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Effects of Low Concentrations of Carbon Nanotubes on Growth and Gas Exchange in Arabidopsis Thaliana

Rajeeva Voleti

EFFECTS OF LOW CONCENTRATIONS OF CARBON NANOTUBES ON GROWTH AND GAS EXCHANGE IN *ARABIDOPSIS THALIANA*

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By

Rajeeva Voleti

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EFFECTS OF LOW CONCENTRATIONS OF CARBON NANOTUBES ON GROWTH AND GAS EXCHANGE IN *ARABIDOPSIS THALIANA*

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ABSTRACT

The effect of pure single-walled carbon nanotubes (SWCNTs) on plant growth and gas exchange was investigated in *Arabidopsis thaliana*. To date there has been no research on the effects of SWCNTs on whole plant physiology. *A. thaliana* seeds were directly grown in growth medium containing SWCNTs concentrations of 24.93µg/ml and 53.55 µg/ml. control plants were grown in media containing distilled water. I determined growth by measuring dry mass of plants. I determined gas exchange by measuring photosynthetic rates, stomatal conductance, transpiration rates, and water use efficiency. I also examined the following physiological mechanisms that would limit plant growth: ATP and NADPH supply to light reactions through photosynthetic light response curves, and rubisco activity through photosynthetic CO_2 response curves. The presence of SWCNTs in the growth medium had no impact on the whole plant dry weight accumulation in any of the six experimental trials I carried out. Plants grown in growth media containing SWCNTs of a concentration of 24.93 µg/ml (4 experimental trials, n=12) and 53.55 µg/ml (1 trial, n=3) did not significantly influence any gas exchange variable after 21 days of growth. I also examined gas exchange variables after 7, 14, and 21 days of growth (1 trial, n=3). In this trial, there was a statistically significant treatment and time effect on photosaturated photosynthetic rate, photosynthetic efficiency and water use efficiency. My study illustrates that pure SWCNTs at realistic environmental conditions have no serious negative effects on plant growth and gas exchange; however, they may affect plant developmental rates. These findings have implications for plant and animal health, public awareness, and environmental remediation.

KEYWORDS: *Arabidopsis thaliana*, Single-walled carbon nanotubes, growth, gas exchange, physiology

This abstract is approved as to form and content

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Dr. D. Alexander Wait
Chairperson, Advisory Committee
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TABLE OF CONTENTS

INTRODUCTION 1
Overview Of Nanotechnology 1
Types Of Nanoparticles 1
Uses Of Nanoparticles In Environmental Science 2
Uses Of Nanoparticles In Agriculture 3
Uses Of Nanoparticles In Medicine 3
Uses Of Nanoparticles In Electric And Electronics 4
Uses Of Nanoparticles In Textiles 5
Uses Of Nanoparticles As An Adsorbent 5
Uses Of Nanoparticles In Sports 5
Negative Effects Of Nanoparticles On Micro-organisms And Animal Cells 6
Negative Effects Of Nanoparticles On Plants 7
Carbon Nanotubes 8
Arc Discharge Method. 8
Pulsed Laser Deposition. 8
Chemical Vapor Deposition. 9
Negative Effects Of CNTs In Higher Organisms 9
Positive Effects Of CNTs On Plants 10
Negative effects of CNTs on plants 10

RESEARCH GOALS 13

MATERIALS AND METHODS 14
Experimental Procedures 14
Study Organism 15
Experimental Methods 16
Preparation Of Holding Containers For Seeds Using Wax Paper. 16
Distributing And Weighing Seeds. 16
Sterilizing Seeds. 16
Preparing The Murashige And Skoog (MS) Medium. 16

Combining Components Of The CNT Medium. 17
Plating Of Seeds. 17
Incubation Of Plants. 17
Gas Exchange Measurements Using A Licor LI-6400 XT Photosynthetic System.. 18
Photosaturated Photosynthetic Rate (A_{max}). 18
Ambient Photosynthetic Rate (A_{amb}). 18
Stomatal Conductance (g). 18
Transpiration (E). 19
Photosynthetic Responses To Light. 19
Photosynthetic Responses To Carbon Dioxide. 20
Biomass. 21
Statistical Analyses 21

RESULTS 22

DISCUSSION 27

REFERENCES 31

TABLES……...38

FIGURES……...52

LIST OF TABLES

Table 1. Mean (±SE) and coeffiecient of variation of photosaturated photosynthetic rate (A_{max}), stomatal conductance (g), transpiration (E), intrinsic water use efficiency (A_{max}/g) and water use efficiency (A_{max}/ E) at a photosynthetically active radiation (PAR) of 400 µmol m^{-2} s^{-1} recorded after 21 days of growth in *A. thaliana*, grown in medium without (Control) (n=12) and with carbon nanotubes (CNT) (n=12) of concentration of 24.93 µg/ml 38

Table 2. Mean (±SE) and coeffiecient of variation of ambient photosynthetic rate (A_{amb}), stomatal conductance (g), transpiration (E), intrinsic water use efficiency (A_{amb}/g) and water use efficiency (A_{amb} / E) at a photosynthetically active radiation (PAR) of 150 µmol m^{-2} s^{-1} recorded after 21 days of growth in *A. thaliana*, grown in medium without (Control) (n=12) and with carbon nanotubes (CNT) (n=12) of concentration of 24.93 µg/ml. 39

Table 3. Mean (±SE) photosaturated photosynthetic rate (A_{max}), photosynthetic efficiency (slope), and light compensation point (LCP) obtained from light response curves after 21 days of growth for *A. thaliana* plants grown without (Control) (n=12) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=12).. 40

Table 4. Mean (±SE) photosaturated photosynthetic rate (A_{max}), carboxylation efficiency (slope), and carbon dioxide compensation point (CO_2CP) obtained from carbon dioxide response curves after 21 days of growth for *A. thaliana* plants grown without (Control) (n=12) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=12). 41

Table 5. Mean (±SE) and coeffiecient of variation of photosaturated photosynthetic rate (A_{max}), stomatal conductance (g), transpiration (E), intrinsic water use efficiency (A_{max}/g) and water use efficiency (A_{max}/ E) at a photosynthetically active radiation (PAR) of 400 µmol m^{-2} s^{-1} recorded after 21 days of growth in *A. thaliana*, grown in medium without (Control) (n=3) and with carbon nanotubes (CNT) (n=3) of concentration of 53.55 µg/ml. 42

Table 6. Mean (±SE) and coeffiecient of variation of ambient photosynthetic rate (A_{amb}), stomatal conductance (g), transpiration (E), intrinsic water use efficiency (A_{amb}/g) and water use efficiency (A_{amb} / E) at a photosynthetically active radiation (PAR) of 150 µmol m^{-2} s^{-1} recorded after 21 days of growth in *A. thaliana*, grown in medium without (Control) (n=3) and with carbon nanotubes (CNT) (n=3) of concentration of 53.55 µg/ml 43

Table 7. Mean (±SE) photosaturated photosynthetic rate (A_{max}), photosynthetic efficiency (slope), and light compensation point (LCP) obtained from light response curves after 21 days of growth for *A. thaliana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 53.55 µg/ml) (n=3). 44

Table 8. Mean (±SE) photosaturated photosynthetic rate (A_{max}), carboxylation efficiency (slope), and carbon dioxide compensation point (CO_2CP) obtained from carbon dioxide response curves after 21 days of growth for *A. thaliana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 53.55 µg/ml) (n=3). 45

Table 9. Mean (±SE) and coeffiecient of variation of photosaturated photosynthetic rate (A_{max}), stomatal conductance (g), transpiration (E), intrinsic water use efficiency (A_{max}/g) and water use efficiency (A_{max}/ E) at a photosynthetically active radiation (PAR) of 400 µmol m^{-2} s^{-1} recorded after 7, 14 and 21 days of growth in *A. thaliana*, grown in medium without (Control) (n=3) and with carbon nanotubes (CNT) (n=3) of concentration of 24.93 µg/ml.. 46

Table 10. Mean (±SE) and coeffiecient of variation of ambient photosynthetic rate (A_{amb}), stomatal conductance (g), transpiration (E), intrinsic water use efficiency (A_{amb}/g) and water use efficiency (A_{amb} / E) at a photosynthetically active radiation (PAR) of 150 µmol m^{-2} s^{-1} recorded after 7, 14 and 21 days of growth in *A. thaliana*, grown in medium without (Control) (n=3) and with carbon nanotubes (CNT) (n=3) of concentration of 24.93 µg/ml.. 48

Table 11. Mean (±SE) photosaturated photosynthetic rate (A_{max}), photosynthetic efficiency (slope), and light compensation point (LCP) obtained from light response curves after 7, 14 and 21 days of growth for *A. thaliana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3). 49

Table 12. Mean (±SE) photosaturated photosynthetic rate (A_{max}), carboxylation efficiency (slope), and carbon dioxide compensation point (CO_2CP) obtained from carbon dioxide response curves after 7, 14 and 21 days of growth for *A. thaliana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3). 51

LIST OF FIGURES

Figure 1. Steps for preparing holding containers for *A. thaliana* seeds using wax paper ..... .52

Figure 2. Photo of *A. thaliana* plants after 21 days of growth. 53

Figure 3. Representative light response curve for a C3 plant 54

Figure 4. Representative ACi curve for a C3 plant 55

Figure 5. Mean photosynthetic rate as a function of photosynthetically active radiation (PAR) after 21 days of growth for *A. thialiana* plants grown without (Control) (n=12) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=12)…….. 56

Figure 6. Mean photosynthetic rate as a function of carbon dioxide concentration (Ci) after 21 days of growth for *A. thialiana* plants grown without (Control) (n=12) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=12)…… 57

Figure 7. Mean (±SE) dry weight of *A. thaliana* plants after 21days of growth inControl grown (n=12) and CNT grown plants (24.93 µg/ ml, n=12) 58

Figure 8. Mean photosynthetic rate as a function of photosynthetically active radiation (PAR) after 21 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 53.55 µg/ml) (n=3) 59

Figure 9. Mean photosynthetic rate as a function of carbon dioxide concentration (Ci) after 21 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 53.55 µg/ml) (n=3). 60

Figure 10. Mean photosynthetic rate as a function of photosynthetically active radiation (PAR) after 7 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3) 61

Figure 11. Mean photosynthetic rate as a function of photosynthetically active radiation (PAR) after 14 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3)…… 62

Figure 12. Mean photosynthetic rate as a function of photosynthetically active radiation (PAR) after 21 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3)…… 63

Figure 13. Mean photosynthetic rates as a function of carbon dioxide concentration (Ci) after 7 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3)... 64

Figure 14. Mean photosynthetic rates as a function of carbon dioxide concentration (Ci) after 14 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3)... 65

Figure 15. Mean photosynthetic rates as a function of carbon dioxide concentration (Ci) after 21 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3). 66

Figure 16. Mean (±SE) dry weight of *A. thaliana* plants after 21days of growth inControl grown (n=3) and CNT grown plants (24.93 µg/ ml, n=3) 67

INTRODUCTION

Overview of Nanotechnology

Nanotechnology encompasses the concepts of science, engineering, and technology to fabricate products at nanoscales. Nanotechnology can be defined as manipulation of matter at atomic or molecular level. Engineered particles that are of 1 to 100 nm in size in at least one dimension are called "nanoparticles" (Rasmussen *et al.*, 2010). The prefix 'nano' was derived from the Greek word *nannos* which means "very short man" and in today's world its scientific value is 10^{-9} or one-billionth (0.000000001) of the base unit. This nanoscale and its extent of size can be easily imagined and familiarized with some daily life examples in a descending order of their value – an ant is on the order of 5 mm in size, a dust mite is 200 µm in size, red blood cells are about 8 µm in diameter, ATP synthase of our cells is 10 nm in diameter and finally the double helix of DNA on nanoscale is about 2 nm wide (Allhoff *et al.*, 2010). The physicist, Richard Feynman, described nanotechnology in a talk "There's Plenty of Room at the Bottom" at an American Physical Society meeting at the California Institute of Technology on December 29, 1959 that paved the way to the ideas and concepts of nanotechnology. He described how individual atoms and molecules can be manipulated and controlled. A decade later, Professor Norio Taniguchi, while working with ultraprecision machining, coined the term "nanotechnology".

Types of Nanoparticles

Since their discovery, several types of nanoparticles have been manufactured, such as whiskers and fibers. These are several nanometers to several hundred microns in width, and are a smaller version of nanowires. Other types include nanotubules, nanocables, and nanotubes of less than 100 nm width. Most nanoparticles are synthesized by either physical vapor deposition (PVD), which transfers growth species from source to a substrate where it deposits these nanoparticles to form a structure; or, chemical vapor deposition (CVD), where chemical reaction in the vapor phase creates the nanostructure (Allhoff *et al.*, 2010). Various compounds of carbon, zinc, silicon, cadmium, or titanium are used at smaller dimensions to create various nanostructures, nanomaterials, and nanotubes whose properties are different from the same materials of larger dimension.

With the advent of manufacturing nanoparticles, nanotechnology has been used in a wide range of applications in many fields: environmental science, agriculture, molecular biology, atomic physics, organic chemistry, medicine, batteries, textiles and manufacturing industries, electronics, fuels, cosmetics, and sports accessories.

Uses of Nanoparticles in Environmental Science

The following are some the uses of nanoparticles in various industries. Nanoclays and nanomembranes, which are made of carbon nanotubes (CNTs), are used to filter organisms and molecules out of water, and perform better than bacterial and viral filters (Buzea *et al.,* 2007). Quantum dots, which are semiconducting particles exhibiting quantum mechanical properties, are a source of cheap renewable energy (Murray *et al.*, 2000). Nanoparticles like cerium oxide (CeO_2) and titanium dioxide (TiO_2) dispersed in

soil act as catalytic agents and convert harmful substances to less harmful or harmless substances, and also hematite catalysis formation of various minerals help in absorbing many heavy metals from both water and soil in the environment (Rai *et al.*, 2015).

Uses of Nanoparticles in Agriculture

Nanoporous fertilizers disperse easily in the solvent and seep through soil like water under correct formulation and by using stabilizing agents (Rai *et al.*, 2015). So, these are used in agriculture to increase resistance against pests and improve overall crop yield. Nanofertilizers function is slow and targeted for efficient release of fertilizer into soil, and sometimes contain nutrients and growth promoters to stimulate growth of the plants. For instance, *Brassica juncea* (mustard) seeds treated with a low concentration (23 µg/ml and 46 ug/ml) of nanoparticles, such as multi- walled carbon nanotubes (MWCNTs) of diameter 30 nm, have shown to have higher germination rates and increased root and shoot growth (Mondal *et al.*, 2011). Nanosensors are used to detect pathogens in the field, and also monitor environment and crop health (Milani *et al.*, 2012).

Uses of Nanoparticles in Medicine

As nanoparticles are of smaller scale (nanoscale) than body cells, these particles can easily approach the cells and can be functionalized for easy drug and gene delivery, or can be tagged with fluorescent biological labels to manipulate the biological targets (Salata *et al.*, 2004). Likewise, nanomachines are used as vehicles during surgery to deliver substances to a target. Another example is tissue engineering with titanium implants coated with hydroxyapatite particles (HA), which provide stability and compatibility to the bone.

Also, HA coated with radiolabeled calcium (^{45}Ca) is used for imaging of dental implants after surgery. Radiolabeled nanoparticles can detect proteins, pathogens, and tumors; and, they can probe DNA structure and enhance contrast during MRIs for imaging of internal organs (Salata *et al.,* 2004). Zinc oxide (ZnO) nanoparticles help in selective destruction of tumors (Rasmussen *et al.,* 2010). Similarly, magnetic and metal based nanoparticles with alternating magnetic and shortwave radiofrequency create hyperthermia around the nanoparticles for thermal destruction of the tumors (Rasmussen *et al.,* 2010). As a final example, exposure of tobacco cell cultures to MWCNTs significantly upregulated the gene expression of tobacco aquaporin gene and marker genes for cell division and cell wall extension (Khodakovskaya *et al.,* 2012).

Uses of Nanoparticles in Electric and Electronics

In the case of electronics, silicon nanowires are used as semiconductors. Zinc Sulphide (ZnS) is used in thin film for electroluminescent displays (ELDs). TiO2 thin films are used as electrodes in photo voltaic cells (Allhoff *et al.,* 2010). In electronic devices, nanoparticles provide a high rate of electric conductivity. Semiconducting CNTs are used to manufacture field effect transistors (Postma *et al.,* 2001) and also nanoradios (Jensen *et al.,* 2007). Owing to their dense nature, gold nanoparticles are used as probes in transmission electron microscopy (Sun *et al.,* 2011).

Uses of Nanoparticles in Textiles

Zinc nanoparticle's property of absorbing UV rays can be used by applying these particles on textiles (Sun *et al.,* 2011). Metal nanoparticles contain surface plasmons, which are used for imparting different colors to textiles (Nadanathangam *et al.,* 2010). CNTs used in textiles show antistatic properties and self-cleaning or water repellent properties. SiO_2 and ZnO increase the durability of the textile and TiO_2, SiO_2 and, silver (Ag) nanoparticles used in textiles have antibacterial properties (Siegfried *et al.,* 2007). Smart clothes are entering into market, which can monitor a person's body functions like respiration rate, breathing frequency, body temperature, and blood pressure (Mecheels *et al.,* 2004). Textiles in the future might contain not only sensors to detect pathogens, but also warn the person by changing their color on simple wiping (Siegfried *et al.,* 2007).

Uses of Nanoparticles as an Adsorbent

Silver nanoparticles are used in the manufacturing of toothpastes, soaps, and face creams as they have an ability to kill bacteria on skin. TiO_2 does not penetrate beyond the epidermis of skin, so it is used in sunscreen lotions providing protection again UV rays. Also, self-cleaning windows use a 15 nm thick coating of activated TiO_2 engineered to be highly water-repellent so that rainwater just flows off the surface, washing away the dirt (Patel *et al.,* 2011).

Uses of Nanoparticles in Sports

CNTs stiffen the shaft and head of some tennis racquets and bicycles, which claim to be of higher strength and are lighter in weight. They also decrease rolling resistance and

increase durability of tires in automobiles. Nano-nickel used in golf balls increase moment of inertia. Swimsuits that use nanotechnology not only allow swimmers to move more quickly through the water but also mimic shark's skin. Nanoclay reduces weight and increases the speed of water boats. Lastly, "Opportunities for Nanomaterials in Sporting Applications – 2008-2013: Trend, Forecast and Competitive Analysis", mentioned that silica nanoparticles are used to decrease torsion index in skis and also increase hoop and flex strength of rods in fly-fishing.

Negative Effects of Nanoparticles on Micro-organisms and Animal Cells

Nanoparticles have many useful applications as described above; however, nanoparticles may also have negative effects on various life forms and the environment. Research examining the potential negative effects of nanoparticles is lagging behind the development of new nanoparticles. For example, Ng *et al.,* (2015) showed that metal oxide compounds of tin (Sn), iodine (In) and aluminium (Al) exhibited low toxicity when interacted with the surface of cell walls of the bacteria *Escherichia coli* and the diatom *Skeletonema costatum*. Titanium nanoparticles have been shown to damage nucleus and cell membranes, along with chloroplasts and internal organelle in the fresh water microalgae of *Chlorella sp.* (Iswarya *et al.,* 2015). Silver nanoparticles have been shown to affect the cellular functions of *Bacillus subtilis* species, and also kill *Azotobacter vinelandii* at low concentrations (Gambino *et al.,* 2015). Silver nanoparticles between 40-100 nm in ionic and in bulk form have caused oxidative stress, genotoxicity, and disruption of actin cytoskeleton in mussel hemocytes and gill cells (Katsumiti *et al.,* 2015). Kidney

epithelial cells exposed to ZnO nanoparticles between 12.5-50.0 µg/ml have been shown to cause DNA damage (Uzar *et al.,* 2015).

Negative Effects of Nanoparticles on Plants

Oukarroum *et al.,* (2015) showed that both nickel oxide nanoparticles and nickel (II) oxide at a concentration of 1000 µg/ml caused cellular oxidative stress and strongly inhibited Photosystem II (a protein complex in the thylakoids membrane of plants where the light energy is converted into the motion of energized electrons) quantum yield in the aquatic plant *Lemna gibba*. Exposure of *A thaliana* plants to TiO_2 nanoparticles and Ag nanoparticles compromised their transcriptional responses to microbial pathogens; and, increased bacterial infection and reduced root hair formation on leaves (Garcia-Sanchez *et al.*, 2015). Barley plants exposed to different concentrations (0, 125, 250, and 500 mg/kg) of cerium oxide nanoparticles failed to from grains (von Moos *et al.*, 2015). ZnO nanoparticles of 500 mg/kg reduced germination of alfa alfa seeds by 50%; and, root and shoot biomass by 80% and, 25% respectively. Zinc chloride ($ZnCl_2$) nanoparticles where observed to reduce catalase activity in stems and leaves of *Medicago o sativa L.* (alfalfa), which was symbiotically associated with *Sinorhizobium meliloti* in soil (Bandyopadhyay *et al.,* 2015). Seed germination was inhibited in ryegrass by nanoparticles of zinc and in corn by nano ZnO_2 at a concentration of 2000 µg/ml. Seed germination was inhibited by nano zinc of 50 µg/ml in radish and of 20 µg/ml concentration in rape and ryegrass (Lin *et al.,* 2007).

Carbon Nanotubes

Of all the nanoparticles discussed above, CNTs are used in many applications because of their high surface area to weight ratio. They are light weight and highly elastic as compared to carbon fibers and deliver higher surface area for increased chemical interaction in their specific application. Wang *et al.,* 2009, described that carbon nanotubes (CNTs) are constructed with length-to-diameter ratios of up to 132,000,000:1 and are allotropes of carbon. They exhibit extraordinary strength and unique properties, and are efficient conductors of heat. There are two main types of CNTs. SWCNTs can be conceptualized as wrapping one-atom-thick layer of graphite called graphene into a seamless cylinder and have a diameter close to one nanometer. MWCNTs are multiple rolls or concentrics of SWCNTs of different diameters.

These CNTs are manufactured mainly by three way- arc discharge method, pulsed laser deposition, chemical vapor deposition.

Arc Discharge Method. This is the easiest and simplest way of producing CNTs. Two carbon rods separated by 1mm distance are filled with inert gas at low pressures. A direct current of 50 to 100 A and a potential difference of 20 V creates higher temperatures between the electrodes. A small rod shaped deposit is formed on the electrodes after the discharge vaporizes their surface (Wilson *et al.,* 2002).

Pulsed Laser Deposition. Graphite rods containing 50:50 catalyst mixture of cobalt and nickel undergo laser vaporization at 1200^{o}C, and heat treatment in vacuum at 1000^{o}C. A second laser pulse is applied to vaporize target uniformly, and to break the larger particles formed during the first laser pulse, which builds them into rope-like nanotube structures of 100 µm in length (Wilson *et al.,* 2002).

Chemical Vapor Deposition. A classical method that is cost effective and produces good quality CNTs. Carbon nanostructures are deposited over a metal catalyst by catalytic chemical vapor deposition of acetylene over cobalt and iron catalysts at 545^{o}C. Chemical vapor deposition synthesizes bundles of SWCNTs as well as MWCNTs over carbon/zeolite catalyst (Wilson *et al.,* 2002).

Apart from uses already discussed above, CNTs have general uses in many industries. CNTs are used in the manufacturing of high efficient solar panels (Guldi *et al.,* 2005), power and data transmission using electrical cables and wires (Dawid *et al.,* 2014), in storing hydrogen (Dillon *et al.,* 1997), used as a paint on aircrafts to absorb incoming radar signals (Bourzac, 2011), used as building blocks in manufacturing of biomedical implants (Sitharaman *et al.,* 2013), in improving physical and mechanical properties of textiles (Shim *et al.,* 2008) and also used in optical power detectors in military equipment for defusing unexploded mines (Pop *et al.,* 2005).

Negative Effects of CNTs in Higher Organisms

Research on CNT toxicity in plants, when discharged in to the environment, is lacking (Chen *et al.,* 2010). However, the scientific community has realized that understanding the fate of nanomaterials from cradle to grave is essential to the sustainability of nanotechnology (Chen *et al.,* 2010). Kolosnjaj *et al.,* 2007, showed that CNTs crossed membrane barriers in T cells and induced harmful effects like inflammation and fibrotic reactions. Lam *et al.,* 2006, showed that CNTs accumulated in the cytoplasm of human cells caused cell death. Serious occupational health hazard related to air polluted cardio-pulmonary disease were produced when CNTs were chronically inhaled. CNTs

impaired respiratory functions, induced atherosclerotic lesions in the brachiocephalic artery, damaged mitochondrial DNA in aorta, and increased aortic plaque in the heart. In rodents, SWCNTs collectively effected and produced inflammation, epithelioid granulomas, and fibrosis in lungs. The needle shape of CNTs has been shown to lead to pleural mesothelioma of the lungs.

Positive Effects of CNTs on Plants

In maize, SWCNTs have been shown to accelerate seminal root growth (Yan *et al.*, 2013). Plants localized with SWCNTs within the lipid layer of their chloroplasts tripled their photosynthetic rates, showed increased electron transport rates in leaves, and suppressed the reactive oxygen species as compared to Control plants (Giraldo *et al.*, 2014). Tomato plants grown in soil with MWCNTs doubled their flower and fruit production as compared to the plants grown in Control soil (Khodakovskaya *et al.*, 2013). Growth of tobacco cell cultures increased by 55-64% when grown in medium containing MWCNTs between 5-500 µg/ml concentrations. (Khodakovskaya *et al.*, 2012).

Negative Effects of CNTs on Plants

Begum *et al.*, 2014, showed that CNTs had significant negative effects on plants including reduction in root and shoot length, cell death and electrolyte leakage. Plant cells exposed to C70–NOM (natural organic matter) and $C_{60}(OH)_{20}$ of 10–110 µg/ml produced cell lysis due to exhaustive endocytosis and necrosis (Chen *et al.*, 2010). MWCNTs adversely effected red spinach (*Amaranthus tricolor L*) roots and leaves via cell damage and oxidative stress (Begum and Fugetsu, 2012). Red spinach, lettuce and cucumber

treated with 1000 and 2000 µg/ml of MWCNTs significantly decreased their root and shoot lengths (Begum *et al.,* 2012). Uptake of MWCNTs particles by *Allium cepa* root cells resulted in altered cellular morphology and, membrane integrity, compromised the function of mitochondria, induced DNA damage and chromosomal aberration, finally leading to apoptotic cell death (Ghosh *et al.,* 2015). MWCNTs decreased dry weights, viability of cells, chlorophyll content, and superoxide dismutase activity of *A. thaliana* T87 suspension cells (Lin *et al.,* 2009). MWCNTs inhibited the growth of algae as a result of oxidative stress and agglomeration (Long *et al.,* 2012) and also reduced cell viability, decreased intracellular ATP levels and also triggered the production of reactive oxygen species (Pereira *et al.,* 2014). In tomato seedlings grown in a medium containing single walled carbon nanotube-quantum dot conjugates, the carbon nanoparticles of 50 µg/ ml concentration accelerated leaf senescence of plantlets, inhibited root formation, reduced chlorophyll content by 1.5 fold in leaves, and decreased the total weight of root system by four times compared to Controls (Alimohammadi *et al.,* 2011). Non functionalized CNTs inhibited root elongation in tomato, enhanced root elongation in onion and cucumber for 0, 24, and 48 hours following exposure (Canas *et al.,* 2008).

Due to their uses in many industries as described above, they will be disposed in high rates into the environment; therefore, their toxicity needs to be determined. As they disintegrate slowly, CNTs may be found in living systems. To understand some of the effects of CNTs on the environment and humans, they need to be studied in plants, as terrestrial plants serve as links connecting all food chains. Any source of CNTs disposed into the environment will reach the soil and start disintegrating slowly, and possibly enter the food chain, which was evident from some recent epidemiological studies which showed

a strong correlation between particulate air pollution levels, respiratory and cardiovascular diseases, various cancers, and mortality. (Buzea *et al.,* 2007). As they disintegrate slowly, CNTs may be found in human and other animal cells. Directly in the environment, CNTs may negatively affect plant physiological processes, such as gas exchange and growth.

The accumulation of carbon nanotubes levels in air and soil may affect growth and gas exchange in plants. In this research, I examined important gas exchange processes (photosynthesis, stomatal conductance, transpiration and biomass) in a model plant system. To my knowledge, all previous studies finding negative effects of CNTs on plants used carbon nanotubes (SWCNTs or MWCNTs) at concentrations that are well above those expected in nature. The prevalence of high concentrations (1000-2000 µg/ml) of carbon nanotubes as reported in Khodakovskaya *et al., (*2012) and Begum *et al.,* (2012), is not realistic, as carbon nanotubes exist in very low concentrations when released into the environment. There is no current literature available which reports the realistic concentrations of carbon nanotubes in the environment. It is essential to initiate studies of CNTs in their pure form and at realistic concentrations to determine if and how they impair any physical or physiological functions in plants. Therefore, I used low concentrations of carbon nanotubes (24.93 µg/ml and 53.55 µg/ml) in my study, and to date, there has been no research on the effects of low concentrations of pure SWCNTs on gas exchange and growth in plants.

RESEARCH GOALS

The goal of my research was to study the effects of realistic environmental concentrations of pure SWCNTs on *Arabidopsis thaliana*'*s* gas exchange and growth. I hypothesized that CNTs at low concentrations would have a negative effect on gas exchange leading to decreases in photosynthetic rate, stomatal conductance, and transpiration. CNTs would also affect the growth of *A. thaliana* leading to a decrease in biomass accumulation. My hypothesis were based in part on Alimohammadi *et al.,* 2011 findings that SWCNTs of a concentration of 50 µg/ml reduced the total weight of the root system of tomato plants by 75% as compared to tomato plants grown on regular media (controls).

MATERIALS AND METHODS

Experimental Procedures

Description of Individual Experiments. Experimental trials were divided into three categories depending on the concentration of CNTs used, and when during the growth period gas exchange measurements were recorded.

Experiment I was conducted with *A. thaliana* plated on medium containing 24.93 µg/ml CNTs and measured after 21 days of growth. The procedure in the experimental methods described below was followed. Four independent growth trials were performed where gas exchange was measured after 21 days of growth. Each trial included Control plates (n=3) and CNT plates (n=3). Plants in a plate not used for gas exchange measurements were harvested after gas exchange measurements for biomass accumulation.

Experiment II was conducted with *A. thaliana* plated on medium containing 53.55 µg/ml CNTs and measured after 21days of growth. This experiment was performed to see if a higher concentration of CNTs would have a negative effect, as the lower concentration did not result in negative effects (see Results). The procedure in the experimental methods described above was followed. One independent growth trial was performed where gas exchange was measured after 21 days of growth. The trial included Control plates (n=3) and CNT plates (n=3). Plants in a plate not used for gas exchange measurements were harvested after gas exchange measurements for biomass accumulation.

Experiment III was conducted with *A. thaliana* plated on medium containing 24.93 µg/ml CNTs and gas exchange was measured after 7, 14 and 21 days of growth. This experiment was performed because previous trials indicated no negative effects on gas

exchange after 21 days of growth, but a reduction in growth of 19% in CNT grown plants compared to Control grown plants. Although this difference in growth was not statistically significant, I wanted to examine if CNTs were having a phonological effect on gas exchange. The procedure in the experimental methods was followed in one individual trial that included Control plates (n=3) and CNT plates (n=3). Plants in a plate not used for gas exchange measurements were harvested after gas exchange measurements for biomass accumulation.

Study Organism

Arabidopsis thaliana belongs to the *Brassicaceae* family, along with species such as cabbage and radish. *A.thaliana* is a small flowering annual and a native of tropical Afroalpine ecosystems and temperate Northern Hemisphere (Hedberg *et al.,* 1957). Its life cycle is about 6 weeks, and is a prolific seed producer that can be cultivated easily in restricted spaces. Extensive genetic and physical m aps of all five chromosomes are available with 157 Mb of its genome sequenced and annotated (Bennett *et al.,* 2003). A large number of mutant lines and genomic resources are also available. Transformation can be efficiently performed utilizing *Agrobacterium tumefaciens* (Valvekens *et al.,* 1988). Owing to these factors, *A. thaliana* offers important advantages for basic research in genetics and molecular biology.

Study Material

I used pure CNTs suspensions that contained $\geq$ 75% SWCNTs of average length of ~0.4-0.6 µm manufactured by arc discharge method are obtained from the Brewer Science,

Rolla, Missouri These pure SWCNTs are of low ion content and have pure CNT fabric without any polymers. Therefore, these CNTs can be easily suspended in water based formulations without forming aggregates. I used these CNTs at low concentrations- 135 µg/ml and 290 µg/ml, which when mixed with a plant growth medium were at a final concentration of 24.93 and 53.55 µg/ml in growth media, respectively.

Experimental Methods

Preparation of Holding Containers for Seeds Using Wax Paper. Wax paper was folded diagonally as shown in diagram A of Figure 1. The creased corner of triangle was folded to the opposite creased corner forming a right triangle as shown in diagram B in Figure 1. The sides of the triangle were taped (which touched) to prevent unfolding. The unsealed side was pulled apart to open creating a cone as shown in diagram C of Figure 1.

Distributing and Weighing Seeds. A wax paper was folded into half for distribution of the seeds. Small amount of seeds were tapped into this folded wax paper and were transferred to the wax containers and 0.004 grams (4 mg) of seeds were weighed per container. These containers were placed carefully into a beaker.

Sterilizing Seeds. Seeds packed in the wax container were placed in a dessicant jar along with a beaker containing 100 ml of bleach. This set up was placed in a hood and 3 ml of HCl was added to 100 ml bleach beaker. The desiccant jar was sealed and was allowed to stand for 2 hours.

Preparing the Murashige and Skoog (MS) Medium. MS salts of 0.1625 g were dissolved in 50 ml deionized water and pH was adjusted to 7. The volume of the solution was made up to 60.40 ml with deionized water and 0.6 g of agar was added. The culture

flasks with the MS medium were autoclaved for 20 minutes and the flasks were kept in a 55^{o}C water bath to prevent the melted agar from solidifying.

Combining Components of the CNT Medium. The CNT suspension of 1,108 µL was added to a culture flask containing 4,826 µL of sterile autoclaved MS medium to prepare CNTs plates, and 1,108 µL of deionized water was added to a culture flask containing 4,826 µL of sterile autoclaved MS medium to prepare Control plates. The flasks were submerged into a sonicator containing water at ~55^{o}C for ~ 2 minutes. Sonication ensured the uniform homogenous distribution of CNTs with the MS medium thus preventing any aggregation or clumps of CNTs. Amphotericin (fungicide) of 60 µL and carbenicillin (antibiotic) of 6 µL were added to the flask. Approximately 25 ml of the resulting medium was poured into each petri plate. The medium was allowed to solidify before plating it with *A.thaliana* seeds.

Plating of Seeds. Plating was done in a fume hood disinfected with ethanol. The wax containers were placed in a hood and were disassembled. Seeds were pressed with the thumb and were sprinkled on to the solidified medium in the plate. The petri plates were wrapped with parafilm and were refrigerated at -4^{o}C for 48 hours. Refrigeration is a critical step for the seeds so as to imbibe uniformly into the agar medium.

Incubation of Plants. Petri plates were removed and transferred into a growth chamber (Percival Scientific incubator, model I-36VL) that was maintained at 28^{o}C with 12 hours of light and 12 hours of dark. Minimum photosynthetically active radiation levels of 150 µmol m^{-2} s^{-1} were maintained in the chamber. The seeds started germinating into plantlets within five to seven days of incubation and full foliage was achieved by 21 days (Figure 2).

Gas Exchange Measurements using a Licor LI-6400 XT Photosynthetic System. Langjun *et al.,* 2006, describes the Licor LI-6400 XT (manufactured by LI-COR Biosciences at Lincoln, Nebraska, USA) as a porTable photosynthetic system used for taking gas exchange measurement of fresh leaves. The Licor measures photosynthetic rates (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}), and transpiration rates (mol H_2O m^{-2} s^{-1}) at various light and CO_2 levels. I used the LICOR to measure photosaturated photosynthetic rates, ambient photosynthetic rates, stomatal conductance, transpiration, and responses of these variables to photosynthetically active radiation and CO_2.

Photosaturated Photosynthetic Rate (A_{max}). A_{max} is the rate of carbon assimilation at a PAR of 400 μmol m^{-2} s^{-1}. Any further increase in the amount (wavelength) of light striking the leaf does not cause an increase in the rate of photosynthesis and the amount of light is said to be 'saturating' for the photosynthetic process (Wareing *et al.,* 1968). This is measured to examine the potential for carbon gain, whereas ambient photosynthetic rate measures the actual carbon gain during the light period in the growth chamber.

Ambient Photosynthetic Rate (A_{amb}). A_{amb} is the rate of carbon assimilation at growth light levels, which were of 150 μmol m^{-2} s^{-1}. Measuring at growth PAR indicates the efficiency at which the plant assimilates CO_2 at growth light levels in the chamber during the 21 days of growth.

Stomatal Conductance (g). Stomatal conductance is a measure of the rate of CO_2 entering or water vapor exiting through the stomata of the leaf. The opening and closing of stomata is regulated by the guard cells. Stomatal conductance is directly related to the concentration gradient of the water vapor from the leaf to the atmosphere.

Transpiration (E). Transpiration is the loss of water through aerial parts of the plants like leaves, stems, and flowers into the atmosphere. This process also occurs through the stomata of the leaf along with CO_2 conductance. Water use efficiency (WUE) is the ratio of photosynthetic rate to the rate of transpiration. Transpiration is a function of both g and vapor pressure, based on this WUE can be calculated as a ratio of A/g (intrinsic WUE) or A/E (instantaneous WUE).

Photosynthetic Responses to Light. Light response curves illustrate a plant's responses to photosynthetically active radiation. Light curves are generated to examine the underlying photosynthetic processes of light-dependent and light-independent reactions, the efficiency at which light is utilized by photosynthesis, and the rate of O_2 uptake. A light response curve (Figure 3) can be used to interpret the rate at which O_2 evolution levels off, light levels below which there is no net O_2 evolution, and how efficiently solar energy is converted into chemical energy.

The light response curve (Figure 3) gives the photosynthetic rate (CO_2 assimilation) as a function of irradiance level (PAR). At under low-light levels, the rate of photosynthesis increases with the irradiance level. At a particular light intensity, the rate of CO_2 assimilation levels off, this point is called "light saturation point". Any more light striking the leaf does not further increase the rate of CO_2 assimilation or photosynthesis, this is called the "saturating point". At "light compensation point", the rate of CO_2 taken up by the stomata (photosynthesis) is equal to the rate of CO_2 evolved (respiration). The efficiency at which the solar energy is converted into chemical energy is given by the slope of the response curve and represents the "photosynthetic efficiency" or "quantum yield".

In order to interpret the above variables from the light curve, a linear equation for the light curve was obtained by fitting a line to initial three points, for each repetition or individual sample measured. Photosaturated photosynthetic rate (A_{max}) was obtained at saturating photosynthetically active radiation (400 µmol m^{-2} s^{-1}). The slope of the linear equation illustrates the photosynthetic efficiency, and the light compensation point (LCP) indicates the photosynthetically active radiation level where photosynthesis and respiration are equal (Table 3).

Photosynthetic Responses to Carbon Dioxide. CO_2 response curves (Figure 4) illustrate a plants response to CO_2 concentrations. The data can be used to assess maximum potential photosynthetic rates, maximum rates of Rubisco carboxylation and maximum rates of electron transport for Rubisco bisphosphate (RuBP) regeneration (Cen *et al.,* 2005).

In Figure 4, the rates of photosynthesis that would be achieved depending on whether Rubisco, RuBP (Rubisco biphosphate), or TPU (triose phosphate utilization) are limiting, as indicated. The actual photosynthetic rate (solid line) at any given Ci is the minimum of these three potential limitations (Long *et al.,* 2003). At the CO_2 compensation point (CO_2CP), the net CO_2 assimilation becomes zero (respiration rate equals photosynthetic rate).

In order to interpret the above variables from the CO_2 response curve, a linear equation for the curve was obtained by fitting a line to initial three points, for each repetition or individual sample measured. Photosaturated photosynthetic rate (A_{max}) was obtained at saturating light levels (400 µmol m^{-2} s^{-1}), which illustrates the "saturation point". The slope of the linear equation illustrates the maximum rate of Rubisco

carboxylation and finally the carbon dioxide compensation point (CO_2CP) was given by the negative intercept on y axis of the CO_2 response curve divided by the slope of the CO_2 response curve (Table 4).

Biomass. Plants were harvested between 21 and 28 days of growth and wet weights were recorded. Plants were then dried in a hot air oven at 100^oC for 48 hours and reweighed.

Statistical Analyses

General linear model in ANOVA (Minitab Student version 14) was used for analyzing the gas exchange variables: photosynthetic rate, stomatal conductance, transpiration and water use efficiency (Tables 1, 2, 5, 6, 9 and 10), light-response curve variables: photosaturated photosynthetic rate, photosynthetic efficiency and compensation point (Tables 3, 7 and 11) and CO_2 response curve variables: photosaturated photosynthetic rate, maximum rate of Rubisco carboxylation, and carbon dioxide compensation point (Tables 4, 8 and 12). Fixed effects (day and treatment) and random effects (treatment x day interaction) in ANOVA were analyzed for the data in Tables 1-12 for *A. thaliana* Control grown and CNT media grown plants. Pairwise comparisons were performed using Tukey's test (Tables 1-12) and the threshold of significance was set to $p = 0.05$ for all the analyses.

RESULTS

In the case of growing plants in medium containing SWCNTs of a concentration of 24.93 µg/ml (experiment I), there were no statistically significant differences found in mean (± SE) photosaturated photosynthetic rate, ambient photosynthetic rate, stomatal conductance, transpiration, intrinsic water use efficiency, and instantaneous water use efficiency between *A. thaliana* plants grown without (Control) and with carbon nanotubes (CNT) of 24.93 µg/ml concentration (Tables 1 and 2). Similarly**,** in case of growing plants in medium containing SWCNTs of concentration of 53.55 µg/ml (experiment II), mean photosaturated photosynthetic rates, ambient photosynthetic rate, stomatal conductance, transpiration, intrinsic water use efficiency and instantaneous water use efficiency were also not significantly different between *A. thaliana* plants grown without (Control) and with carbon nanotubes (CNT) of 53.55 µg/ml concentration (Tables 5 and 6). These non-significant trends are consistent across trails as indicated by low and consistent coefficient of variation (CV) values among the treatments for gas exchange variables and also between the experiments with different carbon nanotubes concentrations (24.93 µg/ml and 53.55 µg/ml). Mean dry weights of the Control grown plants was 19% greater than the mean dry weights of CNT grown plants in experiment I (Figure 7). But this does not represent a statistically significant difference in mean biomass accumulation.

Light curves of *A. thaliana* Control grown and CNT grown plants were almost identical to each other (Figure 5 and 8), which indicates no effects of carbon nanotubes at lower concentrations of 24.93 µg/ml and 53.55 µg/ml on light compensation points, photosynthetic efficiencies and photosaturated photosynthetic rates. Mean photosaturated

photosynthetic rates (A_{max}), photosynthetic efficiencies (slope) and light compensation points (LCP) between Control grown and CNT grown plants were not significantly different as illustrated in Tables 3 and 7. But the light compensation point (LCP) was in Control grown plants was significantly higher ($p<0.01$) as compared to the CNT grown plants as shown in Table 7. CO_2 response curves of *A. thaliana* Control grown and CNT grown plants were almost identical (Figure 6 and 9), which illustrates no effects of carbon nanotubes at lower concentrations of 24.93 µg/ml and 53.55 µg/ml on carbon dioxide compensation points, maximum rates of rubisco carboxylation (slopes) and photosaturated photosynthetic rate. CO_2 response curves of Control grown and CNT grown plants run parallel to each other and for both the curves the rate of Rubisco carboxylation and rate of electron transportation for RUBP regeneration are limiting factors at Ci of 450 ppm (Figure 6) and 480 ppm (Figure 9) in experiments I and II respectively. Mean photosaturated photosynthetic rate (A_{max}), maximum rate of Rubisco carboxylation (slope) and carbon dioxide compensation points (CO_2CP) between Control grown and CNT grown plants of *A. thaliana* were not statistically significant (Tables 4 and 8).

In experiment III, ANOVA results for all gas exchange variables did not show any significant differences between the treatments over time (7, 14, and 21 days of growth) except for the mean photosaturated photosynthetic rate A_{max}. This indicated statistically significant effects of A_{max} as a function of treatment and day ($p<0.05$) and, also as a function of treatment x day interaction ($p=0.03$) (Table 9). Therefore, pairwise comparisons between mean values of these variables (Tukey test) were performed Mean A_{max} readings taken after 7 days of growth of *A. thaliana* Control grown were significantly different from mean A_{max} of CNT grown plants measured after 7, 14 and 21 days of growth.

Mean A_{max} measured after 7, 14 and 21 days of growth significantly differed between the treatments (Table 9). Similarly ANOVA results for all gas exchange variables did not show any significant differences between the treatments over time (7, 14, and 21 days of growth) except for the mean intrinsic water use efficiency (A_{amb}/g) and the mean instantaneous water use efficiency (A_{amb}/E). This illustrates significant effects of A_{amb}/g ($p=0.030$) and A_{amb}/E ($p=0.037$) as a function of treatment x day interaction (Table 10). Therefore, pairwise comparisons between mean values of these variables (Tukey test) were performed. Mean intrinsic water use efficiency of *A. thaliana* Control grown significantly differed with time (7, 14 and 21 days of growth). Mean intrinsic water use efficiency measured after 7 days of growth of *A. thaliana* Control grown were significantly different from mean intrinsic water use efficiency of CNT grown plants measured after 14 and 21 days of growth. Mean intrinsic water use efficiency measured after 21 days of growth significantly differed between the treatments (Table 10). The light curves of *A. thaliana* Control grown and CNT grown plants almost run parallel to each other (Figure 10, 11 and 12). However, the ANOVA results showed significant day effect ($p=0.023$) and also treatment x day effect ($p=0.049$) for mean photo saturated photosynthetic rate (A_{max}) (Table 11). CO_2 response curves of A. thaliana Control grown and CNT grown plants almost run parallel to each other (Figure 13, 14 and 15) and for both the curves, rate of rubisco carboxylation and rate of electron transportation for RUBP regeneration are limiting factors at Ci of ~500 Pa. However, ANOVA results for the mean Rubisco carboxylation rate (slope) showed significant treatment effect ($p=0.023$) and also treatment x day effect ($p=0.039$) (Table 12). Mean carboxylation efficiencies of *A. thaliana* Control grown significantly differed with time (7 and 14 days of growth). Mean carboxylation efficiencies

measured after 7 and 14 days of growth significantly differed between the treatments (Table 12). The significant differences of gas exchange variables between days and treatments describe that the physiology of *A. thaliana* was affected by phenological factors during the time course experiment III. Mean dry weight of *A. thaliana* Control grown plants was not significantly different than the mean dry weight of CNT grown plants during the time course experiments (Figure 16).

DISCUSSION

Studies to date have reported both positive and negatives effects of carbon nanotubes on plants. But to my knowledge, there has been any reports on the effects of SWCNTs on gas exchange and growth in *A. thaliana*. In my study, I examined the effects of SWCNTs on gas exchange variables (photosynthetic rate, stomatal conductance, transpiration and water use efficiency) and growth (biomass accumulation) using *A. thaliana.* Also many studies (Khodakovskaya *et al.,* 2012 and Begum *et al.,* 2012) from the literature reported the effects of higher concentrations (1000-2000 µg/ml) of carbon nanotubes on plants. But in my study I examined the effects of low concentrations of SWCNTs (24.93 µg/ml and 53.55 µg/ml) on *A. thaliana* plants. The use of low concentrations of carbon nanotubes in my study demonstrated more realistic concentrations of carbon nanoparticles in the environment. Results from my study indicate no significant toxic effects of carbon nanotubes on gas exchange variables (Tables 1-8). However, the time course experiment indicated that carbon nanotubes may affect developmental rates (Table 9-12). The low coefficient of variance values between the treatments and among various experiments in my study demonstrated consistency in the results.

My growth methods and sample sizes were constrained by growing plants in sterile media for genetic analysis by other researchers. In addition, growth chamber space was limited. However, rates of growth and gas exchange were consistent with other studies. Control grown and CNT grown plants of *A.thaliana* in all my experiments were grown at light levels of 150 µmol m^{-2} s^{-1}. These values were consistent with the light levels for growth (60 µmol m^{-2} s^{-1}) reported by Zentgraf *et al.,* (2003) in *A.thaliana* L.ecotype

Columbia plants. At saturating light levels, A_{max} in Control grown and CNT grown plants increased from >5 to >7 µmol CO_2 m^{-2} s^{-1} from 7 to 14 days of growth (Table 9). These values were consistent with the results reported by Flexas *et al.,* (2007), where A_{max} slightly increased from 14 to > 15 µmol CO_2 m^{-2} s^{-1} from 28 to 34 days of growth in *A. thaliana* plants. The overall photosynthetic rates values of Control grown plants reported in my study ranged from >3.0 to > 7.0 µmol CO_2 m^{-2} s^{-1} (Table 1, 2, 5, 6, 9 and 10) , and these rates are consistent with the values reported by Dow *et al.,* (2014), which ranged from 2.2 to 8.2 µmol CO_2 m^{-2} s^{-1}. Overall A_{max} values of Control grown plants reported in my study ranged from 4 to 7 µmol CO_2 m^{-2} s^{-1} (Tables 3, 7 and 11), and these were similar to values reported by Tanaka *et al.,* (2013) in Control plants that ranged from 6 to 8 µmol CO_2 m^{-2} s^{-1}. The values of A_{max} of Control grown plants at a CO_2 partial pressure of 400 µmol m^{-2} s^{-1} in CO_2 response curves reported in my study ranged from 3 to 5 µmol CO_2 m^{-2} s^{-1} (Tables 4, 8 and 12) and these rates were consistent with the values reported by Tanaka *et al., (*2013), which ranged from 6 to 8 µmol CO_2 m^{-2} s^{-1}. The values for intrinsic WUE reported in my study ranged from 2 to 15 (Table 2 and 9), were similar to the values reported by Dow *et al.,* (2014). Similarly, instantaneous WUE values reported in my study ranged from 0.28 to 1.2 (Table 2) and these were consistent with the values reported by Dow *et al.,* (2014), which ranged from 1.0 to 4.0. The above findings illustrate that the gas exchange values obtained in my study are of normal ranges and consistent with the previous literature with respect to *A. thaliana*.

Mondal *et al.,* (2011), reported higher germination rates and increased root and shoot growth in *Brassica juncea* seeds grown in low concentrations of MWCNTs (23 µg/ml and 46 µg/ml). Similarly, Yan *et al.,* (2013), reported an accelerated root growth in

maize grown in SWCNTs. In contrast, Begum *et al*., (2014), reported a significant reduction in root and shoot length, cell death, and electrolyte leakage, when exposed to higher concentrations (1000-2000 µg/ml) of MWCNTs. Begum *et al.,* (2012) reported a significant decrease in root and shoot lengths of Red spinach, lettuce, and cucumber when exposed to higher concentrations (1000 and 2000 µg/ml) of MWCNTs. But my study reported no visible effects in germination rates, root, and shoot growth (data not shown) in *A. thaliana* plants grown in low concentrations (24.93 µg/ml and 53.55 µg/ml) of SWCNTs. Lin *et al.,* (2009), reported decreased dry weights in *A. thaliana* T87 suspension cells when grown in agglomerates of MWCNTs. Similarly, Bandyopadhyay *et al.,* (2015), reported reduced germination, shoot, and root biomass in alfa alfa seeds when exposed to ZnO nanoparticles of 500 µg/ml concentration. In my study, no negative effects were observed in dry weights or biomass accumulation in *A. thaliana* plants grown in low concentration (24.93 µg/ml) of SWCNTs (Figure 7 and 16). Begum and Fugetsu, (2012), reported cell damage in root and leaves via oxidative stress in *Amaranthus tricolor L* exposed to MWCNTs. In contrast, I observed no effects on leaves and roots cells in *A. thaliana* when grown in a media containing SWCNTs of concentration 24.93 µg/ml (Figure 2). Giraldo *et al.,* (2014), reported an increased photosynthetic rates (tripled) when SWCNTs were inserted into the chloroplasts in plants. Since my study had neutral effects on the photosynthetic rates, this suggests that SWCNTs were not entering into the chloroplasts of *A. thaliana* plants. *Oukarroum et al.,* (2015), reported cellular oxidative stress and decreased quantum yield in *Lemna gibba* when exposed to both nickel oxide and nickel (II) oxide nanoparticles at a concentration of 1000 µg/ml. In my study, the results from Tables 3, 7 and 11 illustrate no changes in quantum yield or photosynthetic efficiency

in *A. thaliana* CNT grown plants as compared to the Control grown plants when exposed to SWCNTs of low concentrations (24.93 µg/ml and 53.55 µg/ml).

By studying the effects of pure SWCNTs at low concentrations on different gas exchange variables, growth and developmental patterns in *A. thaliana*, I conclude that my study provides no evidence of low concentrations of pure SWCNTs effecting whole plant gas exchange. However, my study can be further extended by using higher concentrations of SWCNTs to check when the shift actually starts where physiological variables and biomass will be negatively affected by CNTs. Second, by using aged CNTs, which are exposed to the UV rays for ~ 300 hours, to check if aged nanoparticles have any significant effect on *Arabidopsis* physiological variables and biomass. Another area of research interest would be to test the effect of functionalized groups, which are attached to florescent tags. This last experiment would demonstrate if the CNTs are internalized into the plant system using with fluorescent microscopy. Fourth, by using MWCNTs of lower concentrations, we can compare their effects on gas exchange variables and growth in *A. thaliana* with SWCNTs.

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TABLES

Table 1. Mean (±SE) and coeffiecient of variation of photosaturated photosynthetic rate (A_{max}), stomatal conductance (g), transpiration (E), intrinsic water use efficiency (A_{max}/g) and water use efficiency (A_{max}/ E) at a photosynthetically active radiation (PAR) of 400 µmol m^{-2} s^{-1} recorded after 21 days of growth in *A. thaliana*, grown in medium without (Control) (n=12) and with carbon nanotubes (CNT) (n=12) of concentration of 24.93 µg/ml.

Variable	Treatment	Mean	SE	Coefficient of Variation
A_{max} (µmol CO_2 m^{-2} s^{-1})	control	5.20	0.42	25.47
	CNT	5.00	0.44	24.80
g (mol H_2O m^{-2} s^{-1})	control	0.38	0.09	66.11
	CNT	0.38	0.09	71.09
E (mol H_2O m^{-2} s^{-1})	control	5.56	0.77	43.55
	CNT	5.32	0.58	30.89
A_{max}/g	control	5.56	0.77	43.55
	CNT	5.32	0.58	30.89
A_{max}/E	control	1.11	0.15	44.09
	CNT	1.00	0.11	30.87

Table 2. Mean (±SE) and coeffiecient of variation of ambient photosynthetic rate (A_{amb}), stomatal conductance (g), transpiration (E), intrinsic water use efficiency (A_{amb}/g) and water use efficiency (A_{amb} / E) at a photosynthetically active radiation (PAR) of 150 µmol m^{-2} s^{-1} recorded after 21 days of growth in *A. thaliana*, grown in medium without (Control) (n=12) and with carbon nanotubes (CNT) (n=12) of concentration of 24.93 µg/ml.

Variable	Treatment	Mean	SE	Coefficient of Variation
A_{amb} (µmol CO_2 $m^{-2}s^{-1}$)	Control	3.93	0.28	22.25
	CNT	3.76	0.29	22.19
g (mol H_2O m^{-2} s^{-1})	Control	0.39	0.09	79.21
	CNT	0.36	0.09	71.31
E (mol H_2O m^{-2} s^{-1})	Control	5.50	0.79	45.05
	CNT	4.96	0.59	33.66
A_{amb}/g	Control	17.42	3.71	67.31
	CNT	15.46	3.45	63.11
A_{amb}/E	Control	0.85	0.13	46.00
	CNT	0.80	0.094	33.39

Table 3. Mean (±SE) photosaturated photosynthetic rate (A_{max}), photosynthetic efficiency (slope), and light compensation point (LCP) obtained from light response curves after 21 days of growth for *A. thaliana* plants grown without (Control) (n=12) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=12).

Treatment	A_{max} (µmol CO_2 m^{-2} s^{-1})	Slope (µmol m^{-2} s^{-1})	LCP (µmol mol)
Control	5.46±0.38	0.030±0.003	28.45±3.93
CNT	5.03±0.79	0.026±0.003	24.24±4.21

Table 4. Mean (±SE) photosaturated photosynthetic rate (A_{max}), carboxylation efficiency (slope), and carbon dioxide compensation point (CO_2CP) obtained from carbon dioxide response curves after 21 days of growth for *A. thaliana* plants grown without (Control) (n=12) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=12).

Treatment	A_{max} (µmol CO_2 m^{-2} s^{-1})	Slope (µmol m^{-2} s^{-1})	CO_2CP (µmol mol)
Control	4.22±0.15	0.013±0.001	134.43±9.60
CNT	4.86±0.27	0.014±0.001	119.73±6.17

Table 5. Mean (±SE) and coeffiecient of variation of photosaturated photosynthetic rate (A_{max}), stomatal conductance (g), transpiration (E), intrinsic water use efficiency (A_{max}/g) and water use efficiency (A_{max}/ E) at a photosynthetically active radiation (PAR) of 400 µmol m^{-2} s^{-1} recorded after 21 days of growth in *A. thaliana*, grown in medium without (Control) (n=3) and with carbon nanotubes (CNT) (n=3) of concentration of 53.55 µg/ml.

Variable	Treatment	Mean	SE	Coefficient of Variation
A_{max} (µmol CO_2 m^{-2} s^{-1})	Control	5.56	0.45	16.22
	CNT	5.45	0.78	24.93
g (mol H_2O m^{-2} s^{-1})	Control	0.78	0.07	17.20
	CNT	0.90	0.06	10.7
E (mol H_2O m^{-2} s^{-1})	Control	8.64	0.32	7.46
	CNT	9.16	0.27	5.06
A_{max}/g	Control	7.18	0.26	7.17
	CNT	6.17	1.19	33.30
A_{max}/E	Control	0.64	0.03	10.34
	CNT	0.74	1.23	12.23

Table 6. Mean (±SE) and coeffiecient of variation of ambient photosynthetic rate (A_{amb}), stomatal conductance (g), transpiration (E), intrinsic water use efficiency (A_{amb}/g) and water use efficiency (A_{amb} / E) at a photosynthetically active radiation (PAR) of 150 µmol m^{-2} s^{-1} recorded after 21 days of growth in *A. thaliana*, grown in medium without (Control) (n=3) and with carbon nanotubes (CNT) (n=3) of concentration of 53.55 µg/ml.

Variable	Treatment	Mean	SE	Coefficient of Variation
A_{amb} (µmol CO_2 m^{-2} s^{-1})	Control	4.05	0.38	18.90
	CNT	3.90	0.51	22.66
g (mol H_2O m^{-2} s^{-1})	Control	0.72	0.07	19.58
	CNT	0.84	0.07	13.39
E (mol H_2O m^{-2} s^{-1})	Control	8.22	0.31	7.55
	CNT	8.82	0.29	5.73
A_{amb}/g	Control	5.60	0.13	4.63
	CNT	4.75	0.88	32.28
A_{amb}/E	Control	0.49	0.03	12.28
	CNT	0.44	0.06	22.32

Table 7. Mean (±SE) photosaturated photosynthetic rate (A_{max}), photosynthetic efficiency (slope), and light compensation point (LCP) obtained from light response curves after 21 days of growth for *A. thaliana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 53.55 µg/ml) (n=3).

Treatment	A_{max} (µmol CO_2 m^{-2} s^{-1})	Slope (µmol m^{-2} s^{-1})	LCP (µmol mol)
Control	6.19±0.60	0.039±0.003	33.71±4.91
CNT	6.792±0.76	0.023±0.004	2.17±0.97

Table 8. Mean (±SE) photosaturated photosynthetic rate (A_{max}), carboxylation efficiency (slope), and carbon dioxide compensation point (CO_2CP) obtained from carbon dioxide response curves after 21 days of growth for *A. thaliana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 53.55 µg/ml) (n=3).

Treatment	A_{max} (µmol CO_2 m^{-2} s^{-1})	Slope (µmol m^{-2} s^{-1})	CO_2CP (µmol mol)
Control	6.75±0.62	0.021±0.001	94.52±6.14
CNT	5.40±1.21	0.015±0.004	127.60±25.40

Table 9. Mean (±SE) and coeffiecient of variation of photosaturated photosynthetic rate (A_{max}), stomatal conductance (g), transpiration (E), intrinsic water use efficiency (A_{max}/g) and water use efficiency (A_{max}/ E) at a photosynthetically active radiation (PAR) of 400 µmol m^{-2} s^{-1} recorded after 7, 14 and 21 days of growth in *A. thaliana*, grown in medium without (Control) (n=3) and with carbon nanotubes (CNT) (n=3) of concentration of 24.93 µg/ml. For all variables, time was a statistically significant variable, but treatment and treatment x day interaction was not statistically different except for Amax; therefore, no pairwise comparisons were performed except for Amax. Means for Amax that do not share a letter are statistically significantly different from each other ($p<0.05$) as determined by pairwise comparisons using Tukey's method.

Variable	Day	Treatment	Mean	SE	Coefficient of Variation
A_{max} (µmol CO_2 m^{-2} s^{-1})	7	Control	5.12[a]	0.69	23.57
		CNT	4.37[a,b]	0.53	20.91
	14	Control	7.25[c]	0.28	6.62
		CNT	5.34[a,b]	0.28	9.08
	21	Control	5.72[a,b,c]	0.10	0.178
		CNT	6.26[b,c]	0.12	3.26
g (mol H_2O m^{-2} s^{-1})	7	Control	1.36	0.14	17.70
		CNT	1.36	0.25	32.09
	14	Control	0.96	0.03	5.07
		CNT	0.88	0.09	16.44
	21	Control	0.64	0.08	0.14
		CNT	0.56	0.04	13.12
	7	Control	13.14	0.98	12.95
		CNT	13.18	2.11	27.76
	14	Control	9.81	0.02	0.36

Variable	Day	Treatment	Mean	SE	Coefficient of Variation
		CNT	9.46	0.49	9.01
	21	Control	7.23	1.14	27.20
		CNT	6.63	0.29	7.69
A_{max}/g	7	Control	3.83	0.51	23.15
		CNT	3.27	0.20	10.36
	14	Control	7.60	0.50	11.30
		CNT	6.24	0.75	20.83
	21	Control	9.22	1.14	21.42
		CNT	11.21	0.85	13.07
A_{max}/E	7	Control	0.39	0.05	21.95
		CNT	0.34	0.02	8.28
	14	Control	0.74	0.03	6.80
		CNT	0.57	0.05	14.61
	21	Control	0.83	0.11	23.67
		CNT	0.95	0.06	10.77

Table 10. Mean (±SE) and coeffiecient of variation of ambient photosynthetic rate (A_{amb}), stomatal conductance (g), transpiration (E), intrinsic water use efficiency (A_{amb}/g) and water use efficiency (A_{amb} / E) at a photosynthetically active radiation (PAR) of 150 µmol m^{-2} s^{-1} recorded after 7, 14 and 21 days of growth in *A. thaliana*, grown in medium without (Control) (n=3) and with carbon nanotubes (CNT) (n=3) of concentration of 24.93 µg/ml. For all variables, time was a statistically significant variable, but treatment and treatment x day interaction was not statistically different except for Amax; therefore, no pairwise comparisons were performed except for Amax. Means for Amax that do not share a letter are statistically significantly different from each other ($p<0.05$) as determined by pairwise comparisons using Tukey's method.

Variable	Day	Treatment	Mean	SE	Coefficient of Variation
A_{amb} (µmol CO_2 m^{-2} s^{-1})	7	Control	3.75	0.25	11.43
		CNT	3.50	0.53	26.39
	14	Control	4.63	0.52	19.54
		CNT	3.80	0.48	21.95
	21	Control	3.75	0.05	2.39
		CNT	4.41	0.20	8.16
g (mol H_2O m^{-2} s^{-1})	7	Control	1.29	0.13	16.87
		CNT	1.26	0.25	34.18
	14	Control	0.87	0.02	3.05
		CNT	0.81	0.07	15.92
	21	Control	0.64	0.10	25.92
		CNT	0.49	0.04	12.25
	7	Control	12.64	0.86	11.79
		CNT	12.46	2.04	28.39
	14	Control	9.31	0.18	3.33

Variable	Day	Treatment	Mean	SE	Coefficient of Variation
		CNT	9.00	0.45	8.57
	21	Control	7.05	1.14	27.99
		CNT	5.88	0.56	16.37
A_{amb}/g	7	Control	2.97[c]	0.37	21.35
		CNT	2.82[c]	0.24	14.74
	14	Control	5.31[b,c]	0.63	20.47
		CNT	4.79[b,c]	0.81	29.31
	21	Control	6.15[b]	0.93	26.09
		CNT	9.04[a]	0.31	5.91
A_{amb}/E	7	Control	0.30[c]	0.03	16.67
		CNT	0.28[c]	0.02	11.98
	14	Control	0.50[b,c]	0.05	18.81
		CNT	0.42[b,c]	0.06	23.66
	21	Control	0.56[a,b]	0.08	23.95
		CNT	0.76[a]	0.04	8.47

Table 11. Mean (±SE) photosaturated photosynthetic rate (A_{max}), photosynthetic efficiency (slope), and light compensation point (LCP) obtained from light response curves after 7, 14 and 21 days of growth for *A. thaliana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3).

Day	Treatment	A_{max} (µmol CO_2 m^{-2} s^{-1})	Slope (µmol m^{-2} s^{-1})	LCP (µmol mol)
7	Control	4.39±0.18	0.021±0.011	29.94±6.42
	CNT	3.89±0.39	0.021±0.011	14.15±4.48
14	Control	6.21±0.78	0.031±0.011	17.23±6.81
	CNT	4.56±0.84	0.024±0.002	13.16±3.69
21	Control	5.12±0.14	0.025±0.003	35.25±1.14
	CNT	6.31±0.18	0.031±0.002	18.30±2.82

Table 12. Mean (±SE) photosaturated photosynthetic rate (A_{max}), carboxylation efficiency (slope), and carbon dioxide compensation point (CO_2CP) obtained from carbon dioxide response curves after 7, 14 and 21 days of growth for *A. thaliana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3). For all variables, time was a statistically significant variable, but treatment and treatment x day interaction was not statistically different except for Amax; therefore, no pairwise comparisons were performed except for Amax. Means for Amax that do not share a letter are statistically significantly different from each other ($p<0.05$) as determined by pairwise comparisons using Tukey's method.

Day	Treatment	A_{max} (µmol CO_2 m^{-2} s^{-1})	Slope (µmol m^{-2} s^{-1})	CO_2CP (µmol mol)
7	Control	4.93±0.02	0.014±0.002**a,b**	104.88±7.55
	CNT	3.76±0.30	0.011±0.001**b**	114.37±8.19
14	Control	4.93±0.02	0.016±0.002**a**	118.11±35.70
	CNT	4.34±0.35	0.011±0.001**b**	115.4±12.30
21	Control	4.01±0.15	0.013±0.001**a,b**	149.52±4.58
	CNT	5.10±0.21	0.014±0.001**a,b**	116.36±7.30

FIGURES

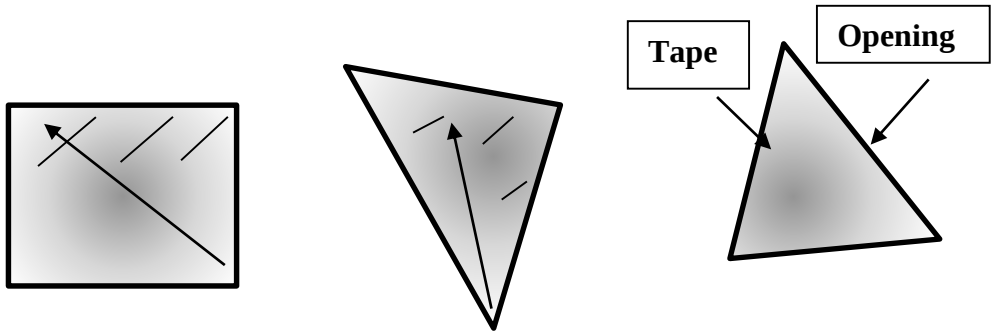
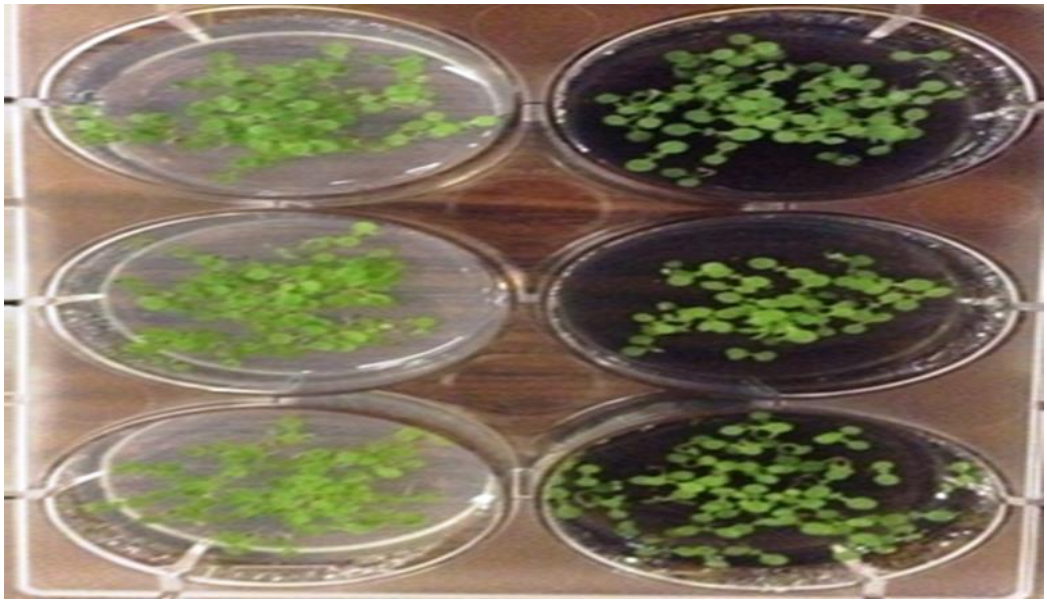


Figure 1. Steps for preparing holding containers for *A. thaliana* seeds using wax paper



Control plates **CNT plates**

Figure 2. Photo of *A. thaliana* plants after 21 days of growth. Petriplates on the left (L) side contain medium without CNTs (Control plates) and on right (R) side contain medium with CNTs (CNT plates).

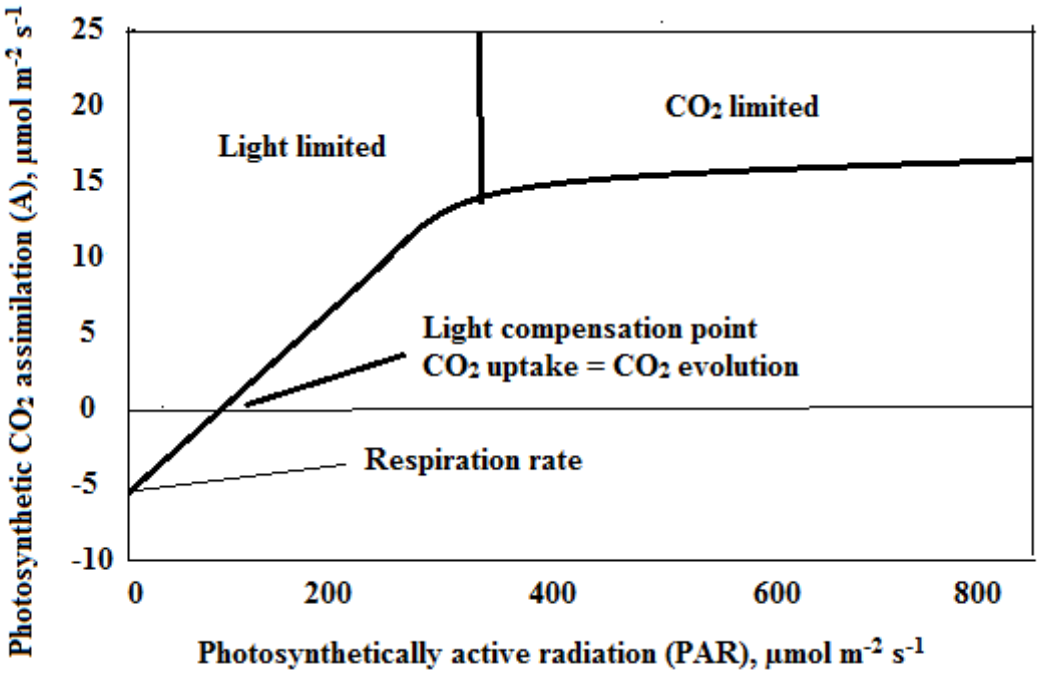


Figure 3. Representative light response curve for a C3 plant.

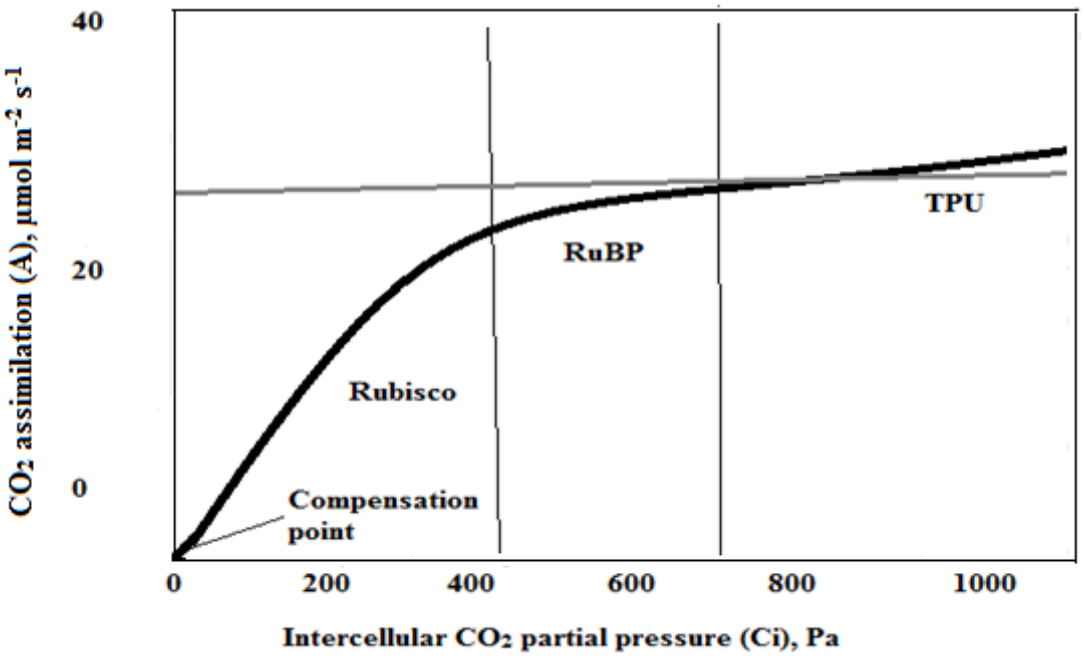


Figure 4. Representative ACi curve for a C3 plant.

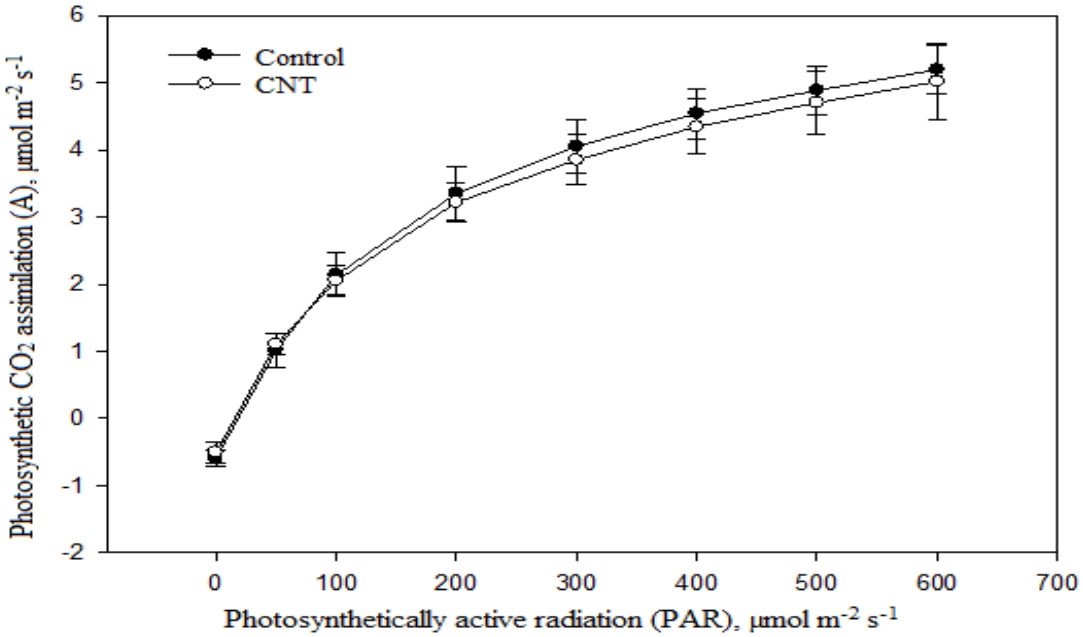


Figure 5. Mean photosynthetic rate as a function of photosynthetically active radiation (PAR) after 21 days of growth for *A. thialiana* plants grown without (Control) (n=12) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=12)

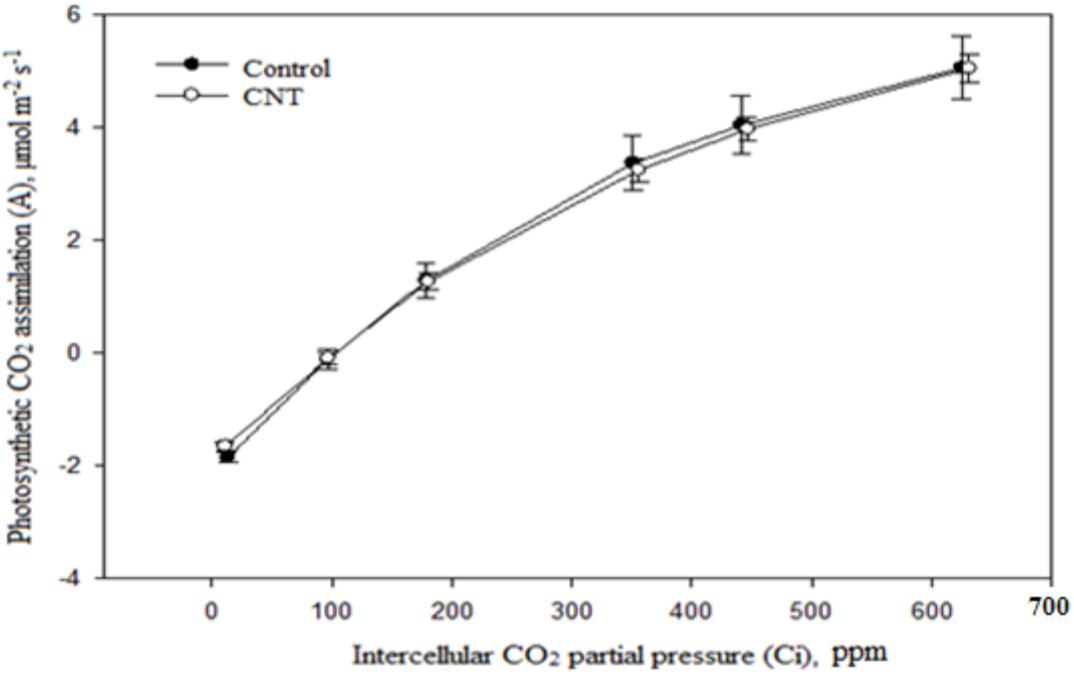


Figure 6. Mean photosynthetic rate as a function of carbon dioxide concentration (Ci) after 21 days of growth for *A. thialiana* plants grown without (Control) (n=12) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=12).

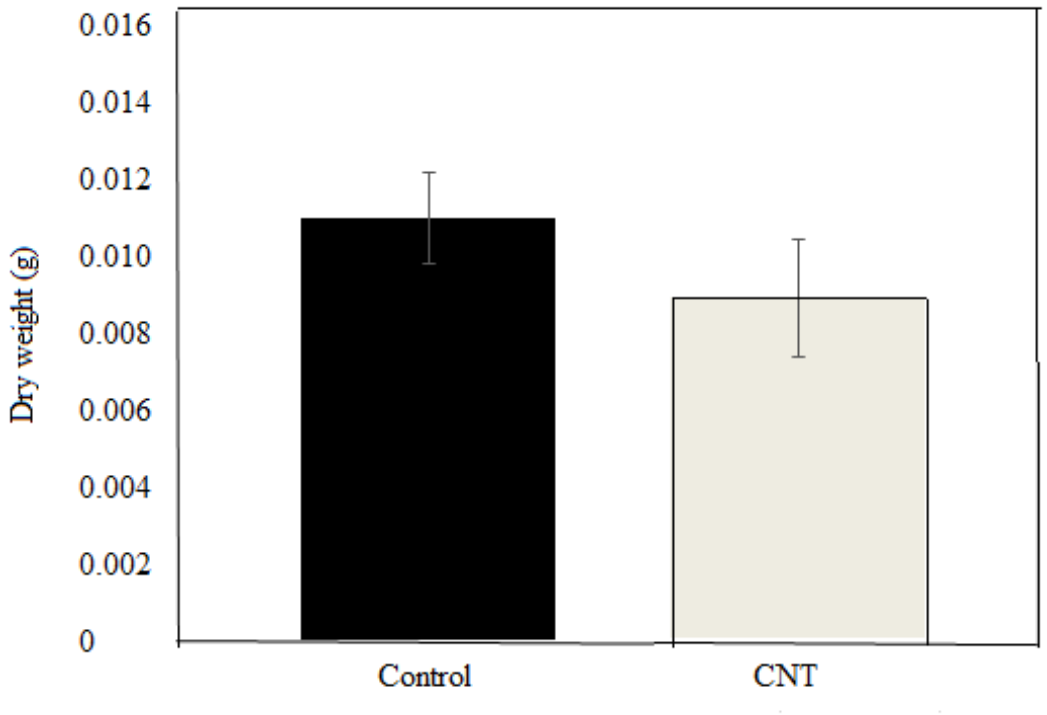


Figure 7. Mean (±SE) dry weight of *A. thaliana* plants after 21 days of growth in Control grown (n=12) and CNT grown plants (24.93 µg/ ml, n=12).

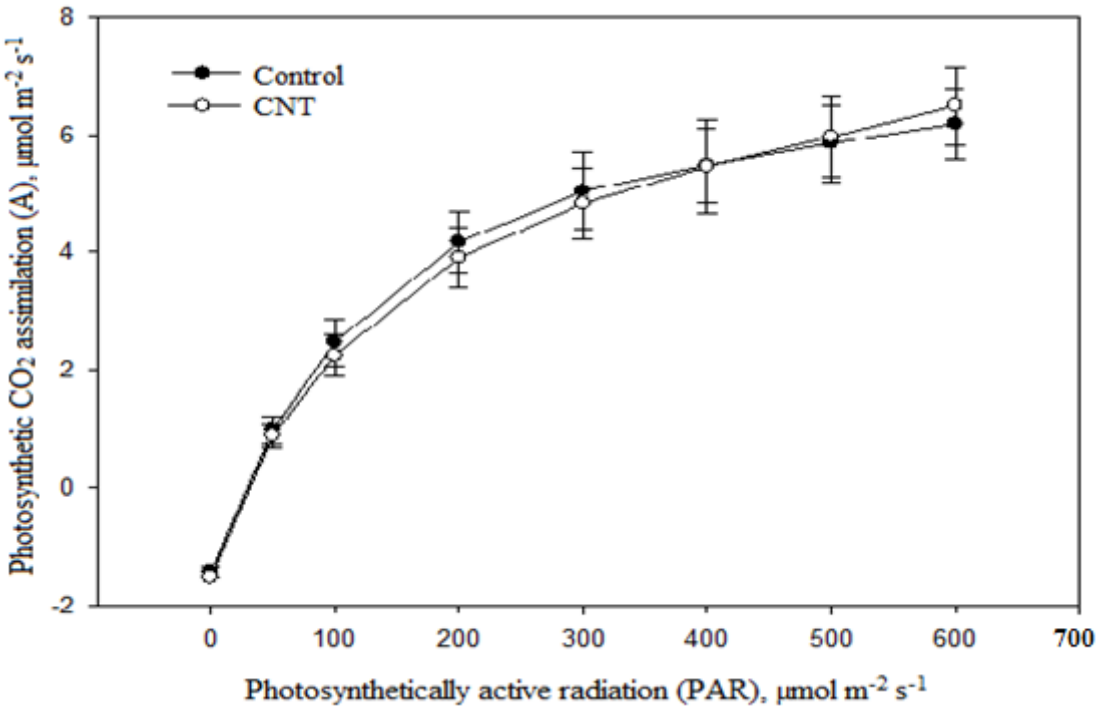


Figure 8. Mean photosynthetic rate as a function of photosynthetically active radiation (PAR) after 21 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 53.55 µg/ml) (n=3).

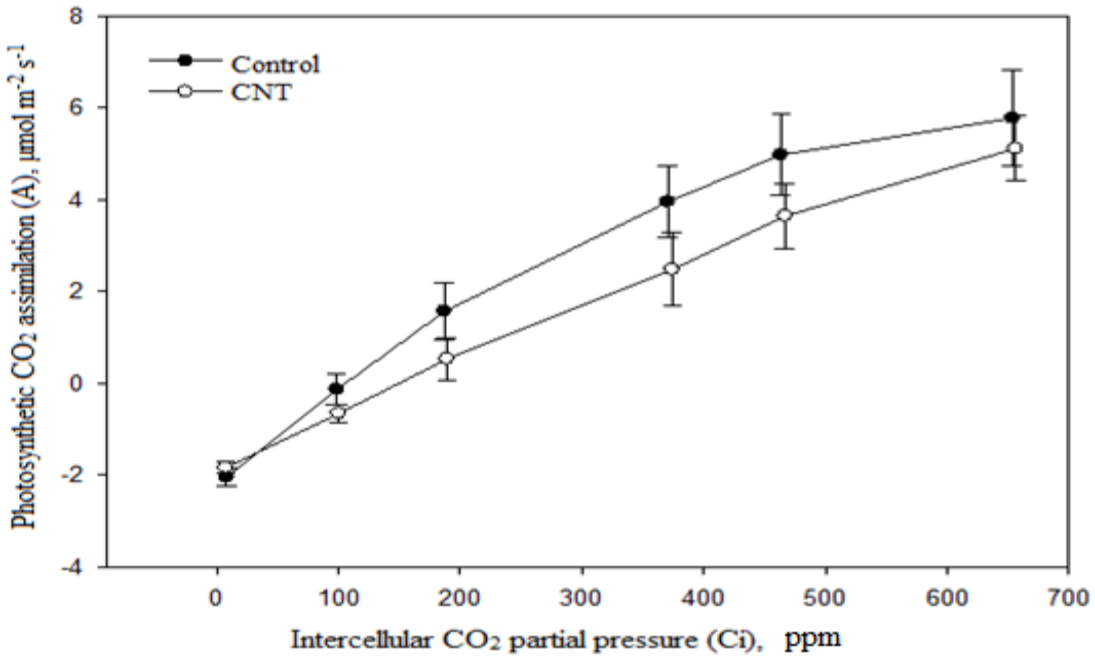


Figure 9. Mean photosynthetic rate as a function of carbon dioxide concentration (Ci) after 21 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 53.55 µg/ml) (n=3).

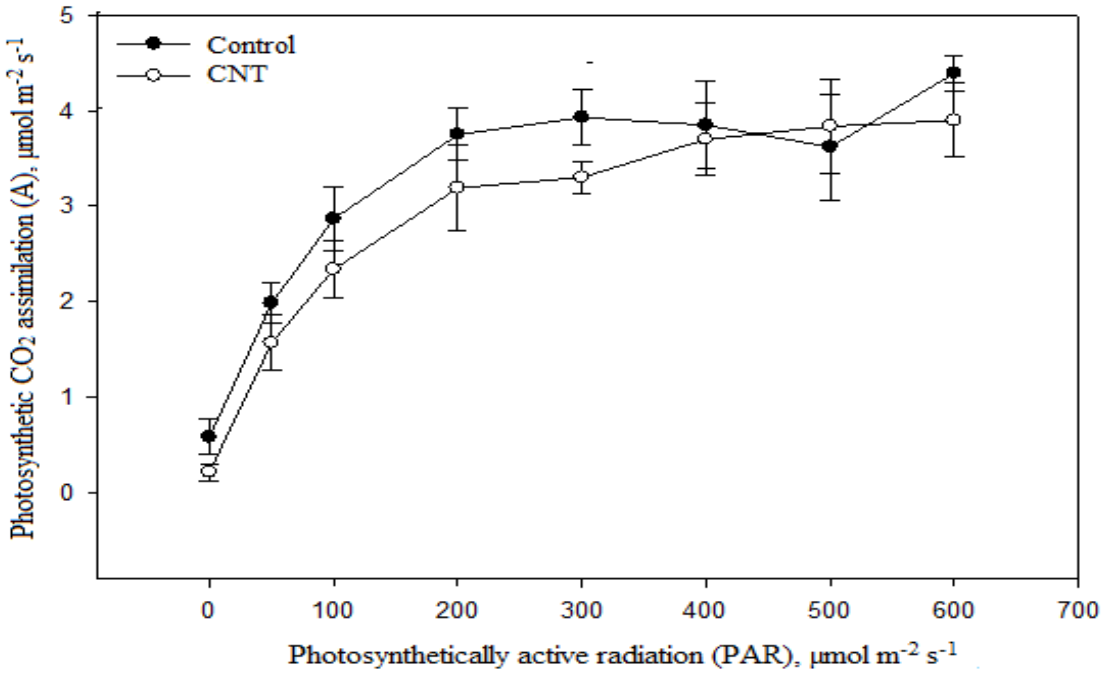


Figure 10. Mean photosynthetic rate as a function of photosynthetically active radiation (PAR) after 7 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3).

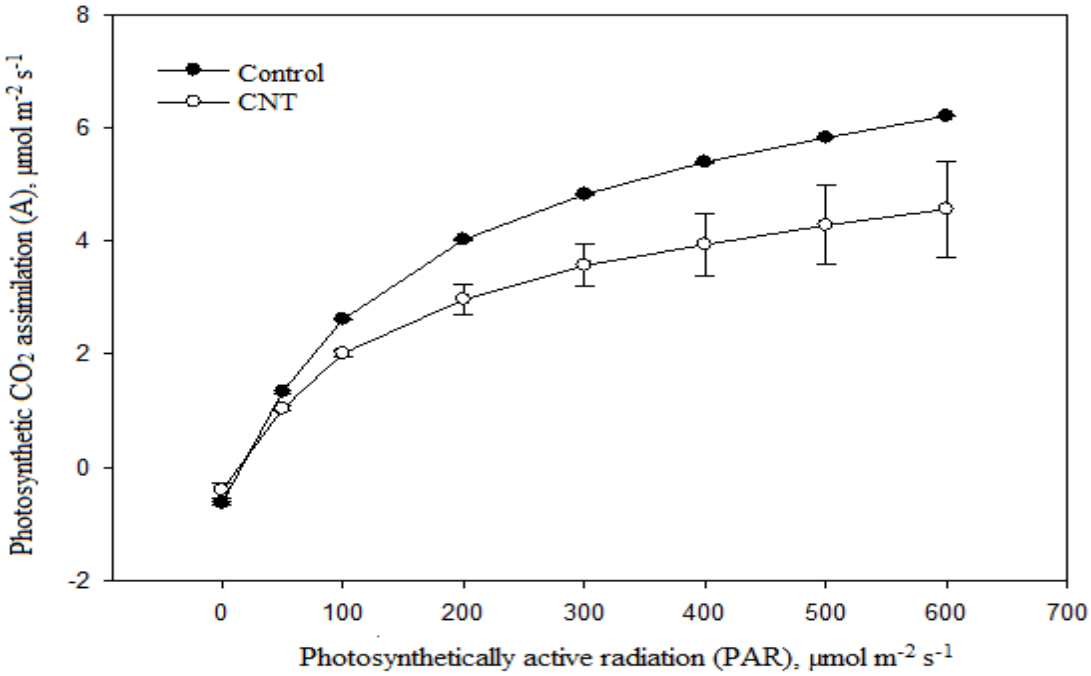


Figure 11. Mean photosynthetic rate as a function of photosynthetically active radiation (PAR) after 14 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3).

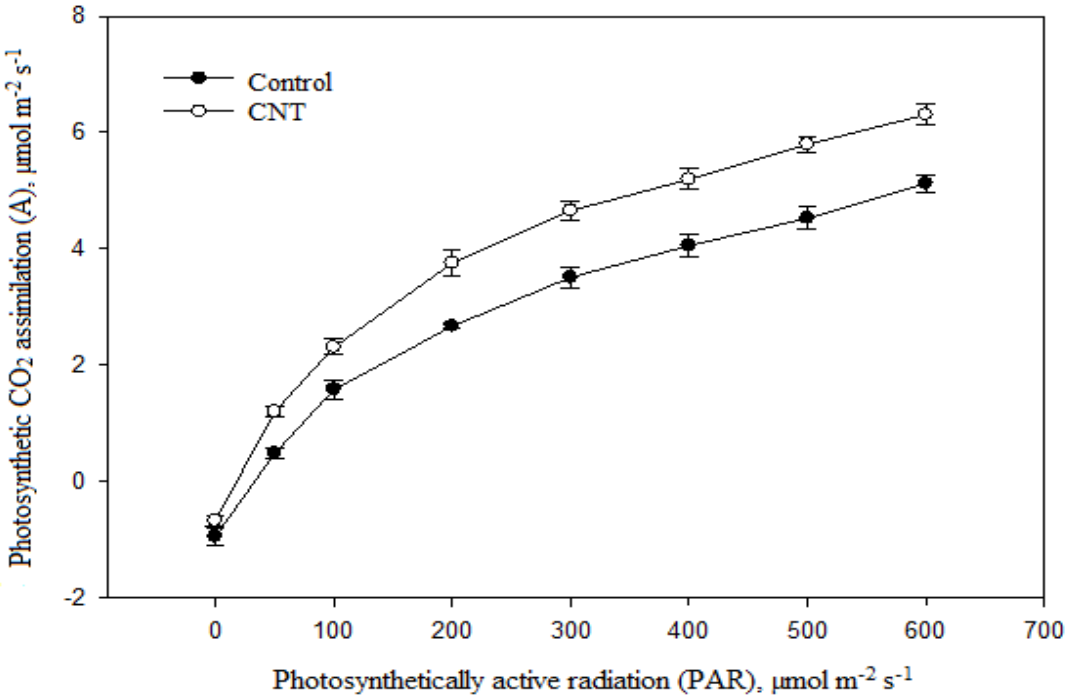


Figure 12. Mean photosynthetic rate as a function of photosynthetically active radiation (PAR) after 21 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3).

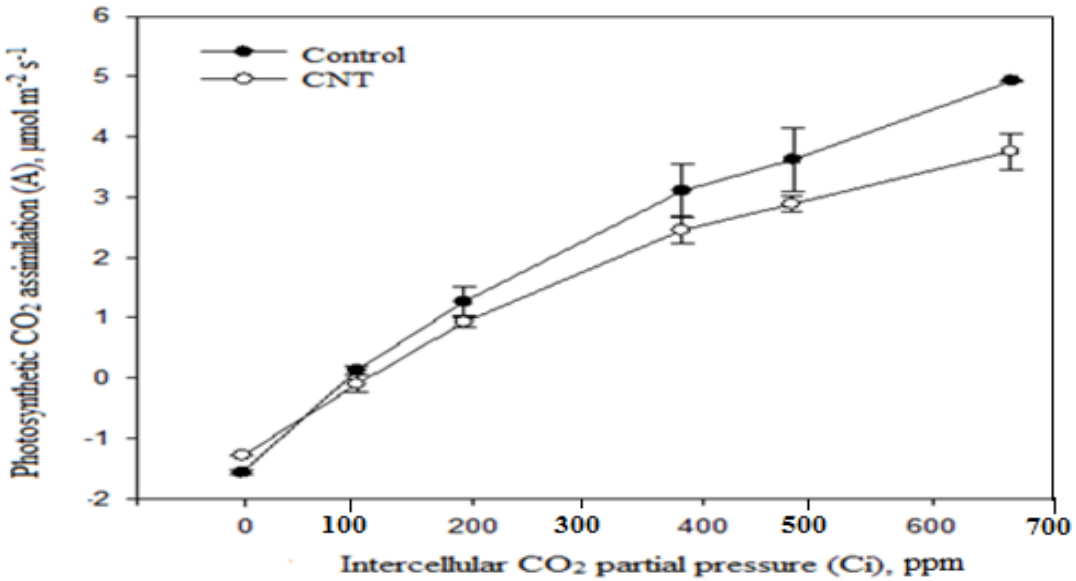


Figure 13. Mean photosynthetic rates as a function of carbon dioxide concentration (Ci) after 7 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3).

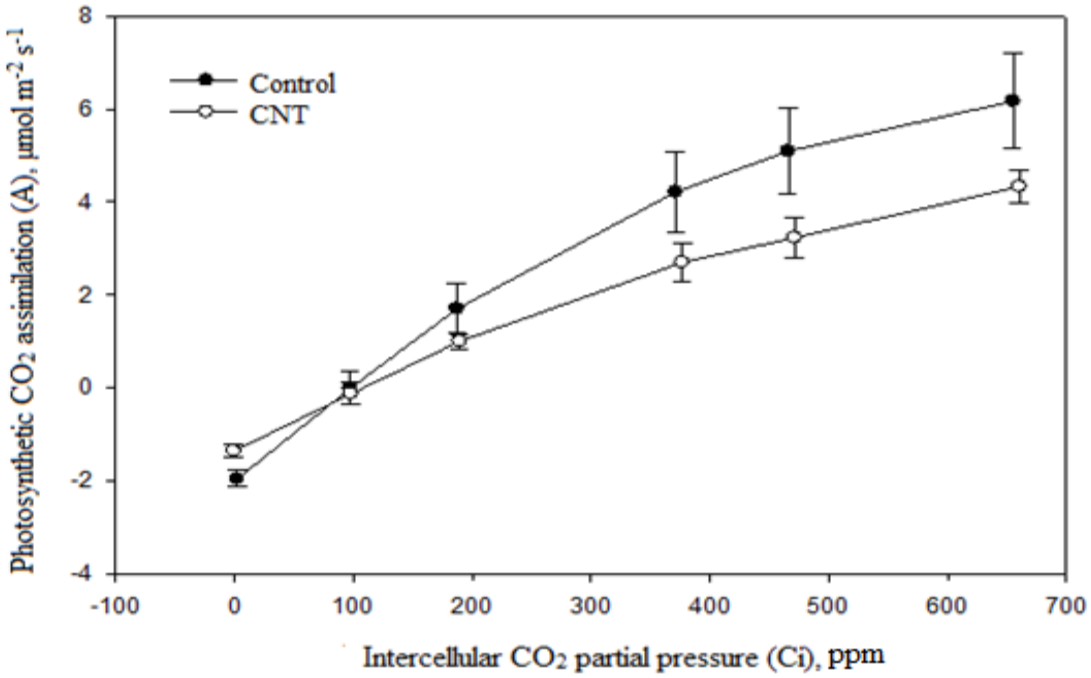


Figure 14. Mean photosynthetic rates as a function of carbon dioxide concentration (Ci) after 14 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3).

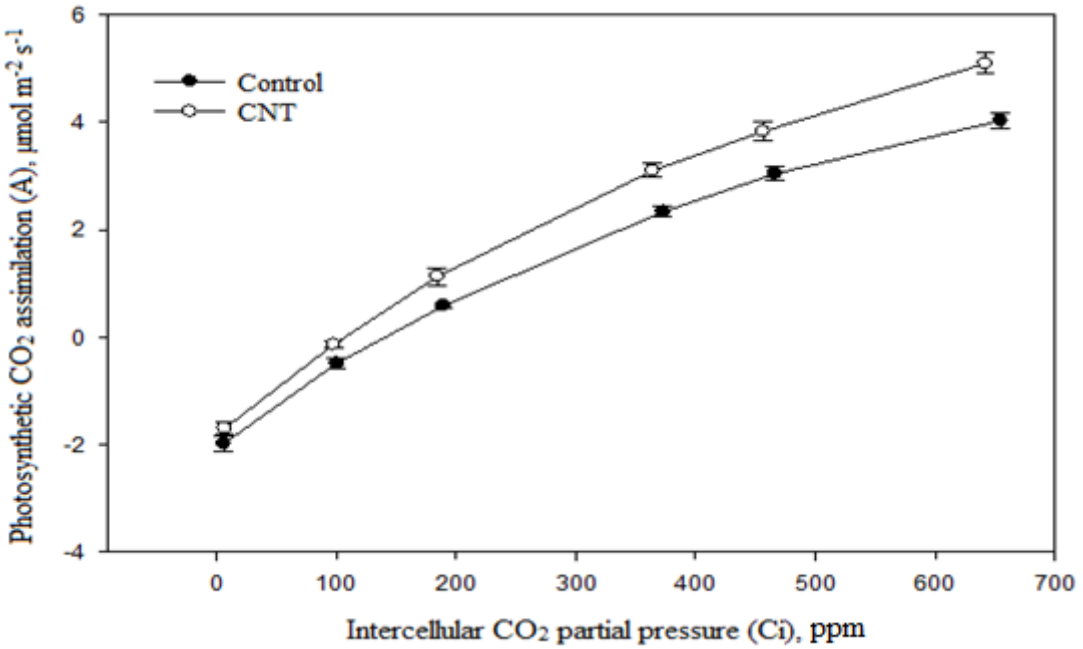


Figure 15. Mean photosynthetic rates as a function of carbon dioxide concentration (Ci) after 21 days of growth for *A. thialiana* plants grown without (Control) (n=3) and with carbon nanotubes (CNT, 24.93 µg/ml) (n=3).

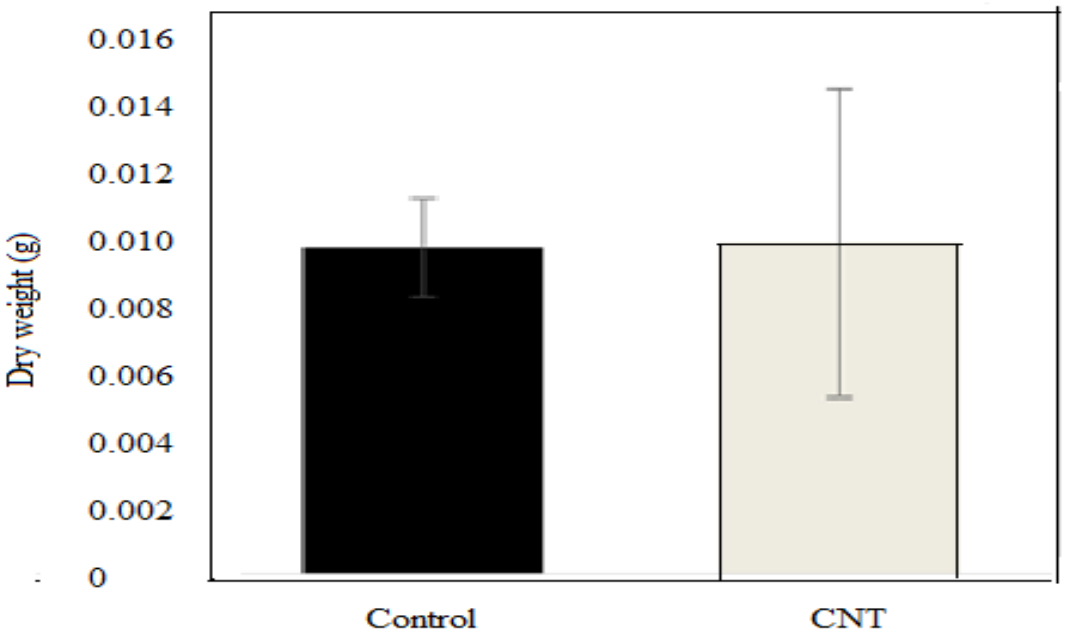


Figure 16. Mean (±SE) dry weight of *A. thaliana* plants after 21 days of growth in Control grown (n=3) and CNT grown plants (24.93 µg/ ml, n=3).