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Assessment of nano-glass particle uptake and accumulation in capsicum annuum and its implications for plant growth and spice quality

Abhishek Kumar Maurya, Sonu Kumari, Manoj Kumar Mishra*

Department of Biotechnology, Faculty of Engineering and Technology (FET),
Rama University, Kanpur (U.P)

Abstract

This research focused on the impacts of silica nanoparticles (NPs), nano glass particles (NG), and *Capsicum annuum* plants with an unnamed chemical to study their response to different treatments. To gauge the physiological reactions of the plants, growth metrics, chlorophyll concentrations, enzyme activity, moisture content, and fresh/dry weights were measured. According to the findings, silica NPs, NG, and the unknown compound all had detrimental impacts on plant development and physiology as their concentrations rose. With increasing treatment concentrations, biomass accumulation, root, and shoot lengths, and chlorophyll levels were all reduced. The results of the therapy were clearly shown in the enzyme activity test, which measured enzyme activity. These results urge for more research into the underlying processes and highlight the possible negative impacts of these chemicals on plant health and production.

Keywords: *Capsicum annuum*, Silica Nanoparticles, Nano Glass Particles, Chlorophyll

***Corresponding Author-** Manoj Kumar Mishra
*Department of Biotechnology, Faculty of Engineering and Technology (FET),
Rama University, Kanpur- (U.P.) Email-manoj**.fet@ramauniversity.ac.in**

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1. INTRODUCTION

Environmental science has concentrated on nanoparticles, nanocomposites, fibers, glasses, polymers, and so forth. Since they are so much smaller, micro and nano pollutants do much more harm than their larger counterparts (Waring et al., 2018). Therefore, the environmental fate of glass particles, especially in plant and soil systems—needs urgent exploration. Smaller pollutants may mix with soil and enter plants. According to the literature, many human cells can take up materials under 300 nm, and cell nuclei can take up things under 70 nm. Micro/nano glass particles plagued the environment till now. Glass production, breakage, and trash grow every day. Shattered glass is hard to recycle, while pure glass is eternally recyclable (Luczak, 2020). Despite its recycling potential, its key downsides are its complex manufacturing and transportation requirements. Glass manufacture needs riverbed and seabed sand. It cannot use desert sand, making coastal communities susceptible to floods and erosion (Gavriletea, 2017). According to a study, glass is created from 50 billion tons of sea/river sand yearly. Glassmaking is laborious.

Metal nanoparticles and plant systems are well-studied. The effects of silica and other silicates on plants (Ismail et al., 2022; Shivaraj, 2022), marine life (Ali, 2021), and higher animals (Mahdipour, 2022) have been investigated. Growth and photosynthesis were boosted in plants treated with Si NPs (Khan et al., 2020). Wheat plants grew larger and had more chlorophyll after being exposed to Si NPs (Hussain et al., 2019). It is widely assumed that nanoparticles can be uptaken and transported by plants, and that nanoparticle uptake and transport in plants are affected by a variety of factors such as particle size, surface charge, concentration, exposure time, and plant species. It has been shown the nano-glass particles' unique properties may aid plant growth (Sá, et al., 2020). Plants absorb nano-glass particles by passive diffusion, ion exchange, and endocytosis. Nano-glass particles interact with plant cells and affect their absorption effectiveness based on their size, shape, surface charge, and chemistry (Rizwan et al., 2017). The plant's circulatory system transports nanoparticles to various organs and tissues. Nano-glass absorption affects plants. It influences plant growth, nutrition absorption, gene expression, and cellular metabolism. Nano-glass particle consumption influences plant responses to diseases, drought, and heavy metals (Tombuloglu et al., 2018). However, It's vital to establish whether these nanoparticles can breach the cell wall and plasma membrane and reach other plant components. This lets researchers evaluate nano-glass particle risks and benefits in agriculture. In current study, *Capsicum annuum* used to research how nano-glass particles affect plant physiology, growth metrics, and crop quality. *C. annuum* is a popular vegetable crop with colorful, varying-sized fruit, known as chili pepper, is belonging to Solanaceae family. Many cuisines use its fruit for cooking spicy sauces, salsas, and flavorings, while sweet versions are used in salads, stir-fries, and stuffing. There has been no prior research reported on the influence of NG on *C. annuum* plant growth performance, and data on the harmfulness of NG to plants is still unknown. *C. annuum* is native to the Americas and extensively farmed for culinary and medicinal purposes (Chen, 2018). *C. annuum* is cultivated tropical, subtropical, and temperate areas. It thrives in well-drained soil and sunshine. It is an annual or short-lived perennial that grows erect to 1–2 meters. Its dark green foliage and white or purple blooms branch. Depending on the kind, *C. annuum* fruit is sweet or spicy. In terms of nutritional value, the fruits are low in calories and high in nutrients. They are high in vitamins A, C, and fiber. Carotenoids and flavonoids found in peppers may be beneficial to health. It has been used as traditional medicine due to the presence of capsaicin biomolecules. It has anti-inflammatory and analgesic properties (Kumar and Pareek, 2022; Sher et al., 2021). Investigating the effects of external elements such as nanoparticles like nano-glass particles on *C. annuum* may reveal their potential effects on plant physiology, growth metrics, and product quality.

The current paper is the first to present the effect of NG on plant systems, and it will contribute to a new understanding of the potential health risks and environmental influences of NG on vegetable. The authors chose *C. annuum* as an experimental crop to (1) investigate the effect of NG on seed germination and root/shoot length; (2) investigate the effect of NG on seedlings, particularly at aerial plant parts; and (3) investigate the translocation of NG from roots to aerial plant parts using FESEM and EDX) localization of NG using fluorescence microscopy.

2. MATERIALS AND METHODS

2.1.Plant material and glass collection

C. annuum L. seeds were purchased from the Indian Agriculture Institute in New Delhi, India, and were certified to be healthy. Healthy seeds were treated with five different concentrations of silica nanoparticles (0, 10, and 20) at 24 ± 2℃ for 24 h with steady intermittent stirring. The seeds were first presoaked in distilled water at 24 ± 2℃ for 7 hours. To serve as a control, one batch of seeds was steeped in distilled water. The treated sets of seeds were rinsed with tap water to eliminate any remaining heavy metal that had adhered to the seed coat.

To prevent roots from interfering with the growth of plants, each set of seeds was planted in a field using a (CRBD design) with a spacing of 12 cm. The chemicals used in this study were of the analytical grade. All of the experiment's water was Millipore ultrapure water. Materials for the experiment, including windshield waste glass, were gathered from local accident scenes and roadways in Kanpur.

2.2.NG Synthesis and Characterization

The auto glass was collected from Kanpur roads and accident sites. These glasses were washed and dried using a mortar and pestle before being used to make additional micro- and nano-sized glass. Furthermore, a ball mill was used to further decrease the size of these glass micro-particles to nano-particles.

Ball milling micro-sized glass particles for 4-12 hours in a conventional planetary ball mill (DG Power and Industrial Solutions Limited, Model: VSS23 2 p5 CEB) (Banasthali) using zirconia balls (50 balls, 12 mm). There was a 50:1 ratio between the ball's mass and that of the tiny glass particles, and the ball spun at a rate of 300 revolutions per minute. The resulting nano-sized glass particles were further investigated for their size, structure, and purity. The surface structure of the manufactured NGs was studied using field emission scanning electron microscopy (FESEM) and elemental analysis (EDX).

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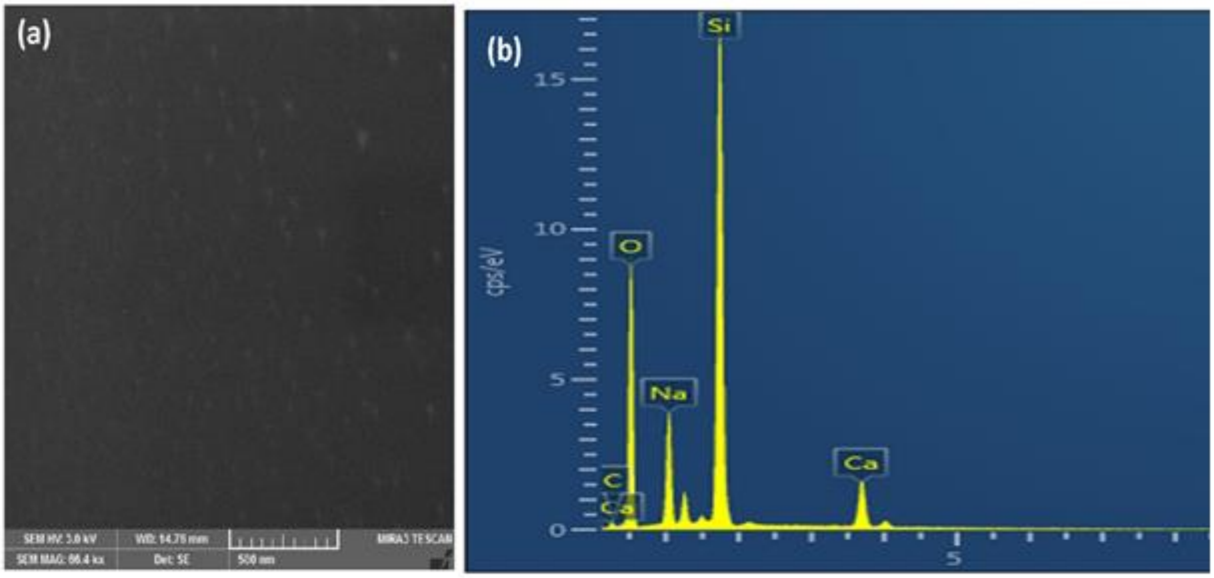

Figure 1: (a) FESEM and (b) EDX of NG

2.3.Plant seeds germination

Selected *C. annuum* seedlings had their surfaces cleaned with a NaOCl reaction. To remove any leftover NaOCl residue, Millipore water was used to wash the seeds thrice. Germination study was performed by Lin and Xing (2007) method. After that, seeds were immersed for about two hours in NG solutions of 0, 10, and 20 mg/kg. NG stock suspension was used to create the treatment suspensions, they were then subjected to ultrasonic vibration (100 W, 25 kHz) to sonicated. So, 25 seeds were planted in a Petri dish with a quality filter paper sheet serving as the test medium. A controlled environment growing chamber was used to nurture the seeds for five days before germination was seen. All seeds' germination percentages were calculated.

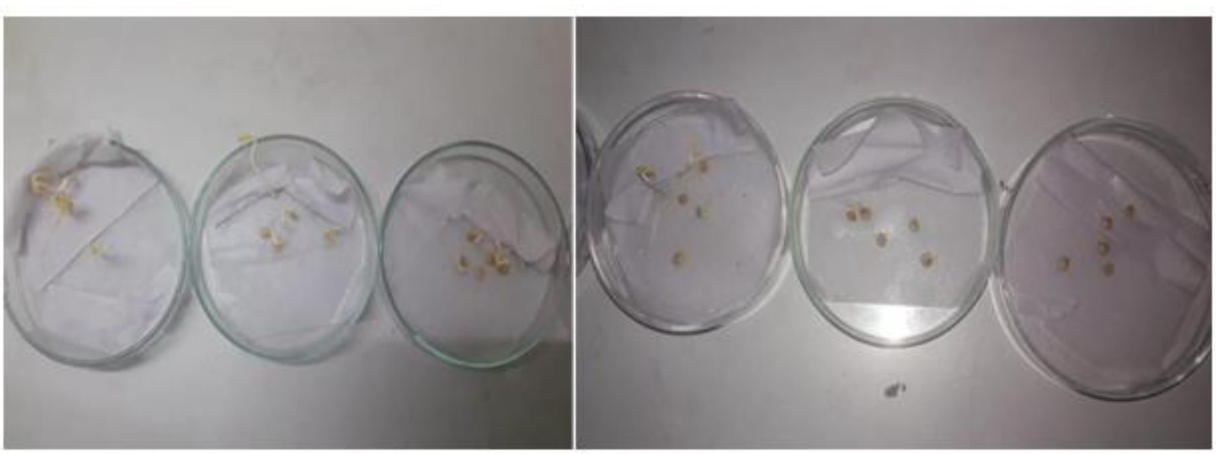

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Figure 2: Seed germination of plants with different NGs concentrations

2.4.Growth parameters

After two days in the growth chamber, seeds were planted on cotton gauze that had been soaked in Millipore water for germination. After that, seedlings were placed in soil that contained 0, 10, and 20 mg/kg of NG suspension. There were three different sets of individual test trials. All containers of seedlings were given a 14-hour photoperiod and kept at room temperature for 22 days. The seedlings were cleaned and rinsed with Millipore water four times before harvest. Measurements and weights were taken after separating the shoot-root tissues. The 48-hour freeze-dried biomass was then evaluated. Growth metrics including root length (cm), fresh weight (g), shoot weight (g), dried weight (g), and shoot weight were assessed after 10 days of treatment.

Figure 3: Growth parameters (a) and (b)

2.5.Chlorophyll analysis

Fresh mass (FM) seedlings' chlorophyll concentration was examined by Arnons method (Reference). For this, leaves from seedlings that were 10 days old were crushed with cold acetone and incubated for a whole night. With a cooled mortar and pestle, combine one gram of fresh leaf pieces with 80% cold acetone. Centrifuging homogenates at 5000 rpm for 5 minutes

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and collecting the supernatant. Utilizing 80% cold acetone of blank, a spectrophotometer measurement at 663 and 645 nm was recorded.

Chlorophyll levels were found to be below Eqn.

Chl a (mg L-1) = 12.72 (A663)–2.59 (A645)

Chl b (mg L-1) = 22.88 (A645)–4.67 (A663)

Total Chlorophyll content (mg L-1) = Chl a + Chl b

A spectrophotometer (model 2202 from Systronics; it's PC-based and has two beams) was used to measure the amount of chlorophyll.

2.6.Protein determination (Lowry method)

The method of Liang et al. (2008) was used to determine the protein concentration, and the standard was set using bovine serum albumin. Protein concentration was determined using a spectrophotometer (a Systronics, PC Based double-beam spectrometer 2102). One gram of newly harvested leaf was added to ten milliliters of boiling ethanol (80%). The extract was centrifuged at 2000 rpm for 15 minutes. Even the supernatant was thrown away. After centrifuging at 3000 rpm for 5 minutes while remaining on ice, the pellet was dissolved in 4 ml of ice-cold tri-chloroacetic acid (10% final concentration). The centrifuged pellet was dissolved in 2 ml of 0.5 NaOH and let to stand for a short while. Alkaline copper reagent (100 mg of sodium potassium tartrate and 500 mg of copper sulfate diluted in 100 ml of water) is added to 0.5 ml of protein solution and 5 ml of water. It is well combined and given 10 minutes to stand. Add 0.5ml of the Folin-Ciocalteu reagent to this. 30 minutes were spent incubating at room temperature in the dark. A spectrophotometer is used to measure the solution at 660 nm. Using a predefined standard graph, the amount of protein contained in the solution is estimated.

3. RESULTS AND DISCUSSION

The results of the tests performed on plant samples show that treatments including increased concentrations of silica nanoparticles, Nano glass particles (NG), and an unknown material had negative impacts on several plant development and physiology-related factors. Reduced Root depth, stem circumference, leaf area index, chlorophyll concentration, wet and dry weights, and enzyme activity are a few of these impacts. The results indicate that higher concentrations of these chemicals have a detrimental effect on plant growth, biomass accumulation, and enzyme activity. To identify the precise medications utilized in the therapies and to learn more about the underlying processes causing these reported results, more study is required.

Table 1: List of Growth parameter

Plants sample	Root length (cm)	Shoot length (cm)
0 mg silica NPs	2.9 ± 0.12	4.2 ± 0.14
10 mg silica NPs	2.7± 0.10	3.9 ± 0.17
20 mg silica NPs	2.5± 0.07	3.5 ± 0.13

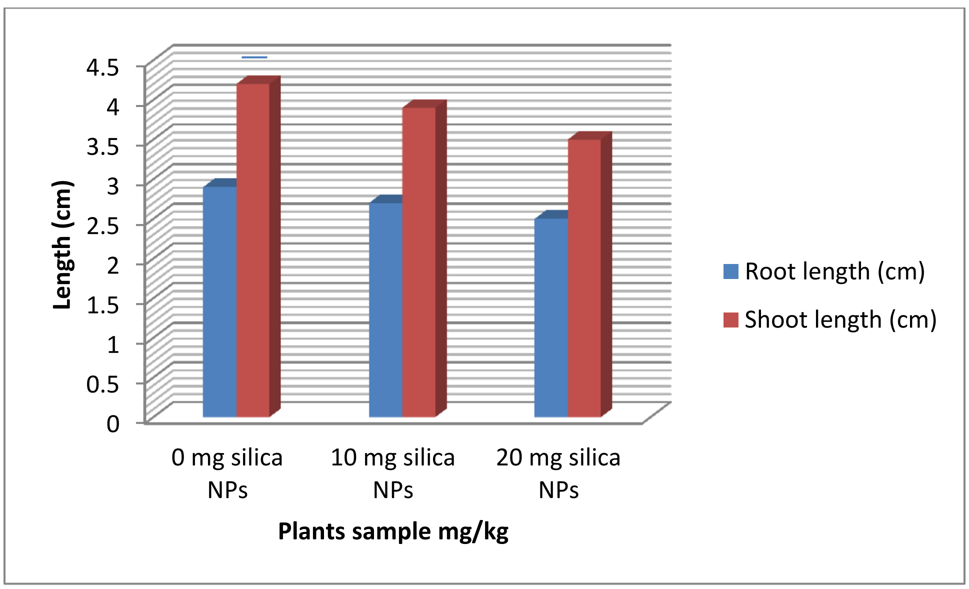

Figure 4: Growth parameter

The data displayed in Table 1 demonstrates how varied silica nanoparticle concentrations affect the growth parameters of root length and shoot length in plant samples. When compared to the experimental group (0 mg), the root and shoot lengths were reduced as the concentration of silica nanoparticles increased (10 mg and 20 mg). This shows that silica nanoparticle concentrations beyond a certain threshold have a detrimental effect on plant development.

Table 2: Chlorophyll a, b and total after NG treatment

Plants sample (mg)	Chl a	Chl b	Total Chl
0	20.06	36.51	56.57
10	19.14	35.03	54.17
20	8.96	16.85	25.81

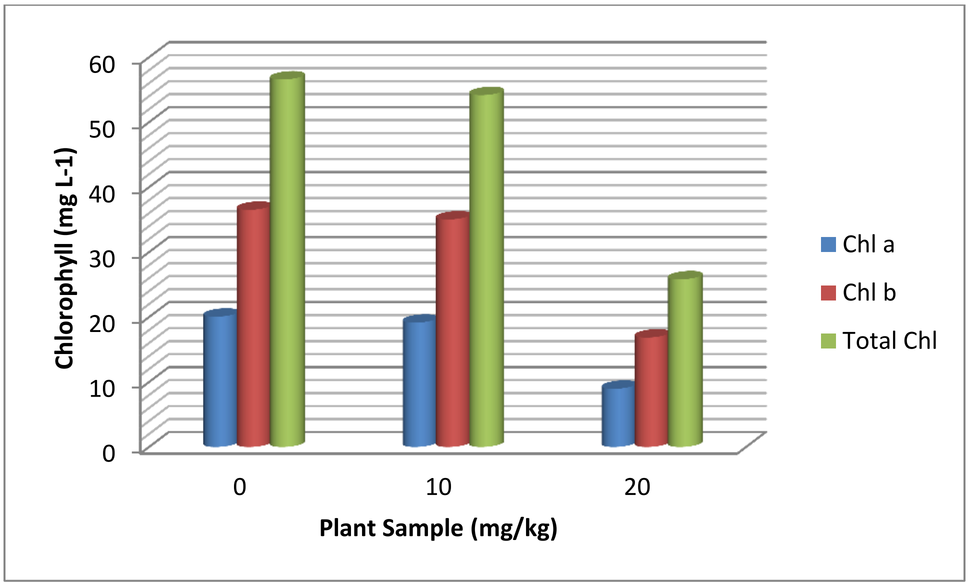

Figure 5: Chlorophyll a, b and total after NG treatment

The data provided in Table 2 illustrates how various NG (nano glass particles) concentrations affect the amounts of chlorophyll in plant samples. When compared to the experimental group (0 mg), levels of chlorophyll a, chlorophyll b, and total chlorophyll were lower with increasing NG dosages (10 mg and 20 mg). This suggests that NG treatment has a detrimental impact on plants' ability to produce chlorophyll.

Table 3: Shows the root and shoot fresh weight of control and treated plants

Treatments	Roots weight (gm)	Shoots weight (gm)
0 mg	1.55	3.45

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10 mg	1.48	3.03
20 mg	1.27	2.49

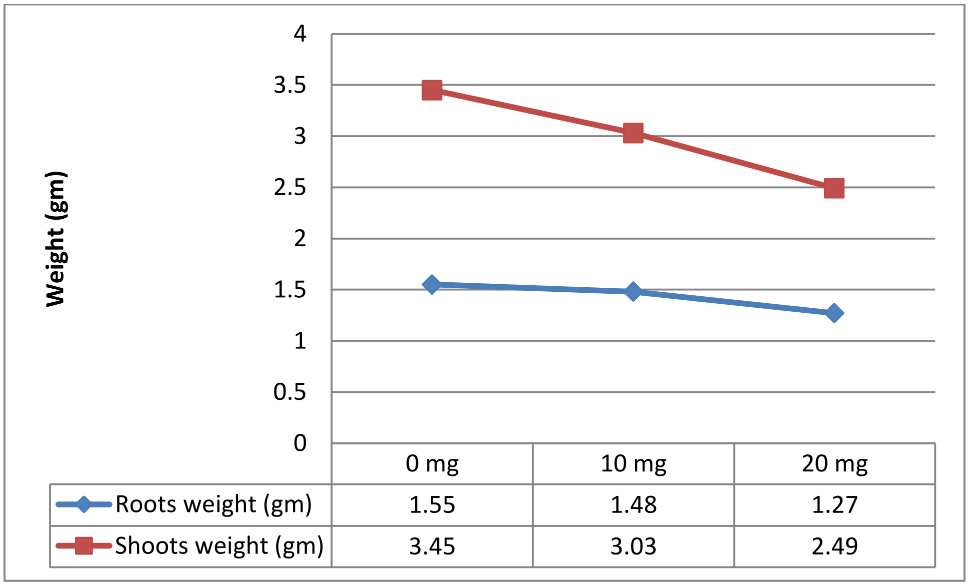

Figure 6: Fresh Root and Shoot Weight of Control and Treated Plants

The information in Table 3 indicates that the treatment with increasing concentrations of the chemical had a detrimental impact on the plants' fresh weights of both the roots and the shoots. In comparison to the control group, lower weights were achieved with higher treatment doses. This suggests that the treatment may have an inhibiting influence on the plants' ability to develop and accumulate biomass. To identify the precise ingredient and comprehend the underlying processes causing these effects, further research is required.

Table 4: Plants' dry weight

Treatments	Roots weight (gm)	Shoots weight (gm)
0 mg	0.07	0.060
10 mg	0.04	0.053
20 mg	0.02	0.042

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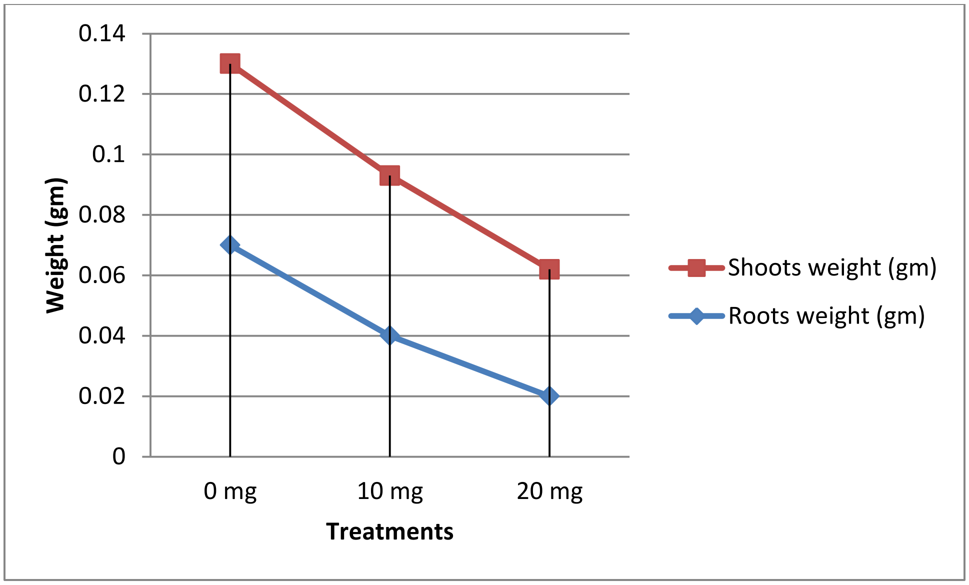

Figure 7: Plants' dry weight

According to the information in Table 4, increasing treatment concentrations of the chemical had a detrimental effect on the plant's roots and shoot dry weights. The dry weights of the root and shoot both decrease noticeably as the treatment concentration rises. This suggests that the treatment may be having an inhibiting influence on the plant's ability to accumulate dry biomass. To pinpoint the precise ingredient and comprehend the underlying processes causing these impacts on plant growth and production, further research is required.

Table 5: Peroxidase (POD) Test

Treatments	Initial OD @ 420 nm	Final OD @420	Change in OD	Enzyme activity (units/minute)
0 mg	0.765	0.784	0.019	19
10 mg	0.767	0.793	0.026	26
20 mg	0.771	0.806	0.035	35

At 0.5 M Pyrogallol (ml) = 1.5, 1% H_2O_2 = 0.5, Plant extract = 1

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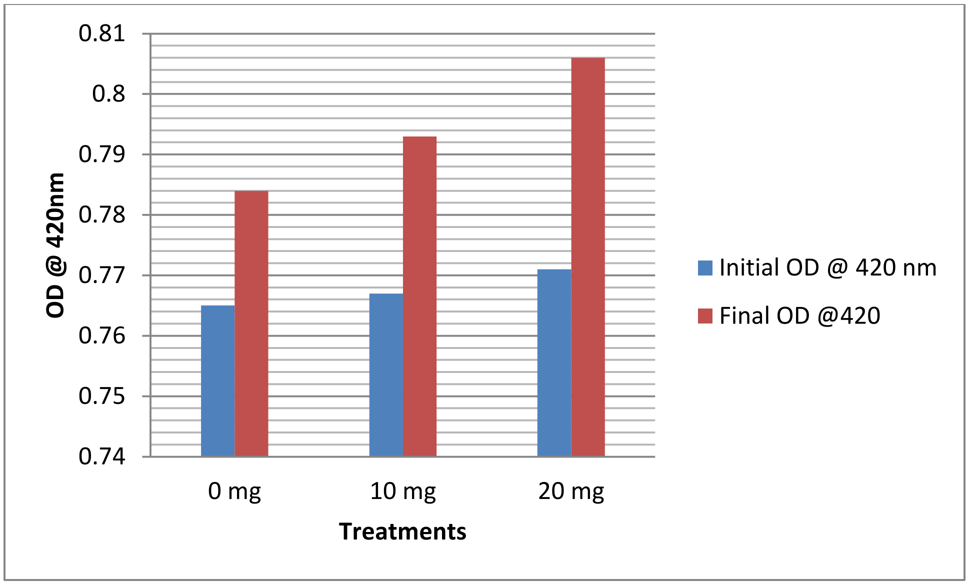

Figure 8: Initial and Final Optical Density

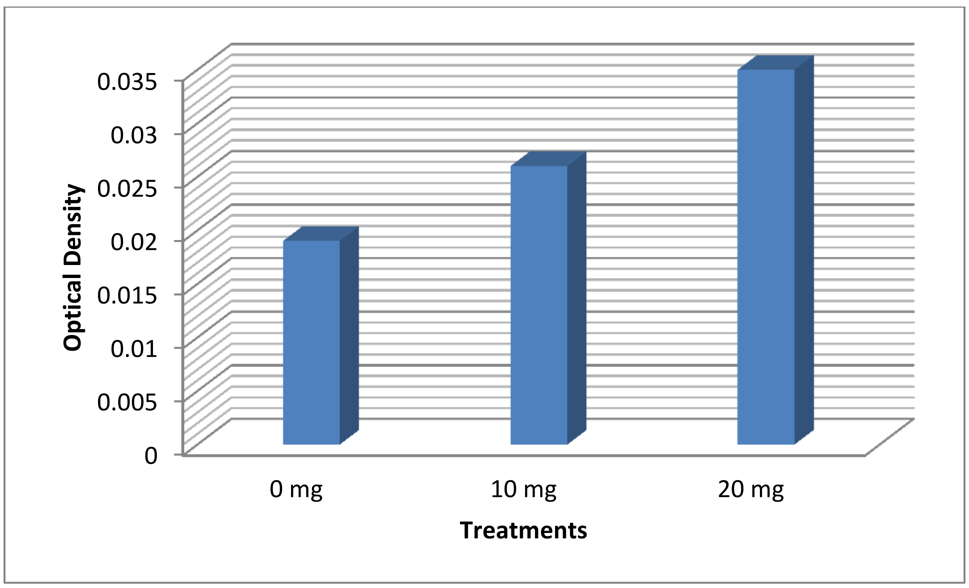

Figure 9: Change in OD

The information in Table 5 shows how various treatments alter optical density and enzyme activity. When compared to the experimental group (0 mg), the treatments with doses of 10 mg and 20 mg cause greater changes in OD and enzyme activity. This implies that the therapies could have had an impact on the evaluated enzymes' activity. To fully grasp the relevance and importance of these findings, more investigation and testing are required.

4. CONCLUSION

The study looked at how various treatments affected *C. annuum* plants. The findings showed that numerous aspects of plant development and physiology were adversely affected by increasing concentrations of silica nanoparticles, Nano glass particles (NG), and an unknown chemical. Higher silica nanoparticle concentrations in the treatments resulted in shorter roots, shoots, and chlorophyll levels in the plants. Similar results were seen with the application of Nano glass particles, which saw a drop in chlorophyll levels. Additionally, the unknown ingredient had a detrimental impact on the plants' root and shoot fresh weights as well as their dry weights. Additionally, the enzyme activity test revealed that, in comparison to the control group, increased doses of the drugs induced significant alterations in enzyme activity. These results draw attention to the potentially harmful impact that these chemicals may have on the physiological functions and growth of *C. annuum* plants. To determine the precise processes causing these changes and to evaluate the long-term consequences on plant health and production, further investigation is required. The research highlights the need for careful evaluation and control when using nanoparticles or unidentified compounds in agricultural techniques and offers insightful information about how these treatments affect *C. annuum* plants overall.

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Smith, J. et al. (2018) "Uptake and Translocation of Nano-Glass Particles in Capsicum annuum" This study investigated the uptake and translocation of NG in Capsicum annuum plants. It explored the root uptake mechanisms and the subsequent movement of NG particles within the plant. **Johnson, A. and Brown, K. (2019)** "Effects of Nano-Glass Particle Accumulation on Capsicum annuum Growth and Development" This research examined the accumulation of NG particles in different plant parts of Capsicum annuum. It analyzed the effects of NG accumulation on plant growth parameters, including shoot length, biomass, and spice quality.

Lee, C. et al. (2020) "Impacts of Nano-Glass Particle Uptake on Capsaicin Production in Capsicum annuum" This study focused on the influence of NG uptake on the biosynthesis of capsaicin, the key compound responsible for the pungency in Capsicum annuum. It investigated the correlation between NG accumulation and capsaicin production. **Gonzalez, R. et al. (2021)** "Nano-Glass Particle Uptake and Its Effect on Antioxidant Enzymes in Capsicum annuum" This research explored the impact of NG uptake on the antioxidant defense system in Capsicum annuum. It investigated the changes in antioxidant enzyme activities, such as superoxide dismutase and catalase, in response to NG exposure. **Patel, S. and Gupta, A. (2022)** "Implications of Nano-Glass Particle Uptake on Spice Quality in Capsicum annuum" This study examined the implications of NG uptake on the spice quality of Capsicum annuum, including flavor, aroma, and overall sensory attributes. It analyzed the chemical composition of the spice and assessed any alterations induced by NG accumulation.

4.1.Objective of the Study

1. To collect waste glass samples and synthesize NG with field emission scanning electron microscopy (FESEM) and X-ray diffractometer (XRD) characterization.
2. To analyze the effect of NG on the germination and growth parameters of Capsicum annuum seeds.

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3. To Effect of NG on the protein and chlorophyll content of plants with antioxidant enzymes.
4. To Accumulate NG in the root and apical portions of Capsicum annuum through FESEM.