

**RESPONSE OF MAIZE (*Zea mays* L.) TO GREEN
SYNTHESIZED ZnO NANOPARTICLES IN A CALCAREOUS
VERTISOL**

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SYNTHESIZED ZnO NANOPARTICLES IN A CALCAREOUS
VERTISOL**

*Thesis submitted to the
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IN
SOIL SCIENCE**

By

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CERTIFICATE

This is to certify that the thesis entitled "RESPONSE OF MAIZE TO GREEN SYNTHESIZED ZnO NANOPARTICLES IN A CALCAREOUS VERTISOL" submitted by Miss. AMRUTHA S. AJAYAN for the degree of MASTER OF SCIENCE (AGRICULTURE) in SOIL SCIENCE to the University of Agricultural Sciences, Dharwad is a record of research work carried out by her during the period of her study in this University, under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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1. INTRODUCTION

Maize is one of the cereal crops which is widely cultivated throughout the world and has the highest production potential among all the cereals. The crop has tremendous genetic variability, which enables it to thrive in tropical, subtropical and temperate climates.

Maize is the third most important cereal crop in India after rice and wheat with an area of 9.23 million hectares, production 23.67 million tonnes and productivity 2,564 kg ha⁻¹. In Karnataka, it is cultivated in an area of 1.34 million hectares with a production of 3.91 million tonnes and an average productivity of 2,921 kg ha⁻¹ (Anon., 2016). Maize accounts for nearly nine per cent of total food grain production of the country. The grains are rich in vitamins A, C and E, carbohydrates, proteins, dietary fiber and essential minerals. It is an important staple food in many countries and is also used in animal feed and has many industrial applications. Poultry industry is heavily dependent on maize as it forms 50-60 per cent of the input required for broiler feed and 25-35 per cent for layer feed. Besides serving as human food and animal feed, it is a source for a large number of industrial products such as starch, oil, baby corn, popcorn, dairy feed, poultry feed and so on.

Zinc (Zn) is the second most abundant transition metal in plants and organisms after iron and the only metal represented in all six enzyme classes. It is an essential micronutrient for humans, animals and plants. Higher plants generally absorb Zn as a divalent cation (Zn²⁺) which acts either as the metal component or as a functional or structural or a regulatory co-factor of a large number of enzymes (Prasad *et al.*, 2012).

Zinc is the most widespread deficient micronutrient in the soil world over. In India, Zn is considered as the fourth most important yield-limiting nutrient after nitrogen (N), phosphorus (P) and potassium (K). In India, 40-42 per cent cultivated lands show Zn deficiency which is causing considerable reduction in yield. Zinc deficiency is very common in Vertisols (calcareous or non calcareous black soils) due to the precipitation of Zn as ZnCO₃ or Zn (OH)₂. Hence, there is a need to follow suitable management practices to protect crop plants from zinc deficiency.

Zinc deficiency in soil results in retardation of plant growth, decreased grain yield, protein synthesis and nutritional value of crops. Zinc deficiency is caused in human beings and animals due to the lower content of zinc in grains. Zinc is required for chlorophyll production, pollen function, fertilization and germination and also plays an important role in biomass production and drought tolerance. It was found that application of zinc fertilizers enhanced the growth and yield of crop plants with better economic benefits. But, the use efficiency of zinc carriers is low. Hence, there is a need to enhance fertilizer use efficiency of applied fertilizers and also to control environmental pollution due to excess application of fertilizers.

Nanotechnology is a novel, innovative, interdisciplinary scientific approach that involves designing, development and application of materials and devices at nanometre scale *i.e.*, at least one dimension ranges from 1 to 100 nanometres. In 21st century, it has to deal with concerns like sustainable use of natural resources, depleting nutrients in soil and environmental issues like runoff and accumulation of fertilizers and pesticides.

There is a need to adopt such a technology that can shape the modern agriculture in a more productive fashion which would ultimately lead to precision farming in a cost-effective way with the delivery of just the right amount of input at the right time. Among the latest technological innovations in the field of agriculture, nanotechnology occupies a distinguished position in remodeling agriculture and food production to fulfill the demands in an efficient and cost-effective way (Ali *et al.*, 2014).

Green nanotechnology is an innovative branch of nanotechnology to produce nanomaterials and products without harming the environment or human health. It uses existing principles of green chemistry and green engineering to synthesize nanoparticles (NPs) without toxic ingredients, at low temperatures using less energy and renewable inputs. Green synthesis also known as plant mediated synthesis of metal NPs is gaining more importance owing to its simplicity, rapid rate of synthesis, diverse morphology of synthesized nanoparticles and elimination of elaborate maintenance of cell cultures. Increasing awareness towards green chemistry and biological processes has led to the development of an eco-friendly approach for the synthesis of nanoparticles (Gnanasangeetha and Thambavani, 2014).

Unique properties of nano-fertilizers are due to their high surface area per unit volume. Nanoparticle is having its entire dimension or at least one dimension in nano scale, *i.e.*, less than 100 nm. Nano-fertilizers make nutrients more available to nano scale plant pores resulting in enhanced nutrient uptake and its use efficiency. Greatest advantage of nano-fertilizers over conventional fertilizers is that their requirement is in small quantity and thus easy to handle and transport.

Zinc oxide is an inorganic compound with the molecular formula ZnO. It appears as a white powder and is nearly insoluble in water. ZnO NPs are having high catalytic efficiency and adsorptive ability and have potential to enhance crop yield. The colloidal solution of zinc oxide nanoparticles is used as fertilizer. Zinc oxide nano formulations can be synthesized by green methods using various plant extracts. Green synthesis of metallic nanoparticles employs dried biomass extract of the plants as bio-reducing agents and a metallic salt as the precursor.

Zinc oxide nanoparticles stand out as one of the most versatile materials due to their diverse properties, functionalities and applications. ZnO NPs exhibit tremendous physical and optical properties. The green synthesis of ZnO NPs is much safer and eco-friendly compared to chemical synthesis because it does not lead to formation of toxic by-product chemicals.

The plants such as *Ananas comosus*, *Azadirachta indica*, *Calotropis procera*, *Hibiscus subdariffa*, *Chrysanthemum morifolium*, *Chrysopogon zizanioides*, *Cylindrocladium floridanum*, *Eucalyptus chapmaniana*, *Euphorbia hirta*, *Leucas aspera*, *Mimusops elengi*, *Morinda citrifolia*, *Nelumbo nucifera*, *Ocimum sanctum*, *Phoenix dactylifera*, *Sida acuta*, *Tephrosia purpurea*, *Tinospora cordifolia*, *etc.*, are the some of the plants which can be considered for green synthesis of metallic nanoparticles.

Characterization of nanoparticles can be done using sophisticated techniques such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), Atomic force microscopy (AFM), field emission scanning electron microscopy (FE-SEM), dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS), X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), energy dispersive atomic X-ray analysis (EDAX), selected area diffraction pattern (SAED) and high resolution transmission electron microscopy (HRTEM) and UV–visible spectroscopy. Characterization of nanoparticles is carried out to determine its shape, size, structure, stability and purity. The formation of metallic NPs can be confirmed by simple UV–visible spectroscopic analysis. FTIR analysis is performed to identify the bio-molecules, which play a key role in the reduction of metal ions in to nanoparticles. XRD confirms the structure of nanoparticles. Scanning electron microscopy determines the size and shape of nanoparticles. EDAX determines the purity of metal nanoparticles and estimated zeta potential values tells about the stability of nanoparticles in the aqueous system.

Green synthesis of metallic nanoparticles and its applications in agriculture to enhance the crop yield is gaining importance in present days. Keeping these things in view, a study was initiated with the following objectives:

1. Green synthesis of ZnO nanoparticles using different plants extracts and their characterization.
2. To compare the response of maize to green synthesized nano ZnO formulations vs. conventional zinc sulphate in a Vertisol.
3. To study the effect of nano ZnO spray formulations on the uptake of macro and micro nutrients by maize.

2. REVIEW OF LITERATURE

A comprehensive review of literature is an essential step in any research endeavor for developing a road map for further research and working out a basis for interpretation of findings. Keeping this in view, relevant literatures were reviewed under different headings to understand and support the research findings of a study on “Response of maize to green synthesized ZnO nanoparticles in a calcareous Vertisol”.

- 2.1 Green synthesis and characterization of ZnO nanoparticles
- 2.2 Use of nanoparticles as fertilizers
- 2.3 Comparative performance of nano ZnO formulations vs. conventional Zn carriers on crop growth and dry matter production
- 2.4 Effect of Nano ZnO spray formulations on uptake of macro and micro nutrients by crops
- 2.5 Effect of ZnO nanoparticles on soil properties and post-harvest available nutrients status of soil

2.1 Green synthesis and characterization of ZnO nanoparticles

Green synthesis is the plant mediated synthesis of nanoparticles where the active principles present in the plant extracts (polyphenols, flavanoids, terpenes, carbohydrates, proteins, *etc.*) are used to synthesis nanoparticles from its bulk molecules (precursor).

Lakshmi *et al.* (2012) synthesized zinc oxide nanoparticles (ZnO NPs) using biological and chemical reducing agents. In biological method, hot and cold *Aloe vera* leaf extract and in chemical method, sodium hydroxide was used as reducing agent. Nanoparticles synthesized by the two methods were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM). It was evident from SEM images that particles obtained by biological method were rod shaped and those synthesized by chemical method were spherical in nature.

Kshatriya *et al.* (2013) reported that plant mediated synthesis of metal nanoparticles (NPs) was gaining more importance owing to its simplicity, rapidity, eco-friendly nature, elimination of elaborate maintenance of cell cultures, economic viability and synthesize nanoparticles of diverse morphology.

Sindhura *et al.* (2013) green synthesized ZnO NPs using leaves of *Parthenium hysterophorous*. The characteristics of the synthesized ZnO NPs were studied using UV–visible spectroscopy, scanning electron microscopy and particle size analyzer. ZnO NPs were observed to be spherical in shape with size ranging from 16 to 108.5 nm. The measured zeta potentials varied from 100.4 to 117.20 mV indicating high stability of the ZnO NPs.

Vidya *et al.* (2013) conducted a study to green synthesize ZnO nanoparticles from zinc nitrate using leaf extract of *Calotropis gigantea*.

The ZnO NPs obtained were having size ranging from 30-35 nm. ZnO NPs were characterized by scanning electron microscopy (SEM) and X-ray diffraction (XRD). The particles obtained were spherical in shape and the X-ray patterns showed hexagonal crystal type for ZnO particles.

An attempt was made by Raut *et al.* (2013) to green synthesize ZnO NPs using the leaves extract of *Ocimum tenuiflorum*. Synthesized ZnO nanoparticles were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). SEM image showed hexagonal shape of nanoparticles with diameter ranging from 11-25 nm.

ZnO nanoparticles (ZnO NPs) synthesized using leaf extract of *Hibiscus rosa-sinensis* were characterized by UV-visible spectroscopy and SEM. In UV-visible absorption spectra showed that ZnO NPs had their absorption peak at UV wavelength (358-375 nm) due to its surface plasmon resonance. In SEM analysis, size of ZnO NPs ranged from 40 to 56 nm (Divya *et al.*, 2013).

Sangeetha and Kuppusamy (2013) attempted bio-synthesis of zinc oxide nanoparticles using marine resources. Sea weeds such as *Caulerpa peltata*, *Hypnea valencia* and *Sargassum myriocystum* were used for synthesis of zinc oxide nanoparticles. The preliminary screening of physico-chemical parameters such as concentration of metals, concentration of sea weed extract, temperature, pH and reaction time revealed that sea weed *S. myriocystum* was able to synthesize zinc oxide nanoparticles. It was confirmed through the initial colour change of the reaction mixture and by UV-visible spectrophotometer. The average size of bio-synthesized zinc oxide nanoparticles was 36 nm which was further characterized by techniques such as dynamic light scattering (DLS), atomic force microscopy (AFM), scanning electron microscopy (SEM), energy dispersive atomic X-ray analysis (EDAX), transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR).

Gnanasangeetha and Thambavani (2013) reported that the aqueous leaf extract of *Coriandrum sativum* can be used as an eco-friendly agent for the synthesis of zinc oxide nanoparticles along with zinc acetate and sodium hydroxide as a surrogate for chemical methods. Characterization of synthesized ZnO NPs was done by XRD, SEM, FTIR and EDAX.

Zinc oxide nanoparticles (ZnO NPs) have remarkable optical, physical and anti-microbial properties and therefore have great potential to enhance crop yield. Sabir *et al.* (2014) synthesized ZnO NPs by chemical methods such as precipitation method, vapour transport method and hydrothermal process. They also synthesized ZnO NPs by using different plant extracts such as *Coriandrum sativum*, *Calotropis gigantea* and *Acalypha indica*. According to them, green synthesis was quite safe and eco-friendly compared to chemical synthesis.

FTIR spectroscopy of green synthesized NPs revealed that the main plant metabolites involved in the synthesis of metal NPs were terpenoids, flavonoids, reducing sugars and amino acids. The diversity of plant extracts, types of metal salts, pH of leaf extract and temperature were different factors which influenced the size and shape of synthesized NPs (Makarov *et al.*, 2014).

Devi and Gayathri (2014) green synthesized ZnO NPs using the chemical zinc nitrate and leaf extract of *Hibiscus rosa-sinensis*. They reported green synthesis as more rapid and reliable method for synthesis of nanoparticles than chemical method. Characterization of synthesized nanoparticles was done by scanning electron microscopy (SEM) and X-ray diffraction (XRD).

Senthilkumar and Sivakumar (2014) carried out green synthesis of ZnO NPs using the aqueous extract of green tea (*Camellia sinensis*) leaves. The UV-visible spectrum was recorded to monitor the formation of the nanoparticles, which exhibited a blue shifted absorption peak at 325 nm. The XRD pattern revealed well-defined peaks appearing at 2 θ positions corresponding to the hexagonal wurtzite structure of ZnO nanoparticles. The average size of the nanoparticles calculated using XRD data was 16 nm. FTIR spectra were recorded for the green tea extract and for the synthesized ZnO nanoparticles to identify the bio-molecules involved in the synthesis process. The higher percentage of phenolic compounds, with antioxidant potential, provided the reducing action on the metal oxides and amino acid, protein and lipids helped to stabilize the growth of the nanoparticles.

Chemical profile of aqueous extract of *Calotropis procera* leaf contained moisture (10.92 %), crude protein (28.53 %), fat (20.42 %), carbohydrate (24.13 %), ash (6.50 %) and crude fibre (9.40 %). Minerals such as Mg, K, Ca, Na and P were found in appreciable amounts. The extract contained 11 phytochemicals which include alkaloids, tannins, glycosides, saponins, steroids, antraquinones, terpenoids, phenols, phlobatannins, cardenolides and chalcones in different amounts (Ajiboso *et al.*, 2015).

Noorjahan *et al.* (2015) synthesized ZnO nanoparticles using aqueous neem (*Azadirachta indica*) leaf extract. The aqueous leaf extract acts as a solvent with manifold roles as promoter, stabilizer and template for the synthesis of nanoparticles. The synthesized ZnO nanoparticle was characterized by using FTIR spectroscopy and SEM analysis. FTIR analysis of green synthesized nanoparticles revealed the presence of bio-molecules such as polyphenols, carboxylic acid, polysaccharides, amino acids and proteins. SEM image of green synthesized ZnO NPs showed their shape as nano-flakes.

Bala *et al.* (2015) synthesized zinc oxide nanoparticles using *Hibiscus subdariffa* leaf extract. Formation of NPs was confirmed by UV-visible spectroscopy, Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD). Electron microscopy was used to study the morphology and size distribution of the synthesized particles. FTIR spectra showed presence of characteristic bands for several functional groups in aqueous extract of *H. subdariffa* being involved in ZnO NPs synthesis. EDAX analysis of green synthesized NPs revealed the presence of carbon and other elemental impurities and concluded that these organic molecules were responsible for the stability of green synthesized NPs.

Parthiban and Sundaramurthy (2015) bio-synthesized zinc oxide nanoparticles (ZnO NPs) using *Pyrus pyrifolia* leaf extract. The structural, morphological and optical properties of the synthesized nanoparticles were characterized by UV-visible spectroscopy, XRD, FTIR, AFM and FE-SEM with EDX analysis. The synthesized ZnO NPs were having absorbance peak at 376 nm in the UV-visible spectrum.

FTIR confirmed the presence of functional groups in the leaf extracts in synthesized ZnO NPs. The morphology of ZnO NPs was analyzed by FE–SEM. High purity of zinc and oxygen and crystalline nature were identified in EDAX. The size and topological structure of the ZnO NPs was measured by AFM. XRD pattern revealed that synthesized NPs were having wurtzite hexagonal structure with an average grain size 22 nm.

Shah *et al.* (2015) conducted a study to synthesize ZnO nanoparticles using the aqueous extract of green tea (*Camellia sinensis*) leaves. The synthesized ZnO nanoparticles were characterized by UV-visible spectroscopy (absorbance peak at 330 nm), particle size analyzer and scanning electron microscopy. The average size recorded for the particle was 853 nm.

Raj and Jayalakshmy (2015a) synthesized zinc oxide nanoparticles by a biological method using the stem extract of *Tinospora cordifolia* using zinc acetate and sodium hydroxide. The synthesized zinc oxide nanoparticles were characterized using scanning electron microscope (SEM), energy dispersive X-ray analysis (EDX) and Fourier transform infrared spectroscopy (FTIR). SEM results revealed that the shape of ZnO NPs as spherical and the average size ranged from 37-42 nm. EDX analysis provided the elemental composition of synthesized ZnO NPs and showed high purity.

Raj and Jayalakshmy (2015b) conducted a study on the green synthesis of ZnO nanoparticles using the aqueous root extracts of *Zingiber officinale* (ginger). The root extracts were found to be rich in flavonoids which enhanced the biogenic synthesis of ZnO nanoparticles. The scanning electron microscopy with the energy dispersive X-ray studies was used for characterization ZnO NPs. The average size of the nanoparticles was found to be 30-50 nm. The FTIR analysis showed the important functional groups from plant extract which involved in the synthesis of ZnO NPs.

The formation of silver nanoparticles using plant leaf extracts of *Clitoria ternatea* and *Solanum nigrum* was viewed by the colour change from colourless to yellowish brown. The shape and size of nanoparticle were characterized by X-ray diffraction and scanning electron microscopic studies. The XRD studies revealed that silver nanoparticles were polydispersed and size ranged from 10 to 50 nm (Krithiga *et al.*, 2015).

Manokari *et al.* (2014) green synthesized zinc oxide nanoparticles from zinc nitrate hexahydrate $[Zn(NO_3)_2 \cdot 6H_2O]$ using the aqueous extracts of a medicinal plant *Micrococca mercurialis*. The plant was found rich in primary and secondary metabolites such as proteins, steroids and alkaloids, which are responsible for the biogenic synthesis of ZnO nanoparticles. Leaves, stems, roots and fruits of *M. mercurialis* were used to prepare the aqueous extracts. The characterization and confirmation of synthesized ZnO nanoparticles was done by UV-visible spectrophotometer. The reaction mixtures showed significant and sharp UV absorbance peaks at 305 nm, 299 nm, 311 nm and 302 nm corresponding to the aqueous extracts of leaf, stem, root and fruits reacted with zinc nitrate, respectively.

Chudarkodi and Rajalakshmi (2016) revealed through their study that *Sesbania grandiflora* leaf extract can act as promoter, capping agent and reductant for the synthesis of ZnO nanoparticles.

ZnO NPs were characterized by UV-visible spectrophotometer, XRD and TEM. The size of the ZnO nanoparticles was about 43.09 nm as estimated by Debye-Scherrer equation.

Manokari *et al.* (2016) investigated a simple eco-friendly approach for synthesis of ZnO NPs using aqueous extracts of leafy vegetable plants such as *Peperomia pellucida* and *Celosia argentea*. The plant extracts acted as reducing and capping agents, while zinc nitrate hexahydrate [$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$] as a precursor. The formation of ZnO nanoparticles was confirmed by visual observation followed by UV-visible spectral analysis. The absorbance peaks for *P. pellucida* leaf, stem and roots were 299, 299 and 293 nm, respectively. The absorption peaks located at 342 nm, 304 nm and 308 nm were obtained for leaf, stem and roots of *Celosia argentea*, respectively. The results confirmed that these two herbs could be exploited for the production of valuable ZnO nanoparticles at room temperature, which can overcome the hazardous issues related to the chemical, physical and conventional methods of synthesis of nanoparticles.

Devasenan *et al.* (2016) made attempt to green synthesise zinc nanoparticles using a medicinal plant *Andrographis paniculata*. Zinc sulphate was used as the precursor for synthesis. The structural characterization of synthesized nanoparticles was carried out by using XRD, EDAX and SEM. The optical characterization was carried out by using UV-visible and FTIR analysis. The results showed that the leaf extract was suitable for the synthesis of zinc nanoparticles.

2.2 Use of nanoparticles as nanofertilizers

Nanofertilizers are either nano-materials which can supply one or more nutrients to the plants resulting in enhanced growth and yield or those which facilitate for better performance of conventional fertilizers, without directly providing crops with nutrients (Liu and Lal, 2015)

A mixture of SiO_2 and TiO_2 NPs at low concentrations increased activity of nitrate reductase in the rhizosphere of soybean and consequently accelerated soybean germination and growth (Lu *et al.*, 2002).

Calcium carbonate NPs (20–80 nm) at a concentration of 160 mg l^{-1} as Ca supplied to peanut (*Arachis hypogaea*) seedlings grown in Hoagland solution showed that Ca-NPs significantly improved the seedling growth in comparison to control (Liu *et al.*, 2005).

Lin and Xing (2007) reported that application of 2 mg l^{-1} of ZnO NPs enhanced root elongation of germinated radish (*Raphanus sativus*) and rape (*Brassica napus*) seeds over the control. ZnO NPs at higher concentrations ($>2 \text{ mg l}^{-1}$) had inhibitory effects on these seedlings.

Lin and Xing (2007) also reported that an application of 2 g l^{-1} multi-walled CNTs increased root elongation of germinated seeds of rye grass, rapeseed and corn compared with their respective control.

Shah and Belozeroval (2009) reported that soils amended with metallic Cu-NPs (130 and 600 mg kg^{-1}) significantly increased lettuce seedling growth by 40 and 91 per cent, respectively. But, higher concentrations (200 to $1,000 \text{ mg l}^{-1}$) of metallic Cu-NPs caused toxic effects on seedling growth of mung bean, wheat and yellow squash.

Su *et al.* (2009) indicated that Ti-NPs application could increase plant-photosynthesis, enzyme activity and thereby enhanced plant growth.

Severe phytotoxicity of ZnO NPs was assessed in *Arabidopsis thaliana* at higher concentrations of ZnO NPs ranging from 400 to 2000 mg l⁻¹. At lower concentrations (< 400 mg l⁻¹) there was no toxicity (Lee *et al.*, 2010).

Carbon nanotubes (CNTs) and zinc oxide nanoparticles can penetrate tomato (*Lycopersicon esculentum*) plant roots or seed tissues which indicated that a new nutrient delivery system can be developed through exploiting the nano-scale porous domains on plant surfaces (De-Rosa *et al.*, 2010).

Mahajan *et al.* (2011) observed that ZnO NPs enhanced growth of mung bean and chickpea (*Cicer arietinum*) seedlings at low concentrations. For the mung bean seedlings, the best growth response for root (42 % increase in length and 41 % in biomass) and shoot (98 % in length and 76 % in biomass) was observed at a concentration of 20 ppm over control and for the chickpea seedlings, concentration of 1 ppm caused significant increases in root (53 % length and 37 % in biomass) and shoot (6 % length and 27 % in biomass) growth compared to control.

A three day incubation study using a type of water weed indicated that low concentrations (0.25 ppm Cu) of Cu-NPs stimulated plant photosynthesis rate by 35 per cent compared with that of the control (Nekrasova *et al.*, 2011).

Arora *et al.* (2012) conducted an experiment to study the effect of gold-nanoparticles on growth and yield of *Brassica juncea*. They reported that foliar spray of gold-nanoparticles positively affected the plant height, stem diameter, number of branches, number of pods and seed yield.

Phosphate based nanoparticles could be used as P-nano-fertilizer as well as for remediation of heavy metal, by subjecting them to highly insoluble and stable phosphate compounds (Liu and Lal, 2012).

Tarafdar *et al.* (2012) revealed that foliar application of nano-phosphorus @ of 640 mg P ha⁻¹ (40 ppm spray concentration) gave a yield in cluster bean and pearl millet equivalent to the application of 80 kg P ha⁻¹ through conventional fertilizers under arid environment.

Song *et al.* (2012) reported that Ti-NPs at concentration of 0.5 g l⁻¹, enhanced elongation and fresh weight of duckweed (*Lemna minor*) compared with the bulk TiO₂ solution. However, concentrations higher than 0.5 g l⁻¹ had an inhibitory effect on plant growth.

Raliya and Tarafdar (2013) studied effect of biologically synthesized ZnO NPs on phosphorous-mobilizing enzyme secretion and gum contents in clusterbean. A significant improvement in plant biomass (27.1 %), shoot length (31.5 %), root length (66.3 %), root area (73.5 %), chlorophyll content (276.2 %), total soluble leaf protein (27.1 %), rhizospheric microbial population (11–14 %), acid phosphatase (73.5 %), alkaline phosphatase (48.7 %) and phytase (72.4 %) activity was observed over control in 6 week old plants due to application of nano-ZnO.

Ghafariyan *et al.* (2013) reported that low concentration of super paramagnetic Fe-NPs significantly increased the chlorophyll contents in sub-apical leaves of soybeans in a green house test under hydroponic conditions, indicating that Fe-NPs could be used as source of Fe to reduce chlorotic symptoms of Fe deficiency.

Pradhan *et al.* (2013) reported that metallic Mn-NPs were a better micronutrient source of Mn than the commercially available manganese sulphate salt. Mn-NPs enhanced growth of mung bean (*Vigna radiata*) and augmented its photosynthesis.

Pandey *et al.* (2014) studied the impact of three concentrations (100, 500 and 1,000 ppm) of silver nanoparticles (Ag-NPs) and two concentrations (100 and 500 ppm) of silver nitrate in *Brassica juncea* var. Varuna to assess the toxicity of these particles. It was found that Ag-NPs induced slight increase in root to shoot length and this effect was correlated with increase in chlorophyll and protein content at all the concentrations compared to control (without Ag-NPs). They also examined the effect of silver nitrate (AgNO₃), which showed that at all the tested concentrations had inhibitory effects with respect to root-shoot length, chlorophyll and protein content.

Liu and Lal (2014) synthesized a hydroxyapatite NPs of 16 nm in size and assessed fertilizing effect of the NPs on soybean in inert growing medium (50 % perlite and 50 % peat moss). The data showed that application of NPs increased the growth rate and seed yield by 33 and 20 per cent, respectively compared to those with a regular P fertilizer. The production of biomass was enhanced by 18 and 41 per cent for the above and below ground portions, respectively. The data indicated that soybean roots can absorb hydroxyapatite NPs as an effective P nutrient source and maintained healthy growth and high yield.

Chaitra (2015) studied the effect of nano ZnO formulations on the growth of maize plants and revealed that spraying of nano zinc oxide up to 750 ppm had positive effects on growth of maize plant and further increase in the spray concentrations had inhibitory effects.

Razzaq *et al.* (2016) reported the effect of silver nanoparticles on germination, seedling growth and yield of wheat cultivar. Silver nanoparticles (10-20 nm size) were synthesized by chemical reduction of silver nitrate with tri-sodium citrate. Number of seminal root, leaf area, root biomass, fresh weight and dry weight increased significantly with 25 ppm, followed by 50 ppm compared to control. Higher concentrations of silver nanoparticles in solution and longer exposure proved toxic to the seedlings.

Manasa (2016) reported that growth and yield of ground nut plants were enhanced by foliar application of nano iron oxides up to 1,000 ppm concentrations and further increase in the concentrations had inhibitory effects.

Raliya *et al.* (2017) studied adsorptive and photocatalytic removal of methyl orange dye by different oxide nanomaterials such as titanium dioxide (TiO₂), zinc oxide (ZnO) and graphene oxide (GO). In the study ZnO NPs were found to be superior in degrading the most dye, followed by TiO₂ and GO. It was also found that TiO₂ removes dye through adsorption at higher concentrations of dye and through photocatalysis at its lower concentrations. The effect of the size of a particle on photocatalytic dye degradation was tested by varying the size of ZnO NPs and smaller particle sizes (< 25 nm) were found to be most efficient.

2.3 Performance of nano ZnO formulations vs. conventional Zn carriers on crop growth and dry matter production

Potarzycki and Grzebisz (2009) reported that growth as well as yield of maize plant can be increased by external application of zinc. Yield attributes such as cob length, number of rows per cob, number of kernels per cob and thousand kernel weight as well as nitrogen use efficiency of maize found to be improved due to the application of zinc.

Sheykhabglou *et al.* (2010) studied effect of five levels of nano-iron oxide (0, 0.25, 0.5, 0.75 and 1 g l⁻¹) on soyabean and showed that at 0.75 g l⁻¹ concentration leaf and pod dry weight increased. But, the highest grain yield was obtained with 0.5 g l⁻¹ concentration.

Avinash *et al.* (2010) studied the effect of ZnO NPs on seed germination and root growth of *Cicer arietinum* and concluded that it was due to the high reactivity and efficiency of oxygen in ZnO NPs which resulted in the increased accumulation of IAA and increased root growth in seedling.

Mahajan *et al.* (2011) studied the effect of nano ZnO particles on the root and shoot growth of mung (*Vigna radiata*) and gram (*Cicer arietinum*) seedlings. It was observed that up to 20 ppm concentration, it promoted plant growth and beyond that increase in the concentration retarded plant growth.

Boonyanitipong *et al.* (2011) investigated the effects of zinc oxide nanoparticles on rice (*Oryza sativa*) roots. The results showed that nano-ZnO had detrimental effects on rice roots at early seedling stage. Nano-ZnO was found to stunt root length and reduce number of roots. This study indicated that engineered NPs (ENPs) exert physical or chemical toxicity depending on their chemical composition, size, surface energy and species of plant.

Prasad *et al.* (2012) conducted an investigation to examine the effects of nano-scale zinc oxide particles on plant growth and development. Peanut seeds were separately treated with different concentrations of nano-scale zinc oxide (ZnO) and chelated bulk zinc sulphate (ZnSO₄) suspensions, respectively and the effect of treatment on seed germination, seedling vigour, plant growth, flowering, chlorophyll content, pod yield and root growth were studied. Application of nano-scale ZnO (25 nm mean particle size) @ 1,000 ppm concentration promoted both seed germination and seedling vigour and in turn showed early establishment in soil manifested by early flowering and higher leaf chlorophyll content. These particles proved effective in increasing stem and root growth. Pod yield per plant was 34 per cent higher compared to chelated bulk zinc sulphate.

Salama (2012) studied the effects of silver nanoparticles on plant growth parameters such as shoot and root length, leaf surface area, chlorophyll, carbohydrate and protein contents in common bean (*Phaseolus vulgaris* L.) and corn. Five levels of silver nanoparticles (20, 40, 60, 80 and 100 ppm) were used. After germination, daily supply with 15 ml from each concentration was carried out for 12 days during plant growth. The results showed that at lower concentrations (20 to 60 ppm) of silver nanoparticles had a stimulating effect and at higher concentrations had inhibitory effect on the growth of plants.

Nano sized TiO₂ at suitable concentrations (2 to 10 ppm) could promote the seed germination of wheat in comparison to bulk TiO₂, but higher concentrations had inhibitory effects on wheat (Feizi *et al.*, 2012).

Application of Ag NPs increased the growth parameters (shoot and root length and leaf area) and bio-chemical attributes (chlorophyll, carbohydrate and protein contents antioxidant enzymes) of mustard, common bean and corn (Salama, 2012; Sharma *et al.*, 2012).

Priester *et al.* (2012) reported that the soybean plants grown on the soils mixed with ZnO and CeO₂ nanoparticles showed a significant increase in their pod and seed biomass when compared to that of control. ZnO nanoparticles enhanced plant growth and biomass accumulation whereas CeO₂ nanoparticles had inhibitory effect and reduced plant growth.

Wang *et al.* (2012) observed that the oxidized multi-walled carbon nanotubes (o-MWCNT) increased the root growth and biomass production and concluded that this effect was due to enhancement in the activity of dehydrogenase enzyme in the roots when the seeds were treated with o-MWCNT.

Adhikari *et al.* (2013) studied effect of nano SiO₂ (10-20 nm) and Mo (<100 nm) particles on rice seed germination and revealed that SiO₂ nanoparticles showed no toxic effects on rice growth (up to 100 ppm concentration), whereas root growth and elongation were arrested with Mo nanoparticles after 50 ppm. Greater adsorption of Mo nanoparticles into the root system was responsible for the toxicity.

Burmana *et al.* (2013) studied effect of ZnO nanoparticles on chickpea (*Cicer arietinum*) seedlings and reported that at lower concentration of 1.5 ppm ZnO NPs had positive effect and 10 ppm concentration showed inhibitory effect on growth and dry matter accumulation.

Sedghi *et al.* (2013) studied the effect of nano zinc oxide on germination of soybean seeds under drought stress. Results showed that nano zinc oxides treatment increased germination per cent over control under stress condition which indicated that treatments were effective for utilizing seed reservoirs for seedling growth.

Zinc sulphate spray @ 0.25 per cent during 8 and 12 leaves stage of maize plant significantly increased grain and biological yield over the control plant (Ghalejoghi *et al.*, 2013).

Nano TiO₂ significantly increased chlorophyll, carotenoids and anthocyanins content of maize plant which can facilitate an increase in crop yield (Morteza *et al.*, 2013).

Laware and Raskar (2014) reported that by spraying ZnO NPs at the rate of 20 and 30 ppm on onion plants at 15 days interval showed better growth and flowered 12-14 days earlier than the control and produced healthy seeds.

Jayarambabu *et al.* (2014) reported that seed germination, seedling growth and dry matter production in mung bean enhanced with ZnO NPs at a lower concentration of 20 ppm and inhibitory effects at higher concentrations.

Ngo *et al.* (2014) reported that the germination rates of soybean seeds treated with zero valent nano Cu, Co and Fe were 65, 80 and 80 per cent, respectively, whereas 55 per cent germination was observed in the control. In the field experiment, soybean treated with nano scale metals showed an increase of 7-15 per cent in chlorophyll index, 20-49 per cent in number of nodules and 16 per cent in yield in comparison with control.

Helaly *et al.* (2014) tested the effect of ZnO nanoparticles in the *in vitro* cultures of banana. Nanoparticles treated soma clones accumulated with more proline, chlorophyll and antioxidant enzymes and developed more dry matter than the control and also regenerated plantlets with well-formed root systems.

Exposure of citrate stabilized gold nanoparticles (GNPs) ranging from 100 to 400 ppm caused a decrease in overall growth of *Brassica juncea* due to the increase in free radical stress, supported by a general increase in biomarkers such as antioxidative enzymes, proline and hydrogen peroxide caused by the formation of reactive oxygen species (Gunjan *et al.*, 2014).

Kisan *et al.* (2015) reported that spinach plants sprayed with ZnO NPs at the concentration of 500 and 1,000 ppm increased leaf length, width, surface area and colour with higher values of protein and dietary fiber content in comparison to control.

The morphological and biometric analysis of sesame plants revealed that plants that were treated with biologically synthesized ZnO nanoparticles showed less toxic effects when compared with chemically synthesized ZnO nanoparticles (Narendhran *et al.*, 2016).

Adhikari *et al.* (2015) carried out investigations to study the effect of zinc oxide nanoparticles (<100 nm) on growth of maize in a solution culture system. Various concentrations of zinc (Zn) were applied through nano-zinc oxide (ZnO) particles (<100 nm) in suspension form and in ionic form through zinc sulphate (ZnSO₄) salt in Hoagland solution culture. Plant height, root length, root volume, and dry matter weight were improved due to application of zinc oxide nanoparticles than conventional fertilizers.

Seghatoleslami and Forutani (2015) conducted a study to evaluate yield and water use efficiency of sunflower due to ZnO nanoparticles under water stress condition. The results indicated that application of nano ZnO increased seed yield and water use efficiency by 43.6 and 40.2 per cent, respectively

Kumar *et al.* (2015) carried out experiments to study the effect of zinc oxide nanoparticles on nitrogenase activity in legumes. Nodulated roots of cluster bean, moth bean, green gram and cowpea were dipped in Hoagland solution containing 1.5 and 10 ppm of nano-ZnO for 24 hrs. Nitrogenase activity in cluster bean, green gram and cowpea roots increased after dipping in solution containing 1.5 ppm nano-ZnO, but decreased in roots dipped in solution containing 10 ppm nano-ZnO. But, in moth bean roots, nitrogenase activity decreased after dipping in solution containing either concentration of nano-ZnO.

Mosanna and Behrozyar (2015) conducted an experiment to study the effect of zinc nano-chelate foliar and soil application on morpho- physiological characteristics of maize.

The results showed that the soil application of nano-chelate zinc had the highest plant height, 100-grain weight and seed yield. Foliar application of nano-chelate Zn had 94 per cent more seed yield per plant. Soil application and foliar application of Zn-nano chelate had 51 per cent more harvest index (HI).

El-Nasr *et al.* (2015) determined the effect of foliar sprays with magnetite nanoparticles (MNPs) on the growth parameters and some chemical components of pear saplings and found that MNPs @ 250 ppm highly improved, biomass parameters (sapling height, stem diameter, leaf area and dry weight) and leaf bio-chemical (carbohydrates, amino acids, nitrogen, iron, chlorophyll and carotenoids) contents than the same concentration of traditional iron chelate.

Yang *et al.* (2015) studied the phytotoxicity of seven metal oxide nanoparticles such as titanium dioxide (nTiO_2), silicon dioxide (nSiO_2), cerium dioxide (nCeO_2), magnetite (nFe_3O_4), aluminum oxide (nAl_2O_3), zinc oxide (nZnO) and copper oxide (nCuO) on crop plants such as maize and rice. The results showed that seed germination was not affected by any of the seven metal oxide NPs. At a higher concentration of 2000 mg L^{-1} the root elongation was significantly inhibited by nano CuO (95.7 % for maize and 97.3 % for rice), nano ZnO (50.45 % for maize and 66.75 % for rice). Minor phytotoxicity of nano Al_2O_3 was observed in maize and no obvious toxic effects were found for other four metal oxide NPs.

Hojjat (2015) studied effect of four levels of silver nanoparticles (10, 20, 30 and $40 \mu\text{g ml}^{-1}$) on fenugreek and reported that AgNPs at lower concentrations promoted seed germination and early seedling growth. However, higher concentrations ($> 20 \mu\text{g ml}^{-1}$) resulted in slight adverse effects.

Burke *et al.* (2015) investigated the effect of positively and negatively charged Fe_3O_4 and TiO_2 nanoparticles (NPs) on the growth of soybean plants and their root associated soil microbes and found that plant growth was significantly lower with the application of TiO_2 compared to Fe_3O_4 NPs. It indicated that the type of NPs can affect plant growth and nutrient content in an agriculturally important crop species and the charge of these particles can influences the colonization of the root system by nitrogen-fixing bacteria.

Zhao *et al.* (2015) studied changes in physiological and agronomic parameters of corn plants when sprayed with engineered nanoparticles (nano CeO_2 or nano ZnO) @ 400 and 800 ppm. Results showed that nano CeO_2 at both concentrations did not show any effect on corn growth, while nano ZnO at 800 ppm reduced net photosynthesis by 12 per cent, stomatal conductance by 15 per cent, relative chlorophyll content by 10 per cent at 20 days after application and finally yield was reduced by 49 per cent. At nano ZnO at 400 ppm, enhanced plant growth by increased photosynthetic rate, chlorophyll content and resulted in high yield.

Siva and Benita (2016) compared effect of EDTA chelated iron and iron oxide nanoparticles on ginger and concluded that ginger roots supplemented with iron oxide nanoparticles showed an enhanced plant growth, *i.e.*, increased plant height, number of leaves, chlorophyll, carotenoid, protein and iron content and was an efficient solution to avoid chlorosis compared to chelated form.

Janmohammadi *et al.* (2016) studied the effects of foliar spray of micronutrient nano-fertilizer (iron and zinc) and nano-titanium dioxide (nTiO₂) solution on grain yield of barley plants and reported that foliar application of nanofertilizers, improved nutrient uptake and resulted in high grain yield and harvest index.

Moghaddasi *et al.* (2017) reported cucumber grown in a gel addition of 1 mg l⁻¹ ZnO into gel chamber enhanced root and shoot biomass and the shoot growth was higher in the presence of NPs compared to its bulk counterpart.

2.4 Effect of Nano ZnO spray formulations on uptake of macro and micro nutrients by crops

The potential contribution of nanofertilizers in improving growth and development of crops lies on its ability for greater absorbance and high reactivity. Nanofertilizers can enter the plant cells directly through the sieve-like cell wall structures as the particle sizes are smaller than the sizes of cell wall pores (Benzon *et al.*, 2015).

Khodakovskaya *et al.* (2009) reported that tomato seeds when exposed to multi-walled carbon nanotubes (MWCNTs), seed germination and vegetative biomass increased significantly due better uptake of water and nutrients.

Nanofertilizers or nano-encapsulated nutrients have properties that are beneficial to crops, released the nutrients on demand, controlled release of chemicals fertilizers that regulate plant growth and enhanced target activity (De-Rosa *et al.*, 2010; Nair *et al.*, 2010).

Yuvakkumar *et al.* (2011) found more silica content in the maize leaves when provided as nano than as bulk molecules, indicating that nanoparticles have better uptake by plants than its bulk molecules.

Suriyaprabha *et al.* (2012) studied the response of maize to applied nano and bulk silica particles. Growth characteristics were much influenced with increasing concentration of silica NPs up to 15 kg ha⁻¹ whereas at 20 kg ha⁻¹, no significant increments were noticed. Silica accumulation in leaves was high (0.57 and 0.82 %) at 10 and 15 kg ha⁻¹ concentrations of SNPs. The observed physiological changes showed that the expression of organic compounds such as proteins, chlorophyll and phenols favored to maize treated with nano-silica @ 15 kg ha⁻¹ compared with bulk silica and control.

Nalwade and Neharkar (2013) reported that multi-walled carbon nanotubes increased dry matter accumulation in cotton seedlings by improving the growth and nutrients uptake by seedlings.

Tiwari *et al.* (2013) found that the low concentration (20 ppm) of multi-walled carbon nanotubes improved water as well as nutrient uptake by seedlings which resulted in better germination and growth of seedling, while the higher concentrations were detrimental to the seedlings growth.

Jaberzadeh *et al.* (2013) studied the effect of titanium dioxide nanoparticles on wheat plants under water deficit stress and revealed that these nanoparticles had a positive influence on yield components such as ear number, seed number per square meter, 1,000 seed weight, highest biomass and harvest index and enhanced the nutrient uptake by crop plants.

Van *et al.* (2013) studied the effect of chitosan nanoparticles in enhancing the uptake of nutrients and concluded that nitrogen, phosphorous, potassium, calcium and magnesium in the robusta coffee plants significantly increased due to nano spray compared to control.

Jahanara *et al.* (2013) reported that foliar application of iron nano-composites significant enhancement in the seed yield and yield attributes of common bean (*Phaseolus vulgaris*) indicating positive effect of nano spray on nutrient uptake and dry matter accumulation.

Ashrafi *et al.* (2013) reported that an increase in seed yield of soybean when they treated with nano silver particles compared to the control indicating the positive influence of nanoparticles in enhancing plant metabolic activities and nutrients uptake.

Harsinia *et al.* (2014) reported that application of nano iron oxide enhanced yield and quality of wheat plant compared to iron chelate and iron sulphate due to better uptake and assimilation of nutrients.

Afshar *et al.* (2014) studied the effects of different spraying concentrations of nano zinc oxide on wheat crop. Results revealed that nano-compounds are quickly absorbed by plants in large amounts and hence it can be toxic on plants and plant products when it applied particularly at higher concentrations.

Taheri *et al.* (2015) studied effect of three different physical forms of ZnO particles (ZnO nanocolloid, ZnO nanoparticles and micrometric ZnO) on the growth of corn hybrid. These particles were applied through irrigation water. The concentration of nanoparticles in irrigation water was 2 ppm. The results showed that the treatment with ZnO nanoparticles increased the shoot dry matter and leaf area indices by 63.8 and 69.7 per cent, respectively over the control due the better uptake and assimilation of nutrients.

Hussien *et al.* (2015) reported that application of nano-P improved the nutrients uptake under stress conditions as well as regular irrigation. The interaction effect of nano-fertilizer and drought through some growth stages of cotton plants indicated that application of nano-P @ 500 and 1,000 ppm promoted uptake of macro (N, K, P, Ca and Mg) and micronutrients (Zn, Fe, Cu and Mn) by cotton.

Valadkhan *et al.* (2015) showed that application of nano-iron chelate fertilizer, increased seed number per pod, pod number per plant, 100 seed weight and grain yield compared to control treatment. Improvement in yield components was due to the enhanced photosynthetic and other metabolic activities, which resulted in more dry matter accumulation and nutrient uptake.

Posic *et al.* (2016) conducted a study to evaluate the effects of soil application of nano cerium oxide (nCeO₂) and nano titanium oxide (nTiO₂) at 0, 500 and 1,000 ppm concentration on the nutritional parameters of barley.

Nano CeO₂ and nano TiO₂ had an impact on composition and nutritional quality of barley kernels in contrasting ways that kernels were negatively affected by nano CeO₂ while nano TiO₂ had potentially beneficial effects by improving accumulation of nutrients such as Zn and Mn.

2.5 Effect of ZnO nanoparticles on soil properties and post-harvest available nutrients status of soil

The most important reaction pathways which may affect fate and behaviour of nanoparticles in soil are dissolution, aggregation, partitioning between solution and solid phase and mobility. Soil properties such as pH, ionic strength, clay and organic matter content affect the dissolution of nanoparticles through their effect on aggregation. High surface area and charge of manufactured nanoparticles result in strong adhesion of nanoparticles to the reactive surface of soil (Milani, 2011).

Kim *et al.* (2011) revealed that concentration of zinc in the soil treated with zinc nanoparticles were more than that of treated with soluble zinc. This indicated that in soil there would be better retention of nanoparticles which reduces phototoxicity and increases the reserve of zinc nutrient in the soil.

Improper handling and disposal of NP-containing wastes could result in environmental contamination. NPs have the potential for harmful effects on plants and plant associated soil bacteria (Dimkpa *et al.*, 2011).

Du *et al.* (2011) investigated the effect of TiO₂ and ZnO nanoparticles on wheat growth and soil enzyme activities under field conditions. Both nanoparticles reduced the biomass of wheat. The nanoparticles also induced significant changes in soil enzyme activities, which are bio-indicators of soil quality and health. Soil protease, catalase and peroxidase activities were inhibited in the presence of the nanoparticles while urease activity was unaffected.

At sub-lethal concentrations, NPs variably modify the production of bacterial secondary metabolites involved in plant growth and productivity (Dimkpa *et al.*, 2012).

Moshe *et al.* (2012) studied effect of metal oxide nanoparticles (CuO and Fe₃O₄) on various soil properties. It was found that the nanoparticles did not change the total amount of organic materials in the soil or the total organic carbon in the soil extract. Denaturing gradient gel electrophoresis (DGGE) fingerprinting revealed that nanoparticles affected the soil bacterial community composition, but had little impact on the macroscopic properties of the soil.

Kim *et al.* (2013) investigated effects of CuO and ZnO nanoparticles (NPs) and microparticles (MPs) on soil microbial toxicity, phytotoxicity and bioaccumulation in two crops (*Cucumis sativus* and *Zea mays*) and found that NPs were more toxic than MPs to microbes and plants in the soil ecosystem. They also found that the soil enzyme activity and plant biomass were inhibited to the greatest extent by CuO NPs than ZnO NPs.

Subbaiah (2014) reported that the post harvest nutrient availability of zinc in the soil increased due to the foliar nano ZnO spray and maximum content was recorded with the application of nanoparticles @ 1,000 ppm indicating that the translocation of zinc from the leaves to the soil through the plant body system and accumulated in the soil. The soil zinc content had shown high significant differences among treatment.

Shen *et al.* (2015) investigated the eco-toxicological effect of ZnO-NPs on soil microorganisms testing respiration, ammonification, dehydrogenase activity, and fluorescent diacetate hydrolase activity in soil. The study revealed that soil type determines the toxicity of ZnO NPs in soil and toxicity was highest in the acid soil, followed by the neutral soil. The toxicity was relatively low in the alkaline soil. The toxicity was not accounted for by the Zn^{2+} released from the ZnO-NPs but due to the direct interaction of ZnO-NPs with biologic targets.

Watson *et al.* (2015) studied the effects of soil properties on phytotoxicity and Zn bioavailability from the NPs in an acidic and a calcareous alkaline soil and concluded that in the acid soil, the ZnO NPs caused dose-dependent phytotoxicity while no phototoxicity in calcareous soil showing that ZnO NPs formulations to be used as a soil amendment need to be tuned to soil properties to avoid phytotoxicity.

Schlich and Hund-Rinke (2015) investigated the effects of silver nanomaterials (Ag-NMs) on five well-characterized soils with distinct physico-chemical properties using two standardized test systems. The carbon transformation test (OECD 217) showed minimal sensitivity whereas the ammonia oxidizing bacteria test (ISO 15685) showed extreme sensitivity over 28 days of exposure. Ag-NM toxicity was compared with the physico-chemical properties of the soils, revealing that toxicity declined with increasing clay content and increasing pH. Ag-NM toxicity did not appear to be affected by the organic carbon content of the soil.

Simonin *et al.* (2016) reported that soil exposed to TiO_2 -NPs (1 and 500 mg kg^{-1} dry soil) showed a decrease in nitrification enzyme activities and abundance of ammonia oxidizing microorganism and concluded it as emerging pollutant to affect the soil health.

Rui *et al.* (2016) studied the effectiveness of iron oxide nanoparticles (Fe_2O_3 NPs) on the growth and development of peanut to replace traditional chelated Fe-fertilizers. The results showed that Fe_2O_3 NPs promoted the growth of peanut by increasing root length, plant height, biomass and SPAD values and regulating phytohormone contents and antioxidant enzyme activity. The iron content in the soil was quantitatively determined by energy dispersive X-ray spectroscopy (EDS) showed that the Fe_2O_3 NPs adsorbed on to sandy soil improved the availability of Fe to the groundnut plants.

You *et al.* (2017) suggested that metal oxide nanoparticle incubation could influence soil enzyme activities and change soil bacterial community. ZnO NPs had a stronger effect on soil enzymatic activities than nTiO_2 , nCeO_2 , and nFe_3O_4 and saline-alkali soil was more susceptible to metal oxide nanoparticles than black soil.

Kwak *et al.* (2017) investigated the long-term effects of ZnO NPs at concentrations of 50 and 500 mg kg^{-1} on the activities of six exo-enzymes in planted soils such as dehydrogenase, fluorescein diacetate (FDA) hydrolase, urease, acid phosphatase, aryl-sulfatase, and β -glucosidase. These effects included both decrease and increase in enzyme activity. However, results suggested that ZnO NP treatments of 50 and 500 mg kg^{-1} can adversely affect soil enzymes, particularly acid phosphatase and urease and thus have implications for phosphorous and nitrogen cycles in the soil.

The review done in this thesis reveals that plant mediated synthesis of nanoparticles is an efficient, effective and eco-friendly method. Moreover, it is simple and cheap and can be used as an alternative for chemical synthesis. Nanoparticles are more bio-available and bio-accessible than their bulk molecules due to their smaller size and high specific surface area. Thus they have high nutrient use efficiency. Nanoparticles are very effective at their lower concentrations than higher concentrations as enhanced bio-availability can lead to nutrient toxicity.

3. MATERIAL AND METHODS

A green house experiment was conducted during 2016-17 to study the effect of green synthesized ZnO nanoparticles on the growth and dry matter production of maize. The details regarding the synthesis of ZnO nanoparticles using different leaf extracts and materials and methods followed for conducting the green house experiment are briefed in this chapter under the following headings:

- 3.1 Location
- 3.2 Soil characteristics
- 3.3 Green synthesis of zinc oxide nanoparticles
- 3.4 Characterization of ZnO nanoparticles
- 3.5 Pot culture experiment
- 3.6 SEM analysis of maize leaf
- 3.7 Chemical analysis of soil samples
- 3.8 Chemical analysis of plant samples
- 3.9 Zinc content in index leaf (mg kg^{-1})
- 3.10 Uptake of nutrients (mg plant^{-1})
- 3.11 Statistical analysis and interpretation of data

3.1 Location

The synthesis of ZnO nanoparticles was carried out at the laboratory of Department of Soil Science and Agricultural Chemistry, College of Agriculture, Dharwad and pot culture experiment was conducted in the green house situated at MARS, Dharwad.

3.2 Soil characteristics

A surface (0-15 cm depth) composite soil sample was collected from MARS, Dharwad (F block, plot no. 136). Soil sample was processed and analyzed for important physical and chemical properties by employing standard methods as given in Table 1. The soil used for pot culture experiment was calcareous in nature (CaCO_3 - 6.36 %) with clay texture, alkaline in reaction (pH - 7.64) and low in salt content (EC - 0.33 dS m^{-1}). The soil organic carbon content was medium (6.2 g kg^{-1}) with low available nitrogen (150 kg N ha^{-1}), medium available phosphorus ($42 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$) and high in available potassium ($420 \text{ kg K}_2\text{O ha}^{-1}$) and sulphur (48 kg S ha^{-1}). Among the available micronutrients, zinc and iron were deficient and manganese and copper were sufficient in soil.

3.2.1 Analysis of soil samples for physical properties

3.2.1.1 Particle size distribution

The particle size distribution in soil was determined by international pipette method as proposed by Piper (2002).

Table 1. Physical and chemical properties of the experimental soil

Sl. No.	Particulars	Value	Method adopted
I	Physical properties		
1.	Particle size distribution		
	Sand (%)	20.53	International pipette method (Piper, 2002)
	Silt (%)	27.50	
	Clay (%)	51.97	
	Textural class	Clay	
2.	Bulk density (Mg m^{-3})	1.36	Clod method (Black, 1965)
3.	Maximum water holding capacity (%)	68.70	Keen Raczkowaski brass cup method (Sankaram, 1960)
II.	Chemical properties		
4.	pH (1:2.5 soil: water suspension)	7.64	Potentiometric method (Sparks, 1996)
5.	Electrical conductivity (dS m^{-1}) (1:2.5 soil water extract)	0.33	Conductometric method (Sparks, 1996)
6.	Organic carbon (g kg^{-1})	6.20	Wakley and Black wet oxidation method (Sparks, 1996)
7.	Free calcium carbonate (%)	6.36	Rapid acid titration method (Piper, 2002)
8.	Cation exchange capacity [$\text{cmol (p}^+) \text{ kg}^{-1}$]	52.50	Sodium saturation method (Jackson, 1967)
9.	Available nitrogen (kg N ha^{-1})	150.00	Modified alkaline permanganate method (Sharawat and Burford, 1982)
10.	Available phosphorus ($\text{kg P}_2\text{O}_5 \text{ ha}^{-1}$)	42.00	Olsen's method with 0.5 M sodium bicarbonate as extractant (Sparks, 1996)
11.	Available potassium ($\text{kg K}_2\text{O ha}^{-1}$)	420.00	Flame photometer method after extraction with 1N NH_4OAc (Sparks, 1996)
12.	Available sulphur (kg ha^{-1})	48.00	Extraction with 0.15 % $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ followed by turbidimetric method (Sparks, 1996)
III	Micronutrient cations (mg kg^{-1})		
13.	Available Mn	2.31	Atomic Absorption Spectrophotometer method after extraction with DTPA (Lindsay and Norvell, 1978)
14.	Available Fe	2.18	
15.	Available Zn	0.55	
16.	Available Cu	0.39	

3.2.1.2 Maximum water holding capacity (MWHC)

The maximum water holding capacity was determined by using Keen's cup as described by Sankaram (1960) and expressed in percentage (%).

3.2.1.3 Bulk density

The bulk density of soil was determined by clod method as proposed by Black (1965) and expressed in megagram per cubic meter (Mg m^{-3}).

3.2.2 Analysis of soil samples for chemical parameters

3.2.2.1 Soil reaction (pH)

The pH of soil was determined in soil:water (1:2.5) suspension after stirring intermittently for half an hour, using a pH meter (Sparks, 1996).

3.2.2.2 Electrical conductivity

The electrical conductivity (dS m^{-1}) of the soil was determined in the supernatant of soil:water suspension (1:2.5) using conductivity meter (Sparks, 1996).

3.2.2.3 Organic carbon

The organic carbon content (g kg^{-1}) in soil was estimated by Walkley and Black's wet oxidation method (Sparks, 1996).

3.2.2.4 Cation exchange capacity (CEC)

Cation exchange capacity ($\text{cmol (p}^+) \text{ kg}^{-1}$) of soil was determined by sodium saturation method as described by Jackson (1967).

3.2.2.5 Free calcium carbonate

Rapid acid titration method (Piper, 2002) was used for determination of free calcium carbonate content in soil sample was expressed in percentage (%).

3.2.2.6 Available nitrogen

Available nitrogen (kg ha^{-1}) was estimated by modified alkaline potassium permanganate method (Sharawat and Burford, 1982).

3.2.2.7 Available phosphorus

The available phosphorous (kg ha^{-1}) in soil was extracted with 0.5 M sodium bicarbonate with pH adjusted to 8.5 and intensity of blue colour was recorded at 660 nm (Sparks, 1996).

3.2.2.8 Available potassium

The available potassium (kg ha^{-1}) was determined by extracting the soil with neutral normal ammonium acetate solution and potassium in the extract was estimated with the help of flame photometer as outlined by Sparks (1996).



Plate 1a: Datura



Plate 1b: Calotropis



Plate 1c: Neem



Plate 1d: Tulasi



Plate 1e: Tea

Plate 1. Plant leaves used for green synthesis of ZnO nanoparticles



Plate 2. Leaf extracts (10 %) used for the green synthesis of ZnO nanoparticles



Plate 3. Green synthesized ZnO nanoparticles

3.2.2.9 Available sulphur

The available sulphur (kg ha^{-1}) in the soil was extracted using 0.15 per cent $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ solution and sulphur in the extract was determined after developing precipitate as proposed by Sparks (1996).

3.2.2.10 DTPA-extractable micronutrients

Available micronutrients (mg kg^{-1}) in the soil were extracted and quantified by atomic absorption spectrophotometer as proposed by Lindsay and Norvell (1978).

3.3 Green synthesis of zinc oxide nanoparticles

Zinc oxide (ZnO) nanoparticles were synthesized from five different plant leaf extracts using zinc nitrate ($\text{Zn}(\text{NO}_3)_2$) as the precursor. The plants used for green synthesis were calotropis (*Calotropis procera*), neem (*Azadirachta indica*), tea (*Camellia sinesis*), tulasi (*Ocimum tenuiflorum*) and datura (*Datura stramonium*) (Plate 1).

3.3.1 Preparation of leaf extracts

The leaves were washed in running tap water to remove dirt and dust and dried under shade for two days. The leaves were then oven dried at 30°C for six to eight days. After complete drying, the leaves were ground to powder using a mixer grinder and powder of each leaf samples were stored separately in air tight polythene bags.

Ten gram powder of each of plants was extracted with 100 ml distilled water (1:10) and boiled for 30-45 minutes with constant stirring and filtered to get 10 per cent extract (Plate 2). The leaf extracts were stored in a refrigerator.

3.3.2 Synthesis of zinc oxide nanoparticles using leaf extract

Ten ml of 10 per cent leaf extract was boiled on a hot water bath. When the leaf extract started boiling, one gram zinc nitrate was added and stirred constantly. The mixture was boiled till paste was obtained. The paste was then transferred to silica crucible and heated at high temperature of 400°C for two hours in a muffle furnace and cooled. Zinc oxide nanoparticles obtained as white powder (Plate 3) were preserved in plastic vials for further characterization.

3.4 Characterization of ZnO nanoparticles

The green synthesized zinc oxide nanoparticles were characterized for their size, shape and stability. The preliminary confirmation of ZnO nanoparticles was done with UV-visible spectroscopy. The average size and morphology of ZnO nanoparticles were determined by particle size analyzer (PSA) and scanning electron microscopy (SEM). Energy dispersive atomic x-ray analysis (EDAX) was used for determination of chemical composition of green synthesized ZnO nanoparticles.

3.4.1 UV –spectroscopy

UV-spectrophotometer analysis was done for preliminary confirmation of green synthesized ZnO nanoparticles (Fig. 1a to 1e). Small amount of green synthesized ZnO NPs was dispersed in water by ultrasonication for 30 minutes. Dispersed sample was fed to UV- visible spectrophotometer and absorption peak of the sample was recorded in UV-visible range (200-700 nm) with help of connected PC and the software SP-UV5. The green synthesized particles which exhibited their absorbance peaks in UV wavelength range (280-375 nm) were considered as nanoparticles.

3.4.2 Particle Size Analyzer (PSA)

Particle size analyzer was used for the proximate size determination of green synthesized ZnO NPs, because all the particles may not have single and same dimension. It determines the size distribution of nanoparticles in its dispersed solution (Fig. 2a to 2e) and average size of nanoparticles was calculated from frequency distribution curve or cumulative distribution curve along with their standard deviation.

Small amount of green synthesized ZnO NPs was dispersed in water using ultrasonication for 30 minutes. Dispersed sample was fed to particle size analyzer to determine the size distribution of nanoparticles in the dispersed solution. From the frequency distribution curve obtained against particle size, the average size of the particles was determined with standard deviation.

3.4.3 Scanning Electron Microscopy (SEM)

Scanning electron microscopy of green synthesized ZnO nanoparticles was done to determine the morphology (size and shape) of synthesized nanoparticles (Plate 4a, 4b, 4c, 4d and 4e).

SEM analysis was done using SEI-1130 machine (at TNAU, Coimbatore). Thin films of the sample were prepared on a coated copper grid by just placing a very small amount of the sample on the grid. Then, the film on the SEM grid was allowed to dry and the images of nanoparticles were taken to determine size and shape of nanoparticles.

3.4.4 Energy dispersive atomic x-ray analysis (EDAX)

EDAX analysis was done at TNAU, Coimbatore to determine the chemical composition of green synthesized nanoparticles. EDAX confirms the presence of zinc and oxygen, in the synthesized particles by analyzing their optical absorption peak (Fig. 3a to 3e).

3.5 Pot culture experiment

A pot culture experiment was conducted to study the “Response of maize to foliar spray of green synthesized nano ZnO formulations in a calcareous Vertisol”. The details of the experiment are given below.

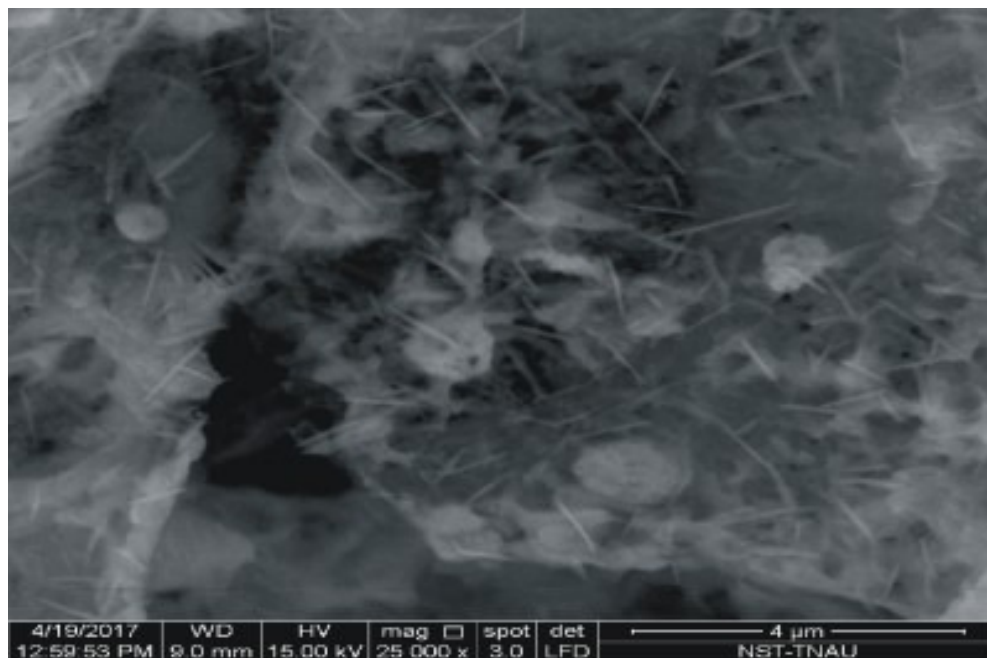


Plate 4a. SEM image of ZnO NPs synthesized using calotropis leaf extract

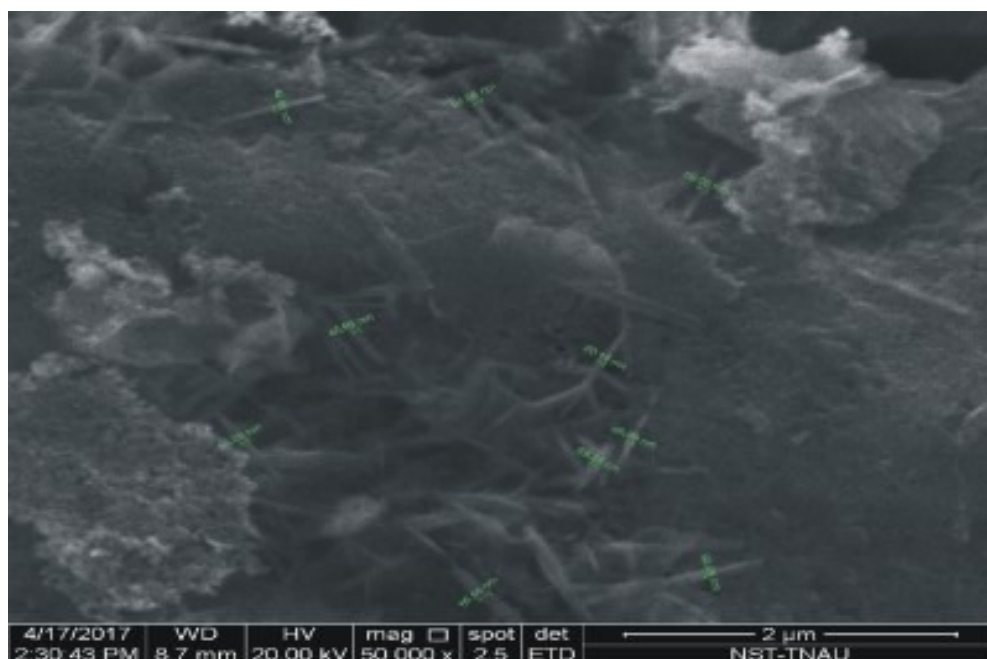


Plate 4b. SEM image of ZnO NPs synthesized using tea leaf extract

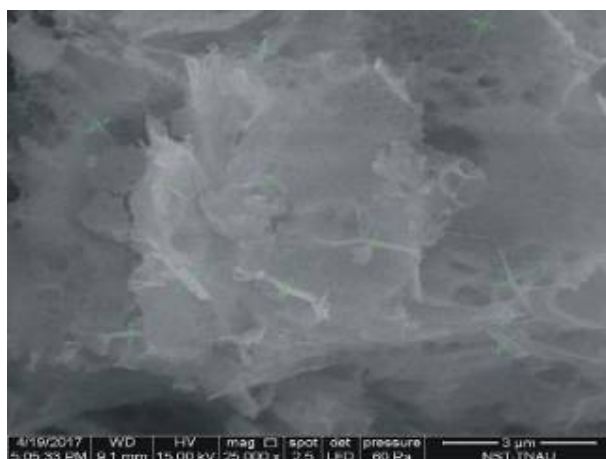


Plate 4c. SEM image of ZnO NPs synthesized using neem leaf extract

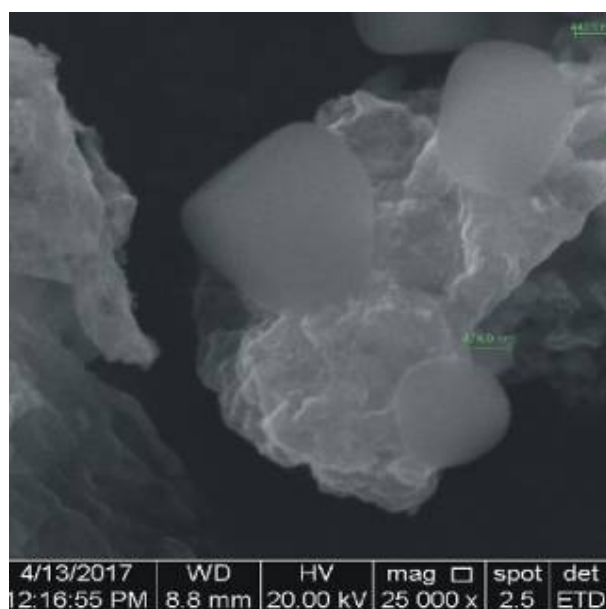


Plate 4d. SEM image of ZnO NPs synthesized using tulasi leaf extract

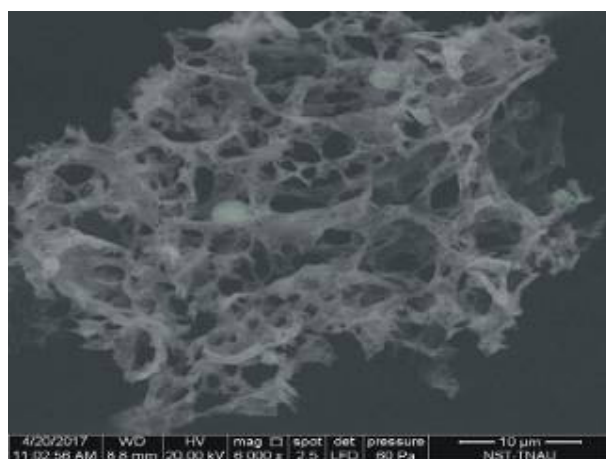


Plate 4e. SEM image of ZnO NPs synthesized using datura leaf extract

Table 2. Details of pot culture experiment

Sl. No.	Particulars	Details
1	Design	Completely Randomized Design (CRD)
2	Treatments	Twelve (12)
3	Replications	Five (5)
4	Amount of soil used	12 kg
5	Size of nano ZnO	< 75 nm (45-75 nm)
6	Crop	Maize cv. NK 6240
7	Date of sowing	23/11/2016
8	Date of harvest	21/01/2017
9	Duration of the crop	60 Days
11	Treatment details	T ₁ : Absolute control (Only water spray at 30 DAS)
		T ₂ : ZnSO ₄ .7H ₂ O spray @ 0.5 % at 30 DAS
		T ₃ : Foliar nano ZnO spray @ 50 ppm at 30 DAS
		T ₄ : Foliar nano ZnO spray @ 100 ppm at 30 DAS
		T ₅ : Foliar nano ZnO spray @ 150 ppm at 30 DAS
		T ₆ : Foliar nano ZnO spray @ 200 ppm at 30 DAS
		T ₇ : Foliar nano ZnO spray @ 250 ppm at 30 DAS
		T ₈ : Foliar nano ZnO spray @ 500 ppm at 30 DAS
		T ₉ : Foliar nano ZnO spray @ 750 ppm at 30 DAS
		T ₁₀ : Foliar nano ZnO spray @ 1,000 ppm at 30 DAS
		T ₁₁ : Foliar nano ZnO spray @ 1,500 ppm at 30 DAS
		T ₁₂ : Foliar nano ZnO spray @ 2,000 ppm at 30 DAS

NOTE:

1. Spraying at 30 DAS
2. Recommended fertilizers and manures were not applied to treatments T₁ to T₁₂
3. ZnO nanoparticles synthesized from calotropis leaf extract were used for spraying
4. ZnO nanoparticles were dispersed in water by ultrasonication.



Plate 5. Plan of layout of pot culture experiment

3.5.1 Selection of soil

About one ton of surface (0-15 cm depth) calcareous soil was collected from plot No. 136, F block MARS, Dharwad. The soil was mixed thoroughly and powdered and used for pot culture studies.

3.5.2 Experimental details

The pot culture experiment was conducted with twelve treatments involving various concentrations of nano ZnO particles in completely randomized design with five replications after filling each pot with 12 kg of soil. The details of the experiment are presented in Table 2 and the plan of layout of experiment in Plate 5.

3.5.3 Preparation of nano ZnO spray formulations

Green synthesized ZnO nanoparticles (from calotropis leaf extract) with size less than 75 nm (45-75 nm) were used for spraying. Bulk $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (AR grade) solution (@ 0.5 %) was used as a reference material along with the absolute control. A known amount of nano ZnO powder was weighed and dispersed in water by ultrasonication (Plate 6) for 30 minutes to obtain nano ZnO formulations at concentrations @ 50, 100, 200, 250, 500, 750, 1,000, 1,500 and 2,000 ppm. Two hundred microlitres of tween-20 was added to each sample before ultrasonication for the effective dispersion of ZnO nanoparticles and to ensure better stability of nanoparticles for longer time in solution and to prevent the agglomeration and settling of nanoparticles after sonication.

3.5.4 Sowing

Twelve kg of soil was filled in each pot as per treatment details (Table 2). Two maize seeds were sown in each pot and after one week one healthy seedling was retained by thinning. Plants were not supplied with any fertilizers and manures through external application.

3.5.5 Foliar application of ZnO nanoparticles

As per treatment details, ZnO nanoparticles formulations and zinc sulphate @ 0.5 per cent were sprayed to maize at 30 DAS (Plate 7). Only water spray was taken up in absolute control treatment.

3.5.6 Method of spraying

The foliar spray was taken up at 30 DAS by using small hand sprayer. Ten ml of nano ZnO formulations and zinc sulphate solution were sprayed as per treatment details.

3.5.7 Observations and sampling procedures

Periodical observations (15, 35, 45 and 60 DAS) on growth parameters and chlorophyll content in leaf were recorded. The dry matter production was also recorded at 30 and 60 DAS by carefully uprooting the plants. Nutrient concentrations, uptake of major and micronutrients and zinc concentration in index leaf were also recorded at 35 and 60 DAS.



Plate 6a. Ultra-Sonicator

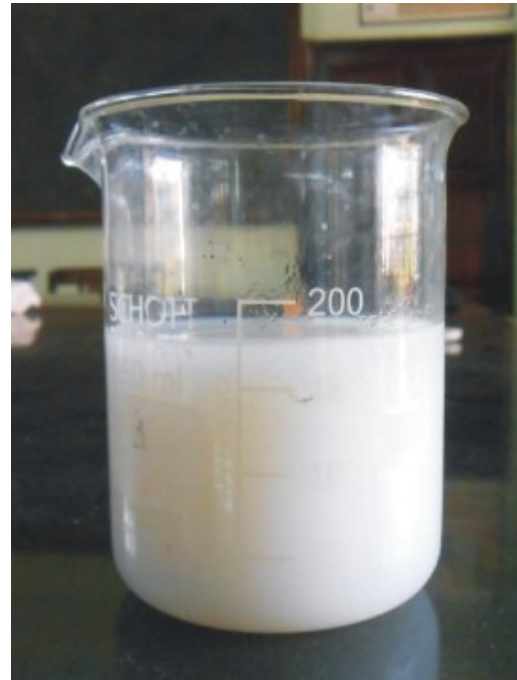


Plate 6b: ZnO NPs dispersed in water

Plate 6. Dispersion of ZnO NPs in aqueous solution by ultrasonication



Plate 7. spraying of nano ZnO formulations at 30 DAS

3.5.8 Growth parameters

3.5.8.1 Plant height

The plant height was measured from the base of the plant to base of the fully opened top leaf and expressed in centimeter (cm).

3.5.8.2 Number of leaves plant⁻¹

The total number of green leaves produced per plant in each treatment was counted and expressed as the average number of leaves per plant.

3.5.8.3 Leaf area

Leaf area of all the green leaves was measured by adopting Stickler's linear measurement method (Stickler and Pauli, 1961) and it was expressed in square centimeter per plant (cm² plant⁻¹).

$$\text{Leaf area (cm}^2\text{)} = L \times B \times 0.747$$

Where,

L = Length of leaf (cm)

B = Breadth of leaf (cm)

The length of fully opened leaf lamina was measured from the base to the tip and leaf breadth was measured at the widest point of the leaf lamina.

3.5.8.4 SPAD Chlorophyll Meter Reading (SCMR)

SPAD (Soil Plant Analytical Development) chlorophyll meter (SPAD 502; Minolta Company Ltd.) measures the greenness or relative chlorophyll content of the leaves. SCM readings were taken at 15, 35, 45 and 60 DAS. The third leaf from the top was used for measuring SCMR which was recorded on one side of leaf blade at the widest point of the leaf lamina. The observations were made between 10.00 and 12.00 hours of the day.

3.5.8.5 Dry matter production

At 35 and 60 DAS, the plants were uprooted and washed thoroughly and chopped into small pieces to enable complete drying. Oven dry weight (at 70 °C to a constant weight) of whole plant including leaf and stem from each pot was recorded and expressed in gram per plant (g plant⁻¹).

3.6 SEM analysis of maize leaf

The maize leaf samples at 60 DAS were dried and analyzed by scanning electron microscope (SEM) to determine the size of pores on the leaf surface (Plate 8a, 8b, 8c and 8d).

3.7 Chemical analysis of soil samples

3.7.1 Collection and preparation of soil samples

After the harvest of crop, soil samples (0-15 cm) were collected from all the treatments and brought to the laboratory in polythene bags. The soil samples were air dried and analyzed for pH, EC, organic carbon, available major, secondary and micronutrients by adopting standard procedures as indicated in Table 1. The powdered soil samples were ground again in an agate mortar and passed through 0.2 mm sieve particularly for organic carbon analysis.

3.8 Chemical analysis of plant samples

3.8.1 Collection and preparation of plant samples

The plant samples collected for recording dry matter production at 35 and 60 DAS were used for chemical analysis. Plants were washed thoroughly with distilled water and dried under shade. Then, samples were dried in hot air oven at 65 °C till a constant weight was obtained. Dried plant samples were powdered in a Willey mill and stored in polythene bags for further chemical analysis.

3.8.2 Total nitrogen content

Total nitrogen content (%) in plant samples was estimated by Kjeldahl digestion and distillation method as outlined by Tandon (1998) after digestion of plant sample with concentrated H_2SO_4 in the presence of digestion mixture.

3.8.3 Digestion of plant sample

A known weight of powdered plant sample was treated with concentrated HNO_3 over night for pre-digestion. Next day, the pre-digested sample was treated with di-acid mixture ($\text{HNO}_3:\text{HClO}_4$) (9:4 ratio) and digested on a sand bath at low temperature initially and then at high temperature till solution become colourless and on cooling white precipitate was obtained. The residue was dissolved in six normal (6 N) HCl and filtered and then extract was made to known volume by using distilled water. This extract was used for further analysis.

3.8.4 Phosphorus content

The phosphorus content (%) in the di-acid plant digest was determined by vanadomolybdo-phosphoric acid yellow colour method (Tandon, 1998) using spectrophotometer at 430 nm wavelength.

3.8.5 Potassium content

The potassium content (%) in the di-acid plant digest samples was determined by flame photometer method (Tandon, 1998).

3.8.6 Sulphur

The sulphur (%) in the aliquot of di-acid plant digest was estimated by turbidimetric method as explained by Tandon (1998).

3.8.7 Micronutrients

Zinc, copper, iron and manganese contents (mg kg^{-1}) in the aliquot of plant digest extract were estimated by using atomic absorption spectrophotometer (AAS) as described by Tandon (1998).

3.9 Zinc content in index leaf

The zinc content (mg kg^{-1}) in the aliquot of plant digest extract of index leaf (third fully opened leaf from top) was estimated by using atomic absorption spectrophotometer as described by Tandon (1998).

3.10 Uptake of nutrients

The uptake (mg plant^{-1}) of nutrients was worked out and expressed in mg plant^{-1} using the following formula.

$$\text{Nutrient uptake (mg plant}^{-1}\text{)} = \frac{[\text{Nutrient concentration (\%)} \times \text{Dry matter plant}^{-1} \text{ (mg)}]}{100}$$

3.11 Statistical analysis and interpretation of data

The statistical analysis and interpretation of data were done using the Fischer's method of analysis of variance technique as described by Gomez and Gomez (1984). The level of significance used in 'F' and 't' test was $P = 0.05$. Critical difference values were calculated between various treatments wherever the 'F' test was found significant treatments mean were compared by Duncan's multiple range test (DMRT). Statistical analysis was done only for the data at 60 DAS as at 35 DAS observations were recorded from single replication only.

4. EXPERIMENTAL RESULTS

A laboratory study was conducted to green synthesize and characterize zinc oxide nanoparticles from analytical grade reagent zinc nitrate using five different plant extracts, followed by a green house experiment to study the response of maize to green synthesized zinc oxide nanoparticles. The results of experiments are briefed below under following headings:

4.1 Lab Experiment-I

4.1.1 Green synthesis and characterization of ZnO nanoparticles

4.2 Lab Experiment-II

4.2.1 Effect of green synthesized nano ZnO foliar spray on growth, chlorophyll content and dry matter accumulation of maize

4.2.2 Effect of green synthesized nano ZnO foliar spray on nutrients uptake by maize

4.2.3 Effect of green synthesized nano ZnO foliar spray on post harvest available nutrient status of soil

4.1 Lab Experiment-I

4.1.1 Green synthesis and characterization of ZnO nanoparticles (cf. Table 3)

Green synthesized zinc oxide nanoparticles (ZnO NPs) were characterized through UV-spectroscopy, particle size analyzer (PSA) and scanning electron microscope (SEM) to determine their size and shape.

4.1.1.1 UV-spectroscopy

UV-spectrophotometer analysis was done for preliminary confirmation of green synthesized nanoparticles. Absorbance peak of bulk molecules shifted from higher wavelength to UV-wavelength range (280-375 nm) confirmed the presence of ZnO nanoparticles. Absorbance peak of green synthesized ZnO NPs obtained in UV-wavelength range (280-375 nm), which confirmed their size in nano range.

Absorbance peak in UV-spectroscopy (cf. Table 3)

In UV-spectroscopy, zinc oxide nanoparticles synthesized from different plant extracts showed difference in their absorbance peaks (Fig. 1). Among the five extracts, ZnO NPs synthesized from calotropis had their absorbance peak at the lowest wavelength (345 nm) and datura had at the highest wavelength (368 nm). For other three leaf extracts (tea, neem and tulasi), synthesized ZnO NPs exhibited absorbance peaks in between these two extremes (355, 359 and 368 nm, respectively).

ZnO NPs synthesized from calotropis exhibited their absorption peak at the lowest wavelength were supposed to have the smallest size and those synthesized from datura were of comparatively larger size due to their absorbance peak at higher wavelength.

4.1.1.3 Particle Size Analyzer (PSA) (cf. Table 3)

The analytical data of particle size analyzer clearly indicated that ZnO NPs obtained with calotropis were having the least size (diameter 79.6 nm) and nanoparticles (NPs) synthesized by datura recorded the highest size (497.4 nm). The NPs obtained with other leaf extracts like tea, neem and tulasi recorded size in between these values (99.6, 101.6, 122.4 nm, respectively) (Fig. 2).

4.1.1.4 Scanning Electron Microscope (SEM) (cf. Table 3)

Scanning electron microscopy of green synthesized ZnO nanoparticles determined the morphology (size and shape) of synthesized nanoparticles (Plate 4). In SEM also, ZnO NPs synthesized from calotropis recorded least size ranging from 45-75 nm, followed by tea (45-90 nm) and they were of rod shape. ZnO NPs synthesized from neem extract were like nano-flakes or flowers and size ranged from 100-300 nm. But tulasi and datura synthesized NPs were in spherical shape and size ranging from 150-500 and 1,000-2,000 nm, respectively. Among the five leaf extracts, datura synthesized ZnO NPs recorded the highest particle size.

4.1.1.5 Energy Dispersive Atomic X-ray Analysis (EDAX) (cf. Table 4)

The elemental composition of green synthesized nanoparticles was determined in EDAX (Fig. 3). ZnO NPs synthesized with calotropis leaf extract were found to have maximum purity with 28.13 and 26.41 per cent of the sample with zinc and oxygen, respectively while rest of sample weight was with other foreign elements present in the leaf extracts. Tea leaf extract synthesized ZnO NPs recorded 5.07 per cent of sample weight as zinc and 58.65 per cent as oxygen. Whereas, datura, tulasi and neem synthesized ZnO NPs had a high per cent of carbon content (79.98, 72.27 and 67.82 per cent, respectively) and that of zinc content was low (0.53, 0.65 and 4.14 per cent, respectively). ZnO NPs obtained with datura recorded minimum purity.

4.2 Lab Experiment-II

The results of pot culture experiment conducted to study "Response of maize to green synthesized nano ZnO formulations in a calcareous Vertisol" are given below.

4.2.1 Effect of green synthesized nano ZnO foliar spray on growth, chlorophyll content and dry matter accumulation of maize

4.2.1.1 Plant height (cf. Table 5)

The height of maize plants was uniform up to 30 days and significantly differed at 35, 45 and 60 DAS due nano zinc oxide spray. Among the treatments, maize plant which received nano ZnO spray @ 250 ppm (T_7) recorded the highest plant height (17.5, 25.17 and 35 cm) and the lowest was recorded with absolute control (T_1) which received only water spray (12.7, 18 and 24 cm) at 35, 45 and 60 DAS, respectively. Treatments T_8 and T_9 (nano spray @ 500 and 750 ppm, respectively) were on par with treatment T_7 . Treatment T_2 (zinc sulphate solution spray @ 0.5 %) recorded plant height (32.3 cm at 60 DAS) on par with low concentration of nano ZnO sprays @ 100, 150 and 200 ppm at 60 DAS (31.8, 32.3 and 32.5 cm, respectively). Treatments T_{10} , T_{11} and T_{12} (nano spray @ 1,000, 1,500 and 2,000 ppm, respectively) recorded lower plant height (28.7, 29.3, 28.3 cm at 60 DAS, respectively).

Table 3. UV-absorbance peak, size and shape of green synthesized zinc oxide nanoparticles

Sl. No.	Plant leaf extract	Concentration of leaf extract (%)	Absorption peak (nm)	Size of ZnO NPs (nm)		Shape of NPs in SEM
				Particle size analyzer (PSA)	Scanning electron microscopy (SEM)	
1	Calotropis	10	345	79.6	45-75	Rod shape
2	Tea	10	355	99.6	45-90	Rod shape
3	Neem	10	359	101.6	100-300	Flakes or flower like
4	Tulasi	10	364	122.4	70-500	Spherical or ovoid
5	Datura	10	368	497.4	1,000-2,000	Spherical

4.2.1.2 Number of leaves plant⁻¹ (cf. Table 6)

At all the stages of growth, number of leaves per maize plant did not differ significantly due to the nano ZnO spray formulations. Number of leaves per maize plant was almost same for all the treatments.

4.2.1.3 Leaf area plant⁻¹ (cf. Table 7)

Leaf area of maize plants differed significantly at 35, 45 and 60 DAS due to the nano ZnO spray. Highest leaf area expansion was recorded in T₇ (117.28, 185.75 and 276.29 cm² at 35, 45 and 60 DAS, respectively) which received nano ZnO spray @ of 250 ppm. Treatments T₈ (nano @ 500 ppm) and T₉ (nano @ 750 ppm) recorded leaf area (267.8 and 263.32 cm²) on par with treatment T₇ at 60 DAS. Conventional zinc sulphate spray @ 0.5 per cent (T₂) recorded leaf area (216.13 cm²) on par with treatment T₅ (226.09 cm²) receiving nano ZnO spray @ 150 ppm. Lower leaf areas were recorded with higher concentrations of nano spray @ 1,000, 1,500 and 2,000 ppm (214.68, 193.47 and 187.34 cm², respectively). The lowest leaf area was recorded in absolute control (75.63, 107.57 and 156 cm² at 35, 45 and 60 DAS, respectively).

4.2.1.4 Chlorophyll content of leaf (SPAD value) (cf. Table 8)

Chlorophyll content in maize leaves varied significantly due to nano ZnO spray. At 30 DAS, the plants recorded less chlorophyll content compared to 15 DAS. However, at 35 DAS chlorophyll content slightly increased over 30 DAS and at 45 DAS a noticeable increase in chlorophyll content was recorded. The plants which received nano ZnO spray @ 250 ppm (T₇) recorded maximum chlorophyll content at 45 and 60 DAS (42.67 and 38.1, respectively). Treatments T₈ and T₉ (nano spray @ 500 and 750 ppm, respectively) were on par with T₇ at 45 and 60 DAS. Treatment T₂ (zinc sulphate spray @ 0.5 per cent) recorded chlorophyll content on par with treatment T₄ and T₅ (nano spray @ 100 and 150 ppm, respectively) at 60 DAS.

4.2.1.5 Dry matter production (cf. Table 9)

The dry matter production at 60 DAS significantly influenced by nano ZnO spray. Maximum dry matter was recorded in T₇ which received nano ZnO spray @ 250 ppm (16.73 g plant⁻¹). T₈ and T₉ (nano @ 500 and 750 ppm) recorded dry matter on par with T₇ (15.46 and 15.35 g plant⁻¹, respectively). At higher concentrations (nano @ 1,000, 1,500 and 2,000 ppm) produced lower dry matter (14.5, 13.67 and 13.50 g plant⁻¹, respectively) at 60 DAS. At 60 DAS, zinc sulphate spray @ 0.5 per cent (T₂) recorded dry matter (9.63 g plant⁻¹) on par with nano spray @ 50 ppm (9.27 g plant⁻¹).

4.2.2 Effect of green synthesized nano ZnO foliar spray on nutrients uptake by maize

4.2.2.1 Nitrogen (cf. Table 10)

Nitrogen concentration in the plant tissue varied from 1 to 1.86 per cent at 35 DAS and 0.42 to 0.95 per cent at 60 DAS due to nano ZnO spray.

Table 4. Chemical composition of green synthesized ZnO nanoparticles obtained with EDAX

Leaf extract	Elemental composition of green synthesized ZnO NPs (wt %)											
	Oxygen (O)	Zinc (Zn)	Carbon (C)	Aluminium (Al)	Silicon (Si)	Sulphur (S)	Potassium (K)	Phosphorus (P)	Sodium (Na)	Calcium (Ca)	Beryllium (Be)	Others
Calotropis	26.41	28.13	-	-	-	-	-	-	-	10.15	10.0	25.31*
Tea	58.65	5.07	-	6.89	4.03	3.20	2.58	3.20	16.25	-	-	-
Neem	21.70	4.14	67.82	2.81	0.06	0.21	0.55	-	2.71	-	-	-
Tulasi	26.77	0.65	72.27	0.12	-	0.02	0.14	-	-	-	-	-
Datura	18.90	0.53	79.98	0.26	-	0.14	0.06	-	-	-	-	-

NOTE:

*others are heavy metals like Platinum (Pt), Bromine (Br), Niobium (Nb), Molybdenum (Mo), Palladium (Pd), Indium (In), Antimony (Sb), Iodine (I), Samarium (Sm) and Gadolinium (Gd)

Table 5. Plant height of maize as influenced by foliar application of nano ZnO formulations at different growth stages

Treatment	Plant height (cm)				
	15 DAS	30 DAS	35 DAS	45 DAS	60 DAS
T ₁ : Absolute control (water spray only)	5.3 ^a	11.33 ^a	12.70 ^f	18.00 ^f	24.00 ^d
T ₂ : Foliar ZnSO ₄ .7H ₂ O spray @ 0.5 %	5.2 ^a	10.83 ^a	13.60 ^{d-f}	19.00 ^{ef}	32.33 ^b
T ₃ : Foliar nano ZnO spray @ 50 ppm	5.2 ^a	11.33 ^a	12.90 ^{ef}	20.00 ^e	28.00 ^c
T ₄ : Foliar nano ZnO spray @ 100 ppm	5.2 ^a	11.50 ^a	13.80 ^{c-e}	22.17 ^b	31.83 ^b
T ₅ : Foliar nano ZnO spray @ 150 ppm	5.4 ^a	11.00 ^a	14.70 ^{bc}	22.67 ^{bc}	32.33 ^b
T ₆ : Foliar nano ZnO spray @ 200 ppm	5.2 ^a	11.33 ^a	15.10 ^b	22.67 ^{bc}	32.50 ^b
T ₇ : Foliar nano ZnO spray @ 250 ppm	5.3 ^a	10.83 ^a	17.50 ^a	25.17 ^a	35.00 ^a
T ₈ : Foliar nano ZnO spray @ 500 ppm	5.3 ^a	10.83 ^a	16.80 ^a	24.17 ^{ab}	33.33 ^{ab}
T ₉ : Foliar nano ZnO spray @ 750 ppm	5.4 ^a	11.00 ^a	16.70 ^a	23.83 ^{a-c}	33.17 ^{ab}
T ₁₀ : Foliar nano ZnO spray @ 1,000 ppm	5.4 ^a	11.16 ^a	15.10 ^b	20.50 ^c	29.67 ^c
T ₁₁ : Foliar nano ZnO spray @ 1,500 ppm	5.4 ^a	11.33 ^a	14.50 ^{b-d}	19.17 ^{ef}	29.33 ^c
T ₁₂ : Foliar nano ZnO spray @ 2,000 ppm	5.3 ^a	11.5 ^a	14.10 ^{b-d}	18.50 ^{ef}	28.33 ^c
S.Em. ±	0.11	0.23	0.32	0.57	0.67
C.D. (p = 0.05)	NS	NS	0.91	1.66	1.96
C.V. (%)	4.75	3.58	4.83	4.60	3.77

NOTE:

1. Spraying at 30 DAS
2. Recommended fertilizers and manures were not applied to treatments T₁ to T₁₂
3. ZnO nanoparticles synthesized from calotropis leaf extract were used for spraying
4. NS- Non significant

Table 6. Number of leaves plant⁻¹ in maize as influenced by foliar application of nano ZnO formulations at different growth stages

Treatment	No. of leaves plant ⁻¹				
	15 DAS	30 DAS	35 DAS	45 DAS	60 DAS
T ₁ : Absolute control (water spray only)	3 ^a	4.7 ^a	4.7 ^a	8.3 ^a	8.3 ^a
T ₂ : Foliar ZnSO ₄ .7H ₂ O spray @ 0.5 %	3 ^a	5.0 ^a	5 ^a	8.7 ^a	8.7 ^a
T ₃ : Foliar nano ZnO spray @ 50 ppm	3 ^a	5.0 ^a	5 ^a	8.3 ^a	8.3 ^a
T ₄ : Foliar nano ZnO spray @ 100 ppm	3 ^a	5.0 ^a	5.0 ^a	9.0 ^a	9.0 ^a
T ₅ : Foliar nano ZnO spray @ 150 ppm	3 ^a	4.7 ^a	4.7 ^a	8.3 ^a	8.3 ^a
T ₆ : Foliar nano ZnO spray @ 200 ppm	3 ^a	5.0 ^a	5.0 ^a	9.0 ^a	9.0 ^a
T ₇ : Foliar nano ZnO spray @ 250 ppm	3 ^a	5.0 ^a	5.0 ^a	9.0 ^a	9.0 ^a
T ₈ : Foliar nano ZnO spray @ 500 ppm	3 ^a	5.0 ^a	5.0 ^a	9.0 ^a	9.0 ^a
T ₉ : Foliar nano ZnO spray @ 750 ppm	3 ^a	5.0 ^a	5.0 ^a	9.0 ^a	9.0 ^a
T ₁₀ : Foliar nano ZnO spray @ 1,000 ppm	3 ^a	5.0 ^a	5.0 ^a	9.0 ^a	9.0 ^a
T ₁₁ : Foliar nano ZnO spray @ 1,500 ppm	3 ^a	5.0 ^a	5.0 ^a	9.3 ^a	9.3 ^a
T ₁₂ : Foliar nano ZnO spray @ 2,000 ppm	3 ^a	5.0 ^a	5.0 ^a	9.0 ^a	9.0 ^a
S.Em. ±	0.18	0.14	0.14	0.27	0.27
C.D. (p = 0.05)	NS	NS	NS	NS	NS
C.V. (%)	10.01	4.77	16.02	5.34	5.34

NOTE:

1. Spraying at 30 DAS
2. Recommended fertilizers and manures were not applied to treatments T₁ to T₁₂
3. ZnO nanoparticles synthesized from calotropis leaf extract were used for spraying
4. NS- Non significant

Table 7. Leaf area plant⁻¹ of maize as influenced by foliar application of nano ZnO formulations at different growth stages

Treatment	Leaf area plant ⁻¹ (cm ²)				
	15 DAS	30 DAS	35 DAS	45 DAS	60 DAS
T ₁ : Absolute control (water spray only)	16.81 ^a	73.77 ^a	75.63 ^g	107.57 ^f	156.00 ^g
T ₂ : Foliar ZnSO ₄ .7H ₂ O spray @ 0.5 %	16.25 ^a	73.77 ^a	80.61 ^f	131.47 ^e	216.13 ^{de}
T ₃ : Foliar nano ZnO spray @ 50 ppm	16.06 ^a	74.70 ^a	77.19 ^{fg}	126.69 ^e	153.38 ^g
T ₄ : Foliar nano ZnO spray @ 100 ppm	16.25 ^a	74.70 ^a	87.40 ^e	140.75 ^d	207.75 ^e
T ₅ : Foliar nano ZnO spray @ 150 ppm	16.43 ^a	73.45 ^a	88.70 ^e	146.85 ^d	226.09 ^d
T ₆ : Foliar nano ZnO spray @ 200 ppm	15.69 ^a	74.70 ^a	89.99 ^{de}	158.61 ^b	238.89 ^c
T ₇ : Foliar nano ZnO spray @ 250 ppm	16.62 ^a	75.01 ^a	117.28 ^a	185.75 ^a	276.29 ^a
T ₈ : Foliar nano ZnO spray @ 500 ppm	16.06 ^a	74.39 ^a	106.15 ^b	179.48 ^a	267.80 ^{ab}
T ₉ : Foliar nano ZnO spray @ 750 ppm	16.62 ^a	74.70 ^a	101.79 ^c	177.89 ^a	263.32 ^b
T ₁₀ : Foliar nano ZnO spray @ 1,000 ppm	16.73 ^a	75.01 ^a	90.96 ^{de}	155.13 ^{bc}	214.68 ^e
T ₁₁ : Foliar nano ZnO spray @ 1,500 ppm	16.62 ^a	74.39 ^a	94.20 ^d	152.08 ^{bc}	193.47 ^f
T ₁₂ : Foliar nano ZnO spray @ 2,000 ppm	16.25 ^a	74.39 ^a	91.28 ^{de}	151.64 ^{bc}	187.34 ^f
S.Em. ±	0.30	0.52	1.41	2.94	3.70
C.D. (p = 0.05)	NS	NS	4.11	8.57	10.79
C.V. (%)	3.13	1.20	2.66	3.36	2.95

NOTE:

1. Spraying at 30 DAS
2. Recommended fertilizers and manures were not applied to treatments T₁ to T₁₂
3. ZnO nanoparticles synthesized from calotropis leaf extract were used for spraying
4. NS – Non significant

Table 8. Chlorophyll content (SPAD values) in maize leaf as influenced by foliar application of nano ZnO formulations at different growth stages

Treatment	Chlorophyll content (SPAD values)				
	15 DAS	30 DAS	35 DAS	45 DAS	60 DAS
T ₁ : Absolute control (water spray only)	37.57 ^a	30.67 ^a	30.03 ^e	33.63 ^e	31.50 ^e
T ₂ : Foliar ZnSO ₄ .7H ₂ O spray @ 0.5 %	38.27 ^a	30.67 ^a	31.67 ^{de}	39.43 ^d	34.6 ^d
T ₃ : Foliar nano ZnO spray @ 50 ppm	38.33 ^a	31.17 ^a	33.133 ^{cd}	39.70 ^d	32.03 ^e
T ₄ : Foliar nano ZnO spray @ 100 ppm	38.23 ^a	32.20 ^a	33.43 ^{b-d}	39.77 ^d	33.83 ^d
T ₅ : Foliar nano ZnO spray @ 150 ppm	38.17 ^a	30.20 ^a	33.87 ^{bc}	40.03 ^{cd}	33.83 ^d
T ₆ : Foliar nano ZnO spray @ 200 ppm	37.43 ^a	31.17 ^a	34.20 ^{bc}	40.13 ^{cd}	35.30 ^{b-d}
T ₇ : Foliar nano ZnO spray @ 250 ppm	38.13 ^a	31.83 ^a	38.87 ^a	42.67 ^a	38.10 ^a
T ₈ : Foliar nano ZnO spray @ 500 ppm	37.93 ^a	31.47 ^a	38.00 ^a	41.67 ^{ab}	37.73 ^{ab}
T ₉ : Foliar nano ZnO spray @ 750 ppm	37.37 ^a	29.93 ^a	37.40 ^a	41.43 ^{a-c}	37.33 ^{a-c}
T ₁₀ : Foliar nano ZnO spray @ 1,000 ppm	37.67 ^a	32.17 ^a	35.33 ^{bc}	40.47 ^{b-d}	36.87 ^{a-c}
T ₁₁ : Foliar nano ZnO spray @ 1,500 ppm	38.43 ^a	31.2 ^a	34.40 ^{bc}	40.87 ^{b-d}	35.33 ^{b-d}
T ₁₂ : Foliar nano ZnO spray @ 2,000 ppm	37.83 ^a	31.93 ^a	33.87 ^b	40.73 ^{b-d}	35.17 ^{cd}
S.Em. ±	0.45	0.64	0.65	0.46	0.65
C.D. (p = 0.05)	NS	NS	1.90	1.34	1.90
C.V. (%)	2.05	3.57	3.27	1.99	3.18

NOTE:

1. Spraying at 30 DAS
2. Recommended fertilizers and manures were not applied to treatments T₁ to T₁₂
3. ZnO nanoparticles synthesized from calotropis leaf extract were used for spraying
4. NS- Non significant

Table 9. Dry matter production (g plant⁻¹) of maize as influenced by foliar application of nano ZnO formulations at different growth stages

Treatment	Dry matter production (g plant ⁻¹)	
	35 DAS	60 DAS
T ₁ : Absolute control (water spray only)	1.15	8.78 ^h
T ₂ : Foliar ZnSO ₄ .7H ₂ O spray @ 0.5%	1.35	9.63 ^g
T ₃ : Foliar nano ZnO spray @ 50 ppm	1.27	9.27 ^{gh}
T ₄ : Foliar nano ZnO spray @ 100 ppm	1.28	13.23 ^f
T ₅ : Foliar nano ZnO spray @ 150 ppm	1.35	14.0 ^{de}
T ₆ : Foliar nano ZnO spray @ 200 ppm	1.38	14.83 ^{bc}
T ₇ : Foliar nano ZnO spray @ 250 ppm	1.60	16.73 ^a
T ₈ : Foliar nano ZnO spray @ 500 ppm	1.53	15.46 ^b
T ₉ : Foliar nano ZnO spray @ 750 ppm	1.53	15.35 ^b
T ₁₀ : Foliar nano ZnO spray @ 1,000 ppm	1.43	14.50 ^{cd}
T ₁₁ : Foliar nano ZnO spray @ 1,500 ppm	1.40	13.67 ^{ef}
T ₁₂ : Foliar nano ZnO spray @ 2,000 ppm	1.38	13.50 ^{ef}
S.Em. ±		0.19
C.D. (p = 0.05)		0.56
C.V. (%)		2.53

NOTE:

1. Spraying at 30 DAS
2. Recommended fertilizers and manures were not applied to treatments T₁ to T₁₂
3. ZnO nanoparticles synthesized from calotropis leaf extract were used for spraying
4. Single plant observation at 35 DAS

Nitrogen uptake was maximum ($130.67 \text{ mg plant}^{-1}$) in T_8 (nano @ 500 ppm) and treatments T_7 (@ 250 ppm) and T_9 (@ 750 ppm) found on par with T_8 (129.12 and $128.62 \text{ mg plant}^{-1}$, respectively) at 60 DAS. Nitrogen uptake in T_2 (zinc sulphate spray @ 0.5 %) was on par with T_4 (nano @ 100 ppm). Lower uptake ($38.06 \text{ mg plant}^{-1}$) was recorded in absolute control (T_1) at 60 DAS. Higher concentrations of nano sprays (@ 1,000, 1,500 and 2,000 ppm), reduced nitrogen uptake (127.05 , 127 and $118.11 \text{ mg plant}^{-1}$) by maize at 60 DAS.

4.2.2.2 Phosphorus (cf. Table 10)

Phosphorus concentration varied from 0.068 to 0.192 and 0.054 to 0.121 per cent at 35 and 60 DAS, respectively.

Phosphorus uptake by maize plant differed significantly amongst the treatments. Maximum phosphorus uptake was recorded in treatment T_7 ($21.12 \text{ mg plant}^{-1}$), which received nano ZnO spray @ 250 ppm followed by T_8 ($16.82 \text{ mg plant}^{-1}$) and T_9 ($16.0 \text{ mg plant}^{-1}$). Lowest uptake was recorded in absolute control ($4.74 \text{ mg plant}^{-1}$). Conventional zinc sulphate spray recorded uptake ($8.01 \text{ mg plant}^{-1}$) on par with nano spray @ 150 ppm. At higher concentrations nano sprays, (@ 1,000, 1,500 and 2,000 ppm) phosphorus uptake by maize decreased (13.97 , 11.86 and $11.33 \text{ mg plant}^{-1}$, respectively).

4.2.2.3 Potassium (cf. Table 10)

Potassium concentration varied from 4.30 to 6.93 and 2.6 to 3.6 per cent at 35 and 60 DAS, respectively.

Plants receiving 250 ppm nano spray (T_7) recorded maximum potassium uptake ($609.29 \text{ mg plant}^{-1}$) followed by T_8 ($561.56 \text{ mg plant}^{-1}$) and T_9 ($562.87 \text{ mg plant}^{-1}$). Lowest potassium uptake ($232.76 \text{ mg plant}^{-1}$) was recorded in absolute control (T_1). Zinc sulphate spray (T_2) recorded potassium uptake ($413.75 \text{ mg plant}^{-1}$) on par with nano sprays @ 100 ppm and 2,000 ppm (439.06 and $441.83 \text{ mg plant}^{-1}$, respectively).

4.2.2.4 Sulphur (cf. Table 10)

Sulphur concentration in maize plants varied from 0.15 to 0.37 and 0.127 to 0.166 per cent at 35 and 60 DAS, respectively.

The highest sulphur uptake ($24.22 \text{ mg plant}^{-1}$) was recorded in T_7 (nano @ 250 ppm) at 60 DAS. Treatments T_8 (@ 500) and T_9 (@ 750 ppm) recorded sulphur uptake on par with T_7 (23.67 and $23.53 \text{ mg plant}^{-1}$, respectively). T_2 (zinc sulphate spray) showed sulphur uptake ($17.01 \text{ mg plant}^{-1}$) on par with T_4 and T_{12} (nano @ 100 and 2,000 ppm, respectively). For T_{10} , T_{11} and T_{12} (nano @ 1,000, 1,500 and 2,000 ppm), sulphur uptake reduced (19.17 , 18.07 and $17.32 \text{ mg plant}^{-1}$, respectively) at 60 DAS.

4.2.2.5 Zinc (cf. Table 11)

Zinc concentration varied from 39 to 179.69 ppm and 30 to 78 ppm at 35 and 60 DAS, respectively. Highest zinc concentration was recorded in T_{12} (nano spray @ 2,000 ppm) and lowest was in absolute control (T_1).

Table 10. Nitrogen, phosphorus, potassium and sulphur uptake by maize as influenced by foliar application of nano ZnO formulations at different growth stages

Treatment	Uptake (mg plant ⁻¹)							
	Nitrogen		Phosphorus		Potassium		Sulphur	
	35 DAS	60 DAS	35 DAS	60 DAS	35 DAS	60 DAS	35 DAS	60 DAS
T ₁ : Absolute control (water spray only)	12.00 (1.0)	38.06 ^h (0.42)	0.69 (0.068)	4.74 ^h (0.054)	50.15 (4.3)	232.76 ^h (2.6)	1.81 (0.15)	11.55 ^e (0.127)
T ₂ : ZnSO ₄ .7H ₂ O spray @ 0.5 %	17.94 (1.28)	56.50 ^g (0.58)	0.97 (0.081)	8.01 ^{ef} (0.082)	70.10 (5.13)	413.75 ^f (4.17)	5.10 (0.37)	17.01 ^d (0.166)
T ₃ : Foliar nano ZnO spray @ 50 ppm	15.51 (1.18)	40.18 ^h (0.43)	1.41 (0.112)	5.06 ^h (0.054)	56.18 (4.33)	295.79 ^g (3.1)	3.51 (0.27)	12.10 ^e (0.127)
T ₄ : Foliar nano ZnO spray @ 100 ppm	16.50 (1.25)	68.30 ^f (0.51)	1.63 (0.125)	7.22 ^f (0.054)	61.05 (4.7)	439.06 ^{ef} (3.27)	3.52 (0.27)	17.41 ^d (0.130)
T ₅ : Foliar nano ZnO spray @ 150 ppm	18.63 (1.35)	76.38 ^e (0.54)	1.80 (0.130)	8.81 ^e (0.062)	71.07 (5.2)	467.33 ^d (3.3)	3.92 (0.29)	18.43 ^{cd} (0.131)
T ₆ : Foliar nano ZnO spray @ 200 ppm	21.03 (1.46)	94.97 ^d (0.63)	2.05 (0.137)	11.00 ^d (0.067)	76.15 (5.47)	503.13 ^c (3.3)	4.23 (0.31)	20.29 ^b (0.134)
T ₇ : Foliar nano ZnO spray @ 250 ppm	24.02 (1.47)	129.12 ^{ab} (0.77)	2.67 (0.150)	21.12 ^a (0.121)	109.27 (6.8)	609.29 ^a (3.63)	4.77 (0.31)	24.22 ^a (0.152)
T ₈ : Foliar nano ZnO spray @ 500 ppm	23.85 (1.47)	130.67 ^a (0.81)	2.65 (0.154)	16.82 ^b (0.110)	108.85 (6.83)	561.56 ^b (3.6)	4.70 (0.31)	23.67 ^a (0.151)
T ₉ : Foliar nano ZnO spray @ 750 ppm	23.92 (1.52)	128.62 ^{ab} (0.85)	2.60 (0.145)	16.00 ^b (0.107)	108.03 (6.93)	562.87 ^b (3.6)	4.58 (0.30)	23.53 ^a (0.15)
T ₁₀ : Foliar nano ZnO spray @ 1,000 ppm	27.02 (1.86)	127.05 ^b (0.87)	2.38 (0.185)	13.97 ^c (0.717)	103.03 (7.0)	507.44 ^c (3.53)	3.92 (0.28)	19.17 ^{bc} (0.128)
T ₁₁ : Foliar nano ZnO spray @ 1,500 ppm	25.53 (1.8)	127.00 ^b (0.95)	2.23 (0.191)	11.86 ^d (0.088)	90.53 (6.4)	461.59 ^{de} (3.4)	3.81 (0.28)	18.07 ^{cd} (0.126)
T ₁₂ : Foliar nano ZnO spray @ 2,000 ppm	25.01 (1.78)	118.11 ^c (0.88)	2.16 (0.192)	11.33 ^d (0.16)	88.80 (6.33)	441.83 ^{de} (3.3)	3.61 (0.27)	17.32 ^d (0.125)
S.Em. ±		0.77		0.29		8.86		0.47
C.D. (p = 0.05)		2.25		0.83		25.86		1.38
C.V. (%)		1.41		4.33		3.35		4.40

NOTE:

1. Spraying at 30 DAS
2. Recommended fertilizers and manures were not applied to treatments T₁ to T₁₂
3. ZnO nanoparticles synthesized from calotropis leaf extract were used for spraying
4. Single plant observation at 35 DAS
5. The values in parenthesis indicate nutrient concentration (ppm)

Table 11. Zinc, iron, copper and manganese uptake by maize as influenced by foliar application of nano ZnO formulations at different growth stages

Treatment	Zinc (mg plant ⁻¹)		Iron (mg plant ⁻¹)		Copper (mg plant ⁻¹)		Manganese (mg plant ⁻¹)	
	35 DAS	60 DAS	35 DAS	60 DAS	35 DAS	60 DAS	35 DAS	60 DAS
T ₁ : Absolute control (water spray only)	0.047 (39.00)	0.27 ^f (30.00)	0.120 (96.67)	0.78 ^j (87.00)	0.034 (27.00)	0.21 ^j (24.00)	0.110 (97.67)	0.77 ^g (87.33)
T ₂ : ZnSO ₄ .7H ₂ O spray @ 0.5 %	0.173 (124.33)	0.65 ^b (62.37)	0.250 (176.67)	1.55 ^h (150.67)	0.057 (39.93)	0.34 ^h (35.27)	0.154 (114.33)	1.07 ^e (106.33)
T ₃ : Foliar nano ZnO spray @ 50 ppm	0.070 (48.00)	0.30 ^f (31.00)	0.191 (145.00)	1.267 ⁱ (133.00)	0.042 (32.33)	0.25 ⁱ (27.00)	0.134 (105.67)	0.91 ^f (96.33)
T ₄ : Foliar nano ZnO spray @ 100 ppm	0.074 (52.67)	0.42 ^e (32.00)	0.207 (156.00)	1.82 ^g (134.33)	0.044 (33.00)	0.36 ^{fg} (27.67)	0.136 (106.67)	1.42 ^d (99.67)
T ₅ : Foliar nano ZnO spray @ 150 ppm	0.080 (55.00)	0.49 ^d (34.00)	0.232 (168.00)	2.18 ^f (145.67)	0.049 (35.00)	0.39 ^{de} (28.33)	0.148 (111.83)	1.51 ^{cd} (102.67)
T ₆ : Foliar nano ZnO spray @ 200 ppm	0.090 (58.00)	0.57 ^c (37.67)	0.253 (180.67)	2.73 ^e (175.00)	0.052 (36.67)	0.43 ^c (29.00)	0.159 (115.33)	1.62 ^c (105.00)
T ₇ : Foliar nano ZnO spray @ 250 ppm	0.116 (73.33)	0.68 ^b (41.67)	0.457 (281.33)	4.33 ^a (274.67)	0.069 (42.00)	0.55 ^a (33.33)	0.198 (123.67)	1.96 ^a (117.67)
T ₈ : Foliar nano ZnO spray @ 500 ppm	0.117 (75.67)	0.65 ^b (42.00)	0.443 (280.67)	4.25 ^{ab} (268.00)	0.066 (40.33)	0.46 ^b (31.00)	0.186 (122.00)	1.83 ^b (115.33)
T ₉ : Foliar nano ZnO spray @ 750 ppm	0.120 (77.67)	0.66 ^b (43.33)	0.435 (277.33)	4.06 ^b (262.67)	0.063 (39.67)	0.44 ^c (29.67)	0.185 (121.00)	1.78 ^b (114.00)
T ₁₀ : Foliar nano ZnO spray @ 1,000 ppm	0.195 (134.33)	1.05 ^a (72.00)	0.381 (268.67)	3.71 ^c (250.00)	0.055 (36.67)	0.41 ^d (28.33)	0.175 (119.00)	1.62 ^c (108.00)
T ₁₁ : Foliar nano ZnO spray @ 1,500 ppm	0.240 (167.33)	1.05 ^a (75.67)	0.373 (263.67)	3.51 ^{cd} (250.33)	0.051 (35.67)	0.38 ^{ef} (27.67)	0.168 (118.00)	1.52 ^{cd} (106.67)
T ₁₂ : Foliar nano ZnO spray @ 2,000 ppm	0.250 (179.67)	1.06 ^a (78.00)	0.343 (245.33)	3.42 ^d (244.67)	0.049 (35.00)	0.35 ^{gh} (26.00)	0.165 (117.00)	1.49 ^d (105.33)
S.Em. ±		0.011		0.077		0.007		0.035
C.D. (p = 0.05)		0.032		0.22		0.019		0.10
C.V. (%)		2.90		4.74		2.98		4.23

NOTE:

1. Spraying at 30 DAS
2. Recommended fertilizers and manures were not applied to treatments T₁ to T₁₂
3. ZnO nanoparticles synthesized from calotropis leaf extract were used for spraying
4. Single plant observation at 35 DAS
5. The values in parenthesis indicate nutrient concentration (ppm)

Highest zinc uptake was obtained in treatments such as T₁₀, T₁₁ and T₁₂ (nano spray @ 1,000, 1,500 and 2,000 ppm) and which recorded 1.05, 1.05, 1.06 mg plant⁻¹, respectively. This was followed by treatment T₇ (0.68 mg plant⁻¹). Treatments such as T₂ (zinc sulphate spray), T₈ and T₉ (nano @ 500 and 750 ppm, respectively) were on par with T₇ (0.65, 0.65 and 0.66 mg plant⁻¹ respectively). Lowest uptake was recorded in absolute control (0.27 mg plant⁻¹).

4.2.2.6 Iron (cf. Table 11)

Iron content in maize plants ranged from 96.67 to 281.33 and 87 to 274.67 ppm at 35 and 60 DAS, respectively.

Iron uptake recorded maximum (4.33 mg plant⁻¹) in T₇ (nano spray @ 250 ppm) while T₈ (4.25 mg plant⁻¹) and T₉ (4.06 mg plant⁻¹) recorded uptake on par with T₇. At higher concentrations of nano sprays such as 1,000, 1,500 and 2,000 ppm (T₁₀, T₁₁ and T₁₂, respectively) significant reduction in iron uptake (3.71, 3.51 and 3.42 mg plant⁻¹, respectively) was noticed compared to T₇. Lowest uptake was recorded in absolute control (0.78 mg plant⁻¹).

4.2.2.7 Copper (cf. Table 11)

Copper concentration varied from 29.37 to 42 ppm and 24 to 35.27 ppm at 35 and 60 DAS, respectively.

Maize plant receiving nano spray @ of 250, 500 and 750 ppm recorded higher copper uptake (0.55, 0.46, 0.44 mg plant⁻¹) followed by T₁₀, T₁₁ and T₁₂ (0.41, 0.38 and 0.35 mg plant⁻¹). Treatment T₂ (ZnSO₄ @ 0.5 %) recorded uptake (0.34 mg plant⁻¹) on par with T₄ (nano @ 100 ppm). The lowest uptake was obtained for absolute control (0.21 mg plant⁻¹).

4.2.2.8 Manganese (cf. Table 11)

Manganese concentration in maize plants ranged from 97.67 to 123.67 and 117.67 to 87.33 ppm at 35 DAS and 60 DAS, respectively.

Maximum uptake was recorded in T₇ (1.96 mg plant⁻¹) followed by treatments T₈ (nano spray @ 500 ppm) and T₉ (nano spray @ 750 ppm) which were on par with T₇. Absolute control (T₁) recorded lowest uptake of copper (0.77 mg plant⁻¹).

4.2.2.9 Zinc in index leaf (cf. Table 12)

Zinc content in the index leaf differed significantly at 60 DAS due to nano zinc oxide spray. With the increase in the nano ZnO spray concentrations, zinc content in index leaf increased. At 35 and 60 DAS, maximum zinc content was recorded in T₁₁ and T₁₂ (nano spray @ 1,500 and 2,000 ppm, respectively). Lowest zinc concentration was recorded in absolute control (27.60 mg kg⁻¹).

Table 12. Zinc concentration in index leaf of maize as influenced by foliar application of nano ZnO formulations at different growth stages

Treatment	Zinc (mg kg ⁻¹)	
	35 DAS	60 DAS
T ₁ : Absolute control (water spray only)	38.67	27.60 ^g
T ₂ : Foliar ZnSO ₄ .7H ₂ O spray @ 0.5 %	119.33	58.04 ^c
T ₃ : Foliar nano ZnO spray @ 50 ppm	46.33	29.00 ^{fg}
T ₄ : Foliar nano ZnO spray @ 100 ppm	52.00	31.67 ^f
T ₅ : Foliar nano ZnO spray @ 150 ppm	53.67	32.00 ^f
T ₆ : Foliar nano ZnO spray @ 200 ppm	56.00	36.67 ^e
T ₇ : Foliar nano ZnO spray @ 250 ppm	73.00	37.33 ^e
T ₈ : Foliar nano ZnO spray @ 500 ppm	75.00	41.00 ^d
T ₉ : Foliar nano ZnO spray @ 750 ppm	78.33	43.33 ^d
T ₁₀ : Foliar nano ZnO spray @ 1,000 ppm	129.33	67.33 ^b
T ₁₁ : Foliar nano ZnO spray @ 1,500 ppm	162.00	76.00 ^a
T ₁₂ : Foliar nano ZnO spray @ 2,000 ppm	176.00	77.33 ^a
S.Em. ±		1.02
C.D. (p = 0.05)		2.98
C.V. (%)		3.81

NOTE:

1. Spraying at 30 DAS
2. Recommended fertilizers and manures were not applied to treatments T₁ to T₁₂
3. ZnO nanoparticles synthesized from calotropis leaf extract were used for spraying
4. Single plant observation at 35 DAS

4.2.3 Effect of green synthesized nano ZnO foliar spray on post harvest available nutrient status of soil

4.2.3.1 Chemical properties of soil (cf. Table 13)

The pH, EC and organic carbon contents in soil were not influenced by nano ZnO spray formulations.

4.2.3.2 Available nitrogen (cf. Table 14)

Available nitrogen status in harvest soil was recorded maximum in absolute control (56.22 mg kg⁻¹). Lowest nitrogen status (36.08 mg kg⁻¹) was recorded in treatment T₈ (nano spray @ 500 ppm) followed by T₇ (nano @ 250 ppm) and T₉ (nano @ 750 ppm) which were 38.28 and 39 mg kg⁻¹, respectively.

4.2.3.3 Available phosphorus (cf. Table 14)

Phosphorus content in soil at 60 DAS, recorded highest for absolute control (T₁) (19.13 mg kg⁻¹) and it was on par with T₃, *i.e.*, nano spray @ 50 ppm. Treatment T₇ (nano @ 250 ppm) showed lowest available phosphorus status (14.65 mg kg⁻¹) whereas T₈ and T₉ (nano @ 500 and 750 ppm, respectively) were on par with T₇ (15.15 and 15.53 mg kg⁻¹, respectively). Conventional zinc sulphate spray (T₂) recorded available phosphorus status (17.57 mg kg⁻¹) same as that of nano spray @ 150 ppm.

4.2.3.4 Available potassium (cf. Table 14)

Available potassium status was minimum in treatment T₇ (149.67 mg kg⁻¹) and treatments such as T₆, T₈ and T₉ (nano spray @ 200, 500 and 750 ppm) were on par with T₇ (154.5, 150.0 and 153.50 mg kg⁻¹, respectively). T₂ recorded potassium (162 mg kg⁻¹) status on par with T₄, T₁₁ and T₁₂ (nano @ 150, 1,500 and 2,000 ppm). However, the highest value was recorded in absolute control (173.17 mg kg⁻¹).

4.2.3.5 Available sulphur (cf. Table 14)

Sulphur availability after harvest was maximum in absolute control (23.07 mg kg⁻¹) and minimum (17.13 mg kg⁻¹) in T₇ (nano @ 250 ppm). Treatment T₈ (nano @ 500 ppm) and T₉ (nano @ 750 ppm) were on par with T₇ (17.20 and 17.37 mg kg⁻¹, respectively). T₂ (zinc sulphate spray @ 0.5 %) recorded sulphur status on par with T₄ and T₁₂ (21.90 and 21.40 mg kg⁻¹).

4.2.3.6 Available micronutrients (zinc, iron, copper and manganese) (cf. Table 15)

Available zinc status after harvest (60 DAS) was recorded lower content and was same for treatments such T₇, T₈, T₉, T₁₀ (nano spray @ 250, 500, 750 and 1,000 ppm respectively). Highest status was recorded in treatments T₁ and T₃ (absolute control and nano spray @ 50 ppm). Treatments T₂, T₅ and T₆ recorded almost same available zinc status. Similarly treatments T₁₁ and T₁₂ recorded on par available status.

Iron, copper and manganese status was recorded higher in absolute control (2.08, 0.352 and 2.22 mg kg⁻¹, respectively) and lowest was (1.75, 0.32 and 2.04 mg kg⁻¹, respectively) in T₇ (nano @ 250 ppm) which was on par with T₈ and T₉. Conventional zinc sulphate spray recorded Fe, Cu and Mn status (1.98, 0.342 and 2.16 mg kg⁻¹, respectively) on par with 100 and 150 ppm nano ZnO spray.

Table 13. Soil pH, electrical conductivity and organic carbon content of soil after the harvest of maize (at 60 DAS) as influenced by foliar application of nano ZnO formulations

Treatment	pH	EC (dS m ⁻¹)	OC (g kg ⁻¹)
T ₁ : Absolute control (water spray only)	7.34 ^a	0.42 ^a	4.1 ^a
T ₂ : Foliar ZnSO ₄ .7H ₂ O spray @ 0.5 %	7.24 ^a	0.41 ^a	4.3 ^a
T ₃ : Foliar nano ZnO spray @ 50 ppm	7.30 ^a	0.43 ^a	4.4 ^a
T ₄ : Foliar nano ZnO spray @ 100 ppm	7.20 ^a	0.41 ^a	4.3 ^a
T ₅ : Foliar nano ZnO spray @ 150 ppm	7.20 ^a	0.42 ^a	4.5 ^a
T ₆ : Foliar nano ZnO spray @ 200 ppm	7.20 ^a	0.42 ^a	4.7 ^a
T ₇ : Foliar nano ZnO spray @ 250 ppm	7.30 ^a	0.40 ^a	4.5 ^a
T ₈ : Foliar nano ZnO spray @ 500 ppm	7.30 ^a	0.41 ^a	4.4 ^a
T ₉ : Foliar nano ZnO spray @ 750 ppm	7.30 ^a	0.41 ^a	4.2 ^a
T ₁₀ : Foliar nano ZnO spray @ 1,000 ppm	7.32 ^a	0.40 ^a	4.7 ^a
T ₁₁ : Foliar nano ZnO spray @ 1,500 ppm	7.32 ^a	0.40 ^a	4.5 ^a
T ₁₂ : Foliar nano ZnO spray @ 2,000 ppm	7.30 ^a	0.41 ^a	4.6 ^a
S.Em. ±	0.04	0.008	0.12
C.D. (p = 0.05)	NS	NS	NS
C.V. (%)	0.903	3.20	4.78

NOTE:

1. Spraying at 30 DAS
2. Recommended fertilizers and manures were not applied to treatments T₁ to T₁₂
3. ZnO nanoparticles synthesized from calotropis leaf extract were used for spraying
4. NS – Non significant

Table 14. Available nitrogen, phosphorus, potassium and sulphur in soil after the harvest of maize (at 60 DAS) as influenced by foliar application of nano ZnO formulations

Treatment	Available nutrients (mg kg ⁻¹)			
	N	P	K	S
T ₁ : Absolute control (water spray only)	56.22 ^a	19.13 ^a	173.17 ^a	23.70 ^a
T ₂ : Foliar ZnSO ₄ .7H ₂ O spray @ 0.5 %	51.72 ^b	17.57 ^{bc}	162.00 ^c	21.07 ^{b-d}
T ₃ : Foliar nano ZnO spray @ 50 ppm	55.00 ^a	19.00 ^a	168.50 ^{ab}	22.53 ^{ab}
T ₄ : Foliar nano ZnO spray @ 100 ppm	50.05 ^c	18.52 ^{ab}	167.00 ^b	21.90 ^{bc}
T ₅ : Foliar nano ZnO spray @ 150 ppm	47.50 ^d	17.57 ^{bc}	160.50 ^c	21.57 ^{b-d}
T ₆ : Foliar nano ZnO spray @ 200 ppm	45.36 ^e	16.15 ^{de}	154.50 ^{d-f}	20.27 ^{c-e}
T ₇ : Foliar nano ZnO spray @ 250 ppm	38.28 ⁱ	14.65 ^f	149.67 ^f	17.13 ^g
T ₈ : Foliar nano ZnO spray @ 500 ppm	36.08 ^h	15.15 ^{ef}	150.00 ^{ef}	17.20 ^g
T ₉ : Foliar nano ZnO spray @ 750 ppm	39.00 ^h	15.53 ^{def}	153.50 ^{d-f}	17.37 ^{fg}
T ₁₀ : Foliar nano ZnO spray @ 1,000 ppm	40.34 ^g	16.15 ^{de}	155.17 ^{de}	19.00 ^{ef}
T ₁₁ : Foliar nano ZnO spray @ 1,500 ppm	40.00 ^{fg}	16.58 ^{cd}	158.17 ^{cd}	19.97 ^{de}
T ₁₂ : Foliar nano ZnO spray @ 2,000 ppm	41.50 ^f	17.43 ^{bc}	161.00 ^c	21.40 ^{b-d}
S.Em. ±	0.72	0.36	1.66	0.58
C.D. (p = 0.05)	2.08	1.05	4.84	1.69
C.V. (%)	2.74	3.67	1.80	4.94

NOTE:

1. Spraying at 30 DAS
2. Recommended fertilizers and manures were not applied to treatments T₁ to T₁₂
3. ZnO nanoparticles synthesized from calotropis leaf extract were used for spraying
4. Available N, P, K and S in initial soil were 75, 21, 210 and 24 mg kg⁻¹ soil, respectively

Table 15. Available micronutrients status in soil after the harvest of maize (at 60 DAS) as influenced by foliar application of nano ZnO formulations

Treatment	Available nutrients (mg kg ⁻¹)			
	Zn	Fe	Cu	Mn
T ₁ : Absolute control (water spray only)	0.52 ^a	2.08 ^a	0.352 ^a	2.22 ^a
T ₂ : Foliar ZnSO ₄ .7H ₂ O spray @ 0.5%	0.48 ^{ab}	1.98 ^{bc}	0.342 ^{a-c}	2.16 ^{b-d}
T ₃ : Foliar nano ZnO spray @ 50 ppm	0.52 ^a	2.07 ^a	0.350 ^{ab}	2.19 ^{ab}
T ₄ : Foliar nano ZnO spray @ 100 ppm	0.50 ^{ab}	2.00 ^{ab}	0.341 ^{a-d}	2.18 ^{a-c}
T ₅ : Foliar nano ZnO spray @ 150 ppm	0.48 ^{bc}	1.95 ^{b-d}	0.332 ^{b-e}	2.17 ^{a-d}
T ₆ : Foliar nano ZnO spray @ 200 ppm	0.48 ^{bc}	1.90 ^{c-e}	0.327 ^{c-f}	2.14 ^{b-e}
T ₇ : Foliar nano ZnO spray @ 250 ppm	0.46 ^c	1.75 ^g	0.320 ^{ef}	2.04 ^g
T ₈ : Foliar nano ZnO spray @ 500 ppm	0.46 ^c	1.76 ^g	0.311 ^f	2.08 ^{fg}
T ₉ : Foliar nano ZnO spray @ 750 ppm	0.46 ^c	1.77 ^g	0.321 ^{d-f}	2.09 ^{e-g}
T ₁₀ : Foliar nano ZnO spray @ 1,000 ppm	0.46 ^c	1.81 ^{fg}	0.331 ^{b-e}	2.12 ^{d-f}
T ₁₁ : Foliar nano ZnO spray @ 1,500 ppm	0.47 ^c	1.87 ^{e-g}	0.332 ^{b-e}	2.13 ^{c-f}
T ₁₂ : Foliar nano ZnO spray @ 2,000 ppm	0.47 ^{bc}	1.89 ^{d-f}	0.333 ^{a-e}	2.12 ^{c-f}
S.Em. ±	0.01	0.028	0.006	0.018
C.D. (p = 0.05)	0.03	0.081	0.018	0.053
C.V. (%)	3.58	2.54	3.13	1.47

NOTE:

1. Spraying at 30 DAS
2. Recommended fertilizers and manures were not applied to treatments T₁ to T₁₂
3. ZnO nanoparticles synthesized from calotropis leaf extract were used for spraying
4. Available micronutrients (Zn, Fe, Cu and Mn) in initial soil were 0.55, 2.18, 0.39 and 2.31 mg kg⁻¹ soil, respectively

5. DISCUSSION

A laboratory study to green synthesize ZnO nanoparticles (NPs) using different plant extracts, followed by a pot culture experiment under green house condition to compare the performance of synthesized ZnO NPs vs. conventional zinc sulphate on maize plants were undertaken during 2016-17. The results obtained during the course of investigation are discussed below under the following headings:

5.1 Lab Experiment-I

5.2 Lab Experiment-II

5.1 Lab experiment-I

The results regarding the laboratory study conducted to green synthesize and characterize zinc oxide nanoparticles from analytical grade reagent zinc nitrate using five different plant extracts are discussed below in detail.

5.1.1 Green synthesis of ZnO NPs using different plant extracts

Plant extracts which are rich sources of polyphenols, alkaloids, terpenes, flavanoids, proteins *etc.*, can reduce bulk molecules into nano size particles, besides acting as capping and stabilizing agents. Nanoparticles synthesized from plant extracts were more stable, smaller in size and cheaper than that of chemically synthesized nanoparticles. Moreover green synthesis is eco-friendly and no toxic by-products are generated compared to chemical methods. FTIR spectroscopic studies revealed that phyto-chemicals such as terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids are responsible for reduction of bulk molecules into nanoparticles during green synthesis. A wide range of plants could be used to synthesize nanoparticles which include *Ananas comosus*, *Azadirachta indica*, *Calotropis procera*, *Camellia sinensis*, *Ocimum sanctum*, *Hibiscus subdariffa*, *Chrysanthemum morifolium*, *Chrysopogon zizanioides*, *Cylindrocladium floridanum*, *Eucalyptus chapmaniana*, *Euphorbia hirta*, *Leucas aspera*, *Mimosa elengi*, *Morinda citrifolia*, *Nelumbo nucifera*, *Phoenix dactylifera*, *Sida acuta*, *Tephrosia purpurea*, *Tinospora cordifolia* *etc.* Nanoparticles of diverse morphology can be synthesized through plant mediated synthesis. Recently, green nanotechnology is gaining importance as an attractive branch of nanotechnology.

Zinc is an essential nutrient and only one element that is represented in all the six enzyme systems. Even though its requirement for crop plants is very low, it is considered as the fourth important yield limiting nutrient after NPK. This is mainly due to the intensive farming with high yielding cultivars, application of high analysis straight fertilizers and reduced use of organic manures which caused zinc deficiency in Indian soils. Moreover, the use efficiency of applied Zn carriers is very low (5-20 %), the moment they are applied to the soil, get precipitated into insoluble forms, particularly in calcareous Vertisols. Zinc application to crop plants in the form of nanoparticles will serve as an efficient nutrient delivery system which reduces the quantity of nutrient required and increases the effectiveness of applied nutrients.

Research results have clearly demonstrated that when nutrient molecules were supplied as nanoparticles enhanced plants growth and yield compared to the traditional bulk chemical fertilizers. Thus, nano-fertilizers reduce environmental pollution and increase crop productivity.

Keeping all these things in mind, leaf extracts of five plants viz., calotropis (*Calotropis procera*), neem (*Azadirachta indica*), tea (*Camellia sinensis*), tulasi (*Ocimum tenuiflorum*) and datura (*Datura stramonium*) were used to green synthesize ZnO NPs from its bulk molecules. Analytical grade zinc nitrate was used as the precursor to synthesis ZnO nanoparticles. The active principles present in the extract especially polyphenols and other principles which are rich in alcoholic functional groups reduced the bulk zinc molecules to nanoscale size particles. Protein and other organic molecules act as capping agents which prevent the size increase of these particles and keep them in nano range and also stabilize dispersed solution to avoid agglomeration of nanoparticles in aqueous solution. Ten per cent leaf extracts were used to synthesize ZnO nanoparticles.

5.1.2 Characterization of green synthesized ZnO nanoparticles

5.1.2.1 Characterization through UV-spectroscopy

UV-spectrophotometer analysis was done for the preliminary confirmation of green synthesized nanoparticles. Absorbance peak of bulk molecules shifted from higher wavelength to UV-wavelength range (280-375 nm) when the particles were reduced to nanoscale particles. This confirmed the presence of zinc oxide nanoparticles (Fig. 1a to 1e).

Among five leaf extracts, ZnO NPs synthesized from calotropis exhibited the lowest absorbance peak (345 nm) showing comparatively greater reduction in size of bulk molecules i.e., least size for synthesized ZnO NPs. While ZnO NPs synthesized from datura showed comparatively higher wavelength absorbance peak (368 nm), which indicated comparatively lower reduction of bulk size molecules than that of other leaf extracts. Zinc oxide nanoparticles synthesized from other three leaf extracts (tea, neem and tulasi) were having their absorbance peaks in between calotropis and datura (355, 359 and 368 nm, respectively) indicating that their size is intermediate to that of synthesized from calotropis and datura. Similar results were reported by Divya *et al.* (2013) that ZnO NPs synthesized using leaf extract of *Hibiscus rosa-sinensis* had their absorption peak at UV wavelength (358-375 nm) and size ranged from 40 to 56 nm. Parthiban and Sundaramurthy (2015) characterized ZnO NPs synthesized using *Pyrus pyrifolia* leaf extract and obtained absorption peak at 376 nm and XRD pattern revealed that synthesized NPs were having wurtzite hexagonal structure with an average size 22 nm. Bala *et al.* (2015) synthesized ZnO NPs from *Hibiscus subdariffa* which showed its absorbance peaks at 370 nm and recorded dumbbell shape with 30-50 nm as diameter. Senthilkumar and Sivakumar (2014) reported a blue shifted absorption peak of ZnO NPs at 325 nm and their average size in accordance with XRD data was 16 nm.

5.1.2.2 Characterization through particle size analyzer (PSA) and scanning electron microscope (SEM)

Particle size analyzer determines the size distribution of nanoparticles in dispersed solution and the average size is determined from the frequency distribution curve (Fig. 2a to 2e). Particle size analyzer gives us an idea about particle distribution and their average size.

ZnO NPs synthesized from calotropis leaf extract recorded lowest size (diameter 79.6 nm), which was followed tea extract (99.6 nm), neem extract (101.6 nm), tulasi extract (122.4 nm) and datura extract (497.4 nm) as per particle size analyzer.

Scanning electron microscope was also used to characterize morphology (size and shape) of green synthesized ZnO nanoparticles (Plate 4a, 4b, 4c, 4d and 4e). ZnO NPs synthesized from calotropis and tea was of rod shape and their size ranged from 45-75 nm and 45-90 nm, respectively. ZnO NPs synthesized from neem extract were like nano-flakes (or flower) and their size ranged from 100-300 nm. Nanoparticles obtained with tulasi were ovoid in shape with diameter ranging from 70-500 nm and that synthesized from datura were spherical with size ranged from 1,000 - 2,000 nm

Calotropis procera is a desert plant which is as known milky weed due to the presence of latex. According to Poovizhi and Krishnaveni (2015) the milky latex secreted by calotropis consists of several biologically active compounds, such as proteins, amino acids, carbohydrates, lipids, vitamins, alkaloids, resins and tannins and predominantly alkaloids viz., calotropin, catotoxin, calcilin, gigatin etc. FTIR spectroscopy of green synthesized NPs showed presence of hydroxyl, aromatic, amine and carbonate groups. This revealed that the active principles in the leaf extracts were responsible for the synthesis of ZnO NPs. The data indicated that size of nano ZnO particles obtained with calotropis was the lowest, as determined by particle size analyzer and SEM. The lowest size of particles could be due to high reducing power of bio-molecules present in the leaf extracts of calotropis particularly the alcoholic functional group which can bring the greater reduction. Those extract which are rich in alcoholic groups or polyphenols efficiently and effectively reduces bulk molecules into nanoparticles.

In particle size analyzer, average size of calotropis synthesized nanoparticles was recorded as 79.6 nm. On the other hand, the size recorded with SEM was in the range 45-75 nm and particles were rod like. Thus, size of NPs produced with calotropis was found to be less than 100 nm. Similar results were reported by Poovizhi and Krishnaveni (2015). They synthesized ZnO NPs using calotropis leaf extract and size of NPs ranged from 100-200 nm with granular shape.

Tea leaf extracts are rich in phenolic compounds and antioxidants which can act as effective reducing agents to get nanoparticles from bulk molecules and amino acid, protein and lipids present in the extract, help to stabilize the growth of the nanoparticles. Senthilkumar and Sivakumar (2014) synthesized ZnO NPs using tea leaf extracts which exhibited their absorption peak at 325 nm and average size in accordance with XRD data was 16 nm. The results of present investigations are almost similar to result of earlier workers. In the present study somewhat higher peak and size were recorded.

Fig. 1. Characterization of green synthesized ZnO NPs using UV-spectroscopy

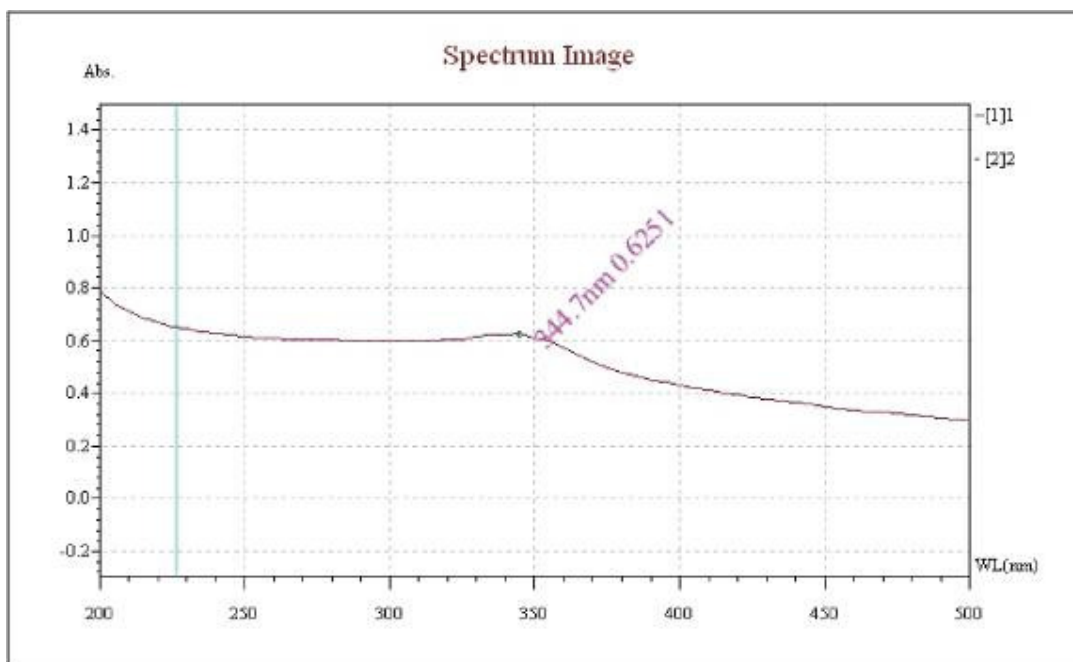


Fig. 1a. Absorbance peak of ZnO NPs synthesized using calotropis leaf extract

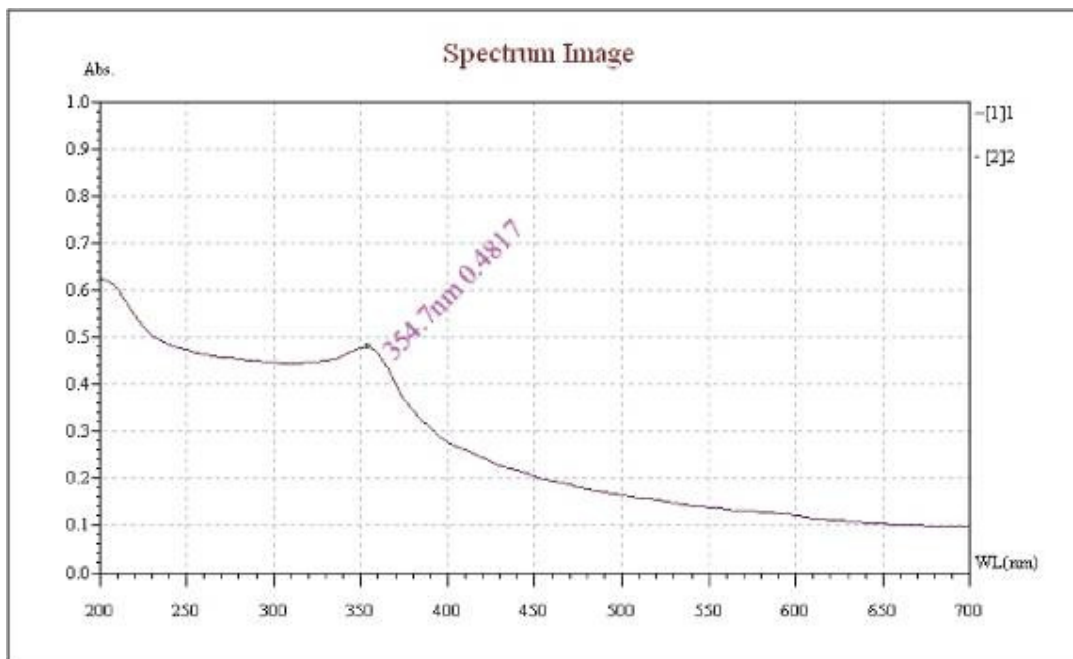


Fig. 1b. Absorbance peak of ZnO NPs synthesized using tea leaf extract

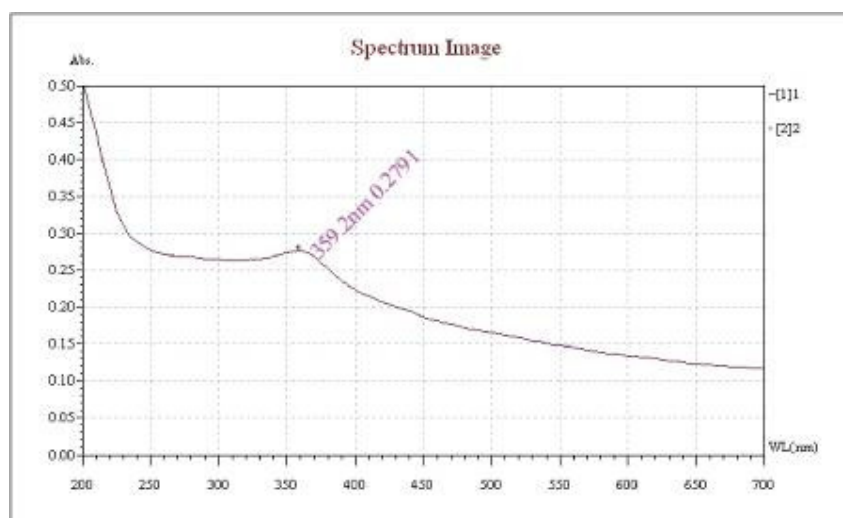


Fig. 1c. Absorbance peak of ZnO NPs synthesized using neem leaf extract

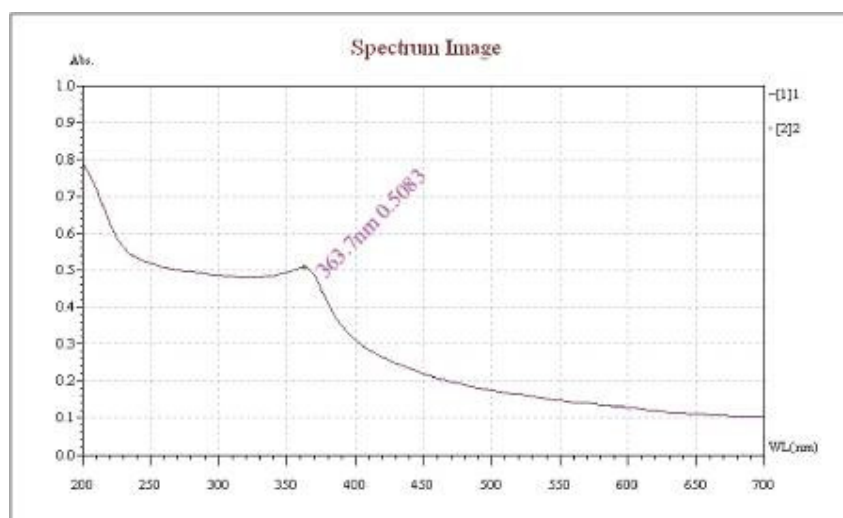


Fig. 1d. Absorbance peak of ZnO NPs synthesized using tulasi leaf extract

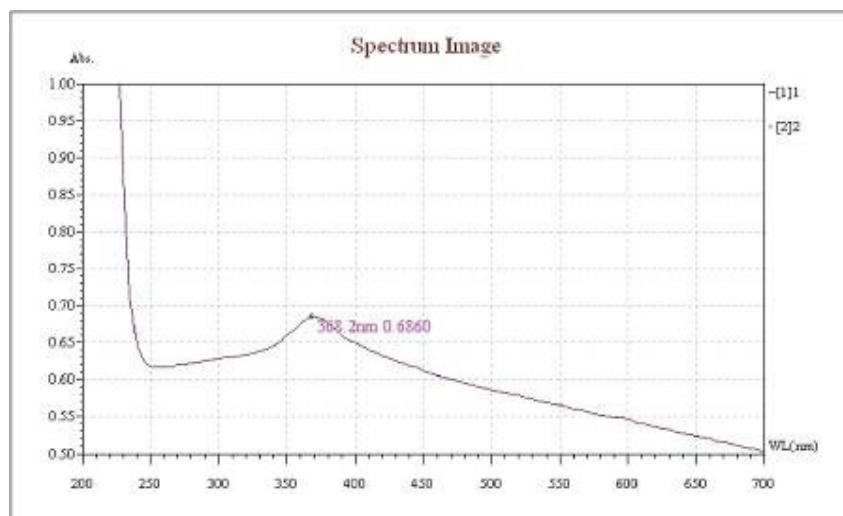


Fig. 1e. Absorbance peak of ZnO NPs synthesized using datura leaf extract

Fig.2. Charactrization of green synthesized ZnO NPs using particle size analyzer

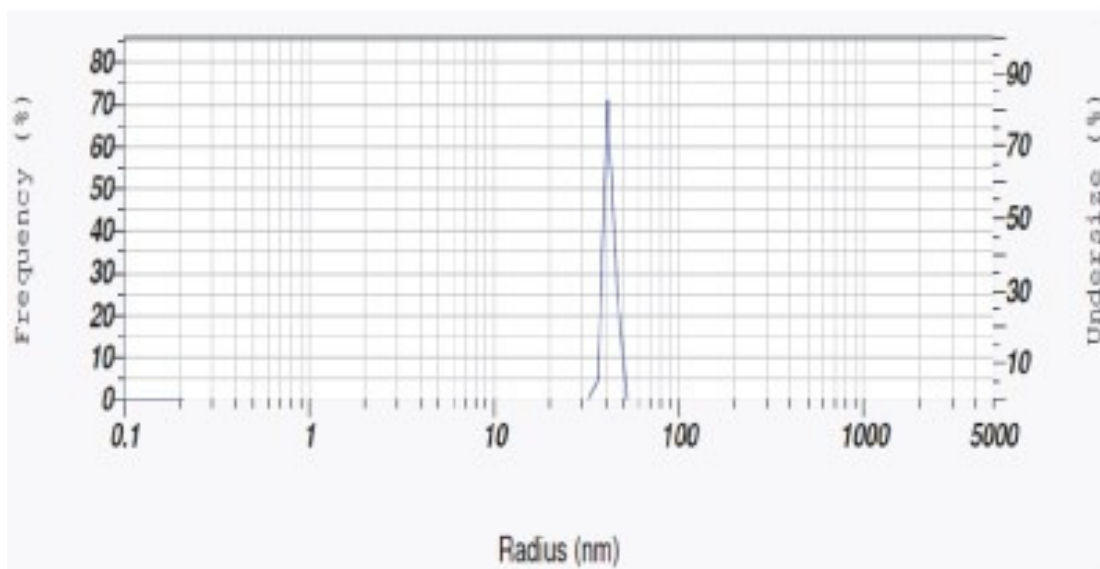


Fig. 2a. Size of ZnO NPs synthesized using calotropis leaf extract

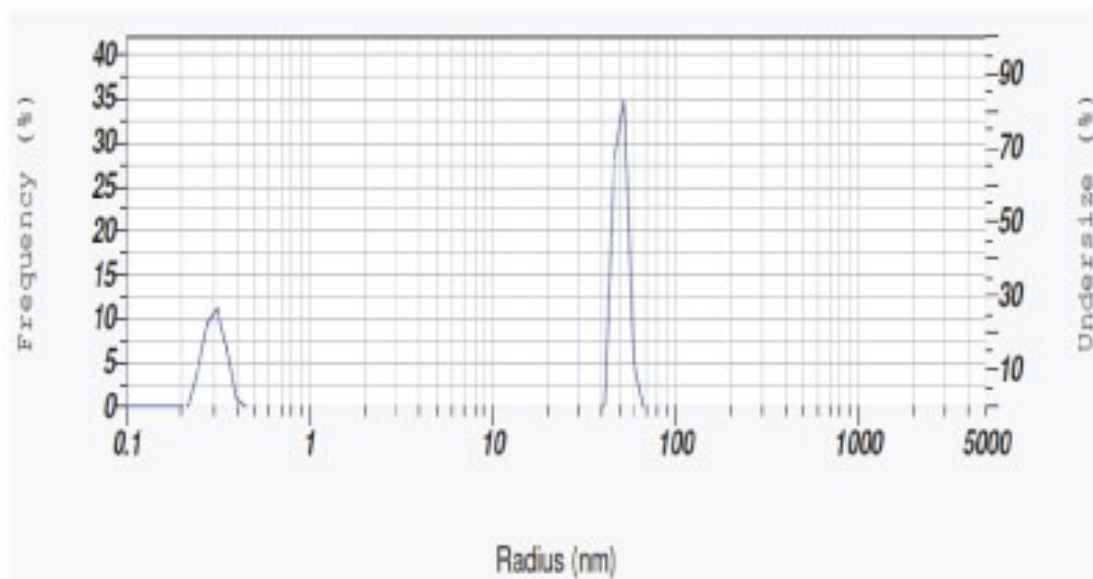


Fig. 2b. Size of ZnO NPs synthesized using tea leaf extract

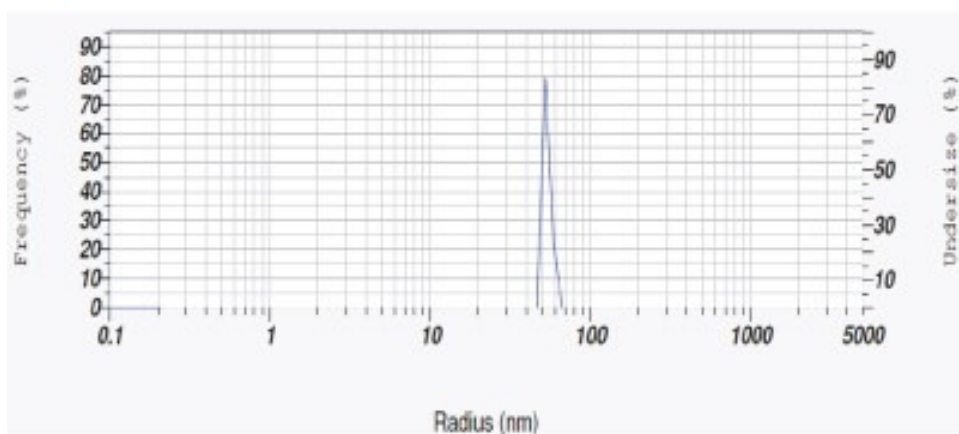


Fig. 2c. Size of ZnO NPs synthesized using neem leaf extract

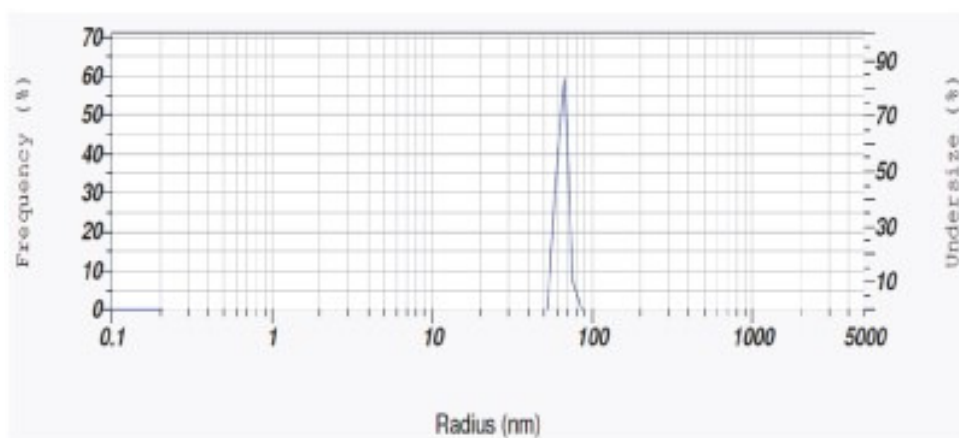


Fig. 2d. Size of ZnO NPs synthesized using tulasi leaf extract

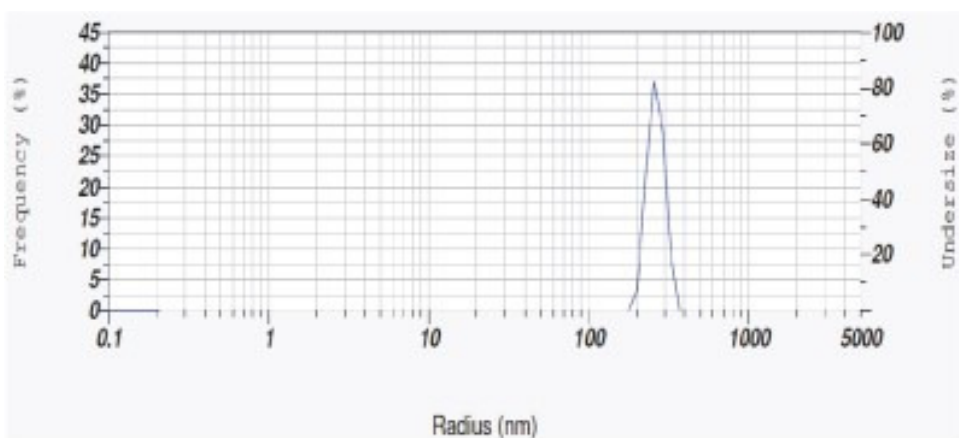


Fig. 2e. Size of ZnO NPs synthesized using datura leaf extract

Tea extracts with high polyphenol content act as both chelating or reducing and capping agents for nanoparticles (Kharissova *et al.*, 2013). Sindhura *et al.* (2013) synthesized ZnO NPs using leaves of *Parthenium hysterophorous* and recorded size ranging from 16 to 108.5 nm and shape was spherical.

Gnanasangeetha and Thambavani (2014) reported neem mediated synthesis of ZnO NPs. They obtained particles of flower morphology and size 100-200 nm. Also Oudhia *et al.* (2015) attempted synthesis of ZnO NPs using neem leaf extract and obtained NPs with size 25 nm and tubular shape. Raut *et al.* (2013) synthesized ZnO NPs from tulasi and obtained hexagonal shape nanoparticle with diameter range 11-25 nm. Thus, the results of present study corroborate findings of earlier studies. In the present study, datura leaf extract synthesized nanoparticles showed larger size compared to other leaf extracts. It recorded an average size 497.4 nm in particle size analyzer and 1,000-2,000 nm in SEM. The shape was found to be spherical.

Further, scanning electron microscope (SEM) images revealed uniform distribution of zinc oxide nanoparticles on the surface without agglomeration which indicated the better stability of green synthesized nanoparticles.

5.1.2.3 Characterization through energy dispersive atomic X-ray spectroscopy (EDAX)

The elemental composition of green synthesized nanoparticles was determined by EDAX to know the purity of synthesized nanoparticles (Fig. 3a to 3e). From the data, it appeared that synthesized ZnO NPs were not pure. They were interfered by carbon content. So, the particles need to be calcinated at a much higher temperature for much longer duration to achieve higher purity in the case of datura, tulasi and neem synthesized nanoparticles while, for calotropis and tea synthesized NPs, impurity were due to other elements present in the leaf extract.

It was found that ZnO NPs synthesized using calotropis leaf extract contained 28.13 and 26.41 per cent zinc and oxygen, respectively while, remaining composition was due to other foreign elements present in the leaf extracts. Among the five leaf extracts, maximum purity for synthesized ZnO nanoparticles was obtained with calotropis and minimum with datura. In tea leaf extract synthesized ZnO NPs recorded 5.07 per cent of sample weight as zinc and 58.65 per cent as oxygen. Datura, tulasi and neem synthesized ZnO NPs recorded a high per cent of carbon content (79.98, 72.27 and 67.82 %, respectively) and whereas zinc content was low (0.53, 0.65 and 4.14, respectively). Ajiboso *et al.* (2015) reported high mineral (K, Mg, Na and Ca) contents in calotropis leaf and 6.5 per cent as ash content. Calotropis plant is a heavy metal accumulator and can accumulate heavy metals from soil leading to the presence of foreign elements (mineral and heavy elements) in green synthesized nanoparticles. Bala *et al.* (2015) reported the presence of carbon and other elemental impurities in small amount and concluded that the stability of green synthesized nanoparticles could be due to organic molecules. Dispersed aqueous solution of green synthesized nanoparticles showed better stability due to the presence of organic molecules. Hydrophobic nature of organic molecules prevents agglomeration of nanoparticles and causes its effective dispersion and stabilization in aqueous solution.

Fig. 3. Characterization of green synthesized ZnO NPs using energy dispersive X-ray analysis (EDAX)

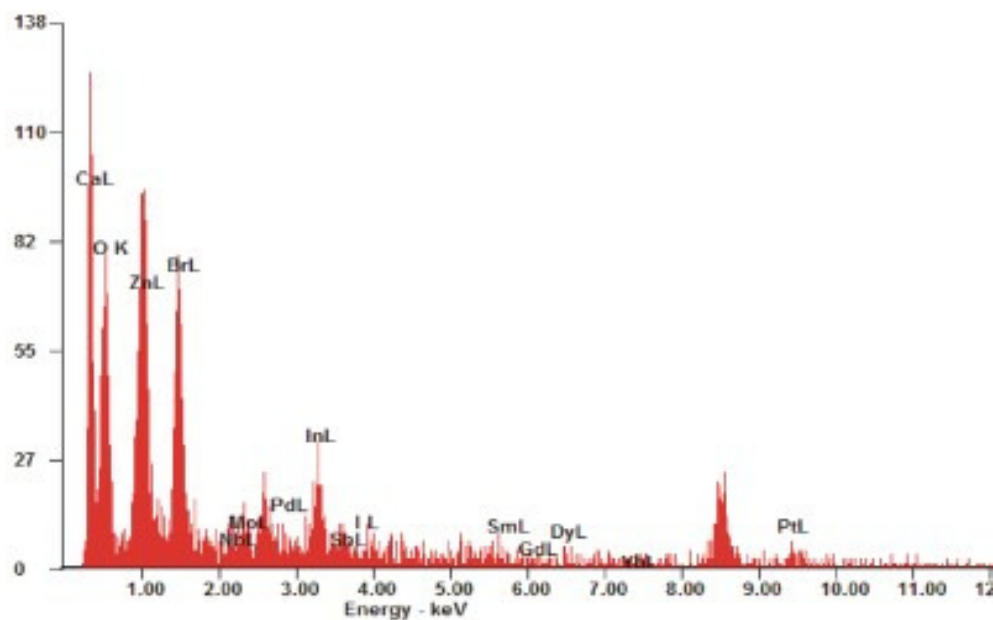


Fig. 3a. EDAX analysis of ZnO NPs synthesized using calotropis leaf extract

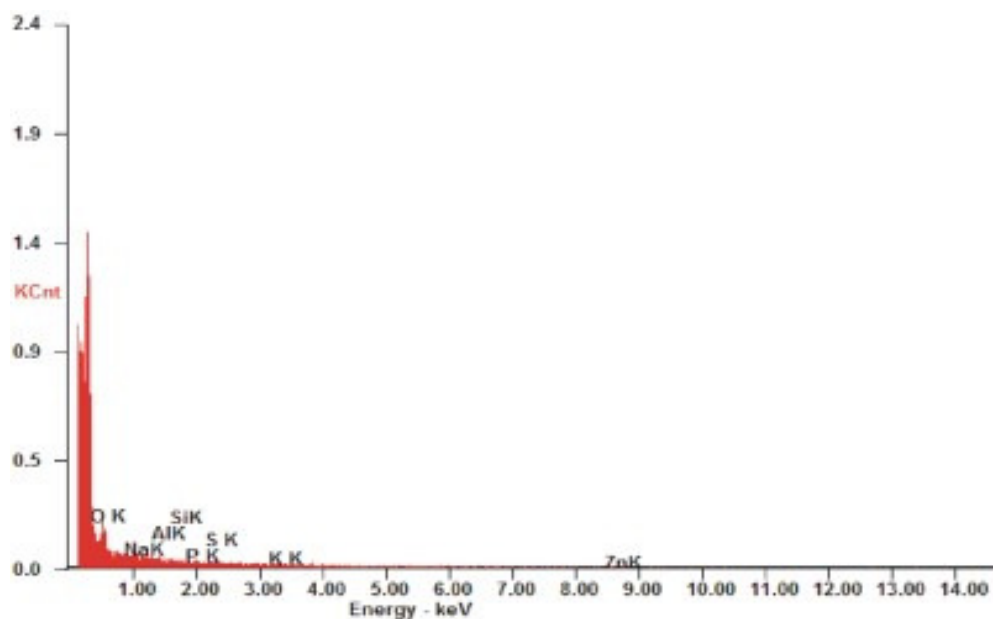


Fig. 3b. EDAX analysis of ZnO NPs synthesized using tea leaf extract

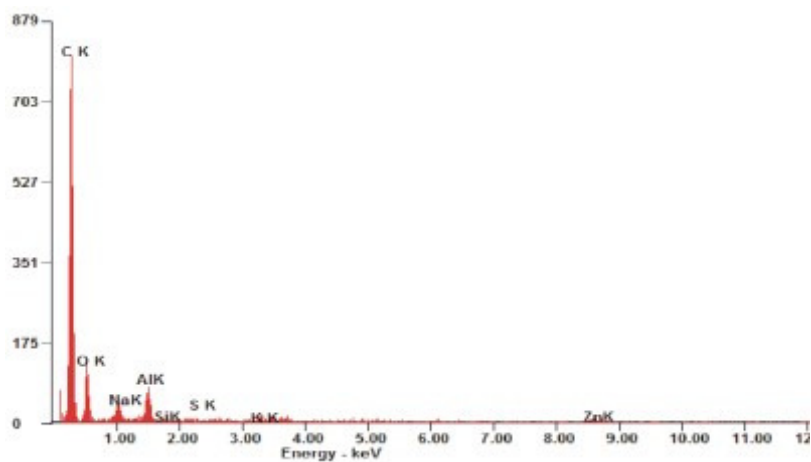


Fig. 3c. EDAX analysis of ZnO NPs synthesized using neem leaf extract

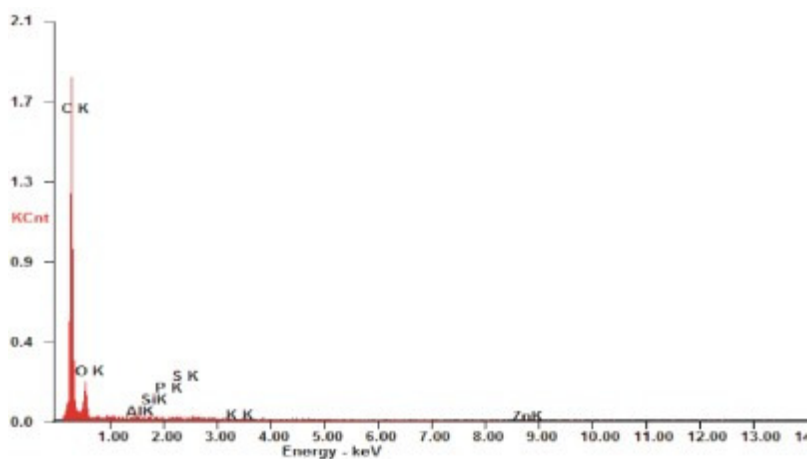


Fig. 3d. EDAX analysis of ZnO NPs synthesized using tulasi leaf extract

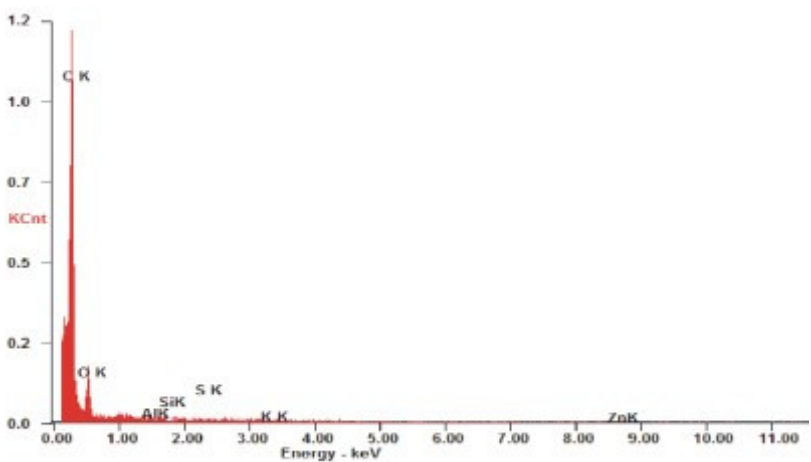


Fig. 3e. EDAX analysis of ZnO NPs synthesized using datura leaf extract

5.2 Lab experiment-II

The results of pot culture experiment conducted to study the “Response of maize to green synthesized nano ZnO formulations in a calcareous Vertisol” are discussed below in detail.

5.2.1 Effect of green synthesized nano ZnO foliar spray on growth, chlorophyll content and dry matter accumulation of maize

The height of maize plants was uniform up to 30 DAS but differed significantly at 35, 45 and 60 DAS due to the foliar application of nano ZnO formulations (Fig. 4). Treatment receiving foliar spray of nano ZnO @ 250 ppm (T_7) recorded the highest plant height at 35, 45 and 60 DAS. At 60 DAS, T_7 recorded 45.8 per cent increase in plant height over absolute control and eight per cent increase over conventional sulphate spray. Treatments T_8 (nano @ 500 ppm) and T_9 (nano @ 750 ppm) recorded plant height on par with T_7 . The lowest plant height was recorded for absolute control (24 cm) at 60 DAS. For treatments such as T_{10} , T_{11} and T_{12} (nano spray @ 1,000, 1,500 and 2,000 ppm), plant height reduced (8, 9 and 12 %, respectively over conventional zinc sulphate spray) significantly due to toxic effects of ZnO nanoparticles. Thus, it is quite evident that nano ZnO spray at lower concentrations enhanced plant height and at higher concentrations plant height was found reduced due to zinc toxicity and its detrimental effects. Conventional zinc sulphate spray @ 0.5 per cent recorded plant height on par with nano spray concentrations @ 100, 150 and 200 ppm. But, nano ZnO spray @ 250 ppm was significantly superior over conventional zinc sulphate spray. However, nano sprays @ 500 (T_8) and 750 (T_9) ppm recorded plant height on par with 250 ppm nano spray (T_7). Further increase in the nano spray concentrations reduced plant height due to inhibitory effects of ZnO NPs. Jayarambabu *et al.* (2014) observed improvement in seed germination, seedling growth and dry matter production in mung bean at a lower concentration of ZnO NPs (20 ppm) and inhibitory effects on plant growth at higher concentrations (> 20 ppm). Adhikari *et al.* (2015) also reported that plant height, root length, root volume and dry matter weight were improved due to application of zinc oxide nanoparticles than conventional fertilizers. Salama (2012) reported that silver nanoparticles at lower concentrations (20 to 60 ppm) had a stimulating effect and at higher concentrations an inhibitory effect on the growth of the plantlets (common bean and corn). In the present study also the maize plant height was significantly increased at lower concentrations, up to 250 ppm and thereafter plant height started to decline. Thus, the results of present investigation corroborate the findings of earlier workers. But, the number of leaves per maize plant was uniform for all the treatments and was not influenced by nano ZnO formulations.

Leaf area expansion of maize plants significantly varied due to foliar application of green synthesized nano ZnO particles (Fig. 5). Maximum leaf area was recorded in treatment T_7 which received nano ZnO spray @ 250 ppm (43.53 % increase over absolute and 28 % increase over conventional zinc sulphate spray) followed by T_8 and T_9 which received nano ZnO sprays @ 500 and 750 ppm, respectively. Kisan *et al.* (2015) reported similar results that spinach plants sprayed with ZnO NPs @ 500 and 1,000 ppm recorded an increase in leaf length, width and surface area compared to control. Similarly, Adhikari *et al.* (2015) observed higher leaf expansion in maize plants received with nano ZnO fertilizers than conventional zinc sulphate fertilizers at same concentration.

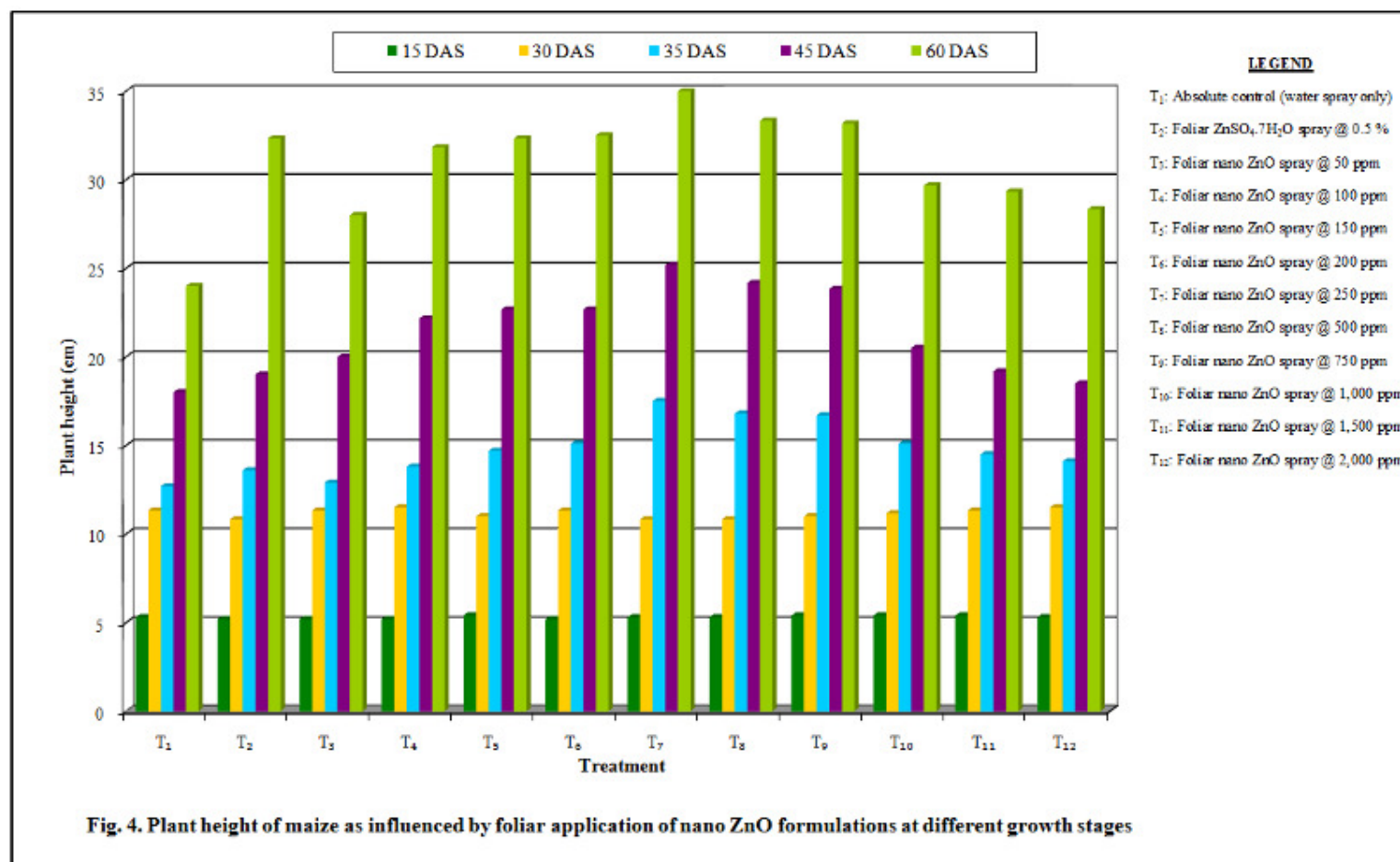


Fig. 4. Plant height of maize as influenced by foliar application of nano ZnO formulations at different growth stages

The increased in leaf area expansion may be due to effective supply of zinc at optimum concentration and in efficient form. Nanoparticles due to their higher surface area per unit volume easily get absorbed and assimilated in the plant system. SEM image of maize leaves showed that the pore size (stomatal openings) in the leaf surface had diameter of 15-40 micrometres (Plate 8a, 8b, 8c and 8d), whereas ZnO NPs being smaller in size can be easily absorbed through these pores. At higher concentrations of nano sprays, lower leaf area expansion recorded due to greater absorption of ZnO NPs leading to zinc toxicity, which had detrimental effects on growth and development of maize plants.

Chlorophyll content in the maize plants varied significantly due to various treatments (Fig. 6) and maximum (21 % increase over absolute control and 10 % increase over conventional zinc sulphate spray) was recorded for treatment T₇ receiving nano ZnO spray @ 250 ppm followed by T₈ and T₉ which received nano spray @ 500 and 750 ppm, respectively. Zinc is an essential nutrient and has a direct role in chlorophyll synthesis. Efficient assimilation of zinc results in better chlorophyll production. Thus, nano ZnO sprays at lower concentrations found to be a better source compared conventional zinc sulphate spray. Chlorophyll content found to be reduced due to nano spray at higher concentrations (1,000, 1,500 and 2,000 ppm) due to greater assimilation of zinc resulting in phytotoxicity. Similar results were reported by Prasad *et al.* (2012) that chlorophyll content in peanut was higher with nano ZnO spray @ 1,000 ppm than at 2,000 ppm. Although zinc is an important element for many metabolic processes, at higher concentrations can interfere photosynthesis (Chaney, 1993) and generate oxidative stress (Prasad *et al.*, 1999). Similar results were also reported by Zheng *et al.* (2005) that application TiO₂ NPs at a lower concentration, enhanced chlorophyll content, photosynthetic activity and dry matter accumulation of spinach plant. The results obtained on chlorophyll content in maize were identical with the observations made by these earlier works.

Dry matter accumulation of maize plants varied significantly due to ZnO NPs spray formulations at 60 DAS (Fig. 7). Dry matter accumulation recorded maximum with nano spray @ 250 ppm (90 % increase over absolute control and 73 % increase over conventional zinc sulphate spray) and lowest in absolute control. At higher nano concentration sprays, plant growth and dry matter accumulation decreased due to zinc toxicity. It may be due to the unique properties of nanoparticles such as higher surface area and presence of more reactive oxygen which enhance the photosynthetic rate and metabolic activities aggravating enzymatic activities. Zinc is only one essential element, represented in the entire six enzyme systems and support anabolic metabolism which results in better dry matter accumulation. Thus, when zinc is supplied as zinc oxide nanoparticles there was better availability and assimilation of zinc nutrient, while higher concentration sprays resulted in the more availability and absorption causing in zinc toxicity. Taheri *et al.* (2015) reported that application of ZnO nanoparticles @ 2 ppm increased the shoot dry matter and leaf area indices of corn by 63.8 and 69.7 per cent, respectively over the control. Similar results were also reported by Prasad *et al.* (2012) and Jayarambabu *et al.* (2014). Mahajan *et al.* (2011) reported that nano ZnO @ 20 ppm enhanced root (40.89 %) and shoot (76.04 %) biomass in mung bean over control. The toxicity of metal oxide NPs involves three distinct mechanisms such as release of respective metal ion, surface modification and interaction with media and disruption of bio-molecules (Brunner *et al.*, 2006) which resulted in reduced dry matter accumulation and reduced yield.

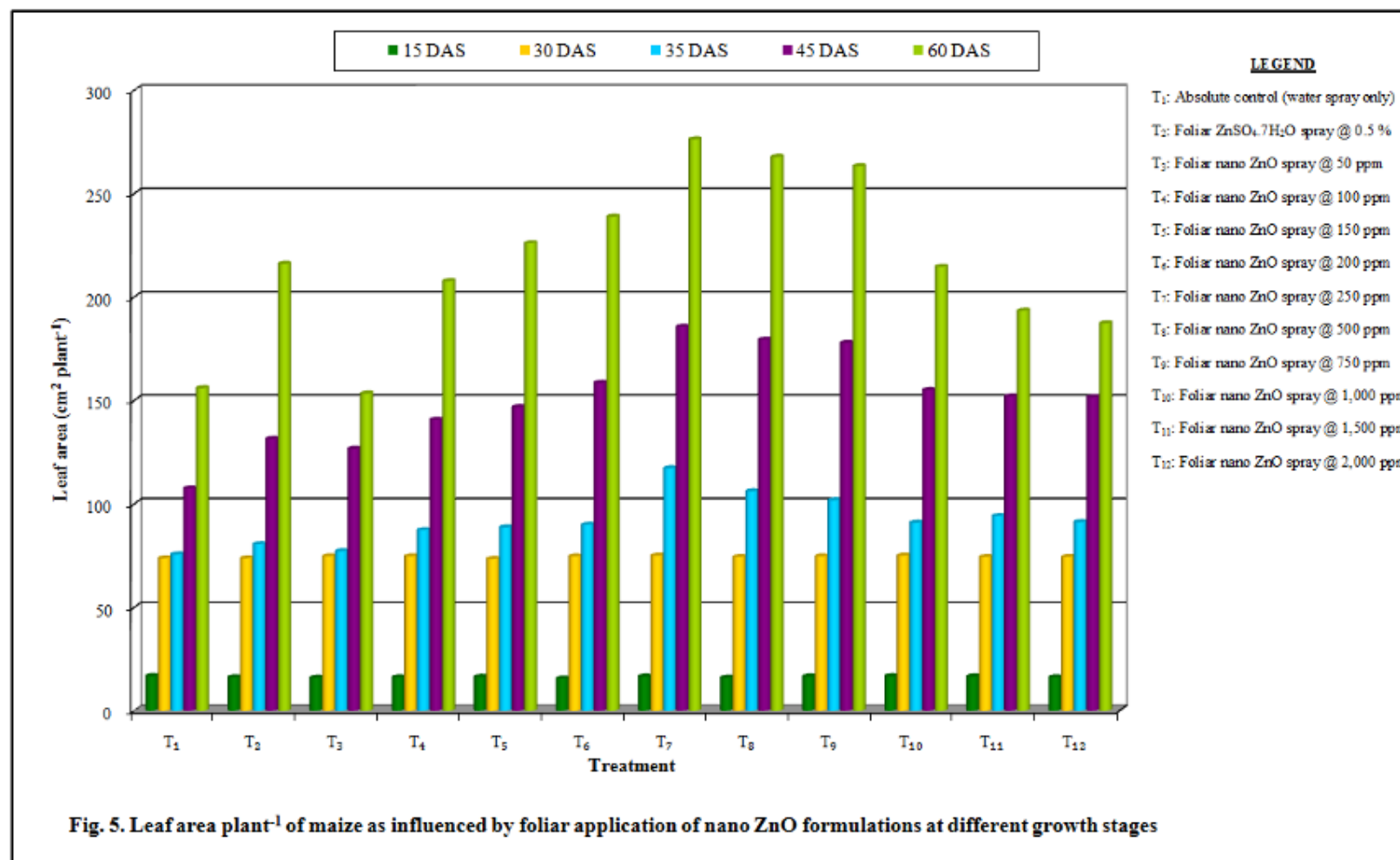


Fig. 5. Leaf area plant⁻¹ of maize as influenced by foliar application of nano ZnO formulations at different growth stages

Titanium dioxide nanoparticles (nTiO_2) act as efficient nutrient source for plants to improve biomass production due to enhanced nitrogen assimilation, photo-reduction activities of photosystem II and electron transport chain and scavenging of reactive oxygen species (Morteza *et al.*, 2013). The results of the present study are also in conformity with the findings of these earlier workers.

5.2.2 Effect of green synthesized nano ZnO spray on the uptake of nutrients by maize

Nutrients uptake by maize plants was significantly influenced by the nano ZnO spray formulations. Nutrient uptake by crop plants is directly related to the dry matter production as well as the nutrient concentration in the tissue. Nanoscale ZnO particles which are less hydrophilic and being more dispersible in lypophilic substances can penetrate plant system through the leaf surface (Da Silva *et al.*, 2006) compared to bulk ZnSO_4 . According to Prasad *et al.* (2012), the bioavailability of the nanoparticles is comparatively higher than chelated ZnSO_4 because of its smaller size and lower water solubility.

For nitrogen, maximum uptake was recorded in treatment T_8 , nano ZnO spray @ 500 ppm (131 % increase over conventional zinc sulphate spray) at 60 DAS and followed by T_7 (128 % increase over conventional zinc sulphate spray) and T_9 (127 % increase over conventional zinc sulphate spray). However, conventional zinc sulphate spray @ 0.5 per cent (T_2) recorded lower uptake compared to nano zinc oxide sprays. Up to 500 ppm concentration of nano spray, nitrogen uptake by maize increased and further increase in spray concentrations (1,000, 1,500 and 2,000 ppm) the uptake decreased due to zinc toxicity (Fig. 8). These observations corroborate the findings of earlier studies. Kisan *et al.* (2015) reported that increase in protein content in spinach leaf with foliar application of nano ZnO @ 500 and 1,000 ppm. Salama (2012) reported that application of silver nanoparticles increased chlorophyll, carbohydrate and protein contents up to 60 ppm concentration and further increase in Ag NPs concentration caused toxic effects.

The maximum phosphorus uptake was recorded in treatment T_7 , nano spray @ 250 ppm (163 % increase over conventional zinc sulphate spray) at 60 DAS and followed by T_8 (109 % increase over conventional zinc sulphate spray) and T_9 (99 % increase over the conventional zinc sulphate spray) (Fig. 8). Conventional zinc sulphate spray (T_2) recorded very low phosphorus uptake compared to nano sprays. Nano spray up to 250 ppm positively influenced phosphorus uptake and further increase in concentration decreased phosphorus uptake due to the antagonistic interaction between phosphorus and zinc and also due to zinc toxicity. Van *et al.* (2013) reported that chitosan nanoparticles enhanced the uptake of nutrients such as nitrogen, phosphorous, potassium, calcium and magnesium in the robusta coffee plants compared to control.

Potassium uptake was influenced by nano ZnO spray and its concentration in the plant tissue varied from 4.3 to 6.93 and 2.6 to 3.63 per cent at 35 and 60 DAS, respectively. Among the nutrients, plants recorded the highest concentration and uptake of potassium indicating the phenomenon of 'luxury consumption'. Among the treatments T_7 (nano spray @ 250 ppm) recorded maximum potassium uptake (47 % increase over zinc sulphate spray) followed by T_8 (nano @ 500 ppm) and T_9 (nano @ 750 ppm).

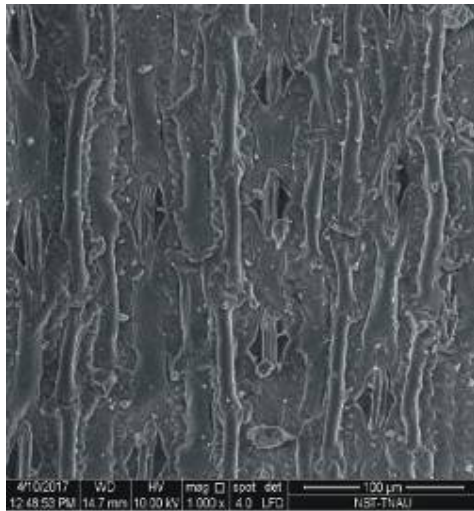


Plate 8a. Surface view of maize leaf

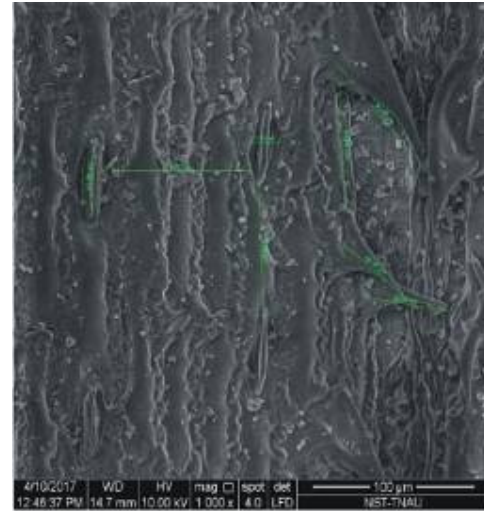


Plate 8b. Surface view of maize leaf

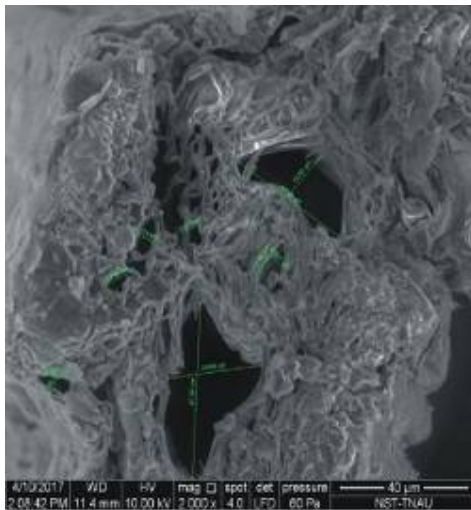


Plate 8c. Cross sectional view of maize leaf

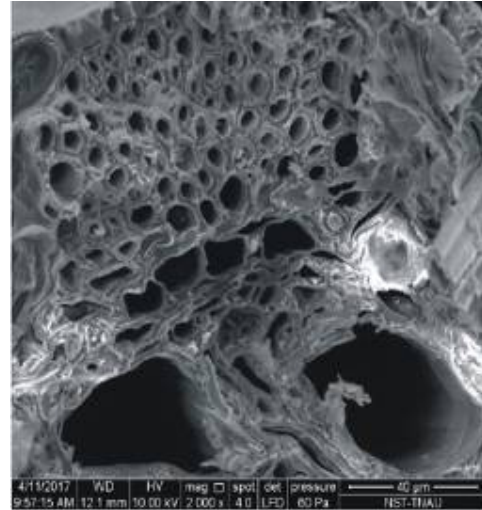


Plate 8d. Cross sectional view of maize leaf

Plate 8. SEM image of maize leaf

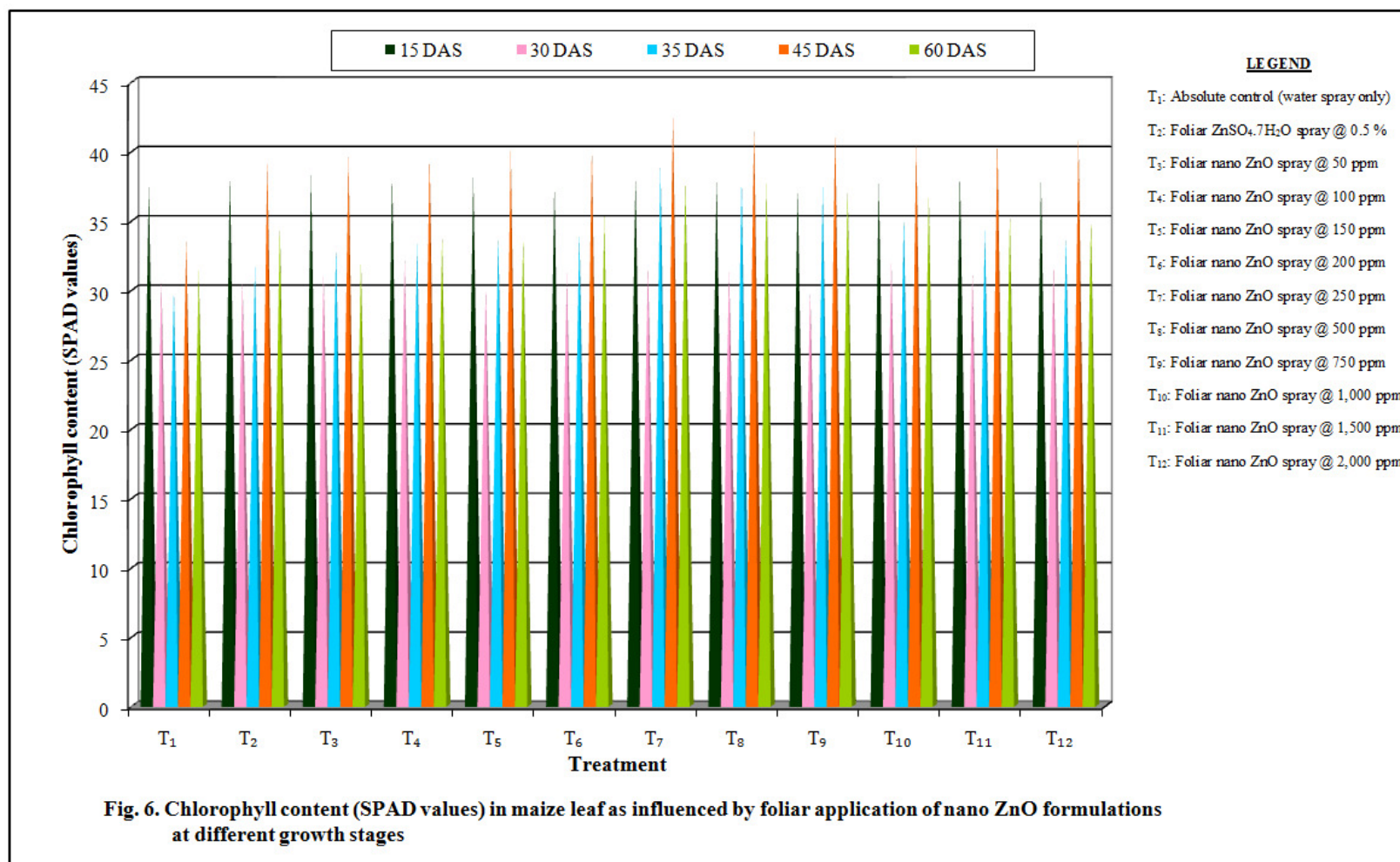


Fig. 6. Chlorophyll content (SPAD values) in maize leaf as influenced by foliar application of nano ZnO formulations at different growth stages

Up to 250 ppm of nano ZnO spray, potassium uptake increased and further increase in concentration uptake decreased due to the zinc toxicity. Conventional zinc sulphate spray resulted in uptake similar to nano spray @ 100 ppm (Fig. 8). All these observations indicated that nano ZnO spray at lower concentrations enhanced nutrient uptake than the conventional spray and nano sprays at higher concentrations (1,000, 1,500, 2,000 ppm). Similar findings were also reported by Sheykhbaglou *et al.* (2010) that among the five levels of nano-iron oxides (0, 0.25, 0.5, 0.75 and 1 g l⁻¹) sprays on soyabean, highest grain yield was obtained with 0.5 g l⁻¹ concentration and further increase in concentration reduced yield due to reduced uptake of iron. Harsinia *et al.* (2014) reported that application of nano iron oxide enhanced yield and quality of wheat plant compared to iron chelate and iron sulphate due to better uptake and assimilation of nutrients. Jahanara *et al.* (2013) reported that foliar application of iron nanocomposites enhanced nutrient uptake and dry matter accumulation in common bean. Nalwade and Neharkar (2013) reported that multi-walled carbon nanotubes increased dry matter accumulation in cotton seedlings by improving the growth and nutrients uptake by seedlings.

Similarly, for sulphur, maximum uptake was recorded with nano ZnO spray @ 250 ppm (42 % increase over conventional zinc sulphate spray), but at higher concentrations of nano spray, sulphur uptake reduced due to zinc toxicity which adversely affected the growth of plants (Fig. 8). Even though conventional zinc sulphate @ 0.5 per cent recorded higher sulphur concentration in plant tissue, uptake was low due to low dry matter production. In nano ZnO sprayed plants, comparatively higher dry matter production was recorded due to enhanced photosynthetic rate and enhanced accumulation of bio-molecules such as carbohydrates and proteins. Similarly, for nano treated plants uptake was high, but concentration in tissue was low due to the dilution effect. Taheri *et al.* (2015) reported that treatments with ZnO nanoparticles increased the shoot dry matter and leaf area indices of corn by 63.8 and 69.7 per cent, respectively over the control due to the better uptake and assimilation of nutrients.

Zinc concentration in the plant tissue showed significant differences (Fig. 9) due to different nano ZnO spray. Highest zinc content (179.67 ppm) was recorded with nano spray @ 2,000 ppm (T₁₂), which was toxic to plants as evident from toxic symptoms on plant growth. Similarly nano spray @ 1,500 (T₁₁) and 1,000 ppm (T₁₀) also recorded zinc concentrations in tissue at toxic level. Even though high zinc content (124.33 ppm) was recorded with zinc sulphate spray (T₂), maize plant did not exhibit any toxic symptoms which could be due to its lower uptake and assimilation by plants. But, higher concentrations of nano ZnO spray (@ 1,000, 1,500 and 2,000 ppm) resulted in severe toxicity symptoms in maize plants such as stunted growth, necrosis, wrinkling of leaves, unfurling of leaves, leaf tip turned to blunt and changed to white (Plate 10). Afshar *et al.* (2014) studied the effects of different spray concentrations of nano zinc oxide on wheat crop. Results revealed that nano-compounds are quickly absorbed by plants in large amounts and hence it can be toxic to plants and plant products when applied particularly at higher concentrations. The toxic effects of nanoparticles depend on its size, surface area ratio, morphology, nature, composition and reactivity (Zaka *et al.*, 2016).

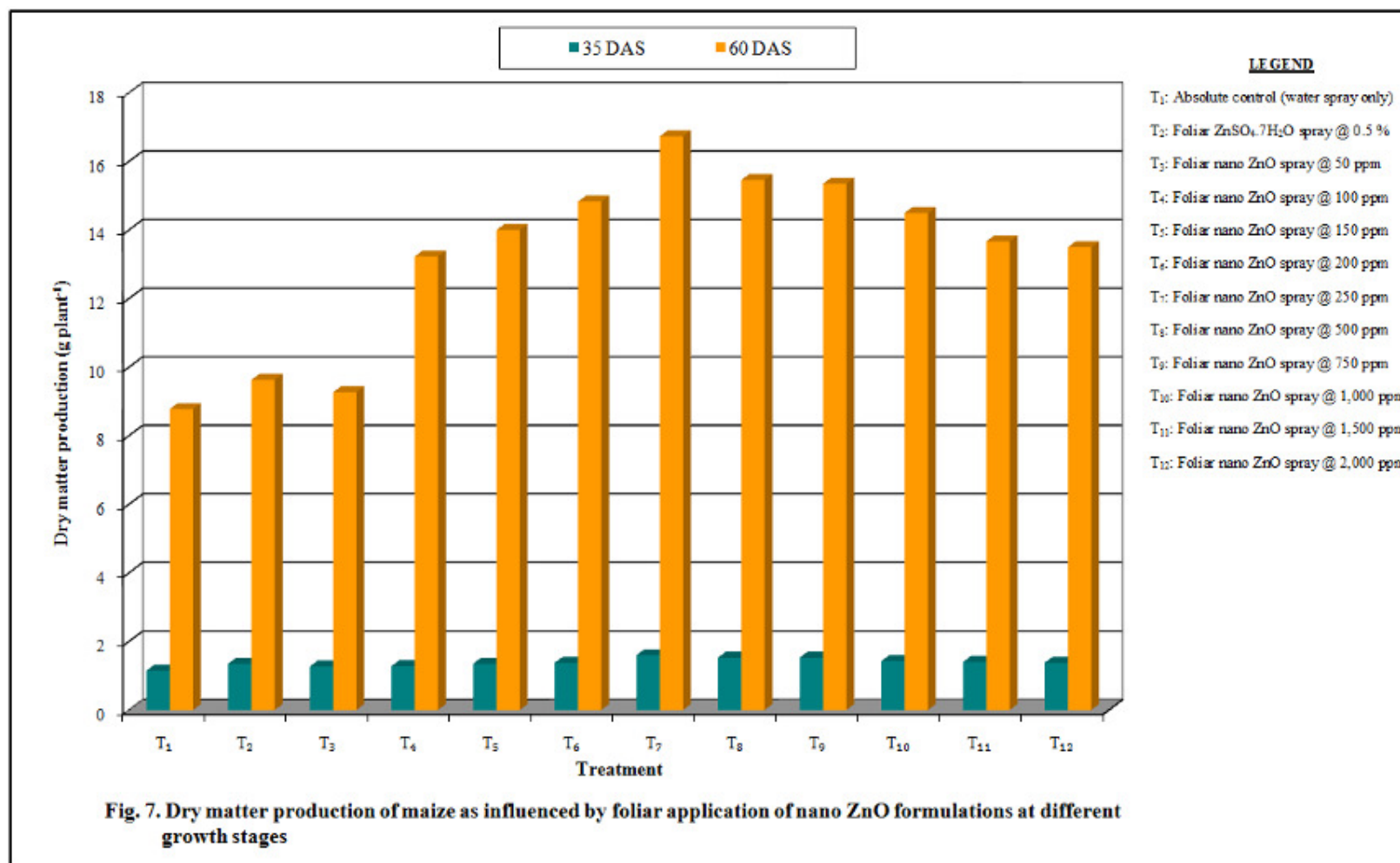


Fig. 7. Dry matter production of maize as influenced by foliar application of nano ZnO formulations at different growth stages

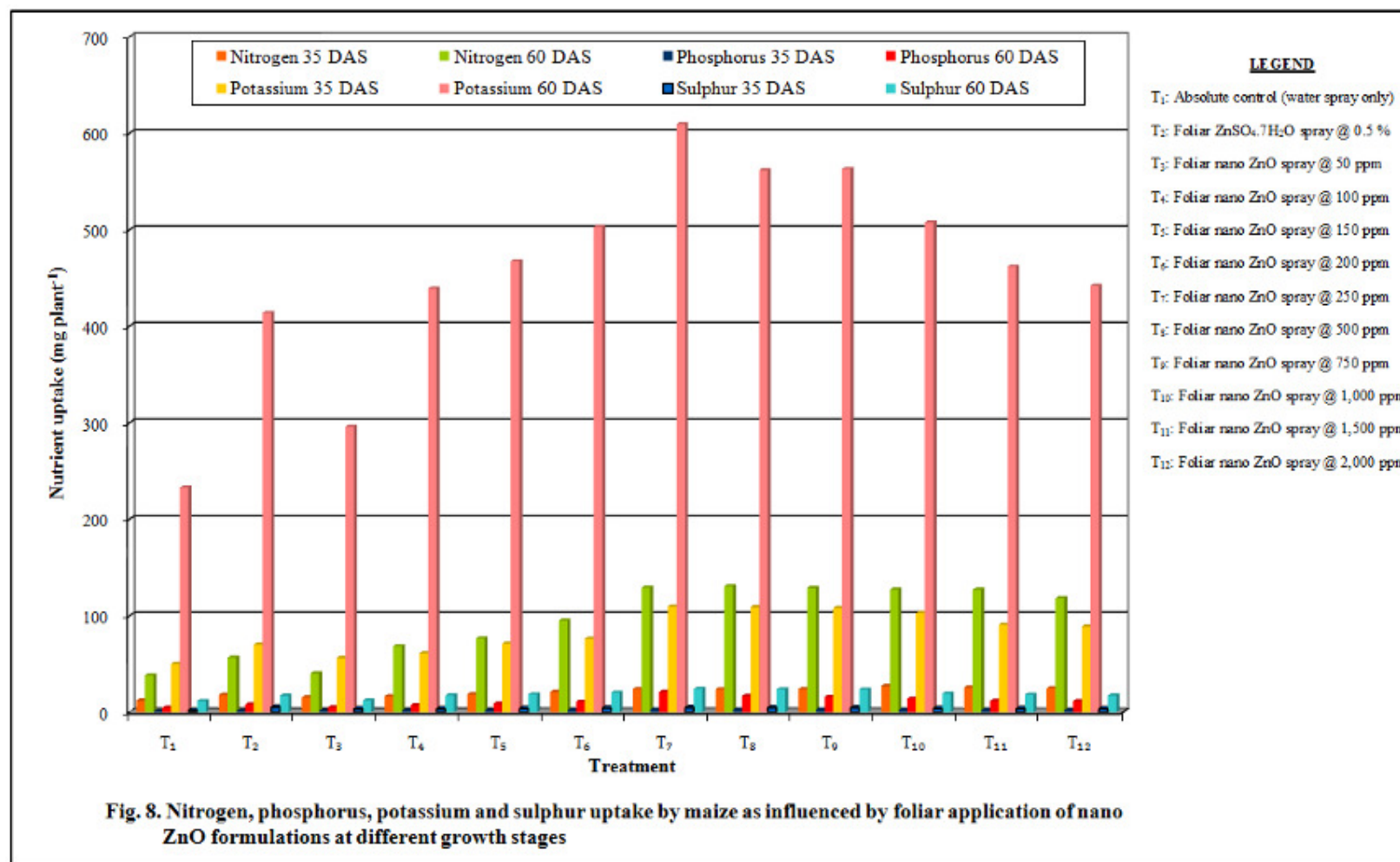


Fig. 8. Nitrogen, phosphorus, potassium and sulphur uptake by maize as influenced by foliar application of nano ZnO formulations at different growth stages

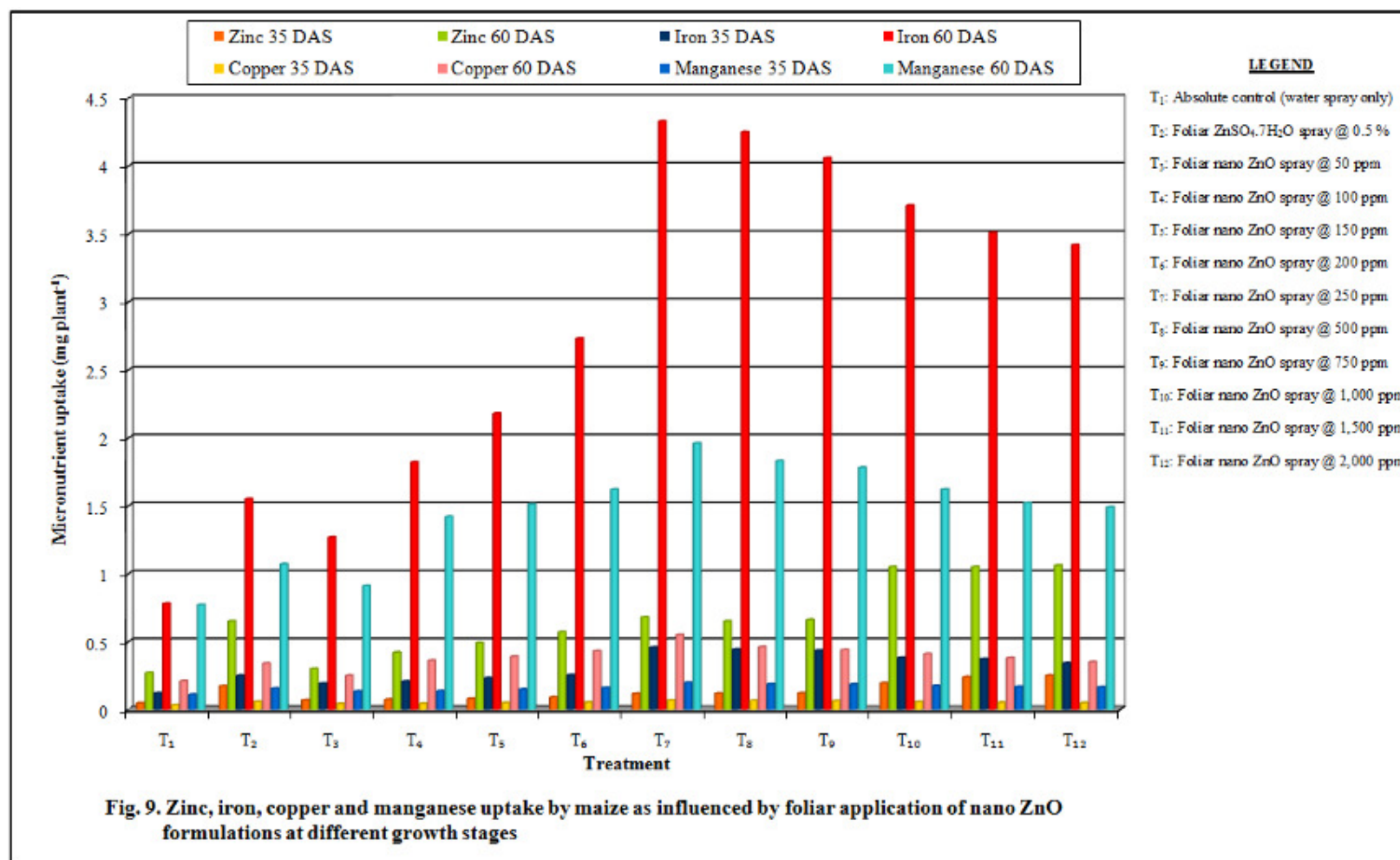


Fig. 9. Zinc, iron, copper and manganese uptake by maize as influenced by foliar application of nano ZnO formulations at different growth stages

At 60 DAS, the concentration of zinc in the tissue decreased due to dilution effect caused by dry matter accumulation and was at non-toxic level. The treatments which received lower concentrations of nano spray (@ 250, 500 and 750 ppm) produced healthy plants and recorded optimum zinc concentration in the tissue. At 60 DAS treatment T₁₂, T₁₁ and T₁₀ (nano @ 1,000, 1,500 and 2,000 ppm, respectively) recorded higher uptake which was followed by T₇, T₈ and T₉ (nano @ 250, 500 and 750 ppm, respectively).

Similarly for other micronutrients also, maximum uptake was recorded in treatment T₇ (Fig. 9), receiving nano spray @ 250 ppm due to the higher dry matter accumulation. Treatment T₈ and T₉ were found on par with T₇. Yuvakkumar *et al.* (2011) found more silica content in the maize leaves, when provided as nano than as bulk molecules, indicating that nanoparticles have better uptake by plants than its bulk molecules.

5.2.3 Zinc concentration in the index leaf of maize

Zinc content in the index leaf varied significantly at 35 and 60 DAS due to ZnO NPs. At 35 DAS maximum Zn content in index leaf was recorded in treatment T₁₂, followed by T₁₁, T₁₀ and T₂. At 60 DAS, also similar trend was followed, but zinc concentration in leaves lowered due to dilution effect and was at non-toxic level. According to Lin and Xing (2007), plants like corn, lettuce, radish, ryegrass and cucumber were sensitive to ZnO nanoparticles. The toxicity is due to internalization of NPs, their accumulation in root tissue and surface, dissolution of zinc ions from NPs along with other physico-chemical properties (Ma *et al.*, 2010). ZnO nanoparticles were supplied as foliar sprays resulted in Zn accumulation in leaves and the upper portion of plants due its intermediate mobility within plants. Thus, main processes like photosynthesis and other metabolic functions got interfered due to zinc toxicity than root inhibition. In the present study also toxicity symptoms were associated with leaves and shoot portions as already quoted under text 5.2.2.

5.2.4 Chemical properties of soil after the harvest of maize (60 DAS) as influenced by nano ZnO spray

Chemical properties such pH, EC and organic carbon content did not differ significantly among the treatments indicating that foliar application of nano ZnO had no effect on chemical properties of soil. Moshe *et al.* (2012) also reported that soil organic content and macro-properties of soil were not influenced by the application of nano metal oxide nanoparticles (CuO and Fe₃O₄).

5.2.5 Available macro and micro nutrient status of soil after the harvest of maize (60 DAS) as influenced by nano ZnO spray

Maximum availability of macronutrients such as nitrogen, phosphorus, potassium and sulphur (56.22, 19.13, 173.17 and 23.7 mg kg⁻¹ of soil, respectively) was recorded in absolute control (T₁) indicating less uptake of these nutrients from soil. For nitrogen, lowest availability status (36.08 mg kg⁻¹ of soil) was recorded in the treatment T₈ whereas phosphorus, potassium and sulphur (14.65, 149.67 and 17.13 mg kg⁻¹ of soil, respectively) status was low in treatment T₇ (nano spray @ 250 ppm) indicating maximum uptake of these nutrients from the soil due to the greater dry matter production.



Plate 9a. Comparison between conventional zinc sulphate spray Vs nano ZnO spray

Plate 9a. Comparison between conventional zinc sulphate spray Vs nano ZnO spray



Plate 9b. Growth of maize plant as influenced by conventional zinc sulphate spray Vs nano ZnO formulations at different concentrations

Plate 9b. Growth of maize plant as influenced by conventional zinc spray Vs nano ZnO formulations at different concentrations

Plate 9. Pot culture experiment at 35 DAS



Plate 10a. Necrosis



Plate 10b. Wrinkling of leaves



Plate 10c. Leaf tip turned to blunt and white



Plate 10d. Unfurling of leaves



Plate 10. Toxicity symptoms in maize plants treated with nano ZnO formulations at 1,000, 1,500 and 2,000 ppm concentrations



Plate 11a. Plants treated with nano ZnO formulations at higher concentrations (1000, 1500 and 2000 ppm)



Plate 11a. Plants treated with nano ZnO formulations at higher concentrations (250, 500 and 750 ppm)



Plate 11c. Comparison between conventional $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and nano ZnO spray

Plate 11. Pot culture experiment at 60 DAS

Maximum post harvest available micronutrients (Zn, Fe, Cu and Mn) status was reported in absolute control (0.52, 2.08, 0.35 and 2.22 mg kg⁻¹ of soil, respectively) and lower status in treatment T₇ which received nano spray @ 250 ppm (0.46, 1.75, 0.32 and 2.04 mg kg⁻¹ of soil, respectively). Nano ZnO spray indirectly affected the post harvest nutrient status of the soil by increasing the uptake of these nutrients from soil resulting in their low available status particularly in the treatments T₇, T₈ and T₉ (nano @ 250, 500 and 750 ppm). Janmohammadi *et al.* (2016) studied the effects of foliar spray of micronutrient nano-fertilizer (iron and zinc) and nano-titanium dioxide (nTiO₂) solution on grain yield of barley plants and reported that foliar application of nanofertilizers, improved nutrient uptake and resulted in high grain yield and harvest index. Valadkhan *et al.* (2015) reported that improvement in the yield components was due to the enhanced photosynthetic and other metabolic activities, which resulted in the production of more dry matter and greater nutrient uptake. Hussien *et al.* (2015) reported that application of nano-P improved the nutrients uptake under stress conditions as well as regular irrigation. Application of nano-P @ 500 and 1,000 ppm promoted uptake of macro (N, K, P, Ca and Mg) and micronutrients (Zn, Fe, Cu and Mn) by cotton. Almost identical results were obtained in the present study. Thus results of present study corroborate the findings of earlier studies.

Practical utility of the results

1. The results of present investigation indicated that maximum growth and development of maize can be obtained through the foliar application of green synthesized ZnO NPs particularly at a lower concentration of 250 ppm.
2. Zinc nutrition of crop plants is getting importance due to its deficiency in Indian soils, especially in alkaline and calcareous soils. So, foliar spray of ZnO NPs at a lower concentration can be recommended for maize crop to enhance its growth and productivity.
3. Nanofertilizers can serve as an efficient nutrient delivery system which reduces the quantity of nutrient required and increase the effectiveness of applied nutrients as well as nutrients in the labile pool.

Future line of work

1. Studies need to be conducted to enhance purity of green synthesized ZnO NPs by adding NaOH as it provides sufficient number of functional OH groups.
2. Based on the nature and type of leaf extract, calcination time needs to be standardized to reduce carbon content.
3. The thorough penetration mechanism of the nanoparticles inside the plants and their transformation in the plant cells need to be understood.
4. Study on response of other crops to green synthesized ZnO NPs need to be conducted.

6. SUMMARY AND CONCLUSIONS

A laboratory experiment was conducted to green synthesize ZnO nanoparticles using different plant extracts followed by green house experiment to study the effect of green synthesized ZnO nanoparticles on maize plant in a calcareous Vertisol.

6.1 Lab experiment-I

- ZnO nanoparticles (ZnO NPs) were green synthesized using the leaf extracts of five different plants (calotropis, tea, neem, tulasi and datura) from analytical grade reagent zinc nitrate as precursor (bulk molecule).
- The preliminary confirmation of green synthesized ZnO NPs was done with UV-spectrophotometer, using the principle that the reduction of bulk molecules in to nanoscale size, its absorbance peak in dispersed aqueous solution shifted from higher wavelength to UV- range (200-375 nm) and nano ZnO particles exhibited absorbance peaks between 340-365 nm.
- ZnO NPs synthesized from calotropis exhibited absorbtion peak at 345 nm, tea at 355 nm, neem at 359 nm, tulasi at 363 nm and datura at 368 nm. This confirmed the presence of nano ZnO particles in the green synthesized materials.
- Green synthesized ZnO NPs were characterized for their size, shape and chemical composition by different analytical methods such as particle size analyzer (PSA), scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDAX).
- The average size of ZnO NPs recorded with PSA was 79.6 nm for calotropis, 99.6 nm for tea and neem, tulasi and datura recorded 101.6, 122.4 and 497.4 nm, respectively.
- Using scanning electron microscopy (SEM), size and shape of green synthesized ZnO particles were recorded. Calotropis synthesized nanoparticles recorded the lowest size ranging from 45-75 nm and that of tea, neem, tulasi and datura synthesized NPs were in the range of 45-90, 100-300, 70-500 and 1,000-2,000 nm, respectively.
- On the other hand, the shape of green synthesized ZnO NPs was rod like for calotropis and tea. In case of neem, tulasi and datura, synthesized particles appeared as flakes-like, ovoid and spherical shape, respectively.
- The chemical composition of green synthesized ZnO NPs was determined with EDAX and it was observed that synthesized particles were not so pure, but contaminated with other elements present in the leaf extracts. The main contaminant was carbon which constituted a major part of green synthesized NPs while other elements were in traces.

- ZnO NPs synthesized from calotropis leaf extract contained 28.13 and 26.41 per cent by weight as zinc and oxygen, respectively and that of tea synthesized particles had 5.07 and 58.65 per cent zinc and oxygen, respectively. Remaining per cent of weight were occupied by other foreign elements present in the leaf extracts.
- Datura, tulasi and neem synthesized ZnO NPs had a high per cent of carbon content (79.98, 72.27 and 67.82 per cent, respectively) and that of zinc content was low (0.53, 0.65 and 4.14 per cent, respectively).
- ZnO NPs obtained with calotropis recorded maximum purity and minimum purity was with datura synthesized particles.

6.2 Lab experiment-II

- In the pot culture experiment, it was found that the growth as well as nutrients uptake of maize plant were influenced by the nano ZnO spray given at 30 DAS. The growth parameters such as plant height, leaf area, chlorophyll content and dry matter production significantly varied among the treatments at 35 and 60 DAS due to ZnO NPs spray.
- The highest plant height (17.5, 25.17 and 35 cm), leaf area (117.3, 185.75, 276.29 cm²), chlorophyll content (38.87, 42.67 and 37.73) were recorded at 35, 45 and 60 DAS and dry matter production (1.6 and 16.73 g plant⁻¹ at 35 and 60 DAS, respectively) was recorded in treatment T₇ which received nano ZnO spray @ 250 ppm.
- Nitrogen uptake by maize at 60 DAS was maximum in treatment T₈ (nano @ 500 ppm) and for other macro nutrients such as phosphorus, potassium and sulphur, maximum uptake was recorded with nano ZnO spray @ 250 ppm (21.12, 609.29 and 24.22 mg plant⁻¹, respectively).
- Maximum uptake of micronutrients such as Fe, Cu and Mn was recorded maximum with nano spray @ 250 ppm (4.33, 0.67 and 1.96 mg plant⁻¹, respectively) while the highest zinc uptake was recorded with nano spray @ 2,000 ppm.
- Conventional zinc sulphate spray @ 0.5 per cent, recorded growth attributes and uptake on par with lower concentrations of nano sprays (@ 50, 100 and 150 ppm).
- At higher concentrations of nano spray such as 1,000, 1,500 and 2,000 ppm (T₁₀, T₁₁ and T₁₂, respectively), plant growth parameter as well as uptake reduced compared to lower concentrations of nano sprays such as 250, 500 and 750 ppm (T₇, T₈ and T₉, respectively) due to zinc toxicity and its detrimental effects on maize plant.
- Soil chemical properties such as pH, EC and organic carbon content after harvest of maize were not influenced by nano ZnO spray.
- Maximum post harvest macro and micro nutrients status of soil was recorded in absolute control indicating minimum uptake of these nutrients by crop plants and minimum status recorded for nano spray @ 250 ppm indicating maximum uptake of these nutrients by maize from the soil.

Conclusions

- ZnO nanoparticles can be green synthesized from analytical grade reagent zinc nitrate using different plants such as calotropis, tea, neem, tulasi and datura. The bio-molecules present in the leaf extract act as reducing, capping and stabilizing agent and convert bulk molecules into its nano-scale size particles.
- Among the five leaf extracts, calotropis and tea synthesized nanoparticles with size range less than 100 nm, while that of neem and tulasi were less than 500 nm. Datura synthesized ZnO nanoparticles were at 1,000-2,000 nm range.
- Results on pot culture experiment revealed that foliar application of nano ZnO at lower concentration of 250 ppm (T₇) was very effective in enhancing growth as well as nutrients uptake of maize plants than the conventional zinc sulphate spray @ 0.5 per cent. Nano ZnO spray @ 500 ppm (T₈) and 750 ppm (T₉) were on par with nano ZnO spray @ 250 ppm (T₇).
- Nano ZnO spray at higher concentrations (@ 1,000, 1,500 and 2,000 ppm) reduced growth and nutrients uptake of maize plants compared to treatments receiving nano sprays @ 250, 500 and 750 ppm due to the zinc toxicity and its detrimental effects on plant growth.

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RESPONSE OF MAIZE (*Zea mays* L.) TO GREEN SYNTHESIZED ZnO NANOPARTICLES IN A CALCAREOUS VERTISOL

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ABSTRACT

A laboratory study was conducted at the Department of Soil Science and Agricultural Chemistry, UAS, Dharwad during 2016-17 to green synthesize zinc oxide nanoparticles (ZnO NPs) from analytical grade zinc nitrate using five different plant leaf extracts viz., calotropis, tea, neem, tulasi and datura. The green synthesized ZnO NPs were characterized for their size and shape. In UV-spectroscopy, absorbance peaks of particles ranged from 345-370 nm confirming their size in nano range. In particle size analyzer and scanning electron microscopy calotropis leaf extract synthesized nanoparticles recorded smallest size (79.6 nm) and datura the highest (497.4 nm). The shape ZnO NPs varied from rod to spherical. The chemical composition of green synthesized ZnO NPs was determined with energy dispersive atomic X-ray analysis which revealed that synthesized particles were not pure, but contains other elements from leaf extracts.

A pot culture experiment was conducted with 12 treatment combinations and 60 days duration to study the response of maize to green synthesized ZnO NPs. The study revealed that foliar application of nano ZnO at lower concentrations of 250, 500 and 750 ppm (T_7 , T_8 and T_9 , respectively) were very effective in enhancing growth and nutrients uptake by maize plants than the conventional zinc sulphate spray @ 0.5 per cent. The growth parameters such as plant height, leaf area, chlorophyll content and dry matter production recorded maximum for treatment which received nano ZnO spray @ 250 ppm. Treatments T_8 and T_9 were on par with T_7 . Nano ZnO spray at higher concentrations (@ 1,000, 1,500 and 2,000 ppm) reduced growth and nutrients uptake of maize compared to lower concentrations (250, 500 and 750 ppm) due to zinc toxicity.