seeds were plated in the same well. The plates were kept in incubator and seeds were allowed to grow into tiny plants in about 10 days. These plants were used to study parameters like maximum photosynthetic rate, stomatal conductance and transpiration using Licor 6400 XT photosynthetic system. At a time 15-20 plants were used to measure these parameters. Biomass measurements were recorded for the remaining plants with a time lag of 10 days after these experiments. Wet weights were recorded followed by drying these plants in hot air oven at 100^o F for a day.

3 RESULTS



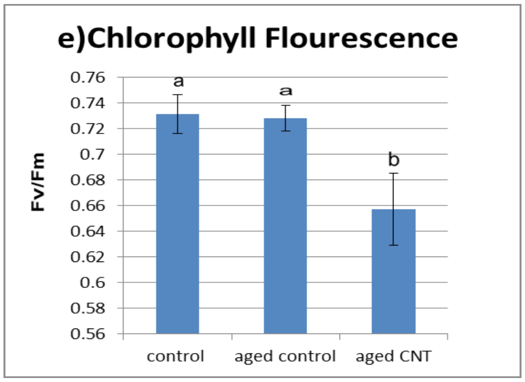


Figure 1: The effects of pure carbon nanotubes on growth (a), net photosynthesis (b), and photosynthetic responses to light (c) and carbon dioxide concentration (d) in *Arabidopsis thaliana* compared to control or positive control grown plants; and, the effects of a pure CNT aged by UV radiation on chlorophyll fluorescence (e).

4 CONCLUSION

We found that photosynthesis in *Arabidopsis thaliana* grown in CNTs was significantly reduced during the first seven days of growth compared to controls and positive controls. Ultimately, however, there was no significant effect of CNTs on photosynthesis, stomatal conducatance or transpiration compared to control or positive controls. Nonetheless, maximum total carbon gain was reduced by 15% in plants grown in CNTs versus control plants. Responses to light and carbon dioxide were likewise mostly unaffected by growth in pure CNTs. However, the carbon dioxide compensation point, as determined by ACi curve analysis, was significantly higher in CNT grown plants than control plants. Plant fresh weight was 32% lower in plants grown in CNTs compared to control plants, while dry weight was not significantly affected. In addition, the dry weight of plants grown in aged CNTs were significantly lower than control plants. Finally, we did find significant reduction chlorophyll fluorescence (Fv/Fm, 11% reduction) between plants grown in aged CNTs versus control media. We will examine other pure, waste stream and functionalized CNTs in future research, but the pure CNTs we examined appear to not be “toxic” to plants at the whole plant level even though some physiological processes appear to be mildly negatively affected.

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