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Effect of Plant-Mediated TiO_2 Nanoparticles on Growth and Yield of Tomato (*Solanum lycopersicum* L.) Under Induced Biotic Stress

By

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Abstract

Agriculture is the backbone of Pakistan and provides food, income, and employment. Agriculture has a main role in a country's economy. Solanum lycopersicum is the second largest crop in the world and is grown and eaten all over the world, but in Pakistan, tomato consumption is quite high. It increased from 1.86 kg in 2001 with an average growth rate of 7.5% per annum. According to a 2020 report by the Planning Commission of Pakistan, the country's tomato trade deficit has ballooned over time. S. lycopersicum crop is highly affected by biotic stress, and it is estimated that 1/3 of the global crop is lost by pathogenic attack. Utilization of manufactured fertilizer and pesticides is economically expensive, damages soil, and harms the environment. Nanotechnology was introduced by Laureate Richard in 1959, and its applications in agriculture are noteworthy and fruitful. The current work investigates the potential significance of TiO2 NPs in inducing biotic stress in S. lycopersicum. TiO_2 enhanced morphological, physiological, and biochemical profiling of S. lycopersicum within induced biotic stress. $TiO_{2\ is}$ not only good for plants, but it is also an active ingredient in sunscreen, where it works as a UV filtering ingredient to help block the sun's ultraviolet light that can cause sunburn and is linked to skin cancer. The presence study was conducted to check the effect of plant-based TiO_2 nanoparticles on S. lycopersicum under induced biotic stress. Plant-based Synthesis of TiO_2 nanoparticles was done, followed by characterization. The application of TiO2 to different Concentrations concluded that it had a positive effect on the plant. TiO_2 nanoparticles have elicited 75%, 175%, 17%, 173%, and 45% increases in the diameter of the stem, area of the leaf, relative water content, phenolic content, and total sugar content, respectively. The study concluded that $TiO_{2\ plant\text{-}based}$ nanoparticles can change plant metabolic profile and cope against biotic stress.

Keywords: *TiO2 NPs, Plant-mediated synthesis, Tomato (Solanum lycopersicum L.), Biotic stress, Nanotechnology in agriculture, Antimicrobial activity of TiO2 NPs, Growth enhancement*

Introduction

The agrarian segment proceeds to play a crucial part in advancement, particularly in low-income nations where the division is huge both in terms of total wage and up to labor drive (Timmer et al., 1988). Horticulture contributes to both salary development and destitution in creating countries by producing pay and business in country ranges and giving nourishment at sensible costs in urban ranges. Agribusiness gives nourishment, pay, and occupations, and consequently can be a motor of development in agriculture-based nations and a viable device to decrease destitution in changing countries (Alderman 2008). Over-the-top utilization of manufactured fertilizers causes financial burdens, expanding soil, water, and climatic contamination. Nano-fertilizers have appeared to have the awesome potential for their feasible

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employment in soil ripeness and edit generation with the least or no natural tradeoffs (Ur Rahim et al., 2021). Nano-fertilizers are of submicroscopic sizes, have a huge surface zone to volume proportion, can have supplement epitome, and have more noteworthy portability; thus, they may increase plant supplement get to and trim abdicate. Due to these properties, nano-fertilizers are respected as deliverable ‘smart frameworks of nutrients. Be that as it may, the issues within the agroecosystem are broader than existing advancements. For example, supplement delivery of different physicochemical properties of soils, moisture, and other agroecological conditions is still a challenge (Boeraeve et al., 2020).

Nanotechnology includes both nanoscience and nano-engineering in its concrete form. The impact of subsequent advancements in instrumented and computational materials science and their application in practical studies is analyzed (Chuah et al., 2014). Subsequent developments in cement-based material nano-engineering and nanomodification are demonstrated. Defined as the comprehension, manipulation, and reconstruction of matter at the nanoscale (i.e., less than 100 nm) to create materials with essentially current capabilities and attributes (Roco 2007). Nanotechnology encompasses two basic methods: (i) the "top-down" method, which breaks down larger structures into their smaller, composite pieces or reduces them to the nanoscale while maintaining their distinctive characteristics without atomic-level control (such as in the case of gadget shrinking) (Ikumapayi et al., 2024) and (ii) the "bottom-up" strategy, often known as "molecular manufacturing" or "molecular nanotechnology" (Drexler, Peterson et al. 1991), whereby materials are created via self-assembly or assembly from atoms or atomic components. Atomic nanotechnology presents enormous promises for advancements in materials and fabrication, devices, pharmaceuticals and healthcare, vitality, biotechnology, data technology, and national security. In contrast, the majority of contemporary inventions rely on the "top-down" approach (Singh et al., 2024). To date, nanotechnology uses, and advances within the development and construction materials fields have been unequal (Bartos 2009). The commercial use of nanotechnology in concrete is still limited, with few results being successfully transformed into eye-catching products. The field of cementitious material nanoscience has made the biggest advancements (Scrivener and Kirkpatrick 2008, Bittnar, Bartos et al. 2009) with growth in knowledge and comprehension of the fundamental wonders of cement at the nanoscale (e.g., the composition and mechanical characteristics of the majority of the hydrate stages, the causes of cement cohesiveness, cement hydration, concrete interface, and corruption components). Recent advancements in tools for sensing and measurement at the nanoscale are providing an abundance of untapped and unique information about concrete, some of which are puzzling conventional wisdom. Important earlier summaries and collections of emerging nanotechnology may be located in (Sobolev and Gutiérrez 2005, Scrivener and Kirkpatrick 2008, Bittnar, Bartos et al. 2009, Sanchez and Sobolev 2010).

TiO2 NPs are considerably more central than other nanoparticles since they significantly advance plant development and reduce surrender. The cosmetics business also makes use of them. It is shown that TiO2 NPs are more beneficial for the physiological, morphological, and biochemical characteristics of several edit plants (Gohari, G. et al. 2020). Furthermore, it has been shown that TiO2 NPs accelerate the rate of photosynthetic reaction, chlorophyll actuation, antioxidant protein accumulation, and rubisco action. Furthermore, a thorough analysis of the beneficial effects of TiO2 NPs on broad bean plants in response to salinity has been conducted (Abdel Latif et al., 2018). They contribute to the advancement of plant growth, increase proline content, provide a strong antioxidant defense component, and reduce the levels of MDA and H2O2 in broad bean plants. In addition, a different study found that the exogenous application of TiO2 NPs to maize crops resulted in improvements in phenolic generation, agronomical parameters, antioxidant arrangement, and trim creation, as well as a decrease in salt stretch (Mustafa, N. et al. 2021). This analysis of the literature reveals that TiO2 NPs have effects on trim plants that are both beneficial and detrimental (Qasim et al., 2024). Tall dosages of TiO2 nanoparticles are destructive to plants due to the amassing of ROS atoms, which causes a diminishment in chlorophyll substances (Gohari, G. et al., 2020). whereas moo dosages upgrade the development properties and edit efficiency (Gohari, Mohammadi et al. 2020). Since there is currently less information available on the beneficial and toxicological effects of biosynthesized TiO2 NPs on wheat crops under saline settings, the current research was designed to examine the role of TiO2 NPs on wheat under salt push. According to this research, biogenic TiO2 NPs are essential for plant resilience under specific stress situations. Access to restricted data on these NPs' atomic components is available. Investigating the atomic instruments of these NPs in plants requires advanced thinking (Butler et al., 2017). This research project aims to produce and analyze TiO2 nanoparticles mediated by Pteris vittata leaf extract, apply TiO2 nanoparticles and spore inoculation to tomato, and monitor tomato physio-morphological, agronomical, and biochemical parameters.

MATERIAL AND METHODS

Synthesis and Characterization of *Pteris vittata* Leaf Extract Mediated TiO_2 Nanoparticles.

Preparation of Leaf Extract

Plants that were sound and not in use were removed from Pteris vittata and cleaned with tap water. Afterward, they were cleaned with refined water to remove any last bits of unwanted material. The clears out were divided into bits and dried at 25°C. After being weighed and transferred to holders holding 200 milliliters of refined water, over 40 grams of clears were bubbled on the hotplate for 20 minutes. The extract was filtered twice using Whatman no. 21 filter paper before being removed and placed in a 250 mL Slant container to start a clear, particle-free process. The endeavor was always

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carried out in sterile settings to guarantee the precise result (Banerjee, Satapathy et al. 2014).

Green Synthesis of Titanium Dioxide Nanoparticles

By combining plant extracts with the salt arrangement, TiO2 NPs were created. By dissolving titanium dioxide salt in purified water to a concentration of 0.8 g/L, a titanium dioxide (10 mM) arrangement was created. Using a seductive stirrer, the mixture was stirred for four hours at fifty degrees Celsius. The process was then measured by measuring the number of plant removal drops. The mixture was then put into an Eppendorf tube and centrifuged for 30 minutes at 8,000 rpm after its color had changed to a smooth white. After centrifuging at 8,000 rpm for 30 minutes, the supernatant was collected, and TiO2 NP pellets were suspended in deionized water. The approaching TiO2 nanoparticles were suspended in deionized water (Hussain, Raja et al. 2017).

Characterization of Nanoparticles

The bio-synthesized TiO2 NPs (nanoparticles) were characterized using distinctive characterization strategies, namely, the UV–visible spectrophotometer (UV752PC) with a wavelength run of 150–600 nm. Color change, UV.

Application of Titanium Dioxide Nanoparticle and Spore Inoculation to Tomato Sowing:

We took two varieties of tomato plant seedlings, Sahil and Zulay, from the Agriculture Research Institute Dera Ismail Khan. These two assortments were sown in 20 plastic crates. Out of these 20 crates, ten were chosen for each variety.

Treatment			Sahil			Zulay		
T0	Control	Before the spray of *Aspergillus flavus*	R1	R2	R3	R1	R2	R3
T1	10ppm							
T2	20ppm							
T3	30ppm							
T4	40ppm							
T5	50ppm							
T0	Control	After the spray of *Aspergillus favus*						
T1	10ppm							
T2	20ppm							
T3	30ppm							
T4	40ppm							
T5	50ppm							

Inoculation

To begin with, we get *Aspergillus flavus* spore culture from Arig College of Islamabad. We have subcultured it in Govt. Degree Collage # 1 Dera Ismail Khan. When the *Aspergillus flavus* Spore is shaped in a new culture, we make a 104 spore /ml arrangement. We shower this arrangement into each plastic crate.

Application

Ten plastic crates were chosen for Sahil, which were treated with the *Aspergillus flavus* spore. Of these, one was selected as control, 1 chose 50ppm of Titanium dioxide nanoparticles, and the two of the remaining eight crates were treated with 10ppm, 20ppm, 30ppm, and 40ppm individually. The 10ppm, 20ppm, 30ppm, 40ppm treatment was given before and after the *Aspergillus flavus* spore spray.

Parameter

Determination of Morphological Parameters

- **Plant Height:** The height of the plant was measured with the help of a meter rod.
- **Stem Diameter:** The diameter of the stem was measured with the screw gauge.
- **Leaf Area:** The area of the leaf was measured with the help of a measuring scale.
- **Numbers of Leaves per Plant:** The number of leaves per plant counted randomly.
- **Number of Flowers per Plant:** The number of flowers counted randomly.
- **Number of Fruits per Plant:** The number of fruits counted randomly.

Determination of Physiological Parameters

Estimation of Chlorophyll Contents

With some modest adjustments, Arnon's [30] technique was used to determine the chlorophyll concentration (Manolopoulou et al., 2016). After thorough consideration, leaf materials were homogenized in 80% cold acetone. After filtering the resulting solution, the absorbance at three specific wavelengths, 633, 645, and 663 nm, was measured using a spectrometer. The following formula was used to compute total chlorophyll, chlorophyll a, and chlorophyll b:

Chlorophyll a 12.7 x A_{663}-2.7 x A_{645}

Chlorophyll b = 22.9 x A_{645}-4.7 x A_{633}

Total Chlorophyll =20.2 xA_{645} +8.02 xA_{663}

Relative Water Content

An approximate weight of 0.2 grams was extracted from each treatment to determine the proportion of water contained in a tomato variety's leaf. First things first: after the leaf was incubated for 24 hours in a Petri dish with distilled water, its fresh weight was determined. Following an estimate of the leaves' turgid weight, they were dried at 70°C in an oven, and a week later, the dry weight was determined (Weatherley 1950). The relative water content was determined by the formula given below.

$$RWC = \frac{\text{(Fresh weight - Dry weight)}}{\text{(Saturated weight - Dry weight)}} \times 100$$

Determination of Biochemical Parameters

Total Soluble Sugar (TSS)

Using the Dubois et al. (1956) approach, the total soluble sugar content of leaves was determined. A 0.2-gram sample of fresh leaf material was ground in 10 milliliters of 80% ethanol and filtered. A mixture of 0.5ml distilled water, 1ml 18% phenol solution, and 0.5ml of the filtered extract was used. After one hour at room temperature, the mixture was shaken down, and 2.5 milliliters of sulfuric acid were added to each sample. At 490mm, the absorbance was measured. The total amount of soluble sugar in plant leaves was calculated using the formula below.

Sugar µg/ml = Absorbance of sample X K value X dilution factor/ Weight of sample

Total Phenolics content

Total phenolic determination was performed using Fu et al.'s (2011) technique. For the quantification, 0.2g of powdered plant material was crushed with 80% ethanol, and 100µl of plant extract was combined with 0.75 ml of folate ciocalteu reagent. This mixture was then incubated at 21° for 10 minutes. The mixture was then added 0.75 ml of sodium bicarbonate solution and stored at 21∨ for 90 minutes. The absorbance at 725 nm was then measured using a UV752PC visible spectrophotometer.

RESULT & DISCUSSION

Morphological Parameter:

The morphological parameters were analyzed in this study, which can be defined under the following heading.

Plant Height:

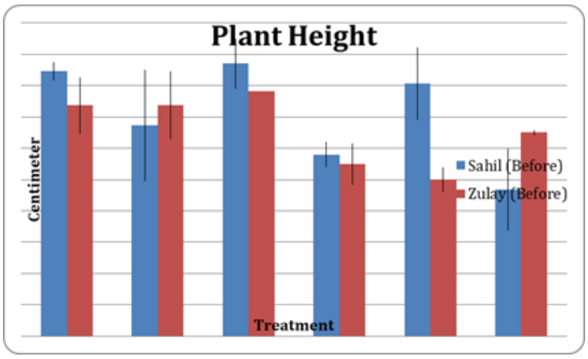

Figure 1: Figure showing plant height of varieties.

When the plant reached maturity, its height was measured. Biologists utilize the plant height parameter extensively. The control replica of Sahil and Zulay had the highest plant height, measuring 84.65 cm. In contrast, the treatment replica of Sahil and Zulay had the lowest plant height, measuring 46.75 cm and 50.03 cm before and after treatment at 50 ppm and 40 ppm, respectively. The lowest plant height in the treatment replica was 45.25 cm and 48.56 cm in 20 ppm and 20 ppm of Sahil and Zulay, respectively.

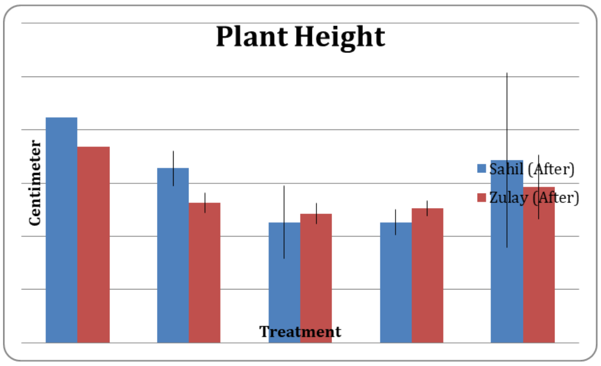

Figure 2: Figure showing Plant height of varieties.

Stem Diameter:

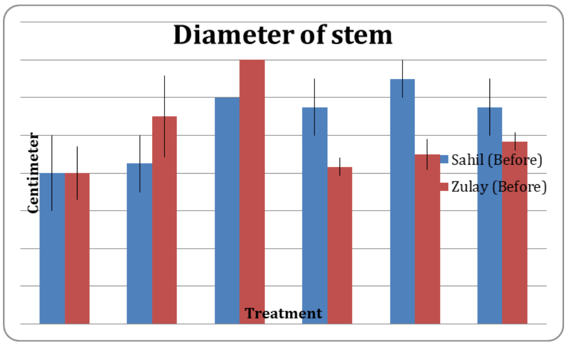

Figure 3: Figure showing Stem diameter of varieties.

The diameter of the stem was measured at maturity, a parameter also widely used among biologists. The diameter of the stem was 0.4cm and 0.4cm observed in control replicas of Sahil and Zulay, respectively. The Highest stem diameter was 0.65cm and 0.7cm before the treatment of 40ppm and 20ppm of Sahil and Zulay, respectively.

The lowest stem diameter was 0.4cm and 0.41cm before treatment of 20ppm and 30ppm of Sahil and Zulay respectively while the highest stem diameter after treatment was 0.57cm and 0.53cm in 10ppm and 40ppm of Sahil and Zulay respectively and the lowest stem diameter in after treatment was 0.40cm and 0.36cm in 20ppm and 20ppm of Sahil and Zulay respectively.

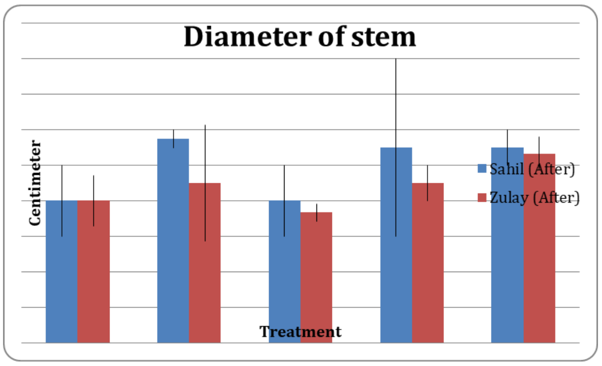

Figure 4: Figure showing Stem diameter of varieties.

Leaf Area:

The area of the leaf was measured at maturity. The area of leaf parameters is also widely used among biologists. The area of the leaf was 8.60m^2 and 15.5m^2 observed in the control

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replica of Sahil and Zulay respectively and the Highest Area of a leaf was 23.73m^2 and 23.08m^2 before treatment of 20ppm and 50ppm of Sahil and Zulay respectively. The lowest Area of a leaf was 10.14m^2 and 13.64m^2 before treatment of 30ppm and 30ppm of Sahil and Zulay respectively while the highest Area of a leaf after treatment was 21.09m^2 and 17.27m^2 in 30ppm and 10ppm of Sahil and Zulay respectively and the lowest Area of leaf in after treatment was 7.4m^2 and 10.7m^2 in 20ppm and 30ppm of Sahil and Zulay respectively.

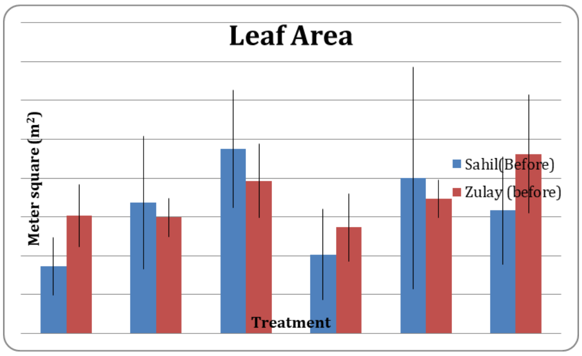

Figure 5: Figure showing leaf area of varieties.

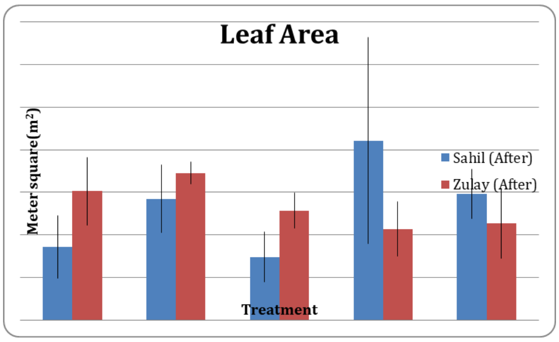

Figure 6: Figure showing leaf area of varieties.

The highest Area of leaf after treatment was 21.09m^2 and 17.27m^2 in 30ppm and 10ppm of Sahil and Zulay respectively and the lowest Area of leaf after treatment was 7.4m^2 and 10.7m^2 in 20ppm and 30ppm of Sahil and Zulay respectively.

Numbers of Leaves per Plant:

The number of leaves was measured at maturity. Several leave parameters are also widely used among biologists. The number of leaves 14 and 10 were observed in the control replica of Sahil and Zulay respectively and the Highest Number of leaves was 28 and 17 before treatment of 20ppm and 20ppm of Sahil and Zulay respectively.

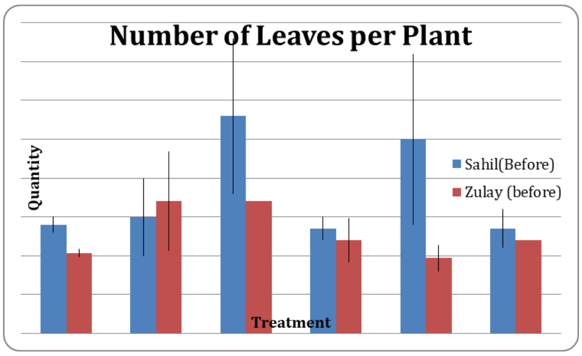

Figure 7: Figure showing number of leaves per plant of varieties.

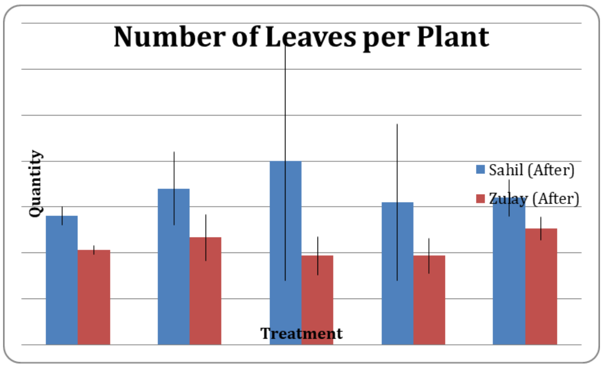

Figure 8: Figure showing number of leaves per plant of varieties.

The lowest Number of leaves was 13.5 and 9.6 before treatment of 30ppm and 40ppm of Sahil and Zulay respectively while the highest Number of leaves after treatment was 20 and 12.6 in 20ppm and 40ppm of Sahil and Zulay respectively and the lowest Number of leaves in after treatment was 15.5 and 9.6 in 30ppm and 30ppm of Sahil and Zulay respectively.

Number of Flower per Plant:

The number of flowers was measured after the floral bud opening. The number of flower parameters is also widely used among biologists. The number of flowers was 15.5 and 6.6 observed in the control replica of Sahil and Zulay respectively and the Highest Number of flowers was 20.5 and 12 before treatment of 10ppm and 10ppm of Sahil and zulay respectively. The lowest number of flowers was 12.5 and 6.6 before treatment of 50ppm and 30ppm of Sahil and zulay respectively while the highest number of flowers after treatment was 15 and 11.3 in 10ppm and 30ppm of Sahil and zulay respectively and the lowest Number of flower in after treatment was 11 and 6 in 30ppm and 20ppm of sahil and zulay respectively.

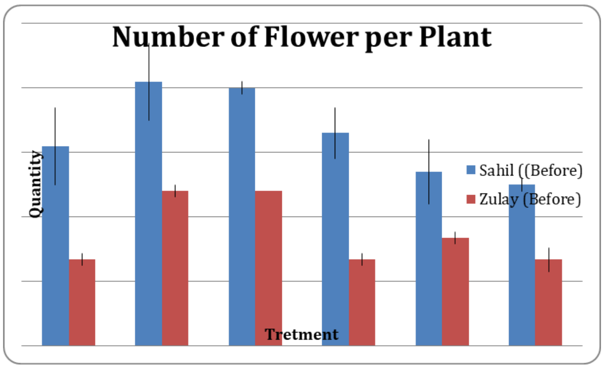

Figure 9: Figure showing number of flowers per plant of varieties.

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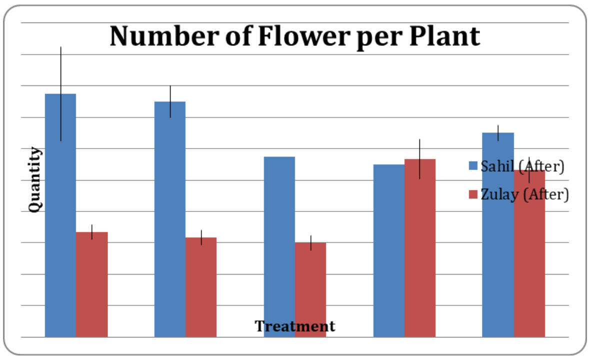

Figure 10: Figure showing number of flowers per plant of varieties. Number of Fruits per Plants:

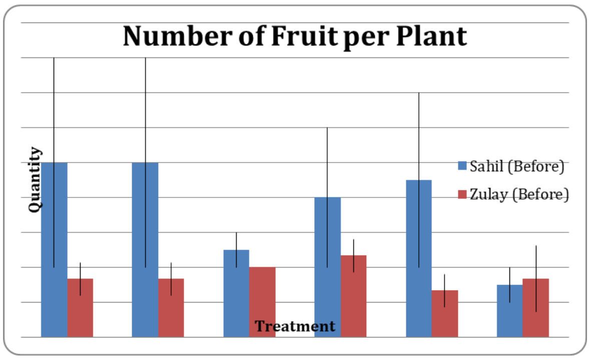

Figure 11: Figure showing some fruit per plant of varieties.

Several fruits were measured after ripening. The number of fruits parameter is also widely used among biologists. Many fruits 5 and 1.6 were observed in the control replica of Sahil and zulay respectively. The Highest Number of fruits was 5 and 2.3 before treatment of 10ppm and 30ppm of sahil and zulay respectively. The lowest number of fruits was 1.5 and 1.3 before treatment of 50ppm and 40ppm of Sahil and zulay respectively while the highest number of fruits after treatment was 5 and 2.3 in 20ppm and 10ppm of Sahil and zulay respectively and the lowest Number of fruits in after treatment was 3 and 2 in 10ppm and 40ppm of sahil and zulay respectively.

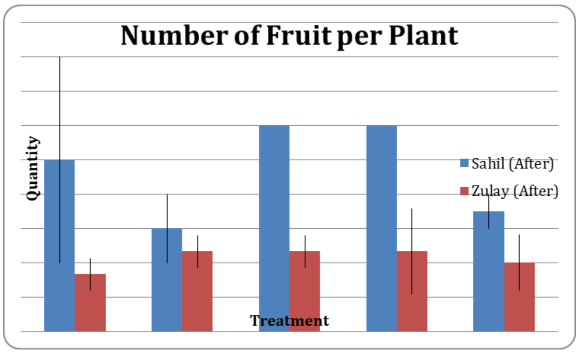

Figure 12: Figure showing number of fruits per plant of varieties.

Physiological Parameter

Chlorophyll Content:

Chlorophyll content was measured by leaf extract. The chlorophyll content parameter is also widely used among biologists. Chlorophyll content was 0.9498 and 1.3955, Chlorophyll b content was 2.4986 and 3.6872 and Total Chlorophyll content was 3.4486 and 4.9117 was observed in the control replica of Sahil and zulay respectively. The Highest Chlorophyll content was 9.1702(in T3) and 3.2937(in T3), Chlorophyll b content was 27.34(in T3) and 8.2145(T3) and Total Chlorophyll content was 36.51(in T3) and 11.21(in T3) was observed in before treatment of Sahil and zulay respectively. The lowest Chlorophyll content was 2.1714(in T1) and 1.0287(in T1), Chlorophyll b content was 8.2488(in T5) and 2.49(T1) and Total Chlorophyll content was 11.57(in T4) and 3.43(in T1) was observed in before treatment of Sahil and zulay respectively. The Highest Chlorophyll content was 13.81(in T4) and 4.3175(in T4), Chlorophyll b content was 9.0133(in T2) and 7.4633(in T4) and Total Chlorophyll content was 16.7163(in T4) and 11.4885(in T4) was observed in after treatment of Sahil and zulay respectively. The lowest Chlorophyll content was 0.7419(in T3) and 1.3323(in T1), Chlorophyll b content was 3.9284(in T3) and 4.0454(in T1) and Total Chlorophyll content was 4.95904(in T3) and 5.17854(in T1) was observed in After treatment of Sahil and zulay respectively.

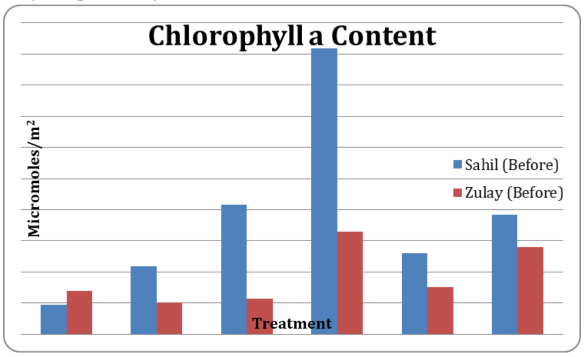

Figure 13: Figure showing chlorophyll content of varieties.

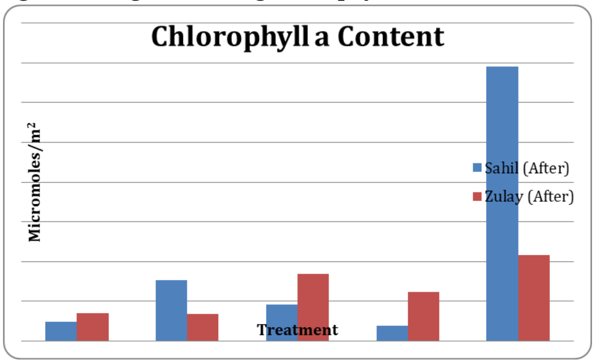

Figure 14: Figure showing chlorophyll content of varieties.

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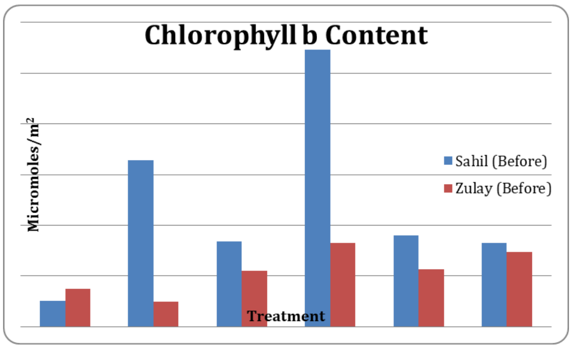

Figure 15: Figure showing chlorophyll b content of varieties.

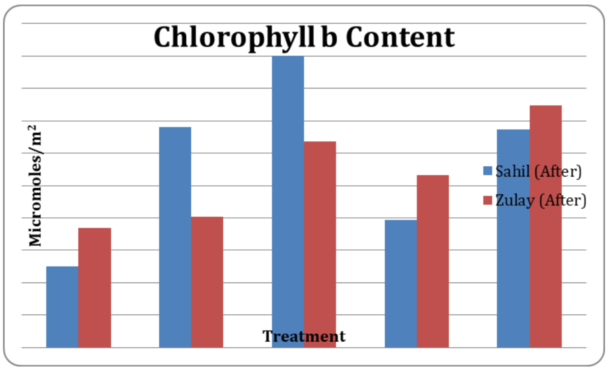

Figure 16: Figure showing chlorophyll b content of varieties.

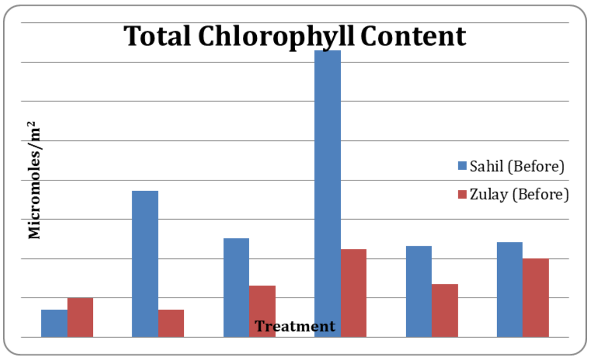

Figure 17: Figure showing the total chlorophyll content of varieties.

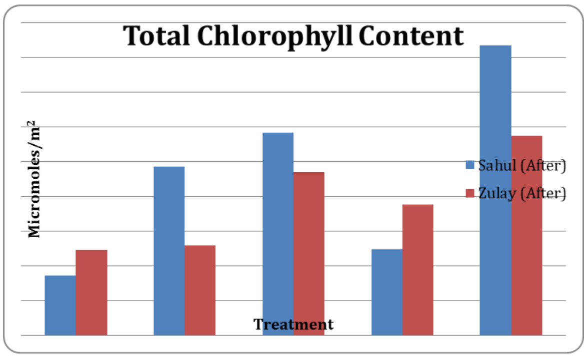

Figure 18: Figure showing the total chlorophyll content of varieties.

Relative Water Content:

The relative water content was measured by taking the leaves of plants. The relative water content parameter is also widely used among biologists. The relative water content was

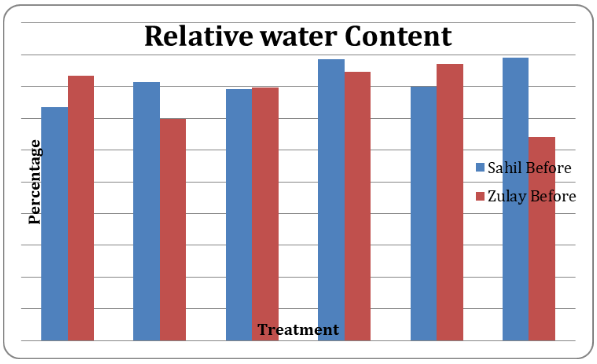

Figure 19: Figure showing relative water content of varieties.

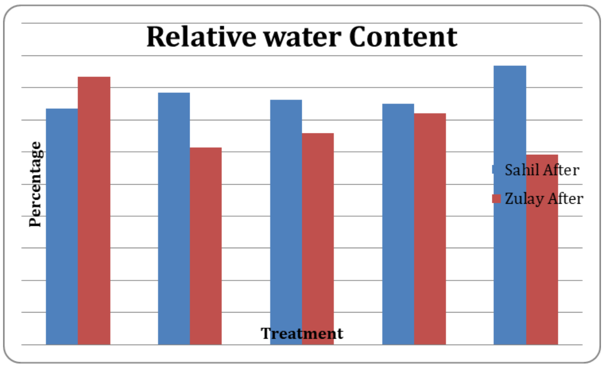

Figure 20: Figure showing relative water content of varieties.

73.5823 and 83.3945 were observed in the control replica of Sahil and zulay respectively. The Highest relative water content was 89.03402 and 87.07483 before treatment of 50ppm and 40ppm of Sahil and zulay respectively and the lowest relative water content was 79.14508 and 69.70437 before treatment of 20ppm and 10ppm of Sahil and zulay respectively while the highest relative water content in after treatment was 86.89065 and 72.10164 in 40ppm and 30ppm of Sahil and zulay respectively and the lowest relative water content in after treatment was 74.86611 and 59.08883 in 30ppm and 40ppm of sahil and zulay respectively.

Biochemical Parameter

Total Sugar Content:

The total sugar content was measured by taking the leaves of plants. The total sugar content parameter is also widely used among biologists. The total sugar content was 10.695 and 6.52 was observed in the control replicas of Sahil and zulay respectively. The Highest total sugar content was 9.545 and 9.745 before treatment of 10ppm and 50ppm of sahil and zulay respectively and the lowest total sugar content was 6.39 and 5.655 before treatment of 30ppm and 40ppm of sahil and zulay respectively while the highest total sugar content in after treatment was 9.75 and 11.725 in 40ppm and 10ppm of sahil and zulay respectively and the lowest total sugar content in after treatment was 3.1 and 6.015 in 20ppm and 30ppm of sahil and zulay respectively.

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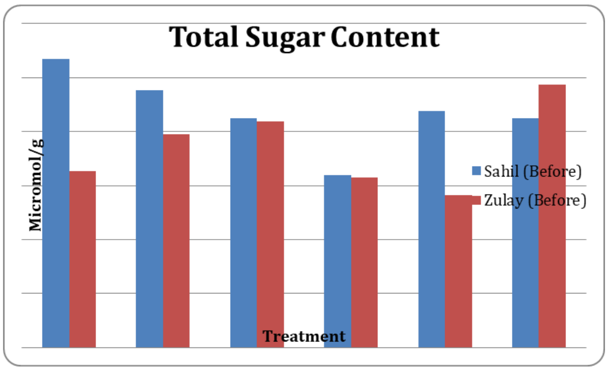

Figure 21: Figure showing the total sugar content of varieties.

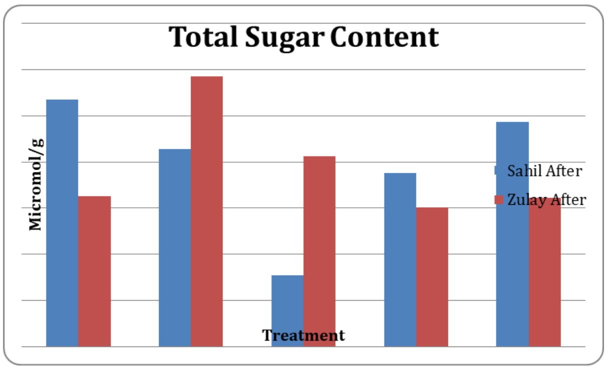

Figure 22: Figure showing the total sugar content of varieties.

Phenolic Content:

The Phenolic content was measured by taking leaves of plants. The Phenolic content parameter is also widely used among biologists. The Phenolic content was 0.34 and 0.22 were observed in the control replica of Sahil and zulay respectively. The Highest total

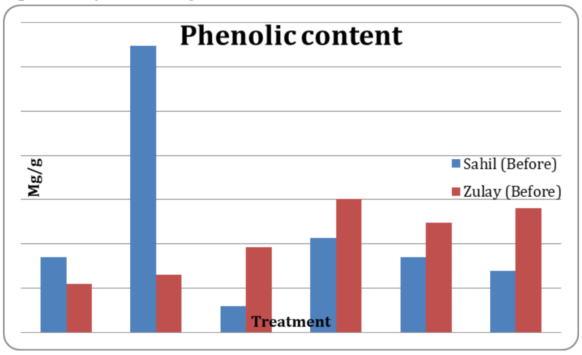

Figure 23: Figure showing the total phenolic content of varieties.

Phenolic content was 1.2955 and 0.602 before treatment of 10ppm and 30ppm of Sahil and zulay respectively and the lowest Phenolic content was 0.1185 and 0.26 before treatment of 20ppm and 10ppm of Sahil and zulay respectively while the highest Phenolic content in after treatment was 0.6545 and 0.8345 in 30ppm and 10ppm of Sahil and zulay respectively and the lowest Phenolic content in after treatment was 0.278 and 0.465 in 10ppm and 30ppm of Sahil and zulay respectively.

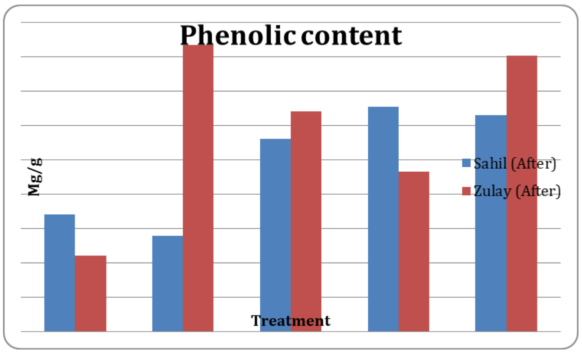

Figure 24: Figure showing the total phenolic content of varieties.

Conclusion

It can be concluded that TiO_2 Nanoparticles can be synthesized by green nanotechnology. Plant-mediated TiO_2 nanoparticles have a significant effect on plant growth. TiO_2 nanoparticles have elicited 75%, 175%, 17%, 173%, and 45% increases in the diameter of the stem, area of the leaf, relative water content, phenolic content, and total sugar content respectively. TiO_2 nanoparticles have also shown a positive impact on plant growth under induced biotic stress in most cases as plant growth increases with the increase of TiO_2 nanoparticles. Furthermore, it can be concluded that TiO_2 nanoparticles have both positive and negative impacts on tomato plants. The following recommendations for future studies are; As due to a lack of equipment nanoparticle characterization should be done through advanced techniques such as Zeta, DLS, SEM, TEM, XRD, etc. As this study was conducted via a spectrophotometer process further proteomics and genomics studies should be done. A mechanistic study should be done to evaluate the mode of the action of TiO_2 nanoparticles.

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