

**GREEN SYNTHESIS OF TITANIUM NANOPARTICLES FROM AERVA JAVANICA
FOR ENHANCED CROP GROWTH – TOWARDS SUSTAINABLE BIOFERTILIZER
DEVELOPMENT**

PROJECT WORK

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Science degree in Microbiology

By

KIRUTHIKA T (41300019)



**SCHOOL OF BIO & CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY**

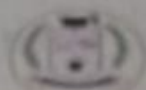
SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of KIRUTHIKA T (41300019) who carried out the project "GREEN SYNTHESIS OF TITANIUM NANOPARTICLES FROM AERVA JAVANICA FOR ENHANCED CROP GROWTH TOWARDS SUSTAINABLE BIOFERTILIZER DEVELOPMENT" under our supervision from January 2024 to April 2024.

Internal Guide

Dr. M. Bavanilatha

Head of the Department

Dr. V. RAMESH KUMAR

Submitted for Viva-voce Examination held on 26.04.2024

Internal Examiner

External Examiner

DECLARATION

I, KIRUTHIKA T(41300019) hereby declare that the Project report entitled "GREEN SYNTHESIS OF TITANIUM NANOPARTICLES FROM AERVA JAVANICA FOR ENHANCED CROP GROWTH:TOWARDS SUSTAINABLE BIOFERTIIZER DEVELOPMENT " done by me under the guidance of Dr.Bavanilatha at Sathyabama Institute of Science and Technology, Chennai. Submitted in partial fulfilment of the requirements for the award of a Bachelor of Science degree in Microbiology.

DATE: 26.04.2024

PLACE:CHENNAI

T. Kiruthika
SIGNATURE OF THE CANDIDATE

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ABSTRACT

The increasing population of the world requires novel techniques to feed everyone, which can replace or work along with traditional methods to increase production of agricultural crops. Plant cells are frequently challenged with a wide range of adverse environmental conditions that restrict plant growth and limit the productivity of agricultural crops. Using nanoparticles through seed treatment, foliar spraying on plants, nano-fertilizers for balanced crop nutrition, nano-herbicides for effective weed control, nano-insecticides for plant protection, early detection of plant diseases and nutrient deficiencies using diagnostic kits, and nano-pheromones for efficient pest monitoring, nanotechnology is currently regarded as a promising and emerging approach to be incorporated into agriculture to improve productivity of various crops. This study reports on the use of TiO_2 nanoparticles to enhance germination and seedling in *Abelmoschus esculentus*. Studies on invigoration have been carried out invitro on *Abelmoschus esculentus* seeds treated with varying concentrations (0,1,3, 5, 7, 15 and 20 mg/L) of TiO_2 nanoparticles from *Aerva javanica* prepared in distilled water and sonicator for 15 minutes. The purpose of the treatment was to observe early seedling growth and characteristics of *Abelmoschus esculentus* and to investigate the effect on bioavailability of seed germination. Under greenhouse circumstances, the experiment was conducted. ladies finger seed quality has significantly improved when exposed to nanoparticles at TiO_2 20mg/l. TiO_2 nanoparticles from *Aerva javanica* were created using a wet chemical technique, and their properties were assessed by FTIR, UV, SEM, and XRD spectroscopy. As the concentration of the nanoparticles grew, the shoot and root length Increased.

KEYWORDS: Green synthesis, Titanium nanoparticles, *Aerva javanica*, Sustainable biofertilizer.

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LIST OF ABBREVIATIONS

UV	Ultra Visible Spectroscopy
FT-IR	Fourier Transform Infrared Spectroscopy
SEM	Scanning Electron Microscopy
EDAX	Energy- Dispersive X-ray Analysis
XRD	X-Ray Diffraction
RSG	Relative Seed Germination Rate
RRG	Relative Root Growth
NPs	Nanoparticles
TiO ₂	Titanium dioxide

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CHAPTER-1

INTRODUCTION

1.1 TITANIUM DIOXIDE (TiO₂)

Because of their potential application in cutting-edge medical treatments, metallic and metal oxide nanoparticles (NPs), such as titanium dioxide NPs, among polymeric NPs, liposomes, micelles, quantum dots, dendrimers, or fullerenes, are gaining importance. The inorganic substance titanium dioxide (also known as titania, TiO₂, or titanium(IV) oxide) has gained recent attention in science due to its photoactivity. Following UV light exposure in aqueous media, TiO₂ generates a variety of reactive oxygen species (ROS). The ability to generate reactive oxygen species (ROS) and consequently trigger apoptosis has been employed in photodynamic therapy (PDT) to address a variety of illnesses, ranging from cancer to psoriasis.

The use of titanium dioxide nanoparticles (NPs) in the treatment of malignant tumors and the photodynamic inactivation of bacteria resistant to antibiotics has been investigated. In PDT, TiO₂ NPs alone, in their composites, and in conjunction with other compounds or biomolecules can all be effectively employed as photosensitizers. Furthermore, hybrid materials can be created by grafting different organic molecules onto TiO₂ nanoparticles. These nanostructures have the potential to demonstrate enhanced light absorption, which will enable targeted therapeutic applications in medicine. Several methods using titanium dioxide were tested to increase effective anticancer and antibacterial medicines.

1.2 TITANIUM DIOXIDE (TiO₂) IN BIOFERTILIZER FIELD

The wide range of uses of titania in technology and medicine, including dye-sensitized solar cells, photodynamic therapy, and water remediation, have been the subject of numerous studies recently. These nanoparticles' exceptional photochemical qualities and good biocompatibility make it feasible. Furthermore, TiO₂ particles are readily available and reasonably priced components. The quantity of active sites on the surface of these materials often determines their photosensitizing qualities and production costs. Following radiation, they may

release reactive oxygen species, which disperse from the nanoparticles and cause nearby tissues' cells to die. As a result, there has been much research done on TiO₂ nano- and microparticle uses in photodynamic treatment. TiO₂ nanoparticles can also be used as drug carriers, which enable medication to enter diseased parts of the body without damaging healthy tissues. As a result, great care must be taken to create innovative formulations that limit side effects while directing the active ingredients toward the intended cells. Numerous researches mentioned above suggest that tailored drug delivery methods and nanocomposites can be used to boost the therapeutic efficiency of TiO₂. It is feasible because TiO₂ NPs' surface can be marked with markers or antibodies to direct medication delivery to specific, sick regions. Some of the disadvantages of TiO₂ NPs should be resolved in order to use them more widely. The fact that TiO₂ nanoparticles require UV light for activation places restrictions on their potential uses in photodynamic treatment. Thankfully, a variety of techniques for functionalizing and doping TiO₂ NPs have been established. These changes cause the absorption band of NPs to move toward longer wavelengths, which is advantageous for PDT. Titania can also avoid using UV light directly by combining with other nanoparticles, such as up-conversion nanoparticles. The solubility of titanium dioxide particles is hampered by their tendency to aggregate at physiological pH levels. It was suggested that surfactants or the functionalization of TiO₂ with large substituents like PEG chains be used to avoid this undesirable problem. Additionally, it might make it possible for the nanoparticles to be distributed uniformly, resulting in repeatable dosage with a positive therapeutic outcome. Lastly, it is necessary to address the critical question surrounding the application of TiO₂ in the medical field. The body's fate and the toxicity of the TiO₂ particles are not fully described by the facts that are currently available. Thus, there is an urgent need for in-depth research in this field.

Titanium dioxide nanoparticles (TiO₂ NPs) display an array of effects from negative, to neutral and positive when applied on plants. Importantly, when the right concentrations are selected, these nanoparticles have positive effects on seed germination, the vigor of young and mature plants and can also increase crop yield and nutritional value. They stimulate the production of certain enzymes and proteins, enhance chlorophyll content and photosynthesis, promote nutrient uptake, and

increase stress tolerance. Yet, the underlying mechanism of these beneficial effects is still not fully understood.

1.3 SURFACE MORPHOLOGY AND SHAPE OF TiO₂ NPS

The TiO₂ NPs synthesized by green method can be characterized by SEM, EDAX and PSA methods for shape and surface analyses and various results confirmed the round shape and clustered form of TiO₂ NPs. By using SEM analysis, it is revealed that TiO₂ NPs are in a spherical shape with small pores and normally found in the cluster that forms the bunch type surface (Sethy *et.al.*, 2020, Irshad Abisharani *et al.*, 2019;). Considering that anatase, brookite, and rutile are bulk materials that have tetragonal, big crystal, and rhombus-like crystal shapes, respectively. The majority of the time, TiO₂ NPs have the aforementioned properties; but, as shown in the work by Irshad *et al.* (2020), the TiO₂ NPs derived from *T. portulacastrum* had distinct surfaces and shapes. Green TiO₂ NPs have an intriguing and distinctive surface shape and are made up of porous structures with relatively big crystals. TiO₂ nanotubes have revolutionary possibilities in the environmental business because of their porous properties. The plant material's leaf extracts contain phytochemicals such as terpenoids, steroids, polyphenols, flavonoids, alkaloids, antioxidants, free amino acids, and tannins, which are responsible for the structure's uniqueness. Shimpi *et al.* (2016) recently produced TiO₂ NPs using TTIP solution as a precursor and *Murraya koenigii* (Curry tree) leaf extract. In order to create TiO₂ NPs, he used both ethanolic and aqueous plant leaf extracts. He then compared the results with standard parameters and observed that, in both samples, the nanoparticles prepared from the etholic leaf extract had a perfectly spherical size of 2–15 nm, while the nanoparticles prepared from the aqueous leaf extract had a scattered spherical crystallite shape and a larger size of 15–30 nm. The former's (3.26 eV) band gap was found to be larger than the latter's (3.17 eV) band gap. In addition, the author compared TiO₂ NPs produced using chemical and environmentally friendly methods, finding that the variations in band gap energy were 4.5 eV and 4.3 eV, respectively (Shimpi *et al.*, 2016). he percentage of the finished product that contains the prepared material's ally elements is taken into account as a metric to gauge how well the synthesis technique performed in analyzing the atomic structure of the final product using spot EDX analysis, as reported by several studies. The end product's (TiO₂ NPs)

incorporation of companion elements is the fine parameter used to assess the sustainability and effectiveness of the synthesis process, including whether it is environmentally hazardous or cost-effective. Numerous investigations revealed that TiO₂ NPs somewhat display companion atoms in the finished product. For example, Solano et al. (2019) synthesized green TiO₂ NPs; in their findings, the percentage of Ti and O atoms was 40% and 60%, respectively, while the percentage of Fe atoms was 2%. Ca and K peaks were only slightly visible. The research of Irshad et al. (2020), which showed that TiO₂ NPs had trace amounts of certain contaminants, also showed the same pattern. However, what is known for sure is that green TiO₂ nanoparticles (NPs) have a high absorption spectrum that is specific to green TiO₂ and lies between 2.9 and 4.7 keV (Santhoshkumar *et al.*, 2014).

In the chemical synthesis process, the proportion of ally is greatest, while the percentage of TiO₂ NPs is lower. Tiny amounts of precursor (K) are an ally element in the relatively green production TiO₂ NPs with plant extracts (*T. portulacastrum*, *C. quinoa*). It is imperative to develop a precursor-free method or minimize the usage of synthetic chemicals if achieving the highest proportion of TiO₂ NPs from green material is desired (Sethy *et al.*, 2020,).

1.4 AERVA JAVANICA

Aerva javanica is an important medicinal plant belonging to the family of *Amaranthaceae*. About 20 species of genus *Aerva* have been reported from Asian countries and most of them are used for medicinal purposes (Burkill, 1985). *A. javanica* is locally known as Tuwaim in Arabic and distributed over a broad range of sandy sediments. Various chemical constituents such as steroids, triterpenes, lipids, flavonoids, tannins, saponins, alkaloids, sulphates, carbohydrates and glycosides have been isolated from this species (Emam, 1999, Khan *et al.*, 1982). Traditionally *A. javanica* has been widely used for its therapeutic effects (Deshmukh *et al.*, 2008). It is applied externally to reduce swelling and encourage wound and ulcer healing. The seeds are thought to relieve headaches, and the blossoms and roots are used to treat rheumatism and kidney issues (Muhammad *et al.*, 2011). Gonorrhea, skin diseases, and dysentery are treated with the plant decoction. Besides this, *A. javanica* is drought resistant, good soil binder and help to control soil erosion. According to the criteria of Global Sustainability Assessment System the plant has been categorized among the native species that could be used for

urban landscaping (Global, 2014). Subsequently, propagation and conservation initiatives of *A. javanica* will help to protect its diversity in their natural habitat besides maintaining the natural environmental conditions.

1.5 INADEQUACY OF NUTRIENTS

A widespread deficiency of nutrients in the soil negatively affects soil health, reduces the nutritional content of agricultural goods, and costs farmers money. Large volumes of fertilizer are sprayed to boost crop yields, yet crops usually only consume about half of the fertilizer since most macronutrients are difficult for plants to absorb. Furthermore, plants are unable to use most macronutrients since they are not soluble in soil. The leftover amounts of the applied residual fertilizer either leach out or run off, contaminating the land, the air, and the water. Therefore, using chemical fertilizers to boost productivity may be profitable in the short run, but it will be detrimental to the agroecosystem in the long run (Chugh *et al.* 2021).

Overuse of chemical fertilizers destroys the microflora and health of the soil, upsetting underground food webs and causing genetic mutations that alter the ecology of ecosystems (Rai *et al.* 2015). The general nutritional state of the plant is influenced by a multitude of elements, including temperature, moisture, leaching, nutrient immobilization, surface runoff, overuse of pesticides and herbicides, soil erosion, and poor soil fertility (Mohanty *et al.* 2019). Globally, the addition of different micronutrients and macronutrients including N, P, and K is progressively impeding plant growth and development in agricultural fields (Jha *et al.* 2011). Therefore, in order to improve the functional value of crops and the biota that supports them, sustainable alternatives to chemical fertilizers must be created. These alternatives must build on basic knowledge and use innovation to improve nutrient uptake, value-adding, and environmental remediation. Food security and productivity are two agricultural fields where nanotechnology can systematically overcome obstacles (Pradhan *et al.* 2017). Both macronutrients and micronutrients play important roles in defending plants from illness. Plants' nutritional status can be improved with NPs to increase yields, survive stress, and fend off disease attack (Zhao *et al.* 2020). In order to effectively address nutrient deficiency in agricultural soils, this highlights the necessity for highly focused, site-specific nanotechnological interventions that release the carrier material (nutrient) in a regulated manner.

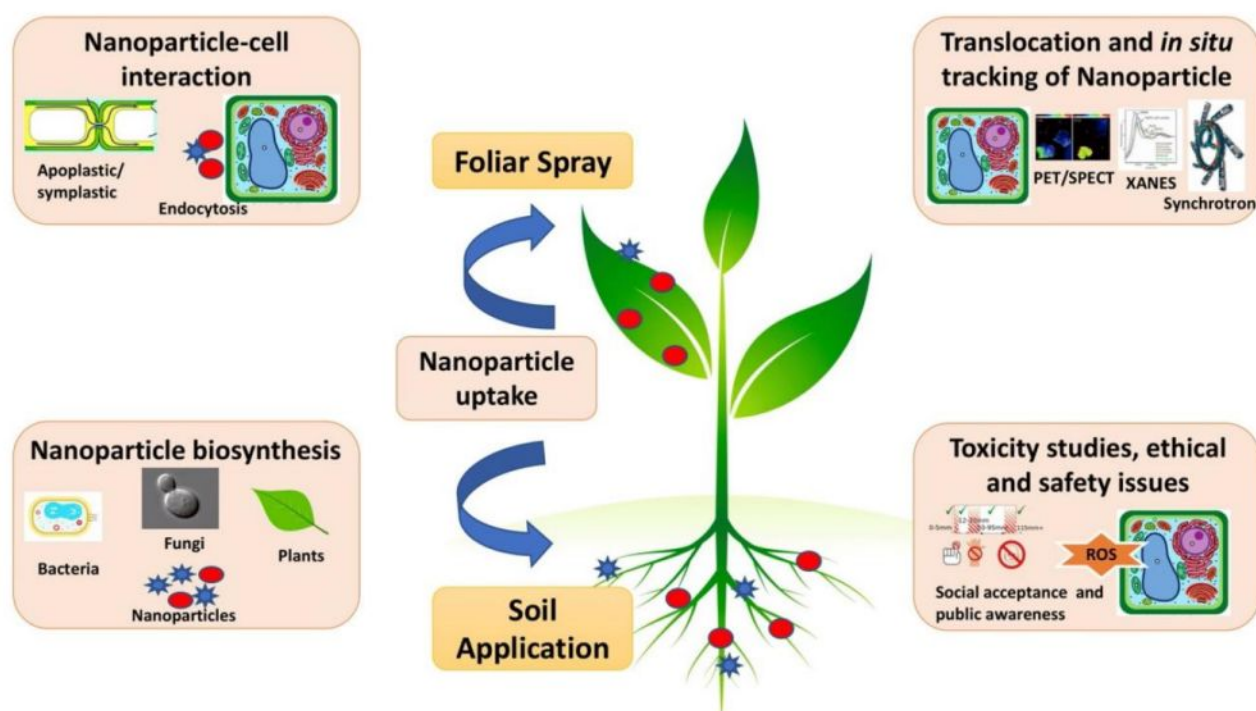


Fig 1.1: Application of Nanobiotechnology in agriculture for combating nutrient deficiencies with nanotoxicity challenge.

1.6 NANO TOXICOLOGY STUDY

It is imperative to conduct a comprehensive and methodical physicochemical characterisation of nanomaterials before examining their potential toxicity *in vivo*. Ensuring the precision and repeatability of the reported cytotoxic effects with the investigated nanoparticles is one of the primary motivations. The physicochemical characteristics of nanoparticles, including size, shape, surface charge, charge density, composition, density of structure, presence of pores, and surface activation sites, greatly impact their toxicity. Correlating the reported biological consequences *in vivo* thus becomes dependent on recording the properties of the nanoparticle being assessed for *in vivo* toxicity. Understanding the characteristics of nanoparticles is essential to comprehending how they behave biologically in a complicated *in vivo* setting. Crucially, characterisation information is necessary to make it easier to compare the toxicity outcomes of a particular nanoparticle with those of other nanoconstructs.

Over the last ten years, significant advancements have been made in the development of instrumentation techniques to adequately characterize the

physicochemical properties of nanoparticles. These techniques include mass spectrometry, elemental analysis, zeta potential measurements for relative particle charge, electron (scanning and transmission) microscopy for six qualitative analyses of size and shape, nuclear magnetic resonance for precise chemical structure determination, X-ray photoelectron spectroscopy for measuring chemical composition, electronic and chemical states, elemental analysis, mass spectrometry, and spectroscopy methods that quantify wavelength or frequency absorption, emission, or scattering. When describing nanomaterials, it's crucial to use caution and assess these attributes in physiologically relevant settings. (Greish et al. 2012; Oberdörster et al. 2005).

1.7 NANOPARTICLE UPTAKE MECHANISM

Abelmoschus esculentus seeds were washed using 2.5% of sodium hypochloride solution for 15 min. After washing the seeds, they were rinsed in distilled water three times and were soaked in various concentrations (3, 5, 7, 15 and 20 ppm) of aqueous titanium oxide nanoparticles for soaking period of 24 hrs at laboratory condition ($30 \pm 1^\circ \text{C}$, 63% RH) in an incubator (dark condition). A thin layer of cotton was placed on each petri dish (90 mm X 15 mm), 3 ml of each nanoparticles suspensions or distilled water were added. 20 seeds were transferred to each petri dish and then it was covered with parafilm and kept for incubation at room temperature.

Following three days of incubation, the length of the roots and shoots was measured, and the number of germination-producing seeds was counted. Three duplicates of each treatment were used, and the mean values were noted. Through endocytosis, aquaporin, and ion channels, nanoparticles attach to the carrier protein to create new pores that allow them to enter the plant cell. Once within a plant cell, nanoparticles can move between cells by means of plasmodesmata via either an apoplast or a symplast. The process of endocytosis, which is responsible for the uptake of nanoparticles by cellular systems, is impacted by the size, shape, an

surface chemistry of the particles as well as the experimental settings used.

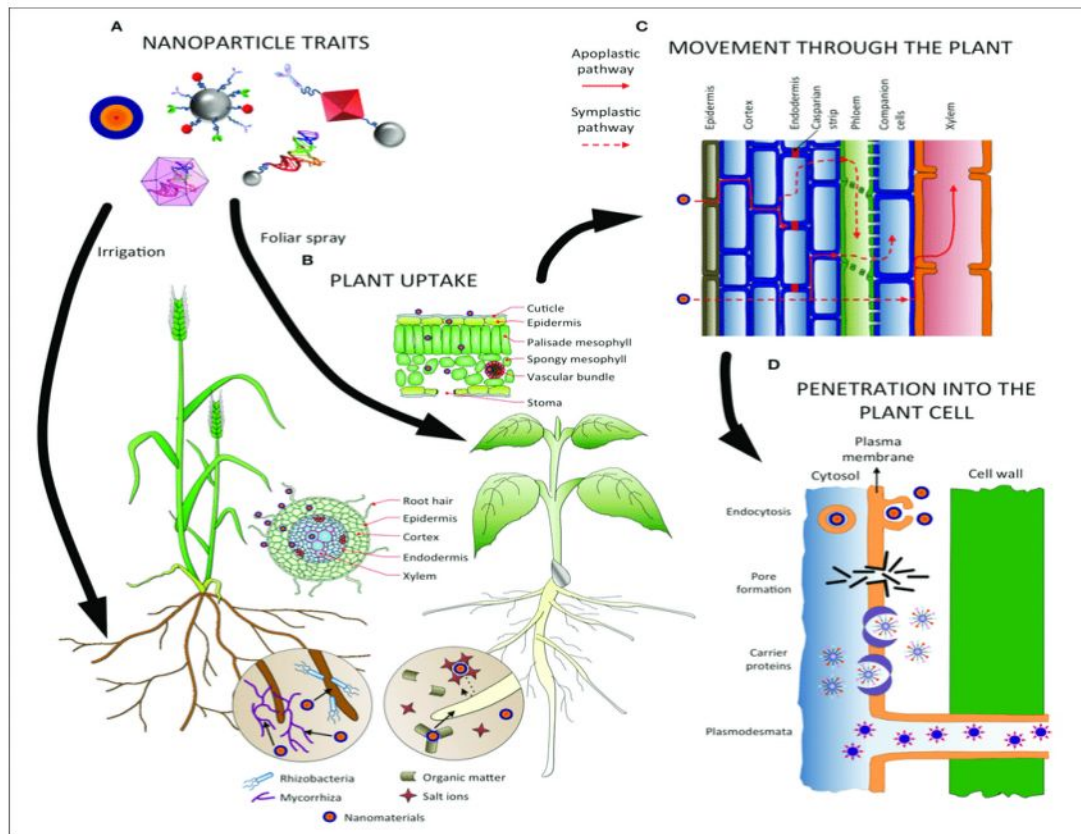


Fig1.2: Factors influencing absorption, uptake, transport and penetration of nanoparticles in plants.

The characteristics of nanoparticles influence both the application technique and how well they are absorbed and transported throughout the plant. (B) Interactions between nanoparticles and compounds in the soil may help or hinder the absorption of such substances. Depending on the entry point (roots or leaves), a number of tissues (epidermis, endodermis, etc.) and barriers (Casparian strip, cuticle, etc.) must be passed before reaching the vascular tissues. (C) Nanomaterials can migrate radially to switch between one pathway and the other, as well as up and down the plant using the apoplastic and/or symplastic pathways. (D) Various methods have been suggested to explain how nanoparticles enter cells, including pore formation, endocytosis, carrier protein-mediated processes, and plasmodesmata.

CHAPTER-2

LITERATURE SURVEY

2.1 TITANIUM DIOXIDE (TiO₂) IN BIOFERTILIZER FIELD

For the synthesis of TiO₂ NPs, chemical vapor deposition, chemical precipitation, micro emulsion hydrothermal, and sol-gel methods have typically been used. However, these synthesis methods have limitations because they require high energy pressure and temperature, as well as the use of toxic chemicals. TiO₂ NPs must thus be developed using environmentally friendly and less expensive synthesis techniques in order to be produced on a wide scale. By using biological extracts as reducing agents in green synthesis processes, it is possible to produce TiO₂ NPs in an environmentally friendly and cost-effective manner, which can also result in large-scale NP production (Edmundson *et al.*, 2014, Chen *et al.*, 1995).

2.2 TITANIUM DIOXIDE NANOPARTICLES

Large volumes of titanium dioxide (TiO₂) nanoparticles are produced globally for usage in a variety of applications. Because of its amazing potential in photocatalysis, solar cells, and the remediation of environmental pollution, nanostructured TiO₂ has attracted a lot of attention in the energy and environmental sectors. TiO₂ nanoparticles also have the benefits of being widely accessible, reasonably priced, and having little toxicity. The information currently known about titanium dioxide's chemical and physical characteristics, manufacture, application areas, health consequences, exposure pathways, limits, and testing methods is compiled in this review. (Castranova V *et al* (2013))

2.3 CHARACTERIZATION OF TiO₂ NPs

After a Successful synthesis using chemical, physical instruments are always utilized to develop sustainable nanomaterials. Authentic characterisation in relation to set material standards is greatly aided by the employment of these specialized tools. demonstrated the promise of cutting-edge methods for the thorough characterisation of TiO₂ NPs.

The round shape and clustered form of TiO₂ NPs produced using the green approach were confirmed by a variety of data obtained from shape and surface examinations using SEM, EDAX, and PSA methods. TiO₂ NPs are spherical in shape, have small pores, and are typically found in the cluster that produces the bunch type surface, according to SEM examination (Sethy *et al.*, 2020)

The majority of the time, TiO₂ NPs have the aforementioned properties; nevertheless, the study's TiO₂ NPs from *T. portulacastrum* had varied surfaces and shapes. Green TiO₂ NPs have an intriguing and distinctive surface shape and are made up of porous structures with relatively big crystals. TiO₂ nanotubes have revolutionary possibilities in the environmental business because of their porous properties. The plant material's leaf extracts contain phytochemicals such as terpenoids, steroids, polyphenols, flavonoids, alkaloids, antioxidants, free amino acids, and tannins, which are responsible for the structure's uniqueness. (Irshad *et al.* (2020))

2.4 SYNTHESIS OF TITANIUM DIOXIDE

TiO₂ nanoparticles are made using a variety of chemical synthesis procedures, including the sol-gel, solvo-thermal, co-precipitation, and hydrothermal methods. Despite the fact that the chemical method of creating TiO₂ nanoparticles is quite popular because it is simple to use and allows for precise control over NP size and shape, there are a number of drawbacks, including high energy costs, high temperature and pressure, ecotoxicity, and sustainability in the environment. Additionally, this restricts their ability to be produced in large quantities and used in a variety of industries. Numerous investigations on chemically produced TiO₂ NPs have been conducted, and their drawbacks and restrictions have also been covered. (Nadeem *et al.*, 2018).

created the TiO₂ NPs using TTIP solution as a precursor and *Murraya koenigii* (Curry tree) leaf extract. In order to create TiO₂ NPs, he used both ethanolic and aqueous plant leaf extracts. He then compared the results with standard parameters and observed that, in both samples, the nanoparticles prepared from the etholic leaf extract had a perfectly spherical size of 2–15 nm, while the nanoparticles prepared from the aqueous leaf extract had a scattered spherical crystallite shape and a larger size of 15–30 nm. The former's (3.26 eV) band gap was found to be larger than the

latter's (3.17 eV) band gap. In addition, the author compared TiO₂ NPs produced using chemical and environmentally friendly methods, finding that the variations in band gap energy were 4.5 eV and 4.3 eV, respectively (Shimpi *et al.*, 2016).

2.5 GREEN SYNTHESIS OF TITANIUM DIOXIDE

The percentage of the finished product that contains the prepared material's ally elements is taken into account as a metric to gauge how well the synthesis technique performed in analyzing the atomic structure of the final product using spot EDX analysis, as reported by several studies. The end product's (TiO₂ NPs) incorporation of companion elements is the fine parameter used to assess the sustainability and effectiveness of the synthesis process, including whether it is environmentally hazardous or cost-effective investigations revealed that TiO₂ NPs somewhat display companion atoms in the finished product. For example, synthesized green TiO₂ NPs; in their findings, the percentage of Ti and O atoms was 40% and 60%, respectively, while the percentage of Fe atoms was 2%. Ca and K peaks were only slightly visible.(Solano *et al.* (2019))

2.6 ENVIRONMENTAL AND ADVANCED SUSTAINABLE APPLICATIONS OF TIO₂ NPs

In agriculture, the main determinants of crop yield and fruit quality are the environmental conditions. However, over the last few decades, climate change has led to a significant increase in the temperature of TiO₂, which has severely muddled the original environment. Other effects include high light intensity, dry and humid winds, irregular precipitation patterns, and a maximum rate of water evaporation and evapotranspiration from the soil. Numerous abiotic challenges, including heat, drought, nutrition, and salt stresses, were brought on by these environmental changes. Crop damage caused by environmental pressures is far more severe than crop damage caused by physiochemical, mechanical, and biochemical causes combined. When plants are exposed to such adverse environmental conditions, they initiate a range of physiological responses and develop the ability to biosynthesize distinct phenolic acids, which fully release their higher antioxidant capacity and, as a result, enhance plant performance in difficult environments. (Baiocco *et al.*, 2011, FAO, 2008).

2.7 TITANIUM DIOXIDE NANOMATERIALS ON PLANTS GROWTH

This chapter discusses how TiO₂ NPs nanoparticles' characteristics such as their size, shape, crystal structure, and surface coating affect plants. Plants are exposed to and interact with these nanoparticles through three main entry pathways: foliar uptake, soil uptake, and seed coating. The impact of varying nanoparticle concentrations is carefully investigated. It has been discussed how exposure duration and pathways impact concentrations that promote plant development. TiO₂ NPs provide numerous advantages for plant development and yield, both when used alone and in complex formulations. A review of these advantages is given. The majority of the information about TiO₂ NPs' effects is derived from transient, high-concentration studies. More long-term studies in more realistic, environmentally relevant settings are required in the future to assess plant growth, nutritional value, the impact of several generations, and the ideal timing and circumstances for application. Although there is still much to learn about the safe use of TiO₂ NPs in agriculture, a lot of effort needs to be done to ensure both agricultural applications and environmental safety.

2.8 SEED GERMINATION AND GROWTH PERFORMANCE OF AERVA JAVANICA

Aerva javanica is a significant member of the Amaranthaceae family of medicinal plants. There are over 20 *Aerva* species known to exist in Asian nations, and the majority of them are utilized medicinally. *A. javanica* is found throughout a wide variety of sandy substrates and is locally known as Tuwaim in Arabic. The seeds are said to be a headache remedy, and the blooms and roots are used to treat rheumatism and renal issue. Gonorrhea, skin diseases, and dysentery are treated with the plant decoction. In addition, *A. javanica* resists drought, is an excellent soil binder, and aids in reducing soil erosion. Based on the guidelines provided by the Global Sustainability Assessment System, the plant is classified as a natural species suitable for urban landscaping. In addition to preserving the natural environmental conditions, *A. javanica* conservation and propagation efforts will aid in safeguarding the species' variety in its natural habitat. In light of this, the current study aims to: (i) create a procedure for *A. javanica* seed propagation in both

laboratory and nursery settings; and (ii) evaluate the growth and survival rates of the chosen plant species for sustainable landscaping.(Muhammad *et al.*, 2011).

2.9 CHEMICAL COMPOSITIONS OF THE AERVA JAVANICA LEAVES AND STEMS

For the first time, the composition of the essential oil of *Aerva javanica* leaves and stems extracted by dry steam distillation is reported in this work. The volume and composition of essential oils and other associated chemicals from aroma-active plants are determined using a variety of extraction techniques. A review of the literature found that hydrodistillation is a popular technique for determining the contents of essential oils. However, because of the potential for compound transformation processes brought on by the method's exposure to heat, steam, and pH, this technique may not be appropriate for non-volatile aroma-active compounds. But with the dry steam distillation method, only steam that is at atmospheric pressure comes into touch with the biomass. The product that prevents the potential of the plant constituents decomposing cannot be ruined or built up to a temperature where it can.(King Saud Univ., 18 (2005) 51).

2.10 ROLE OF NANOPARTICULES IN GROWTH AND DEVELOPMENT OF PLANTS

An examination. Minimize nutrient losses, boost yields, and lessen the need for plant protection measures are the three main purposes of nanoparticles in agriculture. Because of their various qualities, uses, and functions, zinc oxide nanoparticles rank among the most versatile materials. ZnO nanoparticles have amazing optical, physical, and antibacterial qualities. Higher seed germination occurs at lower ZnO NP concentrations. NP concentrations determine how NPs affect germinatio.(Farooqui *et al.* 2016)

2.11 NANOPARTICLES:SYNTHESIS MORPHO PHYSIOLOGICAL EFFECTS AND PROTEOMIC RESPONSES OF CROP PLANTS

On agricultural soil, nanoparticles can have both beneficial and detrimental impacts. Particle size, concentration, and chemistry of NPs, together with the kind of plant species utilized, are the primary determinants defining the type and severity of the cellular responses, according to morphophysiological and proteomic investigations on NPs-induced phytotoxicity(Hossain *et al.* 2020)

2.12 GREEN SYNTHESIS OF COPPER OXIDE NANOPARTICLES USING AERVA JAVANICA LEAF EXTRACT

Thus, environmentally benign processes including using plant extract, microbes, and algae to synthesize metal oxide nanoparticles gained attention. The use of plant extract, however, was preferred over other biological techniques because it is simple to handle, readily available, affordable, and compatible with biomedical applications such drug delivery, cancer treatment, antibacterial and antifungal agent, and insecticide treatment. With the phytosynthesis method, plant extracts are used as a capping and reducing agent to synthesize nanoparticles. The synthesis of CuO-NPs is regarded as a potential NP among metal oxide NPs, such as Ni, Cu, Zn, Au, and Fe; they are less expensive than other Nobel metals. Because of their biocidal qualities, metal oxide nanoparticles (NPs) like CuO have drawn attention and could be a useful component of many biomedical applications, including medication administration, cellular delivery, biomedical imaging, and disease treatment. (M. T. Rahimi *et al.*)

2.13 SYNTHESIS OF METAL NANOPARTICLES IN LIVING PLANTS

By synchronizing the release of nutrients with crop absorption, nanotechnology may be able to create nano-fertilizers that prevent nutrient losses and lower the danger of groundwater pollution. In addition, the nutrients should be released by the nano-fertilizers as needed, avoiding their early conversion into chemical or gaseous forms that are indigestible to plants. This can be accomplished by keeping nutrients apart from the soil, water, and microbes, and releasing them only at the precise moment when the plant is able to absorb them directly. (Marchiol *et al.* 2012)

2.14 TiO₂ NANOPARTICLES CAN IMPROVE MUNG BEAN GERMINATION AND SEEDLING GROWTH

The size and surface characteristics of the nanoparticles affect the absorption rate. Via the cortex and central cylinder, nanoparticles may reach the xylem and gather in the vacuole. The optimal dose limit for seedling growth was revealed by the increase in seed germination and seedling growth at a particular concentration of nanoparticles.(Priya et al. 2020)

2.15 METAL NANOPARTICLE SYNTHESIS,CHARACTERIZATION AND APPLICATIONS

Scientists were able to create metal nanoparticles (NPs) by both top-down and bottom-up methods, involving physical, chemical, and biological processes, by utilizing their metallic properties. The least hazardous and most environmentally friendly techniques were determined to be biological ones. The synthesized NPs can be analyzed and characterized using a variety of analytical techniques, such as size, shape, surface area, functional groups, size distribution, aggregation, dispersion, structure, binding hydrodynamics, and confirmation, to obtain sufficient data to determine the best functionalization or application for these NPs. The primary approaches for identifying the properties of nanoparticles (NPs) have been elucidated and enumerated. These techniques encompass optical, spectroscopic, and microscopic methods such UV spectroscopy, FTIR, SEM, ESEM, TEM, XRD, FCS, SERS, TERs, ZP, CD, MS, STM, DLS, and AFM.(Shnoudeh *et al.* 2019)

2.16 GREEN NANOPARTICLE PRODUCTION,CHARACTERIZATION AND APPLICATION

FTIR, SEM, XRD, and UV spectroscopy were used to analyze the synthesized metal oxide nanoparticles.The range of measured peak values for zinc oxide nanoparticles is 350-380 nm, while the range for copper oxide and iron oxide nanoparticles is 220-280 nm.(Pal *et al.* 2019)

2.17 IMPACT OF METAL NANOPARTICLE-MEDIATED INTERVENTION ON SEVRAL CROPS SEED QUALITY CHARACTERISTICS

It is determined that the type, chemical composition, size, density, preparation technique, and application of nanoparticles all affect how hazardous they are to crops and the environment. Furthermore, a crop's age and kind, as well as the length of time a particular nanoparticle is treated with it, can all have a significant impact on how harmful it is.(N. Singh *et al.* 2021)

2.18 THA APPLICATION OF NANOFORMULATIONS AND NANOPARTICLES IN AGRICULTURE

There is a lot of potential for nanoparticles to increase agricultural output. Because the nanoparticles are frequently insoluble in agricultural goods that humans consume, human toxicity must also be considered.(Pestovsky *et al.* 2017)

CHAPTER-3

AIM AND OBJECTIVES

3.1 AIM

To study Green Synthesis of Titanium Nanoparticles from Aerva Javanica for Enhanced Crop Growth: Towards Sustainable Biofertilizer Development

3.2 OBJECTIVES

- To study toxicity of nanoparticles From Aerva javanica
- Develop a novel biofertilizer by synthesizing titanium nanoparticles from Aerva javanica extract, aiming to enhance crop growth while minimizing environmental impact
- Characterization of nanoparticles; their size, shape, structure, and composition using UV spectrophotometry, SEM, EDAX, XRD, FTIR
- To check the stability of nanoparticles

CHAPTER- 4

MATERIALS AND METHODS

4.1 PLANT COLLECTION AND EXTRACTION

The auxiliary buds of *A.javanica* were collected from way side thickets, Thuraiyur Town, Thiruchirapalli District, Tamilnadu, India, During the month of October 2012. The nomenclature of the plant was authenticated by Botanical survey of India (Government of India, Coimbatore Tamilnadu ,India (BSI/SRc/5/23/2013-2014/Tach/2083).The herbarium specimen was deposited in the department (PU-Boitech-NDRL_04)for futher reference.

4.2. SYNTHESIS OF NANOPARTICLES

4.2.1 Synthesis of *Aerva javanica*

First we took the leaf powder of 2mg from *Aeriva javanica* and boiled it at 100°C,filtered it for 2 times. Extract 20ml from this preparation and for Titanium dioxide solution add TiO₂ of 1.599mg and dissolve in 100ml of distilled water.Took 80ml from this to which we added 20ml of extract which I prepared before and made up to 100ml.Later it was kept in waterbath at 60°C for about 30 min.Kept it in magnetic stirrer for 24hours.Looked for colour change lter centrifuge it at 6000rpm for about 30 min. Pellet was collected and dissolved in 10ml of water and it was powdered. It was characterized using UV,FTIR,XRD,EDAX and SEM and biofertilizer is developed.

4.3 CHARACTERIZATION OF TITANIUM DIOXIDE NANOPARTICLES OF AERVA JAVANICA

The synthesized Titanium oxide were characterized by using spectral-analytical techniques including X-ray Diffraction (XRD) Analysis, UV- Visible Spectrometry, Scanning Electron Microscopy (SEM) Analysis, and Fourier Transform Infrared (FTIR) Microscopy.

4.4 CHARACTERIZATION OF METAL OXIDE NANOPARTICLE

4.4.1 PRINCIPLE OF UV- Visible Spectrometry

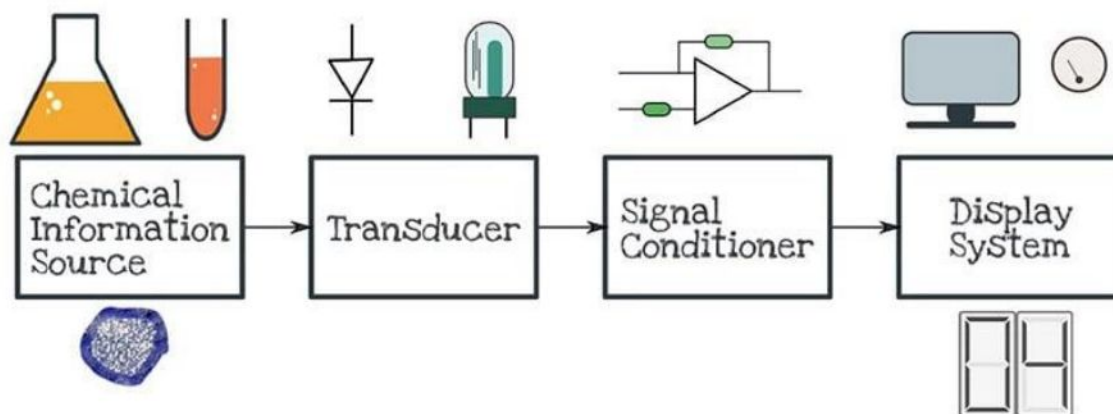


Fig:4:1 Principle of UV- visible spectrometry

The components of this approach include a detector, a monochromator, sample and reference beams, a tungsten lamp for the visible area and a deuterium lamp for the UV. The UV spectrum is obtained by subjecting the material to UV radiation. Cuvettes are held inside the device and are used to hold samples that are to be introduced into the light path. Cuvettes can be made of quartz, silica, glass, or plastic. Glass and plastic cuvettes absorb light below 360 nm, therefore absorbance measurements below that range are not possible with them. Because quartz cuvettes are transparent to wavelengths over 180 nm, they are therefore utilized for measurements of absorption in the UV range.

4.4.2 PRINCIPLE OF XRD

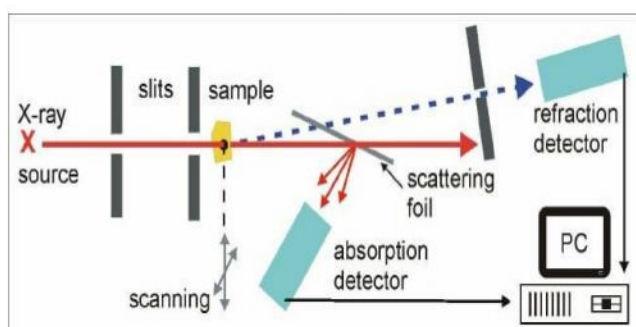


Fig:4:2 Principle of XRD

The most common uses of X-ray diffraction are in the identification of crystalline phases through comparison with a pre-existing database, measurement of atomic spacings (using suitable standards), measurement of the structural characteristics of the crystalline phases that are present (such as preferred orientation and particle size), and investigation of atomic arrangements in amorphous materials. Chemical analysis is frequently used to help identify crystalline materials by determining which components are present. It is possible to ascertain the mass fractions of the phases included in a sample by comparing it to established standards. XRD is frequently one of the first techniques used in the characterisation of nanoparticles because it is a rather rapid and nondestructive procedure. Since diffraction happens when the Bragg condition is met, the electron diffraction pattern resembles the X-ray diffraction pattern (explained below). The electrons' wavelength (λ), the electron beam's angle (θ) with regard to a set of diffracting planes, and the distance (d) between those planes—which is dictated by the material's crystal structure—are related by the Bragg equation ($n\lambda = 2d \sin \theta$). One difficulty with electron diffraction is that the sample frequently multiplies the electrons' dispersed patterns, making it more difficult to comprehend the patterns.

4.4.3 PRINCIPLE OF FTIR

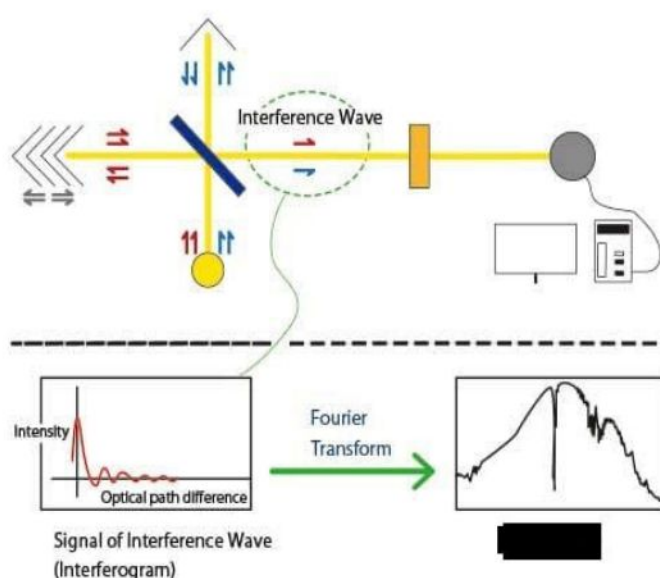


Fig 4.3 :Principle of Fourier Transform Infrared Spectroscopy

In the context of "Green Synthesis of titanium nanoparticles from *Aerva javanica* for enhanced crop growth: Towards sustainable biofertilizer development," Fourier Transform Infrared Spectroscopy (FTIR) refers to a technique used to analyze the chemical composition and structure of the synthesized titanium nanoparticles and the *Aerva javanica* extract. FTIR involves subjecting samples to infrared radiation and measuring the absorption and transmission of the radiation, which provides information about the functional groups and molecular bonds present in the materials. This analysis helps understand the synthesis process, the chemical constituents involved, and the potential interactions between nanoparticles and plant growth-promoting substances, contributing to the development of sustainable biofertilizers.

4.4.4 PRINCIPLE OF SEM

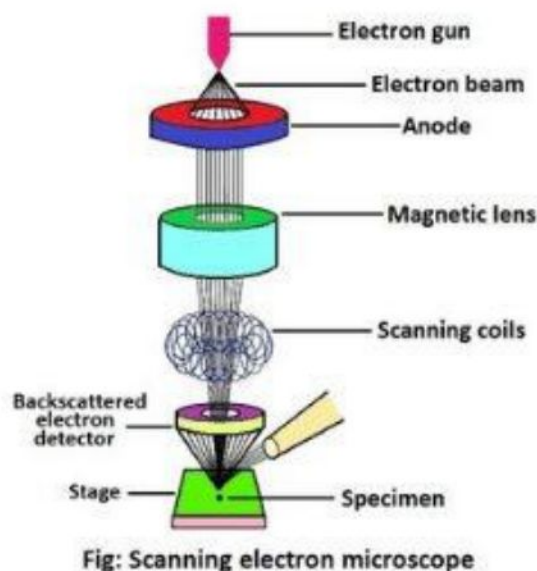


Fig:4:4 Principle of Scanning electron microscope

Unlike an optical microscope, which uses a light beam to see the object, scanning electron microscopy (SEM) uses an electron beam. An electron cannon at the top of the apparatus shoots an extremely focused electron beam. The two primary forms of electron guns are thermionic guns, which heat the filament until the electrons stream out, and field emission guns, which produce a powerful electric field that tears electrons from the atom. High-energy electron beams from the SEM are used to scan the sample's surface. As a result, SEMs are not like traditional light microscopes, which produce a magnified image using light waves. In a scanning

electron microscope, the specimen surface interacts with the electron beam when it impacts it.

4.5 SEEDLING EXPOSURE

The seeds were suspended in double-distilled water to test their viability. The seeds that sank to the bottom were chosen for additional research. After three rounds of rinsing in double-distilled water, the seeds were surface sterilized. Using a sonicator device, the seeds were subjected to a 3-hour sonication in nanoparticle solutions that contained 3, 5, 7, 15, and 20 mg/l. Pots were ready when the seeds were moistened. Study is done on the growth observation

4.6 SEED GERMINATION TEST

This test was conducted on Lady's finger. The seed germination rate (RSG) and relative root growth (RRG) were calculated using the equation and germination index (GI) (Jayarambabu et al. 2014)

$$\text{Relative seed germination Rate} = (SC \backslash SS) \times 100$$

$$\text{Relative root growth} = (RS \backslash RC) \times 100$$

$$\text{Germination index} = (RSG \backslash RRG) \times 100$$

Where SS is the number of seed germinated in sample, SC is the number seed germinated in control, RS is the average root length in sample and RC is the average root length in control

CHAPTER- 5

RESULTS AND DISCUSSION

5.1 SYNTHESIS OF AERVA JAVANICA



Aerva javanica



Plant extract



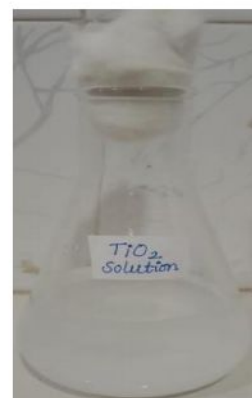
100°C Celsius boil filter



Powderization



Pellet



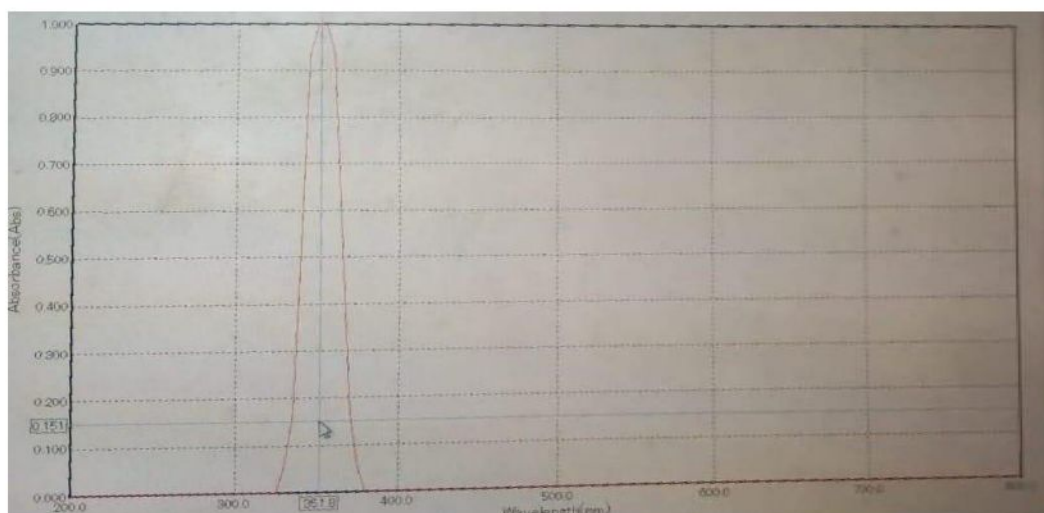
TiO₂ Solution

5.2 CHARACTERIZATION STUDIES

5.2.1 Analysis of Synthesis Titanium dioxide Nanoparticles using UV spectrophotometer

It is generally recognized that UV-Vis Spectroscopy could be used to examine size shape-controlled nanoparticles in aqueous suspensions. Double beam UV-Vis Spectrophotometer (SpectrascanUV-2700, Thermo scientific) was used to analyse the sample

Aerva javanica extract were acted as blank. Small aliquot was prepared by mixing sample solution to sterile distilled water. It was then scanned under UV from 800nm to 200nm. Graph 1 shows the UV-Vis spectra recorded from the reaction mixture after 2 hours. Absorption spectra of Titanium dioxide Nanoparticles formed in the reaction media obtained from aerva javanica has absorbance peak at 360 nm.



UV-Vis of Water solution

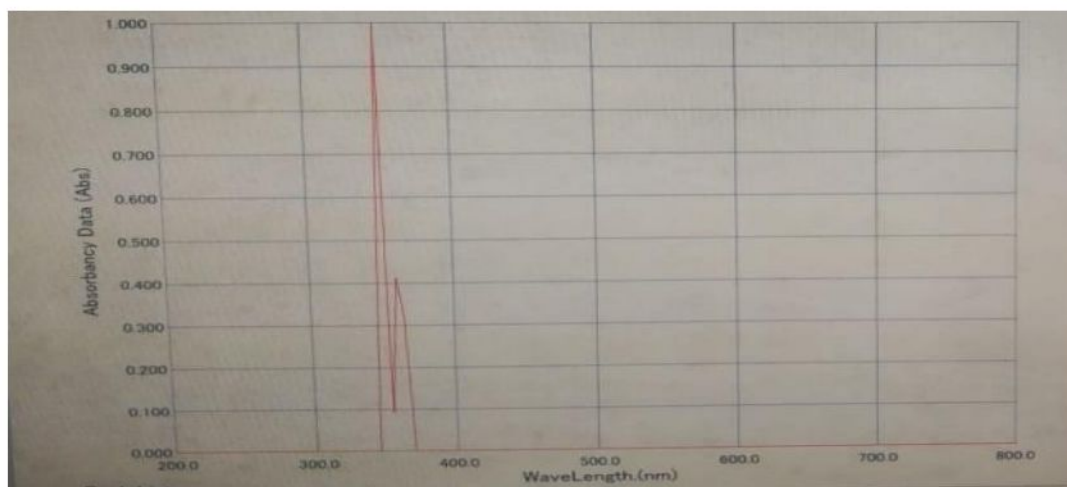


Fig 5.2 UV – Visible Spectroscopy of TiO_2 Nanoparticles

5.2.2 Characterization of synthesized Titanium dioxide nanoparticles by using FTIR

Characterization of synthesized Titanium dioxide nanoparticles by using plant extract of aerva javanica and confirmed under crystallographic structure of the material and the possible compounds in each of the sample was identified using FTIR.

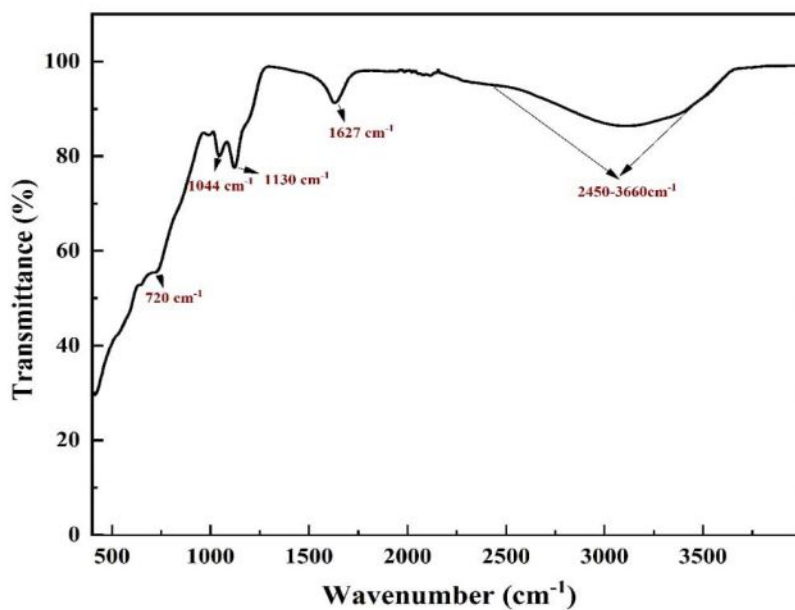


Fig:5.3 FTIR pattern of Titanium dioxide nanoparticles

5.2.3 Characterization of synthesized Titanium dioxide nanoparticles by using XRD

Characterization of synthesized Titanium dioxide nanoparticles by using plant extract of aerva javanica and confirmed under crystallographic structure of the material and the possible compounds in each of the sample was identified using XRD

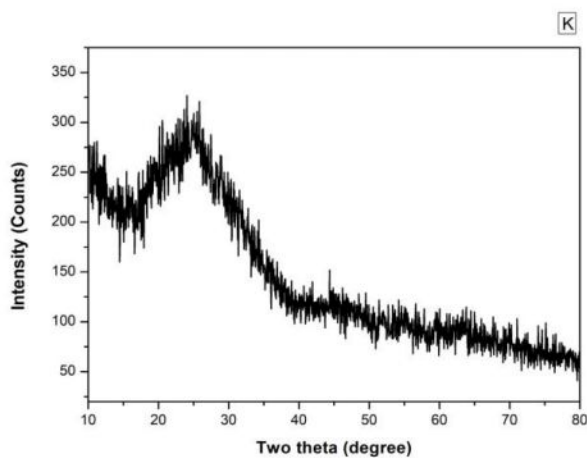


Fig:5.3 XRD Pattern of TiO₂ Nanoparticles

5.2.4 Characterization of synthesized Titanium dioxide nanoparticles by using SEM AND EDAX

Moreover, scanning electron microscopy (SEM) analysis, consistent with the methodologies described in (Lee et al. 2019) and (Kumar et al. 2021), was utilized to examine the morphology and size distribution of the synthesized titanium nanoparticles. The bio-synthesized titanium nanostructure by employing plant extract of *Aerva javanica* was further confirmed by structural view under the scanning electron microscopy and the possible compounds in each of the sample was identified using

EDAX. Using EDAX, the percentage of titanium present in the synthesized titanium nanoparticle solution from *Aerva javanica* is 38.12%.

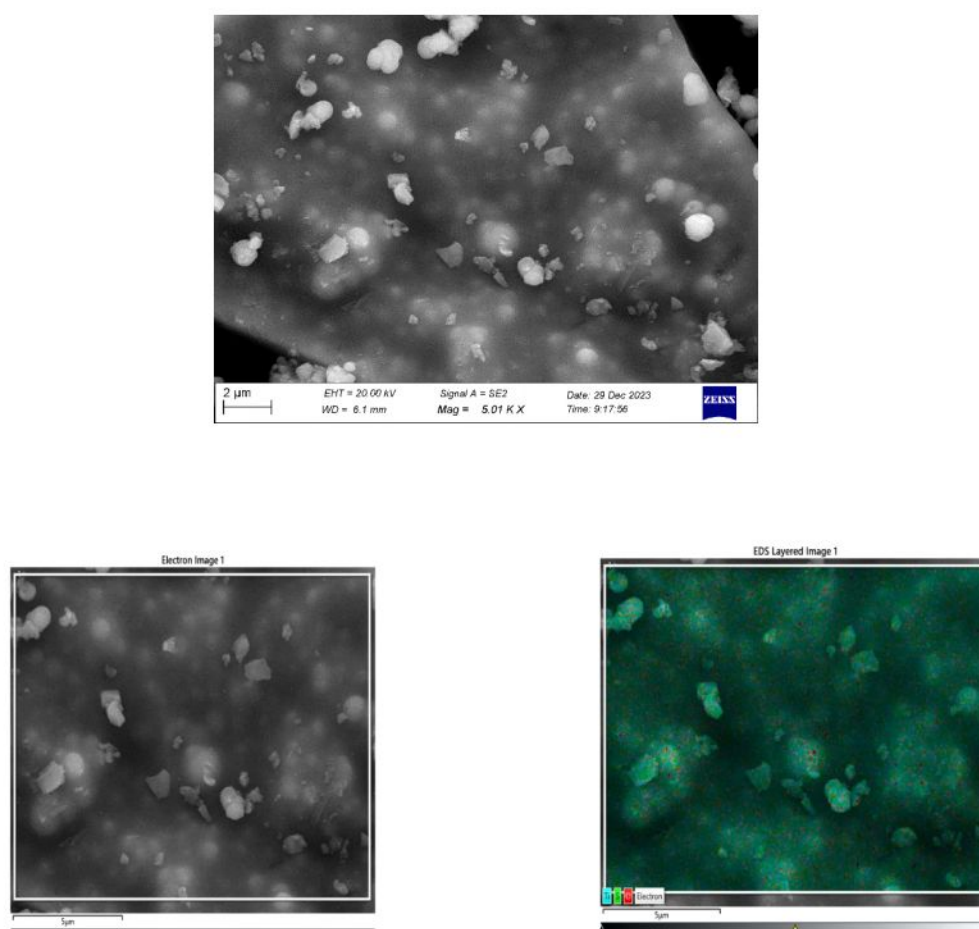


Fig:5.4: SEM analysis of titanium and other compounds from *Aerva javanica*

EDAX

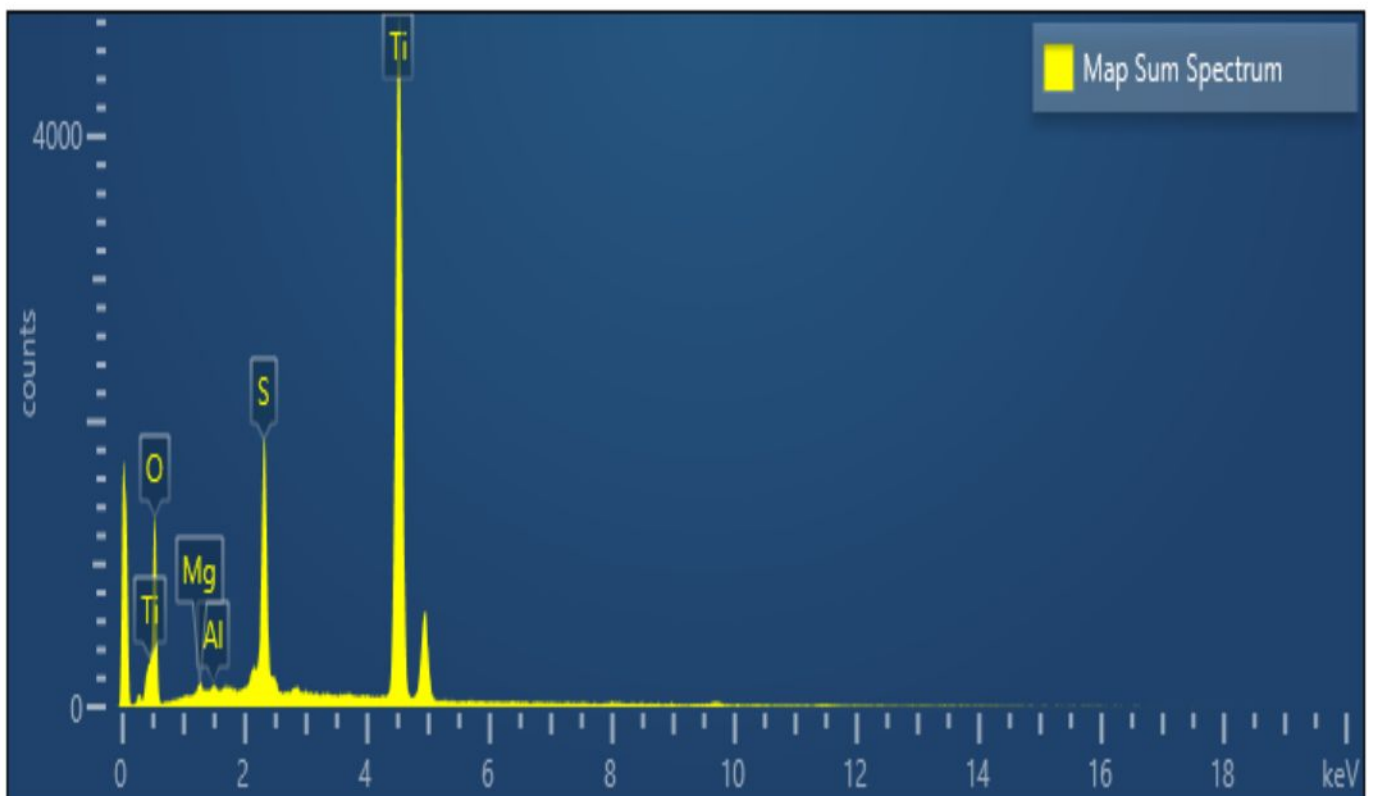
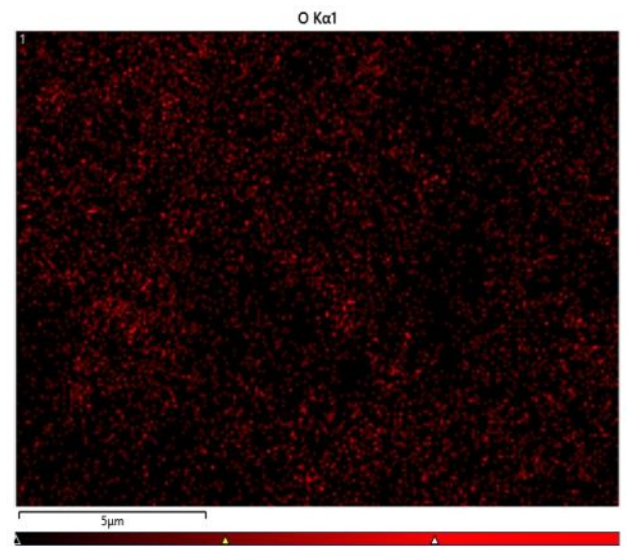
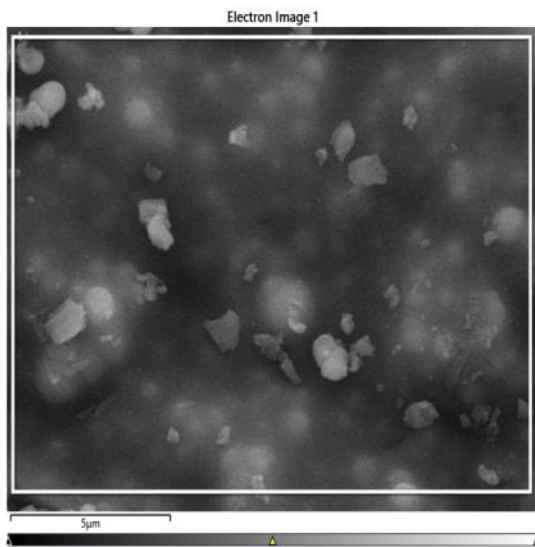


Fig:5.5:EDAX analysis of titanium and other compounds from aerva javanica

**Table 5.1 IR Absorption Frequencies of Organic functional groups Of Aerva
Javaica**

Map Spectrum	Sum			
K series	45.68	Weight %	Weight % Sigma	Atomic %
K series	0.41		0.51	70.03
K series	0.23		0.07	0.42
K series	7.33		0.05	0.21
S			0.12	5.61
Ti	K series	46.34	0.45	23.73
Total		100.00		100.00

5.2.6 SEEDLING EXPOSURE



(a)

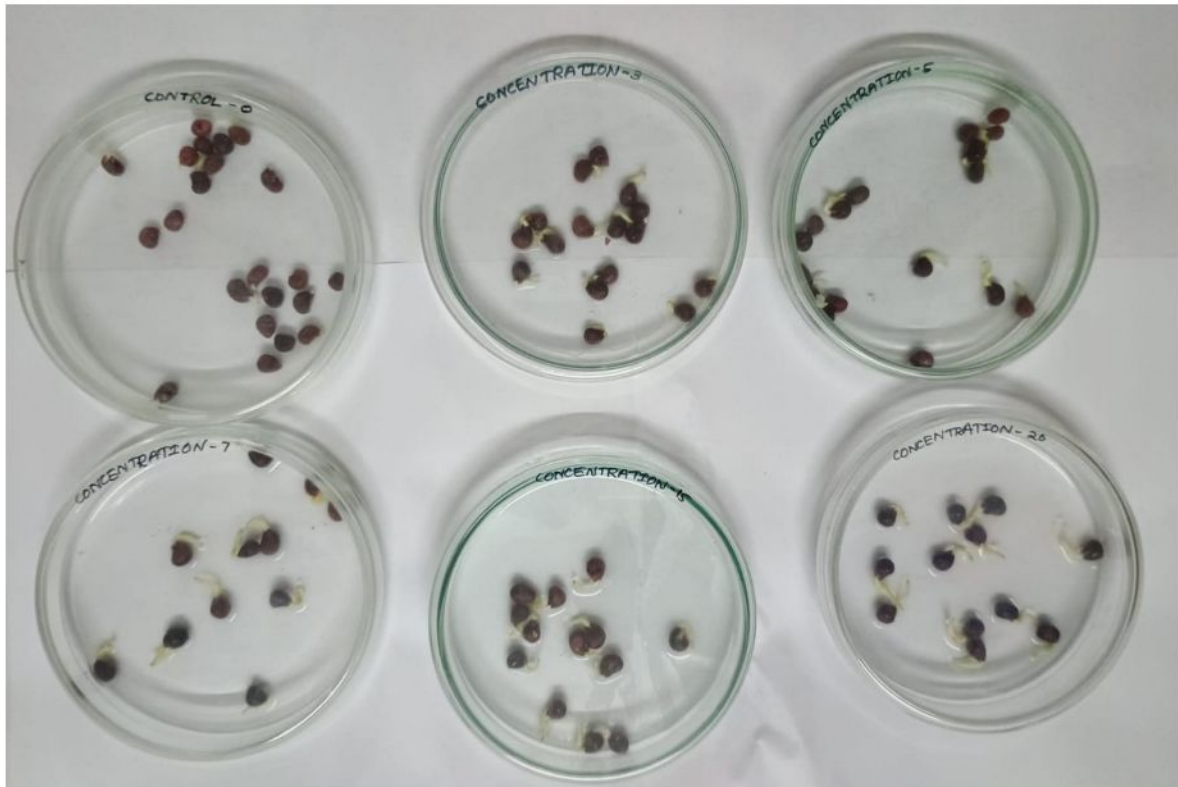


Fig 5.6: seeds soaked in different concentrations of nanoparticles

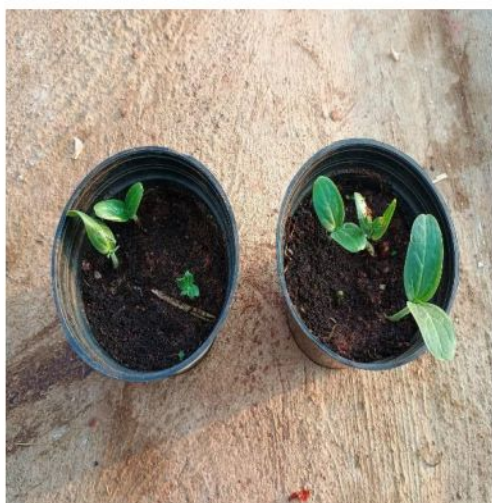
5.2.7 POTTING SEEDS

Nanoparticle treated seeds were transferred to pots. Each pot were labelled with oxides of nanoparticles and their concentration. *Abelmoschus esculentus* seeds were allowed to grow for 20 days and morphological changes were noted



Fig 5.7 germinated seeds were transferred to pot

5.2.8 GERMINATION RATE OF POTTED SEEDS



CONTROL



CONCENTRATION(1,3)



CONCENTRATION(5,7)



CONCENTRATION(15,20)

Fig:5 .8germination of *Abelmoschus esculentus* in different concentrations of nanoparticle

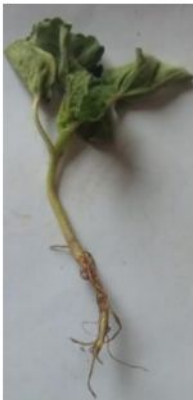
5.2.7 ROOT AND SHOOT LENGTH

Root length was taken from the point below the hypocotyls to the end of the tip of the root. Shoot length was measured from the base of the root-hypocotyl transission zone up 23 to the base of the cotyledons. The root and shoot length was measured with the help of a thread and scale. (Parisi et al. 2015)



(a) CONTROL

5.2.7 ROOT AND SHOOT LENGTH



Concentration (3)



Concentration (5)



Concentration(7)



Concentration(15)



Concentration(20)

Fig 5.9 : shoot growth after 20 days (a) 7mg/l (b) different shoot length

Table 5.2 Measurement (in cm) growth characteristics of Ladys finger at different concentration of TiO₂ NPs (within 8 days)

No. of seeds	C1	C3	C5	C7	C15	C20
1	1	1.5	3	4	5	7
2	1	2	7	7	8	9.5
3	2.2	2.5	7.5	7.8	9.5	10
4	3.2	4	8.3	8	10.8	10.2
5	4.5	7	9	10	11.2	11
6	5	9	11.5	12	11.5	13
7	8	12	14.5	15.5	12.6	15.5
Average length	3.6	5.4	8.6	9.2	9.8	10.9

C1: 1mg/l; C3: 3mg/l; C5: 5mg/l; C7: 7mg/l, C15:15mg/l, C20: 20mg/l

Table 5.3 Average root length (cm)

Nanoparticle/ concentration	C1	C3	C5	C7	C15	C20
Titanium dioxide	3.6	5.4	8.6	9.2	9.8	10.9

90% of the seedlings germinated when the concentration of artificial TiO₂ oxide nanoparticles was 20 mg/l. As the concentration of the nanoparticles rose, the length of the shoots and roots increased. After evaluating the effects of different nanoparticle concentrations on seed germination, found that the highest concentration of TiO₂ nanoparticles improved seed germination as well as the production of shoots and roots. (Hatami *et al.* (2014))

CHAPTER- 6

SUMMARY AND CONCLUSION

Titanium nanoparticles were successfully synthesized using *Aerva javanica* plant extract, with confirmation achieved through a noticeable color change from colourless to white. This visual alteration indicated the formation of nanoparticles a common characteristic observed in many green synthesis methods. Upon characterization using UV -Visible double beam spectrophotometer, the synthesized titanium nanoparticles solutions exhibited a broadening peak at 360nm. This broadening suggests the presence of nanoparticles of varying sizes, indicating a poly-dispersed nature. Furthermore, detailed characterization techniques such as scanning electron microscopy (SEM) and energy -dispersive X-ray analysis (EDAX) were done, revealed the size distribution of nanoparticles with titanium content measured at 38.12% .The SEM images reveal the morphology and size distribution of the nanoparticles, while EDX analysis confirmed the presence of titanium and oxygen elements, further corroborating the successful synthesis of titanium dioxide nanoparticles. Further characterization techniques, including Fourier -transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) also performed. These analyses provided insights into the structural and chemical composition of the synthesized titanium nanoparticles, aiding in a comprehensive understanding of their properties. The XRD patterns obtained revealed distinct peaks corresponding to the crystalline phase of titanium dioxide, affirming the successful synthesis of crystalline nanoparticles. This similarly in the structural characterization underscores the reliability and reproducibility of the green synthesis method across different research studies. The FTIR spectra showed characteristic peaks corresponding to functional groups, providing insights into the chemical composition and surface properties of the synthesized nanoparticles.

Using the chemical reduction approach, TiO_2 from *Aerva javanica* was produced and studied. The effects of varying nanoparticle concentrations (1, 3, 5, 7, 15, and 20 mg/l) on the germination of *Abelmoschus esculentus* seeds and the length of the root and shoot were investigated. Metal oxide nanoparticles are swiftly taken up by the plant and integrated into its metabolic activities. When compared to

other concentrations and the control in this experiment, it was found that the *Abelmoschus esculentus* seeds germination at a 20mg/l concentration suspension solution produced good shoot and root growth outcomes.

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