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Foliar Application of Titanium Dioxide Accelerates Net Photosynthesis in *Ocimum basilicum*

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ABSTRACT

In recent years, atmospheric concentrations of carbon dioxide (CO2) have risen to alarming levels, exacerbating environmental crises such as ocean acidification and global warming (United Nations, n.d.). Plants, through photosynthesis, slow but do not halt the progression of these issues, absorbing roughly 30% of carbon emissions each year (Friedlingstein et al., 2022). However, it is theoretically possible to enhance plants' CO_2 uptake using some catalyst for photosynthesis (Department of Energy, n.d.). The photocatalyst titanium dioxide (TiO_2) is a promising candidate because of studies demonstrating its ability to boost plant growth and to photocatalyze water splitting and CO_2 reduction, both rate-determining steps in photosynthesis (Li et al., 2022; Tao et al., 2022, Rehman et al. 2022). To investigate the potential of TiO_2 as a photosynthetic catalyst, 0, 0.352 and 0.652 g of TiO_2 were sprinkled atop three pots of *Ocimum basilicum* (basil). Placed in sealable containers with CO_2 meters, the plants' consumption of CO_2 was measured twice every day. Although previous literature has looked into The average consumption for the 0.652 g TiO_2 group was 223.875 ppm, almost 15% times the average for the 0 g TiO_2 control, which came out to be 151.625 ppm. Surprisingly, the 0.326 g TiO_2 group was around 62% of the control, with an average consumption of 94.625 ppm.

Introduction

A chemical reaction can take years or seconds depending on the frequency of collisions between reactants, the energy of those collisions, and the orientation of the reactants during those collisions (Laidler, n.d.). Catalysts can increase the rate of a reaction by affecting one or multiple of these factors (Department of Energy, n.d.). Plants already rely on biological catalysts, called enzymes, to photosynthesize, but with the addition of another catalyst, photosynthesis can be sped up even further (Alberts et al., 2002; Lin et al., 2022). During photosynthesis, plants use light energy to transform inorganic carbon dioxide into organic carbohydrates.

TiO_2 is also practical as a potential catalyst for photosynthesis because of its natural abundance, low cost, nontoxicity, and functionality at standard temperature and pressure (PubChem, n.d.; Bono et al., 2021). One limitation with using TiO_2 is that it is a photocatalyst, meaning that it requires light to catalyze reactions.

Specifically for TiO_2, this light must have a wavelength in the ultraviolet (UV) spectrum shorter than 405 nm (Ghamarpoor et al., 2023). In outdoor growing conditions, this is not a concern as sunlight contains UV, but because this experiment was conducted indoors to better control temperature, weather, and humidity, both a grow light and UV flashlight were employed. When TiO_2 absorbs a photon of UV light, it ejects electrons, which can reduce CO_2 into carbon monoxide, methanol, methane, and other organic compounds (Eidsvåg et al., 2021). The positively charged hole that gets left behind can attract water molecules, oxidizing it into oxygen (Eidsvåg et al., 2021).

However, TiO_2 is also known to photocatalyze the decomposition of organic matter, as the electron release in photocatalysis can contribute to the formation of hyper-reactive oxygen species (Azizi-Labadi, 2019; Certified Germ Control, 2020).

Methods

Ocimum basilicum was chosen for this experiment as it is a common plant for consumption, adding to the discussion of photosynthetic catalysts in agriculture. The actual plants used were obtained from the Home Depot Garden Center where they were grown under controlled conditions.

First, 0.326 and 0.652 g of TiO_2 from Pantai USA Inc. were measured out with measuring spoons and dusted over two of the basil plants using a sieve. The TiO_2 was applied directly to the leaves of the basil, allowing it to enter the plants through their stomata.

Figure 1. Dusting TiO_2 over basil leaves.

A Charity Leaf bamboo plate was put underneath each plant to absorb excess water from leaking. When watering the plants, Kirkland bottled water was used over tap water to avoid fluctuations in mineral concentration and purity. No strict watering schedule was followed, and water was given whenever deemed necessary, with dry bamboo plates, dry-looking soil, or wrinkled leaves as indicators of thirst. However, each group was watered at the same time and with the same quantity. For example, if the 0 g TiO_2 group was watered 30 ml, the 0.362 and 0.652 g TiO_2 groups would also receive 30 mls. To prevent washing off TiO_2 from the leaves of the basil when watering, water was poured directly into the soil of the pots for the roots to soak up. Accurate volumes of water were measured with a 50 ml graduated cylinder with precision down to one tenth of a milliliter.

Figure 2. First watering of basil groups.

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Figure 3. 0.652 g TiO_2 group setup complete.

Afterwards, the plants were moved into their own Vtopmart clear storage container. A Danoplus CO_2 sensor was stuck into the box through a handle and sealed with tape and saran wrap. The container's lid allowed access to the plant for watering and checkups while also being able to create an airtight system to measure CO_2. A GooingTop grow light was secured with its clip to a heavy book and was positioned to shine through the container lid and stimulate photosynthesis even in a closed system. The GooingTop light was a combination of white and red LED light. Because white light contains all wavelengths in the visible spectrum, the total range of wavelengths was 400 to 700 nm, but the red light added emphasis on the 630 to 700 nm wavelengths. A UV Beast flashlight was balanced on top of the lids for the 0.362 g and 0.652 g TiO_2 groups and turned on when measuring. The UV light contained wavelengths between 385 and 395 nm, within the range for TiO_2 photocatalytic excitation.

Figure 4. Box setup

The grow lights were turned on at 9:00 am off at 12:00 am every day to mimic the light-dark cycles plants grown outdoors experience. This also relieves the plants from having to photosynthesize all the time.

An additional Danoplus sensor was placed in the same room as the plants, but not in a container. No measurements were taken from that sensor and it was only used as a reference for ambient CO_2 concentrations.

Every day at 10:00 am, the lids for all three containers were closed, preventing gas exchange between the plants and the room. Measurements from all three sensors were also taken, and UV lights turned on for the TiO_2 groups at this time. One hour later, at 11:00 am, the CO_2 sensor values were recorded again, UV light turned off, and the container lid taken off. Through simple subtraction, the consumption of CO_2 over a period of one hour could be calculated. This procedure was repeated again later in the day, during a 4:30 to 5:30 pm window. One thing to note with this method is that it cannot distinguish between the causes of CO_2 concentration changes. Photosynthesis is not the only biological process in plants that change CO_2 concentrations, although it is the only one that lowers it. Photorespiration and cellular respiration occur at the same time as photosynthesis, and both increase CO_2 concentrations. As such, the perceived rate of photosynthesis in this experiment will be an underestimate as it is competing with opposing processes. This is net photosynthesis, which is the measure of carbon fixation minus the CO_2 production through respiration.

group	date	time	co2_initial	co2_final	initial_final_difference
1	6/24	10:00-11:00	857	651	206
1	6/24	16:30-17:30	896	794	102
1	6/25	10:00-11:00	817	528	289
1	6/25	16:30-17:30	578	514	64
1	6/26	10:00-11:00	623	463	160
1	6/26	16:30-17:30	614	612	2
1	6/27	10:00-11:00	762	492	270
1	6/27	16:30-17:30	641	521	120
1	6/28	10:00-11:00	826	452	374
1	6/28	16:30-17:30	637	444	193
2	6/24	10:00-11:00	846	642	204
2	6/24	16:30-17:30	893	817	76
2	6/25	10:00-11:00	821	642	179
2	6/25	16:30-17:30	595	568	27
2	6/26	10:00-11:00	620	534	86
2	6/26	16:30-17:30	614	611	3
2	6/27	10:00-11:00	744	586	158
2	6/27	16:30-17:30	633	609	24
2	6/28	10:00-11:00	798	636	162
2	6/28	16:30-17:30	631	599	32
3	6/24	10:00-11:00	883	530	353
3	6/24	16:30-17:30	915	630	285
3	6/25	10:00-11:00	846	644	202
3	6/25	16:30-17:30	602	431	171
3	6/26	10:00-11:00	648	465	183
3	6/26	16:30-17:30	629	491	138
3	6/27	10:00-11:00	779	435	344
3	6/27	16:30-17:30	656	541	115
3	6/28	10:00-11:00	836	553	283
3	6/28	16:30-17:30	651	517	134

Figure 5. Data table. CO_2 measurements in ppm, time in military time format. Group 1 corresponds to 0 g TiO_2 control, group 2 to 0.326 g TiO_2, and group 3 to 0.652 g TiO_2.

Results

The data was analyzed with a one-way analysis of variance test (ANOVA) and plotted on a histogram and density plot. The average consumption for the 0.652 g TiO_2 group was 223.875 ppm, almost 15% times the average for the 0 g TiO_2 control, which came out to be 151.625 ppm. Surprisingly, the 0.326 g TiO_2 group was around 62% of the control, with an average consumption of 94.625 ppm. Thus, TiO_2 appeared to have accelerated CO_2 consumption in basil.

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        One-way analysis of means

data:  initial_final_difference and group
F = 4.711, num df = 2, denom df = 27, p-value = 0.01758
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Figure 6. ANOVA performed on CO_2 differences of each group

The p-value of 0.01758 signifies the unlikelihood of the null hypothesis from the collected data. The histogram and density plots also display clear differences between each group, with the 0.652 g TiO_2 group having the greatest consumption of CO_2.

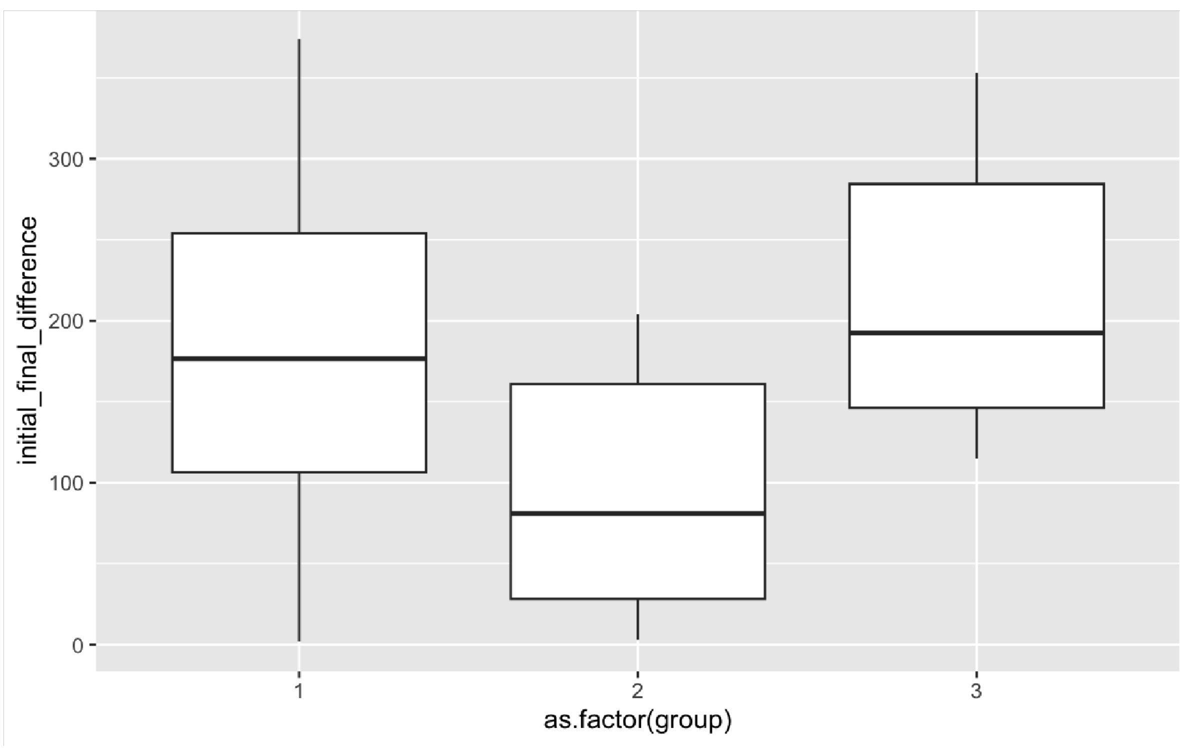
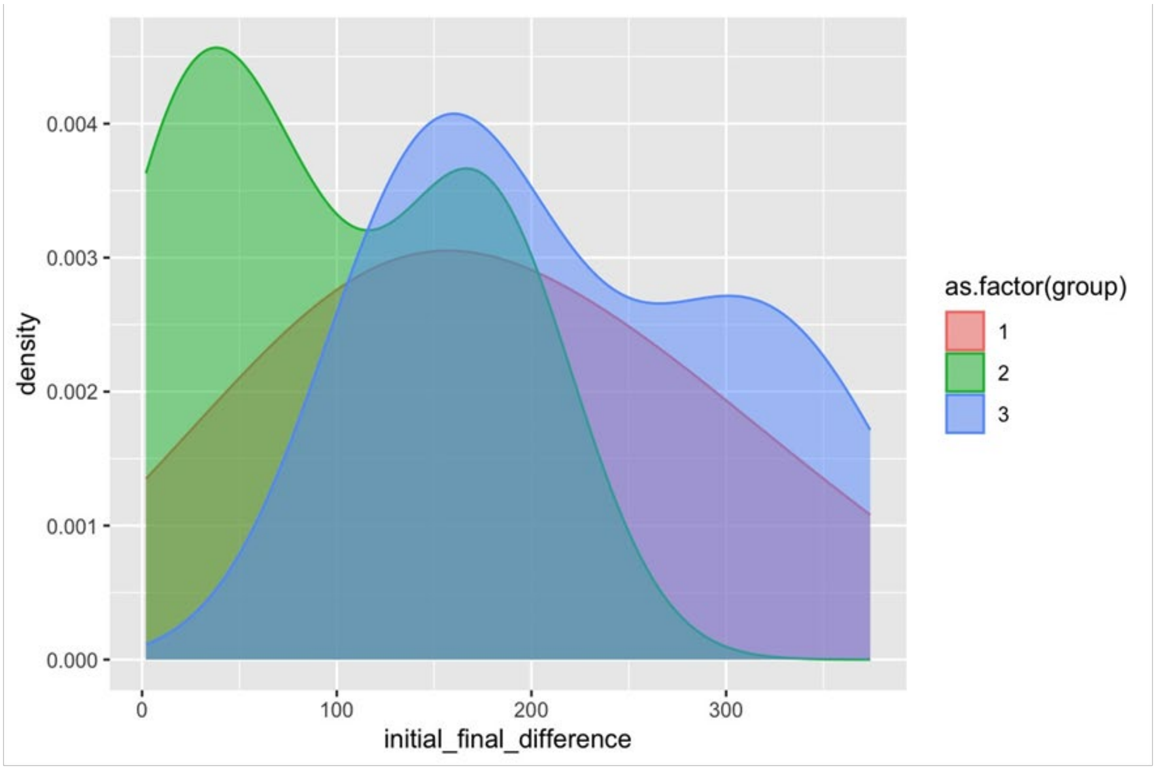


Figure 7. Boxplots generated from ANOVA. initial_final_difference refers to CO_2 consumption. Group 1 corresponds to 0 g TiO_2 control, group 2 to 0.326 g TiO_2, and group 3 to 0.652 g TiO_2.

Slight overlap between the boxplots may raise concerns of the reliability of the data, but the low p-value seems to validate the results of the experiment.



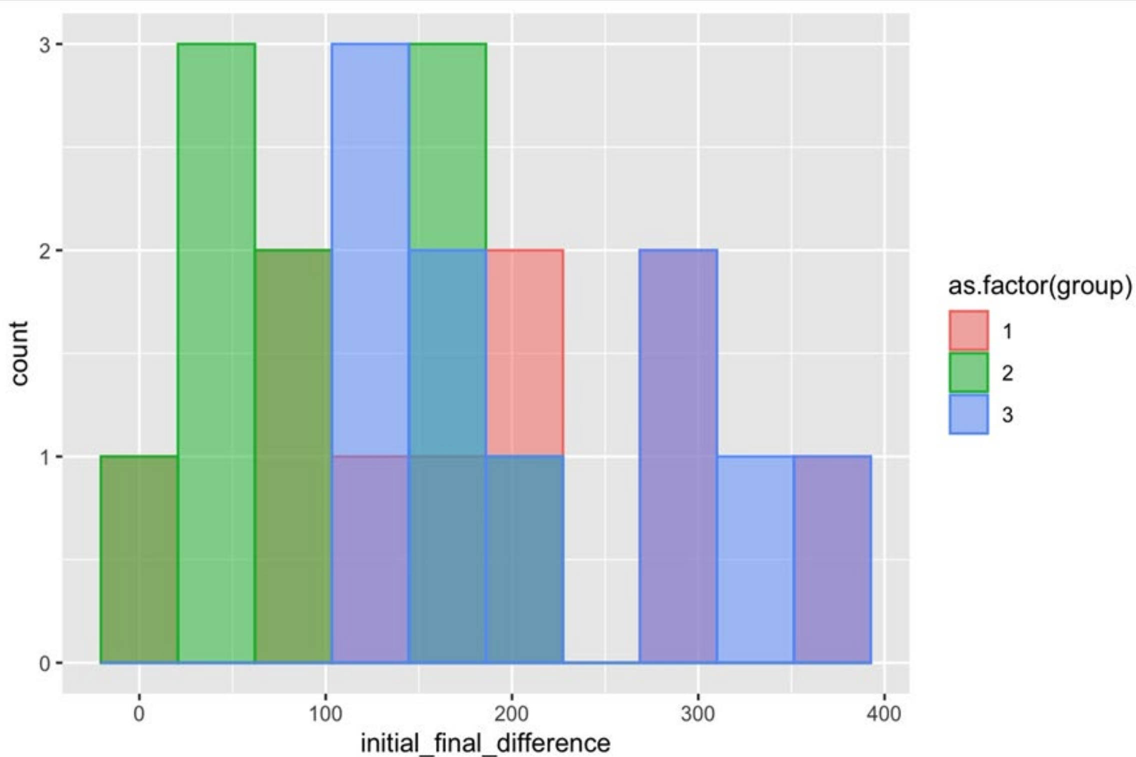


Figure 8. Density plot and histogram of data. initial_final_difference refers to CO_2 consumption. Group 1 corresponds to 0 g TiO_2 control, group 2 to 0.326 g TiO_2, and group 3 to 0.652 g TiO_2.

Discussion

Because only the change in CO_2 concentration was measured in the experiment, it is unclear by what mechanism TiO_2 catalyzed photosynthesis. Photosynthesis is a complex, multi-step reaction, and TiO_2 could have played a role in any one of those steps. It is also possible that TiO_2 indirectly affected photosynthetic rates by catalyzing the decomposition of pathogens inhibiting photosynthesis or taking the plant's resources. TiO_2 is known to have antimicrobial properties due to its ability to form reactive oxygen species (ROS) when excited with UV light (Prakash, 2022). When TiO_2 absorbs a UV photon, it ejects electrons, which can attach to oxygen molecules in the air to become the superoxide radical. The now-positive hole left behind attracts a water molecule, taking an electron from it and transforming it into the hydroxyl radical. These radicals can rapidly decompose bacteria and viruses.

Before the experiment, all of the Danoplus CO_2 sensors were recalibrated outdoors, where the CO_2 concentration would be more or less constant. Despite this, the sensors did diverge slightly, but these small differences were not considered significant in comparison to the roughly 100 ppm decreases observed. Additionally, because just the difference in CO_2 was being measured, errors in calibration would be canceled out.

Recent research suggests that too high doses of TiO_2 can be detrimental to plants (Li et al., 2022). The results of this experiment contradict this idea, as the 0.652 g TiO_2 group had a greater increase in CO_2 consumption than the 0.326 g TiO_2 group, and the 0.326 g TiO_2 group consumed less CO_2 on average than the control. One possible explanation for this is the UV lights. Extended duration to high-energy UV is known to cause cell damage, and this was even physically observed with dark scorch marks on some of the basil leaves in the TiO_2-containing groups. While the administered dosage of TiO_2 in the 0.326 g group may have stimulated photosynthesis, the damage caused by the UV outweighed it and slowed down photosynthesis. Meanwhile, the 0.652 g of TiO_2 in group 3 likely enhanced photosynthesis enough to counteract the damage from the UV. Identifying exactly what the optimal amount of TiO_2 and UV light for *Ocimum basilicum* and other plants would advance the field of photosynthetic catalysis even further.

If TiO_2 as a catalyst for photosynthesis becomes a widely adopted idea, risks for bioaccumulation of TiO_2 will increase. Different sources make different claims about the health effects of TiO_2 on humans, with some saying that it is completely toxic and others saying that it is a carcinogen (Grande et al., 2016; Skocaj et al., 2011). However, major bioaccumulation studies of TiO_2 have yet to be conducted.

Additionally, this research shows that there are still improvements to be made with biological carbon capture and storage (CCS). Because it consumes CO_2 at a very fast rate and it does not divert energy from clean sources, investment in biological CCS is arguably more worthwhile than expensive and energy-intensive artificial CCS. However, one tradeoff with plants is that they consume high amounts of water.

In the future, TiO_2 might be applied akin to a fertilizer in industrial farms so food could be grown simultaneously to benefitting the environment. Overall, TiO_2 is a promising way to accelerate photosynthesis in plants, and may play a role in the development of both artificial and biological CCS technologies.

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