IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)
An International Open Access, Peer-reviewed, Refereed Journal

Effect of ZnO nanoparticles based fertilizer on the growth parameters of maize and wheat plants

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Abstract

Zinc oxide nanoparticles (ZnO NPs) possess remarkable physical, optical, and antibacterial properties that have great potential for improving agriculture. The purpose of the present investigation is to enhance fertilizer response of ZnO NPs. These nanoparticles are synthesized using co-precipitation method to study its physicochemical properties using X-ray diffraction, field emission scanning electron microscopy, X-ray photoelectron microscopy, etc. The synthesized ZnO sample shows hexagonal wurtzite type crystal structure. The FESEM images show the particle-flake like mixed morphology with average grain size of about 30 to 35 nm. Herein, we report effect of fertilizer response of ZnO NPs on Maize and Wheat plant. The plants were sprayed 4 times at the interval of 7 days with different concentrations of ZnO NPs. The growth parameters like seed germination, root and shoot length, height of plant, width of root and stem, number of leaves and dimensions of leaves were studied with respect to control. The outcome of this study is promising with average enhancement in the growth properties of plants with ZnO NPs as fertilizers of about 20% compared to control.

Keywords: Zinc oxide, Nanotechnology, Fertilizer, Plant growth

Introduction

The food production and its supply have been emerged as the major issues before the world which has been raised due to increasing population consequently resulting in ever-growing demand for food. Moreover, the crop production and food demand were adversely affected by the relentless conflict, global warming and weather extremes. The issue pertaining to food production can be tackled only with increase in crop productivity which can be enhanced by improving the quality of soil, rotation of soil, seeds, early planting and planting practices, appropriate water drainage, weeding and use of fertilizers with advanced technology. The fertilizers help to keep the essential nutrients in the soil for the healthy growth of the crops. It not only promotes the growth of crop but enhances the productivity by manifolds. However, the use of accustomed fertilizers does not necessarily result in the proper crop production. The conventional fertilizers have a long history and generations starting from manure to chemical products that have headed to negative impacts on the soil, eco and aquatic systems along with an enormous waste of implanted material and energy resources. For example, the chemical fertilizers get washed away by water and suffer from leaching that cause soil and water pollution. Moreover, the excessive use of chemical fertilizers harms the microbes that affect soil and probably results in changing the nature of soil to make it more acidic or basic. Similarly, the cost of farming increases due to such low efficient chemical fertilizers [1]. In order to turn towards more efficient and cost-effective technologies, instead of traditional crop production methods, which will increases production value, boost farmer earning, and create value chains that benefit rural communities and promote pollution-free environments, the nanotechnology will emerge as a new hope.

The nanotechnology will optimistically reduce the cost of farming; diminish the chemical wastage and efforts, and will prevent environmental damage. The use of the nano-sized fertilizers is attracting an attention of not only researchers but also of the end users or farmers as it can improve the nutrient use efficiency, plant productivity along with a reduction in the environmental issue. Technically, the nano-fertilizers have superior ability of transport and delivery of nutrients to the crop via plasmodesmata owing to their nanoscale size in the range of 10 to 50 nm, comparable to the channels between the cells [2]. The nano-fertilizers may contain the Nitrogen, Phosphorus, Potassium, Calcium, etc. as macro or zinc, boron, iron, copper as micronutrients. Among the various micronutrients, the zinc (Zn) plays an important role for the protein synthesis, membrane integrity, water uptake, plants growth, and the development in plant tissues [3-5]. Additionally, it can drive metabolic reactions in the plants and deficiency of Zn can lead to reduction in the formation of chlorophyll, carbohydrates. However, the Zn is fairly reactive metal which easily reacts with oxygen to form the zinc oxide (ZnO). Therefore, it is mostly delivered either in the form of zinc-oxides (ZnO) or zinc-sulphates ($ZnSO_4 \cdot H_2O/ZnSO_4 \cdot 7H_2O$. The ZnO is also essential micronutrients for the crop production. Interestingly, the oxides of zinc are more suitable than its salt form as Zn in the form of salt is more soluble in water; so have the chance of more leach out and sulphide form is relatively complex compared to its oxide counterpart [6]. In general, the application of ZnO as fertilizers is an effective and safe approach for overcoming Zn deficiency [7]. ZnO is absorbed by plants in the form of divalent cations (Zn^{2+}) and it also showed an enhanced antimicrobial activity against bacterial and fungal species [8]. Meanwhile, the ZnO is an inorganic compound and non-toxic material present in earth's crust as a mineral zincite. It has a distinctive electronic and photonic wurtzite n-type semiconducting property with a wide direct band gap and a high exciton binding energy [9-10]. It is multifunctional material with unique physical and chemical properties, such as high chemical stability [11-12]. ZnO nanoparticles were synthesized by different techniques such as vapour-liquid-solid (VLS) [13], electrochemical deposition [14], hydrothermal growth approach [15], mechano-chemical method [16], chemical precipitation method [17], sol-gel [18], etc.

In the present work, we have synthesized ZnO nanoparticals via cost effective co-precipitation method. This method offers a high yield of the nanoparticles. The physico-chemical characterization of the synthesized ZnO was studied using the appropriate analysis techniques and the outcome shows the phase pure nanostructured zinc oxides powder. Further, we have studied the effect of ZnO nanoparticles on the seed germination, chlorophyll content, shoot and root length and growth of the plant. Here in this work, we selected two major crops as wheat and maize because both maize (Zea mays) or corn and wheat (Triticum) are the most staple food across the globe. Both wheat and maize along with the rice provide foods over half of the population of the world. Meanwhile, the wheat is one of major crop for about 5 billion people in the world. The examination of the literature reveals that the sufficient amount of zinc in maize or wheat crop is beneficial for the pollen viability and is a carbohydrate source [19]. Therefore, we have applied ZnO NPs based fertilizers at regular interval of the time and the growth parameter of the plants was studied.

Methods and Material:

The chemicals such as zinc acetate [$Zn(CH_3COO)_2.2H_2O$], sodium hydroxide (NaOH) were purchased from S. D. Fine Chemicals, India and used as received. A co-precipitation method was employed to prepare ZnO nanoparticles, which has been inspired by the report found in the literature [20]. In brief, certain molar amount of zinc acetate was prepared in 100 ml of deionized water and the beaker containing this solution was placed on the magnetic stirrer. Then, 0.4 M of NaOH prepared in 100 ml deionized water separately and were added drop-wise in the zinc acetate solution. Here, the sodium hydroxide is used as a reducing agent. The reaction mixture was stirred for 2 h at room temperature. Initially, the solution was clear and dense then as a function of the reaction time, a white precipitates began to appear. After completion of the reaction time, the product was washed thoroughly and the settled powder was collected and dried in a hot air oven at 60 °C for 5 h to get ZnO powder. The final product was annealed at 300 °C for 1 h to form the ZnO phase. During the experimental procedure, we have varied the Zn precursors (zinc acetate) concentrations as 0.1, 0.2, 0.3 and 0.4 M and the prepared product was denoted as ZnO-1, ZnO-2, ZnO-3 and ZnO-4, respectively.

The morphology of the ZnO nanoparticles was analysed using the JEOL-JSM 6360 scanning electron microscope (SEM) operating at 5 keV and structural characterization of as synthesized ZnO was performed *via* a X-ray diffraction (XRD) using a Rigaku Miniflex XRD with a $\lambda_{Cu\ K\alpha} = 1.54$ Å with a scan rate of 5° min^{-1}. X-ray photoelectron spectroscopy (XPS) experiments were performed using Thermo Scientific K-Alpha (Al Kα source) surface analysis equipment.

Results and Discussion

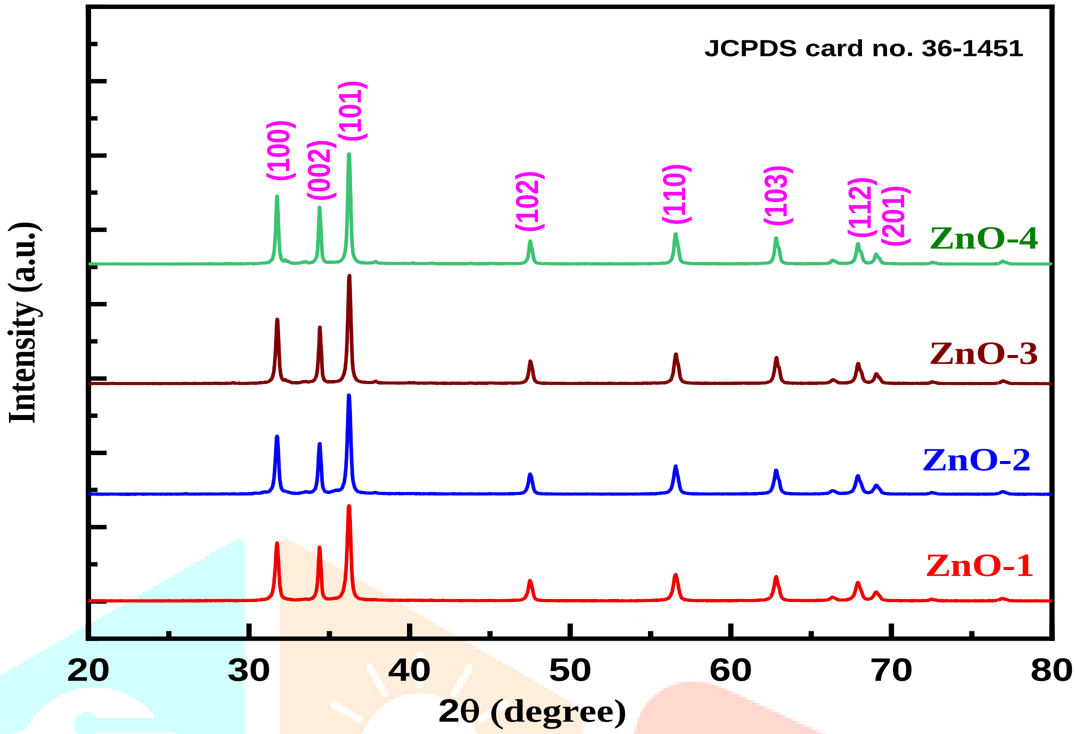


Fig. 1: XRD pattern of ZnO NPs synthesized at different Zn precursor concentrations

The structural analysis of synthesized ZnO NPs is studied by using XRD spectra. Fig. 1 represents the XRD spectra of synthesized ZnO NPs with different Zn precursor concentrations and annealed at temperature of about 300^{o}C. The diffraction pattern of all ZnO NPs illustrates three distinct diffraction peaks located at angle of 31.76, 34.42, and 36.24°; and can be indexed with the planes (100), (002), and (101) of the hexagonal wurtzite type structure of the ZnO (JCPDS card No. 36–1451) [21]. The higher peak intensity of the diffraction peaks assigned to (101) plane suggests the particles-like nature of the prepared product [22]. Meanwhile, no other peaks were detected indicating a high purity level of the synthesized ZnO NPs [23]. The average crystallite size of the NPs was determined by using following Debye–Scherrer formula.

$$D = \frac{0.9\,\lambda}{\beta\ Cos\ \Theta} \qquad (1)$$

In the formula, 'D' is average crystalline size of the particles, ' λ' is a wavelength of Cu-Kα radiation (λ = 1.5406A^{o}) , 'β' is a FWHM (full width at half maximum) of the diffraction peak and 'Θ' is a diffraction angle. The average crystallite size of all ZnO NPs samples is given in Table 1. The crystalline size of all ZnO NPs is almost similar which shows that the concentration of Zn precursor within the experimental limit, and does not significantly influence the structural properties of the ZnO NPS.

Table 1: Crystallite size of ZnO sample for different concentration

Sample	Crystallite size (D) nm
ZnO - 1	32.45
ZnO - 2	36.37
ZnO - 3	33.47
ZnO -4	37.45

The identification of elements present in the ZnO NPs and their chemical composition along with the bond configuration is studied with the help of X – ray photoelectron spectroscopy (XPS) technique. A typical survey spectrum of synthesized ZnO NPs (sample ZnO-2) is shown in Fig. 2 (a). The survey spectrum of ZnO NPs shows the presence of 'Zn' and 'O' peaks along with the peaks assigned to the carbon (C). As the carbon is present in the atmosphere, its nano-level layer might be deposited on ZnO sample during synthesis or XPS analysis. Meanwhile, Fig. 2 (b) shows core level spectra Zn elements. It exhibits double peaks i.e. $Zn2p_{3/2}$ and $Zn2p_{1/2}$ at binding energies of 1021 and 1044.10 eV. The separation between spin orbital coupling of $Zn2p_{3/2}$ and $Zn2p_{1/2}$ is found to be 23.10 eV which is in good agreement with the literature [24]. Fig. 2 (c) represents 'O1s' peaks of pure ZnO nanorods. The oxygen peak of ZnO sample shows two prominent peaks corresponding to the binding energies of 529.5 and 531.2 eV. The 'O1s' peak appears at 529.5 eV indicating the presence of O_2^- in the Zn-O bond of core level ZnO crystal structure and the shallow peak appears at 531.2 eV is corresponds to the O^{2-} from the wurtzite crystal structure of the ZnO lattice [25].

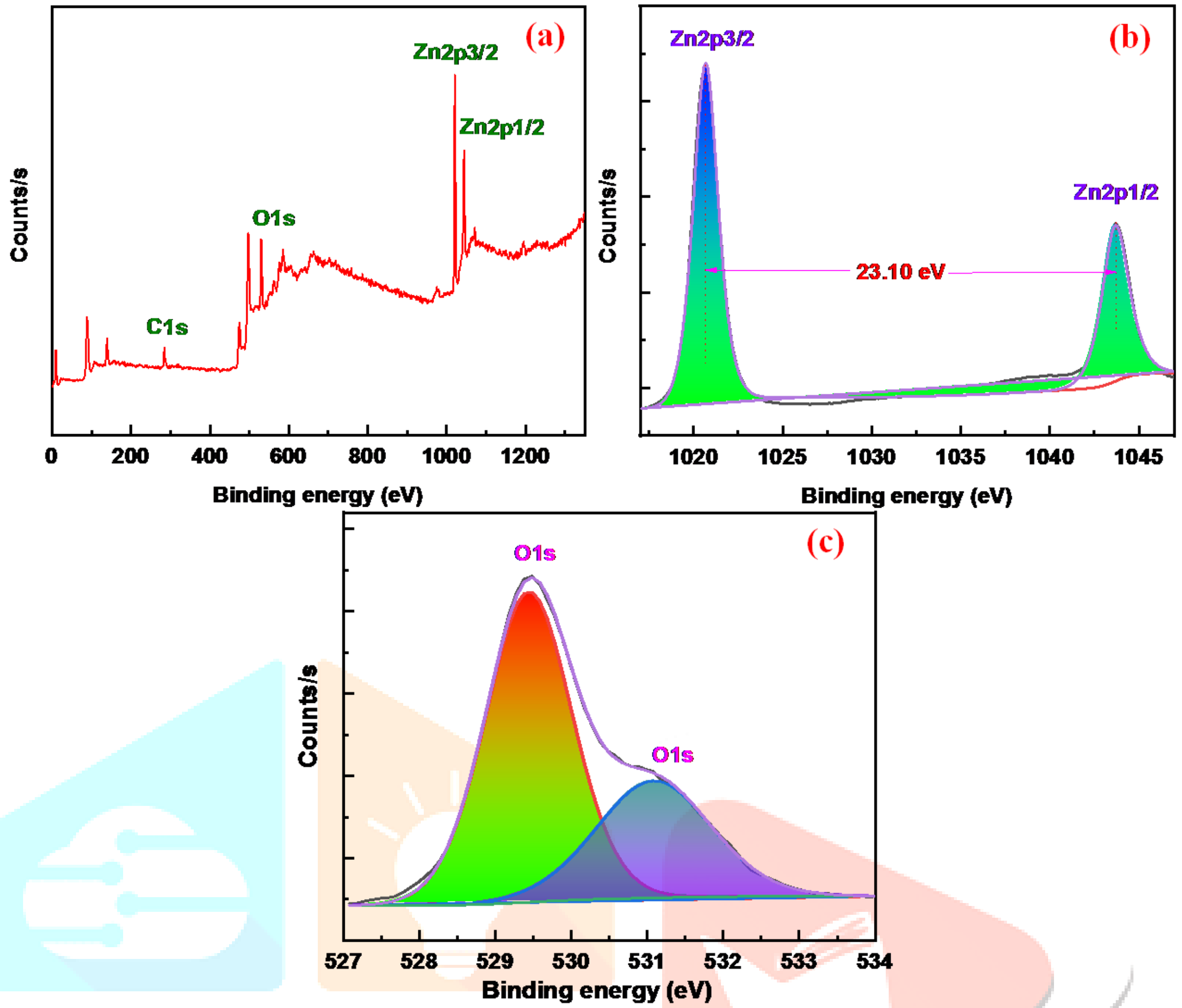


Fig. 2: XPS spectrum of ZnO NPs (a) Full spectrum of ZnO NPs (b) Zn spectrum of ZnO NPs and (c) O spectrum of ZnO NPs

The scanning electron microscopy is used to study the surface morphology of the prepared powder and to calculate its size. The corresponding SEM images of all ZnO powder samples are presented in Fig. 3. The SEM images show the nanostructured agglomerated particles of synthesized ZnO. The grain size ranging from 30 to 35 nm is observed for the sample ZnO-1 to ZnO-4 which is less than the size of plasmodesmata; therefore, absorption of the ZnO NPs will be easy between the channels cells. However, the particles are non-uniformly distributed, but the overall size of ZnO is in the nanoscale regime. Moreover, the ZnO NPs reveals the porous morphology which offers a higher surface area. The nanostrctured particles with higher surface area will be beneficial for the contact and ease in the absorption of NPs by the plant root or leaves. BET analysis is used in order to find the surface area of the ZnO NPs which found to be 11.4, 9.4, 5.6 and 4.4 m^2/g .The BET graphs are shown in Fig. 3 (inset).

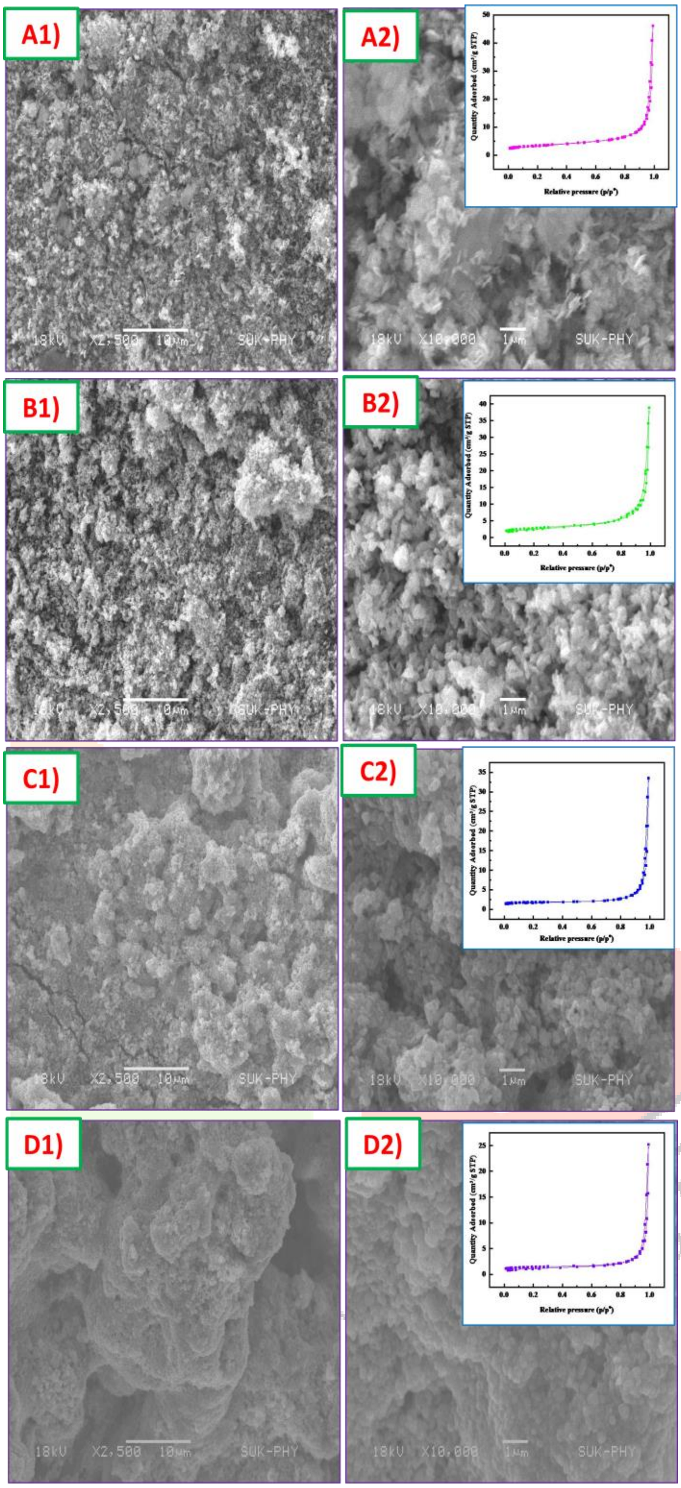
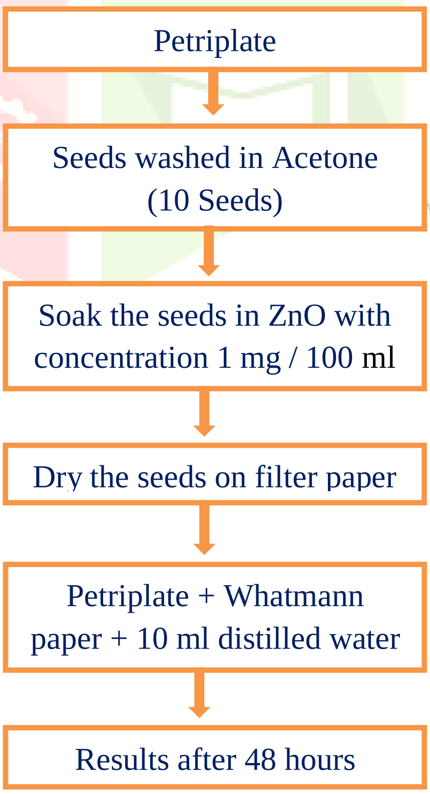


Fig 3: FESEM images of ZnO NPs samples with low (1) and high (2) magnifications of (A1 and A2): ZnO-1, (B1 and B2) ZnO-2, (C1 and C2) ZnO-3 and (D1 and D2) ZnO-4. Inset of Fig. 3 shows the BET analysis of ZnO NPs samples

Overall, the XRD and XPS analysis confirm the presence of zinc and oxide in the prepared product with desirable concentrations, and the FESEM images proved the nanoscale size of the ZnO. Therefore, the ZnO NPs is used to the various plants such as wheat and maize as a fertilizer. It is well known that the fertilizers, whether in bulk or nano-size form, are primarily used as nutrients to promote growth and improve production as well as yield of the crops. Like conventional fertilizers, nanofertilizers can be applied directly to the soil and *via* foliage. Owing to the small size of the ZnO particles, a foliar type of applications are used to the selected crop as it can penetrate into the pores of the leaves. Here, the prepared ZnO NPs are readily soluble in the soil, which are also helpful for its foliar type of applications. Before the application of ZnO NPs to the selected plants, the growth pot is filled with nearly 5 kg of black soil which consists of silicates of metal. A black soil is used as it has unique qualities and concentration of mineral and nutrients. The seeds of maize (Zea mays) are sown 2 cm depth in the pots. For foliar application of nanoparticles, four treatments are carried out respectively with plant control (without applying ZnO NPs) and plant with ZnO NPs as fertilizer. The concentration of ZnO nano-fertilizer is optimized as 1 mg/100 ml (dose 1), 5 mg/100 ml (dose 2), 10 mg/100 ml (dose 3) and 15 mg/100 ml (dose 4) for the period of seven days. Meanwhile, the stock solution of nano-fertilizer is made in 100 ml distilled water and is sonicated for 1 hr each time before the application. The foliar applications are carried out after 1 week of germination. The four dosages are given especially in the morning at the interval of 7 days. The impact of ZnO NPs on maize and wheat with respect to different parameters such as seed germination, root and shoot length, height of plant, width of root and stem, number of leaves and dimensions of leaves as a function of fertilizer dosage is studied.

The first step for the plant is the seed germination; therefore, the ZnO based fertilizer is applied at the stage of the germination. The seed of wheat and maize is washed thoroughly with water followed by acetone to avoid germination inhibitors. Then the seeds are put in a solution containing 1 mg of ZnO NPs in 100 ml of distilled water to soak the ZnO and after 1 day the seeds are taken out, dried using filter paper and kept in the air drying for the period of 48 hrs. Table 2 shows the Protocol followed for the seed germination of maize and wheat plant.

Table 2: Protocol/flowchart representing the seed- germination of maize and wheat



The ZnO NPs based fertilizer is applied to the maize plants. The seed of maize is treated with the nanoparticles before sowing; and the outcomes of the corresponding germination of the seed with and without ZnO NPs are presented in Table 3. The ZnO NPs treated seeds show a significant germination as compared to the control. About 80% seed germination is observed for the ZnO NPs treated maize seeds. After the germination, the maize seeds are planted in a pot containing black soil. After each dose of nanoparticles, the plants show significant improvements in the morphological characteristics compared to the control (Fig.4).

Fig. 4: Response of different concentration of ZnO nano fertilizer on growth of maize plant with respect to control after (a) first dose, (b) second dose, (c) third dose and (d) fourth dose. LHS are the plants without ZnO NPs based fertilizers while RHS with fertilizers

The physical and morphological parameters are enhanced with the application of ZnO NPs based fertilizer. Table 4 provides the statistical data of the ZnO NPs treated as well as control maize plants. After careful observation of the Table 4, it can be concluded that the growth of maize plant with ZnO NPs fertilizer is superior to the control; and the growth parameters continue to enhance after every dosage of nanoparticles.

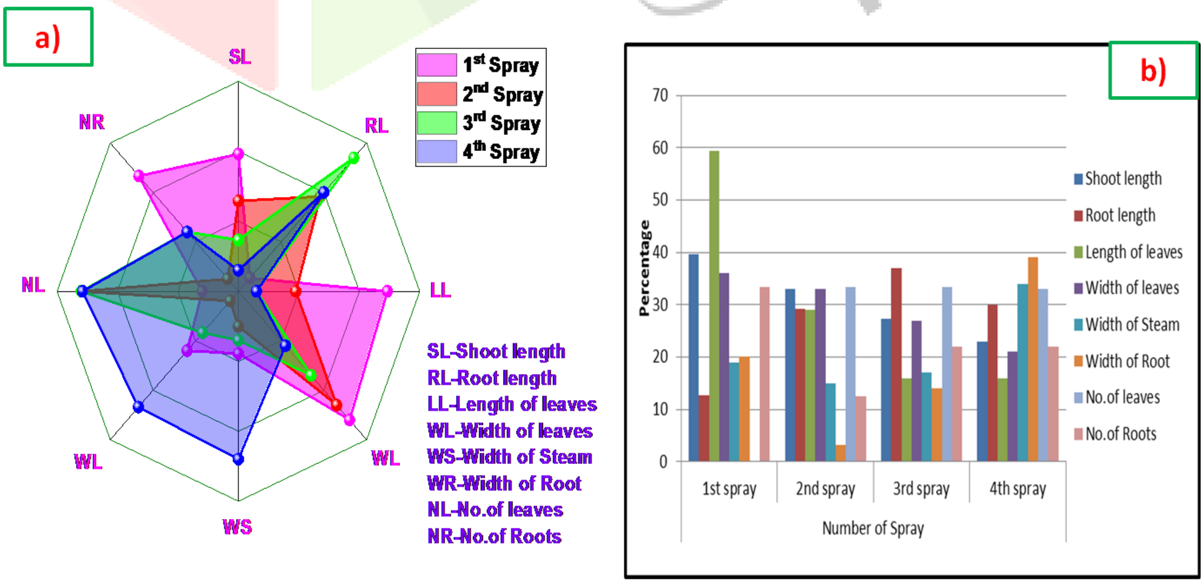


Fig. 5: Impact of sprays of different concentration on different characters of maize Plant (a) radar graph showing the improvement in the growth parameter of maize plant as a function of ZnO NPs dose and (b) the percentage improvement in physical and morphological parameters with respect to spray duration

Fig. 5 represents the impact of sprays of different concentration on characters of maize plants. Interestingly, the length of leaves of nanoparticle treated maize plant is prominent during the first week of dose. About 60% enrichment in the leave length is observed after applying first dose of nanoparticle based fertilizer. However, such growth appears to be a stagnation stage in the order of 20 to 30% during second to fourth dosage of ZnO NPs based fertilizer. After the application of first dose of ZnO NPs based fertilizers, not only lengths of the plants' leaves but shoot length, width of leave as well as stem and root is improved compared to control. Yet it is also noted that the root length of the plants is not developed after first dose of nanofertilizer which can be the result of early stage of growth of the maize plant. Yet, with the increment in the dosage of fertilizers, the length of root was drastically enhanced. The statistical data (Table 4 and Fig. 4 (b)) clearly shows that the development of root length from about 10 % to around 40% is observed in the time interval of 1 to 3 dosages of fertilizers and width of root is at peak after application of 4th dose. The similar observations are found for the upper part of the maize plant. The shoot length remains almost steady for each dose of fertilizer, but the width of the steam of maize plant is improved after third dose of ZnO NPs. It is generally observed that the suppression of seedling growth, loss of water uptake, chlorosis, leaf size reduction and loss of fertility are the results of deficiency in zinc level during plant growth [26]. But, the carefully administered dosages of ZnO NPs based fertilizers show enhanced growth of maize plants since the beginning of germination. In fact, the ZnO NPs not only promotes the seed germination but the plant growth, root-shoot length, number of leaves, dimensions of leaves and width of stem.

Table 3: Effect of ZnO NPs on seed germination in maize with percentage (out of 10 seeds)

Sample	Number of germinated seeds	Number of non - germinated seeds	Percentage of germination	Percentage of enhancement in the germination by treatment of ZnO NPs
ZnO	8	2	80%	
Control	6	4	60%	20%

Table 4: The summary of effect of ZnO nanofertilizer on different parameters of maize plant

Number of Spray	Concentration of ZnO NPs	Sample	Height of plant		Number of leaves per plant	Dimensions of leaves		Width of Stem in cm	Number of roots per plant	Width of root in cm
			Shoot length in cm	Root length in cm		Length in cm	Width in cm			
First	1mg / 100ml	ZnO	31	7.1	3	23.1	1.1	0.370	8	0.240
		Control	22.2	6.3	3	14.5	1.5	0.440	6	0.200
Second	5mg / 100 ml	ZnO	43.2	9.3	4	27.1	1.6	0.560	9	0.320
		Control	32.5	7.2	3	21	1.2	0.410	8	0.310
Third	10 mg /100ml	ZnO	47.5	11.1	5	35	1.9	0.610	11	0.410
		Control	37.3	8.1	4	30.2	1.5	0.520	9	0.360
Fourth	15mg / 100ml	ZnO	52.3	13.3	5	39	2.3	0.820	11	0.530
		Control	42.5	10.2	4	33.5	1.9	0.610	9	0.380

During the experiment, the ZnO NPs fertilizers are also applied to wheat plant. The similar treatment and procedures are followed at the time of experiment using wheat plants. The seedlings of wheat are processed with ZnO NPs before sowing that resulted in nearly 80% of seed germination compared to the control. The observations during the first week of dose show more width of the leaves compared to control.

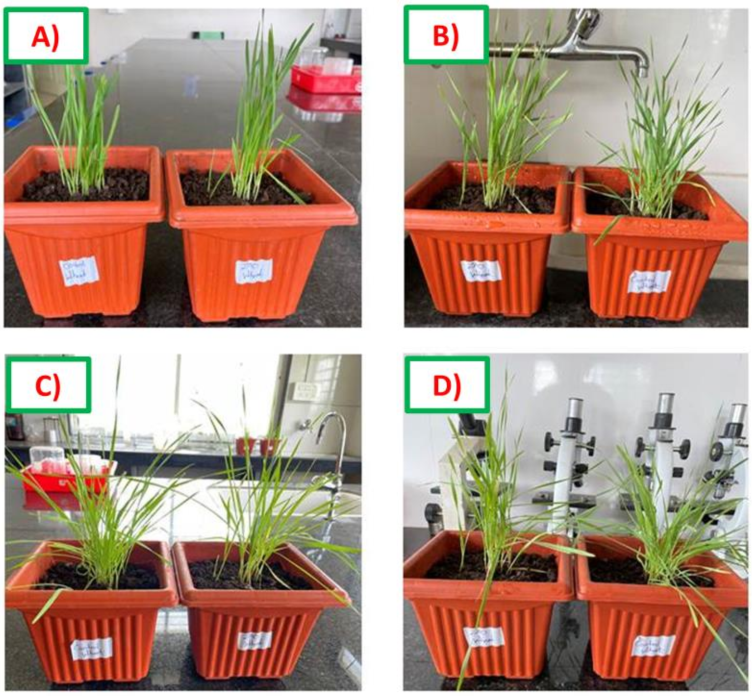


Fig. 6: Response of different concentration of ZnO nanofertilizer on growth of wheat plant with respect to control (spray duration : 7 days)

Fig. 6 (A) represents the improvement in the overall growth of wheat plants treated with ZnO NPs based fertilizers as compared to control. The length of the plants is increased and plants continue to grow after the first dose. Similarly, the leaves get elongated and 100% improvements in the width of leaves are observed after the use of ZnO NPs. There is also an increment in the number of leaves observed after first dose of the fertilizer; however, second and third dose show no response in increment of number of leaves. The fourth dose of fertilizer shows a little improvement in the number of leaves. It can be said that the trend is similar with the maize plant. The following Table No. 5 summarises the effect of ZnO NPs based fertilizer on different parameters of wheat plant. A small percentage of increment in shoot length is observed after the first dose of fertilizer which has reached to its height after the application of the second dose. It is also observed that there is a slow rate of increase of length of leaves.

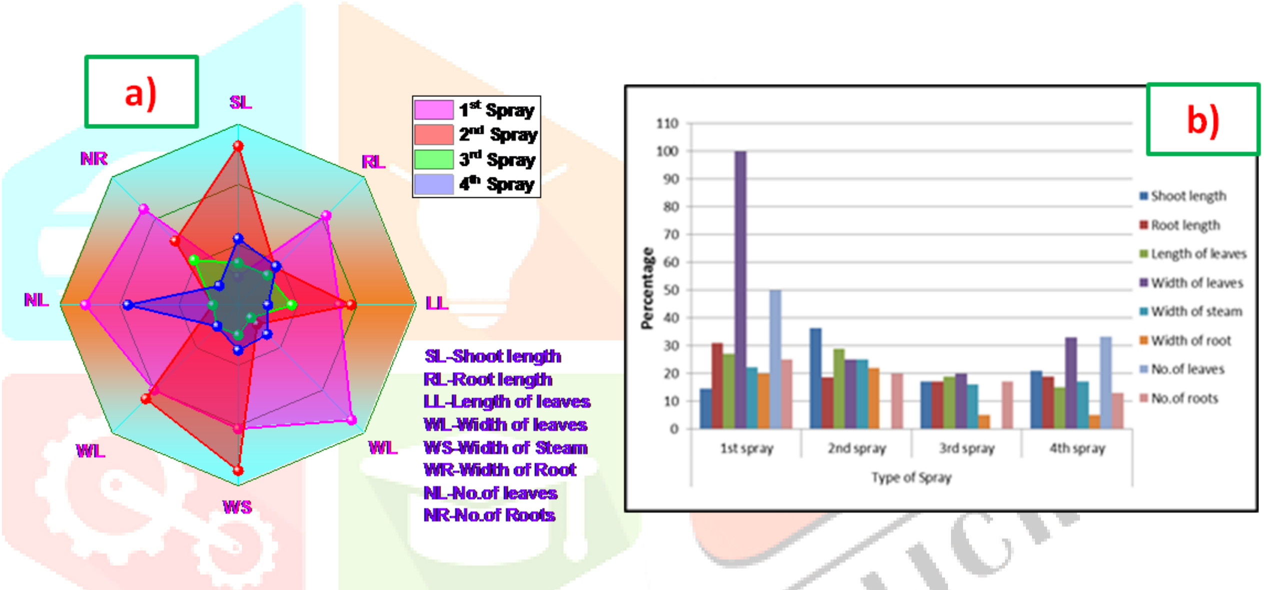


Fig. 7: Impact of sprays of different concentration on different characters of Wheat Plant (a) radar graph showing the improvement in the growth parameter of wheat plant as a function of ZnO NPs dose and (b) the percentage improvement in physical and morphological parameters with respect to spray duration

Figure 7 shows the impact of sprays of different concentration on different characters of wheat plant. Thus, it is observed throughout the period of experiment that ZnO NPs treated wheat plat showed significant enhancement from seed germination to overall growth of the wheat as well as maize plants.

Table 5: The summary of effect of ZnO nanofertilizer on different parameters of wheat plant

Number of Spray	Concentration of ZnO NPs	Sample	Height of plant		Number of leaves per plant	Dimensions of leaves		Width of Stem in cm	Number of roots per plant	Width of root in cm
			Shoot length in cm	Root length in cm		Length in cm	Width in cm			
First	1mg / 100ml	ZnO	17.2	5.5	3	14	0.4	0.220	5	0.130
		Control	15	4.2	2	11	0.2	0.180	4	0.150
Second	5mg / 100 ml	ZnO	28.1	5.7	3	23.1	0.5	0.250	6	0.210
		Control	20.6	4.8	3	19.5	0.4	0.200	5	0.180
Third	10 mg	ZnO	32.2	6.1	3	26.2	0.6	0.290	7	0.200
		Control	27.5	5.2	3	22.1	0.5	0.250	6	0.190
Fourth	15mg /	ZnO	35.1	7.5	4	28.1	0.8	0.340	9	0.230
		Control	28.9	6.3	3	24.5	0.6	0.290	8	0.220

Conclusion

The ZnO nanoparticles are created using a simple and cost–effective co-precipitation method. The physico-chemical properties of ZnO samples are studied using different techniques. The nanoscale ZnO powder has non-uniform distribution of the particles with average size in the range of 30-35 nm, which is beneficial for the plant cell uptake. As zinc deficiency is one of the most common micronutrient deficiencies in plants and soil, the application of ZnO nanoparticles to maize and wheat plants as a fertilizer show radical change in the development of the plants which can lead the crops to better yields ensuring increased productivity. For foliar application and after every spray concentration, the plants characteristics are enhanced by about 20% with respect to the control. The ZnO as nano-fertilizer shows promising efficiency for the enhancement of growth and yield on different characters of plants.

Acknowledgement

This research work is funded by Shivaji University's Research Initiation Scheme 2020-21 (SU/C&U.D. section/Proposal number 101/1397 with PI S. A. Vanalakar). Authors VLP, SRB and RRB greatly acknowledge the research grants from the Chhatrapati Shahu Maharaj Research Training and Human Development Institute (SARTHI), Pune (Government of Maharashtra) India for the financial support under the Chhatrapati Shahu Maharaj National Research Fellowship-2019 and 2021.

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