The Effects of Different Solid Content Carbon Nanotubes and Silver Quantum Dots on Potential Toxicity to Plants through Direct Effects on Carbon and Light Reactions of Photosynthesis

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Abstract: - We examined the effects of two types of carbon nanotubes (CNTs) and one type of silver quantum dot (Ag-QD) on potential plant toxicity through effects on plant gas exchange across four different experiments. First, *Arabidopsis thaliana* seeds were directly grown in growth medium containing 75% solid content CNTs at concentrations of 24.93µg/ml and 53.55 µg/ml in petri dishes. Second, *A. thaliana* seeds were directly grown in growth medium containing 95% solid content CNTs at concentrations of 4µg/ml; or third, 18 nm Ag-QDs at a concentration of 4µg/ ml. Fourth, we grew *A. thaliana* in soil for 6 weeks and added the 95% solid content CNT suspension at increasing concentrations of 10, 30, 90, 150, 190, 250 µg/ ml each week. The 75% solid content CNT, and the CNTRENE® C100LM material production waste produced for disposal, had no negative effects on growth or gas exchange. We found that gas exchange in petri dish grown *A. thaliana* was greatly negatively affected by the Ag-QD, and relatively marginally negatively affected by the 95% solid content CNT. There were significant reductions in photosynthesis rates and related light and carbon fixation reactions in both the Ag-QD and 95% solid content CNT *A. thaliana* grown in petri dishes. We found that gas exchange in soil grown *A. thaliana* was unaffected by 95% solid content CNTs, even at very high concentrations. These findings have implications for understanding toxicity of engineered nanoparticles on plant and animal health, public awareness, and environmental remediation.

Key-Words: - Engineered nanoparticles; plants; toxicity; photosynthesis

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1 Introduction

Engineered nanoparticles (ENPs) are increasingly being used in consumer products and electronic devices [1], [2]. Because they are so useful, more and different types are rapidly being developed and manufactured. ENPs are now found in drugs, electronic devices, and many such commonly products as sunscreens, cosmetics, health and fitness, automotive, food, home and garden, clothing, footwear, and eyeglass/lens coatings [2]. Since manufacturing of ENPs has increased [2], organisms being exposed to them in nature is inevitable, but their toxicity to organisms is not well characterized [2]. Our research focused on how three different ENPs, single walled carbon nanotubes produced with 75% or 95% solid content (hereafter, CNTs) and silver quantum dots (AgQDs), affected plant gas exchange using the model plant Arabidopsis thaliana. There is very little research on the effects of ENPs on plant gas exchange; however, negative effects on plant gas exchange could be considered as a toxic side effect and inferences on effects on higher trophic levels can be made for on-going research.

For example, it has been recommended for a decade that scientists consider plants in their studies when they track carbon nanotube (CNTs) movement in the environment [3], although standard methods and model systems are still lacking [2]. Consistent with this recommendation and recent literature [4], there is evidence that CNTs may be toxic to plants at concentrations more than 20 mg/l because of the barriers that CNT causes when they aggregate around the cells where water is delivered. As a result, they may impede the capillary action for water transportation which would result in negative effects on carbon fixation reactions, and ultimately primary and secondary plant quantity and quality.

Studies that have examined the effects of ENPs (engineered nanoparticles) on plant physiological processes provide some evidence that ENPs can have toxic effects. There are few studies, however, on the effects ENPs on plant carbon reactions. For example, a recent review on impacts of

nanoparticles on the physiology of food crops does not provide evidence directly associated with positive, negative or neutral effects via photosynthesis [5]. Our research aims were to identify the effects of CNTs and Ag-QDs on carbon fixation rates by examining light reaction and Calvin cycle processes in A. thaliana. The data presented in this study offer new evidence on the gas exchange responses of A. thaliana when exposed to CNTs and Ag-QDs; and, importantly, a rapid petri dish method for detecting effects was developed. The resulting information can be applied to the estimation of environmental risks related to the exposure of plants to ENPs.

CNTs were selected because they are used in high quantities in nanotechnology products and have been considered prominently in literature to evaluate their effect on plants. On the other hand; in our knowledge, Ag-QD effects on higher plant photosynthesis has not been tested, although these nanoparticles are used in applications related to increasing light absorption efficiency or have been shown to be toxic via photosynthesis in algae [6].

2 Materials and Methods

We examined the effects of ENPs on gas exchange in wild type *Arabidopsis thaliana* Columbia-0 (Col-0) plants purchased from Lehle Seeds company (Waltham, Massachusetts). Three experiments were carried out in petri dishes and one in pots with soil grown in a greenhouse as follows.

2.1 Seed and Medium Preparation for Petri Dishes Experiments

A. thaliana seeds (4 mg) for each petri dish were sterilized by placing them on a cone into a sterilized chamber. In a fume hood, a beaker containing bleach (100 ml) and Hydro chloric acid (HCL) (3 ml) was placed in the sterilized chamber. The sterilized chamber was kept in the fume hood for two hours to allow seeds being sterilized by the elevated chlorine gas from the beaker.

Agar (0.2g) was added into a flask, and distilled water was added for a total volume of ENP up to 5ml with concentrations stated below. In a separate beaker, 3-Morpholinopropane-1-sulfonic acid (MOPS) buffer (0.225g) and MS salts (0.4875g) were dissolved in 135 ml of distilled water. The pH of the solution was adjusted to 7.0 by adding 100 ml of mM KOH and distilled water in a final volume of 180 ml. The solution (20 ml) was added to flasks containing agar. Flasks were autoclaved at 121°C for 20 minutes. Agar flasks were then placed in warm water bath set at 55 °C. For flasks with water only, nanoparticle was added to each flask. Flasks with unsterilized nanoparticles were supplemented with Amphotericin B and carbencillin to avoid bacterial or fungal contamination. The flasks were sonicated, and the agar containing flasks were poured into the flasks that contain the mixed nanoparticle with distilled water and held in the water in the sonicator to make sure that nanoparticles were evenly distributed within the medium. After 2 minutes, the flask composition was poured into plates and left to cool at room temperature.

Seeds were sprinkled evenly onto each petri dish plate. The plates were sealed with parafilm and then placed in a refrigerator. After 3 days, the plates were taken out of the refrigerator and the parafilm was removed from each plate. An open zip-lock bag was used to cover the plates to prevent water loss from the medium or bacteria or fungi growth in the medium. The plates finally were placed in the growth chamber (Conviron Model Adaptis A1000-AR Chamber) at 21°C, 150 μmol m^{-2} s^{-1}, short day cycle (10 hours light and 14 hours dark). Plates were rotated randomly each day within the growth chamber to avoid the difference effect associated with plate position within the chamber.

In the first experiment, we used a CNT suspension that contained $\geq$ 75% CNTs of average length of ~0.4-0.6 μm manufactured by arc discharge method obtained from Brewer Science, Rolla, Missouri. These CNTs 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. We used these CNTs at concentrations of 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 petri dishes on MS (Murashige and Skoog) medium. At three different growth days (14, 22, and 30), physiological and growth measurements were recorded for all sets of a petri dishes. Only significant effects throughout the entire growth period are reported, otherwise we considered there to be no negative or positive effects.

In the second and third experiment single wall carbon nanotubes; purity>95%, diameter 1.5nm, length 1-5 microns, and surface area 1020.48 M2/gram obtained from Nanolab; and, Ag-QDs, diameter 18.5±3.4 nm, surface area 29.0 m^2/g, and Ag mass concentration 0.021 mg/ Ml obtained from 20 nm Pelco® Citrate NanoXact™ Silver were assayed. In this second and third experiment, *A. thaliana* was again grown in petri dishes (three

replicates of controls, CNTs (4μg/ml), and Ag-QDs (4μg/ml)) on MS (Murashige and Skoog) medium. At three different growth days (14, 22, and 30), measurements were recorded for all sets of a petri dishes as stated above.

2.2 Greenhouse Experiment

In the fourth experiment for the 95% content CNT added to soil we first plated *A. thaliana* seeds on 500 μl of 0.08% agar poured into six microcentrifuge tubes. The tubes were covered with tin foil and kept in a refrigerator for two days. To prepare soil for planting, we filled a pot with mixed potting soil that was obtained from Sun-Gro® Horticulture (San Diego, California). We washed the soil with water to remove fungi and other materials that might exist in the soil as described by Lehle Seeds instructions. This step was repeated three times, and then the soil was left soil to dry. After the cleaning process, potting soil was placed Arraysystem pots, and five seedlings were transferred from the gel to the soil. We grew *Arabidopsis* using Arasystem which is designed by Arasystem for *Arabidopsis*. This system included tray, pots, baskets, inverted cons, and con tubes. Some advantages of this system are that it reduces the effects of plant competition and enhances plant growth. Thirty-six pots were used for planting *A. thaliana* (18 replicate pots were prepared for controls and 18 replicate treatments of CNTs). We added five seeds per pot, covered the pots and tray with plastic, and grew *A. thaliana* on benches under photosynthetically active radiation of 150 μmol m^{-2} s^{-1} and under a cycle of 11hours light/13hours dark. After two weeks of germination, we reduced the number of seedlings in each basket to two plants. The plants were fertilized once a week until harvest. The baskets were moved around randomly to minimize the effect of confounding variables that might interact with the treatment.

2.3 Gas Exchange, Carbon and Light Reactions

Gas exchange was measured using a LI-6400XT Portable Photosynthesis System equipped with 6 cm^2 leaf chamber (Li-Cor, Lincoln, NE, USA). Flow rate in chamber was set to 300 μmol s^{-1} and flow speed set to slow. Block temperature was controlled to be as same as leaf temperature. For light curve measurements, data were recorded at three light level (150, 500, 0 μmol m^{-2} s^{-1} respectively) and CO_2 mixture of reference was maintained at 400 μmol CO_2 mol^{-1} air. On the other hand, CO_2 level was set at 400, 700, or 0 μmol CO_2 mol^{-1} air, and light intensity was maintained at 500 μmol m^{-2} s^{-1} for A-Ci curve measurements. Leaf area was set depending on how much of chosen *A. thaliana* sample filled the space of the Licor cuvette.

A curve fitting program, which is available online for free with instructions for use, was used to estimate variables associated with light and Calvin cycle reactions [16]. For light response curves, the users needs to enter T leaf (leaf temperature), P_{atm} (atmospheric pressure), Rd (day respiration), ambient O_2, g_m values, A (photosynthesis rate), Ci (intracellular concentration) and light intensity. The mean values of T leaf, photosynthesis rate, intracellular CO_2 concentration, which were recorded by the Licor for each treatment in each day, were entered in this Excel sheet. Light intensity (0, 150, and 500 μmol m^{-2} s^{-1}) was assigned next to each data point. Rd was assigned as the data points measured at the lowest light intensity (PAR=0 μmol m^{-2} s^{-1}). P_{atm} =101.3kPa at 0 elevation, O_2 =21kPa, and g_m =2 μmol m^{-2} s^{-1} Pa^{-1} were kept constant for all treatments; note that it is better to indicate g_m values that were directly measured or estimated by other methods. This program estimates Jmax (electron transport rate at highest light level), Φ>= 0.5 (initial slop for modeled J), and Θ >= 1(convexity factor).

For A/Ci response curves, the users enters Tleaf, P_{atm}, O_2, A, Ci as they are indicated in the light response curve. In addition to these values, limiting factors are assigned as follows: rubisco=1, RUBP regeneration=2, and TPU=3. After assigning those values, solver in Excel calculates the following: Vcmax, J, TPU, Rd, and g_m (the maximum carboxylation rate of Rubisco, rate of electron transport for the given light intensity, rate of triose phosphate use, day respiration, and mesophyll conductance, respectively).

For the pot experiment, gas exchange was measured at growth photosynthetically active radiation (PAR), which was 150 μmol m^{-2} s^{-1} and at saturating PAR (600 μmol m^{-2} s^{-1}). Flow rate in chamber was set to 300 μmol s^{-1} and flow speed set to slow. Six cm^2 of leaves were placed in the cuvette chamber.

2.4 Statistical Methods

We used ANOVA to examine the effects of CNTs and Ag-QDs on dry weight, photosynthesis, intracellular CO_2, stomatal conductance, and transpiration. For the pot experiment, each of these variables were applied as fixed factors, but growth days was a random factor because measurements were taken randomly on different growth days when plants attained 6 cm^2 of leaf area. For the petri dish experiments, the variables were applied as response,

while treatments (control, CNT, and Ag-QD), and growth days (14, 22, and 30 day) were applied as fixed factors. The interactions between treatments and growth days for each of the variables were also tested. Tukey's test for multiple comparison was run if a *P*-value was significant (α=0.05).

3 Results

3.1 Effects of 75% solid content CNTs in Petri Dish Experiment

We found no significant negative effects of the 75% solid content CNTs at concentrations of 24.93µg/ml and 53.55 µg/ml in petri dishes. The color of the medium and plant growth is illustrated in Figure 1. There were no significant effects on any gas exchange variables in the first CNT experiment, which includes maximum photosynthetic rates, and photosynthetic responses to light or CO_2 (data not shown). Therefore, as would be predicted, we found no significant effects on growth rates and choose to illustrate those data. However, Figure 2 and Figure 3 control curves are statistically the same as found in this first experiment.

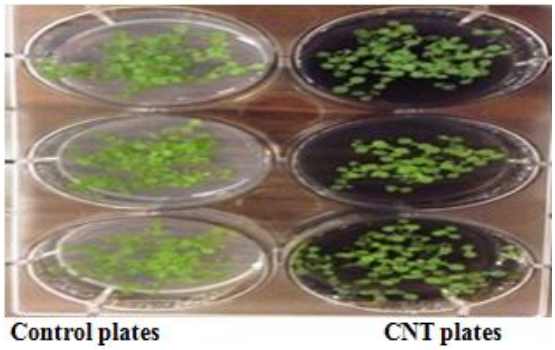

Fig. 1: Photo of A. thaliana plants after 21 days of growth. Petri plates on the left (L) side contain medium without CNTs (Control plates) and on right (R) side contain medium with 95% CNTs (CNT plates).

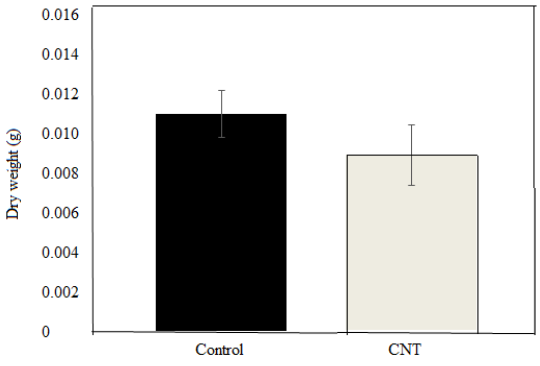

Fig. 2: Mean (±SE) dry weight of A. thaliana plants after 21 days of growth in Control grown (n=12) and CNT grown plants (24.93 µg/ ml, n=12).

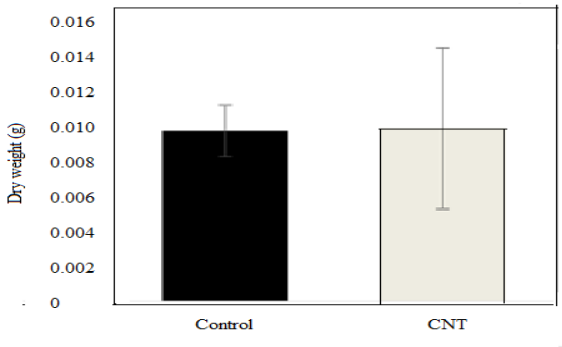

Fig. 3: Mean (±SE) dry weight of *A. thaliana* plants after 21 days of growth in Control grown (n=12) and CNT grown plants (53.55 µg/ ml, n=12).

3.2 Effects of 95% Solid Content CNTs and Ag-QDs in Petri Dish Experiments

Similar to 75% CNTs, 95% CNTs did not have statistically negative effects on plant photosynthetic rates; although there were marginally significant reductions that lead us to examine gas exchange in detail. However, the effects of Ag-QDs on plant photosynthetic rates was very negative. Therefore, we provide a detailed examination of the relative toxicity of these two engineered nanotubes on photosynthetic reactions, even though they are not toxic to the point of killing the plants**.** Carbon assimilation rate for *A. thaliana* treated with Ag-QDs was significantly decreased, with a 56% reduction compared to control grown plants, when measured at PAR 150, and 500 µ mol m^{-2} s^{-1} (Table 1). Carbon assimilation rate for CNT grown *Arabidopsis* was lower by 21% when measured at PAR 150 µ mol m^{-2} s^{-1} and by 23% at PAR 600 µ mol m^{-2} s^{-1} (Table 1). Carbon assimilation rate reduction was identified further by the results that were obtained from intracellular CO_2 concentration (Table 1). Intracellular CO_2 concentration was significantly higher in Ag-QD treated plants compared to controls and CNTs; however, CNTs did not statistically affect intracellular CO_2 concentration.

Table 1. Mean carbon assimilation rate and intracellular CO_2 concentration

Variables	Control	CNT	Ag-QD
$^1A_{amb}$ (µ mol CO_2 m^{-2} s^{-1})	4.54 ± 0.315 a	3.55± 0.224 a	1.96 ± 0.127 b
$^2A_{max}$ (µ mol CO_2 m^{-2} s^{-1})	5.92 ± 0.456 a	4.52 ± 0.266 a	2.59 ± 0.171 b
$^3Ci_{amb}$ (µ mol CO_2 mol^{-1} air)	364.00 ± 2.90 a	364.33 ± 2.42 a	376.08 ± 2.02
$^4Ci_{max}$ (µ mol CO_2 mol^{-1} air)	357.70 ± 3.37 a	358.87 ± 2.52 a	371.25 ± 2.44

$^1A_{amb}$, ambient photosynthesis at light intensity PPFD=150 µmol m^{-2} s^{-1}; ^{2}Amax, maximum photosynthesis at PPFD=500 µmol m^{-2} s^{-1}; $^3Ci_{amb}$, intracellular CO_2 concentration at PPDF= 150 µmol m^{-2} s^{-1}; $^4Ci_{max}$, intracellular CO_2 concentration at PPDF=500 µmol m^{-2} s^{-1}.

The indicated variables for *A. thaliana* grown in petri dishes show significant difference ($P < 0.05$) between treatments (control, CNT at 4µg/ml, and Ag-QD at 4µg/ml). Values are means ± SE (n=83) and the treatments that do not share the same letters are significantly different.

A. thaliana grown in CNTs and controls had about the same compensation points, and the rate of carbon assimilation matches the rate of respiration (Figure 4). Plants grown in Ag-QDs required a slightly higher light level than plants grown in controls and CNTs to reach the compensation point. Quantum efficiency of photosynthesis, which is represented by the curve slope, and the saturation points (rate of A at maximum light intensity) were marginally lower in CNT treated plants, while they were significantly lower than controls in Ag-QDs grown plants (Figure 4).

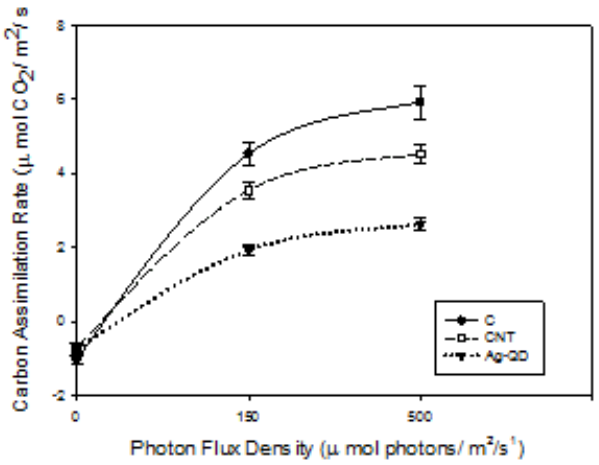

Fig. 4: Mean carbon assimilation responses of *A. thaliana* (n=30) as a function of treatment and photon flux density. Bars are standard errors. C represents the control plants

Plants grown in Ag-QDs had significantly lower Jmax compared to control plants (p-value=0.001); however, Jmax in CNT grown plants was not statistically different from controls. In addition, quantum efficiency and convexity factor of Jmax were not statistically affected by these ENPs (Table 2)

Table 2. Mean (±SE) for estimated parameters from light response curves.

Variables	Control	CNT	Ag-QD
Jmax (µmol m^{-2} s^{-1})*	43 ± 4.38 a	33 ± 2.97 ab	21 ± 2.25 b
Φ*	0.4358 ± 0.0169 a	0.3973 ± 0.0381 a	0.3373 ± 0.0461 a
Θ*	0.5850 ± 0.0641 a	0.4994 ± 0.0590 a	0.3815 ± 0.0606 a

*Jmax, maximum rate of electron transport at saturating light; Φ, initial slope of J; Θ, convexity factor. *A. thaliana* (n=30) grown in petri dishes is significantly different ($P < 0.05$) between treatments (control, CNT at 4 µg/ml, and Ag-QD at 4 µg/ml) in Jmax, but there is no significant difference between treatments in Φ and Θ.

A. thaliana grown in CNTs reached their compensation point at the same concentration of CO_2 as controls, but the compensation point occurred at higher supplemented rate of CO_2 in Ag-QDs treated plants than in the other treatments. Carbon assimilation rate response to partial pressure CO_2 at 400 and 700 µ mol m^{-2} s^{-1} was decreased in Ag-QD treated plants more than CNTs (Figure 5).

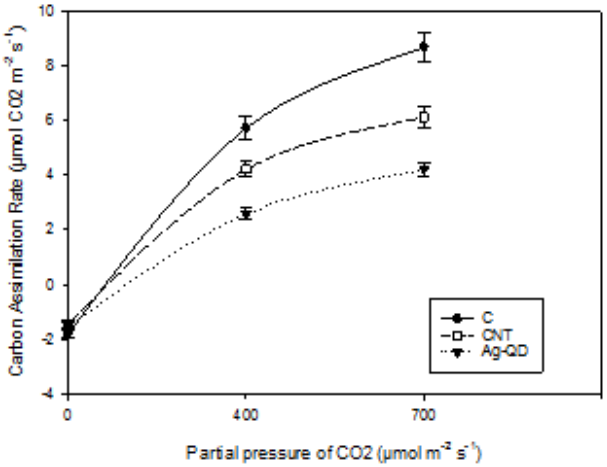

Fig. 5: Carbon assimilation rate response of *A. thaliana* (n=30) in controls (solid slope), CNTs (dashed slope), and Ag-QDs (dashed slope) in all growth days (14,22, and 30) plotted against partial pressure of CO_2 (0, 400, and 700 µ mol m^{-2} s^{-1}). SE are shown at each symbol (circle for controls,

square for CNTs, and triangle for Ag-QDs) that were measured at each light level.

The estimated parameters (J and TPU) from A-Ci-curve were significantly lower than controls in both CNTs and Ag-QDs grown plant, but Vcmax was significantly low in only Ag-QDs grown plants (Table 3).

Table 3. Mean Values for Estimated Parameters from A-Ci-Curve Fitting Program

Variables	Control	CNT	Ag-QD
Vcmax (μmol m^{-2} s^{-1})	56 ± 0.18 a	53 ± 2.79 a	45 ± 2.62 b
J (μmol m^{-2} s^{-1})	64 ± 1.96 a	53 ± 2.79 b	39 ± 2.12 c
TPU (μmol m^{-2} s^{-1})	4.4 ± 0.21 a	3.5 ± 0.19 b	2.8± 0.16 c

3.3 Effects of 95% Solid Content CNTs on Plants Grown in Soil in Greenhouse

The results of gas exchange measurements indicate that *A. thaliana* grown in soil was not statistically negatively affected by CNTs. While carbon assimilation rate at growth (150 μmol m^{-2} s^{-1}) and saturating light (600 μmol m^{-2} s^{-1}) was lower by 15% and 12%, respectively, in CNT grown plants relative to controls, the rates were not statistically significantly different. Similarly, intracellular CO2 concentration at PAR= 150 and 600 μmol m^{-2} s^{-1} was not statistically affected by CNTs.

4 Conclusion

Our results indicate that two different CNTs have no to mild toxicity with respect to photosynthetic gas exchange. We can conclude that crop species in the mustard family would probably not be negatively affected in the quantity or nutrient quality of biomass for consumption, which would be consistent with recent published research on *A. thaliana* [7]. For example, decreases in protein given apparent limited effects on rubisco. Indirect effects on secondary chemicals affecting food quality cannot be ruled out per se because the products of photosynthesis could be diverted beyond the light and carbon reactions. Some important results specific to photosynthetic reactions should, however be considered. Photosynthesis rate was slightly decreased compared to controls by 15% and 12% at ambient and maximum light intensity respectively for *A. thaliana* grown in soil and by 21% and 23 at ambient and maximum light intensity respectively for *A. thaliana* grown in agar. A similar response of *Polyboroides radiatusand* and *Sorghum bicolor* has been reported [8] where plants grown in agar were more susceptible to nanotube toxicity effects than plants grown in soil, which is also similar to the findings for *Sorghum bicolor* [9]. We conclude that the reductions in photosynthetic rates were mainly due to effects on Calvin cycle reactions, but the sample size was not large enough to detect statistically significant reductions.

On the other hand, we found that Ag-QDs had a significantly negative effect on variables that limit photosynthetic assimilation. The negative effect of Ag-QDs may occur inside plant leaves. This is unsurprising since QDs have been found to be absorbed through roots leading to leaf stress [10]. Furthermore, It has also been shown that ZnO reach plant leaves possibly by traveling from root endoderm via apoplastic path way or plasmodesmata in *Lolium perenne* [11], and that may be the case for Ag-QDs. In addition, arginine-rich intracellular delivery peptides were identified as way for QDs to travel inside a plant cell [12]. Therefore, in contrast to CNTs, Ag-QDs may negatively impact both the quantity and quality of crop species, which has been established in some research [8], [11]. For example, Ag-NPs was reported to have a negative effect on absorbing nutrients by blocking intracellular communication or presence of Ag^{+} ions, which were released from Ag-NPs, affecting nutrient carrier proteins function [13]. It is possible that Ag-QDs affect nutrient uptake if they aggregate around plant cells, which is consistent with our data showing significant decrease in the photosynthetic activity.

We found that Ag-QDs reduced carbon assimilation rates by 56% (Table 1). This would potentially be consistent with effects of ZnO NPs [14]. In that study, chlorophyll a and b contents, net rates of photosynthesis, intercellular CO2 concentration, leaf stomatal conductance and transpiration rate were reduced by more than 50% in *A. thaliana* grown in soil containing ZnO NPs (300 mg/L) for 6 weeks. In addition, they reported that genes associated with oxidative stress and toxicity caused the reduction in chlorophyll expression and carbon assimilation. Consistent with this, our data for *A. thaliana* grown in MS medium indicated reduction of carbon assimilation rate by 56%, but intracellular CO_2 concentration is significantly increased in Ag-QDs treated plant which means that CO_2 is not captured efficiently, and this is supported by estimated parameters calculated from A/Ci curves.

Responses of photosynthesis to light can be explained by the estimated parameter of Jmax which can be determined by the equation:

$$J = \frac{(A + R_d)(4C_c + 8G_*)}{(C_c - G_*)}.$$

$$J = \frac{J_{max} + fi - \sqrt{(J_{max} + fi)^2 - 4QJ_{max}fi}}{2Q}$$

Jmax provides information about a theoretical maximum electron transport rate that supports NADP+ reduction [15]. We found that Jmax was reduced by 51% (Table 2); thus, Ag-QDs probably affected electron carrier's occupation and induce inhibition in NADPH production. Electrons that are not delivered to $NADP^+$ go to the Mehler reaction and this causes an increase in reactive oxygen species and PS1 photoinhibition. Beside the effect of Ag-QDs on NADPH, Ag-QDs probably affected ATP generation which is an important reaction for producing H^+ that generate a chemismotic gradient in the grana lamella and permits ATP synthases for interaction between ADP and Pi to make up ATP.

The source of energy (NADPH and ATP), which is regenerated from the light reaction, is the component for running the Calvin cycle. Due to the inhibition of producing NADPH induced by Ag-QDs, the expected response from Calvin cycle is to fix carbon inefficiently. RUBP-regeneration is the limited photosynthesis associated with electron transport rate that used to support NADP+ reduction [16]. Thus, RUBP-regeneration limited photosynthesis is affected by light condition. The reduction of TPU could be related to genes that were down regulated and involved in transporting carbohydrate. A decrease in this gene expression probably affected the use of TPU for exporting sugar. Therefore, TPU declined in the treated plants.

Importantly, there is no literature reporting the effects of engineered nanoparticles on Calvin cycle reactions. We found that, by using A/Ci response curves that Rubisco, RUBP- regeneration, and TPU activity were inhibited under treatment of Ag-QDs. The three limiting photosynthetic factors in *A. thaliana* grown in CNT media were slightly reduced compared with Ag-QDs effect. We also present a relatively novel, easily replicable method for rapid testing of engineered nanoparticles on plant light and carbon reactions, and possibly leaves to feed herbivorous invertebrates [7].

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Conflicts of Interest

The author(s) declare no potential conflicts of interest concerning the research, authorship, or publication of this article.

Contribution of individual authors to the creation of a scientific article (ghostwriting policy)

The author(s) contributed in the present research, at all stages from the formulation of the problem to the final findings and solution.

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