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Synthesis, characterization and effect of Mn doped ZnS nanoparticles on the growth of onion (*Allium cepa* L.) plant

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Abstract
Agriculture is always the backbone of many developing countries. In agriculture the main reason to use fertilizers is to providing macro and micro nutrients to soil for plant growth. Since, large scale uses of chemical fertilizer result in an irreversible damage to soil structure, microbial flora, plants mineral cycles and harmful effect on food chain. Nanotechnology is most innovative field and extensive research is going on for commercializing agricultural products throughout the world. Among other metal nanoparticles, zinc sulfide nanoparticles (ZnS-NPs) are very much important due to their application in agriculture. Zinc sulfide Nano particles have great potential to enhance agriculture production. This paper elaborates the synthesis, characterization, properties and application of Mn doped ZnS-NPs as Nano-fertilizer on onion plant growth. Synthesis of Mn doped ZnS nanoparticle was done by the co-precipitation method, while morphological properties had been studied using X-ray diffraction (XRD) spectroscopy and transmission electron microscope (TEM) to define particle size. The average particle was nearly ~3 nm calculated using XRD which is very close to TEM study. Onion (*Allium cepa* L.) belongs to family Liliaceae is one of the most important vegetable crops in India. The onion plants were sprayed four times at interval of 15 days with graded concentration of Mn doped ZnS-NPs as nano-fertilizer along with control. The growth parameter like plant height was determined at the time of flowering. Nano-fertilizer treated plant showed increase weight and quality of crop yield. Since fertilizers are main concern and developing nano based fertilizer would be a new technology in this field.

Keywords: Mn doped ZnS-NPs, XRD, TEM characterization, co-precipitation method, *Allium cepa*

Introduction

The global population increasing immensely and the cultivated area are decreasing day by day due to the urbanization and industrialization (Kaya and Higgs) [14]. To nourish the increasing people there is need to concern more yield per unit area in per unit time with maintaining the economic, social and ecological sustainability (Rani *et al.*, 2015; Singh *et al.*, 2021a) [23, 31]. Similar to the other issues it is analyzed that in the world and Indian soil zinc is the most wide spread deficient micronutrient (Singh *et al.*, 2020a) [29]. Zinc and sulphur (S) are essential micro-nutrient which required for metabolic reactions functioning in the plant system like zinc required for synthesis of indole acetic acid (IAA), synthesis of protein, chlorophyll, proteins and enzymes production, pollen germination, pollen development including in metabolic processes (Cakmak, 2008; Singh *et al.*, 2018a; Singh *et al.*, 2018b) [6, 27, 28]. Hence, to increase nutrient use efficiency nano-fertilizers being preferred because the nano-nutrient particles easily penetrate into the plant from applied surface, require less doses and give more surface area for different metabolic reaction in the plant system which enhance growth and development of crop (Rani *et al*., 2018a; Anjali, 2021) [1, 24]. Now day's nano-fertilizers are being used for site specific nutrient management to reduce the doses of fertilizer, cost of nutrient management and increase yield and nutrient use efficiency (Singh *et al.*, 2021b) [32]. Farm lands are losing their fertility due to human activities on them and societal change in lifestyle. This invariably affects the production of crops and could lead to famine and hunger, thus concerted efforts are necessary to improve plants for enhanced production (Singh *et al.*, 2021c; Singh *et al.*, 2021d) [33, 34]. Nanotechnology serves as the latest technology for precision agriculture, whereby strategies are formulated and channeled towards meeting with food demands and health of the increasing human population (Yadav *et al.*, 2016; Singh *et al.*, 2020b; Upadhyay *et al.*, 2021a; Upadhyay *et al.*, 2021b) [39, 30, 37, 38]. There is a diversion from the traditional ways of crop production to technologies that could increase agricultural productivity with required nutrients, cost effective and efficient resource use that guarantees nutrient security, uplifts the value of production, boosts farmers' economy, delivers agri-value chain to rural partakers and supports pollution free environment (Subramanian and Tarafdar, 2011; Rani *et al.*, 2018b) [36, 25]. Nanotechnology (NT) is a multidisciplinary science that has gained importance in agriculture and other economic activities including industries such as the textile, cosmetics, medicine, electronics, food production and hydraulics industries (Korkin *et al.*, 2010) [15]. Zinc (Zn) plays a key role in enzymatic activation for protein synthesis in plants. It is considered an essential microelement because it is required in small quantities but it is also crucial for vegetative development (Pandey *et al.*, 2010) [20]. Zinc sulfide (or zinc sulphide/ ZnS) is a white colour, soluble in water, and inorganic compound, non-toxic and non-oxide semiconductor exhibiting excellent properties (Bhargava *et al.*, 1994; Hullmann, 2006) [4, 13]. Different properties of ZnS have been improved with doping of different metals like Ag, Co, Ni, Cu, Mn etc. (Divya *et al*., 2011; Boxi *et al*., 2014;

Pourmoslemi *et al*., 2020) [11, 5, 21]. The doping of various metals in ZnS-NPs has been widely studied, depending on the research area. Mn doped zinc sulphide NPs used as a window for visible optics, infrared optics and optical foldable device (Dixit *et al*., 2009; Bala *et al*., 2021) [12, 2]. In addition to the Mn doped ZnS nanoparticles have great attention from the past few decades in biological field (Labiadh *et al*., 2016) [16]. Nano-fertilizer are synthesized or modified form of traditional fertilizers, fertilizers bulk material or extracted from different vegetative or reproductive parts of the plant by different chemical, physical, biological, method with the help of nanotechnology use to improve soil fertility, productivity and quality of crop (Mottaleb *et al*., 2021) [18]. Therefore, in this study authors synthesized and characterized Mn doped ZnS nanoparticles analyzed by XRD and TEM analysis. The effect of ZnS nano-ferlilizer on the growth of onion plant was determined for improved production to fulfill the need of population and country economy based on agriculture.

Materials and Methods

a. **Synthesis of Mn doped zinc sulfide (ZnS) nanoparticles:** Mn doped ZnS nanoparticles were synthesized by using co-precipitation method. In this method ZnS can be synthesized by using molar concentration 1:3 ratio. In a typical synthesis, 10 g of manganese acetate dissolve in 50 mL distilled water and stirring for 30 minutes on the other hand sodium sulfide (Na_2S) is dissolved in 200mL of distilled water and is kept under constant stirring for 30 minutes for complete dissolution and added drop wise to the other solution containing zinc acetate in 200ml of distilled water with constant stirring for 15 minutes to allow complete formation of nanoparticles. For the growth of particle this solution added in above solution and stirring for 15 minutes. Finally precipitating solution turns whitish cloudy. Precipitates was centrifuged and then washed 3-4 times with double distilled water. Obtained powder was dried for 12h in vacuum oven at 60°C. The particles were de-agglomerated in pestle-mortar to get a very fine powder was obtained. Calcination of the obtained product was done at 160ºC in hot air oven for 2 hr (Bera *et al*., 2018) [3].
b. **Characterization of Mn doped ZnS nanoparticles:** Characterization of Mn doped ZnS was done by XRD and TEM analysis. For the confirmation of phase formation, X-ray diffraction patterns was recorded on PAN analytical X'pert PRO between 10-75° (2θ) with a step size of 0.02° and matched with ICDD card with the help of X'pert High Score software. For optical measurements, Hitachi U-3900H (double beam UV–visible spectrophotometer) and Cary Eclipse Fluorescence spectrophotometer (G9800A) was used to obtain optical absorbance (200–800 nm) and emission spectra (340–600 nm with λexc=330 nm) of synthesized samples, respectively. Further, morphological features were recorded on JEOL 2100 F (200 kV; transmission electron microscope).
c. **Selection of plant:** The terrestrial plants were selected because of the following reasons: Easily available in plenty. Onion species are useful as a vegetable and medicinal purpose. Onion is an important source of natural vitamins, minerals, iron, phosphorus and protein quality. Onion (*Allium cepa* L.) belongs to family Liliaceae is one of the most important vegetable crops in India as well.
d. **Sample collection:** In the present study, the terrestrial NHRDF Red (L-28) plant was collected from the nursery, Karnal 2020. Before taking seedlings the zonation and varieties of plant was noted. Sample was collected from randomly selected spot of seedling bed preparation from the nursery. Stem were plucked from freshly collected seedling and the roots are dipped in water overnight.
e. **Land preparation for onion plant:** Prior to transplanting, field should be ploughed properly to eliminate debris and soil clods. Organic manures was incorporated at the time of last ploughing and beds with appropriate size (4.5ft) width and (3.5ft) length were prepared for onion after leveling. However, flat bed should be avoided by prevent water logging during kharif or rainy season.
f. **Transplanting of onion seedling:** The seedlings were transplanted on the side of ridges in soil. A pre-soaking irrigation is given 3-4 days prior to transplanting. Before planting seedling should be dipped in water for 2 hr. Proper care should be taken while selecting seedlings for transplanting. Over and under aged seedling should be avoided for better establishment. At the time of transplanting, one third of the seedling top should be cut for good result. The onion seedling should be transplanted after dipping roots in water. The optimum spacing is 15 cm b/w the rows and 10cm b/w plant to plant.
g. **Preparation of Mn doped ZnS Nano-fertilizer suspension:** Chelated bulk Mn doped ZnS nano-fertilzer was used for Zn source; the prepared materials were suspended directly in deionised water and dispersed by spray for 30 min on plant. Different concentrations (0.5, 1.0, 1.5 and 2.0 g/l) of solutions were prepared. Magnetic bars were placed in the suspensions for stirring to avoid aggregation of the particles. The nano-scale suspensions as expected too appeared as clear solutions. The pH of all the prepared suspensions was found to be 6.8-7.0. A control was also maintained, corresponding to pure water.
h. **Foliar exposure of plant to Mn doped ZnS nanofertilzer:** The impact of Mn doped zinc sulfide nano-fertilizer (Mn/ZnS NPs) foliar sprays at four different concentrations *viz.* 0.5, 1.0, 1.5 and 2.0 g/l at 15 day interval on onion plant were recorded. The NHRDF Red (L-28) as variety of onion was selected and transplanted into ploughed soil, and after two weeks, foliar spray done on15 February, 2020. The second foliar spray of nano-fertilizer on onion plants was performed on 12 March, 2020 using 1.0 g/l concentration after 15 days interval. Third foliar spray of Mn doped ZnS nano-fertilizer on onion plant was practiced on 28h March, 2020 using 1.5g/l concentration of ZnS nanofertilizer. The fourth foliar exposure of Mn doped ZnS nano-fertilizer on onion plant was done on 12 April, 2020 by sprayin 2.0g/l concentration of nano-fertilizer.
i. **Physical parameter of soil:** Various physical parameter of soil such as pH, electric conductivity and moisture content were analyzed according to standard protocol.

j. **Harvesting of onion:** Harvesting of onion bulbus was done at the maturity of crop, after Mn doped ZnS nanofertlizer application quantity and quality of onion were incresed.

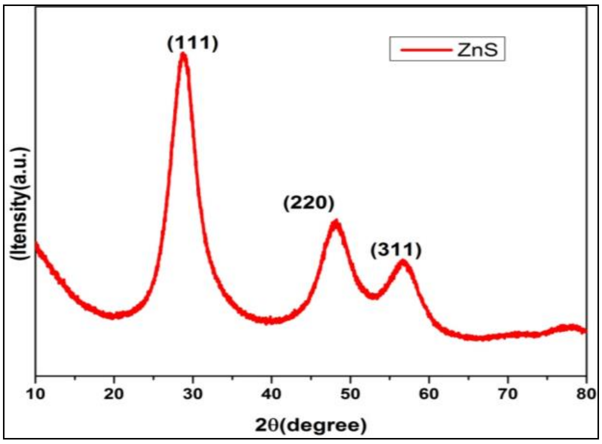

Fig 1: X-ray diffraction spectra of Mn doped ZnS nanoparticles.

Results and Discussion

a. **X-ray diffraction spectroscopy:** The X-ray diffraction (XRD) spectra of the prepared Mn doped ZnS nanoparticles showed three broad peaks at 2θ = 28.69°, 48.23° and 56.72° indicating the formation of nanostructure. These XRD peaks correspond to the (111), (220) and (311) planes of ZnS suggests cubic zinc blende phase [JCPDS card no. 5–0566] (Fig. 1). The size of the particle has been computed from the width of the first peak using Debye–Scherrer formula ($d = \frac{k\lambda}{\beta Cos\theta}$) after Mohamed (2020) [17]. Where K is constant (K = 0.9), λ the wavelength of X-ray, by the full width at half maximum and h is Bragg angle. The particle sizes obtained from XRD were in the range 2–3 nm which was well matched with literature (Zhu *et al.*, 2007; Murugados *et al.*, 2010; Chandrakar *et al.*, 2015) [44, 19, 7].

b. **TEM studies and particle size distribution:** A typical HRTEM (High resolution transmission electron microscope) image of Mn doped ZnS nanoparticles obtained from HRTEM image was found to be in the range of ~3 nm (Fig. 2a). This value was consistent with the XRD results. The size distribution of the particles were worked out (Fig. 2b) and the average size of the particle was 3.89 nm using gauss fit (Singhal *et al.*, 2012)[35].

c. **Foliar spray of Mn doped ZnS nano-fertilizer:** This research focused on chemical synthesis of ZnS nanoparticles for eco-friendly agriculture management for the improved production. The onion (L-28) seedlings were transplanted in the soil and foliar spray was performed 15 days interval at four different concentration of nanoparticles to check the efficency of nano-fertilizer (Fig 3). The soil collected from the roots of plants was good type of soil to prevent root diseases. The elongation of root, shoot and seedling of plants were recorded as total growth (27>17cm.), (32 >20cm.), (34 >29cm.) and (38 >31cm.) after the periodical sprays of Mn doped ZnS nanoparticles compared to control. The application of Mn-doped ZnS-NPs significantly improved the growth, yield and yield-attributing characters in onion. In the soil, pH value was mostly around 7, however, moisture content was around 50% in soil that necessary to maintain plants vitality (Table 1).

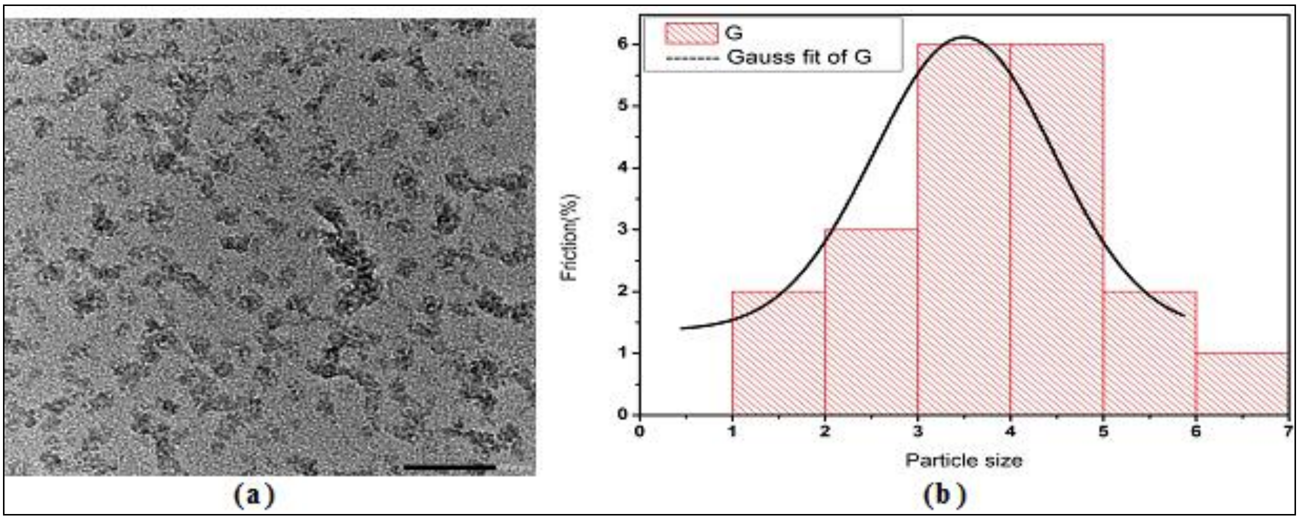

Fig 2: TEM image of Mn doped ZnS nanoparticles (a) and particle size distribution of Mn doped ZnS nano-particles (b).

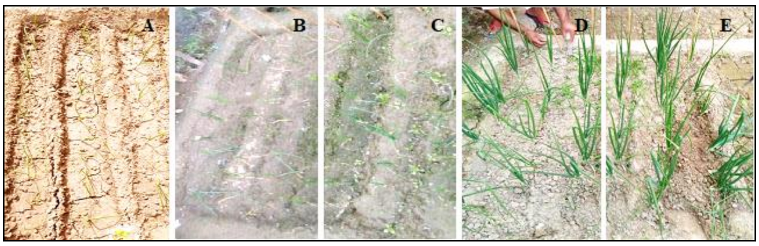

Fig 3: Foliar spray of Mn doped ZnS nano-fertilizer and its growth promoting effect on onion plants.

Table 1: Effect of Mn doped ZnS nano-fertilizer on onion plant growth.

Type of experiment	Total hight of plant (cm) after spray				
	Seedling planted on 15th Feb, 2021	1st spray on 1st March, 2021	2nd spray on 12th March, 2021	3rd spray on 28th March, 2021	4th spray on 12th April, 2021
Control	10-18 (15)	15-20 (17)	18-24 (20)	26-30 (29)	28-33 (31)
Mn doped ZnS nano-Fertilizer	10-18 (15)	24-30 (27)	29-34 (32)	33-36 (34)	35-39 (38)

Physical Parameters of Soil and Water

Experimental soil was analyzed according to procedure and characterize for their physicochemical parameters (Table 2). These samples were processed and analyzed for various physico-chemical parameters such as pH, Electrical conductivity EC, moisture content, etc. summarized in Table. 2. The conductivity of water was estimated 4.56 S/m. The pH, EC (electrical conductivity) estimation of the soil samples revealed an increase in the pH 6.10 to 7.55 after nano-fertilizer treatment of soil. The increase in pH ($p>0.05$) of the samples may be due to breakage of hydrogen bond and electrostatic interactions. The EC was observed 0.38 S/m and 0.23 S/m in onion control and onion nano-fertilizer treated soil respectively. Moisture content was analyzed to estimate total moisture percent in these soil samples. It was found that the highest moisture content 1.52% for control and 1.44 % for nano-fertilizer treated onion plant soil (Table 2).

Table 2: Physical characteristic of soil.

Soil sample	pH	Electric conductivity (Siemens)	Moisture (%)
Onion control	6.10	0.38 S/m	1.52 %
Onion Nano-fertilizer	7.55	0.23 S/m	1.44 %

Nano-fertilizer is any product that is made with nano-particles or uses nanotechnology to improves nutrient efficiency studied the effect of liquid organic fertilizer supplemented with chelated micronutrients (containing Zn) on red pepper and observed increased growth and yield (Deore *et al.*, 2010) [10]. Similarly Datir *et al.* (2010) [8] studied the effect of organically chelated micronutrients (containing Zn) on growth and productivity in okra and reported increased growth and yield due to chelated micronutrient fertilizer (Prasad *et al.*, 2012) [22] observed higher bioavilability of the ZnO-NPs in peanut because of their nano size and lower water solubility. The effect of ZnO-NPs on seed germination and seedling growth in onion observed that seed germination was increased in lower concentration of ZnO-NPs but showed decreas in values at higher concentration (Raskar *et al.*, 2014) [26]. The effect of liquid organic fertilizer supplemented with organically chelated micronutrients (Zn, Cu, Fe) in red pepper and tomato reported beneficial effects on seed germination and early seedlings growth, they attributed these favourable effects to the availability of micronutrients to seed during seed germination. Zinc enhances cation-exchange capacity of the roots, which in turn enhances absorption of essential nutrients, especially nitrogen which is responsible for higher protein content (Deore and Laware, 2011) [9]. These facts indicate that the availability Zn to seed or high Zn content within the seeds during seed germination had very important physiological roles in seed germination and early seedling growth. The higher percent seed germination and significantly more seedling length in onion seedling was observed in seed lots obtained from ZnO-NPs treated plants can be attributed to shipping of Zn from leaf tissues through phloem to the seed at the time of seed development and maturation process (Deore and Laware, 2011; Raskar *et al.*, 2014) [9, 26]. Nano-fertilizers worked as carrier based material which increase the soil-fertility.

Conclusion

Mn doped ZnS Nanoparticles stand out as one of the most versatile materials, due to their diverse properties, and applications. As far as synthesis of Mn doped ZnS Nanoparticles is concerned they can be synthesised by chemical method and their usage is concerned nanoparticle play a significant role in agriculture. Application of these nanoparticles to crop, increase their growth and yield. As food demand is increasing day by day the yield of staple food crop is much low. So it is need to commercialize metal nanoparticles for suistainable agriculture.This study confirmed the potential of foliar application of Mn doped Zinc Sulfide Nanofertilizer for growth of onion upto optimum applied concentration. They also have inhibitory effect on crop plant if concentration is more than the optimum which result reduces growth and yield of crop. This application of nano-fertilizer in agriculture should have a greater concern to society. It can be conclude that the using Mn doped ZnS-NPs is a cost effective, simple and easy method. That excludes the hazards arising out of harmful chemical fertilizers. That's why nano-fertilizer is beneficial and natural product, less toxic to chemical fertilizer and improve the soil fertility at a large scale.

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Conflicts of Interest

The authors claim no conflicts of interest because none financial support was received from any government, non-government agency or organization to conduct this research work.

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