Silicon DiOxide is The Most Abundant Mineral in the Earth's Crust: How Toxic is It?

Ankita Prakash

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Advisor: Baohong Zhang, Ph.D
East Carolina University
Department of Biology

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I hereby declare I am the sole author of this thesis. It is the result of my own work and is not the outcome of work done in collaboration, nor has it been submitted elsewhere as coursework for this or another degree.

Signed: Ankita Prakash Date: 4/ 26/ 2023

Ankita Prakash

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by

Ankita Prakash

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Ankita Prakash

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Approved by:

Dr. Baohong Zhang

ECU Department of Biology – Thomas Harriet College of Arts and Sciences

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Ankita Prakash

Department of Biology, East Carolina University, Greenville N.C. 27858-4353, U.S.A.

ABSTRACT

Silicon DiOxide, commonly known as silica, is the most abundant mineral in the earth's crust, and ubiquitous in commercial use. Although harmless in its native state, its nanoparticle counterpart can be highly toxic. Nanoparticles are materials <100 nm in at least one dimension and highly sought after for commercial purposes. The biomedical, pharmaceutical, cosmetic, and energy industries are just a few amongst many others that conventionally utilize nanoparticles in some form (Biswas and Wu, 2005). Despite their prevalence, little is known about their possible toxic effects on biotic factors. Due to their recent emergence, their bioavailability remains largely unknown. Bacterial and living cells have proven to be able to uptake nanoparticles from their environment, which initiates a bioaccumulation ladder (Biswas and Wu, 2005). This study aims to understand the effects of increasing environmental abundance of SiO_2 on *Camelina sativa* plant growth morphology and cellular oxidative stress. It is hypothesized that SiO_2 significantly hinders *C. sativa* growth by increasing the accumulation of Reactive Oxygen Species (ROS) in cells. Five experimental groups were prepared with increasing SiO_2 concentrations of 0%, 0.01%, 0.05%, 0.1%, and 0.5%. The seeds were sterilized and allowed to germinate for 14 days. Statistical data analysis proved a higher abundance of silica to significantly decrease quantity, weight, and length of shoots and roots. Significant increase in hydrogen peroxide accumulation and peroxide dismutase activity, and significant decreases in root vigor activity indicated increased production of ROS.

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Introduction

Nanoparticles are materials with <100 nm at least one dimension, harboring unique physical and chemical properties due to their high surface area to volume ratio (Khan, 2017). They have been used by humans for millennia, with sources reporting the appearance of nanofibers for creation of the color "Egyptian Blue" over 4500 years ago (Martinez, 2020). Although they have been around for ages, they saw a boom in production and usage with the emergence of a new frontier: nanotechnology. Nanotechnology explores ways to manipulate and maximize the potential of nanoparticle uses possible due to their unique properties. As aforementioned, nanotechnology is nowadays conventionally used in lucrative industries, however, the life path and possible side effects of these molecules remains largely unknown. In recent years, the number of investigations conducted on the release of nanoparticles into the environment has dramatically increased. The potential toxicity of nanoparticles is exacerbated by their ability to travel vastly and aggregate in aquatic, terrestrial, as well as atmospheric habitats (Biswas and Wu, 2005). In the environment, nanoparticles can be readily taken up by bacterial, plant, and animal life.

Nanoparticles go through four stages: product fabrication, product employment, disposal and recycling, and transformation. Although the first three steps can cause unintentional nanoparticle release, recent studies suggest that transformed nanoparticles are almost exclusively the ones that will infiltrate the environment (Martinez, 2020). Transformation mechanisms result in nanoparticles with differing properties compared to their original composition. As an example, silver nanoparticles, often utilized in clothing production, can undergo redox reactions when washed which dramatically increases their toxicity and simultaneously reduces their effectiveness on the product (Martinez, 2020).

Silicon DiOxide

Silicon DiOxide (Silica) is a common food additive, used to improve the taste and durability of packaged foods as well as an anti-caking agent. It is also commonly used in mold-release agents, waxes, concrete, glass materials, and contact lenses. Due to its surface chemistry and stability, silica has become a prime candidate in nanotechnology advances (Prabha, et. al, 2020). Silica nanoparticles easily dissolve in water, and through uptake by microbial life forms, they biomagnify and eventually bioaccumulate in apex predators and humans. A study approximated that humans ingest approximately ~35 mg of silica per day (Guo et. al., 2018). Supporting literature has demonstrated Silica nanoparticles to induce significant formation of ROS, cause cell membrane damage, and modify DNA leading to chain destruction in human skin cells (Fu et. al., 2014). Nanomaterial Silica production surpasses production of all other engineered nanoparticles, in addition to its pervasiveness in natural environmental forms. In 2014, 1400 kilotons of SiO_2 nanoparticles were produced globally (Yang et. al., 2019). These materials pose detrimental risks to human health, including inflammation of the respiratory system, autoimmune diseases, and lung cancer (Yang et. al., 2019).

Increasing uses of SiO_2 have raised questions over their biological effects and potential health hazards. A study by Gong et. al. revealed morphological cell shrinkage, cell wall degradation, increase in ROS levels, DNA damage, and apoptosis in SiO_2 exposed wheat cells. The main method of SiO_2 cytotoxicity is presumed to be through increased oxidative stress and formation of ROS species.

Camelina Sativa

An important biofuel crop *Camelina sativa* was used for this study. *C. sativa* is an oilseed, meaning it has potential to be used as an industrial oil crop (Kagale et. al., 2014). It has a highly conserved genome structure that mimics wheat and cotton genomes, additional economically valuable crops. Agronomic advantages of *C. sativa* include early maturity, low need for water and nutrients, and resistance to environmental pathogens. These characteristics make *C. sativa* fit to be grown in a lab setting. The biofuel crop has a plethora of possible uses, with capabilities of serving as a feedstock, as a source of essential fatty acids, and even the possibility of being used as jet fuel (Kagale et. al, 2014). The plant's genome has been mapped in its entirety, and it is regularly used as a test organism in genetic and CRISPR based research studies. As a result, *C. sativa* makes a suitable organism for the purposes of this study.

Biochemical Factors

Root vigor is an indicator for evaluating healthy root function. High vigor correlates with stronger physiological root function and seed viability (Zhang et al., 2019). The compound triphenyl tetrazolium chloride (TTC) is a commonly known assessor of root vigor. Colorless TTC is reduced to a pinkish red formazan by healthy roots through the plant electron transport chain (Zhang et al., 2019). Assessing root vigor will determine if nanoparticle presence hinders root function, which would in turn affect the plant's ability to acquire nutrients, store food, and provide structural support.

H_2O_2 is a signaling molecule involved in transduction pathways, and a common known indicator of the increased transcription of resistance genes when cells are faced with environmental stressors (Niu and Liao, 2016). 3,3'-diaminobenzidine (DAB) is oxidized by

H_2O_2 to create a dark brown precipitate in the presence of dismutase enzymes (Daudi and O'Brien, 2012). A visual analysis of the precipitate indicates the presence and density of H_2O_2 present.

Superoxide dismutase (SOD) and peroxide dismutase (POD) are antioxidant defense enzymes that combat the buildup of oxidative stress by eliminating ROS in cells. SODs convert a superoxide radical, a form of ROS, into hydrogen peroxide and water. PODs in turn convert hydrogen peroxide into water (Weydert and Cullen, 2009). Combined, these enzymes transform toxic compounds into useful products for cellular usage. An increase in SOD and POD activity would indicate an increased formation of ROS and the inability of the cell to be able to detoxify itself efficiently.

Methods

Experimental design

Five experimental groups were created with increasing nanoparticle SiO_2 concentrations of 0%, 0.01%, 0.05%, 0.1% and 0.5%, respectively. Each experimental group contained five biological replicates to account for variation and increase precision in results. Each replicate (petri dish) contained 25 seeds, planted in a 5x5 matrix. In total, 625 seeds were planted per trial, yielding a large data set to minimize experimental and analytical errors.

Seed preparation and planting

The plant agar solidified medium was prepared using a conventional recipe in one liter diH_2O. Five grams agar was added to solidify the mixture to a suitable consistency. 20 grams of sucrose served as the source of carbohydrates for optimal plant growth. Lastly, 4.3 grams Murashige and Skoog Medium was added to cultivate healthy plant development. Appropriate SiO_2 amounts were added. The pH was checked via a pH Meter and set to 5.8 using HCL and NaOH solutions.

The agar solutions, along with 200 mL diH2O, and a PYREX petri dish used to contain sterilized seeds were autoclaved to avoid contamination. All plating was done under a fume hood. 25 petri dishes were labeled and filled with approximately 50 mL of sterilized agar according to their experimental group. After 10 minutes, the plates were checked to ensure the agar solidity.

Camelina sativa seeds were first sterilized using 70% of ethanol for one minute, then by 5% bleach for three minutes, followed by three washes using sterilized diH_2O. This ensured that any external impurities that could affect plant growth would be removed before

the seeds were planted. All instruments utilized for plating were sterilized using a spirit lamp flame.

Using a set of tweezers, the seeds were planted one at a time in a 5x5 matrix. It is imperative to place the seeds in approximately the same locations on each plate and maintain a similar distance between each seed plated. The plates were sealed using Parafilm and grown under 14/10 light/dark cycle at 26± 2ºC for 14 days.

Morphological Data Analysis

After 14 days of germination, data was collected for each seedling and compared using statistical tests to determine if there was any significant statistical difference in growth morphology between experimental groups. The following parameters were recorded for each seedling: 1) number of leaves, 2) longest leaf length, 3) shoot weight, 4) number of roots, 5) longest root length, 6) root weight, 7) total plant length, and 8) total plant weight.

The raw data was collected in an Excel document. An ANOVA test was conducted for each parameter at a 95% confidence rate to determine if there was a statistically significant difference in the mean values of all experimental groups. A T-test was used to determine significantly differing means in between two selected groups. A T-test was conducted between every group permutation possible.

Biochemical Testing

Root vigor, H_2O_2 accumulation, SOD, and POD activities were measured to quantitatively assess biomolecular impacts of SiO_2 according to a previous method (Zhang et. al, 2021). ANOVA and T-tests were employed to determine statistically significant differences.

A) Root Vigor

A 0.1M PBS buffer was prepared with 0.6% triphenyl tetrazolium chloride (TTC) at pH 7.4. The buffer contained 1 L diH_2O, 11.4 g Na_2HPO_4 * 2 H_2O, 2.61 g NaH_2PO_4 * H_2O, and 6 g TTC. Whole roots were collected and cut into approximately 1 cm pieces. After their weight was recorded, they were placed in a 15 mL tube and mixed thoroughly with 10 mL PBS buffer. The roots were placed in the dark for 24 hours. The next day, the solution was cleared, and the roots were washed three times using diH_2O. 5 mL of 95% ethanol was added to extract triphenyl tetrazolium formazan (TTF) from the roots. The roots were placed in a water bath for 10 minutes at 85 °C. 2 mL of the resulting solution was placed in a spectrophotometer to measure the optical density of the solution and quantify the amount of TTF present.

B) H_2O_2 accumulation

The 3,3'-diaminobenzidine (DAB) staining system was used to assess H_2O_2 accumulation in the leaves. A 1 mg/mL DAB solution was prepared at pH 3.8. Young, fresh leaves were picked from each dish and placed in a petri dish. Only one leaf was placed per plate. 15 mL DAB solution was added to the plates. The plates were allowed to incubate in the dark for 8 hours at 28 °C. Next, the DAB solution was cleared, and the leaves were submerged in 95% ethanol to remove chlorophyll pigmentation. The leaves were left for another 24 hours at room temperature. Images of the leaves were taken to observe dark brown spots indicating H_2O_2 accumulation.

C) SOD Content

To quantify the amount of SOD's present, the enzymes must first be extracted from the leaves. One gram of leaves was weighed and homogenized in a mortar. The mortar was pre-treated at -80 °C. The leaves were ground with 5 mL of 50 mM sodium phosphate

buffer with 0.1 mM ethylenediaminetetraacetic acid (EDTA) at pH 7.8. The homogenate was centrifuged at 6000 rpm for 20 minutes. The supernatant was transferred into a new tube and stored on ice to halt enzyme activity. A 3 mL reaction mixture was created containing 50 mM sodium phosphate buffer at pH 7.8, 0.075 mM nitroblue tetrazolium (NBT), 13 mM methionine, 1.3 μM riboflavin, and 0.1 mM EDTA to measure SOD activity. 1 mL enzyme extracts were added to the solution and the optical density of the resulting solution was measured at 560 nm. The time (in minutes) was recorded for inhibiting 50% of the NBT photochemical reduction.

D) POD Content

The enzyme extraction was carried out as mentioned in previous section C. To measure peroxidase activity, a 3 mL reaction mixture was made using 0.2 M sodium phosphate buffer at pH 6.0, 0.3% guaiacol, and 0.3% hydrogen peroxide. 0.5 mL enzyme extracts were added to the mixture and the optical density was measured at 470 nm. The oxidation of guaiacol was measured over the span of one minute with increase in absorbance.

Results

Impact of NP SiO_2 *on Plant Growth and Development*

Shoots

A) Number of leaves

A significant decrease in the number of leaves per seedling was observed with increasing SiO_2 concentrations (Fig. 1). The p-value using an ANOVA test at a 95% confidence interval was 1.18E-05. Specifically, significiant differences were noted between the following group: 0% - 0.5%, 0.1% - 0.5%, 0.05% - 0.5%, and 0.1% - 0.5%. Values highlighted in green represent significant differences between groups (Table1).

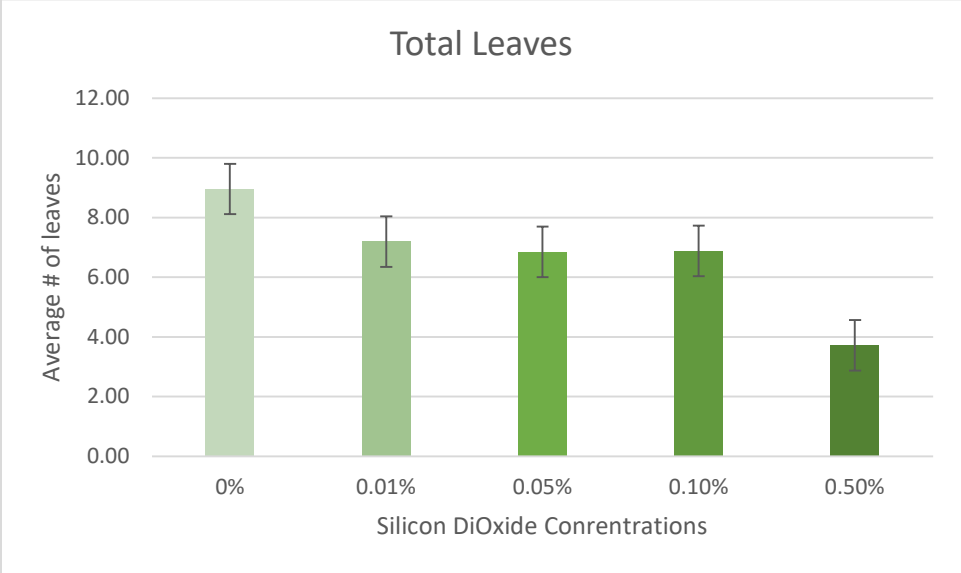

Figure 1: Average total amount of leaves

0-0.01	0-0.05	0-0.1	0-0.5
0.01-0.05	0.01-0.1	0.01-0.5	
0.05-0.1	0.05-0.5		
0.1-0.5			

Table 1: T-test values for average total amount of leaves

B) Shoot length

A significant decrease in the shoot length of seedlings was observed with increasing SiO_2 concentrations (Fig. 2). The p-value using an ANOVA test at a 95% confidence interval was 1.48E-06. Specifically, significant differences were noted between the following group: 0% - 0.5%, 0.1% - 0.5%, 0.05% - 0.5%, and 0.1% - 0.5%. Values highlighted in green represent significant differences between groups (Table 2).

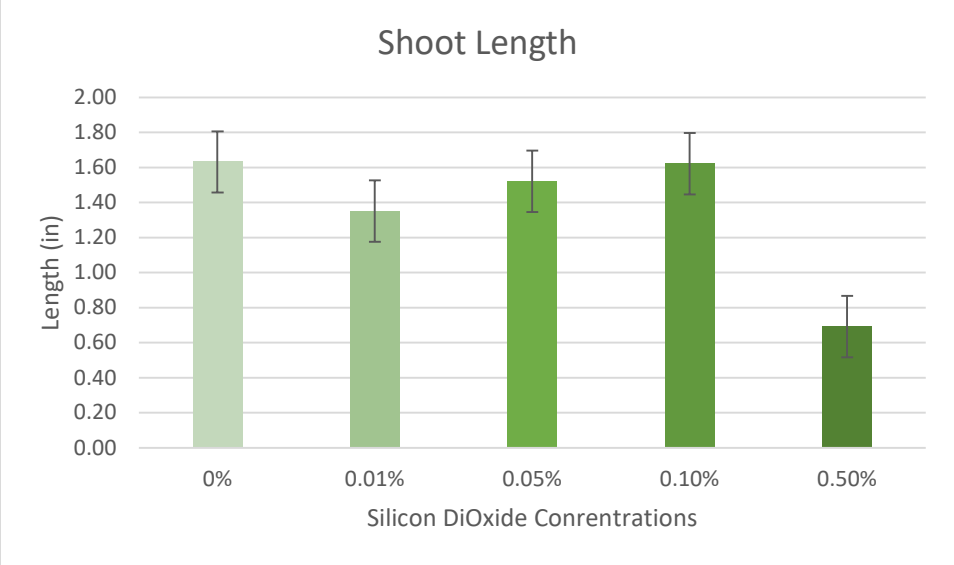

Figure 2: Average shoot length

0-0.01	0-0.05	0-0.1	0-0.5
0.01-0.05	0.01-0.1	0.01-0.5	
0.05-0.1	0.05-0.5		
0.1-0.5			

Table 2: T-test values for average shoot length

C) Shoot weight

A significant decrease in the weight of shoots was observed with increasing SiO_2 concentrations (Fig. 3). The p-value using an ANOVA test at a 95% confidence interval was 5.46E-07. Specifically, significant differences were noted between the following group: 0% - 0.5%, 0.1% - 0.5%, 0.05% - 0.5%, and 0.1% - 0.5%. Values highlighted in green represent significant differences between groups (Table 3).

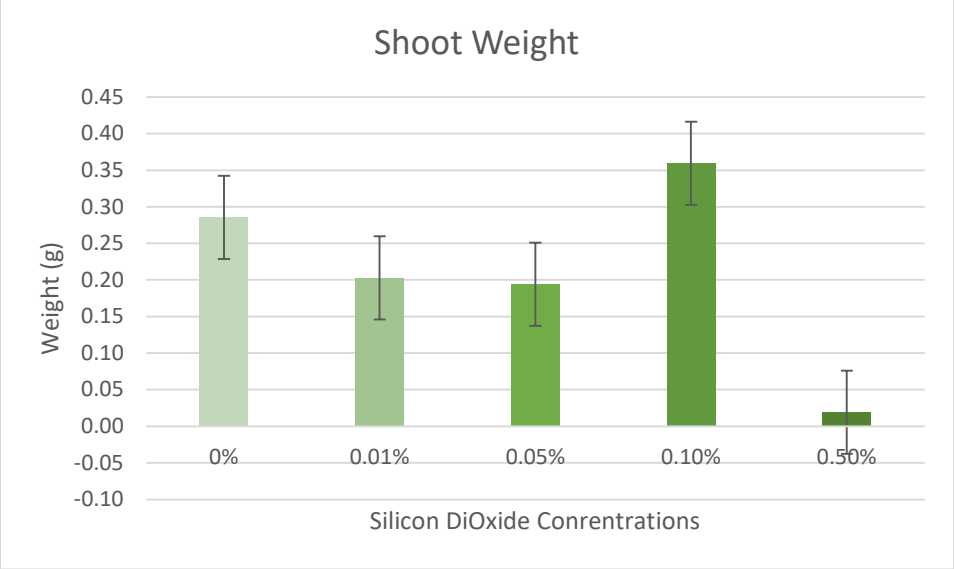

Figure 3: Average shoot weight

0-0.01	0-0.05	0-0.1	0-0.5
0.01-0.05	0.01-0.1	0.01-0.5	
0.05-0.1	0.05-0.5		
0.1-0.5			

Table 3: T-test values for average shoot length

Roots

D) Number of roots

A significant decrease in the number of roots per seedling was observed with increasing SiO_2 concentrations (Fig. 4). The p-value using an ANOVA test at a 95% confidence interval was 0.004. Specifically, significiant differences were noted between the following group: 0% - 0.5%. The value is highlighted in green to represent a significant difference between groups (Table 4).

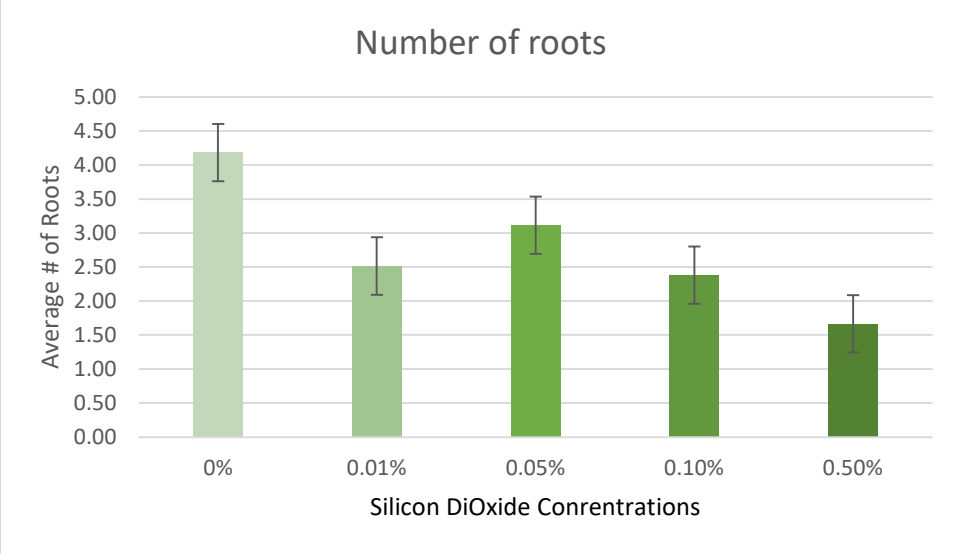

Figure 4: Average total amount of roots

0-0.01	0-0.05	0-0.1	0-0.5
0.01-0.05	0.01-0.1	0.01-0.5	
0.05-0.1	0.05-0.5		
0.1-0.5			

Table 4: T-test values for average toal amount of roots

E) Root length

Root length was not observed to significantly decrease with increasing SiO_2 concentrations (Fig. 5). The p-value using an ANOVA test at a 95% confidence interval was 0.095. No significant differences were noted between groups (Table 5).

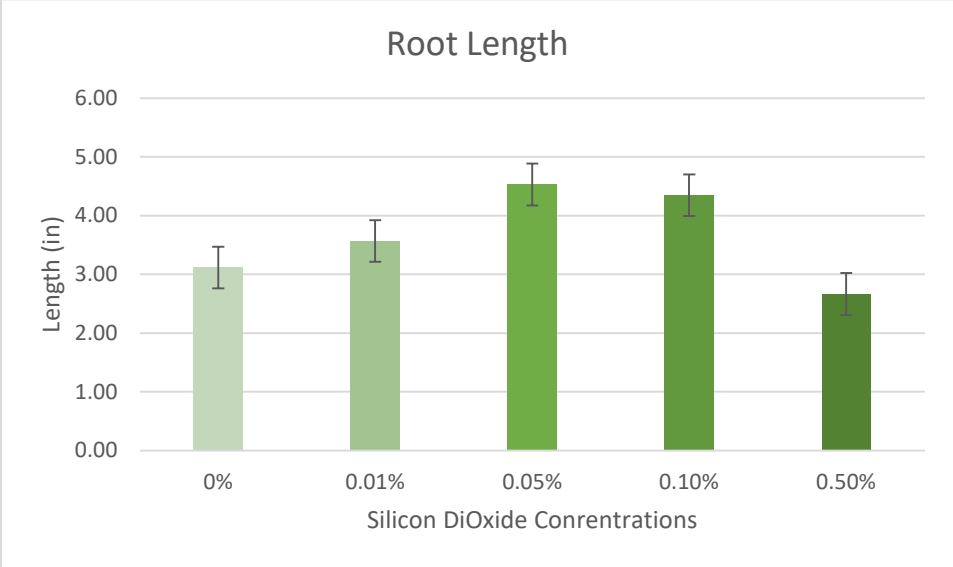

Figure 5: Average root length

0-0.01	0-0.05	0-0.1	0-0.5
0.01-0.05	0.01-0.1	0.01-0.5	
0.05-0.1	0.05-0.5		
0.1-0.5			

Table 5: T-test values for average root length

F) Root weight

Root weight was not observed to significantly decrease with increasing SiO_2 concentrations (Fig. 6). The p-value using an ANOVA test at a 95% confidence interval was 0.010. No significant differences were noted between groups (Table 6).

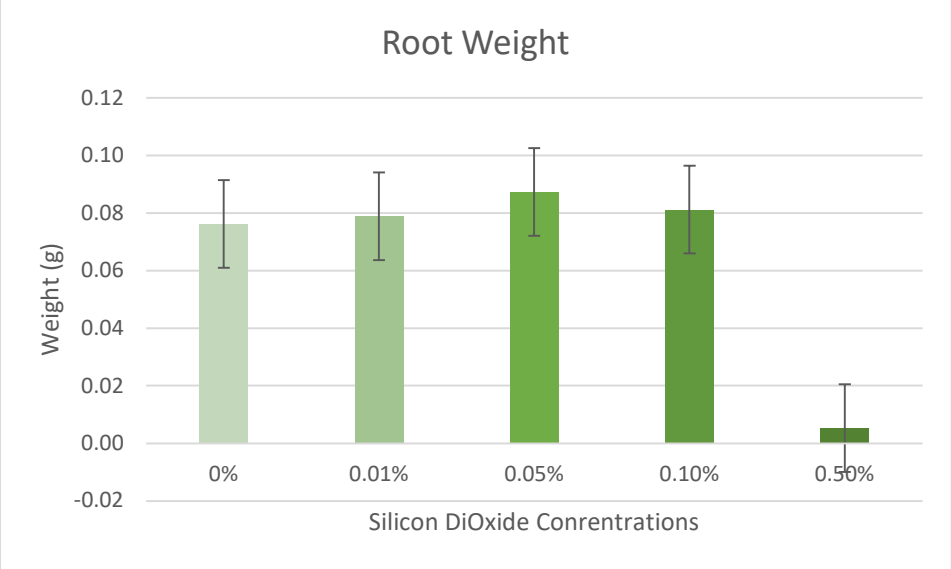

Figure 6: Average root weight

0-0.1	0-0.05	0-0.1	0-0.5
0.01-0.05	0.01-0.1	0.01-0.5	
0.05-0.1	0.05-0.5		
0.1-0.5			

Table 6: T-test values for average root weight

Total Plant

G) Total plant length

A significant decrease in the total plant length was observed with increasing SiO_2 concentrations (Fig. 7). The p-value using an ANOVA test at a 95% confidence interval was 0.001. Specifically, significiant differences were noted between the following group: 0.05% - 0.5% and 0.01% - 0.5%. Values highlighted in green represent significant differences between groups (Table 7).

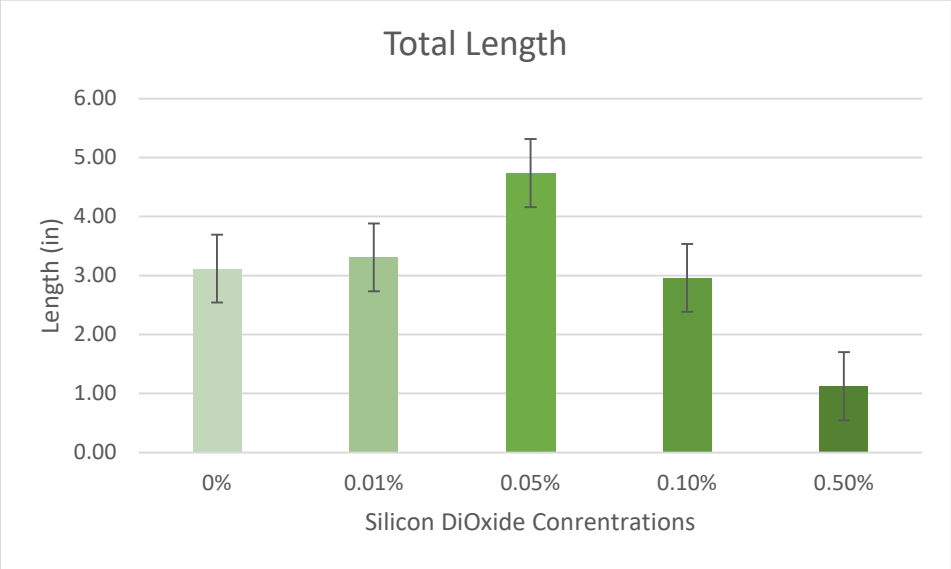

Figure 7: Average total length

0-0.01	0-0.05	0-0.1	0-0.5
0.01-0.05	0.01-0.1	0.01-0.5	
0.05-0.1	0.05-0.5		
0.1-0.5			

Table 7: T-test values for average total length

H) Total plant weight

A significant decrease in the total plant weight was observed with increasing SiO_2 concentrations (Fig. 8). The p-value using an ANOVA test at a 95% confidence interval was 0. 1.64E-05. Specifically, significiant differences were noted between the following group: 0% - 0.5%, 0.1% - 0.5%, 0.05% - 0.5%, and 0.1% - 0.5%. Values highlighted in green represent significant differences between groups (Table 8).

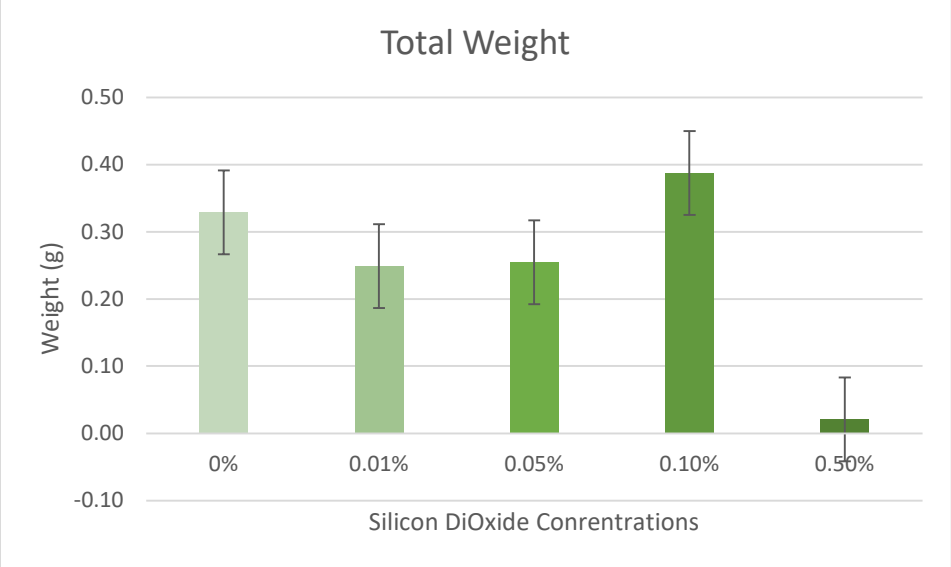

Figure 8: Average total weight

0-0.01	0-0.05	0-0.1	0-0.5
0.01-0.05	0.01-0.1	0.01-0.5	
0.05-0.1	0.05-0.5		
0.1-0.5			

Table 8: T-test values for average total weight

Impact of NP SiO_2 *on Biochemical Activities*

Root Vigor

A significant decrease in Root Vigor was observed with increasing SiO_2 concentrations (Fig. 9). The p-value using an ANOVA test at a 95% confidence interval was 0.019.

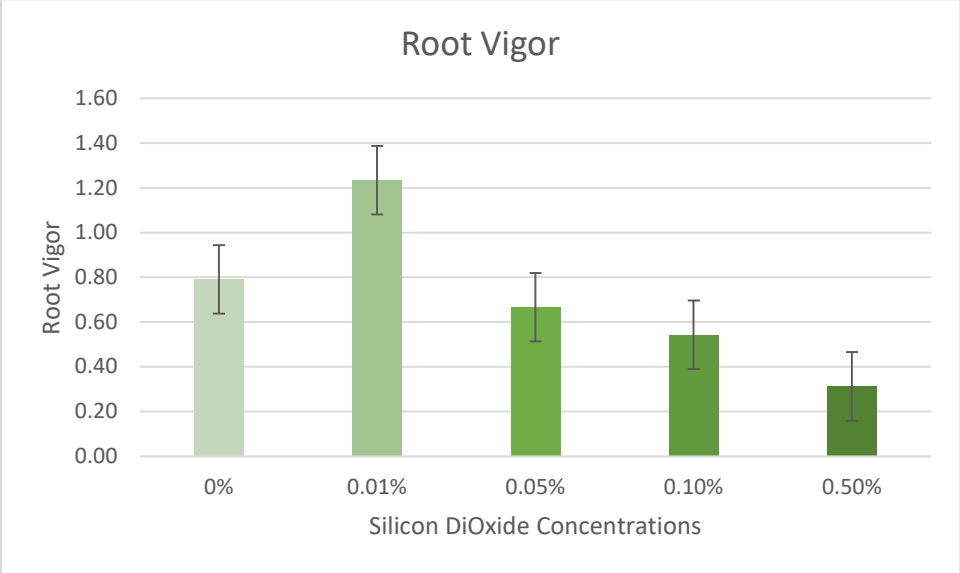

Figure 9: Root Vigor

H_2O_2 Accumulation

Dark brown precipitate on leaves is indicative of hydrogen peroxide accumulation. Control group leaves were not noted to have small brown spots, mostly contained in plant veins. Comparatively, the 0.01% group displayed lower amounts of brown spots with some exceptions of intense brown spots. The 0.05%, 0.1% and 0.5% groups displayed consecutively increasing amounts of brown spots respectively. The 0.5% group visually displayed the highest amount of hydrogen peroxide accumulation.

Experimental Group	Replicate 1	Replicate 2	Replicate 3
Control			
0.01%			

0.05%			
0.1%			
0.5%			

Table 9: H_2O_2 Accumulation in leaves

SOD Activity

A significant increase in SOD activity was not observed. The p-value using an ANOVA test at a 95% confidence interval was 0.740. Although differences between groups were observed, they were not statistically significant. As silica concentrations increased, the amount of time needed to inhibit 50% of the NBT photochemical reaction decreased and the amount of SOD activity increased (Fig. 10). The amount of activity is multiplied by a factor of 1000 so that data pertaining to time in minutes and SOD activity can be displayed on the same scale (Fig. 10).

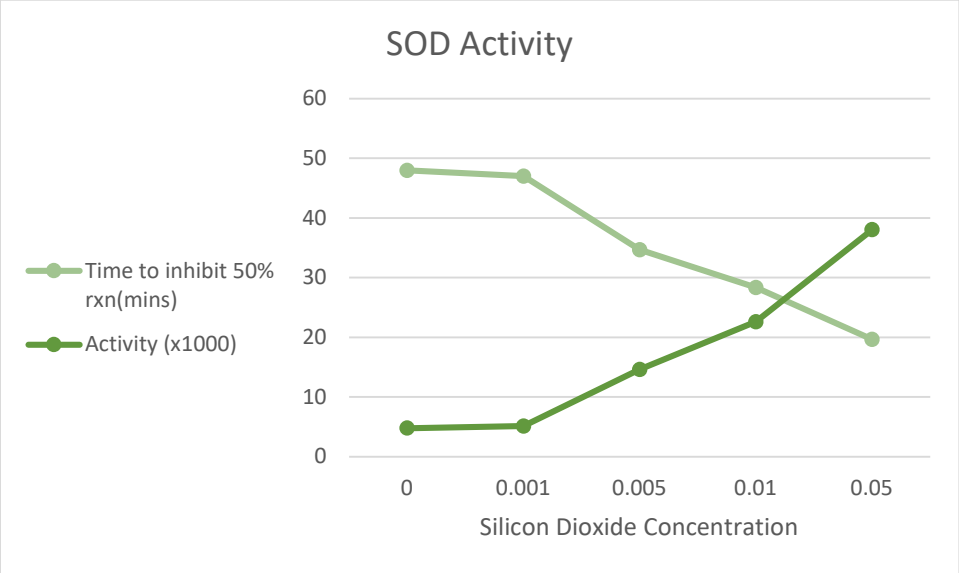

Figure 10: SOD Activity

POD Activity

A significant increase in POD activity was noted with increasing SiO_2 concentrations (Fig. 11). Initial POD activity measurements were taken and then repeated at 60 seconds. A greater difference in POD activity was noted at the end of the time interval with increasing Silica concentrations. The p-value using an ANOVA test at a 95% confidence interval was 1.50E-05. This value is significant.

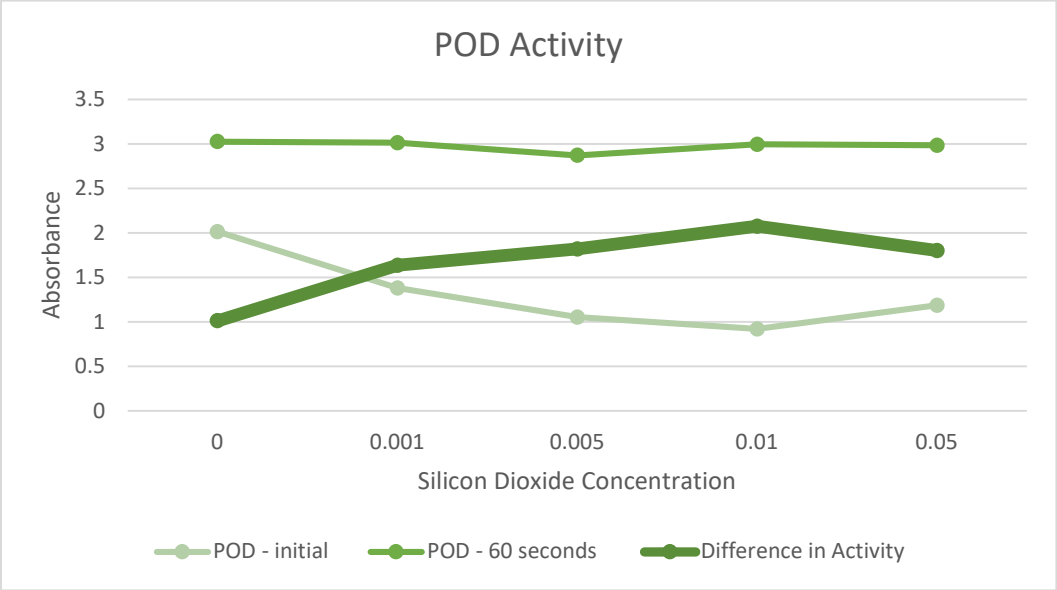

Figure 11: Changes in POD activity over 60 seconds

Discussion

Among the tested concentration range, nanoparticle SiO_2 significantly affected plant growth and development, as evidenced by shoot weight, shoot length, the number of leaves, total plant weight, total plant length, and the number of roots. SiO_2 has greater impacts on shoots compared to roots. This indicates that silica likely induces higher amounts of ROS formation in *C. sativa* leaves compared to roots.

I noticed striking visual differences between experimental groups. The control group plates had abundant leafy growth and the entire physical space inside the plates was taken up at the end of the 14-day germination period. The leaves were soft and able to bend easily to accommodate new growth. Younger leaves were a lighter green and turned darker as they aged (Fig. 12). The roots were highly branched with multiple large roots per seedling. They were thick and full of liquid contents (water and agar). Visual differences between the control and 0.01% group were not apparent. In the 0.05% and 0.1% groups, a decrease in the amount of growth could be visually determined (Fig. 13). There was a drastic visual decline in root presence, and the roots were fuzzy (Fig 14). This could be done by the plants to increase the surface area available for absorption to compensate for the lack of amount of roots present. The leaves were not as soft and thicker than the previous groups. The 0.5% group had the most obvious disparities in plant growth. There was ample empty space in the plates and approximately half of the seeds failed to germinate. The leaves that grew were brittle and broke upon attempts to straighten them out. Root growth was fuzzy, sparse, and noted to grow in a spiral pattern.

Figure 12: Shoot growth: 0%, 0.01%, 0.05%, 0.1% and 0.5% (left to right)

Figure 13: Root growth: 0%, 0.01%, 0.05%, 0.1% and 0.5% (left to right)

Figure 14: Fuzzy roots and brittle leaves in 0.5% group

Although differences in root weight and length were not significant, differences in root vigor were significant. Therefore, even though increasing silica may not affect the plant growth morphology and physical characteristics, it is detrimental towards healthy root function. In turn, this directly impacts the plant's ability to absorb nutrients needed for healthy growth and impacts structural integrity.

Visual changes in H_2O_2 accumulation were apparent. The control group's leaves were notably lighter than the rest of the groups. There were minimal brown spots noted. The 0.01% group had clear brown spot markings. Replicate 2 in the 0.01% group is an excellent example of hydrogen peroxide accumulation in localized spots (Table 9). These spots tend to be located towards the tips of the leaves. 0.05% group leaves were brown throughout as compared to their stem side. They were notably darker than the previous groups. There was not much discernable difference amongst the 0.1% and 0.5% groups. However, compared to all the groups, the 0.1% and 0.5% groups displayed the highest amount of H_2O_2 present. The leaves were entirely covered in a dark brown precipitate. Replicate 1 form the 0.5% group displayed the most intense H_2O_2 accumulation (Table 9).

Although the amount of SOD activity did not significantly change with increasing silica abundance, POD activity did. This indicates that nanoparticle affected cells may not be as efficient at clearing out superoxide radical anions and converting them to hydrogen peroxide. However, the cells are able to efficiently detoxify the amount of hydrogen peroxide that is already present and convert it to water.

Sources of Error

Many factors in this study were out of my control and could alter the results. The *C. sativa* seeds I used were approximately 2 years old. Although they grew as normally expected, there

is a possibility that seed function and viability may have declined over time. There were two plates in my experiment that displayed signs of contamination which were immediately thrown out. However, there exists a possibility that the contamination was transferred between plates before they were removed. Every plate was placed under the same conditions, however, differences in lighting and temperature on a daily basis were not recorded and could alter plant growth rates.

Future Directions

Peers in Dr. Zhang's lab are conducting similar research with varying nanoparticles, namely Aluminum DiOxide, Tin DiOxide, and Magnesium DiOxide. Upon completion, a cross study analysis could be used to determine if the effects of Silicon Dioxide on *C. sativa* are analogous with effects of nanoparticles in general, or if this study shows a unique relationship. Results from a cross study analysis could provide generalizable data about the impacts of greater impacts of nanoparticles on plants in our environments.

Additionally, a CRISPR gene editing study could be done to create a crop line resistant to toxic nanoparticle effects. First, the specific genes affected by silica would have to be identified and then "knocked-out." This would ideally create a resistant crop line and allow sustainable agronomic outcomes. Such advances would prove to be immensely beneficial in the agricultural sector with ever increasing rates of environmental nanoparticle abundance.

Literature Cited

Choudhury, F.K., Rivero, R.M., Blumwald, E. and Mittler, R. (2017), Reactive oxygen species, abiotic stress and stress combination. Plant J, 90: 856-867. https://doi.org/10.1111/tpj.13299

Daudi, A. and O'Brien, J. A. (2012). Detection of Hydrogen Peroxide by DAB Staining in Arabidopsis Leaves. Bio-protocol 2(18): e263. DOI: 10.21769/BioProtoc.263.

Fu PP, Xia Q, Hwang HM, Ray PC, Yu H. Mechanisms of nanotoxicity: generation of reactive oxygen species. J Food Drug Anal. 2014 Mar;22(1):64-75. doi: 10.1016/j.jfda.2014.01.005. Epub 2014 Jan 30. PMID: 24673904; PMCID: PMC9359151.

Gong C, Tao G, Yang L, Liu J, He H, Zhuang Z. The role of reactive oxygen species in silicon dioxide nanoparticle-induced cytotoxicity and DNA damage in HaCaT cells. Mol Biol Rep. 2012 Apr;39(4):4915-25. doi: 10.1007/s11033-011-1287-z. Epub 2011 Dec 18. PMID: 22179747.

Guo Z, Martucci NJ, Liu Y, Yoo E, Tako E, Mahler GJ. Silicon dioxide nanoparticle exposure affects small intestine function in an in vitro model. Nanotoxicology. 2018 Jun;12(5):485-508. doi: 10.1080/17435390.2018.1463407. Epub 2018 Apr 18. PMID: 29668341; PMCID: PMC6157813.

Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities, Arabian Journal of Chemistry, Volume 12, Issue 7, 2019, Pages 908-931, ISSN 1878-5352, https://doi.org/10.1016/j.arabjc.2017.05.011.

Martínez G, Merinero M, Pérez-Aranda M, Pérez-Soriano EM, Ortiz T, Begines B, Alcudia A. Environmental Impact of Nanoparticles' Application as an Emerging Technology:

A Review. Materials (Basel). 2020 Dec 31;14(1):166. doi: 10.3390/ma14010166. Erratum in: Materials (Basel). 2021 Mar 31;14(7): PMID: 33396469; PMCID: PMC7795427.

Biswas P & Wu CY (2005) Nanoparticles and the Environment, Journal of the Air & Waste Management Association, 55:6, 708-746, DOI: 10.1080/10473289.2005.10464656

Prabha, S., Durgalakshmi, D., Rajendran, S. et al. Plant-derived silica nanoparticles and composites for biosensors, bioimaging, drug delivery and supercapacitors: a review. Environ Chem Lett 19, 1667–1691 (2021). https://doi.org/10.1007/s10311-020-01123-5

Radi, R. (2018). Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. PNAS. https://doi.org/10.1073/pnas.1804932115

Weydert CJ, Cullen JJ. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc. 2010 Jan;5(1):51-66. doi: 10.1038/nprot.2009.197. Epub 2009 Dec 17. PMID: 20057381; PMCID: PMC2830880.

Younus H. Therapeutic potentials of superoxide dismutase. Int J Health Sci (Qassim). 2018 May-Jun;12(3):88-93. PMID: 29896077; PMCID: PMC5969776.

Zhang, Z., Zhang, B. (2021). Biochemical and Physiological Toxicity of Nanoparticles in Plant. In: Pan, X., Zhang, B. (eds) Environmental Toxicology and Toxicogenomics. Methods in Molecular Biology, vol 2326. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-1514-0_16

Zhang, G., Yang, C., Liu, R. et al. Effects of three phenolic compounds on mitochondrial function and root vigor of cotton (*Gossypium hirsutum* L.) seedling roots. Acta Physiol Plant 41, 60 (2019). https://doi.org/10.1007/s11738-019-2851-8