GREEN SYNTHESIS OF TIO_2 NANOPARTICLES AND TO STUDY ITS IMPACT ON THE ENHANCEMENT OF SOIL PARAMETERS BY NANO PRIMING TOMATO SEEDS (SOLANUM LYCOPERSICUM L.)

Shailaja Gupta, and Preeti Jain

Keywords: Titanium dioxide, XRD, UVVis, FESEM, FTIR, Moringa oleifera (Drumstick), Solanum lycopersicum L (tomato).

In the present study, green synthesized titanium dioxide nanoparticles were used to nanoprime tomato seeds to study the enhancement of soil parameters. Spectroscopic analysis of nanoparticles was conducted to assess the crystallite structure, particle size, morphology and polymorphism. X-ray diffraction analysis showed an intense peak at 25.27° which corresponds to the (101) plane and confirmed the formation of anatase polymorph having tetragonal structure of TiO2 NPs. Average crystallite size of titanium dioxide nanoparticles was calculated using Scherer's formula and found as 59.6 nm. FE-SEM showed a mix of cuboidal and irregular shapes of the NPs. Energy bandgap was found to be 3.26 eV using UV-Vis spectroscopy which shows the formation of anatase phase. Seed germination indices of the treated seeds were higher than the controlled seeds, and this confirmed the role of titanium dioxide nanoparticles in the formation of reactive oxygen species that formed nanopores on the testa and enhanced the imbibition. Reactive oxygen species, enhanced role of gibberellic acid responsible for the seed germination and availability of nitrogen for absorption in the soil, resulting in enhancement of chlorophyll and rate of photosynthesis leading to increased nutrient uptake, biomass and crop yield.

*Corresponding author Email: preeti.jain@medicaps.ac.in (Preeti Jain)

Department of Chemistry, Medi-Caps University, Indore, 453331, Madhya Pradesh, India, E-mail: shailajarakibe457@gmail.com

INTRODUCTION

Nanotechnology is an expanding technology of the 21st century and transition metals and their oxides exhibit unique physico-chemical properties in their nano form. Titanium dioxide nanoparticles (TiO_2 NPs) produce reactive oxygen species (ROS) that can exhibit astonishing results in agriculture in plant growth parameters and yield of certain plant species such as spinach, sunflower, wheat, mung bean, coriander etc. They have the ability to enhance seed germination vigor, enhance soil quality indices and facilitate the availability of soil macro and micronutrients for uptake by roots. They can also facilitate controlled delivery of fertilizers at specific sites, pest management, photocatalytic degradation of pesticides, increase tolerance to abiotic stress, post-harvest crop management, photoremediation of heavy metals such as Cd, Cu, As, and Hg from soils and act as biosensors to detect plant pathogens and nutrient deficiencies. Plant mediated NPs synthesis is simple, economic, less toxic, environmental friendly and provides better alternative to the traditional pathways.[1–15]

For the experiment, S-22 cultivar of Solanum lycopersicum L. (tomato) was selected since it has a special tangy flavour and is a main ingredient of Indian cuisine. They are generally grown in temperate climates across the world and can grow upto 1-3 meters in height throughout all seasons of the year. Seed priming with nanoparticle solution can enhance the seed germination indices and all the parameters of plant growth leading to early harvest as compared to the controlled plant. TiO_2 NPs stimulate the plant hormones responsible for seed germination and enhance the nitrogen metabolism responsible for increase in chlorophyll and photosynthesis.[16]

EXPERIMENTAL

Materials and Method

Anhydrous $TiCl_4$99%, the titanium precursor, was procured from Spectrochem Pvt. Ltd. Mumbai, India of analytical grade and double distilled water (DDW) that was prepared in the laboratory. The experiment was conducted on the simple principle of precipitation method and carried out in two major parts, firstly green synthesis of TiO_2 NPs using $TiCl_4$ as the precursor and aqueous extract of Moringa oleifera leaves as the stabilising and capping agent. The second part was to study the impact of green synthesized TiO_2 NPs on Solanum lycopersicum L. (tomato) seeds.

Synthesis of TiO_2 NPs

Aqueous extract was obtained by digesting 10 gm of shade dried Moringa leaves in 1 litre DDW for 30 minutes. The extract was cooled, filtered using Whatman filter paper of grade No.1 and used for synthesis of NPs. The pH of the extract was measured and found between 5~6.$TiCl_4$ solution was poured into the plant extract, with continuous mixing at 2000 rpm, using a magnetic stirrer. The particles were washed with DDW, filtered with Whatman filter paper of grade No.1 and calcined in a muffled furnace at 500 °C.[2,4,14,17]

Characterization

The different attributes of TiO_2 NPs size and morphology were characterized using various spectrometric techniques using X-ray diffraction pattern analysis, UV-visible spectroscopy, Fourier Transform Infra-Red spectroscopy and Field Emission-Scanning Electron Microscopy.

Results of Characterization

Figure 1 shows the X-ray diffraction of TiO_2 NPs with intense and broad peaks at the center indicates very small crystallite size.

[Figure 1 about here.]

An intense peak at 25.27 ° indicates to (101) plane and other peak positions corresponds to the tetragonal, anatase phase of TiO_2 NPs. The mean crystallite size calculated by Scherer’s equation was found to be 59.6 nm. Figure 2 shows a strong UV-Vis absorption band between 200-340 nm, that was extrapolated at the edge, and the absorption wavelength was found to be 377 nm.

[Figure 2 about here.]

Using Figure 3 energy bandgap (E_g) of the green synthesized TiO_2 NPs was calculated to be 3.262 eV, by extrapolating the curve on X-axis using Tauc's plot, that confirmed the anatase phase.

[Figure 3 about here.]

Figure 4 depicts the FE-SEM analysis of green synthesized TiO_2 NPs shows a mix of cuboidal shape and irregular geometry. Figure 5 indicates the mean length of TiO_2 NPs, calculated as 3.184 μm

[Figure 4 about here.]

[Figure 5 about here.]

Figure 6 shows a prominent wide band between 400-530 cm^{-1}that relates to Ti-O stretching band and is the characteristic peak of TiO_2.

[Figure 6 about here.]

Soil Chemical Analysis

The black soil of Malwa plateau is rich in calcium, magnesium, aluminium, iron and potassium. Soil analysis was carried out to know about the soil quality in terms of pH, electrical conductivity (EC), organic carbon (OC), the percentage of macro and micro-nutrients present in soil. Table 1 shows all the important parameters of soil analyzed before and after amendment.

[Table 1 about here.]

The soil was deficient in nitrogen which was enhanced by mixing organic compost, pH was neutral which was treated by lemon juice to decrease the pH to 6.5 as tomato plants grow well in slightly acidic soil. EC of soil increased by decreasing the pH.

Laboratory Experiment (Nanopriming and Sowing of seeds)

Laboratory experiments were conducted on S-22 cultivar of tomato for three successive seasons June 2022 (summer), October 2022 (fall) and January (winter) 2023. 10-12 seeds were soaked in 0.1 % TiO_2 NPs colloidal solution and specified as treated seeds and the seeds that were soaked in DDW were specified as controlled seeds. They were kept in indirect sunlight for 4-5 hours before sowing in tubs. The tub soil (1 Kg) was amended with 1 ml of 0.1 % TiO_2 NPs colloidal solution and mixed thoroughly. Six seeds each for treated and controlled seeds were sown in two different tubs and replicated. The seeds were monitored for the germination vigour, growth parameters of seedlings, size and number of leaves, length of root and shoots, foliage, flowering and fruit formation. The average day/ night temperature of Mhow, Indore, in summers (June) was 37 °C /27 °C, fall (october) 33 °C /18 °C and winters was (January) 25 °C/ 19 °C.

RESULTS AND DISCUSSION

1. Nanoprimed tomato seeds exhibited enhanced seed germination vigour, rapid seedling growth, increase in root and shoot length, leaf size, early flowering, larger fruit size and weight and early harvest

Nanoprimed seeds as shown in Figure 7 showed rapid and complete germination as compared to the controlled seeds as shown in Figure 8 for two successive experiments but in winter sowing, cold stratification helped controlled seeds to germinate at equal vigour and rate. TiO_2 NPs treated seeds germinated 4-5 days earlier than the untreated controlled seeds. The germination potential and stress tolerance of seeds were both improved by TiO_2 NPs.[18] Rapid seed imbibition (absorption of water and nutrients), embryo growth and radicle emergence was observed due to hydroxyl radicals (• OH) and other ROS that created tiny pores on the surface of testa (seed Coat) to enhance the water, oxygen and nutrients absorption to facilitate rapid growth of embryo.[19]

[Figure 7 about here.]

[Figure 8 about here.]

Furthermore,gibberellic acid (GA), a plant growth hormone, structure shown in Figure 9 facilitated release of seed dormancy by H_2O_2 could have enhanced the production of gibberellic acid (GA) in the embryo to activate and mobilize the enzyme reactions of hydrolase, lipases and protease on starch, fats and proteins respectively present inside the endosperm to stimulate the germination, promote early growth of embryo and that of seedlings.[18,20,21]

[Figure 9 about here.]

During imbibition, non-covalent linkages such as H-bonds, between cellulose and hemicellulose are disrupted, that allows cell elongation of the radicle, loosening of the cell wall and emergence of radicle from the endosperm of nanoprimed tomato seed which could have been enhanced by the ROS. It also results in DNA repair and abiotic stress tolerance of the seedling.[22]

2. Increase in the nitrogen metabolism, enhanced photosynthesis, leaf size, plant growth early flowering and fruit formation.

Increase in the number and size of leaves in the treated plants suggest enhanced nitrogen metabolism in the soil that might have increased the absorption of nitrates (inorganic nitrogen) from soil and rapidly converted into organic nitrogen needed for chlorophyll production. The enzymes involved in nitrogen metabolism are nitrate reductase, glutamic-pyruvic transaminase, glutamine synthase and glutamate dehydrogenase that aids plants to absorb nitrate. This increased the rate of photosynthesis of the treated plant as compared to the control plants, thereby enhancing the length of roots and shoots, foliage, leaf size, early flowering and fruit bearing activity and biomass of tomato plants. An overall morphological growth of treated and controlled tomato plants with their yield are shown in Figure 10 (a-d).

[Figure 10 about here.]

A comparative bar graphs of all the growth parameters of the three experiments have been shown in Figures 11, 12, 13, 14 and 15 .[12,20,23]

[Figure 11 about here.]

[Figure 12 about here.]

[Figure 13 about here.]

[Figure 14 about here.]

[Figure 15 about here.]

CONCLUSION

Green synthesized TiO_2 NPs using aqueous extract of Moringa oleifera leaves were found to be in anatase phase with an average diameter of 59.6 nm. Nanoprimed tomato seeds displayed enhanced germination rate and vigour, plant shoot and root length, number of foliage, leaf size, early flowering, early harvest and yield as compared to the controlled plants. TiO_2 NPs produced active oxygen species, hydroxyl radical (• OH) and $H_2O_2$when exposed to UV radiation that created tiny pores on the seed coat (testa), weakening and loosening the cell walls that enhanced imbibition of water and nutrients, activation of enzymes which helped in early seed germination of the treated seed of tomato as compared to the controlled ones. It also enhanced the nitrogen availability in soil leading to enhanced nitrogen metabolism and formation of chlorophyll. This increased the plant biomass, the foliage and all plant growth parameters. This can also help in the early harvest in abiotic stress affected soils, such as drought and flood prone soils. Hence, TiO_2 NPs can be very promising in the future agricultural practices to benefit society and the environment, reduce input costs and increase production, improve seed quality, by creating employment opportunities.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION S

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below: 0000-0003- 3849-6606 (Preeti Jain), and0000-0003-1224-5980 (Shailaja Gupta).

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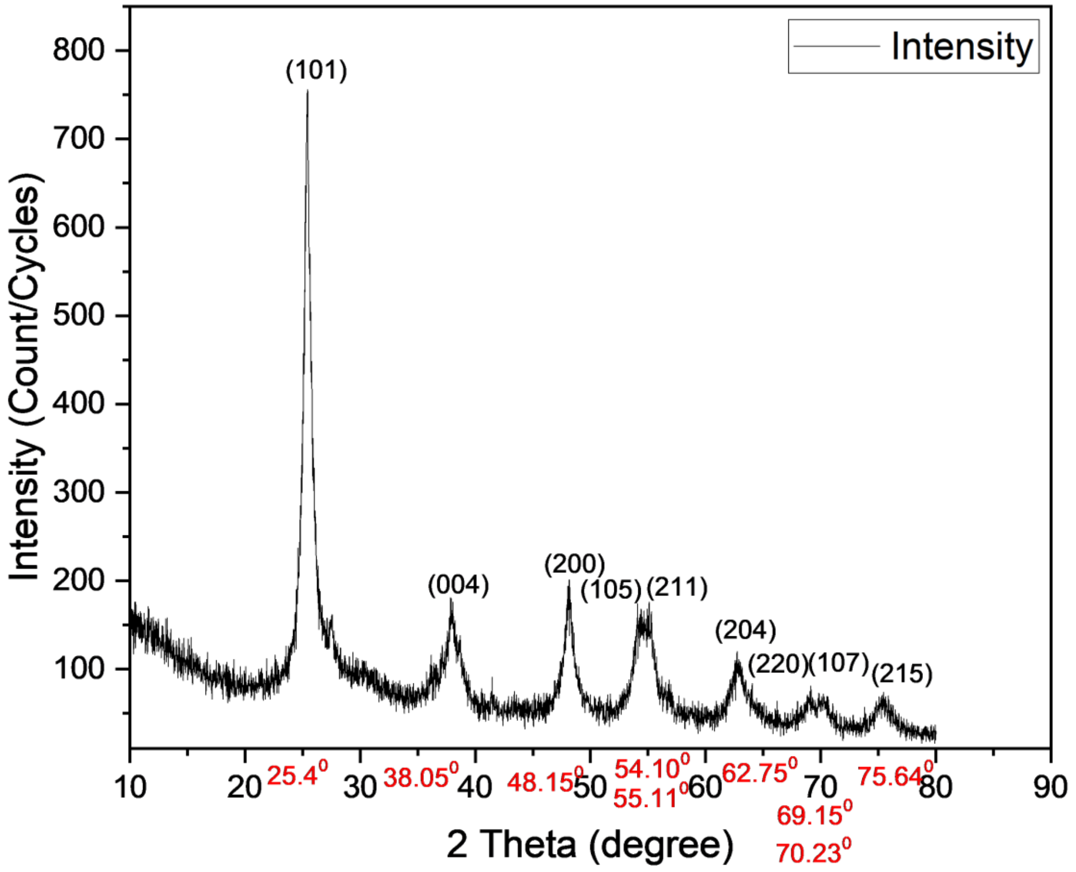


Figure 1. P-XRDgraph of TiO$_2$ NPs

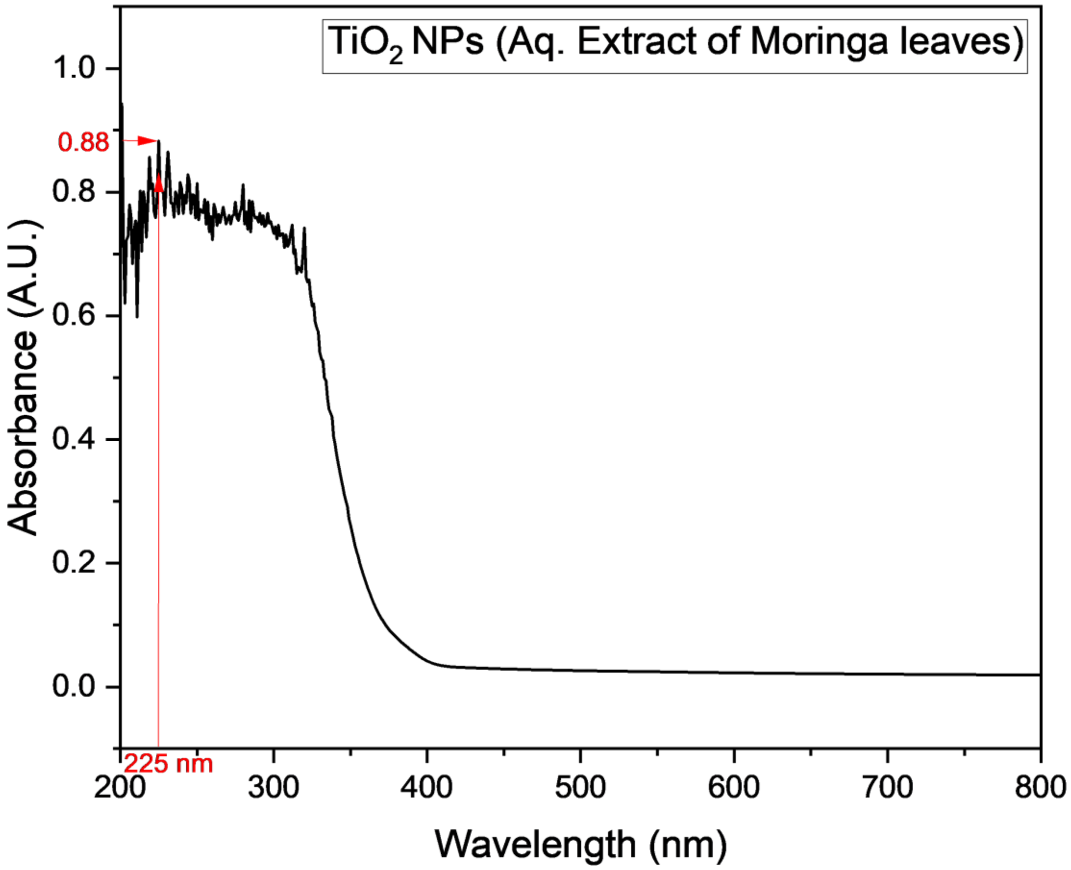


Figure 2. UV-Vis spectra of TiO_2 NPs

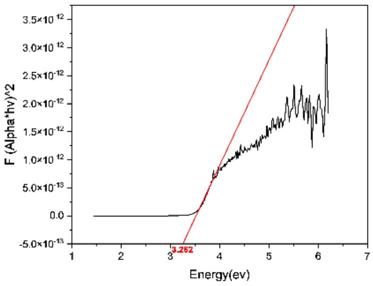


Figure 3. Energy band gap using Tauc’s plot

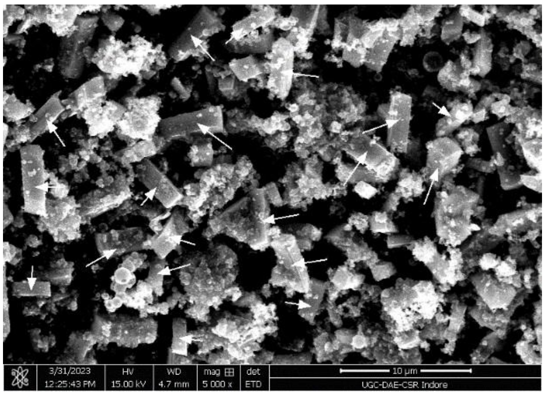
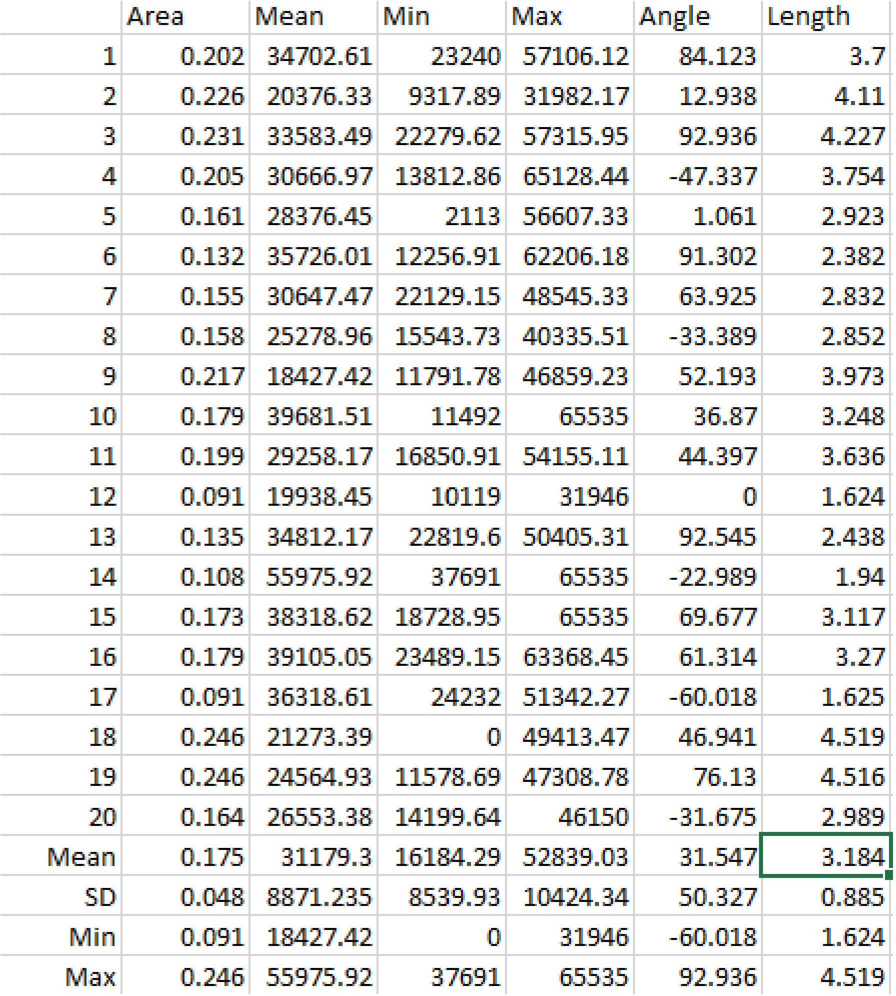


Figure 4. FE-SEM image of TiO$_2$ NPs at 10μm scale



	Area	Mean	Min	Max	Angle	Length
1	0.202	34702.61	23240	57106.12	84.123	3.7
2	0.226	20376.33	9317.89	31982.17	12.938	4.11
3	0.231	33583.49	22279.62	57315.95	92.936	4.227
4	0.205	30666.97	13812.86	65128.44	-47.337	3.754
5	0.161	28376.45	2113	56607.33	1.061	2.923
6	0.132	35726.01	12256.91	62206.18	91.302	2.382
7	0.155	30647.47	22129.15	48545.33	63.925	2.832
8	0.158	25278.96	15543.73	40335.51	-33.389	2.852
9	0.217	18427.42	11791.78	46859.23	52.193	3.973
10	0.179	39681.51	11492	65535	36.87	3.248
11	0.199	29258.17	16850.91	54155.11	44.397	3.636
12	0.091	19938.45	10119	31946	0	1.624
13	0.135	34812.17	22819.6	50405.31	92.545	2.438
14	0.108	55975.92	37691	65535	-22.989	1.94
15	0.173	38318.62	18728.95	65535	69.677	3.117
16	0.179	39105.05	23489.15	63368.45	61.314	3.27
17	0.091	36318.61	24232	51342.27	-60.018	1.625
18	0.246	21273.39	0	49413.47	46.941	4.519
19	0.246	24564.93	11578.69	47308.78	76.13	4.516
20	0.164	26553.38	14199.64	46150	-31.675	2.989
Mean	0.175	31179.3	16184.29	52839.03	31.547	3.184
SD	0.048	8871.235	8539.93	10424.34	50.327	0.885
Min	0.091	18427.42	0	31946	-60.018	1.624
Max	0.246	55975.92	37691	65535	92.936	4.519

Figure 5. mean length ofTiO$_2$ NPs is 3.184 μm

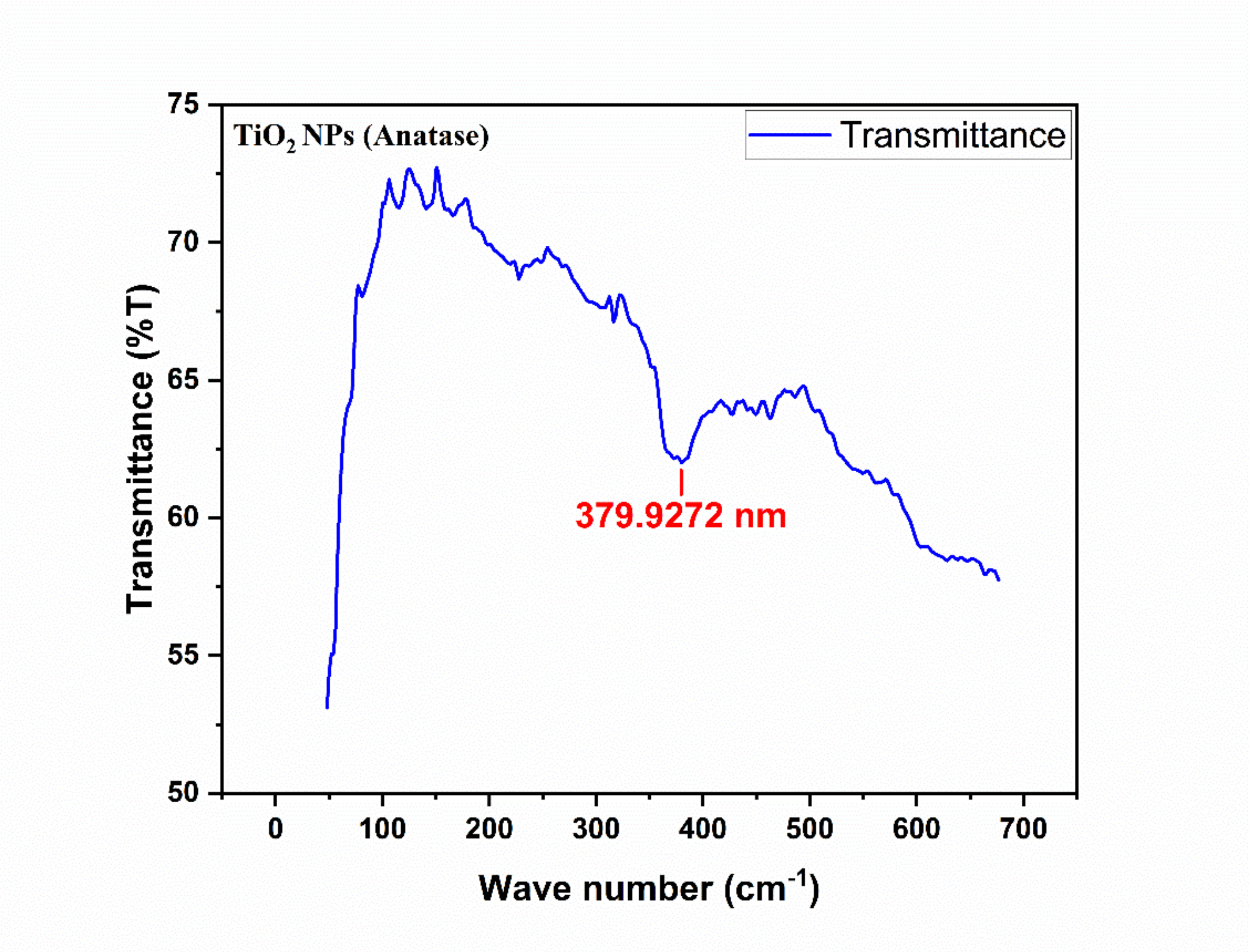


Figure 6. FTIR of TiO$_2$ NPs

Figure 7. Seed germination of TiO$_2$ treated tomato plant

Figure 8. Seed germination of controlled tomato seeds

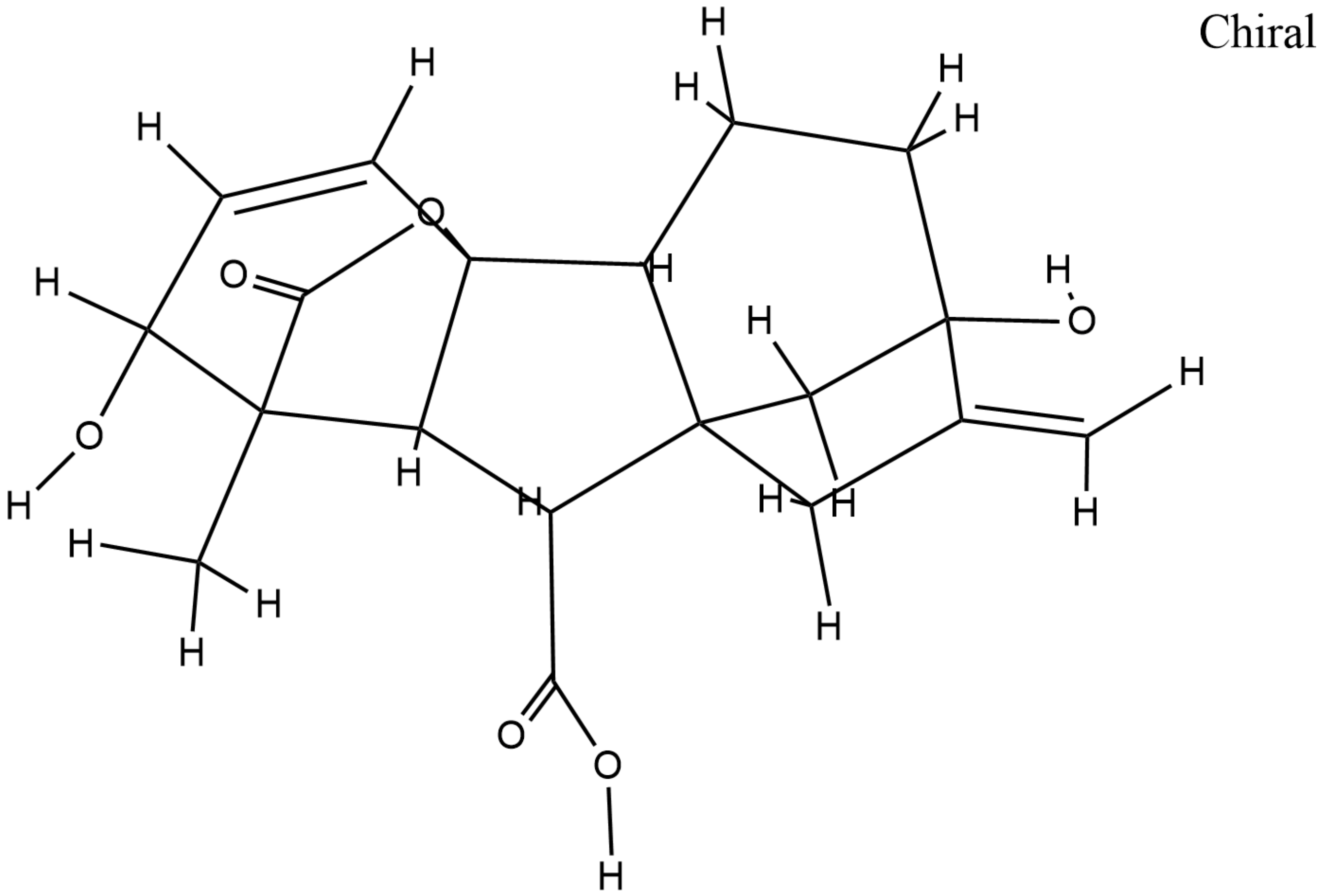


Figure 9. Structure of Gibberellic acid, plant growth hormone

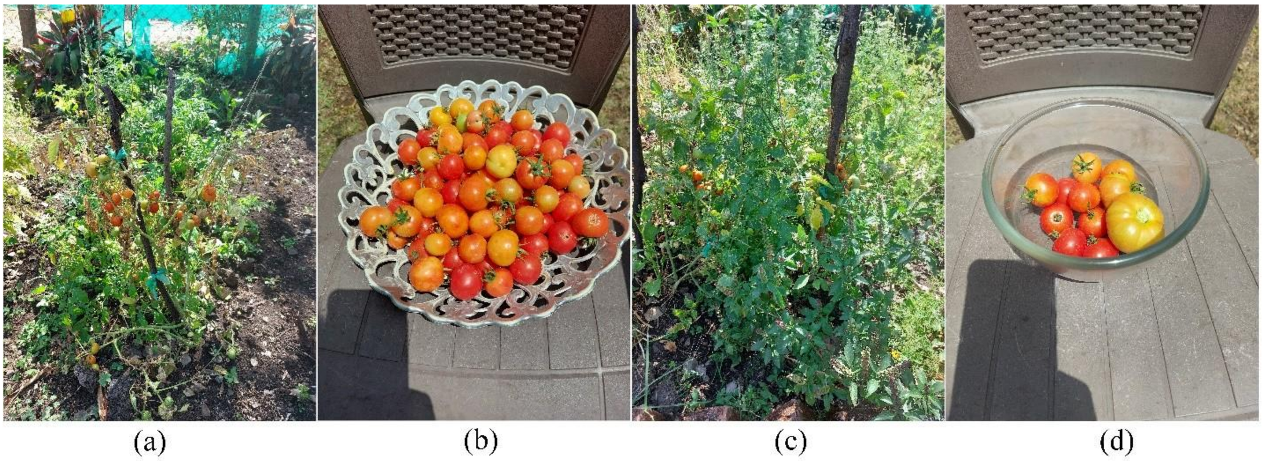


Figure 10. (a) Treated tomato plant, (b) Yield of Treated plant, (c) Controlled tomato plant, and (d) Yield of controlled plant

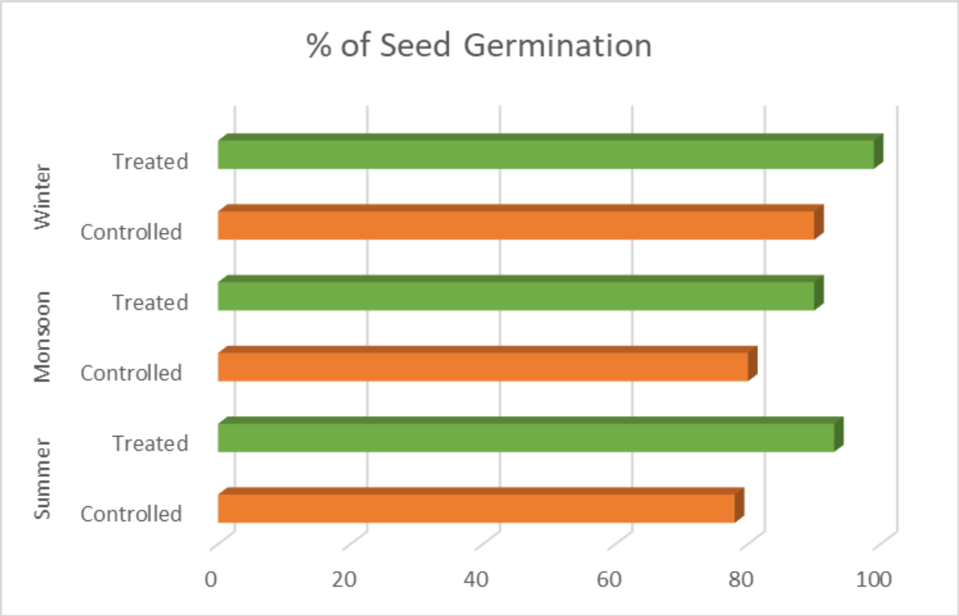


Figure 11. Percentage of seed germination of treated and controlled tomato plants for three seasons

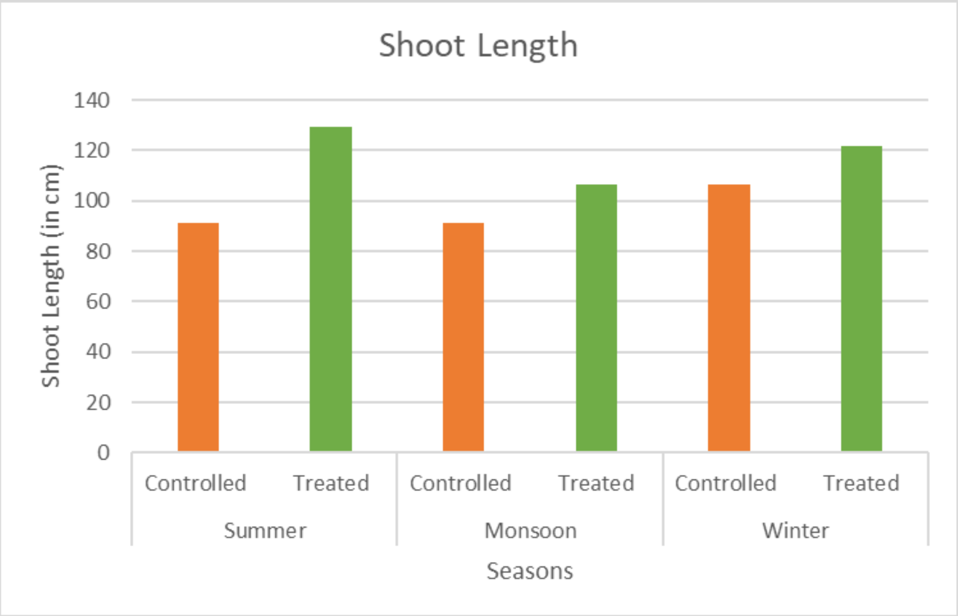


Figure 12. Comparison of shoot length of treated and controlled tomato plants for three seasons

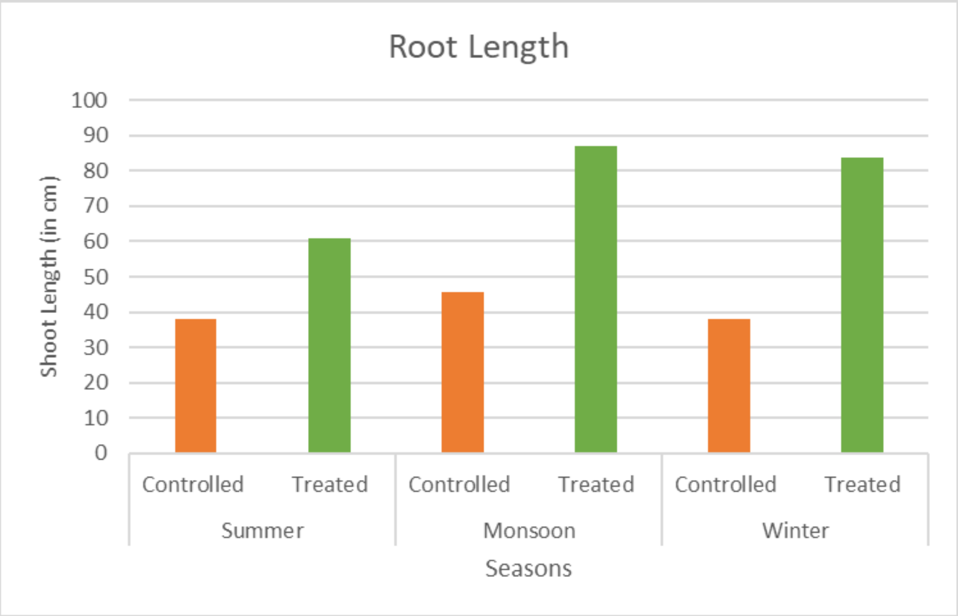


Figure 13. Comparison of root length of treated and controlled tomato plants for three seasons

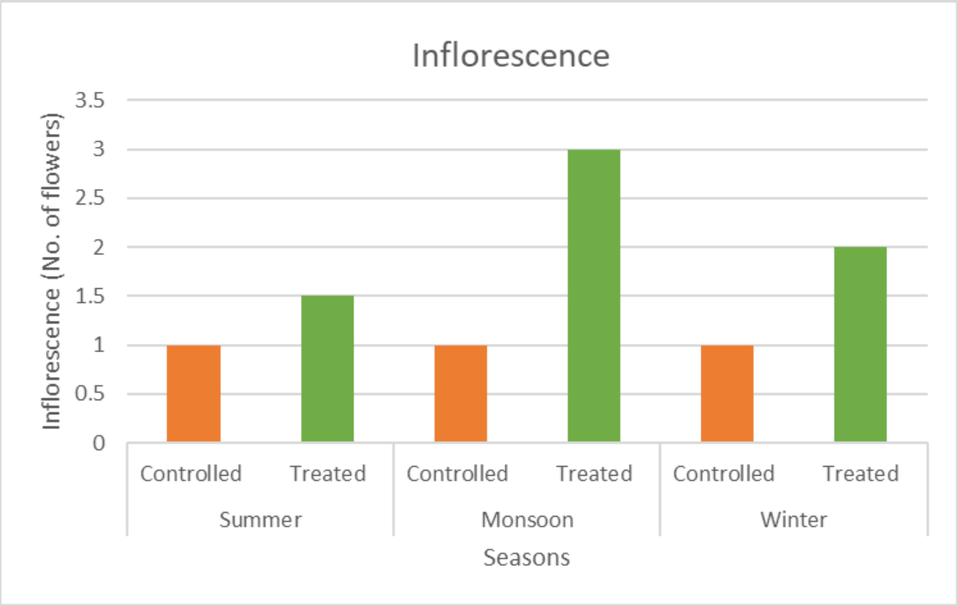


Figure 14. Comparison of inflorescence of treated and controlled tomato plants for three seasons

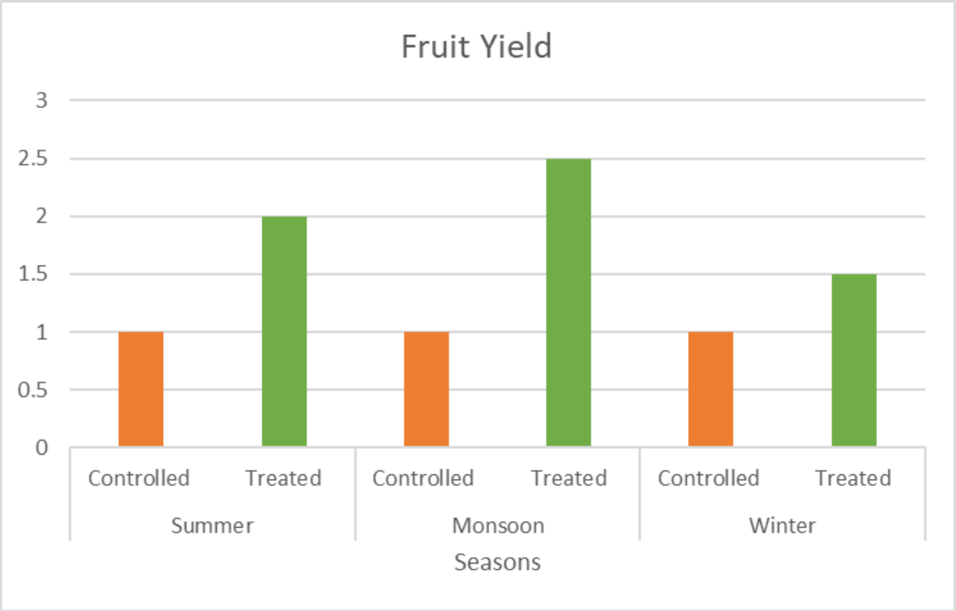


Figure 15. Comparison of fruit yield of treated and controlled tomato plants for three seasons

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Table 1. Analysis of Soil Chemical and Physical parameters of Malwa region, Central India

Measurements (unit)0.22	**Black Malwa soil June 2022**	**Amended soil (2022)**
Soil Depth (cm)	0-60	0-60
pH	7.34	6.5
E.C. (dS/m)	0.47	0.82
Organic carbon (%)	0.60	0.70
Nitrogen (Kg/h)	210	262
Phosphorous (Kg/h)	15.20	15.0
Potassium(kg/h)	433	404
Sulphur (Kg/h)	9.4	9.14
Zinc (ppm)	2.3	2.4
Iron (ppm)	5.2	5.22
Copper(ppm)	0.2	0.2