

CAPITAL UNIVERSITY OF SCIENCE AND
TECHNOLOGY, ISLAMABAD



Cumin Mediated Synthesis of
Silica Nanoparticles and Their
Effects on Green Lettuce
(*Lactuca sativa*)

by

Aiman Fatima

A thesis submitted in partial fulfillment for the
degree of Master of Science

in the

Faculty of Health and Life Sciences

Department of Bioinformatics and Biosciences

2024

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I dedicate my thesis to Prophet Muhammad (SAW) and my parents. They have been a beacon of hope and tremendous joy in my life. I would also like to dedicate this thesis to my future self; for enduring so much hardships and never giving up, for getting back up after every fall, for making it through the darkest nights and loudest storms. Congratulations future Aiman Fatima, you have achieved your life long goal.



CERTIFICATE OF APPROVAL

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Acknowledgement

I am very grateful to Allah Almighty for his countless blessings bestowed upon me. I am grateful for all that I have received and will receive in my life. I am ever so thankful for the countless miracles that took place just for me. I am thankful for the infinite guidance and support of **my parents**. They have been a wonderful blessing for me.

I'm thankful to **Capital University of Science and Technology**. This has been a second home for me for last 6 years. The environment provided here helped me to discover and develop numerous new skills. I would like to acknowledge the help and support of my supervisor **Dr. Sania Riaz**. Her invaluable advice assisted me in conduction and writing of this thesis.

I also like to acknowledge help of my friends in conduction of this thesis. The bonds that were created and the memories that were made will be cherished forever. The unexpected experiences and redirected paths we took together were priceless. I appreciate the help of **Mr. Nouman Shahzad Malik** as well. My thesis would not have been completed without the existence of my friends.

Special shout out to the infinite amount of tea and coffee provided by **my siblings** to make this journey easier. While I was busy burning the midnight oil, my family provided me with lots of love, care and kindness. They helped me keep the hope alive. They motivated me and lift me up whenever I needed them. Thank you so much for being ever so selfless.



(Aiman Fatima)

Abstract

Increasing population worldwide places a lot of stress on food production. The increasing demand for food has been a concern for a long time. The issue is addressed by use of chemical fertilizers. They increase plant biomass and overall yield. However, they also harm the environment and human health. One of the strategies to address this issue is use of nanoparticles as fertilizers. The aim of this research was green synthesis of silicon dioxide nanoparticles (silica NPs) and check their fertilizer activity on *Lactuca sativa* (green leaf lettuce).

Extract of *Cuminum cyminum* (white cumin) seeds was used for synthesis of silica NPs. These were characterized using UV-Vis spectrophotometry, FTIR, SEM-EDX and XRD. Lettuce plants received foliar application of silica NPs (8g/ L). On day 14th, these were harvested and subjected to morphological and biochemical analysis. ANOVA was used for statistical analysis (including Duncan test, $p < 0.05$).

Absorbance at 241nm in spectrophotometry confirmed formation of silica NPs. The size of particles ranged from 72 - 94nm. They were spherical in shape. XRD and EDX both showed presence of silicon and oxygen. XRD also showed that particles were crystalline in nature. An increase in plant biomass was noted in treated plants. Silica NPs enhanced lettuce plants' leaves number, surface area of leaves, length of roots, diameter of roots, weight of fresh and dry leaves and roots. There was increase in chlorophyll a, b and carotenoids. This showed that plants treated with silica NPs had increase in photosynthesis rate. Proline concentration was however, higher in leaves indicating that plants were under stress. Malondialdehyde concentration was low in roots and leaves indicating elevated antioxidant activity.

Based on morphological and biochemical analysis, silica NPs have good fertilizer properties. These can be used for promoting yield of lettuce plants. Green synthesized silica NPs can be further evaluated for their impact on human health and environment. These can hence be produced on large scale as fertilizers. They can also be used in agriculture as fertilizers for growth of other cash crops.

Keywords: Nanofertilizers, silicon dioxide nanoparticles, silica nanoparticles, green synthesis, morphological analysis, biochemical analysis.

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Abbreviations

Ch_a	Chlorophyll a
Ch_b	Chlorophyll b
EDX	Energy Dispersive X-ray
FTIR	Fourier Transform Infrared
LSPR	Localized Surface Plasma Resonance
MDA	Malondialdehyde
NPs	Nanoparticles
SEM	Scanning Electron Microscopy
Silica NPs	Silicon dioxide Nanoparticles
TEM	Transmission Electron Microscopy
UV-Vis spectrophotometry	Ultraviolet- Visible Spectrophotometry
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray Diffraction

Chapter 1

Introduction

1.1 Background

Efficient and sustainable crop production can be achieved with nanoparticles (NPs) (1-100nm particles). The productivity of NPs is high due to small size, slow nutrient release, and targeted delivery. Different nano-fertilizers like MgO, Fe₂O₃, SiO₂ promote growth by improving uptake of calcium, magnesium, iron, zinc, phosphorous and manganese. The use of nanotechnology in agriculture has shown promising results [1]. However, nanotechnology is still a developing field and vast areas remain elusive.

A wide range of applications are there for silica NPs (SiO₂ NPs). Their unique property of biocompatibility, pore size, surface area, surface reactivity, stability etc. make them ideal candidates for different purposes [2]. They are used in pesticide delivery, disease management, drug delivery, increasing nutrition supply, increasing rate of photosynthesis and rate of germination etc. [3]. The applications are further extended because of ease of surface functionalization (allowing biomedicine, biosensing and targeted drug delivery) [2].

There are different methods (physical and chemical) for making silica NPs. Yet these methods have various disadvantages as well like adverse environmental impacts. Use of green synthesis approach to synthesize NPs circumvent the risks

associated with physical and chemical methods. Here bacteria, virus, fungi, yeast, worms, actinomycetes, industrial waste etc. can be used for production of NPs. These does not require use of harsh chemicals, making it an ecofriendly process [2].

Lectuca sativa L. (lettuce, Asteraceae family) is grown in cold season. This leafy green has short life cycle. It is consumed fresh and is used worldwide mainly in salads and sandwiches [4]. While leaf is the common part used for consumption, other parts of lettuce are also used. Stem of lettuce is also used in cooking, drying, pickling or eaten raw in China and Egypt. Lettuce leaves are also used for making non-nicotine cigarettes. Oil is acquired from lettuce seeds. Stem also produces latex which is used for making sedative. *Lactuca virosa* L. is a relative of lettuce and is used in making sleeping pills by pharmaceuticals [5].

A leading grower of lettuce is China. Europe covers a huge portion of lettuce growers as well. Other countries producing lettuce include Japan, India, Turkey, Mexico etc. It was probably introduced in subcontinent by Portuguese or British in 16th century [5].

Lettuce possesses various varieties based on shape, colour and size. They are categorized based on leaf size, leaf shape, head development and type of stem [5]. Table 1.1 shows different lettuce types and their common names.

Lettuce has about 6 - 19% minerals composition of dry weight. It is also a remarkable source of minerals and vitamins. Its value lies in high content of calcium, vitamin C, Vitamin A and iron. Some research studies have reported that calcium content of lettuce is highest among all other vegetables. Low calorie content of lettuce makes it a nexus of many weight loss diets [6, 7]. It can regulate blood pressure and cholesterol. It has the ability to reduce inflammation. In addition, it can improve digestion and appetite [8]. Studies showed that women with lettuce rich diet are less likely to get hip fractures [5].

Since lettuce is a widely consumed vegetable, it's demand is increasing with each passing day. High market demand is addressed by use of fertilizers. These have a negative impact on both humans and environment [4]. Nitrogen fertilizers are

excessively used to grow lettuce because they directly increase plants' yield and growth rate [9].

The nitrates from nitrogen fertilizers accumulate inside the leaves and enter human body. These are converted to nitrites in human body and are hazardous to human skin [10]. Other impacts on human beings include cancers, thyroid problems, diabetes, blue baby syndrome etc. [11]. The environment is affected in terms of soil acidification, eutrophication, increased ozone levels, climate change etc. The end result is adverse impact on humans and other living beings [12].

TABLE 1.1: Various types of lettuce and their common names [5].

Sr. No.	Type	Common Names
1	Crisphead	Ice berg, Head lettuce
2	Latin	Grassé lettuce
3	Romaine	Cos lettuce or Roman
4	Butterhead	Cabbage lettuce
5	Leaf	Leaf or cutting
6	Stem	Celtuce, asparagus lettuce or stalk

1.2 Hypothesis

Silica NPs have been shown to promote growth of many different plants. Hence, they can be used as fertilizer to promote growth of lettuce plant as well.

1.3 Problem Statement

The demand for increased food is tremendously high. Increasing the yield with chemical fertilizers raise environmental concerns. They also have negative impact on human health. Green synthesis of nano-fertilizers can not only increase plant yield but also presents an ecofriendly approach to address the issue of increasing food demand.

1.4 Gap Analysis

Silica NPs have been developed and used on different species to impart disease resistance, as pesticides, biofertilizers and abiotic stress tolerance [3]. Green synthesis approach allows development of nanoparticles in an eco-friendly manner. While silica NPs have been developed using different plants, to the best of my knowledge they have not been made from *C. cyminum*. The synthesized NPs will then be used for checking their effect on lettuce plant.

1.5 Aims and Objectives

The aim of this research was to synthesize silica NPs using green synthesis approach and test their fertilizer nature. The objectives were:

- To synthesize silica NPs using *C. cyminum* seed extract
- To characterize the silica NPs
- To test the silica NPs on lettuce plants for their fertilizer properties

Chapter 2

Literature Review

2.1 Nanoparticles Overview

Nanoparticles (NPs) can be defined as particle of matter that are 1-100nm in size. They have gained importance in advancement of technology due to their physical, chemical and biological properties which can be modified [13]. The small size provides them more functionality in a given volume. The outcome is altered physical properties which are different from their bulk materials. These properties give nanotechnology an edge over their bulk materials [14].

2.1.1 Properties

Different physico-chemical properties like mechanical strength, surface area, optical activity and chemical reactivity can make NPs suitable for different applications [15]. Some of these important properties are discussed herewith.

2.1.1.1 Electronic and Optical Properties

The electrical and optical properties of NPs depend on one another. For example, there are size dependent optical properties in noble metal NPs. Prominent UV-visible extinction band can be noted in these NPs. These bands are not present

in bulk material. When the incident photon frequency is constant with collective excitation of conduction electrons, it leads to the excitation bands. This is known as localized surface plasma resonance (LSPR) [15]. LSPR's peak wavelength is dependent on size, shape and NPs' interparticle spacing. It also depends on local environment like substrates, adsorbates and solvents [16].

Colloidal gold NPs are cause of rusty look in glass doors and windows. The yellow colour is usually due to silver NPs. The free electrons in these NPs are transportable through the nanomaterials. Gold and silver have mean free path of approx. 50nm. This is more than the size of NPs. Hence, when light is made incident on bulk material no scattering is expected. Instead they undergo resonance conditions leading to LSPR [17].

2.1.1.2 Magnetic Properties

NPs with magnetic traits are a center of attention for researchers and businessmen. These are used in variety of arenas like environmental remediation (e.g. decontamination of water), magnetic fluids, heterogenous/ homogenous catalysis, data storage, biomedicine, and magnetic resonance imaging [15]. NPs performance is outstanding when their size is $< 10\text{-}20\text{nm}$ (critical value) [18]. At such small scale the magnetic properties of NPs become quite prominent. This makes them valuable and useful for many diverse applications [19, 20]. The magnetic property is due to uneven electronic distribution. The magnetic properties also depend on synthesis method/ protocol [21].

2.1.1.3 Mechanical Properties

The main distinguishing NPs properties which are focus of research are nanofabrication, surface engineering and nanomanufacturing. Surface coatings, lubrication and coagulation aid in mechanical properties [22]. The mechanical properties of microparticles and bulk material are quite different from their respective NPs. In lubricated or greased contact, the difference in stiffness between NPs and contact surface (when pressure is high) decides if the NPs are going to be deformed or if

they are indented into plain surface. This gives an idea about NPs performance in contact situation. Acquiring control and command on mechanical traits of NPs' and their respective interactions with different surfaces, is crucial quality of surface and increasing material removal [15]. Acquisition of successful insight into mechanical attributes of NPs depend on hardness, modulus of elasticity, friction, laws of movement, interfacial adhesion and size dependent traits [22].

2.1.1.4 Thermal Properties

Metals have better thermal conductivity in solid form than in fluid state. For example, thermal conductivity of copper is 700 times higher than water at room temperature. Furthermore, this is 3000 times higher than engine oil. Water thermal conductivity is also lower than oxides like alumina (Al_2O_3). Hence, suspension fluids have higher thermal conductivity than those of heat transfer fluids. Nanofluids can be produced by dispersion of nanometric scales like solid particles into liquids like oils, ethylene glycol, water etc. Since the transfer of heat takes place at particles surface, so having large surface area is desirable. The stability of suspension is increased with total surface area [15].

2.1.2 Classification

Nanoparticles can be divided into various classes. The classification can be based on particle size, chemical attributes and morphology etc. [15]. NPs classification is discussed below:

2.1.2.1 Carbon Based NPs

The main categories of carbon based NPs are carbon nanotubes and fullerenes. Nanomaterials are constituents of fullerenes. In fullerenes, allotropes of carbon combine to form cage like hollow structure which is globular in shape. They have wide range of applications and commercial demands because of their structure, tremendous strength, versatility, electricity conduction etc. [23]. The carbon

units are arranged in pentagonal and hexagonal pattern. The Fig 2.1 shows C60 and C70 with 7.114 and 7.648nm diameter, respectively [15].

The carbon nanotubes are elongated tubular structures with 1-2nm diameter [24]. Based on their diameter telicity, they can be metallic/ semiconducting [25]. In terms of structure, they are nothing but graphite sheet rolled on itself (Fig 2.2). The rolled sheets can be categorized based on No. of walls as singled walled, double walled and multi walled carbon nanotubes [26].

A common method of their synthesis is by depositing carbon precursors on to metal particles, by vaporization of graphite via electric/ laser arc. They have also been recently made via chemical vapour deposition method [26]. Their unique physical, chemical and mechanical properties, allow them to be used in their pristine form and also as part of nanocomposites. These can be used as fillers [27, 28], environmental remediators as efficient gas adsorbents [29], and as inorganic/ organic catalysts support medium [30].

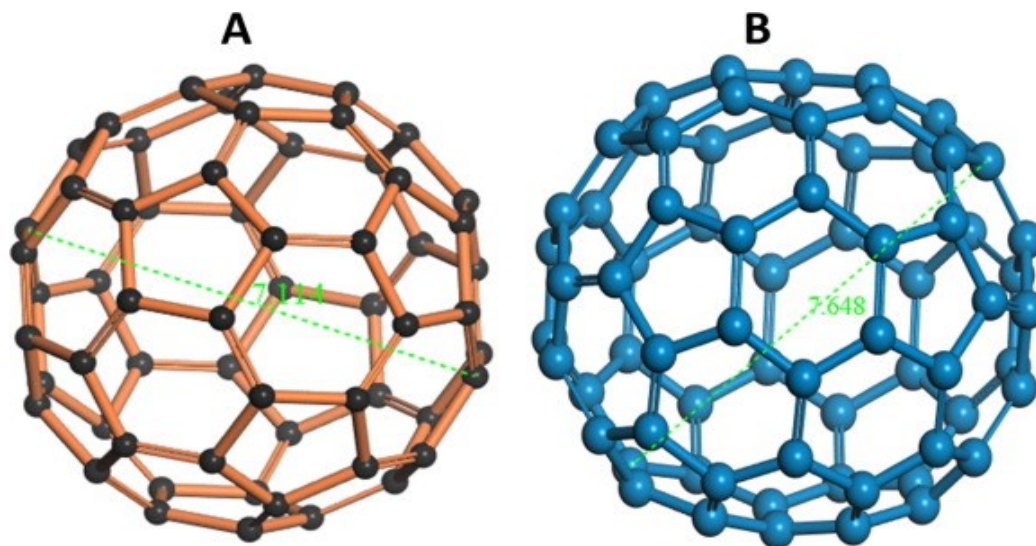


FIGURE 2.1: Fullerene structures of C60 and C70 [15].

2.1.2.2 Metal NPs

They are purely derived from metal precursors. Owing to their localized surface, they possess unique optoelectrical traits. NPs made from noble metals and alkali

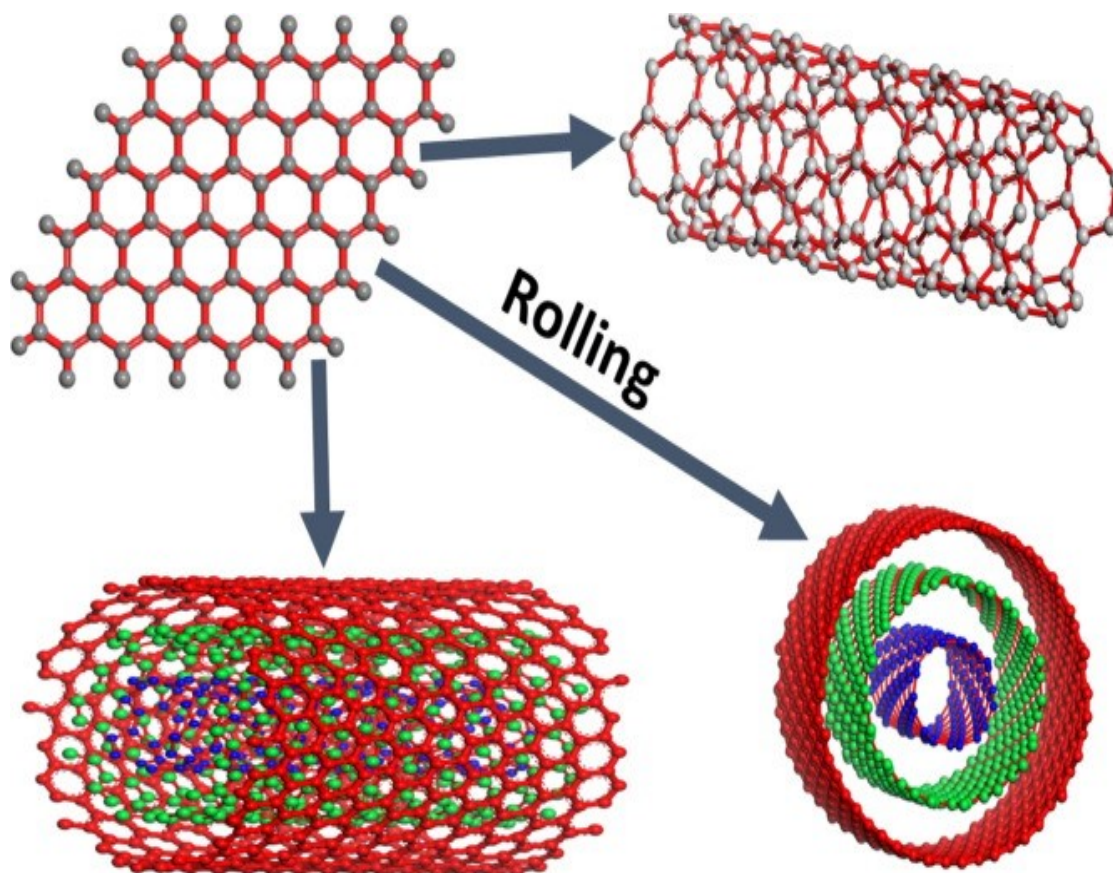


FIGURE 2.2: Graphite sheet which can be rolled to give single and multi walled carbon nanotubes [15].

like silver, copper and gold have absorption bands which are quite broad. These bands fall in electromagnetic solar spectrum's visible zone. In present day technology, the facet, size and shape are very important [31].

Their advance optical properties allow them to be used in diverse fields. Example is use of gold NPs for enhancement of electronic stream, which allows acquisition of high quality SEM image [15].

2.1.2.3 Ceramic NPs

These are non-metal solids which are inorganic. They are synthesized by successive heating and cooling. They can be porous, hollow, amorphous, dense, polycrystalline [15]. These are a center of attention for their applications in photodegradation of dyes, photocatalysis and imaging [32].

2.1.2.4 Semiconductor NPs

Their properties lie between non-metal and metal. This property makes them useful in wide range of applications [33, 34]. Since, their band gaps are wide, there is change in properties with band gap turning. They hence find their applications in electronics, photo-optics, and photocatalysis [15]. Numerous semiconductor NPs are efficient in water splitting because of bandgap and band edge positions [35].

2.1.2.5 Polymeric NPs

They are commonly organic NPs which exists as polymers. Their shape is usually nano-spheric or nano-capsular [36]. Nanospheres which are usually solid particles, have molecules adsorb to the spherical surface at outer boundary. In case of nano-capsules, the solid mass is completely encapsulated by particles [37]. The polymeric NPs find their application in diverse fields due to their ease of functionalization [38, 39].

2.1.2.6 Lipid Based NPs

Lipid moieties are constituents of these NPs. They find their use in many biomedical applications. They generally are spherical in shape and have diameter of 10-1000nm. They have solid core like polymeric NPs. Their matrix has soluble lipophilic molecules. The external core is stabilized via emulsifiers or surfactants [40]. Lipid nanotechnology deals with designing and making of lipid NPs. These are then used as vehicles for drug delivery [41], and transmission of RNA in cancer therapy [42].

2.1.3 Characterization

Various techniques of characterization of NPs exist. Characterization can be done based on morphology, structure, size, surface area and optical properties of NPs.

2.1.3.1 Morphological

Morphology impacts most properties of NPs, and is the center of attention when characterization of NPs is being conducted. Various techniques exist for morphological characterization. The most common ones include polarized optical microscopy (POM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) [15].

All important information is provided by SEM at nanoscale on NPs. It uses scanning electrons for this purpose. It elucidates morphology as well as dispersion of NPs in bulk material [15]. The dispersion of single walled nanotubes in nylon 6 and poly(butylene) terephthalate (PBT) was elucidated via this method [27, 28]. These were also used for POM studies, which revealed star like spherulites of nanomaterials formed. POM also showed that size of material decreased with increasing filling of single walled nanotubes. SEM elucidated morphology of ZnO modified metal organic frameworks (MOFs), in addition to their dispersion under various reaction conditions Fig 2.3 [43].

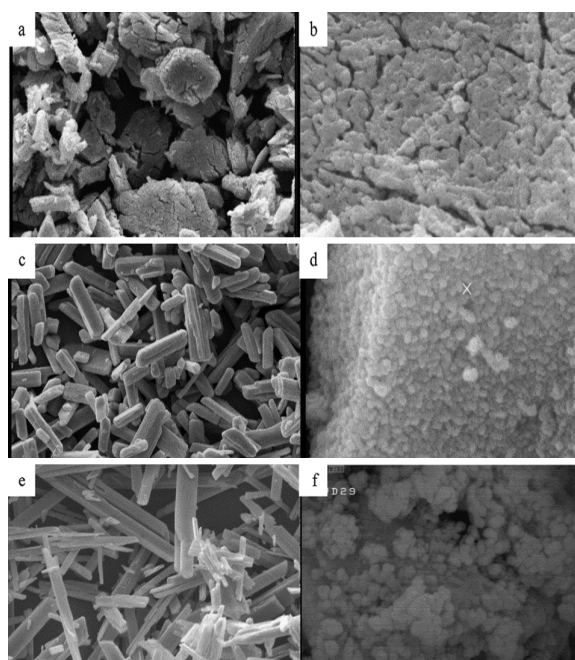


FIGURE 2.3: The images of ZnO modified metal organic framework at different temperatures taken by SEM; a) 180°C, b) Nanostructures at 530°C, c) Micro-rods at 90°C, d) Apohost framework at 550°C, e) Microrods at 550°C, and f) ZnO NPs at 550°C [43].

TEM provides images based on electron transmission. It has good magnification. Various gold morphologies are researched using this technique [44]. Fig 2.4 shows different TEM micrographs. It also gives insight about number of layers of hollow materials, like Co_3O_4 NPs quadrupolar hollow shell structure. These NPs function as anode in Li batteries (Fig 2.5) [15].

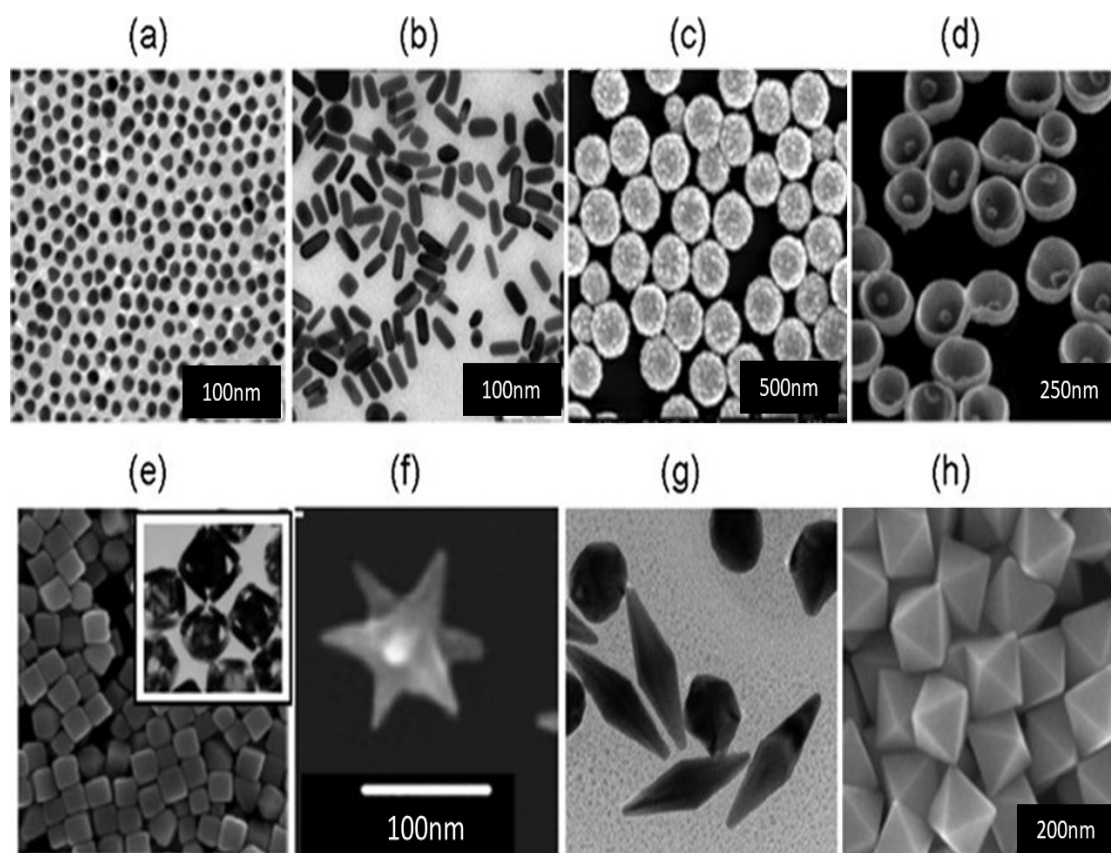


FIGURE 2.4: TEM micrographs of different gold NPs; a) Collidal gold, b) Nanorods, c) Nanoshells, d) Nanobowls with gold seed cargo, e) Ag cubes and Au nanocages, f) Nanostars, g) Bipyramids and h) Octahedrals [15].

2.1.3.2 Structural

Based on composition and type of bonding material, structural traits carry prime importance. Diverse importance is provided about bulk properties of target materials. The techniques involves XRD, Fourier transform infrared (FTIR), energy dispersive X-ray (EDX), BET, raman, zeta size analyzer, X-ray photoelectron spectroscopy (XPS) [15].

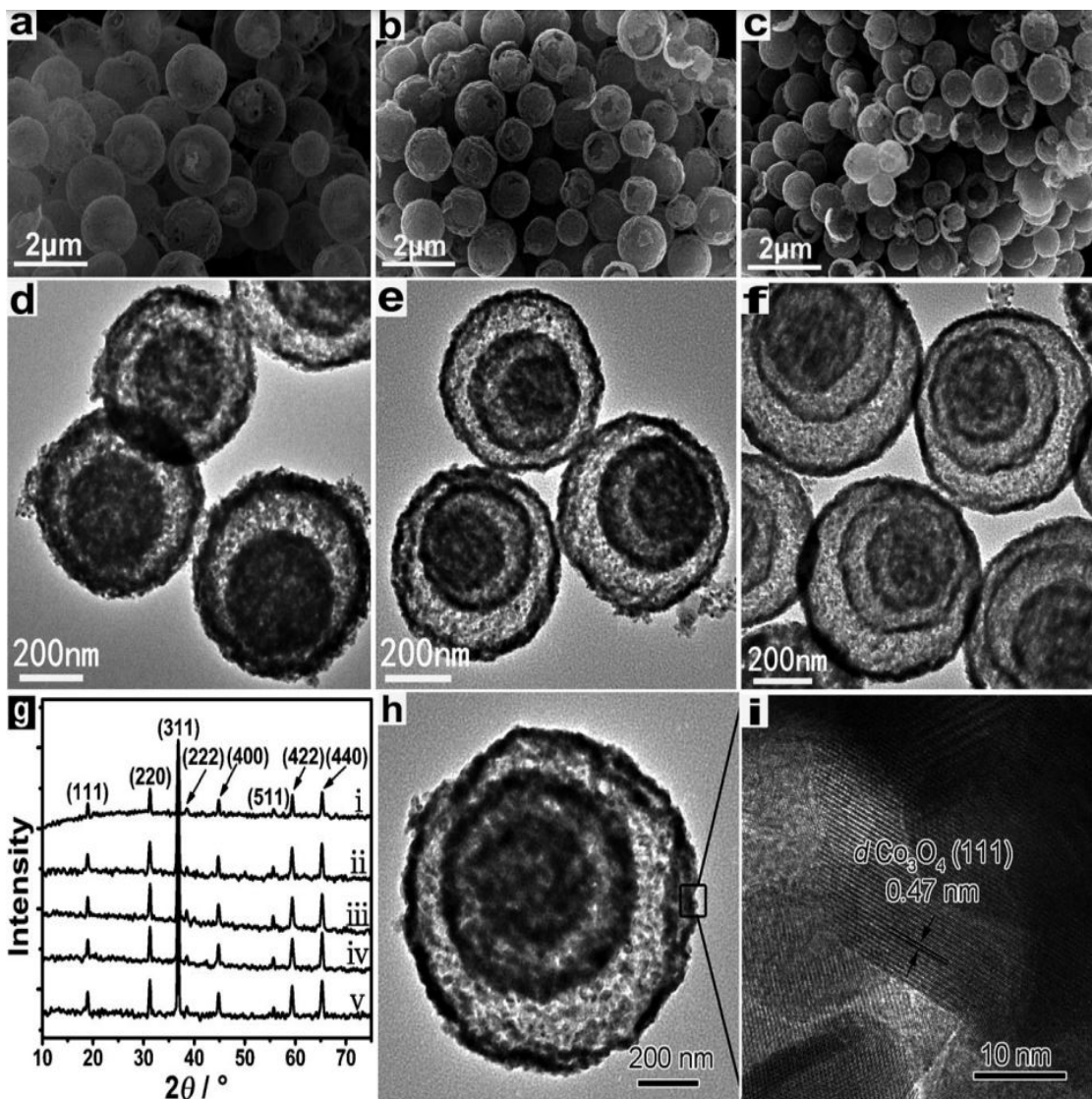


FIGURE 2.5: SEM (a–c, h), TEM (d–f), XRD patterns (g) and HRTEM (i) images of double, triple and quadruple Co_3O_4 hollow shells [45].

For elucidation of structural properties, XRD is a very important technique. It provides information about phase and crystalline nature of NPs [15]. It gives insight regarding size of particles as well via Debye Scherer formula [46]. The technique works great in single/ multiphase NPs characterization [47]. However, when the size is less than $1/100^{\text{th}}$ of an atom, it might be difficult to acquire correct parameters [15].

EDX is used in combination with TEM or Field emission (FE)-SEM. It gives rough idea about elemental composition of NPs. The technique provides support to SEM analysis [48, 49]. It elucidated elemental composition of BiVO_4 NPs (prepared sono-chemically and were pseudo flower shaped) [46]. Similar process

was used to validate elemental composition of graphene impregnated with In_2O_3 . The elemental analysis showed C, In and O as elements of NPs. These NPs were made via hydrothermal technique [50].

The most sensitive and widely used technique is XPS, which determines the precise and accurate element ratio. It also gives insight into bonding nature of elements forming the NPs. The technique has surface sensitivity. It finds its application in compositional and compositional variation analysis. FTIR and Raman spectroscopy can be used for vibrational characterization. These are more developed and feasible than elemental analysis techniques [15]. FTIR showed vibrational peaks of carboxylated C-O 2033 cm^{-1} , conforming the functionalization. It also was used to show broader O-H peak at 3280 cm^{-1} . The red shift values of FTIR bands elucidated the functional groups [51].

2.1.3.3 Size and Surface Area of Particles

Size of NPs can be estimated by different methods. These include TEM, XRD, SEM, dynamic light scattering (DLS), and AFM [52]. DLS and zetapotential analysis can be used to determine particle size at very low levels of NPs. In one study, size variation of silica NPs was carried out using DLS. It was elucidated that the size increased with protein layer acquisition. DLS might prove incapable in case of agglomeration and hydrophilicity. In such a case, one can rely on differential centrifugal sedimentation (DCS) [53].

Another relatively new technique is nanoparticle tracking analysis (NTA). This is helpful when analyzing different biomolecules like DNA and proteins. NTA can be used to see and analyze NPs in liquid medium, which correlates particle size to Brownian motion. NTA allows size distribution profiling with diameter of 10-1000nm in liquid medium [54].

Various applications of NPs rely on large surface area. One of the best ways to determine it is BET (Brunauer–Emmett–Teller). The technique is based on adsorption and desorption principal and BET theorem. It uses gas (N) to create isotherms which are classified into different classes [15].

2.1.3.4 Optical

Photocatalysis is use of light to catalyze a chemical reaction. In photocatalytic applications, optical properties are very important. The characterization is based on principles of light and Beer-Lambert law. The techniques provide insight about reflection, phosphorescence, absorption and luminescence of NPs. Since, metallic and semiconductor NPs have differing colours, they find their use in photo related application. Some common optical instruments are ultraviolet- visible spectrophotometry (UV-Vis), null ellipsometer and photoluminescence (PL). They are employed for observation of optical attributes of light [15].

2.2 Nanoparticles as Fertilizers

The demand for food is increasing with increasing population. This forces farmers to use fertilizers in order to increase plant yield. From 1950 to 2020, the use of fertilizers increased by 13 times from 15 million tons to 194 million tons. Many different chemical fertilizers are used nowadays (Fig 2.6). These enhance the crop yield by supplying different nutrients [55].

The use of chemical fertilizers increases the yield but have adverse effects on environment. Their cost is always waving due to inflation and resource shortage. A safer supplementation is hence, biofertilizers to enhance plant growth. They are eco-friendly and affordable. These include use of plant friendly microbial communities, developed in industries and applies to target crops. These than develop beneficial mutualistic relationships with plants [55].

Agriculture sector can be revolutionized via aid of nanotechnology. Nanofertilizers allow uptake of nutrients by plants. The increased growth of plants is reported by many studies which applied NPs as fertilizers/ growth promoters [56]. The nanotechnology mediated fertilizers usually have coated chemicals on NPs. They allow controlled release and targeted delivery of components, enhance plant production, and decrease environmental pollutants (Fig 2.7) [55].

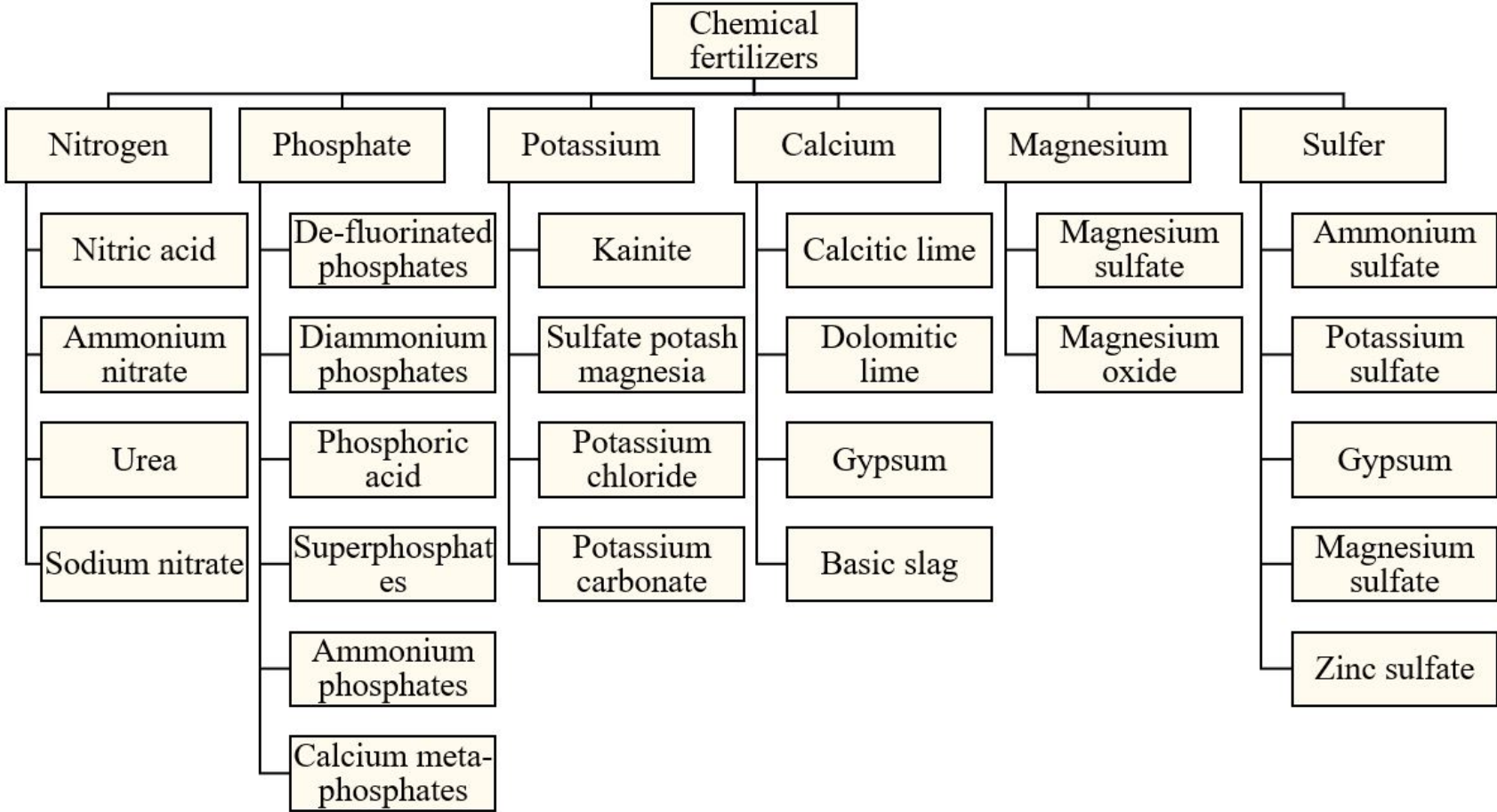


FIGURE 2.6: Various chemical fertilizers used in agriculture sector [55].

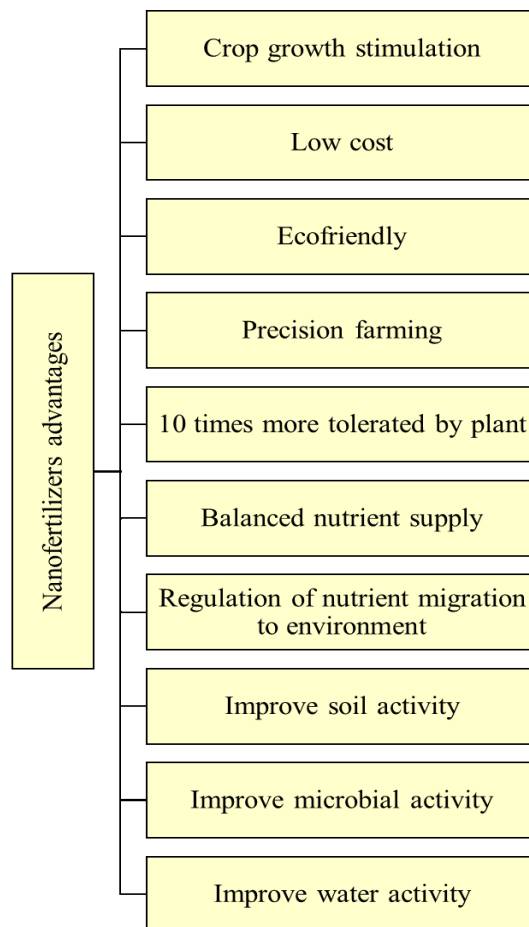


FIGURE 2.7: Advantages of nano-fertilizers [56].

Selenium NPs (Se NPs) are potential candidates to be used as fertilizers. The Se NPs suspension was introduced in soil in concentrations in 5, 10, and 25 $\mu\text{g kg}^{-1}$. At the conc. of 10 $\mu\text{g kg}^{-1}$ the growth of eggplant was twice as compared to control. Tomato showed same results as well. The growth of cucumber leaf plate was 50% higher than that of control. This can be used as fertilizers [57].

A study reported use of carbon nanotubes coated with NPK (nitrogen phosphorous potassium) and chitosan-NPK NPs on French beans. It was speculated that NPs were able to penetrate plant cells via endocytosis. The NPs either alone or in combination were able to increase plant growth and biomass after 30 days. TEM micrographs of French beans leaves showed chitosan-NPK NPs in phloem, while carbon nanotubes were present in both xylem and phloem. Low dosage was apparently enough to improve water absorption and nutrients uptake, in addition to enhancement of plant growth [55]. Other examples of nano-fertilizers can be noted in Table 2.1.

TABLE 2.1: Various nano-fertilizers which can be used for different plants.

NM	Species	Conc., DoT	Application Methods	Results	Refs
MWCN	<i>Hordeum vulgare</i> , <i>Zea mays</i> , <i>Allium sativum</i> , <i>Arachis hypogaea</i> , <i>Triticum aestivum</i> , <i>Glycine max</i>	50-100 $\mu\text{g}/\text{ml}$, 24h	Seed priming	Better growth, water absorption, germination of seedlings and biomass	[58]
ZnO	<i>Coffea arabica</i>	15 mg/L, 40-45 D	Foliar	Increase chlorophyll conc., rate of photosynthesis and biomass	[59]
	<i>T. aestivum</i>	20-25 mg/L, *	Combined with growth substrate	Enhanced yield and biomass	[60]
	<i>Cyamopsis tetragonoloba</i>	10 mg/L, 4-6 weeks	Foliar	Enhanced nutrient conc. and biomass	[61]
FeS ₂	<i>Brassica juncea</i> , <i>Cicer arietinum</i> , <i>Pinacia oleracea</i> , <i>Sesamum indicum</i> and <i>Daucus carota</i>	80-100 $\mu\text{g}/\text{ml}$, 12-14 h	Seed priming	Enhanced germination and yield	[62]
MgO	<i>Solanum lycopersicum</i>	7-10 $\mu\text{g}/\text{ml}$, 5-7 D	Drenching method	Biocontrol for pest (<i>Ralstonia solanacearum</i>)	[63]

TABLE 2.1: Various nano-fertilizers which can be used for different plants (Continued).

NM	Species	Conc., DoT	Application Methods	Results	Refs
CuO	<i>Spinacia oleracea</i>	200 mg/ kg, 60 D	Mixed with soils	Improved physiological processes	[64]
	<i>S. lycopersicum</i>	150-340 μ g/ ml, 11 D	Foliar	Control of dis- ease	[65]
Fe/ SiO ₂	<i>A. hypogaea</i> , <i>Z. mays</i>	15 mg/ kg, 3 D	Mixed with soil	Increased growth of plants and biomass	[66]
TiO ₂	<i>S. oleracea</i>	0.25% S, 48 h and 35 D	Foliar	Increased N, protein, chloro- phyll and biomass	[67]
TiO ₂ / SiO ₂	<i>Oryza sativa</i>	30 mg/ L, 55 D	Foliar	Enhanced growth and inhibition of Cd translocation	[68]
Al ₂ O ₃ NPs	<i>S. lycopersicum</i>	400mg/ L, 20 D	Foliar	Inhibition of <i>Fusarium</i>	[69]
MgO	<i>Solanum lycopersicum</i>	7-10 μ g/ ml, 5-7 D	Drenching method	Biocontrol for pest (<i>Ralstonia solanacearum</i>)	[63]

TABLE 2.1: Various nano-fertilizers which can be used for different plants (Continued)

NM	Species	Conc., DoT	Application Methods	Results	Refs
AgNPs	<i>T. aestivum</i>	50 and 75 mg/ L, -	As fertilizer,	Improved growth and heat tolerance	[70]
	<i>Vigna sinensis</i>	50 mg/ L, 40 D	Foliar	Improved nodu- lation of root and soil micro- biota	[71]
	<i>Vigna unguiculata</i>	30-90 μ g/ ml, 4-7 D	Foliar	Inhibition of growth of pathogens like <i>X. axonopodis</i>	[72]

(NM= Nano-materials, MWCN= Multiwalled carbon nanotubes, DoT= Duration of treatment, D= Days, Refs= References, *=Treatment depends on growth cycle, S= suspension, - = not mentioned).

2.3 Silicon Role in Plant Growth

Silicon is a non-essential/ partly essential element for development and growth of plants [73]. While some plants develop just fine in its absence, others show increased susceptibility to fungal infections in silicon deficient conditions [74]. When it is supplied in growth media to plants as silicic acid, the abiotic stress tolerance of plants increases [75–77].

Plants have aquaporin channels through which silicon is taken up by plants in form of silicic acid [78, 79]. Accumulators, excluders and intermediate type are three categorizations of plants based on presence of biogenic silica presence in their tissues [80].

The silicic acid in some plants have a delayed effect when external stimuli is absent [81, 82]. This latent impact was noted in *Arabidopsis*-powdery mildew pathosystem [81]. In rice, the supplementation of silicic acid triggers major changes. There is upregulation of 35 and down regulation of 121 transcription factors [83]. The difference may simply lie in variation of cell wall types [84], and the significance (structural) of silicon in type II cell walls. Si imparts protection by formation of physical barrier. It precipitates as SiO_2 and incorporates in biological structures like cell wall [73].

2.3.1 Silicon and Abiotic Stress Alleviation

Silicon plays key role in plants against various environmental stresses. The stress resistance is imparted by 1) Biochemical responses and 2) SiO_2 deposition. The biochemical responses trigger metabolic changes while deposition of silicon dioxide provides physical and mechanical protection [85].

There is precision in Si accumulation in different species [73]. In rice, the Si increases the lodging resistance by increasing stem strength [85]. The Si deposition in leaf epidermis induces UV tolerance [86]. It may reduce ultraviolet B radiation (UVB) caused membrane damage [87].

In drought struck plants, silicon influences the water plant relation. Silica cuticle double layer is formed under epidermis of leaf, thereby reducing water loss via cuticular transpiration [88]. As a consequence of Si deposition, there is cell wall modification and reduction of turgor in guard cells [89]. It causes root elongation and allowing water extraction from soil [90] and aquaporin genes upregulation [91], under drought conditions.

Salt stress is also alleviated by silicon. This is done by inhibiting sodium [89] and chloride ions [92] uptake. Si supply reduces the translocation of toxic ions from roots to shoots [93]. In rice, SiO_2 deposition in exodermis and endodermis blocks NaCl toxicity [94]. In salt impacted root tips, silicon nutrition impacts proton pumps, affecting potassium uptake which maintains the K/Na in plants [95].

In soil polluted with metals, it impacts the bioavailability of toxic compounds. The soil pH can become alkaline due to sodium metasilicate or alkaline silicon contamination. This leads to decreased availability of heavy metal conc. in soil [96]. Soluble silicate undergoes hydrolyzation and creates metasilicic acid which sequesters heavy metals [97]. Plants treated with it, exudate phenolics as catechin and quercetin, which have strong aluminum sequestering ability [98]. Al detoxification is also caused by formation of hydroxyl-aluminum silicate in apoplast [99].

In heavy metal tolerance, an important process is compartmentalization of toxic ions. Silicon increases resistance of heavy metal by retention of metals in roots (endodermis accumulation) [100]. In response to Si treatment, Mn was accumulated in shoot's epidermis [101]. Another study reported accumulation of Mn in leaves trichomes after Si treatment [102]. Cd uptake is inhibited by hemicellulose bound form of silicon (negatively charged). This leads to downregulation of *Nramp5*, which codes for Cd transporter [103]. Silicon supplementation, leads to downregulation of other metal transporters (*OsHMA2* and *OsHMA3*) as well which prevent Cu/ Cd uptake [104].

Some studies report that silicon cause heavy metal tolerance by regulation of antioxidant enzymes [105], Overproduction of native antioxidants to mitigate oxidative stress [106], stabilization of chloroplast structures to maintain photosynthesis, integrity of photosystem II, and increase in pigment conc. [107]. Silicon hence, plays crucial role in triggering plant response to adverse environment. These process are still not clearly elucidated and more research is needed on them [73].

2.3.2 Silicon and Biotic Stress Alleviation

Research reported that silicon can improve defense against biotic stresses due to phytopathogens (viruses, bacteria, fungi) and pests (vertebrates and arthropods). The abrasiveness of plant tissue is increased due to silicon deposition. This cause reduction in digestion and palatability for herbivores [108]. It was further elucidated using SEM-EDX that phytolith morphology inside the tissues has more

impact on abrasive nature of plant than conc. of silicon [109]. In addition, using same techniques it was noted that the specific silicon deposition pattern in sugarcane imparts resistance to *Eldana saccharina* [110]. It's accumulation imparts physical strength (mechanical protection), and hence lower susceptibility to infections of *Rhizoctonia solani* [111, 112] or *Bipolaris oryzae* [113].

In order to improve tolerance to biotic stresses, Si improved biochemical/ molecular mechanisms. These include induction/ reinforcements of defensive compounds as momilactones, phenolics and phytoalexins [114]. There is also defensive enzymes activation like peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and lipoxygenase [115]. Treatment with silicon may increase transcription of defensive genes [116].

2.3.3 Silicon and Phytohormones

The impact of silicon in correlation with endogenous phytohormones are commonly analyzed in response to stress conditions. There is increase in abscisic acid initially and then decrease after 14 days in rice exposed to heavy metals. There is also reduction of jamic acid and salicylic acid. The behaviour of abscisic acid is antagonistic to jamic acid and salicylic acid biosynthesis [104]. A reduction in jamic acid was noted in plants in response to wounding by silicon [117]. Gibberellins in silicon treated plants increased under salinity stress [118].

Salicylic acid usually imparts resistance against biotrophic pathogens, while jamic acid and ethylene imparts resistance to necrotrophic pathogens. It was elucidated that in leaves exposed to *Erysiphe cichoracearum*, silicon increased synthesis of jamic acid and ethylene [81]. In same way, tomato plants treated with silicon, when exposed to *R. solanacearum*, showed activation of ethylene and jamic acid pathways to increase resistance [119]. While silicon increases ethylene pathways by activation of associated genes [120], reverse is true for ethylene pathways of pathogens. By interfering with production of fungal ethylene, it imparts resistance against *Cochliobolus miyabeanus* [121]. It also increases cytokinin biosynthesis in *Sorghum* and *Arabidopsis* which delays senescence [122].

2.4 Silica and Silicon NPs

Various silicon and silica NPs, and their impact on different plant species can be noted in Table 2.2.

TABLE 2.2: Silicon and silica NPs as nano-fertilizers and their impact on growth of different plants.

NPs	Species	Conc., treatment and size	Impact	Refs
SiO ₂	<i>Larix olgensis</i>	62, 125, 250, 500, 1000 μ L/L, NP sol. used to soak seedlings, (-)	Increased in root length, height of plant, lateral root No. and chlorophyll conc.	[123]
Si	<i>Pisum sativum</i>	10 μ M, seed and seedlings, treated in petri plate or hydroponic state, 75-125nm.	Protection of seedlings from Cr(VI) phytotoxicity.	[124]
Si	<i>S. lycopersicum</i>	1, 2mM, seedling treated with nutrient sol., (20-35nm).	Reduced salinity stress and increased photosynthesis	[125]
Si	<i>Ocimum basilicum</i>	10ml/L, plant sprayed, (-)	Alleviated the stress of salinity	[126]
Si	<i>O. sativa</i>	1mM, culture sol. received NPs, 19, 48 and 402nm.	Rice cells were able to withstand cadmium toxicity	[127]

TABLE 2.2: Silicon and silica NPs as nano-fertilizers and their impact on growth of different plants (Continued).

NPs	Species	Conc., treatment and size	Impact	Refs
SiO ₂	<i>L. esculentum</i>	2, 4, 6, 8, 10, 12, 14 g/L, seeds treated in petri plate for germination, 12nm	Increased seed germination, vigour of seed, fresh and dry weight significantly at 8g/L	[128]
SiO ₂	<i>Crataegus aronia</i>	10, 50, 100 mg/L, seedling treated via irrigation, 10-30nm.	Drought stress alleviated, increase in photosynthetic pigments, plant growth, and decline in water potential in xylem	[129]
Si	<i>O. sativa</i>	7.5g/ pot, soil mixed with NPs in pot and seeds were placed, (-).	Upregulation of <i>Lsi1</i> and <i>Lsi2</i> which are involved in uptake of silicon. No +ve/ -ve impact was noted on plant after treatment with NPs	[130]
Si	<i>T. aestivum</i>	10 μ M, seedlings, hydroponic, 75-125nm.	Alleviation of negative impact of UVB radiations	[131]
Si	<i>Vicia faba</i>	1.5, 3 mM, seed priming, (-)	Slight improvement in flowering	[132]

TABLE 2.2: Silicon and silica NPs as nano-fertilizers and their impact on growth of different plants (Continued).

NPs	Species	Conc., treatment and size	Impact	Refs
SiO ₂	<i>Trigonella foenum</i>	0-2.5 mM, seedlings were treated, hydroponic, 20-30nm.	Modulation of PST transporters, and increase silica NPs translocation, accumulation of Si and cell wall lignification	[133]
MSi	<i>Triticum</i>	1200, 500, 1000, 2000 mg/ L, seed and seedlings treated in petri plate or hydroponic, 20nm	Facilitation of photosynthesis and plant growth	[134]

(MSi= Mesoporous silicon, - = nil, UVB radiation= Ultra violet B radiations)

2.5 Cumin Mediated NP Synthesis

Various types of NPs have been synthesized using cumin (Table 2.3).

TABLE 2.3: Green synthesis of different nanoparticles using cumin.

Source	NPs	Size (nm)	Description	Refs
<i>C. cyminum</i> (white seeds)	AgNPs	1.84 - 20.57	Non-cytotoxic NPs effective against <i>S. aureus</i> and <i>Escherichia coli</i>	[135]

TABLE 2.3: Green synthesis of different nanoparticles using cumin (Continued).

Source	NPs	Size (nm)	Description	Refs
<i>C. cyminum</i> seeds composited with Hypericum plant	MnO ₂ NPs	10-45	Cytotoxicity against HeLa cell lines. Also showed antibacterial activity against <i>P. aeruginosa</i> and <i>S. aureus</i>	[136]
<i>N. sativa</i> (black cumin) seeds	AgNPs	25.2	Neuroprotective properties against inflammation and oxidative stress associated with diabetes	[137]
<i>C. cyminum</i> seeds	CuNPs	25	Efficacy against <i>Pseudomonas spp.</i> and <i>Penicillium spp.</i>	[138]
<i>C. cyminum</i> seeds	TiO ₂ NPs	15.17	Enhancement in growth rate and seed germination of Mung beans (<i>Vigna radiata</i>)	[139]
<i>C. cyminum</i> leaves	AgNPs NPs	3-20	Antibacterial (<i>Bacillus cereus</i> , <i>E. coli</i> , <i>S. aureus</i> and <i>P. aeruginosa</i>) and antifungal (<i>F. oxysporum</i>) properties	[140]
<i>C. cyminum</i> seeds and <i>Syzygium aromaticum</i>	SeNPs	-	Cytotoxicity against osteosarcoma cell line Saos2 at 40 g/ml	[141]

TABLE 2.3: Green synthesis of different nanoparticles using cumin (Continued).

Source	NPs	Size (nm)	Description	Refs
<i>C. cyminum</i> seeds	Fe ₃ O ₂ NPs	-	Enhancement of germination of wheat, it's biomass and percentage survival in drought conditions	[142]
<i>C. cyminum</i> oil	AgNPs	25-45	Antioxidant efficacy	[143]

(- = Not mentioned)

2.6 Lettuce

A widely consumed and most cultivated leafy vegetable across four corners of the world is lettuce (*Lactuca sativa* L.), with increasing production each year. According to an estimation, the global area harvested in 2018 was > 1.27 million ha, giving approximately 27.3 million tons of production. This belongs to Asteraceae family.

The 12 morphology-based varieties are: 1) Butterhead, 2) Frillice, 3) Cos, 4) Frisée d'Amérique, 5) Gem, 6) Lollo, 7) Novita, 8) Multi-divided, 9) Batavia, 10) Iceberg, 11) Stem and 12) Oakleaf. While most lettuce types are green in colour, some are also semi red or red [144].

2.6.1 Morphology

There are many different types of lettuce, some of which are discussed below:

2.6.1.1 Iceberg

Also known as Crisphead (Fig 2.8) and belongs to *Lactuca sativa var. capitata* family. The spherical leaves form a dense and tight head by folding on one another.

It has thick crisp leaves with quite prominent veins. The colour of leaves range from light green to dark green. Some varieties when mature are mid green. Some genotypes also possess anthocyanins [145].

2.6.1.2 Butterhead

It belongs to *Lactuca sativa var. capitata*. The head is made of spherical leaves and is small (Fig 2.8). The leaves folded are not as tight as in Crisphead. The outer leaves are dark green or brown while inner leaves are creamy green because of absence of light. The leaves are more easily torn or bruised in comparison to Crisphead. Their oily leaf texture and pliability is attractive to consumers [145].

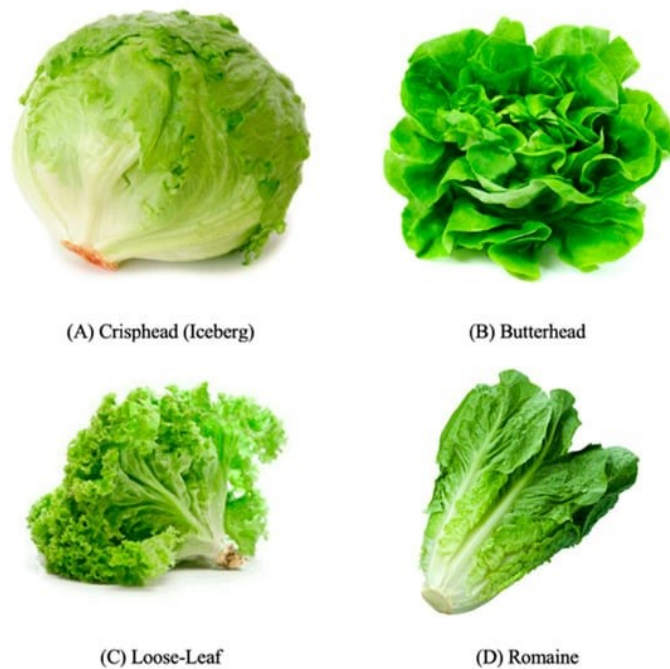


FIGURE 2.8: Different types of lettuce commonly grown [145].

2.6.1.3 Loose Leaf

It is known as leaf (*L. sativa*). They lack heads like Romaine or iceberg lettuce (Fig 2.8). They have centralized leaves with smooth margins and deep lobes. The leaves can be fringed or curled. The number of leaves is large. The leaves in contrast to romaine are softer, shorter and more perishable. The leaf colour ranges from green to greenish yellow and some show shades of red [145].

2.6.1.4 Romaine

This is also called as Cos or *Lactuca sativa* L. It grows upright and forms a head which is oblong shaped. The leaves are long and rigid with midrib. The midrib is upright and can grow up to 30cm. The mid green leaves are outside, while greenish white leaves lie in center (Fig 2.8) [145].

2.6.2 Nutritional Profile

This leafy green has quite a diverse nutritional profile and phytochemicals (Fig 2.9) [146]. Some of the major reported chemicals in lettuce are thymol, thymol acetate, -terpinene, -cymene, -pinene, durenol, caryophyllene, camphene, limonene and spathulenol. The essential oils of lettuce have been reported to contain 4-terpineol, o-methylthymol, L-alloaromadendrene, -terpineol, linalool, -terpinolene, -pinene and viridiflorene [147].

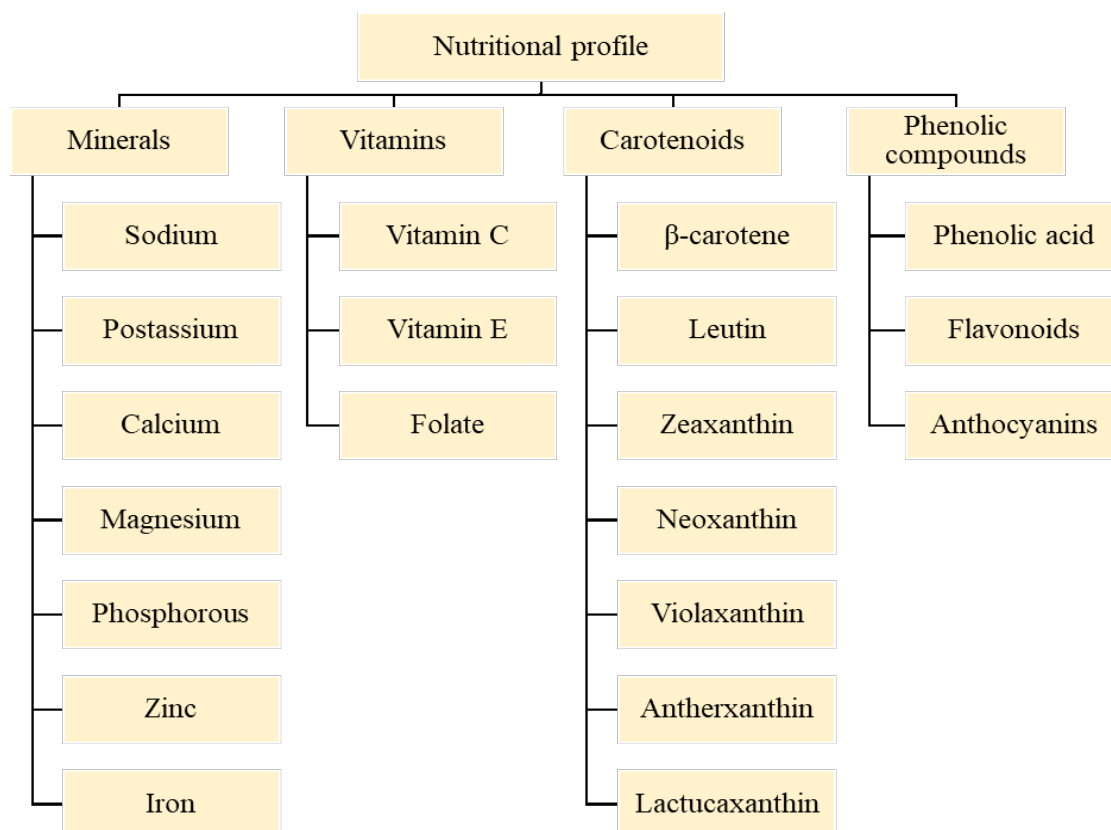


FIGURE 2.9: Nutritional content of *L. sativa* [146].

2.6.3 Pharmaceutical Significance

Lettuce has a very remarkable pharmaceutical significance. These can be noted in Table 2.4.

TABLE 2.4: Pharmaceutical significance of lettuce.

Sr. No.	Pharmaceutical Property	Refs
1	Anti-tumour activity	[148]
2	Antioxidant activity	[149, 150]
3	Hypolipidemic effect	[151]
4	Antidiabetic	[152]
5	Anti-inflammatory	[153, 154]
6	Anti-coagulent	[154, 155]
7	Analgesic	[154]
8	Anti-depressant	[154]
9	Neuroprotective activity	[145]

2.7 Impact of Nitrogen Fertilizers

Nitrogen is a nutrient essential to plants and animals. The increased demand of food is being addressed by increased use of fertilizers. Nitrogen fertilizers are extensively used worldwide. This increases the biomass and overall yield of plants. However, the excess nitrogen is given off into the environment in reactive forms like nitrogen oxides, ammonia and nitrates. These reactive forms enter soil, air and aquatic systems leading to avalanche of harmful effects on environment and living beings [12]. Impacts on environment and living beings are discussed below:

2.7.1 Water

While nitrogen is essential for both animals and plants, its pollution is leading to disastrous results on water bodies. The nitrate (NO_3^-) is quite mobile in soil water.

Successive overuse of nitrogen fertilizer leads to high conc. of nitrate in ground water via leaching. This nitrate radical enters drinking water bodies and enter living beings. High concentration of nitrate in consumed water leads to methemoglobinemia in infants. It also cause formation of carcinogenic nitrosamines in stomach of humans. Furthermore, they also cause hypertension Excessive nitrogen also aggregates in aquatic plants and algae, endangering the whole ecosystem [12].

2.7.2 Air

Facilities involved in manufacture of nitrogen fertilizer emit a lot of green house gases (usually CO_2 and NO). They also release particulate matter (<10 microns in size) which is extremely noxious in atmosphere. Ammonia plants release CO_2 , H_2 , H_2S , and CO which enter the atmosphere [12]. These gases harm the ozone and generate particles which can penetrate deep into the lungs. This results in respiratory and other diseases. NO cause lung irritation. A study reported that NO cause lowering of resistance to influenza and other respiratory infections [156]. Ozone levels affected by these gases hinder the development of plants and cause decline in plant yield [157].

2.7.3 Soil

Excessive use of fertilizers increases soil acidification of crop lands [158]. The use of nitrogen fertilizers while increases plant productivity, contaminate the soil via leaching. The acidification of soil decreases the nutrient availability (boron, phosphate, molybdenum and base cations) and increased metal toxicity (lead, cadmium, aluminum). This leads to limited growth of plants and soil biota [159].

2.7.4 Living Beings

The oxides of nitrogen (NO_x) and ammonia (NH_3) enter the atmosphere and combine, causing health problems. The compounds eventually deposit into ecosystem

after exiting the atmosphere. Nitrogen oxides cause production of ozone that has drastic impact on human health [12]. Excessive use of nitrogen fertilizers can lead to cancer, impaired neural tube development, diabetes, blue babies and reproductive adversities [11].

Excess nitrogen enters the water bodies from soil. This can lead to eutrophication. In addition, it can also cause acidification of waters. The results include algal blooms. The outcome is decline of plant and animal life in water bodies [160]. The algal blooms are linked to shell fish poisoning in humans. Nitrogen pollution can lead to production of allergenic pollen. It can also cause increase in cholera, malaria and schistosomiasis [161].

Chapter 3

Research Methodology

The method selected for synthesis of silica NPs, and testing of their fertilizer nature can be noted in Fig 3.1.

3.1 Extract Preparation

White cumin seeds were washed with distilled water (dH₂O), sun dried and crushed. Finely crushed seeds (5g) were added to 100ml dH₂O and heated on hotplate with constant stirring (40mins, 40°C). This was filtered using Whatman No. 2 filter paper, and the filtrate (plant extract) was stored at 4°C [162].

3.2 Silica NPs Synthesis

In 100ml of plant extract, 20ml of sodium metasilicate was added dropwise with constant stirring. pH value was adjusted to 9 using 1M NaOH and 1M HCl. This was heated at 60°C, for 5h. The final volume of solution was brought to 50ml using dH₂O. The mixture was filtered using Whatman No. 2 filter paper. The filtrate was washed thrice with dH₂O and methanol to remove all the contaminants. Centrifugation (7000rpm, 25mins) was carried out to separate residue from mixture. The pellet was heated at 550°C [162].

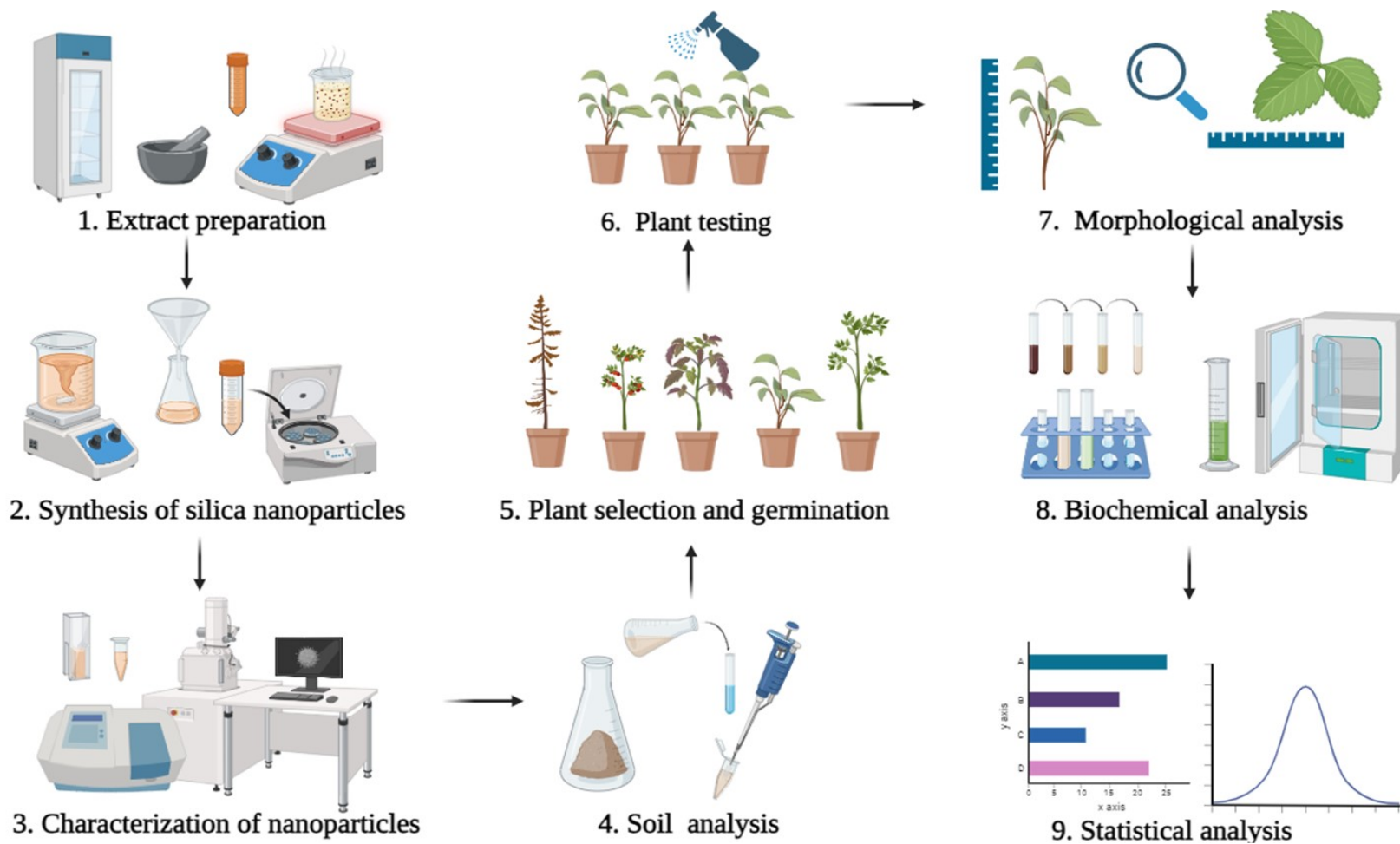


FIGURE 3.1: Methodology for synthesis of silica NPs and their testing on plants.

3.3 Characterization of NPs

There are many techniques for characterization of synthesized NPs. Characterization is important as the synthesis of NPs is occurring at nanoscale and hence, we need to validate first that either the NPs have formed or not. The techniques allow identification of size, shape, elemental composition etc. of Silica NPs [162]. The characterizations techniques used were; 1) UV-Vis spectrophotometry, 2) FTIR, 3) SEM-EDX and 4) XRD. UV-Vis spectrophotometry was carried out at CUST, while FTIR, SEM-EDX and XRD were performed at Institute of Space Technology, Islamabad, Pakistan.

UV-Vis spectrophotometry is an analytical method employed for qualitative and quantitative analysis of chemical compounds/ elements [163]. Here this technique was used for validation purposes of silica NPs formation. Spectrophotometer (UV-1602, Biomedical Technologies) was used. Silica NPs (10mg) were mixed with 5ml of distilled water and analyzed for absorbance from 200-700nm wavelength.

SEM is another technique used for morphological analysis (size and shape) of nanoparticles. EDX highlights elemental composition of sample [14, 48]. Sputter coater (Q150/S by Quorum Technologies) was used to sputter sample of silica NPs with gold particles. This was done to prevent the charging effect. Sample was then placed inside SEM-EDX machine (MIRA 3, TESCAN), and analyzed.

FTIR analyses samples (solid, liquid or gas) and give data about their chemical nature. It gives both qualitative and quantitative analysis of samples [15]. FTIR (Thermofisher Nicolet Summit Pro) was used for analyses of powdered silica NPs as well as plant extract.

XRD gives information about structure of samples. We can acquire information from microscopic traits (crystal component arrangement) to macroscopic traits (size and shape of crystal), using XRD [14]. XRD (RD 50, GNR Explorer) was used to identify structure, size and phase of silica NPs (powder). XRD results were analysed using Origin [164] and Match 3. Grain size was determined using Scherrer equation [165] on XRD data (Eq 3.1).

$$D = \frac{K\lambda}{B \cos \theta} \quad (3.1)$$

Where;

D = Average size of crystal

K = Scherrer constant

λ = X-ray wavelength

θ = Bragg angle

B = Full width half maximum (FWHM) of selected peaks.

3.4 Soil Analysis

The soil (collected from Islamabad, Pakistan) was analyzed for organic matter, soluble salts, pH, composition, cation exchange capacity [166]. This soil was later used for plant germination. The analysis gave insight into soil fertility.

3.5 Plant Selection and Germination

Green lettuce plant was selected because of its availability and growing season (winter). Plant pots were filled with soil and lettuce seeds were sown in Oct, 2023. The pots were placed in greenhouse for controlled environment. When the plants reached four leaf stage and a height of 7cm, the plant testing was done.

3.6 Experimental Plan

Control and experimental groups were designed for plants (Table 3.1). Each group had 3 plants. The application of NPs was foliar. On day 14, plants were harvested and analyzed for different morphological and biochemical traits. The chemical fertilizer used was composed of N, P, K (1:1:1), and its trace elements can be noted in Fig 3.2.

TABLE 3.1: Different plant groups for the experimental plan.

Sr. No.	Groups	Fertilizer Applied	Concentration
1	Control group	Nil	Nil
2	Standard group	Market chemical fertilizer GrowMore	2.5g/ L
3	Treated group	Silica NPs	8g/ L [128]
4	Combined group	Market chemical fertilizer + Silica NPs	Market chemical fertilizer GrowMore (2.5g/ L) + Silica NPs (8g/ L) [128]

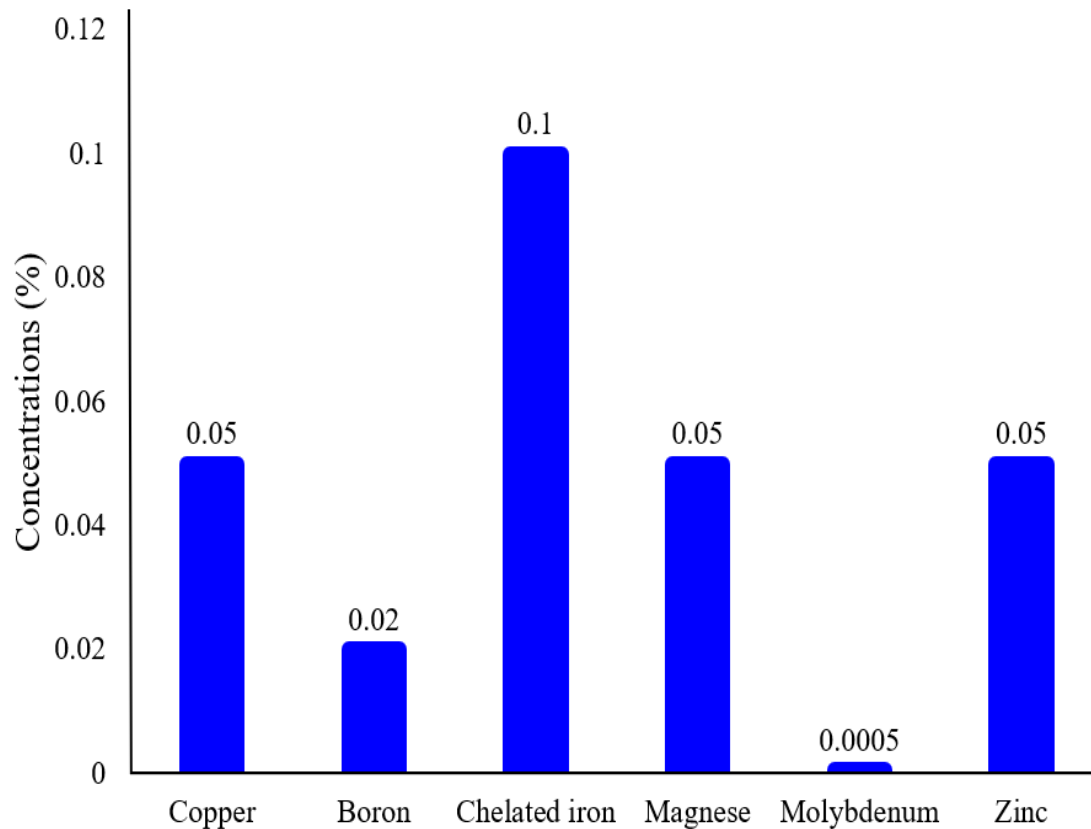


FIGURE 3.2: Percentage composition of different elements in chemical fertilizer GrowMore.

3.7 Morphological Analysis

The plants after harvest were washed gently. The leaves and roots were divided. The morphological traits noted in plants were; No. of leaves, area of leaves, fresh leaf weight, dry leaf weight, fresh root weight, dry root weight, root length and root diameter. No. of leaves were manually counted. The area of leaves, root length and roots diameter were measured with imageJ software [167]. The leaves and roots after harvest were weighed on mass balance, followed by drying (70°C) [168], for acquisition of dry weight of plant.

3.8 Biochemical Analysis

Different biochemical tests were conducted on plants to identify impact of NPs on plant according to the procedures used by Zarafshar et al., [168]. Biochemical profiling was done using spectrophotometer (UV-1602, Biomedical Technologies).

3.8.1 Photosynthetic Pigments

The analysis of plant pigments (chlorophyll (Ch_a), chlorophyll b (Ch_b) and carotenoids) was done by using acetone extraction, followed by spectrophotometry analysis [169–171]. Reagents used for the extraction were 80% acetone solution and 0.1N NH₄OH solution. Reagent grade acetone was added to 0.1N NH₄OH in ratio 1:9 (NH₄OH: acetone) to acquire extraction buffer. Furthermore, reagent grade acetone was mixed with dH₂O (2:8) to get 80% acetone sol.

Leaves of lettuce plant were collected and covered with aluminum foil. These were placed in resealable plastic bags, followed by freezing. The samples were placed in dark to avoid chlorophyll degradation. All the equipment (homogenization tubes, pestals, glassware, sol.(s)) except cuvettes were thoroughly chilled in freezer before starting the experiment. The laboratory lights were dimmed and spectrophotometer was calibrated before start of the experiment.

Each tissue sample was cut to 1000mm² size. The tissue sample was mixed with 2ml of extraction sol. This was grounded over chilled ice using pestle and mortar. The plant material adhered to pestle mortar was washed off using 3ml of extraction buffer. This was placed on ice for 2 hours (refrigerated in dark). Regrinding was done to extract any remaining chlorophyll. All the content was washed off using 5ml of 80% acetone and transferred to centrifuge tube.

The extract was centrifuged (20min, 500g) and supernatant was collected. This was brought to volume (10ml) using 80% acetone. The absorbance was noted at A₄₇₀, A₆₄₅ and A₆₆₃ using spectrophotometer. Blank used was 80% acetone. The equations to calculate conc. of chlorophyll a (Eq 3.2), chlorophyll b (Eq 3.3), total chlorophyll (Eq 3.4) and carotenoids (Eq 3.5) can be noted below:

$$\text{Chlorophyll a (mg/ml)} = 12.7A_{663} - 2.69A_{645} \quad (3.2)$$

$$\text{Chlorophyll b (mg/ml)} = 12.7A_{645} - 2.69A_{663} \quad (3.3)$$

Where;

A₆₄₅ = absorbance at a wavelength of 645nm

A₆₆₃ = absorbance at a wavelength of 663 nm

$$\text{Total Chlorophyll (mg/ ml)} = \text{Chlorophyll a} + \text{Chlorophyll b} \quad (3.4)$$

$$\text{Carotenoids (mg/ml)} = \frac{1000A_{470} - 1.90Ch_a - 63.14Ch_b}{214} \quad (3.5)$$

Where;

A₄₇₀ = absorbance at a wavelength of 470nm

Ch_a = Chlorophyll a

Ch_b = Chlorophyll b

3.8.2 Proline Content

Proline content was measured via protocols of Bates et al., [172]. The protocol involves reaction of proline and ninhydrin, to form red coloured formazone. This takes place in organic solvents like toluene. The absorbance of chromophore at 520nm using spectrophotometer, gives the conc. of proline [168].

Acid ninhydrin was prepared by mixing 30ml glacial acetic acid, 20ml 6M phosphoric acid, 1.25g of ninhydrin. This was agitated on heat, till ninhydrin was completely dissolved. The reagent was stored at 4°C. Leaves fully expanded in sun were collected for analysis. The procedure was standardized using purified proline.

In 10ml of 3% aqueous sulfosalicylic acid, 0.5g of plant material was grounded. This was filtered through Whatman # 2 filter paper. The filtrate (2ml) was mixed with acid ninhydrin (2ml) and glacial acetic acid (2ml) and placed in water bath (1 hour, 100°C). Ice bath was used to terminate the reaction. Toluene (4ml) was added to each tube and mixed vigorously (15-20sec). The chromophore containing toluene was aspirated, brought to room temperature and analyzed at 520nm (spectrophotometer), using toluene as blank. The process was done to determine proline in both plant leaves and roots. The conc. of proline was determined via standard curve and calculated on basis of fresh weight (FW) using Eq 3.6.

$$\mu\text{moles proline g}^{-1} \text{FW} = \frac{(\mu\text{g proline/ml} \times \text{ml toluene})}{115.5\mu\text{g}/\mu\text{mole}} \times \frac{5}{\text{g of sample}} \quad (3.6)$$

3.8.3 Lipid Peroxidation (MDA Test)

A very prominent effect of oxidative stress in biological membranes can be noted through peroxidation of lipids. Fresh plant tissue (1.5g) was homogenized with 3ml of reaction mixture (20% w/v trichloroacetic acid and 0.5% w/v thiobarbituric acid), followed by incubation (30min, 95°C).

Reaction was terminated by placing the homogenate on ice, followed by centrifugation (12,000rpm, 10min). Supernatant was collected and absorbance at 532nm and 600 nm were noted. The process was done for both plant leaves and roots. The amount of MDA (malondialdehyde) is directly proportional to generation of peroxidation level [173]. Extinction coefficient for calculation of MDA concentration, used was $155 \text{ mM}^{-1}\text{cm}^{-1}$. The conc. was calculated using Eq 3.7.

$$\text{MDA (nmol g}^{-1}\text{FW)} = \frac{\text{Absorbance of sample}}{E_o \times L} \times D \quad (3.7)$$

Where;

D = Dilution factor

E_o = Extinction coefficient

L = Light path (cm)

3.8.4 Relative Water Content

Relative water content (RWC) was determined by weighing fresh fully developed leaves. These were immersed in distilled water at 4°C for 24h, and weighed again. Drying was done in an oven at 70°C for 48h, followed by final weighing [174]. RWC was measured using Eq 3.8

$$\text{Relative water content (\%)} = \frac{\text{LFW} - \text{LDW}}{\text{LTW} - \text{LDW}} \times 100 \quad (3.8)$$

Where;

LFW= Leaf fresh weight

LDW= Leaf dry weight

LTW= Leaf turgor weight

3.9 Statistical Analysis

The data obtained from morphological and biochemical analysis was further subjected to statistical analysis using ANOVA. Duncan's test was also conducted using ANOVA with $p < 0.05$.

Chapter 4

Results and Discussion

The increasing challenges associated with crop production and adverse environmental conditions are being addressed with remarkable advancements in technology. Nanotechnology has the potential to address many agricultures related issues and the development of organic and chemical based nano-fertilizers is one of major achievements. Plethora of research has been done on impact of nanotechnology in agriculture. Use of fertilizers play pivotal role in increasing agriculture production. However, chemical fertilizers have hazardous impact on environment. Nano-fertilizers circumvent excessive use of agrochemicals, thereby protecting the environment in addition to enhancement of crops. They also address various environmental stresses, inducing stress tolerance into treated plants. Nanotechnology hence, offers a solution to many problems associated with chemical fertilizers [175]. Silica NPs are widely studied in various emerging fields like medicine, bioimaging, biosensors, agriculture, cosmetics, textile etc. [4]. The current study was conducted to identify impact of silica NPs as nano-fertilizers on growth of *L. sativa*.

4.1 Extract and NP Synthesis

The plant extract was prepared by heating grounded cumin in 100ml distilled water, followed by filtration. This filtrate was collected as extract (Fig 4.1). Silica NPs were made by green synthesis method. After heating the extract and sodium

metasilicate at 60°C for 5h with constant stirring, there was distinct colour change in plant extract. This colour change indicated that NPs have been formed (Fig 4.1).

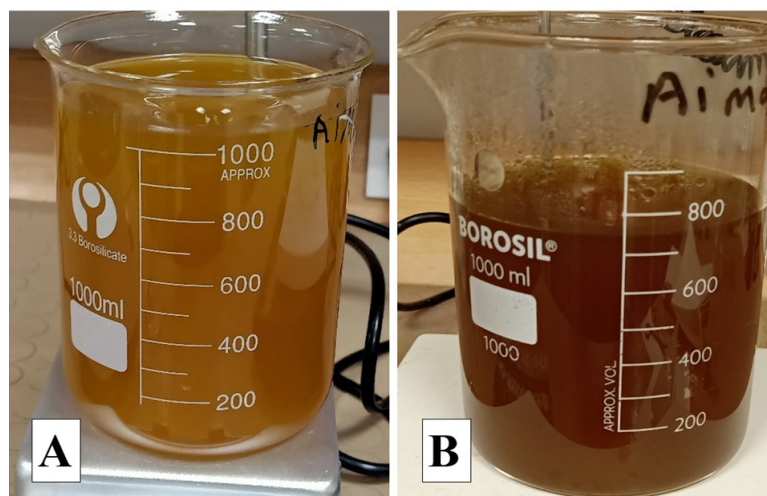


FIGURE 4.1: A) Synthesized extract of grounded white cumin seeds. B) Colour change of plant extract mixed with sodium metasilicate, indicative of NPs formed.

4.2 Characterization

It is important to validate the formation of NPs, since we can't see them with naked eye. For validation different tests were conducted. The characterization of silica NPs was done using UV-Vis spectrophotometry, FTIR, SEM-EDX and XRD.

4.2.1 UV-Vis Spectrophotometry

The silica NPs synthesized via green synthesis approach were first characterized using spectrophotometer. The absorbance was noted from 200 to 700nm. The highest absorbance was noted at 241nm wavelength (Fig 4.2). The absorbance of silica NPs lie from 200nm to 400nm [176]. A sharp peak at 241nm in spectrophotometry spectrum hence, confirmed the synthesis and presence of silica NPs.

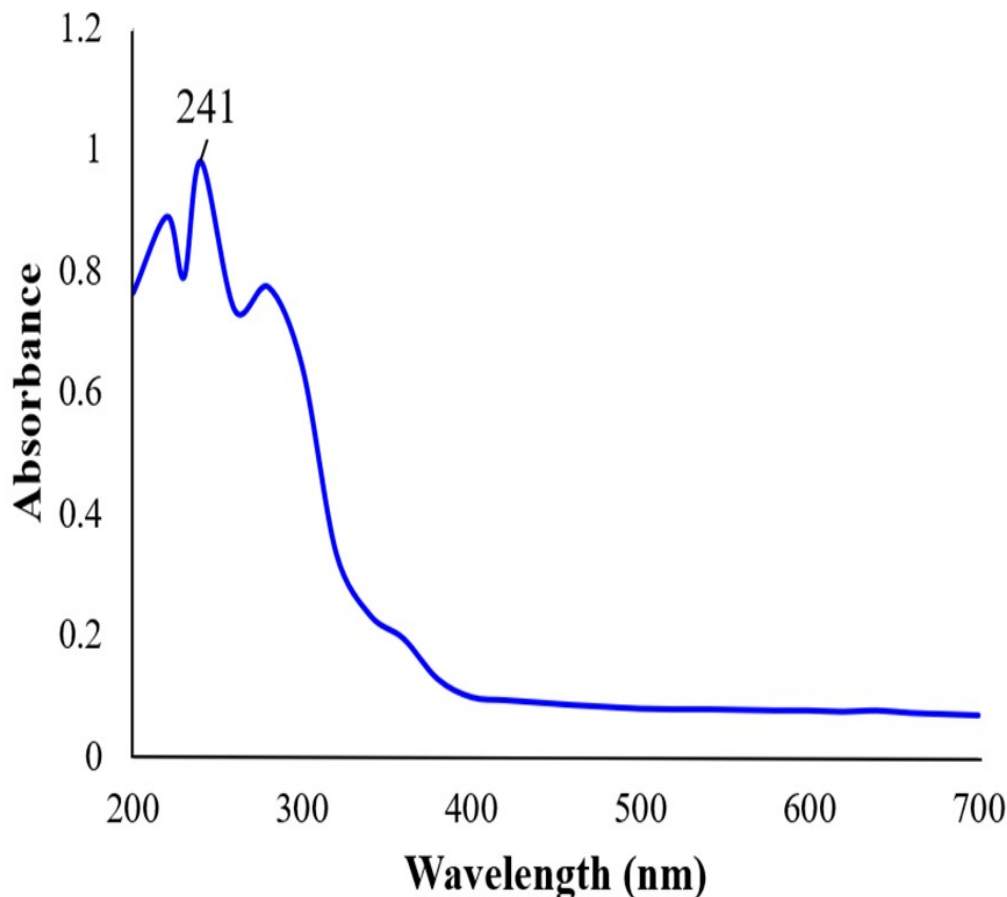


FIGURE 4.2: The absorbance spectrum of silica nanoparticles acquired from spectrophotometry. The peak at 241nm wavelength confirms the presence of silica NPs.

4.2.2 FTIR

The FTIR was performed on both silica NPs and dried plant extract, to identify different functional groups. The peaks can be noted in Fig 4.3 and Fig 4.4 . Different peaks of FTIR found in plant extract can also be noted in silica NPs spectrum.

Different functional groups present in plant extract and silica NPs sample can be noted in Table 4.1. The peaks appearing in both plant extract FTIR spectra and silica NP spectra, correspond to compounds, which were involved in bio-reduction of silica. This reduction allowed synthesis of NPs. Furthermore, these compounds are the ones that are involved in capping of silica NPs. The capping is crucial in preventing aggregation of silica NPs.

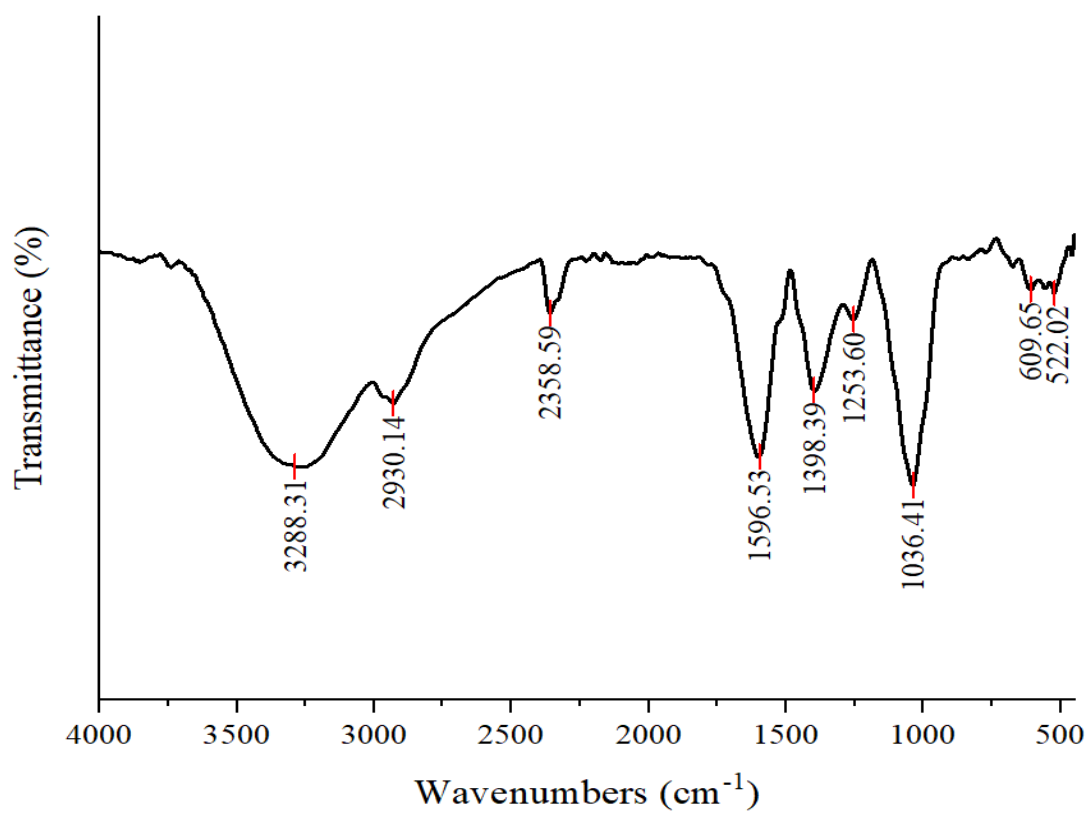


FIGURE 4.3: The FTIR spectrum of dried white cumin extract.

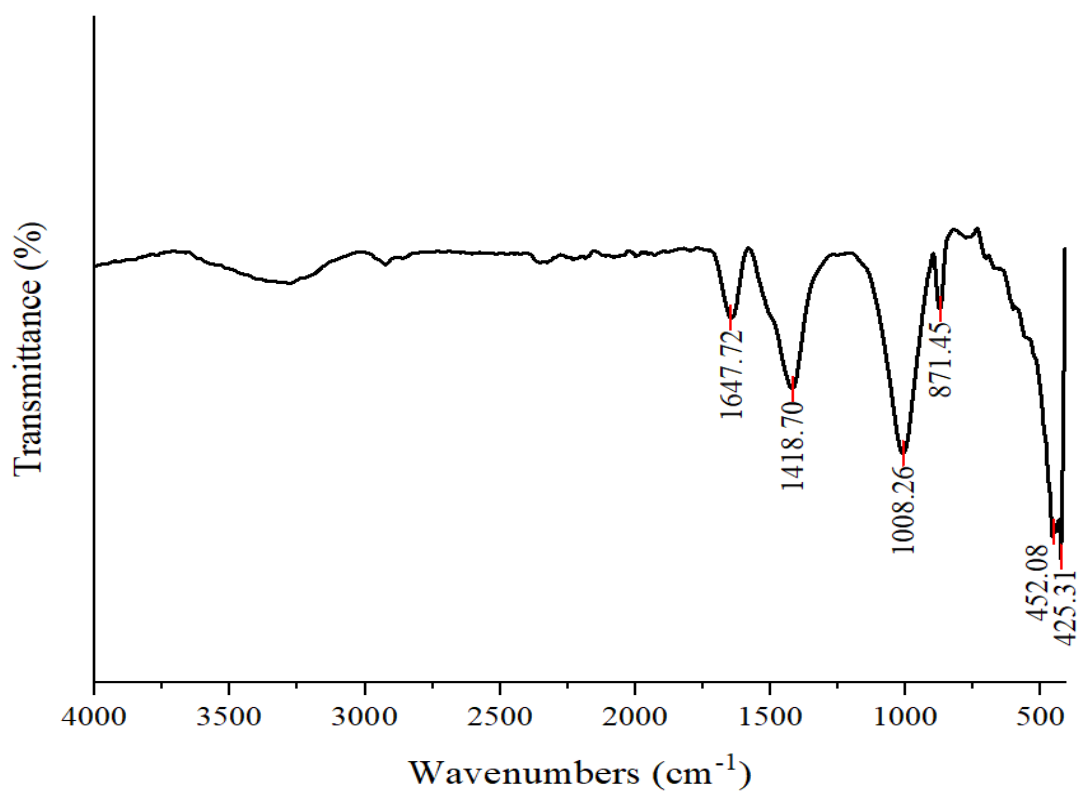


FIGURE 4.4: The FTIR spectrum of synthesized silica NPs.

TABLE 4.1: Sample absorbance wavenumbers and their corresponding functional groups and compounds in plant and silica NPs FTIR.

Sr. No.	Wavenumbers (cm ⁻¹), Source	Functional Groups	Compounds	Refs
1	3288.31, Plant	O-H stretching	Alcohol	[177]
2	2358.59, Plant	C-H bending	Aromatics	[177]
3	1596.53, Plant	O-H bending	Water	[178]
4	1647.72, NPs	O-H bending	Water	[178]
5	1418.7, NPs	C-C stretch in ring	Aromatics	[177]
6	1398.39, Plant	O-H bending	Carboxylic acid	[177]
7	1253.6, Plant	C-O stretching	Alkyl aryl ether	[177]
8	1036.41, Plant	O-H bending	Phenols	[177]
9	1008.26, NPs	O-H bending	Phenols	[177]
10	871.45, NPs	Si-O-Si stretching	Silica NPs	[178]
11	522.02, Plant	C-I stretching	Halo compounds	[177]
12	452.08, NPs	Si-O-Si stretching	Silica NPs	[178, 179]
13	425.31, NPs	Si-O-Si stretching	Silica NPs	[178, 179]

4.2.3 SEM-EDX

SEM showed spherical shape of silica NPs and their size range from 72nm-94nm (Fig 4.5). EDX analysis showed strong peaks corresponding to presence of silica NPs. In addition, the sample analyzed had C, Ca, Mg and P (Fig 4.6). Since, white cumin seed extract was used for synthesis of silica NPs, these elements from cumin extract must have adhered to silica NPs surface.

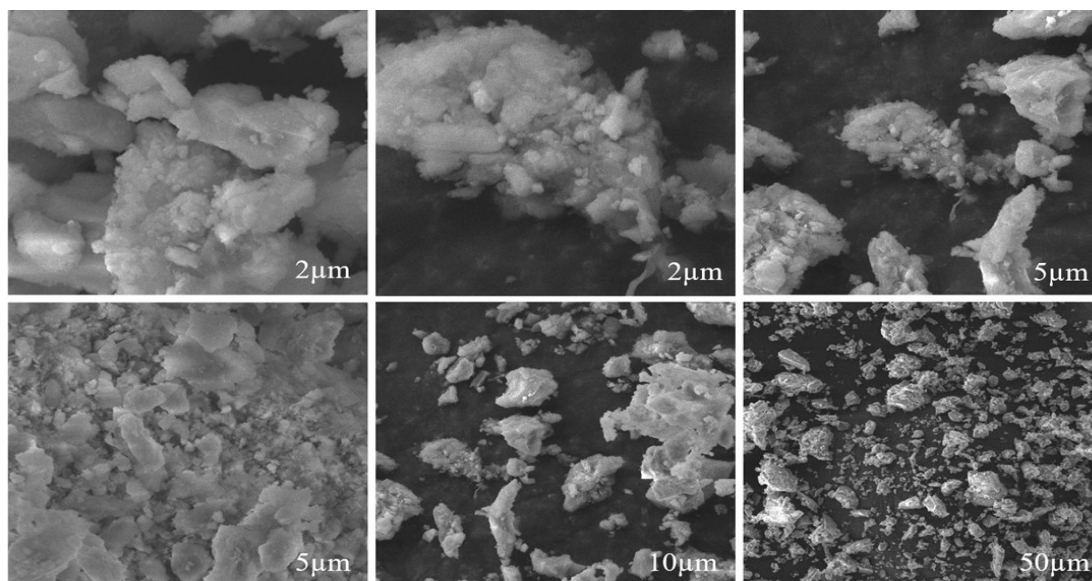


FIGURE 4.5: SEM micrograph of silica NPs.

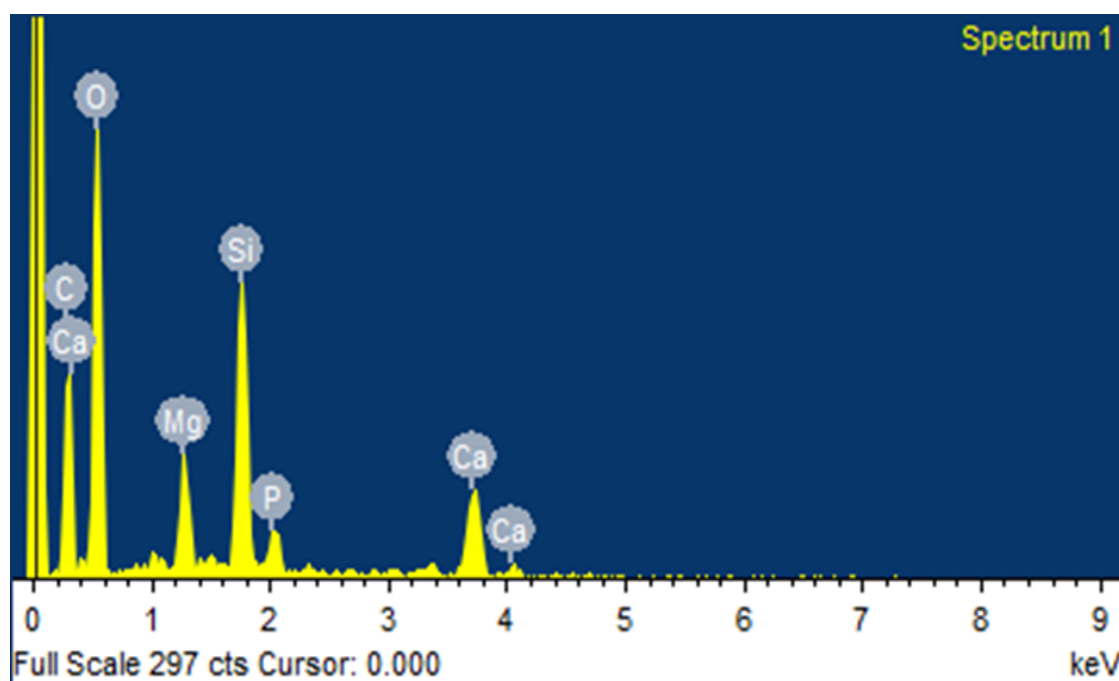


FIGURE 4.6: Elemental composition of silica NPs.

4.2.4 XRD

XRD was performed on dry/ powder state of silica NPs (Fig 4.7). The grain size of crystals was measured to be approx. 83nm using Scherrer Eq 3.1. The angle 2θ had various peaks ranging from 20 - 80nm. The peaks indicated crystalline structure of silica NPs. The phase composition in XRD was determined via Match 3

software. The software confirmed presence of Si, O and Ca. XRD hence validated the results of SEM and EDX.

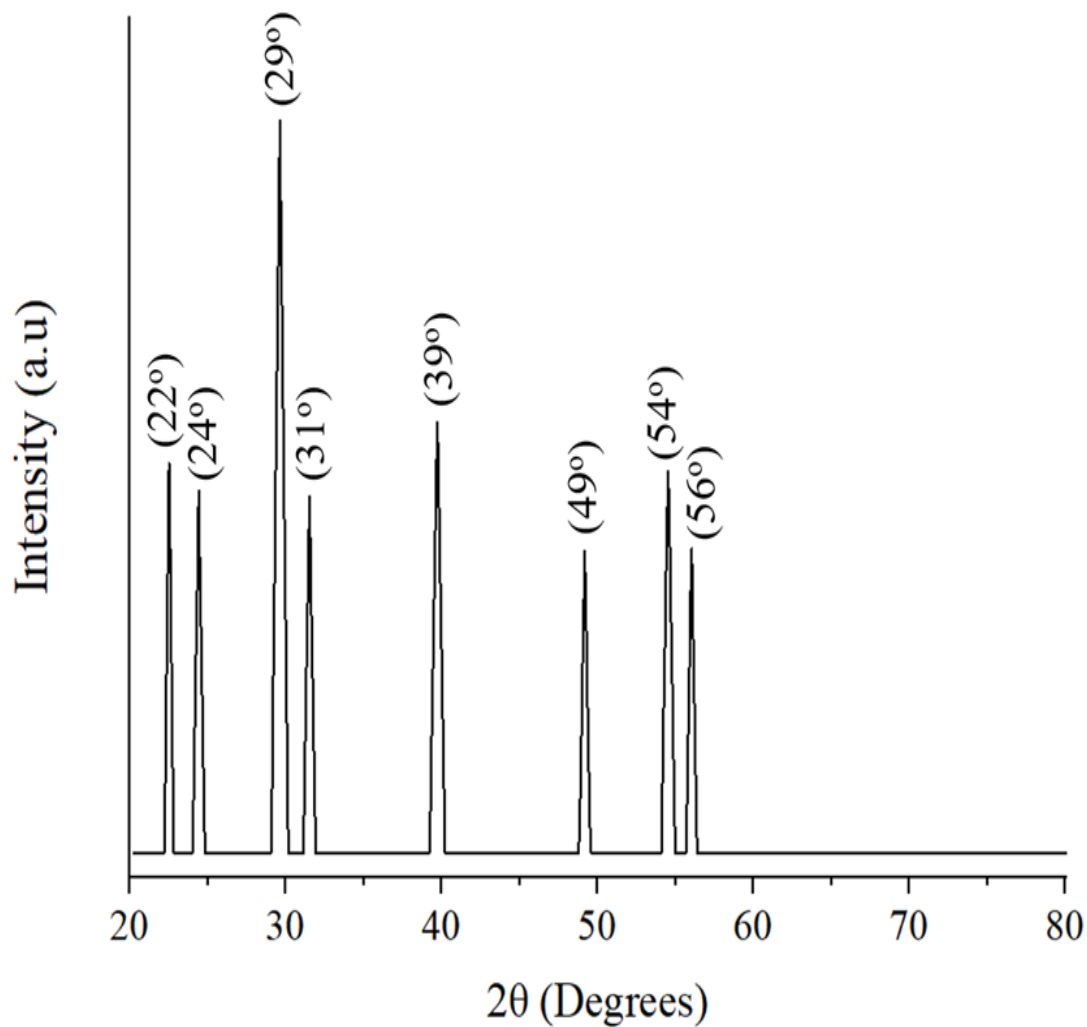


FIGURE 4.7: XRD pattern of silica NPs.

4.3 Soil Analysis

Soil collected from Islamabad, Pakistan was analysed for its elemental composition. Table 4.2. shows different elements present in test soil. The soil was weakly acidic. Phosphorous, boron, copper, zinc, sodium and nitrogen were all low in sample soil. Only calcium and magnesium were present in optimum quantity. The

organic matter in soil was also low. Potassium was present in moderation, while iron and manganese were fairly high. Soil fertility was low.

TABLE 4.2: Soil analysis report of clay soil collected from Islamabad for plant germination.

Sr. No.	Soil Attributes	Results	Description
1	Soil pH	6.9	Weakly acidic
2	Phosphorous	16	Low
3	Potassium	107	Medium
4	Calcium	1200	Optimum
5	Magnesium	245	Optimum
6	Sulfur	15	Medium
7	Boron	0.3	Very low
8	Copper	0.5	Low
9	Iron	57	Very high
10	Manganese	60	Very High
11	Zinc	1.5	Low
12	Sodium	45	Low
13	Total N	32	Low
14	Organic matter	2.5 % ENR 88	Slightly low
15	Cation exchange capacity	8.1meq/ 100g	Low

4.4 Morphological Analysis

L. sativa (green lettuce) was used as test plant to elucidate nano-fertilizer activity of silica NPs. The application of silica NPs was foliar. After application, plants were harvested for their morphological and biochemical analysis. The study had four main plant groups; 1) Control, 2) Standard, 3) Treated and 4) Combined. Control was given no fertilizer at all. Standard was given a basic NPK (1:1:1) fertilizer (GrowMore). Treated group was given synthesized silica NPs. Combined group was given both NPK fertilizer and silica NPs. Each group had three plants.

Morphological differences between four groups (control, standard, treated and combined) can be assessed in Fig 4.8 and Table 4.3. The overall phenotype of control and standard was much inferior to that of treated and combined. The number of leaves of control and standard were equal. Treated group had high number of leaves than control and standard. Highest number of leaves were noted in combined group (Fig 4.9).



FIGURE 4.8: *L. sativa* plants harvested on 14th day of treatment. Control plants were given no treatments at all. Standard plants received basic NPK fertilizer (1:1:1). Treated plants were subjected to foliar application of silica NPs. Combined plants received both NPK fertilizer and foliar spray of silica NPs. A clear visual difference can be noted in roots and leaves development, number of leaves, area of leaves and length of roots.

TABLE 4.3: Morphological parameters along with standard error of green lettuce (*L. sativa*) groups control, standard, treated and combined.

Parameter	Control	Standard	Treated	Combined
No. of leaves	3.7±0.33a	3.7±0.33a	4.7±0.88a	5±0.00a
Area of leaf(cm ²)	12.33±1.42a	11.5±2.52a	25.33±2.53a	28.83±2.24a
Fresh leaf weight(g)	1.43±0.11ab	1.18±0.01a	1.9±0.07bc	2.14±0.12c
Dry leaf weight(g)	0.29±0.02ab	0.24±0.02a	0.38±0.07bc	0.46±0.03c
Root length(cm)	8.35±2.02a	7.85±1.01a	16.69±0.31b	14.69±2.26b
Root diameter(cm)	0.21±0.2a	0.31±0.3a	0.37±0.5b	0.37±.3b
Fresh root weight(g)	1.42±0.11a	1.42±0.09a	2.31±0.06b	2.72±0.03b
Dry root weight(g)	0.2±0.11a	0.2±0.05ab	0.4±0.04bc	0.5±0.04c

(The data presented is average readings followed by standard error. Different letters in front of different readings in same row show significant difference in accordance with Duncan test ($p < 0.05$).)

ImageJ software was used to calculate area, length of roots and leaves, and diameter of roots. The area of leaves in control and standard was quite small in comparison to that of treated and combined. The combined group had biggest area of leaves (Fig 4.10). Fresh and dry weight was significantly higher in treated and combined group, in contrast to control and standard groups in leaves (Fig 4.11) and roots (Fig 4.12).

Length of roots in control was higher than standard. However, treated group had greatest length of roots. Combined group also showed root length higher than that of control and standard but lesser than treated groups (Fig 4.13). Diameter of roots was least in control. Treated and combined groups both showed higher diameter of roots (Fig 4.14). This indicated that overall Silica NPs had a positive impact of morphology of lettuce plant.

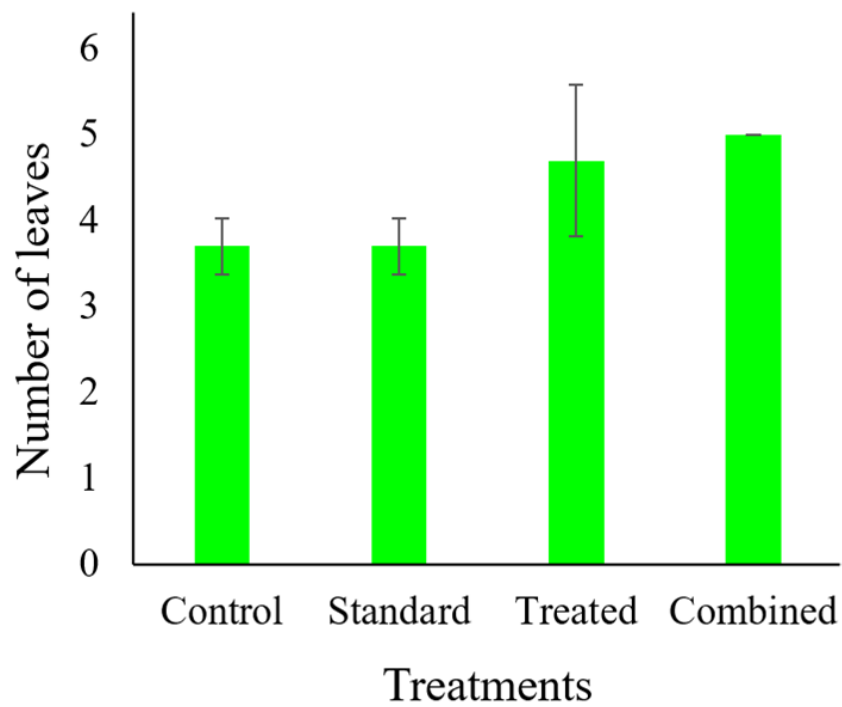


FIGURE 4.9: Number of leaves of different groups of *L. sativa*. Data shown is mean of three replicates per group. Error bars show standard error.

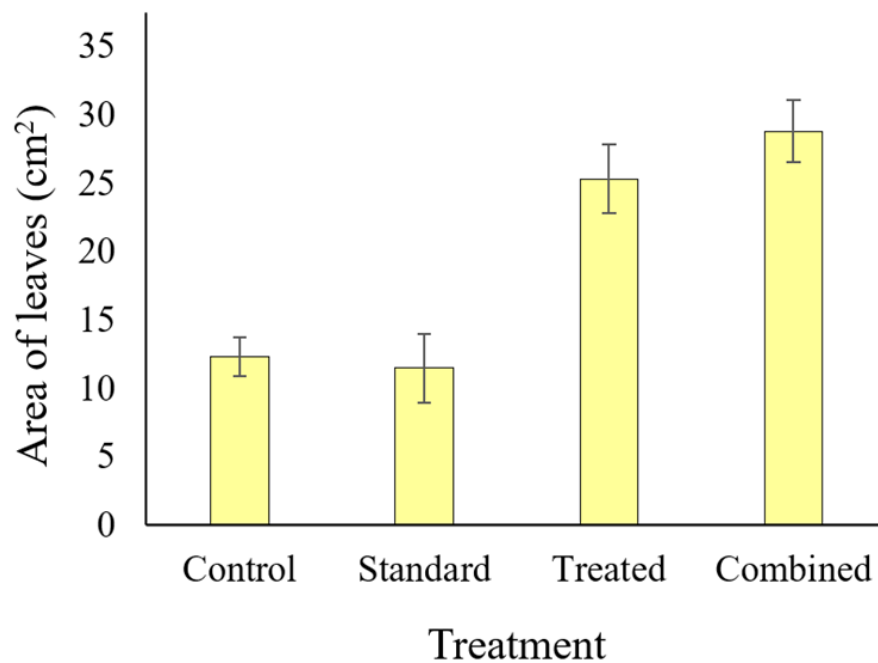


FIGURE 4.10: Area of leaves (cm²) of *L. sativa*. Data shown is mean of three replicates per group. Error bars show standard error.

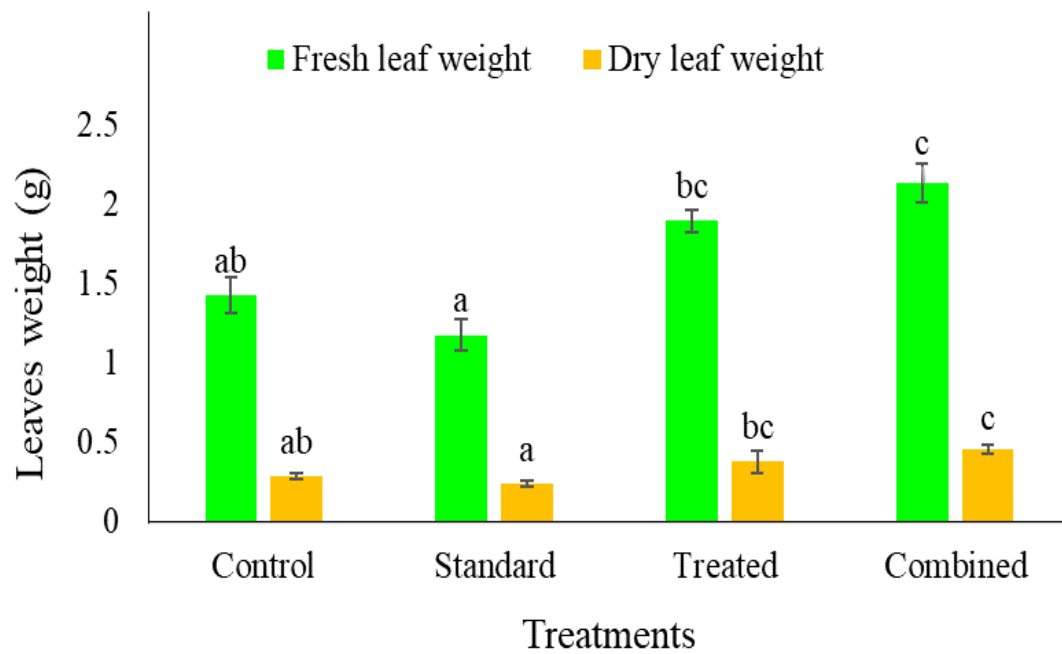


FIGURE 4.11: Weight of leaves of *L. sativa*. Green bars show weight of fresh leaves and brown bars show dry weight. Data shown is mean of three replicates per group. The error bars show standard error of data. Significant difference is indicated by different letters on top of bars according to Duncan test ($p < 0.05$).

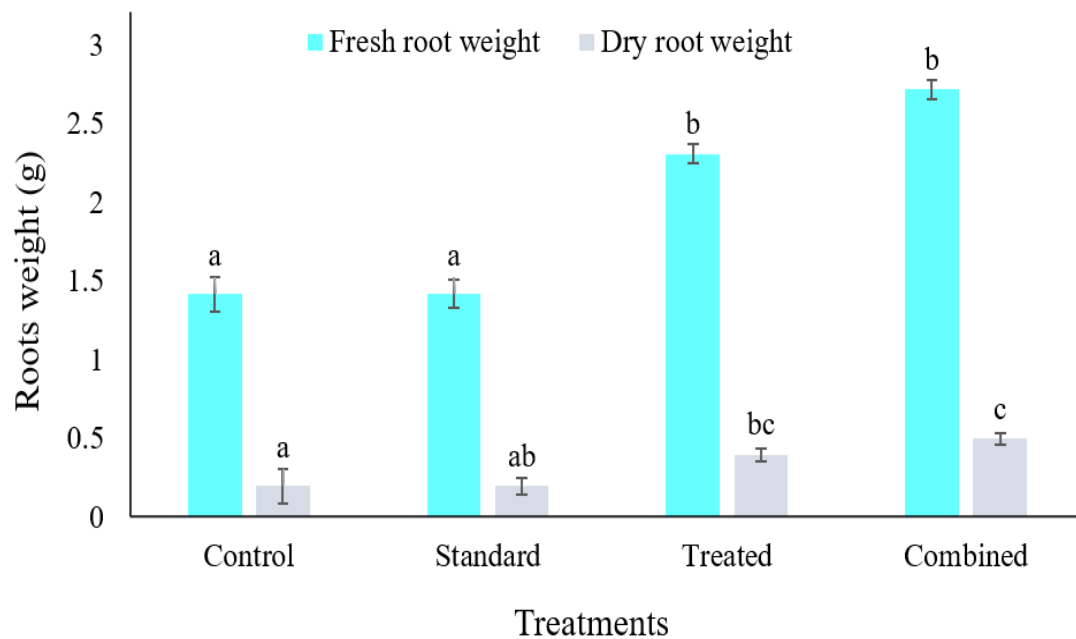


FIGURE 4.12: Weight of roots of *L. sativa*. Blue bars show weight of fresh roots and gray bars show dry weight. Data shown is mean of three replicates per group. The error bars show standard error of data. Significant difference is indicated by different letters on top of bars according to Duncan test ($p < 0.05$).

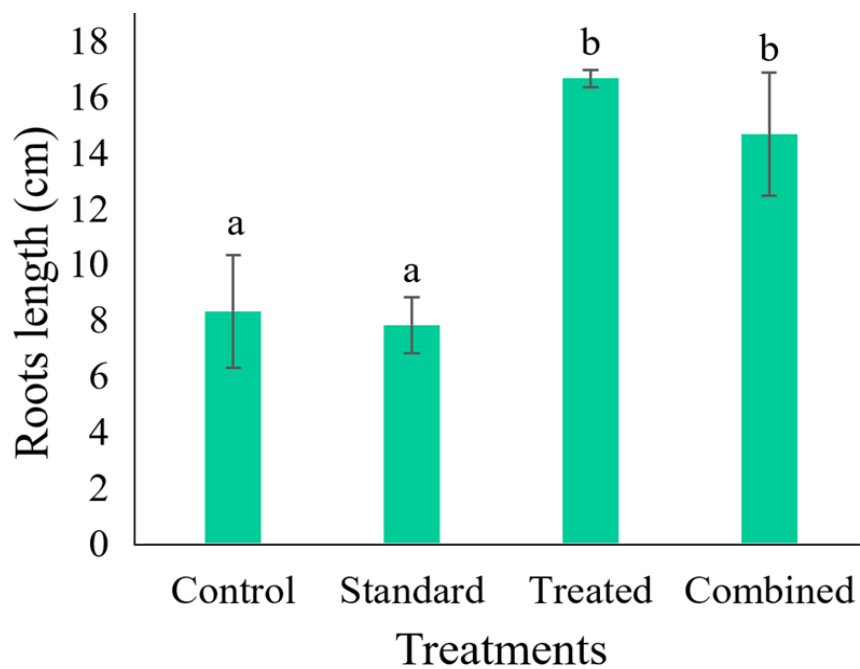


FIGURE 4.13: Length of roots of *L. sativa*. Data shown is mean of three replicates per group. The error bars show standard error of data. Significant difference is indicated by different letters on top of bars according to Duncan test ($p < 0.05$).

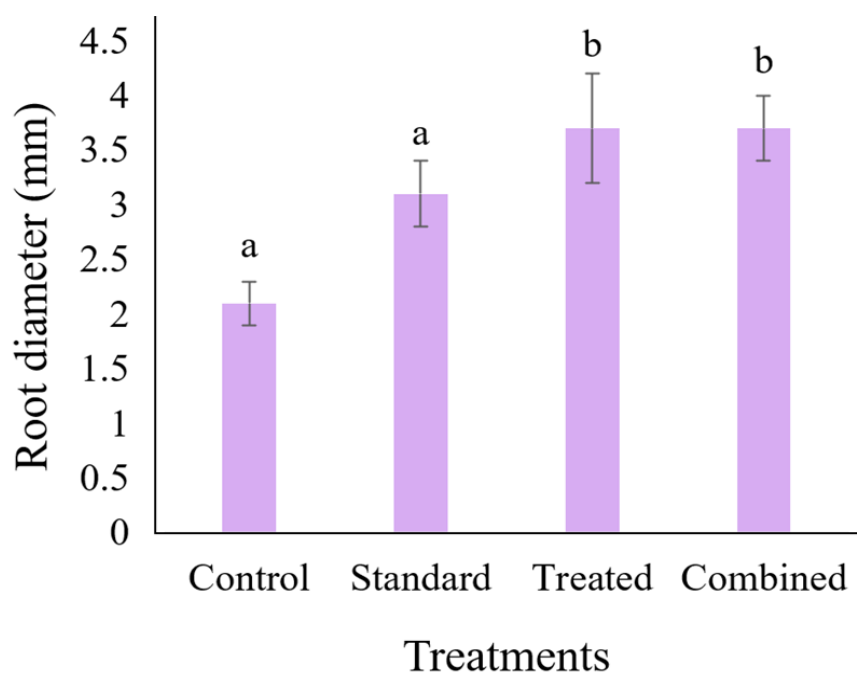


FIGURE 4.14: Diameter of roots of *L. sativa*. Data shown is mean of three replicates per group. The error bars show standard error of data. Significant difference is indicated by different letters on top of bars according to Duncan test ($p < 0.05$).

In light of above data on morphological traits, it is safe to conclude that application of silica NPs had direct impact on lettuce plants' leaves number, fresh and dry weight of leaves and roots, leaf area, root length and root diameter. There was a direct positive correlation between noted morphological attributes and silica NPs usage. This implies that use of silica NPs as nano-fertilizers has direct impact on increasing lettuce plant biomass. Hence, it has good fertilizer properties.

Similar data was reported by other studies as well. An overall increase in plant growth was reported, when silica NPs were applied on *C. aronia* [129]. Application of silica NPs on *L. olgensis* plant increased root length at 500 $\mu\text{L/L}$ conc. [123]. There was increase in fresh and dry weight of *L. esculentum* at 8g/ L conc. of silica NPs [128]. Increase in fresh weight, dry weight, roots length and leaves number was reported in banana plant, when subjected to silica NPs at conc. of 50, 100 and 150 ppm [180].

4.5 Biochemical Analysis

The biochemical analysis of *L. sativa* involved measurement of; 1) Photosynthetic pigments (Ch_a , Ch_b and carotenoids), 2) Proline, 3) MDA and 4) Relative water content (Table 4.4).

4.5.1 Photosynthesis Pigments

Acetone based extraction was used to isolate Ch_a , Ch_b and carotenoids (Fig 4.15), followed by spectrophotometry for measurement of pigments in leaves of *L. sativa*. The concentrations can be noted in Fig 4.16. The photosynthetic pigments in treated group were higher than control and standard groups. However, the combined group had highest levels of photosynthetic pigments. The foliar application of silica NPs had positive impact on these pigments. Similar results have been reported by other studies as well, when plants were subjected to NPs. Increased in chlorophyll conc. was reported in *L. olgensis*, [123], *C. aronia* [129], banana [180] and potato plants [181], indicating increased photosynthesis rate.

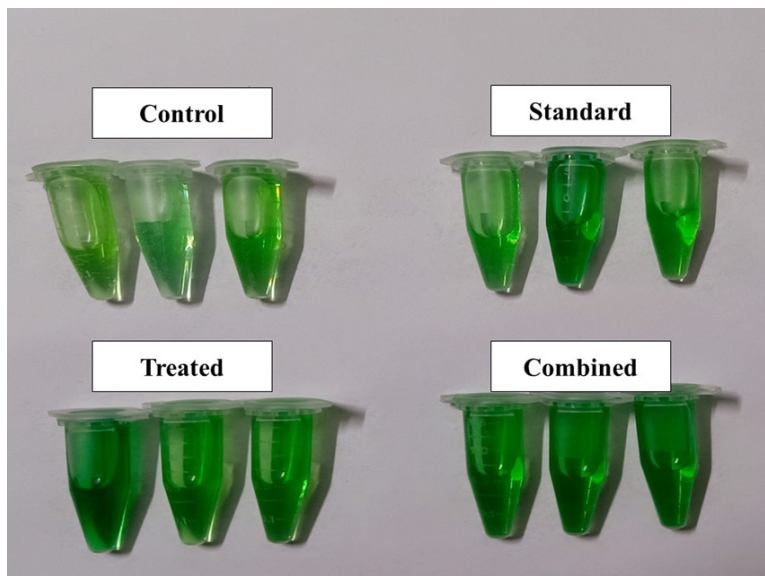


FIGURE 4.15: Acetone based extraction of photosynthetic pigments from *L. sativa* leaves. Control group was given no treatment. Standard group was given NPK fertilizer. Treated group was given synthesized silica NPs. Combined group was given both silica NPs and NPK fertilizer. Each group had three test plants. Colour variation of green can be noted among different vials. The darker the green colour, higher the photosynthetic pigments. Highest chlorophyll is in combined group as shown by darkest green colour. The opposite is true for control group.

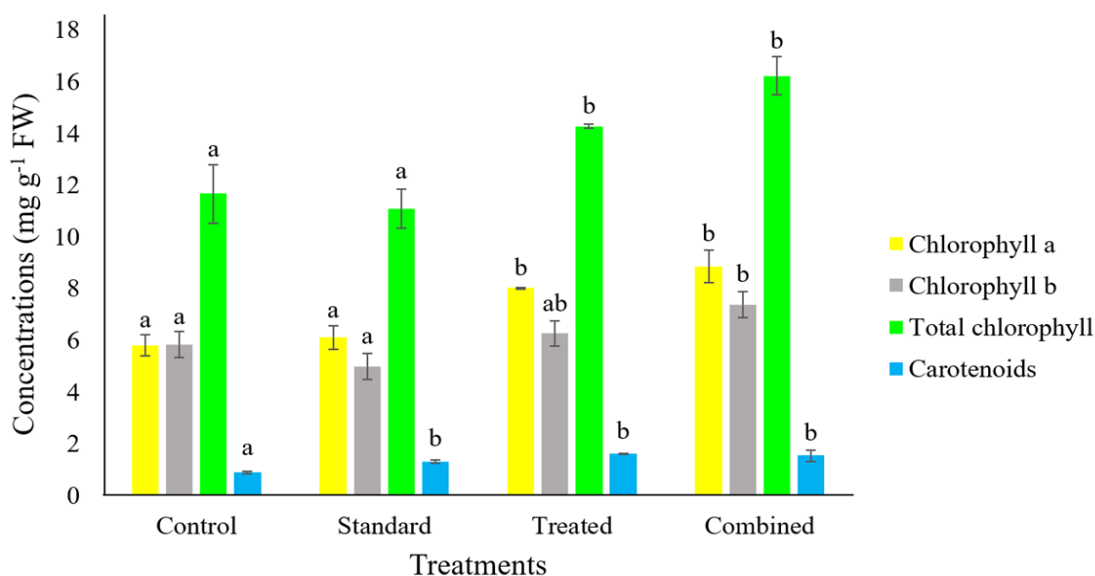


FIGURE 4.16: Photosynthetic pigments of leaves of *L. sativa*. The graph shows A) Chlorophyll a (yellow bars), 2) Chlorophyll b (gray bars), 3) Total chlorophyll (green bars) and 4) Carotenoids (blue bars). Statistical analysis was performed by ANOVA. Data shown is average of three plants per group. The error bars show standard error of data. Different letters at top of bars show significant difference in accordance with Duncan test ($p < 0.05$).

4.5.2 Proline Content

A valuable role is played by proline when plants are under stress. It acts as an osmolyte, signaling molecule, metal chelator, and antioxidant defense molecule [182]. Proline was extracted from leaves and roots (Fig 4.17). The concentration was noted using spectrophotometry. Proline content in leaves was higher in treated group and highest in combined group, in comparison to control and standard groups. On the other hand, in case of roots proline was highest in control group. Treated group proline content was higher than standard and combined groups (Fig 4.18).

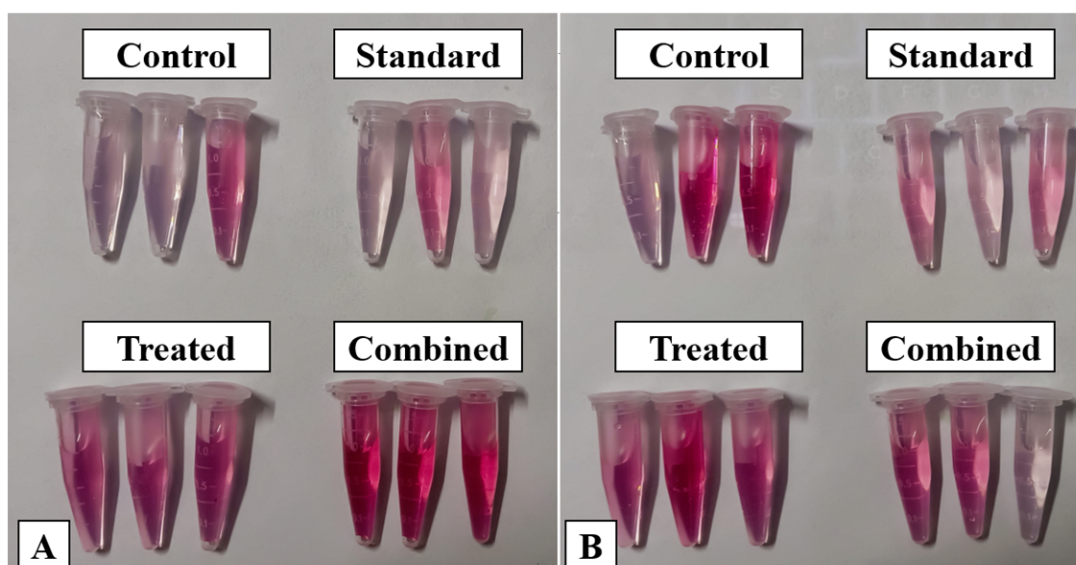


FIGURE 4.17: Proline content isolated from A) Leaves and B) Roots of *L. sativa*. Four groups can be noted in both parts; Control (no treatment), standard (NPK fertilizer), treated (silica NPs) and combined (silica NPs and NPK fertilizer). Each group had three plants. Darker the colour, higher the proline content. A wide range of colour variations can be noted between groups in both leaves and roots. Light pink colour depicting least proline content, while dark pink colour showing highest proline content. (A) Proline content in leaves of combined group is highest. B) Proline content in roots is highest in control group, followed by treated group.

There have been contradictory results related to proline content in plants when treated with silica NPs. *C. aronia* when treated with silica NPs showed lowering of proline content [129]. On the other hand, potato plants showed increase in proline content when subjected to silica NPs [181]. When plants are under stress,

they produce proline. This allows them to better cope with environmental stress [182]. Now considering this research study, high proline content in treated plants indicate that plants were under stress. The plants were not provided any kind of stress in this work. This implicates that either the silica NPs have stressful impact on plant or there was some unknown stressor in plant's environment, causing the increase of proline. More research needs to be done on this particular point.

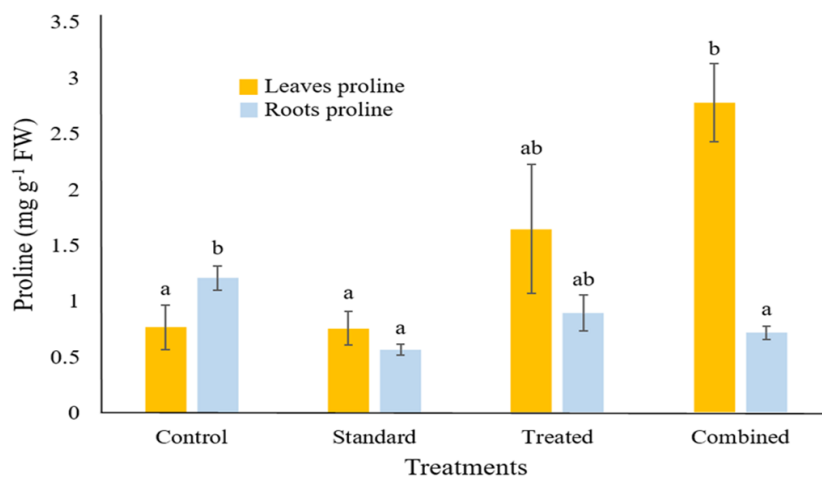


FIGURE 4.18: Proline content in leaves (yellow bars) and roots (blue bars) isolated from *L. sativa*. Statistical analysis was performed by ANOVA. Data shown is average of three plants in each group. Standard error is shown by error bars. Significant difference is indicated by different letters on top of bars according to Duncan test ($p < 0.05$).

4.5.3 MDA Content

MDA test is a major test to elucidate lipid peroxidation in plants. MDA test was conducted on both leaves and roots separately (Fig 4.19). Spectrophotometry was used to identify the concentrations of MDA, by reading absorbance at 532nm and 600nm. The concentrations can be noted in Fig 4.20. The MDA content in control and standard was significantly higher than that of treated and combined groups.

Similar finding have been reported in *C. aronia* when treated with silica NPs [129]. MDA is an indicator of lipid peroxidation and redox status in plants. Lower content of MDA means that plant has higher antioxidative properties and higher drought resistance [183]. Hence, the antioxidative properties in lettuce plants' leaves and roots are high due to treatment of silica NPs.

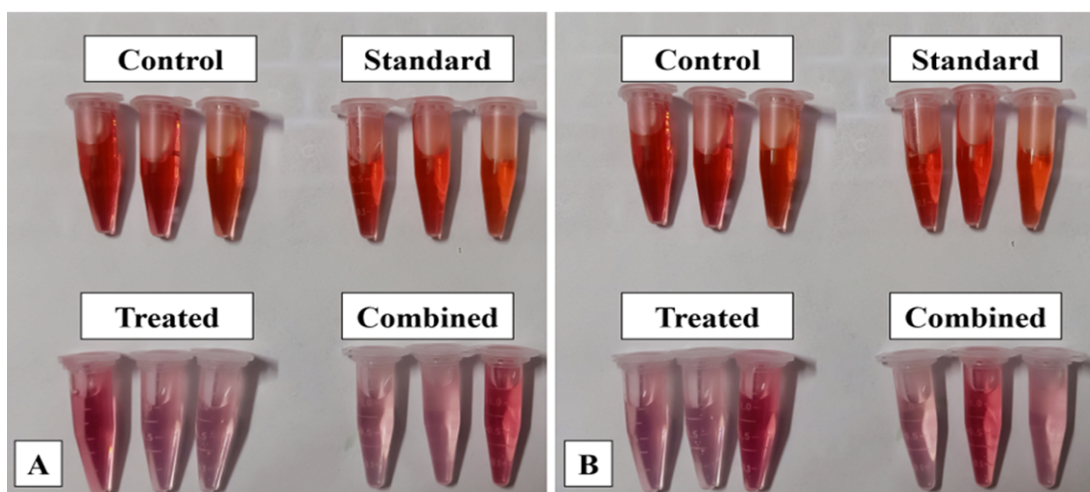


FIGURE 4.19: MDA content isolated from A) Leaves and B) Roots of *L. sativa*. Four groups can be noted in both parts; Control (no treatment), standard (NPK fertilizer), treated (silica NPs) and combined (silica NPs and NPK fertilizer). Each group had three plants. Darker the colour, higher the MDA concentration. The colour range can be noted from very light pink to bright red. The MDA was high in control and standard in case of both leaves and roots. Lower MDA content in treated and combined group of both leaves and roots is depicted by lighter colour.

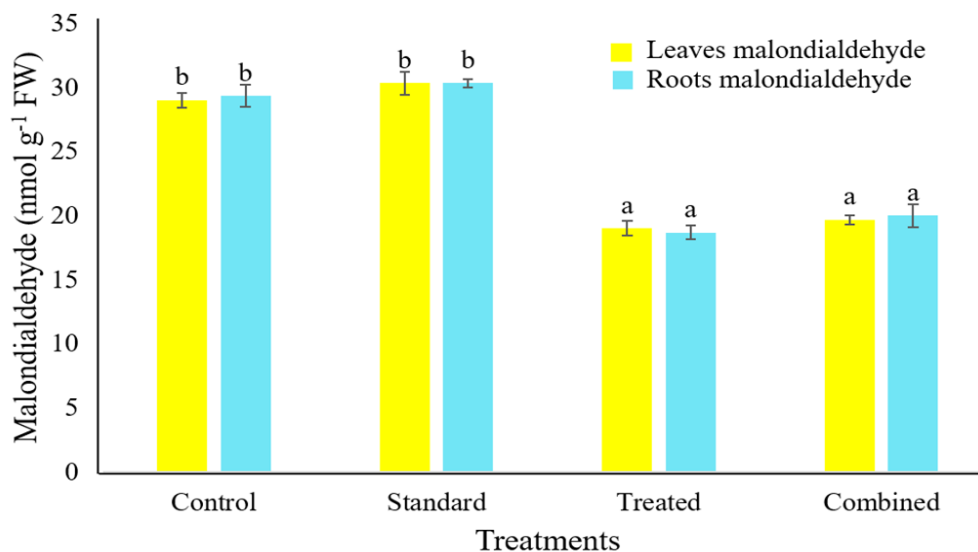


FIGURE 4.20: Malondialdehyde content in leaves (yellow bars) and roots (blue bars) of *L. sativa*. Statistical analysis was done by ANOVA. Data presented is average of three replicates per group. Standard error can be noted by error bars. Different letters on top of each bar indicate significant difference according to Duncan's test ($p < 0.05$).

4.5.4 Relative Water Content

The RWC was measured by using plant leaves fresh weight, turgor weight and dry weight. The percentage RWC can be noted in Fig 4.21. RWC in leaves indicates the status of water in plants. It shows a balance between water supplied to the leaf and rate of transpiration [184]. RWC of treated and combined group was higher in comparison to that of control and standard group.

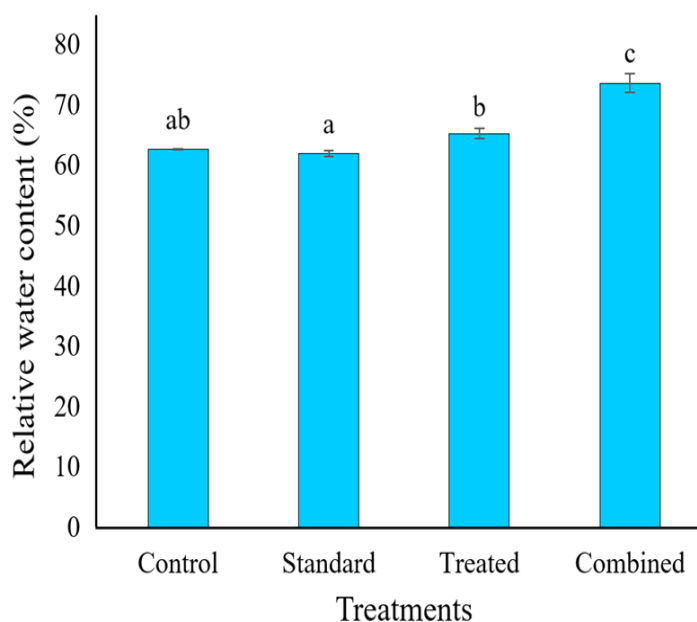


FIGURE 4.21: Relative water content of *L. sativa* leaves. The data is mean of three replicates per group. The standard error per group is shown by error bars. Different letters indicate significant difference according to Duncan's test ($p < 0.05$).

TABLE 4.4: Biochemical parameters along with standard error of *L. sativa* (green lettuce) plant groups control, standard, treated and combined.

Parameters		Control	Standard	Treated	Combined
Chlorophyll (mg g ⁻¹ FW)	a	5.8±0.4a	6.1±0.46a	8.01±.03b	8.85±0.64b
Chlorophyll (mg g ⁻¹ FW)	b	5.82±0.73a	4.97±0.29a	6.25±0.11ab	7.37±0.16b

TABLE 4.4: Biochemical parameters along with standard error of *L. sativa* (green lettuce) plant groups control, standard, treated and combined (Continued).

Parameters	Control	Standard	Treated	Combined
Carotenoids (mg g ⁻¹ FW)	0.87±0.05a	1.29±.07b	1.6±.02b	1.53±0.22b
Proline in leaves (mg g ⁻¹ FW)	0.77±0.2a	0.76±0.15a	1.65±0.58ab	2.78±0.35b
Proline in roots (mg g ⁻¹ FW)	1.21±0.11b	0.57±0.05a	0.9±0.16ab	0.72±0.06a
MDA in leaves (nmol g ⁻¹ FW)	29±0.58b	30.33±0.88b	19±0.58a	19.67±0.33a
MDA in roots (nmol g ⁻¹ FW)	29.33±0.88b	30.33±0.34b	18.67±0.54a	20±0.88a
Relative water content (%)	73.6±1.54ab	65.25±0.88a	62.00±0.58b	62.67±0.33c

(The data presented is average readings (n=3 per group) followed by standard error (ANOVA). Different letters in front of different readings in same row show significant difference in accordance with Duncan test (p<0.05).)

Chapter 5

Conclusion and Future Work

Agriculture sector faces many challenges worldwide. The increase in yield is a major goal for agribusiness. Chemical fertilizers do address this issue, but at the expense of human health and environment. Nano-fertilizers can address this issue by increasing plant production, while staying ecofriendly. Green lettuce (*L. sativa*) is a widely consumed plant worldwide. The objective of this research was to identify impact of biogenic silica NPs on growth of lettuce plant.

Silica NPs were made by green synthesis approach. The particles ranged in size from 72-94nm. They had crystalline structure and spherical shape. The lettuce plants subjected to silica NPs (foliar application) showed positive response in terms of growth. Silica NPs increased leaves number, surface area of leaf, length of roots, diameter of roots, weight of fresh weight and dry leaves and roots. The overall increase in morphological traits was noted.

Biochemical profiling showed that lettuce plants treated with silica NPs had increase in chlorophyll a, b and carotenoids, which indicated increase in photosynthesis. An increase in concentration of proline was noted in roots and leaves. Lowered MDA content in roots and leaves elucidated increased antioxidative abilities of lettuce plants.

Based on morphological and biochemical analysis, the overall impact of silica NPs was positive on lettuce plants. This means that green synthesized silica NPs can be

used as nano-fertilizer. Further experimentation can be conducted to evaluate their impact on soil, microbiota of rhizosphere, endosphere and phyllosphere. These can be used in agriculture to increase plant growth.

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